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MYASTHENIA

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Editorial

This ailment is also known as the Erb-Goldflam disease, because in 1879 *Erb, and in* 1891 - 1893, *Goldflam made a very precise and accurate description of their main clinical and evolutive features.*

From the historical point of view we ought to say that the first clinical description of myasthenia was performed by Thomas Willis in 1672 *and Wilks made the first anatomo-clinical report of this illness in* 1877.

In 1895 *Jolly suggested that this disease ought to be known as pesudoparalitic grave myasthenia, and he established that the myasthenic reaction to the faradic stimulation that induces to a rapid muscular exhaustion is similar to the one produced experimentally by curare on the striated muscle.*

The first thymectomies on myasthenics with tumoral thymus were performed by Sauerbruck in 1917 *and by Blalock in* 1936, *who for the first time in* 1941 *made the extirpation of a thymus exempt of any tumor in a myasthenic patient.*

Thévenard in 1942 *proposed the bilateral enervation of the carotid sinus according to the technique developed by Leger in* 1938.

Myasthenia is a common disease. Kurland affirmed that in United States the prevalence rate is 3 *per* 100.000. *Garland and Clark stated that in England there is a similar prevalence.*

It is a muscular disease that may be started at any age, but generally it has its onset between 15 *and* 20 *years of age.*

It has been observed that besides the disturbances of the skeletical muscles there exist subtle alterations in smooth muscles, heart, lung, and central nervous system, which indicates that myasthenia gravis is a systemic disorder.

It has been accepted that this is a genetically determined autoimmune disease generated by perturbations in the lymphoid system. When it starts in the adult age the frequency is the same in both sexes. But if it has its onset in youthfulness, the feminine sex is more frequently affected there being statistically five women for one man. In women it may be initiated during puberty or pregnancy.

It starts as a muscular weakness that appears by making an effort and disappears at rest. This condition generally becomes worse in the afternoon and evening. In some patients the troubles can be worse when waking up in the morning.

The illness generally starts without there being any apparent reason; but sometimes it is preceded by disturbances that make the disease evident, as an infection, or an intoxication, an emotional stress, an excessive fatigue, an endocrinal factor in the woman (puberty, menstruation, pregnancy), a trauma, an anesthesia, an antitetanic serotherapy, followed by seric accidents, a medication (quinine, hydantoine, chlorpromazine, procainesterase, morphine, streptomycine, neomycine, kanamycine) a thymectomy due to a tumor of the thymus.

The muscular weakness in myasthenia has preference for determined sectors. Its more frequent localization is in ocular musculature, producing transitory diplopia, asymmetrical palpebral ptosis, imperfect oclussion of the eyes. The iris sphincter is commonly involved in myasthenia.

Rapid eye movements, having high velocity and low amplitude are common in myasthenic patients. Probably this is pathognomonic of myasthenia gravis and they are called quiver movements. The preservation of these rapid eye movements, even when an ophtalmoplegia exists, is due to the fact that muscle fibers that are responsible of the rapid movements are not much affected.

Following in frequency to the eyes, the face is the part more affected with difficulty for doing mimicand to whistle and to blow. The masticatory muscles leave the jaw dropped during the meals. There is dysphagia and dysarthria.

In some cases the myasthenia gravis shows only one muscle affected. We will refer to the external rectus and superior obliquous of one eye or an isolated grievance such as occurs in jaw ptosis from the inability to oclose the mouth.

The muscles of the neck reveal its weakness.

Concerning the members the proximal muscles are generally affected.

Following them in frequency and intensity are the muscles of the arm and thigh, of the hand gluteal area. Finally the abdominal, intercostal and distsl muscles of the inferior limbs are affected. In myasthenia the distal muscles are relatively preserved. This may be explained by the fact that in myasthenia there exists a relative sparing of faster twitch fibers and distal muscles which have a higher percentage of fast twitch fibers than the proximal muscles.

Among the muscles of the trunk special attention must be given to the intercostal muscles, the diaphragm in a radioscopic examination and the accesory inspiratory muscles. A spirographic observation of pulmonar ventilation before and after prostigmine is very useful.

The weakness of these muscles is in the origin of the respiratory crisis that determines the gravity of myasthenia Muscular disturbances may be symmetrical, but frequently they are asymmetrical. The arm and the leg of the dominant side are the more affected.

Deep tendon reflexes are generally exalted sometimes with clonus. But if the reflex is repeatedly excited it may be observed that the reflex progressively diminishes until it disappears.

Some patients evidence signs that reveal disturbances in CNS, Uni or bilateral Babinski sign, Hoffman sign, cross adductor reflexes. Generally these manifestations disappear when the disease is controlled by the therapeutic prescribed.

It may be the case that the patient has the overlap syndrome, in which clinical and laboratory findings of myasthenia gravis and multiple sclerosis coexist.

Other nervous disturbances have been observed in patients with myasthenia gravis, for instance, abnormal sleep and decreased REM time, ageusia anosmia

transitory trigeminal anesthesia and we have not mentioned all the nervous disturbances that may be concomitant.

It has been demonstrated that 5 % of myasthenic are also epileptics. It has been thought that antiepileptic drugs may be a factor for the appearance of myasthenia. Particularly hydantoins agravate myasthenia. The graveness of myasthenia is related to the onset of respiratory crisis.

There are two types of crisis, the myasthenic crisis produced by aggravation of the disease and the cholinergic crisis produced by an overdose of anticholinergic drugs.

The sudden death of the myasthenic patient is not rare; it has been seen above all in myasthenia with an acute evolution and it is produced by a myasthenia access of the respiratory and pharyngeal muscles. The process of myasthenia gravis is associated with immunological disturbances and with changes in the thymus.

By X-Ray studies a tumor of the thymus may be evidenced that may be of a benign or a malign type and it is much more frequent in the adult myasthenic than in the young one.

At the radiological studies contrast methods have been proposed, gaseous mediastinumgraphy, thymic arteriography, selective thymic phlebography.

It has been observed that even if there is no evidence of a tumor, the thymus in myasthenia gravis frequently shows changes in its structure. 80 % of the myasthenics show thymus hyperplasia.

Thymoma is present in 25 % of the patients. Hyperplasia is a pre-malignant lesion, that may evolve into a thymoma.

From an immunological point of view it has been observed that myasthenia gravis is an autoimmune disease which generates antibodies that act against the individual own structures concretely against the acetylcholine receptors of the muscles.

The acetylcholine receptors may be indentified in situ by α bungarotoxin that is a neurotoxin with a specific curarizing action.

In human motor plate the number of acetylcholine receptors and their distribution may beestablished with radioactive derivates of the α bungarotoxine. They are particularly concentrated in the summit of the folds of the postsynaptic membrane. It has been observed that in each neuromuscular joint there exists about 20 million of molecules.

In myasthenia gravis with this technique a reduction of 80 % of the amount of the acetylcholine receptors has been virified.

Experimentally an autoimmune myasthenia can be obtained in animals injecting acetylcholine receptor protein extracted from the electric fish.

The serum of myasthenic patients contains antibodies to acetylcholine receptors that if they are incorporated to mice causes in these animals disturbances similar to that of the myasthenic patient, diminution in the number of acetylcholine receptors per neuromuscular junction, reduction in miniature end plate

potential, also decremental responses to repetitive nerve stimulation.

Myasthenia gravis may be accompanied by other autoimmune diseases, like acute lupus erythematosus, chronic evolutive polyarthritis, thyroids autoimmunes diseases, and chronic lymphocytic leukemia. It is observed in the myasthenia myocardial abnormalities, also it has been verified smooth muscle involvement that improve after thymectomy.

With certain frequency it appears along with polymyositis and with myopathic syndrome.

It has been established that 9 % of the myasthenic men and 18 % of the myasthenic women evidence thyroid disturbances.

Hyperthyroidism is the thyroid ailment more frequently associated with myasthenia

Hyperthyroidism aggravates myasthenia.

Myasthenia associated with hypothyroidism is more rare.

Thyroiditis may also appear with the myasthenia.

Another hemopathies besides leukemia may be present associated with myasthenia, it has been pointed out hemolytic anemia, Biermer anemia, aplastic erytroblastopenic anemia

Alterations in the respiratory function, bronquial asthma can be observed.

Cancer is also more frequent in these patients specially when thymectomy has not been done with therapeutic purpose.

In men the most frequent localization is colo-rectal, for women is in the breast.

These conditions frequently are accompanied with leucopenia and deficiency in IgA levels.

It is characteristic of myasthenia and many other autoimmune diseases that if the patient is under psychological stress the process is aggravated.

Probably in myasthenia the stress acts by means of the corticotropin-releassing hormone and adrenocorticotropin-cortisol axis with its effects on suppressor T lymphocytes and immune surveillance.

Cortico-steroides reduce aggressivity of T lymphocites and the facts indicate that the stress may reduce immune surveillance to somatic mutations that result in malignancy in humans.

BIOLOGICAL STUDIES

Antinuclear antibodies are positive in uncomplicated myasthenia gravis in about 18 % ofcases and in 54 % of myasthenia associated with thymoma.

Antistriated muscle antibodies can be found in a proportion of 11 % of not complicated myasthenia and in all of myasthenics with thymoma.

A very important serologic test in myasthenia is the Ig G antibody directed against the acetylcholine receptor.

Related with myasthenia is the complex system of antigens bound to the hystocompatibility, they are represented as HL-A that means histocompatibility

locus-A and it corresponds at least to 30 antigens that are distinguished by numbers. It has been proved that patients with myasthenia gravis and thymoma show a higher frequency of AL-A_2 and a lower frequency of HL-A_1 and HL-A_8 that show the patients with myasthenia gravis without thymoma.

Frequently there exists a clear diminution of changeable potassium.

Also is frequently observed augmentation of serum gammaglobulin and diminution of serum albumins.

ANATOMY

In anatomic studies changes are verified in the myasthenic muscle, and inflammatory manifestations appear in some fibres of variable intensity. On observe also deterioration in the motor plates.

ELECTRICAL STUDIES

The electromyography reveals the existence of the myasthenic block to the stimulation with progressive diminution of the potential amplitude. Other clinical tests may be considered useful to evaluate neuromuscular transmission, they are repetitive stimulation of nerve, recording the compound evoked muscle action potential, single fiber electromyography and pharmacological tests.

PHARMACOLOGICAL TESTS IN MYASTHENIA GRAVIS

Tensilon is employed as a test for diagnosis of miasthenia gravis. An initial IV dose of 3 mgr, is given and if there are no changes in 60 seconds 8 mgrs are added.

The neuromuscular blocking action of curare is used as a test and it has been observed that myasthenic patient are 10 to 100 more sensitive to the drug than normal persons.

Negative tests have been observed in cases of purely ocular myasthenia and in cases of generalized myasthenia in remission.

PUPILLOMETRIC TESTING - AUDIOMETRIC TESTING

The clinical value of these testing are investigated.

ERGOGRAPHY

The record of the muscular contraction with the adecuate equipment allows us to visualize the myasthemic fatigue.

ESOPHAGEAL BARITED TRANSIT

It may be studied, before and after prostigmine.

RESPIRATORY FUNCTIONAL EXPLORATION

Its importance is linked to the danger of the respiratory failure in Myasthenia.

It must be also done before and after prostigmine.

RADIOGRAPHY

Radiography of the chest is necessary to recognize a thymoma.

Contrast medium methods can be used, gaseous mediastinography, thymic arteriography, selective thymic phlebography.

TREATMENT

For treating the myasthenia the neuromuscular junction must be stimulated to compensate the relative deficiency of acetylcholine.

It is also necessary to suppress the abnormal immune basis of the disease.

To improve neuromuscular transmission the anticholinesterase drugs are employed, such as pyridostigmine and neostigmine.

The Mestinon is widely used. It has few cholinergic lateral effects.

With the end to act upon immune mechanism ACTH and adrenocorticosteroids are used.

There is a tendency to employ ACTH in patients in which there is no response to treatment with thymectomy, anticholinesterases and oral corticosteroids.

Most of the cases of Myasthenia gravis benefit from oral corticosteroids. It is necessary to increase the dose of prednisone from 25 mgrs orally every other day to 100 mgrs every other day.

When the patient feels better during three months, the doses of prednisone may be decreased by 5 mgs each month until reaching the lowest dose necessary to maintain the patient in good shape.

THYMECTOMY

With the experience acquired in treating Myasthenia Gravis the thymectomy nowadays is considered the best treatment for all cases of autoimmune myasthenia.

When the thymectomy is performed at the onset of the illness, the pronostic is generally better, and there are less changes at the hystological study in the removed thymus-

Patients that have thymoma, to be benefited after the thymectomy, ought to receive treatment with prednisone or immunosupression therapy.

We must realize that with thymectomy the patient is benefited in different and important aspects, the medication is reduced, the probability of a remission of the disease is more possible, it is difficult that the thymoma or a systemic cancer may appear. It is also performed immunosuppressive treatment of myasthenia gravis that is effective and may cure the patient. It is advised cyclophosphamide to a daily dose of 100 mgs. This treatment is reserved to be employed in those cases with thymoma in which common therapy is not successful. Older patients may be benefited by intramuscular injections every three weeks of 10 to 20 ml. of human gammaglobulin.

In case that they do not respond to the therapeutic resources that we have

mentioned in a desirable level, a thoracic duct drainage is employed in myasthenia gravis. Plasmapheresis is a technique that is experimentally used with good preliminary results. There is a tendency to consider it a treatment generally effective. The procedure conduces to the elimination of antibody and immune complexes producing receptor damage.

MYASTHENIC SYNDROMES

We will refer now to Myasthenic Syndromes originated by different causes. They may be observed in cancer metabolic disturbances, infectious diseases, endocrinopathies, intoxications and other muscular diseases. In these illnesses, conditions are generated that affect the neuromuscular transmission.

MYASTHENIC SYNDROME OF EATON AND LAMBERT

Generally in this Syndrome the weakness starts in the proximal muscles of the lower extremities. Compromising symptoms of extraocular and bulbar muscles are very few times observed. The deep tendon reflexes are absent or diminished.

It is very frequent that this myasthenic syndrome appears in patients suffering from carcinoma and is 5 times more frequent in men, than in women.

In this syndrome the defect in presynaptic release of Ach happens as much in the skeletal muscles as in the smooth muscles and in this way are originated symptoms of autonomic nervous system. Anti-Ach RAb absent.

The Eaton-Lambert Syndrome can be also observed in patients with pernicious anemia, Sjögren's syndrome, thyroiditis, hypothyroidism and hyperthyroidism, perhaps produced by an autoimmune mechanism.

Guanidine or anticholinesterases may benefit the patient.

ENGEL'S DISEASE

The patient looks like a non progressive congenital myasthenia. Ant-AchRab absent. Very small nerve terminals, absence of endplate acetylcholeniesterase and small amounts of postsynaptic acetylcholine receptors have been observed.

In studies made with nerve stimulation repetitive muscle action potentials from single nerve stimulus are observed.

A decremental response at all frequencies of stimulation. It is irresponsive to anticholinesterases. Prednisone 50 mg. daily improves the patient.

CONGENITAL MYASTHENIA

Extraocular muscles are principally affected with little involvement of corporal muscles, antiacetylcholine receptor antibodies are not found in serum, which indicates that this syndrome is related to genetic defects.

Congenital Myasthenia never remits. It may be familial.

Electrodiagnostic tests show small short duration poliphasic motor unit potentials. Repetitive stimulation produces decremental responses at low rates

with post exercise exhaustion. It responds to anticholinesterases. There is no response to thymectomy.

NEONATAL MYASTHENIA

Nearly 12 % of the children born from myasthenic mothers show this myasthenia which starts within 72 hours of birth.

It has a short duration that varies from 3 to 47 days and afterwards disappears.

Anti-acetylcholine receptor antibodies are present. It esponds to anticholinesterase and exchange transfusion.

FAMILIAL INFANTILE MYASTHENIA

No myasthenia exists in the mother. Familial myasthenia is observed in other siblings.

Antiacetylcholine receptor antibodies are absent. It has been differentiated from congenital myasthenia by the lack of ophtalmoplegia, severe respiratory and feeding difficulties and a tendency to spontaeous remissinon.

Anticholinesterase is considered the best treatment.

BOTULISM

This illness has a toxic origin for poor food processing techniques, also may be originated by wound or gastrointestinal infection by clostridium botulism. The effect of the exotoxin is produced by blocking the release of acetylcholine at the terminal axons of the neuromuscular junctions.

In the adult with intoxication appears ptosis palpebral, diplopia, dysphagia, dysphonia, dyspnea, dysmimia, resporatory difficulty, quadriplegia. Profound reflectivity decreases or disappears, sensitivity disturbances do not exists. Mind is not affected.

Needle EMG results in small duration potentials.

In children botulism is produced by colonization of the gastro intestinal tract by clostridium botulism. The child presents hypotonia, hyporeflexia, weakness of cranial and corporal muscles and respiratory failure. Internal or external ophtalmoplegia may be observed. Needle EMG results in small duration potentials.

It is considered also the wound botulism that appears in young persons who are injured ou doors. Clinically on observe diplopia, dysartria and dysphagia. May be observed paralysis of the four members.

Infection in man is generally caused by Clostridium botulism in type A, less frequently by type B and rarely by type E. Guanidine is of benefit in botulism.

MAGNESIUM RELATED NEUROMUSCULAR DISTURBANCE

Magnesium is the body's fourth most abundant cation and the second most abundant intracellular ion. It is very much related to the neuromuscular system and its changes have repercousion in it.

It is important in synaptic transmission and membrane excitability.

Magnesium exists in bone, liver, brain and muscle.

It is actively transported accross the blood brain barrier. It maintains the activation of many enzymatic reactions, ion transport, phophate transfer, glycolysis.

Magnesium reaches de organism by foods, particularly vegetables and meats.

If magnesium is found a large proportion in an internal medium, it blocks neuromuscular transmission, interfering with presynaptic release of acetylcholine and promoting a lowering of postsynaptic sensibility to acetylcholine.

Repetitive stimulation studies evidence decreases as they are observed in myasthenia gravis.

The etiological diagnosis of the clinical picture is made by the observation of increased levels of serum magnesium and the acetylcholine receptors antibody.

ENDOCRINOPATIES

It is possible to observe myasthenic syndromes in adrenal insufficiency, hipercorticisme, hyperthyroidism.

INFECTIOUS DISEASES

Myasthenic syndromes may be observed in patients with typoid fever diphtheria or epidemic encephalitis.

NEUROGENIC AMYOTROPHIES

Myasthenic Syndromes have been observed in anterior chronic polyomyelitis, in multple sclerosis and in amyotrophic lateral sclerosis.

MUSCULAR DISEASES

Muscular diseases, myositis and myopathy may be accompanied by myasthenic syndrome.

ANTIBIOTIC NEUROMUSCULAR BLOCKADE

This myasthenic syndrome is accompanied by a dilatation of the pupils and the blader becomes atonic.

It is originated by the action of some antibiotices that deteriorate the neuromuscular transmission. It is observed with certain frequency in patients that were submitted to a surgical intervention and that presents a low level of serum calcium and chronic renal disease.

Regarding the antibiotics that causes this syndrome, some interfere with presynaptic mechanisms, others with postsynaptic mechanisms most of them affect both presynaptic and postsynaptic mechanisms.

DRUGS THAT DAMAGE NEUROMUSCULAR TRANSMISSION

Varied drugs conduce to myasthenic picture.

Lithium affects directly transmission mechanisms. Mysoline and penicillamine it is believed that induce autoantibodies directed against the acetylcholine receptor.

In myasthenic patient an increase of muscular weakness is produced if for therapeutic purposes quinidine is used as an antirrythmic drug. There is not accord relative to the action of dilantin. As a consequence of its deprimental effect on membrane excitability, which gives significance to its use as an anticonvulsant, some authors consider that it may be a factor of muscular weakness. This opinion is not accepted by other scientists.

TICK PARALISIS

It has been observed that sometimes poisoning by scrubticks produces a neuromuscular paralysis.

The toxic substance is liberated by the adult pregnant female tick.

The embedded tick injects into the epidermis in scalp hairtoxin loaded of salivary fluid. This toxin makes the release of acetylcholine difficult at the neuromuscular junction and reduces nerve action potentials.

The patient develops a generalized illness with anorexia followed by weakness of the lower limbs. In a few hours an ascending paralysis of four limbs, and the bulbar muscles is observed. The respiratory muscles are affected.

Death happens from respiratory paralysis.

FISH POISONING

Toxins from fish and shell fish may produce grave general diseases- The responsible substance in cignatera fish poisoning is cignatoxin, which in the studies performed it generates a cholinesterase inhibition with its consequence in the neuromuscular transmission, besides affecting directly nerve transmission.

Other toxins that have been described are Tetrodotoxin that blocks action potentials in excitable cells affecting sodium permeability.

In shell fish poisoning saxitoxin inhibits entry of sodium ions into nerve and muscle cells, which negatively affects action potentials.

SCORPION STING

Its toxin in its action on the nervous system is similar to that of spider toxins. It liberates central and peripheral transmitters, producing an excessive nervous system discharge, followed by the blockade of neuromuscular transmission.

SPIDER BITE

Spider toxin acts on the nervous system, it causes the release of neurotransmitters from nerve endings, in sympathetic ganglia, muscarinic cholinergic ganglia and effectors and nicotinic cholinergic end plates followed by blockade of neuromuscular transmission.

This subject of which we have made a synthesis in this Editorial will be treated extensively in this issue of the International Journal of Neurology by very distinguished scientific authorities, eveyone of them an star of first magnitude in this field.

We are very happy and proud to offer our readers the crop of their dedicated lives.

To everyone of them our most expressive thanks, for their kind and valuable cooperation with our publication.

It is an honour and a pleasure to announce that Dr. Murray Goldstein from United States is incorporated to our Editorial Board.

We give him our warmest wellcome, in the certainty that he as other members of our Board, will be engaged in perfecting our publication in spreading knowledge at its best and strengthening our motto "Fraternity Through Science".

Prof. Dr. VICTOR SORIANO.

Antibody to Acetylcholine Receptors in Human MG

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INTRODUCTION.

Demonstration that rabbits immunized with acetylcholine receptor purified from electric eels developed muscular weagness [1] suggested that an autoimmune response to acetylcholine receptors could impair neuromuscular transmission and produce symptoms resembling myasthenia gravis. This revived a suggestion made years before [2] that antibodies to receptor acting as curare-like antagonists of receptor might be responsible for MG in humans. Subsequent studies of rabbits [3] provided methods for detecting and quantitating anti receptor antibodies in serum, but showed that, although antibodies could impair receptor function, most of the antibodies in the serum were not curare-like in their effect on receptor. Studies of rats immunized with purified receptor [4-11] showed that chronic "experimental autoimmune myasthenia gravis" was a good model for pathological mechanisms impairing neuromuscular transmission in human MG, though not, of course, a model for whatever mechanisms are responsible for the induction of MG in humans. These studies showed that impairment of transmission by the autoimmune response to receptor was largely humorally mediated, but that the mechanisms involved were far more complex than simply a curare-like effect of anti-receptor antibody.

Assay of anti-receptor antibodies

Several assay methods have been published for the assay of antibodies to receptor in the sera of patients with MG [12-15].

The most sensitive of these methods use the immunoprecipitin technique developed [3], for detection of antibodies to acetylcholine receptor in the serum of rabbits immunized with receptor purified from electric organs. Receptor from both human [14,15], and animal [13] muscle, has been used as antigen in these assays. Because human antireceptor antibodies [5,16], are highly species specific, the use of human receptor has the advantages that antibodies can be detected more sensitively, and hence in a larger proportion of MG patients, and that all antibodies to receptor present are detectable. The use of receptor from animal muscle as antigen detects only that fraction of antibodies which cross react, but has the advantage that receptor from animal muscle is more readily obtained than receptor from human muscle. Since, as will be discussed below, detection of the presence of antibodies and measurement of changes in the relative amount present is probably more important than determination of the absolute titer of antibodies present, the chief advantage of using receptor from human muscle in the assay for serum antibodies to receptor is the increased sensitivity of the assay.

The assay method we use [14,17] has the virtues of being sensitive, quantitative, reproducible and rapid. Human muscle is obtained from amputations. The muscle is homogenized and the particulate fraction pelleted. The pellet is extracted with detergent to solubilize receptor from the muscle

membranes. The detergent extract is incubated with ^{125}I α bungarotoxin, which binds which great specificity and affinity to the acetylcholine binding site receptor. As a control, another aliquot of extract is first incubated with a high concentration of the antagonist benzoquinonium to prevent specific binding or the ^{125}I α bungarotoxin, which is added subsequently. Aliquots of serum are incubated in the presence of ^{125}I toxin labeled receptor, then goat anti-human γ G is added to precipitate the serum γ G along with any antibody-^{125}I toxin-receptor complexes present. ^{125}I in the washed precipitate is measured and a background value for samples using the benzoquinonium extract is subtracted. When the amount of antibody present is much less than the amount of receptor present in the assay mix, the amount of ^{125}I toxin-labeled AChR precipitated is directly proportional to the amount of antibody added. Antibody titers are reported as moles of toxin binding sites precipitated per liter of serum. Thus, antibody to receptor in human serum can be measured in the same units used to measure antibody to receptor in rats, units which can be directly compared with the moles of toxin binding sites of receptor which can be extracted from human [18] or animal muscle [10,11].

Incidence and amount of serum antibodies to receptor in patients with MG

We have been able to detect antibodies to human acetylcholine receptors in about 90 % of patients [14,17]. The reasons for the failure to detect antibodies in the sera of the remaining patients thought to have MG may include that antibodies are present in these patients at concentrations too low to detect or that the muscular weakness in these patients is caused by different mechanisms. Antibodies to receptor were not detected in patients with other neuromuscular or autoimmune diseases.[17] This indicates that production of autoantibodies to receptor is specifically associated with myasthenia gravis and not an epiphenomenon of muscle degeneration.[11]

Concentration of anti-receptor antibodies varied over a wide range in the patients studied.[17] We defined the minimum significant titer as four standard deviations above the mean nonmyasthenic serum assay value.[17] Titers observed have ranged from near this minimum value of 0.6×10^{-9}M to more than 1000×10^{-9}M. The average titer is around 50×10^{-9}M. We found an average receptor concentration in normal human intercostal muscle of 2.9×10^{-13} moles/gm [18], and in patients with MG this amount was reduced to an average of 1.0×10^{-13} moles/gm. Thus an MG patient with a titer of 50×10^{-9}M might contain of the order 10 fold more antibody in his serum than necessary to bind all the receptor in his muscles.

Correlation of antibody titer with severity

Although patients with ocular signs only had significantly lower average titers than other MG patients, there is not a close correlation between serum anti-receptor antibody titer and severity in the populations of MG patients seudied.[17] However, there is some evidence that antibody titer of individual patients can change according to the severity of their disease. Neonatal myasthenia is associated with transplacental transfer of maternal anti-receptor antibodies, and remission of a babies' weakness was associated with clearing of maternal antibodies.[19] Plasmaphoresis [20,21] and immunosuppressive drug therapy produces a reduction in serum anti-receptor titer [21] which is associated with reducer severity of MG. Increased titers were associated with periods of exacerbation in some of these patients.[21] One patient given the immunosuppressive drugs, but no plasmaphoresis, showed neither much of a reduction in titer nor a reduction in severity,[21] while another who did show some improvement on drug therapy alone also showed a decrease in titer. There is other evidence consistent with the idea that antibodies to receptor are important effectors of the autoimmune response in MG. For example, Drachman and co-workers have shown that antibodies to receptor from MG patients can induce myasthenic signs in mice.[22]

Why is antibody titer not closely associated with apparent severity of MG in a population of MG patients? One possibility is that in some patients cell mediated immune responses may play a more important role. Another possibility is that total anti-receptor titer is not so important as the titer of anti-receptor antibodies of certain specificities or activities. For example, antibodies directed at determinants on the re ceptor molecule facing the interior of the cell would be detected by assays using solubilized receptor as antigen, but these antibodies would not be able to bind to receptor *in vivo*. Antibodies of one specificity might very effectively inhibit the activity of the receptors to which they are bound, while antibodies of another specificity might bind to parts of the receptor molecule that did not effect its function. Antibodies might also differ in their ability to induce antigenic modulation of receptor or interact with complement, both mechanisms which might be important in MG, as will be discussed later. Another source of variation is the ability of the endplate to withstand the various forms of immunological assault. The safety factor for neuromuscular transmission is usually quite large. For example rats with experimental autoimmune myasthenia gravis might lose nearly 90 % of their functional acetylcholine receptors before signs of clinical weakness become evident.[18] The MG patients which showed marked improvement after plasmaphoresis[21] did not have antibody titers reduced to normal levels, but simply reduced to ~ 30 % of the starting value. It is probable that these patients still had substantial loss of receptor, and that many of their remaining receptors were labeled with antibodies, but that reduction in antibody titer shifted the balance between rate of receptor synthesis and destruction sufficiently to insure more effective transmission. Variations between patients in ability to compensate for the immune assault might contribute to differing severities of impairment produced by the same concentration of serum antibody.

Usefulness of the assay

Apart from any significance the absolute concentration of anti-receptor antibodies in serum may have, the detection of anti-receptor antibodies provides a sensitive and objective diagnostic test for MG. Hopefully this assay will see increasing use to screen patients suspected of having MG and provide earlier and improved diagnosis. Because immunosuppressive therapy of one form or another seems a rational approach to the treatment of MG, and because measurement of anti-receptor titer seems a rational approach to measuring the effectiveness of this therapy, assay of anti-receptor titer may see increasing use to monitor treatment of those diagnosed as having MG.

Specificities of antibodies to receptor

The assay described[14] detects only antibodies directed at determinants on the receptor protein other than the acetylcholine binding site, because the binding site is occupied by ^{125}I toxin. This represents most, if not all, of the antibodies present.[17] Even antibodies bound to receptor *in vivo* do not prevent ^{125}I toxin binding to antibody-receptor complexes solubilized from muscle.[6,18]

Antibodies to receptor in the sera of MG patients are species specific.[6,16] Antisera from MG patients, in general, cross react with receptor from squirrel monkey muscle to the extent of nearly 50 %.[16] In general, a descending order of cross reaction is seen with receptor from calf muscle or the mouse cell line BC3H1, much less cross reaction is seen with receptor from rat muscle or torpedo electric organ, and negligible cross reaction is seen with receptor from eel electric organ.[16]

The extent of cross reaction varies extensively between patients. A practical implication of these results is that receptors from monkey or calf muscle are reasonable alternatives to receptor from human muscle for assaying sera from MG patients. Receptor from denervated rat muscle is a better antigen for detecting antibodies in MG patients than is receptor from normal muscle.[13] A theoretical implication is that the immunogen in MG patients has properties that would be expected of human receptor. However, if the immunogen in MG patients

is receptor, not all antigenic determinants on receptor are immunogenic. This is shown by the fact that animals immunized with receptor from eel electric organ produce antibodies that cross react with receptor from human muscle, although the human antisera don't react with eel receptor.[16]

Pathological Effects of Antibodies to Receptor

Antibodies to receptor purified from electric organs can inhibit the activity of receptor in electric organ cells [3,23] or muscle.[24] Presumably, antibodies in MG patients could have the same effect. From studies of acetylcholine noise in rat muscle treated with anti-receptor sera, it was found that net conductance of functioning receptors was inhibited only about 30 %.[24] This is consistent with the observation that at least most of the antibodies bind at determinants other than the acetylcholine binding site, and thus need not produce a curare-like all or none block of receptor function. Binding of antibodies to 67 % of the receptors in a normal rat under conditions in which no complement mediated destruction can occur produces no electromyogram decrement.[25] This is the result expected from the observations of only partial blockage of receptor function [24] and a large safety factor for transmission.[9] However, presumably, in both rats or humans suffering from the extensive receptor loss characteristic of MG,[10,18] the further loss of a substantial fraction of remaining activity due to bound antibody would seriously impair transmission.

Intercostal muscle biopsies from MG patients averaged 36 % the receptor content of biopsies from normals.[18] An average of 51 % of the remaining receptors were bound with antibodies.[18] These results are consistent with an earlier study [26] that interpreted a decrease in toxin binding in normal muscle biopsies from MG patients as resulting from loss of receptor. Impairment of transmission as measured by miniature endplate potential amplitude correlated with receptor content, and even better with the content of receptor remaining unbound by antibodies.[18] Relative receptor content as measured histochemically has also been shown to correlate with mepp amplitude in muscle biopsies from MG patients.[27] These results suggest that the most important factor impairing neuromuscular transmission in MG is loss of functional receptors.

Antibody-dependent, complement-mediated focal destruction of the postsynaptic membrane is a mechanism which could contribute to the receptor loss and postsynaptic membrane structure alterations observed in MG. Andrew Engel and coworkers [28] have demonstrated the presence of antibody and C3 in association with receptor on the postsynaptic membrane in biopsies of muscle from MG patients. Toyka and coworkers [22] have shown that γ G from MG patients injected into mice causes loss of receptor and decreased miniature endplate potentials. Further, they showed that the C3 component of complement contributed to this effect, but that the C5 component did not. In rats, inhibition of C3 using cobra venom factor prevents the receptor loss and phagocytic invasion of endplates otherwise associated with passive transfer of anti-receptor antibodies to a normal rat.[25]

Antigenic modulation is another mechanism by which antibodies could contribute to the loss of receptor from muscle observed in MG patients. Antibodies to receptor from both MG patients [29,30] and animals with experimental autoimmune MG [24] cause increased rates of receptor destruction in muscle cells in tissue culture. The receptors on cultured muscle cells are extrajunctional, and, even in the absence of antibody, turn over at a much faster rate than junctional receptors.[31] Antibody binding to normal junctional receptors does not induce antigenic modulation,[25] at least not on time scales anywhere near those observed with cultured cells. However, in a myasthenic endplate whose architecture has already been ravaged by complement mediated mechanisms, receptor may not be so metabolically static as in a normal endplate. The degree to which either complement mediated destruction or antigenic modulation are responsible for the observed receptor loss *in vivo* remains to be determined.

In conclusion, evidence suggests that antibodies to acetylcholine receptors are lar-

gely responsible for impaired neuromuscular transmission in patients with MG. Antibodies specific for receptor, are found both in serum and bound to receptors in muscle. In addition to directly inhibiting receptor function, mechanisms are known whereby antibodies might cause receptors to be destroyed, and thereby account for the decrease in receptor content observed in muscle from patients with MG. Loss of functional receptors is probably the most important single factor impairing neuromuscular transmission in MG.

Acknowledgement: I thank Jean Rivier and Gunther Dennert for preparing French and German translations of the summary.

SUMMARY

Evidence is consistent with the idea that MG is basically an antibody mediated autoimmune disease in which the primary lesion impairing neuromuscular transmission is loss of functional acetylcholine receptors. Antibody to acetylcholine receptors can be detected in the serum of 90 % of patients diagnosed as having MG. Detection of antibodies to receptor in serum provides a sensitive diagnostic test for MG. The antibodies to receptor found in MG patients are mostly directed at antigenic determinants on the receptor molecule which are species specific and located outside the binding site for acetylcholine. Antibody concentrations measured in a population of MG patients do not show a close correlation with severity of MG. However, changes in antibody concentration with time in a patient can reflect changes in disease intensity. Studies using animals have suggested several mechanisms by which antibodies may act to impair neuromuscular transmission. These are 1) partial or complete inhibition of receptor function by antibody binding (by mechanisms other than a curare-like block of the acetylcholine binding site). 2) antibody dependent, complement-mediated membrane destruction resulting in receptor loss and altered postsynaptic membrane architecture, 3) antibody induced increases in the rate of receptor turnover, resulting in receptor loss due to antigenic modulation. Muscle from patients with MG contains reduced amounts of acetylcholine receptor, and many of the receptors which remain have antibodies bound. Decrease in receptor content of muscle from MG patients is directly proportional to decrease in acetylcholine sensitivity, as measured by miniature endplate potential amplitude. Reduction in serum anti-receptor antibody concentration in patients with MG by plasmapheresis and immunosuppressive drug therapy is associated with clinical improvement.

RESUMEN

Parece ahora evidente que la MG es esencialmente una enfermedad antoinmune en la cual la lesión primaria es la pérdida de receptores funcionales de acetilcolina. El suero del 90 % de los pacientes en los cuales un diagnóstico de MG ha sido establecido contiene anticuerpos dirigidos contra los receptores de acetilcolina. El hecho de detectar anticuerpos contra estos receptores constituye un medio de diagnóstico muy sensible para la MG. La mayoría de los anticuerpos encontrados en los pacientes afectados de MG están dirigidos a determinantes antígénicos en la molécula receptora que son específicos de especie y están localizados fuera del sitio en que se fija la acetilcolina.

Las concentraciones de anticuerpos medidas en pacientes afectados de MG no están en proporción a la severidad de la enfermedad. No obstante los cambios en la concentración de anticuerpos en la evolución en un paciente pueden señalar cambios en la intensidad de la afección. Estudios realizados en animales han sugerido varios mecanismos para explicar cómo los anticuerpos pueden actuar para afectar la transmisión neuromuscular. Ellos son: 1) una inhibición parcial o total de la función del receptor por los anticuerpos, que no pondría en causa en bloqueo tipo curare del sitio

de fijación de la acetilcolina, 2) una destrucción de la membrana, dependiente de los anticuerpos y mediada por el complemento que conduciría a la pérdida de los receptores y una modificación de la arquitectura de la membrana post sináptica, 3) un aumento causado por los anticuerpos del "turnover" del receptor que resultaría en una pérdida de receptores debido a una modulación antigénica. Los músculos de los pacientes afectados de MG contienen una cantidad reducida de receptores de acetilcolina y muchos de los receptores que están presentes están unidos a los anticuerpos. La disminución del número de receptores en los músculos de pacientes afectados de MG está en proporción directa a una disminución de la sensibilidad a la acetilcolina, tal como ella puede ser valorada por la amplitud de los potenciales de la placa terminal en miniatura. La disminución en el suero de la concentración de los anticuerpos antirreceptores en los pacientes con MG por plasmaferesis y terapia por drogas inmunosupresivas está asociada con una mejoría clínica.

RÉSUMÉ

Il semble actuellement évident que la MG est essentiellement une maladie autoimmune à base humorale dans laquelle la lésion primaire est la perte de récepteurs fonctionnels de l'acétylcholine. Le serum de 90 % des patients chez lesquels un diagnostic de MG a été posé contient des anticorps dirigés contre les récepteurs de l'acétylcholine. La mise en evidence d'anticorps contre ces récepteurs permet un diagnstic aisé de la MG. La plupart des anticorps trouvés chez les patients atteints de MG sont spécifiques d'espèce et sont localises au dehors du site de liaison de l'acétylcholine. Les concentrations d'anticorps mesurées chez des patients atteints de MG ne sont pas proportionnelles à la sévérité de la maladie. Cependant des variations temporelles dans les concentrations d'anticorps reflètent parfois des changements dans la sévérité de la maladie. Des études faites chez l'animal ont suggeré plusieurs mécanismes permettant d'expliquer comment ces anticorps bloquents la transmission neuro-musculaire. Ils comprennent: 1) une inhibition partielle ou totale de la fonction du récepteur par les anticorps, qui ne mettrait pas en cause un bloquage type curare de la liason de l'acétylcholine, 2) une destruction de la membrane, dépendante de l'anticorps et mediée par le complement, qui menerait à la perte de récepteurs et une modification de l'architecture de la membrane postsynaptique, 3) une augmentation, causée par les anticorps, du turnover du récepteur qui resulterait en une perte de récepteurs due à une modulation antigénique. Les muscles de patients attients de MG contienent une quantité réduite de récepteurs de l'acétylcholine, et bien des récepteurs présents sont liés à des anticorps. La diminution du nombre de récepteurs dans les muscles de patients atteints de MG est directment proportionnelle à une diminution de la sensibilité à l'acétylcholine telle qu'elle est mesurée par l'amplitude des potentiels miniatures (mepp). L'abaissement de la concentration d'anticorps anti-récepteurs dans le serum de patients souffrant de MG qui suivient lors de plasmaphérèse et de traitement avec des remèdes immunosuppressifs est associé à une amélioration clinique.

ZUSAMMENFASSUNG

Experimentelle Epidenz unterstützt die Hypothese dass M.G. eine Autoimmunkrankheit ist, die durch Antikörper hervorgerufen wird. Antikörper specifisch für Azetylcholinrezeptoren findet man in 90 % der Seren von Patienten die M.G. haben. Die präsenz von Antikörpern bietet zugleich die Möglichkeit M.G. zu diagnostizieren. Diese Antikörper erkennen Determinanten die ausserhalb der Bindungsstelle für Azetylcholin liegen. Der Antikörper Titer korreliert nicht mit der Stärke der klinischen

Symptome, jedoch können Fluctuationen im Antikörper Titer eines Patienten sich im Krankheitsbild wiederspiegeln. Experimente im Tier haben gezeigt, dass es mehrere die Nerv-Muskel Interaction stören kann: Mechanismen gibt, mit denen Antikörper 1) partielle oder komplette Inhibition der Rezeptorfunktion durch Bindung von Antikörper en den Rezeptor. Diese Inhibition ist unähnlich der von Curare hervorgerufenen. 2) Antikörper und Komplement abhängige Lysis der Membran und Verlust von Receptor. 3) Verlust von Receptor hervorgerufen durch Antigen Modulation. Muskel von M.G. Patienten enthält subnormale Mengen von Acetylcholinrezeptoren die Antikörper adsorbiert haben. Muskel mit geringen Rezeptorgehalt zeigen geringe Azetylcholin Sensitivität, die durch das MEPP gemessen wird. Patienten, die durch Plasmaphorese oder Behandlung mit immunosuppressiven Substanzen klinische Besserung erfahren, zeigen sinkende anti-Rezeptor Antikörper Titer.

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Myasthenia Gravis and Acetylcholine Receptor: Autoimmunity and Steroid Effects

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Immunologic Abnormalities in Myasthenia Gravis

The possibility that myasthenia gravis (MG) might be an autoimmune disease was postulated in 1960 by Simpson [1] on the basis of indirect evidence. The association between MG and other putative autoimmune disorders, such as thyroiditis or thyrotoxicosis,[2] rheumatoid arthritis,[1] systemic lupus erythematosus (SLE) [3,4] and multiple sclerosis,[5] suggested the involvement of the imune system in MG. Some clinical and epidemiological features of MG, including the age of appearance, female predominance, variable course and absence of a known cause, were shown to be similar to those encountered in other disorders thought to be autoimmune in origin.[6] The most compelling clinical evidence was the demonstration of either thymoma or hyperplasia of the thymus, the central lymphoid organ responsible for the development and control of cell mediated immunity,[7,8] in 70 to 90 percent of patients with MG.[9] The beneficial effects of thymectomy,[10,11] thoracic duct drainage of lymphocytes,[12] immunosuppresent [13] and corticosteroid medication,[14–17] were related to their interference with immune processes.

Further experimental and immunological studies provided other circumstantial evidence for the role of the immune system in MG. Goldstein [18] reported that sensitization of animals with thymus or muscle tissue results in an autoimmune "thymitis"; inflamation of skeletal muscle similar to the muscle "lymphorrhages" seen in MG; electrophysiological evidence for partial neuromuscular block; and antimuscle antibodies. Similar antibodies directed against skeletal muscle were demonstrated in the sera of 30 percent of myasthenics and 90 percent of myasthenics with thymona.[4,19,20] These antibodies bind to the sarcoplasmatic reticulum [21] and have been found to cross react with the "myoid" or muscle-like cells of the thymus.[22] The demonstration of such antibodies could not explain the abnormal neuromuscular conduction in MG, nor could the demonstration of other autoantibodies such as antinuclear, antithyroglobulin, antineural, positive SLE preparations and increase in rheumatoid factor.[4,23,24] Other immunological findings have also indicated the possible involvement of autoimmune mechanisms, but could not also be related to the functional defect at the neuromuscular junction (MMJ). These findings included the capacity of muscle extracts to inhibit the in vitro migration of peripheral blood buffy coat cells and thymic cells of myasthenics; [25,26] the cytotoxic effect of myasthenics' thymocytes on fetal muscle cultures; [26] the increased responsiveness of the thyroid cells to mitogens and an increased percentage of B lymphocytes in the myasthenic thymus.[27] More recently, an increased incidence of the serologically defined histocompatibility linked antigen

HLA-8 has been reported in MG, especially in young females with thymic hyperplesia.[28,29] An increased incidence of certain HLA types has also been reported in other putative autoimmune diseases,[30] and interpreted as being compatible with genetically determined susceptibility to specific viral infection and/or clinical diseases with autoimmune features.

The Postsynaptic Acetylcholine Receptor in Myasthenia

Our view on MG has changed considerably during the last years. We now know that MG is indeed an autoimmune disease where the postsynaptic nicotinic acetylcholine receptor (AChR) at the NMJ is the major autoantigen. This discovery has come largely from the availability of neurotoxins derived from venoms of elapid snakes,[31] which bind specifically to the nicotinic AChR and made possible the isolation and characterization of this receptor. The α-Bungarotoxin (α-BT) binds irreversibly to the active sites and has therefore been used as a probe to measure the number of AChR sites. The α-cobra (Naja) toxins bind reversibly and have therefore been used for purification of the glycoprotein receptor especially from the electric organ (electroplague) of electric eels and fish, which is tre richest source of AChR.[32,33]

Postsynapticlocalization for the NMJ defect in MG was previously suggested by electron microscopic observations of an altered morphology of the end-plate.[34,35] Poorly developed and shallow postsynaptic folds, sparse simplified synaptic clefts and a widened distance between the nerve terminal and the postsynaptic membrane, have been found to be prominent features of the myasthenic NMJ. The presynaptic nerve terminals are somewhat reduced in size, but they contain normal numbers of full-sized acetylcholine Vesicles. Further and definite support for the concept that MG is a disease of the postsynaptic membrane of the NMJ, came from studies showing abnormalities of the postsynaptic receptors. In 1973, Fambrough and Coworkers[36] demonstrated by autoradiography a decreased number of ^{125}I-labeled α-BT binding sites in myasthenic muscles biopsies. The myasthenic muscles showed 70 to 90 percent reduction in the number of receptors per NMJ, as compared with the control muscles. Similar findings were also described by other investigators,[37,38] using toxin binding assays and quantitative measurements of acetylcholine sensitivity. Bender and coworkers[39,40] demonstrated the normal distribution of AChR at the peaks of the postjunctional folds of the muscle sarcolemmal membrane, by immunoperoxidase method for the localization of α-BT binding sites. Reduction of AChR in the descructed postsynaptic membrane of the NMJ was demonstrated by Engel and coworkers[41] in muscle biopsies obtained from myasthenics. Blocking of α-BT binding to normal muscle was demonstrated when normal muscle biopsies were preincubated with serum from MG patients.[39] Satyamurti and coworkers[42] showed that a reduction in AChR sites per se is likely to play an important role in the pathophysiology of the NMJ defect, since clinical and physiological features characteristic of myasthenia could be reproduced *in vivo* in rats by experimental blockade of AChR with α-cobra toxin. A similar blockade of neuromuscular transmission in MG, is a result of an autoimmune attack against the AChR at the postsynaptic membrane.

Experimental Autoimmune Myasthenia Gravis

An experimental model for MG, denoted experimental autoimmune myasthenia gravis (EAMG), can be induced in laboratory animals by the injection of purified AChR in complete Freund's adjuvant (CFA).[43-52] The clinical, histopathological, pharmacological and imunological findings in animals with EAMG closely parallel the manifestations in patients with MG. The experimental model indicates that MG is an autoimune disease and the postsynaptic nicotinic AChR is the autoantigen.

Several animal species, including rabbit, monkey, rat, mouse, guinea pig, etc., develop marked muscular weakness of legs, trunk and head with respiratory insufficiency. Acute as well as chronic phases of

EAMG have been described. Similar to patients with MG, animals with EAMG show improvement after treatment with anticholinesterase agents,[43,45] decremental muscle response on repetitive nerve stimulation,[43,45,51] reduction of miniature endplate potentials (EPP) and EPP amplitudes,[53] and decrease in available AChR sites.[46] Ultrastructural changes, namely destruction and simplification of the postsynaptic membrane with widening of the synaptic cleft have been described in both EAMG and MG.[41]

Several lines of evidence point to the important key role of anti-AChR antibody in the pathogenesis of EAMG and MG. Animals injected with purified AChR produce anti-AChR humoral antibodies before the appearance of the disease.[52] Engel and coworkers [54] have demonstrated by the use of a novel immunoperoxidase technique, the presence of IgG and C3 at the postsynaptic membrane of the neuromuscular junction. Myasthenic manifestations can be produced in recipient animals by transfer experiments of an immunoglobulin fraction isolated from animals with EAMG [55] as well as from patients with MG.[56,57] However, several strains of mice injected with AChR develop high titer of anti-receptor antibody without clinical signs of EAMG.[58] Therefore, it is feasible that antibodies are necessary, but not sufficient, to explain the autoimmune basis of the disease. Indeed, animals immunized with AChR develop cell mediated immunity to the receptor with some correlation to the severity of the disease,[52,59] and the disease itself can be passivly transfered in inbred strains by lymphocytes.[60]

Immune Response to Acetylcholine Receptor in Myasthenia Gravis

Anti-AChR antibody in myasthenics serum was identified by several different methods during the recent years.[39,61-63] The most sensitive radioimmunoassay is based on the binding of antibody to ^{125}I-α-Bungarotoxin labeled AChR preparation.[64-69] In several patients, the titers correspond loosely, but not exactly, to the severity of the clinical signs. However, patients can have very high antibody levels without any symptoms.[76] Nevertheless, the radioimmunoassay is the best diagnostic tool for myasthenia gravis, since 80-95 % of patients have receptor-binding antibodies by this method.

In addition to the humoral immune response, patients with MG have cell mediated imune response to AChR. Peripheral blood lymphocytes from myasthenic patients were found to be stimulated in the blood transformation technique when incubated in the presence of purified AChR preparation.[71-76] Only one report indicates a correlation between the *in vitro* stimulation index of the lymphocytes and the severity of the disease.[73]

Effect of Steroids

Many series reported during the last years [14-17, 77-84] emphasis the beneficial effect of corticosteroids on myasthenia gravis. It seems that corticosteroid treatment is the best therapeutic measure for this disease today. However, differences of opinion still exist concerning the indications for such therapy, the treatment regimens, and the association with other kinds of treatments such as thymectomy of plasmapheresis.[85] Most authors recommend Seybold and Drachman's regimen,[17] namely, gradually increasing dose of prednisone in order to avoid exacerbetion of the weakness during the first days of the treatment. The initial dose is 10-25 mg of predenisone every day, and the optimal therapeutic dose is 60-120 mg/day (or 100-240 mg when prednison is given every other day), for a period of a few months. Under such a treatment, clinical improvement has been noted in 70 to 100 percent of patients, with good to excellent results in 60 to 100 percent. When improvement has reached a maximum, the dose of prednisone is lowered gradually to a maintenence dose of 10-30 mg/day.

Some investigators [86-88] have demonstrated a direct effect of corticosteroids at the neuromuscular junction, and claimed that the beneficial effect of steroids on MG may be attributed at least in part to a direct action on neuromuscular transmission. Others [89,90] could not confirm such findings and

concluded that steroids do not owe their therapeutic efficacy in MG to action at the neuromuscular junction, but to systemic, possibly immunosuppressive effects. Further support for this possibility stems from immunological studies in MG and EAMG. Clinical improvement of MG by corticosteroid therapy is frequently associated with a decrease in anti-AChR antibody in both high and low-titer patients.[84,91] Abramsky et al.[72] and Morgutti et al.[76] demonstrated marked dimination of the blast transformation cellular immune response to AChR in patients who improved clinically with prednisone treatment. In some cases, the transient clinical deterioration during the first days of prednisone treatment was accompanied by a transient increase of the lymphocyte stimulation index.[72] Similar effects of corticosteroids were demonstrated by Abramsky et al.[92] in rabbits immunized with purified AChR. The effect seems to depend on the regimen. Administration of hydrocortisone in gradually increasing doses starting at the time of immunization prevented the appearance of EAMG. On the other hand, when hydrocortisone was administered in high doses from the beginning, EAMG appeared earlier and in a more severe form than in the control animals. The effect of hydrocortisone in suppressing EAMG was shown to be accompanied by parallel decrease of the cellular immune response to AChR. Therefore, it appears that the therapeutic action of steroids in both EAMG and MG, is by an immunosuppressive mechanism. This may involve reduction and/or inhibition of anti-AChR antibodies, as well as suppression of cytolitic effects by sensitized lymphocytes. The early harmful action of steroids may be by an enhancement of immune mechanisms. It was demonstrated that corticosteroids may act to enrich cultures with specifically sensitized lymphocytes both by killing immunologically non reacting lymphocytes and by enhancing the proliferation of the sensitized cells.[93,64] Similarly, *in vivo* activities may occur in clinical and experimental myasthenia.

SUMMARY

The possibility that myasthenia gravis might be an autoinmune disease was postulated in 1960 by Simpson.

The most compelling clinical evidence was the demonstration of either thymoma or hyperplasia of the thymus, the central lymphoid organ responsible for the development and control of cell mediated immunity, in 70 to 90 percent of patients with MG.

An increased incidence of the serologically defined histocompatibility linked antigen HLA-8 has been reported in MG especially in young females with thymic hyperplasia.

We now know that MG is indeed an autoimmune disease where the postsynaptic nicotinic acetylcholine receptor (ACHR) at the neuromuscular junetion (NMJ) is thee major autoantigen.

The α-Bungarotoxin (α-BT) binds irreversibly to the active sites and has therefore been used as a probe to measure the number of ACHR sites.

Poorly developed and shallow postsynaptic folds, sparse simplified synaptic clefts and a widened distance between the nerve terminal and the postsynaptic membrane, have been found to be prominet features of the myasthenic.

The myasthenic muscles showed 70 to 90 percent reduction in the number of receptors per NMJ, as compared with the control muscles.

An experimental model for MG, denoted experimental autoimmune myasthenia gravis (EAMG), can be induced in laboratory animals by the injection of purified ACHR in complete Freund's adjuvant (CFA).

The experimental model indicates that MG is an autoimmune disease and the postsynaptic nicotinic ACHR is the autoantigen.

The radioimmunoassay is the best diagnostic tool for myasthenia gravis, since 80-95 %, of patients have receptor-binding antibodies by this method. Many series reported during the last years emphasis the beneficial effect of corticosteroids on myasthenia gravis. It seems thte corticosteroid

treatment is the best therapeutic measure for this disease today.

Clinical improvement of MG by corticosteroid therapy is frequently associated with a decrease in anti-ACHR antibody in both high and low titer patients.

It appears that the therapeutic action of steroids in both EAMG and MG, is by an immunosuppressive mechanism. This may involve reduction and/or inhibition of anti-ACHR antibodies, as well as suppression of cytolitic effects by sensitized lymphocyts.

RESUMEN

La posibilidad de que la Miastenia gravis pudiera ser una enfermedad autoinmune fue postulada en 1960 por Simpson. La evidencia clínica de más jerarquía fue la demostración de que tanto el timoma como la hiperlasia del timo· el órgano linfoideo se observan en el 70 a 90 por ciento de pacientes con Miastenia gravis.

Una incidencia creciente del Serologicamente definido antígeno conectado con la histocompatibilidad HLA-8 ha sido publicado, especialmente en mujeres jóvenes con hiperplasia tímica.

Sabemos ahora que la miastenia gravis es verdaderamente una enfermedad autoinmune, el mayor autoantígeno es el receptor (ACHR) post-sináptico nicotímico de acetilcolina de la unión neuromuscular (NMJ).

La α-Bungarotoxina (α-BT) se conecta en forma irreversible con los puntos activos y de este modo ha sido empleado como un recurso para valorar el número de emplazamiento de AChR.

Se ha observado como característica prominente de la miastenia, entre la terminal nerviosa y la membrana post sináptica una distancia mayor, hendiduras sinápticas simplificadas esparcidas pobremente desarrolladas y poco profundos los pliegues postsinápticos.

Los músculos miasténicos mostraron una reducción del 70 al 90 por ciento en el número de receptores en cada unión neuromuscular (NMJ) en comparación con músculos de control.

Un modelo experimental de MG, puede ser inducido en animales de laboratorio por la inyección del ACHR purificado en adjutor de Freund completo (CFA).

El modelo experimental indica que la Miastenia Gravis es una enfermedad autoinmune y el autoantígeno es el ACHR nicotínico post sináptico.

La prueba radioinmune es el mejor instrumento diagnóstico para la mistenia gravis, desde que el 80 - 95 % de los pacientes tienen por este método anticuerpos que se fijan a los receptores.

Muchos estudios mencionados en los últimos años insisten acerca del efecto benéfico de los corticoesteroides en la miastenia gravis. Se hace evidente que el tratamiento con corticoesteroides es la mejor medida terapéutica actual para esta enfermedad. La mejoría clínica de la MG por la terapia corticoesteroide está asociada frecuentemente con un decrecimiento en anticuerpos anti-AChR.

Los esteroides actuarían tanto en la EAMG como en la MG por un mecanismo inmunosupresivo. Esto puede involucrar reducción y/o inhibición de anticuerpos anti-AChR, así como supresión de efectos citolíticos por linfocitos sensibilizados.

RÉSUMÉ

La possibilité de que la myasténie gravis puisse être une maladie autoimmune a éte postulée en 1960 par Simpson. L'évidence clinique de plus grande valeur a été la démonstration du fait que aussi bien le timome que l'hyperplasie du thymus, organe lymphoide central responsable du control de l'immunité dûe aux cellules -s'observe dans 70 à 90 % des malades atteints de Myasténie gravis.

On a publié la coincidence de L'antigene sérologique avec l'histocompatibilité HLA-8, surtout chez les femmes jeunes présentant une hyperplasie du thymus.

Nous savons à présent que la myasthénie gravis est une maladie autoimmune, le

plus grand autoantigène est le recepteur (ACHR) post synaptique nicotinique de l'acétylcholine dans l'union neuromusculaire (NMJ).

La α-bungarotoxine αBT est en connexion de façon irreversible avec les points actifs, ce qui a permis de l'employer pour compter les emplacements de ACHR.

On a observé, comme caractéristiques importantes dans le cas de la myasthénie une plus grande distance entre la limite nerveuse et la membrane post synaptique, des fentes synaptiques simplifiées disperses peu développées et des plispost synaptiques peu profonds.

Les muscles myasthéniques ont révélé une réduction de 70 à 90 % du nombre de récepteurs dans chaque union neuromusculaire (NMJ) en comparaison avec les muscles de contrôle.

Un modèle expérimental de MG peut être réalisé avec des animaux de laboratoire en injectant de l'ACHR purifié nicotinique de Freund complet (CFA).

Le modèle expérimental indique que la myasthénie gravis est una maladie autoimmune et l'antiène est l'ACHR nicotinique post synaptique.

Le preuve radioimmune est le meilleur instrument diagnostic pour la myasthénie gravis puisque 80 à 95 % des patients ont, par cette méthode, des anticorps qui ne fixent pas les récepteurs.

Dans de nombreuses études réalisées au cours de ces dernières années on insiste sur l'effet bénefique des corticostéroides dans la myasténie gravis. Il est évident que le traitement par les corticostéroides est la meilleur mesure thérapeutique actuelle pour cette maladie.

L'amélioration clinique de la MG par la thérapie corticostéroide est souvent associée à une décroissance des anticorps anti ACHR.

Les stéroides agiront autant dans la EAMG que dans la MG par un mécanisme immunosuppresseur. Ceci peut entrainer la réduction et/ou l'inhibition des anticorps anti-ACHR de même que la suppression des effets cytolytiques par des lymphocytes sensibiliés.

ZUSAMMENFASSUNG

Die Möglichkeit, dass die Myasthenia gravis eine autoimmune Krankheit wäre wurde 1980 durch Simpson angegeben. Die klinische Evidenz von grösserer Bedeutung war die Demonstration, dass sowohl der Thymus als auch die Hyperplasie des Thymus, das zentrale lymphoide Organ für die Entwicklung und die Kontrolle der Immunität messbar durch Zellen verantwortlich ist, bei 70-90 % bei Patienten mit Myasthenia Gravis beobachtet werden.

Ein wachsender Begriff des serologisch bestimmenden Antigens, das mit der Histokompatibilität HLA-8 verbunden ist, ist veröffentlicht worden, speziell bei jungen Frauen mit Hyperplasie des Thymus.

Wir wissen nun heute, dass die Myasthenia gravis wirklich eine autoimmune Krankheit ist: das grösste Autoantigen ist der Rezeptor (ACHR) postsynaptikal Picothymikal von Acethylcholin der neuromuskulären Union (NMJ).

Das α-Bungarotoxin (α-BT) konnektiert sich in unveränderbarer Form mit den aktiven Punkten, und durch diese Art ist es gebraucht worden als ein Rekurs, um die Zahl der Anordnungen ACHR zu bewerten.

Man hat bebbachtet als prominentes Charakteristikum der Myastenie, zwischen dem nervösen Therminal und der postsynaptischen Membran eine grössere Distanz, synaptische vereinfachte vereinzelte Verästelungen, armselig entwickelt und wenig vertiefte postsynaptische Falten.

Die myasthenische Muskeln zeigten eine Reduktion von 70-90 % in der Nummer der Rezeptoren bei jeder Neuromuskulären Union im Vergleich mit den Kontrollmuskeln.

Ein Modell im Experiment von MG, das in Laboratoriumstieren durch Injection von ACHR, purifiziert durch das Verfahren komplett von Freund (CFA), kann durch geführt werden. Das experimentale Modell zeigt an, dass die Myasthenia gravis eine autoimmune Krankheit ist und dass das Autoantigen das nicothymische postsynaptische ACHR ist.

Der radioimmune Beweis ist das grösste diagnostische Instrument für die Myasthenia gravis, da 80-95 % der Patienten durch diese Methode Antikörper haben, die sich an die Rezeptoren anschliessen.

Viele der in den letzten Jahren erwähnten Studien berufen sich auf den Wohltuenden Einfluss der Certicoesteroiden bei der Myasthenia gravis. Es ist klar dass die Behandlung mit Corticoesteroiden die beste therapeutische Massnahme ist, um heute diese Krankheit zu behandeln.

Die klinische Besserung der MG durch die Corticoesteroide Therapie ist häufig assoziiert mit einer Abnahme der Antikörper AntiACHR. Die Esteroide sind aktiv sowohl in der EAMG als auch in der MG durch einen Immunosuppresor.

Dieser kann eine Reduktion oder Inhibition des Antikörpers AntiACHR hervorrufen, oder eine Suspression der citolitischen Effekte durch eine sensibilisierte Lymphocitis.

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The Immunopathology of Myasthenia Gravis

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BACKGROUND

Several lines of evidence indicate that acquired form of myasthenia gravis (MG) is caused by an autoimmune reaction to the nicotinic postsynaptic acetylcholine receptor (AChR) protein. Experimental animals immunized with highly purified AChR derived from the electric organs of fish develop a syndrome which resembles MG clinically (weakness and easy fatigability on exertion), pharmacologically (response to anticholinesterase drugs and increased curare sensitivity), electrophysiologically (decreased amplitude of the miniature end-plate potential) and morphologically (degeneration and simplification of the postsynaptic region).[1–10] In both MG [11,12] and in experimental autoimmune MG [12,13] there is decreased α-bungarotoxin binding by motor end-plates which indicates a decreased number of AChRs. Uutrastructural studies that localize AChR on the postsynaptic membrane with peroxidase-labelled α-bungarotoxin show decreased amounts of AChR in MG [12] and the decreased amplitude of the miniature end-plate potential correlates linearly with morphometric [12] and radiochemical [14] estimates of the amount of AChR remaining on the postsynaptic membrane. Approximately 90 % of patients with MG have detectable circulating antibodies to human AChR [15] and both the human [16] and the experimental disease [17] can be passively transferred to normal animals with immunoglobulin G (IgG) from affected donors. Transient neonatal MG is due to the transplacental transfer of anti-AChR antibodies and the infants recover as the antibodies to the receptor disappear from the circulation.[18] All these observations imply that the synaptic dysfunction in acquired and in transient neonatal MG is caused by antibody-mediated interference with, or destruction of, end-plate AChR, but to 1977 immune complexes have never been directly demonstrated on the postsynaptic membrane in MG. In fact, some workers had concluded that immunoglobulin deposits were not present at the myasthenic end-plate.[19]

In 1977, my colleagues and I described, for the first time, the reliable localization of antibody and the third component of complement (C3) at the end-plate in MG and correlated the morphologic data with clinical and electrophysiological observations in our patents.[20] This paper describes the light microscopic and ultrastructural localization of IgG and C3 at the myasthenic end-plate and considers the manner in which the anti-receptor antibodies can cause a deficiency of AChR and impair neuromuscular transmission in MG.

METHODS

A basic aim of the study was to obtain satisfactory ultrastructural localization of

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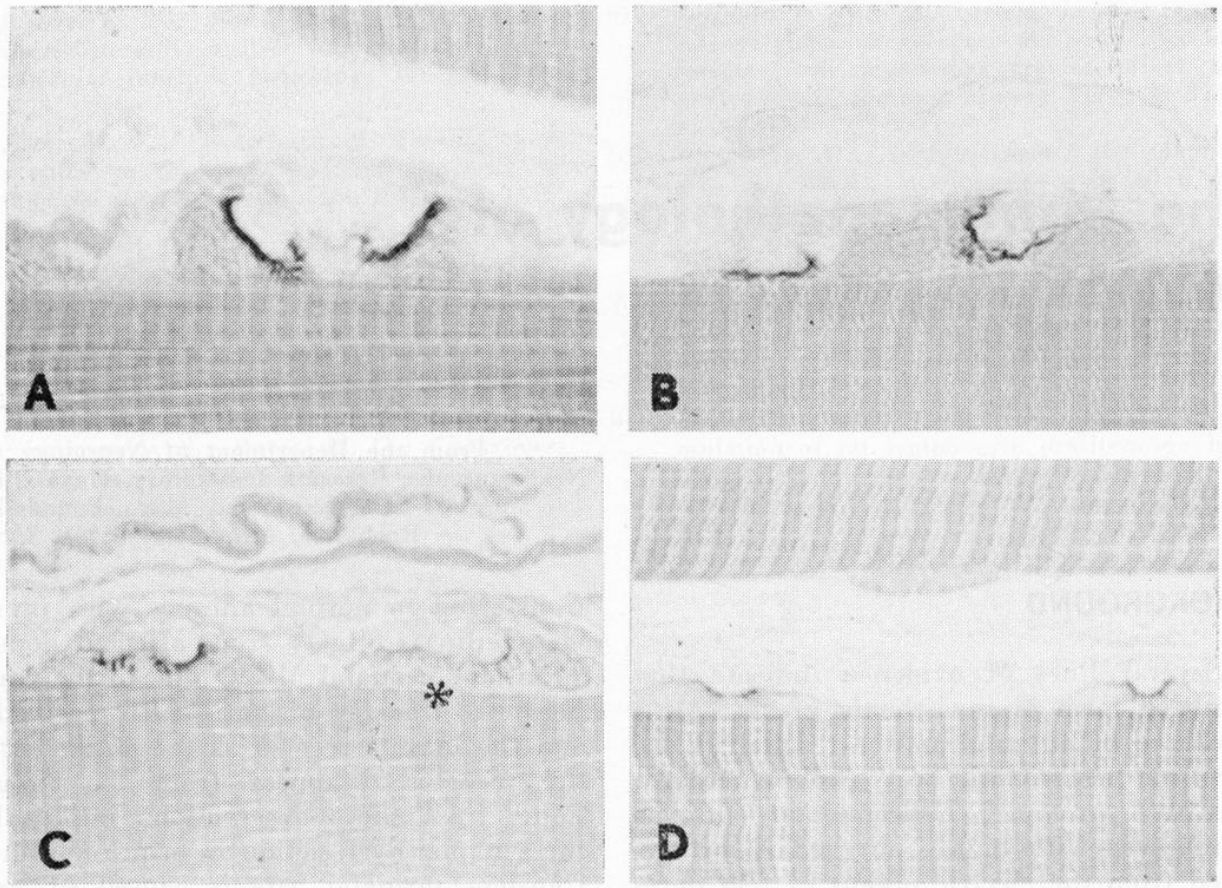

Fig. 1. — *Semi-thin section showing localization of IgG* (A *and* C) *and* C3 (*B and* D) *in mild* (A *and* B) *and more severe* (C *and* D) MG. *Reaction at end-plates is more intense in mild than in more severe* MG. *One of two end-plate regions in* C (*asterisk*) *displays only trace of IgC. Background staining is absent.* X 1.500. *Reproduced from Engel et al.* (*reference* 20) *by permission.*

IgC and C3 at the end-plate with minimum background staining and with optimal preservation of fine structure. This was accomplished by (1) the use of peroxidase-labelled staphylococcal protein A for the ultrastructural localization of IgG, (2) the use of peroxidase-labelled rabbit antihuman C3 for the ultrastructural localization of C3 and (3) the application of optimal concentrations of the immunoreagents to fresh, oxygenated, external intercostal muscle strip, intact from origin to inscrition, which were wellrinsed before and after exposure to the immunoreagents, followed by glutaraldehyde fixation.

Peroxidase-Labelled Staphylococcal Protein A. — The light and electron microscopic localization of IgG was first attempted with peroxidase-labelled goat or rabbit anti-human IgG. However, these reagents gave excessive background staining which precluded the reliable localization of IgG at the motor end-plate. This could not be prevented by extensive rinsing of the specimens or lowering the concentration of the immunoreagents in the reaction system. For this reason, highly purified staphylococcal protein A (Pharmacia Fine Chemicals), which binds to the Fc portion of human IgG subclasses 1, 2 and 4,[21,22] was tested in the reaction system. Protein A is relatively highly reactive toward human and rabbit IgG but is much less reactive toward rat or goat IgG.[23] The specificity and sensitivity of the reagent (and hence the intensity of the reaction at the end-plate versus background staining) are further enhanced by protein A having an increased affinity for IgG molecules bound to antigenic sites.[24]

Five milligrams of horseradish peroxidase (Sigma type VI) was conjugated with 5 mg (an equimolar amount) of protein A by the method of Nakane and Kawaoi [25]

and the conjugate mixture was purified by chromatography using an Ultrogel AcA-44 column.[20] Those fractions representing the 80,000-dalton peak, and presumed to contain the peroxidase-protein A complex in a 1:1 molar ratio, were pooled and used in

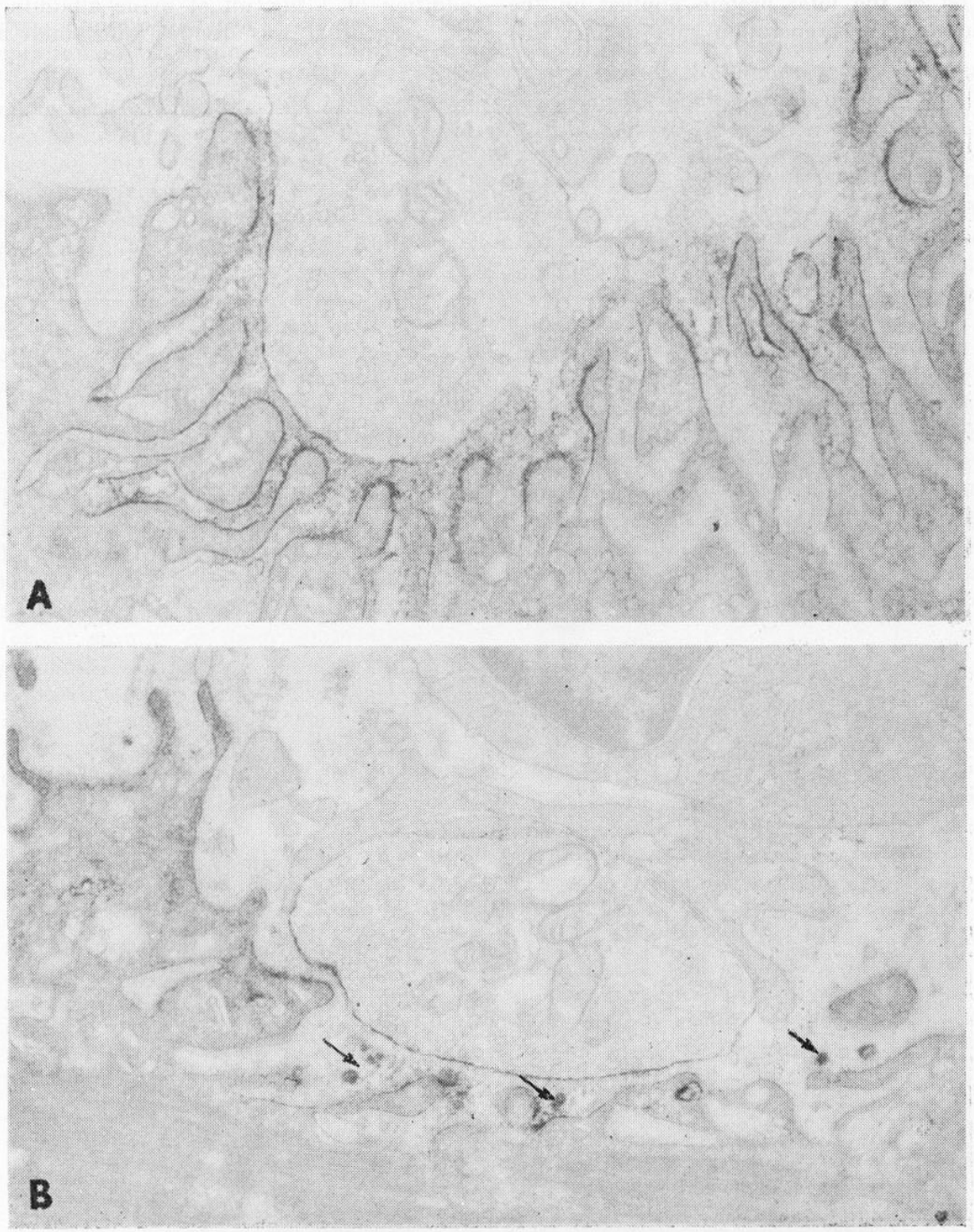

Fig. 2. — *Ultrastructural localization of IgG in mild* (A) *and more severe* (B) MG. *In* A, *postsynaptic region is well preserved and terminal expansions of most folds bind IgG. In* B, *postsynaptic region is highly samplified and junctional folds are small and sparse. IgG deposits occur on short segments of highly simplified postsynaptic membrane and on degenerate material in widened synaptic space* (*arrows*). A, X 29,500; B, X 23,600. *Reproduced from Engel et al.* (*reference* 20) *by permission.*

subsequent immunocytochemical studies. This pool contained 0.36 mg/ml of peroxidase-labelled protein A and represented 65 % of the total protein applied on the column. The preparation gave a detectable reaction for IgG on glomeruli of a kidney from a patient with systemic lupus erythematosus in dilutions up to 1:60. After the addition of 1 mg/ml of ovine albumin to the reagent it was divided into small ali-

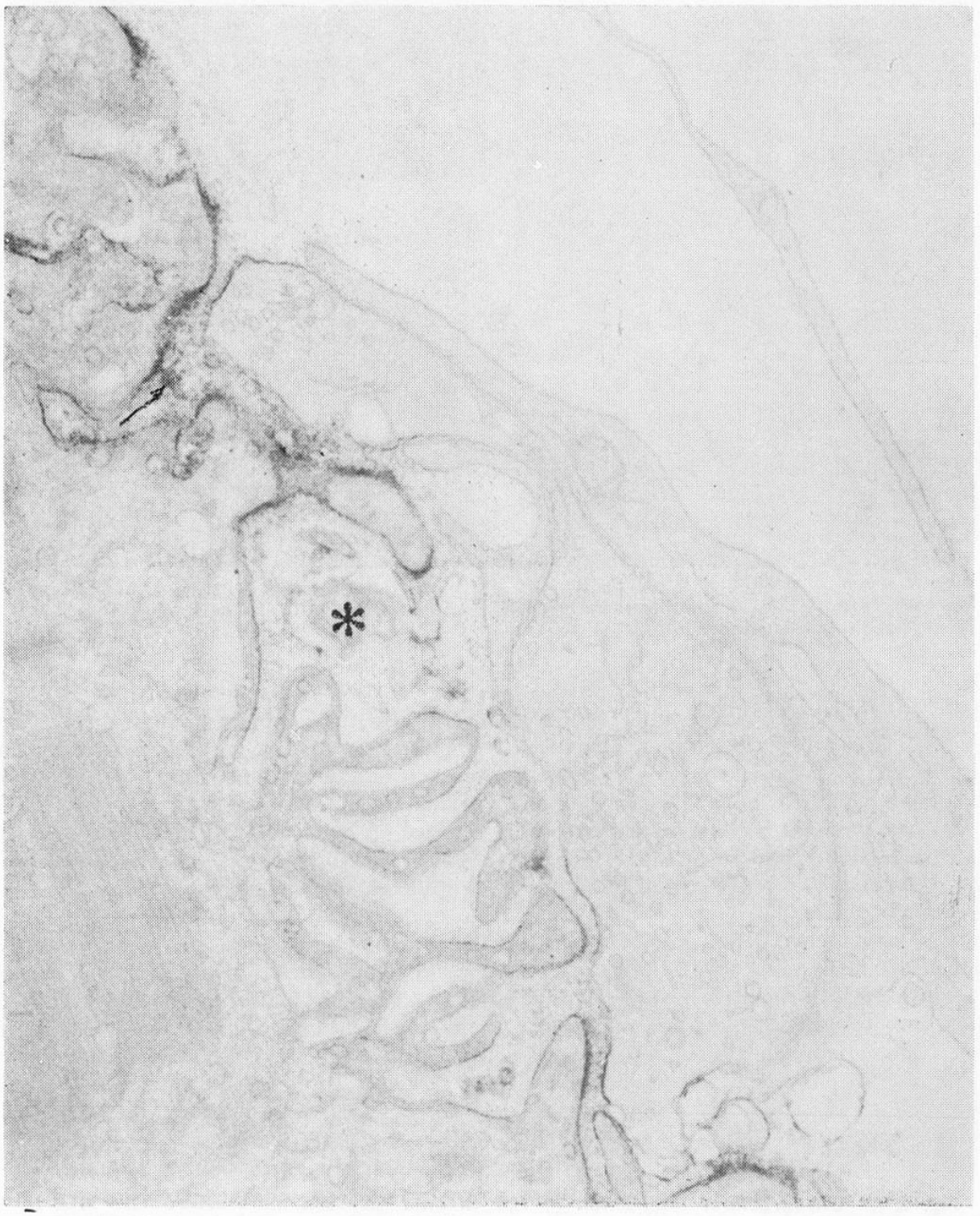

Fig. 3. — *IgG localization at an MG end-plate. IgG deposits have patchy distribution on junctional folds and also appear on debris in synaptic space (arrow). In one region there is degeneration of junctional folds (asterisk). Note reciprocal staining of presynaptic membrane where it faces reactive segments of post synaptic membrane.* X 33,000. *Reproduced from Engel et al. (reference 20) by permission.*

quots and stored at - 20° C. Each aliquot was thawed only once, immediately before use.

Peroxidase-Labelled Anti-Human C3. — Pure immunoglobulin fraction of rabbit anti-human C3 serum was purchased from Bio-Rad Laboratories. The preparation was monospecific for human C3 by immunoelectrophoretic criteria. With the procedure of Nakane and Kawaoi,[25] 5.86 mg of the antibody (equivalent to 0.34 mg of the antigen) was conjugated with 5.86 mg of peroxidase and the conjugate was further purified by ammonium sulfate precipitation.[20] The purified conjugate solution was divided into small aliquots and stored at - 20° C. Each aliquot was thawed only once, immediately before use.

Light Microscopic Localization of Immune Complexes in Cryostat Sections. — Fresh-frozen sections of muscle were rinsed for 15 minutes with phosphatebuffered saline, gently blotted, laid flat in a moist chamber and then treated with drops of immunoreagents of the desired concentration for 30 minutes at room temperature. Subsequently the sections were rinsed for 15 minutes and then reacted with the diaminobenzidine medium [26] for 30 minutes. Next they were rinsed with distilled water, dehydrated, cleared and mounted under Permount.

Ultrastructural localization of Immune Complexes. — Thin strips of fresh muscle, intact from origin to insertion, were pinned to perforated wax plates and rinsed for 3 hours in a large volume of well-oxygenated, ice-cold Tyrode's solution.. The strips were then immersed into Tyrode's solution which also contained 2 % bovine serum albumin shaken on ice for 30 minutes, and again washed with cold, oxygenated Tyrode's solution for 30 minutes. Subsequently they were incubated at room temperature for 2 hours with the desired concentration of the imunoreagent in oxygenated Tyrode's solution 2 hours. Following this the strip were rinsed for two hours with two 500-ml changes of chilled, oxygenated Tyrode's solution and then fixed for 2 hours with 2 % glutaraldehydo buffered with cacodylate at pH 7.3. After an overnight rinse in the same buffer, small pieces of muscle which contained end-plates were isolated from the strips by needle dissection.[12] The dissected pieces were reacted with the diaminobenzidine medium and then prepared for electron microscopy according to previously described procedures.[12]

Specificity Controls. — These consisted of (1) omission of the labelled immunoreagents, (2) omission of H_2O_2 from the diaminobenzidine medium, (3) treatment of peroxidase-labelled protein A with an excess of human IgG. (4) absorption of peroxidase-labelled anti-human C3 with fresh human plasma or with human IgG.

Morphometric Analysis of Electron Micrographs. — The relative abundance of the postsynaptic membrane reacting for IgG or C3 at an end-plate region (IgG and C3 indices) was evaluated by measuring the length of the postsynaptic membrane reacting for IgG or C3 and dividing that length by the length of the primary synaptic cleft.

RESULTS

Light Microscopic Localization of IgG and C3. — Both IgG and C3 were demonstrated at end- plates in cryostat sections of intercostal muscles of 12 patients with MG but not at end-plates of 12 control subjects. Discrete reaction at the end-plate with minimum background staining was obtained with 18 to 36 micrograms per ml of peroxidase-labelled protein A and with 10 to 20 micrograms per ml of peroxidase-labelled anti-human C3. Under these conditions there was no background staining with peroxidase-labelled anti-human C3, but some background staining, which varied from case to case and in different regions of a given specimen, still persisted with peroxidase-labelled protein A. With both immunreagents, the reaction at the end-plate was generally more intense in less severe than in more severe cases of MG.

Immune complexes were again observed by light microscopy in resin sections in tissues that were prepared for electron microscopy. Both IgG and C3 were demon-

strated at the end-plates of 5 patients with MG whose muscles were studied in this manner (Fig. 1) but not at end-plates of control subjects. Background staining was negligible with either immunoreagent and the reaction product was sharply localized to the upper region of the junctional sarcoplasm. As in cryostat sections the reaction for either IgG or C3 was more intense in less severe (Figs. 1 A and B) than in more severe (Figs. 1 C and D) cases of MG.

Ultrastructural Localization of IgG and C3. — The optimal concentration of peroxidase-labelled protein A for the ultrastructural localization of IgG was 18 to 36 micrograms per ml. With higher concentration of the immunoreagent, background staining became noticeable and there was faint staining of the entire postsynaptic membrane of control end-plates. The optimal concentration of peroxidase-labelled anti-human C3 was 12 to 18 micrograms per ml. Higher concentrations of this immunoreagent did not give backround staining but the reaction at the MG end-plates became too intense for precise localization of the reaction product. With the optimal concentrations of the immunoreagents, both IgG and C3 were demonstrated at the end-plates of five patients with MG studied in this manner but not at end-plates of the control subjects.

More abundant immune complexes were detected at the end-plate in less severe (Fig. 2 A) than in more severe (Fig. 2 B) cases of MG. The reaction product was localized on the postsynaptic membrane (Figs. 2, 3 and 4) as well as on degenerate material in the synaptic space (Figs. 2 B, 3 and 4). In addition, there was less intense staining of the presynaptic membrane where it faced reactive segments of the postsynaptic membrane (Fig. 2, 3 and 4). For reasons detailed in a previous publication, staining of the presynaptic sites was considered secondary to the diffusion of the reaction product from the postsynaptic membrane.

At those end-plates where the junctional folds were relatively well preserved, the immune complexes were localized on the terminal expansions of the junctional folds (Figs. 2 A, 3 and 4) where the AChR is known to be located. At end-plates which showed simplification of the postsynaptic

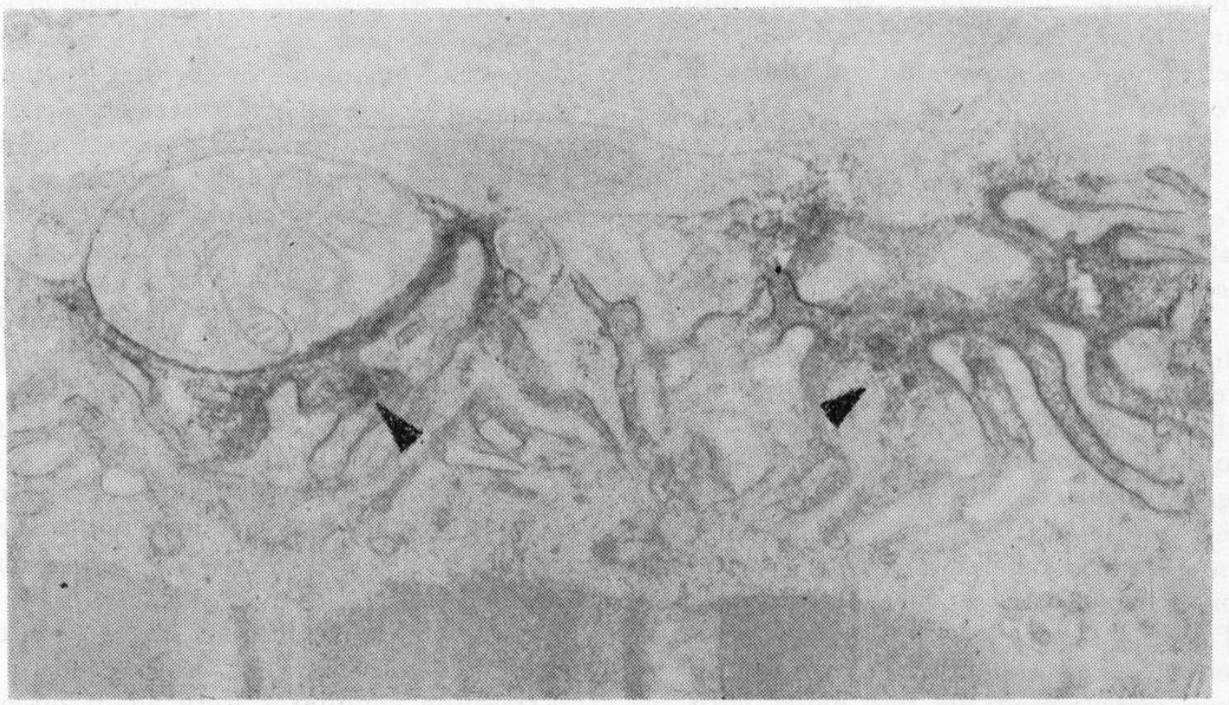

Fig. 4. — *Ultrastructural localization of* C3 *at an* MG *end-plate. The reaction product appears on terminal eypansions and on deeper regions of junctional folds and on degenerate material (arrowheads) in the synaptic space. Reaction product has diffused into the synaptic space and presynaptic membrane is also stained.* X 19,300.

region, the immune complexes were observed on discrete patches of the simplified postsynaptic mebrane. In general, patients with less severe MG had better preserved end-plates and more abundant immune complexes on the postsynaptic membrane than patients with more severe MG. In the most severely affected patients, some end-plate regions bound neither IgG nor C3, while at other end-plates only traces of IgG or C3 were found on segments of the postsynaptic membrane and on degenerate material in the synaptic space (Fig. 2 B).

In each patient the relative abundance and pattern of distribution of IgG and C3 deposits were similar. Further, the distribution of the immune complexes on the postsynaptic membrane resembled the previously reported distribution of AChR in MG cases of varying severity.

Omission of the immunoreagents during the preparation of the tissues for light or electron microscopy or of H_2O_2 from the diaminobenzidine medium, or the preincubation of peroxidase-labelled protein A with human IgG abolished the reaction at the MG end-plates. Absorption of peroxidase-labelled antihuman C3 with fresh human plasma abolished the reaction at the end-plate but absorption of this immunoreagent with human IgG was without effect.

Morphometric Analysis of Electron Micrographs. — IgG and C3 indices were determined in the 5 cases of MG studied by electron microscopy. The values thus obtained were compared with the mean miniature end-plate potential amplitudes in the external intercostal muscles (determined by Dr. E. H. Lambert). Less severly affected patients had higher IgG and C3 indices and higher miniature end-plate potential amplitudes than did the more severely affected ones. For any one patient the IgG and C3 indices were not significantly different (Table I). Regression of either the IgG or of the C3 index on the miniature end-plate potential amplitude revealed a significant correlation of either index with the miniature end-plate potential amplitude. The best fitting regression lines were linear with correlation coefficients of 0.960 ($P<0.01$) and 0.955 ($P<0.02$) for the IgG and C3 lines, respectively. The slopes of the two regression lines were not significantly different.

TABLE 1. — MORPHOMETRIC AND ELECTROPHYSIOLOGIC DATA *

(Mean ± SE)

Case	IgG index	C3 index	mepp amplitude, mV
1	1.75 ± 0.08 (19)	1.58 ± 0.11 (13)	0.59 ± 0.08 (32)
2	1.38 ± 0.11 (21)	1.24 ± 0.07 (14)	0.30 ± 0.03 (19)
3	1.00 ± 0.08 (21)	0.97 ± 0.13 (11)	0.19 ± 0.08 (19)
4	1.17 ± 0.10 (17)	1.08 ± 0.08 (13)	0.18 ± 0.02 (19)
5	1.07 ± 0.16 (14)	1.07 ± 0.10 (18)	0.11 ± 0.01 (28)

* Figures in parentheses indicate number of fibers in which miniature end-plate potential (mepp) amplitudes were measured or number of end-plate regions analyzed by electron microscopy. More than one region can be found in a end-plate.

DISCUSSION

The above observations clearly establish the presence of IgG and C3 at the motor end-plate and provide evidence for a destructive autoimmune reaction involving the postsynaptic membrane in MG. In general, the immune complexes were more abundant in the less severely affected patients than in the more severely affected ones, and more highly degenerate postsynaptic regions bound less IgG or C3 than did bet-

ter preserved ones. On well preserved junctional folds the immune complexes were localized on the terminal expansions of the folds where the AChR is known to be located.[12] Finally the relative abundance of the immune complexes on the postsynaptic membrane was proportionate to the amplitude of the miniature end-plate potential. All these findings are consistent with the assumption that the immune complexes at the end-plate bound to the AChR remaining on the postsynaptic membrane. This is also consistent with the previous finding that the miniature end-plate potential amplitude in MG patients is a linear function of the length of the postsynaptic membrane reacting for AChR.[12]

From these observations it also follows that the primary reason for the small miniature end-plate potential, and hence for the impaired neuromuscular transmission, is a deficiency of the postsynaptic AChR and that the binding of antibody to AChR does not completely block the effect of acetylcholine on AChR in MG. This in itself is not surprising because anti-AChR antibodies are known to be directed predominantly at determinantes other than the acetylcholine binding site.[15]

Antibodies to AChR might adversely affect neuromuscular transmission in a nomber of ways. The first effect might be an immunopharmacological blockade of AChR. This could happen if the antibodies allosterically hindered the attachment of acetylcholine to AChR, or prevented acetylcholine from opening ion channels, or if antibodies on AChR reduced the conductance or open time of the ion channels. However, recent studies suggest that the conductance and open time of the acetylcholine-induced ion channels are normal in MG.[27] The relative contribution of a pure immunopharmacological blockade to synaptic dysfunction in MG can be best assessed in mild cases of the disease, as in one of our patients.[20] In this patient the miniature end-plate potential amplitude was only 40 % less than normal; most of the junctional folds were well preserved, and most bound immune complexes. If in this case immunopharmacological blockade was the only reason for the reduced miniature end-plate potential amplitude, it follows that such a blockade could reduce the amplitude by 40 %. This, however, might be an overestimate, for in this case some end-plates had normal miniature end-plate potential amplitudes, but all end-plates observed with the electron microscope displayed immune complexes on the postsynaptic membrane.

A second possible effect of antibody binding to AChR could be modulation, consisting of accelerated internalization and intracellular destruction of the AChR-antibody complex. Evidence for modulation of AChR of cultured muscle fibers by myasthenic immunoglobulin has been previously obtained.[28–30] However, the AChR of cultured muscle fibers resembles the extrajunctional AChR of denervated muscle fibers which has a faster turnover rate than the junctional AChR.[30,31] The capacity for modulation of junctional and extrajunctional membranes might be different, and the in vitro system, where modulation plays an important role, lacks complement. The presence of complement as well as of antibody on the postsynaptic membrane in vivo might favor membrane lysis rather than modulation. Finally, if modulation were the only reason for the postsynaptic AChR deficiency in MG, one would not expect to see destructive changes at the end-plate. However, such changes do exist.[8,12,32]

A third and probably important consequence of antibody binding to AChR is destruction of AChR containing segments of the postsynaptic membrane. This destructive reaction is consistent with previous morphologic observations in MG which include degeneration of the terminal expansions of the junctional folds, accumulation of degenerated residues of the folds in widened synaptic spaces, highly atrophic postsynaptic regions and postsynaptic regions denuded of their nerve terminals.[8,12,32] These degenerative changes are associated with a progressive loss of AChR [12] and with a progressive decrease in the abundance of immune complexes on the postsynaptic membrane. The fact that immune complexes are also associated with degenerate material in the synaptic space clearly indicates that segments of the folds that had bound antibody and complement had been shed into the synaptic space.

The possible role of the complement system in human and experimental myasthenia requires additional comment. We have demonstrated C3 (as well as IgG) on the postsynaptic membrane in MG [20] as well as in experimental autoimmune MG [33] and in passively transferred experimental autoimmune.[34] The presence of C3 on the postsynaptic membrane indicates that the assembly phase of the complement reaction sequence (via C1, C4 and C2) has been completed.[35,36] The membrane fixed fragment of C3, C3b, could act as an opsonizer, targeting membranes for destruction by macrophages, as in the acute phase of the experimental and rarely in the human disease; or C3b together with C4 and C2 could act on C5, initiating the membrane attack phase of the complement reaction sequence which leads to the binding of C8 and C9 (via C5 through C7) and irreversible membrane defects.[35,36] The degree of injury to the postsynaptic membrane may therefore depend on the extent to which the attack phase of the complement reaction sequence becomes activated. That the complement system does play a role in damaging the postsynaptic membrane is further supported by the observation that depletion of C3 with cobra venom factor prevents the clinical and electromyographic signs and the deficiency of end-plate AChR in rats with passively transferred experimental autoimmune MG.[37]

Regardless of the relative importance of the three mechanisms (antibody-dependent interference of AChR interaction with acetylcholine, antibody-dependent modulation of AChR, and antibody-dependent complement-mediated lysis of the post-synaptic membrane) the immunoelectron microscopic studies afford unambiguous evidence for the assumption that the synaptic dysfunction in acquired MG is caused by an autoimmune reaction to the nicotinic postsynaptic AChR.

SUMMARY

This poper describes the ultrastructural and light microscopic localization of immunoglobulin G (IgG) and of the third component of complement (C3) at the motor end-plate in acquired myasthenia gravis (MG). IgG was localized with peroxidase-labelled staphylococcal protein A and C3 with peroxidase-labelled rabbit anti-human C3. The methods that were finally adopted gave excellent localization of the immune complexes with minimum background staining and optimal preservation of fine structure. Both IgG and C3 were localized on segment of the postsynaptic membrane and fragments of the junctional folds in the synaptic space. Immune complexes were more abundant in less severely affected patients than in the more severely affected patients. The abundance of the immune complexes was proportionate to the miniature end-plate potential amplitude, and hence to the amount of the acetylcholine receptor (AChR) remaining at the end-plate. Antibody-dependent complement-meriated injury to the postsynaptic membrane probably plays an important role in causing AChR deficiency at the MG end-plate. Immunopharmacological blockade of AChR by antibody and antibody-induced modulation of AChR may also contribute to the synaptic dysfunction in MG.

RESUMEN

Este trabajo describe la localización microscópica a la luz y ultraestructural de Inmunoglobulina (IgG) y del tercer componente de complemento (C3) en la placa terminal motora en la miastenia gravis adquirida (MG). La IgG fue localizada con peroxidasa rotulada proteína A estafilocóccica y C3 con peroxidasa rotulada C3 antihumana conejo. Los métodos que fueron adoptados dieron excelente localización de los complejos inmune con un mínimo de coloración de fondo y una óptima preservación de la fina estructura. La IgG y el C3 fueron localizados en segmentos de la membrana postsináptica y fragmentos de los pliegues de unión en el espacio sináptico.

Complejos inmunes eran más abundantes en los pacientes menos severamente afectados que en pacientes más severamente afectados. La abundancia de los complejos inmune fue proporcional a la amplitud del potencial en miniatura de la placa terminal, y por lo tanto a la cantidad del receptor de acetilcolina (ACHR) que queda en la placa terminal. Daño dependiente de anticuerpos, mediado por complemento a la membrana post sináptica probablemente juega un papel importante el determinar deficiencia de ACHR en la placa terminal de MG. Bloqueo inmunofarmacológico de ACHR por anticuerpos y modulación inducida por anticuerpos de ACHR pueden también contribuir a la disfunción sináptica en la MG.

RÉSUMÉ

Dans ce travail on décrit la localisation microscopique à la lumière et l'ultrastructure de l'Immunoglobuline G (IgG) et du troisième composant de complement (C3) dans la plaque terminale motrice dans la cas de la myasthénie gravis acquise (MG). La IgG a été localisée grâce à la peroxydase marquée proteine A staphylococcique et C3 l'a été avec la peroxidase marqu-e C3 antihumaine lapin. La méthode adoptée a donné d'excellents résultate avec localisation des complexes d'immunisation avec une légère coloration de fond et une excellent préservation de la structure fine. La IgG et le C3 ont été localisés dans des fragments de la membrane postsynaptique et des parties des plis d'union dans l'espace synaptique. Les complexes immuns étaient plus abondants chez la patients les moins affectés que dans ceux que l'étaient sévèrement. L'abondance des complexes immuns était proportionnelle l'amplitude du potentiel en miniature de la plaque terminale, et par conséquent à la quantité de rècepteur d'acétylcholine ACR qui reste dans la plaque terminale. Ceci est indépendant des anticorps et dû aux compléments. La membrane post-synaptique doit jouer un rôle important en déterminant le manque de ACHR dans la plaque terminale dans le cas de MG. Le blocage immunopharmacologique de ACHR par des anticorps et la modulation produite par des anticorps de ACHR peuvent aussi contribuer au mauvais fonctionement synaptique dans le cas de MG.

ZUSAMMENFASSUNG

Diese Arbeit beschreibt die mikroskopische Lokalisation und Ultrastruktur des Immunoglobulins G (IgG) und der dritten Komponente des Komplements (C3) in der Terminalplakette motorischen bei Myasthenia gravis durch Infektion (MG). Das IgG wurde durch Peroxydase, durch Staphylocokken entwickeltes Proteins A und C3, mit Peroxydase bezeichnete C3 anti-humane Kaninchen. Die Methoden, die angewandt wurden, gaben eine ausgezeichnete Lokalisation del immunen Komplexe mit einem Minimum der Koloration des fundus und eine optimalen Präservation der feinen Struktur. Das IgG und C3 wurden in Segmenten der postsynaptischen Membran und Fragmenten der Falten der Union in dem synaptischen Raum lokalisiert. Immune Komplexe waren zahlreicher verhanden bei den weniger stark affektierten Patienten als bei den ernsten Erkrankten. Der Überfluss der immunen Komplexe war proportional zu der potentiellen Grösse in Miniatur zu der Endplatte, und dadurch zu der Menge des Rezeptors von Acetylcholin (ACHR), die in der Endplatte blieb. Schaden, der von Antikörpern abhängt, komplett gemessen an der postsynaptschen Membran, spielt eine wichtige Rolle bei der Bestimmungder Mangels von ACHR in der Endplatte von MG.

Immunopharmakologischer Block von ACHR durch Antikörper und Modulation hervorgerufen durch Antikörper von ACHR können auch zur synaptischen Dysfunktion in der MG beitragen.

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Experimental Myasthenia Gravis: A Model of Receptor Disease

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Presynaptic and postsynaptic hypothesis for the pathogenesis of myasthenia gravis

Myasthenia gravis is a disease caused by an abnormality in the neuromuscular transmission by acetylcholine, but a controversy exists as to whether the primary site of pathology is the nerve terminal or the muscle endplate. The presynaptic hypothesis was supported by evidence that in biopsied myasthenic muscles, the amplitude of miniature endplate potential (representing the spontaneous release of single quantum of acetylcholine) was much reduced,[1-3] while the time course and extent of the endplate depolarization produced by the bath application of acetylcholine analogues and the response to iontophoretic microapplication of acetylcholine were normal.[1,2] Based on these studies, the prevalent concept for many years was that the primary defect of myasthenia gravis involved the nerve terminal in which there was a reduced amount of acetylcholine per quantum [2] or a defect of acetylcholine synthesis.[4] In the past several years, however, exciting new studies have raised the postsynaptic hypothesis. Histometric analysis of the ultrastructure of the neuromuscular junction by Engel and Santa in 1971 [5] showed that major abnormality in myasthenia gravis appeared to be at the postsynaptic region. The postsynaptic membrane profile concentration was decreased while the nerve terminal showed only minor alteration and the synaptic vesicle diameter and count per unit nerve terminal area were not significantly affected. A direct quantitative measurement of acetylcholine receptor has become possible after elucidating the neuropharmacologic action of α-bungarotoxin, a snake venom from Bungarus multicinctus. By means of technique which utilizes iodine-labeled α-bungarotoxin binding to receptor, Fambrough et al [6] determined in 1973 that the number of available acetylcholine receptors was reduced in myasthenic junctions as compared with the controls.

Postsynaptic theory based on experimental myasthenia due to α - bungarotoxin

The above-mentioned recent studies raised the question whether the decrease of available acetylcholine receptors, per se, could acount for the physiopathological defects of myasthenia gravis. To explore this question, Satyamurti et al [7] and we [8] studied changes in the neuromuscular transmission by pharmacological blockade of acetyl-

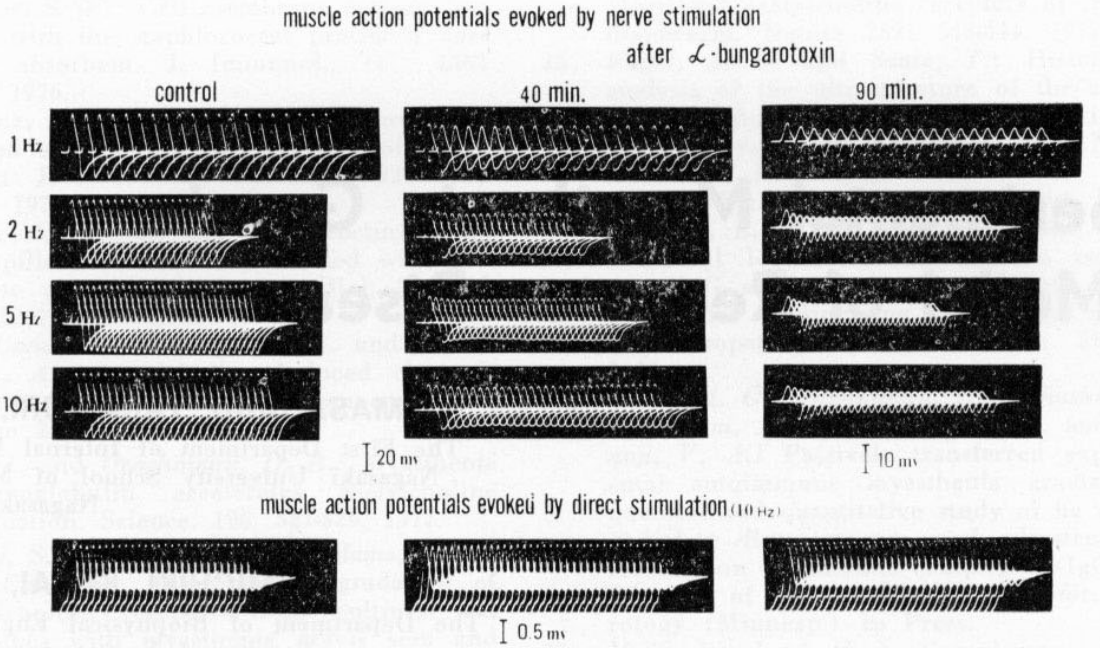

Fig. 1. — *Successive muscle action potentials evoked by nerve stimulation* (1, 2, 5 *and* 10 Hz) *and by direct stimulation of muscle* (10 Hz) *before and after the injection of α-bungarotoxin.*

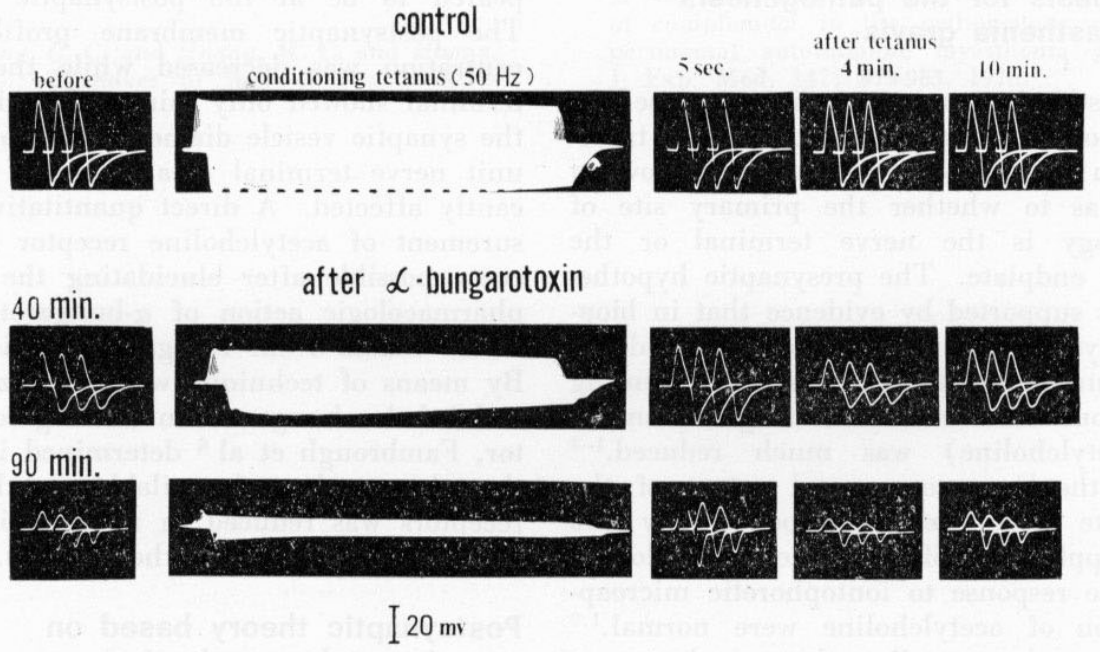

Fig. 2. — *Effects of prior tetanization on* 2 Hz *triple-evoked muscle action potentials: post-tetanic potentiation* (5 *seconds after a tetanus*), *post-tetanic exhaustion* (4 *minutes after a tetanus*), *and recovery* (10 *minutes after a tetanus*). *Records were obtained before and after the injection of α-bungarotoxin.*

choline receptors in animals and compared them with the abnormality occurring naturally in human myasthenia. The former used the α-toxin isolated from the venom of the Formosan cobra, Naja naja atra, and analyzed the data obtained during the period of recovery from complete paralysis. The latter, we, used the α-bungarotoxin isolated from the venom of an Elapidae snake from Southeast Asia, Bungarus mul-

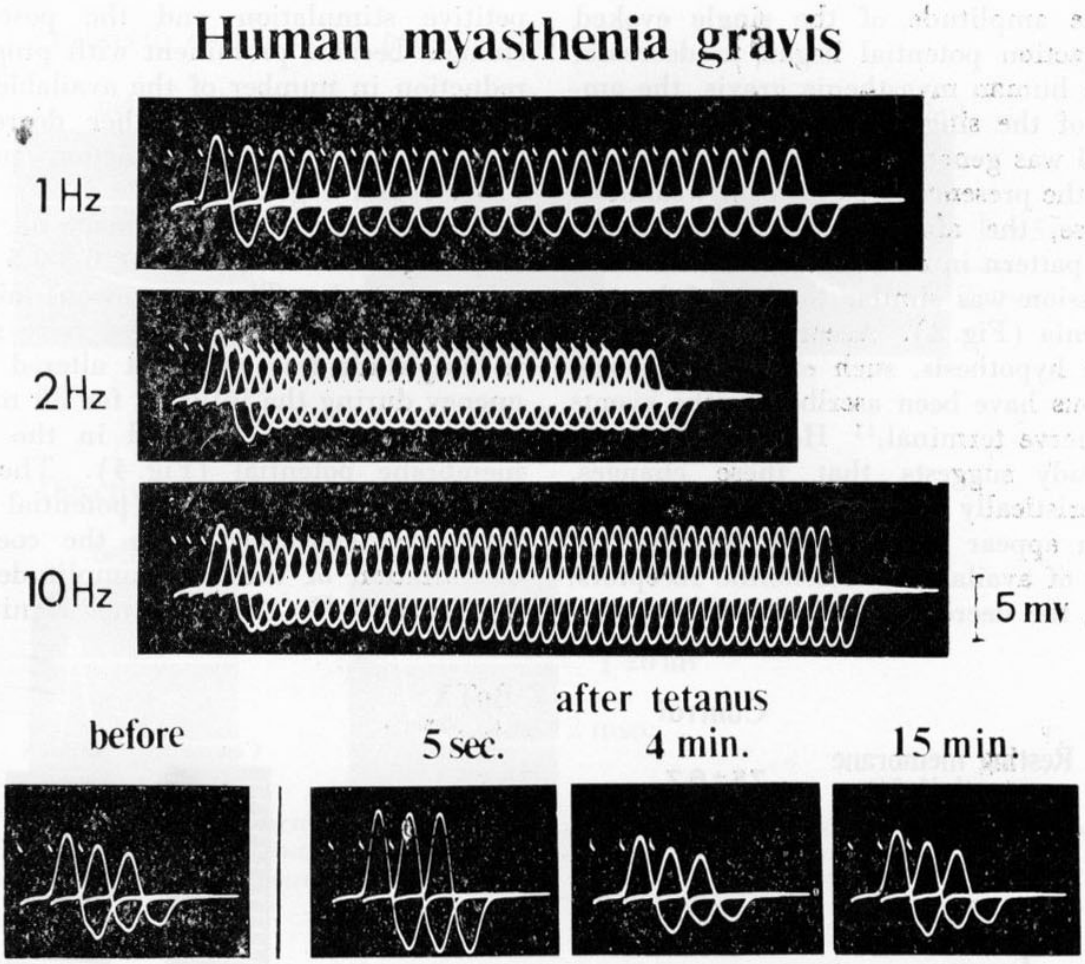

Fig. 3. — *Successive evoked muscle action potentials and post-tetanic changes of* 2 Hz *triple evoked muscle action potentials in a patient with myasthenia gravis. Record were obtained from the adductor pollicis muscle with stimulation of the ulnar nerve.*

ticinctus, and studied the data in the course of the torin-induced paralysis. The α-bungarotoxin, as well as cobra toxin, has been known to interact specifically with acetylcholine receptor.[9]

The toxin which we used consisted of single polypeptide chain of 74 amino acid residues cross-linked by five disulfate bridges; its molecular weight was estimated, giving a formula weight of 7,983.[10] Using this toxin, by which available acetylcholine receptors were presumed to be reduced in number, we undertook the investigation in the peroneal nervetibialis anterior muscle of rabbit in vivo and the phrenics-diaphragm preparation from rat in vitro. In the in vivo study, after control records were obtained, an injection of α-bungarotoxin (80 μg/kg resolved in physiologic saline) was given intravenously under the artificial respiration by tracheostomy. Electrophysiologic studies were then repeated during the intoxication. As the single evoked muscle oaction potential became decreased in amplitude, 1) even at the slow rates of repetitive nerve stimulation, the amplitude of successive evoked muscle action potentials decreased progressively until the 4th or 5th response and then was followed by a maintained plateau or a return toward its initial amplitude (Fig. 1). 2) After a conditioning tetanus, an increase in amplitude of the evoked muscle action potential occurred (post-tetanic potentiation) and was followed thereafter by a decrease (post-tetanic exhaustion) (Fig. 2). 3) Repetitive direct stimulation of the muscle produced no change in amplitude of muscle action potentials (Fig. 1). In the toxin-induced paralysis, features 1) and 2) appeared only

after the amplitude of the single evoked muscle action potential began to decrease, while in human myasthenia gravis, the amplitude of the single evoked muscle action potential was generally in the normal range even in the presence of significant weakness. Otherwise, the aforementioned electrophysiologic pattern in change of neuromuscular transmission was similar to that of human myasthenia (Fig. 3). According to the presynaptic hypothesis, such electrophysiologic alterations have been ascribed to the events in the nerve terminal.[11] However, the present study suggests that these changes, characteristically seen in human myasthenia, can appear if there is a reduction in number of available acetylcholine receptors. In fact, the decrement in amplitude by repetitive stimulation and the post-tetanic changes became prominent with progressive reduction in number of the available receptors as suggested by further decrease in amplitud of the single action potential (Fig. 1 and Fig. 2).

The in vitro study was made on the rat diaphragm at concentrations 0.3-0.5 μg/ml α-bungarotoxin. The spontaneous miniature endplate potentials (MEPPs) were reduced in amplitude but were not altered in frequency during the infusion for 30 minutes; no alteration was recorded in the resting membrane potential (Fig. 4). The quantum content of the endplate potential (EPP) was also determined form the coefficient of variation of the EPP amplitude distribution at 1 Hz; this did not significantly

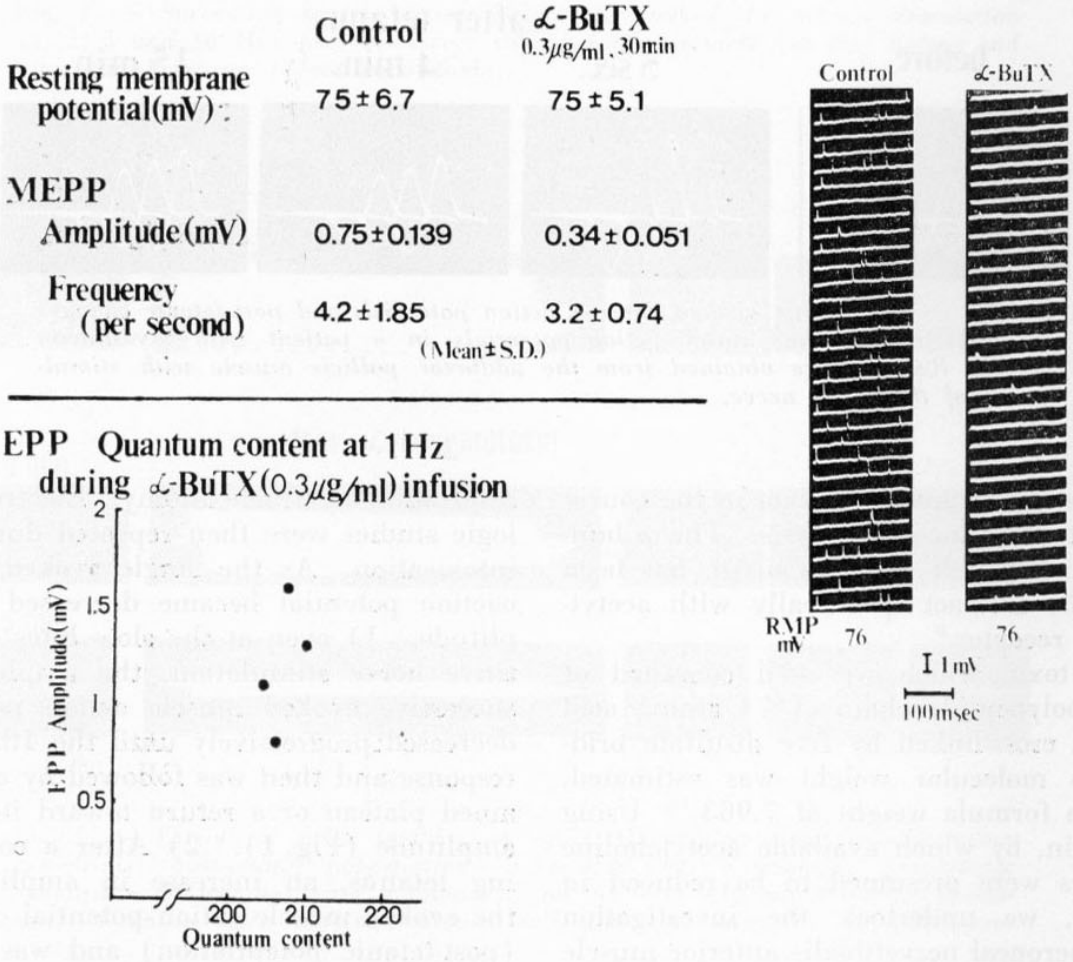

Fig. 4. — *Microelectrode studies in the rat diaphragm before and during the infusion of α-bungarotoxin.*

decrease with the progressive reduction in number of the available receptors as suggested by progressive decrease in the single EPP amplitude (Fig. 4), confirming the lack of presynaptic effect of the toxin. The toxin-induced block was antagonized with

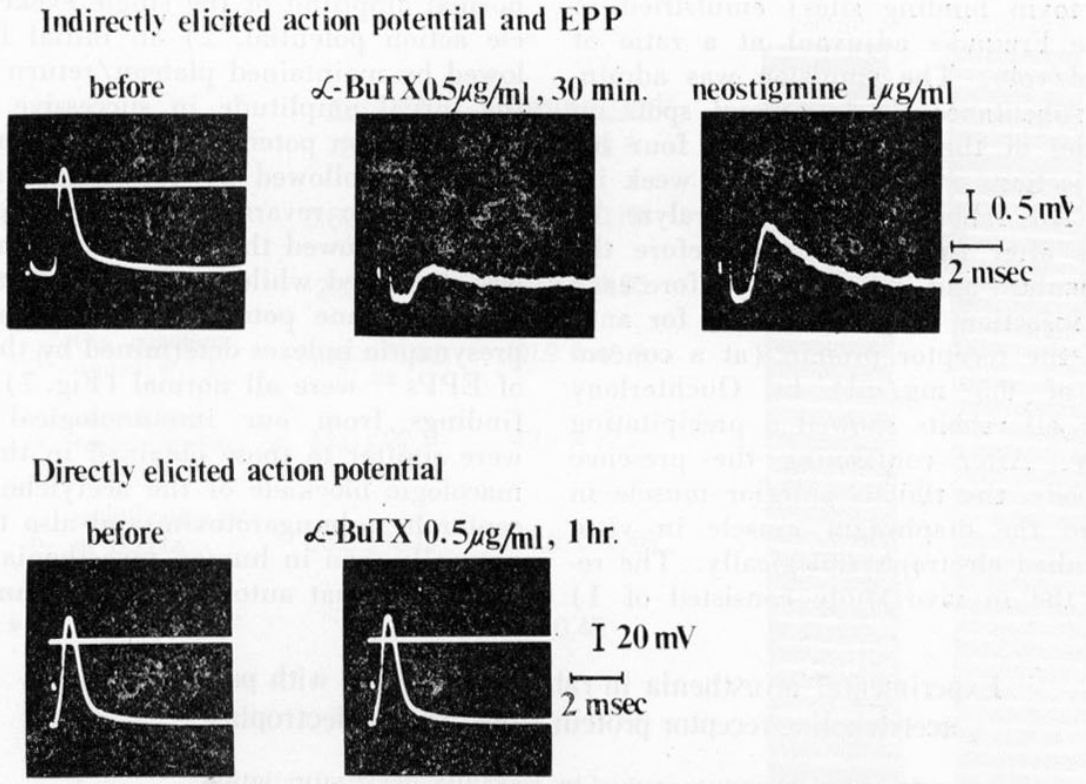

Fig. 5. — *Microelectrode studies in the rat diaphragm: Toxin-induced block antagonized by neostigmine, and no effect of toxin on the directly elicited muscle action potential.*

anticholinesterase, as evidenced by increase in the EPP amplitude and duration by neostigmine (Fig. 5). The amplitude and rates of rise and fall of the directly elicited action potential were not affected by toxin (Fig. 5), suggesting the lack of toxin effect on the extrajunctional membrane. These findings obtained with microelectrodes, as well as the aforementioned data from the in vivo study, provide a similarity to human myasthenia gravis,[3] and support the idea that the receptor abnormality may be of fundamental importance in the pathogenesis of myasthenia gravis.

Immunological model: Experimental myasthenia in animals immunized with purified acetylcholine receptor protein

Further impressive have been the immunological studies concerning the question of how the receptor abnormality comes about in myasthenia gravis. Simpson [12] in 1960, and Lennon and Carnegie [13] in 1971 proposed that myasthenia gravis might be the consequence of an autoimmune response to postsynaptic acetylcholine receptor. Not until 1973, was this idea confirmed in an animal model by Patrick and Lindstrom [14] and Sugiyama et al.[15] They reported experimental induction of myasthenia in rabbits immunized with purified acetylcholine receptor protein from the electric organ of Electrophorus electricus in association with raised antbodies against the receptor protein. This observation has since been confirmed by other groups [16,17] and in other species.[18-22] We solubilized the acetylcholine receptor protein from the electric organ of Narke electroplax japonica with the detergent Triton X-100, and purified by affinity chromatography on a conjugate of cobra toxin (Naja naja siamensis) coupled to agarose. Receptor protein was diluted in a solution containing 0.5 % Triton X-100, 0.5 M NaCl, 0.01 M Tris-HCl pH 7.4 and 0.02 % NaN_3. Receptor concentrations were 0.7-3.2 mg/ml or 3.2-15.7 nmoles toxin binding sites/ml. Five female New Zealand White rabbits weighing 1.8-2.2 kg were injected with receptor protein (1,600

pmoles toxin binding sites) emulsified in complete Freund's adjuvant at a ratio of 1:1 (vol/vol). The emulsion was administered subcutaneously in several spots on both sides of the spine. Two to four booster injections were given at one week interval. All rabbits began to paralyze by 5 weeks after first inoculum. Before the first inoculum and immediately before each booster injection, sera were tested for antibody to the receptor protein (at a concentration of 0.5 mg/ml) by Ouchterlony method; all rabbits showed a precipitating antibody. After confirming the presence of antibody, the tibialis anterior muscle in vivo and the diaphragm muscle in vitro were studied electrophysiologically. The result of the in vivo study consisted of 1) normal amplitud of the single evoked muscle action potential, 2) an initial fall followed by maintained plateau/return toward the initial amplitude in successive evoked muscle action potentials. 3) post-tetanic potentiation followed by exhaustion, and 4) edrophonium revarsal (Fig. 6). The in vitro study showed that the MEPP amplitude was decreased while its frequency and resting membrane potential were normal; the presynaptic indexes determined by the study of EPPs [23] were all normal (Fig. 7). These findings from our immunological model were similar to those obtained in the pharmacologic blockade of the acetylcholine receptor by α-bungarotoxin, and also to those naturally seen in human myasthenia gravis, suggesting that autoimmune mechanism di-

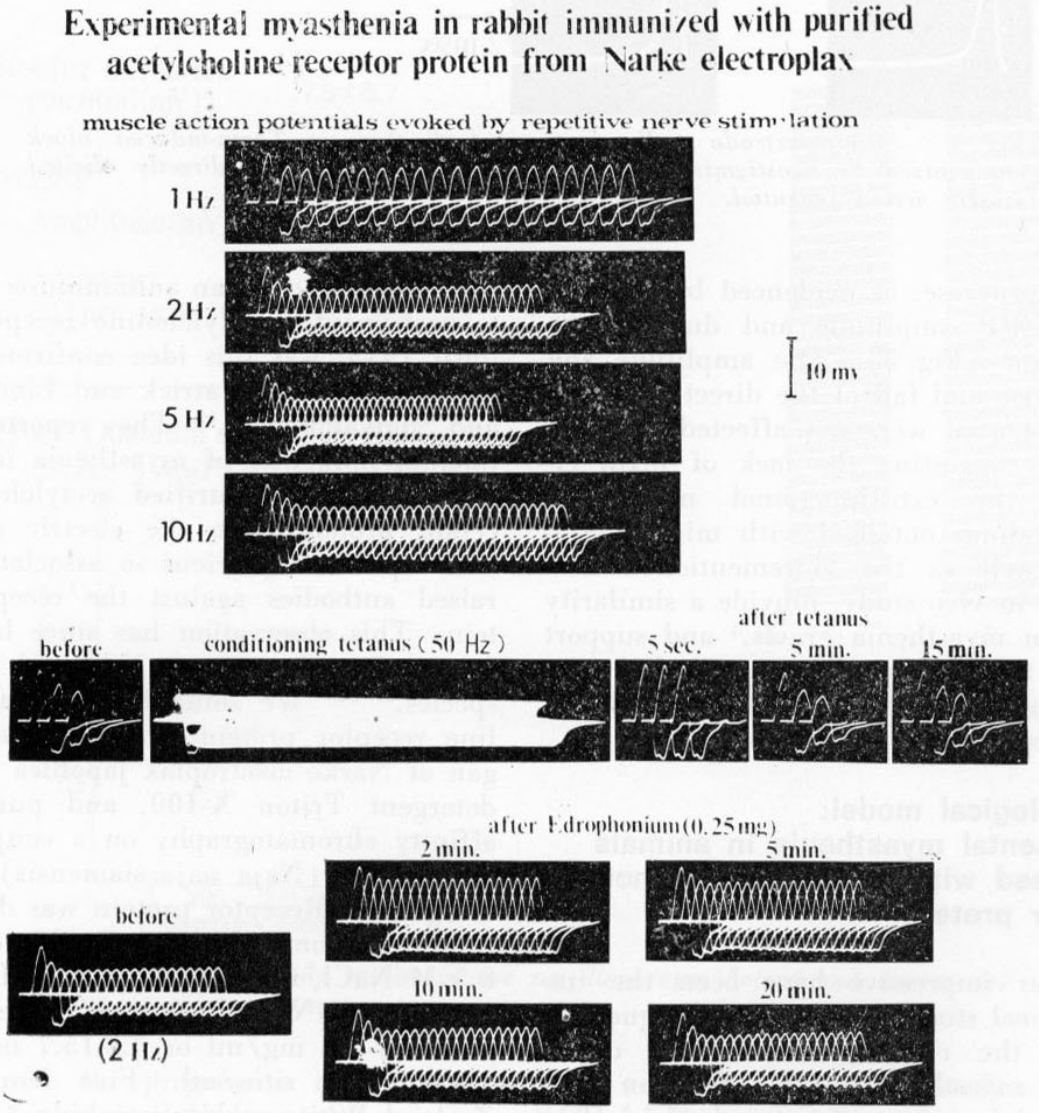

Fig. 6. — *Electrophysiological study in the tibialis anterior of rabbit immunized with purified receptor protein.*

		control	immunized
1.	resting membrane potential (mv)	72 ± 4.8	73 ± 5.5
▶ 2.	mepp amplitude (mv)	0.60±0.063	0.34±0.101
3.	mepp frequency (Hz)	2.2±0.81	2.3 ± 0.95
4.	epp, quantum content at 1 Hz	183.6±75.82	191.9 ± 46.9
5.	immediately releasable store, quanta	44.0±22.6	42.1 ± 12.7
6.	mobilization rate at 100 Hz (quanta/sec)	56.0 ± 13.19	47.8 ± 12.46
▶ 7.	d-tubocurarine (μg/ml)	1.2	0.45

Fig. 7. — *Microelectrode studies in experimental induction of neuromuscular block in rabbit by immunization with purified receptor protein. Records were obtained from control and immunized rabbit diaphragms.*

rected against the acetylcholine receptor may play a role in the pathogenesis of myasthenia gravis. This concept is supported by observations that anti-receptor antibodies were demonstrated in sera of many patients with myasthenia gravis [24–28] and experimental autoimmune myasthenia [18–22] by various assay methods, and that the immunoglobulin fraction (IgG) of myasthenic sera was shown to effect passive transfer of the disease from man to mouse [29] and from rat to rat.[30] In fact, the reduced density of acetylcholine receptor was visualized ultrastructually by the decrease in staining of α-bungarotoxin binding in human myasthenia and its experimental autoimmune model; [31,32] a destructive autoimmune reaction involving the postsynaptic membrane in human myasthenia was evidenced by the ultrastructural and light microscopic localization of IgG and C3 at the motor endplate.[33]

How is receptor activity blocked by antbody?

Anti-acetylcholine-binding site antibody would be expected to prevent binding of α-toxin to acetylcholine receptor. In human myasthenia gravis and its experimental autoimmune model, anti-acetylcholine receptor was not detected significantly by the toxin inhibitory assay but was highly demonstrated by the immunoprecipitation assay which utilized solubilized receptors labeled with ^{125}I-α-bungarotoxin and was sensitive for detecting antibodies directed to determinants outside the acetylcholine - binding site.[24,26,34] These reports suggest that antibody can impair the acetylcholine receptor molecule without blocking its acetylcholine-binding site, and also that blockage of receptor activity may result from either allosteric effect on acetylcholine binding or interference with the regulation or function

of the ionophore (ion conductance modulator system). This concept is supported by our protection experiment utilizing electrophysiological technique as such. Sera were obtained from two controls and five rabbits which, as mentioned above, were immunized with purified receptor protein and showed anti-receptor antibodies and physiologic features similar to human myasthenia. γ-globulin fractions were prepared by precipitation with ammonium sulfate al 33 % final saturation and dialysis against Ringer solution (24 mg/ml). Conventional microelectrode technique was employed for recording from the rat diaphragm preparation. When the preparation was exposed to α-bungarotoxin (5 μg/ml) for 30 minutes, the indirectly elicited action potential and endplate potential decreased below the noise level, and the following 4 hr washing produced a minimum recovery. In contrast, a block induced by d-tubocurarine (10 μg/ml, 30 minutes) was fully recovered during washing. Exposure of the preparation to d-tubocurarine (10 μg/ml) for 30 minutes and subsequent treatment with a combination of d-tubocurarine and α-bungarotoxin (5 μg/ml) for another 30 minutes followed by 4 hr washing resulted in a complete recovery to the control level in action potential amplitude (Fig. 8). This indicates that acetylcholine-binding site was protected by d-tubocurarine against α-bungarotoxin. When γ-globulin (10 mg/ml) from immunized rabbits, instead of d-tubocurarine, were added prior to α-bungarotoxin, the action potential was not affected by this, but abolished with subsequent treatment with α-bungarotoxin. During 4 hr washing, the action potential did not recover (Fig. 8). The same procedure using γ-globulin from control rabbits gave

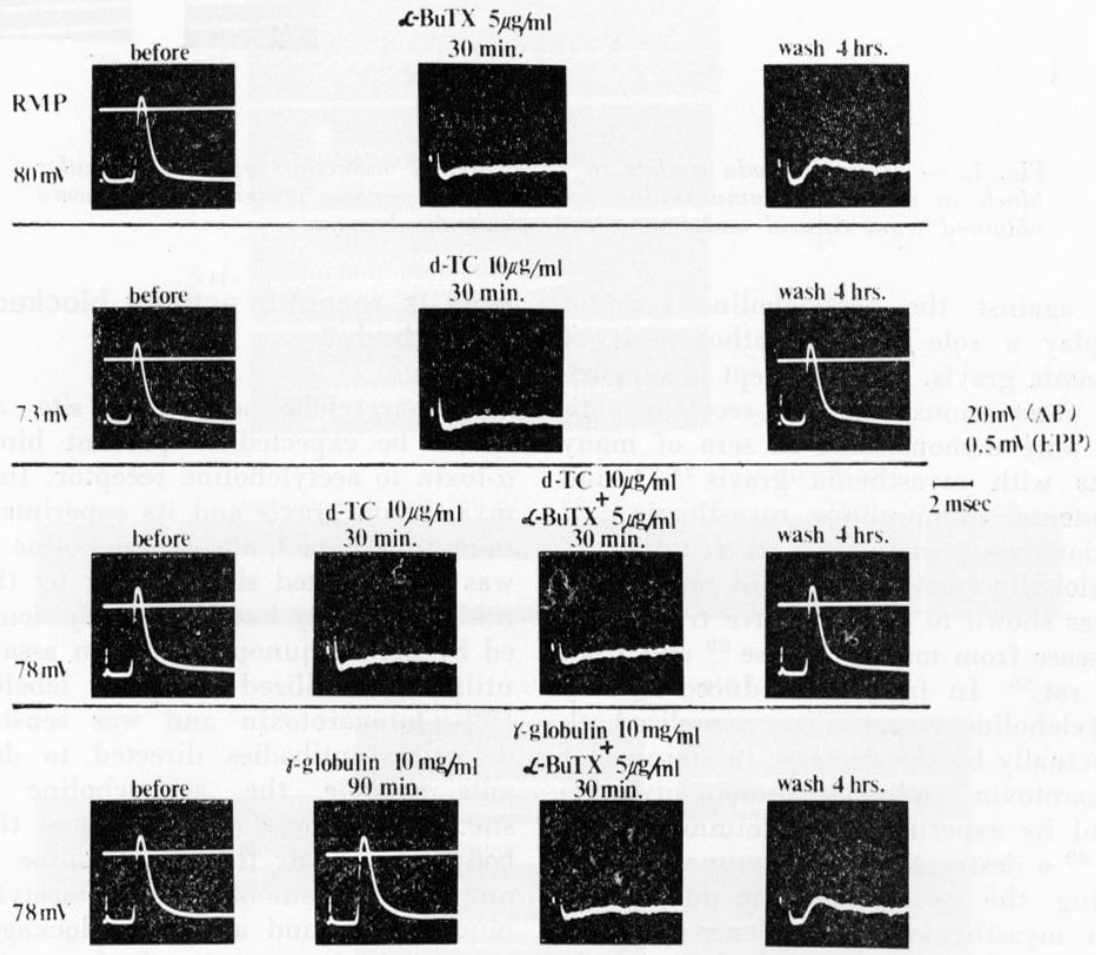

Fig. 8. — *Protection studies with d-tubocurarine, α-bungarotoxin, and γ-globulins obtained from sera of rabbits immunized with purified receptor protein. Records were taken from the rat diaphragm by microelectrode technique.*

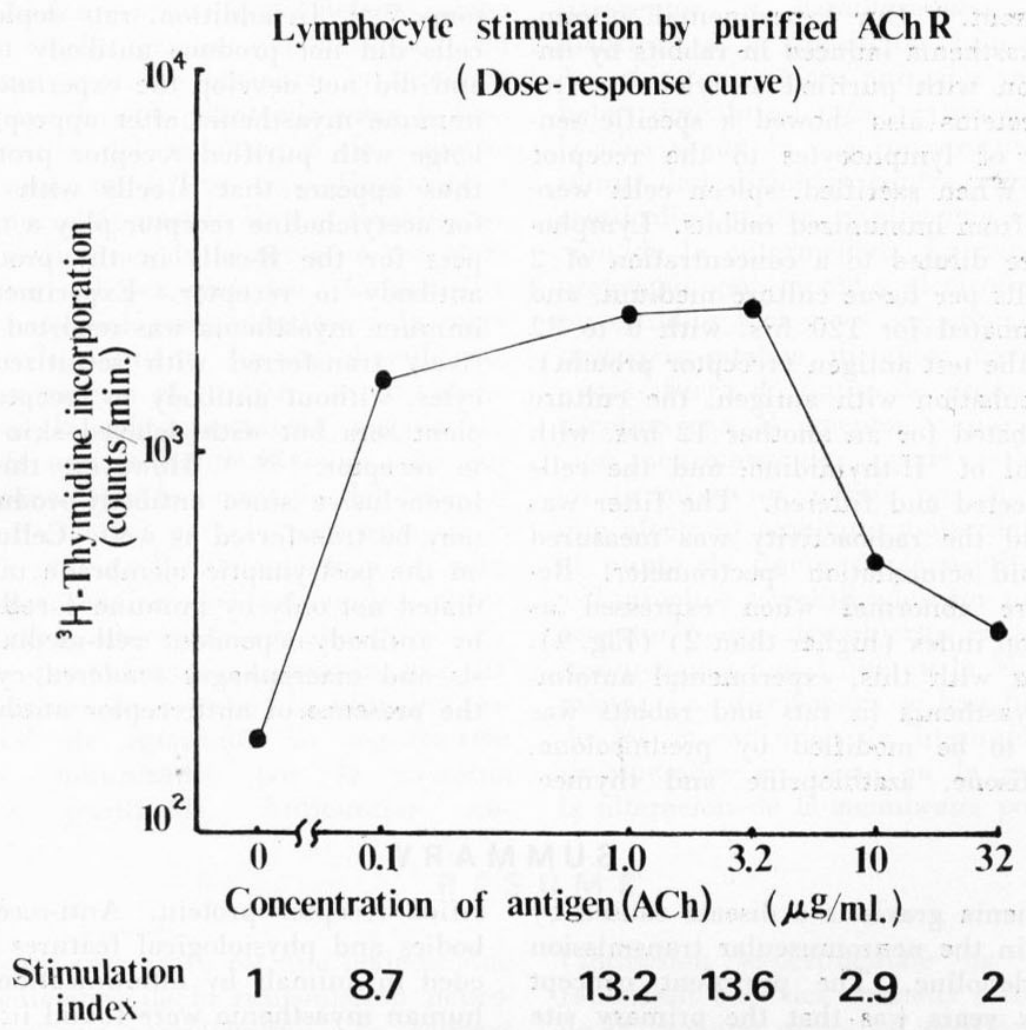

Fig. 9. — *Cellular hypersensitivity to the acetylcholine receptor protein demonstrated by lymphocyte transformation technique in experimental autoimmune myasthenia (rabbit).*

similar results. Resting membrane potential was not altered with any of the agents applied. These finding suggest that γ-globulin from immunized rabbits is not able to protect against α-bungarotoxin block of the acetylcholine-binding site and the neuromuscular transmission may be impaired through action involving the adjacent molecular environment of the acetylcholine-binding site of the receptor protein. γ-globulins had no effect on directly elicited action potential and resting membrane potential, suggesting that the extrajunctional membrane is without the scope of immunological implication. The same protection experiment utilizing human myasthenic sera also resulted in similar conclusions.[35] A recent report, however, showed a rapid reduction in acetylcholine sensitivity when the preparation used more labile receptors such as those in tissue cultured cells.[36] Histrionicotoxin [37] and ceruleotoxin [38] may be useful in studying further the properties of the acetylcholine receptor ionophores in pathologic muscle.

Cellular immunity to acetylcholine receptors

Peripheral blood lymphocytes from myasthenic patients were reported to exhibit specific sensitization when cultured in vitro with eel acetylcholine receptor proteins. A significant correlation between the severity of the disease and the degree of lymphocyte sensitization to eel receptor was noted,[39] and such a sensitization was reduced under steroid treatment in association with clinical

improvement.[40] Our experimental autoimmune myasthenia induced in rabbits by immunization with purified acetylcholine receptor proteins also showed a specific sensitization of lymphocytes to the receptor protein. When sacrified, spleen cells were obtained from immunized rabbits. Lymphocytes were diluted to a concentration of 2 X 10^6 cells per tissue culture medium, and were incubated for 120 hrs. with 0 to 32 g/ml of the test antigen (receptor protein). After incubation with antigen, the culture was incubated for an another 12 hrs. with 0.5 Ci/ml of ^{3}H-thymidine, and the cells were collected and filtered. The filter was dried, and the radioactivity was measured in a liquid scincillation spectrometer. Results were abnormal when expressed as stimulation index (higher than 2) (Fig. 9). Consistent with this, experimental autoimmune myasthenia in rats and rabbits was reported to be modified by prednisolone, hydrocortisone, azathioprine and thymectomy.[41,42] In addition, rats depleted of T-cells did not produce antibody to receptor and did not develop the experimental autoimmune myasthenia after appropriate challenge with purified receptor proteins.[43] It thus appears that T-cells with specificity for acetylcholine receptor play a role as helpers for the B-cells in the production of antibody to receptor. Experimental autoimmune myasthenia was reported to be passively transferred with sensitized lymphocytes, without antibody to receptor in recipient sera but with delayed skin reactivity to receptor.[19,30,43] However, this remains inconclusive since antibody-producing cells may be transferred as well. Cellular attack on the postsynaptic membrane may be mediated not only by immune T-cells, but also by antibody-dependent cell-mediated cytolisis and macrophages rendered cytotoxic in the presence of antireceptor antibodies.[30,43,44]

SUMMARY

Myasthenia gravis is a disease caused by a defect in the neuromuscular transmission by acetylcholine. The prevalent concept for many years was that the primary site of the defect was on the side of the nerve terminal. In the past several years, however, the postsynaptic hypothesis has been raised on the basis of the development in isolating α-neuro toxins capable of binding specifically to acetylcholine receptors and purifying the receptor protein from electric fish organs. Pharmacophysiological features similar to human myasthenia gravis were found when the number of available acetylcholine receptor was reduced by α-bungarotoxin isolated from the venom of an Elapidae snake. This suggests that the receptor abnormality may be essential for the pathogenesis of myasthenia gravis. In parallel with the reports that antibodies to receptor were detected in sera of myasthenic patients by various assay methods, experimental induction of myasthenia was succe-

rified receptor protein. Anti-receptor antibodies and physiological features similar to eded in animals by immunization with puhuman myasthenia were found in immunized animals. These findings support the idea that autoimmune mechanisms directed against the acetylcholine receptor may play a role in causing the disease. The protection experiment by electrophysiological techniques and the biological studies reported suggest that antibodies were directed to determinants outside the acetylcholine-binding site of receptor, and that the neuromuscular transmission may be impaired in myasthenia gravis through action involving the adjacent molecular environment of the acetylcholine-binding site. Lymphocytes sensitized with receptor proteins were detected in immunized animals as well as human myasthenia. Discussions were made on cellular immune mechanisms involved at least in part in amplifying impairment of the postsynaptic membrane.

RESUMEN

La miastenia gravis es una enfermedad causada por un defecto en la transmisión neuromuscular por acetilcolina. El concepto sostenido durante muchos años fue el

de que el asiento primario del defecto estaba asentado en la terminación nerviosa. En los últimos años, no obstante, la hipótesis postsináptica ha sido planteada sobre la base del desarrollo del aislamiento de α-neurotoxina capaz de fijarse específicamente a los receptores de acetilcolina y también sobre la purificación de la proteína receptora de órganos del pez eléctrico. Características farmaco-fisiológicas idénticas a la miastenia gravis humana fueron descubiertos cuando se redujo el número de receptores útiles por la α-bungarotoxina aislada del veneno de una serpiente Elapidae. Lo que hace suponer que la anomalía del receptor puede ser esencial para la patogenia de la miastenia gravis. Paralelamente a las referencias que señalan que anticuerpos al receptor fueron observados en los sueros de pacientes que sufren de miastenia, por distintas técnicas de estudio, la indución experimental de miastenia se lograba en animales inmunizados por la proteína respectiva purificada. Anticuerpos antirreceptor y caradterísticas fisiológicas idénticas a la miastenia humana fueron descubiertas en estos animales inmunizados. Estos descubrimientos permiten confirmar la idea según la cual mecanismos de autoinmunidad dirigidos contra el receptor de acetilcolina tienen implicancia en la aparición de la enfermedad. La experiencia de protección por técnicas electrofisiológicas y los estudios biológicos sugieren que los anticuerpos estaban dirigidos hacia determinantes fuera de punto de unión de la acetilcolina con el receptor y que la transmisión neuromuscular puede estar afectada en la miastenia gravis por el mecanismo que afecta el ambiente molecular adyacente al punto de unión de la acetilcolina.

Linfocitos sensibilizados por proteínas del receptor fueron descubiertos en los animales inmunizados, igual que en la Miastenia humana. Las discusiones se han hecho acerca de los mecanismos de inmunidad celular involucrados en parte en la extensión de la alteración de la membrana postsináptica.

RÉSUMÉ

La myasthenie grave est une maladie due à une déficience de la transmission neuromusculaire par acetylcholine. Le concept qui a prévalu pendant de nombreuses années était que l'emplacement originel de la déficience se trouvait sur le côté du nerf terminal. Ces dernières années, cependant, l'hypothèse postsynaptique a été posée en se basant sur le développement de l'isolation de α-neurotoxine capable de se lier spécifiquement aux récepteurs d'acetylcholine et en se basant aussi sur la purification de la protéine réceptive en provenance d'organes de poisson électrique. Des caractéristiques pharmaco-physiologiques identiques à la myasthenie grave humaine furent décelées quand l'indice de récepteur d'acetylcholine disponible fut réduit par l'isolation de α-bungarotoxine provenant du venin d'un serpent Elapidae. Ce qui laisse supposer que l'anomalie du récepteur peut être essentielle pour la pathogenèse de la myasthenie grave. Parallèlement aux rapports signalant que des anticorps au récepteur étaient décelés dans les sérums de malades souffrant de myasthenie, par diverses méthodes d'essai, l'induction expérimentale de myasthenie reussissait chez des animaux immunisés par de la protéine réceptive purifiée. Des anticorps anti-récepteur et des caractéristiques physiologiques identiques a la myasthenie humaine furent décelés chez ces animaux immunisés. Ces découvertes permettent de confirmer l'idée selon laquelle des mécanismes d'auto-immunition dirigés contre le récepteur d'acetylcholine jouent un rôle dans l'apparition de la maladie. L'expérience de protection par des techniques électrophysiologiques et les rapports des études biologiques laissent suggérer que des anticorps étaient dirigés vers des déterminants hors de l'emplacement de liaison d'acetylcholine du récepteur et que la transmission neuro-musculaire peut être altérée, dans la myasthenie grave, par le mécanisme entraînant l'environnement moléculaire adjacent de l'emplacement de liaison d'acetylcholine. Des lymphocytes sensibilisés par des protéines du récepteur furent décelés chez les animaux immunisés de même que dans la myasthenie humaine. Les discussions portèrent sur les mécanismes d'immunition

cellulaire qui jouent en partie un rôle dans l'extension de l'altération de la membrane postsynaptique.

ZUSAMMENFASSUNG

Myasthenie Gravis ist eine Frankheit, die von einen Defekt in der Neuromuskulären Transmission durch Acetylcholin erregt wird. Jahrenlang war die herrschende Meinung die, dass die primäre Stelle des Defekts an der Seite des Nervterminals lag. In den letzten paar Jahren, aber, ist die postsynaptische Hypothese aufgeworfen worden, und zwar auf der Basis der Fortschritten, erstens der Isolierung des sich spezifisch an Acetylcholinrezeptoren zusamnenschiliess enfähigen α-Neurotoxins, und, zweitens, der Reinigung des Rezeptorenproteins von den Organen elektrischer Fische. Als der Zahl der vorhandenen Acetylcholinrezeptoren reduziert wurde, durch das vom Gift einer Elapidaenschlange isolierte α-Bungarotoxin, wurden pharmako-physiologische Eigenschaften festgestellt, die den des Myasthenie Gravis des Menschen ähnlich sind. Dies deutet darauf hin, dass die Abnormalität der Rezeptoren vielleicht für die Pathogenese von Myasthenie Gravis unentbehrlich sein kann. Parallel mit den Berichten, dass nach verschiedenen Untersuchungsmethoden, Antikörper gegen Rezeptoren in der Sera myasthenischer Patienten festgestellt wurden, wurde Myasthenie in Versuchstieren durch Immunisierung mit gereinigtem Rezeptorenprotein erfolgreich erregt. Antikörper gegen Antirezeptoren und physiologische Eigenschaften, die die Eigenschaften Myasthenie in Menschen ähnlich sind, wurden in den immunisierten Tieren festgestellt. Diese Feststellungen unterstützen den Begriff, dass selbstimmune Mechanismen, die gegen die Acetylcholinrezeptoren gerichtet werden, in der Krankheitserrugung eine Rolle spielen. Die Beschützungsprobe nach elektrophysiologischen Methoden und die berichteten biologischen Studien deuten darauf hin, dass die Antikörper an Determinanten gerichtet wurden, die ausserhalb der Stelle der Acetylcholinzusammenschließens der Rezeptoren waren, und dass die neuromuskuläre Transmission in Myasthenie Gravis beeinträchtigt werden kann, durch eine Einwirkung, die die benachbarte molekulare Umgebung der Stelle des Acetylcholinzusammenschliessens betrifft. Lymphozyten, die von Rezeptorproteinen sensibilisiert waren, wurden sowohl in immunisierten Tieren, als auch in myasthenischen Patienten festgestellt. Man hat über die Zellenimmunmechanismen diskutiert, die, wenigstens teilweise, an der Vergrösserung der Beeinträchtigung der postsynaptische Membrane beteiligt sind.

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Myasthenia Gravis and Associated Diseases

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Myasthenia gravis is sometimes associated with other diseases. Many of them, like myasthenia, are suspected or have been demonstrated, to arise from an immunological disorder.

Most publications expose isolated case histories.[39,40] Studies evaluating incidence of diseases related to myasthenia in large series of patients are rare.[55,57]

This study is undertaken to evaluate this problem among 125 myasthenia patients.

MATERIALS AND METHODS

—*Materials*: our study includes 125 myasthenia patients admitted in the intensive care of "Hôpital Raymond Poincaré" (Garches) during the past ten years. General characteristics of this group were as follow:

—Sex: 34 males and 91 females

—Age at onset of myasthenia: 64 over 35 years of age; 61 under 35

—Severity of the disease (using Oserman's classification): grade I: 9 (7 %): grade II A: 11 (9 %); grade II B: 21 (17 %); grade III: 24 (19 %); grade IV: 60 (48 %).

—69 patients underwent thymectomy: 28 of those patients were found to have a thymoma at operation. Among non surgical patients, roentgengraphic study and scintigraphy using selenomethionine [11] led to suspicion of thymoma in 8 patients.

—*Methods*: all but five patients underwent the following studies:

- Arterial blood gases, electrolyte determinations, blood urea nitrogen, glycemia
- Plasma electrophoresis and immunoelectrophoresis
- Combs test and L. E. Cells test
- Rhumatoid factor determination. Waaler Rose and latex reaction
- Complement fractions assays by radial immunodiffusion (laboratoire d'Hématologie, Hôpital Raymond Poincaré de Garches).
- Antiorgan antibody assays: striated muscle, stomach, thyroid, smooth muscle, nucleus, mitochondria, thymic myoid cell using immunofluorescence; antithyroglabulin antibody. assay using passive hemagglutination (Pr EYQUEM, Institut Pasteur, Paris; Dr ANDRE, service du Prof. SULTAN, Hôpital Mondor à Creteil).
- Lymphoblastic transformation test in the presence of phytohemagglutinin (Dr PAPIERNICK, Service du Prof. BACH, Hôpital Necker à Paris).
- Electromyogram.

Older patients underwent systematic exploration in search of a neoplasm using pulmonary tomography, intraveinous pyelography, gastroduodenal series and barium enema.

For the past ten months, several new studies have been performed in a systematic basis:

- H.L.A. group determination by lymphocytotoxicity (Centre National de Transfusion Sanguine du Val de Marne): 56 patients.
- Gastric tubage and histamine test with intrinsic factor assay (I.F.) using the technics of Gottlieb and Coll [22] as well as

TABLE I

N°	Name	Sex	Grade	Age at onset of myasthenia	HLA	Striated muscle	Anti bodies anti-thyroid	stomach	nucleus	IF	Surgery	Thymoma	Associated disease
1	CRE...	F	IIB	57	–	+ 1/5	1/3125	–	1/100	–	+	–	Rhumatoid arthritis Hypothyroid
2	LEB...	F	IV	30	–	–	1/80	–	–	–	+	–	Rhumatoid arthritis goiter
3	MAR...	F	IV	49	29x/12y	+1/500	1/250	–	1/1000	–	+	+	Systemic lupus erythematosus
4	CLOU..	M	IV	38	W24–W32/8W25	+1/100	–	–	1/2000	–	–	–	Systemic lupus erythematosus Severe infection
5	LEG...	F	IIA	37	10-29/7–8	–	1/640	–	1/20	–	–	–	Hyperthyroidy
6	MAR...	F	IV	40	W29/12–W 27	1/500	1/640	1/20	1/50	+	+	+	Pernicious anemia
7	TAL...	F	IV	66	3-2/8–12	1/500	1/50	1/50	–	+	–	+	Low-Intrinsic factor
8	NIE...	F	IV	36	11–x/5W22	1/100	–	–	–	+	+	+	Low-Intrinsic factor
9	AMA..	F	IV	21	3-29/5–14	–	–	–	1/50	–	+	–	Thrombopenia
10	SAV..	F	III	78	–	1/200	–	–	–	–	–	–	Y2K2 paraproteinemia
11	DIE...	M	IIB	37	–	+1/5	–	–	–	–	+	+	chronic mucocutaneous candidiasis severes infections
12	HU...	F	IV	30	1W26/8W35	1/2000	–	–	1/20	–	+	+	Naevocarcinoma
13	SEV..	M	IIB	62	–	+1/5	–	–	–	–	+	+	Cancer
14	DAV..	F	IIB	62	–	1/50	–	–	–	–	–	–	Cancer
15	CYP..	F	IIB	71	W26–W32/7w22	–	1/25	–	–	–	–	–	Cancer Transient hyperthyroidy
16	BLAN.	F	IIB	57	3-9/7 W15	–	–	–	–	–	–	–	Cancer
17	FLEU.	F	IV	71	W24/W35W39	1/2000	–	–	–	–	–	+	Melanoma

TABLE II

Comparison between our series and Simpson's [55] and Souadjian's [57]

	our Results	Simpson (1964)	Souadjian (1974)
Cancer	3	○	9
Dysthyroidia	3	87	2
Systemic lupus erythematosus	2	1	○
Rhumatoïd arthritis	2	18	○
Pernicious anemia	1	9	2
Cytopenia	1	1	1
Hemolytic anemia	○	2	○
Raynaud's Syndrom	○	16	1
Polymyositis Dermatomyositis	○	○	2
Severe or mycotic infection	2	○	1
Sarcoidosis	○	1	1
Total number of myasthenic patients	125	491	186

We note a significant difference between this three series concerning the number of patients with thyroid disfunction.

an anti-intrinsic factor antibody assay using the method of Samloff and Coll [49] (Dr. Zitoun, Service du Prof. Sultan, Laboratoire d'Immunologie, Hôpital Henri Mondor a Creteil(: 40 patients.

RESULTS

Intercurrent disease was present in 17 patients (table 1). The following characteristics of this group were undistinguishable from those of the total group:

—Sex: 3 males and 14 females

—Antiorgan antibodies levels: similar in both groups

—Average time since onset of myasthenia.

On the other hand, several characteristics distinguished these 17 patients from the parent group:

—Age at onset of myasthenia was higher: 14 patients were over 35 years old.

—Seriousness of the disease: absence of class I and II A.

—Frequency of thymoma was greater: 8 out of 17 (47 %) versus 24 % in the remaining 108 patients.

—H.L.A. determination was performed in 56 patients: 21 were H.L.A. 8; 14 of these were under 25 years of age and 7 over. Of 11 myasthenia patients with associated disease tested 4 were H.L.A. 8 while all 11 patients were over 25 years old The H.L.A. 8 group is often correlated with autoimmune disease.[12,18]

STUDY OF THE DIFFERENT ASSOCIATIONS

—Myasthenia and rheumatoid arthritis [1-38-55]

Two patients in our series presented this association.

Case report n° 1:

Onset of rheumatoid arthritis at age 20 in 1936 (latex test 1/80· Waaler rose 1/40, rheumatoid factor 1/518). The patient received corticosteroid therapy from 1967 to 1972 followed by D penicillamine (30 mg/kg/day) in December 1972. Improvement was noted in the arthritis but 10 months later, myasthenia appared. The drug was discontinued after 14 months of administration. Symptoms of myasthenia nevertheless persisted and an increase of anticholinesterase drugs was necessary. Following a relapse of the arthritis in April 1974, D penicillamine was resumed. Aggra-

vation of the myasthenia was noted in July requiring mechanical ventilation by endotracheal tube. In August, immuno assay demonstrated antithyroid antibodies in a titer of I/3125 with hormone tests demonstrating hypothyroid (low T3 and T4, low radioactivity iodine 131 fixation curve). The patient died in October 1974. Autopsy findings included chronic thyroiditis with lymphocytic and plasmocytic infiltration. Only vestigial thymic tissue was present.

Case report n° 2:

Onset of rheumatoid arthritis at age 28 (latex 1/80, Waaler rose negative). Onset of myasthenia two years later at 30. At this time an euthyroid goiter was also diagnosed. Antithyroid antibodies were present in a titer of 1/80 and thyroid biopsy failed to demonstrate any infiltration of the Hashimoto type. At age 48, thymectomy was performed ant thymus hyperplasia was found. Improvement was initially obtained by slow actings corticotropin. Stabilisation of myasthenia has been maintained by Ambenonium chloride; the patient is leading a normal life, however the tracheostomy has not been closed because of tracheal compression by the goiter. The patient is receiving no treatment for the rheumatoid arthritis.

We note a higher frequency of this association in females: Oosterhuis series of 142 myasthenia patients included 7 associations of this type, all occuring in females; of the three associations in Aarli's series, 2 were females.

The chronological order of onset of the 2 diseases varies: arthritis appeared initially in two patients in each of the 3 series.[1,38]

Cases of myasthenia with onset during treatment by D penicillamine have been reported occuring in 2 patients with Wilson's Disease [33] and in 6 patients with rheumatoid arthritis, including one of our own.[8,13,21] Discontinuance of the drug led to disparition of the myasthenia in most patients after a delay of 15 days to 7 months. One patient improved somewhat while 2 patients presented no change one in our series and one reported by Masters.

The responsability of D penicillamine appears certain. The physiopathologic mechanism appears to be merely an unmasking of a pre existing desorder rather than a direct cause. Nevertheless antistriated muscle and anti acetylcholine receptor antibodies appeared with no clinical symptoms of myasthenia in some patients treated with D penicillamine.[33] D penicillamine has also been implicated in other complications: disseminated lupus erythematosus polymyositis Goodpasture's syndrome nephrotic syndrome.

—Myasthenia and disseminated lupus erythematosus [2,14,19,28,51]

Two of our patients presented this association.

Case report n° 3:

Thymectomy for thymoma at age 49. Onset of myasthenia several months later with stabilisation under treatment with Ambenonium chloride. Relapse at 54 requiring tracheostomy with mechanical ventilation. Further exploration at this time revealed the presence of lupus with positive. L. E. Cells test and antinuclear antibodies in a titer of 1/1000. Subsequent treatment with prednisone followed by imuran resulted in improvement of the myasthenia but a leucopenia forced the discontinuation of this agent. The patient has been treated with Plaquenil and prednisone since age 55 with 2 relapses followed by a stabilisation of the myasthenia for 5 years (tracheostomy, mechanical ventilation). No cutaneous or renal abnormalities have been detected thus far.

Case report n° 4:

Onset of myasthenia at age 38. Treated with Ambenonium chloride, with satisfactory results. Relapse at 48 requiring tracheostomy with mechanical ventilation. A lupic syndrom was then diagnosed then L.E. cells and antinuclear antibodies in a titer of 1/500 were detected. At the same time a serious eye infection arose quickly leading to irreversible damage and abcess of the eye itself. Subsequent enucleation was performed. Imunological exploration then revealed a depressed cellular immunity

with a negative intra dermo reaction to tuberculin and varidase.

The myasthenia improved with treatment by Plaquenil and Ambenonium chloride No cutaneous or renal abnormalities have been detected thus far.

Considering the rarity of lupues and myasthenia, their association cannot be a result of random chance. This association generally occurs in females; no cases involving male patients were found in the literature. Our patient however was male. The order of onset of the 2 diseases is variable: lupus is most often discovered after onset of myasthenia, as was seen in two patients each of Aarli's study and our series, one of Oosterhuis and Schoener's two patients, as well as others reported in the literature.

The etiology of these two diseases in uncertain. Both patients had elevated serum antibody titers suggesting a possible immunological disorder. Systematic studies in patients with lupus carried out by Mac Kay [30] concluded in abnormal histologic findings on the thymus glands of 13 patients.

The diagnosis of lupus was established in our patients only on the basis of biological signs. No visceral pathology was ever found. Immunosuppressive therapy did not alter the course of the myasthenia, significantly.

—Myasthenia and thyroid disfunction [4,5,6,13,17,41,47,53,55,56,62]

• Patients, in ours series, often have elevated antithyroid antibodies' but only 4 patients present thyroid disfunction.

Case report: nº 1 - 2
Case report nº 5:

Onset of myasthenia at age 37. At 48, the patient developped clinical and biological hyperthyroidy. Antithyroid antibodies were positive in a titer of 1/640. Satisfactory results were achieved with neomercazol and ambenonium chloride therapy.

Case report nº 15:

Previous history included breast cancer treated surgically at age 64. Onset of myasthenia was at the age of 71. Satisfactory control was obtained with prednisone and ambenonium chloride.

Hyperthyroid was diagnosed at the same time: thyroxinemia 18 gamma per 100 ml (N. 6.11) then 92.6 gamma per 100 ml; the antithyroid antibodies were presents in a titer of 1/25.

A third thyroid study one month later turned out anormal, with negative antithyroid antibody assay. Thyroid biopsy was not performed.

A follow up examination 3 years later finds the myasthenia stabilised with ambenonium chloride. Thyroid findings are unremarkable with a negative antithyroid antibody assay.

The incidence of this association varies with the different author's series: Simpson[55] reports 18 % in his series of 491 myasthenia patients, Van der Geld reports 10 % in his series of 111 patients, Sahay [47] reports only 8 patients (3 %) with thyroid disfunction in his series of 260 patients. Our own study agrees with the latter one: 4 out of 125 myasthenia patients had thyroid disfunction.

The diversity of results can probably be attributed to lack of standardised diagnostic criteria. Only those used by Sahay are identical to our own.

The association of these 2 diseases arises in both sexes with equal frequency. Nevertheless, those in our series were exclusively women.

The association of myasthenia and hyperthyroid was recognized as early as 1908 when Rennie described a case of exophtalmic goiter in a myasthenia patient. Histology of the thymus of patients with Hashimoto's thyroiditis and the thyroid of patients with myasthenia often detects various abnormalities. Thus Becker [4] reports 19 % in his series of 32 myasthenia patients with such findings.

The chronological order of onset is variable. In reviewing the literature, we note that onset of the two diseases is often paral-

lel. The evolution is most often independant. In the few cases where improvement was parallel, the problems of a hyperthyroidian myopathy can be posed.

The association of myasthenia with hypothyroid is much less frequent. Feinberg in 1963 described 3 cases, Storm Mathison in 1961 had two cases in a series of 90 myasthenia patients and Sahay [47] reported 5 cases in his series of 260. The single patient in our series presenting this association had histological signs indicating thyroiditis.

—Myasthenia and hematologic diseases

• Pernicious anemia occurring in a myasthenia patient was first described in 1956 by Rowland. Since then, many case histories have been published [17,40,56] but this association remains rare among studies done on large series of patients:

—Osserman in 964 described two cases of pernicious anemia in a series of 687 myasthenia patients (0.3 %).

—Simpson in 1964 reported 9 cases in a series of 491 patients (1.8 %).[55]

—Soudjian in 1974 reported two cases in a series of 186 patients (1 %).[57]

Our series of 125 myasthenia patients included one patient with anemia, and histamine résistant gastric achlorhydria (0.8 %).

Case report nº 6:

Female with a negative history until age 34 (1955) when a mediastinal tumor was discovered during a routine examination. Onset of myasthenia was at age 40 in 1965. At age 44, the patient was hospitalised in our service with grade II B myasthenia. Surgery was performed but only partial thymectomy was possible. Radiotherapy was there-fore undertaken during the post surgical convelescence. Progressive improvement was noted and the patient was discharged in February 1966 with a normal CBC and negative antistomach antibodies.

The patient was regulary seen with no detectable pathological findings until 1971 when antistomach antibodies were detected. In July 1973, she was hospitalised for digestive problems, glossitis, stomatitis, dyspnea and vertigo. A CBC revealed anemia fith 1 300 000 RBC and 5.8 g of hemoglobin. Findings included a reticulocyte count at 2 %, rich bone marrow, gastric tubage showing a histamino resistant achlorhydria, low vitamin B 12 assay (80 pg/ml; N = 200 — 400) and normal folate level. A Schilling test confirmed the existence of pernicious anemia. Intramuscular vitamin B 12 injections were followed by a rise in reticulocytes to 15 % in 4 days with progressive normalisation of the blood count.

With continued supplemental vitamin B 12 therapy, the patient's CBC remained normal. Further tests detected the presence of anti intrinsic factor antibody (1976) Sudden death occurred after discharge in 1977.

Other autoimmune diseases occurring in the presence of pernicious anemia have been reported, notably thyroid disfunction and systemic lupus erythematosus.[15,25]

All myasthenia patients in our service now undergo test for the presence of anti intrinsic factor antibodies. Of 60 patients thus tested, its presence was detected in 3, including the patient just described.

Case report nº 7:

Onset of myasthenia at age 66 with hospitalisation one year later. Upon arrival in our service, the patient presented a grade IV myasthenia. Chest roentgenogram showed the presence of a thymoma but surgery was ruled out because of chronic respiratory insufficiency. Anti stomach antibodies were positive in a titer of 1/200 histamine sensitive achlorhydria existed and intrinsic factor level was low with a positive serum anti intrinsic factor antibody. Shilling's test was normal and no anemia was present.

During the next year, acute deterioration occured on 3 occasions, requiring tracheostomy with mechanical ventilation. The disease is now stabilised with persistance of an anti intrinsic factor antibody and absence of anemia.

Case report n° 8:

Onset of myasthenia at age 36 with subsequent thymectomy a few months later. Stabilisation was achieved and maintained for 10 years with anticholinesterase therapy. Deterioration occured in 1976 requiring tracheostomy with mechanical ventilation.

Test at this time detected the presence of a serum anti intrinsic factor antibody, low gastric intrinsic factor level, histamine sensitive achlorhydria, negative Shilling test and normal findings after gastric fibroscopy and biopsy. The patient's condition improved ved under corticosteroid therapy. No change immunologic findings was observed after one year. No anemia has been detected.

Wheter or not the presence of anti intrinsic factor antibodies indicates the eventual development of pernicious anemia can only be determined by long term observation. These antibodies may be considered either specific of the disease [63,64] or indicative of a different pathologic process of the type found in auto-immune diseases.[26,44]

• Cytopenia.

—Only one patient in our series had a transitory thrombopenia.

Case report n° 9:

Onset of myasthenia at age 21. Initial radiotherapy was followed by thymectomy. Tracheostomy with mechanical ventilation was required. Slow progressive improvement was observed under anticholinesterase therapy. The patient's condition permitted closing of the tracheostomy in 1968 at the age of 31. Stabilisation of the myasthenia has been maintained thus far.

During a routine examination in March 1977, a thrombopenia with 50.000 platelets per mm3 was discoverd. The patient was rehospitalised in June for further test on platelet function with normal findings.

Segal has reported a case of thrombopenic purpura in a patient who developed myasthenia a few years later. In fact this association seems quite rare.[53]

The literature describes a more frequent association between thymoma and erythroblastopenia, and myasthenia, thymoma and erythroblastopenia. We have not had the occasion to observe such a case, but numerous case reports have been published.[9,10,20,23,31,34,43,52,54] In 1928, Mahas and Priesil reported a patient with thymoma and aplastic anemia and they hypothesized the presence of an erythropoietin blocking factor secreted by the thymic tumor.

• Finally, a few cases of hemolytic anemia occurring in myasthenia patients have been reported.[16,36,55]

—Myasthenia and gammapathy

Systematic immunoelectrophoresis in all our patients has led to the discovery of only one monoclonal gammapathy.

Case report n° 10:

Myasthenia onset at age 78 with quick progression to grade III requiring tracheostomy and mechanical ventilation. Little improvement was noted with anticholinesterase drugs. No thymoma was found. Immunoelectrophoresis detected a γ 2 K 2 paraprotein and there were no anti organ antibodies.

Improvement and stabilisation in the patient's condition was achieved using corticosteroid therapy (25 mg/day prednisone). Death ensued at age 81 from a pulmonary infection.

The literature contains few cases of type G or M gammapathy [35,45] occuring in myasthenia patients. Admittedly this associations is possibly a mere coincidence considering its rarity. Nervertheless, the thymus gland's influence in myasthenia and on the immune system suggests a possible common pathophysiology in the two diseases.

The association of myasthenia· thymoma and hypo-gammaglobulinemia has also been reported in the literature.[36,42,60]

—Myasthenia and infection

Infection occured in our myasthenia pa-

tients with no greater frequency than in other patients in our service who had tracheostomy and mechanical ventilation.

Two case reports are singled out:

—*Case report n°* 4: occurring in a patient with lupus

—*Case report n°* 11:

Onset of myasthenia at age 37 while the patient was under treatment for toxoplasmosis. Thymectomy for invasive thymoma as performed the same year.

Cutaneo mucus candida infection was diagnosed for the first time 3 years after the thymectomy with presence of digestive pharyngeal and ungual moniliasis. Lingual granuloma biopsy confirmed the diagnosis. We noted also the presence of anticandida precipitins. Treatment by nystatin followed by 5 fluorocytosine was ineffective. Septicemia with enterobacter also developped.

Immunity tests revealed a negative tuberculine intra dermo reaction and a positive candida intra dermo reaction at I/1000 dilution. A lymphoblast transformation test in the presence of phytohemagglutinin was normal, and positive in the presence of candidin. A deficit in the oxigen dependent bactericide system was detected (Laboratoire d'Hematologie, Hôpital Bichat, Prof. Hakim). Leucocyte peroxydase was normal and no G6PD deficit was found.

The thymoma reappeared 6 years after the thymectomy and candidiasis persisted. A second operation followed by radiotherapy was successful in stabilizing the myasthenia. The patient died 1 year later afterleaving the service.

While humoral immunity disfunction has been cited in myasthenia patients, serious infection can be the result of cellular immunity disfunction.

The association of myasthenia and serious infection is not frequent. Souadjian reported a 6 % incidence among patients with thymoma.

On the other hand, chronic mucocutaneous candidiasis of the adult is almost exclusively associated with thymoma and myasthenia (3 out of 6 cases in the literature).[32,37] Cellular immunity disfunction is often detected. Still, the precise role of the thymus gland in protection from candida infection remains to be defined.[48,50]

—Myasthenia and cancer

Systematic search for neoplasm in older myasthenia patients led to an occasional positive finding.

Case report n° 12:

Onset of myasthenia at age 30. Discovery of an invasive thymoma was followed by excision and radiotherapy in 1959. Five years later a presternal noevo-carcinoma developed and was treated by wide excision. Myasthenia remained stabilised throughout this episode. Aggravation of the myasthenia did occur on 2 occasions, one in 1968 and one in 1969. The naevocarcinoma reappeared in 1970 with no change in the myasthenia. 1976 saws an aggravation of the myasthenia and discovery of metastases of an undetermined nature in the sternum, axillary nodes and brain. Death ensued a few months after the patient had been discharged.

Case report n° 13:

Onset of myasthenia at age 62. Findings included an invasive thymoma which was treated by excision followed by radiotherapy. The myasthenia was stabilised for a few months with anticholinesterase therapy but aggravation soon occurred. Further tests found an elevated urinary 5 HIAA level of 13.9 mg/day (N = 2-8). Death ensued soon after. A left lung tumor was found at autopsy.

Case report n° 14:

Onset of myasthenia at age 61. Past history included breast cancer treated by surgery and radiotherapy. The myasthenia has been stabilised for the last 4 years using anticholinesterase drugs and no reappearance or the neoplasm has occured.

Case report n° 15:

Case report n° 16:

Onset of myasthenia in 1972 at age 57. Stabilisation was achieved with pyridostigmine-bromide which was discontinued after 18 months. In 1973, breast cancer was

discovered and treated by surgery and radiotherapy. No aggravation of the myasthenia was noted. In 1977, aggravation did occur and stabilised with ambenonium chloride. No reappearance of the neoplasm was taken place.

Case report nº 17:

Onset of myasthenia at age 71. Thymoma was present but surgery was ruled out because of the patient's coronary artery and respiratory post history. A melanoma was found in June 1974 and treated surgically. The thymic region received radiotherapy in August 1974. The myasthenia improved under anticholinesterase medication. However chronic respiratory insufficiency secondary to pulmonary fibrosis compromised the patient. Despite stabilisation of the myasthenia and absence of reapparition of the melanoma, death insued in 1976 frompulmonary infection. No tumor was found at autopsy.

The thymus gland has often been linked to carcinogenesis bothexperimentally and in human studies. Souadjian [59] reported a histological study of the thymus in 348 patients and his findings included a significant difference between patients deceased from neoplasm and those deceased from neoplasm and those deceased from other causes. We also reported that 31 out of 146 patients with thymoma eventually developed neoplasms during a 20 years period of observation versus only 8% in a control group.[58] However in 1974 he found only 9 out of 186 myasthenia patients having thymomas. Simpson [55] reported no cancer. Other authors [7-24] reported only isolated cases.

Other diseases have also been described with myasthenia: endocarditis, polymyositis,[27] dermatomyositis,[46] vitiligo.[16]

The associations of these various diseases with myasthenia are not random phenomenon, but rather a disfunction of the immunologic system. Other associations with myasthemia have been described however, which do appear to result from coincidence (epilepsy and diabetes mellitus).

For this reason, we have addressed ourselves only to those associations which are suspected of having common etiology.

SUMMARY

Among 125 myasthenia patients admitted in the intensive care unit during the ten past years, 17 had associated diseases which can be due to a disfunction of the immunologic system: 2 rheumatoid arthritis, 2 disseminated lupus erythematosus, 3 thyroid disfunction, 1 pernicious anemia, 1 cytopenia, 1 gammapathy, 2 serious infections and 3 cancer. In all these case reports: myasthenia starts after 35 years of age, the grade is more serious, there are more thymomas and HLA 8 group there in the patient group.

After having told every case, we discuss about each association with a review of literature.

It seems that these associations cannot be random phenomenon but rather a disfunction of the immunologic system.

RESUMEN

Entre 125 miasténicos hospitalizados y seguidos en diez años en el servicio de tratamiento intensivo, 17 tenían enfermedades asociadas las que podían estar en relación con una disfunción del sistema inmunita: rio: 2 poliartritis reumatoidea, 2 lupus eritematoso diseminado, 3 disfuncion tiroidea, 1 anemia perniciosa, 1 trombocitopenia, 2 infecciones severas, 1 gammapatía y 3 cáncer. En todos los casos la miastenia comenzó después de los 35 años de edad, y es más severa, se aprecia una tasa más elevada de timomas y grupos HLA8.

Después de haber mencionado cada caso,

discutimos cada asociación a la luz de casos aparecidos en la literatura. Parece que todas estas asociaciones mórbidas no son fortuitas, sino que evidencian probablemente una disfunción del sistema inmunológico.

RÉSUMÉ

Sur 125 myasthéniques hospitalisés et suivis en dix ans dans le service, 17 ont eu des maladies pouvant relever d'un dysfonctionnement du système immunitaire: 2 polyarthrites rhumatoides, deux lupus érythémateux disséminés, 3 dysthyroïdes, 1 anémie pernicieuse, une thrombopénie, une gammapathie, deux infections sévères et trois cancers. Dans tous les cas, la myasthénie a débuté après 35 ans, et est plus sévère, on note un taux plus élevé de thymomes et de groupes HLA 8.

Après avoir exposé chaque observation, nous discutons les associations à la lumière des cas parus dans la littérature.

Il semble que toutes ces associations morbides ne soient pas fortuites, mais révèlent probablement un dysfonctionnement du système immunologique.

ZUSAMMENFASSUNG

Zwischen den 125 hospitalisierten und seit zehn Jahren beobachteten Myasthenikern mit Intensivbehandlung, hatten 17 assoziirte Krankheiten, die auf eine Dysfunktion des immunitärischen Systems in Zusammenhang gebracht werden konnten: 2 rheumatische Polyarthriden, 2 Lupus erytematosis disseminadas, 3 thyreoide Dysfunktion, 1 Anämia perniciosa, 1 Thrombocitopenie, 2 schwere Infektionen, 1 Gammaminapathie und 3 Cancer . En allen Fällen begann die Myasthenie nach dem 35. Lebensjahr, und war schwerer, wenn man eine erhöhte Taxe von Thymome und Gruppe HLA8 schätzt.

Nach Erwähnung jedes Falles, haben wir jede Association je nach der Veröffentlichung der Fälle in der Literatur diskutiert. Es scheint, dass jede dieser Associacionen der Krankheiten nicht forziert sind, sondern dass sie wahrscheinlich aus einer immunologischen Dysfunktion ihren Ursprung haben.

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End-Plate Acetylcholinesterase Deficiency Associated with Small Nerve Terminals and Reduced Acetylcholine Release. A New Syndrome.

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We have recently observed a unique, congenital disorder of neuromuscular transmission different clinically, physiologically and morphologically from myasthenia gravis and from the Lambert-Eaton myasthenic syndrome.[1]

CLINICAL DATA

The patient, a Hindu boy, was the product of a normal pregnancy and delivery. When 5 days old he was noted to have ptosis of the eyelids that temporarily disappeared after he slept. A prostigmine test at age 6 months and numerous subsequent Tensilon tests were negative. The patient sat up at 7 months, stood with support at 16 months, and walked alone at 3 years of age. Throughout his life he had intermittent strabismus and generalized weakness worsened by exertion. Treatment with Mestinon (pyridostigmine) before the age of 5 had no clinical effect. The patient was first seen at the Mayo Clinic in 1974, when he was 14 years old. At this time he weighed 52 kg and stood 152 cm tall. He had small muscle bulk, lordosis and kyphoscoliosis. There was weakness of all external ocular as well as of other cranial, cervical, trunk and limb muscles. The weakness in the limbs was greater proximally than distally and in all muscles weakness increased during exertion. The deep tendon reflexes were absent in the upper limbs and could be barely elicited in the lower ones. Serum enzymes of muscle origin were normal. The serum contained no antibodies directed against the human acetylcholine receptor (AChR) protein.

A Tensilon test had no effect on the patient's weakness or on his neuromuscular transmission defect. Guanidine (20 mg/kg/day) failed to improve the patient's strength or the electromyographic abnormalities. Prednisone treatment was started in June 1975. Initially, this consisted of 50 mg daily for three months, and the dose was then tapered to 40 mg, alternating with 10 mg every other day. The patient reported subjective improvement a few days after therapy was started. After 9 months of treatment the patient could rise from the squatting position unassisted and walk 90

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m without stopping, a slight but objective improvement over his previous condition. However, the electromyographic abnormalities remained unchanged.

ELECTROPHYSIOLOGICAL STUDIES

Electromyographic studies showed a repetitive muscle action potential in response to stimuli applied to nerve, in both proximal and distal muscles (Fig. 1, left upper panels). This was like that seen after anticholinesterase drug overdose, but the patient was taking no drugs. The evoked compound muscle action potential showed a decremental response at both slow and rapid rates of stimulation (Fig. 1, right upper panels). Moderate postactivation facilitation followed by slight postactivation exhaustion were also noted. During voluntary activity the configurations and amplitudes of the motor unit potentials varied abnormally, and the proportion of polyphasic potentials was greater than normal. Spontaneous electrical activity was absent. Sensory and motor nerve conduction velocities were normal.

In vitro microelectrode studies were done on external intercostal muscles of the patient in October, 1974 and May, 1976, according to previously described methods.[2,3] The recorded miniature end-plate potentials (mepps) were corrected for nonlinearity of the end-plate response and to a standard membrane potential of 85 mV. Mepps with less than 1.5-ms rise times were used for measurements. The number of quanta released by nerve impulse was determined from measurements of the end-plate potentials (epps) during stimulation at 1 Hz, using the eleventh to fifty-eighth epps. There was good agreement between quan-

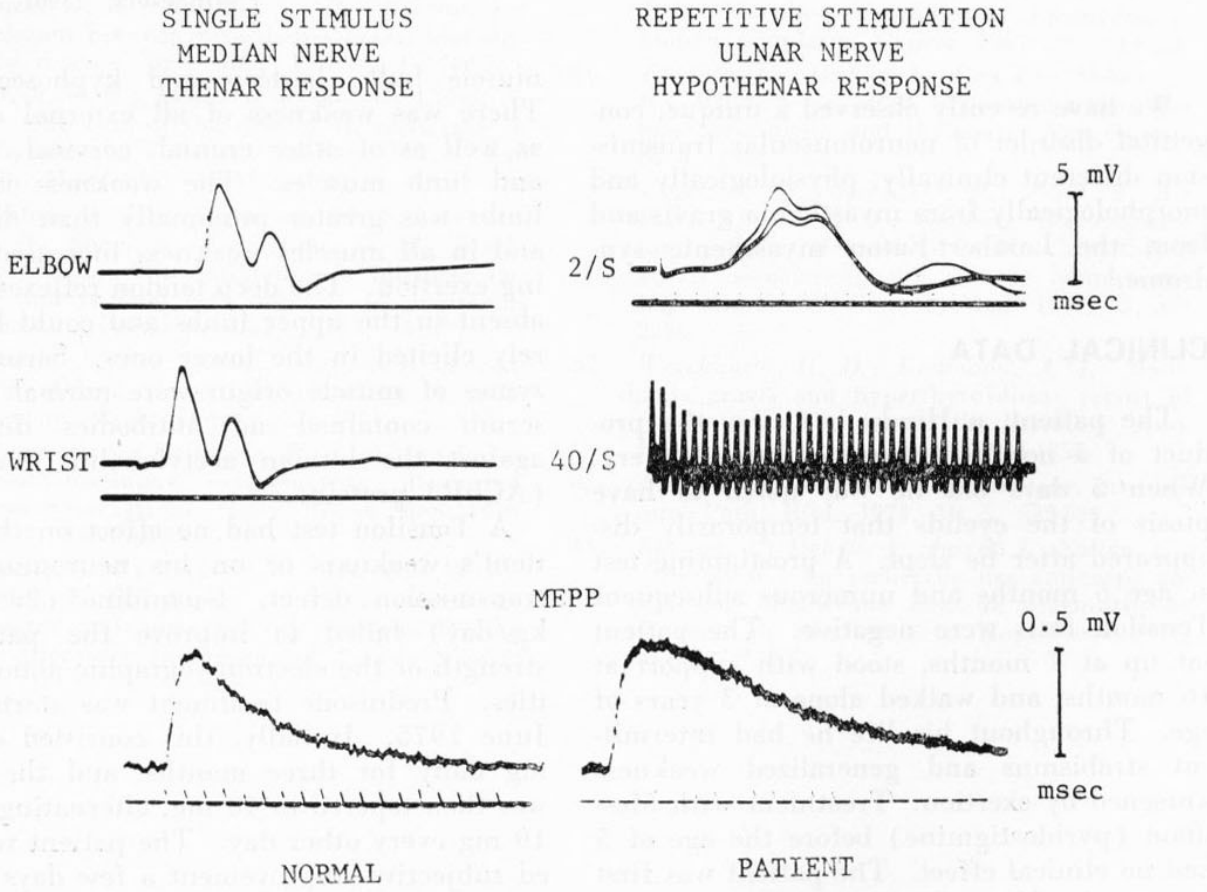

Fig. 1. — *Electromyographic and in vitro intracellular microelectrode studies. Left upper panels: repetitive response to single nerve stimulus. The second response is of lower amplitude than the first. Right upper panels: decremental response of muscle action potential at slow and rapid rates of stimulation. Lower panels: patient's MEPP is of longer duration and has longer half-decay time than normal control. (Reproduced from Reference 1, by permission of Little, Brown and Co.)*

tum content determined by the three methods employed by Elmqvist and co-workers.[4]

The amplitude of the mepp was 0.72 ± 0.04 mV (mean + SE) (n = 52 fibers) in October, 1974 and 0.73 ± 0.05 mV (n = 32 fibers) in May, 1976. In 27 control subjects the mean mepp amplitudes ranged from 0.75 to 1.35 mV. Although the mepp amplitude was in the low-normal range the duration and halfdecay time were abnormally prolonged (Fig. 1, lower panels). The addition of up to 8×10^{-6} gm/ml of neostigmine methyl sulfate to the bath had no additional effect on the patient's mepps while in a normal muscle one-fifth of this dose significantly prolonged the rise time and half-decay time of the mepps.

The mean mepp frequency was 14 % of the normal mean in October, 1974 and 17 % of the normal mean in May, 1976. The quantum content of the epp was only 12 ± 1.1 (mean ± SE) (n = 53 fibers) in October, 1974 and 26 + 4.8 (n = 25 fibers) in May, 1976. In 17 control subjects the mean values for the quantum content of the epp ranged from 36 to 103.

Although the epp quantum content (m) was abnormally low, as it is in the Lambert-Eaton syndrome, m decreased at higher frequencies of stimulation as in normal muscle [5] and myasthenia gravis. In contrast, m increases in the Lambert-Eaton syndrome at higher frequencies of stimulation.[2]

The store of acetylcholine (ACh) quanta immediately available for release and the probability of release by nerve impulse were estimated by the methods of Elmqvist et al [5,6] in the patient, normal controls, myasthenia gravis and Lambert-Eaton syndrome. The store of quanta immediately available for release was approximately one-sixth of normal in the patient, while it was not decreased in Lambert-Eaton syndrome or in myasthenia gravis. The probability of release was normal in the patient and in myasthenia gravis but was about one-seventh of normal in the Lambert-Eaton syndrome.

Depolarization of the nerve terminals by increasing the external potassium concentration [7,8] from 5 to 20 mM increased the mepp frequency in the patient's muscle 33-fold while in 4 control muscles the corresponding factor was 36 ± 7 (mean ± SE). By contrast, in 10 patients with the Lambert-Eaton syndrome, the corresponding factor was only 11 ± 2. These findings were also consistent with the assumption that the small m and the low mepp frequency in the patient's muscle were not due to a decreased probability of quantal ACh release.

HISTOPATHOLOGICAL OBSERVATIONS

Specimens of external intercostal muscles (October, 1974 and May, 1976) and a motor point biopsy specimen from the left common finger extensor muscle (October, 1975) were studied.

Light Microscopy and Routine Histochemistry.

No abnormalities were found in paraffin sections. Routine histochemical studies of fresh frozen sections also showed no abnormality. In these sections muscle fiber diameter ranged from 24 to 78 μm. Isolated or groups of atrophic fibers were absent, and there was a normal distribution of histochemical fiber types.

Light Microscopic Studies for the Demonstration of End-plate Acetylcholinesterase.

Specimens from all three biopsies were reacted for acetylcholinesterase (AChE). Fresh and formalin postfixed frozen sections as well as glutaraldehydefixed strips of muscle were incubated with three substrates: α-naphthy acetate; [9] dithio-bis-acetic acid; [10] and acetylthiocholine iodide.[11] No reaction could be demonstrated at the patient's end-plates with any of the substrates even when the periods of incubation were increased ten-fold over that required to show enzyme activity at the normal end-plate, except for faint traces at a few sites with α-naphthyl acetate as substrate. Even this was not seen in the presence of 10^{-5} M iso-OMPA, and inhibitor of nonspecific AChE (Fig. 2). Traces of non-specific esterase activity also occur at normal end-plates.[12]

Preincubation of fresh-frozen sections

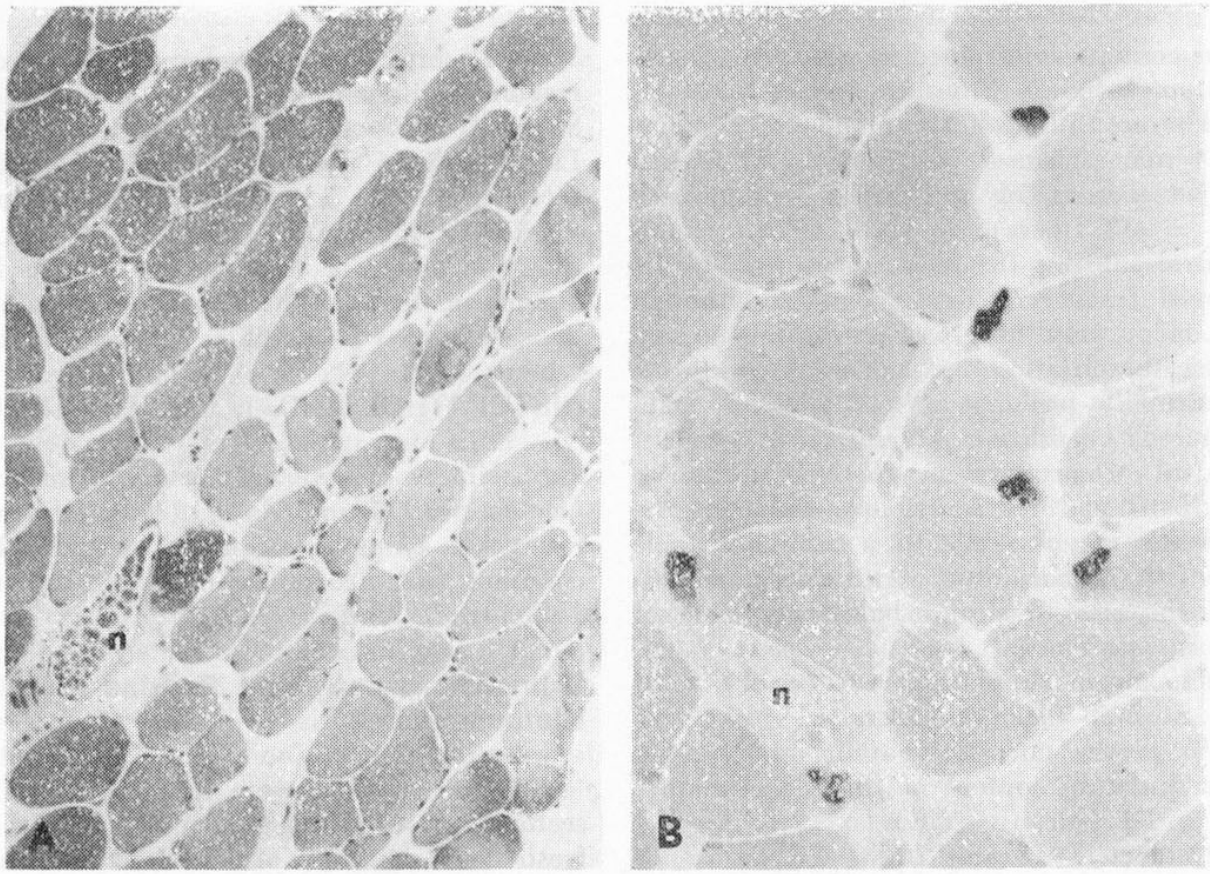

Fig. 2. — *Fresh-frozen sections reacted for AChE with α-naphthyl acetate in the presence of* 10^{-5} *M iso-OMPA. Patient* (A) *shows no end-plate AChE after* 10 *minutes. Control* (B) *displays strong reaction after* 1 *minute. n: intramuscular nerve. A, reduced from X* 145; *B, reducer from X* 360. (*Reproduced from Reference* 1, *by permission of Little, Brown and Co.*)

with 10^{-3} M pyridine-2-aldoxime methiodide (2-PAM), an agent that protects the active site of AChE,[13] did not restore enzyme activity at the patient's end-plates. To exclude the presence of a circulating AChE inhibitor, fresh frozen sections of normal intercostal muscle were incubated with the patient's serum for 30 minutes before being reacted for the enzyme. This had no effect on the AChE activity of the normal end-plates.

Light Microscopic Demonstration of AChR at the End-plates.

AChR was localized at the end-plates with peroxidase-labelled α-bungarotoxin.[14] The patient's end-plates were readily visualized by this method. In other experiments the same end- plates were first reacted for AChR and then for AChE. In normal muscle the brown stain for AChR became covered with a heavy, red reaction product for AChE after one minute of incubation with α-naphthyl acetate. In the patient's muscle the brown stain for AChR persisted, and there was no reaction product for AChE even after 10 minutes of incubation.

Electron Microscopic Observations.

In all three specimens, ultrastructural abnormalities were found at the end-plates but not elsewhere in the muscle fibers. The pathologic changes involved the nerve terminals, the junctional folds and the junctional cytoplasm.

In most end-plates the nerve terminals were smaller than normal. The small nerve terminals were applied to only a fraction of the post-synaptic region (Fig. 3). Frequently, even the small nerve terminals were often nearly completely surrounded by Schwann cells so that the surface of the presynaptic membrane through which ACh could be released was minimal. Apart from their small size, the nerve terminals appeared normal. They contained abundant mi-

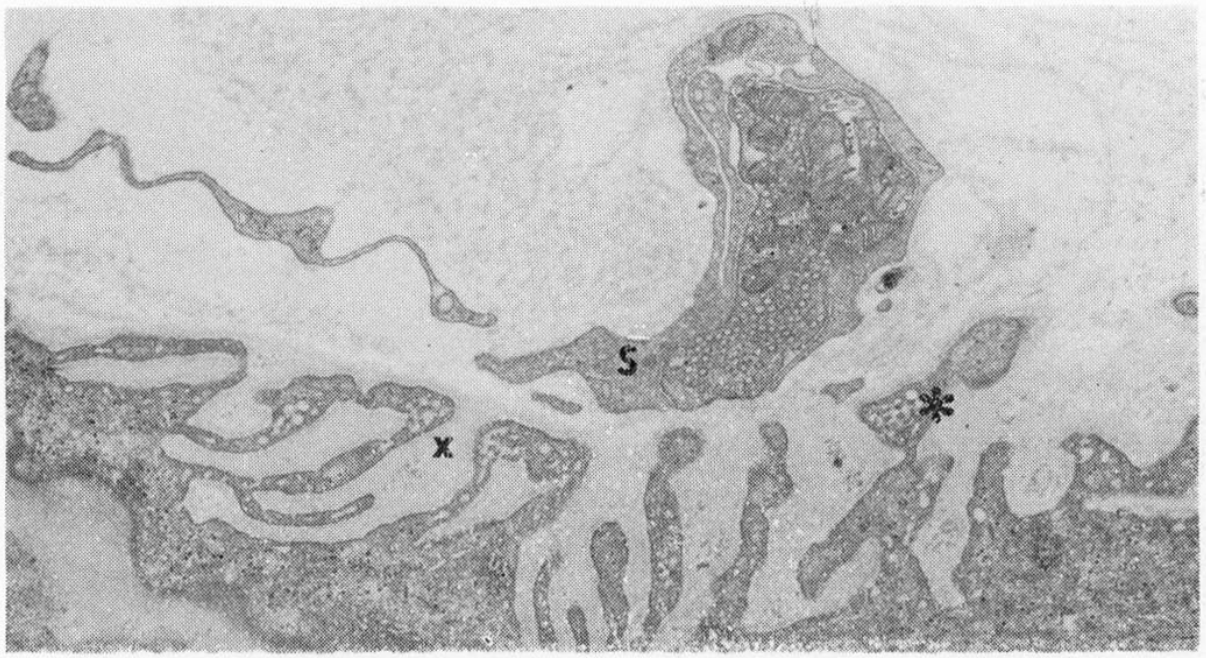

Fig. 3. — *Electron micrograph of an end-plate region. The small nerve terminal is almost completely surrounded by Schwann cell* (S) *and is applied against only a small part of the postsynaptic region. Residues of atrophied junctional folds* (X) *occur in widened synaptic space. The folds contain numerous labyrinthine membranous networks.* X 14,500. (*Reproduced from Reference* 1, *by permission of Little, Brown and Co.*)

tochondria and synaptic vesicles and never showed signs of degeneration. The intramuscular nerves were also normal.

At most end-plates the junctional folds contained abundant labyrinthine membranous networks (Fig. 3). These resembled those known to be of transverse tubular origin. In the junctional folds the networks communicated with the extracellular space via pinocytotic vesicles. At some end-plates the junctional folds were sparse and showed signs of degeneration (Figs. 38 and C). At about 20 percent of the end-plates the junctional sarcoplasm contained small autophagic vacuoles, dilated sarcotubular components, an increased number of lipid droplets, and debenerating nuclei.

Morphometric Analysis of End-plates.

The analysis was done a previously described method.[15,16] All end-plates located in the two external intercostal muscles and in the finger extensor muscles were analized. The results were compared with those obtained in the same muscles of control subjects.[16] These studies revealied that the mean nerve terminal area in the patient's muscles was one-third to one-fourth of the normal value; (2) the synaptic vesicle density of the nerve terminals was significantly greater than normal; (3) the postsynaptic membrane density was significantly lower than normal; and (4) the same abnormalities were found in all three biopsy specimens.

Ultrastructural Localization of the AChR.

The ultrastructural localization of AChR was with peroxidase-labelled α-bungarotoxin[14] in the patient's external intercostal muscle. In the patient, as in the control subjects, AChR was localized on the terminal expansions of the junctional folds and, at times, on the stalks of the folds. In addition, there was less intense staining of the presynaptic membrane where it faced reactive portions of the junctional folds (Fig. 4) Abundant AChR was also present on simplified junctional folds or when the folds showed signs of degeneration. This was in contrast with findings in myasthenia gravis in which simplification or degeneration of the folds is associated with a marked

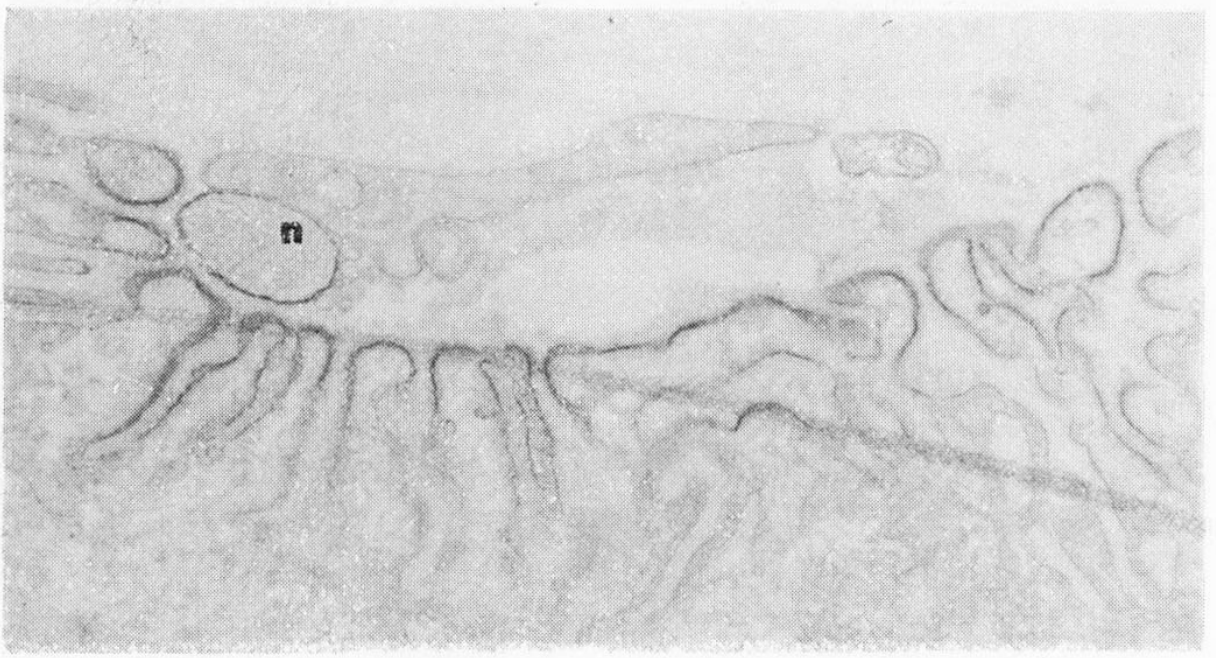

Fig. 4. — *Ultrastructural localization of AChR. The nerve terminal is very small and covers only a fraction of the postsynaptic area. Abundant AChR is associated with the postsynaptic membrane.* X 23,400. (*Reproduced from Reference* 1, *by permission of Little, Brown and Co.*)

decrease in the amount of demonstrable AChR.[14] None of the patient's muscle fibers showed extrajunctional spread of AChR.

Electron Cytochemical Studies to Demonstrate AChE at the End-plates.

A glutaraldehyde-fixed portion of the patient's finger extensor muscle was reacted for AChE by the method of Gautron with dithio-bis-acetic acid as substrate.[10] Normal human intercostal muscles served as controls. In normal muscle, a reaction product detectable by electron microscopy appeared on the postsynaptic folds after 7 minutes of incubation at 4°C. When the incubation was at room temperature or for longer periods, an excessive reaction product accumulated in the synaptic spaces and also in regions adjacent to the junctional folds. The patient's end-plates showed no reaction to AChE even after 1 hour of incubation at room temperature (Fig. 5A). A normal end-plate incubated for 30 minutes at room temperature is shown for comparison in Figure 5B.

BIOCHEMICAL STUDIES

In the rat diaphragm 40 percent of the total AChE is associated with the end-plate. with the remainder distributed diffusely along the rest of the muscle fiber.[17] In this muscle there are three species of AChE, with 4 S, 10 S, and 16 S sedimentation constants on sucrose gradient centrifugation. The 4 S and 10 S species are distributed throughout the muscle while the 16 S form is confined to the end-plate region. According to Massoulié, in human muscle the 16 S component is not confined to the end-plate region but is also distributed diffusely throughout the muscle fiber (personal communication from J. Massoulié). It was thus of interest to determine the amount of sedimentation constants of AChE in the patient's entire muscle.

The patient's intercostal muscle (May, 1976) and normal human intercostal muscles were used. The specimens (initially intact from origin to insertion) were extracted for AChE as described by Hall.[17] AChE was assayed radiochemically using 1-^{14}C-acetylcholine as substrate at 37°C for 20 minutes to 4 hours [17,18] in the presence

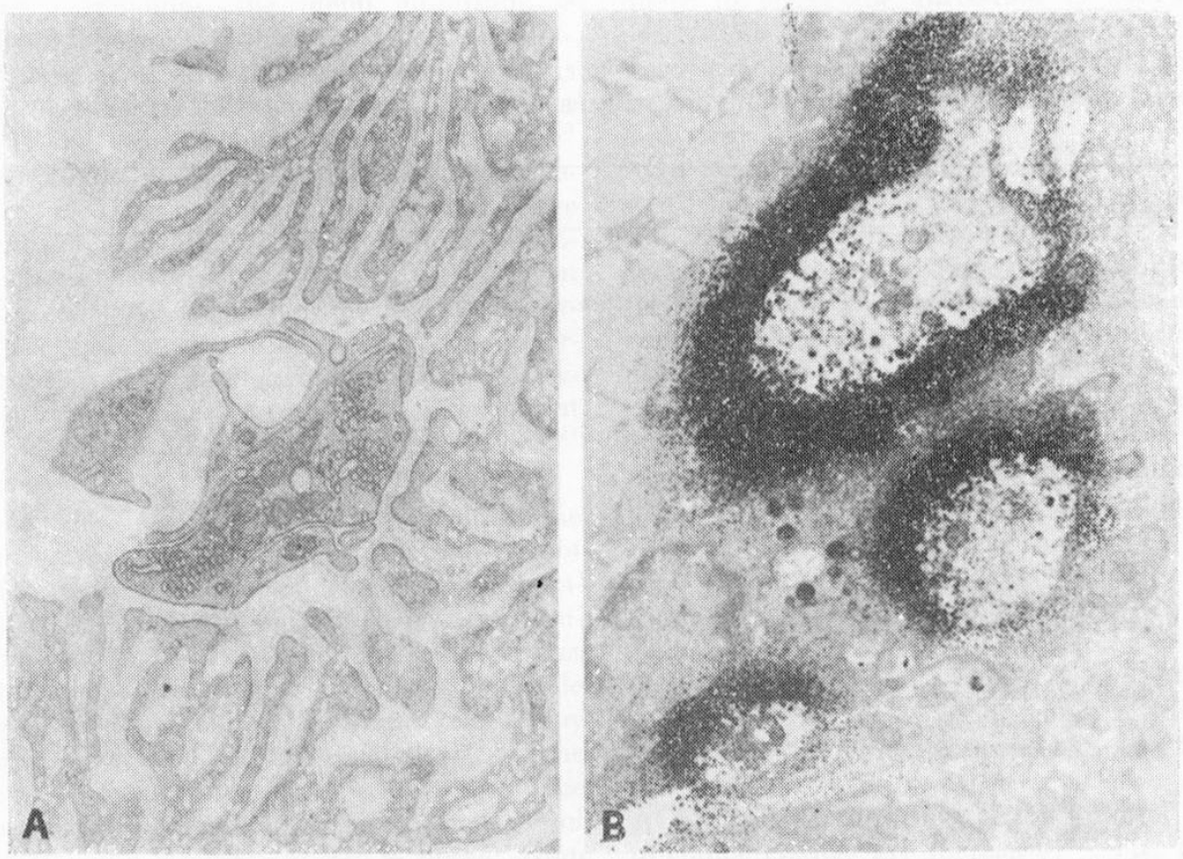

Fig. 5. — *Electron cytochemical localization of AChE. In A, patient's end-plate shows no reaction after 1 hour incubation at room temperature. In B, control end-plate is greatly over-reacted after 30 minutes of incubation at room temperature. Here the black reaction product (lead sulfide) completely covers the junctional folds and has spread into adjacent regions. A, reduced from X 20,500; B, reduced from X 8,900. (Reproduced from Refere nce 1, by permission of Little, Brown and Co.)*

of 10^{-5} M Iso-OMPA with and without 5 x 10^{-6} M BW-284C51, a specific AChE inhibitor.[19]

The total muscle AChE activity for the patient was 2.47 nM/min/mg of protein. For nine control intercostal muscles the corresponding value was 11.05 ± 0.87 (mean ± SE) (range, 8.42 to 16.95). For both the patient and the controls, the addition of BW-284C51 inhibited all the iso-OMPA-resistant enzyme activity, indicating that this activity represented specific AChE.

AChE extracted from the patient's intercostal muscle and from the control intercostal muscle obtained at thoracotomy was analyzed by sucrose gradient centrifugation. Samples containing 1 mg of protein in 0.15 ml were layered on 4.5 ml of a linear sucrose gradient (5-20 percent) made up in the extracting solution. Centrifugation was for 10 hours at 38,000 rpm at 3°C in an SB 283 (IEC) rotor. Enzymes with known sedimentation constants (β-galactosidase (16 S), catalase (11.3 S) and alkaline phosphatase (6.1 S) were added to each sample prior to sedimentation. The reference enzymes were assayed by the methods employed by Hall.[17] The normal muscle specimen contained two major ACh components peaking at 6 S and 16 S respectively. By contrast, for the patient's muscle the 16 S component was absent and the 6 S component was considerably smaller than that in the control muscle.

DISCUSSION

The clinical, electromyographic, electrophysiological, ultrastructural, cytochemical, and biochemical features of the new myasthenic syndrome described here distinguish it from myasthenia gravis and from the

TABLE I

COMPARISON OF FEATURES OF PRESENT PATIENT, LAMBERT-EATON SYNDROME, AND MYASTHENIA GRAVIS*

Features	New Syndrome	Lambert-Eaton Syndrome	Myasthenia Gravis
Clinical			
Weakness and fatigability	+	+	+
Increased strength during beginning of voluntary contraction	−	+	−
Hyporeflexia	+	+	−
Bulbar symptoms	+	−,(+)	+,(−)
Improved by anticholinesterase drugs	−	±	+
Improved by guanidine	−	+	±
Sometimes associated with carcinoma	−	+	−
Circulating antibodies to AChR	−	−	+
Electromyographic			
Repetitive response to single nerve stimulus	+	−	−
Decrementing EMG at slow rate of stimulation	+	+	+
Incrementing EMG at rapid rate of stimulation	−	+	−, (+)
Postactivation facilitation and exhaustion	+	+	+
Electrophysiological			
Small-amplitude mepp	−	−	+
Increased duration and half-decay time of mepp	+	−	−
Decreased mepp frequency	+	−	−
Small epp quantum content at 1/sec	+	+	−
Increased epp quantum content at 40/sec	−	+	−
Decreased store of immediately releasable quanta	+	−	−
Decreased probability of quantal release	−	+	−
Ultrastructural			
Nerve terminal small relative to postsynaptic region	+	−	−
Absolute decrease in nerve terminal size	1/3 to 1/4 of normal	−	2/3 of normal
Increased synaptic vesicle density	+	−	−
Focal degeneration of junctional folds	+	−	+
Labyrinthine membranous networks in junctional folds	+	−	−
Hypertrophy of postsynaptic area	−	+	−
Degenerating nuclei in junctional sarcoplasm	+	−	−
Cytochemical and biochemical			
Decreased postsynaptic AChR	−	−	+
AChE absent from end-plate	+	−	−
16 S AChE absent from muscle	+	...	...

+ = present; − = absent; (+) = occasionally present; (−) = occasionally absent; ± = variable.

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Lambert-Eaton syndrome. Table I compares the divergent aspects of the three syndromes.

In myasthenia gravis the defect of neuromuscular transmission is associated with a deficiency of postsynaptic AChR [12,20,21] is conditioned by an autoimmune reaction directed against the AChR.[21,22] The junctional folds show signs of degeneration and bind anti-AChR antibodies and complement.[22] The physiological hallmark of the disease is the small amplitude of the mepp while *m* is essentially normal.[2,4,21,22] Anticholinesterase drugs usually improve the defect.

In the Lambert-Eaton myasthenic syndrome, which is sometimes associated with small cell lung carcinoma and rarely with

other carcinomas, the mepp amplitude is normal but *m* is abnormally small at low frequencies of stimulation.[2,3] The defect is improved at high frequencies of stimulation as is the case in magnesium poisoning.[2,3] Decrease in *m* is attributed to a low probability of release of quanta from the nerve terminal and not to a lack of quanta immediatelly available for release. Electron microscopy shows a normal density of synaptic vesicles in the nerve terminal, hipertrophy rather than atrophy of the postsynaptic folds [23] and there is a normal amount of AChR on the postsynaptic membrane.[21] Guanidine usually improves the defect.

In the new myasthenic syndrome the neuromuscular transmission defect is not affected by anticholinesterase drugs or guanidine. The defect of neuromuscular transmission is associated with a decrease in *m* which is due to a lack of quanta available for immediate release. Electron microscopy shows a marked decrease in the size of the nerve terminals, degenerative changes in the postsynaptic region, and normal amounts of postsynaptic AChR. AChE is absent from the end-plates (which never occurs in myasthenia gravis or in the Lambert-Eaton syndrome) and the total muscle AChE level is markedly reduced.

Normal neuromuscular transmission depends on the generation of an epp of sufficient amplitude to fire a propagated action potential. Te amplitude of the epp is affected by *m*, by the number of ACh molecules in the individual quantum, and by the availability of postsynaptic AChR. The size of *m* is affected by the number of quanta available for immediate release from the nerve terminal and by the probability of release. The transmission defect in our patient was caused by a decrease in the store of immediately releasable quanta and this was attributed to the smallness of the nerve terminals. A direct relationship between *m* and the size of the nerve terminal has been previously observed in newly formed synapses and has been attributed to the low number of quanta available for release rather than to a low probability of release.[24] Although the number of synaptic vesicles per unit nerve terminal area was increased by approximately 50 percent, this was not adequate to offset the three-fold to four-fold decrease in nerve terminal size. However, *m* was normal at some end-plates, and at some end-plates the nerve terminals were of normal size.

In our patient, AChE was invariably absent from all end-plates histochemically electron cytochemically. The absence of the enzyme from the end-plate readily explains the patient's total unresponsiveness to anticholinesterase drugs, the repetitive muscle action potential in response to a single impulse applied to nerve,[25] the abnormal duration and prolonged half-decay time of the mepp [26] and the failure of prostigmine to have any additional effect on the mepp in vitro.

At the normal end-plate AChE limits the number of collisions between ACh and AChR and hence the total duration of the quantal conductance change. When AChE is absent, removal of ACh occurs by diffusion from the synaptic space and the total number of collisions between ACh and AChR and the duration of the quantal conductance change are increased.[26] The peak amplitude of the quantal current response also increases but less markedly, presumably because the number of AChRs with which ACh can collide before it begins to diffuse laterally does not change.[27] In our patient, the duration of the mepp was prolonged, as would be expected in the absence of AChE, but its amplitude was only low normal. This could not be attributed to a lack of AChRs on the postsynaptic folds, but it might have been related to focal atrophy of the folds which would favor dilution or diffusion of ACh. In addition, the decreased or absent reuptake of choline into nerve terminals for synthesis of new ACh might curtail ACh synthesis and the labyrinthine networks in the junctional folds that communicate with the synaptic space could act as conduits for diffusion of ACh from the immediately adjacent AChRs.

An anticipated consequence of a total lack of end-plate AChE would be a life-threatening block of neuromuscular transmission due to receptor desentitization, as in a cholinergic crisis caused by anticholinesterase drug intoxication. This did not occur in our patient, probably because the

lack of AChE at the end-plates was offset by reduced ACh secretion from the nerve terminals. Accordingly, the smallness of the nerve terminals, the decrease in *m*, and the structural changes in the postsynaptic folds might have been reactive, or compensatory, to protect AChR from desensitization and the patient from a perpetual cholinergic crisis.

An alternative possibility would be that the basic abnormality was a presynaptic disturbance and the total absence of AChE from the end-plate a secondary phenomenon. A large proportion of end-plate muscle AChE is under neurotrophic regulation. Totally denervated rat muscle shows a 60 percent decrease in end-plate AChE seven days postoperatively.[28] There is also evidence that axoplasmic flow is involved in the regulation of end-plate AChE. Blockade of this flow in the cat hypoglossal nerve decreases AChE activity in end-plate-enriched segments of the genohyoid muscle by 50 percent after 10 days.[29] However, neither surgical denervation nor interference with axoplasmic flow has yet been shown to result in a total loss of end-plate AChE, and end-plate AChE can be detected histochemically even eight months after denervation.[30] In our patient, there was no hint that the muscles were totally denervated, AChE was completely absent from the end-plates histochemically and electron cytochemically, and fibrillation potentials and extrajunctional spread of AChR, which develop after blockade of axoplasmic flow,[31] were not observed.

While AChE activity at the end-plate and elsewhere in muscle is partly under neural regulation, there is also postsynaptic genetic control of this activity. Aneurally cultured muscle fibers synthesize AChE. Activity is present in myoblasts and increases with the formation of myotubes.[32–34] In cultured muscle fibers initially a low-molecular-weight form of the enzyme is synthesized and secreted into the medium. Subsequently, higher-molecular-weight forms are assembled and retained by the cell.[35] A similar transition from low- to higher-molecular-weight isomers also occurs during mouse embryogenesis.[36]

The different molecular forms of AChE have been well characterized in electric organs of fish. Globular subunits of the enzyme tend to aggregate into tetramers and higher-molecular-weight forms arise when two or three tetrameric units combine. The larger species are also associated with a tail-like structure which is presumed to attach the enzyme to membrane.[37,38] Since collagenase treatment disoociates AChE from the end-plate,[39,40] it seems likely that attachment of the enzyme to the postsynaptic membrane depends on the presence of a collagen-like tail. Accordingly, the lack of AChE from the patient's end-plates and the decrease in the total muscle AChE might have been caused by at least four mechanisms: (1) a structural abnormality of enzyme subunits preventing their aggregation; (2) a structural abnormality of subunits or tetramers, preventing their combination with the tail part; (3) defective biosynthesis of the tail part; or (4) a structural abnormality of the tail part, preventing its combination with the enzyme or its attachment to the postsynaptic membrane.

An interesting aspect of the present case was the absence of any signs of AChE deficiency in tissues other than muscle. There was no autonomic dysfunction, and erythrocyte AChE activity was normal.

The new syndrome has two different aspects: decreased ACh release from the nerve terminal and no inactivation of released ACh by AChE. Further studies of this disorder might shed additional light on trophic interactions between nerve and muscle and the biological significance of the different molecular forms of AChE.

SUMMARY

A new myasthenic syndrome is described in a patient whose symptoms began soon after birth and included generalized weakness increased by exertion, easy fatigability, hyporeflexia, and refractoriness to anticholinesterase drugs. Electromyography showed a decremental response at all frequencies of stimulation and a repetitive response to single nerve stimulation. Miniature end-plate potentials (mepps) were of normal

amplitude but of decreased frequency. The mepp duration and half-decay time were prolonged, and prostigmine was without any additional effect. The quantum content of the end-plate potential was decreased due to a reduced store of quanta immediately available for release, but the probability of release was normal. Quantitative electron microscopy demonstrated a three-fold to four-fold decrease of nerve terminal size and reduced postsynaptic membrane density. The postsynaptic folds showed focal degeneration, and many were distended by labyrinthine membranous networks that communicated with the synaptic space. Degenerating nuclei were found in the junctional sarcoplasm. The ultrastructural localization of the acetylcholine receptor protein was normal. Acetylcholinesterase (AChE) was absent from the motor endplates by histochemical and electron cytochemical criteria. Biochemical studies indicated a marked decrease in total muscle AChE and an absence of the 16 S species of the enzyme. A congenital defect in the molecular assembly of AChE or in its attachement to the postsynaptic membrane might represent the basic abnormality and condition the morphological and physiological alterations.

RESUMEN

Se describe un nuevo síndrome miasténico en un paciente en el cual los síntomas se hicieron presentes poco tiempo después del nacimiento. Estos síntomas eran debilidad generalizada acrecentada por el ejercicio, fácil fatigabilidad, hiporreflexia y era refractario a las drogas anticolinesterasa.

La electromiografía puso en evidencia una respuesta disminuida para todas las frecuencias de estimulación y respuesta repetida a estimulación única de nervio.

Potenciales en miniatura de placa terminal (mepps) eran de amplitud normal pero de frecuencia disminuida. La duración de "mepp" y el tiempo medio de declinación eran prolongados y la prostigmina no tenía efecto agregado. El contenido quantum del potencial de la placa terminal estaba disminuido debido a una reserva reducida de quanta aprovechable inmediatamente para ser liberada, pero la posibilidad de liberación fue normal, la microscopía electrónica cuantitativa demostró un decrecimiento triple o cuádruple de la magnitud de la terminal nerviosa y una densidad de membrana postsináptica reducida. Los pliegues postsinápticos mostraron degeneración focal y muchos estaban distendidos por redes membranosas laberínticas que comunicaban con el espacio sináptico. Núcleos en degeneración fueron hallados en el sarcoplasma de la confluencia de las vías. La localización ultraestructural del receptor de acetilcolina era normal. La Acetilcolinesterasa (ACHE) estaba ausente de las placas terminales motoras de acuerdo a un criterio histoquímico y citoquímico electrónico. Estudios bioquímicos indicaron un decrecimiento marcado en el total de la ACHE del músculo y en una ausencia de las 16 S especies de la enzima. Un defecto congénito en el agrupamiento molecular de ACHE o en su adherencia a la membrana postsináptica puede representar la anormalidad básica y condiciona las alteraciones morfológicas y fisiológicas.

RÉSUMÉ

Il est décrit un neuveau syndrome myosthénique chez un patient où les symptômes se sont manifestés peu de temps après la naissance. Ces symptômes étaient: faiblesse géneralis-e augmentée par l'exercice, facile fatigue, hiporéflexibilité et étaient réfractaires aux droques à anticholinestérase.

L'électromygraphie a mis en évidence une réponse repétée à une unique stimulation du nerf.

Les potentiels miniature de plaque terminales (mepps) étaient d'amplitude normale mais de fréquence diminuée. La durée du "mepp" et le temps moyen de diminution étaient prolongés et la prostygmine n'avait pas d'action suppleémentaire.

Le contenu quantun du potentiel de la plaque terminale était diminué a cause d'une réserve réduite de quanta utilisable immédiatement pour être liberée, mais la possibilité de libération a été normale. La microscopie électronique a montré una décroissance triple ou quadruple de la taille de la terminaison nerveuse et una densité réduite de membrane postsynaptique. Les plis postsynaptique ont montré une dégénération focale et beaucoup etaient distendus par des filets membraneux labyrintiques qui communiquaient avec l'espace synaptique.

Des noyans de dégénération on été trouvés dans le sarcoplasme de la confluence des voies.

La localisation ultra structurelle du récepteur d'acétylcholine était normale. L'acétylcholinestérase (ACHE) était absente des plaques terminales motrices d'accord avec un critérium hystochimique et cytochimique électronique.

Des études biochimiques ont indiqué une croissance marquée dans l'ensemble de ACHE du muscle et une absence des 16 S sortes de l'enzime. Un défaut congénital dans le groupement moléculaire de ACHE ou dans son adhérence à la membrane post synaptique peut représenter l'anomalie basique et conditionne les alterations morphologiques et physiologiques.

ZUSAMMENFASSUNG

Es wird ein neues myasthenisches Syndrom bei einem Patientes beschrieben, bei dem sich die Symptome kurze Zeit nach seiner Geburt bemerkbar machten. Diese Symptome waren generalsierte Schwäche, verstärkt durch Übungen, leichte Ermüdung, Hyperreflexibilität und refraktäre Reaktion auf die Drogen von Anticholesterinase.

Die Elektromyographie zeigte deutlich eine verminderte Antwort auf alle Frequenzen der Stimulation und wiederholte Antwort auf einzige Stimulation des Nerven.

Potentiale in Miniatur der Terminalplatte (mepps) waren von normaler Amplitut, aber von verminderter Frequenz. Die Dauer des "mepp" und die mittlere Zeit der Deklination waren verlängert und das Prostigmin hatte keine deutlichen Effekt. Das ständige Quantum des Potential der Terminal-platte war vermindert infolge einer reduzierten Reserve der nutzbaren sofortigen Menge, die frei werden sollte, aber die Möglichkeit der Freisetzuung war normal. Die quantitative elektronische Mikroskopie zeigte ein dreifaches oder vierfaches Abnehmen der Brösse des nervösen Terminal und eine Dichte der postsynaptischen reduzierten Membran. Die postsynaptischen Falten zeigten eine fokale Degeneration, und manche waren durchzogen von laberintisschen membranösen Netzen, die mit dem synaptischen Raum in Verbindung standen. Kerne in Degeneration wurden in dem Sarkoplasma des Zusammen reffen der Wege gefunden. Die ultrastrukturelle Lokalisation der Rezeptoren von Acetylcholina war normal. Die Acethylcholine sterase (ACHE) war abwesend von den motorischen Terminalplatten entsprechend einem histochemischen und cytochemischen elektronischen Kriterium. Biochemische Studien zeigten an ein markiertes Abnehmen im Total des ACHE des Muskels und eine Abwesenheit des IGSraum des Enzyms. Ein angeborener Defekt bei der Gruppierung der Achemolekei oder seine Adherenz mit der synaptischen Membran kann die basische Anormalität darstellen und die morphologischen und physiologischen Anderungen bedingen.

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Clinical Statistics of Myasthenia Gravis in Japan

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Myasthenia gravis is a disease of skeletal muscle caused by the disturbance of neuromuscular transmission having physical characteristics of easy fatigability, weakness and diurnal fluctuation of clinical symptoms. Most of the patients follow a course of long term chronic and a small per cent of them can recover completely. They often encounter an unexpected crisis and complications in the course of illness showing a high mortality rate.

In Japan, myasthenia gravis has been designated as a specific (intractable) disease since 1972 and the goverment shares in the expenses of its study and treatment. The Ministry of Health and Welfare Myasthenia Gravis Research Committee, Japan (Chairman: M. Uono, M.D., 1972-1975) [14] is making every effort in epidemiological research, elucidation of pathophysiology (etiology), early diagnostic methods, and development of competent treatment.

As for epidemiological research, a first step investigation has been made to discover the round figure of the patients reported by about 7,000 hospitals all in Japan. Secondly, a survey composed of 55 items (age at onset, sex, natural history, symptoms, treatments, process, prognosis, complications, etc.) has been made and 1.430 cases (male 467, female 963) have been studied. The results were treated by computer analysis and investigated thoroughly to find there exist approximately 5.000 patients with myasthenia gravis in Japan after adjustment by the actual prevalence rate. The adjustment has been applied because the answers were obtained from a limited period of time, one year, and selected hospitals.

In the third place, study was made of patients with confirming diagnosis of the relation between pathological observations of thymus, immunological findings and effects of treatment. Epidemiological study has been made of childhood ocular myasthenia, frequently found in Japan, at the fourth stage of investigation. As the result of the above serial pursuance of the cases, we have obtained very important fact-finding materials, that is, for knowing the actual condition of myasthenia gravis, elucidation of its etiology, and their diagnosis or treatment, etc.

Now we want to refer to the present state of the study in our country as follows:

1. Age and sex of patients

Age at onset reaches to it's peak in the twenties for females and in the thirties for males respectively, in 70 % of all cases. It is noticeable that among females from 15 to 29 years of age it occurs four or five times as much as in males. Moreover, younger children suffer more from ocular myasthenia than the average. It reaches as 21.6 % to our surprised conclusion (Fig. 1). This suggests that early diagnosis and treatment is very important to avoid beforehand the relapse of the disease as a generalized form after adolescence (the author attached importance to this type calling it

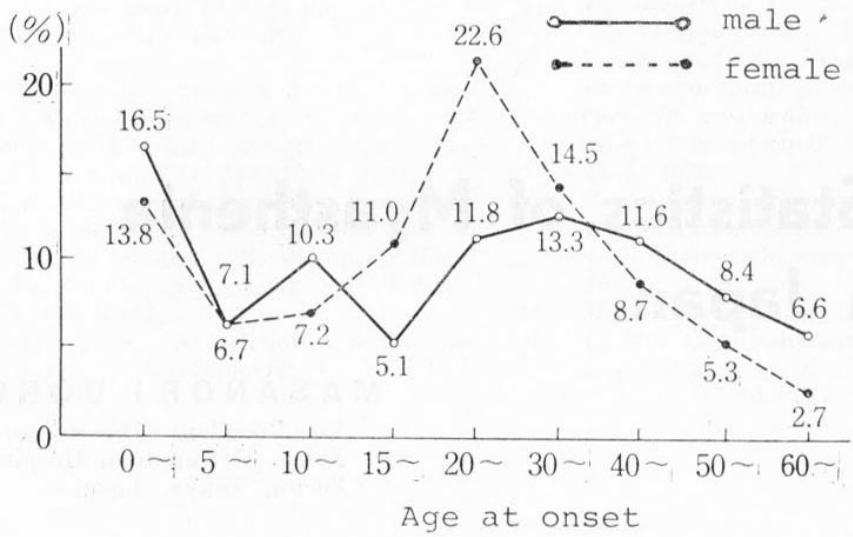

Fig.1 Age at onset of myasthenia gravis(1430 cases)in Japan.

"intermittent form"). On the other hand, we can not observe in Japan the high percentage onset of advanced age, fifties to seventies, as prevails in the U.S.A. This has already been reported by Perlo et at [10] (1966). This phenomenon means the fact that only a small number of Eaton-Lambert syndrome accompanied by lung or bronchial cancer occurs in Japan, and we must also consider the different frequency of myasthenic myopathy. This is the task to be pursued in the future.

Regarding the comparison of sex of the patients, it comes to 1 for male and 2.1 for female and this fact is almost the same as in Europe and U.S.A.

2. Process and prognosis

Analizing the process of 1.430 cases suffering from myasthenia gravis in the latest one year (Jan.-Dec., 1973), we found the following; Recovery rate 5.1 % and improvement 40.8 %, against no change 30.3%, aggravation 7.7% and death 3.7%. There is no difference in sex and age at onset but the recovery rate is as good as 12.8 % in the cases of onset ranging from

Table 1 Process and prognosis of myasthenia gravis(1430 cases).

	male	female	total
recovery	25(5.4%)	48(5.0%)	73
improvement	182(39.0%)	402(41.7%)	584
no change	140(30.0%)	294(30.5%)	434
aggravation	38(8.1%)	72(7.5%)	110
death	17(3.6%)	36(3.7%)	53
miscellaneous	4(0.9%)	14(1.5%)	18
unknown	61(13.1%)	97(10.1%)	158
total	467(100%)	963(100%)	1430

10 to 14 years old. It was also revealed that most of the patients had been suffering more than 4 years and their prognosis is no good as a whole (Table 1).

3. Territorial distribution

Climate, natural features, living circumstances were found to have no relation to territorial distribution except for the fact that a slightly larger number of patients under 15 years of age come from northern areas. There is no explanation for this fact (Fig. 2).

4. Familial myasthenia gravis

Familial occurence is very rare for myasthenia gravis, nor is its mode of inheritance clear. European and American literature records under 6 % (average 3.5 %) and Japanese statistics show only 2% (28/1.430 cases) for familial myasthenia occuring for the one year of 1973.

The author [13] found in 1977, 19 cases in 8 pedigrees out of 423 cases during the last 23 years (1954-1977), mother and daughter; sister, younger sister and her niece; elder and younger sister of dizygotic twins; sisters of monozygotic twins; elder and younger sister and brother; sister and younger brother; brothers; findings its frequency to be 4.5 %. Analyzing 19 cases of familial myasthenia, we find in every case that the age at onset is under 20 years old (mostly under 10 years of age), and female's predominate, as much as 5 times the male rate compared with cases of non-familial myasthenia. There were 12 cases of generalized form, 2 of form with muscular atrophy and single case of neonatal form, and their process is long and prognosis rather good in general, finding 3 cases of complete recovery for infants. Thymus abnormalities (hyperplasia 4 cases, thymoma 1 case) were found in 5 cases but generally there are few immunological findings (thyroid test, microsome test, antimuscle antibody, antinuclear antibody, anti-DNA antibody, dysgammaglobulinemia, etc.).

In the case of elder and younger brother with muscular atrophy, it combined unusual scoliosis at the same time of their onset with scant ocular symptoms on the contrary. They were both completely cured of

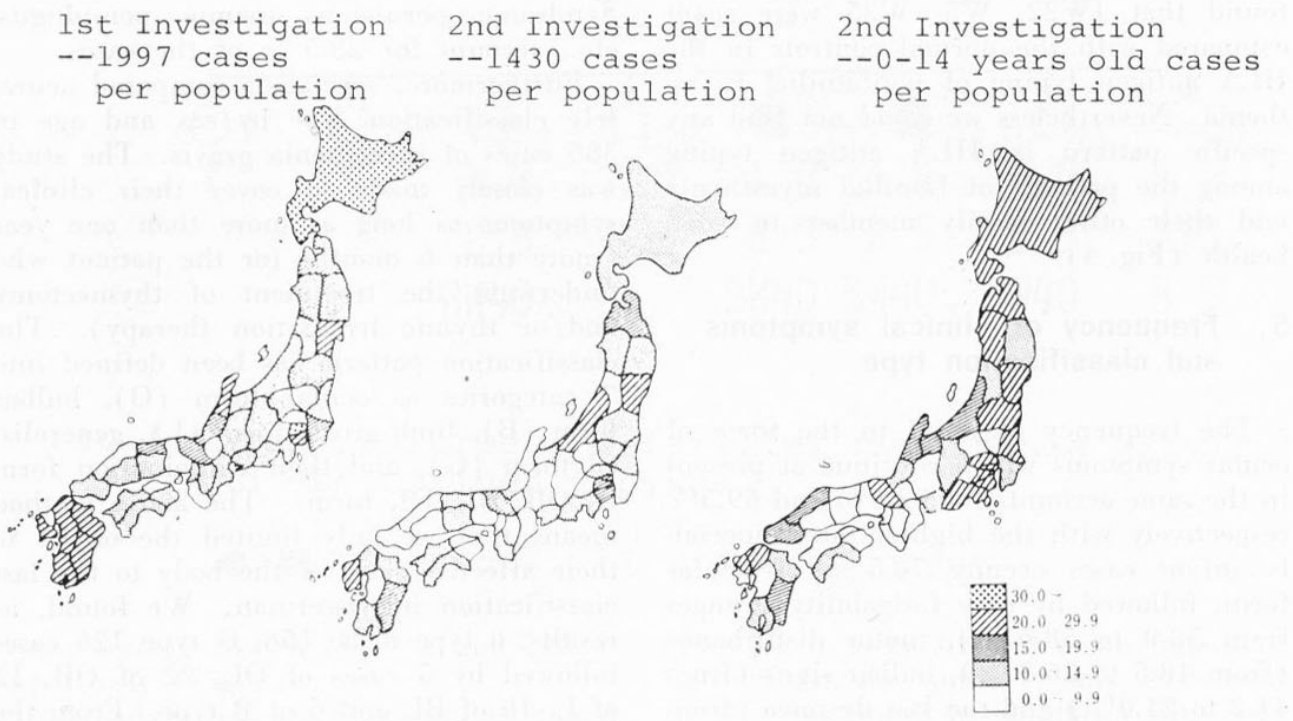

Fig.2 Distribution maps of the patients with myasthenia gravis in Japan.

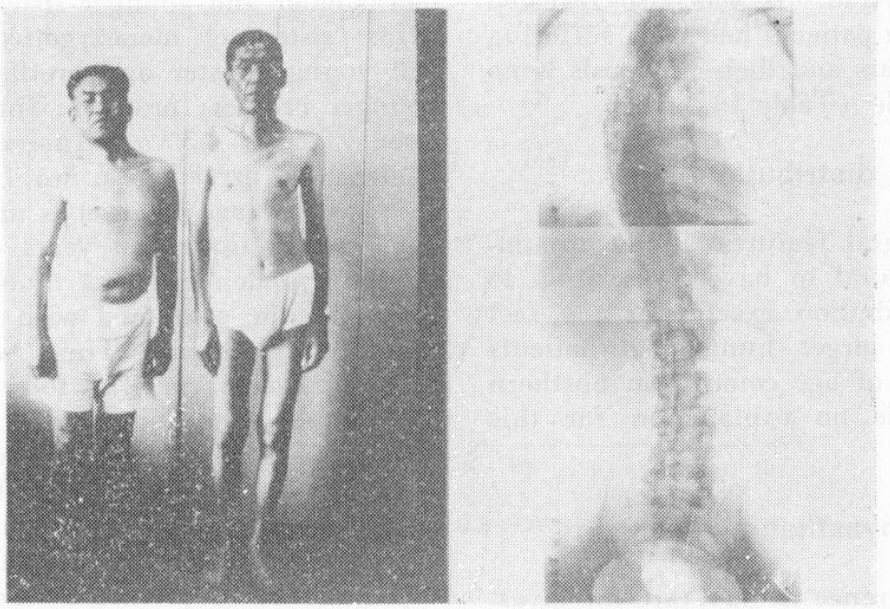

Fig.3 Familial myasthenia cases with muscular atrophy and scoliosis. (lt:36 and 34 years old males, rt:X-ray of the vertebra)

their myasthenic syndrome by thymectomy (Fig. 3). The attack of the disease was found monozygotic twins in both members and it in only one member for dizygotic twins.

Autosomal recessive mode of inheritance was perceived and on the other hand it was found that JW22, W5, W35 were scant compared with the normal controls in the HLA antigen typing of nonfamilial myasthenia. Nevertheless we could not find any specific pattern in HLA antigen typing among the patients of familial myasthenia and their other family member in good health (Fig. 4).

5. Frequency of clinical symptoms and classification type

The frequency of onset in the form of ocular symptoms which continue at present in the same accounts for 92.6% and 69.5% respectively with the highest rate, especially infant cases occupy 70.5 % of ocular form, followed by easy fatigability (ranges from 58.0 to 58.6 %), motor disturbance (from 48.5 to 36.2 %), bulbar signs (from 41.2 to 24.0%) and the last dyspnea (from 12.8 to 9.0 %). Diurnal fluctuation of their symptoms are seen to decrease with the corresponding treatments (from 71.1 to 46.0 %) (Fig. 5). The case of combining thymoma is rather high among male patients and the appearance of 16 % of crisis. Combination of diseases other than thymona, e.i. dysthyroidism, rheumatism, lupus erythematodes, periarteritis nodosa, sclerodermia, dermatomyositis, Sjogren's Syndrome, pernicious anemia, pemphigus, etc., account for 28.5 % of the cases.

Furthermore, specialists compared acurately classification type by sex and age in 385 cases of myasthenia gravis. The study was closely made to cover their clinical symptoms as long as more than one year (more than 6 months for the patient who underwent the treatment of thymectomy and/or thymic irradiation therapy). The classification pattern has been defined into 7 categories as ocular form (O), bulbar form (B), limb girdle form (L), generalized form (G), and their combination form ie. OB, BL, OL forms. The above method means that we only limited the object to their affected parts of the body to the last classification by Osserman. We found, as results, 0 type to be 156, G type 126 cases followed by 5 cases of OL, 22 of OB, 11 of L, 10 of BL and 6 of B type. From the point of sex, females occupy the larger part of the cases as a whole (69.8 %), especially we can find this fact in G, OB and L

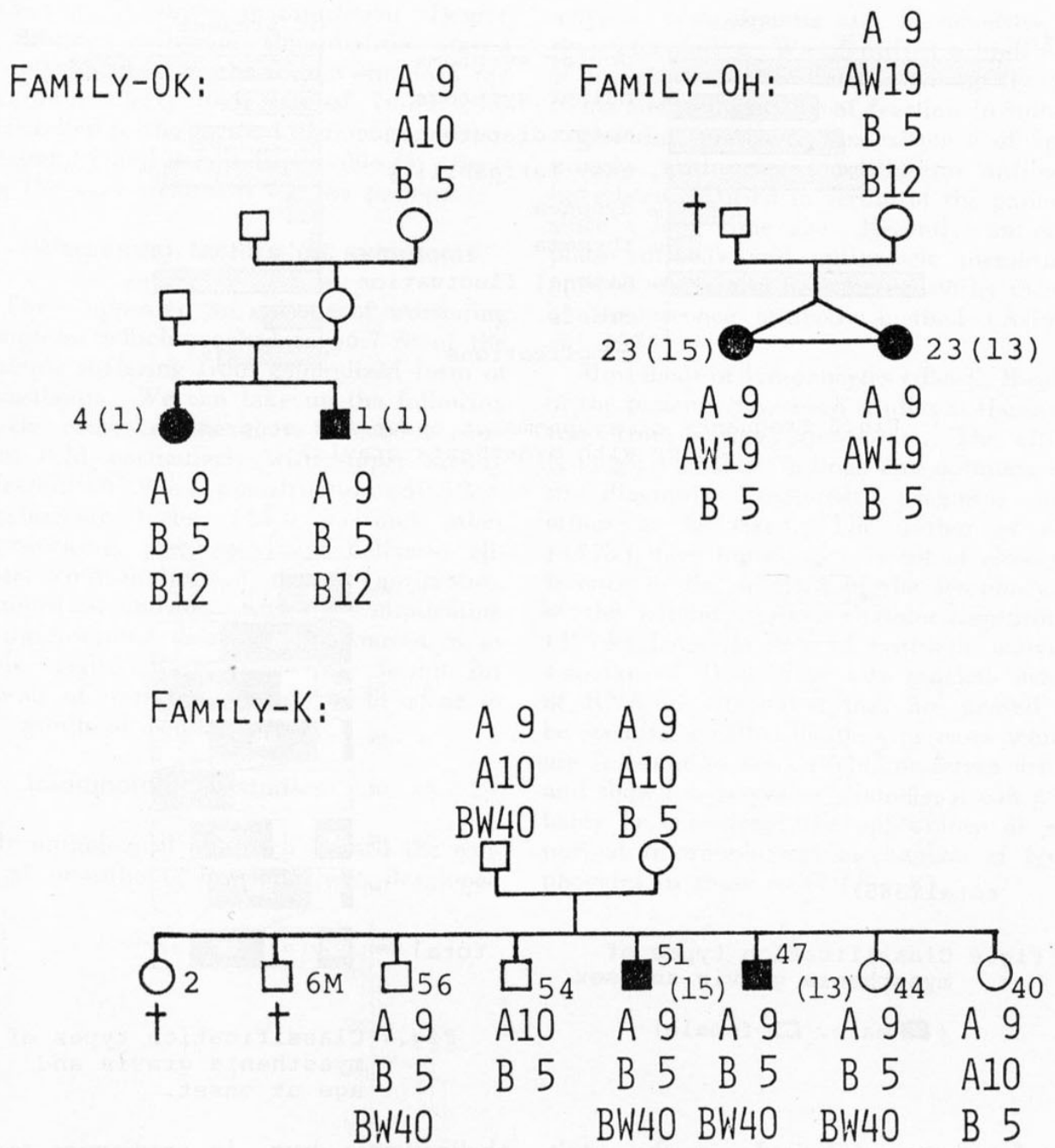

Fig.4 Three pedigrees of familial myasthenia gravis and HLA antigen typings.

● ■ :patients, ( ):age at onset

types covering 78.6 %, 77.3 % and 100 % respectively (Fig. 6).

Next, observing age at onset, we found 70.5 % of 0 type occured in their infancy, especially the fact that 35.9 % of the said percentage is occupied by infants under 5 years old is to be stressed as an important finding. On the contrary, only 10.3 % of G type was found in childhood with very small percentage of 3.2 % under 5 years of age. Now it goes without saying, as a conclusion, that all other types than 0 type

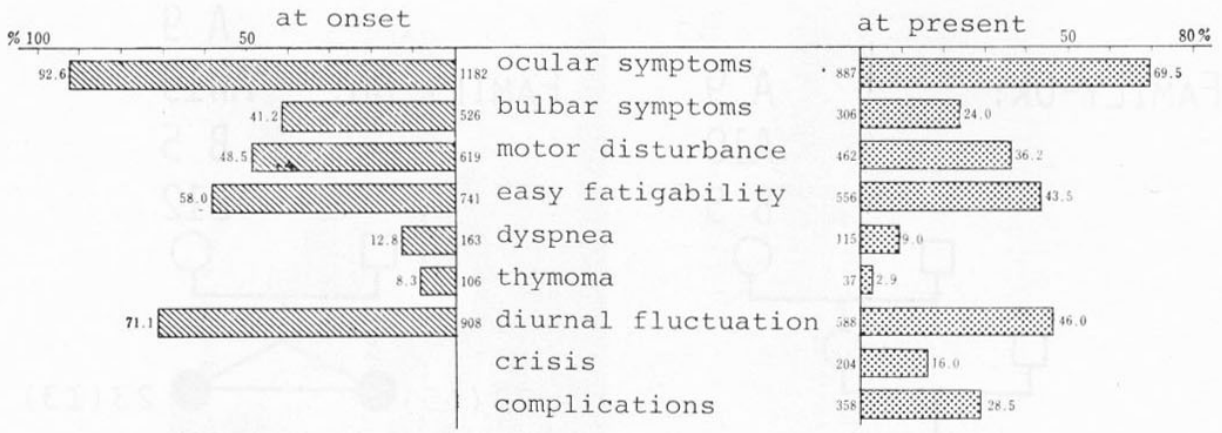

Fig.5 Frequency of symptoms at onset and at present.
(1277 cases with myasthenia gravis)

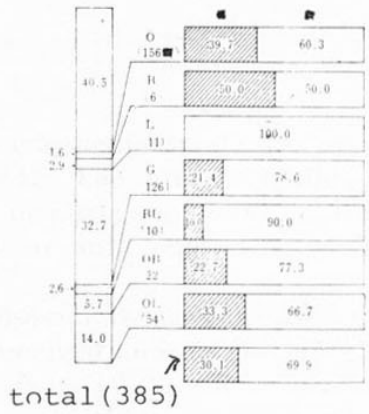

Fig.6 Classification types of myasthenia gravis and sex.

(male, female)

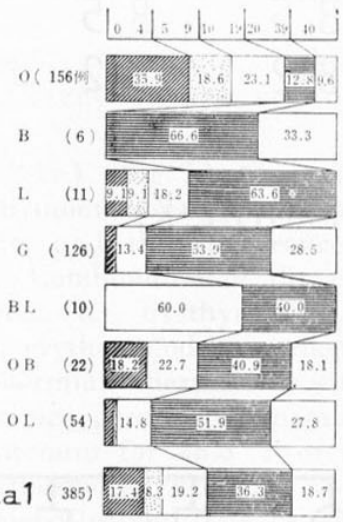

Fig.7 Classification types of myasthenia gravis and age at onset.

are found overwhelmingly in the adult (Fig. 7).

6. Diagnosis

As regards diagnosis of myasthenia gravis, clinical findings only will do generally if they are done minutely. But in many cases they are followed by tensilon test as a routine, resulting in showing positive reaction in as many cases as 95.4 %. A noteworthy fact is that, in these cases, sometimes tensilon effect would not be realized exactly for he long term chronic patients whose type is brittle and resistant to anticholinesterase drug. In prostigmine tests, positive reactions also show a lesser percentage of 89.

It is one of the important methods of diagnosis to catch electromyographically the waning phenomena in the affected muscle of myasthenia patients. We perceive, at times some negative findings in the period of progression and treatment of disease.

Pneumomediastinography has come into wide use in these several years in Japan. It is an excellent way of catching location and size of thymus abnormalities and the discrimination between thymoma and thymusmus hyperplasia easily together with a

rather simple way of manipulation. Degree of finding of thymus abnormalities stayed at only 15-30 % by the former simple X-ray technique. Very high rate of 70-80 % is distributed to the method of pneumomediastinography and it is indispensable for selecting the very treatment for the patients.

7. Worsening factors on symptoms

There appeared an episode of worsening symptoms which reached to 66.7 % of the patients suffering from generalized form of myasthenia. We can take up the following as the causes of the above effects; a common cold particularly with upper airway infection (67.9%), menstruation (48.8%), psychogenic factor (25.0 %) and other overworking, pregnancy and delivery, climate, contraindication drugs (antibiotics, tranquilizer and etc.) and the complication of autoimmune diseases join sometimes as their origin. Rare cases were found for episode of worsening symptoms to occur in the group of ocular form.

8. Immunological studies

Immunological approach toward the causes of myasthenia has long been developed actively with thymus and lymphocytes as its center object. We identified a high rate of hypergammaglobulinemia among the patients and abnormality of fraction in immunoglobulin, moreover the existence of antimuscle-, antinuclear-, antithymus antibody have been certified in serum of the patients since a long time ago. Recently, antiendplate antibody and antimuscle membrane antibody have also been perceived by means of fluorescence antibody method (Arimori,[1] 1975).

Movement of lymphocytes (T-cell, B-cell) of the patients have been studies at the same time from various viewpoints. The effort is to get valuable findings and opinions for its diagnosis, treatment, prognosis and others to be fixed. The author et al [7] (1973) have found, as a result of close reference to the reaction of the lymphocytes of the patient against phytohemagglutinin (PHA) from the side of synthetic activity (uptake of H^3-uridine into nucleic acid) of RNA in vitro, that they are proved to be specific so-called brittle type cases which are resistant to the anticholinesterase drugs and show low response. Namely, it can probably be considered the appearance of abnormal immunological mechanism of lymphocytes in these cases (Fig. 8).

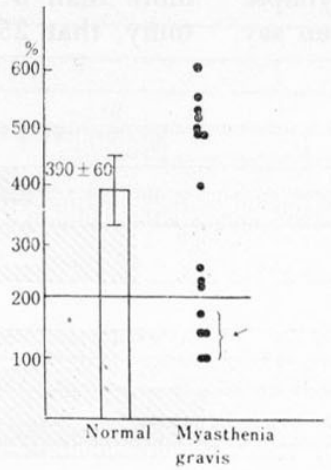

Fig. 8 PHA-induced increase of RNA synthesis in lymphocytes of patients with myasthenia gravis.
(〃: brittle type patients)

Recently, a study on histocompatibility antigen as one of the inheritance factors of the disease is in the process of development. There are some reports that in the case of myasthenia in Caucasians, HLA-Al and A8 (Behan et al,[2] 1973), HLA-A2 and A8 (Feltkamp et al,[4] 1974) or HLA-A3 and A8 (Fritze et al,[5] 1974) show high rate of

frequency. However, the author et al [8] (1977) performed on HLA typing in 105 Japanese patients with myasthenia and in 128 controls, incidences of both HLA-Al and B8 were 1/105 (0.95%) in myasthenia patients compared with 3/128 (2.3%) and 0/128 (0%) in controls, respectively. These antigens were very rare among Japanese as compared with Caucasians. In patients with myasthenia a decreased frequency of HLA-BW35 was also established (1.9 % vs. 18.8 % in controls, $P<0.0005$) and an increased frequency of HLA-A10 was observed (26.7 % vs. 11.7 % in controls, $P<0.019$). No differences were found between thymoma or thymus hyperplasia group, sex, age at onset, Osserman's classification type and HLA typings. Yoshida et al [16] (1977), on the other hand, have got the following data, assorting 63 cases of Japanese patients with myasthenia, that many HLA-B12 can specially be seen in the female group whose age at onset is rather young and it has some connection with thymus hyperplasia. HLA-B5, on the contrary, has a close relation to thymoma.

Autoimmune theory on myasthenia in this way is now developing to get to the heart of the matter on the level of histo- and cytoimmunology. Moreover, immunological study on motorendplate or synapse itself is progressing these days. We can say that the experimentation by means of bungarotoxin started by Fambrough et al [3] (1973) pulled the very trigger of this study. Pathology of synaptic membrane has been clarified by these experimental findings. We presume on this occasion that there is a great possibility of the myasthenia occurrence perhaps by immunological disturbance on acetylcholine receptor itself, and their radical treatment, therefore, would not be long before its very development.

9. Treatment

Reviewing the situation of the treatments for the myasthenia patients during one year of 1973 in Japan, medication therapy Occupys 9.4 %, thymectomy 15.5 % and thymus irradiation 7.0 % respectively. Most of thymectomy has been done at the time of from 5 to 14 years after their onset, with gradual shortening of the period these days. It is noticeable that their postoperative process and its prognosis are favorable. Namely, the cases of healing and improvement with the treatment of thymectomy reached as much as 63.6 % and showed it is better than that of medication or radiation therapy (Fig. 9). Papatestas et al [9] (1971) reported, in their observation of more than 5 years the cases after thymectomy, that 25 % of cure and 50 % of im-

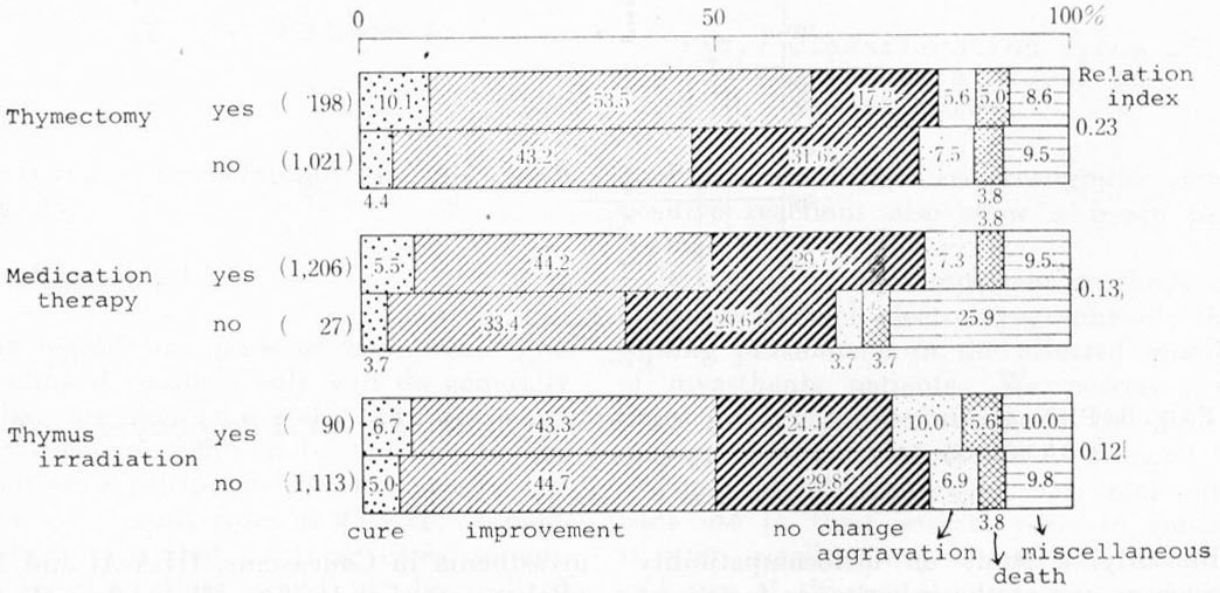

Fig.9 Treatments and process of myasthenia patients during a year.

provement respectively and better prognosis in cases of no germinal center in thymus. The author et al [12] (1977) found, on the other hand, in the 31 cases observed long time after the operation that effective ratio 71 %, variety in prognosis, few cases of remission right after the operation, postoperative corticosteroid therapy was needed in many cases of male, a small number of the patients exposed themselves to crisis as long as 4 or 5 years. In addition to the above, we found that effective ratio is low for autoantibody positive cases, no difference between thymona and hyperplasia in their effect, and that it is much more efficient in the cases having germinal center and or Hassall's body in thymus. In their effective cases, T-cell/B-cell ratio were found to be high. Prognosis proved to be better in the case of performing operation first and making corticosteroid as secondary treatment compared with the opposite way (Fig. 10).

Corticosteroid has recently become to our attention again as medication therapy by both Griggs [6] (1968) and Warmolts et al [15] (1970).

The author et al [11,12] (1976-77) made a long term observation for 5 years on the case of high dosis of prednisolone therapy for patients to whom anticholinesterase drug has no effect or diagnosed to have thymus abnormalities. We found consequently as follows; ultimate effective ratio to be 61 %, many cases of no effect shared by younger female, no relation between duration of illness and effectiveness, alternate-day treatment is more fruitful than every day treatment and perceived maximum dose of prednisolone is 50-120 mg/day. We found also initial worsening (transient) in 70 % of the patients observed.

On the day corticosteroid was given, lymphocytes and cortisol in blood decreased particularly at first and it increased reboundly, and it proved that decrease of T-cell is more than that of B-cell (Fig. 11). Moreover all of serum gamma-globulin, IgG, IgA and IgM decreased in effective cases and autoantibodies were perceived to tend to negativeness. But, as there is a possibility that corticosteroid acts directly on acetylcholine recepter protein and corticosteroid and anticholinesterase drug are in

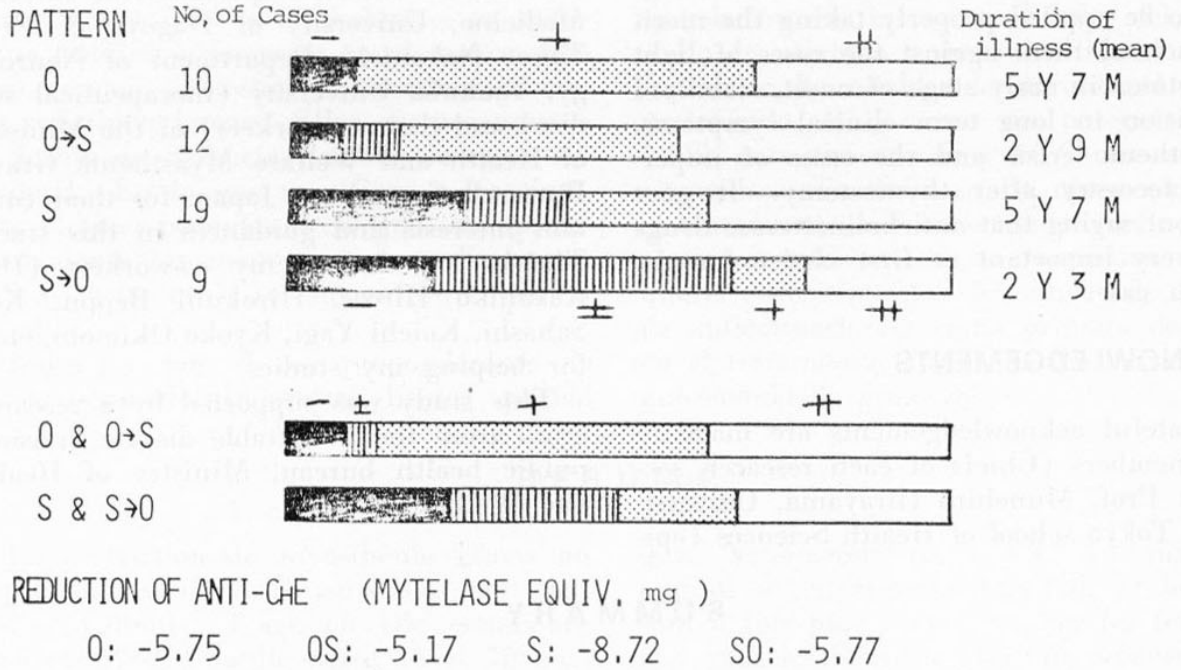

Fig.10 Antecedent therapy (thyme-tomy or steroid) and clinical effect of myasthenia patients.
O:thymectomy, S:corticosteroid
卄:dood, +:fare, ±:no change, —:aggravation

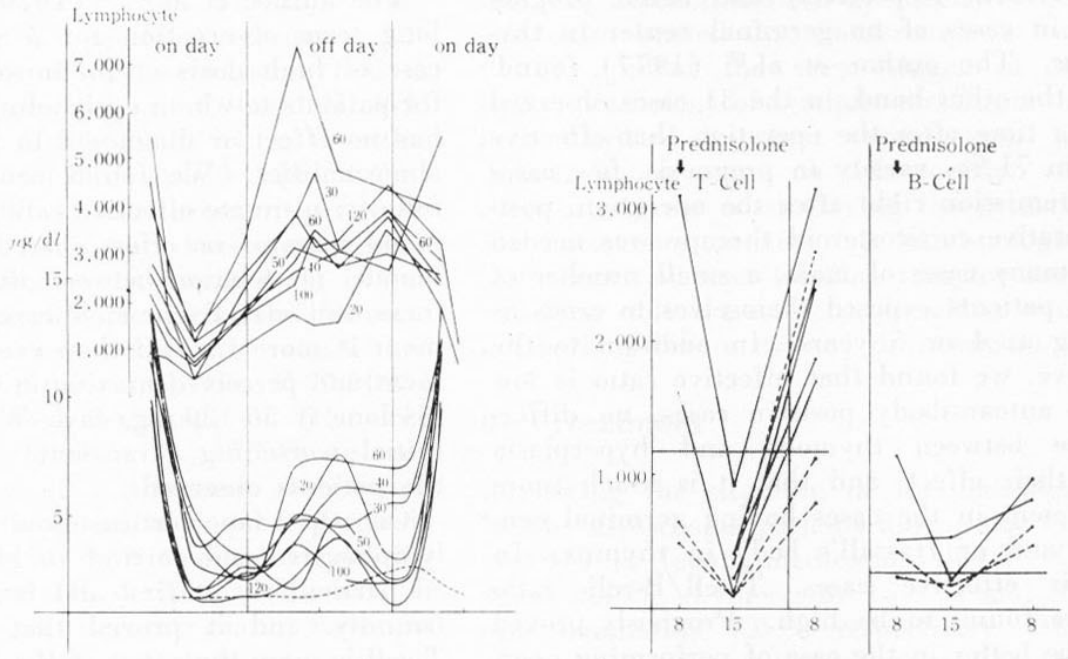

Fig. 11 Changes of lymphocyte count (upper) and cortisol (lower) in the blood under treatment by prednisolone in M. G. (prednisolone : mg/day)

Changes of T-, B-cell count in the blood under treatment by prednisolone in M. G. (count at 8 a.m., 3 p.m., ·····thymectomized patients.)

the relation of antagonism, full care is to be taken at the time of applying both of them.

Various kinds of anticholinesterase drugs are to be applied properly taking the merit of each of them against the cases of light symptoms in early stage of onset, stabilized condition in long term clinical symptoms, myasthenic crisis and the cases of imperfect recovery after thymectomy. It goes without saying that anticholinesterase drugs are very important as first choice for the virgin case.

ACKNOWLEDGEMENTS

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SUMMARY

The prevalence rate of myasthenia gravis in Japan is presumed to be 5 persons per one hundred thousands of the nation. Age at onset has its peak at ages from 20 to 30 and also indicating high frequancy among infants under 10 years old. Cure and mortality rate shows 5.1 % and 3.7 % respectively and they generally trace the process of long term chronic.

We find 4.5 % of the diseased in fami-

lial myasthenia and female cases show five times as much as male cases with their rather good prognosis on the average. They follow the mode of inheritance called autosomal recessive.

As regards clinical symptoms we can say they are about the same as in Europe and U.S.A. and the crisis are found in 16 % of them. By dint of the prevalence of pneumomediastinography, we have come to be able to find out thymus abnormalities in more than 70 % of all the myasthenia patients.

We could find the lymphocytes with decreased synthetic activity of RNA in the brittle type myasthenia patients. In HLA typing of Japanese myasthenia, many HLA-A10 are found and few HLA-BW35 on the other hand. HLA-B5 in the patients with thymoma and HLA-B12 in the thymus hyperplasia have their relation with each of thymus abnormalities.

In the aspect of treatments in Japan, effective ratio by 70 % in thymectomy and 60 % in corticosteroids have been assessed in the long term course of process observation. And also it has been found that treatment with thymectomy prior to corticosteroids produce better prognosis than the contrary case. The value of applying anticholinesterase drugs as first choice in the treatment for myasthenia gravis is still high.

RESUMEN

La proporción en que prevalece la miastenia gravis en el Japón se calcula en 5 personas de cada cien mil habitantes. La edad de comienzo en general es entre los 20 y 30 años, también es muy frecuente entre niños menores de 10 años. Proporción de cura y mortalidad es respectivamente de 5.1 % y 3.7 % y por lo general se relacionan a un prolongado proceso crónico. Encontramos 4.5 % de los enfermos en miastenia familiar y los casos en el sexo masculino, con un pronóstico en términos generales más bien bueno. Ellos corresponden a la forma de herencia llamada recesiva autosomal. En lo que se refiere a los síntomas clínicos podemos decir que son aproximadamente los mismos que en Europa y U.S.A., y las crisis se encuentran en 16 % de ellos.

Inducidos por el uso preponderante de la neumomediastinografía, hemos podido hallar anormalidades del timo en más del 70 % de todos los pacientes de miastenia.

Pudimos hallar los linfocitos con actividad sintética disminuída de RNA en el tipo frágil de pacientes miasténicos. En el tipo HLA de miastenia japonesa, se encuentra muchos HLA-AIO y pocos HLA-NW35. HLA-B5 en los pacientes con timoma y HLA-B12 en la hiperplasia del timo tienen su relación con las anormalidades del timo.

En lo atinente a los tratamientos en el Japón, se ha establecido durante el curso prolongado del proceso un porcentaje de 70 % para timectomía y 60 % en corticoesteroides. También se ha observado que el tratamiento con timectomía previo a los corticoesteroides tiene un pronóstico mejor que el orden opuesto. El empleo de drogas de anticolinasterasa como primera decisión en el tratamiento de la miastenia gravis sigue siendo de gran valor.

RÉSUMÉ

La proportion de myasthénie gravis au Japon est estiméeà 5 personnes sur cent mille habitants. L'àge où elle commence à se manifester oscille entre 20 et 30 ans: elle est également fréquente chez des enfants de moins de 10 ans.

La proportion de guérison par rapportà la mortalité est de 5.1 % et 3.7 % et s'associe en général à un long processus chronique. Nons avons trouvé 4.5 % de malades atteints de myasthénie familiale et les cas sont 5 fois plus fréquents chez les femmes que chez les hommes avec un pronostic en général favorable.

Ces cas correspondent à la forme d'hérédité appelée récessive autosomale.

Pour ce qui est des symptômes cliniques nows pouvons dire qu'ils sont à peu pres

les mêmes qu' en Europe et Etats Unis et les crises se manifestent chez 16 % d'entre eux.

En utilisant surtout la pneumomédiastinographie nous avons constaté des anomalies du thymus dans plus de 10 % des malades atteints de myasthénie.

Nous avons contaté que l'activité synthétique des lymphocytes quant au RNA était diminuée dans le genre fragile de malades myasthéniques. Dans le genre HLA de myasthénie Japonaise on trouve beaucoup de HLA-A10 et pen de HLA-NW35.

En relation avec les anomalies du thymus on trouve HLA-B5 chez les patients affectés de thymone et HLA-N12 chez ceux qui sont porteurs d'hyperplasie du thymus.

Quant aux traitements appliqués au Japon, on a pu établir an cours d'un processus prolongé un porcentage de 70 % de thymectomie et 60 % de traitement avec corticosteéroides. On a pu observer également qu'il vaut mieux effectuer la thymectomie avant d'appliquer les corticosteroides que d'observer l'ordre inverse. L'emploi de drogues à anticholinestérase comme premiere décision dans le traitement de la myasthémie gravis est toujours de grande valeur.

ZUSAMMENFASSUNG

Die Ausbruchsratio der Myasthenia gravis in Japan wird 5 Personen pro ein Hundert Tausend der Population vermutet. Das Alter des Auftretens ist zwischen dem 20. und dem 40. Alterjahr und es kommt auch in hoher Frequenz in Kindern im Alter von unter 10 Jahren vor. Die Heilung- und Mortalitätsratio ist 5.1 % und 3.7 %. Sie nimmt meistens einen langen und chronischen Verlauf.

Wir finden 4.5 % familiären Myasthenia in dieser Krankheit. In der familiäre Myasthenia wurden häufiger Frauen als Männer betroffen, und sie zeige gute Prognose mit autosomalrezessiver Vererbung.

In bezuge auf die klinischen Symptome können wir sagen, dass sie ähnlich wie das klinische Bild in Europa und USA sind, und die Krise in 16 % gefunden wurde. Von der Ausbreitung des Pneumomediastinalgraphie, haben wir Abnormalität des Thymus in mehr als 70 % in der Patienten mit dem Myasthenia gravis finden können.

Wir konnten die Lymphozyten mit verminderter synthetischer Aktivität von RNA in den Myasthenia Patienten mit Brittle Typ finden. In HLA Typ der japanische Patienten, viel HLA-A10 werden gefunden und weniger HLA-BW35. HLA-B5 in der Patienten mit Thymoma und HLA-B12 in der Thymushyperplasie haben ihre Beziehung mit der Thymus Abnormalität.

In Japan haben wir eine Effektsratio von 70 % in Thymektomie und 60 % in Kortikosteroid Behandlung. Man hat auch gefunden, dass die Behandlung mit Thymektomie vor Kortikosteroid bessere Prognose als den kontralen Fall zeigt. Der Wert der anticholinesterase Mittel zum Anfang der Behandlung für Myasthenia gravis ist noch hoch.

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Teaching Neurology in France

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Teaching methods are the reflection of a nation's traditions and customs, and although neurology has a proper language which is shared from one country to another, the methods employed to teach it are diversified.

France still maintains the tradition of anonymous competitive examinations. This examination constitutes the basis for the selection of the "Internes des Hopitaux" (hospital residents) from whom, after their training, will be recruted future hospital "Chefs de Service" and professors in neurology and other medical disciplines.

Before speaking of specialist training, it would be best to first examine the problem of teaching basic neurology to the group of students who will become general practitioners.

Medical School Instruction of Neurology

Although the various medical schools and different units of education and research which are responsable for the formation of future general practitioners, and which were created from reforms in medical education dating from 1958, enjoy a certain autonomy in their teaching methods, they continue to have certain general points in common.

Neurology is taught to all students in two stages during the second cycle of medical studies which culminate with the degree of Doctor of Medicine. During the first year of this cycle (DCEM 1 - which follows the two-year first cycle), theoretical instruction of semiology is assured by either a full professor or a university-approved lecturer and then reviewed with the teaching assistants in small groups at clinical bed-side seminars; these seminars are destined to illustrate and solidify the theoretical concepts of the lectures as well as to aid the student acquire experience in physical examination. This second-year program also includes courses in neuroanatomy and neurophysiology. Subsequently, during the fourth and final year of the second cycle (DCEM 4), neurological nosology is taught either as an independant "Certificat of Neurology" —taught concurrently with "certificats" of other disciplines— or as an "Integrated certificat" which includes the elements of other subjects which pertain to diagnosis and therapy in neurology (neuropharmacology, neuropathology, neurobiology, functional rehabilitation, etc.).

This curriculum aims to provide the foundation needed by the future practitioner to diagnose a current affection, to reasonably select the necessary complimentary examinations, to establish a course of treatment, to follow-up the patient, and to propose an eventual hospitalisation if necessary. Pedagogical committees established at each medical school are focusing their attention on the problem of defining this program's objectives, because, although these schools do no t share a standard teaching program, they must still try to find the means to assure uniform levels of instruction and formation.

Some schools continue to dispense "traditional" instruction where syndromes and diseases are enumerated with little effort made to clarify, simplify, or adapt to the demands of the daily practice of general medicine. Thus certain programs still consecrate more time to precious rarities of the neurological museum than to syndromes such as headaches which are so essential in general practice. One innovating tendancy

consists of dismantling this concept of discipline. Neurology for example might no longer be taught as a highly specialized discipline; consequently only the most frequent syndromes which are within the realms of general practice need be retained.

This "required" knowledge, however, still remains to be precisely defined. Each discipline requires that the student acquire a limited number of elements; this basic information, however, must be perfectly assimilated by every student and should be verified with continuous control examinations which could be given as easy-to-grade semi-automated "short-answer" tests.

Does this profound transformation lead to the conclusion that the traditional humanist-imbued education to which the student added his personal effort is no longer possible? Must it be replaced by mass education?

Although the teaching population has increased to keep up with the ever-growing number of future physicians, the student-teacher ratio remains the same; each teacher, therefore, has before him the same number of students as his predecessors, and these students are as capable of personal effort and reflection as their elders.

What has markedly changed is the volume of information which must be assimilated in order to comprehend each discipline and its techniques. It would be illusory, however, to try and dispense encyclopedic knowledge, and it is wise to limit student instruction to clear and coherent information which can subsequently be applied to the practical demands of their future profession.

Post-Graduate Instruction in Neurology

Recycling of physicians is assumed as part of the post-graduate educational program, and each university's physiognomy and teaching practices are defined by the local group of general practitioners. The teaching methods which are employed are numerous and preclude detailed discussion. They include: conferences covering current topics and bringing into focus new techniques and treatments, case presentations, audio-visual methods, etc.

Formation of the Neurologist

Until World War II, admission to this specialty was limited; neurologists were recruted exclusively from the Internes des Hôpitaux de Paris (Paris hospital residents) and from residents of medical schools located in a few other large cities. In several hospitals— among which the Salpetrière Hospital was at the forefront— future neurologists received their education in the tradition of the great professors such as Charcot, Babinski, Pierre-Marie, Dejerine, Guillain, and Lhermitte, who brought fame to the French School of Neurology. During his residency, the future neurologist was assigned to various departments where his theoretical knowledge was refined as he listened to case presentations and weekly lectures. To this was added an intense personal effort in the wards, at the library, and in the laboratory, as well as an ardent rivalry at the heart of groups which grew out the residents' affinities and mutual feelings. For these residents, the enthusiasm of these early years and the fraternal atmosphere in which they worked remain their richest and most beautiful memories.

From ward to ward and school to school, the resident accomplished a veritable "compagnonnage" prior to becoming attached to a "Patron" by mutual consent. In this small world, every resident was known by his own capabilities as well as by the reputation of his professor who acknowledged the resident as *his* "pupil" and guaranteed his qualification, thereby rendering the attribution of a diploma unnecessary.

The "Internat" still constitutes the royal road to neurology and to a first step in the hospital and university hierarchy. The increasing number of candidates for this specialty, however, prohibits using the "Internat" as the only means of forming neurologists. The "Certificat d'Etudes Spéciales" (C.E.S. - Certificat of Special Studies) in neurology has therefore become a reality. It includes four years of theoretical and practical education in a third cycle of university studies which follows the medical school curriculum. These C.E.S. students take rotations in clinical wards, neuroradiology, functional exploration laboratories, and

neuropathology, where they are accorded limited responsabilities and a few experimental projects. One year's training must be spent in a Psychiatric Department.

Theoretical courses in neurology are given as bi-weekly exposés. The program is divided into four sections designed to cover the entire scope of neurology in four years. All students in the C.E.S. program regardless of the year of their nomination, attend the same courses. In this way the program rotates each year; at the end of four years, the student has covered the entire neurological curriculum.

A volontary control examination takes place each year, but only the final nationally-organized examination given at the end of these four years sanctions the C.E.S.

New laws pertaining to medical specialty education have been proposed which contemplate combining the "Internat" and C.E.S. These laws would enable all C.E.S. students to have access to the same diagnostic and therapeutic responsabilities as the residents. Many educators remain aprehensive, however, and sense that these laws will increase the size of the "Internat" thereby denaturing it and effacing in the process the traditional education which benefited our residents and formed physicians who were both specialists in neurology and competent in internal medicine.

The C.E.S.'s educational content will be modified because of pressure resulting from: the increasing difficulty of dispensing practical training to this growing number of trainees, the development of diagnostical techniques, and the application of therapeutics to an ever-increasing number of afflictions. For these reasons, the clinical examination may wane in importance and eventually be relegated to a secondary role. With this change in status, instruction of this examination may soon be neglected, omitted and subsequently forgotten until the day when perhaps semeiology will regain its priority and medicine will again become the affair of doctors rather than technicians. In certain areas of internal medicine, this evolution towards technicalization has taken place several decades ahead of neurology, which until now, has been able to avoid these tendencies. Keeping clinical considerations at the forefront in neurology is not retrograde and does not suggest that we must neglect the immense fields of exploration and discovery which are opening up before us and auguring great strides ahead in diagnostic accuracy and therapeutic efficacy. It is still true, however, that it is this inestimable clinical experience that constitutes the strength of senior medical men and enables them to complete these new scientific discoveries while at the same time, lessening the ardure of younger doctors who might tend to over-estimate the importance of these innovations.

We would like to close this article with a wish that might one day come true.

We have learned in France to no longer limit our projects and pedagogical efforts to the university; instead, objectives, programs, and methods are coordinated on a national level at the heart of the "College of Educators in Neurology". A similar exchange might be possible on an international scale. A delegation could be assigned the task of studying the programs which already exist in various countries and then propose solutions for their harmonization. This would sooth certain disparities which appeared in Europe when national frontiers were opened to allow liberal circulation of doctors in the european community. Consequently, each country might benefit from the experience, traditions, and pedagogical techniques of their neighbors thereby enriching their own teaching programs.

History of Medicine

ARNOLD KLEBS AND HARVEY CUSHING AT THE 1st INTERNATIONAL NEUROLOGICAL CONGRESS AT BERNE IN 1931 *

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I

International gatherings of scientific men often have an importance which far transcends that of the formal reports of the meeting itself; and this was notably true of the Neurological Congress held at Berne in the autumn of 1931. There had previously been other international congresses of physicians, and even of neurologists, but to neurology, and to medicine generally, the meeting at Berne had peculiar vitality and significance. It was the first time after the World War of 1914-18 that neurologists from Germany, France and England, as well as of other countries of the world, had found it possible to have a joint meeting, and it proved to be one that was little marred by politics or the old animosities of war. But the gathering was also remarkable for other and quite different reasons; these had to do with personalities. In the first place the meeting was held at Berne, the birthplace of Arnold Klebs (Fig. 1), and there were present many of his oldest and most intimate friends: William Welch, Harvey Cushing, Charles Sherrington, Otfrid Foerster and many others including Bernard Sachs, the President of the Congress, Hugh Patrick an old friend of Klebs's Chicago days, and Llewellys Barker whom he had known at the Hopkins.

On the opening day of the Congress, Arnold Klebs, in the true spirit of Swiss hospitality, gave a dinner [1] to which he invited many old friends, and a good many new and younger friends to meet one anoher, and also to meet his Swiss colleagues, Professors Sahli, de Quervain, Wegelin and Asher. The seating was as follows:

Dr. Ramsay Hunt
Dr. Gaston De Coppet
Dr. Ernest Sachs
Mr. Geoffrey Jefferson, F.R.C.S.
Dr. B. W. Th. Nuyens
Dr. Walter Timme
Dr. Reymond de Saussure
Prof. Vittorio Putti
Prof. F. de Quervain
Prof. Harvey Cushing
Sir Charles Sherrington
Prof. Otfrid Foerster
Prof. Leon Asher
Prof. Llewellys F. Barker
Dr. Charles Dubois
Dr. Foster Kennedy
Dr. Ign. Oljenick
Prof. Louis Michaud
Prof. Hugh Patrick
Mr. Norman Dott, F.R.C.S.
Dr. C. P. Symonds
Dr. R. H. Meagher
Dr. Henry Alsop Riley
Prof. Otto Marburg
Prof. John F. Fulton
Prof. B. Brouwer
[Prof. Hermann Sahli]
Mr. Hugh Wilson, U.S. Minister
Prof. William H. Welch
Dr. Arnold C. Klebs

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* From the Laboratory of Physiology, Yale University School of Medicine.

[1] Dr. Klebs had planned the dinner with the utmost care—truly Halstedian—and had had reprinted for the occasion Lücke's *Der Mohr von Bern* (1868), a volume which caricatured all the prominent members of the Berne medical faculty of 1868, including Edwin Klebs.

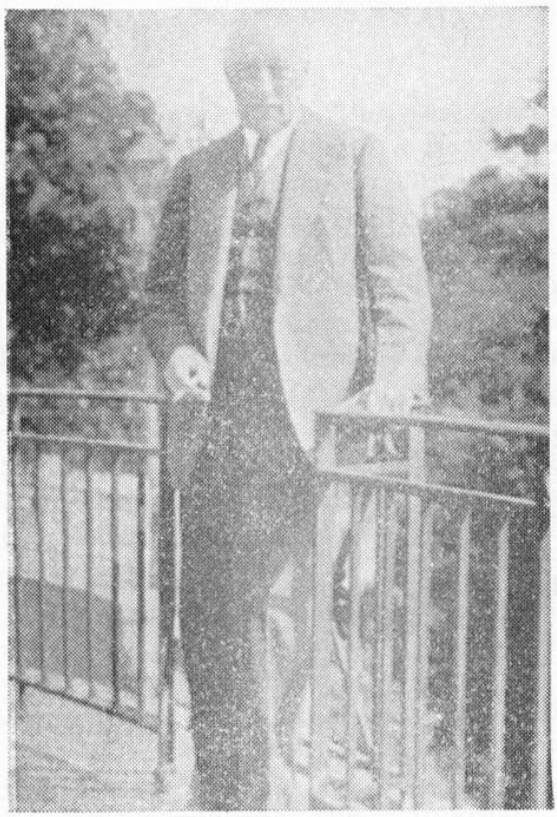


Fig. 1.

Arnold Klebs on terrace of Hotel Bellevue, Berne, September 2, 1931.

(All photographs taken by Richard U. Light)

Prof. Bernard Sachs
Prof. A. von Eiselsberg
Prof. Carl Wegelin
Dr. Percival Bailey
Dr. R. U. Light
Mr. George Armitage, F.R.C.S.
Dr. Franc Ingraham
Mr. Hugh Cairns, F.R.C.S.
Dr. Daniel O'Brien

At the end of the dinner Dr. Klebs, to whom I like to refer as "A.C.K.," made a speech of welcome, which no one present, dinner or no dinner, could possibly forget. I stole the text, copied it, and I give it to you here without securing the permission of the speaker, It began as follows without formal salutation:

" It is a singular privilege to welcome you, friends from across the sea, to Berne which I call my home-town, not only because of the accident of birth in it, but because of many precious bonds past and present.

" Welcome to you, Dr. Sachs, and thanks that you have brought together from many corners of the earth busy and distinguished workers, who, while they know each other well on paper, yet yearn for the living and warm contact which allows them to return to their task with renewed hope and vigor. It has always seemed to me that the real value of congresses lies in these personal contacts fully as much as in the actual business. Scientific thought, Dewey says, and I agree, is a function of society as well as of the individual, just as breathing is a function of the air as well as of the lungs.

" I am happy that my friends from the present medical faculty, Prof. Sahli, de Quervain, Wegelin and Asher, have joined me here to-night to add their welcome to mine. I present to you also their earliest predecessors in a reprint of Lücke's *Der Mohr von Bern*, the original of which has become very rare. It shows them in the lighter vein which apparently did not mar their historical record.

" Welcome to you, Patrick, friend of the old days in Chicago, where the winds are cold sometimes, but hearts ever warm. Welcome to you, Bailey, who brought thither the spirit of the Brigham. Welcome to you, Dr. Barker, though you forsook early the Western Metropolis for Johns Hopkins.

" Johns Hopkins, what precious memories arise out of that name! Osler and Halsted, now gone but unforgetable. When I came first into contact with it my one desire was to become a student again. I had passed through seven universities but here it seemed I might really learn something. I had had splendid teachers before and the best equipment Europe could offer, but I missed what I found, and now can sum up in that inadequate expression 'American spirit.' At Johns Hopkins it was first infused into scientific medicine and from there it spread. We have with us tonight the man who knows better than anybody else how this miraculous infusion is performed. I am afraid he will not tell. President Hoover called him our greatest states- man of public health, but those who have benefitted by his fatherly counsel call him by a less formal but sincerely affectionate title. Dr. Welch, you are at home here as everywhere, we welcome you most cordially.

" In matters of the 'American spirit' I must read you what a recent Dutch critic (Huizinga) has said about it: 'Es besteht

in Amerika ein geistiger Zusammenhang, ein gemeinschaftliches Streben, wie *wir* es nicht kennen... Männer und Frauen der Wissenschaft arbeiten als Alle opfern sie etwas von ihrer eigenen Persönlichkeit... Sie verzichten wenn auch unbewusst, auf die Luftschlösser Ihres eigenen Denkens, um für die andern zu pflügen".' Let us keep this precious gift!

"And now, dear Harvey, what shall I say to welcome you to the town that is as much yours as mine? The letter you wrote home from here in 1901 gives me the key. Your versatility has always amazed me but I did not realize what an excellent medical historian you were already then. The preoccupation with batrachian hearts at Kronecker's and with thyroid complexities at Kocher's did not prevent your going to the Stadtbibliothek to study Haller at the source, in his *Tagebücher*. His unfathomable erudition bothered you and you wanted to discover his human side. It relieved you to find him noting 'perdu au jeu' or a 'bouteille de vin.' You compared him to John Hunter who hated lecturing, but became the guiding star of many followers. while Haller with all his grifts left no personal school. You in your own career have followed Hunter. Your boys from Brigham, some of whom I am delighted to see here with you, will heartily agree that you have succeeded. They are your living history. Some historians can predict but to few it is given to show that they have learned from history. Keep it up, Harvey, give us some more 'from tallow-dip to television.' Don't forget what you said in perfect Bärndütsch: Nit nolo g'wunnt!"

Dr. Welch replied for the guests—with his usual felicity and charm—and he characterized A.C.K. as one who had "the rare gift of friendship." He also spoke of the remarkable letters which all his friends enjoyed and treasured, and of his great bibliographical erudition. No one but "Popsy" could have expressed so effectively what we had all felt in our hearts.

II

But we must now retrace our steps. The 1st International Neurological Congress was held at the Municipal Casino, beginning on Monday morning August 31st. A surprise had been arranged by Professor Asher, then "Rector Magnificus" of the University, in the form of two honorary degrees, one for Harvey Cushing and the other for Charles Sherrington. Neither recipient had been previously informed of the honour, and it had required some little diplomacy to ensure their presence at the meeting without revealing the reason for having them prompt in their time of arrival. Sherrington, as one of the Vice-Presidents of the Congress, sat on the platform. With some difficulty Dr. Cushing was persuaded to sit in one of the front rows. Beside him were William Welch and Arnold Klebs.

The meeting was opened at nine sharp by Herr Haeberlin, the President of the Swiss Federation, who welcomed the delegates in the name of his democratic country. He spoke in a clear, slow French, most happily and to the point. Then came addresses of welcome by four Swiss Professors, Asher, Wegelin, Naville and Bing, each of whom spoke in a different language (English, German, French and Italian). The Rector Magnificus then took the platform. referring first to Pavlov, which called forth an enthusiastic response. He then announced that the University was to do honour to itself by the conferring of two honorary degrees, the first to Harvey Cushing. He then called for H.C., who walked slowly up the steps on the left side of the rostrum and crossed to the opposite side to face Asher. A brief statement was then read in Latin in which H.C.'s achievements were described . Dr. Cushing meanwhile stood facing Professor Asher, profile to the audience, without moving an eyelash and his face, although almost immobile, gave evidence of suppressed pleasure and emotion. After all this, Asher turned to him, grasped his and, and spoke spiritedly and in perfect English somewhat as folows:

"Harvey Cushing, when you first came to Berne, thirty-one years ago, Hugo Kronecker and I saw in you the future master of neurological surgery. You have more than lived up to our expectations, for in your field you have no peer. We are proud to think that some at least of your inspiration came from the Laboratory of Physiology at Berne and we are even more proud now to welcome you as a member of our University."

Asher also referred to H.C.'s relations with

Kocher and to all of his many friends of early days, male and female, who recalled him vividly and were proud to have known him. The diploma in a large red case was then presented to him.

Professor Asher next called upon Sir Charles Sherrington who,like Cushing, was quite dumbfounded for the moment; and repeated much the same performance first in Latin, followed by a speech in perfect English vernacular. His references to Sir Charles were particularly happy and were terminated by the phrase: "Sir Charles, we look upon you as the supreme philosopher of the nervous system."

The opening proceedings were terminated by Bernard Sachs's excellent Presidential Address. His references to Switzerland as the home of human liberty seemed especially well timed. After the formal part of the morning session the scientific program began with a number of important papers by Clovis Vencent, Percival Bailey, Wilder Penfield and several others.

On Monday afternoon the 31st, Dr. Cushing read, as the third speaker on the scientific program, his celebrated report on 2,000 verified tumours of the brain. At least a thousand people attended, and for the first time (and probably the only session during the Congress), all of the side foyers were empty. Dr. Cushing, speaking slowly into a microphone which seemed perfectly tuned to his voice, began with references to his early physiological experiments in Berne carried out in 1900-1901, mentioning that he had now come to give an account of the work he had done in the interval. He referred to the various factors which had led to the dramatic fall in mortality rate for cerebral operations. "Younger men," he went on to say, "picking up where I leave off, can reduce the mortality still further." He reviewed the life history of the various categories of brain tumour and then ended with a somewhat unexpected climax: "Gentlemen, this will be the last report on the statistical results of brain tumours as a whole which I shall ever publish." [2]

[2] Dr. Cushing's full report was later published in monograph form under the title: *Intracranial tumours. Notes upon a series of two thousand verified cases with surgical-mortality percentages pertaining thereto.* Springfield,Ill.; Charles C. Thomas, 1932. 8vo. xii, 150 pp.

For a moment there was complete silence. then a burst of prolonged applause. Professor Ariëns Kappers, who was presiding, broke the precedent by offering a vote of thanks to Dr. Cushing in the name of the Congress for placing before them in this inspiring way the brilliant results of his life's work.

On Monday evening, Arnold Klebs gave the dinner for Dr. Cushing and his pupils to which I referred at the beginning of this account.

TUESDAY, SEPTEMBER 1st

The following day began with a morning session devoted to muscle tone and the postural reflexes, with Sherrington in the chair. There were many papers. Little new, however, was brought forward and the session was chiefly remarkable for the nationalities represented and for the brilliant summing-up at the end by Sherrington. He expressed his objection to the word 'tonus' itself and voiced the hope that the term would ultimately be dropped; so long as it is employed an element of mystery will hang about it and since we now know that it represents postural contraction, it were better to use this latter term.

In the afternoon there were demonstrations and no end of fascinating anecdotes. Dr. Welch, for example, told us of his student days in Vienna, Berlin and Breslau (1876-1877). He knew Rokitansky and had informed Cohnheim how to stain tissues with haematoxylin and eosin; at Ludwig's laboratory he discovered the bipolar ganglia in the frog's heart, but Ludwig had not encouraged him to publish the observations. He knew Roy and attempted with him to establish an Anglo-American journal of experimental pathology. There seemed no end during these days of fascinating reminiscences, but H.C., even more than Welch, formed the centre of attention. He was besieged by his old friends, many of them by now dignified matrons, and also his lodging-housekeepers of 1900 and 1901, booksellers, reporters and foreign physicians.

A wealthy family made a pilgrimage from Hungary with a child that had an odd-shaped head. They camped on his doorstep until he had to see them. As the baby was the son and heir he was never allowed out of his mother's sight. H.C. sized up the

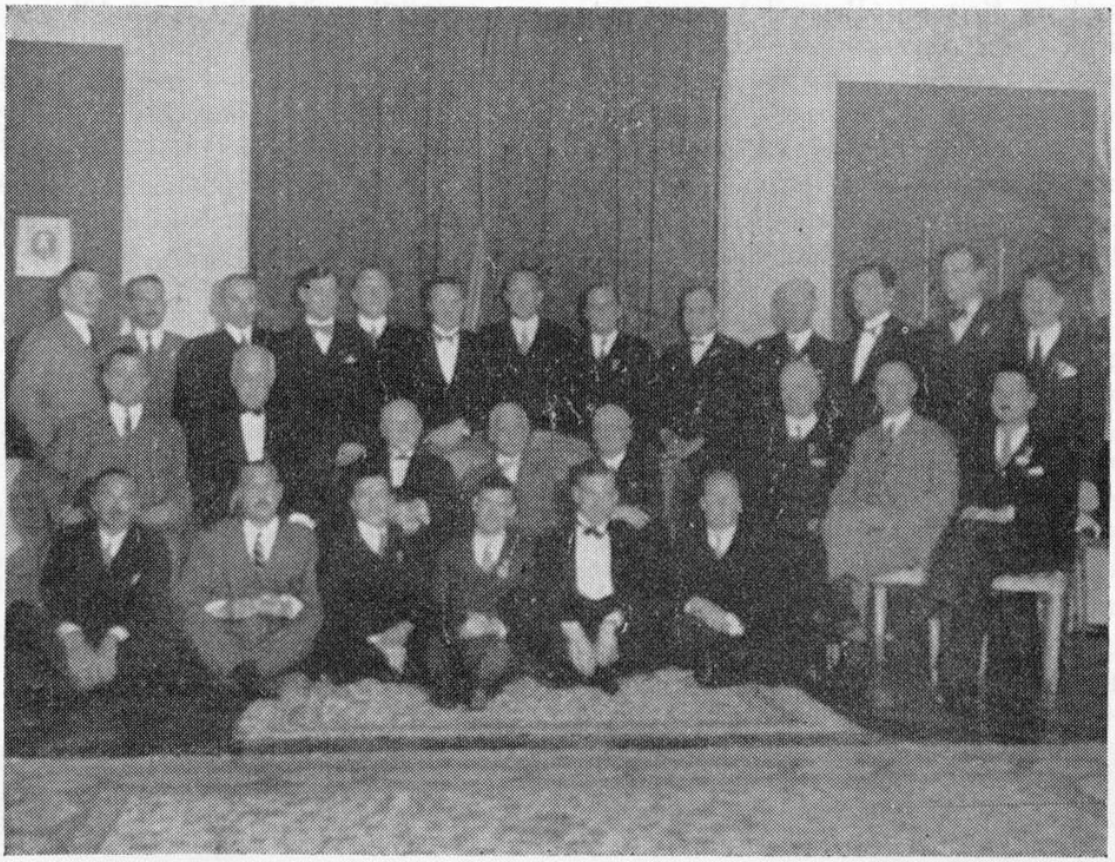


Fig. 2.

Dr. Cushing, his teachers and his pupils. September 1st, 1931. From left to right, *Above*: Drs. Armitage, Petit-Dutailles, Bremer, Martin, Oljenick, Bailey, Penfield, Schreiber, Bagdazar Olicecrona, Morelle, Schaltenbrand, Ingraham; *Middle*: Drs. Francis Grant, Arnold Klebs, William Welch, Harvey Cusring, Charles Sherrington, Thierry de Martel, Geoffrey Jefferson, Tracy Putnam; *Below*: Drs. De Coppet, Meagher, Fulton, Dott, Cairns, Fremont-Smith.

situation in a flash, saying: "You need not come to Boston; you have only to let your child run and play with other children." Whereupon he took the child down the hotel corridor, making him chase a ball. The mother, the sisters, the cousins and aunts remained in the room aghast and wide-eyed; presently the docile infant crept back and startled them all with a vociferous 'Boo!' In five minutes H.C. had thus cured the family as well as the child. The astonished but obviously delighted father enquired what his fee would be; H.C. said there would be none, whereupon the father protested. H.C. then asked, "Do you know Jeremiah Smith?" Their faces all brightened. They had known him well. "He is one of my best friends," said H.C. "I feel towards Hungary as he does." At this they all made a low bow and retired.

Dr. CUSHING'S DINNER [3]

" On the spur of the moment this morning, H.C. decided to invite all of his former pupils to dinner to meet his 'masters,' Welch, Sherrington, de Martel and Klebs I had to dash around and find them in the course of the morning and everyone accepted—28 in all—and it proved a memorable occasion. The seating, which H.C. slaved over for nearly two hours (writing all the cards himself), was as follows (Fig. 2):

H.C.
Thierry de Martel (Paris)
Paul Martin (Brussels)
Daniel Petit-Dutailles (Paris)
Tracy Putnam (Boston)
Jean Morelle (Louvain)
John Fulton (New Haven)
Dr. Welch (Baltimore)
Richard Meagher (Boston)
Francis Grant (Philadelphia)
Norman Dott (Edinburgh)
Frank Freemont-Smith (Boston)

[3] From a diary kept during the Congress.

Frédéric Bremer (Brussels)
Sir Charles Sherrington (Oxford)
Dimitri Bagdazar (Bucharest)
Percival Bailey (Chicago)
Georges Schaltenbrand (Hamburg)
Frederic Schreiber (Detroit)
Richard Light (Boston)
Herbert Olivecrona (Stockholm)
Dr. Klebs (Nyon)
Hugh Cairns (London)
Ignaz Oljenick (Amsterdam)
George Armitage (Leeds)
Gaston DeCoppet (Berne)
Franc Ingraham (Boston)
Geoffrey Jefferson (Manchester)
Otfrid Foerster (Breslau)
Wilder Penfield (Montreal)

" I had the good fortune to sit beside 'Popsy,' who remarked in a quiet way that the gathering had a far-reaching significance since it meant that someone in America had at last founded a 'school,' and that the 'school' was exerting an influence perhaps even greater abroad than at home. The followers of Cushing were fired with much the same enthusiasm that had fired the students of Ludwig nearly a century before. There was much in this vein and many reminiscences. 'Popsy' never repeats himself and he has never told a dull anecdote.

" As the dinner was drawing to an end, 'Popsy' suggested that I propose the health of 'The Chief'—who had meanwhile moved to the end of the table, exchanging places with Penfield. His response was moving, witty and very simple, and began as most of his speeches do, with a humorous twist:

Child : Papa, where were you born?'
Papa : 'In Berlin.'
Child : 'Mama, where were *you* born?'
Mama: 'In Paris, my dear.'
Child : 'Where was I born?'
Mama: 'In Baltimore. Don't you remember?'
Child : 'Isn't it funny how we all got together!'

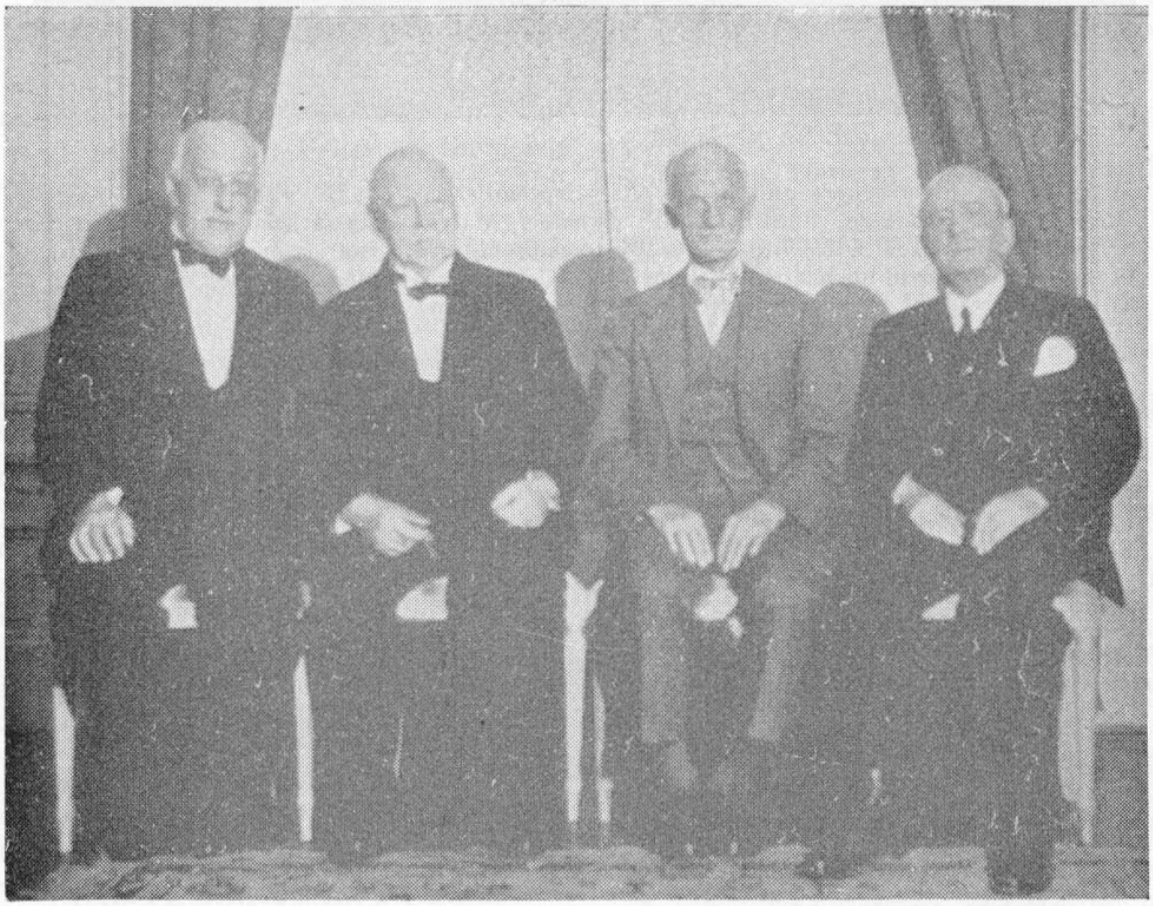


Fig. 3.

Arnold Klebs, William Welch, Harvey Cushing and Charles Sherrington, September 1st, 1931.

Then he went on somewhat as follows:

" Everything has a small beginning. Henry Head once said that there would have been no Great War had he not once looked through a peep-hole in a girl's school. We could not be sitting here had a Baltimore saloonkeeper not shot his wife in the neck in a moment of extreme emotion in September 1896. She by chance was a patient of mine and had haematomyelia with some of her dermatomes knocked out. This led me to Kocher's book and I decided to come to work under him. In 1900 I sailed with the desire to work with three people: the man at my left (Sherrington, Fig. 3), which explains his being here; with Horsley, who unhappily has joined his fathers; and with Kocher of Berne. The only note of introduction was a letter to Horsley which Dr. Welch (Fig. 3) had given me the night before I left. Then as now, Welch spent his life seeing how he can make the way easy for other people (burst of applause). Horsley was delightful, but was running for Parliament and I decided to return when he had either gotten in or gotten out! I went to Paris and then to Berne, but waited a month for Kocher to give me an *Arbeit*. Then I found myself in Kronecker's laboratory. This brief story accounts for everyone here except Jefferson, de Martel and Olivecrona, who speak for themselves. So it is natural that we should foregather here in Berne. I hope we shall go out to see De Coppet operate on Friday; if things go well, we shall rejoice with him if not, we shall all know that each of us has done a much worse job himself.

" Funny how we all got together!

" Hold together. Keep up your friendships—form societies where you can meet together frequently to exchange ideas, and to criticize one another's technique. Keep your finger on neurology, but don't get lost in scientific minutiae—a meduloblastoma or a perineurial fibroblastoma by any other name is just as sweet! Don't let neurological surgery get too far away from general surgery. It is the greatest possible compliment that we have been adopted by the neurologists and accepted as one of them. Be this as it may, we did not grow wholly out of neurology, for our roots are in the fertile soil of general surgery. I like to think that our specialty is perhaps the richest in the field of Medicine, and it will be if you make it so."

THURSDAY, SEPTEMBER 3rd

Wednesday, the 2nd, had been devoted to excursions and nothing worthy of special record took place except that a good many of us were glad of the opportunity to reco-

Fig. 4.

Dr. Cushing speaking beside the monument of Theodor Kocher. September 3rd, 1931.

ver from the excitement of the previous two days. On Thursday, at noon, after the morning session, a group of some thirty people went out to the Berne Cemetery for an unforgetable ceremony.

III

THE LAYING OF THE WREATHS

In the pouring rain, Welch, Sherrington, Cushing and Arnold Klebs, along with several Swiss physicians (de Quervain, De Coppet and Fischer) went out with a number of the younger American physicians to lay wreaths on the graves of Kocher, Kronecker and Edwin Klebs. The ceremony resulted from Dr. Cushing's inspiration and nothing could have been more touching to the hearts of the people at Berne. The group first went to the tomb of Theodor Kocher which lies at the far end of the cemetery. The rain continued and H.C., picking up a large wreath went slowly up to the tablet and laid it at its foot. He came back to the plot and spoke for about ten minutes, coatless, his head bared (Fig. 4) and the rain soaking him, as follows:

Dr. CUSHING ON KOCHER

" In my younger days I had the good fortune to come under the influence of two men who were outstanding surgeons of their generation—William S. Halsted and Theodor Kocher. I may couple their names together for they had much in common. They were engaged in similar problems; they were friends and correspondents; they were equally fastidious in their operative craftsmanship and at the same time, by precept and example, exerted a profound influence as investigators on the scientific aspects of their art. They represented the European and American surgery of their day at its very best.

" So in placing this wreath, in loving tribute, on the grave of one of these masters, I feel that I am merely the agent of the other, his admiring friend, whose memory I hold in equal affection and gratitude.

" The most precious heritage of our profession lies in its noble traditions. What has been accomplished does not die, but too often, alas, the personality of those who have handed the torch from one generation to another soon fades into oblivion. So for those of you—his spiritual grandchildren—who have gathered here and to whom Theodor Kocher is little more than the name of a street which you have frequently traversed the past few days, I would like to give at least an impression of what he was in life—a slight, spare man of personal neatness, of quick step and alert bearing, of unfailing courtesy and dignity, precise and scrupulous in all his dealings professional, public and personal—a man to trust.

" His clinic at the Inselspital had long been a surgical Mecca and when in 1900 I came here hoping to find an opportunity to work under his guidance he finally gave me a problem which took me away from the clinic to the physiological laboratory. When sometime later on he chided me for my failure longer to appear in his operating room I awkwardly replied that after witnessing a few of them, to learn his methods, I found his operations singularly uninteresting; but I hastened to add that for this very reason I would unhesitatingly and with perfect confidence put myself in his hands, should I at any time happen to be in need of an operation myself. For a moment he looked mystified and then said: 'I shall interpret your absence then as a compliment. Surgeons who take unnecessary risks and operate by the clock are exciting from the onlookers' standpoint but they are not necessarily those in whose hands you would by preference choose to place yourself.'

" We think of America as the land where young men are given their chance; but in 1872, at the unusual age of 30, Kocher succeeded Lücke in the Chair of Surgery here. And here to the end, a loyal Bernei Burgher, he remained in spite of tempting calls to posts in other universities possibly of greater renown. It is given to few surgeons continuously and actively with ever increasing reputation to hold a professional chair for so long a time as forty-five years. I last saw him on his seventieth birthday when in 1912, shortly after he had been made a Nobel laureate for his contributions to our knowledge of the thyroid gland, a *Jubeleum* was held here in his honour and at this great festival tributes from every part of the world poured in upon him. From hard work and responsibility surgeons are prone to burn themselves out comparatively young, but Kocher had been blessed

with an imperturbability of spirit or had cultivated these habits of self-control which enabled him to bear his professional labours, his years, and his honours with equal composure to the very end. The current of his long and active life was as steady, cool and uninterrupted as that of the Aare encircling his beloved Berne."

Hugo Kronecker. From Kocher's tomb we came slowly to Kronecker's and that of Edwin Klebs, which are together in an enclosure. Sir Cherles laid the wreath on Kronecker's monument and reminisced most felicitously about him (Fig. 5). Just as he started, however, there was a loud clap of thunder which caused him to look quizzically at the sky as if annoyed with the Almighty. He told the story of Kronecker's habit of being late and of Holmgren's celebrated remark when awaiting him at a committee: "Der kronische Verspätung!" Sir Charles spoke as follows:

SHERRINGTON ON KRONECKER

" We, coming, some of us from homes far distant, offer here together our token of respect and affection to memories intimately with us in this place. Our thoughts turn to three names which, in their time—the generation immediately preceding our own,—contributed to adorn Berne as a source of medical culture; the names of three men who lived to ennoble medicine both as art and science by their work and their example. Of them at this spot where we now stand lie two; and for one of them, Hugo Kronecker, the tributary wreath we bring to him, now rests against his grave.

" His name was early familiar to the young physiologists of my generation because so often was it on the lips of our teachers; and always with a note of special warmth of admiration. Later I had, through many good years, the privilege and pleasure of personal contact with him. So it came that I experienced myself the charm of his gifts and character. To know him was to understand the universal regard in which he was held. A circumstance which favoured our friendship was the organizing of the International Congresses of Physiology. Those meetings could not have found a more devoted or unselfish collaborator than he. At Berne, in the 'Hallerianum,' as he loved to style the laboratory, he presided over the Congress of 1895; to its outstanding success his own personality contributed no small share.

" As for his services to his science, they were many. Research, in which the action of cardiac muscle was perhaps his favourite

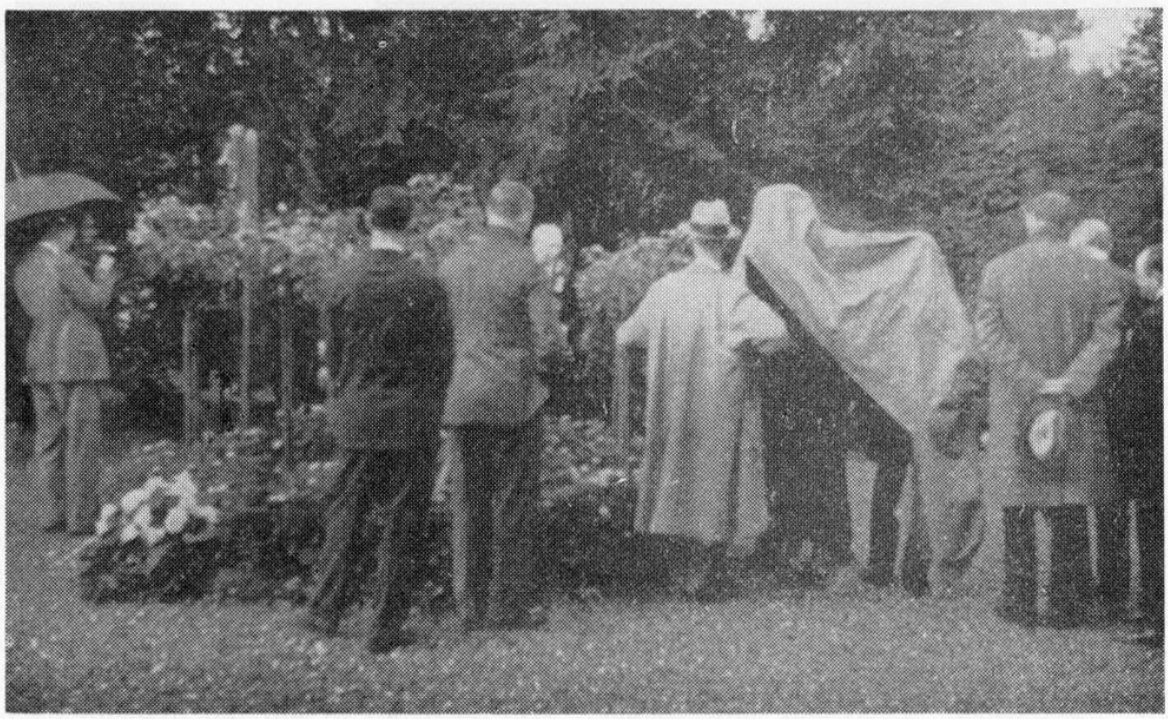


Fig. 5.

Sir Charles Sherrington speaking at the grave of Hugo Kronecker. September 3rd, 1931

theme; apparatus, his ingenious instruments made his name a household word in many laboratory outside his own and thirdly, he typified a fine tradition of laboratory comradeship, and so genially and happily that he stood as its very embodiment for us all. He had a genius for friendship, with old and young alike—even with children. I can still see him in a schoolboy's room, during a visit to England, standing on a chair fixing to the roomwall a clock brought with him from Berne and pieced togeher by him to delight the young friend with its jubilant 'cuckoo.' His silvered head will be remembered with affection not only by those many who recognized him as an honoured master of our science, but by many who, apart from all that, knew and loved him for his nature as a man."

Dr. WELCH ON EDWIN KLERS

Dr. Welch, standing before the tomb of Edwin Klebs—utterly oblivious of the drenching rain spattering on his bald head —spoke for fifteen minutes extemporaneously of Klebs's life and his achievements (Fig. 6). No one, alas, was there to take it down and he had no notes—just the resources of a memory which seemed never to fail. He recalled Klebs's full name: Theodor Allbrecht Edwin, and that he had been born 97 years earlier, *i. e.*, on February 6, 1834. "Popsy" described him as a restless man of profound prescience, whose many peregrinations entitled him to be called a "wandering scholar." For he had lived successively in Königsberg, Berne, Prague, Zürich, Karlsruhe, Asheville (North Carolina), Chicago, Hannover, Berlin, Lausanne; and finally he returned to Berne to spend his declining years. Of his achievements he mentioned the joint discovery with Loeffler of the diphtheria bacillus, his description of acromegaly in 1884, and that fifteen years before Metchnikoff's studies he had inoculated monkeys with the exudate of syphilitic lesions. Mention was also made of his introduction of solid culture media before Koch, and of his isolation, prior to Eberth, of typhoid bacilli from cases of typhoid fever.

Much else was said, but words left less of an impression than vivid memory of an occasion so unique—everyone was deeply moved, Arnold Klebs perhaps more than anyone else and certainly more than he ever confessed.

Otfrid Foerster who, during his rich and productive life, has always profoundly admired Kronecker, Kocher and Klebs, remarked: "Harvey Cushing was inspired to

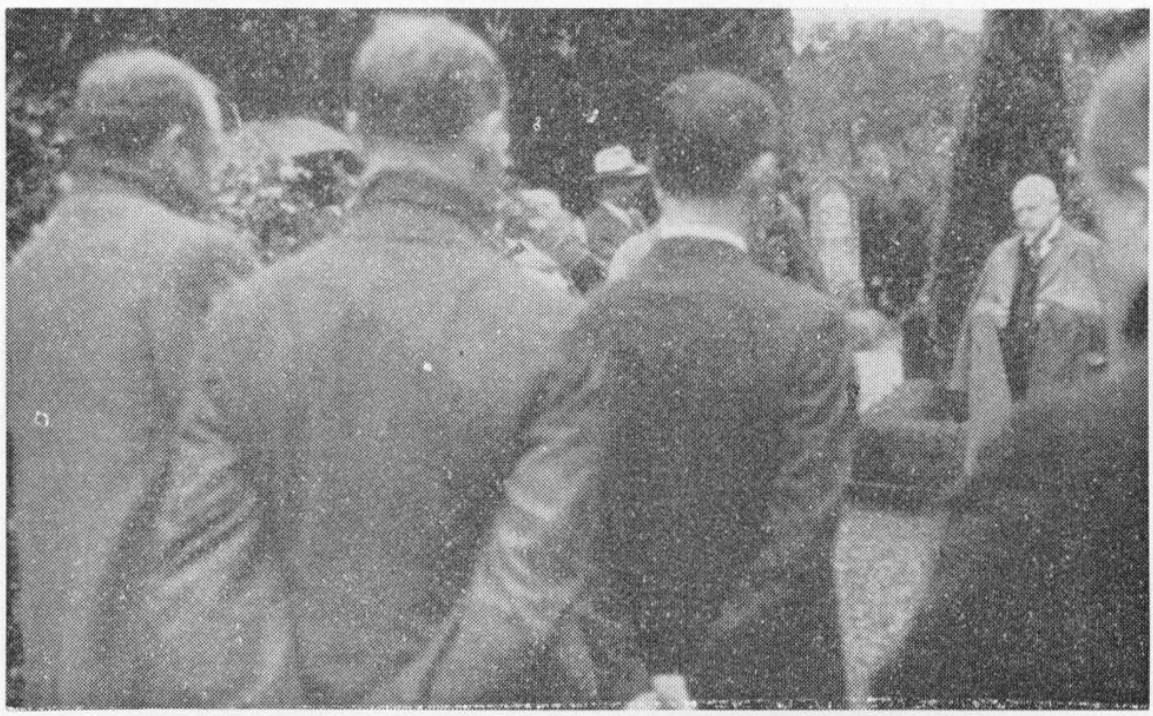


Fig. 6.

Dr. Welch speaking before the grave of Edwin Klebs. September 3rd, 1931.

think of this, and to have been present was the greatest privilege I have ever had."

IV

The Congress went on for another day. After the ceremony at the cemetery Professor and Mrs. Asher gave a memorable luncheon party, to which many went direct from the cemetery. There were present:

Dr. Graham Brown (Cardiff)
Dr. von Weizsäcker (Heidelberg)
Prof. Pavlov (Leningrad)
Frau Asher
Dr. Cushing (Boston)
Dr. Springer (Berlin)
Dr. Pavlov (son of Prof. P.)
Dr. Sachs (Pres. of Congress)
Prof. Asher (Berne)
Sir Chas. Sherrington (Oxford)
Dr. Welch (Baltimore)
J. F. F. (New Haven)

Pavlov and "Popsy" were the chief centres of attention. Dr. Welch took an active part in the conversation about journals—a topic which had been brought up by Dr. Ferdinand Springer of Berlin—and he told of his early experiences with the *Journal of Experimental Medicine.* They founded it in 1896 in order to give a channel of publication for experimental work in the broad field of the medical sciences (See his preface to the first volume). In the early volumes he used English spelling entirely; later, spelling preferences were allowed; since it has been taken over by the Rockefeller Foundation the conventions adopted have been rather vigorously standardized, but the grotesque A. M. A. spelling conventions have not been adopted. The *American Journal of Physiology* was an offshoot of Dr. Welch's journal.

PAVLOV'S PAPER [4]

" Pavlov had been a little uneasy during lunch as his paper was obviously on his mind. The session was held in the 'Aula' at the University. He was called on at 3:00 and the whole room was thronged; as soon as he mounted the platform they all stood and cheered for several minutes. As usual he looked pleased, but not in the least surprised! He spoke in German on neuroses in animals, briefly and quite effectively. Sir Charles, who has never seen quite eye-to-eye with Pavlov remarked afterwards: 'He has accumulated an enormous body of significant experimental data, but his attempts at interpreting it are infantile!!" I had had no idea that he felt so strongly.

" *The Hallerianum.* After Pavlov's paper Asher took me to his laboratory which was built about 1890 by Kronecker and is known as the Hallerianum in honour of the great 18th Century physiologist. It is a yellow brick structure about the shape of the old Sterling Hall of Medicine at Yale, and it has been very little changed since it was erected. He took me into his office and showed me first the conventional engraving of Ludwig. 'That,' he said, 'was the Ludwig who taught Bowditch; and this,' pointing to a bust in profile over his desk, 'is the Ludwig who taught me!' And the spirit of Ludwig somehow permeates the laboratory, and it is very refreshing; Asher is almost the last of those who can call themselves Ludwig pupils. The laboratory is fairly well equipped with apparatus, but Asher talks poverty constantly; despite this a surprising amount of work is going on, and each year he has pupils collaborating with him from all over the world. He showed me with considerable emotion the corner room in which Dr. Cushing had worked in 1900-1901."

In the evening of September 3rd came the official dinner of the Congress with, alas, speakers from every country. H.C. and "Popsy" both spoke but unfortunately they were scarcely audible. Foerster, however, male a brief but most eloquent and audible plea for the recognition of Neurology as a separate discipline, and as far as formal utterances were concerned it was the high point of the entire Congress. Since it has not previously been published I venture to give it here in full.

Herr Präsident!
Meine Damen und Herrn!

Als vor 2 Jahren der Aufruf zur Teilnahme an den Internationalen Neurologen-Congress auch an Deutschland erging, da haben alle deutschen Neurologen diesem Rufe begeistert zugestimmt. Und wenn trotzdem heute die Gesellschaft Deutscher

[4] From a diary kept during the Congress.

Nervenärzte leider nur durch eine verhältnismässig geringe Zahl ihrer Mitglieder hier vertreten ist, so wollen Sie darin bitte nicht einen Mangel an Interesse an diesem Congress erblicken, der durch die grosszügige Initiative der American Neurological Association inauguriert worden und bisher so glücklich verlaufen ist. Nicht Mangel an Interesse, nur die bittere Not der Zeit ist es, die so viele Deutsche Neurologen heute von hier ferne halt. Aber im Geiste weilen sie alle hier und mit den herzlischsten Wünschen begleiten sie den Verlauf dieses Congresses vom ersten bis zum letzten Augenblicke.

Und in der Tat! kann es denn für uns ein schöneres, ein erhabeneres Ziel geben, als wenn wir, die Erben eines Romberg, eines Leyden, eines Wilhelm Erb, Hermann Oppenheim, eines Hitzig, Flechsig, Wernicke, uns zusammenfinden mit den Erben des unsterblichen Hughlings Jackson, mit den Erben eines Sir William Gowers, Sir Victor Horsley, Sir David Ferrier, Sir Byrom Bramwell, eines Bastian; als wenn wir zusammenkommen mit dem Mann, der wie ein Zauberkünstler in die geheimnisvollen Zusammenhänge des nervösen Geschehens hinein geleuchtet hat wie kein anderer, mit dessen Namen die Physiologie des Nervensystems steht und fëllt, Sir Charles Sherrigton, als uns zusammenzufinden mit den Erben eines Brown-Séquard, eines Duchenne de Boulogne, des grossen Charcot, eines Brissaud, eines Dejerine, eines Sicard, eines Foix, eines van Gehuchten, eines Wertheim-Salomonson, eines Bolk, eines Retzius, Salomon Eberhard Henschen, Carl Petrén, eines Ernst Alexander Homén, eines Korsakow, Kojewnikow, eines Roth, eines Jendrassik, eines Pilcz, eines Golgi, Mingazzini, Camillo Negro; als zusammenzukommen mit den Jüngern des greisen Titanen, der den Ariadnefaden, der in das Labyrinth des Nerven-systems hineinführt und wieder daraus hinausführt, an silberner Spindel auf- und abgesponnen hat, Ramon y Cajal, als zusammenzukommen mit den Erben eines Dubois, eines Monakow, eines Forel, als einzuschlagen in die Bruderhand der Erben eines Meynert, eines Nothnagel, eines Heinrich Obersteiner, eines Emil Redlich, als uns zusammenzufinden mit den Erben der grossen Neurologen aus der Neuen Welt, eines Weir Mitchell, eines Dercum, eines Charles Mills, eines Sir William Osler. Und kann es für uns deutsche Neurologen überhaupt ein schöneres, ein erhabeneres Ziel geben, als dass auch wir, voll aufrichtigster Dankharkeit und feuriger Inbrunst, zusammen mit allen anderen Neurologen, dem Manne begeistert zujubeln, welcher der prominenteste Eckpfeiler unserer Wissenschaft ist, dem unser Sonderfach, die Neurologie, überhaupt erst die Stellung einer eigenen selbständigen Disziplin verdankt, dem grossen Forscher, dem gesegneten Helier der Menschheit, "der die Blinden sehend und die Lahmen gehend macht," der wundervollen Persönlischkeit Harvey Cushings!!!

Meine Damen und Herrn! Wenn wir uns hier zusammenfinden und wenn wir, vom Geiste dieser grossen Männer einen Hauch verspürend, hier in diesem gottbegnadeten Lande, der Schweiz, dem Lande, da einst der Freiheit Wiege stand, dem Lande, das Hort und Hüter höchster Kulturgüter stets war, noch ist, und immer sein wird, am Fusse der gewaltigen Bergriesen, die, vor Aconen von Jahren erstanden, noch immer ihr schnee- und firnbedecktes Haupt zum Himmel emporheben, wenn wir uns hier gleichsam einen "Rütlischwur" einander in die Hand leisten, dass wir das Erbe, das wir von unseren Vätern übernommen haben, getreu bewahren und vermehren wollen und dass wir diese heilige Pflicht auch unseren Schülern und Nach- folgern ins Herz legen wollen, ich meine, dann erscheinen wir als Teile eines grossen Organismus, der ewigen Bestand hat, und dann erleben wir zugleich einen jener seltenen und grossen Augenblicke, in denen Zeit und Ewigkeit sich paart.

Meine Damen und Herrn! Die medizinische Wissenschaft gehört zu den Kulturgütern, die Gemeingut aller Nationen sind. Sie ist mit an erster Stelle dazu berufen, ein gemeinsames Band um alle Völker zu schlingen. Sie ist der Boden, auf welchem Angehörige der verschiedensten Rassen, mögen ihre Charaktere und Anschauungen auch noch so verschieden sein, mögen ihre sonstigen Ziele und Wünsche auch noch so weit divergieren, zu einer grossen Synergie zusammentreten. Es will mir scheinen, dass unter den Vertretern unseres Sonderfaches, der Neurologie, von jeher ein besonders gutes Einvernehmen geherrscht hat, ein besseres gegenseitiges Verstehen gewaltet hat

als in anderen Sonderdisziplinen der Medizin. Ich glaub deshalb, weil das Fach der Neurologie von jeher ein besonders exaktes gewesen ist, weil die Forschung sich immer und überall auf den gleichen grossen Linien bewegt hat. Ich bin überzeugt, dass dies immer so bleiben wird, so lange die Neurologie den festen Boden der Tatsachen nich unter den Füssen verliert, so lange die anatomische und die physiologisch-analytische Beobachtungsweise das Feld beherrscht, so lange die Vertreter unseres Faches sich nicht in die Gefilde der Mystik verlieren, nicht abschweifen in die nebligen Sphären einer nach freiem Belieben interpretierenden Symbolik, sich nicht an Worten einer oft schwer verständlichen Terminologie berauschen, uneingedenk eines der vielen tiefsinnigen Worte des grössten Neurologen aler Zeiten, Hughlings Jackson: "Words fetter our thoughts as well as define them."

Meine Damen und Herrn! Wenn wir einen Rückblick werfen auf den bisherigen Verlauf dieses Congresses, so dürfen wir wohl ohne Ueberhebung sagen: Dieser erste Internationale Neurologische Congress ist ein grandioser Erfolg! Er ist eine geradezu imposante Demonstration der grossen Bedeutung und des riesigen Umfanges, den die Neurologie heute erlangt hat. Aber wir haben nicht nur unsere Anschauungen und Kenntnisse über die wichtigsten schwebenden Probleme und Fragen gegenseitig ausgetauscht. Wir sind uns auch menschlich einander näher getreten, haben neue Bekanntschaften geschlossen und alte Freundschaften erneuert. Wir haben Männer, deren Werk und Namen uns seit langem bekannt, von Angesicht zu Angesicht geschaut, von Mund zu Ohr vernommen. Ich glaube das bedeutet viel. Befruchtend ist und segenvoll der Regenstrom, der aus der dunklen Wolke niedergeht, doch zieht der Wanderer, der nach der Wahrheit lechzt, den hellen Quell ihm vor, der aus der aus des Felses scharf umrissener Spalte stürzt!

Dieser Congress, ich wiederhole es, ist ein beispielloser Erfolg. Trotz des schier babylonischen Sprachengewirrs, das hier herrscht, stehen wir doch alle hier, als ein einig Volk von Brüdern, zu unserer Alma Mater Neurologica! Möchte dies immer so bleiben! Hoffen wir, dass dieser erste Internationale Neurologische Congress bald von weiteren gefolgt sei, auf denen die Grundlagen, die hier gelegt wurden, weiter ausgebaut werden, zum Heile und Segen der Wissenschaft, der wir alle mit gleicher, heisser Liebe dienen!

Zum Angenblicke möcht ich sagen: "Verweile doch, du bist so schön!" "Dann wird die Spur von unsren Erdentagen nicht in Aconen untergehn!"

V

And so the Neurological Congress of Berne drew to a close. Arnold Klebs, through his warm affection for Harvey Cushing and his unusual capacity for brinding people together, had made it an event with few parallels in the history of such gatherings. A new "school" in the ranks of Medicine had come suddenly to have international recognition; and the *esprit de corps* which this has fostered in the group itself has found substantial expression in countless ways—but in none more poignantly than in the elaborate organization in all European countries now at war for dealing with head injuries. In each country those responsible for these services have been trained by Dr. Cushing or by the pupils which they in turn have inspired; but Harvey Cushing's influence has touched nearly every sphere of modern Medicine.

The Bahamas

On Vacation

THE BAHAMAS. — It is known that some ancient kind of glass, after it is buried for a certain length of time, acquires a rainbow-like iridiscence that transforms it into a real gem, and its value increases.

The same happens with our experiences. I cannot write a diary while on vacation; its nearness blockades our inner vision. We are functioning at a different level, collecting, feeling, sensing, hiding in our memory treasures to be recounted afterwards in solitude. Some months have passed since we went to Nassau. Winter is bitter cold and the wind's glacial embrace makes us shudder; then, memories of these experiences return, beautiful, iridiscent and with all the nuisances that accompany a trip erased.

Then came to our mind the word of Bliss Carman, the poet who best expressed the charm of these Islands.

"Where I can buy the magic charm
Of the Bahamian sea
That fills mankind with peace of mind
And soul's felicity".

NASSAU. — The minute we arrived we were enveloped by a soothing atmosprere. It was as if someone took a heavy overcoat off our shoulders burdened with preocupations and cares, and with its pockets loaded with undone tasks. Then suddenly we felt light, free, skin to skin with Nature.

It was a deliverance from the complicated womb of civilization, to a wonderful environment.

A band in the airport gave us a gay welcome; among the passengers were newly weds who were elated.

The people of Nassau are friendly, and proud of their heritage.

A recent proclamation of Independence and the booming of their wonderful Summer resort make their faces beam with justified pride.

A wonderful place in which to rest. On the sand, under the sun, or in the sea, the frontiers that separate man and nature are in a peaceful calm smelted, until man is restored to his true environment. As Walt Whiteman statess

"Man and Nature shall be disjoin'd and diffused no more
The true Son of God shall absolutely fuse them."

To rest is to change to break a repetitious pattern. And the Bahamas gave us two choices: the simplicity of nature with its soothing atmosphere and warm embrace, and man's sophisticated way of enjoying life.

Nassau has the traditional charm of an old city with its Government buildings, colonnial style and shops with goods from different countries in the world, which are displayed on Bay Street.

Nassau honors the name of William III, formerly William of Orange - Nassau. Strolling along its streets is like turning the pages of a history book. Surrounded by Parliament Buildings there stands the statue of Queen Victoria,


THE BAHAMAS.

whose marble frozen countenance stares with absent eyes the flood of people that go back and forth along Bay Street, while dressed like tropical Bobbies, the policemen reflect the English influence, and direct in their garb the unending traffic stream of tourists and islanders. Queen Victoria is loved for her gesture to abolish slavery.

Not far from there stands a statue full of dynamic and aggressive movementss one hand presses the handle of a pistol while the other threatens with a sword. It represents Woodes Rogers, the Bahama's First Royal Governor, who in 1718 *drove out the pirates who for more than* 70 *years infested the islands that were a place of choice for burglars and pirates.*

You can also find their traces in Nassau, where the Famous Blackbeard inflicted terror in the Bahamian waters in the Blackbeard Tower, a site now in ruins which it is claimed to have been used by the pirate to peer the horizon to detect coming ships that may give them a rich aftermath.

The tiny winding streets, the time-enduring forts, with rusty cannons, and many still beautiful chalets dating from 1800 *coupled with the once famous Royal Victoria Hotel built in* 1862, *which is mentioned in the beautiful novel, "Gone with the wind", in its historical aspect as being used during the Civil War to give shelter to the* blockade-runners once caroused, *brings a nostalgic feeling for old times.*

The Royal Hotel is now closed; it must hold many happy remenbrances to Miss Lorraine Onderdonck, regarded as the first lady of the Bahamas Hotel, who was running it in 1937 *when Nassau was a humble fishing village.*

The present is omnipotent; like an unconquerable avalanche it fills all the paths and crevises of the past.

Tourists invade everything; they are particularly attracted by a stroll through the Woodes Rogers Walk. The beauties and fruit of the sea are there.

The most dazzling shells and corals are displayed, while open booths offer different kinds of fish. There are also boats selling fish, specially a mollusk that hides its flabby and gellatinous nature in the most beautiful and big sea shell. Customers standing on the border of the walk deal with the fishermen. Once the shells are sold, he kills the animal with a stab and extracts it from its enclosure. A soft pink mass, that he wraps up and hands to his customer. The shells are thrown into the sea. Under the clear waters thousands of shells demonstrate the fruitfulness of the business.

Wood carving is performed on the street and the craftsman displays his interesting work. More popular and numerous are the straw markets, where hats, dolls, mats and baskets are displayed with beautiful colours and designs all along Bay Street and Rawson Square.

Tourist's children love to wear straw hats with the name of Nassau embroidered on them. Some ladies buy dolls for presents, and wait while the stitches of the vendor are inscribing the name of John, Ann, or whoever the destinatary may be.

Shopping is a big lure for the vacationists the more you see, the more you want. Bay Street will satisfy all the needs.

There is also shopping at night, but the most rewarding spectacle is the

once a week Goombay Parade that invades the streets of Nassau.

Man horrows the most beautiful feathers from the birds and from the flowers, their most exciting colours.

Trembling muscles echo the drumming, the players' hands beat the parch with dexterity, first in the center, afterwards on the side; the void glottis grunts: "Goom! ... Bah!" which gives this festival its name.

The Bahamian Concertina is added to this leading sound and the vibrations of the scraping of saws with long nails. Beautiful Bahamian girls move gracefully like exotic tropical birds. The fever of music is contagious. Nerves tune rhythms, muscles vibrate and everybody is happy. Some tourists join the joyful stream of dancing people.

A little coloured girl is attracted by the music and enters into the parade instinctively moving and twisting to the sound of music, her beautiful round eyes wide open, shine with excitement and the mouth is open in an ecstatic smile. She was the most authentic example of the magic power at the Goombay elating musical rhythm.

PARADISE ISLAND. — The glow of Paradise Island induces many visitors to cross the bridge over the harbor that separates it from the center of Nassau to enjoy a vacation that may be quite restful or full of excitement, or alternating, as his mood requires.

Nature's attractions are counterbalanced by man's sophisticated way of seeking exciting experiences. The most luxurious new hotels, a wonderful Casino and a Night Club represent an investment of $ 600 millions dollars.

In one of these fabulous hotels, the Britania Beach Hotel, where the elusive Howard Hughes hides from the world, there is a passage which connects directly with the Casino and also with five restaurants.

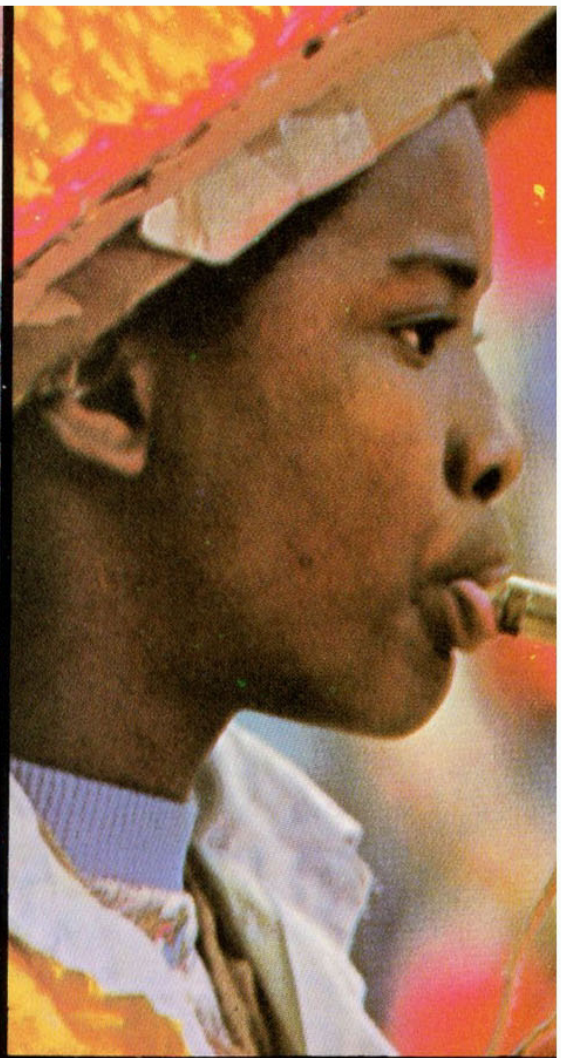
Our main purpose in going to the Bahamas was to rest, but we found the people's different ways of life very interesting to watch.

Besides, the Bahamians make gaiety bubble in the air. Hotel hostesses are very charming and encouraging. Music never ends: at the airport, on the terrace where you are eating, or in the Hotel Hall, where a fascinating fashion show is taking place. Elegance, originality in the beautiful afro prints, is displayed by the elegant models who with dancing steps and graceful movements charm the public.

For a short period the model freezes in a beautiful movement to allow some of the audience to perpetuate in a picture its exotic beauty.

Resting near the sea side is really like feeling the calmness of the tropics, our perceptions become keener, every sense is sucking from nature like a bee the nectar of life. Enjoying the soft caress of the trade winds, while feeling the sun on our skin, warm and compassionate, patiently washing away the whitish tint of winter. Looking at the sea whose brilliance and peacefulness is invaded by yachts of colorful sails swelled by the wind, and inflated platforms that offer private decks where they are happily rocked by the waves.

Men, women and children, all feel the joy of the sea. Life returns to its ancient dwellings.



Around the island

Like a bronze statue with all its muscles tightened, firmly pressing the feet on the water, the skier rushes past pulled by a tense cable tied onto a speeding motor boat, while the sea puts brilliant transparent wings at the side of his feet making him an Olympic God like Mercury.

The morning is crystal clear. The Atlantic waters like a wrinkled silver foil shimmers to the sun. Several tourists are getting ready to explore the submarine wonders which in these regions compete in splendour with those that adorn the surface of the earth. It is a jolly group; laughing and joking they adjust their plastic shoes which seem like the lower extremities of a batracian, transforming them into "frog-men". On this excursion they take a sort of knapsack something that is more valuable than food - the air tank.

Adjusting their round, transparent visors, there they go! one after the other, carrying their personal port-hole through which to peer at the marvelous submerged gardens whose magnificence reach the highest at the Bahamas.

Coming from the beach salted and summed, how rewarding it is to find a huge punch bowl courtesy of the Hotel, for its customers. It was not only refreshing, but a delicious Bahamian mixture. The night was hanging its twilight decor before enveloping us in a silvery blue darkness. The moon began to shimmer on the placid waters.

Seated in the hall of the hotel, after a long day, divided between shopping in Nassau and swimming in Paradise Island we were comfortably resting and listening to an orchestra whose contagious rhythms made us unconsciously tap the floor.

Two middle-aged couples from New Jersey began to talk to us. One of the ladies urged her reluctant husband and all of us to go to the Casino. She was the nervous type, blonde with glasses and of medium height. Under her insistence we relinquished to her wishes. Just to see I explained, it is fun! The bus is free.

A green little bus was waiting at the hotel door.

It goes back and forth all the day long to the Casino open round the clock, but the major games start at 1 p.m. and ends at 5 a.m.

It was one of the most luxurious casinos I have ever seen (I must confess that I have seen few of them) but I suspect that not too many of them can boast of having an immense red carpetted saloon, lighted with dazzling chandelliers, six scarp tables, six roulette wheels, and nearly 300 slot machines of different values from 25 cents to a dollar.

The little lady from New Jersey stopped in front of one of them with a bunch of nickels in her hand. The rest of us just watched. She placed the nickel into the slot and pressed some buttons. In a line of quadrantal different kinds of fruit appeared. I did not understand the game but it seems to be that she ought to obtain four of the same kind. Strawberries, strawberries, next a pear and an orange, signaled her defeat. Several times the machine swallowed the nickel and mocked at her. She was getting nervous, and looked angrily at the machine. At her side a colored lady, tall, and physically generously equipped, was playing, disdainfully looking at the machine and chewing gum; she seemed to master her mechanical opponent. And it paid! A cascade of nickels like an exorcism

was thrown out of the machine's entrails.

The lady from New Jersey felt humiliated, affronted. She told us she was going to change to another machine. She eagerly went to another one with a more amiable look, followed by us. Then a peach appears, another peach, and another —she was trembling with anticipated joy. A Nut! And this was really driving her nuts!

She cast us a suspicious look. We understood what she meant. Somebody ought to be guilty of her bad luck. Then she said goodbye, and went to a different machine in another corner of the Casino.

We began to wander about and it was for us a surprise to learn that they have a roulette table especially for ladies. Two of them were playing at that time.

One of the ladies was about thirty years old, round faced, with a plump body loosely seated and wrinkling her satin dress. At her side a young man was standing, a few years older than her. She seemed to be morally unsure, like one that is recovering from some mental maladjustment. She took the chips hesitantly and looked at him with an almost pleading look, asking for a decision. He affectionately encouraged her to choose a number.

The other lady was just the opposite. Thin, sophisticated, with artificial lashes that hid an incisive look. Self controlled, she circled her glass of whiskey with tightened fingers, with the same grasp with which a parrot holds its balancing stick. In the other hand she held a cigarette with a long cigarette holder, and did not leave it when with almost surgical sureness, with the available fingers, she placed the chips. Sipping from the tall tumbler of whiskey, she followed the whirling of the wheel with the corner of her eye under the long lashes. Lady Luck does not like defiant people. She lost. Just an imperceptible contraction of the corner of her mouth revealed that she felt the stoccade.

Prof. Dr. VICTOR SORIANO

News

TOURETTE SYNDROME ASSOCIATION

NEWSLETTER
MEMBERSHIP MEETING HELD
OCTOBER 25, 1980

DISCUSSION OF "TOURETTE SYNDROME IN YOUNG ADULTHOOD

Elaine Shapiro, Ph.D., moderated a panel of six young adults who have Tourette Syndrome. The panelists introduced themselves, and Dr. Shapiro them posed questions to the group.

Pat has had T.S. since the age of ten, and was diagnosed when she was twenty-eight years old. Phyllis is now twenty years old. She has had T.S. since the age of nine, and was diagnosed at nineteen. Currently a junior in college, the hopes to be a special education teacher, and is doing well on Haldol. (One other panelist was using Haldol, two were on Clonidine, and one did not use any medication.)

Kathy, a young woman of twenty-two, is working at present after having attended college for a time. She expects to return to school to prepare for a career in social welfare. Although her symptoms began at the age of seven, she was not diagnosed until she was fifteen.

Orrin just received his Bachelor's Degree. He is working part-time and applying for entrance to medical school. Orrin has had Tourette's all of his life, and has not been helped by any medication. At twenty-seven, Marco is married and works full time. His symptoms started in childhood but it was not until two years ago that he learned the name of his affliction. Twenty-three year old Mark works full time, and has known he had T.S. since the age of sixteen.

Dr. Shapiro noted the lengthy time which had elapsed between the onset of T.S. and proper diagnosis. She asked the panelists how they felt when they were growing up and did not know that they had T.S.

Mark responded that he was constantly teassed by other children. His parents had taken him to many physicians who all said he was a normal, healthy, "hyperactive" child who had "tics." Many doctors assured the family that the tics would go away. Mark wondered at times if he was "crazy," and found his parents support and assurances very helpful.

Phyllis was also taken to many physicans to no avail. When her peers teased her she reacted by becoming reculsive. She often locked herself in her room, and when she joined the family to watch T.V. she consciously sat behind the people in the room in order not to disturg them.

Kathy reported that growing up was a devastating experience. She was taken to many doctors because her family thought that seeking advice of physicians was the best thing to do. However, one doctor advised her parents to punish and imitate her because tics were part of a "stupid childhood game." She became withdrawn, but this began to change in high school when she finally began to find some acceptance.

When asked by Dr. Shapiro how to deal with the negative experiences of childhood, Pat recalled that a teacher had labelled her an "animal," and she advised parents of T.S. children to inform the teacher of their child's illness. She regretted that she was always "left out" as a child because her peers thought of her as "different." An informed teacher could aid the T.S. child. Phyllis agreed that an understanding teacher was most important in order to help alleviate the problems of these youngters.

Marco reported that gangs of children would beat him and call him "retar d" during his school years. One teacher had tied him to a chair and put a gag in his mouth because of his bizarre behavior. He could not turn to his family for help during this time because they too believed he was a behavior problem and would punish him for his symptoms. Even the teachers would make fun of him in school and eventually he learned to pretend that he was sick so that he could stay home. At times he would have a tutor come to his home and he recalls this as the happiest time because he didn't have to endure the ridicule of peers and teachers. Marco's problems, which are due to T.S., still persist. As a newlywed in his first apartment he found that neighbors misunderstood his noises and thought he was violent. He had to move into an apartment with thick walls.

Dr. Shapiro asked, "Who should tell the class about T.S.?' Phyllis thought the child who has T.S. should tell the class. Mark

felt that children are disposed to tease other children if they are fat, short, wear glasses, etc., and only a teacher who is informed about the illness can minimize the hurt. Orrin stressed the importance of educating everyone in the school, teachers, administrators, nurses, etc. If a child is teased by his peers, at least the professionals, who know about the illness, will be able to help.

Recalling school experiences, Orrin said that, when he was young, nobody beat him up, but neither would anyone be his friend. During college his circumstances were improved. He told his teachers about his condition before the term began, so that they would understand. He reported that his biggest problem involved studying. He began using recorded books from the Library for the Blind and Physically Handicapped. This, coupled with extended time on exams, has helped him to do well. Orrin feels attending school in a large city has advantages because "in the city everyone is crazy and used to bizarre behavior," and so it is easier to cope.

Kathy disagreed. She went to a small college in a rural area and found her professors very sympathetic, and encountered few problems.

Mark spoke about the loneliness of growing up mith T.S. He felt isolated and has always wanted to be treated like the other kids. He thought each patient should decide for himself whether to ask for special arrangements in school.

Kathy felt that the most important factor is growing up with T.S. is having understanding parents. She reported that she still has problems with middle age adults in stores or on the street. They do not tolerate echolalia or coprolalia which is also hard for parents to accept. She advised parents to ignore symptoms and avoid asking if there is any way they can help. Offers of concern or assistance from parents only make it worse, she said. She also spoke about her inability to control her temper and how she seems to get angry over inconsequential things. She advised parents to ignore tantrums because one must have an outlet for anxiety.

Marco said he worked with 40 and 50 year old employees who teased him. He was forced to speak to his boss about them and the boss approached the employees telling them if they persisted in making fun of him they would be fired. Even his parents, who try to understand, cannot cope with his symptoms when he makes loud noises.

One panelist stated that at times his parents would tell him that they understood that he had to say those words but pleaded to "try not to say it so loud." He said it was not possible to modify the symptoms and he advised parents to act as if they didn't notice them.

Mark felt that people with T.S. needed a friend (this might be parents or a doctor) to whom they could express their feelings. Kathy felt that she did not understand why she loses her temper so often, and it's nice to know there is someone nearby to help out. But she questioned how people are to know when she does or doesn't want help.

Dr. Shapiro asked how the panelists dealt with job seeking problems? Pat said she told her first employer, "I jump and twitch but it doesn't interfere with my work." She was employed there for 5 years. Later she did run into employment problems. Large companies required a medical check-up, so she decided to put T.S. on her employment records. It hasn't harmed her chances for a job, she feels, because most doctors don't know what T.S. is. She advises people with T.S. to "play it by ear," tell some employers and not others.

Mark got his first job through a local agency which helps handicapped youth. His first employer knew he had T.S. and what T.S. is. When he was hired for his present job he did not tell his employer about T.S. but, a week later, when his ties got worse, he did tell him. His employer has been very accepting.

Dr. Shapiro asked how T.S. has weakened or strengthened each panelist? Kathy felt she was weakened socially and academically, but it has helped her now that she understands Tourette Syndrome. She feels that she has tremendous insight due to T.S. and is going into the field of social welfare as a result of her experiences. She feels that she has a "sixth sense" and can tell immediately whether a person she meets will accept her or reject her. Her suffering has made her want to help others.

Phyllis feels that she is no longer outgoing. Although she understands and ac-

cepts much more than when she was younger, her social life in college is limited. But T.S. has helped her to decide that she should be a special education teacher because she can accept children for what they are, not for what they could be.

Pat said that as a child she was made to feel as if she was "bad." She also has a "sixth sense" and can judge people's acceptance immediately. She, too, has a limited social life.

Mark believes he's socially "behind" his peers and does not have as many friends, as he would like to. He feels he has more compassion for other people's feelings than the average person.

The audience asked about the panelists dating experience. "Do you tell your date you have T.S. or wait awhil?" Kathy remarked that she dated a lot, but could not bring herself to tell a special boyfriend about T.S. Finally, when she did, he felt this information was trivial and didn't matter. She feels that the person with T.S. should understand and accept himself/herself. If you are not ashamed, she advised, other people will accept it.

Orrin sometimes waits several dates before he brings up the subject of T.S., but most girls he dates know him because they are classmates. He also feels the more the person with T.S. accepts T.S., the more the people around him will accept it.

Another question asked was: "Do both of your parents accept T.S. or does one parent accept it more than the other?" One panelist said while she was growing up her symptoms were considered as bad behavior. Her mother would get angry which in turn made her father angry. When she was diagnosed her mother cried, and she could perceive her parents' anger at physicians as well as their guilt for not having accepted T.S. Today they both accept it and have become active in the Association. Another panelist reported that her father supported her but her mother could not accept it. A third panelist admitted that both parents can't cope with T.S. even though it is now diagnosed.

A question probing the panelists' feelings about parenting, now that there is a suspected genetic component in T.S., brought forth the response that, having experienced the illness, they would expect to be compassionate parents. Whether or not to have children was a difficult decision, not yet resolved.

An audience participant asked if any of the panelists had relatives with T.S. Two panelists reported that their grandmothers had unusual movements and one girl stated that all of her grandmother's sisters were diagnosed as having "St. Vitus dance."

Panelists were asked about the value of psychotherapy in accepting the diagnosis of T.S. Mark felt that it was important to have a good friend who is unbiased. Another panelist felt that it is important to have someone around to talk to and help build up your confidence.

One member of the audience suggested that patients and parents get involved with their regional chapters so that we all have someone to talk to, people who understand because they've had similar experiences.

Dr. Shapiro felt that we could all agree that there is a lot of hope. The enthusiasm and accomplishments of the panelists are very impressive and they deserve our thanks and admiration.

Abbey Meyers

THE PHARMACOTHERAPY OF TOURETTE SYNDROME

Eric D. Caine, M.D.

With the exception of the initial description of Tourette Syndrome, the documentation of haloperidol's effectiveness in treating many T.S. patients may be the most significant contribution thus far to understanding this ill-defined disorder. Clarification of the role of this potent compound has had profound therapeutic and investigative impact.

Despite the effectiveness of haloperidol for many individuals, it can be a difficult medication to use. I will briefly consider my approach to treating Tourette patients, where haloperidol is no doubt the mainstay, and discuss in general terms my philosophy for employing pharmacotherapeutic agents. Ideally, patients with mild cases of Tourette Syndrome, who have made a good adaptation in their lives, can avoid the use of any medications for their symptomatology.

I cannot stress forcefully enough my bias toward the view that medication must be

an integral part of a thoughtfully considered approach to managing the personal difficulties which occur with Tourette Syndrome. The symptoms, which are often viewed as bizarre, provide a seed for the development of additional difficulties, such as low self-esteem, social isolation, self-demeaning behavior, or misdirected anger, which may present complex therapeutic issues. Although suppression of motor and vocal tics may prove effective in reducing these unwanted consequences, 'behavioral fall-out' can take on a 'life of its own.' Although I view these problems from my vantage point as a neuropsychiatrist, I have found them to be most remediable by taking a common sense, 'physicianly' approach, utilizing informal counseling and education rather than structured psychotherapy. Occasionally patients have been referred for more extensive psychological treatment, but this has been necessary rarely.

I begin haloperidol therapy with a bedtime dose of 0.25-0.50 mg. At these low doses, I have not often encountered side effects warranting the use of other medicines such as benztropine (Cogentin), although I discuss the use of additional medications with patients and parents. It has been possible to avoid the use of benztropine in most individuals treated with haloperidol, if the dosage is increased slowly. I raise the dose by 0.25-0.50 mg every 4-7 days; patients and parents must be thoroughly educated about how to judge the severity of symptoms, the effectiveness of medication, and the appearance of side effects. (Early in the course of treatment is a time for frequent telephone calls!)

The maximum dose utilized depends on achieving a " tolerable " suppression of symptoms. Many respond to 2-4 mg daily, 8-10 mg is generally the most I give. "Tolerable" is determined by the nature of the symptoms (for example, coprolalia is usually less tolerable than eye tics) and the ability of an individual to exercise voluntary modulation of his/her sounds and movements. Some youngsters may have relatively few manifestations in school but a great many at home (in my view, *home is a haven* where one doesn't have to hold symptoms in check), thus allowing less overall use of medication.

It is also essential that patients and family understand the ever changing nature of T.S., as medication must be adjusted in many individuals, increasing with symptom upsurge and decreasing during periods of relative remission. It is essential for effective dose adjustment that "indicator symptoms" be present at all times. If an individual is treated with enough haloperidol to suppress all movements, one can never know when tics decrease spontaneously. In the long run, my goal is to use as little medication as possible. (I have been accused of repeating *"less is best"* like a broken record).

Many individuals are aware of the side effects of haloperidol, but I will briefly review them and my approach of dealing with each. The *motor side effects* to haloperidol can be distressing. These motor symptoms can be divided into acute or short-term problems, intermediate difficulties, and long-term abnormalities. The *acute dystonic reaction* is not uncommon in psychiatric patients who use high doses of haloperidol; the powerful muscle contractions which characteriz these involuntary movements are frightening but completely reversible. They cause no lasting ill effects. (Intravenous or intramuscular treatment with benztropine is a commonly available therapy which can be sought at any emergency room.) *Symptoms which resemble Parkinson's disease* (tremor, slowed movements, masked face, increased muscle tone, abnormal gait, drooling, etc.) are caused by haloperidol and other similar compounds. They begin after several days to a few weeks of therapy. They too are treated with "anti-Parkinson" drugs such as benztropine, trihexphenidyl (Artane), etc.; at the low doses of haloperidol utilized by T.S. patients, Parkinsonian symptoms are relatively uncommon. A slowness of movement and a decrease in facial expression may occur, however, requiring either a lowering of haloperidol or the institution of counteractive therapy. Another movement disorder which can develop is *akathisia*, a motor restlessness which may be evident on observation and extremely distressing subjectively. (One patient described it as if, "My legs are going to run away with me.") This can be alleviated with anti-Parkinson agents in some instances, although I have encountered several individuals who found no relief

beyond discontinuing haloperidol . . .*Tardive dyskinesia*, or late abnormal movement, is a concern of many. While I was at NIMH, my colleagues and I observed one adolescent with a full blown disorder. His medication was discontinued initially but later required reinstitution because of psychotic symptoms. Two other youngsters were observe with abnormal movements typical of tardive dyskinesia; these became apparent while they were taking maintenance haloperidol therapy. One had been on a dose of 6 mg per day for 6 months, while the other had been taking 5-7 mg per day for 2 years. In both instances, discontinuation of medication led eventually to a total remission of symptoms. These abnormal movements were distinctly different from Tourette symptoms and they did not wax and wane in the same manner as T.S. tics. As a precaution for detecting tardive dyskinesia, I see patients at regular intervals, usually 3-4 times per year; whenever possible, they reduce their medication or discontinue it completely 4-5 days before coming for an evaluation.

A common unwanted side effect is drowsiness. This can be countered somewhat by taking a once daily dose near bedtime. Coffee, tea, or cocoa in the morning may provide a sufficient counteracting stimulant effect (if it is necessary at all). In line with a "less is best" philosophy. I make every attempt to avoid utilizing other medications for preventing drowsiness. If a bedtime regimen fails, it has proved possible most often to lessen this problem by decreasing the haloperidol dose, or by dividing the dose (in late afternoon and evening), or by using an alternating regimen, with more one day and less another. Occasionally, drowsiness is accompanied by an irritable disposition. For this, a decrease in the dose of haloperidol has proved most effective.

A distinctly different side effect has been reported by Dr. Ronald Polinsky on NIMH and myself. We have treated several individuals (three with a definite disorder and three 'probables') who developed a dose-related depression, described by one as "a shade comming down." This was a most unmistakable syndrome, where individuals developed tearfulness, unaccountable sadness, decreased energy, loss of joy, and other signs of a depressive disorder. Symptoms disappeared after medication was decreased, often with the maintenance of substantial therapeutic effectiveness at a lower dose. Interestingly, there was no evidence of other side effects, including subtle Parkinsonian symptoms such as slowness and masked face, or drowsiness and mental dulling.

Several youngsters have noted a robust increase in appetite with haloperidol treatment, leading to excessive food intake and significant weight gain. It may be necessary to establish a strong dietary regimen for an affected individual, as well as promoting a vigorous daily exercise routine. Its significance cannot be underrated, as there may be numerous aftereffects of gaining 56-60 unwanted pounds.

There are other medications which have been described as effective for treating some T.S. patients (e.g., clonidine, fluphenazine HCl, pimozide). There is increasing support for the idea that T.S. is a heterogeneous syndrome, where the ultimate expression of symptoms may be a "final common pathway" for different disorders in the brain. Clearly, haloperidol seems almost magically effective for some patients and frustratingly ineffective for others. It is quite probable that new medications will be found to more effectively treat some or many T.S. patients.

The ability of a medication to help one individual but not another may reflect differences in drug metabolism. With new technology, it is often possible to measure how much drug is available in a person's blood. There may be differences between individuals in the way they absorb a compound from the gastrointestinal tract or break it down in the liver. Whenever possible, measurement of blood drug levels should be a part of a new investigation. Differences in response may actually reflect differences in the amount of drug available in the brain; this may be indicated by the blood level. Increasingly rigorous standards will be necessary to evaluate the effectiveness of new agents for combating T.S. symptoms. This effort should move forward in a systematic and sophisticated fashion, in combination with studies to discern the fundamental disorders which lead to the expression of T.S.

Clinicians experienced in the treatment

of T.S. are not readily available throughout the country. Less experienced doctors may nonetheless provide valuable therapeutic assistance. The administration of haloperidol is not in itself complex. Rather, it is the overall therapeutic effort which requires most time and attention, evaluating effectiveness and side effects, and blending the use of pharmacotherapy into a thorough program of management, education, and gradual personal adjustment.

Dr. Caine is Assistant Professor of Psychiatry, Neurology, Pharmacology and Toxicology at the University of Rochester Medical Center, Rochester, New York 14642

NINCDS Notes

National MS Survey Reports Preliminary Data

A national survey of the incidence, prevalence, and cost of multiple sclerosis, conducted by the NINCDS Office of Biometry and Field Studies, is nearing completion. Preliminary findings indicate that there were 122, 873 cases of multiple sclerosis in the conterminous United States as of January 1, 1976. This is equivalent to a rate of 57.8 cases per 100.000 population.

These findings are similar to preliminary data reported from the 1978 Health Interview Survey conducted by the National Center for Health Statistics, after these latter results were adjusted for individuals who were unaware that they have multiple sclerosis.

According to the NINCDS study, the estimated average annual incidence of multiple sclerosis for the period 1970-1975 was 8,793 cases, or 4.2 cases per 100,000 population. Both prevalence and incidence rates were higher among females than males and higher among whites than nonwhites. Rates for individuals whose residence was above the 37th parallel were higher than the rates for those residing below the 37th parallel.

A series of articles presenting detailed information on the results of the NINCDS survey will be forthcoming.

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Otitis Media Research Center Funded

NINCDS is stepping up efforts to improve recognition and treatment of otitis media. Latest in a series of actions toward this end is a 5-year grant to the University Health Center of Pittsburgh for an otitis media research center. To be headed by Dr. Charles D. Bluestone, Professor of Otolaryngology at the University of Pittsburgh. the center will conduct basic and clinical research into every aspect of Middle ear infections, the most common childhood illness diagnosed by physicians in the United States.

Studies that center scientists will undertake include comparing the effectiveness of different treatments of otitis media, such as myringotomy and removal of tonsils and adenoids.

Over 50 percent of all children at some time experience middle ear infections, and 20 percent of school age children show evidence of having had significant middle ear disease. Left untreated, middle ear infections can have extremely serious sequellae and impact. Among these are:

- hearing loss
- physical morbidity (ear drum perforation, ossicular destruction or fixation, chronic tympanomastoiditis, cholesteatoma, labyrinthitis, and meningitis)
- language dysfunction
- cognitive dysfunction
- compensatory behavior
- aberrant social interactions
- reduction in educational achievement

Even when diagnosed and controlled before any of these consequences occur, otitis media costs the Nation some $ 1.5 billion in yearly medical bills. The new center is expected to make important contributions toward settling some of the controversies surrounding treatment of otitis media, ultimately reducing its human and economic costs.

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Reye's Syndrome Conference Set For March

"The Diagnosis and Treatment of Reye's Syndrome" will be the subject of an NIH Consensus Development Conference March 2-4, 1981, at NIH.

NINCDS is the lead Institute for the conference; other sponsors are the National In-

stitute of Allergy and Infectious Diseases, the National Institute of Arthritis, Metabolism and Digestive Diseases, the National Institute of Child Health and Human Development, the National Institute of Environmental Health Sciences, and the NIH Division of Research Resources. Collaborating agencies are the Center for Disease Control and the National Center for Health Statistics.

The purpose of the conference is to reach agreement on criteria for diagnosis and treatment of Reye's Syndrome. Key questions to be addressed include:

- What is Reye's syndrome?
- What symptoms should alert parents?
- What other conditions may cause similar symptoms?
- When should parents seek medical help?
- Which tests are helpful in diagnosis?
- What early treatments are useful for non-comatose patients?
- What are the indications for and risks of intensive care.
- What are the indications for monitoring intracranial pressure?
- Is barbiturate coma useful?

For information, contact:

Dr. Joseph S. Drage, Chief
Developmental Neurology Branch, NINCDS
Rm 816 Federal Bldg.
Bethesda, Md. 20205
(301) 496-6701

★ ★ ★

Criteria Set for Spinal Cord Regeneration Experiments

To facilitate research and minimize misleading claims, an NINCDS Advisory Task Force has developed five criteria for evaluating spinal cord regeneration experiments. The Task Force recomends that the criteria must all be fulfilled before an investigator can conclude that functional regeneration of spinal neurites has occurred in an experimental or test situation.

The five criteria are:

- The experimental lesion must cause disconnection of nerve processes;
- Processes of central nervous system neurons must bridge the level of injury;
- The regenerated fibers must make junctional contacts;
- The regenerated fibers must generate postjunctional responses;
- Changes in function must derive from regenerated connections.

The criteria, with commentary, appear in *Experimental Neurology*, 69, 1-3 (1980). Reprints are available from NINCDS.

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Tentative Consensus Reached On Brain Dissection Issues

An NINCDS-NIMH sponsored workshop on human brain dissection was held in Cambridge, England, September 4-6, as part of a continuing effort to develop international standards for the collection, dissection, and chemical analysis of human nervous system specimens. Methods currently used are extremely diverse, complicating comparisons of data between laboratories and impeding attempts to map transmitter systems in the brain.

Scientists reached a tentative consensus in several controversial areas such as the boundaries of the nucleus accumben and use of GABA or tryptophan levels as indicators of the degree of postmortem change. Necessary future tasks were outlined. A formal report of the workshop will be published.

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'Centers Without Walls' Established

The National Institute of Neurological and Communicative Disorders and Stroke has awarded initial grants of $ 1,367,000 to establish two "Centers Without Walls" for research on Huntington's disease and related neurological disorders.

Grant recipients are The Johns Hopkins University School of Medicine and a consortium of medical institutions in Boston, headed by Harvard University Medical School.

Unlike the traditional idea of specialized disease center consolidated under one roof, Centers Without Walls do not emphasize a central location for research. Instead, a

Center Without Walls consists of investigators engaged in basic or clinical research in different departments within a university, or at different universities and medical centers. Patients and their families may be seen in clinical research facilities at any of the institutions comprising the center.

In Boston, a first-year award of $ 782,198 will support investigators in various departments within Massachusetts General Hospital, McLean Hospital, Boston University Tufts New England Medical School, the Boston Veterans Administration Hospital, and the University of Massachusetts.

This Center Without Walls will support 10 research investigations, including severalal projects to map and measure levels of brain neuroendocrines and neuropeptides. Other investigations focus on developing bet ter methods for analyzing brain tissues to detect changes characteristic of degenerative brain disorders.

As part of the Boston center's studies, a team of molecular geneticists at Massachusetts General Hospital will be using recombinant DNA techniques to pinpoint the chromosomal location of the Huntington's disease gene and pursue mapping of the human genome.

Dr. Joseph B. Martin of Massachusetts General Hospital, a Harvard affiliate, is the Boston center's director.

The Johns Hopkins University will receive a first-year award of $ 585,110 for a center to be directed by Dr. Marshal F. Folstein. This center will oversee nine basic and clinical research projects, including efforts to identify and examine all Huntington's disease patients in Maryland and support of research on genetic counseling techniques. Among other projects, investigators will study abnormal eye movements and swallowing difficulties in Huntington's disease patients and will seek more detailed understanding of the role of the basal ganglia in the pathophysiology of Huntington's disease and other motor disorders.

The Johns Hopkins Center Without Walls will involve a number of departments and programs within the university-among them, psychiatry, neurology, genetics, epidemiology, and public health. Patients will enter the center's programs through the J. Earle Moore Medical Genetics Clinic at Johns Hopkins.

Establishment of Centers Without Walls fulfills a major recommendation of the Commision for the Control of Huntington's Disease and Its Consequences. The commission reported its findings to Congress and the President in October 1977.

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Director Named for Stroke and Trauma Program

Dr. Michael D. Walker has been named director of the Stroke and Trauma Program, National Institute of Neurological and Communicative Disorders and Stroke. In this position he will oversee a $ 41 million program of extramural grants and contracts supporting research on stroke, cerebrovascular disorders, brain tumors, brain and spinal cord trauma, and regeneration.

Dr. Walker has served as acting director of the Stroke and Trauma Program since 1979. He came to NINCDS from the National Cancer Institute, where he had been an associate director of the Division of Cancer Treatment since 1973. Prior to that, at NCI's Baltimore Cancer Research Center, he established the Section of Neurological Surgery and subsequently served as the center's deputy chief, acting chief, then chief and director.

Dr. Walker received his B.A. degree in psychology from Yale University in 1956 and his M.D. degree from Boston University School of Medicine in 1960. After completing an internship at Massachusetts Memorial Hospital, he took a neurosurgical residency at Boston City Hospital, which included a year's fellowship in neurosurgery at the Lahey Clinic. He also served as a clinical instructor in neurosurgery at Harvard Medical School.

The author of more than a hundred scientific publications, Dr. Walker has had a long-standing interest in the diagnosis and treatment of malignant brain tumors. He has carried out research on the blood-brain barrier, the pharmacodynamics of drug delivery to the brain, and the design and analysis of controlled clinical trials, and has served as chairman of the National Cancer Institute's Brain Tumor Study Croup for the past 10 years.

Dr. Walker holds memberships in a num-

ber of professional societies, among them the Congress of Neurological Surgeons, the American Association for cancer Research, the American Academy of Neurology, the Research Society of Neurological Surgeons, the American Society of Clinical Oncology, and the Electron Microscopic Society of America. He is a fellow of the Royal Society of Health.

Dr. Walker holds an appointment as assistant professor of neurological surgery at The Johns Hopkins University School of Medicine and has served on numerous advisory boards and committees.

In 1974 he received the Superior Service Honor Award of the U.S. Department of Health, Education and Welfare.

★ ★ ★

Huntington's Disease Focus in Venezuela Under Study

Under a new contract between NINCDS and the University of Zulia, Venezuela, studies have begun on a focus of Huntington's disease patients and their relatives living in several small fishing villages along the shore of Lake Maracaibo. The population under research scrutiny is thought to be one of the largest concentrations of Huntington's disease families in the world.

The Lake Maracaibo HD focus holds unique research potential. The affected families trace their ancestry to a single individual, which greatly enhances the prospects for informative genetic linkage studies. Moreover, the HD patients, because of social ostracism and proximity to other affected individuals, sometimes marry each other and have children, some of whom can be expected to be homozygous for the Huntington's disease gene. If such homozygotes have survived, they may be ideal subjects to reveal the exact manner in which the genetic defect manifests itself.

The new collaborative research project has four major objects:

(1) Ascertainment of the Huntington's disease population and identification of potential homozygotes as well as families in which two and three generations of choreics are living.

(2) Intensive study of families identified as most potentially informative subjects for research.

(3) Collection of tissue samples for use by Venezuelan and U.S. investigators and donation to tissue banks.

(4) Pursuit of genetic linkage studies.

The contract for the collaborative project was signed in Venezuela in July by Dr. F. J. Brinley, director of the NINCDS Neurological Disorders Program, and Dr. Humberto J. La Roche, rector of the University of Zulia. Launching of the project fulfills a major recommendation of the Commission for the Control of Huntington's Disease and Its Consequences, which reported its findings to Congress and the President in October 1977.

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Urban Epilepsy Program Launched

The National Institute of Neurological and Communicative Disorders and Stroke has awarded a three-year contract, totaling nearly $ 4 million, for establishment of a comprehensive epilepsy program serving a large urban area with a high proportion of minorities among the population.

The new program will be carried out by the University of California at Los Angeles for Los Angeles Country, which includes the Watts-Compton and Torrance areas.

The UCLA investigators will undertake a number of advanced clinical and laboratory research projects on diagnosis, prognosis, prevention, and treatment of epilepsy. They will develop accurate epidemiological and vital statistics data on epilepsy in the Los Angeles Country area, and will assure that medical, social, educational, and rehabilitative services for patients are integrated into the program.

A Human Neurospecimen Bank for brain tissue from epilepsy patients will be developed under the program. Other important projects include demonstration of the Latest research and treatment advances to physicians and other health professionals and development of a broad program of public education about epilepsy.

Principal investigator for the UCLA program is Dr. A. V. Delgado-Escueta. Coinvestigators are Drs. Richard D. Walter, Paul Crandall, and Jerome Engel, Jr.

Other medical institutions in the Los An-

geles County area that will be involved in the UCLA program include the University of Southern California School of Medicine, the Harbor General Hospital in Torrance, and the Charles Drew-Martin Luther King, Jr., Medical Center.

In addition to its support of the new program for Los Angeles County, NINCDS has for several years funded comprehensive epilepsy research programs at the University of Minnesota, Minneapolis; the Good Samaritan Hospital, Portland, Oregon; the University of Virginia, Charlottesville; the University of Washington School of Medicine, Seattle; and the Medical College of Georgia, Augusta. These programs bring together physicians and patients, conduct clinical and epidemiological research, and offer intensive diagnostic treatment and rehabilitative help to patients in the research setting.

This information is prepared monthly by the Office of Scientific and Health Reports, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bldg. 31, Rm 8A06, Bethesda, MD 20205; tel: (301) 496-5751.

NATIONAL SOCIETY FOR AUTISTIC CHILDREN

SUITE 1017

1234 MASSACHUSETTS AVENUE, N.W.

WASHINGTON, D.C. 20005 (202) 783-0125

Dedicated to the education and welfare of children and adults with severe disorders of communication and behavior

Call For Papers

"Hope Through Research and Education," an International Symposium on Autism and Related Disorders of Communication and Behavior, will be held on July 14 and 15, 1981, at the Park Plaza Hotel, Boston, Massachusetts. The symposium is a part of the 13th Annual Meeting and Conference of the National Society for Autistic Children, U.S.A.

EMPHASIS:

Day One: Recent findings in applied (behavior/social/educational) research.

Day Two: Recent findings in basic (medical/physiological) research.

CRITERIA:

Submissions may include individual papers, coordinated sets of papers or complete symposia. Each submission must include an abstract of not more than 400 words, typed, double spaced; a cover letter which includes the name and affiliations of the principal investigator, the names and affiliations of co-investigators, and full addresses of each potential presentor.

Findings must be directly applicable to children and adults with autism and related disorders of communication and behavior, and must not have been published previously in any scientific journal.

DEADLINE:

December 1, 1980

SUBMIT ABSTRACTS TO:

International Symposium on Autism
National Society for Autistic Children
1234 Massachusetts Avenue, N.W.
Suite 1017
Washington, D.C. 20007
Attn: Frank Warren
Phone: (202) 783-0125

PROCEEDINGS:

Manuscripts of not more than 30 pages, typed, double spaced, will be required of those accepted by the scientific panel. A monograph of the proceedings will be published for distribution within the scientific community and among interested lay people.

MIGRAINE SYMPOSIUM

AT

INTERNATIONAL CONGRESS OF NEUROLOGY

KYOTO, JAPAN

22nd September, 1981

Abstracts (less than 100 words) not later than 31st March to

Dr. F. Clifford Rose
Princess Margaret Migraine Clinic
Charing Cross Hospital
London W6 8RF

12th WORLD CONGRESS OF NEUROLOGY

The Japanese Society of Neurology takes peasure in announcing the 12th Congress of the World Federation of Neurology, 1981, in the scenic and historical city of Japan. Kyoto.

The National Organizing Committe welcomes al of you to Kyoto.

General Information

Dates:

September 20 (Sun.) - 25 (Fri.), 1981

Congress Site:

Kyoto International Conference Hall
Takara-ike, Sakyo-ku
Kyoto 606, Japan
Telephone: (07') 791-3111

Scientific Program

A. Main Themes

The fou main themes below were selected at a cerent meeting of National Deegates of the World Federation of Neurology.

1. Cerebral vascular diseases
   Chairman:
   Prof. Masakuni Kameyama
   Department of Geriatrics and Neurology
   Kyoto University, School of Medicine,
   Kyoto, Japan
2. Hemispheric specialization in man
   Chairman:
   Prof. François Lhermitte
   Clinique de Neurologie et de Neuropsychologie,
   Hôpital de la Salpêtrière
   Paris, France
3. Neurotransmitter and neuropeptide dysfunction in relation to neurological disease
   Chairman:
   Prof. André Barbeau
   Department of Neurobiology
   Clinical Research Institute of Montreal
   Montreal, Canada
4. Viral infections of the nervous system
   Chairman:
   Prof. Richard T. Johnson
   Department of Neurology
   Johns Hopkins University
   School of Medicine
   Baltimore, U.S.A.

The chairmen of each main theme will seect and invite internationaly acknowledged experts in these fields to present a mosaic of pertinent contributions. These will be published in the Congress Proceedings.

B. Free Communications

The Organizing Committee welcomes your submission of *Free Paper* abstracts.

You wil be notified of the method of preparation and submission in the next announcement. A *Poster session* will be organized concurrently with patform presentations.

Languages

The official languages of the Congress wil be English, French, German, and Spanish. Simulaanenus interpretation in these languages will be provided during the main

Exhibitions

During the Congress, the folowing exhibitions wil be held.

— A scientific exhibition
— A technical exhibition of medical equipment, books, and pharmaceuticals.

You wil be notified of the details concerning the preparation and submission of exhibits in the next announcement.

Social Program

There will be an informal welcome gatherin, a Ladies' Program, a Theater Event (a visit to see the *Miyako Odori* or *Kabuki*), and a Banquet.

Preliminary Registration

If you are interested in attending this Congress, please complete and return the enclosed postcard to the Secretariat *by June* 30, 1980. The next annoncement, together with the official registration form will be mailed in June, 1980 to anyone who returns the provisional registration form.

Travel Arrangements

Japan Travel Bureau (JTB) has been appointed the official travel agency. Arrangements are being made for special all inclusive tours to the Congress. Details will

be announced in the next announcement.

We are planning to provide the Congress participants with reduced air fares and hotel accommodations at a reasonable cost.

Transportation

Japan Air Lines (JAL) has been appointed the official carrier of the Congress. For information concerning your air-transportation to Kyoto, please contact your nearest JAL office.

Meetings During the Congress

The folowing satellite symposia of the WFN Research Committees will be accommodated during the Congress.

Extrapyramidad diseases
Migraine and headache
Neuropharmacology
Cerebrospinal fluid
Neurotoxicology
Neuroepidemiology
Multiple Sclerosis
FULTON SOCIETY:
Developmental Neurobiology

Related Information

- Tenth International Congress of Electroencephalography and Clinical Neurophysiology.
  Kyoto, Japan
  12-18 September, 1981
- Epilepsy International Congress 1981.
  Kyoto, Japan
  17-21 September, 1981

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Prof. H. NARABAYASHI

Kyoto Officer:
Prof. M. KAMEYAMA

The symbol of The 12th World Congress of Neurology is a design made out of the Japanese term "(*shin-kei*: nerve)". *The* term *shin-kei* was first introduced in 1774 by Genpaku Sugita (1733-1817), a pioneer of modern medicine in Japan, when he joined togeher tre first letters of (*shin-ki*: spirit) and (*kei-myaku*: paths).

VI International Symposium on Posturography, Kyoto.
16 - 20 September 1981.

• Book Reviews

MEDICAL NEUROLOGY

Prof. John Gilroy-Prof. John Stirling Meyer-Third Edition-Macmillan Publishing Co. Inc. New York-1979, vol. 787 pages.

A beauaiful, very well impressed book that brings up to date the stuly of Neurology.

We will reproduce part of the preface of the authors in which is very well expressed the purpose of this new edition of the book of to describe up to date all the advances in neurological sciences which are remarkable for developing the knowledge of the multiple topics that integrate the normal and pathological conditions of the Nervouss System.

However such rapid increase in knowledge and publications places considerable demands on the authors of a textbook of clinical neurologs in an effort to maintain a practical and equitable balance of the contents. This has been the prime consideration in the preparaation o the third edition of Medical Neurology which remains a pragmatic textbook for the clinical practice of neurology, placing particular emphasis in newer diagnostic and therapeutic developments in the specialty.

The neurologic history and clinical examination still remain the keystone to the practice of neurology.

Many recent changes in pediatric neurology are reflectad in the addition of much new material. The section on mental retardation has been expanded. The developmental defects and metabolic disorders have been extensively revised in light of the many advances made in these areas in the last few years.

There have alzo been mayor advances in the understanding of the childhood encephalopathies.

The demyelinating diseases have been reclassified to correlate the hereditary leukodystrophies and lipidoses with underlying biochemical abnormalities. Newer techniques of diagnosis in the demyelinating diseases are now available and the role of computed tomography and evoked potentials is illustrated.

The sugestion that a multidisciplinary approach to diagnosis and treatment of the dementía is desirable.

New and improved concepts of disorders such as hepatic coma, hepatic encephalopathy, uremic encephalopathy and dialysis encephalopaathy and their treatment have now been defined, improvement in the understanding of the mechanisms involved in the varions types of headache and migraine has produced a more rational therapeutic approach to these diorders.

The treatment of sezures has been revised, imcorporating a more rational approach to therapy that utilises determinations of serum levels of anticonvulsant druge to ensure adequate dosage.

Chapter 7 has been revised to conform to modern practice in the treatment of bacterial infection and its complications including brain abscess, edema and bacterial meningitis. Advance in the diagnosis and treatment of the viral encephalitides have also been added in this chapter.

The recognition of diffuse brain damage offers a logical and useful concept of brain injury followinghead trauma. New ideas and newer techniques including computed tomography, have radically altered the diagnosapproach to the head injured patient and are described in logical order.

The problem of cerebro vascular disease has been revised with emphasis on the profilaxis of cerebro vascular insufficiency. Much can be done for the patient with transient ischemic attacks by treating those conditions that contribute to acelerated atherosclerosis.

There is an established place for surgical treatment in cerebro vascular insuficiency and both conventional and newer innovations in surgical techniques have been induced in the chapter.

The advent of computed tomography has resulted in a profund change in the early diagnosis of brain tumours.

A number of additions have been made to reflect better understanding of entrapment neuropthies. The classification of some of the familial neuropathies adds to the understanding of these conditions.

Chapter 12 has been extensively rewritten to cover the many advances in the diagnosis and treatment of muscle diseases.

The new emphasis on modern techni-

ques, coupled with continued emphasis on traditional neurologique practice on care in history taking and precision in examination, has been stressed throughout this edition, reflecting a modern approach to neurology.

This is a book that in a clear and concise way expound all the topies that approaches, up dating them in a clear and precise way providing the readers all the extraordinary contributions done in the last years to the knowbedge of the nervous System in its normal and pathological aspects.

It is a very useful book for the neurologists and for all those interested in the comprehension of the ways and doing of the nervous System.

Prof. Dr. VICTOR SORIANO

CURRENT NEUROLOGY

Editions H. Richard Tyler M. D., David M. Dawson, M. D. - Houghton Mifflin Professional Publishers - Medical Division - Boston.

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The Editors outstandings Professors of Neurology from Harvard Medical School, have reunited in this book the contribution of a brilliant group of personalities each one of them an authority in the different topics integrated in this Neurology. The fast pace of progress in science is successfully covered by the authors which offer to the readers the latest news in each subject with a deeper knowledge gathered in novel investigations. These books are in themselves "A continuing education" and the specialists may easely follow through them the advancement of science.

This means a better knowledge that may be applied in understanding the Patient's problem, in giving a lecture, or further future investigations.

Summarizing, this a collection of books that maintain up to the Specialist knowled. ge in varioous aspects of medical Neurology.

Prof. Dr. Víctor Soriano