The equilibrium blood sugar level of the hypophysectomized-pancreatectomized animals is slightly more variable than that of normal animals. In a group of 20 depancreatized animals the blood sugars of which were taken at 24 to 42 days after hypophysectomy the mean level was 97.87 with a coefficient of variation of about 9% and extreme values of 84 and 117 mg. % respectively.

In attempting hypophysectomies to be followed by pancreatectomies we noted that a number of the hypophysectomized rats died within a few days and the rest within a few weeks. These deaths proved to be due to hypoglycemia, for in a group of hypophysectomized animals observed at 8 days after hypophysectomy and raised upon our standard diet the mean blood sugar was 73.02 mg. % whereas in a control group operated on at the same time but given additional sugar in the diet the mean value was 102.18 mg. %.

Summary: A constant hyperglycemic blood sugar level of about 215 mg. % is attained in about 2 weeks after removal of the tail, body, and most of the head portion of the pancreas of month-old rats. Hypophysectomy restores the blood sugar level to about 98 mg. % which is in the normal range (102 mg. %). Hypophysectomy without pancreatectomy results in hypoglycemia unless sugar additional to that used in the diet of normal rats is added.

8645 C

Bacteriologic Studies in Myasthenia Gravis.

EDWARD C. ROSENOW AND FORDYCE R. HEILMAN.

From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.

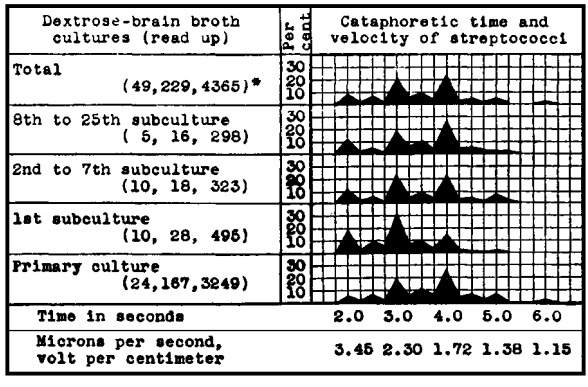
Boothby and his associates[1] have reported on their metabolic studies in cases of myasthenia gravis and on the beneficial effect of treatment with glycine and ephedrine. They have called attention to the fact that not infrequently the disease begins, or is made worse, following upper respiratory infections. We have coöperated in the study of this peculiar syndrome and have carried out bacteriologic studies in 49 cases by methods similar to those previously described.[3, 4] A streptococcus which was very similar in

[1] Boothby, W. M., *J. Am. Med. Assn.*, 1934, **102**, 259.

[3] Rosenow, E. C., *Internat. Clin.*, 1930, **2**, 29.

[4] Rosenow, E. C., and Jensen, L. B., *J. Infect. Dis.*, 1933, **52**, 167.

the different cases has been consistently isolated from the nasopharynx and other foci of infection, and sometimes from the muscles and urine of patients with myasthenia gravis. This streptococcus, although resembling in its morphologic and cultural characteristics green-producing streptococci isolated from similar sources in cases of diseases other than those affecting muscles and nerves, differed markedly from these green-producing streptococci in its cataphoretic velocity (Fig. 1) and in its specific virulence.



*The figures in parenthesis indicate respectively, the number of strains, cultures, and streptococci timed in each group studied.

FIG. 1.
Cataphoretic time and velocity of the streptococcus of myasthenia gravis.

The primary blood-agar plate-cultures of material from the nasopharynx and other foci of infection were not characteristic, but the number of colonies of green-producing streptococci was usually far greater than in like cultures from well persons. This was true before, as well as after, weakness of muscles of the pharynx became evident. The distribution of cataphoretic velocities of the streptococci, as isolated in dextrose-brain broth, was of the "neuromyotropic" type (Fig. 1), in practically all cases and varied but little according to season.

We have isolated the streptococcus in pure culture from excised pieces of muscle in 3 acute cases of myasthenia gravis during the patients' lives, and from 3 pieces of muscle excised 1½ hours after the patient's death in a fourth case. We have also demonstrated diplococci[2] within or adjacent to lesions in the muscles in each of 5 severe cases of myasthenia gravis (Fig. 2). Cultures and sections of muscles in a series of controls proved negative.

[2] Butt, H. R., *Arch. Path.*, 1936, **21**, 27.

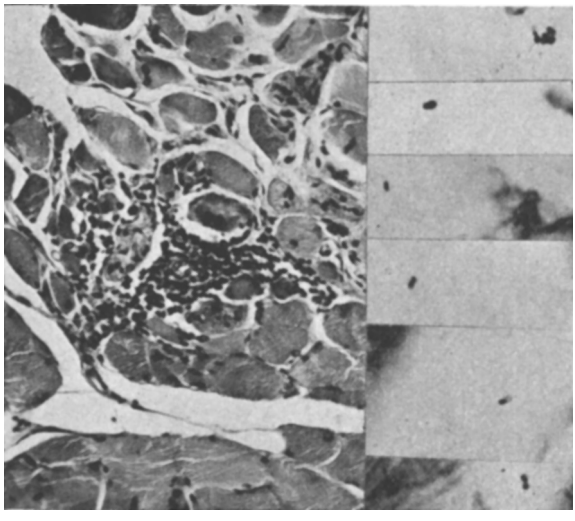


FIG. 2.
Section of muscle from a patient who had myasthenia gravis. A circumscribed area of lymphocytic collections (lymphorrhagia) is evident (hematoxylin and eosin stain, x 175) and diplococci can be seen in, or adjacent to, the lesions (Gram-Rosenow stain, x 1000).

The most striking characteristics of the streptococcus on isolation in dextrose-brain broth were its marked tendency, following intravenous and intracerebral injection, to localize and to produce lesions in muscles, often involving nerve fibrils, to grow slowly, and to survive for a long time in muscular tissue, causing soon or long after injection progressive weakness, undue fatigue on exertion, and characteristic microscopic lesions in the muscles. The streptococcus was isolated from and demonstrated in the lesions of muscles in monkeys (Fig. 3) and rabbits (Fig. 4) when the blood and other tissues were free from streptococci as long as 4 months after intravenous injection, but this was true only in animals that showed symptoms resembling myasthenia gravis. The heat-killed streptococcus, filtrates of cultures, and solutions of the partially-purified specific carbohydrate of the streptococcus, just as the living culture, electively produced characteristic lesions in muscles following intravenous and intracerebral injection; the weakness and undue fatigue on exertion, however, were transient. The resistance of animals decreased following repeated intravenous and intracerebral injection of the living streptococcus but increased following repeated injections of the heat-killed streptococcus and of filtrates of cultures and filtrates of glycerin-salt solution extracts of the streptococcus. Patients with myasthenia gravis were found to be ex-

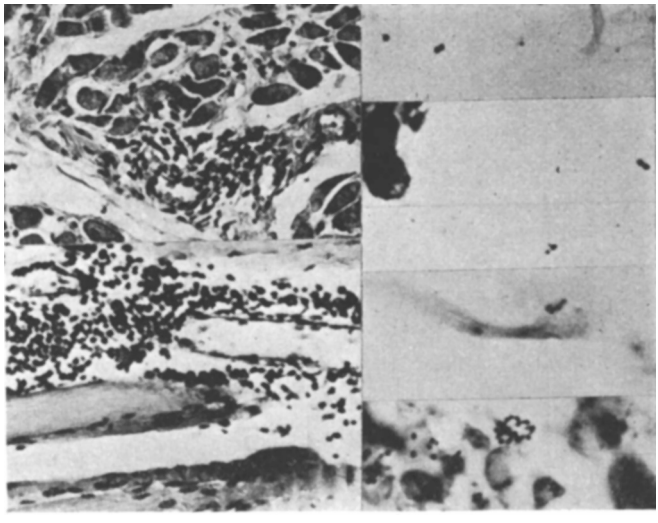


FIG. 3.

Cross and longitudinal sections of muscles of the monkey referred to in the protocol. Areas of lymphocytic collections (lymphorrhagia) are evident (hematoxylin and eosin stain, x 175), and diplococci can be seen in lesions in skeletal muscles and myocardium (Gram-Rosenow stain, x 1000).

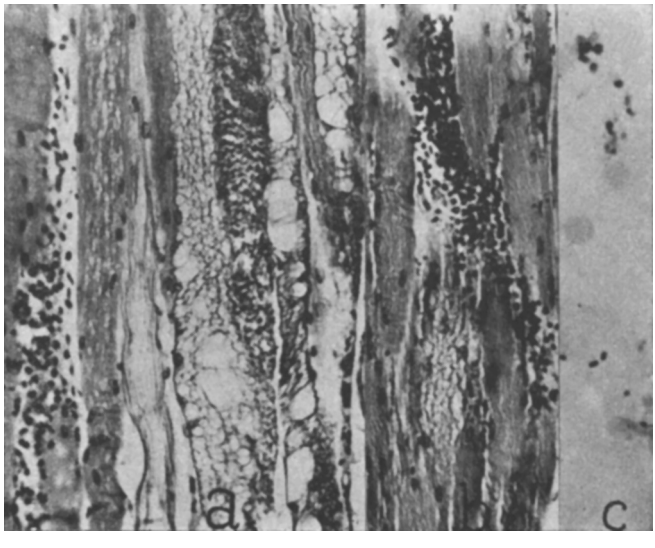


FIG 4.

Granular, hyaloid, and fatty degeneration and lymphocytic collections in and between muscle fibers (*a. b.* hematoxylin and eosin stain, x 175), and diplococci in the lesions in muscles of the rabbit referred to in the protocol (*c*, gram stain, x 1000).

tremely sensitive to therapeutic subcutaneous injections of the heat-killed streptococcus and filtrates. The manifestations following injection of the heat-killed streptococcus and of filtrates of cultures occurred promptly and were attributed to a specific toxin or poison of the streptococcus and not to "virus."

The living streptococcus on isolation produced symptoms of myasthenia gravis and characteristic lesions in the muscles of monkeys and rabbits, after many (25) rapidly repeated subcultures and after from one to 4 passages through animals. Affinity of the streptococcus for muscles was greatest in the strains isolated from excised pieces of muscle, and this affinity increased on passage of such strains through rabbits if the streptococcus was isolated consecutively from the muscles of positive animals. Specific localizing power and other distinctive properties of the streptococcus usually disappeared promptly on cultivation in ordinary mediums.

Control animals injected intravenously and intracerebrally with streptococci from diseases not involving muscles never developed symptoms or lesions in muscles such as those following like injection of the streptococcus of myasthenia gravis.

The results obtained following intravenous injection of the living streptococcus of myasthenia gravis into monkeys and rabbits are well illustrated in the following protocols:

1. A *Macacus rhesus* monkey was given intravenous injections on 3 occasions, 3 days apart, of 40 cc. of the dextrose-brain-broth culture of a strain, after one passage through a rabbit and 14 subcultures in dextrose-brain broth, from the muscle of a patient. Aside from moderate weakness, from which the animal recovered soon after these injections, it remained apparently well for 3 months. Rapidly progressing weakness and undue fatigue on exertion then developed. Four months after injection, when the animal was anesthetized, it was entirely unable to stand or to chew or swallow its food, even banana, on account of profound weakness. Diarrhea and generalized edema of the subcutaneous tissues had developed. The muscles of the jaw had become so weak that the lower jaw could easily be pressed open with one's fingers. Consciousness was normal and the animal tried repeatedly to move about when prodded. Aside from marked atrophy of the muscles and subcutaneous edema no lesions were found at necropsy. Cultures from the brain and blood remained sterile. Dextrose-brain-broth cultures of pieces of muscle excised under sterile precautions yielded green-producing streptococci which had the characteristic cataphoretic velocities and which produced progressive weakness

and characteristic lesions in the muscles of rabbits following intravenous injection. Sections of muscles revealed typical mild, diffuse, and dense, sharply-circumscribed areas of lymphocytic accumulations (lymphorrhagia) and few diplococci (Fig. 3).

2. A white rabbit was given an intravenous injection August 30, 1934, of 12 cc. of the primary dextrose-brain-broth culture of the streptococcus from the tonsil of a patient who had myasthenia gravis. The animal seemed well 3 days later, September 2, when the injection was repeated. On September 3 it appeared well but, on slight exercise, the front legs spread outward owing to weakness and fatigue. September 4 it was weaker and more easily exhausted. On September 5 it again appeared well but after only moderate exertion it became completely exhausted. September 7 it appeared well when sitting in its cage but again became easily exhausted on exertion. It was given 2 cc. intravenously, and 2 cc. subcutaneously, of the same strain as before, but after this strain had been passed through 2 rabbits. On September 11 the animal was still easily exhausted. Eight cubic centimeters of the dextrose-brain-broth culture were injected intravenously. On September 19 the animal was weaker, became very easily fatigued, and lay with its legs outstretched after slight exertion. September 22 it was very weak; slight exercise exhausted it completely and it lay on its back unconcerned when placed there. By October 17, the animal had become extremely weak and almost powerless in its hind extremities. On November 1 it was almost powerless in its hind extremities but could move them. On November 3 there was almost complete absence of strength in the hind extremities and there was marked weakness of the fore extremities and of the muscles of the neck; it could not hold its head erect. It attempted repeatedly to get up on its legs but was entirely unable to do so on account of marked weakness. It was therefore etherized. On examination there was marked generalized atrophy of muscles, in some of which soft, edematous, whitish, friable areas were found. Cultures from the blood, brain, fluid from knee-joints, and urine remained sterile; those from the muscles revealed a pure culture of the streptococcus. Sections of muscles revealed marked hyaloid, fatty, and granular degeneration of muscular fibers associated with edema and lymphocytic collections and few diplococci (Fig. 4).

Summary. By the methods which have been successfully used in a study of the etiology of other diseases a streptococcus of low general virulence, having characteristic cataphoretic velocity, has been found constantly associated with cases of myasthenia gravis.

The living and heat-killed streptococcus and its toxins have specific affinity for muscles. The progressive weakness, fatigability, and the pathologic lesions of myasthenia gravis have been reproduced in monkeys and rabbits.

8646 C

Sulfur Partitions in Cat Urines Following Injections of Monobromobenzene, Cystine or Methionine.*

ROBERT W. VIRTUE. (Introduced by H. H. Beard.)

From the Department of Biochemistry, Louisiana State University Medical Center, New Orleans.

Monohalobenzenes have been found to be conjugated with cysteine and excreted as mercapturic acids in the urine of the dog, rabbit, rat[1] and mouse.[2] The work herein reported was undertaken to see whether the cat could also effect such a synthesis. It also became of interest to carry out distributions of urinary sulfur after administration of cystine or methionine.

Cats were kept in metabolism cages, and urines collected at 24-hour intervals by gentle abdominal pressure. In the analysis, sulfur determinations were run by Folin's method, nitrogen by Kjeldahl, and disulfide compounds according to Virtue and Lewis.[3] The cats were put on their dietary regime for 7 days before collection of urine was begun, and urines were analyzed 4 days before injections were made. To insure that a constant supply of the material whose metabolism was to be studied would be present in the body, all injections were subcutaneous, and were given as follows: half the amount 24 hours before the end of the metabolism period, one-fourth 6 hours before the end, and the remaining fourth 3 hours later. The metabolism period was limited to 24 hours because the animals were sacrificed at the end of that time to obtain information for other work. Thirty-three animals were used in the series. Typical results are shown in Table I.

As will be seen in the figures for cats 29 and 37, the organic

*Part of these data were presented before the American Society of Biological Chemistry at Washington, D. C., March 25-28, 1936.

1 Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037.

2 Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 115.

3 Virtue, R. W., and Lewis, H. B., *J. Biol. Chem.*, 1934, **104**, 415.