(demonstrated to be unlikely by Milhorat[3]); (b) defects in the ability of muscle to utilize acetylcholine (disproven by Lanari[4] and Harvey and collaborators[5,6]); and (c) defects in the synthesis of acetylcholine.

The most plausible hypothesis is that there exists a fundamental defect in the synthesis of acetylcholine in patients with myasthenia gravis. Dr. Otto Loewi suggested that such synthesis be investigated. We are immeasurably indebted to Dr. Loewi for his enthusiastic interest in the development of the problem and for his valuable advice on technique.

Method: The acetylcholine synthesis of mixtures containing standard amounts of frog brain and serum from control subjects was compared with the acetylcholine synthesis of mixtures of frog brain and of serum of patients with myasthenia gravis. Using a modified method of Quastel, Tennenbaum and Wheatley,[7] the mixtures were incubated for a standard period at a standard temperature and the acetylcholine content ascertained by means of the rectus abdominis muscle of frog (Riesser,[8] Chang and Gaddum[9]). The amount of acetylcholine synthesized during the incubation was calculated by subtracting from the content of each incubated sample the acetylcholine content of identical non-incubated samples.

Results: Mixtures containing serum and frog brain synthesized more acetylcholine than mixtures containing Ringer's solution and frog brain. Freshly prepared frog brain-serum mixtures contained 0.38 ϒ acetylcholine per 100 mg of tissue. As a result of incubation 1.45 ϒ acetylcholine was produced. In contrast the acetylcholine synthesized from the mixtures of frog brain and serum obtained from patients with advanced myasthenia gravis was approximately one third as much (0.53 ϒ). Six patients with myasthenia gravis have been compared with forty-eight control subjects.

At least some of the agents which modify acetylcholine synthesis are dialyzable.

Comment: The decrease of acetylcholine synthesis is apparently specific for myasthenia gravis, since it does not occur with other diseases presenting debility, cachexia, immobility and prostration. Also the magnitude of defect in acetylcholine synthesis is related to the severity of the myasthenia gravis.

Conclusion: The defect in acetylcholine synthesis in patients with myasthenia gravis probably explains the fatiguability and weakness of these patients.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CORTICOTROPIN OBTAINED BY ULTRAFILTRATION OF PITUITARY EXTRACTS[1]

IT is generally believed that hypophyseal corticotropin is protein in nature, as are the other hormones of the anterior pituitary, and consequently non-dialyzable. Purification has been directed towards the isolation of a protein, and dialysis has been used as a means for removing impurities of smaller molecular size. However, the stability of certain of the anterior pituitary hormones when exposed to drastic chemical procedures suggests that they may be of small molecular size.

In recent experiments it was found that dialysates of hog pituitary extracts contained a factor which increased the size of the adrenals in hypophysectomized rats. The observation was first made with a dialysate of a glacial acetic acid extract[2] of acetone-dried hog pituitary powder. The material had dialyzed for a period of two weeks against an equal volume of water, and the dialysate was used directly for injection into immature hypophysectomized male rats. In subsequent experiments extracts were ultrafiltered through Cellophane membranes (Visking sausage casing) under the pressure of a water column of approximately six feet. The ultrafiltrates were perfectly clear and almost colorless. The active material was precipitated by adding solid sodium chloride to the ultrafiltrate at a pH 4.0 until a molarity of 4.5 was reached. The subsequent addition of ammonium sulfate to a molarity of 1.5 brought about further precipitation of active material from the supernatants of the ultrafiltrates previously nearly saturated with sodium chloride. The salt precipitates were dried with ether. Another effective procedure for obtaining the activity from the original ultrafiltrates was found in the freezing-drying technique.

The amount of active substance obtained was determined by assay[2] in hypophysectomized rats. The relation of dose to response followed the parabolic assay curve which was based on the activity of hog whole pituitary powder. As the ultrafiltrates showed a

[3] *Jour. Clin. Invest.*, 5: 649, 1938.
[4] *Rev. Soc. Arg. Biol.*, 13: 239, 1937.
[5] Harvey and Lilienthal, *Bull. Johns Hopkins Hosp.*, 69: 566, 1941.
[6] Harvey, Lilienthal and Talbot, *Jour. Clin. Invest.*, 21: 579, 1942.
[7] *Biochem. Jour.*, 30: 1668, 1937.
[8] *Arch. für exper. Path. u. Pharmacol.*, 91: 342, 1921.
[9] *Jour. Physiol.*, 79: 255, 1933.

[1] Under the auspices of the Committee on Pharmacotherapy, Harvard University. Supported by the National Research Council.

[2] Details of the extraction procedure and assay method will be published elsewhere.

biuret reaction, the intensity of which closely paralleled its hormonal activity, the amount of active substance was expressed in equivalents of protein as determined by photoelectric colorimetry. The amount of protein equivalents relative to the solids obtained by the above procedures and the activity in units, as defined, are given in Table 1. The yield of corticotropin obtained by ultrafiltration of a glacial acetic acid extract for six days, with change of the membrane every 24 hours, is expressed in Table 2 in percentage of the activity of the extract. Nearly half of the solids of the extract passed through the membrane and contained 38 per cent. of the total activity of the extract. The part remaining within the dialysis bag contained 28 per cent. of total activity. The powders obtained from the ultrafiltrates showed a better solubility in water than the original material or the residue after ultrafiltration. The solutions also contained less color. The biuret, Hopkins-Cole, Millon and Sakaguchi reactions were positive. A 0.1 per cent. solution of the material showed immediate precipitation upon addition of phosphotungstic or phosphomolybdic acid, but not with trichloracetic or picric acid. The activity was not destroyed by boiling the solution for ten minutes. The ultrafiltrates of glacial acetic acid extracts were free from gonadotropic and thyrotropic hormones.

TABLE 1

CORTICOTROPIN OBTAINED BY SALTING OUT OR BY FREEZING AND DRYING ULTRAFILTRATES OF EXTRACTS OF HOG PITUITARY

Number of animals	Material obtained from ultrafiltrates	Mgm. protein* equivalents per 1 gm of solids	Units† per 1 gm of protein equivalents	Units† per 1 gm of solids
8	(a) 4.5 M NaCl precipitate	484	120	58
5	(b) 1.5 M $(NH_4)_2SO_4$ precipitate from supernatant of (a)	255	102	26
9	(c) Total solids obtained by freezing and drying	286	125	36

* Amount equivalent to protein as measured photoelectrically by the biuret reaction.

† 1 unit is equivalent to the activity of 12 mgm of acetone-dried hog pituitary powder which elicits a 100 per cent. increase of the adrenal weight in the hypophysectomized rat over a five-day injection period.[2]

TABLE 2

YIELD OF CORTICOTROPIN OBTAINED BY FREEZING AND DRYING AFTER ULTRAFILTRATION FOR 6 DAYS OF A GLACIAL ACETIC ACID EXTRACT OF ACETONE-DRIED HOG PITUITARY POWDER. CHANGE OF THE MEMBRANE EVERY 24 HOURS

	Solids gm	Activity units	Percentage of total activity
Glacial acetic acid extract; 2 M NaCl precipitate	4.00*	205	100
Material obtained by ultrafiltration; frozen and dried	2.18	79	38
Residue after ultrafiltration; 2 M NaCl precipitate	2.69	58	28

* As calculated from the weight of the precipitate of one ninth of the extract.

The fact that a corticotropic substance passes through a Cellophane membrane indicates the probability that its molecular size is smaller than has been assumed. In order to determine whether the extraction with glacial acetic acid resulted in the splitting of an active group from a larger molecule or whether there were active groups of smaller molecular size in the original acetone-dried powder, the following experiments were performed. The acetone-dried powders of sheep and beef pituitaries were suspended in water and adjusted to pH 3.0 with hydrochloric acid. Suspensions of hog pituitary powder were similarly adjusted to pH 3.0 and to pH 9.0 and 10.0, respectively, with sodium hydroxide. Corticotropic activity was demonstrated in the ultrafiltrates of all five of these solutions, showing that the unextracted acetone-dried powder of the pituitary contained under those conditions a corticotropic substance of a relatively smaller molecular size.

Summary: A substance with corticotropic activity has been obtained by dialysis and by ultrafiltration of pituitary extracts.

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