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Effect of Adrenotrophic Hormone of Pituitary Gland on Ability of Tissue to Synthesize Acetylcholine.*

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In the presence of serum[1,2] and spinal fluid[3] from patients with myasthenia gravis less acetylcholine was synthesized *in vitro* than in the presence of serum and spinal fluid from persons without myasthenia gravis. Patients with myasthenia gravis may have enlarged thymus and lymphocytic infiltration of certain tissues and are sometimes benefited by removal or irradiation of the thymus. Also, ether extracts of thymus decrease the synthesis of acetylcholine both *in vivo* and *in vitro*.[4]

It is known that there is a reciprocal relationship between the size of the adrenal cortex and thymus.[5-11] Thymic atrophy[11-17] and decrease in the amount of lymphoid tissue[16-18] following administration of the adrenotrophic hormone of the pituitary gland has also been reported. In the following experiments an attempt was made to ascertain whether injection of the adrenotrophic hormone of the pituitary gland into living animals modifies the synthesis of acetylcholine.

Experimental. The adrenotrophic hormone of the pituitary gland was prepared according to the method of Sayers, White, and Long.[19] Mice (18 to 20 g each†) were injected subcutaneously daily with 0.5 mg adrenotrophic hormone in 0.5 cc aqueous solution for 15 days. Non-injected mice and mice injected with albumin served as controls. The activity of the adrenotrophic hormone was tested by ascertaining the morphological changes induced by the hormone during the 15 days of injection (Table I). The changes observed in the thymus, adrenal glands and lymphatic tissue were similar to those found by Dougherty and White.[16]

After 15 days of injection the animals were killed by separating the head from the body. The brain was excised, 200 mg samples were weighed, finely minced and incubated for assay of the synthesis of acetylcholine. The synthesis of acetylcholine was ascertained according to the method of Quastel, Tennen-

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† All animals received "Big Rid" dog pellets (G. L. F. Farms, Inc., Ithaca, N.Y.), and water *ad libitum*.

TABLE I.
Effect of Adrenotrophic Hormone on Weight of Some Tissues of Mice.

Mice	No. of animals	Tissue wts as mg per 100 g of initial body weight: Thymus	Paired axillary nodes	Paired mesenteric nodes	Paired adrenals
Controls	45	165.0 ± 12.0	63.0 ± 2.9	135.1 ± 2.5	18.2 ± 1.6
Adrenotrophic hormone injected	25	75.9 ± 7.0	52.7 ± 3.5	95.3 ± 4.7	27.0 ± 1.3

TABLE II.
Effect of Adrenotrophic Hormone on the Ability of Brain to Synthesize Acetylcholine.

Mice	No. of animals	Amounts of acetylcholine synthesized in μ per 100 mg brain: Free acetylcholine	Total acetylcholine
Control	45	0.66 ± 0.024	1.48 ± 0.030
Adrenotrophic hormone injected	25	1.01 ± 0.041	2.28 ± 0.046

baum, and Wheatley[20] with minor modifications.[2] Two hundred mg minced brain, 3 cc mammalian Ringer's solution at pH 7.4, 3 mg physostigmine salicylate, and 4.8 mg glucose were shaken and incubated aerobically for 4 hours at 38°C. Because of the lability of the enzyme involved in the synthesis of acetylcholine, not more than 2 minutes were allowed to elapse between the killing of the animals and the start of shaking and incubation. After incubation the amounts of free and total acetylcholine synthesized were assayed biologically using as indicator the sensitized rectus abdominis muscle of the frog.

The amounts of acetylcholine synthesized by the brains of injected mice as compared to the controls are summarized in Table II. It was found that brains obtained from animals injected with the adrenotrophic hormone synthesized on the average 50% more acetylcholine than did brains obtained from the control animals.

Discussion. The above results indicate that injection of the adrenotrophic hormone of the pituitary gland into living animals induces not only an increase of the mass of the adrenal gland and atrophy of the thymus and the lymphatic tissue, but also causes an increase of the ability of tissue to synthesize acetylcholine.

The adrenotrophic hormone increases the secretion of the adrenal cortex.[21] The increase of acetylcholine synthesis is probably not due to an accumulation of some of the hormones of the adrenal cortex in the brain since addition of desoxycorticosterone and 4,5-dihydro-17-hydroxy-11-dehydro-corticosterone to brains of non-injected animals before incubation does not increase the amount of acetylcholine synthesized *in vitro*.[22]

The increase of the synthesis of acetylcholine may be due to an increased amount of enzyme in the brain, or to changes in the substrate for the enzyme contained in the brain. Due to the effect of the adrenotrophic hormone on metabolic processes, the brain may become a more favorable medium for the enzyme.

Since it is the opinion of the authors that the symptomatology of myasthenia gravis may be due to a decrease of the ability of nerve tissue of these patients to synthesize acetylcholine, the effect of the administration of the hormone to patients with myasthenia gravis is now being investigated.

Summary. The effect of the adrenotrophic hormone of the pituitary gland on the synthesis of acetylcholine was ascertained. Brain obtained from mice injected for 15 days with the adrenotrophic hormone synthesizes on the average 50% more acetylcholine than does brain obtained from control animals. There-

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fore it has been demonstrated that the adrenotrophic hormone of the pituitary gland augments the ability of tissue to synthesize acetylcholine.

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Fractions Derived from Soy Beans and Navy Beans Which Retard Tryptic Digestion of Casein.

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During recent studies observations were made regarding certain factors of navy and soy beans which influence digestion. While following the digestive availability of the starch of navy beans as influenced by various methods of treatment it was observed that a certain fraction of navy and soy beans retards the *in vitro* digestion of milk casein by trypsin. In view of the rather low biological value of the proteins of raw soy[1,2] and navy beans[3] and the increasing interest in their dietary use a preliminary account of some of these findings may be of interest.

Methods. In this particular work, aqueous extracts of the beans or grain in question were prepared from 1% aqueous suspensions of the finely ground materials. After centrifuging the supernatant solutions were employed in digestion experiments or were further fractionated as described.

Ten cc of such extracts or fractions prepared from this volume were added to 10 cc of a 1.52% solution of casein. Following incubation with 2 cc of 0.5% pancreatin and 2 cc of 0.8 M phosphate buffer (pH 7) for 1.5 hours the undigested protein was precipitated with trichloracetic acid and was estimated by nitrogen determination.

Results. The data of Table I represent typical findings. It will be observed that the original aqueous extracts prepared from navy and soy beans considerably retard the digestion of casein under the conditions employed while comparable extracts prepared from wheat and corn have little influence. Each value in the table represents the average of 6 determinations. The retarded digestion caused by the extracts of both types of beans becomes even more apparent when the undigested fraction is expressed in each case in terms of the percent of the total casein which is not digested as the specific result of interference by the extract. In the present series these values are 84, 51, 0.7, and 2.5 respectively for the extracts of navy beans, soy beans, wheat, and corn.

After discarding the proteins which precipitate from the original aqueous extracts of both soy and navy beans at pH 4, the filtrates obtained were found to contain nearly all of the retarding fraction. The protein content of 10 cc of such filtrates is equivalent to approximately 1 mg. Comparing this value with those in Table I it would appear that the retarding influence of the original aqueous extracts of the beans is not a function of their total protein content.

The digestion-retarding fraction can be very largely precipitated from the pH 4 filtrate of the navy bean extract with 90% acetone or ethyl alcohol. A similar precipitation of the soy bean factor may be obtained with acetone but a large part is lost with alcohol. Fractional precipitation further concentrates the material in question to a considerable degree, a small amount serving to retard the digestion of a relatively large amount of casein.

Boiling the supernatant solution of the

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