before androsterone. The "middle," or second group, is made up of androsterone and aetiocholanolone; and the "late," or third group, includes all the compounds eluted after aetiocholanolone. Those substances which are obtained regularly from the urine of normal subjects are indicated by asterisks.

The total amounts of alpha ketosteroids present in each of these groups and also the amounts of the individual components were measured by the Callow procedure based upon the Zimmermann color reaction. The results of the assays are represented graphically by the curves in the figure. The amounts of segregated fractions, expressed as the percentage of the total alpha ketosteroid content, are plotted on the ordinates. On the abscissae the principal substances listed in Table 1 are indicated from left to right in the order of their elution.

The percentage of the total alpha ketosteroid fraction made up by the compounds of the first or early group (I) is indicated by the height of the first peak of the light line; the parts represented by the middle (II) and late (III) fractions by the heights of the second and third peaks. The heavy-lined curves within each group represent the amounts of the individual components of that group.

A pattern of the alpha ketosteroid distribution in the urine of a normal person is given in the figure—A. Similar patterns were obtained from 5 normal men and 5 normal women. The curves resemble each other closely and differ only in minor details. The ratio of androsterone to aetiocholanolone (represented in the patterns as the two large, heavy-lined peaks in the middle group) is about 1:1 except in the case of older individuals.

The figure includes the patterns obtained from individual patients with lymphatic leukemia (E) and cancer (B, C, D). They are abnormal. A similarity is apparent between pattern E (lymphatic leukemia) and those from the patients with cancer. Pattern F obtained from a patient with myeloid leukemia shows only minor differences from the normal. The abnormality of the ketosteroid excretion by patients with cancer has been confirmed by the isolation from their urine of compounds so far not obtained from normal individuals or those with the non-neoplastic disorders investigated.

At present no conclusions can be drawn as to whether the abnormalities are specific for the particular disorders studied. The results indicate an abnormal function of the gonads, the adrenals or both, or possibly a disturbed metabolism of the products of these organs. A dysfunction of the adrenal cortex is suggested by the relatively large amounts of material isolated in the late or third fraction. In this fraction are found the highly oxygenated compounds which are assumed to be metabolites of the adrenal cortical hormones.

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THE NATURE OF MYASTHENIA GRAVIS[1]

IN a recent communication Torda and Wolff[2] reported that the formation of acethylcholine (ACh) from incubated frog brain was significantly reduced in the presence of serum of patients with myasthenia gravis. They concluded from this finding that the defect in ACh synthesis in patients with myasthenia gravis probably explains the fatiguability and weakness of the patients.

A possible correlation between the thymus and myasthenia gravis is suggested by the frequent finding of thymic tumors in these patients. Recently it has been shown by Harvey and co-workers[3] that thymectomy in patients with myasthenia gravis eliminates certain differences between these patients and other individuals in the electro-myographic response to intra-arterial prostigmine injections.

On suggestion of Dr. Otto Loewi the synthesis of ACh from minced brain (after Quastel[4]) has been studied in the presence of thymic tissue obtained from a patient who died from myasthenia gravis and in the presence of serum from patients with myasthenia gravis.

Experiments: In order to be able to run controls from the brain of the same animal, rat brain (because of its larger size) seemed to us more suitable than frog brain. Fresh rat brain was minced in eserine Locke solution and the suspension divided with the pipette into four equal portions. Ground pieces of thymoma tissue were added to half of the samples. One sample with and one without thymoma was extracted immediately with hydrochloric acid, the other two were incubated at 37° and extracted after three hours. The total ACh contents of all the samples were then estimated on the frog rectus muscle.

In an attempt to confirm Torda and Wolff's findings rat brain was also incubated in the manner described above in the presence of serum of six patients with myasthenia gravis and in the presence of serum from

[1] From the Department of Pathology, Columbia University, College of Physicians and Surgeons, New York City.
[2] Torda and Wolff, SCIENCE, 98: 225, 1943.
[3] Harvey, Lilienthal and Talbot, *Jour. Clin. Invest.*, 21: 579, 1942.
[4] Quastel, Teemenbaum and Wheatly, *Biochem. Jour.*, 30: 1668, 1937.

control individuals. The ACh contents were determined before and after incubation. The findings are given in Fig. 1, where it is seen that no significant differences in the amount of synthetized ACh were obtained regardless of whether or not thymus or serum from patients with myasthenia gravis were added to the medium. We also failed to observe significant differences between the amounts of ACh formed in Locke solution and in human serum. The latter findings are in contrast to Torda and Wolff's observations on frog brain.

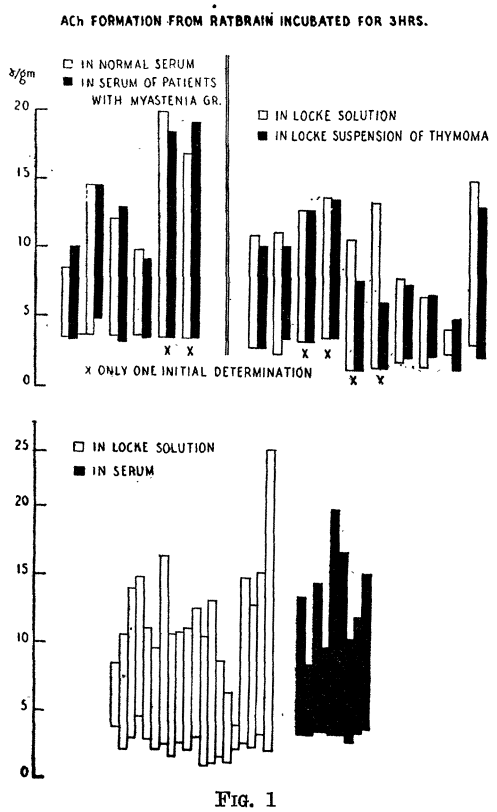

FIG. 1

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ANTITYPHOID ACTIVITY OF Vi ANTIGEN FROM EXTRA-GENERIC SOURCES[1]

LONGFELLOW and Luippold[2] reported a high degree of immunity to large doses (10,000 to 1,000,000 MLD) of *E. typhosa* in mice immunized with vaccines prepared from the V-forms of Salmonella which, aside from their content of Vi antigen, were antigenically alien to the typhoid bacillus. Against such large challenging doses, vaccines prepared with the V-forms of *S. ballerup* and *S. coli* 5396/38 produced an immunity in mice against Vi strains of the typhoid organism which was quite as high as that produced by vaccines prepared in an identical manner with Vi strains of *E. typhosa*. It may be added here that the typhoid cultures used in these experiments and in the more recent investigations reported below consisted of pure V-form organisms, having been lyophilized as such and thereby maintained in their most active immunologic and pathogenic state.

It has recently been found that when mice were immunized with serial dilutions of *E. typhosa* and *S. coli* 5396/38 vaccines and subsequently challenged with small "invasive" doses (50 to 1,000 MLD) of typical Vi strains of *E. typhosa*, the *S. coli* 5396/38 vaccine proved to be significantly *more* effective. In short, *S. coli* 5396/38 vaccine produced a higher degree of immunity to *E. typhosa* than did *E. typhosa* vaccine itself. This anomalous result was obtained repeatedly, even when the typhoid vaccine and the challenging organisms were represented by the identical strain of the typhoid bacillus.

It is believed that this superiority of *S. coli* 5396/38 vaccine is a simple quantitative manifestation—that is, a manifestation of a greater quantity of Vi antigen on the V-form *S. coli* 5396/38 organisms than is present on the V-form typhoid bacilli. Some support of this assumption was obtained from dilute-HCl extracts of these two organisms; for, when these extracts, as cleared supernates, were inoculated into mice, there resulted an even greater dominance of *S. coli* 5396/38 over *E. typhosa* in antityphoid immunogenic potency. Just as, organism for organism, *S. coli* 5396/38 vaccine was the more effective, so was the quantity of available Vi antigen on this organism the greater.

In this way, it was found that the immunogen responsible for this immunity was easily removed from the organisms by solution in diluted HCl, from which it could be precipitated with acetone and recovered as a light-brown crystalline powder. Minute amounts of the latter (Vi extract) exhibited marked antityphoid immunogenicity as gauged by the potency of Wakeman's polysaccharide[3] and of Morgan's purified antigen[4].

In comparative mouse-immunization tests with alcohol-insoluble fractions of autolysates (Morgan) or tryptic digests (Wakeman) of the typhoid bacillus, this Vi extract from *S. coli* 5396/38 proved to be more potent per unit of dried material than the typhoid antigens cited above, when opposed by the lower invasive doses (100 to 1,000 MLD) of virulent typhoid organisms. When enormous challenging doses (10,000 to 1,000,000 MLD) of the test organism were given, the antigens prepared from autolysed or digested typhoid bacilli appeared to be somewhat more effective

[1] Preliminary report.

[2] D. Longfellow and G. F. Luippold, *Am. Jour. Hyg.*, 37: 206–210, 1943.

[3] F. B. Wakeman, *Military Surgeon*, 84: 318–338, 452–471, 1939.

[4] H. R. Morgan, *Jour. Immunol.*, 46: 161–180, 1941.