(c) A third portion of the sample was titrated for bacteriophage content.

As can be seen from a protocol of one of the experiments, the culture growing in the presence of active bacteriophage increases in turbidity faster than does the control culture. The curves representing the turbidity changes are in general similar to those representing the changes in bacterial count, except that acceleration of growth in the presence of phage as recorded by the increase in turbidity is apparent earlier than it could be detected by the bacterial count. This we believe is due to the fact that gelatine fails to completely stop all lysis and certain number of bacteria present in the samples undergo lysis after being transferred upon the plates. In spite of this inaccuracy of the method, the results show quite clearly that in the presence of phage the lag period is somewhat shortened, and that bacteria multiply at a greater rate than they do in the absence of phage. As the concentration of phages increases in the culture up to the critical titer (10^{-8} cc.) the massive lysis takes place.

These experiments substantiate by direct evidence our earlier observation that phage stimulates the metabolism of bacteria. In order to elicit this phenomenon, however, it is necessary to adjust the experiment in such a way that lysis of bacteria would be delayed, otherwise the lysis occurring simultaneously with growth of bacteria obscures the results. Essentially analogous results were obtained with *B. coli* and staphylococcus.

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Is Ant. Pituitary Hormone Demonstrable in Urine of Graves Disease, in Urine of Guinea Pigs Injected With Ant. Pituitary Extract?

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Loeb and Bassett[1] have shown that an acid extract of cattle anterior pituitary produces hypertrophy of the thyroid gland of guinea pigs. They noted that this experimentally produced hypertrophy characterized by an enlargement of the acinar cells, softening and absorption of colloid, and an irregular and often slit-like shape of the acini, and by the formation of papillary ingrowths into the

[1] Loeb, Leo, and Bassett, R., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

acini resembles the pathological change in the thyroid gland in Graves disease. In a series of papers from this laboratory this similarity between the effects produced by the injection of extracts of the anterior pituitary gland of cattle and the symptoms of Graves disease has been further emphasized. Loeb and Friedman[2] have produced exophthalmos in guinea pigs by repeated intraperitoneal injections of acid extract of the anterior pituitary. Siebert[3] showed that this extract produced a rise in metabolism which was counteracted by the administration of potassium iodide, observations which accorded with the depressing effect of potassium iodide on the hypertrophy of the thyroid gland elicited by anterior pituitary which had previously been noted by Silberberg.[4] McCordock and Hageman[5] produced tachycardia in guinea pigs by means of this extract. Closs, Loeb, and MacKay[6] in joint investigations from this laboratory and the Scripps Metabolic Clinic in La Jolla, found the changes in the distribution of iodine in the thyroid gland and in the blood called forth by extract of anterior pituitary to be the same as those observed in Graves disease. Notwithstanding the similarity between these two conditions, Loeb[7] believes that while the anterior pituitary may possibly play a certain rôle in the etiology of Graves disease, there is reason for assuming that various other factors may elicit an overaction of the thyroid gland.

With the increased pituitary function occurring during pregnancy an excretion of large amounts of pituitary substance in the urine has been demonstrated by Ascheim and Zondek.[8] However, Loeb[9] has shown that this substance, present in the urine of pregnant women, has no effect on the thyroid gland of guinea pigs. In view of the presence of large amounts of pituitary substance in the urine in association with the increased pituitary function of pregnancy, and considering the similarity which exists between the symptoms of Graves disease and the effects of injections of anterior pituitary

[2] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 648.

[3] Siebert, Walter J., and Thurston, E. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 652.

[4] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 166.

[5] McCordock, H. A., and Hageman, P., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 297.

[6] Closs, Karl, Loeb, Leo, and MacKay, Eaton, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 170.

[7] Loeb, Leo, *Klin. Woch.*, 1932, **51**, 1.

[8] Ascheim and Zondek, B., *Klin. Woch.*, 1927, **28**; 1928, **30**, **31**.

[9] Loeb, Leo, *Endocrinol.*, 1932, **16**, 129.

extracts, it was of interest to determine whether it would be possible to demonstrate the presence of an anterior pituitary substance in the urine of patients with Graves disease. We studied, therefore, the effect of injections of urine of patients showing marked symptoms of Graves disease on the thyroid gland of guinea pigs. Especially did we wish to determine whether such injections would produce hypertrophy of the thyroid gland.

Urine was obtained from 4 such patients before any treatment was instituted and, in addition, from 3 patients in which the exophthalmos persisted several months after thyroidectomy. The urine from each of the 4 untreated patients was injected into at least 2 guinea pigs weighing between 170 and 190 gm. A daily intraperitoneal injection of 3 cc. was given on 10 consecutive days and the animals were killed on the eleventh day. In 3 instances, one additional animal received heated urine. The 3 specimens of urine, specimens II, IV, and V, obtained from patients several months after thyroidectomy were injected into 4, one, and 2 animals respectively. Of the 4 animals receiving specimen II, 2 received 5 intraperitoneal injections of 3 cc. each of unheated urine, and 2 others received 9 similar injections of heated urine. The animals receiving specimens IV and V were given 10 intraperitoneal injections of 3 cc. of unheated urine on consecutive days.

Results. In no instance did the thyroid of the injected guinea pigs show any hypertrophy. We may then conclude that under the conditions of our experiments it has not been possible to demonstrate the presence, in the urine of patients suffering from Graves disease, of a substance stimulating the thyroid gland to active growth. And inasmuch as it has been shown that a correspondence exists between the structure of the thyroid gland and its function, we may furthermore conclude that it has not been possible to demonstrate in the urine of patients suffering from Graves disease a substance stimulating the thyroid gland to increased function.

The question arises as to whether the lack of such a substance in the urine in Graves disease permits the conclusion that such a substance is not present in the body fluids of patients affected by this disease. It is conceivable that such a substance even were it present in the blood might not be excreted in the urine. We therefore carried out several series of experiments in which we tested the degree to which extract of anterior pituitary gland previously injected into the guinea pig is excreted in the urine as well as the rate at which it is excreted. Five series of 2 animals each were injected intraperitoneally with 3 cc. of acid extract of cattle anterior pituitary

daily for five days. About 4 hours following the last injection the animals were killed and the urine collected directly from the bladder. Each day for 5 consecutive days the urine thus obtained, which averaged a little less than 2 cc. per day, was injected into a guinea pig weighing 190 gm. The animal was killed on the sixth day. The characteristic change in the thyroid gland produced by acid extract of anterior pituitary could not be demonstrated in this animal. We repeated this experiment and again obtained negative results.

These experiments suggested that acid extract of anterior pituitary gland of cattle when injected intraperitoneally in guinea pigs is either not at all, or to only a very slight extent, excreted by way of the kidney. However, we tested these conclusions as follows: Instead of a series of injections of 3 cc. of extract of anterior pituitary, we gave one huge intraperitoneal injection of 4 cc. to each of 6 male guinea pigs, each animal weighing approximately 180 gm. This represents a very large dose, larger than any given previously in this laboratory in a single injection. At intervals of 3 and 7 hours following the injection the urine was collected by exerting pressure over the bladder. On the two days following the injection, the urine was collected 3 times at intervals of from 3 to 3½ hours. Three guinea pigs, weighing from 180 to 190 gm., received this urine. One animal was injected intraperitoneally for 5 days with the urine collected on the first day. A second and third guinea pig were similarly injected with the urine obtained on the second and third day respectively. The animals were killed on the sixth day following the beginning of the injections. Our results were as follows: the thyroid of the guinea pig receiving the urine from the first day's collection showed a slight degree of hypertrophy. In many parts of the gland, the epithelium of the acini was somewhat increased in height. The colloid in many acini was somewhat softened as indicated by a paler color and by an increased size of peripheral vacuoles. In the center of the gland, we found a few acini converted into slits. However, in some parts of the gland, hardly any hypertrophy was noticeable. The acini were of normal size and the colloid hard. There was, therefore, in this case a definite although a moderate hypertrophy found. In the animal receiving the urine from the second day there was hardly any change, while in the animal receiving the urine from the third day's collection completely negative results were obtained.

This experiment was repeated. Each of 16 male guinea pigs was given a single intraperitoneal injection of 4 cc. of acid extract of cattle anterior pituitary and the urine collected for 3 days in the same

way as in the preceding experiment. Six female guinea pigs, ranging in weight between 180 and 195 gm., were injected intraperitoneally for 5 consecutive days; 2 receiving the urine from the first day's collection; 2, the urine from the second day's collection; and 2, the urine from the third day's collection. No definite hypertrophy in the thyroids of these 6 animals resulted from these injections.

We may therefore conclude that even when present in the body in large amounts at least only a very small fraction of the hormone contained in the acid extract of cattle anterior pituitary which causes hypertrophy of the thyroid gland is excreted in the urine of guinea pigs. Perhaps this hormone, in contrast to the pituitary-like hormone of pregnant women, which is excreted in the urine in large amounts, is bound to such organs as the thyroid and ovary and then changed in some way before its excretion. The failure to demonstrate such a substance in the urine of patients suffering from Graves disease is, therefore, not inconsistent with a possible increase of pituitary function in this disease.

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Distribution of Vagus and Sacral Nerves to the Large Intestine.

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There still remains considerable disagreement regarding the distribution of vagus and sacral nerves to the large intestine. According to Klee[1] vagus fibers do not reach the large intestine. Carlson[2] has recently reported experiments in which stimulation of the sacral nerves in the dog caused contraction of the circular and longitudinal muscle of the entire colon.

The present experimental anatomical investigation was carried out on cats and dogs. The vagus and sacral nerves respectively were sectioned in 2 groups of animals. Three weeks after operation the animals were killed and the large intestines were removed and studied both macro- and microscopically. Pyridine silver sections were made from 3 levels; viz., the ascending colon, the distal half of the transverse colon, and the middle portion of the descending colon.

[1] Klee, P., *P. A.*, **145**, 594.

[2] Carlson, J. A., *J. Am. Med. Assn.*, **94**, 78.