FURTHER OBSERVATIONS ON THE THYROID GLAND (IX).¹

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IN a previous paper² experiments were related as to the keeping alive of dogs which had undergone total excision of the thyroid and parathyroids by the administration of salts of calcium or by feeding on milk, which contains much calcium.

At the time of writing that paper, three dogs, Nos. 5, 7, and 8, were still alive.

DOG No. 5 was reported as well eight months after operation, and he continued so for five and a half months more, when he was taken off his milk and put on a meat and biscuit diet, but given at the same time crystallised lactate of calcium, in water and otherwise, 13 grms. a day, which corresponds roughly to the amount of calcium in a quart of milk (1·1 litre). The dog became thin and weak, but had no tremors; later he became moribund, and had to be destroyed.

DOG 7. This dog was reported as well fifty-four days after operation. At the present time, fifteen months after operation, he, on milk diet and with calcium lactate added, continues well.

DOG 8. He too remains alive and well fifteen months after total excision,—he also is on a diet of milk.

That two of these three dogs are alive so many months after operation, and that dog 5 remained well thirteen and a half months and until his milk was stopped, makes it probable that on this diet such a dog might live out the natural term of his life. The virtue of the diet of milk probably consists mainly in its being the best method of administering calcium. Adding lactate of calcium to the drinking water or mixing it up with meat does not seem nearly so efficient a method. But it is doubtful if this is the sole cause of the benefit of giving milk. As was stated in the last paper, six cats subjected to total excision of the thyroid and parathyroids were fed on the milk of normal goats, and of the six five survived, whereas of six such cats fed on the milk of thyroidless goats all six died, and this although the milk of thyroidless goats contains the same amount of

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calcium as normal milk. The simplest explanation of these experiments is that the milk of normal goats contains more thyroid and parathyroid secretion than that of thyroidless goats; and that this helps to keep alive the cats or dogs in which total excision had been practised. It was found in the case of one dog thus kept alive that boiling the milk made no difference; but then, suprarenal solution can be sterilised by boiling without destroying its effect.

Further, it seems probable that some compensation occurs elsewhere, for in two out of the three dogs whose lives were saved by the diet of milk with lactate of calcium added, in one it was necessary to give in addition—four times in the first fortnight but not afterwards, and in the other three times in the first month but not afterwards—intravenous injections of 9 grs. (0.6 grm.) of lactate of calcium; where the compensation occurs is not clear. No change in the pituitary was found in the cases examined, but this was not to be expected, on the view that the pituitary is allied with the thyroid proper and not with the parathyroids. The administration of pituitary was tried, but it did not relieve the symptoms in two of these dogs.

It was mentioned that one dog subjected to total excision of both thyroid and parathyroids was found after death to have a large quantity of sugar in the urine in the bladder; accordingly, some experiments were made to test the assimilation of sugar in normal and total excision of thyroid and parathyroid dogs, and also in dogs which had been operated on so as to leave only one parathyroid and a minute portion of thyroid proper. As some of the dogs were on a diet of milk, and as a quart (1.1 litre) of milk contains about 45 grms. of lactose, the effect of a milk diet on a normal dog had to be tested.

It was found that a normal dog on the usual meat and biscuit diet had by Fehling's test no sugar in the urine; that if he was given a pint of milk a day, the other articles of diet being proportionately reduced, he also had no sugar in the urine. But if he was put on a diet consisting wholly of milk, some dogs would, and others would not, have sugar in the urine. Further, if to a normal dog on milk there was given in addition as much as 25 grms. of glucose, there might not be sugar in the urine; on the other hand, there might be with less glucose given, or even when none was given.

The result with two thyro-parathyroidless dogs fed on about a quart of milk a day was that there was no sugar in the urine in either dog. In one of the dogs, when 15 grms. of glucose were added, there was no sugar in the urine; but when 20 grms. were given there was sugar, and this continued when the glucose was reduced to 5 grms. a day, and also for some days after the glucose was stopped. In the other total excision dog there was no sugar in the urine even after 20 grms. of glucose had been added to his milk.

So far, then, as these experiments go they seem to show that a dog

without thyroid and parathyroids, but with the latter of these defects apparently compensated for by the administration of calcium and possibly otherwise, is less prone to sugar in the urine than a normal dog.

An experiment was also made with respect to the parathyroids and sugar in the urine. A normal dog was put on a diet of a quart of milk a day and found not to have mellituria; there was added to the milk 25 grms. of glucose,—still no mellituria. The dog was then subjected to an operation in which the thyroid and parathyroids were removed with the exception of one parathyroid and a minute portion of thyroid proper; after this the dog sometimes had, and sometimes had not, mellituria from his milk without any added glucose. Then the dog was put on a meat and biscuit diet (on which he could live perfectly well, having one parathyroid left); he had no mellituria as tested for by Fehling's method. The removal, then, of the whole of the thyroid and of three out of four parathyroids results in a diminution of the power of assimilating sugar, whereas the complete removal of thyroid and parathyroids with treatment (as we have seen) increases that power.

These experiments are open to the criticism that Fehling's test for sugar does not distinguish between glucose and lactose, and that possibly the thyro-parathyroidless dogs, fed as they were on milk, might possibly have had lactose in the urine from this cause.

In order to distinguish between glucose and lactose, Mr. Twort, the superintendent of the Brown Institution, gave me cultures of two bacilli, one belonging to the typhoid group and the other to the paratyphoid group: the first fermented both glucose and lactose, and the other glucose only. It would have been convenient to have had also a bacillus which fermented lactose only, but if such a bacillus exists I had not a culture of it. Some experiments were made with yeast which were unsatisfactory; but Mr. Twort isolated for me a pure culture, and with this blastomyces experiments were also made.

Fermentation with the two bacilli will not take place in a simple aqueous solution of glucose or lactose; it is necessary to add a medium. There was used for this purpose Gordon's lemco medium, which contains extract of meat; an equal quantity of the urine to be tested and the medium were mixed. When known amounts of glucose and lactose were being tested they were either dissolved in the medium or dissolved in a urine known to be sugarless, which was later mixed with the medium. Although not necessary, the medium was also used for the sake of uniformity with the yeast culture.

The experiments were as a rule made in an Einhorn's saccharometer: this consists of a U-shaped glass tube on a foot, one end of the U is closed for the collection of the gas generated, the other end is open and funnel shaped for the introduction of the solutions. The closed end is graduated both in cubic centimetres and to show under certain conditions the percentage of sugar; only the former readings were noted.

The bacilli solutions were incubated at 37° C., and the yeast cultures at 30° C.

The process of fermentation of glucose is usually described by the equation

and the changes which occur in lactose by

both dextrose and galactose can be further fermented by yeast into alcohol and carbonic acid gas in accordance with the first equation. It must be remembered that the living organisms require to take up carbon for their multiplication, and that therefore the measurement of the amount of CO_2 gas evolved cannot be an accurate measure of the quantity of sugar fermented; also that the gas is liable to be reabsorbed; also that the products of fermentation may inhibit further fermentation. With what was intended to be two identical experiments the readings of the gas evolved differed materially: a 1 per cent. solution of lactose is not fermented in the first twenty-four hours by the yeast culture, so far as the evolution of gas is concerned, but it is in the second twenty-four hours; on the other hand, the paratyphoid bacilli cause no evolution of gas in that solution even in forty-eight hours. It may be added that the bacilli produce a small bubble of gas in solutions of glucose and lactose as weak as 1 in 10,000, and this whether the sugar is dissolved in water or in a urine known to contain no sugar. The yeast culture is a less delicate test; it will be remembered that glucose is said to be present in normal human urine to the extent of 1 in 10,000.

The two bacilli cannot distinguish between a solution of glucose and a mixture of glucose and lactose; but lactose is only to be expected where the dog is fed on milk or when lactose is purposely given.

Experiments were also made with zymin, but this substance seemed less satisfactory.

With these cultures a new series of experiments was made, to determine the effect on normal dogs of the administration by mouth of glucose, lactose, and milk; also the effect of these treatments on dogs which had been operated on, some by the removal of the whole thyroid and three parathyroids, leaving only one parathyroid, some by the removal of the whole thyroid and parathyroids, allowing the dogs to die on a meat and biscuit diet; and on other dogs in which this operation had been performed and the dogs kept alive on a diet of milk (containing much calcium), with, in addition, when necessary, intravenous injections of lactate of calcium.

With respect to normal dogs on a diet of meat and dog biscuit, four dogs were examined. In one on four occasions no sugar was found in the urine; in a second on two occasions no sugar; in a third on one occasion no sugar, and on two occasions glucose; in a fourth on one occasion no sugar and once glycosuria; in a fifth dog on one occasion no sugar. It may be added that one of these animals, a bitch, had pups. When the pups were separated from her for the purpose of collecting the urine, there was found lactose in it; later, after the pups had been weaned ten days, there was no lactose in the urine.

Experiments were also made by giving sugar to normal dogs fed on meat. In the case of three dogs fed on meat only, there was given 5 grms., 10 grms., and 20 grms. a day of glucose, and in no case on

any occasion was glucose or lactose found in the urine. On the other hand, to two normal dogs on a wholly meat diet there was given 5 grms., 10 grms., and 20 grms. of lactose, and in both dogs lactose was found in the urine, on every occasion, in consequence.

With normal dogs fed exclusively on cow's milk, about a quart (1.1 litre) a day, containing 45 grms. of lactose, there was found with one of the three dogs tested (on five occasions), on three occasions lactose, on one glucose, and on one no sugar. In a second dog, lactose was found in both of the two occasions tested, and the same with a third dog. Thus we see that normal dogs on a meat and biscuit diet have as a rule no mellituria, but exceptionally may have glycosuria, but never, as far as these observations go, lactosuria. On the other hand, normal dogs fed on milk have as a rule lactosuria, but may, exceptionally, have glycosuria or no mellituria.

Observations were also made on dogs which had been operated on so as to leave only one parathyroid and a minute portion of the thyroid proper, which latter had been left to avoid the danger of interfering with the blood supply of the parathyroid adjacent. With one of these one-parathyroid dogs on a meat and biscuit diet there was, on testing, no sugar in the urine; when this dog was put on a purely milk diet, out of seven testings there were five times lactosuria and twice no sugar. Another similar dog was tested while on a meat and biscuit diet, and with 5 grms. of glucose added to his diet; on two occasions tested there was no sugar in his urine. It may therefore be inferred that there would be no sugar in this dog's urine when on a meat diet. This dog was now put on milk only; on four occasions lactose was found, and on one occasion there was no sugar in the urine.

Observations were also made on dogs from which the whole of the thyroid and parathyroid glands were removed, and which were fed on a meat and biscuit diet. One such dog died two days after the operation,—in the urine in his bladder after death there was glucose. In another similar dog on the same diet no sugar was found in the urine after death. In another such dog on the same diet the urine was tested four times, and on each occasion there was no sugar in the urine.

A certain proportion of dogs subjected to thyro-parathyroidectomy can be saved by the administration of calcium, and the best mode of doing this is by putting them on a diet wholly of milk, of which they will take about a quart (1.1 litre) a day. In this way they receive an amount of calcium about equivalent to that in 13 grms. of crystallised lactate of calcium. Even this amount of calcium is not sufficient in some cases, and has to be supplemented at first by the intravenous injection of lactate of calcium or by giving it in the milk.

Observations were made on these dogs who would receive in their milk about 45 grms. of lactose a day. In one such dog, as a result of seven observations, lactose was found three times, and on four occasions

no sugar; in another such dog on milk there was found as the result of fourteen observations, lactose seven times and no sugar seven times. In another such dog also on milk, as a result of twelve testings, lactose was found on ten occasions (but on two in very minute quantity), and twice no sugar. In a fourth such dog also on milk, as a result of thirteen observations, there was no sugar in the urine on any of thirteen occasions; and further, when 4 grms. of glucose were added to the milk there was on four occasions no sugar in the urine. Finally in a fifth such dog, where on one occasion 5 grms. of glucose were added to the milk, there was no mellituria.

If these results are compared with those obtained by feeding normal dogs on milk, it will be seen that of the three normal dogs one had three times lactose, once glucose, once neither; and a second and a third had each twice lactosuria. On the other hand, with the thyro-parathyroidless dogs, out of five dogs tested one had lactose oftener than not, two had it as often as not, and two had no lactose in the urine. Further, it may be added that when once or twice simultaneous experiments were made on normal and abnormal dogs on milk, it was seen that the amount of lactose, judging from the amount of gas generated, was less in the abnormal dogs.

In order to be compared with meat-fed normal dogs, some of these thyro-parathyroidless dogs were temporarily taken off their milk and put on meat, lactate of calcium being given at the same time. One of these dogs on four occasions was found to have no sugar in the urine, and in another the same condition was found three times. Further, to such dogs were also given 5 grms. of glucose. As a result of three experiments in one dog there was no mellituria, and in another the same result was found from four experiments; also to the former dog there were given, on one occasion, 5 grms. of lactose, but there was no sugar in the urine. With normal dogs on meat there was found, after 5 grms. (or more) of glucose, no mellituria; but with 5 grms. of lactose there was lactosuria. From the foregoing we may fairly conclude that dogs deprived of both thyroid and parathyroids and kept alive by calcium can assimilate lactose better than can normal dogs. This, however, does not apply to dogs who on a meat diet die after the operation; they, notwithstanding their diet, have occasionally glucose in the urine.

It is well known that suprarenal extract, when administered to dogs and other animals, causes glycosuria. Accordingly experiments were also made with this. A commercial preparation was used, and there was injected, either subcutaneously or intramuscularly, 5 c.c. of a 1 in 1000 solution, either with or without dilution, the latter being much to be preferred. This would be a dose of 5 mgrms. of the extract. Four experiments were made on normal dogs, with the result that in two glycosuria was produced, while in two it was not. The same experiment was made three times in dogs with only one parathyroid left. The result of these three experiments (in two dogs, one

being tested twice) was that on all three occasions there was glucose in the urine. Further, in a pair of experiments in which a normal and a one-parathyroid dog were tested at the same time, although both had glycosuria, the amount of gas evolved with the latter dog's urine was much more than with that of the normal dog.

No experiments were made with dogs which had undergone total excision of the thyroid and parathyroids, and which were kept on a meat and biscuit diet; but as these dogs sometimes have glycosuria without the administration of suprarenal, it may be supposed that they would readily have this after administration. But experiments were made with dogs which had undergone total thyro-parathyroidectomy and had been kept alive by milk feeding. The result of these three experiments, on three separate dogs, was that in no case was there any glucose in the urine; in one there was a trace of lactose, but that could be accounted for by the milk on which the dog was being fed. One of the normal dogs just mentioned was also fed on milk to act as a control; this dog had glucose in his urine, as a result of the suprarenal extract.

It may be added that the total excision dogs seemed to be less affected by the suprarenal injection than the normal or one-parathyroid dogs: to one of the latter there were given 10 (instead of 5) mgrms. of suprarenal extract. This dose produced such severe local reaction that the dog had to be destroyed. At the post-mortem the suprarenal glands were found to be greatly enlarged; this was the only marked change. This increase in the volume of the suprarenals has been previously described (Biedl, 1913¹).

The outcome of these two sets of experiments, by sugar feeding and by suprarenal injection, is that normal dogs assimilate glucose given by the mouth well, lactose not nearly so well; that dogs with hardly any thyroid and only one parathyroid, not so well; and that dogs without thyroid or parathyroid, kept alive by calcium feeding, assimilate lactose better than normal dogs. This is in accord with our general knowledge. Thyroid feeding tends to produce glycosuria, and we may infer that the removal of the gland would act in the opposite direction; on the other hand, Eppinger, Falta and Rudinger (1909³) found that removal of the parathyroids one by one, leaving the thyroid proper, hindered the assimilation of sugar; their presence therefore may be held to favour assimilation, thus acting contrary to the thyroid. It would seem from my experiments on dogs with practically no thyroid and but one parathyroid, that the removal of three parathyroids had a greater hindering effect than the removal of the whole thyroid had a favouring assimilation effect; if this is so, it is to be expected that the complete removal of the parathyroids and thyroid would have a hindering effect, which it has in untreated animals. On the other hand, in these animals successfully treated with calcium the opposite condition prevails; we may explain this by supposing that by treatment the functions of the parathyroids are provided for elsewhere, and

that then the pro-assimilating effect of the removal of thyroid only holds sway.

It is clear that internally secreting glands have much influence on the metabolism of sugar. They may be divided into those that by their absence cause glycosuria, and therefore it is to be presumed by their presence prevent it,—the chief of these is the pancreas;—and those that by their excessive administration cause glycosuria, and are therefore against assimilation,—the chief of these is the suprarenal. On the same side is the thyroid, for its administration causes glycosuria. This also occurs in a certain proportion of cases of Graves's disease. The pituitary also acts in the same way, for glycosuria occurs in some cases of acromegaly, which is attributed to hypersecretion by the enlarged pituitary. The parathyroids, however, seem to act in the opposite manner, favouring assimilation. In total and untreated excision of the thyroid and parathyroids the rapid death is due to the aparathyroidea, and to this should also be attributed the glycosuria when it occurs. Thus on the side of the assimilation of sugar we have the pancreas and the parathyroids, and against it the suprarenals, the thyroid, and the pituitary. Against this view is the fact that Ott (1909⁵) found glycosuria after injecting parathyroid extract.

CONCLUSIONS.

The thyroid gland in dogs hinders the assimilation of sugar: the parathyroids glands favour it.

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