LX. CONTRIBUTIONS TO THE STUDY OF BRAIN METABOLISM. IV.

CARBOHYDRATE METABOLISM OF THE BRAIN TISSUE OF DEPANCREATISED CATS.

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It has recently been shown by Warburg, Posener and Negelein [1924], and by Loebel [1925], that brain tissue is able to break down glucose to lactic acid with great rapidity.

We found that the lactic acid content of the excised brain (rabbit) did not rise appreciably above the "resting" level on incubation of the tissue under anaerobic conditions, although large amounts of lactic acid were formed when glucose was supplied to the tissue [Holmes and Holmes, 1925, 1]. It seemed, therefore, that the failure of the excised brain to make lactic acid must be due to a lack of glucose in the tissue.

A certain amount of reducing substance has been found in the tissue, which we have shown [Holmes and Holmes, 1926, 1] to consist mainly of creatine and creatinine, with about 30 mg. % pentose. There is no evidence for the presence of any glucose. There is a small amount of glycogen in the brain, but it does not give rise to any lactic acid, and is not lessened in amount upon incubation, whether aerobically or anaerobically, at alkaline p_H [Holmes and Holmes, 1926, 2]. E. G. H. has lately been able to show that the glycogen content of the brain tissue does not fall after depancreatisation, when the glycogen in the body tends to disappear, nor after a convulsant dose of insulin, although the brain is then suffering from a shortage of glucose. It appears, therefore, that the glycogen of the brain is in some way immobilised, and does not play the part one would expect in the carbohydrate metabolism of the brain tissue.

From consideration of these various facts we concluded that the brain contained little or no lactic acid precursor. On the other hand, it contains a by no means negligible (about 100 mg. %) amount of lactic acid after death, and the most likely origin of this lactic acid is the glucose of the blood. Though some of this glucose is probably present in the brain during life, it is all converted into lactic acid in the time (about $3\frac{1}{2}$ minutes) elapsing between the death of the animal and the removal and rapid cooling of the brain.

If this is indeed the fact, one might expect the lactic acid content of the brain to be dependent upon the level of glucose in the blood. We have been able to show that this is apparently the case, and thus to support our view of the origin of the lactic acid in the brain.

The experiments involved are described below. The method employed for the estimation of the lactic acid is in no essential different from that of Meyerhof [1920]. Rabbits were used and the blood was obtained from an ear vein immediately before the death of the animal, which was killed by a blow on the neck. The results of the experiments are shown in Table I.

It will be seen from Table I that as the level of the blood sugar falls after the administration of insulin to the rabbit, the brain lactic acid content falls in a corresponding manner. When the hypoglycaemia is severe, the amount of lactic acid in the brain is very small. These results, which indicate that a convulsant dose of insulin produces considerable glucose deprivation in the central nervous system, are described in earlier papers [Holmes and Holmes 1925, 1, 2]. It is well to emphasise the point once more, that under these conditions of carbohydrate deprivation, the glycogen in the brain remains at a normal level. For this reason, too, the decrease in lactic acid content of the brain cannot be due to a local increase of glycogen formation in that organ, as the following figures show—a starved rabbit, just about to convulse after a dose of insulin, showed a brain lactic acid content of 36.5 mg. % and a glycogen content of 25.5 mg. %. The normal lactic acid content is 90–110 mg. %, so that, to account for the drop in lactic acid, the brain would have to contain at least 55 mg. % of extra glycogen, in addition to any which might have been present before the insulin was given.

Table I.

Exp.						Blood sugar mg. %	Brain lactic acid mg. %
1	Killed with chloroform	...	...	...	...	273	163
2	„ „ ether	...	...	...	...	260	159
3	Intravenous injection glucose	...	...	...	...	244	153
4	„ „ „	...	...	...	...	217	146
5	„ „ „ 11 mins. previously	...	...	...	...	208	113
6	Anaesthetised with urethane	...	...	...	...	191	134
8	Intravenous glucose 27 mins. previously	...	...	...	...	183	123
9	Normal	...	...	...	...	160	97.4
10	„	...	...	...	...	150	101
11	Insulin	...	...	...	...	124	96
12	Amytal	...	...	...	...	123	85
13	Insulin. No symptoms	...	...	...	...	107	88
14	„ No symptoms	...	...	...	...	95	76
15	„ Flaccid	...	...	...	...	65	49
16	„ Commencement of convulsion	...	...	...	...	50	45
17	Insulin convulsions well marked	...	...	...	...	—	29
18	Convulsed with insulin: recovered with glucose	...	...	...	...	119	56

The converse of this effect of hypoglycaemia can be seen when the blood sugar level is raised by any means. After the injection of glucose, for instance, the brain lactic acid level is high, as it is after the administration of adrenaline, or after killing with chloroform or ether, all of which processes raise the

blood glucose level. Table I gives some typical examples, showing the correspondence between the blood sugar and brain lactic acid levels.

Gesell [1925] traces a direct relationship between the level of the lactic acid in the brain, and the activity of the respiratory centre. Our experiments do not support this view, since the hyperglycaemic animals showed no hyperpnoea, while animals on the verge of insulin convulsions pant heavily.

It is interesting to notice that in deep anaesthesia the relationship between brain lactic acid and blood sugar still persists. Chloroform, ether, and urethane are known to exert a much greater inhibitory effect on the oxidative processes of brain than upon glycolysis [Loebel, 1925]. The rise in lactic acid might, therefore, be in part due to a failure in removal rather than to an increased production. The figures, however, do not suggest this, falling as they do into series with the others, nor have we been able to find any increase in cases of light amytal anaesthesia, which drug, in moderate doses at least, does not raise the blood sugar.

When the blood sugar has been abruptly raised by the injection of glucose, there is, of course, some delay before the brain lactic acid is raised correspondingly. This, doubtless, accounts for some of the irregularities in brain lactic acid level observed after the intravenous injection of glucose in normal animals, and also for the fact that in these animals the brain lactic acid level does not rise as high as it does in the case of depancreatized animals with the same blood sugar level. Exp. 18 of Table I illustrates this point, the animal having been killed a few minutes after the injection of the glucose.

Since the formation of lactic acid is known to be necessary for the process of muscular contraction, it has long been puzzling to note that there is no marked failure of this process in extreme diabetes. The problem which confronted us was a somewhat similar one. The fact that an animal will fall into convulsions when its brain is deprived of lactic acid precursor by the lowering of the blood sugar after insulin, and will recover from these convulsions when the supply of glucose is restored, indicates that the central nervous system is dependent for some purposes upon its carbohydrate metabolism. Yet, except indirectly through ketosis, there are no marked symptoms of any impairment of the functions of the central nervous system in diabetes. This was very plainly apparent in the case of our depancreatized cats, which showed no ketosis, and remained active and intelligent.

Recent work [Himwich, Loebel and Barr, 1924; Hetzel and Long, 1926] has shown that the formation of lactic acid takes place in diabetic as in normal muscles, and it is simple to show that lactic acid is present in the brain of the depancreatized animal. But whereas it is extremely difficult to determine directly the origin and fate of the lactic acid in the diabetic muscle, it should be far easier to determine the origin, at least, of the lactic acid in an organ like the brain, which has little or no precursor in its own tissues, and is dependent upon an outside supply.

We have considered the possibility that the amount of lactic acid in the

brain may depend upon the level of the lactic acid in the blood, that is, that its presence may be due in part to a passive accumulation from the blood. The figures of Himwich, Loebel and Barr [1924] do not indicate that any rise in the lactic acid level of the blood occurs as a result of diabetes, except after exercise, so that we do not need to give particular consideration to this point in connection with our depancreatized animals. We have carried out a few experiments comparing the blood lactic acid and the brain lactic acid levels, which show that, as far as the experiments on rabbits are concerned, there is no reason to suppose that the changes in the lactic acid content of the brain are due to changes in the lactic acid content of the blood.

Owing to the extreme difficulty of depancreatizing rabbits, it was decided to use cats for the following experiments. We had had no previous experience with cats, and therefore thought it necessary to carry out some determinations of the blood sugar and brain lactic acid levels in normal animals. The results (Table II) show that, in cats as in rabbits, the brain lactic acid level depends upon the blood sugar level. The normal lactic acid values are fairly constant. In two cases only (one normal and one diabetic) was there any increase in the lactic acid content of the brain after incubation; in both of these the brain had been badly injured by the blow which killed the animal. The glycogen of the brain showed no corresponding fall, and we had to assume that the breakdown of the glucose remaining in the brain had in some way been retarded. These cases supported our view that a detectable amount of the glucose entering the brain from the blood is present during life, but is usually broken down directly after death. For comparison with the other animals it seemed best to take the higher value obtained in each of these two cases, and when this was done the results agreed well with those obtained in other experiments.

Both the normal and the depancreatized animals were killed with one heavy blow on the head, death being instantaneous. The brain was removed, and was then cut in halves and one half frozen immediately, or it was quickly chopped and sampled and a portion frozen. The latter method gave the best sampling, and the slightly increased delay before freezing made no difference to the level of the "resting" values obtained. The frozen portions were weighed out and immersed in alcohol whilst still very cold, and were used for the estimations of the "resting" lactic acid content.

The estimations were carried out by the method of Meyerhof, with the following very slight modification. Since the greasiness of the tissue makes filtration through paper impossible, the alcoholic extracts were all filtered through Jena glass filters (porosity < 7 or 5-7).

For experiments dealing with the effect of aeration or of anaerobic incubation upon the lactic acid content of the tissue, the portions of the brain which had not been subjected to cooling were employed.

As we wished the depancreatized animals to be as normal as possible in every other respect, they were given injections of insulin, and sometimes of

glucose also, for the first few days after the operation¹, this treatment facilitating recovery from the anaesthetic and healing of the scar. Cat 5, which was pregnant, and Cat 4 (Table II), refused to eat and were therefore killed three and five days, respectively, after the operation. Two out of the four animals operated on recovered very well (Cats 3 and 7, Table II) and these were killed eight and fourteen days respectively after the operation. At the time of death they appeared to be in good health, took a good deal of exercise, ate enormously, and were perfectly intelligent. Cat 7 excreted 5 g. of glucose per 100 cc. urine the day before its death, although it was fed entirely on lean meat with a little diluted milk. In no case could we detect any ketone bodies, by Rothera's test, in the urine of the animals, and the fact that the diabetes was uncomplicated by ketosis probably accounted in part for the surprisingly good health they displayed.

After death all the animals were very carefully examined for possible residual portions of pancreas, and any suspected fragments of tissue were taken for section. In no case, however, was any pancreatic tissue found.

As we could detect no acetone or acetoacetic acid either in the urine of the depancreatized animals or in the extracts of the brain tissue in which the lactic acid was to be estimated, we made no allowance for these substances in our estimations.

The estimations by Meyerhof's method showed the presence of a considerable amount of lactic acid, and the thiophene test for lactic acid was given by the solutions.

If this lactic acid arises from the glucose of the blood as we had found to be the case in normal animals, and if the power of the brain tissue to break down this glucose is not decreased after depancreatisation, then the greatly increased blood glucose level found in diabetic animals should lead to an increased production of lactic acid by the brain. This we did, in fact, find to be the case (see Table II).

Table II.

	Blood sugar mg. %	Brain lactic acid mg. %
Cat 5. Diabetic. Killed 3 days after depancreatisation. Pregnant; anuric for 24 hours	440	220
Cat 7. Diabetic. Killed 14 days after depancreatisation. Insulin for first 9 days. Subcutaneous injection of glucose 25 minutes before killing. Appeared healthy	317	200
Cat 4. Diabetic. Killed 5 days after depancreatisation. Would not eat. Given glucose and insulin for first 3 days. Subcutaneous injection of glucose 45 minutes before killing	311	187.5
Cat 3. Diabetic. Killed 8 days after depancreatisation. Had insulin for first 3 days. Appeared healthy	245	170
Cat 1. Killed with ether. Blood sugar certainly raised	—	127
Cat 7. Normal	123	104
Cat 8. Normal	—	90
Cat 9. Normal	110	110.5
Cat 2. Insulin. Killed in flaccid stage	—	57

¹ We wish to express our gratitude to Dr H. H. Dale for instructing us in the technique of this operation, and for allowing us to witness a depancreatisation which he himself performed upon a cat.

It will be seen that the brain lactic acid values correspond quite well with those obtained in normal animals when the blood sugar is artificially raised.

Thus, so far as we can judge from the data in Table II the brain tissue of a completely depancreatized cat is still capable of converting the glucose of the blood into lactic acid. A high level of glucose in the blood produces a high level of lactic acid in the brain of depancreatized as well as of normal animals. It is therefore difficult to imagine that the lactic acid which is present in the brain tissue of the depancreatized animals is derived from some unknown and abnormal source. Moreover, the excised brain tissue, taken from a completely depancreatized animal (Cat 3, 8 days after operation) was found to be capable of breaking down large amounts of glucose to lactic acid *in vitro*. The tissue was chopped and placed in Ringer's solution containing 0.25 % glucose, and potassium cyanide was added (final strength $M/650$) to prevent oxidation of the lactic acid. After standing for 24 hours at room temperature, the amount of lactic acid present had increased from 171 mg. % (the "resting" value) to 365 mg. %. Tissue kept under similar conditions, but without added glucose, showed no increase in the amount of lactic acid present.

We feel safe in assuming that in all probability the origin of the lactic acid in the brain of diabetic animals is the same as that in the brain of normal animals. Of the subsequent fate of the lactic acid we know nothing, except that it is removed by some oxidative mechanism when the excised tissue is well aerated, as one would expect from Warburg's work [Warburg, Posener and Negelein, 1924], and that the power to effect this oxidative removal is retained by diabetic tissue.

The figures (Table III) showing the disappearance of lactic acid from normal and depancreatized brain tissue, illustrate this point, and also show the activity of brain tissue in oxidizing preformed lactic acid. The conditions of the experiments were as follows.

The brain was chopped and weighed. One portion of it was then used immediately for the determination of the "resting" level of the lactic acid; the other portion was placed in a stoppered bottle with about 10 cc. of Ringer's solution (p_H 7.4) and shaken in a bath at 37°, to ensure thorough aeration for about 3 hours.

Table III. *Lactic acid values expressed in mg. per 100 g. tissue.*

	"Resting" lactic acid mg.	Lactic acid after aeration mg.	Decrease mg.
Rabbit. Normal	91	15	76
Rabbit. Normal	97	36	61
Cat 6. Normal	104	41	63
Cat 4. Depancreatized	187	92	95
Cat 7. Depancreatized	202	111	91

It can be seen from this table that the power to remove lactic acid by oxidation is in no way decreased after depancreatization.

SUMMARY.

1. The apparent absence of preformed lactic acid precursor from the brain, and the stability of the glycogen store in that organ, are discussed, and evidence is brought forward showing that the lactic acid found in the brain is formed from the glucose supplied by the blood. The lactic acid values fall and rise with the blood sugar, both in hypo- and hyper-glycaemic conditions.

2. Hyperglycaemia due to the administration of anaesthetics is accompanied by increased brain lactic acid values.

3. The brains of completely depancreatized cats show a resting lactic acid value corresponding to the degree of hyperglycaemia existing at the time of death.

4. The brain tissue of diabetic, like that of normal animals, is capable of converting glucose to lactic acid, and of removing lactic acid under aerobic conditions.

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REFERENCES.

- Gesell (1925). *Amer. J. Physiol.* **75**, 170.
Hetzel and Long (1926). *Proc. Roy. Soc. Lond. B*, **99**, 279.
Holmes and Holmes (1925, 1). *Biochem. J.* **19**, 492.
—— — (1925, 2). *Biochem. J.* **19**, 836.
—— — (1926, 1). *Biochem. J.* **20**, 595.
—— — (1926, 2). *Biochem. J.* **20**, 1196.
Loebel (1925). *Biochem. Z.* **161**, 219.
Himwich, Loebel and Barr (1924). *J. Biol. Chem.* **59**, 265.
Meyerhof (1920). *Pflüger's Arch.* **182**, 239.
Warburg, Posener and Negelein (1924). *Biochem. Z.* **152**, 309