XLV. THE RELATIONSHIP BETWEEN SUGAR IN BLOOD AND LACTIC ACID IN BRAIN.

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IN a series of earlier communications [1925, 1, 2; 1927] Holmes and Holmes developed the hypothesis that the brain tissue of the living animal was dependent for the supply of carbohydrate from which it formed lactic acid on glucose supplied to it by the blood stream. Since that time, several workers [Kinnersley and Peters, 1929, 1930; Jungmann and Kimmelstiel, 1929] have, on the basis of their own experiments, questioned the validity of this view. If the hypothesis originally advanced by Holmes and Holmes is the correct one it emphasises the dependence of the brain upon the circulation, not only for oxygen, but also for carbohydrate, and it renders it possible that the train of symptoms shown by the hypoglycaemic animal may be referred directly to deficiency of carbohydrate supply to the cerebral tissue, just as syncope is the result of anoxaemia of those same cells.

It was in the hope of reaching a definite conclusion that the experiments here reported were undertaken: they constitute a re-investigation of the original hypothesis, and we believe that they succeed in demonstrating its validity.

In the original experiments, Holmes and Holmes employed rabbits and cats. The animals were killed, the brains removed, cooled as quickly as possible with ice and salt freezing mixture, and their lactic acid contents compared with the level of the blood-sugar at the time of killing. Since only small changes in lactic acid or carbohydrate content could be observed when the tissue was incubated under anaerobic conditions, it was concluded that the brain contained little or no lactic acid precursor. It was however recognised that the so-called resting values might in reality be "final" values, since 3-4 minutes necessarily elapsed after killing before the brain could be cooled, and a considerable amount of lactic acid formation might take place in that time. Since the original experiments have been performed, the work of McGinty and Gesell [1925], Kinnersley and Peters [1929] and Jungmann and Kimmelstiel [1929] has shown that this was, in fact, the case. These authors, by cooling the tissue in liquid air, obtained "resting" values lower than those of Holmes and Holmes. These low initial values however increased on incubation to values of the same

order as those observed by the last-named workers, all of whose figures represented, evidently, "final" or nearly "final" values. On these grounds, both Kinnorsley and Peters and Jungmann and Kimmelstiel have concluded that the brain contains lactic acid precursor, and therefore are unwilling to admit its dependence on the blood-sugar in this respect. They do not appear however to have attempted to investigate the actual relationship between blood-sugar and brain-lactic acid, that is to repeat the experiments upon which the original hypothesis was founded.

In our re-investigation of the matter, we employed mice. Blood-sugar was lowered when required by insulin and raised by intraperitoneal injections of glucose. Liquid air was used to fix the tissue for the purpose of obtaining "initial" values.

Method.

Mice were used, in groups of six or eight, for each experiment. Low blood-sugar values were secured by injecting insulin after a preliminary period of starvation. For normal values the animals were taken directly from stock. For high values the animals were given an intraperitoneal injection of glucose solution of appropriate strength 15 minutes before being killed. The mice were decapitated with a single stroke of a pair of strong, sharp scissors. Four (or if six animals only were used, three) of the heads dropped directly into a flask of liquid air. They were frozen within 2-3 seconds of decapitation, and the brains were subsequently removed, and worked up for "initial" values. The remaining heads were placed in an incubator at 37° for periods of 1 hour, or sometimes of 30 minutes. Blood was collected from each severed trunk in turn in a small dish containing oxalate: a "pooled" sample from the whole group of animals was thus obtained, and its sugar content determined by the method of Hagedorn and Jensen [1923].

The tissue was worked up in the same way for both sets of values, the method differing somewhat according to the substance or substances to be estimated.

Lactic acid only. The brains were removed from the skulls and weighed. They were then ground with sand in a mortar with 5 cc. of 20 % trichloroacetic acid, and the "brei" poured into a 25 cc. volumetric flask. The mortar was washed out, and the washings, *plus* 10 cc. more of the 20 % trichloroacetic acid, were added to the flask. The contents were made up to volume, and filtered. An aliquot part of the filtrate was treated with 10 % copper sulphate and a 10 % lime suspension in the usual way and filtered, and a suitable volume of filtrate taken for lactic acid estimation by the method of Friedemann, Cotonio and Shaffer [1927].

Lactic acid and total carbohydrate. The method used was based on that of Carruthers [1931]. After weighing, the tissue was ground with 4 cc. of boiling water, and washed into a 15 cc. volumetric flask with two more 3 cc. portions of boiling water. 3.5 cc. of 40 % trichloroacetic acid were added, and the con-

tents of the flask made up to the mark with hot water. After standing for 30 minutes, the material was filtered. 10 cc. of filtrate were pipetted into 25 cc. centrifuge-tubes; 0.66 cc. of conc. HCl was added, and the tubes were heated (with air condensers) in a boiling water-bath for 3 hours. After hydrolysis the fluid was transferred to a 15 cc. flask; 1.0 cc. of 10 % copper sulphate was added, and the fluid neutralised with concentrated sodium hydroxide. After the addition of 2.0 cc. 10 % suspension of lime, the contents of the flasks were made up to volume, transferred to centrifuge-tubes, cooled for 30 minutes in ice and centrifuged. 10 cc. of the supernatant fluid were taken for lactic acid estimations: the precipitate was used for estimation of carbohydrate, by the technique previously described [Holmes and Ashford, 1930]. This method, as is claimed by Carruthers, permits the estimation of the total carbohydrate of the tissue. The hot water extracts "soluble carbohydrate" and glycogen; the trichloroacetic acid precipitates proteins and lipoids. The glycogen having been hydrolysed during the period on the water-bath, the sugar arising from it, together with other carbohydrate, is separated from other substances by the "copper-lime" procedure.

Glycogen. This substance was estimated by the method described by Holmes and Ashford [1930]. The tissue was placed at once in absolute alcohol. Simultaneous estimations of lactic acid were not carried out.

The results of these experiments are presented in Tables I and II, and in Figs. 1 and 2. While it is desired to give the figures actually obtained, the general results can be most conveniently appreciated by an inspection of the curves. They can be summarised quite briefly, as follows. For all values of blood-sugar, the initial value for brain-lactic acid is low, and very fairly constant; except for the lowest, *i.e.* those obtained in severe hypoglycaemia, the average figure is 67 mg. per 100 g. of tissue. This confirms the findings of McGinty and Gesell, Jungmann and Kimmelstiel, and Kinnersley and Peters, and makes it clear that the initial values previously obtained by Holmes and Holmes were too high. The final values on the other hand show an almost linear relationship to the blood-sugar until the latter reaches a value of about 200 mg. per 100 cc.; as long, that is, as it is within what may fairly be looked upon as a normal range of values. When the blood-sugar level exceeds 200 mg. per 100 cc. the curve falls off rather sharply, and the slope becomes much more gradual. An almost exactly similar relationship is to be seen in the figures published for rabbit and cat brain [Holmes and Holmes, 1927].

The explanation of the shape of the curve for "final" values shown in Fig. 1 is afforded by the curve for "initial" total carbohydrate values in Fig. 2. Curve *A*, Fig. 1, falls away after the value of 200 mg. of sugar per 100 cc. of blood has been reached because at higher levels of blood-sugar the total carbohydrate in the brain no longer follows the blood-sugar value, but itself tends to fall away (Fig. 2 *a*). The formation of lactic acid evidently is limited by the lack of precursor, the amount of which depends (1) on the blood-sugar level and (2) on the ability of the brain cells to pick up carbohydrate

Table I.

Blood-sugar mg./100 cc.	Lactic acid mg./100 g.			Total carbohydrate mg./100 g.			Procedure
	Initial	Final	Rise	Initial	Final	Fall	
34	24	30	6	64	33	31	Starved 15 hrs. 4 units insulin subcutaneously
35	49	43	6	—	—	—	"
43	—	48	—	—	—	—	"
48	30.5	47.4	17	—	—	—	"
121	55	114	59	—	—	—	No injection
137	83	108	25	121	33	88	"
139	93	121	28	102	56	46	"
140	59	116	57	—	—	—	"
197	—	—	—	152.2	43.8	108.4	0.5 cc. 10 % glucose killed after 15 mins.
198	54	153	99	—	—	—	"
202	79	128	49	—	—	—	"
263	79	148	69	153	65	88	0.5 cc. 20 % glucose killed after 15 mins.
291	—	—	—	159	62.2	96.8	"
284	41	168	127	—	—	—	"
296	62	173	111	221	62	159	0.5 cc. 30 % glucose killed after 15 mins.
297	63	173	110	—	—	—	0.5 cc. 20 % glucose killed after 30 mins.
487	—	—	—	262	50.4	211.6	0.5 cc. 30 % glucose killed after 15 mins.
543	53	185	132	—	—	—	"
593	67	224	157	210	54	156	0.5 cc. 40 % glucose killed after 15 mins.
618	67	186	119	—	—	—	0.5 cc. 30 % glucose killed after 15 mins.
637	75	193	118	195	78	117	0.5 cc. 40 % glucose killed after 15 mins.
670	—	—	—	225	43	182	"
844	63	210	147	—	—	—	"
946	54	189	135	—	—	—	"

Table II.

Date	Blood-sugar mg./100 cc.	Glycogen mg./100 g. fresh tissue			Injection
		Initial	Final	Fall	
15. i. 32	145	60	40	20	None
26. i. 32	644	33	22	11	None
19. i. 32	653	74	35	39	0.5 cc. 40 % glucose
21. i. 32	880	57	51	6	0.5 cc. 40 % glucose

from the circulating fluid. Curves 1 (*a*) and 2 (*a*) are not superposable, it is true. But there is a considerable difference between the relative simplicity and accuracy of the methods for estimating lactic acid and total carbohydrate respectively. The figures for lactic acid are on the whole surprisingly steady; those for total carbohydrate show a very considerable scatter, and the method which has to be employed is neither short nor simple. Moreover, as has been

pointed out elsewhere, even with the copper-lime technique it is sure that all the material estimated is not glucose.

While these experiments leave little room for doubt that the brain does depend, in a very direct sense, upon the blood-sugar for its lactic acid precursor, they throw no light on the possible steps by which the glucose of the blood is transformed to lactic acid. Ashford and Holmes [1929] have found reason to believe that hexosephosphate formation is not a necessary step in

Fig. 1.

○—○ Initial values ×—× Final values

Fig. 2.

○—○ Initial values ×—× Final values

the production of lactic acid by brain *in vitro*, though it does intervene in the conversion of glycogen into lactic acid. But it is still possible that sugar might be taken from the blood and stored in the living brain as glycogen, to break down rapidly after death.

To test this, glycogen was estimated both in the initial and in the final samples. The results given in Table II, show that

- (1) the initial glycogen values are independent of the blood-sugar;
- (2) the fall in glycogen on incubation is small compared with the total rise in lactic acid;
- (3) the total amount of glycogen in the brain is relatively small.

We are satisfied therefore that glycogen is not an intermediary in the conversion of glucose into lactic acid by the brain.

The "resting" values that we find for lactic acid seem to be independent of the blood-sugar, unless the latter is very low, when they tend to decrease. In such cases, they approximate closely to the final values: there is however always some rise on incubation, which we attribute chiefly to a conversion of some glycogen into lactic acid. It will be noticed that curve *A*, Fig. 1, would cut the ordinate at zero blood-sugar. This is somewhat unexpected, since the method of Hagedorn and Jensen probably gives values appreciably higher than the true glucose content of the blood, and one would expect to find no brain-lactic acid when there was still apparently some sugar in the blood. If, however, as we believe, there is always some glycogen in the brain, no matter how low the blood-sugar may be, this state of affairs may be explained. Curve *A*, of Fig. 2, will in fact cut the ordinates at a level indicating that, at zero blood-sugar, the brain will contain some 35 mg. of total carbohydrate per 100 g.

The "initial" lactic acid value must be the resultant of at least three processes; lactic acid formation, lactic acid removal by diffusion into the blood stream, and lactic acid removal by oxidation *in situ*. It is remarkable that these should balance to give such steady values as they do. It is even more remarkable that such a readily diffusible substance as glucose should apparently fail to be taken up by the brain cells beyond a certain limit of its concentration in the blood. The failure to find an increase in total carbohydrate of the brain cannot be referred to an increased rate of removal of lactic acid (and therefore of sugar) by oxidation, for the oxygen uptake of the brain, even *in vitro*, is for the early period of any experiment almost independent of the lactate concentration [Ashford and Holmes, 1931], showing that the lactic-oxidising enzyme systems immediately after death (and therefore presumably during life) are fully saturated with substrate.

It is evident, both from the figures and the curves, that the fall in carbohydrate is in general greater than the observed rise in lactic acid. In the first few experiments, the reverse appeared to be the case, and a statement to that effect appeared in a previous publication [Ashford and Holmes, 1931]. Little is so far known about the steps whereby glucose is converted into lactic acid by the brain, except that the process does not appear to require the intervention of phosphate, but the discrepancy between these curves suggests that it is probably a complex affair.

While we believe that these results show clearly that the amount of lactic acid precursor in the brain depends upon the blood-sugar, and while they fit well with the lowered O_2 uptake observed for excised brains of hypoglycaemic animals [Holmes, 1930], they do not help us to understand the actual mechanism of hypoglycaemic symptoms, save in so far that it appears from curve 1 (*b*) that at blood-sugar levels at which these symptoms occur there is a definite drop in the true "resting" lactic acid level, *i.e.* in the concentration of lactic acid in the living brain cells. It is conceivable that the function of the

lactic acid is to maintain a stable condition at some intracellular surface, and that its removal results in a profound disturbance of function. This disturbance, of course, does not necessarily show itself in the occurrence of convulsions which, though usual in the hypoglycaemic rabbit, are usually, though not always, replaced by muscular flaccidity and collapse in the mouse, and occur but rarely in the human subject.

SUMMARY.

The relationship between brain-lactic acid and blood-sugar content has been re-examined with the following results.

1. The lactic acid content of mouse brains fixed rapidly in liquid air is invariably low. (This confirms the results of other workers.)

2. The amount of lactic acid formed on anaerobic incubation depends on the blood-sugar level at death, up to blood-sugar values of some 200 mg. per 100 cc.

3. At higher levels there is a smaller increase in lactic acid in proportion to the increase in blood-sugar.

4. This increase is accounted for by a corresponding failure of the brain tissue to take up carbohydrate.

5. At blood levels at which hypoglycaemic symptoms occur, the "resting" brain lactic acid level is decreased.

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REFERENCES.

- Ashford and Holmes (1929). *Biochem. J.* **23**, 748.
— (1931). *Biochem. J.* **25**, 2028.
Carruthers (1931). *Chinese J. Physiol.* **5**, 93.
Friedemann, Cotonio and Shaffer (1927). *J. Biol. Chem.* **73**, 335.
Hagedorn and Jensen (1923). *Biochem. Z.* **135**, 46.
Holmes (1930). *Biochem. J.* **25**, 914.
— and Ashford (1930). *Biochem. J.* **24**, 1119.
— and Holmes (1925, 1). *Biochem. J.* **19**, 492.
— (1925, 2). *Biochem. J.* **19**, 836.
— (1927). *Biochem. J.* **21**, 412.
Jungmann and Kimmelstiel (1929). *Biochem. Z.* **212**, 347.
Kinnersley and Peters (1929). *Biochem. J.* **23**, 1126.
— (1930). *Biochem. J.* **24**, 711.
McGinty and Gesell (1925). *Amer. J. Physiol.* **75**, 70.

Note added April 4th, 1932. Since this paper went to press, a communication by Haldi [(1932) *Amer. J. Physiol.* **99**, 702] has been published, in which appear figures for the time relationships of lactic acid production by (dog) brain tissue. The initial values found are in substantial agreement with those given here, and the maximum lactic acid production is shown to occur in 15–20 minutes at 38°. The final values reached would correspond, in the mouse, to a blood-sugar level of about 200 mg. per 100 cc.