CCXXXIX. OBSERVATIONS ON THE VARIATION OF p_H OF BRAIN TISSUE.

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IN previous communications [Holmes and Holmes, 1927; Holmes and Sherif, 1932], it has been demonstrated that the amount of lactic acid formed by the excised brain after incubation is dependent, within limits, on the level of the blood-sugar at the time of death, while the lactic acid found in brains which have been rapidly fixed in liquid air is constant in amount and independent of the blood-sugar, except when the latter has been reduced by insulin to a low level. It was thought that it would be interesting to see whether these changes in lactic acid content were associated with any demonstrable change in the p_H of the tissue.

Mice were used for the experiments; the blood-sugar was raised, when desired, by the intraperitoneal injection of glucose, or lowered by means of subcutaneous injections of insulin. The animals were decapitated by a single stroke of a pair of strong, sharp scissors, and the heads were either allowed to fall into liquid air (which prevents further lactic acid formation, even after thawing), or were incubated for 1 hour (sometimes 45 minutes) at 37°. For p_H determinations the brains were removed from the skulls, placed in a small beaker or specimen tube, and rubbed to a fine suspension with 5.0 cc. of Ringer's solution. The p_H of the suspension was determined by means of the Cole potentiometer, gold electrodes and quinhydrone being employed. The standard acetate buffer was checked against a calomel cell. While the values obtained probably do not accurately represent the actual p_H of the tissue during life, there seems no reason to doubt that the differences observed were significant; and the results showed a very considerable constancy. It is curious that, in spite of the fact that the determinations were not made in an atmosphere containing a proportion of CO_2 , yet the values observed were consistently lower (*i.e.* the tissue was more acid) than it could reasonably be expected to have been during life. The results are arranged in six groups, as follows.

- (1) Animals with normal blood-sugar, decapitated and the heads frozen.
- (2) Animals with normal blood-sugar, decapitated and the heads incubated.
- (3) Animals decapitated after glucose injection, and the heads frozen.
- (4) Animals decapitated after glucose injection, and the heads incubated.

(5) Animals decapitated during insulin hypoglycaemia, and the heads frozen.

(6) Animals decapitated during insulin hypoglycaemia, and the heads incubated.

It will be seen that the values observed in groups (1) and (3) are about the same. There is a fall (increase of acidity) on incubation in all cases, but in group (4) it is definitely greater than in group (2); in group (6) it is slightly less than in group (2). The values in group (5), where the brains were frozen during hypoglycaemia, are definitely higher (less acid) than in (1) (normals). In a certain number of cases in groups (5) and (6), estimations of the blood-sugar were performed, the blood from several animals, killed at the same time, being pooled for this purpose. It will be seen from the tables that, in general, the lower the blood-sugar, the higher is the p_H , both in brains frozen immediately and in those which have been incubated.

All the estimations of p_H were carried out at room temperature, which varied from 10 to 18°, in different experiments. The correction which would have to be applied, according to Adair, Cordero and Shen [1929], who were working with blood, to correct the values for this would be in such a direction as to make the values for 37° even more acid than those actually observed. In a few experiments which were performed at different temperatures, this

Table I.

<i>Group (1). Normal animals. Decapitated, and heads dropped directly into liquid air.</i>		<i>Group (2). Normal animals. Decapitated, and heads incubated at 37° for 2 hours (Exp. of 5. v. 32) and 45 mins. (Exps. of 6. v. 32 and 19. v. 32).</i>	
Date	p_H observed	Date	p_H observed
5. v. 32	6.868	5. v. 32	6.754
"	7.035	"	6.763
"	6.868	6. v. 32	6.665
12. v. 32	6.823	19. v. 32	6.670
"	6.858		6.677
"	6.888	Mean	6.704
"	6.892		
"	6.920		
18. v. 32	6.923		
Mean	6.897		
<i>Group (3). Animals given 0.5 cc. of 40 % glucose in water by intraperitoneal injection. Decapitated after 15 mins.; heads dropped directly into liquid air.</i>		<i>Group (4). Animals given 0.5 cc. of 40 % glucose in water by intraperitoneal injection. Decapitated after 15 mins.; heads incubated at 37° for 45 mins. (Exp. of 6. v. 32) and 60 mins. (Exp. of 30. v. 32).</i>	
Date	p_H observed	Date	p_H observed
30. v. 32	6.984	6. v. 32	6.510
"	6.984	"	6.440
"	6.958	"	6.391
Mean	6.975	30. v. 32	6.408
		"	6.454
		"	6.464
		Mean	6.444

Table I (*contd.*).

Group (5). Animals starved for 24 hours. Insulin (1 unit) injected subcutaneously. Decapitated after various intervals of time. Heads dropped directly into liquid air.

Date	Interval before killing	Symptoms	p_H observed	Blood-sugar mg./100 cc.	Mean p_H for given blood-sugar
13. v. 32	2 hours	+	6.978	—	7.029
"	"	+	7.042	—	
"	"	+	7.037	—	
"	"	+	7.037	—	
"	"	+	7.055	—	
18. v. 32	15 mins.	—	6.976	61.2	6.955
"	"	—	7.037		
"	"	—	6.853		
"	45 mins.	—	6.966	46.3	7.005
"	"	—	7.028		
"	"	—	7.023		
"	1 hr. 55 mins.	+	7.063	48.5	7.077
"	"	+	7.073		
"	"	+	7.041		
"	"	+	7.019		
"	"	+	7.079		
"	"	+	7.188		

Group (6). Mice starved for 24 hours. Given insulin as indicated in table. Decapitated, and heads incubated 1 hour at 37°.

Date	Insulin units	Interval before killing	Symptoms	p_H observed	Blood-sugar mg./100 cc.	Mean p_H for given blood-sugar
19. v. 32	0.1	15 mins.	—	6.855	74	6.931
"	"	"	—	6.982		
"	"	"	—	6.955		
"	"	45 mins.	—	6.977	39	6.954
"	"	"	—	6.931		
"	0.1 + 2.0	2.0 units given 3½ hrs. after 0.1 unit; killed after 4½ hrs.	+	6.923	26	6.934
"	"	"	+	6.923		
"	"	"	+	6.957		

was found to be the case for the tissue under investigation. No doubt, therefore, some change takes place rapidly in the tissue, immediately after excision, which renders it much more acid than it is during life. This does not necessarily invalidate deductions drawn from differences observed in the p_H of brains of animals which have been subjected to varying experimental treatment, and the results of the experiments do, in fact, fall into line with the previous observations on the behaviour of the brain-lactic acid. It is especially interesting that the values for frozen hypoglycaemic brains are actually higher than those for frozen normal ones, and this may possibly indicate that there is some actual p_H difference during life.

The actual decrease in p_H observed on incubation of the brains is shown in the following summary:

	Frozen	Incubated	Difference
Hypoglycaemic	7.029	6.934	0.095
Normal	6.897	6.704	0.193
Hyperglycaemic	6.975	6.444	0.531

It has previously been shown [Holmes and Sherif, 1932] that there is a rise in lactic acid on incubation of the brains, and that this rise is least with hypoglycaemic, greater with normal, and greatest with hyperglycaemic animals. It is clearly of interest to discover whether these changes in lactic acid content of the brain, which are known to occur, would, in fact, give rise to changes in p_H of the order here observed. Figures are available, both from the experiments already reported and from others performed since, for the increase in lactic acid to be expected in each of the three sets of conditions. In addition, the change in p_H to be expected from a given increase in lactic acid has been determined in the following way. The brains of 3 mice, weighing together approximately 1 g., were suspended in 10 cc. of unbuffered Ringer's solution, and the p_H was determined. 0.01 *N* lactic acid was added to the suspension in amounts of 0.05 cc. at a time, from a micro-burette, and the p_H determined after each addition. Titration curves were constructed, in this

Fig. 1.

way, for the brains of normal and hypoglycaemic animals, both after fixing in liquid air and after incubation. As was to be expected, the initial p_H differed in the different cases, so that the curves in various experiments did not coincide. They were, however, very nearly parallel with each other. Fig. 1 is a curve constructed from the mean values of six of these titration curves. Table II shows the expected increase in lactic acid, the observed fall in p_H , and the fall in p_H read off from the titration curve.

Table II.

	Increase in lactic acid mg. per g. tissue	Increase as cc. 0.01 <i>N</i> acid	Fall of p_H (from curve)	Fall of p_H (observed)
Hypoglycaemic	0.16	0.177	0.093	0.095
Normal	0.54	0.60	0.257	0.193
Hyperglycaemic	1.39	1.54	0.640	0.521

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For convenience, the increases in lactic acid are calculated in terms of cc. of 0.01 *N* acid per g. of tissue.

In the case of the hypoglycaemic animals, the agreement is good; the rise in lactic acid is small, but the relevant part of the titration curve (p_H 7.029 onwards) is steep. In the other two cases, all that can be said is that the changes observed and the changes calculated are of the same order.

SUMMARY.

The p_H values, determined *in vitro*, of mouse brains which have been fixed in liquid air are higher in the case of hypoglycaemic animals than of normal or hyperglycaemic ones. In all cases the p_H of incubated brains is lower than that of those which are frozen immediately.

The changes in p_H observed are of the same order as those calculated from the titration curve of brain tissue and lactic acid, allowance being made for the amount of lactic acid which would be liberated in the various circumstances considered.

REFERENCES.

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