CCIII. STUDIES ON THE METABOLISM OF ANIMALS ON A CARBOHYDRATE-FREE DIET.

III. EFFECT OF ALKALI ON THE SENSITIVITY TOWARDS INSULIN OF ALBINO RATS.

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IN a previous paper [Hynd and Rotter, 1931] it has been shown that the effect of a carbohydrate-free diet on the sensitivity to insulin varies with the species of animal employed for the experiment. Further, as the results obtained pointed to the conclusion that animals became resistant to insulin on diets which were capable of causing a lowering of the alkali reserve, the experiments now described were carried out with the object of obtaining further support for this view.

As Hawkins [1924] has shown that rabbits and guinea-pigs show a great variation in acid-base equilibrium, while rats, on the other hand, exhibit considerable constancy both in p_H and CO_2 content of the whole blood, the rat was the animal selected for our experiments. Moreover, as we have found that piebald rats, though fed on the usual stock diet, vary considerably in their sensitivity to insulin, all the experiments described in this paper were carried out on standard albino rats of 120–160 g. body weight. The basis of the diets employed was bread and milk for the carbohydrate-fed animals, and cheese for the carbohydrate-free animals. To these diets were added varying amounts of different salts as detailed below, and after certain periods the animals were tested for their response to insulin. The experiments were so arranged that the effect of the salts on the response to insulin could be observed simultaneously in carbohydrate- and non-carbohydrate-fed animals, as well as in control animals on a normal diet without the addition of any salts.

EXPERIMENTAL.

(1) *Effect of sodium bicarbonate on cheese-fed rats.* Seven normal rats, which had been receiving the ordinary stock diet of bread and milk, were transferred to a diet of cheese to which was added 0.12 g. $NaHCO_3$ per g. of cheese. After 1 week the animals were tested with insulin: none was resistant.

The diet was continued for another week with 4 of the rats, and again all responded to the injection.

(2) *Effect of potassium bicarbonate on cheese-fed rats.* An experiment similar to the above was carried out with 8 rats, the sodium bicarbonate being replaced by an equivalent amount (0.14 g.) of potassium bicarbonate. On testing the animals with insulin after 1 week on this diet, all were resistant, but after 2 weeks they were found to have lost their resistance.

As the animals in this and in the preceding experiment lost weight, the second dose of insulin, being proportional to the body weight at the time of injection, was somewhat smaller than the first dose, so that the loss of resistance to insulin is all the more striking.

(3) *Effect of sodium bicarbonate on rats previously rendered resistant to insulin by a cheese diet.* Seventeen normal rats were fed on cheese for a fortnight and proved to be resistant to the usual dose of insulin. Each animal was then fed with cheese containing 0.12 g. NaHCO_3 per g. After 2 days on this diet, one animal was tested with insulin and found to be partially resistant. After 3 days, 4 rats were tested and found non-resistant; after 4 days, 7 other animals were tested and found non-resistant, and after 5 days, 5 other rats were also found non-resistant to insulin.

After 3 days, therefore, all the rats responded to insulin, although the behaviour was not quite typical. In almost every case the animals became cold and limp, and convulsions if they occurred were not of a violent type. Moreover, administration of glucose did not bring about a rapid recovery, and 3 of the animals died.

Similar results were obtained in an experiment with mice.

(4) *Effect of potassium bicarbonate on rats previously rendered resistant to insulin by a cheese diet.* Eight rats rendered resistant to insulin by a cheese diet were then given cheese and an amount of potassium bicarbonate equivalent to the sodium bicarbonate used in the preceding experiment.

After 5 days on this diet all the animals were still resistant. Four of the rats were continued on this diet for 9 days and still failed to respond.

Similar results were obtained in an experiment with mice.

(5) *Comparison of effect of (a) NaHCO_3 and (b) KHCO_3 on the blood-sugar, muscle- and liver-glycogen, and liver-fat of cheese-fed rats.* Owing to the striking difference obtained in the response to insulin after 5 days with potassium bicarbonate, as described in Exp. (4), compared with that after 3–5 days with sodium bicarbonate in Exp. (3), it seemed advisable to know the blood-sugar level, the muscle- and liver-glycogen content, and the liver-fat content of the animals in the two series.

Accordingly, 4 rats were kept on a cheese diet for 15 days and assumed to be resistant to insulin, as it seemed inadvisable actually to test the animals, lest the glycogen distribution might be affected thereby. As in 32 experiments we have encountered only 3 rats which under this treatment did not become resistant to insulin, the assumption seemed justifiable. Two of the rats were then fed on cheese containing 0.12 g. NaHCO_3 per g. for 4 days, and the other two on cheese containing the equivalent amount of KHCO_3 per g. for the same

period. That is, the NaHCO_3 pair when analysed would have lost their resistance to insulin, while the KHCO_3 pair would still be insensitive.

The analyses (Table I) show no significant differences.

Table I.

Rat	Diet	Response to insulin	Blood- sugar %	Muscle- glycogen %	Liver- glycogen %	Liver- fatty acids %
1	Cheese + NaHCO_3	+	0.175	1.0	1.4	5.4
2	" "	+	0.145	0.93	1.8	4.2
	Average...	+	0.16	0.965	1.6	4.8
3	Cheese + KHCO_3	-	0.122	0.79	0.87	3.7
4	" "	-	0.16	0.94	2.60	4.9
	Average...	-	0.141	0.865	1.73	4.3

(6) *Effect of sodium bicarbonate on carbohydrate-fed rats. (a) Small dose of NaHCO_3 per os.* Five rats from the stock cages were kept without food for 9 hours. Each animal was then given bread and milk containing 0.5 g. NaHCO_3 , and 15 hours later was tested for its response to insulin.

Four of the rats showed typical symptoms, though the effect, compared with that on a control animal receiving no alkali, was somewhat delayed in its development. Further, after the administration of glucose the animals did not recover well, and one of them, although repeatedly injected with glucose, never recovered sufficiently to be able to take food and died in about 6 hours after the insulin injection. One rat did not react, and, as this animal was still resistant to insulin after the alkali had been continued for a week, it was considered to be abnormal.

(b) *Large dose of NaHCO_3 per os.* Three rats were each given bread and milk containing 2 g. NaHCO_3 , and as in the preceding experiment then tested with insulin. All responded in a normal fashion, though the effect was slightly delayed.

(c) *Subcutaneous injection of NaHCO_3 .* Two rats on a bread and milk diet were injected subcutaneously with 2 cc. of 8 % solution of sodium bicarbonate, and $\frac{1}{2}$ hour later given insulin. In both, typical symptoms ensued very rapidly, as in 1 hour after the insulin injection glucose had to be administered to relieve the convulsions, whereas a control animal which received a corresponding dose of insulin showed no outward sign of hypoglycaemia until $1\frac{1}{2}$ hours later.

(d) *Effect of alkali when continued for 1-2 weeks.* To the ordinary daily ration of bread and milk 0.5 g. NaHCO_3 was added and fed to each of 4 rats. Four other rats each received an equivalent amount of KHCO_3 , and 3 rats were given a larger amount of NaHCO_3 , viz. 2 g. each daily.

The animals took a sufficient quantity of food and remained almost steady in weight. Meyer and Cohn [1911] state that the addition of potassium bicarbonate to the diet of a rat causes the animal to lose weight rapidly, while with sodium bicarbonate rats put on weight. No difference in this respect was detected in the animals fed on the two alkalis. The examination of the urine

collected was rendered of rather doubtful value, as, though the animals took the diet well, there was always a considerable amount of food scattered. The urine in every case was found to be strongly alkaline and to reduce Benedict's solution. In the case of the rats receiving the larger amount of NaHCO_3 , small amounts of acetone bodies were detected.

After 1 week on these diets, the animals were injected with insulin and all responded in a more or less normal manner. The rats which had received KHCO_3 differed from those treated with the sodium salt in that, though they had more violent convulsions, they made more rapid and more complete recoveries. On the other hand, the animals which had received sodium bicarbonate made slow recoveries from the effects of the insulin injection, relapses were frequent, and 2 of the rats were found dead the following morning.

The experiment with the rats on the potassium bicarbonate was continued for a second week and the animals were re-tested with insulin. A normal response again resulted in every case, though the development of symptoms was slightly delayed.

Animals treated similarly to those just described were also employed for determinations of blood-sugar, muscle- and liver-glycogen and liver-fat. The average values obtained are given below in Table II, and for comparison values are quoted from our previous paper [Hynd and Rotter, 1930]. In addition, there is included the result of the following experiment. A rat, weighing 160 g. at the beginning of the experiment, was fed for 1 week on bread and milk to which were added 2 g. NaHCO_3 daily. A control animal of equal weight was fed for 1 week on bread and milk, so adjusted that the two rats were eating practically the same amount of food. Both were then starved for 24 hours before making the analyses.

Table II.

Daily diet contains	Duration of experimental feeding (weeks)	Period of fasting (hours)	Blood-sugar %	Muscle-glycogen %	Liver-glycogen %	Liver-fatty acids %
0.5 g. NaHCO_3	2	0	0.15	0.77	3.4	—
Control	2	0	0.16	0.77	3.4	3.4
2 g. NaHCO_3	1	0	0.14	0.78	2.6	2.4
Control	1	12	0.13	0.70	2.5	3.7
2 g. NaHCO_3	1	24	0.11	0.90	1.1	4.2
Limited ration	1	24	0.135	0.81	1.1	4.5

(7) *Effect of ammonium chloride on carbohydrate-fed rats.* Each of 4 rats was fed on bread and milk containing 1–2 g. NH_4Cl per day's ration. As the animals objected to this diet, the experiment was continued for only 4 days. At the end of this period the rats were tested for their response to insulin. Three of the animals were resistant but showed the early transitory sprawling similar to that previously described for rats rendered insensitive to insulin by a cheese diet. The fourth rat reacted to insulin. This animal had very severe convulsions, from which it made only a partial recovery though injected with the

usual amounts of glucose. It died suddenly 5½ hours after the administration of the insulin.

(8) *Effect of alkali on rats previously rendered resistant to insulin by feeding ammonium chloride.* Four rats were rendered resistant to insulin by feeding ammonium chloride as described in the preceding experiment. Two of the animals were then given bread and milk containing sodium bicarbonate (1.2 g. NaHCO_3 each daily). The other two received the equivalent of potassium bicarbonate. After 2 days the animals were no longer resistant to insulin. All responded in a normal manner, the symptoms being similar in both sodium- and potassium-fed animals.

DISCUSSION.

Several years ago Murlin and Kramer [1913] suggested that mobilisation of glycogen is favoured by an acid medium and its storage by an alkaline medium. The results of the foregoing experiments are in full accord with this hypothesis and prove beyond question that the acid-base balance is an important factor in determining the effectiveness of the pancreatic hormone.

The resistance to the action of insulin, which rats develop when maintained on a carbohydrate-free diet, can be altered by the addition of alkalis to the diet. Animals previously rendered resistant to insulin by a diet of cheese display a more or less normal sensitivity after 3 days' treatment with sodium bicarbonate. On the other hand, an equivalent amount daily of potassium bicarbonate has little, if any, effect even though continued for 9 days. Nevertheless, analysis shows (Table I) that the blood-sugar, the muscle- and liver-glycogen and also the liver-fat of a rat giving a response to insulin after the sodium bicarbonate treatment are practically identical with those of a rat still resistant to insulin after the ingestion of an equivalent amount of alkali in the form of potassium bicarbonate. Again, while sodium bicarbonate prevents a cheese diet from bringing about resistance to insulin, potassium bicarbonate fed along with cheese produces resistance in a shorter period (1 week) than does cheese alone (12–14 days). Continued feeding of potassium bicarbonate, however, eventually destroys the resistance to insulin, so that it appears that this difference between the two salts is only temporary.

Bunge [1873] concluded that the ingestion of large amounts of potassium led to an increased output of sodium, so that though large amounts of salts were being fed, the output might exceed the intake and a lowering of alkali reserve result. However, even though the high intake of potassium were continued, a point would soon be reached at which the body would retain its sodium, so that the alkali reserve would rise. Our results are explicable on this theory. Feeding potassium bicarbonate would produce a temporary period of low alkali reserve, during which the animals would be resistant to insulin, and this would be followed by a gradual increase in the alkali reserve with a consequent return of a more or less normal response. Feeding sodium bicarbonate on the other hand, would lead to a steady increase in the alkali reserve, as no

drastic elimination of potassium results, and consequently no resistant period would be observed. Richards, Godden and Husband [1927] find, however, that large doses of potassium do not lead in growing pigs to an increased elimination of sodium, or to an increased retention of potassium, but cause an increased excretion of calcium. Excess of sodium, on the other hand, was followed by retention of calcium, so that the Na/K ratio of a diet may have a very considerable influence on the calcium assimilation. The fact that Brougher [1927] has shown that insulin increases the blood-calcium may, therefore, be of importance. However, though certain experiments of Geiger and Kropf [1930] led them to form the opinion that a diet with a high calcium content favoured carbohydrate metabolism—a view supported by clinical experience—decreased calcium assimilation may play only a minor part in bringing about the resistance to insulin observed in our experiments. A simpler, although not complete, explanation is that owing to the diuretic effect of the potassium salt there is a smaller retention of total base when potassium bicarbonate is given than when sodium bicarbonate is the alkali used.

Further, it is to be noted that only on the acid-forming cheese diet does this difference between the effects of sodium bicarbonate and potassium bicarbonate become apparent. When rats are fed on the normal diet of bread and milk the only difference detected in the effect of the two salts on the response to insulin is that, as already pointed out, the animals receiving sodium bicarbonate are less liable to have convulsions and recover more slowly and less completely from the insulin symptoms. Probably in this series of experiments sodium bicarbonate increases the alkali reserve more quickly than does the potassium salt, but this point was not investigated as we were concerned mainly with the effect on the response to insulin.

As indicated in the experimental part, with one exception all the rats on the carbohydrate-diet with the addition of either sodium bicarbonate or potassium bicarbonate reacted to the insulin injection. Our results, therefore, are contrary to the findings of Goldblatt [1927], who reported that alkali inhibits the action of insulin. In his experiments, this worker used rats and also a rabbit which was admittedly abnormal. As he did not state the amount of alkali fed to each animal, it remains uncertain whether we have repeated his conditions, and in any case Goldblatt did not report on the action of insulin on rats fed for more than one day with alkali. Neither after a small dose nor a large dose of sodium bicarbonate *per os*, for one or more days, did we find the action of insulin inhibited, and administration of alkali subcutaneously definitely hastened and accentuated the response to insulin. We also failed to confirm the remarkable and almost complete disappearance of glycogen from both liver and muscle noted by Goldblatt to occur as the result of feeding sodium bicarbonate to rats for 3–8 days. Our values for the liver- and muscle-glycogen in rats fed with sodium bicarbonate are somewhat lower than those of controls, but the diminution is not striking, and we attribute the slight decrease to the fact that animals do not take the normal amount of bread and

milk when sodium bicarbonate is added to it. In other words, we are merely studying, in such experiments, the effect of slight undernutrition. This is indicated clearly by the results summarised in Table II, from which we conclude that alkali feeding does not reduce the liver- or muscle-glycogen content of carbohydrate-fed rats.

Goldblatt's contention that alkalis interfere with the action of insulin appears to receive support from the work of Haldane, Wigglesworth and Woodrow [1924, 2]. It is to be noted, however, that, though these workers found the ingestion of alkali to cause hyperglycaemia and glycosuria after less than half the normally tolerated dose of glucose, they did not actually demonstrate resistance to insulin. Moreover, the prolonged hyperglycaemia observed is not necessarily an index of defective insulin action [Cajori, Crouter and Pemberton, 1925], and further, Haldane and his co-workers draw attention to the fact that the symptoms of faulty sugar metabolism do not come on until long after the concentration of sodium bicarbonate in the blood is at its maximum. Goldblatt, further to support his view, also quotes the experiments of Hetényi [1926]. The results obtained by this investigator, however, not only in animal experiments but also with diabetic patients [Hetényi, 1927] point decidedly in the opposite direction, namely that alkali favours the action of insulin. Again Goldblatt's reference to the work of Koehler [1926] is misleading. While it is true that Koehler found huge doses of insulin to have no marked effect in several diabetic patients with marked acidosis (blood- p_H 6.8–7.2), Goldblatt omitted to add that "after alkali in the form of 3 % sodium bicarbonate solution was administered intravenously in just sufficient quantities to return the blood reaction to normal, the blood-sugar decreased very rapidly." Hédon [1925] also, as the result of experiments with a depancreatized dog, came to the conclusion that insulin and sodium bicarbonate are together required to restore the alkali reserve and health in cases of diabetes. Moderate dosage of sodium bicarbonate is also approved of by Campbell and Macleod [1925].

On the other hand, as it has been proved that insulin increases the alkali reserve, it is held by many and emphasised particularly by Joslin, that it is not only unnecessary but harmful to give alkali in diabetes. However, this assumes that the insulin is exerting its effect. If the conditions are such that the action of insulin is weakened in a marked degree, insulin treatment alone evidently will not correct the alkali reserve. In other words, it is essential that the alkali reserve to begin with be not too far reduced, otherwise the secreted or injected insulin will be rendered ineffective.

Further support for the view that the action of insulin is favoured by alkali and inhibited by acid is furnished by our experiments with ammonium chloride. The addition of this salt to the bread and milk diet very quickly produces in rats a severe acidosis, and even though abundant carbohydrate is supplied in the diet, there is established simultaneously a marked resistance to insulin, which, moreover, is easily corrected by replacing the ammonium chloride with either sodium or potassium bicarbonate, although here again the sodium salt

is more effective. At the same time, it must not be forgotten that an ammonium chloride acidosis is accompanied by an excessive excretion of water and also of sodium and potassium ions [Haldane *et al.*, 1924, 1]. Moreover, though the absolute loss of potassium may be less than that of sodium, the relative loss of potassium is much greater than that of sodium. Consequently, the Na/K ratio may be altered greatly. This may have an important effect on the action of insulin, especially as it is here shown that there is, for a time at least, a marked difference in the effects produced by sodium and potassium bicarbonates, both as regards their ability to dispel resistance to insulin and also in the type of insulin response which may be elicited.

In dealing with the resistance to insulin reference should perhaps be made to the well-known fact that fat depancreatized animals are much more difficult to control with insulin than are lean animals. This appears to us not to be due to a hyperglycaemia being maintained by a continuous conversion of fat into carbohydrate [Macleod, 1930], but rather to the tendency which fat animals show to develop acidosis and thereby render the insulin less effective. A similar explanation, that is, a greater tendency to acidity, seems applicable to the so-called resistant types of human diabetes. Lawrence [1927] holds that such cases of diabetes are either infected ones, or complicated by over-activity of the thyroid or suprarenals. While admitting that these glands are recognised antagonists to the pancreas, the conditions indicated are just those which would be accompanied by excessive acidity.

In conclusion it may be pointed out that the results of the present investigation suggest an alternative explanation to that usually given as to why glucose produced endogenously appears less stimulating to insulin production than ingested carbohydrate [Lawrence, 1926]. There is a large body of evidence to support the view that a raised blood-sugar is the normal stimulus to the production of insulin by the pancreas. Why then does the hyperglycaemia occurring in the later stages of starvation, when the metabolism of protein increases [Morgulis and Edwards, 1924], or that which results when a normal animal is maintained on an excess fat diet not stimulate the output of insulin and thereby correct the hyperglycaemic condition? The answer seems evident, for the formation of glucose from sources other than carbohydrate involves the simultaneous production of acid substances. Thus, though the glucose produced furnishes the necessary stimulus to the pancreas and an adequate secretion of insulin occurs, the acid character of the medium will interfere with the effectiveness of the insulin, and, at the same time, as shown by Abderhalden and Wertheimer [1924], will facilitate the action of adrenaline. The net result therefore would be an increased blood-sugar by a further depletion of the glycogen stores. The present tendency to increase the amount of carbohydrate and diminish the amount of fat in the diet of diabetics [Rabinowitch, 1930; Poulton, 1931] is thus shown to be founded on a sound basis. Further, as patients so treated keep well and require much less insulin, the clinical results support the views advanced in this communication.

SUMMARY.

Rats previously rendered resistant to insulin by a cheese-diet soon become responsive after the addition of sodium bicarbonate to the diet, but remain resistant after the ingestion of the equivalent amount of potassium bicarbonate.

Sodium bicarbonate is also more efficient than potassium bicarbonate in preventing the development of resistance to insulin in rats on a carbohydrate-free diet.

This striking difference between the effects of the bicarbonates of sodium and potassium is shown to bear no relationship to the carbohydrate stores of the animal. The acceptance of Bunge's theory of sodium impoverishment by potassium feeding furnishes a possible explanation.

Administration of sodium bicarbonate does not reduce the liver- or muscle-glycogen of carbohydrate-fed rats, and it favours rather than inhibits the action of insulin.

An ammonium chloride acidosis is accompanied by marked resistance to insulin, which readily disappears after the addition of either sodium bicarbonate or potassium bicarbonate to the diet.

The results, taken as a whole, suggest that, even with abundant carbohydrate to stimulate the production of insulin and presumably with a normal secretion of insulin, the activity of the pancreatic hormone will be interfered with if the medium be too acid.

The clinical importance of the results obtained is discussed.

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