CXXXII. THE EFFECT OF ALKALOSIS ON THE EXCRETION OF CHLORIDE AND ON CARBOHYDRATE METABOLISM.

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THE phenomena accompanying alkalosis have received considerable attention in recent years, both because of their clinical bearing in such cases as tetany and because of certain peculiar consequences of theoretical interest.

Apart from its occurrence in pathological conditions alkalosis may be induced by two main methods in the normal subject: (a) by overbreathing, (b) by administration of alkalis. The former method is a severe one for the subject, as if it is persisted in for any length of time marked symptoms of tetany are produced. The expulsion of CO_2 from the alveoli leads to an uncompensated alkalosis and the p_{H} of the blood may change markedly as the attempt to compensate becomes futile. The attempt to compensate consists in lowering the volume of expired air and the excretion of alkali in the urine. In addition, there occurs an excretion of ketonic substances in the urine.

In inducing alkalosis by the administration of alkalis the effects produced are much milder because the compensation of the condition is much more effective. Large doses of sodium bicarbonate or acetate may be taken without any obvious disturbance other than an occasional diarrhoea and, of course, marked thirst. The compensatory mechanism again consists in diminished breathing, retention of CO_2 in the alveoli and excretion of large quantities of alkali in the urine.

No symptoms of tetany have been seen in the experiments in this paper.

Of course, in a case of lowered kidney efficiency, a flooding of the tissues with alkali without rapid excretion may bring about tetanic symptoms. Such cases are on record.

As in the case of alkalosis following overbreathing, the alkalosis following alkali administration is sometimes followed by ketosis.

The production of ketosis in alkalosis has been explained in two principal ways: (1) that the alkali in being excreted carries with it the ketonic acids from the seat of their formation, that is a so-called "washing-out" effect; (2) that it is due to a disturbance of carbohydrate metabolism. It is clear that the determination of the correct view is of the first importance in justifying or condemning alkali therapy in diabetes, or other conditions accompanied by acidosis.

In this paper the method of inducing alkalosis was by administration of alkali by mouth. The questions which are considered are the following.

(1) What is the rate of excretion of sodium bicarbonate in normal man after different doses?

(2) Under what conditions is the alkalosis accompanied by ketosis?

(3) The effect of alkalosis on ketosis in starvation.

(4) The effect of alkalosis on carbohydrate metabolism.

(5) The effect of alkalosis on the action of insulin.

(6) The effect on glycogen formation.

The methods of analysis were the following.

(1) For the determination of bicarbonate in the urine the apparatus of Van Slyke for blood-gas analysis was used. The urine was diluted so that 1 cc. gave not more than about 0.7 cc. of CO_2 on treatment with acid. The volume of CO_2 was found by direct absorption in normal NaOH , corrected and expressed as NaHCO_3 .

(2) Urine- and blood-chlorides were determined by Van Slyke's method.

(3) Blood-sugar estimations were made by MacLean's method. Urine-sugar was estimated by Shaffer-Hartmann's or Bertrand's method.

(4) Glycogen estimations were made by Pflüger's method. The animals used were white rats. They were killed by a smart blow on the head and immediate decapitation. Violent reflex spasms occurred for a few seconds. The liver was quickly removed, dropped into a weighed wide-mouthed flask and quickly weighed to the nearest centigram. With a little practice this can be done so quickly that the loss of water vapour is negligible.

The final filtrate was always made up to 100 cc. and 25 cc. were hydrolysed for 3 hours with HCl , the glucose being estimated by Bertrand's method.

While the liver was being weighed the carcass was rapidly skinned, eviscerated, weighed, minced, and about 40 g. worked up for glycogen. The final filtrate was made up to 100 cc. and 25 or 50 cc. were hydrolysed.

(5) Phosphates in the urine were determined by Briggs' modification of the Bell-Doisy method.

(6) Respiratory quotients were determined by means of the Douglas bag and the Haldane apparatus.

(7) Alveolar CO_2 values were obtained by averaging the analyses of the inspiratory and expiratory samples.

Exp. 1. The excretion of NaHCO_3 in a normal man. The subject was the writer. A meal had been taken at 9.30 a.m. At 12 noon 10 g. NaHCO_3 in 100 cc. water were ingested.

No disturbance of any kind was experienced and there was no ketosis. The concentration of NaHCO_3 in the urine reached a maximum in 2 hours and remained practically constant for the following 2 hours. The total excretion of alkali in $4\frac{1}{2}$ hours was 2.58 g. and 8 hours later the urine was still strongly alkaline to litmus. The excretion of NaCl must be compared with the ordinary excretion after the morning meal.

Urine					
Time	Vol. cc.	NaHCO ₃ g.		NaCl g.	
		%	Total per hour	%	Total per hour
11.47 a.m.	57	—	—	1.485	—
12.00 noon	6	—	—	1.695	0.471
12.30 p.m.	24	0.135	0.065	1.594	0.766
1.00 "	43.5	0.842	0.732	1.083	0.942
1.30 "	42	0.914	0.768	0.960	0.806
2.00 "	42	1.021	0.858	0.906	0.762
2.30 "	32	1.093	0.700	0.854	0.546
3.00 "	26	1.111	0.578	0.765	0.398
3.30 "	26.5	1.111	0.589	0.765	0.404
4.30 "	44	1.052	0.463	0.800	0.352

Exp. 2. The excretion of chloride and inorganic phosphate after the morning meal. Breakfast 9.30 a.m. 100 cc. water at 12.20 p.m. Subject M. W. G.

Urine					
Time	Vol. cc.	NaCl g.		Inorganic phosphate mg. P	
		%	Total per hour	%	Total per hour
11.20 a.m.—	52	1.465	0.762	26.64	13.85
12.20 p.m.	58	1.646	0.955	17.30	10.03
1.20 "	50	1.571	0.789	48.62	24.31
2.20 "	56	1.594	0.893	57.81	32.37
3.20 "	47	1.566	0.736	56.42	26.52
4.20 "	39	1.472	0.574	64.66	25.22

It is now seen that although the kidney was slowly excreting NaHCO₃ in Exp. 1 it was beginning to find difficulty in excreting chloride. This will be seen much more markedly in the next experiment.

Exp. 3. The excretion of NaHCO₃, NaCl and inorganic P after three doses of NaHCO₃ of 10 g. each at 11.38 a.m., 12.33 p.m., 1.35 p.m.; each dose in 40 cc. water. Breakfast at 9.30 a.m.

Urine							
Time	Vol. cc.	NaHCO ₃ g.		NaCl g.		Inorg. P mg.	
		%	Total per hour	%	Total per hour	%	Total per hour
11–11.30 a.m.	31	—	—	1.46	0.90	32.3	20.64
12.30 p.m.	68	0.466	0.317	1.30	0.88	12.8	8.70
1.30 "	53	1.606	0.851	0.56	0.30	30.9	32.75
2.30 "	91	1.820	1.656	0.268	0.24	34.6	31.49
3.30 "	117	2.998	3.508	0.218	0.26	23.4	27.38
4.30 "	90	2.922	2.630	0.206	0.19	26.4	23.76
5.30 "	54	2.656	1.434	0.150	0.08	38.7	20.90
6.30 "	44	2.427	1.068	—	—	56.7	24.95
7.20 "	22	1.898	0.500	—	—	80.0	17.6

In this experiment about 12 g. of NaHCO₃ were excreted in 7 hours and the urine was still alkaline at noon the following day.

There was no ketosis throughout this experiment and the compensation of the alkalosis was perfect. The marked fall in the output of chloride was very striking and has been observed in every experiment in which the kidney

was made to excrete large amounts of alkali. This fall may be variously interpreted and without entering into a discussion on the matter on this occasion we may briefly say that there seem to be two possible views.

(1) The kidney may only be capable of excreting up to a certain limiting concentration of ions, which might be calculable from a summation of the molar concentrations of each electrolyte in the urine multiplied by its ionisation coefficient. The work of Adolph [1923] in this connection is suggestive.

(2) Although we have given our chloride figures as NaCl it must be remembered that after all what is estimated is chlorine ions, *i.e.* negative ions. It may be that the retention of chlorine is a compensatory mechanism to deal with the alkalosis.

No appreciable fall in the excretion of inorganic phosphate has been found in alkalosis. Evidence has also been obtained that there is no interference with the excretion of inorganic sulphate.

The following experiment was carried out to see if any evidence of retention of chlorine in the blood could be obtained in alkalosis.

Exp. 4. Breakfast 9.15 a.m. 30 g. NaHCO_3 in 150 cc. water at 1.15 p.m. Blood taken from arm vein for blood- and plasma-chlorides, collected under oil.

Time	Vol. cc.	Ke- tones	Urine				Blood	
			NaHCO_3 g.		NaCl g.		NaCl g. %	
			%	Total per hour	%	Total per hour	Whole blood	Plasma
10.15 a.m.—								
1.15 p.m.	166	—	—	—	1.715	0.949	0.478	0.598
2.15 „	75	—	0.888	0.666	1.030	0.773	0.447	0.579
3.40 „	170	±	1.844	2.213	0.399	0.479	0.471	0.603
4.55 „	164	+	2.814	3.692	0.297	0.389	—	—
5.30 „	63	+	2.407	2.600	0.220	0.238	—	—
8.15 „	217	+	3.240	2.557	0.208	0.164	—	—
9.55 „	125	±	1.815	1.361	0.246	0.185	—	—

In the 8½ hours of this experiment the total excretion of NaHCO_3 was 19.23 g., the maximum hourly excretion being 3.69 g. It will be observed that the mild ketosis appeared when the concentration of alkali in the urine was 1.84 %. This ketosis was associated with only 4–5 mg. % of acetone. This became more marked as the excretion of alkali continued but at 9.55 p.m. when the concentration of bicarbonate was 1.815 % the reaction was only just perceptible. This matter will be returned to later. The chloride excretion as before fell very markedly, but the blood chlorides, whilst indicating some initial dilution of the blood, soon returned to the original level both in whole blood and plasma.

After a dose of 30 g. of bicarbonate one very soon feels a large accumulation of fluid in the intestine. This fluid is fairly certainly rich in chloride which probably makes a large call on the store of salt in the body. The blood-chloride is, as we have seen, kept up to normal levels and it appears that the kidney is responsible for this by refusing to excrete chloride in these conditions at the same rate as it would normally.

In the next experiment a period of starvation was undergone before inducing alkalosis in order to see how far the diminution in chloride excretion could be pushed.

Exp. 5. (a) Chloride excretion in a normal man after $13\frac{1}{2}$ hours' starvation. 100 cc. water at 11.30 a.m. Subject M. W. G.

Time	Urine		
	Vol. cc.	NaCl g.	
		%	Total per hour
9-11.30 a.m.	86	1.280	0.440
12.30 p.m.	41	1.371	0.562
1.30 "	53	1.443	0.765
2.30 "	42	1.226	0.515
3.30 "	34	1.191	0.405
4.30 "	23	0.938	0.216
5.30 "	23	0.764	0.176

(b) Effect of bicarbonate ingestion on chloride excretion in a normal man after 14 hours' starvation. Subject M. W. G. 25 g. NaHCO_3 in 100 cc. water at 12 noon.

Time	Vol. cc.	Rothera test	Urine			
			NaCl g.		NaHCO_3 g.	
			%	Total per hour	%	Total per hour
12.00 noon	78	—	1.006	0.392	—	—
1.00 p.m.	51	—	0.934	0.476	0.575	0.293
2.00 "	88	—	0.430	0.378	1.978	1.741
3.00 "	68	—	0.340	0.231	2.427	1.650
4.00 "	50	—	0.278	0.139	2.445	1.223
5.00 "	46	—	0.245	0.113	2.481	1.141
5.45 "	32	+	0.232	0.099	2.660	1.135

The fall in chloride excretion in *(b)* is very marked and levels are reached which ordinarily are only attained after 40 hours' starvation. In this experiment the total excretion of alkali was 7.2 g. in 6 hours and the urine was alkaline late the following day. A definite ketosis appeared $5\frac{3}{4}$ hours after the NaHCO_3 was taken, when there was 2.66 % in the urine. The intensity of the ketosis was such as to make it very unlikely that it was due to a "washing-out" effect. In *Exp. 4* the ketosis was more intense when the urine contained 1.84 % NaHCO_3 than when in the above experiment the urinary alkali was 2.44 %.

In fact it seems that the more rapidly the alkali is excreted the less likelihood is there that a ketosis will appear.

Further, the value of the very small quantities of ketones excreted in these conditions is negligible as a neutralising mechanism.

In *Exp. 5 (b)* the minimum hourly output of chloride is seen to be 0.099 g. The following experiment was carried out to see if values below this could be obtained by increasing the period of starvation.

Exp. 6. (a) The excretion of chloride after 38 hours' starvation. Subject M. W. G. 100 cc. water at 8 a.m.

Time	Urine		
	Vol. cc.	NaCl g.	
		%	Total per hour
9.05-10.00 a.m.	24	0.412	0.108
11.10 a.m.	29	0.483	0.120
11.45 "	16	0.395	0.108
12.00 noon	7	0.412	0.116
12.30 p.m.	12	0.412	0.099
1.00 "	13	0.332	0.086
1.30 "	13	0.365	0.095
2.00 "	16	0.355	0.114
2.30 "	13	0.254	0.066
2.45 "	9	0.294	0.106
3.15 "	15	0.370	0.111

The hourly excretion of chloride is seen to be very steady during the 6 hours of the experiment, but this is not always the case. In the following table the chloride output is much higher but the dose of NaHCO_3 brings it down to minimal levels in about 2 hours.

(b) A fast of 41 hours was undergone and 20 g. of NaHCO_3 in 100 cc. water were taken at 11.30 a.m. Subject M. W. G.

Time	Vol. cc.	Urine			
		NaCl g.		NaHCO_3 g.	
		%	Total per hour	%	Total per hour
9.30 a.m.	188	0.295	0.052	—	—
11.00 "	52.5	0.781	0.273	—	—
11.30 "	14.5	0.797	0.231	—	—
12.00 noon	17	0.720	0.245	—	—
12.35 p.m.	32	0.510	0.280	0.950	0.54
1.08 "	51	0.369	0.342	1.127	1.045
1.15-1.45 p.m.	30	0.217	0.130	1.118	0.671
2.10 "	31	0.126	0.094	1.478	1.100
2.41 "	35.5	—	—	2.084	1.480
3.11 "	42	0.152	0.128	2.264	1.902
3.35 "	24	0.171	0.103	2.290	1.374

It is seen that even after 41 hours of complete fasting and when the kidney in addition is excreting as much as 1.9 g. NaHCO_3 per hour the output of chloride is still at the rate of about 0.1 g. per hour. This minimum is rarely diminished and it would appear that the concentration at which the alkali is excreted is limited by this minimum of chloride. Summation of the molar concentrations of the bicarbonate and chloride within certain limits gave figures of some constancy in particular experiments.

For example, the following figures were obtained in one case after 30 g. NaHCO_3 : 0.027, 0.029, 0.033, 0.034, 0.026, 0.023, 0.024, 0.016.

These values were not corrected for ionisation. The failure in constancy occurred generally during the fall in bicarbonate excretion, the recovery in the chloride being much slower than the fall in the alkali.

Exp. 7. Seeing that the ingestion of alkali brings about a markedly diminished excretion of chloride, it was of interest to see if a similar effect could be demonstrated in the stomach.

Mr W. J. Griffiths of this laboratory, who is very expert in gastric analysis, kindly undertook to be the subject of this experiment.

The experiment was started $4\frac{1}{2}$ hours after a moderate breakfast. The stomach-tube was passed and the stomach washed out until the washings were clear. 25 g. of NaHCO_3 were taken in 100 cc. water at 1 p.m. Gastric and urinary samples were taken about every 15 minutes.

Time	Gastric contents		Time	Urine		
	Reaction to Congo red	% NaCl		Vol. cc.	NaCl g. per hour	NaHCO_3 g. per hour
1.15 p.m.	Neg.	0.317	8.30 a.m.—	316	0.621	0.020
1.30 "	Neg.	0.317	1.00 p.m.	55	1.335	0.033
1.45 "	Neg.	0.311	1.15 "	21	0.454	0.063
2.00 "	Neg.	0.355	1.30 "	82.5	1.243	0.833
2.15 "	Neg.	0.437	1.47 "	13.5	0.298	0.703
2.34 "	Neg.	0.466	2.00 "	37	0.789	1.751
2.45 "	Neg.	0.484	2.15 "	40	0.440	1.760
3.00 "	Neg.	—	2.34 "	33	0.363	1.760
3.15 "	Neg.	0.640	2.45 "	26.5	0.389	1.698
3.30 "	Neg.	0.638	3.00 "	22	0.303	1.293
3.45 "	Acid	—	3.15 "	—	—	—
4.15 "	Acid	—	3.30 "	—	—	—

The steady rise in chloride in the stomach, accompanied by a fall in the urinary chlorides, makes it clear that whatever the mechanism for sparing chloride may be the gastric glands do not assist in its maintenance. We may further regard these values of stomach chlorides as corroborating the conclusion that the supply of chlorine from the blood is not interfered with.

THE EFFECT OF ALKALOSIS ON CARBOHYDRATE METABOLISM AND ON KETOSIS.

It was shown by Haldane and his co-workers [1924] that alkalosis had some inhibitory effect on carbohydrate metabolism. Alkalosis, whether induced by overbreathing or alkali ingestion, lowers the normal tolerance to carbohydrate, that is to say, that doses normally producing a good rise in R.Q. and not followed by glycosuria gave rise in alkalosis to glycosuria and practically no change in the respiratory metabolism. Accompanying these phenomena, markedly abnormal blood-sugar curves were found.

The following experiments were designed to throw further light on this question.

Exp. 1. This experiment was carried out to see if the mere presence of alkali in the gut affected sugar metabolism. $5\frac{1}{2}$ hours after a light meal 25 g. of NaHCO_3 in 100 cc. water were ingested and 14 minutes later 50 g. of glucose were taken. Subject M. W. G. Alkali at 2.46 p.m. Sugar at 3 p.m.

Time	Blood-sugar %	Urine			
		Vol. cc.	Rothera test	NaHCO ₃ g.	
				%	Total per hour
2.20 p.m.	—	181	—	—	—
2.45 "	0.087	—	—	—	—
3.00 "	0.090	12	—	0.009	0.001
3.30 "	0.141	19	—	0.119	0.025
4.00 "	0.135	33	—	0.779	0.514
4.30 "	0.090	45	—	1.383	1.224

The blood-sugar values of this subject in analogous conditions without alkali are of the following order: 0.102, 0.150, 0.125, 0.095, 0.090 %. Thus it appears that the mere administration of alkali and an almost immediate ingestion of sugar do not give rise to an abnormal blood-sugar curve; nor was there any appearance of sugar in the urine. The situation is, however, quite different if a period of some hours is allowed to elapse between the taking of the alkali and the sugar. This was done in the next experiment.

Exp. 2. The conditions were similar to those in *Exp. 1.* 30 g. NaHCO₃ were taken at 12 noon and 50 g. glucose at 3.35 p.m. Subject M. W. G.

Time	Blood-sugar %	Urine				
		Time	Vol. cc.	Rothera test	Glucose g. %	NaHCO ₃ g. %
12.00 noon	0.133	—	—	—	—	—
1.30 p.m.	0.115	1.32 p.m.	285	—	—	1.341
3.10 "	0.102	1.50	—	—	—	—
4.5 "	0.146	2.35	113	±	—	2.019
4.35 "	0.171	3.30	76	+	0.034	2.430
5.05 "	0.120	4.07	38	+	0.034	2.502
5.35 "	0.090	4.37	23	+	0.466	1.841
		5.10	34	+	0.140	1.573
		5.41	—	±	0.070	1.090

The blood-sugar curve here produced is clearly of the so-called lag type. There is a smart rise above the threshold and a rapid readjustment to normal levels. With larger doses of alkali the curve can be made to reach higher levels and the return to resting values can be delayed by some hours. Traces of reducing substances were present in the urine between 5.10 and 5.41 p.m. when the blood-sugar was certainly below 0.12 %. Some slight effect on the threshold value of glucose seems to have been produced but it is difficult to be sure about this.

It is to be observed that the storage of sugar was in full activity at a time when 1.84 % of NaHCO₃ was being excreted by the kidney. Further, throughout the whole experiment only 6.4 g. of alkali were excreted, so that, even allowing for the loss which certainly takes place by the bowel, there was still a great deal being absorbed.

It would appear then that the interference with sugar storage takes place most markedly at a time when the excretion of alkali is at a maximum. The effect can pass off rapidly if the removal of the alkali *via* the kidney is sufficiently quick to prevent any accumulation of it above a certain optimum level in the tissues. It is also seen that a definite ketosis appeared in spite of

the administered glucose. This ketosis reached a maximum when the interference with adequate sugar metabolism was most pronounced and almost disappeared as the excess alkali was thrown out.

There can be little doubt that alkalosis when well established produces a definite diminution in the power to store sugar. In the next experiment evidence is presented that there is also some interference with oxidation as indicated by respiratory quotient determinations.

Exp. 3. The effect of alkalosis in a case of markedly increased sugar tolerance. Subject M.

The case chosen was a eunuchoid whose sugar tolerance had been examined on two previous occasions. On the first occasion, 19. iii. 1926, 50 g. of glucose 12 hours after food gave rise to the following blood-sugar values at half-hourly intervals: 0.075, 0.104, 0.113, 0.091, 0.085 %. On the second occasion, 22. ii. 1927, the R.Q. values were also obtained:

Blood-sugar %	0.072	0.100	0.104	0.113	0.081
R.Q. ...	0.88	1.01	1.02	0.99	0.95

On 1. iii. 1927, 40 g. NaHCO_3 were given at 11.0 a.m. and 50 g. glucose at 12.30 p.m. The conditions were similar to those in the previous tests.

Time ...	10.30	11.30	12.00	12.30	1.00	1.15	1.30	2.00	2.30	3.00	3.30
Blood-sugar %	0.085	0.090	0.095	0.106	0.135	0.122	0.120	0.120	0.133	0.111	—
R.Q. ...	0.86	—	0.91	—	0.92	—	0.876	0.895	—	—	—

Such blood-sugar values have never been found in this patient under ordinary conditions and there can be little doubt that the storage mechanism for sugar has in some way been rendered less active than normally. Further, the rise in R.Q. was much below that which occurred in the control experiment and we must suppose that oxidation was interfered with, although by no means completely inhibited.

Exp. 4. The effect of alkalosis in a case of severe glycosuria without ketosis. Subject Mrs V.

The case chosen was a woman of 50 years weighing 15 st. 10 lb. and still gaining weight slowly. A dose of 75 g. of glucose 14 hours after food gave the following blood-sugar values at half-hourly intervals: 0.336, 0.327, 0.400, 0.376, 0.312 %, and in the $2\frac{1}{2}$ hours of the experiment the urine contained 24.5 g. glucose.

This patient constantly excreted between 100 and 120 g. sugar a day even on a restricted diet. She had no ketosis at any time during 12 months' intermittent examination. It must apparently be supposed that in this type of case adequate fat metabolism is maintained by means of carbohydrate formed from either fat or protein or both, and that this is more readily utilisable than ordinary exogenous carbohydrate.

In this experiment a light breakfast was taken by the patient at 8 a.m. and a dose of 30 g. of NaHCO_3 was given at 11.38 a.m.

Time	Urine						
	Vol. cc.	Sugar g. %	Total sugar (g.)	Rothera test	NaCl g. per hour	NaHCO ₃ g. %	R.Q.
10.55 a.m.	185	5.83	10.79	—	0.335	—	0.74
11.35 „	152	8.25	12.54	—	0.748	—	—
11.38 „	30 g. NaHCO ₃ in 100 cc. water						
1.00 p.m.	400	4.70	18.80	+	1.028	0.523	0.70
2.07 „	282	3.30	9.31	+	0.488	1.125	0.72
3.05 „	173	3.15	5.45	+	0.230	1.079	—
4.05 „	142	—	—	+	0.145	1.033	0.70
5.00 „	78	—	—	+	0.083	0.888	—

The blood-sugar values were:

Time ...	...	11.20	12.00	2.05	3.00	4.00	4.50
Blood-sugar %		0.457	0.434	0.347	0.349	0.304	0.316

In 4½ hours 56.89 g. of sugar were excreted after only a very light meal at 8 a.m., but it is seen that even with 8.25 % of glucose in the urine there was no trace of ketonic acids. Judging from the R.Q. very little sugar was being metabolised, but even this little was thrown out of action by the alkali and a ketosis was established 1½ hours after the NaHCO₃ was taken. Still it must be observed that the amount of ketones excreted was not such as one would associate with a complete inability to metabolise sugar. Anti-ketogenic substances must still have been present and in action.

The total bicarbonate excreted was 9.28 g. in 5½ hours which is not less rapid than in the normal subject. It is further to be observed that the excretion of sugar was not interfered with.

As in the previous experiments there occurred a striking fall in the urinary chlorides.

THE EFFECT OF ALKALOSIS ON THE KETOSIS OF STARVATION.

Administration of alkali to diabetics leads to an increased excretion of ketonic acids. The same thing occurs in the ketosis of starvation in normal subjects. The ketosis induced in rats by feeding on a high fat diet is much increased by alkali feeding. Lactic acid may also be increased in the urine in these conditions and it was tentatively suggested by Macleod and Knapp [1919] that these acids might be of importance in the regulation of blood reaction. As a matter of fact the quantities in which these acids are produced are so small that they can only be of very little value as a neutralising mechanism. In our view the increase in ketosis in alkalosis is an expression of an interference with carbohydrate metabolism.

The following experiment is regarded as strong evidence in favour of this view.

Exp. 5. The writer underwent a period of 41 hours' starvation after which a marked ketosis was present. It had been found on many previous occasions that such a ketosis can be completely destroyed by 50 g. glucose in about 1 hour and always in less than 1½ hours [Goldblatt, 1925].

In this experiment 20 g. NaHCO_3 were taken at 11.30 a.m. and 50 g. glucose at 12.45 p.m.

Time	Blood-sugar g. %	Urine				R. Q.	Alveolar CO_2 %	Acetone in expired air per 2 mins. mg.
		Vol. cc.	NaHCO_3 g. %	Glucose g. %	Acetone mg. per hour			
9.30 a.m.	—	188	—	—	10.60	—	—	—
10.12 "	0.075	—	—	—	—	0.67	—	0.286
10.30 "	—	—	—	—	—	—	5.585	—
10.45 "	0.076	—	—	—	—	—	—	—
11.00 "	—	52.5	—	—	35.70	—	—	0.182
11.20 "	—	—	—	—	—	—	5.655	—
11.30 "	—	14.5	—	—	24.36	—	—	—
11.46 "	—	—	—	—	—	0.68	—	—
12.00 "	0.077	17	—	—	24.62	—	—	—
12.20 "	—	—	—	—	—	—	6.075	0.092
12.35 "	—	32	0.95	—	45.42	—	—	—
1.05 p.m.	0.113	—	—	—	—	0.64	—	0.068
1.08 "	—	51	1.127	—	—	—	—	—
1.17 "	0.164	—	—	—	43.40	—	—	0.077
1.22 "	0.175	—	—	—	—	—	—	—
1.45 "	0.187	30	1.118	0.061	23.40	0.74	5.983	—
2.02 "	0.209	—	—	—	—	—	—	0.120
2.10 "	—	31	1.478	0.400	—	—	—	—
2.17 "	0.168	—	—	—	—	—	—	—
2.35 "	0.115	—	—	—	—	—	—	—
3.11 "	0.078	—	2.264	—	23.00	—	—	—

The findings in this experiment may be summarised as follows.

(1) The already diminished tolerance to sugar existing after starvation is accentuated in the alkalosed starving subject.

(2) The ketosis was most markedly increased during the second hour after the alkali was taken and when the concentration of NaHCO_3 in the urine was only about 1 %.

(3) The ketosis had not disappeared and was only slightly diminished 9 hours after the glucose was taken. In the table only the figures up to $2\frac{1}{2}$ hours after the sugar are given, but the nitroprusside test was still strongly positive $6\frac{1}{2}$ hours after this.

(4) The excretion of ketonic substances in the breath is greatly diminished under the influence of alkali.

(5) The rise in alveolar CO_2 is brought in as a compensatory effect and reaches a maximum long before the kidney reaches its maximum excretory function. The alveolar mechanism appears to be the first line of defence until the kidney gets rid of the excess bicarbonate. This fact is shown very well in the following table of an experiment in which the writer took 30 g. NaHCO_3 about 3 hours after a meal.

Time	...	...	11.48	12.45	1.13	1.55	2.35	3.20	4.57	5.40
Alveolar CO_2 %	...	...	5.37	5.58	5.89	6.38	—	6.45	5.94	—
Urinary NaHCO_3 g. %	0		0.744	1.822	2.564	2.676	2.137	3.243	2.764	

The evidence presented in the foregoing experiments leaves little doubt that alkalosis is accompanied by a definite diminution in carbohydrate storage and oxidation. The next logical step seemed to be to find if this effect might be

referred back to an interference with the effectiveness of insulin and further, whether the glycogenic function of the liver and tissues was in any way affected.

THE EFFECT OF ALKALOSIS ON THE ACTION OF INSULIN.

For this purpose rabbits and rats have been used. It will be unnecessary to give more than typical experiments. Similar results to my own have been obtained by Hetenyi [1926], who found that the effect of insulin is strongly delayed by alkali given *per os*. He also found that acid given *per os* had no appreciable effect on the action of insulin. The findings with alkali have been confirmed and we give the following experiment on a single rabbit, which was very striking.

This animal, which had been observed on several occasions, could have its blood-sugar lowered to values of 0.02 % without symptoms. As we had never seen such an animal before experiments were repeated several times with the following results.

Exp. 6. Effect of two units of insulin injected subcutaneously on the blood-sugar of the above rabbit. Weight 2 kg. There were no symptoms. No food, of course, was given during these experiments.

Time ...	...	2.25	2.40	3.10	4.15	4.45
Blood-sugar g. %		0.096	Insulin 2 units	0.062	0.020	0.020

Exp. 7. Same rabbit as above. Blood taken from veins of both ears.

Time ...	12.30	12.35	1.15	2.10	3.08	3.40	4.35	4.40
Blood-sugar g. %	...	Insulin 3 units	0.090	0.030	0.020	0.025	0.125	0.122

Exp. 8. In conditions similar to those of Exps. 6 and 7 the same rabbit was given 3.5 g. of NaHCO_3 by stomach-tube at 12 o'clock and insulin injected as below.

Time ...	11.20	12.00	12.08	12.55	1.35	2.20
Blood-sugar g. % ...	0.111	0.152	Insulin 4 units	0.177	0.131	0.122
Time ...	2.45	3.20	3.50	4.20	6.00	
Blood-sugar g. % ...	Insulin 4 units	0.072	0.056	0.067	No symptoms	

In this last experiment the initial rise of blood-sugar to 0.152 % was due to the passage of the stomach-tube. The first dose of insulin brought the blood-sugar down to 0.122 % in 2 hours 12 minutes. A further dose of four units only lowered it to 0.067 % in a further $1\frac{1}{2}$ hours, a level which could be attained by two units in $\frac{1}{2}$ hour starting at levels between 0.08 and 0.09 %. When it is considered that altogether eight clinical units were injected, it seems clear that the alkali has in some way definitely inhibited the action of the insulin.

It seems feasible to suppose that a similar sort of thing is occurring in the alkalosed human subject and that here lies the explanation of the intolerance to carbohydrate which accompanies alkalosis.

THE EFFECT OF ALKALOSIS ON GLYCOGEN STORAGE.

For this purpose white rats were used. The recent work of Macleod and co-workers [Barbour, Chaikoff, Macleod and Orr, 1927] on the glycogen distribution in rats under the influence of insulin is of great importance in showing how to obtain uniform controls in these animals. For our experiments, however, it soon became evident that absolutely uniform controls were unnecessary as the changes induced by alkali were remarkable.

White rats of between 130 and 140 g. weight were taken and put on a diet of bread and milk. This diet suits them admirably and good deposits of glycogen are soon laid down both in liver and muscles. After the required period of feeding the animals were weighed, tapped smartly on the head and immediately decapitated. The liver was quickly weighed and worked up for glycogen. The carcass was rapidly skinned, eviscerated, weighed and an aliquot part worked up for glycogen.

Exp. 9. Three rats on bread and milk. No glycosuria. No ketosis.

No.	Days on bread and milk	Weight (g.)	Weight of liver (g.)	Weight of skinned carcass (g.)	Glycogen (mg.)	
					Liver	Carcass
1	8	142	6.28	56	104	36
2	13	135	6.39	60	96	69
3	16	152	6.78	61.5	254	114

Exp. 10. Another four rats which had been on the same diet as those in *Exp. 9* were now given NaHCO_3 added to their bread and milk. The periods of alkalisation varied from 3 to 8 days. The animals took the bicarbonate well and passed considerable amounts of alkali in the urine. In one case 0.49 g. of NaHCO_3 appeared and in another 0.66 g. in one day. The following table gives the results of analysis of these animals.

No.	Days on bread and milk	Days on alkaline diet	Weight (g.)	Weight of liver (g.)	Weight of skinned carcass (g.)	Glycogen (mg.)	
						Liver	Carcass
4	29	3	149	5.83	71	Traces	13
5	33	7	135	4.81	60	Traces	Traces
6	34	8	132	4.86	63	26	Traces

The fourth alkalosed rat was now taken off the alkali and put on an ordinary bread and milk diet for 2 days. On the first day after the return to ordinary diet it excreted alkali but no acetone and there were 48 mg. (as glucose) of Cu-reducing substances in the urine. The urine also reduced alkaline safranin so that sugar was probably present. On the second day the urine was amphoteric, acetone was present and the reduction value was 53 mg. The rat was now killed and analysed.

Exp. 11. Rat no. 7.

Days on alkalisied diet	Days of return to ordinary diet	Weight (g.)	Weight of liver (g.)	Weight of skinned carcass (g.)	Glycogen (mg.)	
					Liver	Carcass
8	2	140	7.92	52	156	104

The above Exps. 9, 10 and 11 seem definitely to show that in conditions of powerful alkalosis the animals do not lay down glycogen but the readjustment to normal storage is rapid when the excess alkali is removed.

SUMMARY.

1. Utilising doses of NaHCO_3 up to 30 g. the maximum concentration of bicarbonate in the urine of a normal subject is about 3.2 %. The rise in bicarbonate excretion is constantly associated with a very marked fall in chloride excretion. The fall in chloride excretion is not accompanied by a fall in blood- or plasma-chloride. It is difficult to be sure whether this effect is due to an inability of the kidney to excrete chloride or to a diversion of tissue-chloride so that the "head" of chloride is removed.

2. The fall in urinary chlorides in alkalosis is not accompanied by a similar phenomenon in the stomach, where the secretion of chloride may be quite normal.

3. Alkalosis is accompanied by a definite diminution in carbohydrate tolerance and sometimes by a ketosis. The alkalosis-ketosis is not a mere "washing-out" effect, but is rather to be regarded as a result of defective carbohydrate utilisation.

4. The ketosis of starvation is much increased in alkalosis and the maximum excretion of ketonic acids does not necessarily coincide with the maximum output of alkali in the urine.

5. The ketolytic action of glucose in starvation is markedly diminished by alkali.

6. The action of insulin is diminished by alkali given *per os*.

7. The deposition of glycogen in liver and muscle can be greatly diminished by alkalosis.

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