CLXIX. THE INFLUENCE OF VARIATIONS IN SYSTEMIC ACID-BASE BALANCE UPON CARBOHYDRATE TOLERANCE IN NORMAL SUBJECTS.

By GEOFFREY THOMPSON¹, DAVID MICHAEL MITCHELL
AND LAWRENCE COLEMAN KOLB.

From the Department of Physiology and Biochemistry, Trinity College, Dublin.

(Received June 29th, 1933.)

THE relationship of changes in acid-base balance of the blood to carbohydrate storage has attracted the interest of several workers. Elias [1913], working with dogs, showed that the administration of HCl by mouth was followed by hyperglycaemia and glycosuria. Underhill [1916] showed that administration of acid tended to raise the blood-sugar, whereas alkalis reduced it. Hendrix and McAmis [1924] found that the alkalosis produced in dogs by the injection of hydrazine sulphate was associated with hypoglycaemia. Peters and Geyelin [1917], studying adrenaline hyperglycaemia in three human diabetics and two normal subjects, suggested that the hyperglycaemia and glycosuria were the results of an intermediate acidosis. Haldane [1924] reports "an upset of carbohydrate metabolism like that of diabetes" during the alkalosis induced by over-breathing or alkali ingestion. This observer also states that during ammonium chloride acidosis the ingestion of 100 g. of glucose produced hyperglycaemia and glycosuria.

The subject is one of considerable interest owing to the association of severe acidosis with human diabetes. It seems possible that the acidosis of diabetes, which is incidentally the severest acidosis met with in human beings except for some cases moribund from uraemia, may have a contributory effect upon the changes in carbohydrate metabolism encountered in this disease. Unfortunately most of the experimental work up to the present suffers from the obvious drawback of having been performed on laboratory animals and not on the human subject.

The object of the present investigation was to study carbohydrate tolerance in young healthy male adults during experimental acidosis of a degree comparable to that encountered in severe untreated diabetes. Such an acidosis can readily be induced by the oral ingestion of massive doses of ammonium chloride [Haldane, 1921]. Similar observations were performed on the same subjects during alkalosis induced by the ingestion of large amounts of sodium bicarbonate.

Method. Diet was kept constant throughout, except as regards water and salts. Metabolic changes occurring during acidosis and alkalosis were followed by estimations of the CO₂-combining power of the blood-plasma and of the total daily excretion of acid, chloride and ammonia in the urine. In each case there was a preliminary control period on normal diet, followed by a period of

¹ Rockefeller Research Fellow in Biochemistry.

Table I. *Urine analyses.*

Day	Volume cc.	Total daily output			CO ₂ plasma vols. %	Ammonium chloride or sodium bicarbonate ingested	Remarks
		Acid cc. N/10 NaOH	Ammonia cc. N/10 NaOH	Chlorides g. NaCl			
Subject 1:							
1	1325	239	599	11.6	—	Nil	—
2	1530	201	563	8.75	—	Nil	—
3	1220	220	488	4.9	63	Nil	Control glucose tolerance test
4	1740	400	974	16.2	—	15 g. NH ₄ Cl	Symptoms: headache, insomnia; continuous nausea, no actual vomiting. No dyspnoea. Only 17 g. on account of nausea. Better next day
5	2600	520	1625	27.0	—	20 g. NH ₄ Cl	
6	1960	643	2399	24.5	—	17 g. NH ₄ Cl	
7	1000	376	1972	9.3	33	Nil	Acidosis glucose tolerance test
8	945	208	431	6.9	—	Nil	—
9	760	207	403	4.1	—	Nil	—
10	1090	76	283	5.7	—	20 g. NaHCO ₃	Symptoms: marked thirst, otherwise nil
11	2030	41	81	2.4	—	40 g. NaHCO ₃	
12	2560	Nil	51	3.9	—	70 g. NaHCO ₃	
13	—	—	—	—	68	—	Alkalosis glucose tolerance test
Subject 2:							
1	—	—	—	—	52	Nil	Control glucose tolerance test
2	1020	379	388	9.4	—	Nil	—
3	1130	289	378	13.6	—	Nil	—
4	1025	291	394	10.5	—	Nil	—
5	2320	483	724	18.7	—	15 g. NH ₄ Cl	Salivation; slight nausea
6	2420	600	1142	29.6	—	20 g. NH ₄ Cl	Nausea more marked; watery diarrhoea
7	2030	665	1697	26.1	—	20 g. NH ₄ Cl	Headache; severe nausea; vomited at 3 a.m.
8	—	—	—	—	32	Nil	Acidosis glucose tolerance test
9	870	390	313	9.2	—	Nil	—
10	1390	234	289	14.2	—	Nil	—
11	1680	275	262	14.4	—	Nil	—
12	2050	156	172	10.9	—	20 g. NaHCO ₃	—
13	2510	80	90	14.4	—	20 g. NaHCO ₃	Symptoms nil
14	2690	81	83	14.8	—	20 g. NaHCO ₃	—
15	—	—	—	—	57	Nil	Alkalosis glucose tolerance test
Subject 3:							
1	—	—	—	—	66	Nil	Control glucose tolerance test
2	1130	429	760	15	—	Nil	—
3	1205	414	733	14	—	Nil	—
4	1330	463	734	16	—	20 g. NH ₄ Cl	Symptoms nil except for increased salivation
5	2080	649	1440	30	—	20 g. NH ₄ Cl	
6	2490	936	1972	36	—	20 g. NH ₄ Cl	
7	2170	1068	2930	35	—	20 g. NH ₄ Cl	Acidosis glucose tolerance test
8	2470	929	3359	39	38	25 g. NH ₄ Cl	
9	1385	526	1233	7	—	Nil	—
10	1600	307	678	8	—	20 g. NaHCO ₃	Symptoms nil
11	2310	129	286	13	—	25 g. NaHCO ₃	Symptoms nil
12	2360	Nil	Nil	9	—	30 g. NaHCO ₃	Symptoms: marked tingling in hands and feet
13	—	—	—	—	68	—	Alkalosis glucose tolerance test

acidosis and finally a period of alkalosis, diet being kept constant throughout. Glucose tolerance was estimated during each period by following the changes in venous blood-sugar after the oral ingestion of 100 g. of B.D.H., A.R. standard dextrose dissolved in 250 cc. of water. Specimens of blood were withdrawn at 15-minute intervals during the first hour of the test, and then at 30-minute intervals. Urine specimens were examined for reducing substances at half-hour intervals.

Urine analysis. A. Quantitative.

- (1) Total acidity by Folin's method.
- (2) Ammonia by Malfatti's formaldehyde titration method. This includes amino-acids.
- (3) Chlorides by Volhard's method.

B. Qualitative.

- (1) Protein by salicylsulphonic acid.
- (2) Sugar by Benedict's qualitative method.
- (3) Ketone bodies by Rothera's method.

Blood-sugar was determined in specimens of venous blood by Folin and Wu's colorimetric method. The figures obtained include non-glucose reducing substances [Herbert and Groen, 1929].

Induction of acidosis. This was accomplished by the ingestion of ammonium chloride in 2 % solution. The total amounts taken were 52 g., 55 g. and 105 g. in 3 days.

Induction of alkalosis. Sodium bicarbonate suspended in water was ingested in amounts as shown in Table I. The mixture was stirred up and immediately swallowed. Thus in spite of low solubility 5 or 10 g. could be taken in a small volume of water.

Fig. 1. Blood-sugar curves in subject 1.

Fig. 2. Blood-sugar curves in subject 2.

Fig. 3. Blood-sugar curves in subject 3.

Determination of CO₂-combining power of plasma. In each case this determination was performed on the first specimen of blood (fasting) taken for the glucose tolerance test. About 5 cc. of venous blood were rapidly withdrawn

under oil without stasis, oxalated and rapidly centrifuged under oil. The plasma was sucked off and equilibrated with the subject's own alveolar air. The CO_2 content of 1 cc. of equilibrated plasma was then determined in the Van Slyke apparatus, duplicate determinations being performed in each case. Results, after appropriate corrections for temperature and pressure, are shown in Table I.

DISCUSSION AND CONCLUSIONS.

It was found possible by the ingestion of large amounts of ammonium chloride to reduce the alkali reserve of the blood-plasma to a level comparable with that found in diabetic coma and to raise the urinary excretion of acid by 100–200 % and of ammonia by 300 %. The acidosis so produced caused a definite failure in glucose tolerance, shown by hyperglycaemia and slow return of the blood-sugar to fasting level following glucose ingestion, but without any trace of glycosuria. These points are strikingly shown in subjects 1 and 2, but are less marked though still apparent in subject 3 for whom the curves for venous blood tend to be of a flat type. The absence of glycosuria is surprising in view of the fact that in two of the subjects the blood-sugar level exceeded what is usually regarded as the normal renal threshold.

The ingestion of sodium bicarbonate in large doses, sufficient to reduce the urinary excretion of ammonia and acid to zero, was not followed by marked increase in the CO_2 -combining power of the plasma. In subject 1 the last dose of alkali (10 g.) was taken $2\frac{1}{2}$ hours before the determination of plasma- CO_2 . These observations were carefully performed on three occasions with the same result. Palmer and Van Slyke [1917] found it necessary to raise the plasma-bicarbonate to 75–80 volumes % by alkali ingestion before the sum of urinary ammonia and titratable acid fell to zero. It appears, however, that the CO_2 -combining power of the plasma may sometimes be a misleading guide to the extent of the total upset in acid-base balance following alkali ingestion.

Ingestion of alkali caused very little variation in the blood-sugar response to glucose ingestion. There was a tendency to a sharper rise in the initial phase, but thereafter the curves were little different from normal.

REFERENCES.

- Elias (1913). *Biochem. Z.* **48**, 120.
- Haldane (1921). *J. Physiol.* **55**, 265.
- (1924). *Lancet*, i, 537.
- Hendrix and McAmis (1924). *J. Biol. Chem.* **61**, 45.
- Herbert and Groen (1929). *Biochem. J.* **23**, 339.
- Palmer and Van Slyke (1917). *J. Biol. Chem.* **32**, 499.
- Peters and Geyelin (1917). *J. Biol. Chem.* **31**, 471.
- Underhill (1916). *J. Biol. Chem.* **25**, 463.