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THE EFFECTS OF ALKALOSIS UPON KETONE BODY PRODUCTION AND CARBOHYDRATE METABOLISM IN MAN

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Available evidence from animal investigation suggests that changes in pH can alter carbohydrate and ketone metabolism. Guest and Rawson (1) observed a distinct inverse relationship between blood sugar content and pH in rats. In the fasting animal, alkalosis was found to enhance ketosis and decrease liver glycogen (2). In human studies, Beumer and Soecknick (3) and Porges and Lipschütz (4) noted the early appearance of acetoneuria in patients on subcaloric diets treated with sodium bicarbonate.

The present study was undertaken to define some of the metabolic changes attendant upon the acute administration of large amounts of alkali to normal and diabetic individuals in the postabsorptive state. Because of the known ketogenic activity (5) of acetic acid and the key position occupied by the 2-carbon fragment "acetate" in the intermediary metabolism of various foodstuffs (6), it was decided to compare the effect of alkalosis induced by sodium acetate to that of sodium bicarbonate.

EXPERIMENTAL PROCEDURES AND METHODS

An infusion of an isotonic solution consisting of 20.5 gm. of sodium acetate dissolved in 1600 cc. of distilled water was administered intravenously at a constant rate over a two hour period in all experiments. Eleven of the subjects, four medical students and seven patients hospitalized for minor ailments, were young adult males without evidence of disturbances of hepatic function or carbohydrate metabolism. Of the nine male diabetic subjects selected with ages ranging from 25 to 55 years, two patients, J. Z. and A. C., required no insulin, being controlled by diet alone. Both had undergone leg amputations for peripheral vascular disease six months prior to study. The diagnosis of diabetes mellitus was well substantiated before operation by the usual clinical and lab-

oratory criteria. At the time of investigation both were found to be normoglycemic. The remaining seven subjects received no insulin for 2 to 5 days prior to observation. Several developed various degrees of diabetic ketosis during this period. One had mild associated acidosis. All subjects were maintained on diets containing adequate calories, protein, fat, and 250 grams of carbohydrate for at least one week prior to the study. Tests were routinely performed at 8 A.M. with the subjects in the postabsorptive state.

Analyses were performed before and at 120, 180, 240, and, in some instances, 280, 320, and 360 minutes after the onset of the infusion. Serum electrolytes were measured by methods previously described in publications from this laboratory (7). Citric acid in the serum was determined by the method of Natelson, Pincus, and Lugovoy (8). Blood was analyzed for total ketones, *i.e.*, the sum of acetone, acetoacetic acid, and β -hydroxybutyric acid by the method of Michaels, Margen, Liebert, and Kinsell (9), glucose by the iodometric titration method of Somogyi (10), and pyruvic acid by the technique of Friedemann and Haugen (11). When significant degrees of ketonemia were present, pyruvic acid filtrates were treated in a manner described by Lardy (12). Recoveries for glucose, ketones, citric and pyruvic acids ranged from 95 to 99 per cent in all instances.

Observations were made in two diabetic subjects, utilizing the hepatic vein catheterization technique of Bradley, Ingelfinger, Bradley, and Curry (13). Hepatic blood flow was determined by the bromsulphalein method as outlined by these authors.

Simultaneous samples of peripheral venous and arterial blood were obtained through indwelling Courmand needles placed in the left antecubital vein and brachial artery. Analyses of blood for glucose and ketones were performed in triplicate. The standard deviation of replicate samples for glucose at the 100 mgm. per cent level was ± 1 mgm.; for ketones at 1-2 mgm. per cent, ± 0.064 ; and at 20 mgm. per cent, ± 0.305 mgm. per cent.

Finally, eight paired control studies were performed in two consecutive days on four normal and four diabetic subjects. In one, sodium acetate, and in the other an equimolar solution of sodium bicarbonate was used.

RESULTS

Changes in acid-base balance

The administration of either sodium acetate or sodium bicarbonate solutions to normal and dia-

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TABLE I

*Serum CO₂ and chloride response to the intravenous administration of equimolar quantities of Na acetate and NaHCO₃ in normal and diabetic subjects **

Time min.	Na Acetate						NaHCO ₃					
	Normals			Diabetics			Normals			Diabetics		
	No.	CO ₂	Cl	No.	CO ₂	Cl	No.	CO ₂	Cl	No.	CO ₂	Cl
0	11	25.2 ±0.177	95.1 ±0.812	8	24.7 ±0.453	94.2 ±0.988	6	25.6 ±0.486	95.3 ±1.36	6	24.2 ±0.583	91.4 ±1.88
120		30.5 ±0.291	88.9 ±0.945		29.5 ±0.517	88.7 ±0.877		31.3 ±0.482	89.3 ±1.53		29.1 ±0.538	87.1 ±1.63
240		28.8 ±0.389	90.5 ±1.90		27.7 ±0.692	90.3 ±1.06		29.8 ±0.408	90.5 ±1.65		28.3 ±0.772	89.3 ±0.808

* The mean and standard error.

betic subjects produced comparable degrees of metabolic alkalosis. Table I summarizes the changes observed in all studies including the paired experiments. Alkalosis was maximal at the termination of the infusion (120 minutes) and still persisted at 240 minutes, but to a lesser extent. Simultaneously, the chloride concentration of the serum decreased to a roughly equivalent degree in all cases.

Ketones

Two diabetic patients, L. R. and D. B., were subjected to hepatic vein catheterization prior to the administration of sodium acetate (Figures 1, 2). The initial ketone concentration in the blood of L. R. was slightly elevated. D. B. was in moderate diabetic ketosis at the onset of the procedure. The splanchnic ketone output which was determined by multiplying the hepatic vein-arterial

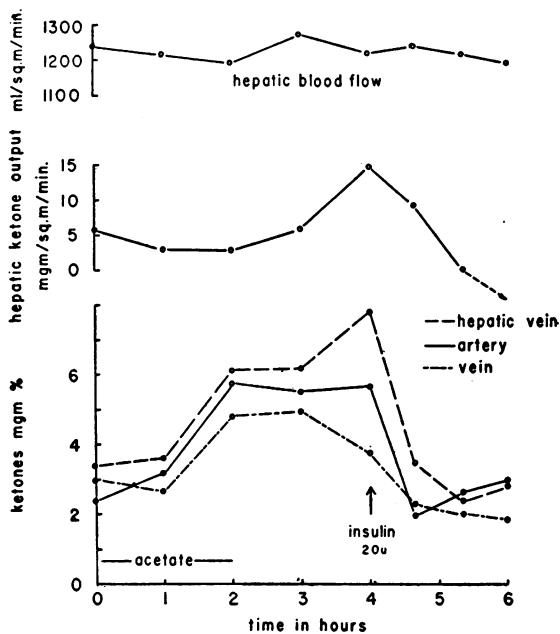


FIG. 1. PATIENT L. R. WITH MILD DIABETES. THE EFFECT OF ACETATE AND INSULIN ON HEPATIC BLOOD FLOW AND HEPATIC KETONE PRODUCTION

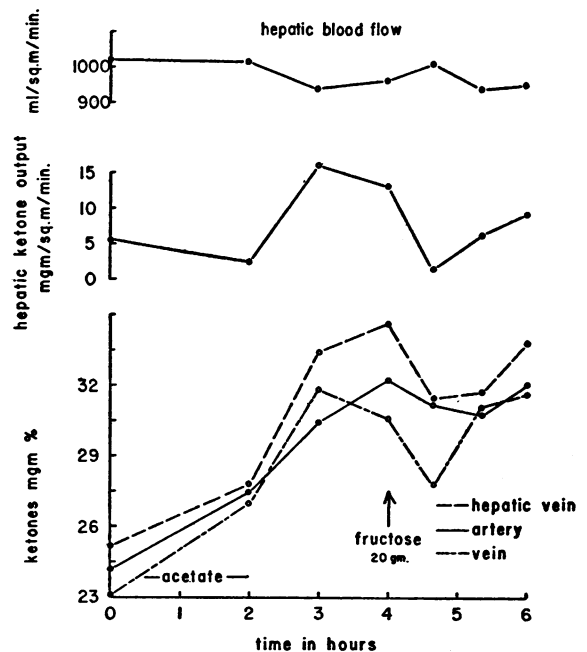


FIG. 2. PATIENT D. B. WITH SEVERE DIABETES. THE EFFECT OF ACETATE AND FRUCTOSE ON HEPATIC BLOOD FLOW AND HEPATIC KETONE PRODUCTION

(HV-A) ketone difference by the estimated hepatic blood flow was quantitatively similar in both instances. Ketone production rose from 5 mgm. per M² per minute to 15 mgm. per M² per minute at 180 (D. B.) and 240 minutes (L. R.) after the onset of the infusion. The greatest HV-A differences were apparent at these points. The largest peripheral arterial-venous (A-V) differences were noted at 240 minutes. The hepatic blood flow remained relatively constant throughout.

Eighty minutes after the intravenous administration of 20 units of regular insulin to subject L. R., splanchnic ketone production as evidenced by HV-A differences fell, approaching zero. The concentration of ketones in the hepatic vein, peripheral artery, and vein fell to initial levels. These effects persisted until the termination of the experiment.

Likewise, 40 minutes after the rapid intravenous injection of 20 grams of fructose to patient D. B., a sharp depression in splanchnic ketone output was noted. The peripheral A-V difference increased significantly at this point, indicating increased peripheral ketone utilization. However, these effects were only fleeting, for 80 and 120 minutes later ketone production was again approaching precarbohydrate injection levels. Similar changes are reflected in the ketones of the hepatic vein, peripheral artery, and vein.

The intravenous infusion of 100 grams of fructose over a 60 minute period to a patient in diabetic ketosis, not treated with acetate, resulted in a similar decline followed by a secondary rise above preinjection ketone concentrations (14).

The response of blood ketones to intravenously administered sodium acetate and sodium bicar-

TABLE II
The blood ketone response to intravenous administration of sodium acetate and sodium bicarbonate in normal and diabetic subjects

Normals	Time in minutes				Diabetics	Time in minutes			
	0	120	180	240		0	120	180	240
	mg. %					mg. %			
Na Acetate					Na Acetate				
					Mild Diabetics				
F. G.	1.6	1.8	1.6	2.9	L. R.	3.0	4.8	5.0	3.8
S. D.	2.0	3.0	4.7	4.3	A. C.	1.3	1.7	2.8	2.9
G. M.	2.1	2.7	3.3	4.0	F. H.*	2.0	2.8	3.8	3.7
H. D.	2.0	2.0	2.9	3.6	L. D.*	3.2	2.8	3.4	4.4
T. H.*	2.0	2.0	1.6	2.9	J. S.*	0.7	1.7	2.5	1.9
E. F.*	1.5	2.0	1.9	2.8	Mean	2.0	2.8	3.5	3.5
F. J.*	1.5	1.6	2.7	2.7	Standard Error	±0.480	±0.565	±0.04	±0.43
K. H.*	1.4	1.2	1.4	2.5	Na Acetate				
Mean	1.8	2.0	2.5	3.2	Severe Diabetics				
Standard Error	±0.102	±0.58	±1.13	±0.235	H. B.	16.0	25.3	26.6	29.2
NaHCO ₃					D. B.*	23.0	26.9	31.8	30.6
R. S.	1.6	1.6	1.7	2.1	D. B.	10.7	14.1	17.2	16.4
L. L.	1.7	2.4	1.9	2.0	L. B.*	27.4	38.8	40.5	46.7
T. H.*	1.8	1.7	1.7	1.4	Mean	19.3	26.2	29.0	30.7
E. F.*	2.4	1.4	1.2	0.9	Standard Error	±3.7	±5.0	±5.0	±6.2
F. J.*	1.4	1.1	0.9	0.8	NaHCO ₃				
K. H.*	1.6	1.5	1.4	1.1	Mild Diabetics				
Mean	1.8	1.6	1.6	1.4	F. H.*	4.1	3.5	5.5	3.1
Standard Error	±0.143	±0.179	±0.163	±0.229	L. D.*	3.2	2.3	3.4	2.7
					J. S.	1.9	2.3	2.4	2.5
					H. B.	3.2	2.3	6.1	7.0
					Mean	3.1	2.6	4.3	3.8
					Standard Error	±0.45	±0.30	±0.72	±1.07
					NaHCO ₃				
					Severe Diabetics				
					D. B.*	35.3	39.3	42.3	42.2
					L. B.*	23.2	26.7	29.4	33.0

* Paired studies.

bonate in normal and diabetic subjects are presented in Table II. In both single and paired observations in the normal group, following acetate infusions, blood ketones increased slightly, but significantly ($p < 0.01$) and consistently. In the control series receiving bicarbonate there were no similar changes. Indeed, in the majority of subjects, ketone concentrations diminished slightly and progressively. A comparison of the mild diabetics at 0 and 240 minutes shows a significant increase in ketones following acetate infusions, but a borderline or insignificant increase following bicarbonate. In severe diabetics, ketones increased appreciably following infusions of either acetate or bicarbonate. The absolute rise was greatest in those individuals who were initially in diabetic ketosis.

Glucose

The effects of acetate on the splanchnic glucose metabolism of the two diabetic subjects, L. R. and D. B., are shown in Figures 3 and 4. In both individuals, splanchnic glucose output decreased

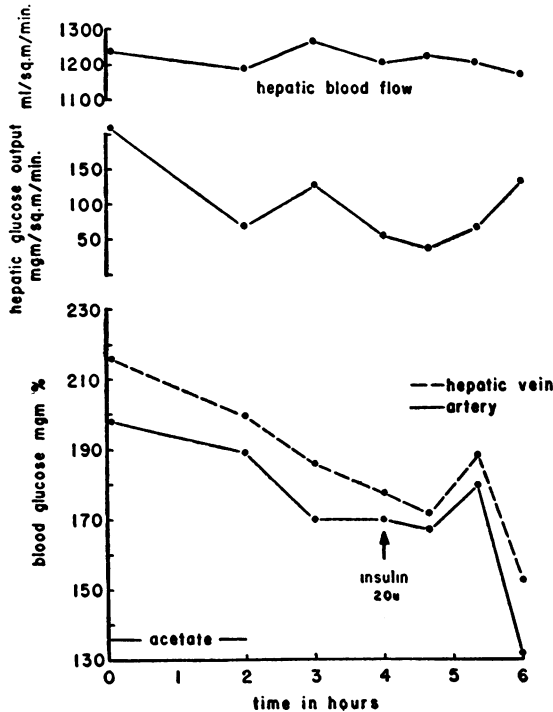


FIG. 3. PATIENT L. R. WITH MILD DIABETES. THE EFFECT OF ACETATE AND INSULIN ON HEPATIC BLOOD FLOW AND HEPATIC GLUCOSE PRODUCTION

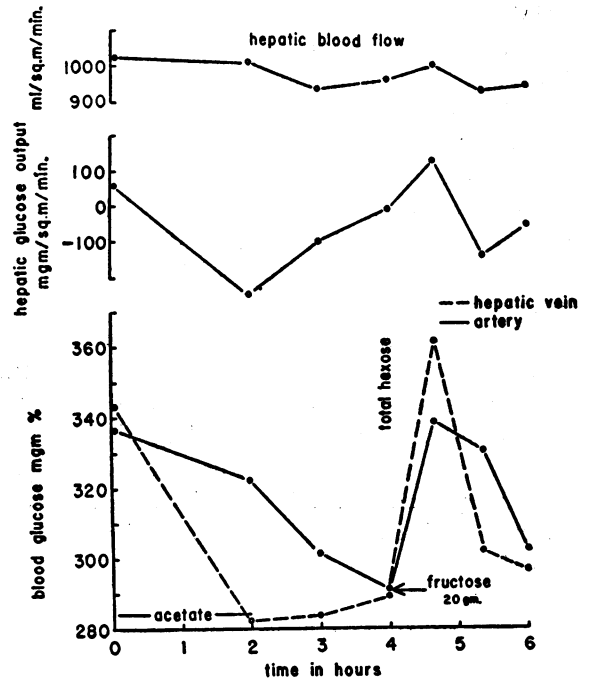


FIG. 4. PATIENT D. B. WITH SEVERE DIABETES. THE EFFECT OF ACETATE AND FRUCTOSE ON HEPATIC BLOOD FLOW AND HEPATIC GLUCOSE PRODUCTION

strikingly. In patient L. R. this was most marked at 120 and 240 minutes. Two hours after the intravenous administration of 20 units of insulin, the blood sugar of this patient had fallen approximately 40 mgm. per cent with an associated increase in splanchnic glucose production. The action of insulin on peripheral blood sugar concentrations and splanchnic glucose output was similar to that observed by Bearn, Billing, and Sherlock (15). In subject D. B. glucose production fell precipitously. The splanchnic bed began to remove glucose from the circulation, producing negative values for HV-A. This was most pronounced at the completion of the acetate infusion and persisted to a lesser degree for an additional 120 minutes. However, with the passage of time, there was a tendency for the glucose concentration in the hepatic vein to rise again above that in the artery. At 240 minutes the rapid intravenous injection of 20 grams of fructose induced an outpouring of hexose into the blood stream by the liver. This phenomenon was dissipated within a 40 minute period, and only after peripheral concentrations began to descend again was an upward trend in output seen.

TABLE III
The response of blood glucose to intravenous administration of sodium acetate and sodium bicarbonate in normal and diabetic subjects

Normals	Time in minutes				Diabetics	Time in minutes			
	0	120	180	240		0	120	180	240
mg. %					mg. %				
Na Acetate					Na Acetate				
W. J.	84	82	84	82	A. P.	201	186	184	174
F. G.	80	82	83	77	L. R.	215	193	178	168
S. D.	67	67	72	70	E. B.	184	164	163	167
G. M.	63	69	73	74	H. B.*	237	203	200	203
H. D.	63	62	71	77	D. B.*	437	382	393	389
T. H.*	74	73	76	82	D. B.	339	323	297	292
E. F.*	78	76	81	81	F. H.*	212	190	176	162
K. H.*	75	70	78	81	L. D.	172	149	143	141
F. J.*	76	73	80	73	J. Z.†	86	87	88	86
					A. C.†	92	88	91	94
Mean	73	73	77	78	Mean	217	196	191	187
Standard Error	±2.17	±2.54	±1.60	±1.47	Standard Error	±33.3	±29.4	±28.9	±28.7
NaHCO ₃					NaHCO ₃				
R. S.	84	86	87	84	H. B.*	241	177	199	186
L. L.	102	106	110	110	D. B.*	436	415	379	367
T. H.*	75	74	71	70	F. H.*	207	157	162	164
E. F.*	82	81	80	84	L. D.*	218	182	180	175
K. H.*	79	77	80	72	J. Z.†	104	101	107	100
F. J.*	72	78	71	69	A. C.†	83	77	79	92
Mean	82	84	83	82	Mean	215	185	183	181
Standard Error	±4.32	±4.73	±5.69	±6.33	Standard Error	±51.7	±49.2	±43.0	±40.5

* Paired studies.

† Normoglycemic at time of study, had substantiated diagnosis of diabetes, and required insulin prior to leg amputations for peripheral vascular disease.

Table III shows the changes observed in the glucose of the blood following the infusion of acetate and bicarbonate to normal and diabetic individuals. The blood sugar of the normal subjects did not change appreciably in single and paired studies after the administration of either solution. In the diabetic group, however, at the termination of the experiment, mean decreases of 30 and 34 mgm. per cent below initial concentrations were noticed after the injection of acetate and bicarbonate, respectively. There was no distinct correlation between the height of the initial blood sugar and the degree of depression at the termination of the experiment.

Citric acid

Citric acid concentrations in the blood increased significantly in both normal ($p < 0.02$) and diabetic subjects ($p < 0.02$) at 240 minutes following the infusion of acetate (Table IV). Small increments noted after the bicarbonate solution were not statistically significant. In this group, at 180

minutes, two subjects showed peak increases of similar magnitude to those seen in the acetate treated series. However, these increments were followed by slight declines at the 240 minute pe-

TABLE IV
Blood citric acid response to the intravenous administration of Na acetate and NaHCO₃ in normal and diabetic subjects *

Time	Na Acetate				NaHCO ₃			
	Normals		Diabetics		Normals		Diabetics	
	No.	Citric acid	No.	Citric acid	No.	Citric acid	No.	Citric acid
0	5	2.30 ±0.157	7	2.28 ±0.098	6	2.09 ±0.204	6	2.33 ±0.094
120		2.42 ±0.295		2.12 ±0.105		1.91 ±0.234		2.09 ±0.229
180		2.68 ±0.241		2.59 ±0.375		2.25 ±0.273		2.39 ±0.114
240		2.80 ±0.085		2.78 ±0.141		2.39 ±0.249		2.58 ±0.081

* Mean and standard error of mean.

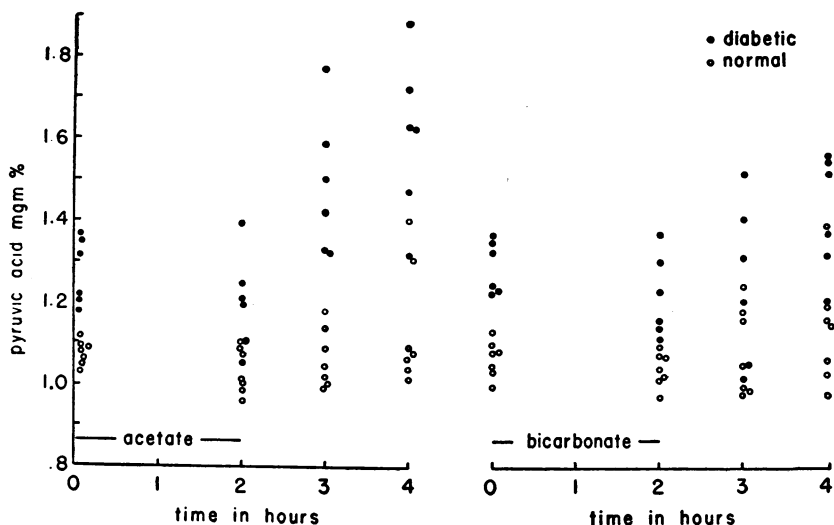


FIG. 5. THE EFFECTS OF ACETATE AND BICARBONATE ADMINISTRATION ON BLOOD PYRUVIC ACID CONCENTRATION IN NORMAL AND DIABETIC SUBJECTS

riod. The initial values of both groups fell within the normal range for blood citric acid concentration.

Pyruvic acid

The administration of acetate or bicarbonate to normal subjects (Figure 5) usually resulted in a slight decline of blood pyruvate at the termination of the experiment. In two instances, using either solution, pyruvate concentrations rose at this point. A more definitive pattern was noted in the diabetic group of patients (Figure 5). Generally, slight depressions in pyruvic acid at the end of the infusion period were followed by subsequent increases above initial concentrations. In the acetate treated group this elevation (12 to 38 per cent) appeared earlier, was higher and more consistent than that noted in subjects receiving bicarbonate.

The patients with diabetes mellitus exhibited slightly higher pre-injection concentrations than the normal group.

DISCUSSION

Acetate as such is not found in the blood stream under normal conditions. The rapidity with which this compound is metabolized in the mammalian body has been demonstrated by Harper, Neal, and Hlavacek (16). Following the rapid intravenous

injection of C^{14} labeled acetate (4.8 mM per Kilo, in 1 to 2 mins.) to dogs, these investigators noted the quick appearance and maximal concentration of C^{14} in the CO_2 of the expired air within 30 minutes after administration. During the same period, the formation of new $C^{14}O_2$ ceased as the tagged acetate rapidly disappeared from the blood. This was taken as presumptive evidence for utilization of this compound. If the data obtained from these experiments are applied to our studies, it can be assumed that at the rate of administration of 15 grams of exogenous acetic acid (20.5 grams CH_3COONa or 3.5 mM per Kilo) over 120 mins., the metabolism of 2C fragments was almost instantaneous. However, the amount of acetate that could be given was limited by the associated sodium load. Under these circumstances the alkalosis that was produced was comparable to that induced by equimolar amounts of $NaHCO_3$. With the rapid utilization of the acetate radical, the associated sodium ions became available for combination with CO_2 and H_2O . The effect in each instance, therefore, was an increase in the concentration of sodium bicarbonate in the serum. Since it is virtually impossible to differentiate the metabolic effects of acetate "loading" from those of concomitant alkalosis, it seems reasonable to conclude that the results obtained in this study probably were related to alterations in the pH of body fluids *per se*.

Alkalinization (NaHCO_3 or Na acetate) in the diabetic subject was associated with a fall in blood sugar and an accentuation of pre-existing ketosis (Tables II, III). In the two diabetic patients (Figures 3-4) subjected to hepatic vein catheterizations, splanchnic glucose production decreased and hepatic ketone output increased following the administration of sodium acetate. From the results of previous animal investigations on the effects of alkalosis on carbohydrate, protein, and fat metabolism (2), it appears that these changes are, for the most part, directly related to a decrease in the transformation of protein to glucose. Since ketosis varies inversely with the availability of carbohydrate, the amount of fat converted to ketone bodies in the diabetic becomes largely dependent upon the extent and rate at which these catabolic processes take place. In the acetate experiments, it is conceivable that the addition of 2C fragments could alter total body metabolism. Fifteen grams of acetate given over two hours would represent an appreciable fraction of the caloric requirement during this period of time. For example, if this rate of administration were continued over 24 hours, 180 grams of acetate could provide over one-half the total fat and carbohydrate intake per day in equivalent weight of carbon chains. Two carbon fragments are rapidly converted into ketone bodies in the uncontrolled diabetic. Although ketone bodies can readily be utilized to meet, in part, the energy requirements of the body, it is improbable that this effect alone could account for the diminution in the production of glucose from various sources, since similar results were noted following bicarbonate administration.

The lack of appreciable changes in glucose concentration noted during alkalosis in the normal subjects, and in the two diabetic patients, J. Z. and H. C., who became normoglycemic following leg amputations for obliterative peripheral vascular disease, remains unexplained. It may be hypothesized that the observed fall occurs only in the presence of either overproduction of glucose from noncarbohydrate sources or inadequate stores of liver glycogen. However, the latter seems unlikely, for in a normal subject fasted for 48 hours and whose liver glycogen was presumably depleted, the blood sugar remained relatively unchanged while ketosis was enhanced with alkalosis (14).

In eight paired studies ketone concentrations in the blood of normal subjects failed to increase following the administration of NaHCO_3 (Table II). The individuals in these experiments were in the postabsorptive state. No prior stimulus for ketosis existed. However, it has been noted that in the presence of depleted carbohydrate stores, alkalosis induced by either NaHCO_3 (3, 4) or hyperventilation (3, 17) will accentuate ketosis. In the latter situation, respiratory alkalosis was associated with an actual decrease in serum bicarbonate. In the acetate treated group of normals the slight but significant increase in ketone bodies probably represents the result of condensation of small excesses of preformed acetate.

It has long been known that shifts in pH toward alkalinity are associated with substantial increments of citric acid in blood and urine (18). The efficiency of the reaction acetoacetate + oxaloacetate \rightarrow citric acid may therefore be considerably increased during alkalosis. Thus in the subjects receiving acetate, the mechanisms for the increased production of citric acid may occur singly or in combination. First, it may be an effect of alkalosis alone; second, it may be due to the availability of excesses of acetate or acetoacetate in the presence of intracellular oxaloacetate. The slight fall in citric acid noted in several studies coincident with maximal alkalosis at the termination of the infusion at 120 minutes probably represents a distribution phenomenon.

Very little is known concerning the pathways of pyruvate metabolism in man. The somewhat variable changes that were noted in blood pyruvate can not be explained at this time in the light of present knowledge.

SUMMARY

1. The administration of large amounts of acetate as the sodium salt to normal and diabetic subjects results in the production of an alkalosis comparable to that induced by equimolar solutions of NaHCO_3 .
2. In normal subjects there were no changes in blood glucose concentrations following the infusion of acetate or bicarbonate.
3. The administration of sodium acetate to diabetic subjects caused depressions in splanchnic glucose output and peripheral glucose concentrations. These effects were associated with simul-

taneous increases in splanchnic ketone production and peripheral blood ketones. Diabetics treated with bicarbonate usually exhibited similar changes in peripheral glucose and ketone levels.

4. The effect of alkalosis *per se* on carbohydrate metabolism is discussed.

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