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Quantitative Relation Between Administered Alkali and Endogenous Citric Acid Production.

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Östberg[1] proposed an hypothesis that, in human metabolism, the citrates of the urine constitute one of the important buffer systems. This suggestion evoked considerable investigation relative to the possible function of citric acid as a "metabolic acid" in much the same sense that ammonia is a "metabolic base." This theory has been supported by the establishment of the endogenous origin of citric acid, as shown by its synthesis in the animal organism from various precursors.[2,3] Furthermore, when citric acid is administered as the free acid to human subjects[4-8] and to experimental animals[9-14] it disappears as such, whereas the ingestion of sodium citrate leads to the appearance of relatively large amounts of citric acid in the urine.[1,7,9] That this difference is due principally to the alkali administered is indicated by the fact that equivalent amounts of sodium, as the bicarbonate, produce similar increases in citric acid excretion.[1,4-7,9] Conversely, the administration of hydrochloric acid and potentially acidic substances, such as ammonium chloride, cause a significant decrease in the excretion of the acid.[1,4,5] It is evident from these investigations that the endogenous production of citric acid is highly responsive to factors known to affect the recognized metabolic adjustments to changes in acid base equilibrium in the body.

In view of the evidence relative to the qualitative stimulation of citric acid production by alkali, research was inaugurated in an attempt to establish a possible direct relationship between the excretion of citric acid and the amount of alkali administered. Seven male albino rats, weighing from 200-250 g, were maintained over the experimental period on either of 2 low-citrate, iso-caloric diets. Diet Cbh contained Lactalbumin (Labco, No. 15-42, Borden) 14.0%, Dextrin 58.6%, Hydrogenated Fat (Crisco) 20%, Cod Liver Oil (Mead, Johnson & Company) 5%, Salt Mixture[15] 2.4%, while diet Pr consisted of Lactalbumin 72.6% and no dextrin—the remainder of the purified ration being the same as diet Cbh. The caloric values were 5.24 cal. per g for the Cbh diet and 5.32 cal. per g for the Pr ration. The daily supplements were 1 cc of a solution containing 80 mg of liver extract (Lilly 343) and 200 mg of Ryzamin-B concentrate (Burroughs Wellcome).

The animals were kept in individual cages placed over large glass funnels containing a 3/16-inch mesh screen and glass wool plugs which separated the feces and food from the

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TABLE I.
Alkali Administration and Endogenous Citric Acid.

Diet	Periods on diet	Mg citric acid per period: Intake* A	Mg citric acid per period: Urinary excretion B	Mg $NaHCO_3$ per period C	Urinary citric acid excretion: Excr. per 100 mg $NaHCO_3$ D	Urinary citric acid excretion: Total incr. over norm E	Urinary citric acid excretion: Incr. per 100 mg $NaHCO_3$ F	Urinary citric acid excretion: Avg incr. per 100 mg $NaHCO_3$ G
Cbh R	5	6.06	8.49	—	—	—	—	—
" R B_1	3	5.99	14.49	150	9.66	6.00	4.00	
" R B_2	3	5.98	23.33	300	7.78	14.84	4.95	4.73
" R B_3	3	6.23	39.90	600	6.65	31.41	5.24	
Pr R	3	26.66	11.96	—	—	—	—	—
" R B_1	3	27.82	23.39	150	15.60	11.43	7.66	
" R B_2	3	27.82	28.83	300	9.61	16.87	5.62	6.16
" R B_3	3	27.82	43.21	600	7.20	31.25	5.21	
							Mean	5.45

* Analysis for Exogenous Citric Acid:
Cbh—0.166 mg per g.
Pr—0.916 mg per g.
Suppl. vitamin—0.51 mg.

urine, which was collected in a flask containing approximately 25 cc 1 N H_2SO_4. The funnels were rinsed down daily with distilled water and the combined urine and washings were diluted to 250 cc. Appropriate aliquots were analyzed for citric acid by the method of Pucher, Sherman, and Vickery,[16] the final colorimetric measurement being made in a photelometer using a filter with maximum transmission at 4250 Å. Quantitative measurements of food consumption, as well as of urinary citric acid, were made at period-intervals of 3 days.

After preliminary "adjustment" periods, to establish the caloric requirement for the individual animal, the rats were placed on a restricted diet "Cbh R and Pr R", so that consumption of food was complete or nearly so. In this series of experiments, the animals were used as "internal controls"—that is, each rat served as its own control. A "norm" was established first on the Cbh R diet after an adjustment period of 10 days. A solution of sodium bicarbonate was then administered orally by incorporation with the vitamin mixture to insure complete consumption. A similar "norm" was established for the Pr R diet and the effect of alkali on endogenous citric acid studied in an analogous manner. During all periods food intakes were iso-caloric for the individual animal. The results of experiment are found in Table I, each of the figures represents a period (3-day) average for the seven animals on the specified diet over the number of periods indicated.

In Table I, Column A represents the total citric acid intake per rat per period, while Column B shows the urinary excretion. In all cases dietary citric acid is relatively constant on the diet employed, while the increase of total urinary citric acid is dependent upon the degree of alkalosis. Calculation of the urinary citric acid excretion per 100 mg of $NaHCO_3$, Column D, illustrates a decrease in the effect of alkali as the amount of bicarbonate is increased. When the urinary citrate is calculated as the increment over the "norm," Column E, the linear relationship between administered alkali and urinary citrate becomes evident. The "norm" value for the Pr diet is higher than expected, due probably to a "carry over" from the previous experiments, even over an intervening adjustment period of 3 weeks. Since, however, the animals served as their own controls, this effect would not alter subsequent results to any major degree. Development of the possible direct relationship between the citric acid and $NaHCO_3$ is

[16] Pucher, G. W., Sherman, C. C., and Vickery, H. B., *J. Biol. Chem.*, **1936, 118, 235.**

particularly evident in Columns F and G, where the average citric acid increments on both the Cbh and Pr diets are of the same order of magnitude.

The foregoing data indicate that there is a direct relation between the amount of alkali administered to the rat and the excretion of endogenous citric acid. It would appear therefore that under the present experimental conditions, citric acid plays an important role in the regulatory mechanism involved in the acid-base balance of the body.

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Metabolic Studies in Patients with Cancer of Gastro-Intestinal Tract. XV. Lipotropic Properties of Inositol.*

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Recent studies from this hospital have demonstrated that the livers of patients with gastro-intestinal cancer almost uniformly are infiltrated with fat.[1] In contrast to this finding, normal fat contents were found in the livers of 11 patients with carcinoma of the gastro-intestinal tract who each received 8 g of lipocaic† during the night prior to laparotomy. The average hepatic fat concentration of this latter group was only 0.46 that of the control untreated patients.[2]

Lipocaic, however, is a crude mixture which contains significantly large amounts of choline and inositol,† both of which are lipotropic alone.[3,4] In experimental animals, the lipotropic effects of lipocaic apparently are not due to its choline alone,[5] and this observation has been confirmed here for human subjects.[2] On the other hand, when about 4 times the amount of inositol present in the 8 g of lipocaic was administered to a group of 8 patients with gastro-intestinal cancer, with but one exception the concentrations of fat in their livers were found to be within normal limits. The average lipid value was 0.42 that of the control group.

TABLE I.
Concentration of Fat in Livers of Patients with Gastro-intestinal Cancer Who Received Preoperatively 280 mg of Inositol.

Patient	Disorder	Conc. of hepatic fat, g per 100 g wet tissue
J.K.	Carcinoma oesophagus	7.2
R.H.	" stomach	8.7
O.S.	" colon	7.1
M.S.	" stomach	10.3
M.T.	" colon	7.8
W.R.	" stomach	5.7
H.J.	" "	5.0
F.M.	" "	15.9
G.M.	" "	5.9
M.J.	" "	8.9
	Avg	8.25

Because it seemed probable then that the inositol alone might account for the physio-

* The authors gratefully acknowledge the support of the National Cancer Institute.

† The lipocaic used in this investigation was supplied through the courtesy of Eli Lilly & Co., Indianapolis, Ind., and the inositol through the kindness of Lederle & Co., Pearl River, N.Y. The concentration of choline and of inositol in the material used was found by analyses to be 2.6 and 3.5 g % respectively. These analyses were made by Dr. W. Wooley of the Rockefeller Institute for Medical Research, New York City, and by the Laboratories of the Standard Brands, Inc., New York City.

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