urinary origin was injected into groups of rats of various ages, after which both measurements of total circulating red cell volume and Fe^{59} red cell incorporation were made. Total red cell volume of young rats throughout the period of neonatal anemia did not increase in response to administration of erythropoietin, whereas it did increase in all older groups. According to this assay, the rat most sensitive to erythropoietin was the oldest rat studied. Using the Fe^{59} red cell incorporation assay, no increase in erythropoiesis was observed in normal young rats (14 days of age) following erythropoietin administration. However, when erythropoiesis was depressed in young rats by hypertransfusion, red cell uptake of Fe^{59} was greatly reduced. When such hypertransfused rats were injected with erythropoietin, red cell incorporation of Fe^{59} was significantly increased.

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Organic Acid Excretion, Enhanced Calcium Absorption and Body Fat of Rats Fed Incompletely Digested Carbohydrates.* (26411)

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A relatively low content of body fat has been found in rats fed diets containing the incompletely digested carbohydrates, lactose, sorbitol, cellobiose or raw potato starch(1). The mechanisms involved are not known. Other characteristic effects associated with feeding of poorly digested carbohydrates include an initial transient diarrhea, enlargement of the cecum, establishment of an aciduric bacterial flora in the intestine, and enhanced gastrointestinal absorption of calcium and other alkaline earth elements(2). Recently Fournier and Digaud(3) have reported an increased urinary excretion of α-ketoglutaric, citric, aconitic, malic and succinic acids but not of pyruvic or hippuric acid. The present study investigated the possibility that the increased excretion of acids of the Krebs cycle is a consequence of metabolic alterations that result in a decreased deposition of body fat.

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Methods. Male weanling Sprague-Dawley rats, individually caged, were fed diets whose basal composition consisted of casein 24, carbohydrate 52, fat mixture 20, salts 4 and adequate amounts of vitamins(1). Twenty-four hour urine samples were collected under toluene. Body fat content was determined by whole carcass analysis(1) or estimated from the weight of the epididymal fat pads. The weight of the cleaned cecum was determined as an index of the digestibility of the dietary carbohydrate. Total organic acids in the urine were determined by the method of Van Slyke and Palmer(4). As this method is not specific, determination of keto acids by the simple and rapid method of Friedemann and Haugen(5) was routinely used to follow the effect of dietary carbohydrate on organic acid excretion. The organic acids were identified by silica gel chromatography. A 10-fold concentration of lyophilized urine in 2 N H_2SO_4 was mixed with an appropriate amount of dry silic acid and placed on top of a column prepared, and then eluted, by the

procedures of Dajani and Orten(6). Urinary Na, K and Ca were determined by standard flame photometric procedures; Ca was first separated as the oxalate. Mg was determined colorimetrically(7).

Results. Rats fed a diet containing lactose excreted 3 to 4 times more organic acids than those fed a glucose diet. Values of 0.89 and 0.84 meq/100 g/day were found for lactose-fed rats at the second and ninth week of feeding. At these same periods the glucose-fed animals excreted 0.26 and 0.22 meq/100 g/day. Excretion of organic acid, expressed as keto acid, was constant in terms of body weight during the first 9 weeks of experiment; keto acid excretion of the lactose group fell in the range of 6.8-10.3 mg/100 g/day, the glucose group 1.7-2.9 mg/100 g/day.

Fractionation of urine on silic acid columns revealed that the predominant organic acids were citric, α-ketoglutaric, succinic and fumaric acids, confirming the observations of Fournier and Digaud(3). A typical chromatographic profile of a urine sample from a lactose-fed rat is presented in Fig. 1. No qualitative difference was noted in the organic acid pattern of urine from lactose and glucose-fed rats although positive identification of all of the 9 or 10 peaks usually found was not attempted.

The relationships between the digestibility of the dietary carbohydrate, organic acid excretion and body fat content are presented in the experiment outlined in Table I. Rats fed the readily digested and absorbed carbohydrates, sucrose, glucose and galactose, excreted relatively small amounts of organic

TABLE I. Dietary Carbohydrate, Body Fat and Organic Acid Excretion.

Carbohydrate	Cecum (g/100)	Keto acid† (mg/day)	Body fat (%)
Glucose	.22	3.1 ± .2‡	14.2 ± .6‡
Sucrose	.24	3.5 ± .1	13.0 ± .4
26% galactose*	.23	2.7 ± .2	13.3 ±1.7
Lactose	.74	10.9 ± .9	9.8 ± .6
15% cellobiose*	.58	5.1 ± .7	9.8 ± .6
26% raw potato starch*	.65	8.9 ± .3	10.8 ± .5

6 wk experiment, 7 rats/group.
* Glucose added to 52% total carbohydrate.
† Avg of 4 daily averages during 2nd to 6th wk.
‡ ± S.E.

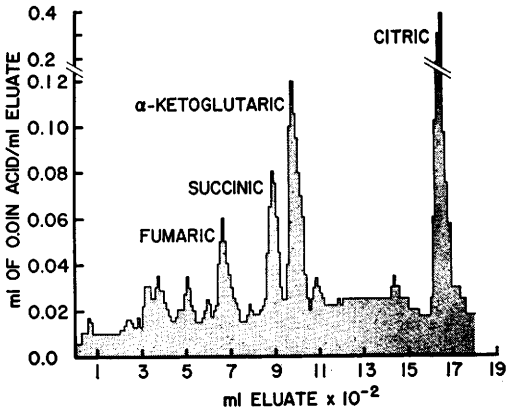

FIG. 1. Silica gel chromatogram of urinary organic acids of lactose-fed rat. 2.2 ml of 10-fold concentrated urine on column 2 × 28 cm, gradient elution, $CHCl_3$ to 45% ter-amyl alcohol in $CHCl_3$, 1 liter each solvent.

acids and had more body fat than rats fed the incompletely digested carbohydrates, lactose, cellobiose and raw potato starch. In every case the differences in body fat content and acid excretion between the readily digested and the incompletely digested carbohydrates were of statistical significance, $p = 0.05$ or less (Student's t test).

The increase in organic acids in urine of the rats fed incompletely digested carbohydrate did not appear to originate from the increased numbers of bacteria in the intestinal tract, since feeding of antibiotics did not lower keto acid excretion. Addition of 50 mg of chlortetracycline or a combination of 200 mg each of bacitracin and polymyxin B to a kilogram of the lactose diet resulted in an average excretion of 20.4 mg/day and 17.1 mg/day of keto acids respectively as compared to 20.9 mg/day excreted by a control group. It had also been observed(8) that supplementation of a lactose diet with chlortetracycline did not influence body fat content.

The increase in urinary organic acid during a metabolic acidosis is characterized by a concomitant increase in ammonia. Rats fed lactose-containing diets excreted less ammonia than glucose controls; 3.9 *vs.* 5.9 mg ammonia N/100 g/day in a typical experiment. The urinary pattern was more suggestive of a compensated alkalosis and since it is well known that the feeding of lactose

TABLE II. Urinary Excretion of Anions and Cations.

Constituent	Lactose (meq/day)	Glucose (meq/day)
Na	.35	.30
K	.99	.74
Ca	.52	.03
Mg	.08	.04
Cl	1.02	.71
HPO_4	.22	.46
SO_4	.13	.08
Organic acid	2.21	.48
Keto acid	.21	.03

Avg of 3 daily averages of 5 rats/group after 5 wk on diet.

will stimulate gastrointestinal absorption of calcium and other alkaline earth elements (2) it seemed likely that the increased organic acid excretion was the result of a renal mechanism for neutralization of the additional calcium excreted. The increased excretion of organic acids, particularly citric acid, resulting from administration of alkalinizing salts, has been extensively studied in the rat(9-13).

An analysis of the principal ionic constituents of urine samples from lactose and glucose-fed rats (Table II) yielded data in accord with this concept. Urine from the lactose-fed rats contained about 15 times more calcium, with little difference in the content of the other cations. Of the anions, only the organic acid fraction was correspondingly increased.

The results of feeding tests (Table III) offered further evidence that the increase in urinary organic acids was involved in acid-base balance. In Exp. 1, excretion of organic acids was shown to be directly related to salt content of the diet. Increasing or decreasing the salt content, irrespective of the nature of carbohydrate, resulted in a corresponding change in organic acid excretion. In Exp. 2 it was demonstrated that the effective ingredient of the salt mixture was $CaCO_3$; 54% of the Hubbell, Mendel, Wakeman salt mixture(14) is $CaCO_3$. Addition to the diet of $CaCl_2$, a calcium salt of a fixed anion in contrast to the metabolizable anion, carbonate, resulted in an organic acid excretion less than that of the control, Exp. 2, Table III. Analysis of the urine of the rats fed additional $CaCO_3$ or $CaCl_2$, Table IV, showed an equal

TABLE IV. Effect of Dietary Calcium Salts on Urine Constituents.*

Salt†	Ca (meq/day)	Cl (meq/day)	Keto acid (meq/day)
2.2% $CaCO_3$	1.0	.6	.28
2.2% $CaCl_2$	1.1	2.8	.04
None	—	—	.16

6 rats/group, 6 wk experiment.

* Avg of 3 daily averages during 5th to 6th wk.

† Added to diet containing lactose and 2% salt mixture.

excretion of calcium but in the rats fed $CaCl_2$, with an excess of anion, chloride, to be eliminated, organic acid excretion was depressed. The effect of feeding other alkalinizing salts, *i.e.*, salts of a fixed base and metabolizable anion, was studied in Exp. 3, Table III. Sodium citrate and sodium acetate greatly stimulated acid excretion; an equivalent amount of acetate as triacetin had no

TABLE III. Effect of Dietary Salts on Organic Acid Excretion.

Diet characteristic	Keto acid (mg/day)* Exp. 1	Exp. 2	Exp. 3
Glucose, 4% salt mixture	4.2	—	
" 6% " "	6.7	—	
Lactose, 2% " "	11.6	16.3	
" 4% " "	23.0	22.6	
" 6% " "	32.9	27.5	
" 2% " " + 2.2% $CaCO_3$	—	20.0	
" 2% " " + 2.2% $CaCl_2$		2.7	
Glucose, 4% " " + 3.6% Na acetate	—	—	45.1
Lactose, 4% " " + 3.2% triacetin	—	—	1.9
" 4% " " + 4.3% Na citrate	—	—	40.2
" 4% " " + 2.6% NaCl	—	—	3.7

* Avg of 4 daily averages during 2nd to 6th wk. 7 rats/group, 6 wk experiment.

TABLE V. Nonrelationship of Body Fat and Calcium and Acid Excretion.

	Diet characteristic	Epididymal fat body (g/100 g)	Carcass fat (%)	Keto acid* (meq/day)	Ca* (meq/day)
Exp. 1	Glucose, 4% salt mixture	2.0	16.6	.04	—
	Idem + 3.6% Na acetate	1.8	16.7	.61	—
	Lactose, 4% salt mixture	1.2	11.6	.21	—
Exp. 2	Glucose, 4% salt mixture	2.0	—	.08	.06
	Idem + 6% salt mixture	2.0	—	.28	.15
	" + 3.5% Ca and Mg salts†	2.0	—	.25	.31

* Avg of 4 daily averages during 3rd to 7th wk.
† Amounts in 6% total salt mixture. 6 rats/group, 7 wk experiment.

effect. The neutral salt, NaCl, yielded the expected low value.

The continuous excretion of abnormally large amounts of organic acids for 6 or 7 weeks was not in itself responsible for the low deposition of carcass fat. In Exp. 1 of Table V, rats fed a glucose diet with added sodium acetate had a high body fat content despite daily excretion of 45 mg of keto acid, about twice that of lactose-fed rats. Neither was the low body fat content of the lactose-fed rat caused by the increased absorption, transport and excretion of calcium. In Exp. 2 of Table V, urinary keto acid and calcium excretion of rats fed a glucose diet was enhanced by increasing the amount of the total salt mixture from 4 to 10% of the diet, or by adding calcium and magnesium salts in amounts supplied by the additional 6% salt mixture. No difference was found in the deposition of body fat as measured by the weights of the epididymal fat pads.

Discussion. The increased urinary excretion of acids of the Krebs cycle, observed in rats fed diets containing incompletely digested carbohydrates, is not a manifestation of an altered metabolism that results in a lowered deposition of body fat but appears to be a renal mechanism for neutralization of increased urinary calcium, an increase resulting from enhancement of gastrointestinal absorption of calcium by these carbohydrates (15,16). The experiments of the present study have shown that organic acid excretion is influenced by the amount of salt mixture in the diet and more specifically, by the nature of the calcium salts in the salt mixture. When calcium is ingested as calcium carbonate, the carbonate is metabolized leaving an excess of base to be neutralized by organic acid. With $CaCl_2$ in the diet, since a major fraction of the calcium is excreted in the feces, chloride is in excess and renal elaboration of organic acid is suppressed. It is known that organic acid excretion plays a more dominant role in acid-base balance in the rat than in man (12); little bicarbonate is found in rat urine. The renal origin of these acids has been indicated by the findings of several investigators that, with the injection of alkalinizing salts, an increase of citrate is found in renal tissue and urine but not in blood(10,11) and recent evidence(13) further suggests that the excretion rate of citrate, and probably all the acids of the tricarboxylic acid cycle, is dependent not on renal-tissue levels but on renal acid-base balance.

The results of the experiment presented here offer no enlightenment on the nature of the relationship between carbohydrate digestibility and body fat content. Attempts to reduce the body fat content of glucose-fed rats by inducing a urinary excretion of organic acid by feeding sodium acetate, and of calcium by increasing the salt content of the diet, had no effect on fat deposition.

Summary. 1. Rats fed diets containing the incompletely digested carbohydrates, lactose, cellobiose and raw potato starch, excreted larger amounts of organic acids and had a lower content of body fat than rats fed glucose, sucrose or galactose. 2. The predominant urinary organic acids were identified by silica gel chromatography as citric, α-ketoglutaric, fumaric and succinic acids. 3. Organic acid excretion could be increased by increasing the amount of dietary salt mixture or by adding $CaCO_3$ but not $CaCl_2$ to the

diet. 4. This latter finding, in conjunction with the results of urinary analysis of ionic constituents, indicated that the increased excretion of organic acid is not a consequence of an altered metabolism that decreases body fat deposition but is a renal acid-base mechanism for neutralization of increased urinary calcium, an increase resulting from the enhancement of gastrointestinal absorption of calcium by incompletely digested carbohydrates.

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Leucinamidase Activity Associated with Influenza-Parasite Complexes in Pigs.* (26412)

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Activity of amino acid amidases is increased in homogenates prepared from bovine kidney monolayered tissue cultures when infected with the virus of infectious bovine rhinotracheitis virus. Leucinamidase was the most active of the 5 enzymes studied(1). Amide-N^{15} from either leucinamide or glutamine was incorporated into cellular nucleic acid in both normal and virus infected cells but activity was greater in the infected cells (2). Studies have been extended to evaluation of serum leucinamidase activity in pigs. The enzymic activity in serum of pigs which were infected with swine influenza virus, *Ascaris suum,* and lungworms (*Metastrongylus pudendotectus* and *M. apri*) which harbor influenza virus is herein reported.

Materials and methods. One week-old pathogen-free, colostrum-deprived pigs housed in modified Horsfall-Bauer isolation units as previously described were used as the experimental host(3). Eight littermate pigs were utilized in pairs for each of 4 experimental conditions. These were: 1) Uninoculated controls; 2) Inoculated with Shope's strain 15 of swine influenza virus; 3) Fed infective eggs of *Ascaris suum;* and 4) Fed infective larvae of swine lungworms originating from influenza-infected pigs. Fifteen days after the lungworm larvae were given to the pigs *Ascaris* eggs were fed to provoke the masked influenza virus(4). Methods used for preparation and inoculation of virus and parasites(5,6,7) and for evaluation of

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