The amount of iodine taken up at this pH in a period of time sufficient to iodinate the enzyme extensively at pH 7 does not cause appreciable lowering of proteolytic activity or hypotensive effect. Likewise, the pH influences the rate of iodination of dilute solutions of histidine and tyrosine. Under conditions similar to those maintained for the iodination of trypsin, the time required for 1×10^{-4} millimoles of tyrosine to decolorize 2 equivalents of iodine per mole increases from 1 minute at pH 7 to 480 minutes at pH 5. The comparable values for histidine under the same conditions were found to be 17 minutes at pH 7 and more than 3000 minutes at pH 5. This suggests further consideration of these groups in the iodination of trypsin, with possible emphasis on tyrosine.

Iodination of a substrate rather than the enzyme, under similar conditions, decreases the proteolytic activity of untreated trypsin. Thus, the digestion of casein which has reacted with 2×10^{-3} milliequivalents of iodine per mg may be limited to about 45% of that of untreated casein. Furthermore, it has been observed that the iodination of an active protein substrate such as insulin serves to increase its resistance to trypsin. If the iodination is limited so that the iodinated hormone retains about one-half (52%) of its original activity it tends to resist digestion and further inactivation (to 44% of the original activity) by trypsin under conditions which markedly reduce the original activity of noniodinated insulin to 3%.

Summary. Iodination serves to decrease greatly the hypotensive effect of intravenously-administered trypsin. Proteolytic activity is decreased to a lesser extent. Iodination of a substrate under similar conditions decreases the ease with which it is digested by untreated trypsin.

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Production of Ketosis in Alloxan Diabetic Rats with Nicotinic Acid.

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There is much evidence to support the belief that niacin is an important constituent of the enzyme systems of the body[1,2] although the reported effects of this vitamin on tissue metabolism and its co-activity with other vitamins lack clarity in certain instances.

Nicotinic acid appears to play a minor role in fat metabolism, resulting in an increase in body fat;[3] in a reduction of the lipotropic effect of choline;[4] and in an increased fatty infiltration of the liver in rats.[5]

The role of niacin in carbohydrate metabolism has been established largely by observations made on glycemic changes in the intact animal. Whether the administration of this vitamin produces hyperglycemia,[7-9]

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2 Axelrod, A. E., and Elvehjem, C. A., *Nature*, 1939, **143**, 281.

3 Gavin, G., and McHenry, E. W., *J. Biol. Chem.*, 1940, **132**, 41.

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5 Foà, P. P., Foà, N. L., and Field, H., Jr., *Arch. Biochem.*, 1945, **6**, 215.

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11 Ledrut, J., *Bull. soc. chim. biol.*, 1940, **22**, 321.

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hypoglycemia[10-14] or is without effect on blood sugar levels,[6] are controversial points still under consideration.

In view of the evidence that niacin influences both fat and carbohydrate metabolism, alloxan diabetic rats were selected as subjects for this study since they are usually on the borderline of ketosis and respond more readily to any treatment that alters carbohydrate metabolism than do normal animals.

Experimental. Twenty adult rats of the Long-Evans strain, 2 females and 18 males, varying in weight from 235 to 400 g, were used in this study. These animals were severely diabetic following intraperitoneal injections of alloxan (125 mg/kg), although there was some variation in the degree of diabetes exhibited by individual rats. Three animals showed evidence of acidosis during the preliminary study period. The rats were checked to make certain the diabetes was stable 2 to 4 months after the onset of diabetes.

During the stabilization period the diabetic animals were fed a synthetic diet in which yeast (Pabst) was the source of the vitamin B complex. In the experimental period, a basic diet was fed in which crystalline B vitamins* were supplied as shown in the following formula:

BASIC DIET

Casein	180 g
Sucrose	300 "
Starch	390 "
Crisco	50 "
Wesson Oil	50 "
Haliver Oil	2 cc
Salt Mixture†	30 g
Choline	1 "
Thiamin Hydrochloride	5 mg
Niacin	10 "
Pyridoxine	5 "
Calcium Pantothenate	15 "
Riboflavin	5 "

The experimental diet high in niacin was a modification of the basic diet containing 1 g niacin/kg instead of 10 mg niacin/kg. The length of time that the animals were allowed to remain on the basic or high-niacin diet was altered during the course of the investigation. The rats ate *ad libitum* and were kept in metabolism cages.

All determinations were made on nonfasting animals. Weights were taken every other day; food intake and urine volume were measured daily. Urine was collected under a few drops of toluol for acetone and urinary sugar studies. Blood sugar and urine sugar determinations were made at 5- to 6-day intervals by Somogyi's micro-method.[15] Because of the high levels of both blood and urine sugar, the blood filtrate was diluted 1:1 with distilled water, and only 1 cc of a 1:500 urine dilution was used in these determinations. No corrections were made for the nonfermentable substances in the urine

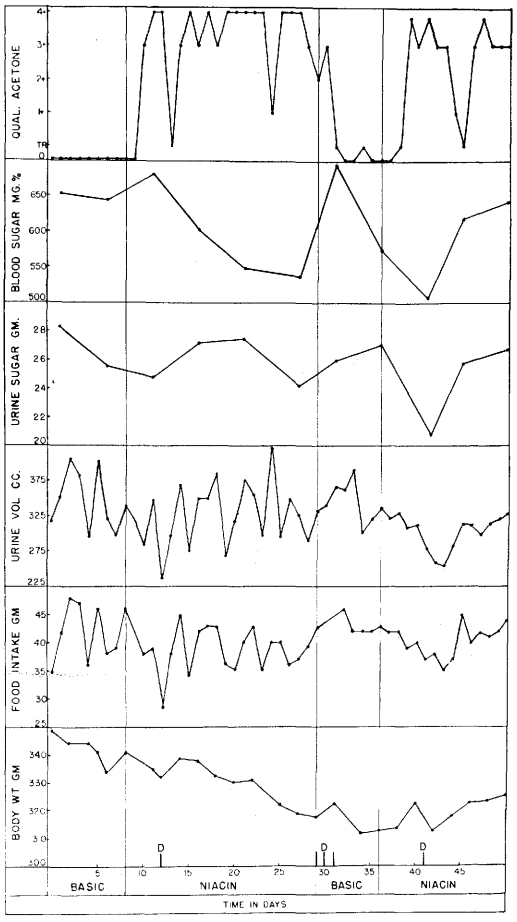

FIG. 1.
Data on rat with Type I ketonuric response. Tr. = trace. D = days when the rat had diarrhea.

* Crystalline vitamins supplied through the courtesy of Dr. J. R. Bright, Gelatine Products Corp.

† Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

[15] Somogyi, M., *J. Biol. Chem.*, 1945, **160**, 69.

since preliminary tests proved them to be negligible. Acetone bodies were estimated daily by the sodium nitroprusside test. Quantitative total urinary acetone body determinations were made for several of the animals at 2- to 5-day intervals by the gravimetric method of Van Slyke.

Results. Eighty % of the diabetic animals fed on high-niacin diet exhibited ketosis of a pathological degree. Four rats showed ketosis during the first 25 hours after the high-niacin diet was fed; 3 during the 2nd day; 2 on the 3rd day; one on the 4th day; 4 on the 5th day; one on the 7th day; and one on the 20th day.

No uniform response pattern of ketosis was exhibited, although most of the rats developed ketonuria in 1-5 days after high-niacin diet was given. The severity and duration varied from animal to animal. The rats were grouped according to their particular ketonuric response to niacin, or their failure to show symptoms of ketosis:

(1) Eight rats which showed ketonuria only while they were on the high-niacin diet. Some of these rats responded rapidly (Fig. 1) while others were slow to reach their peak of ketosis (Fig. 2). Acidosis was decidedly positive as long as these animals were ingesting niacin, but as soon as the niacin was withdrawn it disappeared, becoming evident again when the high-niacin diet was reinstated.

(2) Five rats which developed ketosis on the high-niacin diet, all had a remission before niacin was withdrawn (Fig. 3). Of this group, 3 failed to exhibit ketonuria on the second introduction of high-niacin, made after a period on basic diet.

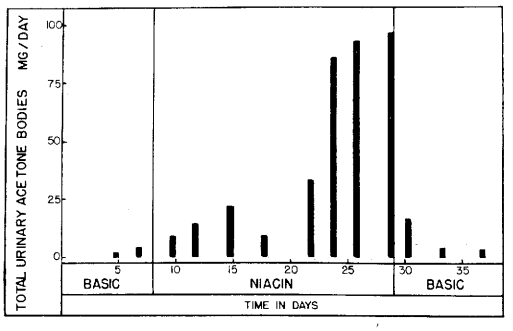

FIG. 2.
Urinary acetone bodies from rat that responded slowly to niacin. Type I response.

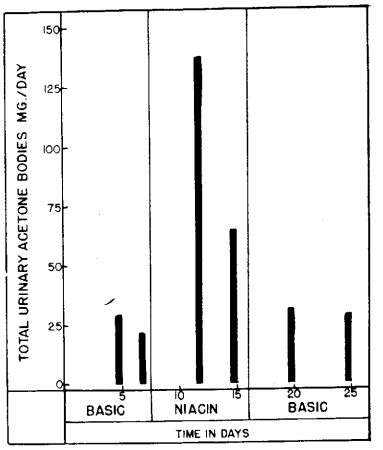

FIG. 3.
Type II response. Rat had mild ketosis before niacin was given; and showed a remission before niacin was withdrawn.

(3) Three rats showed ketonuria while they were on the high-niacin diet, became negative when placed on basic, then developed temporary ketonuria 5 to 7 days afterward, while still on the basic diet.

(4) Four animals were resistant to the formation of excess ketone bodies when placed on high-niacin diet. These rats were severely diabetic, as indicated by the nonfasting blood sugar levels which ranged from 500-650 mg %, and should have been just as vulnerable to acidosis as the other experimental animals.

Niacin intake per 100 g of body weight was calculated for all rats which showed ketonuric response and for those which did not. There seemed to be no relationship between the amount of niacin ingested and the degree of ketonuria exhibited.

The weight of the rats and their food intake were followed closely throughout the experimental period. The animals, particularly those which were most severely diabetic, lost weight during the course of the experiment. This was not unusual since the animals were not treated with insulin. The food intake did not change materially (Fig. 1), either on basic or high-niacin diet, although there was considerable variation from day to day.

The urine volume fluctuated in proportion to the food intake and varied in different groups of animals from an average minimum of 90 cc to an average maximum of 360 cc daily.

Although there was some fluctuation throughout the experiment in levels of blood sugar and in quantities of urinary glucose excreted (Fig. 1) by a single animal, these were directly correlated, in most cases, with the food intake. That is, when the food intake increased, there was a corresponding increase in blood sugar and in urinary glucose excretion. A factor of importance in considering blood sugar fluctuations is that determinations were made on nonfasting animals, in which the time between last ingestion of food and withdrawal of blood for glucose determinations was not controlled.

Discussion. Normal rats are relatively resistant to ketosis. Before urinary acetone bodies appear in appreciable quantities, rats must either undergo long periods of fasting,[16] or high-fat diets must have been previously ingested if shorter periods are to be effective.[17,18] Rats which have been diabetic with optimum amounts of alloxan are generally free from excessive ketone bodies after they have recovered from the immediate effect of this drug. However, these rats are always on the borderline of ketosis when they are severely diabetic.

In the present experiment, ketosis has been produced in 16 of 20 diabetic rats by means of a diet high in niacin. Although the animals showed a somewhat variable pattern, the degree of urinary ketone excretion was great enough to indicate that niacin ingested in large quantities produces some alteration in fat metabolism.

It has been shown that the introduction of relatively large amounts of niacin or niacinamide will cause a depletion of the labile methyl supply in the body,[19,20] as these groups are necessary to the formation of trigonelline (the methyl betaine of nicotinic acid) which is excreted in the urine of the rat after niacin has been fed. In correlating the observed increase in urinary acetone bodies with the intake of large amounts of niacin, it seems possible that the synthesis of choline, requiring methyl groups, is blocked or retarded by the reduction of free methyl groups coincident with high niacin intake. A choline deficiency would, in such a case, become the limiting factor in normal fat metabolism which would then result in an increase of total liver fat deposition and in the production of excessive ketone bodies.

To test this hypothesis, choline was given in large amounts (3 g/kg in certain diets) without altering the ketonuric response. Therefore, it has been concluded that the ketosis observed in this experiment is not caused by the lack of dietary choline.

When total blood fat was determined in some instances, there was no apparent difference between ketonuric and nonketonuric rats. Furthermore, a few observations indicated that the livers of animals which exhibited ketonuria contained no more total fat than those which were free from ketonuria. Lipoid distribution, however, as evidenced in osmic acid preparations, was clumped and sparse in livers of nonketonuric rats but was diffuse and rather fine in livers of ketonuric animals (unpublished data).

Certainly the increased ketogenesis which has been noted in the present experiment was not the result of a decreased food intake. The rats were allowed to feed *ad libitum* and the food intake remained fairly constant whether they were feeding on basic or high-niacin diet. There was no evidence of an alteration in carbohydrate metabolism consequent to the high niacin intake that could be detected from urinary and blood sugar studies.

Summary. A series of 20 rats of the Long-Evans strain which had severe alloxan dia-

[16] Deuel, H. J., Jr., and Gulick, M., *J. Biol. Chem.*, 1932, **96**, 25.

[17] Deuel, H. J., Jr., and Hallman, L. F., *J. Biol. Chem.*, 1941, **140**, 545.

[18] Roberts, S., and Samuels, L. T., *J. Biol. Chem.*, 1943, **151**, 267.

[19] Handler, P., and Dann, W. J., *J. Biol. Chem.*, 1942, **146**, 357.

[20] Handler, P., *J. Biol. Chem.*, 1944, **154**, 203.

betes for a preliminary period of 2 to 4 months, was given a diet with excess nicotinic acid. The diet was adequate but contained 1 g niacin/kg. The most outstanding effect of this diet was the production of ketonuria in 16 of the 20 animals, although the degree of ketonuria and the response pattern was variable. Supplementary choline did not inhibit ketosis. The action of nicotinic acid in altering fat metabolism is discussed.

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Inadequacy of Certain Natural Diets for Guinea Pigs.*

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Ross, Phillips and Bohstedt[1] found that a ration composed of corn, soybean oil meal and alfalfa, which is similar to rations widely used for growing hogs, is inadequate for reproduction and lactation in swine and rats. It was, therefore, of interest to try this ration with guinea pigs in order to determine if the deficiencies in these animals were related to the postulated factors required specifically by guinea pigs.

Experimental and Results. The methods of handling the animals and securing the blood samples have been described previously.[2] Young guinea pigs initially weighing 150 to 200 g were housed in individual screen-bottomed cages, and kept on experiment for 6 weeks.

The basal ration (R-1) consisted of ground yellow corn 75 g, soybean oil meal 17.5 g, ground alfalfa 5 g, and salts 2.5 g. ($CaHPO_4$ 1.18 g, $CaCO_3$ 0.70 g, NaCl (iodized) 0.60 g, $MnSO_4$ 0.03). When supplements were incorporated into the ration, the amount of corn was correspondingly decreased. The letter *c* following the ration number indicates that the ration contained crystalline vitamins in the amounts used in experiments with purified diets:[2] thiamine 0.3 mg, riboflavin 0.5 mg, pyridoxine 0.4 mg, Ca *d*-pantothenate 1.5 mg, niacin 5 mg, choline chloride 100 mg, biotin 0.02 mg, *i*-inositol 50 mg, and 2-methyl-1,4-naphthoquinone 0.2 mg. All animals were given 20 mg of ascorbic acid orally every other day and 1 mg of α-tocopherol and 0.05 cc of haliver oil orally twice a week. The solution of α-tocopherol in corn oil was mixed with the haliver oil just prior to feeding.

The results obtained with Ration R-1 with and without various supplements are summarized in Table I. The results for the group receiving stock ration, a commercial-pelleted guinea pig feed, were described in a preceding publication[2] and are included for comparison. The animals that received Ration R-1 grew very poorly and less than half of them survived the 6-week experimental period. These animals showed a moderate anemia and leucopenia.

The addition of the crystalline vitamins (R-1c) enabled all animals to survive the experimental period and to maintain essentially normal levels of blood elements with a rate of growth ⅓ to ½ that of animals receiving the stock ration. With the work of Krehl, Teply, Sarma and Elvehjem[3,4] in mind, the vitamin mixture was replaced by

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3 Krehl, W. A., Teply, L. J., and Elvehjem, C. A., *Science*, 1945, **101**, 283.

4 Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A., *Science*, 1945, **101**, 489.