TABLE I. Comparative Vasodilation of Coronary Arteries to L-epinephrine and L-norepinephrine.

Exp.	L-epinephrine	L-norepinephrine
714	1.7	3.5
728	4.0	10.0
728	4.3	10.5
729	2.2	5.8

and l-norepinephrine the nor compound produced vasodilation about 2½ times greater than l-epinephrine as measured by this technique. The molecular weight of norepinephrine is 92.3% that of epinephrine hence these studies were not accomplshed on a strictly equimolecular basis, yet the difference in reactivity far exceeds the probable difference attributable to differing molecular weights.

Summary. 1. l-norepinephrine and l-epinephrine produce vasodilation of the isolated surviving coronary arteries of swine.

2. l-norepinephrine produces about 2½ times the degree of vasodilation produced by l-epinephrine in the same artery in the same dose. This difference exceeds the anticipated difference attributable to differing molecular weights.

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Comparative Study of the Ketogenic Activity of Certain Niacin Compounds.* (18160)

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Normal rats, when fed on a niacin deficient diet, probably synthesize sufficient niacin or niacin-like compounds to carry on normal metabolic processes, providing the diet contains adequate amounts of protein(1-3). However, when excessive amounts of niacin are given to diabetic rats or to normal fasting rats, a marked ketonuria is produced, resulting presumably from some alteration in the metabolism of fat(4-5). The present work is concerned with a comparison of the ketogenic action of niacin, niacinamide and the alcohol of niacin (Roniacol) in the non-fasting diabetic rat.

Experimental. Twenty-one adult male rats of the Long-Evans strain, 8 to 10 months of age, were used. After the rats had been fasted for 48 hours, diabetes was produced by a single subcutaneous injection of 125 mg of alloxan/kg body weight. All of the rats were diabetic for at least 2 months before the experiment was started and most of the animals showed a severe diabetes. Qualitative urinary acetone body determinations were made daily by the sodium nitro-prusside method, which gave an estimation of the degree of ketonuria. In the conditions of this experiment, each rat acted essentially as its own control.

Effect of niacin compounds when 10% fat diet was used. Initially, the animals were given the basal diet†(5) for several days in order to determine a baseline for the excretion of urinary acetone bodies. After this was established they were fed an experimental diet which consisted of the basal diet with 0.1%

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† The basal synthetic diet contained 10% fat, 18% protein, 70% carbohydrate with essential vitamins and minerals.

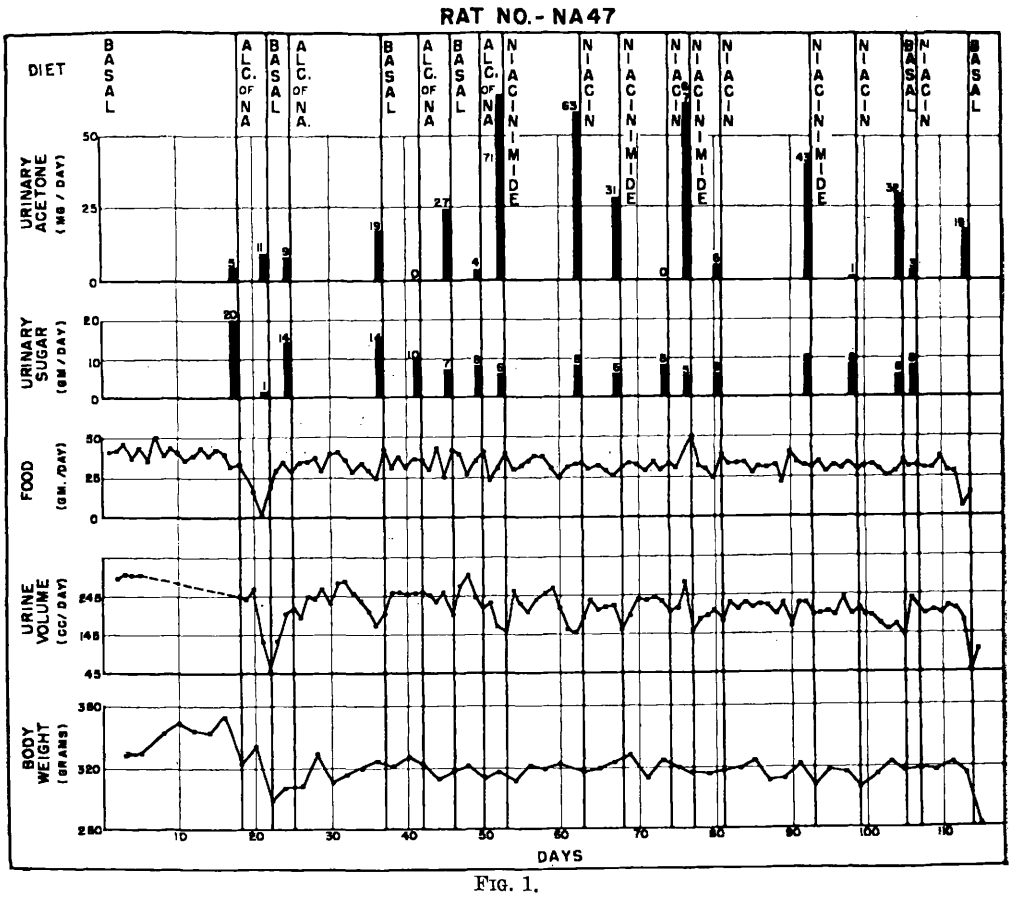

FIG. 1.

The alcohol of niacin, niacin and niacinamide diets were made by adding 0.1% of these compounds, respectively, to the basal diet. The values for urinary acetone and sugar are for the last 24 hours that a particular diet was fed.

of niacin, the alcohol of niacin or niacinamide.‡ The experimental diets were alternated several times with the basal ration during the study. Fig. 1 and 2A illustrate the manner in which the diet was alternated in two of the rats. Usually some increase in the excretion of acetone bodies was noted while the rats were eating diets containing either 0.1% niacin or alcohol of niacin. In many instances, however, several periods of feeding with the niacin compounds were necessary before ketonuria of a pathological degree was seen. When the rats were returned to the plain basal diet for 1 or 2 days, the severe ketonuria disappeared. Usually several days on the basal regimen were allowed to intervene between treatment with different compounds. However, since the niacinamide diet was the least effective in producing a ketonuria, in certain instances (Fig. 1), it was alternated with the diets containing 0.1% niacin or the alcohol of niacin rather than going back to the plain basal diet. Just before a diet was changed, quantitative urinary acetone body determinations were made by the method of Van Slyke and urinary sugar was determined by the micro-method of Somogyi(6). Of the 21 rats studied, 10 showed a rather prompt ketogenic response to niacin or the alcohol of niacin. Data for 2 animals, representative of the group, are shown on Figures 1 and 2A. When animal NA 47 received a supplement of 0.1%

‡ The vitamins were supplied through the kindness of Dr. Elmer L. Sevringhaus, Hoffmann-La-Roche, Inc.

6. Somogyi, M., *J. Biol. Chem.*, 1945, v160, 69.

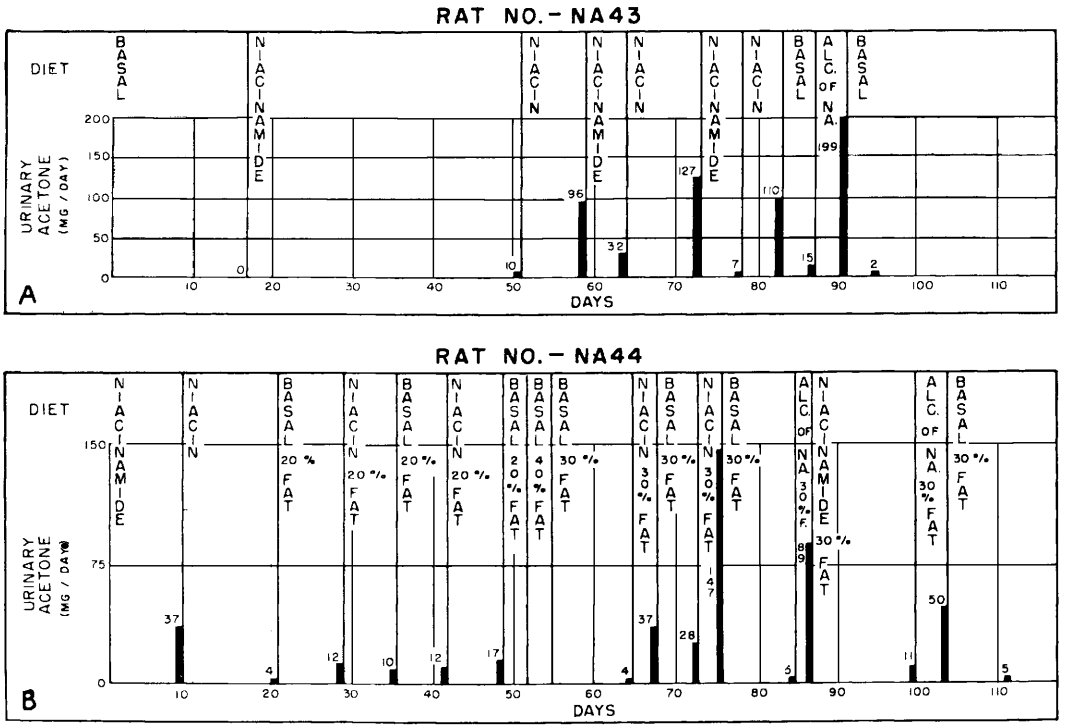

FIG. 2A.
The diets fed to Rat No. NA43 were similar to those given No. NA47 (Fig. 1).
FIG. 2B.
The niacin compounds were added to the high fat diet in the proportion of 0.1 mg to 350 calories of diet.

alcohol of niacin a mild ketonuria became evident on the 37th day of the experiment, and a severe ketonuria was present on the 52nd day. This pathological state persisted for 10 days even though the rat received the ration containing 0.1% niacinamide. The sequence of the alternation of diets is shown in Fig. 1. Towards the end of the study, the rats appeared to have developed some refractiveness to the niacin compounds, since the latter were less effective in producing ketonuria.

It was impossible to detect any ketone bodies in the urine of the second rat (NA 43) during 17 days on the basal regimen. The addition of 0.1% niacinamide caused only small amounts of urinary acetone to be excreted after 33 days on the supplemented diet. However, as soon as 0.1% niacin or alcohol of niacin was introduced, a marked ketonuria was seen.

In addition to urinary acetone values, Fig. 1 shows values for urinary sugar, food intake, urine volume and body weight. These data are quite typical for all the rats used in this experiment. The severity of the diabetes, as indicated by urine sugar values, declined during the course of the experiment. The curves for food intake, urine volume and body weight appeared very similar, since reduced urine volume and loss in body weight accompanied declines in food intake. No explanation can be given for the anorexia which occurred on the 21st day of the experiment. In certain instances, however, loss of appetite appeared to accompany marked ketonuria (not shown in Fig. 1). A reduced food intake is not the cause of the ketonuria, as was shown in previous studies in which rats fasted for as much as 5 days excreted only small amounts of acetone bodies(5).

Effect of niacin compounds when 20 to 30% fat diet was used. When niacin or the alcohol of niacin failed to evoke ketonuria within a period of 2 weeks, the rats under observation were given a diet containing a higher fat content(7), in order to make them mildly keto-

TABLE I. Excretion of Urinary Acetone Bodies. Average values last 24 hours when niacin compounds were given.

	No. diabetic rats	Controls	Niacinamide	Niacin	Alcohol of niacin
Basal synthetic diet	10	6.0(28)*	9.5(13)	27.4(26)	62.1(12)
20 to 30% fat diet	7	11.5(25)	21.7(3)	44.4(8)	72.0(13)

* No. of determinations.

nuric or bring them to the borderline of ketosis. A diet containing 20% fat was first given and the fat content was increased up to 30% when necessary. The protein in the high fat diets was maintained at 19% of the total calories and extra vitamins and minerals were added in proportion to the caloric content in order to prevent deficiencies. One particular level of fat was fed until the excretion of ketone bodies became stabilized. Excessive amounts of the niacin compounds were then added to the diet. Eleven rats were placed on the high fat diets and 7 showed an increased excretion of acetone bodies when niacin or the alcohol of niacin was fed. Three rats responded while on the 20% diet, one on the 25% and 3 on the 30% fat diet. The rats which did not respond to these diets had a milder diabetes and thus could not be brought to the borderline of ketosis. The urinary acetone excretion of one diabetic rat, representative of the group, which was fed the high fat ration is shown on Fig. 2B. Originally this rat showed a mild ketonuria when given the basal diet plus niacinamide, but since niacin was not effective in elevating the urinary ketone bodies the high fat diet was introduced. The 20% fat diet, with or without 0.1% niacin, failed to induce a severe ketonuria but when 0.1% niacin was added to the 30% fat diet a marked ketonuria was produced. Likewise, the 0.1% alcohol of niacin was effective in increasing urinary acetone bodies when it was fed with the 30% fat diet.

There was considerable variation in the ketogenic response shown by the different rats in the niacin compounds. As has been indicated, in certain animals it was necessary to alternate the experimental and basal diets several times before a marked ketonuria developed. Also, in some cases, there seemed to be a peak in the response to the niacin compounds which was followed by a slow but usually gradual decline in the level of ketone body excretion. However, it was possible to compare the effectiveness of the 3 niacin compounds by taking averages of the acetone body excretion just before the diets were alternated. Table I gives average values for mg of acetone excreted in the 17 rats which showed the ketogenic response. Data are shown for those fed the basal and the high fat diets. It is quite apparent from these data that the alcohol of niacin is the most ketogenic, niacin the next, and niacinamide the least ketogenic.

Recently, Banerjee *et al.*(8) gave intravenous injections of 500 mg of niacinamide to normal and diabetic patients. There was no increase of blood ketone bodies during the following two hours. From the data presented in the present studies, however, one would not expect that niacinamide would cause much of a ketosis, particularly during a two-hour period. Unpublished studies from this laboratory have shown that a well-controlled human diabetic may tolerate 1800 mg of nicotinic acid daily, when given orally, without showing a significant rise in urinary acetone bodies.

Summary. Excessive amounts of niacinamide, niacin or the alcohol of niacin were given to 21 diabetic rats. The latter two compounds caused a marked increase in the excretion of ketone bodies in 10 of these animals. The 11 remaining rats were fed a diet containing 20 to 30% fat in order to make them mildly ketonuric or bring them to the borderline of ketosis. When excessive amounts of the niacin compounds were then added to the high fat diets, 7 of the rats

7. Janes, R. G., and Prosser, M., *Am. J. Physiol.*, 1947, v151, 581.

8. Banerjee, S., and Ghosh, N. C., *J. Biol. Chem.*, 1949, v177, 789.

showed an increase in acetone body excretion. It is apparent from the data presented that the alcohol of niacin is the most ketogenic, niacin next and niacinamide the least ketogenic.

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Effect of Variations in Osmotic Pressure on Macrophages in Tissue Culture.* (18161)

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In experiments dealing with the cultivation of the exoerythrocytic forms of *Plasmodium gallinaceum* it became necessary to study the effects of variations of the culture medium. This in turn required a knowledge of the range of osmotic pressure tolerated by the malarial parasite and the host cell, the macrophage. This paper presents the results of experiments on the effect of variations in osmotic pressure on macrophages and parasites.

Methods. The tissue culture methods used in the present experiments have been described in detail(1). The macrophages were derived from chick embryo spleens infected with the exoerythrocytic stages of *Plasmodium gallinaceum*. Macrophages were grown *in vitro* at various constant levels of osmotic pressure for 4 days. These levels varied from 3 to 16 atmospheres of pressure at 37°C. The osmotic pressure determinations were made by means of the cryoscopic method. In previous experiments(1) it was determined that the optimal pH range for malarial parasites was pH 7.2 to 8.0, and for macrophages pH 6.8 to 8.2. For this reason the pH of the medium was kept between pH 7.4 and 7.8 in the present experiments. This was done by means of a continuous flow of CO_2 gas mixtures. The nutrient medium consisted of chicken serum diluted with a balanced salt solution.

The osmotic pressure was varied in two ways: (1) by varying the water content only, keeping the relative proportion of ingredients constant, and (2) by varying the content of NaCl. In the first set of experiments hypotonic solutions were prepared by starting off with a mixture of chicken serum and Tyrode's solution and by the addition of varying amounts of water to produce the desired hypotonic solutions. To achieve similar results with hypertonic solutions, a starting hypertonic solution was made from a mixture of 50% chicken serum and 50% triple concentrated Earle's solution(2).‡ Such a mixture has an osmotic pressure of 16 atmospheres at 37°C. Starting with this solution various hypertonic solutions varying from 16 to 8 atmospheres were made by the addition of water. In this set of experiments, however, the percentage of serum varied from one experimental group to another. This necessitated setting up a parallel series of control groups to determine the effect of diluting the serum at constant osmotic pressure. This was done by mixing various concentrations of serum in Tyrode's to match the experimental

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1. Dubin, I. N., and Yen, C. K., *Arch. Path.*, in press.

2. Earle, W. R., *J. Nat. Cancer Inst.*, 1943, v4, 165.

‡ Triple concentrated Earle's solution was highly alkaline, giving a precipitate of salts. To avoid the precipitate the solution was sterilized by passing through a sintered glass filter under 10 lb pressure of 100% CO_2. It was stored in stoppered bottles under equilibration with the same gas.