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Abstract

In order to see whether isovalthinuria can be induced in animals other than the cat, that was found to excrete isovalthine in normal urine as previously reported[3], using rat, guinea pig, rabbit and dog as test animals, isovaleric acid was adminstered either orally or parenterally and their urine was analyzed for the presence of isovalthine. As the result it was found that the rat, guinea pig, rabbit and dog administered with isovaleric acid orally or parenterally all excreted isovalthine in their urine, which normally does not contain it.

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EXPERIMENTAL ISOVALTHINURIA

I. INDUCTION BY ISOVALERIC ACID

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MIZUHARA *et al.*[1,2] have isolated a new sulfur-containing amino acid named "isovalthine" from the urine of hypercholesterolemic patients. Isovaleric acid residue of isovalthine molecule is known to be an intermediate of cholesterol synthesis and to be formed from leucine or acetic acid via β-hydroxy-β-methyl-glutaryl CoA in the animal body.

So far as examined in this laboratory, only cats excrete isovalthine in their normal urine. A previous communication[3] reported that the administration of leucine to cat caused an increased excretion of isevalthine.

Present experiments will show the experimental isovalthinuria induced by the administration of isovaleric acid to some animals of which normal urine never contains isovalthine. Animals tested are rat, guinea pig, rabbit and dog.

EXPERIMENTALS AND RESULTS

I. Identification of urinary isovalthine:

Urine is collected in a bottle containing toluene and hydrochloric acid and the urinary isovalthine is identified by the method of UBUKA[4], which consists of paper electrophoresis and paper chromatography.

II. Oral administration:

a) Rat: Male rats of about 180 g. body weight are used. Four control rats are fed on MF-solid food (Oriental Yeast Inc., Tokyo). Isovaleric acid-containing diet is prepared by mixing sodium isovalerate and MF-solid food. Four experimental rats are fed for one week on isovaleric acid-containing diet and MF-solid food, so that each rat receives 600 mg. of isovaleric acid per kg. body weight per day. The urine of each group is collected together under toluene for one week. The total amount of urine of control group was 250 ml. and that of experimental group was 210 ml.

Fig. 1 shows the chromatogram of acidic amino acid fraction of the experimental group in which a faint spot of isovalthine is observed. The chromatogram of control group did not show the presence of isovalthine, so the figure

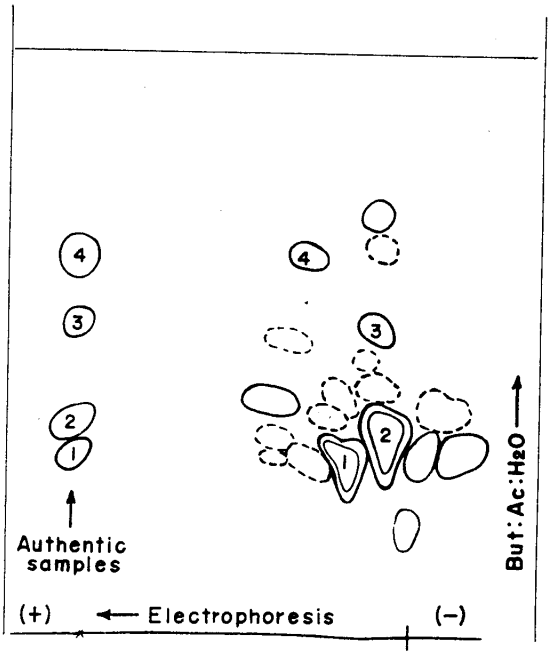

Fig. 1. A chromatogram of acidic amino acid fraction of rat urine after oral administration of isovaleric acid.
Spot number 1: Aspartic acid, 2: Glutamic acid, 3: Isobuteine, 4: Isovalthine.

200mg. of isovaleric acid per kg. body weight per day for four days. Urine is pooled together in each group for four days. The total amount of urine of control group was 270 ml. and that of experimental group was 230 ml.

Fig. 2 shows the chromatogram of urinary acidic amino acid fraction of experimental group. Besides isovalthine, a large unknown spot is seen with a high Rf value on vertical direction. This unknown spot was omitted.

b) Guinea pig : Male guinea pigs of about 370 g. body weight are used. Basal and isovalerate-containing diets for guinea pig are the same as that for rat, and some vegetables are also supplied in this case. Two guinea pigs are used in each group of the control and experimental. Each guinea pig of experimental group receives

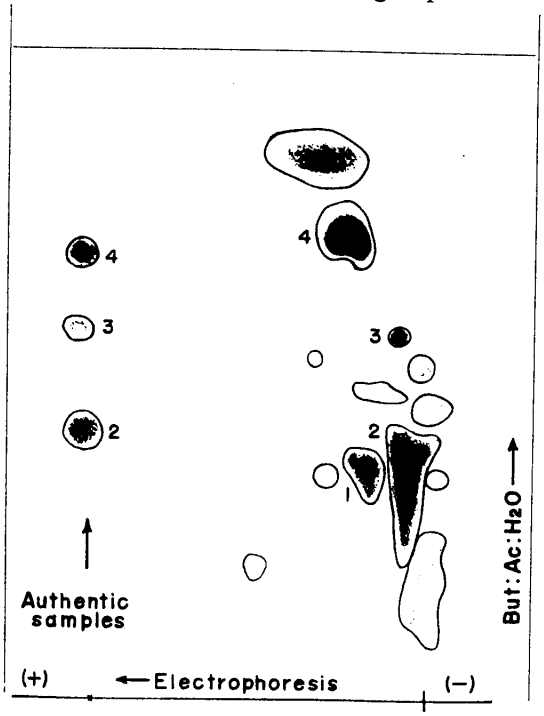

Fig. 2. A chromatogram of acidic amino acid fraction of guinea pig urine after oral administration of isovaleric acid.
Spot number is the same as in Fig. 1.

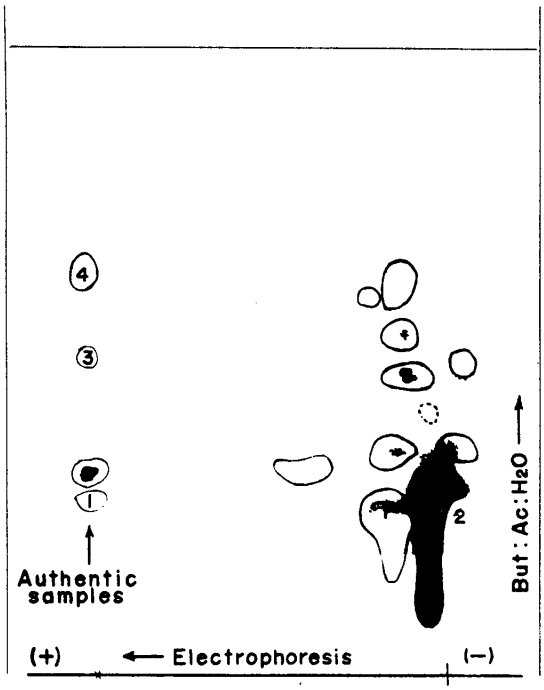

Fig. 3. A chromatogram of acidic amino acid fraction of guinea pig urine (control).
Spot number is the same as in Fig. 1.

is not seen in the urine of control group as shown in Fig. 3.

c) Rabbit: Male rabbits of about two and a half kg. body weight were fed on beancurd refuse. Two rabbits are used as control and one as experimental. Sodium isovalerate is mixed into beancurd refuse and experimental rabbit receives 60 mg. of isovaleric acid per kg. body weight per day for one week. The urine of each rabbit is collected separately for one week. The total volume of urine of each rabbit was about 1000 ml. of which 200 ml. were used for analyses.

Fig. 4 shows the chromatogram of an isovalerate-fed rabbit in which isovalthine is clearly seen. Control rabbits never excrete isovalthine.

d) Dog: Male dogs of about ten kg. body weight are fed on boiled barley and fish. Two dogs are used as control and one as

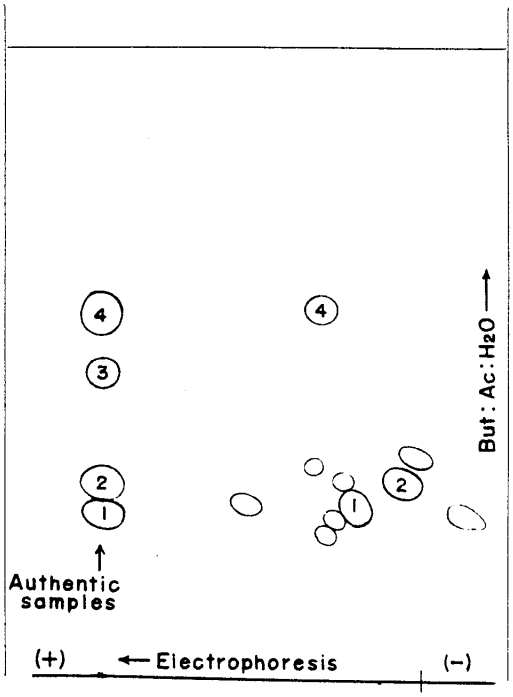

Fig. 4. A chromatogram of acidic amino acid fraction of rabbit urine after oral administration of isovaleric acid.
Spot number is the same as in Fig. 1.

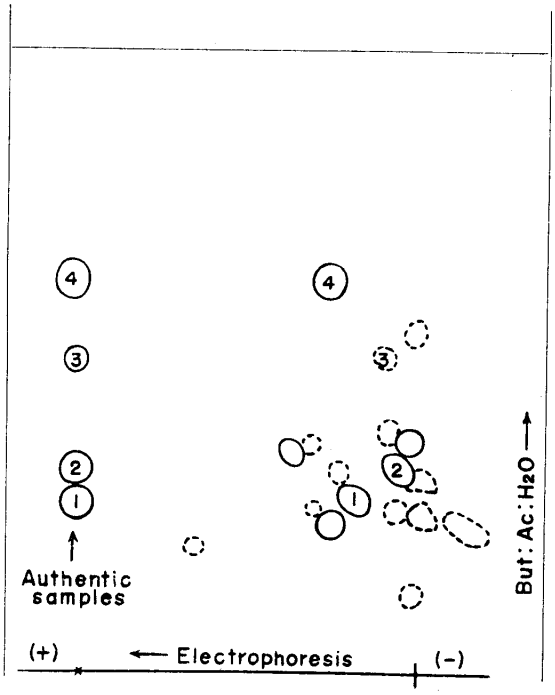

Fig. 5. A chromatogram of acidic amino acid fraction of dog urine after oral administration of isovaleric acid.
Spot number is the same as in Fig. 1.

experimental. Sodium isovalerate is mixed into boiled barley and given to an experimental dog in 100 mg. per kg. body weight per day for five days. Urine was collected for five days and its total volume of each dog was about 1000 ml. Two hundred ml. of the urine was used for analysis. Only the isovalerate-fed dog excretes isovalthine as shown in Fig. 5. The feeding of dogs with isovaleric acid is a quite convenient method for the isolation of fairly large amount of isovalthine.

III. Parenteral administration

a) Dog : A male dog of 4.6 kg. body weight is fed on the same diet as above used in II. d). Isovaleric acid neutralized with sodium bicarbonate is injected intraperitoneally, so that the dog receives the acid in 50 mg. per kg. body weight per day for three days. About 480 ml. of urine which is collected for

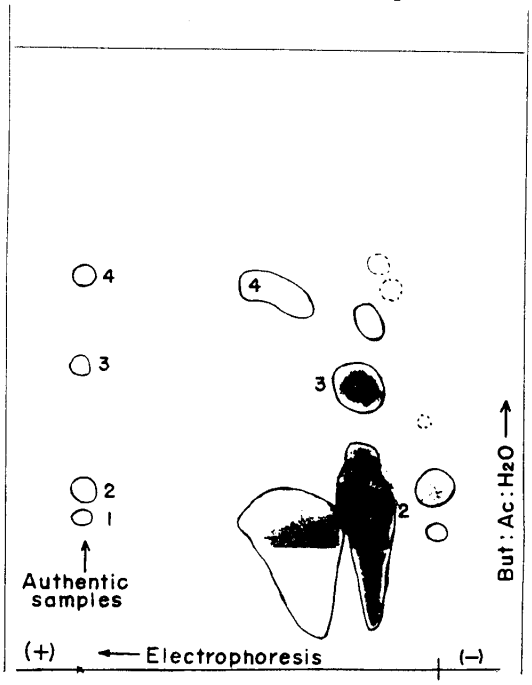

Fig. 6. A chromatogram of acidic amino acid fraction of dog urine after intraperitoneal administration of isovaleric acid.
Spot number is the same as in Fig. 1.

four days after the first injection is used for analysis.

Fig. 6 shows the excretion of isovalthine in the urine of the experimental dog.

b) Rat: Seven rats weighing 200—250 g. are used in this experiment. One ml. of sodium isovalerate solution (50 mg. per ml.) is injected everyday intraperitoneally to each rat for a week. The total volume of urine pooled from seven rats was about 500 ml. after one week.

Fig. 7 shows that isovalthinuria is induced also in rats after intraperitoneal administration of isovaleric acid.

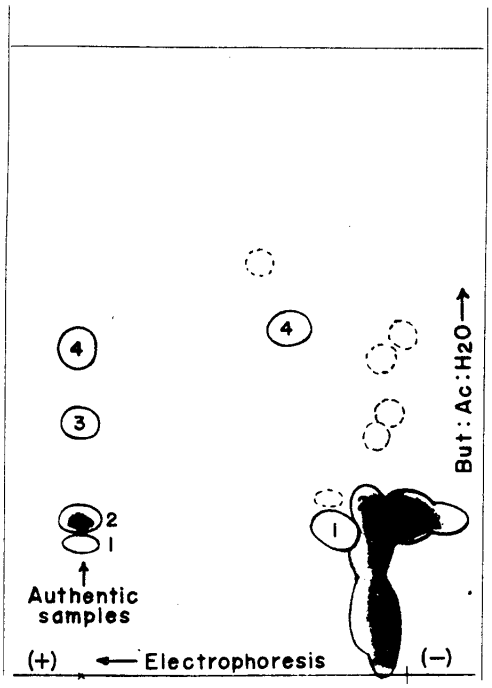

Fig. 7. A chromatogram of acidic amino acid fraction of rat urine after intraperitoneal administration of isovaleric acid.
Spot number is the same as in Fig. 1.

DISCUSSION

The present paper is the first report of experimental isovalthinuria induced by the administration of a normal metabolite to animals, which never excrete isovalthine in their normal urine. A possibility of isovalthine formation by intestinal bacteria will be denied by the parenteral experiment (III).

Although the limit concentration necessary for the induction of isovalthinuria is not determined in each animal, it may be different from animal to animal even in the same species.

It will be deduced from the present experiments that the isovalthinuria will be caused by the accumulation of isovaleric acid or similar precursor of cholesterol biosynthesis in the animal body. But at present the direct precursor of isovalthine is not known.

SUMMARY

In order to see whether isovalthinuria can be induced in animals other than the cat, that was found to excrete isovalthine in normal urine as previously

reported[8], using rat, guinea pig, rabbit and dog as test animals, isovaleric acid was adminstered either orally or parenterally and their urine was analyzed for the presence of isovalthine. As the result it was found that the rat, guinea pig, rabbit and dog administered with isovaleric acid orally or parenterally all excreted isovalthine in their urine, which normally does not contain it.

REFERENCES

1. MIZUHARA, S. and OHMORI, S.: A new sulfur-containing amino acid. *Arch. Biochem. Biophys.* 92, 53, 1961
2. OHMORI, S. and MIZUHARA, S.: Structure of a new sulfur-containing amino acid. *Arch. Biochem. Biophys.* 96, 179, 1962
3. FUKUTOME, K.: Biosynthesis of isovalthine in the cat. *J. Biochem.* (Tokyo) 49, 444, 1961
4. UBUKA, T.: Identification of isovalthine and its related compounds. *J. Biochem.* (Tokyo) 1962, in press