THE EFFECTS OF ACTH AND DEHYDROASCORBIC ACID ON THE SERUM AND URINARY URIC ACID LEVEL IN RABBITS

SHIGERU AONUMA AND KAZUE INOUE

Pharmaceutical Faculty, University of Osaka

There are already many reports describing of the diabetogenic action of alloxan (Goldener *et al.* 1943; Bailey *et al.* 1943). Patterson found that dehydroascorbic acid (DHA) (Patterson, 1950a), dehydroisoascorbic acid (Patterson, 1950a) and dehydroglucoascorbic acid (Patterson, 1950b) had the same action as alloxan in rats. Ascorbic acid also has weaker activity than DHA. It is said that certain structural similarities between alloxan and DHA or its analogues were held responsible for their parallel action (Fig. 1). It is supposed from the fact that both alloxan and DHA can make addition compounds with SH-group and react with amino acids (Strecker reaction) (Patterson, 1959a; Bessey *et al.* 1953). Injection of ACTH is also known to produce diabetes mellitus. Conn

Fig. 1. Structural Relationships of Uric Acid

et al. (1948) found a close correlation between heightened uric acid production as evidenced by increased uric acid excretion and glycosuria in man, following injection of ACTH. It is conceivable that as the result of increased uric acid, intermediaries of uric acid metabolism, similar in their effects to alloxan, are produced and reach a critical intracellular concentration in the beta cells. The authors supposed that uric acid might have some correlation to the diabetogenic effect of DHA, there being some structural resemblance between uric acid and alloxan (Fig. 1). In this paper, the effect of injection of DHA on the serum and urinary uric acid level in rabbits was described.

Fig. 2. Effects of ACTH and DHA on the Serum Uric Acid Level in Rabbits

* 10 U.S.P. units: Armour's Lot. No. K55508.

MATERIALS AND METHODS

Analysis of serum uric acid—Male rabbits weighing 2 to 3 kg were fasted for 24 hrs. before the experiment and injected materials to the intravenous. The blood samples were withdrawn immediately before (control) and 1, 3 and 5 hrs. after the injection. Uric acid content in 1 ml of serum from each animal was determined; protein was removed with 10 per cent tungstic acid solution, and into the filtrate cyanide-urea solution (Newton, 1937) and phosphotungstic acid solution (Brown, 1945) were added. Phosphotungstic acid was reduced with uric acid and then the blue color of WO_3 developed quantitatively and was measured photometrically with the filter S-72 after 1 hr.

Analysis of urinary uric acid—Male rabbits weighing 2 to 3 kg were constantly fed 1 week before the injection and during the experiment. The materials were injected intravenously. Urine was collected for 24 hrs. before and every 24 hrs. after injection following 3 days. Then the urinary uric acid contents were analyzed; it was filtered and diluted as much as ten times. One ml of diluted urine was treated to determine uric acid content likely as serum uric acid. Uric acid excretion per day was given next formula; (Uric acid content $\times$ urine volume per day).

Preparation of DHA solution—DHA solution was prepared immediately before each injection using the method of Patterson (1950a), *i. e.* 1.8 g of ascorbic acid in water (10 ml) was oxidized by shaking with 0.66 g of quinone in ether (10 ml) and the aqueous solution was washed 3 times with an equal volume of ether. Excess ether was removed by suction and the solution was used without further treatment. This solution contained 98 mg of DHA per ml and seldom ascorbic acid.

EXPERIMENTAL RESULTS

Fig. 2 shows the results of determination of serum uric acid, following a single intravenous injection of 100 mg of DHA, 10 mg of DHA and 10 mg of ACTH on rabbits. Serum uric acid of intact rabbits and those injected 1 ml of

0.9 per cent NaCl were also determined. The injections of DHA (10 mg) and ACTH (10 mg) had no effect on serum uric acid, but 100 mg of DHA apparently increased serum uric acid 1 hour after injection and decreased to its normal level after 5 hrs. Then 100 mg of DHA were administered to each of the 6 rabbits and the significant difference was found between the normal values and the values after 1 hour (Table 1).

A hundred mg. of DHA per day was administered for 4 days and serum uric acid was determined during the injection period and the following 4 days. The results are shown in Fig. 3. In this case the blood samples were withdrawn before injection (control) and just 24 hrs. after each injection. By the first injection serum uric acid was in the range of normal level, as supposed from the results of Fig. 2 and Table 1. As injections were repeated, uric acid level gradually increased. When the injection was stopped, it returned to its normal level.

Fig. 3. Effect of the Daily Administration of DHA (100 Mg per Day) for 4 Days on the Serum Uric Acid Level in Rabbits

Table 1. Effect of the injection of DHA (100 mg) on the serum uric acid level in rabbits

Rabbit No.	Serum uric acid mg %			
	Before injection	1 hr.	3 hrs.	5 hrs.
1	0.43	0.76	0.60	0.43
2	0.54	0.66	0.52	0.44
3	0.29	0.54	0.37	0.39
4	0.55	0.71	0.31	0.39
5	0.32	0.78	0.62	0.40
6	0.56	0.76	0.38	0.64

Conn *et al.* (1948) observed uric acid increase in urinary excretion rather than in serum when ACTH was injected on man. The authors therefore determined the urinary uric acid excretion, following injection of 100 mg of DHA and 5 mg of ACTH. As the normal urinary uric acid excretion itself varies in rather a wide range, it was difficult to distinguish the effect of injections. In this experiment, the range of normal excretion was from 10 to 35 mg per day. When the value deviated from this range, it was defined the change caused by injection. Effects of DHA (100 mg) and ACTH (5 mg) were shown in Fig. 4 and Fig. 5 respectively. DHA and ACTH both increased urinary uric acid excretion a day after a single injection and returned to its normal value 2 days after.

Fig. 4. Effect of 100 Mg of DHA on Uric Acid Excretion in Rabbits

Fig. 5. Effect of 5 Mg of ACTH on Uric Acid Excretion in Rabbits

DISCUSSION

From the results that injection of DHA made increase serum uric acid and urinary excretion, it would be proved that uric acid had some correlation to the diabetogenic action of DHA. ACTH had no effect on serum uric acid, but increased urinary uric acid excretion. As regards the latter, the authors got same results as Conn *et al.* (1948). Usually rats are used for the diabetogenic assay of DHA. In this work, the effect of DHA on blood sugar level was measured on rabbits by the method of the authors (Ito, *et al.* 1956). When 100 mg of DHA was injected (a large dose of DHA would make rabbits die (Bhattacharya *et al.* 1956)), it was observed of the significant rise in 5 per cent level on blood sugar. The increase of uric acid excretion in rabbits by daily administration of DHA (100 mg) was observed only 2 in 5 animals. On this point, the authors will discuss in their next report.

SUMMARY

1. Intravenous injection of DHA (100 mg) increased serum uric acid after 1 hr., but injection of ACTH (10 mg) had no effect on serum uric acid.
2. Daily injections of DHA (100 mg per day) gradually increased serum uric acid level only for the period of injections.
3. Injection of DHA (100 mg) and ACTH (5 mg) apparently increased urinary uric acid excretion a day after injections.

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