Influence of Insulin on Liver Vitamin A in Rats

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SUMMARY

Both Regular and globin-type insulins interfered with storage of vitamin A in the livers of rats by all routes of injection. Regular insulin, injected subcutaneously, had the least effect. Injected insulin increased the rate of depletion of previously stored vitamin A from the liver. Alloxan diabetes had no effect upon liver storage of ingested vitamin A nor upon the rate of depletion of vitamin A from the liver. DIABETES *16*:704-07, October, 1967.

A number of factors affect the amount of vitamin A stored in the liver of the rat; among these are sex, nutritional state, and hormone balance. Female rats store comparatively more of the vitamin than do males.[1] In prolonged dietary deficiency of the vitamin, the liver reserve is depleted.[2] Clark and Colburn[3] showed that injections of cortisone cause diminished liver stores of vitamin A in rats. Clinical[4,5] and laboratory[6,7] studies have shown that diabetes mellitus interferes with conversion of carotene to vitamin A, and insulin has been shown to impair vitamin A storage in guinea pigs.[8]

Since both the diabetic state and administration of insulin have been shown to affect vitamin A storage adversely, this study was undertaken to compare the influence of injected insulin on the storage and rate of depletion of vitamin A in the livers of normal and diabetic rats.

METHODS AND MATERIALS

Vitamin A supplements

The purity of the vitamin A acetate samples was determined from the extinction coefficient in 2-propanol using $E_{1\,cm}^{1\%} = 1{,}525$ as the criterion.[9] Vitamin A acetate was dissolved in corn oil to give a concentration of 150 μgm. per milliliter.

Insulin

Regular insulin or a 3:1 mixture of Regular and Protamine Zinc insulin (hereafter referred to as globin-type insulin because it has a duration of action similar to Globin-Zinc insulin) was given daily in injections of 0.5 U. in 0.2 ml. of 0.85 per cent saline solution.

Diet and supplementation

Female Sprague-Dawley rats twenty-one days old were placed on the U.S.P. vitamin A-free diet with feed and water supplied ad libitum throughout the experiments. The rats were depleted of liver vitamin A to produce a higher degree of uniformity of liver vitamin A concentrations, resulting in more uniform storage of vitamin A to be fed during the experimental period. When analysis of livers of rats selected at random revealed that liver vitamin A was depleted, the rats were arranged in groups of six to twelve of approximately equal weight. Depletion usually required about two weeks. Test and control groups were given 30 μgm. of vitamin A daily by mouth. Test groups were given 0.5 U. of insulin by subcutaneous, intramuscular or intraperitoneal injection in addition to vitamin A supplements. All supplements and injections were given between 8:00 and 10:00 a.m. daily. Each experimental period was twelve days, unless otherwise indicated, followed by one day of no supplementation. The rats were sacrificed on the morning of the fourteenth day and the livers were removed, weighed, and placed in flasks containing 30 ml. of 12 per cent alcoholic potassium hydroxide.

Rats used to study the effect of insulin on the rate of depletion of liver vitamin A were fed 60 μgm. of vitamin A acetate daily for fourteen days. On the fifteenth day administration of vitamin A was discontinued, and from the sixteenth through the twenty-seventh day test groups were given Regular or globin-type insulin by subcutaneous, intramuscular or intraperitoneal injections.

To test the influence of alloxan diabetes on depletion of liver vitamin A stores, vitamin A-deficient rats were fed 30 μgm. of vitamin A acetate daily for twelve days. Twelve of these rats were then killed and the vitamin A content of each liver was determined. The remain-

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ing twelve rats were divided into two groups; one group served as untreated controls and the other group was treated with alloxan. Both groups were maintained on the vitamin A-free diet and water without further treatment for the remainder of the experimental period. Twelve days later, both groups were sacrificed and the vitamin A content of each liver was determined.

Diabetes

Alloxan diabetes was produced in rats by intraperitoneal injection of 200 mg. per kilogram body weight of alloxan one week before they were put on experiments. Weight loss and blood glucose levels were used as criteria for determining the diabetic state in the rats.

Liver vitamin A analysis

The rat livers were removed under ether anesthesia and saponified by refluxing with 12 per cent alcoholic KOH. The vitamin A was extracted with peroxide-free diethyl ether. An aliquot of dry ether extract was evaporated to dryness under reduced pressure and the residue was dissolved in 1 ml. of dry chloroform. The vitamin A was estimated by the Carr-Price antimony trichloride reaction[10] with use of the Evelyn Photoelectric Colorimeter and a 620 filter.

RESULTS AND DISCUSSION

Table 1 presents a comparison of the effects of short-acting Regular insulin with globin-type insulin, which has an intermediate duration of action. All groups of insulin-treated rats stored less vitamin A than did the controls. Rats given Regular insulin subcutaneously showed the smallest difference in stored vitamin A; livers from these rats stored an average of 70.8 per cent as much of the vitamin as did controls. Rats given Regular insulin by intramuscular and intraperitoneal injection stored, respectively, 35.5 per cent and 39.8 per cent as much vitamin A as did controls. Only slight variations in liver vitamin A were found among rats given globin-type insulin, ranging from 25.4 per cent to 32.6 per cent of control values. A possible explanation for the large difference in effect on liver vitamin A found between Regular insulin by subcutaneous injection and all of the other combinations of insulin type and route of administration may be related to differences in the circulating level of insulin resulting from differences in rate of absorption from the various routes of injection. It is well known that a substance injected subcutaneously is absorbed more slowly than if injected intramuscularly or intraperitoneally. If rate of absorption were the determining factor, subcutaneously injected insulin would not reach as high a concentration in the bloodstream as insulin injected by other routes, and therefore would have less effect upon storage of vitamin A.

An explanation entirely different from rate of absorption is also possible. Subcutaneously injected insulin, being absorbed more slowly, is subjected for a longer time to the influence of certain insulin-inactivating enzymes.[11] This view is supported by the fact that globin-type insulin exerted a fairly uniform effect by all routes of injection. In globin-type insulin the hormone is bound to a protein and is presumably less vulnerable to the attack of the "insulinase" enzymes. One further point suggesting that rate of absorption is not the determining factor is that globin-type insulin is absorbed slowly from all sites of injection, and yet is as effective in lowering liver storage of vitamin A as Regular insulin given by intramuscular or intraperitoneal injection.

No statistical correlation was found to exist between mean initial body weight and final liver vitamin A, nor between weight gain and liver vitamin A.

The data in table 2 show that either type of insulin, by any route of administration, significantly increased the rate of depletion of vitamin A from the liver. At the

TABLE 1

Comparison of the influence of Regular and globin-type insulin on liver storage of vitamin A in rats fed 30 μgm. of vitamin A acetate daily for twelve days

Daily insulin treatment	Number of rats	Mean initial body weight (gm.)	Mean body weight gain (gm.)	Mean liver vitamin A (μgm.)
None	12	153	25.2	109.3 ± 4.55*
0.5 U. Regular insulin s.c.	12	160	23.3	77.4 ± 3.75
0.5 U. Regular insulin i.m.	12	152	23.8	38.8 ± 2.68
0.5 U. Regular insulin i.p.	12	160	21.6	43.5 ± 3.27
0.5 U. globin insulin s.c.	10	153	23.9	27.8 ± 2.20
0.5 U. globin insulin i.m.	10	153	20.9	34.9 ± 3.21
0.5 U. globin insulin i.p.	12	157	24.5	35.7 ± 1.84

*Standard error of the mean.

TABLE 2

The effect of Regular and globin-type insulin on the rate of depletion of liver vitamin A during a twelve-day period

Experimental group and daily treatment	Number of rats	Mean body weight before depletion (gm.)	Mean body weight gain (gm.)	Mean liver vitamin A (μgm.)
Before depletion*	10	204	—	404.9 ± 16.6†
After depletion untreated	12	181	21.4	205.2 ± 9.41
0.5 U. Regular insulin s.c.	11	186	16.8	139.8 ± 6.63
0.5 U. Regular insulin i.m.	12	189	18.6	127.1 ± 6.88
0.5 U. Regular insulin i.p.	12	192	20.0	86.7 ± 5.39
0.5 U. globin insulin s.c.	12	188	17.7	84.1 ± 5.17
0.5 U. globin insulin i.m.	12	187	20.2	84.1 ± 6.04
0.5 U. globin insulin i.p.	13	184	18.5	70.7 ± 5.09

*All rats had been fed 60 μgm. of vitamin A acetate daily for fourteen days immediately preceding the depletion period.
†Standard error of the mean.

end of the twelve-day depletion period, the livers of control rats still retained 50 per cent of the initial storage level of vitamin A. Livers of insulin-treated rats retained only 17.5 to 35 per cent of the initial vitamin A levels.

Rats given Regular insulin subcutaneously retained 34.6 per cent of the initial vitamin A; rats given Regular insulin intramuscularly retained 31.4 per cent; rats given Regular insulin by intraperitoneal injection retained 21.4 per cent. In rats given globin-type insulin, those injected subcutaneously and intramuscularly each retained 20.8 per cent, while rats injected intraperitoneally retained 17.5 per cent of the initial storage level of vitamin A.

By taking the percentage ratio of mean total liver vitamin A of each insulin-treated group to mean total liver vitamin A of the control group, a pattern of liver vitamin A storage in response to insulin administration can be established. Table 3 shows a general similarity in the storage patterns of rats given insulin and vitamin A simultaneously (cf. table 1) and rats given insulin during a period of depletion of vitamin A (cf. table 2). This suggests that the mechanism by which insulin interferes with storage of vitamin A is the same mechanism by which it mobilizes the stored vitamin. It is not clear whether the effect of insulin is directly upon the vitamin A or upon some other component of the liver.

Alloxan diabetic rats, fed 30 μgm. per day of vitamin A acetate, stored vitamin A in about the same quantities as did controls (table 4). In diabetic rats given globin-type insulin concurrently with vitamin A, liver vitamin A storage was 78 per cent of control levels for rats given insulin subcutaneously, and 68.4 per cent of control levels for rats given insulin intramuscularly. In this study no insulin was given intraperitoneally.

Alloxan diabetes did not affect depletion of vitamin A

TABLE 3

Comparison of the effect of insulin on storage of vitamin A by the liver with the effect of insulin on rate of depletion of stored vitamin A from the liver

Type of insulin	Route of injection of insulin	Storage Mean liver vitamin A (per cent of control level)	Depletion Mean liver vitamin A (per cent of control level)
——	Controls— no insulin	100	100
Regular	Subcutaneous	70.8	68.2
Regular	Intramuscular	35.5	62.0
Regular	Intraperitoneal	39.8	42.3
Globin-type	Subcutaneous	25.4	41.2
Globin-type	Intramuscular	31.9	41.2
Globin-type	Intraperitoneal	32.6	34.5

stored in the liver (table 5). After twelve days on the depletion diet, there was no significant difference in liver vitamin A storage between controls and diabetic rats.

The data in table 1 show that vitamin A storage in rats is inhibited by insulin, as has also been reported for guinea pigs.[8]

Results reported in tables 4 and 5 confirm a report[6] that diabetes does not affect assimilation of preformed vitamin A.

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TABLE 4

Influence of globin-type insulin on liver storage of vitamin A in alloxan diabetic rats fed 30 μgm. of vitamin A acetate daily for twelve days

Experimental group	Number of rats	Mean initial body weight (gm.)	Mean body weight gain (gm.)	Blood sugar* (mg./100 ml.)	Mean liver vitamin A (μgm.)
Normal rats (untreated)	15	67.9	53.4	69.2	106.4 ± 2.86†
Alloxan-diabetic rats (untreated)	11	66.6	25.7	180.9	115.6 ± 5.66
Diabetic† 0.5 U. insulin s.c.	8	71.5	26.9	156.8	83.1 ± 5.30
Diabetic† 0.5 U. insulin i.m.	11	66.5	24.3	106.7	72.8 ± 3.70

*Rats fasted for twelve hours prior to collection of blood; samples collected twenty-four hours after last injection of insulin.

†Standard error of the mean.

TABLE 5

Influence of alloxan diabetes on the rate of depletion of liver vitamin A of rats during a twelve-day period

Experimental group*	Number of rats	Mean body weight before depletion (gm.)	Mean body weight change (gm.)	Mean liver vitamin A (μgm.)
Controls before depletion	12	177.5	—	109.3 ± 4.55†
Controls after depletion	7	177.5	9.1 (gain)	33.6 ± 2.91
Alloxan-diabetic after depletion	5	180.6	35.4 (loss)	37.5 ± 5.18

*All rats had been fed 30 μgm. of vitamin A acetate daily for twelve days immediately preceding the depletion period.

†Standard error of the mean.

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