THE INFLUENCE OF INSULIN AND OTHER PHYSIOLOGICAL FACTORS ON LIVER STORAGE OF VITAMIN A

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I hereby recommend that this dissertation prepared under my direction by William H. Bowles

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TO MY WIFE

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ABSTRACT

THE INFLUENCE OF INSULIN AND OTHER PHYSIOLOGICAL FACTORS ON LIVER STORAGE OF VITAMIN A.

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Injection of 0.5 unit insulin concurrently with oral administration of 120 μg of beta-carotene or 30 μg of vitamin A acetate to vitamin A-deficient rats daily for 12 days caused less vitamin A to be stored in the livers of insulin-treated rats than in non-insulin-treated rats. Administration of insulin to normal rats increased the rate of depletion of previously stored vitamin A from the liver, so that at the end of the depletion period livers of insulin-treated rats contained only 40 to 65 per cent as much vitamin A as did livers of rats given no insulin. Alloxan-induced diabetes caused rats fed carotene to store less vitamin A than did non-diabetic rats. Administration of insulin to alloxan-diabetic rats further inhibited liver storage of vitamin A. Alloxan-diabetes had no significant effect upon storage of ingested vitamin A acetate, nor upon the rate of depletion of vitamin A previously stored in the liver. Cortisone at a dose of 1 mg/day inhibited

storage of vitamin A in the liver of the rat. To test for a possible relationship between insulin and cortisone, cortisone was given to alloxan-diabetic rats along with carotene or vitamin A acetate. Cortisone significantly improved liver vitamin A storage in alloxan-diabetic rats. Adrenalectomized rats stored approximately 75% as much vitamin A as did normal rats and insulin-treated adrenalectomized rats stored only 57% as much vitamin A as did normal rats.

INTRODUCTION

An early record of the treatment of a nutritional disease dates back to about 1500 B.C. Akroyd (1) states that in the Ebers Papyrus, an ancient Egyptian treatise on medicine, roast ox liver or liver from a black cock is recommended as a cure for night-blindness. The famous Greek philosopher, Hippocrates, is said to have also prescribed ox liver for night-blindness but suggested that it be eaten raw after being dipped in honey (1).

Although men have known how to cure night-blindness for hundreds of years, studies by Fridericia and Holm (2) in 1925 were among the earliest planned scientific experiments on the relationship between vitamin A and dark adaptation. They were able to show that dark adaptation was defective in vitamin A-deficient rats, and that the pigment "visual purple" could be formed only slowly in their retinas.

Yudkin (3) first proved the presence of vitamin A in the retinas of normal animals. Wald (4, 5) showed that "visual purple" is a complex of a protein with vitamin A and elucidated the role of vitamin A in dark adaptation.

In 1912 Hopkins (6) showed that diets containing carbohydrates, fats and proteins were inadequate for growth, and that

certain accessory food factors were required for normal growth. McCollum and Davis (7) showed that certain lipids were essential for growth with closely related but independent work by Osbourne and Mendel (8, 9, 10). In 1914 McCollum and Davis (11, 12, 13) announced the isolation of a substance in butterfat that stimulated growth, which they named accessory factor A.

In 1914 Palmer and Eckles (14) showed that the yellow "lipochrome" pigments of some animal fats were not produced by the animal but were absorbed from vegetable sources in the diet. From this and other early investigations of vitamin A, a relationship between vitamin A activity and yellow color was noted and studied by McCollum and co-workers (15). They compared diets made up largely of colored foods with diets made up of largely colorless foods, and showed that yellow-pigmented foods gave better growth than did colorless.

Steenbock (16) concluded that the relationship of yellow color to vitamin A activity was not accidental. But when he tested carotene and xanthophyll, the latter was found to be inactive. Thus, he showed that not all yellow pigments possessed vitamin A activity. Since some animal fats with little or no pigmentation showed vitamin A activity, Steenbock theorized that there must be some carotenoid which could be converted to a colorless form without loss of its vitamin A activity. The accuracy of his theory can readily be seen in the light of present knowledge of the carotene-vitamin A relationship.

Palmer and Kempster (17) compared growth, appearance and reproduction in chickens on a normal diet with chickens on a test diet containing white maize, white summer squash and white onions. Growth and pigmentation were normal in chickens on a normal diet, while chickens in the test group failed to grow, and bleaching of skin, beaks, earlobes and shanks occurred. When the test diet was supplemented with pig's liver, growth was obtained but lack of pigmentation continued, and the hens layed eggs with colorless yolks. These chickens grew, reproduced and appeared healthy on a diet free of "lipochrome." Before the significance of the pig liver became known, it appeared that Steenbock's theory had failed to stand a rigid test.

An important step in the discovery of vitamin A came when Stephenson (18) demonstrated that butterfat retained its vitamin A activity after being decolorized with charcoal. This showed that vitamin A activity was not dependent upon color. Drummond and Coward (19) reported similar findings and conceded that if vitamin A were a member of the lipochrome group, it could have an active leuko-form.

Carotenoids have been known to give a blue-color reaction with concentrated sulfuric acid. Cod-liver oil was also known to give a bright-blue to purple color when treated with sulfuric acid. Rosenheim and Drummond (20) correctly inferred that this color reaction was a direct indication of vitamin A. Drummond and Watson (21) then tested the sulfuric acid on the liver oils of fish,

birds and mammals, as well as butterfat and other body fats. They showed that the reaction was lost in the liver oils of animals which were vitamin A-deficient but occurred in animals which had been cured of vitamin A deficiency. Zilva and Drummond (22) found that all fish liver oils tested proved to be potent sources of vitamin A and reaffirmed the potency of ox liver as a source of vitamin A, as inferred in the Ebers Papyrus. Drummond and Zilva (23) traced the origin of vitamin A in fish oils to the marine algae which is a major food source for the fish.

In 1926 Carr and Price (24) showed that a saturated solution of antimony trichloride in chloroform reacts with vitamin A to give a blue color. This reaction has become known as the "Carr-Price Reaction." Von Euler (25) showed that carotene could also react with antimony trichloride and that the curves for the two reactions were very similar, pointing to the possibility of some structural relationship between carotene and vitamin A. Direct evidence for such a relationship was presented by Von Euler, et al. (26) in which it was shown that as little as 10 μg of crystalline beta-carotene could cure rats suffering from vitamin A deficiency.

In 1929 Moore (27-29) presented conclusive evidence that carotene is converted to vitamin A in the animal body. He fed rats a vitamin A-free diet until no vitamin A could be detected in their livers. Carotene was then fed to some of the vitamin A-depleted rats and the presence of vitamin A was then demonstrated in their livers.

The final proof of the relationship between carotene and vitamin A came when Karrer (30-33) determined the structure of carotene and vitamin A, and demonstrated their structural relationship. Kuhn (34) showed that one mole of beta-carotene could yield two moles of vitamin A, although most carotene feeding experiments have shown a much lower activity for carotene on a weight basis. Koehn (35) claimed quantitative biological conversion of carotene to vitamin A, using what appeared to be an optimum combination of carotene and alpha-tocopherol.

The next problem to be dealt with in the carotene-vitamin A relationship was the determination of the site of conversion of the carotene to vitamin A. Since vitamin A was found in the liver in large quantities, Moore (29, 36) assumed that this organ contained the "carotenase" enzyme. Many experiments were carried out in an effort to prove that the liver is the site of conversion, but results were generally negative (37).

Studies by Sexton, et al. (38) in which they compared conversion of carotene administered orally and parenterally to rats, showed that there was little or no conversion when carotene was injected parenterally even though there was evidence that the carotene reached the liver. The larger amount of vitamin A formed after oral dosing of carotene led to suspicions that the intestine, rather than the liver, might play a major role in carotene conversion.

Mattson, Mehl and Deuel (39) showed that after oral dosing of rats with carotene, vitamin A first appears in the intestinal wall. Glover, et al. (40, 44) conducted similar studies, and demonstrated that carotene and vitamin A were both present in the intestinal wall after oral administration of carotene, but only vitamin A reached the liver. Thompson and co-workers (41, 45, 46) washed out the contents of the small intestine and analyzed both contents and intestinal wall for vitamin A. They concluded that the conversion of carotene to vitamin A takes place in the intestinal wall. In vitro studies by Wiese, Mehl and Deuel (42), Rosenberg and Sobel (53), and Olsen, et al. (54) showed that the isolated small intestine was able to convert carotene to vitamin A.

It was assumed that after ingestion of carotene, the vitamin A formed was absorbed by the lacteals and carried by the lymphatic system into the general circulation via the thoracic duct. Thompson and co-workers (46) were the first to prove that this assumption was correct. They showed that after oral dosing of rats with carotene or vitamin A, the systemic and portal blood showed equal rates of increase in vitamin A concentration. If the lymph was diverted from the bloodstream by cannulation, no increase in vitamin A concentration was found in either systemic blood or liver. Goodwin and Gregory (43) cannulated the thoracic duct of goats and showed that orally-administered carotene produced a rise in lymphatic vitamin A. Alexander and Goodwin (47), using a similar technique in rats, showed that no carotene was present in the

lymphatic fluid. These findings established the route by which vitamin A reaches the liver.

Studies of carotene administration by routes other than oral by Bieri and Pollard (48), McGillivray, et al. (49), Kon and co-workers (55), and in vitro studies by Worker (50) have shown that a large number of tissues in addition to that of the wall of the small intestine possess the ability to convert carotene to vitamin A, although no other organ is as efficient as the small intestine.

The greater efficiency of the small intestine may be related to the lipid-dispersing system of that organ. For example, Hebert and Morgan (51) showed that carotene dispersed in Tween 40 was utilized better than when dissolved in oil. Glover (52) suggested that the small intestine may contain a higher concentration of the enzyme system which carries out the conversion than does any other organ.

Moore (56) was one of the first to recognize the sparing effect of tocopherols on beta-carotene. It was shown by Davies and Moore (57) that in vitamin E deficiency the rate of depletion of vitamin A stores in rats on a vitamin A-free diet was higher than when alpha-tocopherol was given at regular intervals to rats on a vitamin A-free diet. It was also found that administration of alpha-tocopherol increased utilization of ingested carotene and vitamin A, and the tocopherol requirement for optimum utilization of carotene was much greater than for vitamin A. Koehn (35) also demonstrated

the necessity for tocopherol for optimum carotene conversion and concluded that tocopherol acts as an antioxidant. In his work Koehn showed that on a weight basis carotene is equally as active as vitamin A for promoting growth. Johnson and Bauman (58) studied the effects of various levels of tocopherol on carotene utilization as measured by vitamin A storage in the liver of the rat. They showed that 0.5 mg of alpha-tocopherol per day has optimum effect on carotene utilization, while levels higher than 1 mg per day tend to inhibit carotene utilization. No increase in fecal carotene was noted, so it was concluded that high levels of tocopherol interfere with enzymatic conversion but do not decrease absorption of carotene.

The physiological role of bile as an emulsifier in fat absorption is well known, so it was only logical that it should play some role in absorption of fat-soluble vitamins. Greaves and Schmidt (60) were among the first to study the effect of bile on carotene and vitamin A absorption. Irvin, et al. (61) also demonstrated the effectiveness of bile in carotene utilization. Thompson and co-workers (45) showed that carotene is not converted to vitamin A at any point above the entrance of the common bile duct into the small intestine. Olson (62, 63) showed that the salt, sodium glycocholate, was equal to bile as a dispersing agent for beta-carotene, while cholate was less effective and deoxycholate was ineffective.

Tweens have also been widely used as emulsifying agents for carotene preparations. In 1951 Burns, et al. (64) demonstrated the favorable effects of Tween 20 on carotene absorption. Hebert and Morgan (51) showed that Tween 40 increased the amount of vitamin A formed from ingested carotene; and Olson (63) showed that a combination of Tween 20 and sodium glycocholate gave optimum conversion of carotene to vitamin A in rats.

Within the complex structures of a living organism many factors interact to regulate and control the metabolic processes of the organism. The various hormones are often thought of as "chemical messengers" which act to regulate these metabolic processes. For example, Daniel and Bass (65) showed that cortisone inhibits intestinal motility while stimulating gastric secretions. Gray, et al. (66) also cited evidence that cortisone stimulates gastric secretions. Wang and co-workers (67) have shown that cortisone and corticotropin increase serum vitamin A and carotene in children during rheumatic fever attacks.

In 1935 Malmejac, et al. (68) showed that the mobilization of liver vitamin A in dogs by nervous stimulation is mediated by the secretions of the adrenal cortex. In 1955 Clark and Colburn (69) reported that 3 mg per day of cortisone caused loss of vitamin A from the livers of rats treated with this hormone, while untreated rats maintained their liver stores of the vitamin. Cortisone increased the rate of depletion of stored vitamin A and reduced storage of the ingested vitamin. These workers showed that adrenal-

ectomy had no effect on liver vitamin A, nor did it influence the rats' ability to convert carotene to vitamin A. However, when adrenalectomy was accompanied by cortisone treatment, the livers contained only 40 per cent as much vitamin A as pair-fed controls at the end of 15 days. Carotene conversion was inhibited by cortisone but not entirely blocked.

Boland and Headley (70) cited a clinical case in which a diabetic patient with rheumatoid arthritis, who required 10 units of protamine zinc insulin per day, required 30-50 units of protamine zinc insulin per day following initiation of cortisone therapy. After withdrawal of cortisone, the patient reverted back to the original level of 10 units of insulin per day. Other workers also have reported an apparent antagonism between cortisone and insulin. Penhos (71) cited evidence that insulin can partially or totally counteract the inhibitory effects of cortisone on growth, thymus, adrenals, uterus and frequency of estrus. Millen and Woollam (72) studied the effects of cortisone and insulin upon congenital deformities in rats induced by hypervitaminosis A. Sixty thousand international units of vitamin A acetate fed to pregnant female rats from the 8th through 13th days of gestation caused congenital malformations in 7.8% of the offspring. When large doses of cortisone were given along with the vitamin A, congenital deformities in the young increased to 36.6%. These authors attributed the potentiating effect of cortisone on hypervitaminosis A to interference with carbohydrate metabolism, and

led them to study the effects of insulin upon congenital deformities. When 1.5 units of protamine zinc insulin were given with the vitamin A, no brain deformities occurred. When insulin was given in conjunction with vitamin A and cortisone, the high level of malformations was reduced from 36.6% in cortisone-vitamin A rats to 1.2% in insulin-cortisone-vitamin A rats. In a somewhat similar series of experiments, Landauer (73, 74), and DeFranciscis and Landauer (75) studied deformities in developing chick embryos induced by injection of insulin during the incubation period. They found that injection of 2-5 units of insulin into the yolk-sac after 4-8 days of incubation caused shortening of the long bones of the legs (micromelia) and abnormalities of the beak, with severity of the symptoms varying with the amount of insulin. When cortisone was injected during the same period, no malformations occurred. It was noted, however, that the cortisone caused dwarfing of the embryo in accordance with results of Karnofsky, et al. (76). Simultaneous injection of cortisone and insulin into the yolk-sac after 4 days' incubation showed no difference from injection of insulin alone, but injection of both hormones after 8 days of incubation showed that the presence of cortisone lessened the incidence of congenital defects caused by insulin.

These findings all tend to support the idea of an antagonism between cortisone and insulin. However, there is room for doubt as to whether insulin counteracts the effect of cortisone upon vitamin A storage. As cited previously (68, 69), cortisone has been shown to

mobilize stored vitamin A from the liver as well as inhibiting storage of vitamin A from ingested sources. In 1939 Bauereisen (77) reported that insulin adversely affects vitamin A storage. He showed that 0.5 unit of insulin daily caused a lowering of liver vitamin A in guinea pigs. In feeding experiments, in which the animals were given daily doses of vitamin A and subcutaneous injections of insulin, less of the vitamin was stored than in animals given no insulin.

Apparently, no other work has been reported on the effect of insulin upon liver storage of vitamin A. However, a number of workers have reported that in diabetes mellitus there seems to be an abnormal metabolism of carotene or vitamin A (78, 79, 80). Some of these studies have been considered inconclusive because they were based only upon blood levels of carotene and vitamin A (81). However, in vivo studies by Sobel, et al. (82), and Rosenbery and Sobel (83) have confirmed that in diabetes mellitus there is an impairment in carotene conversion.

The study of diabetes mellitus has been greatly facilitated by the discovery in 1926 by Dunn and Polson (84) that diabetes mellitus can be produced artificially by injection of alloxan, a uric acid derivative. More detailed studies by Dunn, et al. (85, 86), Hard and Carr (87), and Goldner and Gomori (88, 89) showed that alloxan produces diabetes mellitus by selective necrosis of the beta-cells of the pancreas where insulin is produced. Experimental diabetes produced in this way is apparently identical in effect to

diabetes mellitus which, according to Mirsky (90), is due to a hereditary defect in tryptophan metabolism. Thus, alloxan has become an invaluable tool in diabetic research.

The object of this paper is to study the influences of insulin, alloxan-diabetes, cortisone and adrenalectomy on storage of vitamin A in the liver of the rat.

EXPERIMENTAL

Animals

It is known that female rats store more vitamin A in their livers than do male rats at the same level of vitamin A intake (91). Therefore, animals used in these studies were female albino rats of the Sprague-Dawley[1] strain. Twenty-one-day-old rats were fed the U.S.P. vitamin A-free test diet and maintained on this diet throughout the course of each experiment. On this diet the vitamin A stores of the rats were depleted within 15-21 days, as evidenced by failure to gain weight and mild xerophthalmia. At this time the rats were placed in individual cages where they were kept throughout the experimental period. The cages were supported in racks containing 60 cages each. The racks were equipped with trays on which excreta could be collected. Temperature was controlled ($80^{\circ}F \pm 2$) and a continuous record of temperature and relative humidity was kept on a Freas Hygrothermograph.

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[1] Sprague-Dawley Farms, Madison, Wisconsin.

Preparation of Vitamin A Supplements

Crystalline vitamin A acetate[2] was used as the source of vitamin A. The extinction coefficient of the vitamin was determined by means of a Beckman DU spectrophotometer, using 2-propanol as a solvent. Compensation for impurities was made by adjusting the concentration of the vitamin A preparation. An oil solution was prepared by dissolving a weighed amount of crystalline vitamin A acetate in a minimum volume of chloroform, which was then added to a volume of corn oil. The concentration of vitamin A in the preparation was then determined colorimetrically by the Carr-Price reaction (24).

Preparation of Carotene Supplements

Commercial beta-carotene[2] was purified by recrystallization from a chloroform-methanol solution. The $E^{1\%}_{1\ cm}$ was determined for the purified compound by means of a Beckman DU spectrophotometer at 450 and 478 mμ to determine the degree of purity of the carotene; readings were also taken in the range of 330 to 350 mμ to check for the presence of cis isomers of carotene (92). The carotene crystals were weighed and dissolved in a minimum volume of chloroform, which was then added to corn oil[3]. Two types of carotene preparations were used during the course of these studies: (1) an oil

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[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[3] Mazola Oil, Corn Products Company, New York.

solution of carotene and (2) an emulsion composed of 5% Tween 80[4], 5% sodium glycocholate[5], 20% corn oil and water. In each preparation 1% alpha-tocopherol[5] was included as an antioxidant. The concentration of carotene in each supplement was determined by saponifying the preparation with 12% alcoholic potassium hydroxide, extracting the non-saponifiable fraction with Skellysolve B and measuring the color intensity of the extract in an Evelyn colorimeter with filter #440.

Feeding and Supplementation of Animals

The animals were supplied feed and water ad libitum in all experiments. The U.S.P. vitamin A-free diet was used except that in experiments with diabetic animals this diet was modified to contain 48% carbohydrate as compared with 65% carbohydrate in the original diet. Corn oil was added to keep the diet isocaloric.

When the majority of the rats ceased to gain weight, two rats taken at random were killed and their livers removed and analyzed individually for vitamin A. The liver was usually found to contain 5 µg of vitamin A or less. At this point the rats were put on experiment. Daily vitamin A and carotene supplements were given orally with a hypodermic syringe equipped with a round-tipped oral needle. The level of vitamin A fed was usually 30 µg per day,

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[4] Atlas Powder Company, Wilmington 99, Delaware.

[5] Mann Research Laboratories, Inc., 136 Liberty Street, New York 6, New York.

although levels ranging from 20 to 60 μg per day were fed in some experiments. Carotene was fed at a level of 120 μg per day.

The rats were divided into experimental groups of 6-12 rats of approximately equal mean weights. In some experiments carotene was fed as the source of vitamin A, while vitamin A acetate was fed in the remainder of the experiments. Cortisone[6] was given by subcutaneous injection at a level of 1 mg per day suspended in 0.2 cc of 0.85% sodium chloride solution. Insulin[7] was injected in a fixed dose of 0.5 unit per day in 0.2 cc of 0.85% sodium chloride solution. This was the highest dose tolerated by the young rats. The effect of insulin was studied using three separate routes of administration namely subcutaneous, intramuscular and intraperitoneal injections. The rats were injected every day between 8:30 and 10:00 a.m. for 8, 10 or 12 days consecutively. The rats were killed with diethyl ether approximately 48 hours after the last treatment. Feed was withdrawn 12 hours before the rats were sacrificed. The livers were removed, blotted on absorbant paper and placed in 125 ml erlenmeyer flasks containing 25-30 ml of 12% alcoholic potassium hydroxide.

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[6] Cortone, Merck & Company, Rahway, New Jersey.

[7] Iletin and protamine zinc insulin, Eli Lilly & Company, Indianapolis, Indiana.

Vitamin A Analysis

The rat livers were saponified by refluxing in 12% alcoholic potassium hydroxide for 15-20 minutes until all the tissue was digested. The nonsaponifiable fraction was extracted three times with 25-30 ml portions of peroxide-free diethyl ether. The ether extracts were combined and washed with distilled water until free of alkali. The samples were dried over granular anhydrous sodium sulfate for about one hour and then made to a volume of 50 ml with dry peroxide-free ether. An aliquot of each sample was pipetted into a colorimeter tube and evaporated to dryness under reduced pressure. The residue was dissolved in 1 ml of anhydrous chloroform. One drop of acetic anhydride was added to each tube and the tube placed in an Evelyn colorimeter. By means of an automatic pipette, 9 ml of Carr-Price reagent (24) were added quickly to each tube and the resulting blue color was read using a 620 mμ filter. The colorimeter readings were converted to vitamin A concentration from a standard curve and the concentration of vitamin A was calculated for each liver.

Fecal Carotene Analysis

Fecal samples for carotene analysis were collected for two days before carotene supplementation was begun. In all cases these fecal extracts were found to be colorless. After carotene supplementation began, total feces were collected daily for 5 to 12 consecutive days from 6 rats in each experimental group. Total

daily feces from each group were pooled and treated as a single sample. For the analysis, each pooled sample was ground in a Servall Omnimixer and then saponified in 12% alcoholic potassium hydroxide. The saponified samples were extracted with 25 ml portions of Skellysolve B until no more yellow color appeared in the solvent layer. The Skellysolve extract of each sample was washed with distilled water until the wash-water was free of alkali. Each sample was dried over anhydrous sodium sulfate and made to a volume of 100 ml with dry Skellysolve B. Aliquots of these solutions were then pipetted into colorimeter tubes and read in an Evelyn colorimeter with filter #440. The colorimetric readings were converted to carotene concentrations from a standard curve for pure beta-carotene.

Production of Experimental Diabetes

Since Gomori and Goldner (88) reported that one intraperitoneal injection of alloxan of 200 mg per kg was "invariably effective" in producing experimental diabetes in rats weighing up to 180 g, this dose level was chosen for the present work. About one week before the anticipated start of experiments with diabetic rats, the rats were fasted 3 to 6 hours and then injected with one intraperitoneal dose of alloxan[8]. Goldner and Gomori (89) reported that death in alloxanized animals is caused by pancreatic discharge

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8 Alloxan Monohydrate, Mann Research Laboratories, 136 Liberty Street, New York 6, New York.

of insulin from the degenerating beta-cells, and showed that administration of glucose protects against the fatal hypoglycemia. Accordingly, 20 mg of glucose was injected subcutaneously immediately following the alloxan injection, and feed was made available.

Blood Sugar Analysis

Blood sugar was determined by the method described by Nelson (93). The rat's tail was warmed in warm water, dried, and a few drops of blood obtained by puncturing one of the tail veins a few millimeters from the tip. The blood was collected on a paraffin block and a 0.05 ml sample of blood was pipetted into 1.55 ml of distilled water. To this mixture 0.2 ml of 0.2 normal $Ba(OH)_2$ was added and mixed, followed in two or three minutes by 0.2 ml of 0.2 normal $ZnSO_4$ to precipitate the proteins from the solution. This mixture was centrifuged and the clear supernatant portion was decanted into a clean test tube. A 1 ml aliquot was taken for the actual sugar determination. One milliliter of copper reagent was added and the sample was heated for 20 minutes in a boiling water bath. After removal from the water bath, the sample was cooled in running tap water. One milliliter of arsenomolybdate reagent was added, mixed thoroughly, and diluted to a volume of 25 ml with distilled water. After a final mixing, the solution was transferred to a colorimeter tube and the absorbance read in a Coleman Jr. spectrophotometer at 520 mμ. These readings were converted to concentration and calculated as mg of glucose per 100 ml of blood.

Adrenalectomy

There is evidence to indicate that insulin may stimulate the adrenal gland to increased cortisone output. Romano, et al. (94) presented cytochemical evidence to show that a single dose of 1 unit of insulin is sufficient to stimulate the adrenal gland. Hausberger (95) demonstrated that in the presence of physiologically excessive amounts of either insulin or cortisone, compensatory increased release of the other hormone of the pair occurs. Therefore, it was of interest to remove the adrenal glands to ascertain whether insulin affects liver vitamin A by causing increased cortisone output.

Young female rats were anesthetized with ether and the adrenals removed through a single ventral mid-line incision. The single ventral incision seemed most appropriate since a small speculum, ordinarily used through two small dorsal incisions, was not available. The incision was closed by two separate sutures with size 0 silk thread; the first closed the muscular layer and the second the skin. On the day following the operation the rats were given normal saline (0.85%) as drinking water. One week after the adrenalectomy the rats were put on experiment.

RESULTS

Influence of Insulin on Vitamin A Storage in the Liver

Three experiments were conducted to determine the influence of insulin on carotene utilization as measured by vitamin A storage in the liver (table 1). The quantity of vitamin A stored during a 12-day period was significantly lower in insulin-treated rats.

Since the action of regular insulin is characterized by relatively rapid onset and short duration of action (96), it was desirable to test the effect of a longer-acting insulin preparation. Globin zinc insulin or a three to one mixture of regular and protamine zince insulin has a maximum duration of action of approximately 24 hours (96). The mixture of regular and protamine zinc insulins was chosen for comparison with regular insulin because its slow onset would tend to minimize the severe hypoglycemia of rapid-acting regular insulin, and its longer duration of action would allow a more uniform response.

The data in table 2 show vitamin A storage in livers of rats fed carotene and injected with globin-type insulin subcutaneously, intramuscularly or intraperitoneally. Rats injected with insulin by the intramuscular or intraperitoneal routes stored significantly less vitamin A than did control rats or rats injected subcutaneously.

Table 1

Comparison of liver vitamin A storage of rats fed 60 μg beta-carotene daily with or without subcutaneous injection of regular insulin

Daily insulin treatment	Number of rats	Mean initial weight	Mean weight gain	Mean liver vitamin A
		g	g	μg
none	7	117	43.8	17.2 ± 0.38[a]
0.5 unit insulin	9	121	48.0	12.1 ± 0.22
none	11	120	27.0	18.5 ± 1.27
0.5 unit insulin	9	120	47.8	11.2 ± 1.26
none	5	107	33.6	21.7 ± 1.58
0.5 unit insulin	6	106	22.1	12.7 ± 1.90

[a] Standard error of the mean

Table 2

Comparison of three routes of injection of globin-type insulin on liver storage of vitamin A of rats fed 120 µg of beta-carotene daily for 12 days

Daily insulin treatment	Number of rats	Mean initial weight	Mean weight gain	Mean liver vitamin A
		g	g	µg
none	15	167	24.1	29.3 ± 0.60[a]
0.5 unit, s.c.	13	168	25.5	29.0 ± 0.84
0.5 unit, i.m.	17	164	23.9	16.7 ± 1.15
0.5 unit, i.p.	12	166	26.4	17.8 ± 0.92

[a] Standard error of the mean

There was no difference between controls and rats injected subcutaneously.

In this experimental liver vitamin A storage was considerably lower than in earlier work reported from this laboratory (97). In both instances the carotene was dissolved in a vegetable oil. In an effort to obtain better storage of vitamin A from ingested carotene, a comparison was made between carotene dissolved in corn oil and emulsified carotene (table 3). In two experiments rats fed emulsified carotene stored 36 and 41 per cent more vitamin A than did rats fed an oil solution of carotene. It was, therefore, decided to feed aqueous emulsions of carotene in subsequent carotene-feeding experiments.

Data in table 4 show comparisons of the effect of regular and globin-type insulins by three routes of injection on carotene utilization for liver storage of vitamin A in rats. All insulin-treated rats stored less vitamin A per liver than did controls. Liver vitamin A storage was approximately the same regardless of the type of insulin; but rats injected subcutaneously with regular insulin stored slightly more vitamin A than any of the other groups but still 50 per cent less than control rats.

The spectrophotometric absorbance curves for recrystallized commercial beta-carotene were determined on a Perkin-Elmer Model 202 Spectrophotometer at several concentrations in Skellysolve B (figure 1). Figure 2-a shows the visible spectrum of fecal extracts from rats fed a carotene-free diet, and figure 2-b shows the visible

Table 3

Comparison of oil solution and water emulsion of carotene for storage as liver vitamin A for a 12-day period

Daily carotene supplement	Number of rats	Mean liver vitamin A
		µg
120 µg carotene in:		
Oil solution	14	30.6 ± 1.90[a]
Water emulsion	12	41.7 ± 4.08
127 µg carotene in:		
Oil solution	12	42.2 ± 2.42
Water emulsion	11	59.6 ± 3.25

[a] Standard error of the mean

Table 4

Comparison of the influence of regular insulin with globin-type insulin on utilization of 120 µg of carotene daily for liver storage of vitamin A in rats

Daily insulin treatment	Number of rats	Mean initial weight	Mean weight gain	Mean total fecal carotene	Carotene excreted	Ingested carotene converted to liver vitamin A	Metabolized carotene converted to liver vitamin A	Mean liver vitamin A
		g	g	µg	%	%	%	µg
None	12	157	27.3	299.0	20.76	3.25	4.10	46.8 ± 2.89[a]
0.5 unit reg. insulin s.c.	12	151	24.6	244.3	16.97	1.53	1.01	22.1 ± 1.58
0.5 unit reg. insulin i.m.	12	162	25.7	232.0	16.11	1.04	1.27	15.4 ± 1.83
0.5 unit reg. insulin i.p.	11	161	29.1	223.0	15.35	0.88	1.04	12.7 ± 1.17
0.5 unit globin insulin s.c.	9	151	31.2	244.0	16.94	0.92	1.11	13.3 ± 1.05
0.5 unit globin insulin i.m.	11	154	26.7	227.5	15.66	0.77	0.92	11.2 ± 0.97
0.5 unit globin insulin i.p.	12	151	28.1	223.6	15.39	1.22	1.45	17.6 ± 1.73

[a]Standard error of the mean

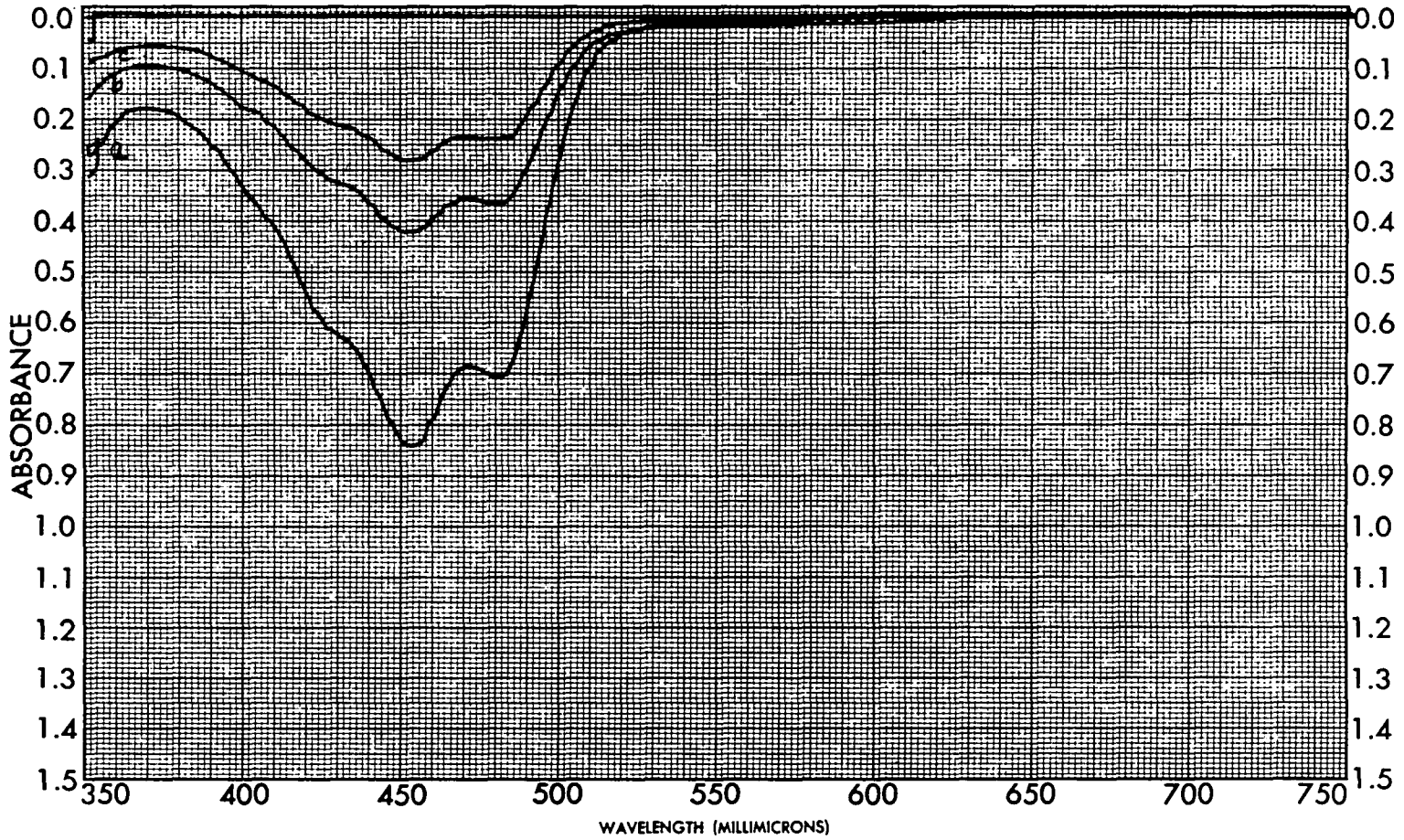

Figure 1. Visible spectrum of beta-carotene in Skellysolve B; Recrystallized all-trans beta-carotene at concentrations of (a) 3.76 µg/ml, (b) 1.88 µg/ml and (c) 1.28 µg ml.

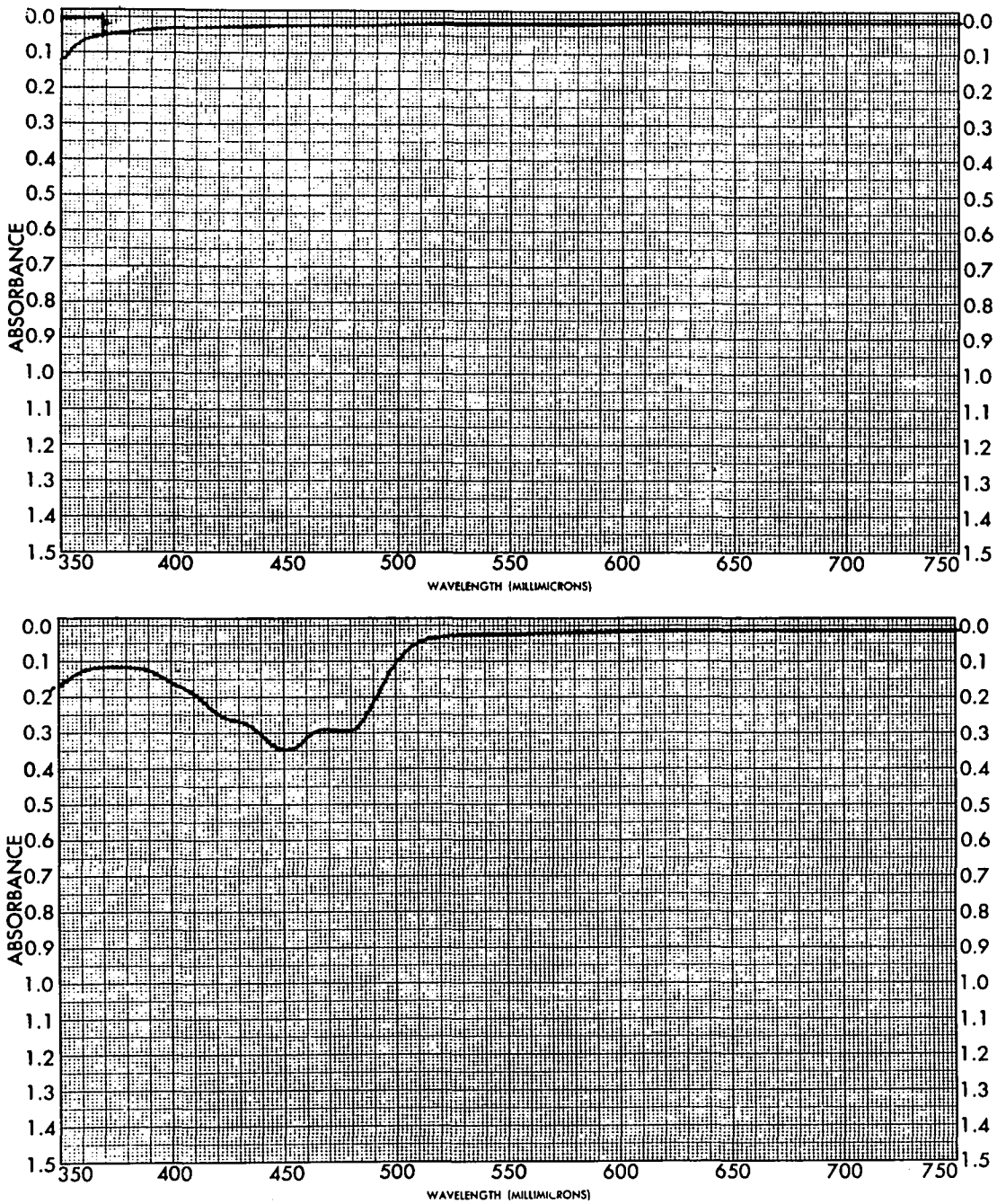

Figure 2. Visible spectrum of fecal extracts in Skellysolve B:

Upper--Fecal extract from rats on a carotene-free diet before supplementation with carotene.

Lower--Fecal extract from the same rats after dietary supplementation with carotene.

spectrum of fecal extracts from the same rats after carotene supplementation.

Fecal carotene was measured (table 4) to determine whether insulin exerts its effect by influencing intestinal absorption of carotene. The control rats stored more vitamin A and also excreted more carotene than did insulin-treated rats. There were no significant differences in the amounts of carotene excreted by rats when different insulins were injected by different routes. Control rats excreted more carotene but utilized the metabolized carotene about four times as efficiently as insulin-treated rats when liver storage of vitamin A was the criterion. From these data it appears that insulin exerts its influence during metabolism of the carotene.

Four experiments are reported in table 5 showing the influence of regular insulin upon liver storage of vitamin A in rats fed vitamin A acetate. In this series rats given subcutaneous injections of insulin consistently stored less vitamin A in their livers than did control rats. Rats given insulin by intramuscular injection showed no consistent pattern of vitamin A storage. In the first trial rats given intramuscular injections of insulin stored more than twice as much vitamin A as did controls. In the second trial insulin was given only by subcutaneous injection; these rats stored less vitamin A than did controls. In the third trial rats injected intramuscularly stored more vitamin A than did controls, but the difference is of doubtful significance. In the

Table 5

The influence of 0.5 unit of regular insulin on storage of ingested vitamin A acetate in the liver of the rat

Experimental treatment	Number of rats	Treatment period	Mean initial weight	Mean weight gain	Mean liver weight	Mean liver vitamin A
		days	g	g	g	µg
30 µg vitamin A	9	12	135	33.5	6.36	106.4 ± 1.40[a]
30 µg vitamin A + insulin i.m.	6	12	121	41.7	7.13	222.8 ± 4.31
30 µg vitamin A	6	10	144	29.8	6.87	72.1 ± 7.04
30 µg vitamin A + insulin s.c.	7	10	125	27.4	6.36	58.1 ± 5.19
40 µg vitamin A	11	10	156	24.6	6.73	83.7 ± 4.55
40 µg vitamin A + insulin i.m.	11	10	168	26.6	7.42	93.5 ± 4.65
40 µg vitamin A + insulin s.c.	10	10	176	23.0	7.34	60.4 ± 3.41
40 µg vitamin A	10	10	125	33.5	----	85.1 ± 4.51
40 µg vitamin A + insulin i.m.	10	10	115	36.9	----	54.3 ± 6.70
40 µg vitamin A + insulin s.c.	10	10	132	27.6	----	66.8 ± 7.36

[a]Standard error of the mean

fourth trial rats injected subcutaneously with insulin stored less vitamin A than did controls, and rats given intramuscular injections of insulin stored less vitamin A than either controls or rats injected subcutaneously. No obvious reason could be found for these differences in rats given intramuscular injections of insulin. In all subsequent experiments intramuscular injection of insulin consistently caused less vitamin A to be stored in the liver.

In table 6 a comparison is made of the influence of regular and globin-type insulins on liver storage of ingested vitamin A acetate. All insulin-treated rats stored less liver vitamin A than did controls, regardless of route of administration or kind of insulin. Rats injected subcutaneously with regular insulin stored approximately 30 μg less vitamin A than did controls. Rats given regular insulin by intramuscular and intraperitoneal injections stored an average of 68 μg less vitamin A than did controls with no significant difference between these two insulin-treated groups. Rats injected with globin-type insulin stored approximately 75 μg less vitamin A than did controls. Differences among groups of globin insulin-treated rats were not statistically significant. Quantities of vitamin A stored by rats given globin-type insulin were similar to quantities stored by rats given intramuscular and intraperitoneal injections of regular insulin.

The effect of insulin on the rate of depletion of liver vitamin A was determined (table 7). Rats were fed 60 μg of vitamin A acetate daily for 14 days. On the fifteenth day vitamin A

Table 6

Comparison of the influence of regular insulin with globin-type insulin on liver storage of vitamin A in rats fed 30 μg of vitamin A acetate daily for 12 days

Daily insulin treatment	Number of rats	Mean initial weight	Mean weight gain	Mean liver vitamin A
		g	g	μg
none	12	153	25.2	109.3 ± 4.55[a]
0.5 unit reg. insulin s.c.	12	160	23.3	77.4 ± 3.75
0.5 unit reg. insulin i.m.	12	152	23.8	38.8 ± 2.68
0.5 unit reg. insulin i.p.	12	160	21.6	43.5 ± 3.27
0.5 unit globin insulin s.c.	10	153	23.9	27.8 ± 2.20
0.5 unit globin insulin i.m.	10	153	20.9	34.9 ± 3.21
0.5 unit globin insulin i.p.	12	157	24.5	35.7 ± 1.84

[a]Standard error of the mean

Table 7

The effects of regular and globin-type insulin on the rate of depletion of liver vitamin A during a 12-day period

Experimental group	Number of rats	Mean weight before depletion	Mean weight gain	Mean liver weight	Mean liver vitamin A
		g	g	g	µg
Control before depletion[a]	10	204	----	7.61	404.9 ± 16.6[b]
Control after depletion	12	181	21.4	7.89	205.2 ± 9.41
Depletion with: 0.5 unit reg. insulin s.c.	11	186	16.8	7.85	139.8 ± 6.63
0.5 unit reg. insulin i.m.	12	189	18.6	7.97	127.1 ± 6.88
0.5 unit reg. insulin i.p.	12	192	20.0	7.17	86.7 ± 5.39
0.5 unit globin insulin s.c.	12	188	17.7	7.65	84.1 ± 5.17
0.5 unit globin insulin i.m.	12	187	20.2	7.30	84.1 ± 6.04
0.5 unit globin insulin i.p.	13	184	18.5	7.18	70.7 ± 5.09

[a] All rats had been fed 60 µg of vitamin A acetate daily for 14 days immediately preceding the depletion period.

[b] Standard error of the mean

administration was discontinued and the rats were given regular or globin-type insulin by subcutaneous, intramuscular or intraperitoneal injections daily for 12 days. Either type of insulin by any route of administration significantly increased the rate of depletion of liver vitamin A. The livers of control rats retained approximately 50 per cent of the initially stored vitamin A, while rats treated with regular insulin by subcutaneous and intramuscular injections retained approximately 33 per cent of the initially stored vitamin. Rats injected with regular insulin intraperitoneally or with globin-type insulin by any of the three routes of injection retained approximately 20 per cent of the initially stored vitamin A.

Alloxan-Diabetes and Vitamin A Storage

Comparisons were made of the effects of insulin-treated normal rats, non-treated alloxan-diabetic rats and of insulin-treated alloxan-diabetic rats on utilization of carotene for storage as vitamin A in the liver (table 8). Insulin was injected by the same three routes used previously. As noted earlier with non-diabetic rats (tables 5, 6 and 7), storage of liver vitamin A was affected less by subcutaneous injection of insulin than by intramuscular or intraperitoneal injections. Untreated diabetic rats stored less vitamin A than control rats. Diabetic rats treated with insulin by intramuscular or intraperitoneal injection showed improved liver storage of vitamin A as compared with untreated diabetic rats or rats injected subcutaneously with insulin, although the improvement was of doubtful statistical significance.

Table 8

Comparison of liver storage of vitamin A in insulin-treated rats, diabetic rats, and insulin-treated diabetic rats fed 120 μg of carotene daily for 12 days

Daily experimental treatment	Number of rats	Mean initial weight	Mean weight gain	Blood sugar[a]	Mean liver vitamin A
		g	g	mg/100 ml	μg
Normal rats (untreated)	12	127	32.7	91.0	56.8 ± 3.15[b]
0.5 unit insulin s.c.	11	135	26.8	----	45.8 ± 3.07
0.5 unit insulin i.m.	12	133	26.2	----	36.5 ± 2.54
0.5 unit insulin i.p.	9	130	35.6	----	32.6 ± 2.68
Alloxan-diabetic (untreated)	8	131	18.7	450.0	40.4 ± 2.60
0.5 unit insulin s.c.	4	131	20.3	----	39.4 ± 1.30
0.5 unit insulin i.m.	5	127	22.4	----	49.3 ± 1.87
0.5 unit insulin i.p.	3	136	25.8	----	45.3 ± 0.43

[a]Blood was collected from non-fasted rats 36 hrs. after administration of alloxan and prior to division into groups.

[b]Standard error of the mean

In one further trial the same type of study was conducted using globin-type insulin instead of regular insulin (table 9). In this trial alloxan-diabetic rats showed blood glucose levels over twice as great as those of control animals. Although vitamin A storage was considerably lower in the rats in this experiment using globin-type insulin, the pattern of storage was approximately the same as with regular insulin except that diabetic rats given globin-type insulin by intramuscular and intraperitoneal injections showed no significant increase in stored vitamin A as was noted in diabetic rats given regular insulin (table 8).

The influence of alloxan-induced diabetes on liver storage of ingested vitamin A acetate was studied (table 10). Diabetic rats stored vitamin A in about the same amounts as did controls. In diabetic rats given globin-type insulin, liver vitamin A was 20 to 25 per cent below control values. The similarity of these differences and the differences between insulin-treated and untreated normal rats (table 6) suggests that excessive insulin alone caused decreased liver storage of vitamin A in both instances.

An experiment was designed to test the influence of the diabetic condition on depletion of liver vitamin A stores (table 11). Vitamin A-deficient rats were individually fed 30 μg of vitamin A acetate daily for 12 days. Twelve of these rats were killed at this time and the vitamin A content of each liver was determined. The remaining rats were divided into two groups; one served as normal untreated controls and the other was treated with alloxan

Table 9

Comparison of liver storage of vitamin A by globin insulin-treated rats, diabetic rats, and globin insulin-treated diabetic rats fed 120 µg of carotene daily for 12 days

Daily experimental treatment	Number of rats	Mean initial weight	Mean weight gain	Blood sugar[a]	Mean liver vitamin A
		g	g	mg/100 ml	µg
Normal rats untreated	12	136	32.9	93.9	28.1 ± 1.64[b]
0.5 unit insulin s.c.	11	140	32.6	----	21.2 ± 1.86
0.5 unit insulin i.m.	12	131	31.4	----	18.7 ± 1.96
0.5 unit insulin i.p.	12	138	32.4	----	21.1 ± 2.04
Alloxan-diabetic untreated	8	129	0.5	219.3	17.2 ± 1.67
0.5 unit insulin s.c.	5	121	0.4	----	18.5 ± 1.23
0.5 unit insulin i.m.	6	122	0.0	----	16.3 ± 0.96
0.5 unit insulin i.p.	5	115	13.0	----	22.5 ± 2.25

[a]Blood was collected from non-fasted rats 36 hrs. after administration of alloxan and prior to dividion into groups.

[b]Standard error of the mean

Table 10

Comparison of liver storage of vitamin A in normal rats, diabetic rats, and globin insulin-treated diabetic rats fed 30 µg of vitamin A acetate daily for 12 days

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

[a] Rats fasted for 12 hrs. prior to collection of blood; samples collected 24 hrs. after last injection of insulin.

[b] Standard error of the mean

Table 11

The influence of alloxan-diabetes on the rate of depletion of liver vitamin A during a 12-day period

Experimental group[a]	Number of rats	Mean weight before depletion	Mean weight change	Mean liver vitamin A
		g	g	µg
Controls before depletion	12	177.5	----	109.3 ± 4.55[b]
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

[a] All rats had been fed 30 µg of vitamin A acetate daily for 12 days immediately preceding the depletion period.

[b] Standard error of the mean

and, thus, rendered diabetic. After 12 days of depletion, there was no significant difference in liver vitamin A between controls and diabetic rats. These data and the data in table 10 show that diabetes did not significantly influence storage or depletion of vitamin A in the liver of the rat. This corroborates a previous report (82) that diabetes has no effect on vitamin A absorption or storage.

Cortisone and Liver Vitamin A

Experiments were conducted to determine the effect of cortisone on liver vitamin A storage (table 12). At levels of 23 to 60 μg of vitamin A per day, 1 mg of cortisone per day inhibited storage of vitamin A in the liver of the rat. This confirms results reported by Clark and Colburn (69).

Since both insulin and cortisone have been shown to inhibit liver storage of vitamin A, it was of interest to determine if the two hormones might be interrelated. Romano, et al. (94), and Hausberger (95) have presented histochemical evidence that the administration of one of these hormones causes compensatory release of the other. To test the hypothesis that only one of these hormones is directly responsible for this inhibiting effect and that the other is only indirectly involved, diabetic rats were treated with cortisone and carotene or vitamin A, and adrenalectomized rats were treated with insulin and carotene or vitamin A.

Table 12

The influence of cortisone on liver storage of ingested vitamin A acetate

Daily supplement	Experimental period	Number of rats	Mean initial weight	Mean weight gain or loss	Mean liver vitamin A
	days		g	g	µg
23 µg Vitamin A	8	12	112	20.9	40.6 ± 3.37[a]
23 µg Vitamin A plus 1 mg Cortisone	8	12	113	-3.5	27.3 ± 1.80
30 µg Vitamin A	12	9	135	33.5	106.4 ± 1.40
30 µg Vitamin A plus 1 mg Cortisone	12	9	132	-20.0	76.0 ± 0.97
50 µg Vitamin A	12	9	121	41.8	198.6 ± 11.18
50 µg Vitamin A plus 1 mg Cortisone	12	8	125	30.4	163.0 ± 10.28
60 µg Vitamin A	12	9	144	40.1	291.2 ± 13.01
60 µg Vitamin A plus 1 mg Cortisone	12	11	137	20.9	241.0 ± 5.88

[a] Standard error of the mean

Alloxan-diabetic rats were fed carotene as the source of vitamin A and were injected with 1 mg of cortisone daily for 12 days. The results were equivocal (table 13). Non-diabetic cortisone-treated rats and alloxan-diabetic rats stored slightly more liver vitamin A than did controls, although the differences were not statistically significant. Diabetic rats given cortisone stored significantly more vitamin A than did controls.

A similar experiment was conducted with the feeding of vitamin A acetate instead of carotene (table 14). Cortisone failed to inhibit liver vitamin A storage contrary to previous experiments. The reason for this deviation is not understood. Livers of diabetic rats stored less vitamin A than did controls, while cortisone-treated diabetic rats stored more of the vitamin than did controls. In both instances the differences were statistically significant.

An experiment was conducted to determine the effect of the absence of cortisone on vitamin A storage (table 15). Adrenalectomized rats stored approximately 25 per cent less vitamin A; and insulin-treated adrenalectomized rats stored 40 to 50 per cent less vitamin A than did control rats.

Table 13

Comparison of liver storage of vitamin A in normal rats, alloxan-diabetic rats and cortisone-treated diabetic rats fed 120 μg of carotene daily for 12 days

Experimental group	Number of rats	Mean initial weight	Mean weight gain	Blood sugar[a]	Mean liver vitamin A
		g	g	mg/100 ml	μg
Normal rats					
Untreated	16	69.1	47.2	78.8	48.1 ± 3.57[b]
Cortisone, 1 mg/day	11	67.3	22.4	--	55.1 ± 3.28
Alloxan-diabetic rats					
Untreated	5	68.6	22.4	120.4	55.3 ± 10.2
Cortisone, 1 mg/day	7	71.7	21.4	--	61.7 ± 4.94

[a] Blood was collected from non-fasted rats 36 hrs after administration of alloxan and prior to division into groups.

[b] Standard error of the mean.

Table 14

Comparison of liver storage of vitamin A in normal rats, alloxan-diabetic rats and cortisone-treated diabetic rats fed 30 μg of vitamin A acetate daily for 12 days

Experimental group	Number of rats	Mean initial weight	Mean weight gain	Blood sugar[a]	Mean liver vitamin A
		g	g	mg/100 ml	μg
Normal rats					
Untreated	16	70.2	55.0	80.7	98.5 ± 4.51[b]
Cortisone, 1 mg/day	15	68.8	28.7	76.5	106.0 ± 7.90
Alloxan-diabetic rats					
Untreated	5	68.6	22.4	219.3	55.3 ± 10.5
Cortisone, 1 mg/day	17	66.7	19.5	379.0	109.7 ± 2.88

[a] Blood was collected from non-fasted rats 24 hrs after the last treatment.

[b] Standard error of the mean.

Table 15

Comparison of liver storage of ingested vitamin A acetate in normal rats, adrenalectomized rats and globin insulin-treated adrenalectomized rats

Experimental group[a]	Number of rats	Mean initial weight	Mean weight gain	Blood sugar[b]	Mean liver vitamin A
		g	g	mg/100 ml	µg
Controls	10	125.4	33.5	75.2	86.8 ± 4.83[c]
Adrenalectomized rats	8	116.6	30.1	51.4	64.6 ± 7.64
Adrenalectomy plus:					
0.5 unit insulin s.c.	6	127.4	33.6	56.4	46.3 ± 6.61
0.5 unit insulin i.m.	6	131.4	26.0	52.2	53.7 ± 5.29

[a] All rats were fed 30 µg of vitamin A daily for 12 days.

[b] Blood was collected from non-fasted rats just prior to final experimental treatment.

[c] Standard error of the mean.

DISCUSSION

Mirsky, et al. (98, 99, 100) have presented evidence of the existence of an insulin-inactivating system in the animals body. Since the rate of absorption of injected materials decreases from intraperitoneal to intramuscular to subcutaneous routes of administration, it seems reasonable that insulin injected subcutaneously might be subject to the insulin-inactivating system for a longer period of time and, therefore, less of the hormone would reach the site of its action on carotene and vitamin A. Alternately, the difference noted in the effect of subcutaneously-injected regular insulin may be due to the slower rate of absorption from this route, in which case the circulating concentration of insulin would be lower than after injection by other routes from which materials are absorbed more rapidly. In either case the circulating concentration of insulin would be affected, and this may explain the difference noted in the effect of subcutaneously-injected insulin upon liver storage of vitamin A compared to the effect of insulin by other routes of injection. Rats injected with insulin subcutaneously stored more liver vitamin A than did rats injected intramuscularly or intraperitoneally (tables 2 and 4) but in most instances stored significantly less vitamin A than non-treated control rats (tables 1 and 4).

Since regular insulin has a rapid onset and a short duration of action, fluctuation of systemic levels of insulin is possible. A 3 to 1 mixture of regular and protamine zinc insulins has a slower onset and a duration of action of about 24 hours. This insulin mixture is comparable to globin-zinc insulin in time of onset and duration of action. This longer-acting insulin preparation was compared with regular insulin (table 4) in its influence on carotene utilization. Fecal carotene analysis showed differences in carotene excretion among the rats injected by the various routes of administration. For a given route of injection the excretion of carotene was essentially the same for both types of insulin. Since control rats stored more vitamin A and also excreted more carotene than did insulin-treated rats, it appears that there is more destruction of carotene in the insulin-treated animals although the evidence for this is indirect.

In studies of the effect of insulin on vitamin A acetate absorption and storage, it first appeared that insulin injected intramuscularly actually increased liver storage while subcutaneously-injected insulin inhibited storage of vitamin A (table 5). However, all subsequent experiments failed to confirm this finding (tables 5, 6 and 7). No plausible explanation could be found for this initial difference in results. Insulin has the same effect on storage of fed vitamin A that it has on utilization of carotene as measured by the quantity of vitamin A stored in the liver (table 6). Although the values reported in table 4 for ingested carotene are lower than

those for ingested vitamin A in table 6, the pattern is almost identical. Rats given regular insulin subcutaneously stored more vitamin A than any of the other insulin-injected groups, regardless of the type of insulin, but still less than controls. Regular insulin by intramuscular and intraperitoneal injections had essentially the same effect as globin-type insulin, which decreased liver vitamin A by all routes of injection.

Insulin did not appear to interfere with the absorption of carotene or vitamin A from the digestive tract but rather inhibited storage in the liver (table 7). The degree to which insulin influenced liver storage of vitamin A seems to be related to the availability of the insulin. Subcutaneously-injected regular insulin exerted the smallest influence on liver storage of vitamin A. Insulin by other routes of injection was more rapidly absorbed and exerted a more pronounced effect. Globin-type insulin is insoluble in body fluids (90) because of the basic protein, protamine. It is absorbed as it is slowly released from the protamine and exerts about the same effect as regular insulin given intraperitoneally. These results seem to agree with the theory that the degree of effect of insulin upon liver vitamin A is related to the effective circulating level of insulin in the animal.

While diabetes has been shown to inhibit conversion of carotene to vitamin A (82, 83), it has no significant effect upon the storage of preformed vitamin A nor upon the rate of depletion of the stored vitamin from the liver (tables 10 and 11). Alloxan-

diabetic rats showed approximately the same degree of liver vitamin A storage as rats injected with insulin when carotene was fed. Since diabetes does not interfere with the storage of preformed vitamin A, it is apparent that diabetes inhibits the conversion of carotene to vitamin A, and that injected insulin interferes with the storage of the vitamin in the liver but has no effect upon the conversion of carotene (tables 8 and 9).

In four experiments (table 12) cortisone was found to inhibit storage of vitamin A in the liver of the rat. This is in agreement with earlier work reported by Clark and Colburn (69). In two experiments to determine if cortisone would inhibit vitamin A storage in the diabetic state, cortisone failed to inhibit liver storage of vitamin A in normal rats while diabetic rats treated with cortisone stored significantly more of the vitamin than did control rats (tables 13 and 14). The only obvious factor in later experiments which differed from the earlier experiments was that the initial weights of the rats were 65 to 70 grams, while in the earlier studies the starting weights were 112 to 144 grams. Although these results were generally equivocal, it appears that cortisone improved vitamin A storage in the diabetic rats.

Adrenalectomized rats with or without insulin stored significantly less vitamin A than did controls (table 15). The degree of inhibition of liver vitamin A storage in insulin-treated rats was of the same order of magnitude as found in most other experiments in which insulin was administered to normal rats. Cortisone and

Studies on fecal excretion of carotene and rate of depletion of liver vitamin A stores showed that insulin interferes with storage of vitamin A in the liver, rather than preventing absorption from the intestinal tract.

In diabetes mellitus there is a depression of conversion of carotene to vitamin A. Injected insulin had no ameliorative effect upon impaired carotene conversion in alloxan-diabetic rats. Alloxan-diabetes had no effect upon liver storage of ingested vitamin A acetate, nor upon the rate of depletion of previously stored vitamin A.

Cortisone interfered with storage of vitamin A in the livers of normal rats. In alloxan-diabetic rats administration of cortisone improved liver vitamin A storage when either carotene or vitamin A acetate was fed.

Adrenalectomized rats stored less vitamin A than did normal rats. Administration of insulin to adrenalectomized rats further decreased liver vitamin A storage.

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