THE INTERRELATIONSHIP OF CORTISONE AND INSULIN TO CAROTENE METABOLISM

by

William Howard Bowles

A Thesis Submitted to the Faculty of the

DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

In the Graduate College

UNIVERSITY OF ARIZONA

1960

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in their judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: William H. Bowles

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

M. G. Vavich — May 9, 1960

M. G. Vavich
Professor of Biochemistry and Nutrition

Date

ACKNOWLEDGMENTS

The author wishes to express great appreciation of the helpful advice, assistance, and criticism given by Dr. M. G. Vavich during the course of these studies and his thanks for the helpful suggestions from Professor A. R. Kemmerer.

Grateful acknowledgments also to all the other members of the Department of Agricultural Biochemistry for their kindness and cooperation during this investigation.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Raica (1) reported that a number of foods and physiological factors influence liver storage of vitamin A in rats when carotene is fed orally. Raica also found that cortisone inhibits the storage of vitamin A in the liver after the administration of carotene, but not when vitamin A acetate was given.

There are a number of examples which can be used to show an antagonism between insulin and cortisone. It is well-known that an excess of insulin causes hypoglycemia, and Ingle (2) demonstrated that the administration of large amounts of cortisone to experimental animals causes hyperglycemia. There is evidence from clinical studies by Boland et al. (3) that in diabetes mellitus the therapeutic use of cortisone increases considerably the amount of insulin required to maintain normal blood sugar levels. A good example of insulin-cortisone antagonism is shown by Mirsky (4) in studies on congenital deformities in rats. Mother rats were given 60,000 units of vitamin A acetate and 20 mg. of cortisone daily from the ninth through the twelfth days of gestation. This caused brain deformities to be increased by 36 per cent in the offspring. When 1.5 units of insulin were given along with the

vitamin A and cortisone during the same period of gestation, the incidence of deformities was reduced to 1.2 per cent. Another example of insulin-cortisone antagonism is found in the work of DeFrancis and Landauer (5) on chick embryos. Insulin injected into chick embryos caused a shortening of the long bones of the legs and malformations of the beak. When cortisone was given concurrently with insulin, a dwarfing of the embryos occurred, but skeletal malformations were prevented.

Clark and Colburn (6) showed that the administration of cortisone to rats on a vitamin A-free diet greatly increases the rate of depletion of vitamin A stores from the liver. Bauereisen (7) found that the administration of 0.5 unit insulin daily to guinea pigs causes rapid depletion of liver vitamin A, and less of the fed vitamin was utilized for storage in the liver. In view of the findings cited, it was of interest to study the interrelationship of insulin and cortisone in rats with respect to liver storage of vitamin A.

EXPERIMENTAL

Preparation of Carotene Supplement

A commercial preparation of 90% beta- and 10% alpha-carotene[1] was purified by recrystallization from a chloroform-methanol solution. The purified carotene crystals were redissolved in a minimum volume of chloroform and cottonseed oil[2] was added to give a final concentration of 3.05 mg. carotene per gram of cottonseed oil. The concentration of carotene was checked colorimetrically with an Evelyn colorimeter.

Preparation of Vitamin A Supplement

Crystalline vitamin A acetate[3] was used as the source of vitamin A. The purity of the vitamin preparation was determined by means of a Beckmann DU spectrophotometer, using isopropanol as a solvent. The $E^{1\%}_{1cm}$ value was 1188 for the preparation used, as compared with

---

[1] Nutritional Biochemicals Corp., Cleveland, Ohio.

[2] Wesson Oil Co., New Orleans, La.

[3] Nutritional Biochemicals Corp., Cleveland, Ohio.

1520 for pure vitamin A acetate in isopropanol. This showed a purity of 78%. Compensation for the impurity was made in preparing the vitamin A acetate solution. The solution was prepared by dissolving a weighed amount of crystalline vitamin A acetate in a minimum volume of chloroform. This solution was added to a volume of cottonseed oil to give a final concentration of 1.53 mg. vitamin A acetate per gram oil. The oil solution was checked colorimetrically by the antimony trichloride reaction (8).

Experimental Animals

The animals used in these studies were albino rats of the Sprague-Dawley strain. At the time of weaning, the young rats were put on the U.S.P. vitamin A-free diet, and maintained on this diet throughout the course of the experiments. The experiments were begun when a majority of the rats exhibited symptoms of hypovitaminosis A. The symptoms used for reference were retardation of growth, muscular weakness in the hind quarters, and cornification of the conjunctival tissues.

Feeding and Supplementation of Animals

The animals were supplied food and water ad libitum and total feed consumption was measured during the 14-day test period. In the carotene studies, one drop of carotene oil solution from a carefully calibrated dropper was given to each rat daily by mouth, so that each rat received 64 micrograms of carotene daily six days of the week for two weeks. The rats were divided into four groups with equal numbers of males and females in each group. Group 1, the control group, received only carotene as a supplement. Group 2 received carotene orally and 1 mg. of cortisone[1] daily. Group 3 was given carotene and 2 units of insulin[2] daily. Group 4 was given carotene and 1 mg. of cortisone and 2 units of insulin daily. In each case, the hormones were given by intramuscular injection.

The carotene supplements and injections of cortisone and insulin were given at approximately the same time each morning, six days of the week for two weeks. At the end of the thirteenth day the rats were fasted for twenty-four hours and sacrificed by ether anesthesia at the beginning of the 15th day. Livers and kidneys were removed, blotted

---

[1] Cortone acetate, Merck, Sharp and Dohme, Philadelphia, Pa.

[2] Iletin (insulin), Eli Lilly & Co., Indiana, Ind.

on filter paper to remove blood, then frozen with dry ice and stored in a deepfreeze at -15°C. until they could be analyzed for vitamin A content. A similar study was conducted using a daily supplement of 32 micrograms of vitamin A acetate in place of carotene.

Analysis of Vitamin A

The frozen livers were allowed to thaw and were then saponified by refluxing with 30 ml. of 12% alcoholic potassium hydroxide for 25 to 30 minutes. The saponified liver was extracted three times with 30 ml. of peroxide-free diethyl ether. The ether extracts were combined and washed repeatedly with distilled water until free of alkali, dried over anhydrous sodium sulfate, and diluted to a volume of 50 ml. with dry, peroxide-free ether. Aliquots were pipetted into colorimeter tubes, evaporated to dryness under reduced pressure and the residue dissolved in 1 ml. of dry chloroform. Two drops of acetic anhydride were added to take up any traces of moisture present. Each tube was placed in an Evelyn colorimeter (filter No. 620) and nine ml. of Carr-Price reagent (22% antimony trichloride in dry chloroform) were added. Vitamin A concentration was measured as a function of color density and calculated from a standard curve obtained with crystalline vitamin A acetate. Analysis of kidneys for vitamin A content was carried out in a manner similar to that employed for livers.

RESULTS

Values for liver vitamin A when rats were fed carotene were lower than expected, on the basis of previous work done in this laboratory (1). However, the differences between the controls and the various test groups were highly significant, as demonstrated by Student's "t" test. Table 1 shows that although the three test groups differed significantly from the controls, no significant difference was found among the test groups themselves. However, it is evident that both insulin and cortisone have a definite depressing effect upon the liver storage of vitamin A when carotene is the food source of the vitamin.

Table 2 gives the responses of rats to treatment with insulin and cortisone when vitamin A acetate was used as a supplement. Large differences were found in liver storage of vitamin A between females of the control group and females of all three test groups. In males, however, very little difference was noted in the group receiving cortisone, but large differences appeared in the group receiving insulin, and in the group treated with a combination of insulin and cortisone.

Table 1

The Effects of Insulin and Cortisone on the Storage of Vitamin A in the Livers and Kidneys of Rats When Carotene Was Fed.

Group	Number of Animals	Sex	Mean Weights		Vitamin A	
			Livers	Kidneys	Livers	Kidneys
			gm	gm	ug	ug
Controls	8	M	7.66	1.63	11.46 ± .29*	9.08 ± .10
	7	F	5.50	1.30	17.18 ± .38	5.01 ± .16
Cortisone	10	M	6.70	1.52	7.07 ± .07	6.94 ± .14
	9	F	5.59	1.44	14.37 ± .20	3.71 ± .16
Insulin	6	M	7.84	1.60	5.75 ± .07	6.90 ± .16
	9	F	6.41	1.43	12.14 ± .22	4.63 ± .07
Cortisone and Insulin	10	M	7.14	1.57	6.62 ± .10	5.81 ± .06
	9	F	5.84	1.36	12.17 ± .38	4.53 ± .07

* Standard error of the mean.

Table 2

The Effects of Insulin and Cortisone on the Storage of Vitamin A in the Livers and Kidneys of Rats When Vitamin A Acetate Was Fed.

Group	Number of Animals	Sex	Mean Weights		Vitamin A	
			Livers	Kidneys	Livers	Kidneys
			gm	gm	ug	ug
Controls	9	M	8.34	1.64	60.83 ± .77*	6.23 ± .07
	9	F	6.36	1.42	106.38 ± 1.40	2.53 ± .03
Cortisone	9	M	5.49	1.28	55.77 ± .50	3.04 ± .03
	9	F	5.00	1.31	75.97 ± .97	2.39 ± .02
Insulin	6	M	7.01	1.61	169.24 ± 4.01	5.80 ± .22
	6	F	7.13	1.72	222.83 ± 4.27	4.35 ± .12
Cortisone and Insulin	4	M	8.69	1.82	153.64 ± 4.47	7.22 ± .49
	10	F	6.20	1.60	151.91 ± 1.04	4.35 ± .04

* Standard error of the mean.

DISCUSSION

Liver Storage of Vitamin A

Bauereisen (7) reported that the daily administration of 0.5 unit insulin to guinea pigs stimulated the conversion of carotene to vitamin A. However, studies on liver storage of vitamin A in this laboratory do not confirm this report, when rats were used as experimental animals. Insulin decreased the amount of liver vitamin A storage in rats given carotene, while insulin-treated rats given vitamin A acetate showed a large increase in storage of the vitamin. This suggests that insulin inhibits the activity of the enzymes involved in the conversion of carotene to vitamin A.

A possible explanation of the depression of vitamin A storage by cortisone is that the hormone reduces tissue permeability and thus decreases the amount of absorption through the intestinal wall. Studies on lipid absorption by Adlersberg (9), which showed that cortisone definitely decreased tissue permeability to lipids and lipid-soluble materials, strongly support this view.

In the carotene-fed rats (Table 1) no significant difference was found between the group receiving a combination of insulin and cortisone

and the groups receiving only insulin or cortisone. This was surprising because it was reasoned that insulin should counteract the effect of cortisone. Therefore it is difficult to speculate what mechanisms are involved in the effects of these hormones on the conversion of carotene to vitamin A. As stated in the "RESULTS", liver vitamin A was lower than expected in the rats which were given carotene. Had these values been higher, a larger difference might have been found between the group given a combination of cortisone and insulin and the groups which were given only insulin or cortisone.

It is well-known that females store greater amounts of vitamin A in the liver than do males. In the vitamin A-fed rats this was found to be true in all cases except when cortisone and insulin were given together. In this case no significant difference was found between males and females. In the group of males treated with cortisone a slight but significant lowering of liver vitamin A was noted, while males of the insulin-treated group showed a great increase in the amounts of the vitamin stored. In males treated with insulin and cortisone together, the amount of liver vitamin A was found to be much greater than in controls, yet significantly lower than in the insulin group. Similar responses were noted in the females. The females responded to treatment with cortisone more markedly than did the males, with a large decrease in the amount of the vitamin stored in the livers, compared to

the control animals. Females of the group treated with both insulin and cortisone also showed a greater decrease in the amount of vitamin A stored in the livers than did the males.

Table 2 shows that the differences in vitamin A storage between males and females of the cortisone group are much less than the differences between the males and females of the control, or insulin groups. The amounts of vitamin A stored by males and females of the group receiving combined hormone treatment are nearly equal. These facts suggest that cortisone exerts an effect upon the hormone balance of the females. That is to say, cortisone may inhibit or antagonize the estrogenic hormones and cause the females to utilize vitamin A in a manner similar to the males.

Table 2 also shows that insulin caused an increase in the amount of liver vitamin A in both males and females. These findings are not in agreement with the work of Bauereisen (7), who found that in guinea pigs the daily administration of 0.5 unit insulin caused less of the fed vitamin to be stored in the liver. The difference in species may account for this difference. The increase in liver stores of vitamin A in both males and females of the group which received insulin suggests that insulin increases the permeability of the intestinal wall, permitting a greater amount of the vitamin to be absorbed and stored. Park et al.

(10) have shown, in studies on the uptake of sugars by rat diaphragms, that insulin increases the transport of sugars across the cell membranes. These findings lend support to the view that insulin increases the permeability of the intestinal wall to vitamin A.

Kidney Storage of Vitamin A

Although the studies show that significant amounts of vitamin A are present in the kidneys of both carotene and vitamin A supplemented rats, differences between test groups and controls were not great enough to be of any real significance. It is interesting to note, however, that in both carotene and vitamin A supplemented rats, the males consistently stored more vitamin A in the kidneys and less in the livers than did the females. Raica (1) reported similar findings. The uniformity of response in these experiments suggests that it is a characteristic sex difference.

CONCLUSIONS

Insulin causes a decrease in the amount of vitamin A stored in the livers of rats receiving carotene as a source of the vitamin. When vitamin A acetate is given, insulin causes a great increase in liver vitamin A. Cortisone decreases liver storage of the vitamin when either carotene or vitamin A acetate is fed. When insulin and cortisone are given in combination, liver vitamin A in carotene-fed rats is not significantly different from rats treated with only cortisone or insulin. Vitamin A-fed rats receiving a combination of the two hormones store more of the vitamin than rats given only cortisone, and less of the vitamin than rats given only insulin, showing that cortisone counteracts to some degree the effect of insulin upon vitamin A storage in the liver.

LITERATURE CITED

1. Raica, N., Jr., Dissertation, The Influence of Foods and Physiological Factors on the Utilization of Carotene by the Rat, 1958.

2. Ingle, D. J., The Production of Glycosuria in the Normal Rat by Means of 17-Hydroxy-11-dehydrocorticosterone. Endocrinology 29:649 (1941).

3. Boland, E. W. and N. E. Headley, Effects of Cortisone Acetate on Rheumatoid Arthritis. J. Amer. Med. Assn. 141:301 (1949).

4. Mirsky, I. A., Insulinase and Diabetes Mellitus. Recent Progress in Hormone Research 13:429 (1957).

5. DeFrancis, P. and W. Landauer, Combined Effects of Cortisone and Insulin on Developing Chicken Embryos. Nature 184: 101 (1959).

6. Clark, I. and W. Colburn, A Relation Between Vitamin A Metabolism and Cortisone. Endocrinology 56:232 (1955).

7. Bauereisen, E., Influence of Insulin on the Carotene-Vitamin A Components of the Liver. Endokrinologie 21:247 (1939).

8. Carr, F. W. and E. A. Price, Color Reactions Attributed to Vitamin A. Biochem. J. 20:497 (1926).

9. Adlersberg, D., Hormonal Influences on the Serum Lipids. Amer. J. Med. 23:769 (1957).

10. Park, C. R., L. H. Johnson, J. H. Wright, Jr., and H. Batsel, Effect of Insulin on Transport of Several Hexoses and pentoses into Cells of Muscle and Brain. Amer. J. Physiol. 191:13 (1957).