THE EFFECT OF THYROID ACTIVITY ON THE CONVERSION OF CAROTENE AND RETINENE TO VITAMIN A AND ON SERUM PROTEINS

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Johnson and Baumann ('47) reported that desiccated thyroid increased liver stores of vitamin A in rats fed carotene, but thiourea or thiouracil lowered the vitamin A reserves in the liver. These findings were confirmed by Kelley and Day ('48). Barrick, Andrews, Beeson and Harper ('48) indicated that very high doses of thiouracil inhibited the conversion of carotene to vitamin A in feeder lambs and it was also shown to be true in sheep by Bolin and Bolin ('49). Cama and Goodwin ('49) showed that desiccated thyroid increased absorption of carotene from the intestines whereas thiouracil decreased the absorption. This finding was supported by the work of Chanda, Clapham, McNaught and Owen ('51) in lactating cows and goats.

Bieri and Schultze ('51) could not demonstrate any effect of thiouracil on the vitamin A in the serum, liver and kidneys of rats fed or injected with aqueous dispersions of carotene. Arnrich and Morgan ('54), using liver storage of vitamin A as the criterion to measure the effect of thiouracil on the conversion of carotene to vitamin A, found that carotene-fed rats, rendered hypothyroid with thiouracil, stored much more vitamin A in the liver than their respective controls. Arnrich ('55) extending the studies to dogs, could not demonstrate increased storage of vitamin A from carotene in the liver of the animals treated with thiouracil. McGillivray, Thompson and Worker ('56) and Worker ('56), found that thyroidectomy or hyper-

thyroidism had no direct effect on the conversion of intravenously injected carotene to vitamin A as observed by similar blood levels and liver storage of vitamin A in treated groups. Clinical studies by earlier workers have been reviewed by Drill ('43).

In the present investigation, assuming that the conversion of carotene to vitamin A *in vivo* takes place in two stages, (a) β-carotene → retinene (Glover and Redfearn, '54) and (b) retinene → vitamin A (Glover, Goodwin and Morton, '48) the effect of hyper- and hypothyroidism on the conversion of retinene to vitamin A in rats was investigated. The fate of intravenous injections of retinene to hyper- and hypothyroid rats was studied. On account of recent conflicting reports, the effect of iodinated casein and thiourea on carotene conversion to vitamin A was also examined.

Electrophoretic analyses of sera from rats were also carried out to study the effect of iodinated casein and thiourea on the different components of serum proteins.

MATERIALS AND METHODS

Preparation of retinene for feeding and injection

The method used for preparing retinene was essentially the same as that described by Ball, Goodwin and Morton ('48), but in the present investigation vitamin A acetate,[1] after saponification, was used as the starting material instead of fish-liver oil. The reaction with manganese dioxide [2] was complete in three days, and the resulting retinene solution was chromatographed three times over 10% alumina.[3] Retinene thus obtained had an $E^{1\%}_{1cm}$ value of 1360 at 370 mμ (light petroleum). It was approximately 80% pure on the basis of the $E^{1\%}_{1cm}$ value of 1690 for crystalline retinene.

To known volumes of retinene and α-tocopherol acetate,[1] a measured quantity of arachis oil was added and the solvent

[1] Obtained from Hoffmann-La-Roche Ltd., Switzerland.

[2] B. D. H. Laboratory reagent.

[3] Alumina, specially prepared for chromatographic adsorption. Obtained from E. Merck, Germany.

removed in vacuo, so that the desired amount of retinene, equivalent to 40 μg of crystalline retinene was present in 0.1 ml of the oil which also contained 0.5 mg of α-tocopherol acetate.

The aqueous dispersion of retinene for injection was prepared by using 2% (w/v) solution of Tween 80 [4] (polyoxyethylene sorbitan monooleate) in physiological saline so that an amount equivalent to 400 μg of crystalline retinene was present in 0.4 ml.

Preparation of carotene for feeding

The oily solution of carotene for feeding was prepared as described above so that 0.1 ml of arachis oil contained 40 to 60 μg of crystalline carotene.[5] For higher dose levels of 400 and 3000 μg, the carotene was present in 0.2 ml of the oil.

For quantitative feeding of oily solutions of retinene and carotene, a micrometer syringe [6] with a blunt needle was used. Very accurate volumes for antimony trichloride colour test were also measured in the same way.

Preparation of animals and design of the experiment

Litter mates of albino rats of both sexes were used in separate groups. When the rats were 5 weeks old and had reached a weight of 25 to 40 gm, they were divided into three groups of the same sex and fed the following vitamin A-deficient diet: casein (ether extracted) 18, cornstarch 59, sugar 10, salt mixture [7] 4, refined arachis oil 8.8, cystine 0.2, plus the following vitamins per kilogram of diet: α-tocopherol acetate 100 mg, pantothenic acid 50 mg, calciferol 100 μg, menadione sodium bisulphite 10.2 mg, thiamine hydrochloride 5 mg, pyridoxine 5 mg, riboflavin 5 mg, niacinamide 50 mg, biotin 0.5 mg, folic acid 0.5 mg, inositol 100 mg, choline chloride 1.0 gm.

Rats in the control group were fed the above mentioned vitamin A-free diet. In another group, they were rendered

[4] Atlas Powder Co., Wilmington, Delaware, U. S. A.
[5] British Drug Houses Ltd., U. K.
[6] Agla, Burroughs Wellcome Ltd., U. K.
[7] Hawk and Oser, 1931.

hyperthyroid by treatment with iodinated casein [8] at a level of 0.125% of the diet. Similarly, in the third group, a hypothyroid state was induced by feeding either thiouracil [9] or thiourea [10] at levels of 0.1 and 0.2% of the diet respectively. The dosage of thiourea at 0.5% of the diet (Johnson and Baumann, '47) was found to be toxic in the experiments in which retinene and thiourea were fed. The rats were therefore treated with thiourea at a level of 0.5% of the diet for the initial three weeks of the experiment and at 0.2% level for the subsequent three weeks.

Each experiment was divided into two periods; one of about 4 weeks on vitamin A-free diet and one of two weeks during which the rats were daily dosed orally with an oily solution of retinene or crystalline carotene. Throughout the depletion period and the period of supplementation with retinene or carotene, the feeding of iodinated casein, thiouracil or thiourea was continued. In all series, a rat was considered depleted when the weighings on three consecutive days indicated that growth had virtually ceased.

For intravenous injections of retinene, the rats were treated as described above. A Tween dispersion containing an amount equivalent to 400 μg of crystalline retinene in 0.4 ml was injected into the tail vein.

Determination of vitamin A in livers and kidneys of rats fed retinene or carotene

Twenty-four hours after the last dose of retinene or carotene, the animals were anaesthetised with chloroform and killed. The livers and kidneys were separately ground with acid-washed sand and anhydrous sodium sulphate,[11] and the tissue lipid was extracted 5 times with light petroleum.[12] The combined extracts were reduced in volume at low pressure.

[8] Boots Drugs Co., U. K.

[9] Nutritional Biochemicals Corporation, U. S. A.

[10] British Drug Houses Ltd., U. K.

[11] B. D. H. Laboratory reagent.

[12] Light petroleum (b.p. 40-60°C), Obtained from Burmah-Shell, is left over $KMnO_4$, washed, dried over $CaCl_2$ and twice distilled before use.

In experiments in which retinene was administered orally or intravenously to rats, vitamin A in the livers was estimated spectrophotometrically in the whole liver extract by the antimony trichloride colour test at 620 mμ (Cama, Collins and Morton, '51). Vitamin A was also estimated by the three-point correction method of Cama et al., '51, using the absorption readings at 310, 325 and 335 mμ (light petroleum) but the results obtained are not presented in table 1 since this procedure gave only the vitamin A esters in the liver as compared to the $SbCl_3$ colour test which gave the total vitamin A (esters + alcohol).

When carotene was administered to rats, the lipid residue, obtained after removal of the solvent in vacuo from the petroleum ether extract of the livers, was saponified with ethanolic KOH. Vitamin A was estimated in the unsaponifiable portions of the liver by the antimony trichloride colour test at 620 mμ.

In all series, vitamin A in the kidneys was determined in the whole kidney extracts by the antimony trichloride colour test.

The significances of the differences between control and treated groups were statistically analysed by the "t" test of significance for paired values as given by Davies ('49).

The results were considered as significant where P was between 5 and 10% or less than 5%, whereas those which had a value for P of more than 10% were insignificant.

Analysis of serum proteins by agar electrophoresis

The procedure followed for the separation of serum proteins was essentially the same as that described by Giri ('56) and Giri and Pillai ('56). The blood was taken from the rat by cardiac puncture under mild anaesthesia and allowed to clot at 37°C for one hour. The clear serum obtained after centrifugation was used for analysis. The serum (30 μl) was applied on the agar [13] gel (0.5%) containing barbital buffer [14]

[13] B. D. H. fine powder.

[14] Barbitone sodium obtained from Bayer Co., Germany.

(pH 8.6, 0.05 ionic strength). Electrophoresis (200 volts, 5 milliamperes, 4 hrs.) was carried out at room temperature (25°C). Subsequently, the agar plates were dried and stained with Amidoschwarz 10 B.[15] The quantitative evaluation of the protein components was carried out using a Photovolt Electronic Densitometer, Model 525. Total nitrogen and non-protein nitrogen were estimated by the micro-kjeldahl method.

RESULTS

Vitamin A in livers of rats fed retinene

The results presented in table 1 indicate that male and female rats fed iodinated casein stored significantly lower amounts of vitamin A in the livers than those of the control group when fed equal amounts of retinene ($P < 0.1$, series I and $P < 0.1$, series III). When the three-point correction procedure was applied to determine vitamin A esters, the differences between the control and treated groups were found to be even more significant ($P < 0.05$ for series I and $P < 0.01$ for series III). The differences in total vitamin A levels in the livers between control and thiouracil-treated rats were not statistically significant (series II and IV).

When thiourea was used to induce hypothyroidism, the differences noted in the vitamin A contents between control and treated groups of male as well as female rats were similar; the data on male and female rats are therefore discussed together in series V. Thiourea treatment significantly increased vitamin A in the liver ($P < 0.02$).

Intravenous injections of retinene

The results of intravenous injections of retinene to control, hyper- and hypothyroid rats are presented in table 1 (series VI and VII). Rats rendered hyperthyroid accumulated as much vitamin A in the livers as those of the control group (series VI), while thiourea treatment increased vitamin A in the livers ($P < 0.05$, series VII).

[15] E. Merck, Germany.

TABLE I

Effect of iodinated casein, thiouracil and thiourea on vitamin A in the livers of rats (a) fed retinene in arachis oil (b) injected with Tween dispersions of retinene and (c) fed crystalline carotene in arachis oil.

SERIES NO.	DOSE	NO. OF OBSERVATIONS AND SEX	GROUP	TOTAL VITAMIN A IN THE LIVER BY $SbCl_3$ COLOUR TEST	MEAN DIFFERENCE (CONTROL-EXP.)	S.E.[1] DIFF.	P (PROBABILITY)
				(I. U.)			
I	40 μg retinene orally for 15 days	5 M	Control	806.8	+52.6	±22.9	S*[2]
		5 M	Iod. casein-fed	754.2			
II	—Do—	7 M	Control	786.57	−32.72	±29.73	N.S.
		7 M	Thiouracil-fed	819.29			
III	—Do—	8 F	Control	701.5	+44	±18.68	S*
		8 F	Iod. casein-fed	657.5			
IV	—Do—	8 F	Control	734.75	−26.75	±42.08	N.S.
		8 F	Thiouracil-fed	761.50			
V	—Do—	8 (3M)	Control	315.63	−51.87	±16.82	S***
		8 (3M)	Thiourea-fed	367.50			
VI	400 μg retinene intravenously 24 hrs. before dissection	6 M	Control	213.0	−17.0	±11.09	N.S.
		6 M	Iod. casein-fed	230.0			
VII	—Do—	5 (4M)	Control	224.8	−23.8	± 8.3	S**
		5 (4M)	Thiourea-fed	248.6			
VIII	40 μg crystalline carotene for 14 days and 3000 μg carotene 24 hrs. before dissection	8 (3M)	Control	39.13	− 6.87	± 2.94	S**
		8 (3M)	Iod. casein-fed	46.00			
IX	60 μg crystalline carotene for 12 days and 400 μg carotene for the last three days	9 F	Control	47.00	+16.0	± 7.65	S*
		9 F	Thiourea-fed	31.00			

[1] Standard error of the difference.
[2] S* significant between 5 and 10% levels.
S** significant between 2 and 5% levels.
S*** significant between 1 and 2% levels.
N.S. not significant.

Oral administration of carotene

In series VIII (table 1), a single dose of 3,000 μg of crystalline carotene in oil was given to all rats 24 hours before dissection, after administration of 40 μg of carotene for 14 days of the supplementation period. In series IX, the dosage of carotene was increased to 60 μg per day for 12 days and three doses of 400 μg each were given to all rats during the last three days of the supplementation period.

Table 1 shows that iodinated casein-treated rats fed carotene accumulated larger amounts of vitamin A in the liver than the controls ($P < 0.05$, series VIII). In contrast, animals treated with thiourea had smaller amounts of vitamin A in the livers ($P < 0.1$, series IX).

Electrophoretic analysis of serum proteins

The percentage distribution of the serum proteins in rats given different treatments is presented in table 2. The values reported represent the average of duplicate experiments conducted under identical conditions and the results were observed to be consistent. It is apparent from the table, that in vitamin A-deficient rats, there is a significant increase in the α_2-, β_2- and γ-globulins and a significant decrease in albumin

TABLE 2

Percentage distribution of serum proteins from vitamin A-deficient, control, hyperthyroid and hypothyroid rats

GROUP	TOTAL PROTEINS	NON-PROTEIN NITROGEN [1]	ALBUMIN	GLOBULINS				
				α_1	α_2	β_1	β_2	γ
	gm %		%	%	%	%	%	%
Vitamin A deficient	6.1	3.6	54.3	4.8	5.1	3.8	20.0	12.0
Control	7.9	4.0	71.1	4.8	3.2	4.4	8.9	7.6
Iodinated casein-treated	7.9	7.2	62.8	4.8	5.1	4.0	18.2	5.1
Thiourea-treated	7.3	4.0	70.3	8.3	1.7	3.0	10.1	6.5

[1] Expressed as percentage of total nitrogen.

as compared to the control group. In iodinated casein-treated rats, the α_2- and β_2-globulins increase, but albumin is considerably lowered. Thiourea-treated rats show a remarkable increase in the α_1 component whereas the α_2 component of the globulins is considerably lowered. There is little change in the β- or γ-globulins or in the albumin fraction of thiourea-treated rats.

No appreciable change in the total serum protein concentration in the different groups of animals could be noted except in the case of vitamin A-deficient rats which show a decrease. The non-protein nitrogen is increased considerably by hyperthyroidism.

DISCUSSION

The demonstration of the conversion of vitamin A aldehyde (retinene) into vitamin A in the intestinal wall of rats by Glover et al. ('48) is of significance, for in all probability retinene may be an intermediate in the conversion of β-carotene into vitamin A. Glover and Redfearn ('54) have, in fact, shown the formation of retinene by stepwise degradation of β-carotene.

The results reported in the present studies show that the effects of iodinated casein and thiourea on rats fed retinene orally are contrary to those on rats fed carotene, on the basis of the liver reserves of vitamin A. Iodinated casein decreases but thiourea increases vitamin A in the liver, while thiouracil does not have any significant effect. When retinene was administered intravenously to hyper- and hypo-thyroid rats, there was no significant difference in the total vitamin A in the livers between the treated groups. [The "t" test of significance for paired values between hyperthyroid and hypothyroid groups (rows 12 and 14; table 1) could be carried out since the rats used in these groups were littermates of the same sex.] This clearly indicates that the thyroid activity does not have any direct effect on the conversion of intravenously administered retinene to vitamin A. Thus our observation is in

line with that of McGillivray et al. ('56) and Worker ('56), who showed that thyroid activity has little influence on the conversion of intravenously administered carotene into vitamin A. However, it is observed that the vitamin A levels in the livers of thiourea-treated groups are higher than those in the control groups (series VII; table 1). It seems probable that these rats in the control groups utilise more and hence store less of vitamin A than the treated ones during the 24-hour period.

It is evident from table 1 that the liver vitamin A reserves of carotene-fed rats are much lower than in those fed retinene. In general, the absorption of carotenoids by mammals is very poor as compared to both vitamin A and retinene and a considerable proportion of any ingested carotenoid is excreted in the feces (Krebs and Hume, '49; Gounelle, Marnay, Cheroux and Raoul, '52).

There is considerable evidence to show that the thyroid hormone has a significant role in the metabolism of carotene. Cama and Goodwin ('49), and Chanda et al. ('51) demonstrated that its effect is mainly on the intestinal absorption of carotene. The claim of Arnrich and Morgan ('54) that neither absorption, transformation nor utilisation of carotene is affected by thyroid activity was re-investigated using the criterion of liver storage of vitamin A. In case of hyperthyroidism, the liver vitamin A levels in rats are significantly high and thiourea treatment significantly lowers vitamin A in the livers (table 1). This confirms the observations reported by Johnson and Baumann ('47) and Kelley and Day ('48). We have thus to attempt a reconciliation between contradictory experiences by groups of workers employing different experimental procedures. It is important to note that the experimental procedures adopted by Arnrich and Morgan ('54) are different from those employed by previous workers. Arnrich and Morgan ('54), in fact, conclude that, in part, the disagreement found on the subject of carotene utilization in hypothyroidism is undoubtedly due to discrepancies in experimental procedures and the criteria used to measure carotene utilization.

The vitamin A reserves in the kidneys of rats fed retinene were, however, higher for hyperthyroid females than controls ($P < 0.01$) and lower for thiourea-treated animals ($P < 0.05$), which is in line with the observations of Kelley and Day ('48) on carotene. Our results also confirm the report of Moore and Sharmann ('50) who observed that males deposit more vitamin A in kidneys than females.

In vitamin A deficiency, protein synthesis is inhibited as indicated by the remarkably low level of albumin and the total serum protein value (table 2). The high value for γ-globulin is indicative of an increased production of antibodies in the system, which is characteristic of any infection. α_2-Globulin is known to increase in cases of inflammatory lesions and in other cases of tissue destruction. From the abnormally high value of α_2-globulin observed in vitamin A-deficient rats, it is reasonable to presume that the animal is in a similar state of metabolic disturbance.

Our results on hyperthyroidism (table 2) confirm the findings of Lewis, McCullagh and Clark ('44) who reported that albumin always remains low, often accompanied by an increase in α-globulins. In the case of hypothyroidism, it is observed that there is a very high increase of α_1-globulin to almost double the concentration of that in the control group but there is little change in albumin concentration. This finding is in agreement with some of the earlier observations made by Moore, Levin and Smelser ('45) and Leathem and Seeley ('47).

SUMMARY

The effect of iodinated casein, thiourea and thiouracil on retinene and carotene metabolism in rats has been investigated.

Iodinated casein decreased whereas thiourea increased the liver storage of vitamin A on feeding retinene, but thiouracil did not show any significant effect. There was no significant difference in total vitamin A in livers between the iodinated casein and thiourea groups, when retinene was injected intravenously. On feeding carotene, hyperthyroid rats stored more

and hypothyroid rats less vitamin A in the liver than the controls.

The changes occurring in the different components of serum proteins, in vitamin A-deficient, hyperthyroid and hypothyroid rats have been shown. In vitamin A deficiency a very low albumin level and a significant increase in α_2-, β_2- and γ-globulin levels are observed. In hyperthyroidism, there is a decrease in the albumin and an increase in the α_2- and β_2-globulins. In hypothyroidism an increase in the α_1-globulin is noted.

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