Absence of Relationship Between Vitamin A Alcohol Levels in Plasma and in Liver of Rats

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A moderately extensive amount of work has been reported in the literature dealing with the relationship between vitamin A levels in the blood and in the liver of various species. Generally speaking, these reports can be classified into two categories. Lewis, Bodansky, Falk & McGuire (1942) believe that plasma tends to resist, to a certain extent, any changes in its vitamin A concentration, despite wide variations in the amount stored in the liver, and this view has been supported by many investigators (Josephs, 1942; Horton, Murrill & Curtis, 1941; Brenner, Brookes & Roberts, 1942; Ralli, Baumann & Roberts, 1942).

On the other hand, Glover, Goodwin & Morton (1947), using a chromatographic separation of free and esterified vitamin A, have claimed that while the level of vitamin A in the blood cannot be deduced from the total concentration in the liver of rats, there is a rough linear relationship between the concentration of vitamin A alcohol in the liver and in the blood.

A qualitative difference in the distribution of vitamin A ester and alcohol and of carotenoids in the plasma of several species has recently been reported from this laboratory, and it has been suggested that specific proteins are involved in the absorption and transport of these compounds (Ganguly, Krinsky, Mehl & Deuel, 1952*b*). On account of the implications of this hypothesis, we have re-investigated the relationship between plasma and liver vitamin A alcohol levels in the rat. A preliminary report was presented to the American Society of Biological Chemists (Ganguly, Krinsky & Deuel, 1952*a*).

EXPERIMENTAL

Animals. Three lots of male rats of the University of Southern California strain were used in these experiments. The first lot (groups 1 and 2, Table 1) consisted of adult stock rats raised on Purina chow; the second lot (group 9, Table 1) was raised on a vitamin A-free diet (*U.S. Pharmacopoeia*, 1950) after weaning, and was used when depletion of vitamin A was indicated by cessation of growth. The third lot consisted of rats previously used for a vitamin A bioassay (*U.S. Pharmacopoeia*, 1950) of margarine samples; during the period of the experiment they had received 1·25–1·5 i.u. of vitamin A daily. These were used either immediately after the completion of the bioassay (animals for Fig. 1) or received weekly supplements of 10–15 i.u. of vitamin A until used (groups 3–8, Table 1). Although rats on a bioassay cannot be expected to grow normally, the small supplements given during the assay were enough to produce appreciable plasma vitamin A levels (see 0 hr. group in Fig. 1).

Supplements. The vitamin A solutions used for dosing were made from a fish-liver oil concentrate suitably diluted with peanut oil, mixed tocopherols, and soya lecithin to contain the requisite amounts of vitamin A, 5 mg. of tocopherols, and 40 mg. of lecithin/ml. of supplement. The solutions were administered by stomach tube.

Analysis. Blood was withdrawn by direct heart puncture, with an oxalated syringe, while the rats were under light Nembutal anaesthesia. The plasma, obtained by centrifugation, was extracted according to the method of Kimble (1939), and the other tissues were immediately excised, and were extracted following the procedure of Thompson, Ganguly & Kon (1949). The vitamin A alcohol and ester were separated by chromatography on alumina as described by Ganguly *et al.* (1952*b*), and were determined by the procedure of Thompson *et al.* (1949), except that a Coleman Junior spectrophotometer calibrated against a standard vitamin A solution was employed.

RESULTS

The rate of appearance of vitamin A alcohol and ester in blood and liver

Vitamin A-deficient rats, previously used for a vitamin A bioassay, were fasted for 12–18 hr. Each was given a single 1 ml. dose containing 14000 μg. of vitamin A ester. Groups were killed at given time intervals after dosing. The averages of the results of vitamin A analysis obtained from individual rats for liver and for plasma are presented in Fig. 1. The plasma ester showed the typical absorption curve, starting at 1 hr., rapidly reaching a peak at 5 or 6 hr., and then dropping sharply by the 15th hr. The liver ester presented a continual increase up to 18 hr., after which no further storage occurred.

The plasma alcohol increased at a slower rate than did the ester and, by the 24th hr., declined to the approximate pre-dosage level. On the other hand, the liver alcohol slowly increased, reaching a maximum at about 5 hr., and maintained a fairly steady

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Table 1. *The relation between vitamin A alcohol in the plasma and vitamin A alcohol and ester in the liver of male rats receiving various diets and supplements*

(The figures in brackets indicate the range of values obtained.)

Group	No. of rats	Weight range (g.)	Age	Dietary regime: Diet	Dietary regime: Treatment	Vitamin A alcohol: Plasma (μg./100 ml.)	Vitamin A alcohol: Liver (μg./g.)	Vitamin A ester. Liver (μg./g.)
1	24	218–435	Adult	Purina chow	None	26·9 (19·9–36·2)	21·7 (1·2–61·4)	473 (154–794)
2	26	245–407	Adult	Purina chow	110000 μg. vitamin A divided into 13 daily doses	26·2 (14·4–38·1)	57·4 (10·9–234·0)	3470 (1595–5520)
3	16*	149–338	3–5 months	Vitamin A-free	3–21 days on Purina chow	23·5 (17·3–28·8)	4·4 (1·5–8·0)	178 (17–307)
4	9*	100–222		Vitamin A-free	110000 μg. vitamin A divided into 4 daily doses	17·4 (13·6–20·2)	169·3 (137·9–186·0)	2033 (1920–2118)
5	20*	110–270		Vitamin A-free	3300 μg. vitamin A in one dose	20·1 (14·1–25·5)	16·3 (5·7–30·0)	332 (182–442)
6	20*	136–255		Vitamin A-free	8300 μg. vitamin A in one dose	24·4 (17·3–29·5)	28·2 (5·6–61·6)	638 (316–1275)
7	20*	127–245		Vitamin A-free	16600 μg. vitamin A in one dose	23·9 (19·1–29·0)	29·3 (8·2–81·0)	824 (477–1258)
8	44*	65–188		Vitamin A-free	22–48 μg. vitamin A in one dose	27·0 (19·4–36·7)	0·1 (0·0–0·4)	0·4 (0·0–1·4)
9	13*	121–203	2 months	Vitamin A-free	None	0	0	0

* Three to four rats pooled.

level throughout the 24 hr. period, in spite of the continual increase in the liver ester. It appears that under these conditions the liver vitamin A alcohol concentration rapidly attains a steady level, even while there is a continual deposition of vitamin A ester in the liver.

from 0·1 to 169·3 μg./g., the plasma vitamin A alcohol remained fairly stable, namely 17·4–27·0 μg./100 ml. It is noticeable that group 4, which had the highest liver vitamin A alcohol content, was accompanied by the lowest plasma level of this component, whereas group 8, in which the livers contained only

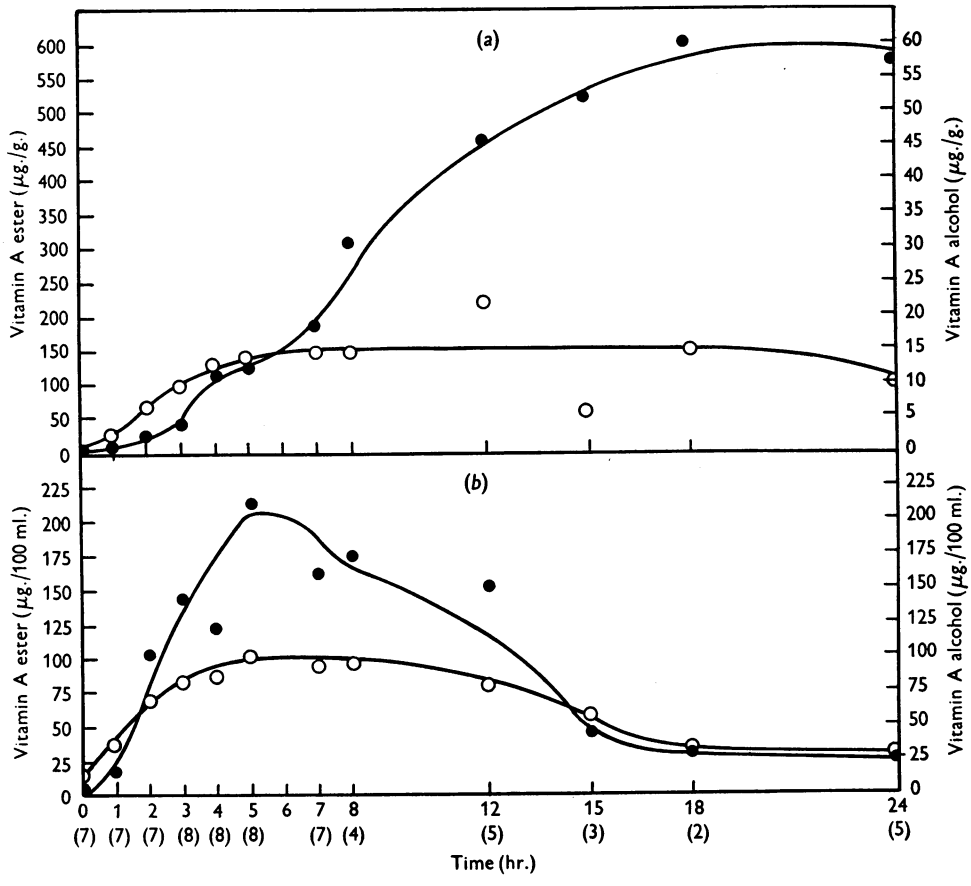

Fig. 1. The rate of appearance of vitamin A ester and alcohol in (*a*) liver and (*b*) plasma of vitamin A-deficient male rats following a single 1 ml. dose containing 14000 μg. vitamin A, 5 mg. mixed tocopherols, and 40 mg. lecithin. The figures in brackets indicate the number of animals upon which the averages were determined. ●—●, Vitamin A ester; ○—○, vitamin A alcohol.

The relationship between plasma and liver vitamin A alcohol

In order to obtain wide variations in the concentration of vitamin A in the liver, several groups of rats were given different diets and supplements, as described in Table 1. All animals were killed not earlier than 3 days after their last supplement following a 12 to 18 hr. fast. The results are summarized in Table 1, in which the averages of the individual values within each group are presented. Despite the variation in the liver vitamin A alcohol traces of vitamin A alcohol, had the highest plasma concentration of this alcohol.

Fig. 2 represents the individual plasma alcohol values plotted against their respective liver alcohol values for the rats used in Table 1. This diagram does not show any direct relationship between liver and plasma vitamin A alcohol. These results, however, confirm the earlier observations of Lewis *et al.* (1942) and Glover *et al.* (1947), that normal plasma vitamin A concentration can be maintained even when there is none in the liver. This blood vitamin A disappears on prolonged depletion.

Vitamin A in kidneys

In normal adult rats, small amounts of vitamin A alcohol, 0·4–1·5 μg./g. tissue, were present in all kidneys, whereas the ester form was found only occasionally, at approximately the same concentration as the free form. Vitamin A alcohol, 5·2 μg./g. and vitamin A ester, 66·9 μg./g., were found in the kidneys of vitamin A-deficient rats 24 hr. after a single dose of 14 000 μg. of vitamin A had been given.

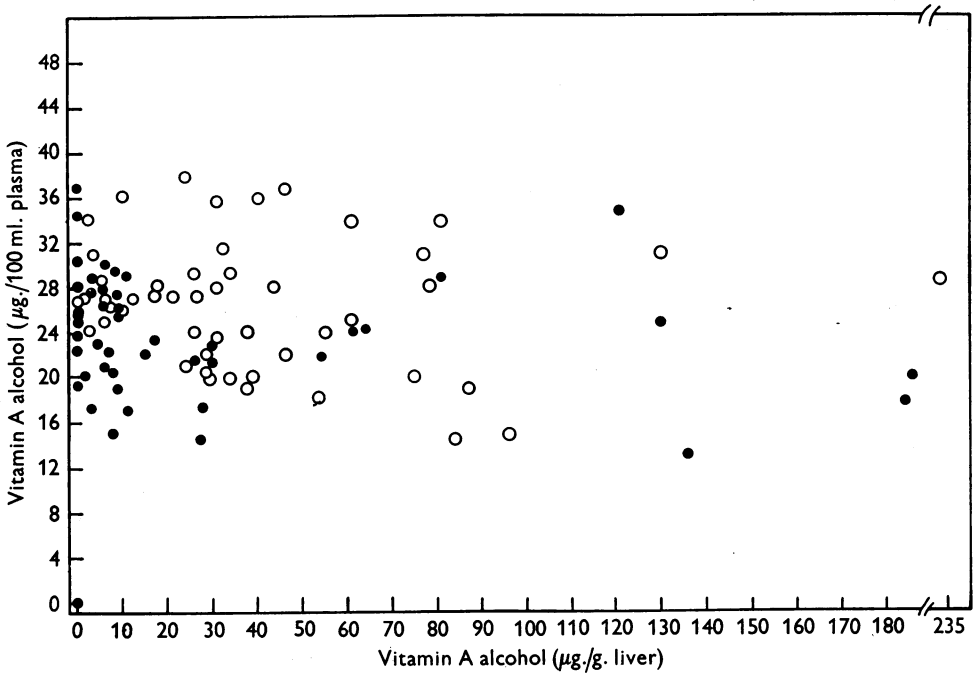

Fig. 2. Relationship between liver and plasma vitamin A alcohol in rats. ○ represents the animals in groups 1 and 2, Table 1; ● represents the animals in groups 3–9, Table 1.

Sex difference

Moore, Sharman & Ward (1951) have recently reported a sex difference in the total vitamin A contents of blood and of liver of rats. The males had more vitamin A in the blood and less in the liver, compared with the female rats. More recently, Booth (1952) has reported a similar sex difference in liver storage in rats. The vitamin A ester and alcohol contents of the plasma and liver of fourteen female stock rats were determined. Despite the fact that comparable amounts of plasma were analysed in both stock male and female rats, the values for the female rats were so low that a definite Carr-Price reaction could be obtained in only one case, whereas in the males all plasma samples gave positive reactions. The average liver alcohol value of the females was 35·8 μg./g. tissue (range 3·1–86·4 μg./g. tissue) and the liver ester value was 714 μg./g. tissue (range 83·0–1168 μg./g. tissue). Although we cannot compare these females directly with the male rats of group 1, Table 1, as their previous dietary intake of vitamin A is unknown, we feel it necessary to emphasize the fact that these females with higher liver vitamin A alcohol stores had lower plasma alcohol values as compared with the males.

DISCUSSION

Our results do not show any direct relationship between the plasma and the liver vitamin A alcohol, as suggested by Glover *et al.* (1947). There are, however, two major deviations in the techniques employed. The first is the method of extraction. We have compared the two methods but found no significant difference. The second involves the instrumental determination of the rapidly fading blue colour developed by the Carr-Price reaction. In our procedure, a direct reading instrument, such as the Coleman spectrophotometer, should give a more precise value than could be obtained with the Beckman spectrophotometer, which necessitates time-consuming adjustments during the measurements.

On the other hand, these results demonstrate that plasma tends to maintain a rather steady vitamin A alcohol level. Although this mechanism is still unknown, one possible explanation is that the plasma concentration is dependent upon the degree of association of vitamin A alcohol with a protein carrier. Variations in this degree of association may explain the temporary increase in plasma vitamin A alcohol immediately following absorption of vitamin A. The sharp increase in the ester form of vitamin A during active absorption may likewise be due to its association with another protein.

SUMMARY

1. The rate of appearance of vitamin A ester and alcohol in plasma and liver, respectively, of vitamin A-depleted male rats following a single dose of vitamin A was studied. In plasma, the ester form presented a typical sharp peak, and the free form showed relatively less increase. In liver, the ester form continued to be deposited up to 18 hr. after dosing, whereas the alcohol form increased for only 5 hr. and was then maintained at a fairly constant level.

2. In separate experiments, normal stock and vitamin A-depleted male rats were used either as such or after vitamin A dosage, to determine the vitamin A content in plasma and in liver. A direct relationship between the plasma and the liver vitamin A alcohol could not be confirmed, inasmuch as, despite wide variations in the concentrations of vitamin A alcohol in the liver, the plasma level remained relatively constant.

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Acetylation of Collagen

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In recent years it has been possible to account for almost the whole nitrogen content of collagen in terms of its constituent amino-acids (Bowes & Kenten, 1948*a*). From the known composition of collagen it is evident that many of its side chains should be distinguished by polar groups, of which the most important are carboxyl, amino, guanidino and aliphatic hydroxyl groups. Before asking which of these groups may be responsible for such complicated changes as occur in reaction with tanning agents, it seems desirable to inquire whether they are free to react quantitatively with simple chemical reagents. It has already been shown by Bowes & Kenten (1949) that the carboxyl groups can be quantitatively esterified, and one of us (Green, 1951) has demonstrated a stoicheiometric relationship when dry hydrogen chloride reacts with the protein. We report here a detailed study of the reaction between collagen and acetic anhydride.

This substance might be expected to react with some or all of the amino, guanidino and hydroxyl groups. Olcott & Fraenkel-Conrat (1947) found that several proteins in aqueous alkaline solution reacted with acetic anhydride in an amount equivalent to their free amino groups. They recommended this reaction as a specific means for the *N*-acetylation of proteins. Several workers have found that, when the conditions are changed and the solid protein is treated with acetic anhydride, both amino and hydroxyl groups are substituted. This was the case