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Significance of Vitamin A Alcohol and Ester Partitioning under Normal and Pathologic Circumstances.*

HANS POPPER, FREDERICK STEIGMANN, ALVIN DUBIN, HATTIE A. DYNIEWICZ, AND FRANK P. HESSER.

From the Hektoen Institute for Medical Research; Departments of Pathology and Therapeutics of Cook County Hospital; Pathology of Northwestern University Medical School; Internal Medicine of University of Illinois College of Medicine, Chicago, Ill.

Previous studies[1] indicated that the low plasma vitamin A level in hepatic diseases and conditions associated with liver damage is at least in part due to disturbed release of vitamin A from the liver. On the basis of fluorescence microscopic observations,[2] this was explained by displacement and retention of vitamin A within the damaged liver cells. Recent investigations[3,4] showed that vitamin A is transported in esterified form from the intestine to the liver where it is stored in this form.[5,6] It is released from the liver to the blood as vitamin A alcohol.[4] The latter represents about 80% of the total plasma vitamin A in the normal fasting human being and in several animals studied.[3,4,5,6] In pathologic conditions, the total plasma vitamin A level may decrease, especially in liver diseases[7,8] or it may be elevated as in recovery from these diseases[9] or in renal alterations.[10] This raises the problem as to the changes of the vitamin A alcohol-ester partition in pathologic conditions.

Material and Methods. Total vitamin A and vitamin A esters were determined in the plasma of 186 fasting patients. In 9 instances the determinations were repeated 2, 3, 6, 24 and 48 hours after administration of vitamin A as non-saponifiable fraction of fish oil, dissolved in corn oil or in an aqueous dispersion.† The latter was prepared with sorbitan monolaurate derivative. In 5 cases, determinations were performed after 3 and 7 days on a diet containing not more than 20 units of vitamin A per day.

For the ester separation,[11] 4 cc of 95% ethyl alcohol were added to 4 cc of oxalated plasma and after mixing, 12 cc of petroleum ether were added. After shaking the mixture for 10 minutes, separation of the layers was permitted in a refrigerator. Then, a 10 cc aliquot of the petroleum ether was transferred to a separatory funnel and extracted seven times with 10 cc portions of 90% methyl alcohol, at a temperature between 10 and 30°C. The petroleum ether fraction was evaporated to dryness under nitrogen in vacuum. The residue was dissolved in chloroform and examined for vitamin A esters as in the total vitamin A determination according to the method of

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TABLE I.

Concentration of Total Vitamin A, Vitamin A Ester, Vitamin A Alcohol in μg per 100 cc Plasma and Vitamin A Ester Percentage of Fasting Hospital Controls and Patients Suffering from Various Diseases.

Diagnosis	No. of cases	Mean total Vit. A	Ester Vit. A			Alcohol Vit. A			Mean ester %
			Mean	Range	T value* compared with hospital control	Mean	Range	T value* compared with hospital control	
Hospital controls	31	51.2	9.5	3.0-15.1		41.7	25.1- 78.0		18.2
Cardiac disease	13	38.7	9.5	2.3-16.8	0.03	29.2	9.0- 47.7	2.71	26.8
Gastrointestinal disease	7	44.6	11.6	6.7-16.7	0.93	33.0	9.9- 48.2	1.48	30.6
Carcinoma without involvement of biliary tract	15	34.8	13.1	2.3-21.0	2.07	21.7	6.1- 54.3	4.54	42.4
Chronic wasting disease	5	29.3	10.3	2.3-21.6	0.32	19.0	5.1- 52.5	3.37	39.5
Malnutrition	4	22.5	13.2	9.7-18.1	1.25	9.3	6.2- 17.9	4 37	56.0
Tuberculosis	11	29.2	10.7	7.6-12.9	0.64	18.5	2.9- 42.4	4.74	44.1
Infections	2	40.3	14.0	11.7-16.2	1.09	26.3	22.4- 30.1	1.51	34.5
Pneumonia	9	27.4	14.6	10.0-20.0	2.38	12.8	2.8- 24.4	5.47	53.4
Pneumonia recovering	10	53.2	12.7	2.1-29.6	1.59	40.5	14.1- 64.5	0.23	24.1
Nephritis	10	67.3	11.1	3.3-17.4	0.81	56.2	31.7-105.8	2.87	17.3
Nephrosis	6	57.0	22.3	14.1-59.2	5.06	34.7	6.5- 55.7	1.12	40.0
Acute infectious hepatitis	14	34.2	11.1	2.3-16.7	0.91	23.1	2.0- 41.0	4.14	38.1
Infectious hepatitis recovering	6	40.8	12.0	8.3-12.4	1.02	28.8	10.9- 57.0	2.07	32.4
Acute toxic hepatitis	6	26.3	12.9	7.8-18.9	1.36	13.4	6.7- 21.7	4.34	46.4
Cirrhosis without jaundice	12	26.7	11.9	5.0-24.7	1.25	14.8	21.1- 29.5	5.67	47.9
Cirrhosis with jaundice	17	23.1	12.0	4.0-24.7	1.51	11.1	1.7- 27.9	7.25	56.9
Obstructive jaundice	8	39.4	11.6	5.0-21.5	0.94	27.8	11.6- 65.0	2.50	31.9

* T test for significance of difference between means (see Fisher, R. A., *Statistical Methods for Research Workers,* Oliver and Boyd, Edinburgh, London, 1948). T value above 2.5 is considered significant.

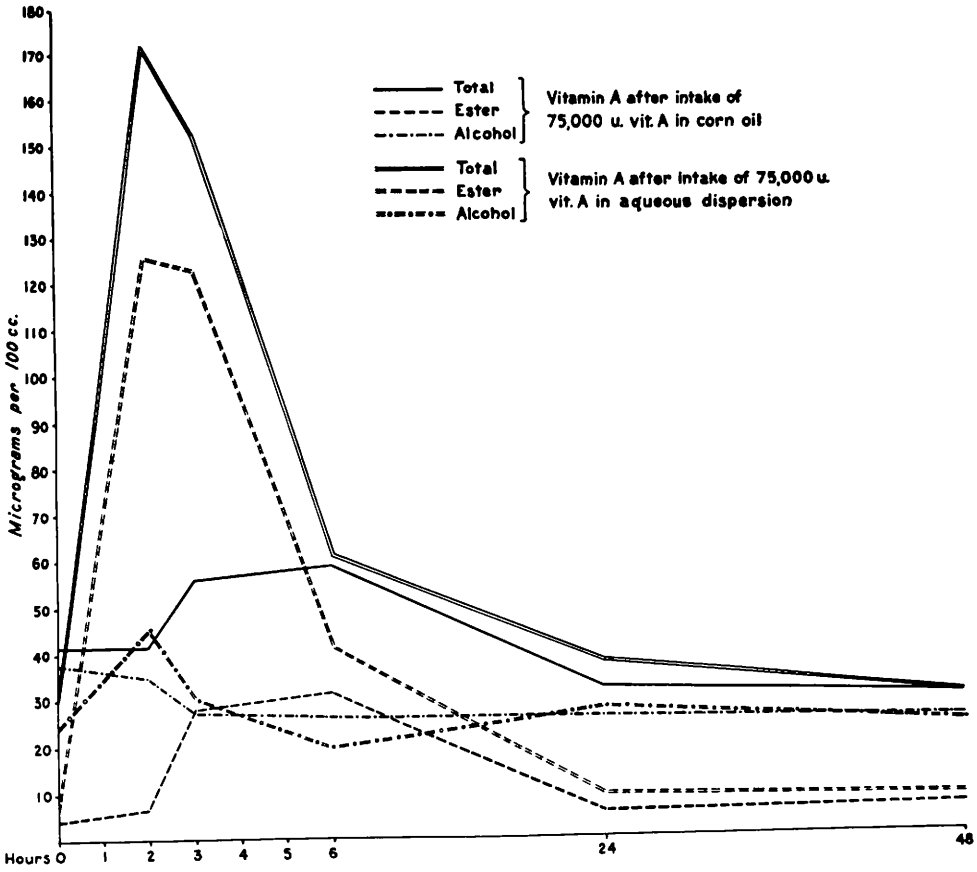

FIG. 1.

Response of total, ester, and alcohol vitamin A concentration in the plasma to the intake of 75,000 units of vitamin A in corn oil and in aqueous dispersion in a hospital control.

Kimble[12] using the Coleman spectrophotometer. The alcohol vitamin A was calculated by subtraction of the ester vitamin A from the total vitamin A concentration.

Results. In hospital controls, about 20% of the total vitamin A was found in the ester form (Table I). The average of the ester fraction revealed, under the examined pathologic circumstances, no statistically significant changes with the exception of the cases of nephrosis in which the ester vitamin A was significantly increased. In carcinoma (even without involvement of the biliary tract), malnutrition, pneumonia and especially in liver diseases, there was a definite trend for elevated ester vitamin A which was brought out by the average as well as by the maximal levels obtained.

The vitamin A alcohol revealed a statistically significant increase in cases of nephritis in which the highest level of this series was recorded. In nephrosis, however, the level appeared on the average lower than normal without statistical significance. A similar, statistically not significant reduction was encountered in gastrointestinal diseases and in infections, whereas a significant reduction was found in cardiac diseases. In carcinoma without involvement of the biliary tract, in malnutrition, in severely sick persons, in tuberculosis and in pneumonia, the levels were low, the lowest of the entire group being found in pneumonia. The esters in these instances were high. After recovery from pneumonia the vitamin A alcohol returned to normal and might even exceed the normal. In acute, infectious or toxic hepatitis there was a significant reduction of vitamin A alcohol with irregular return to normal during recovery. In obstructive jaundice, the re-

TABLE II.
Mean Total Vitamin A, Vitamin A Ester, and Alcohol in μg per 100 cc Plasma and Ester Percentage of 5 Patients Kept on a Vitamin A-Deficient Diet.

Days on Vit. A deficient diet	Total Vit. A	Ester Vit. A	Alcohol Vit. A
0	42.8	11.1	31.7
3	38.2	12.4	24.2
7	31.4	13.9	17.9

duction was less significant. In cirrhosis, especially with jaundice, there was the most marked reduction of the plasma vitamin A level with the lowest values recorded. The esters in these instances were reduced, normal or elevated.

The peak of the vitamin A tolerance curve after feeding of 75,000 units of vitamin A varied in the different examined conditions. It was especially low in liver diseases following the intake of vitamin A in oil. However, the relation between alcohol and ester was the same in the examined normal and pathologic conditions in that the rise was entirely due to the ester fraction, whereas the vitamin A alcohol concentration showed no characteristic variations (Fig. 1). After intake of vitamin A in aqueous dispersion the rise of total and ester vitamin A was far higher than after administering the same amount of vitamin A in oil. During the peak of the tolerance curve the percentage of esters rose from around 20% to 80% in hospital controls.

The plasma vitamin A level dropped slightly in the patients kept for 7 days on a vitamin A deficient diet (Table II). This drop was due to a reduction of the alcohol fraction. The ester fraction dropped in one case slightly, whereas it rose in the other 4. In all instances, the percentage of the esters rose during the duration of the experiment.

Comment. This study confirms on a large number of subjects that in the fasting normal human being only about 1/5 of the total plasma vitamin A is in the esterified form. The ester portion increases after intake of vitamin A as well in aqueous as in oily menstruum in both normal and pathologic cases, while the alcohol does not change. Vitamin A esters are elevated in nephrosis in which cholesterol and other lipids are known to be increased. This elevation, therefore, may be due to increased solubility in the blood. The ester fraction reveals a slight but not regular or statistically significant rise in malnutrition, carcinoma or liver diseases, while the total or alcohol vitamin A level is reduced. One could consider the ester rise in these latter conditions an indication of inability of the liver to absorb esters from the blood with their subsequent piling-up in the blood. However, vitamin A esters rise also while vitamin A is withheld from the diet. One might assume a higher threshold for vitamin A absorption in conditions associated with liver impairment as is the case with glucose and other metabolites. However, the fact that in such conditions the vitamin A ester level may be low militates against this possibility. No evidence is available for increased solubility of vitamin A. The fourth and so far most likely hypothesis would assume a pathologic release of vitamin A in ester form due to liver damage which prevents the hepatic esterase from converting vitamin A ester, the bulk of the stored hepatic vitamin A, into vitamin A alcohol. Only vitamin A alcohol present in small amounts in the liver was found to be in balance with the blood vitamin A alcohol,[6] and is the form in which the liver releases vitamin A normally. With inactivation of the esterase and impaired release of alcohol, esters would enter the blood. This agrees with the observation that after administration of ethyl alcohol either the vitamin A ester[5] or alcohol[4] level may be elevated.

Vitamin A alcohol is increased in nephritis (not in nephrosis) and the reported high blood levels in this condition are due to the alcohol but are still not explained. The vitamin A alcohol also rises in recovery from pneumonia and hepatitis. The hypervitaminemia A in such conditions is probably the

result of increased release of vitamin A from the liver. Since the ester level is not significantly raised in this condition, the hypervitaminemia is apparently not, as it has been assumed,[5] due to reduced uptake of vitamin A by the liver during the recovery period when the absorption has returned to normal but liver damage still persists. The fact that the blood vitamin A alcohol represents vitamin A released from the liver explains also its reduction in malnutrition, infections, hepatic and wasting diseases, *i.e.* conditions in which the hepatic vitamin A concentration is reduced as in chronic liver diseases or malnutrition, or vitamin A release is impaired as in acute diseases (pneumonia or hepatitis). This impairment was previously considered morphologically the result of a displacement of vitamin A in the liver cells[2] and now is believed, physiologically the result of impaired esterification.[6]

The reduction of the vitamin A alcohol concentration under pathologic conditions, often associated with a rise in esters, elevates the ester percentage like the intake of vitamin A, but obviously on an entirely different basis.

Although the total plasma vitamin A level is shown to depend as much on endogenous[7] as on nutritional factors, it is often used as a valuable index of general or vitamin A nutrition. However, the vitamin A alcohol level is a superior index because it reflects much better storage in and release from the liver and is independent of solubility and postprandial rise. Despite the more elaborate method of determination, the vitamin A alcohol level may replace the total plasma vitamin A level as a nutritional index.

Summary. Plasma vitamin A esters rise markedly after intake of vitamin A. They are elevated significantly in nephrosis and slightly and irregularly in conditions associated with hepatic impairment. The plasma vitamin A alcohol level does not change after the intake of vitamin A but is elevated in nephritis and sometimes in recovery from conditions associated with hypovitaminemia A. It is significantly decreased in malnutrition, infections, hepatic and wasting diseases, apparently due to reduced storage in and/or release from the liver. The vitamin A alcohol level, therefore, excels the total vitamin A level as index of hepatic vitamin A storage and vitamin A nutrition.

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Probability of Normal Development after Transplantation of Fertilized Rabbit Ova Stored at Different Temperatures.

M. C. CHANG. (Introduced by D. Rapport.)

From the Worcester Foundation for Experimental Biology, Shrewsbury, and the Department of Physiology, Tufts College Medical School, Boston.

In 1890 and 1897, Heape[1] reported his successful transplantation of rabbit ova to a uterine foster mother. This was mainly a demonstration of the unimportance of somatic tissue to the germ cells. Pincus and Enzmann[2] obtained normal young at term by transplantation of rabbit ova fertilized or cultured *in vitro*. In my last communication,[3] optimal temperature for the storage of ova and the normal development of young after low temperature storage of ova was reported. Since then, I have obtained the following data which show the probability of normal development.

Methods. The ova were obtained from

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