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ON THE DISTRIBUTION OF VITAMIN A IN VARIOUS TISSUES OF THE RAT ADMINISTERED WITH DRY VITAMIN A CONCENTRATE *

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In a previous paper, we demonstrated i) the annual changes of vitamin A and carotene in the bovine blood plasma and milk, and ii) the extent to which the vitamin A level of the milk, as well as of blood plasma, could be affected by the administration of the concentrated cod liver oil. But in the preceding research, unfortunately, no examination of the liver vitamin A could be performed (1). On the other hand, the role of the liver as the main store house for vitamin A reserves in the body has been well established by numerous works (2–8). It would be very effective to obtain richer information as to the changes of vitamin A in the milk and blood plasma, since these changes could be influenced greatly by the storage of vitamin A in the liver, judging from the results of Wise *et al.* (9).

The primary object of the present experiment is in following the works so far published with regard to the vitamin A distribution in the tissues of the rat fed with a higher level of vitamin A (dry concentrate) standard, so as to yield further interpretations on the effect of vitamin A treatment in the cattle. In addition, a test was carried out to show the manner of vitamin accumulation in the liver after days of treatment by different vitamin A levels.

Materials and Methods

i) *Animals*:

Twenty-four mature, nulliparous female rats of the Wistar strain were used in this experiment. Their weights ranged 110–150g in the first experiment, and 180–240g in the second.

* Some data of this paper were presented at the Annual Meeting of the Japanese Society of Veterinary Science, Tokyo, April, 1957.

ii) *Diets*:

Four diets, depending on the vitamin A content (VH, MH, H, and L-as described below in '**Conditions**'), with the following ingredients were used:

1) wheat flour (commercial). ……75 %*
2) milk casein, treated three times with boiling ethanol-ether mixture (3 : 1) to eliminate fat soluble materials ……15 %
3) soy bean oil, oxygenized for a few hours. …… 5 %
4) brewer's yeast ('Ebios', Nippon Beer Co., Tokyo). …… 2 %
5) salt mixture ($NaCl : CaCO_3 = 1 : 1$) …… 2 %
6) vitamin A, added as the concentrated powder "Alvita"**, Takeda Pharmacy Co., Osaka which is equivalent to 10,000 i u/g of vitamin A and also 1,000 i u/g of vitamin D, so as to given the desired i u of vitamin A/15g of the dry matter of the biscuite. Flour per cent was modulated to make the final volume to 100 per cent adding the required amount of vitamin A powder. By a preliminary test, it was shown that a rat consumed daily 20g of the biscuite of which the water content was about 25 per cent.

iii) *Conditions*:

For the first trial, 12 rats were divided into three groups. They were kept on the 10,000, 2,000 and 0 i u/head/day for a fortnight, and were named the very high (VH), moderately high (MH) and low group (L), respectively. The rats ate up the diet almost completely, though small remnants were sometimes found in the cage the next morning. Before the feeding experiments started, the animals were kept on a standard diet in which only casein was replaced by a 'fish soluble' "Viscera S", Nippon Suisan Chem. Co., so that there 576 i u of vitamin A/20g of the biscuite; otherwise, it was of the same composition as the ration mentioned above. The rats for the first test were fed on the standard diet for more than 30 days and the second group for about 10 days.

Next, the increase or decrease of vitamin A in the liver was measured following the time course of the feeding (5, 10, and 15 days) with MH (2,000 i u) H (400 i u) and L (o) rations. Nine rats were allotted to these three groups.

iv) *Estimation*:

The rats after being stunned were sacrificed by bleeding the parotid artery on the next morning after the final feeding, and the serum was prepared. Other tissues were immediately dissected and weighed, and then cut into small pieces so as to be easily sapponified. The mammary gland consisted mainly of the fat tissue. The analytical method for vitamin A was the same as previously described (1): spectrophotometric, in which the technique of destructive irradiation by ultra-violet was employed (10–12), because this method makes it

* Note, later description in 6) 'vitamin A'.

** Dry vitamin, A, D adsorbed to the defatted soy bean powder stabilizing by dibutyl-hydroxytoluene.

possible to estimate such small quantities of vitamin A as would be scarcely measurable by other means.

To know the precision of the method employed, comparison with the GDH method (13) was paralleled analysing vitamin A preparates employed and some of the liver.

Results

i) **Vitamin A concentration in the various tissues at different level feeding:**
The results obtained in the present experiments are summarized in Table 1.

Table 1. Vitamin A concentration (i u) of the various tissues in the rat fed on 10,000 (VH), 2,000 (MH) and 0 (L) iu level.

Group	Serum	Liver	Mammary gland	Ovary	Adrenal	Lung	Kidney	Small intestine	Spleen	Pancreas
a) iu per g fresh tissues										
V H	3.86	15,427	272	193	1,492	1,844	480	2,848	26	137
M H	1.12	3,677	82	34	280	173	11	69	4	15
L	0.51	3,810	13	7	119	25	20	7	3	7
b) total i u										
V H	—	91,400 (6.25)	552 (2.03)	7 (0.040)	71 (0.050)	3,040 (1.65)	600 (1.25)	6,850 (2.41)	17 (0.64)	121 (0.89)
M H	—	19,830 (5.41)	195 (2.44)	2 (0.054)	16 (0.059)	143 (1.12)	15 (1.40)	200 (2.95)	2 (0.58)	13 (0.84)
L	—	21,220 (5.57)	25 (1.96)	0 (0.050)	6 (0.051)	35 (1.42)	29 (1.46)	17 (2.45)	2 (0.79)	5 (69.0)

The parentheses in the column of 'total i u' indicate the weight(g) of organs.

The table indicates the vitamin A concentration on the basis of both i u per g fresh tissue (or *ml* serum) and total units of the whole organ. In general, the liver contained the highest concentration of vitamin A, when expressed as either total content or i u/g tissue, and the apparent differences between the VH and MH or L groups was shown despite some variations in the concentration within a group. Per cent of the liver vitamin A against the total so far estimated showed from 94 to 99 per cent. Moreover, all organs in the VH group indicated a richer value comparing with those of the MH or L. On the basis of vitamin A concentration in the tissues of the VH group, for example, they would be listed: liver-highest by far, in second class-small intestine and lung, in the third class-kidney and mammary gland (mostly of fat tissue) and in the fourth-pancreas, adrenal and so on. The same tendency in the stated order was observed in the MH group. Absolute value in the increase due to vitamin A administration, of course, was predominantly marked in the liver.

On the other hand, no difference was seen in the present experiment between the MH and L groups except for the opposite relation detected in the kidney, although the rise was slight. If we calculate the efficiency of the storage of vitamin A, on basis of the MH and L, about 50 per cent ingested was found in the liver.

Next, let us examine the relative concentration, expressed in i u per fresh weight basis, of the vitamin among the tissues. Almost the same relation with the total was shown in the order of magnitude, indicating uncomparably high value in the liver. The adrenals gave a moderately high potency, so it was ranked next to the lung and placed in the second class. Ovaries showed the same trend of increasing in the concentration, but to a less degree than the adrenals. Serum demonstrated also a remarkable elevation in the VH group, but remained within a normal range both in the MH and the L, although L was considerably lower than MH.

In the deficient condition, a little increase in the kidneys and a relatively slighter decrease in the adrenals seemed to have occurred.

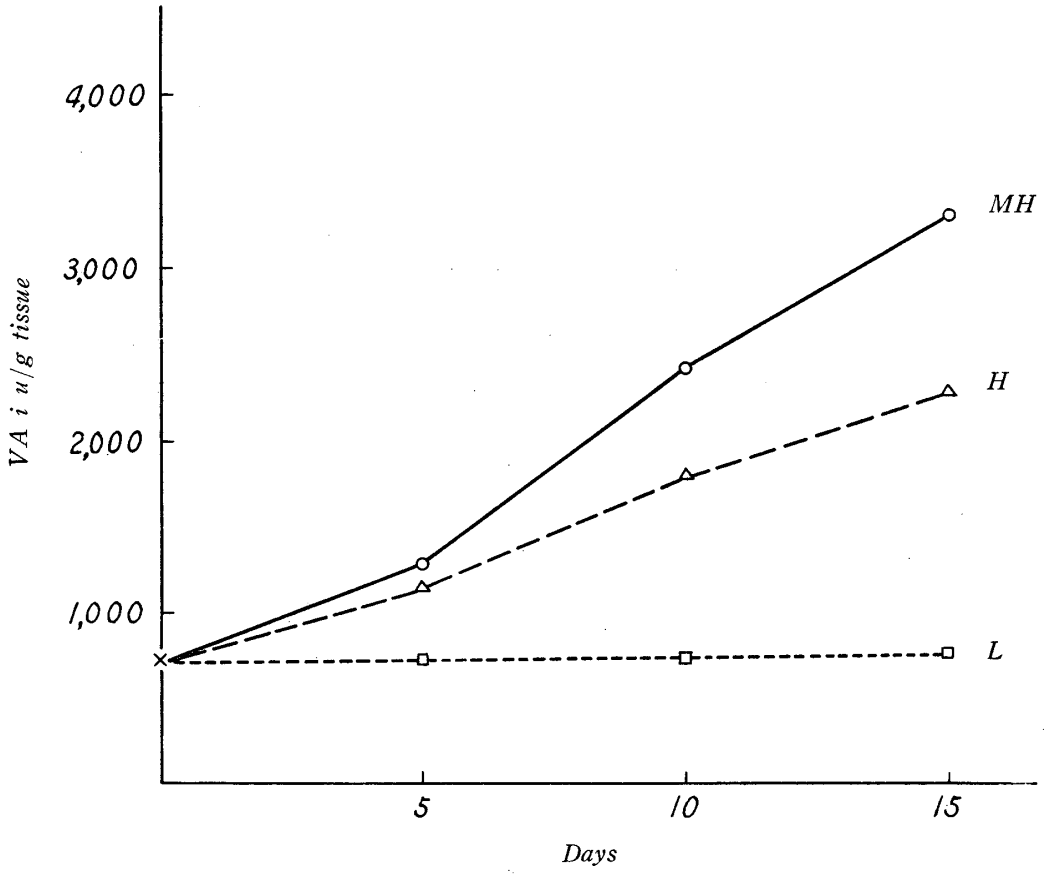

Fig 1. Time course of the accumulation of vitamin A in the liver.

ii) **Changes of vitamin A concentration in the liver due to the days fed with different vitamin dose:**

The vitamin A concentration in the liver of the rat placed on two high and one low levels is illustrated in Figure 1. The usual liver vitamin A concentration of rats fed on standard diets was 750 i u in average of two, and, this was deemed as the initial value. The concentration of the MH liver rose continuously during 15 days and that of L remained at a constant value. With H dose, the increasing curve was located in a situation intermediate between MH and L.

iii) **Comparison of the method employed with that of GDH.**

A test was carried out comparing the method used with the GDH method and demonstrating the availability of the spectrophotometric measurement using the ultra violet destruction of vitamin A in the specimen. The results were as follows:

Method of estimation	Liver (average of 11 estimations) i u/g tissue
UV	2,590
GDH	2,530

So far as the tests went, these two methods resulted in fairly close agreement.

Discussion

It has been well established hitherto that the main distribution of vitamin A in the tissues is in the liver, and that other sites of reservation are the kidneys, lungs and adrenals, although their concentrations remain much less. The present experiments proved the evidences cited above. And it was confirmed by this study that the liver concentration was elevated markedly when an extraordinary amount of the vitamin was given as demonstrated in previous papers (2–8). At this time the concentration in various tissues also increased but the degree was not necessarily the same, that is, there were some tissues in which vitamin content could be much increased (small intestine, lung, kidney and adrenal) but in the others only to a minor extent.

As was mentioned, no reductions were observed in the vitamin A content of the L group both in the first and second experiment. Furthermore, the level of these two L groups differed moderately. These findings may be attributed to the differences in the body weight and the period of pre-experimental feeding since the vitamin A level of the standard diet employed was far higher than the vitamin A requirement of rats. So that the liver had reserved abnormally high vitamin A, especially in the first experiment, and in addition, probably the experimental period was too short to cause any detectable decrease.

The major reason why the 10,000 i u/head/day was adopted was to determine the attitude in the rats of an exceptionally high dose of vitamin A, hoping to obtain evidence as in the case of dairy cows treated with a very high daily amount (1,100,000 i u/head) of vitamin as in our previous work. Similarly, the moderately high dose (2,000 i u/head/day) in the present experiment corresponded to the 220,000 i u feeding with the dairy cows (1). Presuming the rats and cows are administered an equal amount of vitamin A some presumptions can be undertaken, *i.e.* most of the vitamin ingested into the body, when the high dose was given, would be accumulated in the liver and that the plasma level would rise conspiciously only with such huge dosage. The content in the other organs would increase, more or less. The mode of increase in the rat serum showed the same tendency as that in the dairy cows (1,9)—a fairly high elevation in the rats when ten thousands i u was given, whereas a slight augmentation occurred when the cows were treated with about a million i u/head/day. However, it was little with 2,000 or 220,000 i u in rats or cows, respectively.

As is well known, the vitamin A concentration in the blood keeps unchangeable value under normal conditions, even if a moderately high dose is given, although the liver reserves vitamin A nearly proportionally to the dose given in the rat (5, 7, 14, 17, 18, 19). As Wise and his coworkers also demonstrated with the dairy cows, vitamin A intake reflected in the concentration of vitamin A both in liver and milk. Accordingly, it can be said from the experiments with rats and dairy cows that the liver of the cows treated with a high amount of vitamin A stores the vitamin, and this would probably be the cause of the apparent increasing of the vitamin in the milk, as found in the previous investigation (1).

As regards the efficiency of the vitamin ingested, similarly with Davies *et al.* (5), Gray *et al.* (16) and De Man *et al.* (21), our results indicated moderately high values than those of some works (17, 19, 20) and much more than that of Baumann and his collaborators (7). But this figure should be calculated, as a rule, from the experiment with rats in which the liver vitamin was previously depleted (5, 7, 14, 15, 18, 19, 20, 21) and therefore the final decision seemed to need further experiment. In addition, the concentration could be regulated by many factors; namely, the form of vitamin A employed (16, 20, 21, 22), and the physiological conditions of the animal such as sex (13, 23), thyroid function and the dietary constituents (2). One of the most probable factors which can be pointed out here is the better rate of absorption of 'dry vitamin A' concentrate.

The spectrophotometric method is more advantageous, as discussed in the previous paper (1), and in addition, it is possible to apply the method for the small tissue specimen with less vitamin potency. The present results as to

the liver specimens showed close agreement with that of the GDH method.

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Summary

An experiment was carried out to determine i) the distribution of vitamin A in the various tissues under the different levels of vitamin A concentrate and, in addition, ii) the manner of accumulation of the vitamin into the liver following the days treated.

The liver was the major reservoir for vitamin A with absolutely high level, as had been demonstrated previously by numerous works. But on the very high and moderately high levels, 10,000 and 2,000 i u daily, the response in the increasing of vitamin A in the tissues was not necessarily parallel. Next to the liver, the small intestine and lung placed in the total magnitude, and the adrenals ranked second to the liver in expressing i u per g fresh tissue. Blood serum remained within a normal range until the animal was treated with a very high dose, and then it came up to the very high level.

By the moderately high and high doses, 2,000 and 400 i u the storage in the liver was sharply raised during the 15 days.

The results obtained in the present investigation were discussed in relation to those of other previous papers and also to that of our preceding work dealing with the elevation of vitamin A in the milk and blood of the dairy cows.

A test was undertaken to determine the precision of the availability of the method employed for the estimation of the liver vitamin A comparing it with the GDH method, and the results showed good agreement.

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