SPECTROPHOTOMETRIC MICROMETHOD OF DETERMINING THE VITAMIN A CONTENT IN PUNCTURE SPECIMENS OF THE RHESUS MONKEY AND RABBIT LIVERS*

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The vitamin A content in the liver is one of the most important indices used in its quantitative determination in the animal organism. For this purpose the colorimetric methods of Ames [4] and Gallup [7], which require liver specimens weighing at least 1 g, are usually used. This entails laparotomy or sacrifice of the animal, which limits the possibilities of observing the dynamics of the process.

Needle biopsy of the liver of agricultural animals has been used for obtaining smaller specimens (0.7-1 g) [6]; puncture of the human liver has been used for histological investigations [1]. However, these methods are unsuitable for monkeys and rabbits whose liver has a multilobular structure and thin lobes. Therefore it was necessary to find technical methods of puncture which would permit us to obtain microquantities of the liver many times. At the same time the problem arose concerning the method of determining vitamin A in microsamples. The present report is devoted to the solution of these problems.

Reports on the investigation of the vitamin A content in depot blood are lacking. In spite of the fact that even now it is not clear whether these investigations will find practical application, nevertheless, in our opinion they can have a certain theoretical value. In the present report we will touch upon only the methodological problems, therefore, we will refrain from explaining the data pertaining to the problem of the content of vitamin A in the blood and liver of animals although these data are also used for substantiating certain methodological aspects.

The attempt to use the methods of Ames and Gallup for spectrophotometric determination of vitamin A in microquantitites of the liver showed that the first of these (Ames' method) is unsuitable. The reason is that exclusion of the process of saponification of the liver creates the possibility of extraction from the tissue of other compounds besides vitamin A having absorption in the 328 mμ region. Therefore, the Gallup method in which the liver is saponified proved to be more suitable for us in combination with other additions.

It is also necessary to point out that a dissimilar content of vitamin A was found in different lobes of bovine liver [5]. Therefore, it was necessary to check whether this was the case for monkeys and rabbits.

For puncture of the monkey and rabbit liver, V. G. Sotulo, engineer at our institute, suggested a needle-trocar which would permit taking 50-70 mg of liver and 100 mg of depot blood (see figure). The needle consists of a cannula (a) with an inside diameter of 2 mm, a stylet (b), and an attachment (c). Inside, the attachment has a screen which serves to trap the detached parenchyma and clot of depot blood. The attachment is connected through a T-joint to a vacuum pump and the atmospheric air.

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TABLE 1. Average Content of Vitamin A (in μg%) in the Liver

Experimental animal	Investigated material (liver)	M	n	σ±	m±
Rabbit	With blood.	32.4	9	9.0	3.0
	Without blood.	83.2	9	4.4	1.4
	With blood.	49.2	9	16.8	5.6
	Without blood.	74.8	9	5.2	1.6
	With blood.	46.4	9	14.8	4.8
	Without blood.	84.0	9	7.2	2.4
Monkey	With blood.	242.0	8	143.2	50.4
	Without blood.	403.2	8	40.4	14.0
	With blood.	288.4	8	72.0	25.6
	Without blood.	380.4	8	16.4	6.4
	With blood.	296.0	8	154.8	55.2
	Without blood.	416.0	8	25.6	10.4

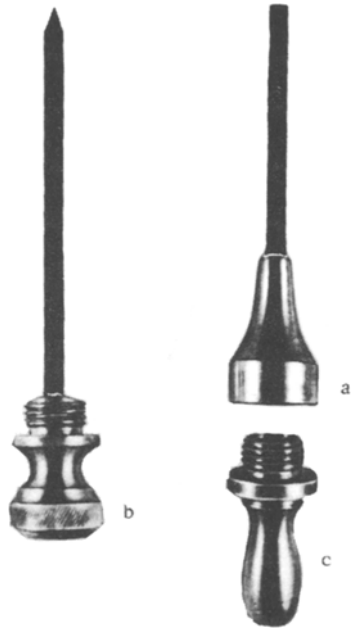
TABLE 2. Comparative Content of Vitamin A (in μg%) in Peripheral and Depot Blood and Liver

Animal	Blood		Liver
	Peripheral	Depot	
Monkey No. 1	2.7	3.8	449.2
" No. 2	2.8	3.8	496.0
" No. 3	1.3	2.2	619.2
Rabbit No. 1	3.2	4.1	8.4
" No. 2	1.6	10.9	14.8
" No. 3	2.6	4.1	11.6
Guinea pig No. 1	1.2	3.2	4.8
" " No. 2	1.0	1.6	10.0
" " No. 3	1.0	1.3	2.8

Puncture Technique

Three days before puncture the animal is subcutaneously injected daily with 1.5% aqueous solution of vicasol in a dose of 0.3 ml/kg. After scrubbing hands and the operation field and anesthesizing the skin, a cutaneous incision is made (0.5-1 cm). For the monkeys the incision is made from the right near the lower margin of the costal arch along the parasternal line, and for the rabbits near the lower margin of the xiphoid process. The puncture needle with the enclosed stylet is inserted into the cutaneous incision, the abdominal wall is punctured, the stylet is extracted from the needle and replaced by the attachment with the screen. A clamp is applied to the free branch of the T-joint communicating with the atmospheric air. With a sharp jab the liver is punctured, the needle advanced 1-1.5 cm into the parenchyma, the vacuum pump turned on and a negative pressure created. After 30-40 sec the needle is extracted from the abdominal cavity. Antibiotics are poured into the incision and a skin suture applied. After treating the suture with iodine, a collodion dressing is applied.

The piece of liver and depot blood (which has generally coagulated) are pushed out of the needle and the clot of depot blood quickly removed from the liver. The liver tissue is dried, placed between sheets of a folded piece of cellophane (to reduce distortion of weight owing to drying out), and weighed on a torsion balance (approximately 50 mg). On a second weighed piece of cellophane the clot of depot blood is weighed. Weighing should be completed before the onset of clot retraction.



Needle-trocar for puncturing the liver. Explanation in text.

Determination of the Vitamin A Content in the Liver

The weighed sample of liver and cellophane are placed in a test tube and a 2 N ethanol solution of NaOH added at a rate of 0.1 ml of solution per each 10 mg of tissue. The ethanol solution of NaOH is prepared from 1 part of 11 N aqueous solution of NaOH and $4^1/_2$ parts of a 0.002% ethanol solution of an antioxidant (butylhydroxytoluene). The test tube is placed in a Dewar vessel with liquid nitrogen, then extracted from it by tweezers and at the moment the last drop of nitrogen changes from a liquid to a gaseous state, it is tightly sealed with a cork stopper. Thus saponification is achieved in a nitrogen atmosphere, which is done on a water bath at 75° for 30 min.

After cooling, an equal volume of distilled water and 0.4 ml of petroleum ether (60-70° fraction) is added to the saponified sample, the test tube is stoppered with a cork, shaken for 30 min, then centrifuged for 2-3 min at 1500 rpm. The concentration of vitamin A in the obtained extracts is measured in plane-parallel microcuvettes (working length 8 mm) of the SF-4 spectrophotometer at a wavelength of 328 mμ. The light flux is diaphragmed at the exit [3]. The measurement is carried out against a blind sample in which water is used in place of of the analyzed material.

The Content of Vitamin A in the Depot Blood was determined by the method proposed earlier for studying the vitamin A and carotene content in whole peripheral blood [2]. The volume of the reagents was changed in proportion to the obtained weighed sample of the blood clot.

RESULTS AND DISCUSSION

Needle biopsy of the liver with 50-60 mg of the material being taken was carried out on 105 monkeys and 10 rabbits. Autopsy of the rabbits (after 48 h) and monkeys (after 7-10 days) showed the absence of blood in the abdominal cavity and a thrombosed lesion of the liver parenchyma. There was no inflammation of the liver or peritoneum. The surgical wound healed by the first intension. Four fold puncture at intervals of 2-3 days did not cause complications either from the liver or from the organism as a whole.

Since it was proposed to determine vitamin A in microsamples, we investigated how much the blood retained in the parenchyma could distort the results of the analysis. The data shown in Table 1 indicates that removal of the blood helps to obtain higher and more accurate values of the vitamin A content. The values of σ and m indicate this. The difference of the arithmetic means is more than 3, which is statistically reliable.

Shortening of the saponification period of the microsamples did not have an adverse effect on the elicited quantity of vitamin A. Thus, saponification at 75° for 30 min made it possible to elicit in the monkey liver an average of 2672 μg% of vitamin A ($n = 8$, $\sigma \pm 17.2$, $m \pm 7.2$). Prolongation to 60 min (Gallup's method) led to losses of vitamin A: its content was 2028 μg% ($n = 8$, $\sigma \pm 23.2$, $m \pm 23.2$, $m \pm 8.0$). The difference is reliable.

The information on the content of vitamin A in the lobes of the liver of the investigated animals is of interest. Thus, in the superior lobe of the monkey liver there was 476.8 μg% of the vitamin ($n = 9$, $\sigma \pm 41.2$, $m \pm 13.0$), in the right 482 μg% ($n = 9$, $\sigma \pm 15.6$, $m \pm 5.2$), and in the left 468.8 μg% ($n = 9$, $\sigma \pm 18.4$, $m \pm 0.6$). In the left lateral lobe of the rat there was 93.2 μg% of vitamin A ($n = 3$, $\sigma \pm 18.0$, $m \pm 10.8$), in the right lateral 110.8 μg% ($n = 3$, $\sigma \pm 20.0$, $m \pm 11.6$), and in the left internal 134 μg% ($n = 3$, $\sigma \pm 32.4$, $m \pm 18.8$). The right lobe of the rabbit liver contained 938.8 μg% of vitamin A ($n = 3$, $\sigma \pm 171.6$, $m \pm 100.0$), the left lobe 1168 μg% ($n = 3$, $\sigma \pm 932.0$, $m \pm 784.0$), and the left internal lobe 1224 μg% ($n = 3$, $\sigma \pm 227.8$, $m \pm 133.6$). The difference between the content of vitamin A in the various lobes of the liver proved to be statistically unreliable in all combination. Thus, the fact of a dissimilar content of vitamin A in different lobes of the liver [5] is apparently valid only with respect to large agricultural animals.

In the depot blood we elicited a larger content of vitamin A than in the peripheral blood, but less than in the parenchyma of the liver (Table 2).

The diveregence in the figures of the vitamin A content in the rabbit liver, which is observed in the tables and in the text, is explained by the dissimilar content in the ration of carrots and greens of these animals, by the different age of the rabbits, and by the condition of their liver.

Thus, saponification of a liver sample in a nitrogen atmosphere and the use of an antioxidant, the experimental grounds for which were given earlier [2], and also the elimination of blood from the tissue sample ensured a more accurate detection of the vitamin A content. These methodological additions in combination with spectrophotometric measurement of the concentration of vitamin A distinguish the proposed modification from Gallup's method.

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