CXXXIX. REACTION OF FATTY EXTRACTS OF CERTAIN ORGANS WITH THE ANTIMONY TRICHLORIDE TEST FOR "VITAMIN A."

(PRELIMINARY NOTE.)

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THE test as described by Carr and Price[1926] is thought to indicate the presence of the fat-soluble vitamin A. It is carried out as follows: 0.2 cc. of a 20 % solution in chloroform of the fat to be tested is added to 2 cc. of a 30 % solution of SbCl_3 in chloroform. A blue colour results which varies in depth with the amount of the reacting substance present; the colour fades rapidly, the time taken for complete disappearance depending on the depth of the original colour.

It appeared to be of interest to examine the comparative amount of the reacting substance contained in the fatty mixture extracted from the organs, in particular the liver, of man and other mammals without necessarily assuming this to be vitamin A.

Method.

(1) From 100 to 200 g. of liver, or other organ, was passed through a mincing machine into three times the volume of 94 % alcohol, the purpose of the procedure being mainly to facilitate the subsequent drying. The alcoholic extract (holding about 77 % alcohol) was found to contain traces of the reacting substance; the amount lost was however negligible.

(2) After 24 hours, during which time the material was occasionally stirred, the residue was strained off, squeezed out in a cloth and dried in a hot-air oven at about 95°.

(3) The dried material was ground to a fine powder in a mill, the powder being then extracted with ether (at room temperature in a flask) for 24 hours. At the end of this time the residue was filtered off and washed with ether on the filter.

(4) The filtrate was collected in a weighed basin, evaporated until there was no further smell of ether and weighed. It may here be mentioned that the yield thus obtained from different specimens of liver was from 1.3 to 2.7 % of the weight of organ dealt with. The method of extraction by infusion in cold ether with occasional shaking appeared to be sufficiently complete for

the purpose aimed at, which was to compare the amounts of reacting substance present in different fatty extracts.

(5) The fatty extract was dissolved in chloroform to make a 2, 10 or 20 % solution, the concentration being determined by the result of a preliminary test, carried out as described below, to show the comparative richness of the extract in the reacting substance.

The extracts were of a brownish yellow colour of varying depth; those from human liver and other tissues had the consistence of a thick oil but those from ox and sheep's liver were solid at 25°.

The intensity of the reaction with SbCl_3 had no relation to the depth of coloration of the extracts.

The liver extracts were tested for the presence of bile pigments with alcoholic iodine, the result being negative. The absence of any change of colour with iodine indicated also that carotene was not present.

In testing the fatty extracts of liver for bile pigment a glass rod wetted with fuming HNO_3 was drawn across a thin layer of the extract on a white porcelain surface; a very deep purple to a bluish pink colour was obtained, fading rapidly to pink and then disappearing. The depth of colour had no relation to the darkness of the brownish yellow coloration of the extracts but was found to be roughly proportional to the intensity of the reaction with SbCl_3 . The reaction (with HNO_3) was therefore used as a preliminary test to determine the probable richness of different extracts in vitamin A.

To obtain a quantitative expression of the comparative amount of the reacting substance in extracts from different specimens, the blue colour produced with SbCl_3 was matched against solutions of indigo-carminé of known concentration. The standard taken was a sample of cod-liver oil which, when tested in 20 % solution, gave a colour matched by a $\frac{1}{5000}$ solution of indigo-carminé. This was given unit value, the content in vitamin A being expressed in multiples or fractions of the unit.

A colorimeter was not used, the comparison being made in test-tubes of the same internal diameter.

It appeared to be of interest to examine the unsaponifiable residue of the liver extracts. Two experiments were done, saponification being effected by boiling with alcoholic potash under a reflux condenser. In the first the saponified material was extracted with ether, the ether evaporated and the residue dissolved in CHCl_3 ; it gave a very strong reaction with both HNO_3 and with SbCl_3 . In the second 15 g. of the mixed fatty extracts from different specimens of liver were saponified and the material was extracted with light petroleum. This was evaporated, an almost colourless residue being obtained of which 0.02 g. was soluble in CHCl_3 . Tested in 0.2 % solution this residue had a unit value of 100. It contained cholesterol, the amount of which was not determined. The material thus obtained, although evidently very rich in the reacting substance, gave only a faint reaction with fuming nitric acid. To find some explanation for this rather unexpected result, the saponified

liquid, which had already been extracted with light petroleum, was further extracted with ether, a yellow residue being obtained on evaporation of the ether which gave a strong colour reaction with HNO_3 and a comparatively slight reaction with SbCl_3 .

In spite therefore of the apparent correspondence of the results of the two tests, when applied to the fatty extracts examined, the reactions appear to be due to the presence of two different substances. Both are found in the unsaponifiable residue, the substance reacting with SbCl_3 being however very much more soluble in light petroleum than is the substance giving a purple colour with HNO_3 .

The method of colorimetry used would not serve for exact measurements; it gives however a comparative numerical expression of the content in vitamin A of the extracts which is approximately correct.

The subjoined table gives the results of observations on the extracts of human or other origin and of some other materials.

Table I.

Various extracts.

Source of extract	Value in vitamin A	Source of extract	Value in vitamin A
Cod-liver oil A	1	Kidney. Human	Trace
„ B	0.5	Liver. Guinea-pig	3.5
„ C	0.25	„ Ox (a)	10
Butter. Foreign	0.05	„ Ox (b)	11
„ Local (Cairo) 2 samples	0	„ Water-buffalo	6
Human Milk (Fats) 8	0.05-0.025	„ Camel	12.5
Heart. Sheep	0	„ Sheep (a)	7.5
Suprarenal. Ox	0.33	„ Sheep (b)	8
Spleen. Human	0	Snail (from 20 glands)	0
Placenta. Human, 2	0		

Extracts of Human Liver.

Source and cause of death	Value in vitamin A	Source and cause of death	Value in vitamin A
1 Child. Bronchopneumonia...	25	13 Man. Pneumonia	2.5
2 Man. Phthisis	25	14 „ Pneumonia	2
3 Child. Burns	16	15 Child. Bronchitis	2
4 Man. Electric shock	12.5	16 Woman. Burns	1.5
5 „ Stenosis of larynx	12.5	17 Man. Pneumonia	1
6 „ Heart failure	10	18 „ Nephritis	0.83
7 „ Pneumonia	6	19 „ Aortic disease (syphil- litic)	0.4
8 Woman. Burns	5	20 Man. Asphyxia	0.25
9 Child age 3 days. Exposure. Meconium from same	0	21 Child. Ill-developed, age 2 months. Corneal ulcers	0.2
10 Child (premature) 8 days. Pneumonia	3.5	22 Man. Pellagra	0.1
11 Child. Acute enteritis	4.5	Unsaponifiable residue of mixed liver extracts	100
12 Man. Heart failure	3.5		

Note. In regard to some of the results recorded in the table it is to be noted that a negative result with Carr's test does not demonstrate the absence of vitamin A, traces which are shown by the AsCl_3 test of Rosenheim and Drummond [1925] not being detected with SbCl_3 .

My colleague Dr A. Hassan has been good enough to carry out some comparative experiments with the two tests which indicate that the former (AsCl_3) is between two and three times as sensitive as the latter but that in regard to the determination of the relative richness in vitamin A of different specimens of fatty extracts the two tests agree in their results.

It would be entirely premature to draw any conclusions as to the importance or otherwise of the amount of vitamin A found in the liver in relation to the diseases tabulated. In most of the cases no information was available as to the nature of the diet. The majority of the individuals had either been in hospital for too short a time for the diet supplied to have had any influence or had died outside hospital under conditions which necessitated a medico-legal *post mortem* examination. It may however be of interest to consider very briefly the cases in which there appears to be a great deficiency of vitamin A in the liver extract. These are numbered 20, 21 and 22.

Case 20. This case was that of a man who was buried by a fall of sand when excavating in a sand-pit. There was no evidence of what disease if any he may have suffered from. He belonged to the poorer class and it is unlikely that his diet differed much from that of many of the other cases recorded in whom the liver was found to be rich in vitamin A.

Case 21. The condition of the cornea in this case was suggestive of a deficiency in vitamin A. The child had scarcely grown, although for a month previous to death it had been under treatment in the Children's Institute at Kasr el Aini Hospital. While in hospital the child received partly artificial, partly breast-feeding (a healthy wet-nurse) with only a temporary increase in weight.

Case 22. This was a case of advanced pellagrous insanity, the man having been an inmate of the Khanka Asylum for a long period before his death. I have to thank Dr Miller, the Director of that institution, for kindly supplying the material. The amount of fatty material extracted was 2 % of the weight of liver taken, about the normal quantity.

The great deficiency of vitamin A in the liver extract need not be assumed to have any etiological connection with the disease (pellagra); to establish this the examination of a sufficient number of early cases would be required. It is in fact more probable that this deficiency is related to the fact demonstrated by Roaf and the writer [1918] in an experiment on five comparatively early cases of pellagra that the absorption of fats from an ordinary mixed diet is deficient to the extent of about 19.5 % of the fat intake. In an advanced case, such as No. 22, the extreme atrophy of the mucosa of the small intestine, in particular of the villi, would doubtless exaggerate the defective absorption.

If the substance giving the reaction be regarded as identical with vitamin A¹, the observations on the livers of animals used for food would indicate the value of liver as a foodstuff, especially for children. In the cases recorded in

¹ Apart from the evidence given by Carr and Price [1926] for the identity of the reacting substance with the vitamin, the following facts are in support of the view: (a) the results with the AsCl₃ and SbCl₃ tests give comparable estimates of the amount of the reacting substance present; (b) the substance giving the reaction is gradually destroyed (in solution) by exposure to diffused daylight and air at the laboratory temperature; (c) the substance is found in the unsaponifiable residue. Wokes and Willimott [1927] in a recent paper on the effect of heat and oxidation on cod-liver oil state that in their observations with different colour tests for vitamin A the AsCl₃ and SbCl₃ tests were in agreement when used quantitatively.

the table (ox, sheep, camel, water-buffalo) the average percentage of fatty extract was 2.5 %, the average value of which in vitamin A was 9.1 %. On the basis of these figures 100 g. of liver would have as regards vitamin A the same value as 22.5 g. of cod-liver oil.

SUMMARY.

Although in many respects the observations must be regarded as of a preliminary nature, the following conclusions appear to be justified.

1. The fatty extract from the human liver contains a substance giving the same colour reactions as vitamin A in an amount which may be equal to 25 times that found in a good sample of cod-liver oil.
2. The amount of vitamin A found in the liver varies within wide limits and may, under some conditions of disease, be no more than $\frac{1}{25}$ of the maximum amount observed to occur.
3. The substance is present in considerable amount in the liver at birth but is not present, in an amount detectable by the test used, in the placenta.
4. The extract of livers of healthy animals slaughtered for food was found to contain from 6 to 12.5 times the amount of vitamin A present in cod-liver oil.

REFERENCES.

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