CXXIV. SOME LIVER OILS YIELDING A STRONG COLOUR REACTION WITH ANTIMONY TRICHLORIDE¹.

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DURING recent years the authors [1928, 1; 1929, 1] have been preparing oils from different raw materials for the purpose of investigating the practical value of the antimony trichloride test for the detection and quantitative estimation of vitamin A [Carr and Price, 1926]. It has been shown that fat from certain marine animals yields a particularly strong blue colour. Our investigations on the biological effect of these products are not yet finished, but the results found are of such interest as to justify a preliminary communication.

Reaction of fish-liver oils with antimony trichloride.

As is known, the antimony trichloride test is carried out by adding 2 cc. of a saturated solution of antimony trichloride in chloroform to 0.2 cc. chloroform containing 0.04 cc. oil. The colour intensity for ordinary cod-liver oil measured with a Rosenheim-Schuster colorimeter amounts to six Lovibond blue units, the time for the reading being 1 minute. Many different kinds of fishes, including both cartilaginous and bony ones, give values of the same order of magnitude. Thus 0.04 cc. of oil obtained by boiling the liver of the porbeagle (*Lamna cornubica* Gmel.)—caught January, 1927—gave six Lovibond blue units. As usual, large quantities of liver oil were obtained by extraction of the remainder with ether, but this fraction showed to our great surprise a very strong colour reaction, viz. several hundred Lovibond blue units for 0.04 cc. (see below). This striking difference shows that the colour-producing substance must be differently bound to the liver cells from the fat obtainable by melting.

Not all liver oils, however, behave in this manner. Thus cod-liver treated with boiling water gave a liver oil that in some cases showed eleven Lovibond blue units for 0.04 cc. Upon extraction, however, the remainder yielded a colour intensity which lay only a few units higher than that of the directly melted part. It appears that in this case colour-producing substances are also

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present in the liver which do not directly follow the melted fat, but the quantity is much higher for porbeagle-liver than for cod-liver.

We have also examined the liver oil from other fishes and particularly from those which do not yield any oil by direct heating of the liver, but only by extraction with a solvent. It then appeared that the liver from the halibut, salmon, red perch, and mackerel upon extraction also gave a strong colour reaction with antimony trichloride. This was true also for whale-liver, which—like that of all mammals—does not yield any liver oil upon heating with water (the fat content did not exceed 4 g./100 g.).

The determination of the colour intensity for these liver oils gives rise to some difficulty. Readings above 30 blue units cannot be effected with the tintometer, and the optimum is between 5 and 15. This necessitates dilution of the samples, the colour, however, is not proportional to the degree of dilution, but somewhat stronger.

Thus, the following values were found for the liver oil from the mackerel:

cc. oil in 0.2 cc. chloroform (2 cc. of the reagent)	Colour intensity	Calculated for 0.04 cc.
0.002	14.7	294
0.001	11.0	440
0.0004	5.8	580
0.0002	3.4	680

At lower concentrations the values for 0.04 cc. lie still higher. Under these circumstances it is impossible to use one reading for the colour obtainable. We have therefore plotted the colour intensities against dilution and read off from this curve the quantity of liver oil that would correspond to six Lovibond blue units, *i.e.* the average value for a good cod-liver oil. From this value is calculated the colour intensity for 0.04 cc. of the oil, all readings being made after exactly one minute. The values found (see Table I) represent minimum values, since the colour in many cases is perceptibly fading after 1 minute. Parallel readings have been made for all dilutions, and these show good concordance. The light conditions are of some influence, and the values obtained at different hours of the day are therefore not always the same.

Table I. *Showing the Lovibond blue units for 0.04 cc. oil.*

Liver oil from the halibut (<i>Hippoglossus hippoglossus</i>), sample FE	700
Liver oil from the salmon (<i>Salmo salar</i>), sample FD ...	200
Liver oil from the salmon (<i>Salmo salar</i>), sample FN ...	250
Liver oil from the mackerel (<i>Scomber scombrus</i> Lin.), sample FL	570
Liver oil from the red perch (<i>Sebastes marinus</i>), sample FG ...	70
Peritoneal fat from red perch (<i>Sebastes marinus</i>), sample FH ...	13
Liver oil from porbeagle (<i>Lamna cornubica</i>), sample HDA melted	1.6
Liver oil from porbeagle (<i>Lamna cornubica</i>), sample HDB extracted	270
Liver oil from porbeagle (<i>Lamna cornubica</i>), sample II a, melted	6
Liver oil from porbeagle (<i>Lamna cornubica</i>), sample II b, extracted	420
Liver oil from whale (<i>Balaenoptera borealis</i>), sample FM ...	150

The values for yellow and red on the Lovibond scale were also determined together with the blue colour, but these results are less trustworthy, so we do not give them here. Attention may nevertheless be called to the fact that

the intensity in yellow and red changes to a marked degree both absolutely and relatively. Some liver oils give a pure blue colour which slowly changes into violet and red, whereas others give a reddish violet tint at once. The tendency towards fading of the colour is also very different for the different materials, and this is also true of the final colour obtained after the blue tint has disappeared. This phenomenon is linked up with the content of unsaponifiable matter, as previously pointed out by the authors. This clearly shows that several substances—not only a single one—react with the antimony trichloride under the conditions prevailing and that the colour observed is produced by all these reactions.

Vitamin A content of extracted liver oils.

The investigations on the content of vitamin A in the samples mentioned here have not as yet been brought to a conclusion, but for all that, the data obtained at the present stage are remarkable. Although not particularly potent the melted liver oil from porbeagle (HDA) proved to be sufficient for a satisfactory growth, when it was applied in daily doses of 1–2 mg. The

Fig. 1. Weight curves of rats, 0.04 mg. halibut-liver oil a day, corresponding to 25,000 units of vitamin A.

same results were also obtained with the extracted liver oil, and we were disposed to conclude from this result that the content of vitamin A in both samples corresponded to 1000–500 units. This proved, however, to be quite erroneous. The extracted liver oil gives still more excellent growth, if it is applied in great dilutions, *e.g.* in doses of 0.1 mg. a day, and the content of vitamin A is therefore in reality not 1000, but about 10,000 units. The liver oil from the halibut is active in doses of 0.04 mg. a day, as is shown in Fig. 1, which gives the curves of growth for five rats fed on this dose. This consequently means that the liver oil from halibut contains not less than 25,000 units of vitamin A.

Similar results have been obtained with liver oil from salmon (FD) which gave normal growth in daily doses of 0.1 mg., *i.e.* the oil contains in reality 10,000 units instead of 600, as we originally found [1928, 2].

Agduhr [1926] published some three years ago very interesting investigations on the poisonous effect of too heavy doses of cod-liver oil. His results exactly confirm experiments which were made in this laboratory five years

ago, but which have not yet been published in detail [see Schmidt-Nielsen, 1929].

Whether the antimony trichloride reaction can be used for the assay of vitamin A in liver oils yielding a strong blue colour will not be discussed here, since the biological analyses are not yet finished.

Effect of puberty on vitamin A requirements of rats.

Mention may here be made of the importance of puberty for vitamin research. Four of the curves drawn in Fig. 1 have a normal slope in the first part of the test period, but then a more or less pronounced break occurs. This break is coincident with the beginning of puberty, *i.e.* the animals are 3 months old. At this age the animals need vitamin A in larger quantities, and since they are also more sensitive a decline or a stoppage of growth will occur if the doses given are not rather high. If the dose is only sufficient for producing a small, constant growth, then it may happen that the animals die during this period, although the doses and the entire procedure remain unchanged. Normal growth of the animals will, however, reappear after 1 to 3 or 4 weeks, if the original dose has been sufficient.

Fig. 2. Influence of puberty. The age of 3 months is marked by a broad, vertical line. The rats from the beginning of the test period had an adequate supply of vitamin A.

This phenomenon is clearly demonstrated in Fig. 2. The age of 3 months is marked by a vertical line, and it will be seen that the curves all bend down at about this point. Then follows a period of normal increase again, and the second part of the curves seems to have the original slope. At first we believed that the periods of decrease were due to a deterioration of the preparations, but the phenomenon appeared also when fresh mixtures were used [Schmidt-Nielsen and Schmidt-Nielsen, 1929, 2].

It is clear that false conclusions may be drawn, if the period of examination is too short, and the 3 months' mark should therefore be drawn in all laboratory journals. The investigations should also be continued for a sufficiently long period, eventually until the rats are 5 months old. A survey of the literature—*e.g.* the results published by various authors in this *Journal*—indicates that most of the curves show similar breaks, and that these occur on the whole at the age of 3 months. But, as far as we know, this fact has not previously been explained as a puberty phenomenon.

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