Chromatographic Fractionation of Whale-Liver Oil

Pritchard, Wilkinson, Edisbury and Morton[1] have already reported striking disagreement between the biological activity and the absorption at 328 mμ of the concentrates of mammalian liver. As we had formerly used the Carr-Price reaction to a great extent for practical determinations, we were interested in its relation to ultra-violet absorption and to biological activity. We therefore fractionated whale-liver oil and determined the blue colour with trichloride of antimony, the curve for the ultra-violet absorption, and the biological activity, of the various fractions thus obtained.

The fractionation was carried out in the following way: The oil was first saponified, after which the part that could not be saponified was given further treatment. (We offer our best thanks to Messrs. Ferrosan and Co., Ltd., Copenhagen, for so kindly supplying a large quantity of the unsaponifiable part of whale-liver oil.) The sterols were eliminated by crystallization from methyl alcohol, first in an ice-box, and then by freezing in solid carbon dioxide and acetone. The soluble part was dissolved in petroleum-ether and submitted to chromatographic separation with calcium hydroxide as an adsorbent. Painting with antimony trichloride, as described by Zechmeister, Cholnoky and Ujhélyi[2], gave a positive reaction for the whole length of the column, and the adsorption column was, therefore, divided arbitrarily into four parts, from which vitamin A was eluted by petroleum-ether containing a little methyl alcohol. The top fraction gave a red-violet reaction towards antimony trichloride, which, according to our standard curve, would have meant about 101,000 I.U. per gm. activity of vitamin A. Biological assay, however, showed that it was quite inert. The next fraction should have contained 3,160,000 I.U. per gm., according to the antimony trichloride reaction, but the biological activity was 1,160,000 I.U. per gm. It has been impossible, unfortunately, up to the present to evaluate the ultra-violet absorption on account of lack of material. All three determinations are, however, available for the third and fourth fractions:

	III	IV
Biological activity	531,000 I.U./gm.	562,000 I.U./gm.
Antimony trichloride value ..	950,000 „	1,160,000 „
Ultra-violet absorption at 328 mμ	384,000	382,000 „

The biological activity is thus considerably lower than would have been expected from the antimony trichloride reaction. The biological activity is greater than might have been expected from the ultra-violet absorption, but not so much, when the number of animals used for the biological assay is taken into consideration. The absorption maxima for the ultra-violet curve are not, however, at 328 mμ, but are displaced towards the short-wave part—more exactly, at 293 mμ.

The investigations of Gillam, Heilbron, Jones and Lederer[3] showed that there is a substance in the liver of freshwater fish with vitamin A activity, the absorption maximum of which is at 344 mμ. This substance does not occur alone, but together with the ordinary vitamin A. It has, however, not been possible to produce the actual substance in pure form, though the English investigators were able to determine the chemical constitution of the new substance by preparing suitable derivatives.

According to our experiments, whale-liver oil does not contain this substance. The ultra-violet absorption at wave-lengths longer than 328 mμ is weak even for our chromatographic fractions, and we cannot find a maximum for the antimony trichloride reaction at 690 mμ. On the other hand, the maxima for the ultra-violet absorption of our fractions III and IV agree well with the results given by Pritchard, *et al.*

It has already been shown many times that the maximum absorption occurs at 570–580 mμ for the antimony trichloride reaction of vitamin A. Chromogen 610 and chromogen 570 were separated by adsorption on to fuller's earth[4] in Wolff's laboratory. Karrer, Morf and Schöpp[5] isolated the so-called hepaxanthin, the absorption maximum of which is at 270 mμ. Hepaxanthin is not thought to have any biological activity.

Since our highly purified products, however, show greater absorption for the antimony trichloride reaction at 570 mμ than at 610 mμ, have a distinct maximum in the ultra-violet field at about 290 mμ, and possess biological activity which, as has been mentioned, is considerably greater than that corresponding to the absorption at 328 mμ, it does not seem to be impossible that our preparations contain a hitherto unknown substance with the biological action of vitamin A.

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Formation of Negative Ions by Negative-Ion Bombardment of Surfaces: a New Process

EXPERIMENTS with a double mass-spectrograph have been described recently by one of us and R. Press[1]. These show conclusively that bombardment of a gas-contaminated surface with positive ions of one species can produce a spectrum of negative ions. In this work particular attention was directed to the process involved. We have now extended these experiments, in the first place, to identify some light ions, the identity of which was not definitely established in the earlier work. By calibrating the mass-spectrograph with a Kunsman alkali ion source, used in such a way that the magnet current did not have to be altered from the value used during the negative ion runs, we have identified accurately the main negative ion peaks, with results in general accord with the early work of Woodcock[2] (which we had previously overlooked), and with that of Arnot and Beckett[3].

We have also studied the emission of negative ions from oxide-coated cathodes, which can be liberated either by heating[4] or by positive ion bombardment[2]. During an investigation of the energy distributions of these ions, an interesting effect was observed. A wire gauze had been inserted between the cathode