Letters to the Editor

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Characteristics of Highly Active Vitamin A

THE recent publications of Karrer, Morf and Schöpp¹, and of Heilbron, Heslop, Morton, Webster, Rea and Drummond², have pointed to the conclusion that the highest concentrations of vitamin A they were able to obtain were almost pure. We have recently been able, by the help of high-vacuum technique, to prepare a much more highly active vitamin A. The physical data relating to our preparation are recorded below.

For the final stages of purification a pumping system with a limiting pressure of 10^{-7} mm. was employed, and the vacuum was of the order of 10^{-6} mm. The conditions of distillation were such as to secure vaporisation with as small a path of traverse as possible for the molecules from the evaporation surface to the condensation surface, the mean distance being 12 mm. for an evaporation area of 30 sq. cm. Provision was made to regulate the heat input so that the end point of distillation of the various fractions might be as sharp as possible, a temperature advance equal to about 5° – 7° C. being necessary to bring about the commencement of distillation of the next higher fraction after removal of the main fraction. By this means it was possible to effect separative distillation over a range of temperatures between 115° and 170° C. The fraction boiling between 136° and 137° C. gave physical constants and figures on analysis which lead to the conclusion that the substance is a purer vitamin A preparation than has hitherto been described. They are as follows:—

The antimony trichloride blue value is 78,000.
Spectroscopic figures:

- (1) From the colour produced with antimony trichloride

E	1 per cent	693 m μ .	465
	1 cm.	617 m μ .	5,000
		580 m μ .	2,600

- (2) Direct spectrophotographic measurement:

E	1 per cent	328 m μ .	1,600
	1 cm.		

The figures for the purest vitamin A previously obtained by Heilbron and his colleagues were:
Antimony trichloride blue value of 65,000.

(1)	E	1 per cent	693 m μ .	450
		1 cm.	617 m μ .	4,650
			580 m μ .	2,550
(2)	E	1 per cent	328 m μ .	1,370
		1 cm.		

Ultimate analysis gave the following figures: C = 83.5 per cent, H = 10.6 per cent, which are in excellent agreement with the formula suggested by Karrer, $C_{20}H_{30}O$, requiring C = 83.9 per cent, H = 10.5 per cent. It has also been found that the product in alcoholic solution shows no optical rotation.

The biological test showed that 0.0006 mgm. a day administered to rats gave slightly better growth than 0.001 mgm. (or 1 unit) a day of International

Standard Carotene. This dose sufficed to cure xerophthalmia.

We desire to express our thanks to Miss K. Culhane for the biological test and to Prof. Heilbron and Dr. Morton for much help with the analysis and physical determinations.

FRANCIS H. CARR.
WILLIAM JEWELL.

The British Drug Houses, Ltd.,
Graham Street, London, N.1.
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¹ *Helv. Chim. Acta*, **14**, 1431.
² *Biochem. J.*, **26**, 1178.

Irradiation of Nucleic Acids and Uracil

GUHA and Chakravorty¹ recently announced the formation of vitamin B₁ by the ultra-violet irradiation of adenine sulphate. We wish to report similar experiments with nucleic acids and uracil.

Studies of the relation of the constituents of the cell nucleus to the effects of ultra-violet light on cells led us to examine the absorption changes of these materials upon irradiation with various ultra-violet wave bands². In the course of these investigations, we were struck by the fact that modifications of the spectra of these materials upon irradiation—more particularly if wave-lengths shorter than 2900 Å. were excluded—brought them into close resemblance to the spectra of certain B₁ concentrates³. The similarity of the sources of B₁ and these materials, and the observation that uracil and adenine acquire the ability upon irradiation of reducing arsenophosphotungstate solutions³—a colour test held by some to be a property of vitamin B₁—suggested that B₁ might be a modified purine or pyrimidine of the type produced by the irradiation of such materials.

In most of the experiments by which we undertook to test this hypothesis, thymus and yeast nucleic acids were used, since, as each contains four nitrogen bases, any activation of either or all of these to any or several of the components of the vitamin B complex might, we thought, be so secured. The injection of irradiated nucleic acids into polyneuritic rats appeared in some instances to indicate moderate vitamin B₁ activity. Indeed, one apparent cure resulted from the administration of irradiated uracil. But careful examination of the results with large numbers of animals made it appear that the positive effects should be attributed to variability of the rats, and it was necessary to conclude that ultra-violet irradiation was probably ineffective in producing vitamin B₁ from these substances. Our irradiated materials were also tested for vitamin B₂ potency, with negative results. A paper by one of us describing these experiments is now in press⁴.

The negative results are borne out by the fact that our more recent spectrographic work^{5,6} indicates vitamin B₁ to be characterised by a maximum at 2600 Å., whereas the absorption changes believed at first to be associated with the production of vitamin B₁ were mainly in the shorter wave region³. Our studies of the absorption spectrum of irradiated adenine sulphate³ show it to be less, rather than more, similar to what we now believe to be the characteristic spectrum of vitamin B₁^{5,6}.

The reported finding of sulphur in the most potent vitamin B₁ concentrates⁷, is another factor which now makes it seem unlikely that any sulphur-free base could yield vitamin B₁ upon irradiation. That irradiation could introduce sulphur as an integral