increased reactivity of newly formed molecules, reactions frequently proceed differently or go further than corresponds to the properties of the ordinary (non-active) forms of the species of molecules taking part therein. The normal saturated dicarboxylic acids in particular offer fine examples of this phenomenon; a number of cases, in which dicarboxylic acids formed by oxidation processes were found to possess a much increased vulnerability to oxidative degradation at the instant of their formation, are enumerated in a paper by P. E. VERKADE [28]). It therefore certainly will not do to apply quantitatively the results of experiments with dicarboxylic acids administered *per os* or subcutaneously, in which the body is flooded from time to time with these substances, to cases in which these acids are formed *in vivo* as intermediate products. A too unfavourable impression of the importance of ω-oxidation and related phenomena is doubtless obtained in such a way.

We are indebted to the VAN 'T HOFF-Fund and the HOOGEWERFF-Fund for financial assistance in the execution of these investigations.

Rotterdam, September 1937.

28) Rec. trav. chim. **46**, 200 (1927).

Chemistry. — *On the analysis of the provitamins A in blood serum.* By A. G. VAN VEEN and J. C. LANZING.

(Communicated at the meeting of October 30, 1937.)

Introduction:

The quantitative determination of vitamin A in blood serum is complicated by the fact that by the side of vitamin A there occur in the serum carotinoids, some of which may serve as provitamin A, since they may be converted into vitamin A, especially in the liver. The qualitative composition of these carotinoids largely depends on the carotinoids in the food consumed.

It is an open question whether one has to reckon only with the concentrations of vitamin A itself or also with the provitamins A present, when wishing to show a relation between the determinations of vitamin A in the blood and the clinical deficiencies (xerophthalmia, hemeralopia) occurring among the population. This relation between clinical deficiencies [1]) and A concentration (both vitamin A itself and provitamin A from the carotinoids) of the blood appeared to us to be much less simple than supposed at first. Perhaps the fact that clinical symptoms develop only some time after the A level of the organism has sunk and that, after a

1) On Java, as also in other tropical countries, ophthalmic deviations as a result of vitamin A deficiency prove to be much more frequent than was formerly supposed.

vitamin A containing diet, they recover more slowly than the A level of the blood, may play a part here. So that one single determination in the blood serum may not be sufficient, but possibly a whole curve is necessary. However this may be, in regard to the intricacy of the problem we thought fit to determine not only the vitamin A really present but also the carotinoids serving as provitamin, without adding up the Int. Units derived from the two separate determinations, as is sometimes done. To be sure it is often sufficient, as e.g. done by L. K. WOLFF [1]) and cooperators, to consider part (i.e. half) of the carotinoids as real β-carotene and thus to take only this part into account (after conversion into Int. Units). This procedure appeared to us undesirable for the problems of interest in this country. As mentioned before, the carotinoid composition of the blood depends in the first place on nutrition. The diet of the European in the tropics is often greatly varied; this is reflected in the blood serum carotinoids which also differ more in quantity than vitamin A.

In 16 Europeans, of a vitamin A reserve varying between 5.9 I. U. per 10 cc serum as lowest and 13.2 I. U. as highest value the carotinoid content varied between 7 γ and 21.8 γ.

Name	I. U. vit. A per 10 cc serum	γ carotinoids (calc. as β-carotene)
Al.	7.4	16.8
d. N.	10.6	7.—
A.	7.4	11.5
P.	6.8	10.—
Bz.	9.2	10.2
P.	9.2	14.—
K.	9.3	7.4
v. V.	10.8	8.4
v. d. P.	6.9	21.8
Ae.	9.—	13.3
V.	11.1	8.4
Be.	12.6	17.5
L.	5.9	9.—
I.	6.7	9.7
L.	9.3	13.2
v. V.	13.2	10.5

[1]) Oral information.

We also found, by means of our serum-carotinoid-analysis to be described below, greatly varying qualitative compositions for the blood of healthy Europeans. Sometimes there was much β-carotene, sometimes hardly any, notwithstanding the large quantity of carotinoids in the serum.

For instance: B has per 10 cc serum 8.9 I. U. vit. A and 11.3 γ carotinoids. The carotinoids consist of 30 % β-carotene, 40 % cryptoxanthene and 30 % xanthophyll. The provitamin here is equal to almost 10 I. U. vit. A and so exceeds the real A value.

L. has per 10 cc serum 9.3 I. U. vit. A and 13.6 γ carotinoids; these consist of very little (not quite a half-gamma) β-carotene, 45 % lycopene, 20 % cryptoxanthene and 30 % xanthophyll. The provitamin value is here only 2 I. U. vit. A. From this it appears that in persons living on a greatly varied diet the provitamin A reserve may be strongly varying and that, therefore, the determination of carotinoids only is of little practical use in this case.

If during some successive weeks the vitamin A and carotinoid reserve of a person who has a fairly regular diet is determined, it appears that the quantity of both is practically constant and that the composition of the carotinoids is also mostly the same.

Moreover, it is "a priori" possible that in persons of an insufficient vitamin A and provitamin A reserve the, in itself low, carotinoid concentration is practically devoid of provitamin A, since the body, driven by its need of vitamin A, rapidly converts this scanty supply of provitamin A into vitamin A. We intend to investigate this point more in detail. It may be that with certain groups of the population, whose diet is very monotonous, (which is of very frequent occurrence in the tropics) the level of these substances is much more constant than with the Europeans examined by us, and thus the determination of provitamin A content gets much more practical value in relation to A deficiency.

An important final consideration is that the population of these countries, whose diet is to a great extent vegetarian, must derive practically all their vitamin A from the carotinoids acting as provitamin A. Consequently a better knowledge of provitamin in the principal Indian foodstuffs and also in the blood serum of the population living on these foodstuffs is of great importance. That is why we felt the necessity of finding and working out a method that allows us to determine qualitatively and roughly quantitatively blood carotinoids and that preferably in small quantities of serum. Once more: if the relation between clinical vitamin A deficiency and the blood level of real vitamin A were simple, and if the fact that the carotinoid fraction of the serum might be a good producer of vitamin A were not a complicating factor, a similar study were superfluous.

Experimental.

The carotinoids from 5 cc. of serum (from 10 cc., however, when in very low concentrations) were analysed chromatographically. For larger quanti-

ties this had been done previously [1]). As there was generally no more than 5 γ [2]) of carotinoid available, microchromatograms were made.

A tube of 20 cm. length and 3 mm. diameter is closed at the bottom with cotton-wool and filled up to 15 cm. with Al_2O_3, from MERCK (standardised after BROCKMANN). At the top it is connected by means of rubber tubing via a washing bottle with a CO_2- bomb or a Kipp apparatus. The tube is fitted with a bored cork in a straight allonge and placed on a suction flask. It is then filled with petrol ether (boiling-point 40—60°) and, if this does not run through quickly enough, it is put under pressure of CO_2. When the whole of the Al_2O_3 column is moistened, the petrol ether extract of about 5 cc. blood serum is run through. (This extract is obtained by saponifying the blood in heat during half an hour with 60 % KOH, extracting it with petrol ether, washing this with water and drying it.) Very plainly visible coloured bands will appear. In the extracts examined by us there was as a rule a narrow yellow band at the top, under this usually one or more bands of a brown, pink or light red hue. When the whole of the extract has run through, "developing" with petrol ether is still continued for some time. Since the whole procedure is only short, isomerising and similar phenomena are practically excluded. With solutions of pure α-, β-carotene and cryptoxanthene and, when necessary, other carotinoids of known strength microchromatograms of some gammas of substance are made for the purpose of comparison, in a similar tube. Half a gamma of cryptoxanthene as well as half a gamma of β- and γ-carotene still yield a clear narrow band, α-carotene is still visible in this quantity, but not very clearly.

When thus through comparison in the chromatogram of the blood-carotinoids the different components and especially the provitamins A have been recognised (which is not difficult after some practice), a quantitative estimation, accurate to about $\frac{1}{2}$ γ can be made at the same time, by measuring the thickness of the different bands. If, in order to separate the bands, the blood chromatogram is "developed" with petrol ether to which an other solvent has been added, this has of course to take place analogously with the artificial chromatogram. By the way it may be observed that, because of the high temperature at Batavia (28—32° C), Ca-hydroxyde is not very serviceable, in contrast with Europe, as the adsorption is too weak. For the same reason the adsorption of the carotinoids to Al_2O_3 is also less strong, in consequence of which a better separation is most likely obtained with this adsorbent than in Europe. In considering the experimental part, the peculiar circumstances under which the work is done here should therefore be remembered.

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[1]) See: ZECHMEISTER u. CHOLNOKY. Die chromatogr. Adsorptions-methode. Wien, 1937, p. 114, 115; and: DANIEL u. BERES: Z. f. Physiol. Chem. **238**, 160 (1936).

[2]) Calculated as β-carotene: thus no error is made for α- and β-carotene and cryptoxanthene, a slight one for xanthophyll, but a rather large one for γ-carotene and lycopene.

α- and β-carotene were mostly determined after development with petrol ether and 2 to 5 % benzene. A benzene concentration of 10 to 25 % causes the α- and β-carotene to disappear entirely from the chromatogram. In this benzene concentration it is the lycopene and the γ-carotene that are seen to broaden into brightly coloured bands, wheras cryptoxanthene is then only just separated from the uppermost yellow edge. A peculiar quality of the cryptoxanthene is that, when developed with a mixture of petrol-ether-benzene 1 : 1 or with pure benzene, it has a brownish-yellow to yellow colour which merges into orange-pink when afterwards petrol ether is run through again. So there are a number of slight indications from which it may be concluded, after some practice, what materials are dealt with.

In order to be absolutely certain whether it was a definite carotinoid, a mixed chromatogram was also often used. To make this, a solution of one or more gammas of pure provitamin is run through the blood chromatogram. If the provitamin concerned is already present in the chromatogram, the relative band broadens, otherwise a new band is formed between (or under or above) those existing. Further checking by means of developing with petrol ether with 10 or more percent benzene or by means of a mixed chromatogram is absolutely necessary. Often, when no or hardly any β-carotene was present, lycopene was taken for β-carotene. β-Carotene, when added, however, would then appear below the supposed β-carotene in the blood chromatogram; when developed with petrol ether with about 20 % benzene, the real carotene would run through rapidly, whereas the lycopene would only broaden into a light red colour characteristic of lycopene.

Results.

By means of this method the qualitative composition of the serum-carotinoids of Europeans, native servants and native prisoners was determined. These last are more especially interesting because their physical condition as well as their nutrition (the composition of which has not been changed since rather more than a year and a half) are exactly known. The provitamin A both in their food and in the blood serum was analysed; vitamin A proper they practically do not get. The uniformity of the provitamin A supply from vegetable food was reflected in the uniformity of the chromatograms. In contrast with what was observed in many (but not all) Europeans (who as a rule take in much vitamin A in the animal food and the dairy produce they consume), α-carotene is mostly absent, β-carotene only present in small quantities (< 15 %); crypto-xanthene, however, (from Indian corn and tjabe = Capsicum) was mostly found in rather large quantities in the blood serum of the native prisoners (25—35 %) of the total carotenoids, though in their food it occurs in much smaller concentration than β-carotene (viz.

about only 10 % of the total vitamin A activity of the provitamins). The average real vitamin A level of prisoners and Europeans, etc. is practically equal, notwithstanding the fact that the former in contrast with the latter have to draw all their vitamin A from he provitamin mentioned. The low α- and β-carotene level may be explained by the fact that much more than in the case of Europeans, etc. this is (and has to be) converted into vitamin A. The conversion of α- and β-carotene into vitamin A is apparently easier than that of cryptoxanthene. It may be questionable whether cryptoxanthene (which in the vegetable foodstuffs of tropical countries occurs much more frequently than in those of colder regions) is indeed a provitamin A for man as it is for the rat. To these and similar questions we shall revert more explicitly in future publications.

Finally we wish to express our thanks to the Institute for Nutrition Research for the financial aid received.

Batavia C., September 1937.

Chemical Department of the Central Medical Laboratory.

Hydrodynamics. — *Preliminary records of the velocity fluctuations in a boundary layer before and after the transition to turbulent motion.* (Mededeeling N°. 33 uit het Laboratorium voor Aero- en Hydrodynamica der Technische Hoogeschool te Delft.) By G. BROERSMA. (Communicated by Prof. J. M. BURGERS.)

(Communicated at the meeting of October 30, 1937.)

1. *Introduction.*

In an earlier communication from the Laboratory for Aero- and Hydrodynamics of the Technical University at Delft [1]) some experiments have been described on the simultaneous recording of the fluctuations of the air velocity in a windtunnel by means of two hot wire anemometers, which were placed at a small distance from each other in the same plane perpendicular to the direction of the motion of the air. At distances of the order of magnitude of 1 cm or less a distinct correlation can be remarked in the records given by the two wires, which correlation gradually disappears when the distance becomes larger. It was then planned to perform similar experiments in the boundary layer along a flat plate, and extensive series of measurements have been performed already in 1924, on which, however, only a short communication has been published [2]). Afterwards, in 1930

[1]) J. M. BURGERS, Experiments on the fluctuations of the velocity in a current of air, Proc. Royal Acad. Amsterdam, **29**, 547 (1926).

[2]) J. M. BURGERS, Experimental investigation of the motion of the air in the boundary layer along a smooth surface (in russian), Journ. of Applied Physics (Moscow), **4**, 7—9 (1927).