ISOLATION OF A NEW "CAROTENOID" FROM RAT LIVER

DURING an investigation of the influence of carcinogenic hydrocarbons upon the hepatic vitamin A stores of mice and rats,[1] there was observed not only a marked difference in response by the two groups of animals, but even an unlikeness in the chemical composition of the unsaponifiable fraction of the livers. A study of this latter interesting dissimilarity has led to the isolation of a new carotenoid-like substance.

The mice and rats were killed by a sharp blow on the head and the livers removed at once. These organs were then macerated, saponified and extracted with peroxide-free ethyl ether, according to the technique of Davies.[2] Chloroform solutions of the unsaponifiable fraction of mouse livers were invariably colorless, while those derived from rats were always a light yellow.

The unsaponifiable fraction of the livers dissolved in chloroform was treated with a saturated solution of antimony trichloride in the same solvent (Carr-Price reaction), and the colored mixture resulting was examined immediately, and at short intervals thereafter, for the appearance and disappearance of spectroscopic absorption bands. Mouse livers usually exhibited only one readily visible absorption band for vitamin A, that at 620 mμ. However, by concentrating the chloroform solution so that 1 cc contained the unsaponifiable fraction of 3 to 6 livers, a faint band at 570 mμ could be seen also.

On the other hand, the unsaponifiable fraction of rat livers showed a stronger band at 565–570 mμ besides the 620 mμ band for vitamin A. The persistence of these bands was somewhat dependent upon the concentration of the unsaponifiable fraction and also upon the amount of added antimony trichloride. Nevertheless, both bands (620 mμ and 565–570 mμ) made their appearance immediately with the addition of the reagent, although the 565–570 mμ band proved more enduring. Although the mice were fed Rockland Mouse Pellets, and the rats received Purina Dog Chow, a reversal of diets failed to alter the above reactions.

The "carotenoid" was isolated as follows: The residue of the unsaponifiable fraction of the livers from 4 to 6 mature rats was taken up in 50 to 70 cc of petroleum ether (30°–60°). This extract was then added to a 500 cc Erlenmeyer flask, containing approximately 50 gm of finely powdered Kaolin (China clay), which had previously been well wetted with petroleum ether. Adsorption of the yellow substance apparently occurred instantaneously, for the Kaolin at once assumed a purple color. However, the mixture was shaken and allowed to stand for a few minutes.

The supernatant petroleum ether solution, now colorless, was decanted and the Kaolin washed three to four times with 50 to 70 cc portions of petroleum ether to remove any unadsorbed unsaponifiable substances. After separation of the greater part of the petroleum ether, the adsorbed "carotenoid" was eluted with absolute methyl alcohol. The colored substance dissolved easily in the remaining petroleum ether-methyl alcohol mixture. Final separation from the Kaolin was accomplished by filtration through Munktell's OA. paper.

When the solvents were removed from the eluate on a steam bath and the Carr-Price reaction applied to the eluate in chloroform at 0°, an absorption band at 585–587 mμ was seen. This band was too transient to be observed at room temperature. It is not known whether isomeric changes occurring during adsorption and elution can explain the shift in absorption from 565–570 to 585–587 mμ.

The "carotenoid" was crystallized as follows: After evaporating the methyl alcohol-petroleum ether mixture from the eluate on the steam bath, the orange-yellow residue was dissolved in 95 per cent. alcohol with heating. The cholesterol was removed by adding a hot alcoholic solution of digitonin and allowing the mixture to cool to room temperature. The cholesterol digitonide was filtered off, and the filtrate evaporated to dryness in a vacuum desiccator. The residue was then dissolved in ethyl ether, any excess digitonin being left behind.

After removing the ether on a steam bath, the residue was dissolved in a few cc of carbon disulfide, and the solution poured into 10–20 cc of hot absolute methyl alcohol. The "carotenoid" crystallized at −12°, and the crystals were separated by filtration at 0°, in a cold room. Approximately 10–12 mg of the new "carotenoid" was obtained from five rat livers. A solution of the "carotenoid" in chloroform did not show any maxima from 350 mμ to 850 mμ when examined spectrophotometrically, which rules out the carotenes. The "carotenoid" is solid at 0° and at this temperature showed an absorption band at 585–587 mμ when treated with a cold solution of antimony trichloride.

Carbon and hydrogen were determined by micro combustion.[3]

Analysis

3.885 mg substance: 4.325 mg H_2O and 11.475 mg CO_2
3.185 " " : 3.580 " " " 9.310 " "

Calculated for $C_{16}H_{30}O$.	C 80.59,	H 12.69
Found.	" 80.52,	" 12.45
	" 80.40,	" 12.70

The "carotenoid" is quite soluble in chloroform, ethyl ether, carbon disulfide and petroleum ether. It

[1] C. Carruthers, *Cancer Research*, 2: 168, 1942.
[2] A. W. Davies, *Biochem. Jour.*, 27: 1770, 1933.
[3] Micro combustions done by Dr. Carl Tiedcke, New York, N. Y.

is less soluble (25°) in absolute methyl alcohol and in ethyl alcohol.

The absorption band at approximately 570 mμ has been observed by others, but its significance was never before determined. Van Eekelen, Emmerie, Julius and Wolff,[4] and Willstaedt and Jenson,[5] have postulated that the 570 mμ "chromogen" is another vitamin A. Karrer and Morf[6] suggested that hepaxanthin may give this same chromogen. On the other hand, Brockmann and Tecklenburg[7] found that oxidation products of vitamin A (*in vitro*) yield an absorption band at 570 mμ.

Further work is in progress to determine the structure of the new "carotenoid." Whether it is an intermediate metabolite of vitamin A or of β carotene, remains to be determined.

SUMMARY

A new carotenoid-like substance has been isolated from rat liver. A method for its separation and some of its properties are given.

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MOCK-DOMINANCE AND HYBRID VIGOR

Two plant varieties, one of which has twice as many internodes of half the length as the other, will be equal in height. A hybrid between these varieties will exceed their height by 12½ per cent. if internode number and length are exactly intermediate in inheritance, *i.e.*, if dominance is lacking. Here, then, is an apparent dominance for plant height, or an example of hybrid vigor in its pristine sense, that is not the result of dominance in its genetic meaning. This effect may conveniently be called *mock-dominance*. It results from the fact that plant height is determined as the product of number and length of internodes and from the relations that obtain between the means of products and the product of means.

These relations are simply shown by considering the products $A'B'$ and $A''B''$. Their mean, of course, is:

$$\frac{A'B' + A''B''}{2} \qquad (1)$$

The product of the means of the two components, on the other hand, is:

$$\frac{A'B' + A''B'' + A'B'' + A''B'}{4} \qquad (2)$$

Now:

$$(1) = (2) \text{ when } A' = A'', \text{ or } B' = B'', \text{ or both} \qquad (3)$$
$$(2) < (1) \text{ when } A' > A'' \textit{ and } B' > B'', \text{ or } \textit{vice versa} \qquad (4)$$
$$(2) > (1) \text{ when } A' > A'' \textit{ but } B' < B'', \text{ or } \textit{vice versa} \qquad (5)$$

The relations indicated by (3), (4) and (5) apply generally to all characters that are the products of component dimensions which, in turn, are intermediate and independent in inheritance. It is evident that (3) describes what frequently may be expected in crosses between similar varieties, and (4) what may be expected when varieties similar in proportions but differing in size are crossed. The mock-dominant effect described by (5) is the expectation for characters in crosses between varieties differing in type with respect to those characters. The importance of this effect will depend upon its magnitude and its generality of occurrence. Adequate data on these points are not available.

Examples of characters in which this effect may frequently be manifest come at once to mind. The yield of grain per plant in cereals is the product of the number of grains and their average weight. Many-seeded varieties often tend to have relatively small grains and *vice versa*. Plant height has been referred to. Individual leaf area is the product of length and width. Longer corn leaves often are narrower than shorter leaves, particularly for extremes in length and width. Crosses between contrasting types should have leaves with larger areas than the mean of the parents. If the parent having leaves with smaller areas has more leaves than the other parent, there would be a cumulative mock-dominant effect on total leaf area per plant. The weight of ear in corn is the product of length, diameter and density. Length of ear, in turn, is dependent on number and length of the cob internodes, and density is even more complex.

The characters mentioned are among those the measurements of which have been largely used in the quantitative determination of hybrid vigor. They also are characters generally conceded to be controlled in inheritance by numerous genes lacking dominance. The same principles will apply to rates which are the products of subsidiary rates. Thus, growth rate is the product of the rates of cell division and of cell enlargement. Again, intense chlorophyll and small leaf area from one parent combined with weak chlorophyll and large leaf area from the other would establish a basis for superior photosynthesis in the hybrid.

[4] M. Van Eekelen, A. Emmerie, H. W. Julius and K. L. Wolff, *Acta Brevia Nederlandica*, 1: 8, 1931.

[5] H. Willstaedt and H. B. Jenson, *Nature*, 143: 474, 1939.

[6] P. Karrer and R. Morf, *Helv. Chim. Acta.*, 16: 625, 1933.

[7] H. Brockmann and M. L. Tecklenburg, *Ztschr. f. Physiol. Chem.*, 221: 117, 1933.