chloroform solution with 1 per cent hydrochloric acid, demonstrating that it was biliviolin and not a bilipurpurin complex.

The zinc complex of biliviolin prepared by oxidation of a pure specimen of bilirubin showed an absorption maximum in methanol at 644 mμ shifting to 568 mμ on addition of hydrochloric acid, the colour of the solution changing simultaneously from green to blue-violet.

Biliviolinoid pigments have been observed in dog bile[1] and in gall-stones[2], but have been considered to be almost certainly secondary oxidation products of bilirubin. It is unlikely that the biliviolinoid pigment found in ox bile arises in this way, as its typical absorption spectrum has been found in association with an undistorted bilirubin curve. This suggests that the pigment occurs *per se* in the bile, as oxidation has been found always to be accompanied by distortion of the bilirubin spectrum. Such a compound would probably occur normally in bile in the form of a trace metal complex, for a number of such elements are known to be excreted mainly in the bile, and the *p*H of ox bile (7–8) is such as to allow the formation of metal complexes.

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Stearolic Acid, an Essential Fatty Acid?

According to Bernhard[1], stearolic acid (Δ^9-octadecynoic acid-1; CH_3—$(CH_2)_7$—C≡C—$(CH_2)_7$—COOH) has vitamin F activity, since this acid, when mixed throughout the diet in the form of its ethyl ester, prolongs the life of vitamin F-deficient rats.

This result is in contradiction with our investigations[2], in particular with the '6,9(term.)-hypothesis'. According to this hypothesis, the presence of two double bonds at the 6,9(term.)-positions of fatty acids (that is, double bonds at the 6 : 7- and 9 : 10-positions when numbered from the terminal CH_3-group) is essential for vitamin F activity. The known essential fatty acids, namely, linoleic, linolenic and arachidonic acid, are all characterized by this 6,9(term.)-configuration. On assaying a large number of fatty acids, it appeared that acids which do not have this characteristic are inactive, and further, three new essential fatty acids were found, namely, $\Delta^{10,13}$-nonadecadienoic, $\Delta^{11,14}$-eicosadienoic, and $\Delta^{6,9,12}$-octadecatrienoic acid, all characterized by the occurrence of double bonds at the 6,9(term.)-positions.

The activity of stearolic acid has now been investigated by means of our new method for the standardization of vitamin F. Using this method, male weanling rats received a limited amount of drinking water—14 ml./day/animal. The food was given in the form of a fat-free sucrose diet. The preparatory period lasted for seven weeks, and was followed by a four-week test period.

Five times per week 0·2 ml. of the various preparations was pipetted to groups of twelve and twenty-four animals respectively, except the control group (eleven animals) which received nothing. The groups forming the reference standard received various doses of sunflower-seed oil, which, if necessary, were made up to 0·2 ml. with fully hardened coconut fat, which contains no linoleic acid. The stearolic acid was administered as the ethyl ester. The stearolic acid ester was prepared from oleic acid by bromination to dibromo-oleic acid, followed by dehydrobromination with aqueous potassium hydroxide. The stearolic acid thus obtained melted at 47·2–47·3° C., was free from bromine and optically pure. It was esterified in ethanol/sulphuric acid.

Probably as a result of the longer preparatory period (normally five weeks) some of the animals died. Moreover, a number of the animals developed lesions at the tip of the tail. In its final stage the lesion amounted to a complete necrosis of the tail tip; this stage was assigned the figure 3, while 2 and 1 represented less serious stages, and 0 the normal tail.

The data on the mortality, the tail lesions, and the change in the body-weight during the four-week test period are collected in Table 1. Owing to the mortality, the data regarding the change in the body-weight relate to the surviving animals.

Table 1. Mortality, Extent of Tail Lesions, and Change in the Body-weight of Vitamin F-deficient Animals receiving Different Doses of Sunflower-seed Oil and Stearolic Acid during a Period of Four Weeks

Preparation	Dosage (day/animal)	Mortality	Mean stage tail lesions	Change in body-weight (gm.)
Control	nil	3/11	0·44	−5
Sunflower-seed oil	10 mgm.	0/24	0·00	+18
,, ,,	50 ,,	0/24	0·00	+70
,, ,,	182 ,,	0/12	0·00	+86
Stearolic acid (ethyl ester)	172 ,,	9/12	0·92	−38

In the sunflower-seed oil groups, no deaths or tail lesions occurred, while a distinct growth reaction was observed. Of the controls, three out of eleven animals died, the mean tail lesion was 0·44, and the body-weight of the group decreased slightly (− 5 gm.). Of the twelve animals which received the ethyl stearolate, nine died (significant with regard to the control group), while the tail lesions (stage 0·92) were more serious than in the control group. The stearolate did not give any improvement in the body-weight, but quite the reverse, for a significant decrease in weight was found.

A similar growth retardation in vitamin F-deficient animals has also been observed by Holman[3] with conjugated linoleic acid, as well as in our own investigations[2] with a number of other fatty acids, both saturated and unsaturated.

Summarizing, the present investigation has made it clear that stearolic acid is not able to improve the survival time, tail lesions or growth of vitamin F-deficient animals; on the contrary, it affects them adversely.

I wish to thank the management of Unilever N.V. for permission to publish this work; and Mr. Sparreboom for the preparation and Mr. Duin for the esterification of the stearolic acid.

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