hypnotic effect. The dextro form, however, is three times less toxic as judged by the greatly reduced incidence of "nirvanol disease."

Allylisopropylhydantoin and β-bromoisopropylhydantoin were prepared and tested.

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[CONTRIBUTION FROM THE C. F. KETTERING FOUNDATION FOR THE STUDY OF CHLOROPHYLL AND PHOTOSYNTHESIS]

OCCURRENCE OF DECOMPOSITION PRODUCTS OF CHLOROPHYLL. I. DECOMPOSITION OF CHLOROPHYLL IN THE DIGESTIVE SYSTEM OF THE COW

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The study of natural porphyrins, especially copro-, uro- and ooporphyrin, was of greatest importance in ascertaining the constitution of hemin. Phylloerythrin, a biological decomposition product of chlorophyll, is for similar reasons of particular interest for studies in the chlorophyll series. L. Marchlewski[1] discovered it in the fresh feces of cows fed on green food. In the same year Loebisch and Fischler[2] isolated it from ox bile under the name bilipurpurin. Marchlewski[3] proved the identity of these two substances and their identity with MacMunn's cholehematin. Marchlewski also showed that phylloerythrin is derived from chlorophyll and not from hemoglobin. The porphyrin nature of phylloerythrin was demonstrated by H. Fischer and H. Hilmer,[4] who also proved D. Kémeri's porphyrin from human feces to be identical with phylloerythrin. By chemical methods H. Fischer and co-workers succeeded in preparing the substance from chlorophyll derivatives such as chlorophyllide $a + b$, pheophytin $a + b$, pheophorbide a, methylpheophorbide a, pheoporphyrin a_5.[5]

In a preliminary note[6] we announced that phylloerythrin occurs in the stomachs of herbivorous animals (cow and sheep). It has the formula I according to H. Fischer and he considers this structure of fundamental importance for the understanding of the nature of chlorophyll a. The study of decomposition products of chlorophyll in the animal body presents many possibilities. (1) Waste products can be examined. This method led to the discovery of phylloerythrin.[1] (2) Examination of the contents

[1] Marchlewski, *Bull. intern., acad. polonaise des sciences et des lettres*, A, 638–642 (1903).

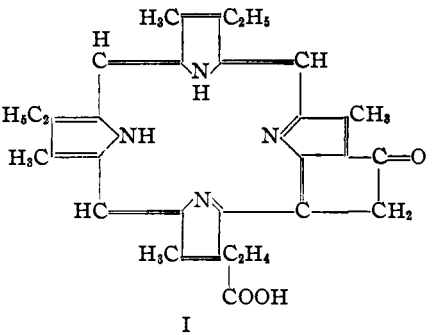
[2] Loebisch and Fischler, *Monatsh.*, 335–350 (1903).

[3] Marchlewski, *Bull. soc. chim. biol.*, **6**, 464–472 (1924).

[4] Fischer and Hilmer, *Z. physiol. Chem.*, **143**, 1–8 (1925).

[5] H. Fischer and O. Süs, *Ann.*, **482**, 225–232 (1930); H. Fischer, O. Moldenhauer and O. Süs, *ibid.*, **486**, 107–177 (1931).

[6] Inman and Rothemund, *Science*, 221 (1931).



I

of the digestive tract of lower animals has been tried: H. Fischer and A. Hendschel[7] examined the changes chlorophyll undergoes in the digestive system of caterpillars and isolated phyllobombycin, a crystallized substance free from magnesium, phytol and methoxyl. Following the suggestion of H. Fischer that the actual location in the animal body where phylloerythrin is formed should be investigated, we used the contents of the digestive tract of the cow and sheep in the present studies.

The contents of the stomachs were collected separately and immediately after the animals were killed. The material thus obtained was extracted within a few hours with a pyridine–chloroform mixture or with glacial acetic acid and ether. Such extraction yielded from the third stomach a dark red ether solution. The character of the absorption spectrum of this solution indicated a mixture of porphyrins. Willstätter's method of hydrochloric acid fractionation led to the isolation of crystallized phylloerythrin and other porphyrins, of which rhodo-, phyllo- and pyrroporphyrin could be identified. Two other substances, probably porphyrins, were found spectroscopically, but have not yet been crystallized. They occur only in minute quantities and we cannot say at the present time whether they are chlorophyll decomposition products already known or new substances.

The place for the formation of these porphyrins seems to be mainly the third stomach. Spectroscopic examination of the contents of the first and second stomachs, extracted according to our method, showed the presence of traces of phylloerythrin. The quantity of material used in these cases was 20 kg. In order to find whether the other porphyrins are present in the first and second stomachs, it would be necessary to extract larger quantities of material than we used. The contents of the fourth stomach of the cow contain the same porphyrins as the third stomach. No evidence was obtained for the presence of pigments like phyllobombycin. While examining the contents of the third and fourth stomachs of sheep we paid special attention to the possible presence of chlorophyll decomposition products of the pheophorbide type in order to determine the place of

[7] H. Fischer and A. Hendschel, *Z. physiol. Chem.*, **198**, 33–42 (1931).

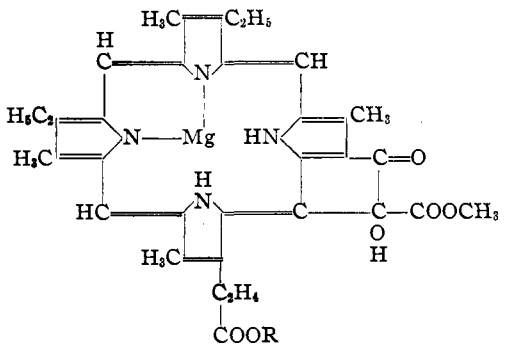
formation of the probophorbides, which H. Fischer[8] has recently isolated from the feces of sheep. Four kilograms of material from the third or fourth stomach is usually sufficient to demonstrate the presence of probophorbides, although it is not possible to isolate the different components *a–d* from the small quantity obtained.

The results of these studies are given in the following table.

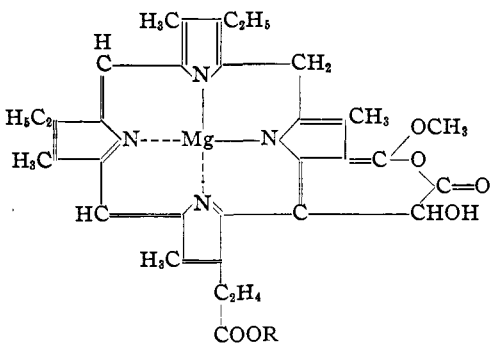
Animal	Stomach	
Cow	1 and 2	Traces of phylloerythrin
	3 and 4	Phylloerythrin, rhodo-, phyllo-, pyrroporphyrin and two other pigments
Sheep	3 and 4	Phylloerythrin and probophorbides
Calf on a milk diet		No porphyrins found

The formula of chlorophyll *a* as suggested by H. Fischer is given in formula II, while J. B. Conant prefers formula III.[9]

II
R = Phytyl



III



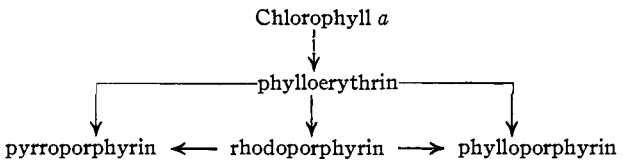
The formation of phylloerythrin from chlorophyll *a* in the digestive tract includes the following reactions: splitting off of the magnesium and the phytol groups, change of the molecule to porphyrin, and decarboxylation,

[8] H. Fischer and A. Hendschel, *Z. physiol. Chem.*, **206**, 255–278 (1932).

[9] Recently the formula of chlorophyll *a* has been changed [Fischer and Siebel, *Ann.*, **499**, 84–108 (1932); Stoll and Wiedmann, *Helv. Chim. Acta*, **15**, 1128–1136, 1280–1285 (1932)]. These changes, however, have retained the five-membered ring attached to the fundamental structure of the molecule.

accompanied by the reduction of an hydroxyl group. This series of changes must be followed by reactions producing the other simpler porphyrins (rhodo-, phyllo- and pyrroporphyrin): opening of the ring attached to the porphin structure, further decarboxylation and an oxidation–reduction reaction.

The decomposition of chlorophyll *a* in the digestive system of the cow may be represented tentatively by the following scheme



The fact that these chemical changes of the chlorophyll *a* molecule take place under normal physiological conditions as they exist in the stomachs of ruminants, seems to the authors to favor the view that the added five-membered ring of phylloerythrin (formula I) is present in the structure of the molecule of chlorophyll *a*, as shown in formula II.

Experimental

The contents of the different stomachs of cows and sheep were collected separately at the slaughter house about fifteen minutes after the animals had been killed; the material was taken to the laboratory and worked up immediately. Two kilograms of stomach contents was drained on a Buchner funnel with a pump and extracted with 2100 cc. of a mixture of chloroform and pyridine containing 1800 cc. of chloroform and 300 cc. of pyridine. The extraction is performed by alternately macerating without the use of the vacuum and draining with but slight suction. Finally the material is sucked dry by the strong suction of the pump.

The extracts of about 40 kg. were distilled *in vacuo* almost to dryness and the residue was dissolved in a small quantity of pyridine, filtered and transferred to a separatory funnel containing 3 liters of ether. Five per cent. hydrochloric acid was added to render the reaction slightly acid to Congo paper and the mineral acid solution was changed into an acetic acid solution ("discolored" reaction on Congo paper) by adding a few cc. of concentrated aqueous sodium acetate solution. The resulting pink ether solution was washed several times with distilled water and fractionated with hydrochloric acid according to Willstätter and Mieg.

The 0.5% HCl fraction was transferred into fresh ether and extracted with 0.35% HCl and the porphyrin from this extract was shaken into ether. After drying and evaporating the ether solution yielded long needle-shaped crystals; yield, 4 mg. recrystallized from ether. The spectrum in ether plus a little pyridine was identical with that of phylloporphyrin (measurement with the Zeiss–Loewe–Schumm spectroscope for chemists, also direct comparison, by means of the comparison prism, with phylloporphyrin prepared from pheophytin).[10] The esterification of the porphyrin was performed with methanolic hydrochloric acid. The ester, recrystallized from pyridine–methanol, melts at 231–232°; a mixture with phylloporphyrin methyl ester (from pheophytin) showed no depression.

The higher hydrochloric acid fractions were worked up similarly to the procedure

[10] H. Fischer, L. Filser, W. Hagert and O. Moldenhauer, *Ann.*, **490**, 35 (1931).

given for the 0.5% fraction. The porphyrin resulting from extraction with 1.3% HCl was identified with pyrroporphyrin from pheophytin by direct spectroscopic comparison. Methyl ester: HCl number, 2.5; m. p. 239–240° (no depression on mixture with methyl ester from pheophytin). Also, rhodoporphyrin from the 4% HCl fraction was identified spectroscopically, by the hydrochloric acid number of the ester and the melting point of the methyl ester (265°, no depression). The amounts of pure porphyrins were: pyrroporphyrin ester, about 5 mg.; rhodoporphyrin ester, 3 mg. Phylloerythrin was found in the 8% HCl fraction. Eleven milligrams of this porphyrin was obtained and identified; m. p. of the methyl ester, 263°; no depression with phylloerythrin ester from bile; m. p. of the mixture with rhodoporphyrin ester from cow stomach, 245° (depression 18°).

No porphyrin was found in the extracts with higher concentrations of hydrochloric acid.

The 6% HCl fraction yielded a substance which shows the following spectrum in pyridine–ether:

I, 646.4–641.0	II, 611.1–574.5	III, 562.8–540.5
643.7	592.8	551.7
IV, 526.6–505.6	E.A. 448	Int. II, III, IV, I
516.1		

Direct comparison with verdoporphyrin shows difference in spectrum.

Spectrum of the 7% HCl fraction in pyridine–ether

I, 634.8–629.2 faint 611	II, 603.4–574.1	III, 563.6–539.3
632.0	588.8	551.5
IV, 525.7–506.5	E.A. 445	Int. II, III, IV, I
516.1		

The spectrum is distinctly different from the spectrum of chloroporphyrin e_6 with respect to the absorption bands II, III and IV, although band I is identical and the hydrochloric acid numbers are the same.

In the cases of the 6 and 7% HCl fraction the yields were too small to prepare the pure methyl esters for characterization.

Instead of the pyridine–chloroform mixture glacial acetic acid may be used for the extraction of the stomach contents. One kilogram of the material was ground up in a large mortar with 600 cc. of glacial acetic acid, the solution was extracted three times with 500 cc. of ether and the ether was decanted; 400 cc. of acetic acid was added and three more ether extractions were made with 500 cc. of ether each time. The ether–acetic acid mixture was poured into the separatory funnel, the acetic acid removed by several washings with water and the ether solution subjected to hydrochloric acid fractionation. The yields obtained by this method are lower than those obtained by pyridine–chloroform extraction.

Summary

1. A series of porphyrins has been found in the stomach of the cow under normal physiological conditions.

2. Phylloerythrin and probophorbide are present in the stomach of sheep.

3. These porphyrins are decomposition products of chlorophyll. The stomach contents of animals on a milk diet do not contain these porphyrins.

4. The occurrence of these porphyrins supports a formula for chlorophyll containing a five-membered ring added to the porphin structure.