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The Conversion of Ferri-porphyrins ("Hemins") to Their Corresponding Porphyrins by Means of Pyruvic Acid

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Several procedures have been tried for the removal of iron from its complexes with porphyrins. Thus metallic iron and formic acid convert protohemin to protoporphyrin [1]. In this reaction substitution of iron by palladium in a hydrogen atmosphere, under certain conditions leads to the formation of mesoporphyrin [1] in theoretical yield [2]. Hydrogen bromide in glacial acetic acid effects de-ironization; if vinyl side chains are present, the hydrogen bromide adduct is obtained [3–5]. Stannous chloride [6] or sodium amalgam [7, 8] have been suggested as reducing agents. Occasionally other methods have been used [*Cf.* 3, 9], but none of them lends itself to convenient microscale work or, if it does, is sufficiently harmless. The de-ironization with ferrous acetate [10] is unsuitable for routine work.

For certain purposes we needed a mild method, suitable for small scale, routine work. We have therefore tried some new procedures. Thiols release iron from its porphyrin complexes, but in the presence of air there is a simultaneous opening of the porphyrin ring [11]. In a few experiments we obtained from protohemin and thioglycolic acid in carbon dioxide atmosphere a mixture of protoporphyrin, small amounts of a sulphur containing porphyrin and considerable quantities of a water soluble, orange coloured pigment. The latter had no absorption bands in the visible region except for a flat maximum at about 490 mμ.

The observation of Schumm and Mertens [12] that pyruvic acid will effectively release iron from hemins seemed to be more promising.

PROCEDURE

After some trials the following procedure was found to be the best. The hemin is dissolved in 0.25 % ammonia (or weaker) and filtered if necessary. 0.6 ml pyruvic acid are added per ml of filtrate to give a final concentration of about 5 *M* acid. The suspension is then placed in a boiling water bath, where porphyrin formation starts within a few minutes. After about 30 min the reaction is finished (*cf.*, however, page 1206) (Table 1). A lower concentration of acid (2.5 *M* final concentration) gives no porphyrin formation within a reasonable time. If the hemins are not initially dissolved in ammonia, the porphyrin formation will take place nevertheless but much more slowly, and stronger acid must be used (Table 2).

Table 1. Yield of protoporphyrin from protohemin. Two series of samples of 0.2—1.0 ml of a solution, containing 375 μg protohemin per ml were diluted to 1.0 ml with 0.25 % ammonia and acidified with 0.62 ml 13 M pyruvic acid and heated for 30 and 60 min. respectively at 100°. The solutions were then diluted to 15 ml with 5 % hydrochloric acid, left for 4 h and centrifuged. No unreacted sedimented hemin could be detected as pyridine hemochrome. Calculated extinctions are based on the value $\beta_{555} = 3.30 \times 10^7\ cm^2 \times mole^{-1}$ *for protoporphyrin in 5 % hydrochloric acid * (Beckman spectrophotometer type DU, slit 0.01 mm, 1 cm cuvettes). The complete curves 380—620 mμ were determined and the wavelengths for the maxima and minima were found to agree with those for pure protoporphyrin. The addition of ferric chloride to the blanks did not influence their optical densities.*

Protohemin μg	Log I_0/I at 555 mμ		
	Reaction time in min 30	60	Calc. for 100 % conversion to protoporphyrin. Blank values not included
0	—	0.002	—
75	0.092	0.092	0.110
150	0.192	0.197	0.221
225	0.283	0.297	0.331
375	0.452	0.532	0.551

* $\beta = \frac{1}{c} \times \frac{1}{d} \times ln \frac{I_0}{I}$;

where c = concentration in moles per ml of the solution.
d = optical depth in centimeters.
I_0 = intensity of incident light.
I = intensity of transmitted light.

Table 2. De-ironization of protohemin with pyruvic acid without previous solution in ammonia. The recalculations of optical densities to μg are based on $\beta_{408} = 61.1 \times 10^7\ cm^2 \times mole^{-1}$ *for protoporphyrin in 5 % HCl, slit 0.025 mm* *.

Protohemin Dry subst. in μg	Pyruvic acid	Time min.	Dil. to ml with 5 %HCl	log I_0/I at 408 mμ	Porphyrin in μg	
					Found	Calc.
135	3 ml 9 M	150	50	0.908	96	116
183	4 » 10 »	150	50	1.330	141	158
112	4 » 10 »	150	50	0.790	84	97
115	4 » 10 »	60	50	0.636	67	100
987	4 » 10 »	210	330	0.650	460	850

The pyruvic acid should be distilled *in vacuo* without fractionation not more than one month before its use, and kept in a dark bottle. The distillate from the commercial preparation is generally about 13 *M*. The pyruvic acid could also be purified by the crystallization of its sodium salt [13]. Air exclusion with a stream of carbon dioxide has no influence, nor has the addition of sodium pyrophosphate. We have made our experiments, unless otherwise stated, in open test tubes. Since pyruvic acid is fairly strong (p*K* 2.5) it can hardly be used for the isoelectric flocculation of the porphyrins. On the other hand, if the porphyrins are extracted with ether after their precipitation with acetate, the major part of the pyruvate will remain in the aqueous phase. Pyruvic acid can be traced by its typical odour, if the ether residues after their extraction with hydrochloric acid are evaporated and the "empty" flasks slightly heated.

The method may be illustrated by the following examples.

The isolation of di-methyl esters from small quantities of ferri-porphyrins

Mesoporphyrin. 6.68 mg mesohemin in 4.1 ml 0.25 % ammonia were acidified with 2.54 ml 13 *M* pyruvic acid and heated for 60 min on the water bath. After dilution with one volume of water and the addition of ammonium acetate, the porphyrin was extracted with ether, and the ether solution washed with 5 × 100 ml water. The first washings contained some porphyrin, which was re-extracted with ether. From the combined ether solutions the porphyrin was extracted with 3 × 25 ml 1 % hydrochloric acid. The acid solution was washed with ether without loss of porphyrin. In this ether the last traces of pyruvic acid were found.

Spectrum in 1 % HCl **:	592,	570,	547				mμ
Mesoporphyrin in 5 % HCl[1]:	591.7,	570.4,	547.8				mμ
Spectrum in ether:	625,	610,	598,	580,	570,	528,	496 mμ
Mesoporphyrin in ether [1]: ***	623.5,	611.0,	596.8,	577.4,	568.2,	527.7,	496.6 mμ

The porphyrin was forced into ether by acetate, and the ether washed, dried and left overnight. Next day the porphyrin had crystallized in nice flocks, which in the microscope showed the typical mesoporphyrin shape. They were not collected, but esterified with diazomethane and crystallized from chloroform-methanol. After one recrystallization from the same system 4.9 mg were obtained (= 81 % yield), m. p. 218° **** (216° [3], 216.5° [2]).

Deuteroporphyrin. 10.72 mg deuterohemin in 4.6 ml 0.25 % ammonia were acidified with 2.85 ml pyruvic acid and treated step by step in the same way as the mesoporphyrin.

Spectrum in 1% HCl: 590, 567, 546 mμ
Deuteroporphyrin in 5 % HCl [3]: 589.5, 569.0, 546.6 mμ
Spectrum in ether: 624, 610, 597, 568, 558, 526, 492 mμ
Deuteroporphyrin in ether[3]: 621.7, 610.8, 595.7, 576.8, 566.8, 557.7, 525.9, 494.1 mμ

The crystals from the ether showed double refraction but were too small to permit any observations of their shape. Yield of twice crystallized dimethyl ester 3.6 mg (37 % yield). M. p. 219° (218°—220° [3]).

Protoporphyrin. 8.2 mg protohemin in 10 ml 0.25 % ammonia + 6.2 ml 13 *M* pyruvic acid were heated in a boiling water bath. Already after 3 min a considerable porphyrin formation had taken place. After 30 min the heating was interrupted and the solution cooled in tap water, diluted with one volume of water and filtered. No unreacted hemin remained on the filter. Ammonium acetate was added and the porphyrin was extracted with 4 × 30 ml ether. The ether was washed with 3 × 1 vol. of water. Hydrochloric acid in a con-

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** Spectroscopic date given in this paper were obtained with a Zeiss prism pocket spectroscope unless otherwise stated.

*** This spectrum is probably not the true spectrum of mesoporphyrin in dry, neutral ether, where only four bands should be seen. Our observation was made on the "combined ether solutions" before the extraction with 1 % HCl. (cf. deuteroporphyrin).

**** Melting points were determined with a Kofler-block and the values are thus to be regarded as corrected.

centration of 0.12 % or 0.18 % extracted no porphyrin from the ether, but with 0.36 % acid a small amount of porphyrin migrated over into the acid. The ether was therefore extracted with 6 × 50 ml 0.5 % hydrochloric acid, the last portion being almost colourless. By far the major amount of porphyrin remained in the ether. The porphyrin, which had been extracted with 0.5 % acid (= fraction I) was forced into ether (50 ml) by ammonium acetate.

Spectrum in ether	631,	605,	572,	535,	500	mμ

A small amount of 1 was taken into 5 % hydrochloric acid, where it gave the spectrum 600, 576, 554 mμ. The residue of I in ether was esterified with diazomethane after washing with water and drying. From chloroform-methanol shapeless, double refracting masses appeared, which melted at 205—220°.

The main bulk of the porphyrin (= fraction II) in the ether was extracted with 3 × 20 ml 5 % acid. After washings as for fraction I this portion gave the spectrum

in ether:	633,	605,	575,	538,	506	mμ
in 5 % HCl	600,	580,	555	mμ		

When standing overnight in dry ether the porphyrin crystallized in nice rhombohedra. They were not collected but esterified with diazomethane. The ester crystallized in the typical rhombohedral form from chloroform-methanol. After one recrystallization from the same system 2.4 mg (= 32 % yield) were obtained. M. p. 226—227°, (228°[2], 230°[3]).

The interposition of the spectrum of fraction I between the spectra of proto- and mesoporphyrins as well as the indefinite melting point indicated a mixture of protoporphyrin and some porphyrin with saturation of the side chains 2 and 4. Since it would be of interest for the understanding of the reaction mechanism to find out whether that porphyrin with meso-spectrum really was mesoporphyrin or not, the following experiment was made.

84 mg twice crystallized[14] protohemin in 40 ml 0.25 % ammonia were acidified with 25 ml acid. After 30 min at 100° no porphyrin could be seen, but after another 30 min the whole preparation had gone into solution and showed a strong cherry-red colour. It was diluted with 1 vol. of water, cooled and left for one hour. The material, which had settled, was washed with 2 × 50 ml 5 % hydrochloric acid. The black residue gave a pure pyridine hemochrome spectrum, corresponding to 19.2 mg unreacted protohemin.

The combined porphyrin solutions were buffered with ammonium acetate and extracted with ether, which was subsequently washed with 6 × 1 vol. of water. No porphyrin could be extracted with 0.1 % hydrochloric acid but with 3 × 1 vol. of 0.4 % acid a considerable amount (fraction I) was withdrawn. After extraction with 4 % acid (fraction II), the ether had a slight yellow colour. The yellow substance was extracted from the ether with 13 % acid in which it gave a green solution with a broad, diffuse band at 570 mμ. This last fraction was rejected. Fractions I and II were separately forced in to ether, washed with water, dried and esterified with diazomethane.

Fraction I. Since mesoporphyrin has the hydrochloric acid number 0.5 [3], it should be found in this fraction if it had been formed. The whole esterified preparation was dissolved in chloroform and put on a 5 cm alumina column from chloroform: petrol ether 1 : 20. With those solvents down to the proportion 1 : 2 nothing could be eluted, but with the ratio 1 : 1.5 a red material came out, which seemed to be homogenous (= IA).

IA. This gave a pure proto-spectrum but could not be crystallized, and was re-chromatographed as above. The first fraction to come out with pure chloroform was red, gave a pure proto-spectrum and crystallized easily from chloroform-methanol with m. p. 230°. Weight 5.3 mg (= IA1). Two small grey fractions migrated down after IA1, but showed no spectrum and were rejected. After them an emerald green compound descended slowly. It absorbed strongly at 675 mμ with weak bands at 510, 540 and 580 mμ.

Nothing more could be eluted with the above mentioned mixture or with pure chloroform, but with chloroform: glacial acetic acid 30 : 1 another red fraction left the column (= IB). Its spectrum was shifted about two mμ towards the blue as compared to protoporphyrin, but it did not crystallize. After IB an almost brown fraction was eluted with chloroform: glacial acetic acid 2 : 1. It showed a mixture of spectra with numerous bands, most of which were diffuse. It did not contain proto- or mesoporphyrin, and was discarded.

Fraction II. As a pilot experiment a part of this fraction was chromatographed on alumina as above with chloroform as eluant. Since the above mentioned green and brown regions were seen, the whole preparation, dissolved in chloroform, was washed with 7.5 % ammonia, which removed a faint brown colour. Upon the addition of methanol to the dried residual solution crystals appeared, which melted indefinitely at 218—223° and were of no regular shape (= IIA). By chromatography on alumina 7 mg of easily crystallizable material of m. p. 230° and with a pure proto-spectrum could be obtained from the mother liquor from IIA (= IIB). IIA was washed with methanol and chromatographed on alumina with chloroform as eluant. The following fractions were obtained:

IIA1. Pure proto-spectrum. Crystallized easily from chloroform-methanol with m. p. 230°. Weight 13 mg.

IIA2. A small yellowish fraction, the diffuse bands of which could not with certainty be attributed to a meso- or proto-spectrum. Rejected.

IIA3. A green fraction with a strong band at 675 mμ, weak bands at 510, 540 and 580 mμ, and a very weak band at 620 mμ.

IIA4. A green region remained at the top of the column and did not move at all until chloroform: glacial acetic acid 4 : 1 was employed. Its spectrum was the same as that of IIA3.

Fraction IIA*1*, which seemed to be pure, was recrystallized very slowly from a dilute chloroform-methanol solution. The very large crystals, which were formed in a week, gave a proto-spectrum, which was free from any band at 675 mμ, and they melted sharply at 230°. They were dissolved in 3 ml of chloroform and illuminated by a common 40-watt tungsten lamp for two hours. Afterwards the solution absorbed strongly at 675 mμ, and chromatography on alumina with chloroform as eluant gave two fractions:

IIA1a. Red, with pure proto-spectrum. Crystals, obtained from chloroform-methanol, melted at 230°. This portion was thus unchanged proto-ester.

IIA1b. Green pigment which descended very slowly with chloroform. It showed bands at 540 and 580 mμ plus the strong band at 675 mμ. Since it appeared on the column as one discrete strip and lacked the band at 510 mμ, which had been found in the other green fractions, it was believed to be pure, and attempts were made to crystallize it from chloroform-methanol and from chloroform-petrol ether, but without success.

Hematoporphyrin. Hematoporphyrin was prepared from protohemin according to the method of Nencki and Zaleski[4] as modified by Fischer[3] and crystallized as the di-hydrochloride. The free porphyrin was obtained, when the crystals were dissolved in 0.1 *M* sodium hydroxide and the porphyrin precipitated with glacial acetic acid. Since the ordinary method for the introduction of iron in glacial acetic acid could be suspected partly to dehydrate the α-hydroxy groups, a modified procedure was used. The hematoporphyrin was dissolved in a mixture of equal parts of sodium chloride saturated 90 % acetic acid and water and boiled for 1 ½ min. with one volume freshly prepared ferrous acetate solution in 50 % ($^{v}/_{v}$) acetic acid. The hematohemin, which crystallized during the cooling, was washed twice with water, twice with dilute hydrochloric acid and once again with water. It was dried overnight at 40° and then to complete dryness in a desiccator. Its pyridine hemochrome seemed to be free of any band at 557 mμ.

31.3 mg of the preparation thus obtained were dissolved in 28 ml 0.25 % ammonia, acidified with 17.5 ml pyruvic acid, and heated for 50 min at 100°.

After that time every trace of the hemin spectrum had disappeared. A muddy bottom layer settled when the mixture was cooled. After centrifugation the settled material was washed with 4 × 200 ml. 2 *M* hydrochloric acid. The combined extracts were filtered and washed with 2 × 1 vol. of ether. The porphyrin was brought into ether with ammonium acetate, the ether washed with water and subjected to fractional extraction with hydrochloric acid.

Fraction I. The major part of the porphyrin could be extracted with 5 × 200 ml of 0.12 % acid. The last portion was almost colourless. In this fraction the hematoporphyrin should be found. The preparation was again taken up into ether, where it showed a meso-spectrum, washed, dried and esterified with diazomethane. A large amount of crystals appeared from chloroform-methanol. After one recrystallization from the same system 17 mg of rhombohedral crystals of uniform size were obtained. They showed a step-ladder spectrum with maxima at 624, 571, 537 and 504 mμ (determined spectrophotometrically). The 537 band, however, was a little too low to give the perfect step-ladder appearance. The Soret band was found at 403 mμ. No band was found in the region 260—290 mμ, where carbonyl groups generally exert a band. However, since the crystals did not melt below 300°, they could not be the hematoporphyrin di-methyl ester.

Chromatography of the mother liquor on alumina with chloroform-petrol ether mixtures revealed five different red compounds in about equal quantities. In addition to these a brown region remained at the top. No green pigment was found in fraction I. Common to all these red pigments was their slower descent with chloroform as compared to the porphyrins from protohemin.

Since the fractions II (0.4 % acid) and III (4 % acid) could contain no hematoporphyrin, they were not examined in more detail. It would not have been very surprising if protoporphyrin had been formed from the dihydric secondary alcohol hematohemin, but neither proto- nor mesoporphyrin could be isolated. Both fractions contained several green pigments, all of which migrated more slowly down the alumina column with chloroform and had the absorption band in the red shifted about 5 mμ towards the blue as compared to the green pigments from protohemin.

DISCUSSION

To facilitate the conclusive identification of the porphyrins we isolated them as their di-methyl esters. The reported yields are therefore minimal values for the yields of porphyrins. Since the dimethyl esters are more sensitive to light than the corresponding free porphyrins, it is reasonable to

assume that the substances which crystallized from the ether solutions before the addition of diazomethane were pure porphyrins.

Fischer and Dürr [15] illuminated a chloroform solution of protoporphyrin dimethyl ester with a light source of about the same intensity as we used in the experiment with fraction IIA1. From the solution they were able to isolate in the crystalline state a green pigment with a sharp band at about 670 mμ. The formation of this pigment was strongly inhibited, in slightly different degrees by acids, alcohol and benzene, and the protoporphyrin spectrum reappeared when a chloroform solution of it was acidified. We could observe the same properties for our preparation (IIA1b).

From these similarities and from the formation of fraction IIA1b from protoporphyrin di-methyl ester we conclude that the green pigments were derived from protoporphyrin di-methyl ester, though we were unable to crystallize them. In agreement with the results in Table 1, the yield of the free protoporphyrin should therefore be much higher than 33—35 %.

According to Schumm [16] there is a reversible equilibrium between proto- and hematoporphyrin in hot hydrochloric acid. Since 0.12 % hydrochloric acid failed to extract any porphyrin from ether (*cf.* p. 1209) in the experiment with protohemin, there is no equilibrium of that kind in pyruvic acid, or, if it exists, it is shifted completely in favour of protoporphyrin when the mixture is cooled.

The procedure which is described in this paper, is unsuitable for large scale preparations unless large quantities of pyruvic acid be used. When about 1 g of protohemin was heated in 30 ml ammonia + 18.6 ml 13 *M* pyruvic acid, no porphyrin formation took place in 60 min and the yield after heating for 2 h was about 16 %. On the contrary, when 10—40 μg protohemin in 1.62 ml solution were warmed in the hand, strong porphyrin bands appeared after a few seconds.

The unsuccessful result with hematohemin is difficult to explain. As already mentioned, no protoporphyrin was formed. They crystals with m. p. above 300° were not analysed. An attempt was made to recrystallize them from chloroform-methanol, when the solution suddenly became brown, the pigment gave diffuse absorption bands, and could not be brought to crystallize again. Bouveault [17] identified some lower alcohols by means of the melting points of the semicarbazones of their pyruvic acid esters. He found a reaction time of 2—3 h at 140—150° to be necessary for the complete esterification. This information, the relatively large water content of the reaction mixture and the absence of any absorption at 260—290 mμ exclude the possibility that the hydroxylic groups in hematohemin could have been esterified by pyruvic acid.

Schumm and Mertens [12] had examined several organic acids for their abilities to de-ironize protohemin. Pyruvic, formic, oxalic and lactic acid were found to be active, while acetic, propionic, malonic, succinic, tartaric and citric acid were inactive. We have confirmed their results except for lactic acid, which we found to give no porphyrin. Acrylic acid as well as glycine and alanine were also inactive. Gompel, Mayer and Wurmser [18] investigated the oxidisability of some organic acids in the presence of activated blood charcoal. Samples of 125 ml of solutions, containing 1 % carbon from the compound to be investigated, were shaken with 5 g charcoal at 40° until carbon dioxide formation ceased. Pyruvic, formic and oxalic acid gave 0.40—0.62 ml gas, while acetic, propionic, lactic, succinic and citric acid, glycine and alanine gave 0.07—0.30 ml. If the concentrations of the first three mentioned acids are recalculated to normalities, it is obvious that there is a linear relationship between carbon dioxide evolution and concentration. The formation of carbon dioxide increased when pH was lowered within the region examined (pH 1—10). In our experiments we found that no porphyrin formation took place when 90 % of the pyruvic acid was neutralized by ammonia but the concentration of (pyruvic acid + pyruvate) still kept at 5 *M*.

The fact that those acids de-iron hemins which give the highest carbon dioxide formation, suggests a relationship between those two properties. In other experiments we found that no porphyrin was formed when protohemin was heated for 2 h with either pure acetaldehyde or with acetaldehyde-water-glacial acetic acid 1 : 1 : 1 in sealed test tubes. There is but little hope of success in attempts to isolate the reaction products from pyruvic acid. On the assumption that the removal of one mole of iron from hemin corresponds to the liberation of one mole of carbon dioxide, the conversion of 260 μg of protohemin to porphyrin would correspond to 10 mm^3 carbon dioxide. This quantity is impossible to determine with any accuracy under our experimental conditions.

A feature which is common to the methods of de-ironization of hemins, is the reduction of the hemin in the presence of an acid. Dilute hydrochloric acid converts pyridine hemochrome to porphyrin very easily [3]. Zeile and Meyer [19] prepared porphyrin c from cytochrome c by hydrolysis with sulphuric acid after the previous reduction of the cytochrome by dithionite. We may therefore suggest as a hypothesis, that the function of the above-mentioned acids is to act as electron donors to the trivalent iron, and that this reaction, perhaps, is in some way coupled to their decarboxylation. The excess of acid will then remove the bi-valent iron. We found that pyruvic acid gave no porphyrin from the copperprotoporphyrin complex, prepared according to Fischer and Pützer [1]. However, there must be some other factors involved in

the reaction; though the pyruvic acid must always be present in large excess of the hemin, nevertheless the ratio pyruvic acid/hemin as we have shown, exerts a profound influence upon the reaction velocity. No porphyrin formation took place when 80 % of the pyruvic acid was replaced by acetic acid, the total concentration of acid still being 5 *M*.

SUMMARY

1. A procedure is described for the conversion of small quantities of iron-porphyrins to their corresponding porphyrins.

2. The over-all yields (removal of iron, isolation of the crude porphyrin, esterification, two crystallizations of the ester) were from 6.68 mg mesohemin 81 %, from 10.72 mg deuterohemin 37 %, from 8.2 mg protohemin > 32 % and from 31.3 mg hematohemin unknown but low.

3. With amounts of hemin less than 100 μg, the reaction seems to give better yields and can conveniently be carried out under mild conditions.

4. The procedure is inconvenient and expensive for large quantities of hemins.

5. If the procedure is to be applied to a small, limited quantity of a valuable material, *e. g.* from an isotope experiment, a pilot experiment should if possible be made with quantities large enough to permit the conclusive identification of the porphyrin which may be formed. This is suggested because we failed to isolate any hematoporphyrin ester from hematohemin.

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REFERENCES

1. Fischer, H., and Pützer, B. *Z. physiol. Chem.* **154** (1926) 17.
2. Granick, S. J. *J. biol. Chem.* **172** (1948) 717.
3. Fischer, H., and Orth, H. *Die Chemie des Pyrrols*, Vol. II : 1, 1st. ed., Leipzig (1937).
4. Nencki, M., and Zaleski, J. *Z. physiol. Chem.* **30** (1900) 423.
5. Fischer, H., and Hasenkamp, J. *Ann.* **519** (1935) 42.
6. Hamsik, A. *Z. physiol. Chem.* **196** (1931) 195.
7. Schumm, O. *Ibid.* **139** (1924) 219.
8. Schumm, O., and Papendieck, A. *Ibid.* **152** (1926) 219.
9. Lemberg, R., and Legge, J. W. *Hematin compounds and bile pigments.* 1st ed. New York (1949).
10. Warburg, O., and Negelein, E. *Biochem. Z.* **244** (1932) 9.
11. Barron, E. S. G., Miller, Z. B., and Kalnitsky, G. *Biochem. J.* **41** (1947) 62.

12. Schumm, O., and Mertens, E. *Z. physiol. Chem.* **177** (1928) 15.
13. Robertson, W. B. *Science* **96** (1942) 93.
14. Fischer, H. *Org. Syntheses.* **31** (1941) 53.
15. Fischer, H., and Dürr, M. *Ann.* **501** (1933) 107.
16. Schumm, O. *Z. physiol. Chem.* **168** (1927) 1.
17. Bouveault, L. *Compt. rend.* **138** (1904 : 1) 984.
18. Gompel, M., Mayer, A., and Wurmser, R. *Ibid.* **178** (1924 : 1) 1025.
19. Zeile, K., and Meyer, H. *Z. physiol. Chem.* **262** (1939) 178.

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