external conditions. This has led to the suggestion that the variability is mainly due to "intangible accidents of development"[2]; but it may be argued that this does not really solve the problem, and the statistical facts need to be brought into relation with what is known about the morphogenesis of chætæ.

The relevant facts are as follows. A pair of small islets of embryonic cells, the hypodermal histoblasts, become visible four hours after formation of the puparium; they spread and form the ventral imaginal hypodermis of each abdominal segment during the next thirty hours. Towards the end of this period, the trichogen cells appear in the hypodermis, enlarge, and each gives rise to a chæta[4]. Each chæta is innervated by a bipolar nerve cell[5,6]. Various macrochætæ have a temperature-effective period in the late larva[7], and this has been taken to indicate that the trichogen cells are already in existence then, long before they can be seen[4]; but the temperature-effective periods for microchætæ do not appear to have been studied. What conclusions can we draw from these facts?

Since most of the external surface carries chætæ in more or less regular patterns, the tendency to produce them must be a general property of imaginal hypodermis; and, in view of the rapid development of the hypodermis from small groups of embryonic cells, it seems probable that the trichogen cells are induced shortly before they become visible, and do not exist earlier. It may be suggested that local irregularities in the newly formed hypodermis cause the induction of the trichogens and that some mechanism prevents them from forming too close together. This mechanism is almost certain to be connected with the rapid growth of the trichogen cells, which would exhaust certain materials in the immediate neighbourhood of each, and thus inhibit the formation of others near by. This leaves us with the problems of what could cause local irregularities in the hypodermis, and what could be responsible for the uncorrelated variations in numbers of chætæ.

Local irregularities could be caused by many factors, including the early presence of the nerve cells which later underlie the chætæ. But nothing is known of the history of these cells, and they may well be induced at the same time as the trichogens, or by them. It seems, in any event, far-fetched to attribute the regularity of the surface patterns to the regularity of underlying nerve cells. Any disturbance of the potential uniformity of the hypodermal tissue might be sufficient to set off the production of trichogen, and the regularity of the chætæ would then be maintained by the inhibitory mechanism postulated above. But we require some additional cause for the variability in number of chæta on each segment. The factors responsible cannot easily be equated with any known environmental variables, for the reasons given earlier, and must be very local in effect.

One agency which may contribute to this variation is somatic mutation. Genetic homogeneity of somatic tissue certainly cannot be assumed; and, since the hypodermis forming each abdominal sternite contains large numbers of cells, sufficient mutations might occur to affect the probability of production of chætæ at any point, and thus to vary the density of chætæ. Another possibility is that random disturbances acting on the newly formed hypodermis might destroy its homogeneity at a number of points, and so affect the siting of the chætæ. Thus, it has recently been suggested[8] that slight random disturbances could set off a chain of reactions in a homogeneous tissue leading to the production of a variable pattern, for example, dappling.

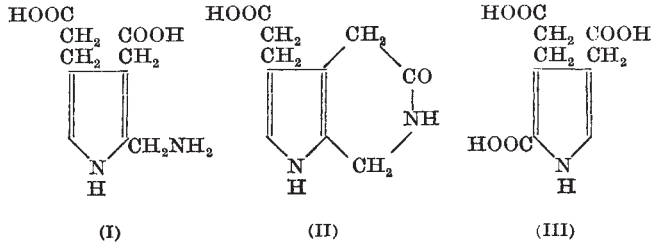
These suggestions are largely speculative, but may serve to encourage further study of the morphogenetic basis of production of chætæ. The facts put forward certainly reinforce our previous conclusion[2] that most of the non-genetic variation in number of abdominal chætæ, under normal culture conditions, is not an 'environmental' variability of the kind we meet in dealing with most quantitative characters. Such chance—or stochastic—variability may well be a property of many pattern characters. [Oct. 28.

[1] Robertson, Forbes W., and Reeve, E. C. R., *Nature*, **170**, 296 (1952).
[2] Robertson, Forbes W., and Reeve, E. C. R., *J. Genetics*, **50**, 414 (1952).
[3] Mather, K., and Harrison, B. J., *Heredity*, **3**, 1 (1949).
[4] Robertson, C. W., *J. Morph.*, **59**, 351 (1936).
[5] Stern, C., *Genetics*, **23**, 172 (1938).
[6] Lees, A. D., and Waddington, C. H., *Proc. Roy. Soc.*, B, **131**, 87 (1942).
[7] Child, G., *Genetics*, **20**, 127 (1935).
[8] Turing, A. M., *Phil. Trans. Roy. Soc.*, **237**, 37 (1952).

PORPHOBILINOGEN

Chemical Constitution

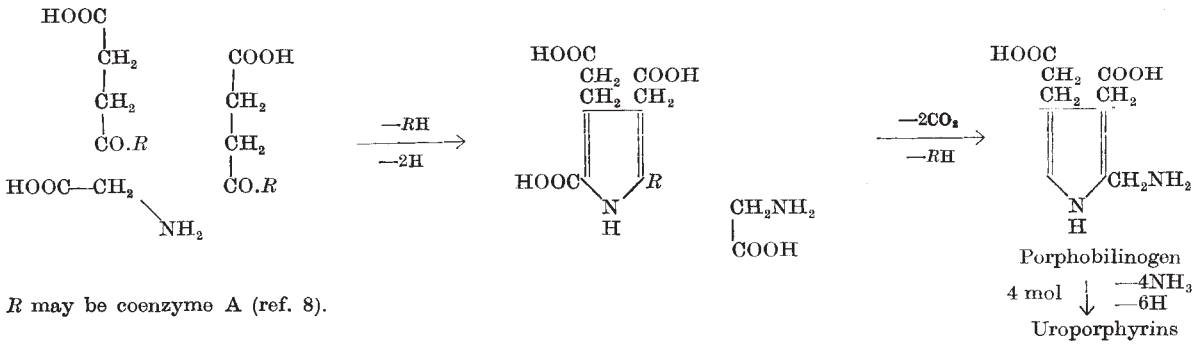
THE urine of patients suffering from acute porphyria contains a compound, porphobilinogen, which gives a positive Ehrlich reaction and which can be converted into a mixture of uroporphyrins on heating with acid[1]. Porphobilinogen was recently isolated in crystalline form by Westall[2], who kindly provided the material for this investigation. The reactions summarized below show that it probably has the structure (I). In an accompanying communication, Mrs. Kennard presents X-ray crystallographic evidence in support of a formula containing a single pyrrole ring.



(I) (II) (III)

Porphobilinogen boiled with phosphate buffer (*p*H 8·5) loses ammonia but does not do so at 40° [2]. 2 : 4-Dinitroaniline has been isolated after treatment of a porphobilinogen solution at *p*H 8·5 and room temperature with 1 : 2 : 4-fluorodinitrobenzene.

When dissolved in strong alkali such as caustic soda or aqueous triethylamine, porphobilinogen gave, on treatment with acetic anhydride or benzoyl chloride, what were assumed to be the corresponding acyl derivatives; owing to their water solubility these could not be isolated. They were recognized on paper chromatograms run in butanol – 20 per cent acetic acid and developed with Ehrlich reagent (acetyl R_F 0·79, benzoyl R_F 0·92). However, when a solution of porphobilinogen in aqueous pyridine was treated with acylating agents, the major product was the lactam (II), which was unchanged by solution in caustic soda and further treatment with acetic



R may be coenzyme A (ref. 8).

anhydride. The lactam (II) (R_F 0·65. Found: C, 57·6; H, 5·9; N, 12·9. $C_{10}H_{12}O_3N_2$ requires C, 57·7; H, 5·8; N, 13·45 per cent) with diazomethane gave its methyl ester, melting point 248–250° (R_F 0·83. Found: C, 59·6; H, 6·6; N, 12·6. $C_{11}H_{14}O_3N_2$ requires C, 59·4; H, 6·3; N, 12·6 per cent). Both (II) and its ester showed a brilliant blue fluorescence under a Wood's lamp, but the only strong absorption in the ultra-violet was at *c.* 210 mμ, ε 7,500, as expected of a pyrrole with no conjugated chromophore[3].

The methyl ester of (II) underwent a Hoesch reaction with acetonitrile and hydrogen chloride to give its α-acetyl derivative. The product of the reaction, which was carried out on only 8·2 mgm., was investigated spectroscopically, the intermediate ketimine hydrochloride having $\lambda_{max.}$ 331 mμ and the acetyl derivative 306 mμ (cf. 2-acetyl-4-ethyl-3 : 5-dimethylpyrrole, 308 mμ[3]). 2·7 mgm. of the pure 2 : 4-dinitrophenylhydrazone were obtained (found: N, 19·2. $C_{19}H_{20}O_7N_6$ requires N, 18·9 per cent).

The infra-red spectra of porphobilinogen, the lactam (II) and its ester, which were kindly measured by Dr. L. A. Duncanson, of Imperial Chemical Industries Butterwick Research Laboratories, are in accordance with the structures assigned to them. The alternative structures with the positions of the β-substituents in (I) reversed, leading to a seven-membered lactam, have not yet been rigidly excluded.

The porphyrin formed when porphobilinogen was heated in acid solution yielded a crystalline octamethyl ester, melting point 255°. On paper chromatography[4] the major part of the material behaved as uroporphyrin III, but at least one other isomer was present. To explain the formation of a mixture of isomeric uroporphyrins *in vitro*, it has been suggested that porphobilinogen itself is a mixture[5]. We regard this as unlikely, X-ray (see accompanying communication) and chemical evidence being in favour of homogeneity. Other examples exist of the formation of mixtures of isomeric porphyrins from single compounds[6].

Isotopic tracer studies of hæm biosynthesis[7,8] have shown that natural porphyrins are synthesized from glycine and an unsymmetrical succinic acid derivative arising from the tricarboxylic acid cycle. Determination of the positions of the labelled atoms in the hæm led to the suggestion of (III) as an intermediate, which was then in some way converted to uroporphyrin, the methene bridges of which were derived from the α-carbon atom of glycine[9]. The structure of porphobilinogen which we now put forward can be immediately fitted into the scheme at the top of the page.

We are carrying out further work on this subject, and our results will be reported in full at a later date.

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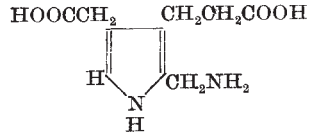
[1] For summary of previous work, see Brockman, P. E., and Gray, C. H., *Biochem. J.*, **54**, 22 (1953); and ref. 2.
[2] Westall, R. G., *Nature*, **170**, 614 (1952).
[3] Cookson, G. H., *J. Chem. Soc.* (in the press).
[4] Falk, J. E., and Benson, A., *Biochem. J.* (in the press).
[5] Waldenström, J., and Vahlquist, B., *Z. physiol. Chem.*, **260**, 189 (1939).
[6] Corwin, A. H., and Andrews, J. S., *J. Amer. Chem. Soc.*, **59**, 1973 (1937). Siedel, W., and Winkler, F., *Ann.*, **554**, 162 (1943). MacDonald, S. F., *J. Chem. Soc.*, 4184 (1952).
[7] Shemin, D., and Wittenberg, J., *J. Biol. Chem.*, **192**, 315 (1951).
[8] Shemin, D., and Kumin, S., *J. Biol. Chem.*, **198**, 827 (1952).
[9] Muir, H. M., and Neuberger, A., *Biochem. J.*, **47**, 97 (1950). Wittenberg, J., and Shemin, D., *J. Biol. Chem.*, **185**, 103 (1950).

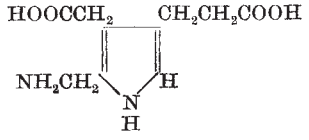
X-Ray Crystallographic Determination of Molecular Weight

A porphyrin precursor, porphobilinogen, was recently isolated by R. G. Westall in the form of the pure crystalline base[1] from the urine of patients suffering from acute porphyria. Chemical evidence by Westall[1] and by G. H. Cookson and C. Rimington (accompanying communication) suggests an empirical formula of $C_{10}H_{16}O_4N_2$ or $C_{10}H_{14}O_4N_2$ respectively. No molecular weight could, however, be assigned to porphobilinogen, and a crystallographic examination was undertaken to determine this and to differentiate between possible pyrrole or dipyrrylmethane structures, Waldenström and Vahlquist[2] having favoured the latter on the basis of diffusion experiments with porphobilinogen concentrates. In addition, it was hoped that the method would indicate whether or not two position isomers (*A* and *B*) were present. Intermediates of this type with unspecified α-substituents were postulated by Neuberger, Muir and Gray[3] in their theory of the biosynthesis of porphyrins.

Crystallographic methods were recently used to differentiate in a similar way between the various porphyrin isomers[4].



(*A*)



(*B*)