preliminary extraction of the tobacco with the series of organic solvents.

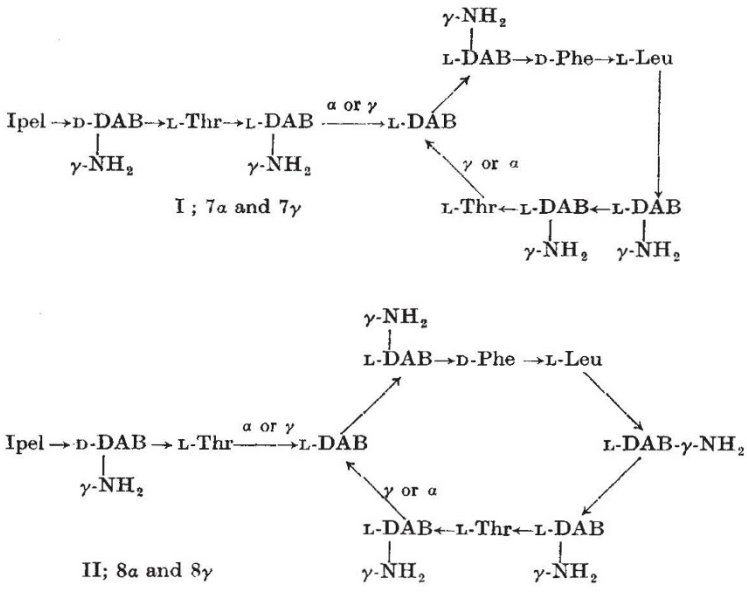
H. E. Wright, jun.
W. W. Burton
R. C. Berry, jun.

Department of Research and Development,
The American Tobacco Co.,
Richmond,
Virginia.

[1] Jean, D. B., *Chem. and Indust.*, 201 (1960).
[2] Wright, jun., H. E., Burton, W. W., and Berry, jun., R. C., *Chem. and Indust.*, 1491 (1961).
[3] Wright, jun., H. E., Burton, W. W., and Berry, jun., R. C., *Arch. Biochem. Biophys.*, **86**, 94 (1960).
[4] Gelotte, B., *J. Chromatog.*, **3**, 330 (1960).
[5] Porath, J., *Biochim. Biophys. Acta*, **39**, 193 (1960).
[6] Flodin, P., *J. Chromatog.*, **5**, 103 (1961).
[7] Jepson, J. B., and Stevens, B. J., *Nature*, **172**, 772 (1953).

Structure of Polymyxin *B*1

In their publications on the constitution of polymyxin *B*1, Hausmann and Craig[1], Hausmann[2] and Biserte and Dautrevaux[3] concluded that the structure of the antibiotic was limited to one of the four alternative formulæ I (7α and 7γ) and II (8α and 8γ), the symbols referring to the size of the ring and the amino-acid group of the αγ-diaminobutyric acid to which the chain is attached.



We have now independently examined the products of total and partial hydrolysis of polymyxin *B*1 and have established the following points:

(1) The optical rotation of the total DAB obtained by complete hydrolysis indicates that D-DAB is not present and that the amino-acid composition is 6 L-DAB : 1 D-Phe : 1 L-Leu : 2 L-Thr.

(2) The foregoing observation was confirmed by isolation of the fragment Ipel$\xrightarrow{\alpha}$DAB by partial hydrolysis. This, on further hydrolysis, gave L-DAB and not D-DAB.

(3) The isolation of the three peptides Thr$\xrightarrow{\alpha}$DAB, Thr$\xrightarrow{\gamma}$DAB and Thr$\xrightarrow{\alpha}$DAB$\xrightarrow{\alpha}$DAB, together with the

$$\begin{matrix} & & & \uparrow\gamma \\ \text{Thr} & & \text{Thr} & \text{Thr} \end{matrix}$$

absence of α-DNP-DAB from the complete hydrolysis of penta-DNP polymyxin *B*1, indicates that, provided the overall amino-acid sequences in formulæ I and II are correct, the structure can be further limited to either I 7α or II 8γ, with amended configuration of the initial DAB. Structures 7γ and 8α are not consistent with the isolation of the foregoing tetrapeptide.

S. Wilkinson
L. A. Lowe

Wellcome Research Laboratories,
Beckenham,
Kent.

[1] Hausmann, W., and Craig, L. C., *J. Amer. Chem. Soc.*, **76**, 4892 (1954).
[2] Hausmann, W., *J. Amer. Chem. Soc.*, **78**, 3662 (1956).
[3] Biserte, G., and Dautrevaux, M., *Bull. Soc. Chim. Biol.*, **39**, 795 (1957).

BIOCHEMISTRY

'Free' Apoferritin and Apoferritin obtained by Reduction of Iron-containing Ferritin

Ferritin prepared from horse-spleen by Granick's method[1] consists of a mixture of a colourless component and a heterogeneous coloured material[2]. The coloured iron-containing material, ferritin, sediments with a widely spread boundary and is separated by starch-gel and polyacrylamide-gel electrophoresis into at least three distinct fractions (α-, β- and γ-ferritin, that is, bands 1, 2 and 3)[3–6]. The colourless iron-free component moves with a sharp boundary in the ultracentrifuge ($S^0_{20,w}$ = 17·6; mol. wt. 465,000) and is homogeneous in starch-gel and polyacrylamide-gel[5]. From ultracentrifugal investigations Rothen[2] concluded that apoferritin obtained by reduction of ferritin with sodium dithionite[7] was identical with the colourless component which he called 'free' apoferritin.

In the following experiments ferritin consisting of α-, β- and γ-ferritin and free of 'free' apoferritin was used, which was prepared by repeated ultracentrifugation (15 consecutive runs, Spinco *L*, rotor 40, 2·5 h, 40,000 r.p.m.) from commercially available horse-spleen ferritin (*N.B.C.*) (preparation *A*). In this preparation no 'free' apoferritin could be detected with the Spinco *E* analytical ultracentrifuge (rotor *AnD*, 39,400 r.p.m., phosphate-buffered salt solution, *p*H 6·9).

'Free' apoferritin was also obtained from *N.B.C.*-ferritin by repeated ultracentrifugation and concentrated by ammonium sulphate precipitation (preparation *B*). In this preparation no iron-containing ferritin could be detected by the Prussian blue reaction and in the analytical ultracentrifuge.

Apoferritin was prepared by reduction of preparation *A* with sodium dithionite according to Behrens and Taubert[8] (preparation *C*).

α-Ferritin (band 1 of iron-containing ferritin) was obtained from preparation *A* either by repeated ammonium sulphate precipitation[4,5], by DEAE-cellulose chromatography or by gradient centrifugation in sucrose[9] (Fig. 1, preparation *D*). Preparation *E* was prepared by reduction of preparation *D* with sodium dithionite.

The five preparations were investigated with the agar-gel electrophoresis technique of Wieme[10], the starch-gel electrophoresis technique of Smithies[11], with the analytical Spinco *E* ultracentrifuge and with the agar-gel double diffusion technique[12].

Rabbit anti-ferritin was obtained by immunization with preparation *A* and anti-'free' apoferritin by immunization with preparation *B* as antigen.

In agar gel all preparations tested migrated in the α-globulin region. Iron-containing ferritin (*A*) and apoferritin (*C*) migrated in a more widely spread zone suggesting heterogeneity, while 'free' apoferritin (*B*), α-ferritin (*D*) and reduced α-ferritin (*E*) seemed to be homogeneous.