Für dieses Gas behielt BLACK den bereits bekannten Namen der «fixen Luft» («fixed air») bei.

BLACK war sich der geschichtlichen Bedeutung des exakten Nachweises eines gasförmigen Stoffes durchaus bewusst, wie aus der rückblickenden Betrachtung in seinen *Vorlesungen über die Grundlehren der Chemie* hervorgeht. Diese «Lectures» wurden 1799 von seinem jüngeren Freund JOHN ROBISON (1739–1805) aus dem Nachlass BLACKS herausgegeben. Erst aus diesem posthumen Werk erhielt die inzwischen völlig veränderte wissenschaftliche Welt offizielle Kenntnis von physiologischen Versuchen, in denen BLACK 1757 gezeigt hatte, dass das von ihm entwickelte Gas «allen Tieren tödlich ist, welche es durch den Mund und die Nasenlöcher einatmen». Ja er erkannte sogar, dass die durch das Atmen eingetretene Veränderung der Luft darin besteht, «dass ein Teil derselben in fixe Luft» umgewandelt wird. Den Beweis für die Richtigkeit dieser Lehre erbrachte er kurz darauf in einem äusserst interessanten Massenexperiment: In eine Dachöffnung einer von 1500 Menschen besetzten Kirche in Glasgow brachte er Lappen, die mit einer Lösung von «kaustischem Mineralalkali» getränkt waren. Durch die ausgeatmete Luft der grossen Menschenmenge wurde nun diese Lösung, wie ROBISON berichtet, in mildes Alkali verwandelt. Auch die Entstehung der Kohlensäure bei der Gärung wurde geprüft.

Es scheint uns, dass diese ersten, bisher kaum allgemein bekannten späteren Untersuchungen im Hinblick auf die weitere Entwicklung von ausserordentlich grosser Bedeutung sind. Sie stellen nicht nur die Anfänge der sogenannten pneumatischen Chemie dar, sondern die *Anfänge chemischer Experimentalforschung in der Biologie.* Als *erste physiologische Wendung in der Chemie* bilden sie eine Vorstufe für das ähnlich gerichtete Lebenswerk des Franzosen LAVOISIER, dessen früheste Versuche dem schottischen Arzt sicher Wesentliches verdanken. Unmittelbaren Einfluss gewannen BLACKS Arbeiten auf den schottischen Chirurgen DAVID MACBRIDE (1726 bis 1778), der dessen Methodik verfeinerte und sie zum Nachweis der Kohlensäure im Blut (1764) heranzog, eine nur wenig bekannte Tatsache unter vielen, welche die auch in der wissenschaftlichen Medizin eingetretene Neuorientierung illustriert. Aus diesem Grunde und weil von BLACK bis zu LAVOISIER die Kette genialer pneumatisch-physiologischer Experimente nicht mehr abreissen sollte, hat die Abhandlung des Jahres 1756 verdient, der Vergessenheit entrissen zu werden.

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Summary

Reference is made to a treatise published in 1756 by JOSEPH BLACK (1728–1799), which was the first work to contain conclusive evidence of a gas bound to solid bodies; and in this connection the historical significance of the earliest studies on carbon dioxide is emphasised. Attention is drawn in particular to a subject about which little has hitherto been known, *i.e.*, the use which BLACK and his contemporaries (notably DAVID MACBRIDE) made of this discovery by applying it to animal and human physiology.

STUDIORUM PROGRESSUS

Synthesis of a Polypeptide with ACTH-like Structure

By R. A. BOISSONNAS, St. GUTTMANN, J.-P. WALLER, and P.-A. JAQUENOUD*

The structure of the main component of hog ACTH has recently been elucidated by BELL *et al.*[1] and by WHITE and LANDMANN[2]. It is a linear polypeptide composed of thirty-nine amino acids residues: seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophanyl-glycyl-lysyl-prolyl-valyl-glycyl-lysyl-lysyl-arginyl-arginyl-prolyl-valyl-lysyl-valyl-tyrosyl-prolyl-aspartyl-glycyl-alanyl-glutamyl-aspartyl-glutaminyl (or glutamyl)-leucyl-alanyl-glutamyl-alanyl-phenylalanyl-prolyl-leucyl-glutamyl-phenylalanine. HOFMANN and JÖHL[3] have confirmed by synthesis the sequence of the first five N-terminal amino acids of this structure.

LI *et al.*[4] have found that sheep ACTH is structurally closely related to hog ACTH, the only differences being located in the carboxyl moiety of the molecule.

Large fragments can be enzymatically or chemically split from the carboxyl end of the ACTH molecules without loss of the biological activity. On the other hand splitting of peptides bonds at the amino end of the molecule is followed by complete inactivation. The biologically active part of the molecule is therefore located in the amino moiety, which presents the same sequence of amino acids for the diverse ACTH molecules hitherto investigated.

We have recently reproduced the sequence of the first twenty N-terminal amino acids of this common moiety of the ACTH molecules by achieving the synthesis of an icosapeptide: seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophanyl-glycyl-lysyl-prolyl-valyl-glycyl-lysyl-lysyl-arginyl-arginyl-prolyl-valine methyl ester. This icosapeptide ester is by four amino acids residues shorter than the smallest biologically active fragment hitherto obtained by degradation of an ACTH molecule. Assayed by the method of SAFFRAN and SCHALLY[5], our synthetic product exhibited a positive, though limited, ACTH activity (2–3 UI/mg). The possibility of a certain amount of racemization at some steps of the synthesis cannot be excluded. We give below a preliminary report on this synthesis.

CBO-arginine[6] was condensed by dicyclohexylcarbodiimide (DCCI)[7] with arginine methyl ester dibromhydrate (V) in dimethylformamide (DMF) to CBO

* Laboratory of Organic and Pharmaceutical Chemistry, University of Geneva and Department of Pharmaceutical Chemistry, Sandoz Ltd., Basle. July 21, 1956.

[1] P. H. BELL *et al.*, J. Amer. chem. Soc. *76*, 5565 (1954); *77*, 3419 (1955).

[2] W. F. WHITE and W. A. LANDMANN, J. Amer. chem. Soc. *76*, 4193 (1954); *77*, 771, 1711 (1955).

[3] K. HOFMANN and A. JÖHL, J. Amer. chem. Soc. *77*, 2914 (1955).

[4] C. H. LI *et al.*, Nature *176*, 687 (1955).

[5] M. SAFFRAN and A. V. SCHALLY, Endocrinology *56*, 523 (1955).

[6] All starting amino acids were of the L-form.

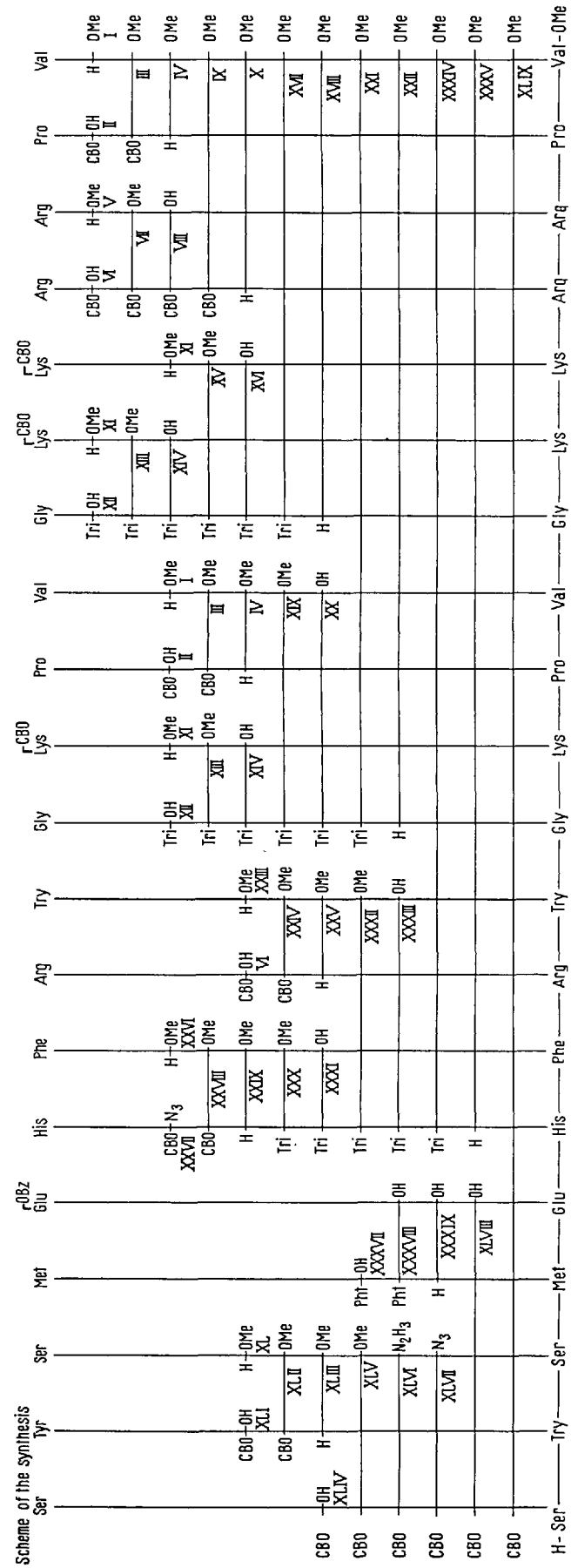
[7] J. C. SHEEHAN and G. P. HESS, J. Amer. chem. Soc. *77*, 1067 (1955).

arginyl-arginine methyl ester dibromhydrate (VII; 58% yield. Rf^*_M 0·30[8]. Analysis calculated for $C_{21}H_{36}O_5N_8Br_2$: N 17·50; Br 24·96. Found: N 17·00; Br 25·40). This was saponified to CBO-arginyl-arginine dibromhydrate (VIII; 98% yield. Rf^*_M 0·09. Analysis calculated for $C_{20}H_{34}O_5N_8Br_2$: N 17·89. Found: N 16·75) and condensed by DCCI and tributylamine (TBA) in DMF with prolyl-valine methyl ester bromhydrate (IV), obtained by HBr/acetic acid scission of CBO-prolyl-valine methyl ester[9] (III), to CBO-arginyl-arginyl-prolyl-valine methyl ester dibromhydrate (IX; 57% yield. Analysis calculated for $C_{31}H_{52}O_7N_{10}Br_2$: N 16·74; Br 19·10. Found: N 16·81; Br 19·98), which was cleaved by HBr/acetic acid into arginyl-arginyl-prolyl-valine methyl ester tribromhydrate (X; 74% yield. Rf_M 0·47. Rf_A 0·52. Analysis calculated for $C_{23}H_{47}O_5N_{10}Br_3$: Br 30·63. Found: Br 30·25).

Di-CBO-lysine[10] was converted by thionylchloride and methanol to ε-CBO-lysine methyl ester chlorhydrate[10] (XI; 72% yield), which was condensed with trityl-glycine[11] by the mixed anhydride method in tetrahydrofurane (THF) to trityl-glycyl-ε-CBO-lysine methyl ester (XIII; 85% yield. M.p. dec. 75°. Rf^*_M 0·79. Analysis calculated for $C_{36}H_{39}O_5N_3$: N 7·08. Found: N 6·91), which was saponified to trityl-glycyl-ε-CBO-lysine (XIV; 85% yield. M.p. 110°. Rf^*_M 0·41. Analysis calculated for $C_{35}H_{37}O_5N_3$: N 7·25. Found: 7·05). This was condensed by DCCI in THF with ε-CBO-lysine methyl ester to trityl-glycyl-ε-CBO-lysyl-ε-CBO-lysine methyl ester (XV. Rf^*_A 0·85. Analysis calculated for $C_{50}H_{57}O_8N_5$; N 8·18. Found N 8·03), which was saponified to trityl-glycyl-ε-CBO-lysyl-ε-CBO-lysine (XVI; 79% overall yield. M.p. 80°. Rf^*_A 0·60. Analysis calculated for $C_{49}H_{55}O_8N_5$; C 69·89; H 6·58; N 8·32; equivalent weight 842. Found: C 69·67; H 6·98; N 8·25; equivalent weight 840).

The latter tripeptide was condensed by DCCI in DMF with the tetrapeptide X to trityl-glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester dibromhydrate (XVII; 45% yield. M.p. dec. 150°. Rf^*_M 0·64. Analysis calculated for $C_{72}H_{99}O_{12}N_{15}Br_2$: N 13·77; Br 10·47. Found: N 13·77; Br 10·31), which was cleaved by hot acetic acid to glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester monoacetate dibromydrate (98% yield. M.p.dec. 133°. Rf_M 0·65. Analysis calculated for $C_{55}H_{89}O_{14}N_{15}Br_2$: N 15·61. Found: N 15·61) and treated with HBr to obtain the tribromhydrate (XVIII; 92% yield. Analysis calculated for $C_{53}H_{86}O_{12}N_{15}Br_3$: N 15·39; Br 17·55; $-OCH_3$ 2·27. Found: N 14·95; Br 17·31; $-OCH_3$ 2·22).

The above trityl-glycyl-ε-CBO-lysine (XIV) was condensed by DCCI in THF with prolyl-valine methyl ester bromhydrate (IV) to trityl-glycyl-ε-CBO-lysyl-prolyl-valine methyl ester (XIX; 93% yield. M.p.dec. 65°. Rf^*_A 0·75. Analysis calculated for $C_{46}H_{55}O_7N_5$: N 8·87. Found: N 8·50), which was saponified to trityl-glycyl-ε-CBO-lysyl-prolyl-valine (XX; 80% yield. M.p. 100°. Rf^*_A 0·65. Analysis calculated for $C_{45}H_{53}O_7N_5$: C 69·65; H 6·89; O 14·43; N 9·03; equivalent weight 776. Found: C 70·62; H 6·91; O 13·91; N 8·74; equivalent weight 769). The latter was condensed with the heptapeptide XVIII



[8] Ascending chromatograms on S. & S. 2040 *b* ausgew.; Rf_A in isoamyl alcohol:pyridine:water 35:35:30; Rf_M in methylethylketone:pyridine:water 65:15:20; * indicates a preliminary scission of the α-amino protecting group.

[9] R. L. M. Synge, Biochem. J. *42*, 99 (1948).

[10] M. Bergmann, L. Zervas, and W. F. Ross, J. biol. Chem. *111*, 245 (1935).

[11] G. Amiard, R. Heymès, and L. Velluz, Bull. Soc. chim. France *1955*, 191.

by DCCI and TBA in DMF to trityl-glycyl-ε-CBO-lysyl-prolyl-valyl-glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester dibromhydrate (XXI; 49% yield. M.p. dec. 150°. Rf^*_A 0·62. Analysis calculated for $C_{98}H_{136}O_{18}N_{20}Br_2$: N 13·71. Found: N 13·72). Successive treatments by hot acetic acid, IRA-410 and three equivalents of HCl gave glycyl-ε-CBO-lysyl-prolyl-valyl-glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester trichlorhydrate (XXII; 82% yield. M.p. dec. 175°. Rf_A 0·68. Analysis calculated for $C_{79}H_{123}O_{18}N_{20}Cl_3$: C 54·30; H 7·10; O 16·48; N 16·03; Cl 6·09. Found: C 54·25; H 7·03; O 17·13; N 15·47; Cl 6·18).

CBO-arginine and tryptophane methyl ester chlorhydrate were condensed by DCCI in DMF to CBO-arginyl-tryptophane methyl ester chlorhydrate (XXIV; 84% yield. Rf^*_M 0·55. Analysis calculated for $C_{26}H_{33}O_5N_6Cl$: N 15·42; Cl 6·51. Found: N 15·20; Cl 6·47), which was converted by HBr/acetic acid (free of bromine and of aldehyde) to arginyl-tryptophane methyl ester dibromhydrate (XXV; 91% yield. M.p. dec. 240°. Rf_M 0·55. Analysis calculated for $C_{18}H_{28}O_3N_6Br_2$: N 15·67; Br 29·80. Found: N 15·64; Br 29·60).

CBO-histidyl-azide[12] (XXVII) was reacted with phenylalanine methyl ester (XXVI) to CBO-histidyl-phenylalanine methyl ester (XXVIII; 82% yield. M.p. 108°. Rf^*_M 0·75. Analysis calculated for $C_{24}H_{26}O_5N_4$: N 12·44. Found: N 12·60), which was cleaved by HBr/acetic acid to the dibromhydrate (XXIX; 98% yield. M.p. 140°. Rf_M 0·75. Analysis calculated for $C_{16}H_{22}O_3N_4Br_2$: Br 33·42. Found: Br 34·0) and tritylated to ditrityl-histidyl-phenylalanine methyl ester (XXX; 91% yield. M.p. 70°. Analysis calculated for $C_{54}H_{46}O_3N_4$: N 6·99. Found: N 6·93). This was saponified to ditrityl-histidyl-phenylalanine (XXXI; 90% yield. M.p. 120°. Rf^*_M 0·37. Analysis calculated for $C_{53}H_{46}O_3N_4$: N 7·12. Found: N 7·11), which was condensed with the dipeptide XXV by DCCI and TBA in DMF to ditrityl-histidyl-phenylalanyl-arginyl-tryptophane methyl ester bromhydrate (XXXII; 89% yield. M.p. 185°. Rf^*_M 0·67. Analysis calculated for $C_{71}H_{71}O_5N_{10}Br$: N 11·44. Found: N 11·56). This was saponified to ditrityl-histidyl-phenylalanyl-arginyl-tryptophane (XXXIII; 88% yield. M.p. 228°. Rf^*_M 0·48. Analysis calculated for $C_{70}H_{68}O_5N_{10}$: N 12·40. Found: 12·13). The latter was condensed with the endecapeptide XXII by DCCI in DMF to ditrityl-histidyl-phenylalanyl-arginyl-tryptophanyl-glycyl-ε-CBO-lysyl-prolyl-valyl-glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester trichlorhydrate (XXXIV; 73% yield. M.p. 200°. Rf^*_M 0·65. Analysis calculated for $C_{149}H_{189}O_{22}N_{30}Cl_3$: C 62·59; H 6·66; Cl 3·72. Found: C 61·25; H 6·48; Cl 2·96). Treatment with hot acetic acid followed by HCl gave histidyl-phenylalanyl-arginyl-tryptophanyl-glycyl-ε-CBO-lysyl-prolyl-valyl-glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester pentachlorhydrate (XXXV; 89% yield. M.p. dec. 220°. Rf^*_A 0·65. Analysis calculated for $C_{111}H_{163}O_{22}N_{30}Cl_5$: C 54·45; H 6·72; O 14·38; N 17·17. Found: C 52·88; H 6·80; O 13·92; N 17·44).

Phtalyl-methionine was condensed by DCCI with the triethylammonium salt of γ-benzyl-glutamic acid to phtalyl-methionyl-γ-benzyl-glutamic acid (XXXVIII), which was cleaved by one equivalent of hydrazine into methionyl-γ-benzyl-glutamic acid monohydrate which was purified by partition chromatography (XXXIX; 26% over all yield. Rf_M 0·63. Analysis calculated for $C_{17}H_{26}O_6N_2S$: N 7·26. Found: N 7·31).

CBO-tyrosine was condensed with serine methyl ester by DCCI in chloroform to CBO-tyrosyl-serine methyl ester (XLII; 70% yield. M.p. 151°. Rf^*_M 0·90. Analysis calculated for $C_{21}H_{24}O_7N_2$: N 6·73. Found: N 6·69), which was cleaved by HBr/acetic acid to tyrosyl-serine methyl ester bromhydrate (XLIII; 98% yield). This was condensed with CBO-serine by DCCI and TBA in DMF to CBO-seryl-tyrosyl-serine methyl ester (XLV; 75% yield. M.p. 165°. Rf^*_M 0·95. Analysis calculated for $C_{24}H_{29}O_9N_3$: N 8·34. Found: N 8·32), which was converted into the hydrazide (XLVI; 68% yield. M.p. 230°. Analysis calculated for $C_{23}H_{29}O_8N_5$: C 54·86; H 5·81; O 25·42; N 13·91. Found: C 55·36; H 6·63; O 24·22; N 14·48). The corresponding azide was reacted in DMF with the triethylammonium salt of the dipeptide XXXIX to CBO-seryl-tyrosyl-seryl-methionyl-γ-benzyl-glutamic acid (XLVIII; 65% yield. M.p. dec. 150°. After scission by HBr in a mixture of acetic acid and methyl-ethyl-thioether[13]: Rf_M 0·60[14]. Analysis calculated for $C_{40}H_{49}O_{13}N_5S$: N 8·34; equivalent weight 840. Found: N 8·33; equivalent weight 816). This was condensed with the pentadecapeptide XXXV by DCCI and TBA in DMF to CBO-seryl-tyrosyl-seryl-methionyl-γ-benzyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophanyl-glycyl-ε-CBO-lysyl-prolyl-valyl-glycyl-ε-CBO-lysyl-ε-CBO-lysyl-arginyl-arginyl-prolyl-valine methyl ester trichlorhydrate (XLIX; 77% yield. M.p. dec. 200°. Analysis calculated for $C_{151}H_{208}O_{34}N_{35}SCl_3$: N 15·34. Found: N 15·72. The hydrolysate presents a correct chromatographic correlation with a synthetic mixture of amino acids). The protecting groups of this icosapeptide were cleaved by HBr in a mixture of acetic acid and methyl-ethyl-thioether[13]. After evaporation in vacuo the residue was dissolved in water. The icosapeptide precipitates from its aqueous solution at pH above 6.

Zusammenfassung

Es wird über die Synthese eines Icosapeptides, das aus den zwanzig ersten Aminosäuren des N-Endes eines ACTH Moleküls aufgebaut ist, berichtet. Das synthetische Produkt besitzt eine schwache ACTH-Wirkung.

[12] R. W. HOLLEY and E. SONDHEIMER, J. Amer. chem. Soc. *76*, 1326 (1954).

[13] To fix the benzyl bromide produced.

[14] In sec-butanol: 3% ammonia it appears between glutamic acid and lysine. *Cf.* footnote 3.