

occur until respiration had lowered the carbon/sulphur ratio to about 50/1. When the proportion of nitrogen in the organic matter was reduced, production of sulphate tended to be more rapid—though the effect did not seem to be nearly as well marked as the effect of reducing the proportion of sulphur on production of ammonium.

These results suggest that mineralization of an element from organic matter depends not only on the proportion of that element in the organic matter but also on the proportion of the other elements. It is probable that a similar generalization applies to immobilization of elements, and in fact other results from these experiments have shown that immobilization of phosphorus was reduced when the proportion of nitrogen or of sulphur in the organic matter was reduced.

The statistical analysis was performed by Mr. P. F. May, of the Division of Mathematical Statistics, Commonwealth Scientific and Industrial Research Organization. It is intended to publish this work in greater detail elsewhere.

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April 16.

¹ Duncan, D. B., *Biometrics*, **11**, 1 (1955).

Absence of γ -Aminobutyric Acid from Brain Extracts containing Factor I

FLOREY¹ and Florey and McLennan^{2,3} have described some of the actions of extracts of brain and spinal cord which possess an inhibitory activity on certain synaptic processes, both in mammals and in invertebrate animals. For example, these extracts, which are said to contain 'Factor I', can block transmission in sympathetic ganglia, can inhibit monosynaptic reflexes, can block neuromuscular transmission and the neurogenic heartbeat of Crustacea, and can inhibit the discharge of crustacean stretch receptor neurones. Bazemore, Elliott and Florey⁴ have reported that an active component of extracts containing Factor I is γ -aminobutyric acid. Since that time it has been shown that γ -aminobutyric acid does have effects of an inhibitory nature, especially in Crustacea⁵ and when applied to the mammalian cerebral cortex⁶. However, a comparison of the actions of Factor I and γ -aminobutyric acid on those structures where the former is active has shown that, except in Crustacea, the effects obtained with the two are not comparable, and it was concluded that they are not identical⁷.

In the present experiments a solution of Factor I was applied to Whatman No. 4 filter paper, and subjected to chromatographic separation for free amino-acids. The solvents used were *n*-butanol/glacial acetic acid/water (4:1:5, v/v/v) in one

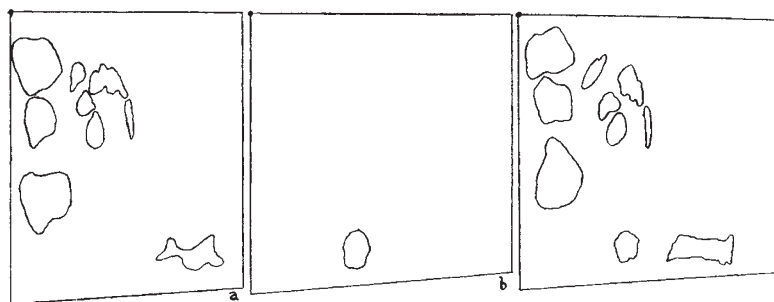


Fig. 1. Tracings of descending chromatograms developed with ninhydrin. In each case the solution was applied to the upper left-hand corner. The horizontal dimension represents movement in the butanol/acetic acid/water solvent, and the vertical the movement in phenol. (a) Factor I solution, 0.025 ml.; (b) γ -aminobutyric acid solution, 0.01 ml. of 1 mgm./ml.; (c) Factor I solution, 0.025 ml.; γ -aminobutyric acid solution, 0.01 ml.

dimension, and 80 per cent (w/w) aqueous phenol in the second. The chromatograms were developed with ninhydrin solution to reveal the amino-acid spots.

Fig. 1a shows the distribution of spots obtained with a solution of Factor I prepared according to Florey and McLennan². Fig. 1b shows the spot obtained when a solution of pure γ -aminobutyric acid was applied to the paper, and Fig. 1c the result when Factor I with added γ -aminobutyric acid was applied. It is evident from these chromatograms, which were all performed simultaneously, that there is no spot in Fig. 1a corresponding to γ -aminobutyric acid, such as appears clearly in Fig. 1c, where the substance was added artificially to the solution of Factor I.

It can be concluded from this experiment that extracts containing Factor I prepared by the method of Florey and McLennan, which possess the whole complex of actions described by those authors, contain no γ -aminobutyric acid, which cannot therefore be the active component of Factor I. Nor can it be argued that the quantity of Factor I applied to the paper in Fig. 1a contains an undetectably small amount of γ -aminobutyric acid, for the amount applied is $2\frac{1}{2}$ –5 times that required to block the crayfish stretch receptor neurone, and is therefore at least $2\frac{1}{2}$ times more than the amount applied in Fig. 1b⁷. A possible explanation for the finding of Bazemore *et al.*⁴ that γ -aminobutyric acid was present in the extracts containing Factor I is that it was split off from some larger molecule in the course of the relatively drastic extraction procedures which were used. It may perhaps be conjectured that this postulated larger molecule, of which γ -aminobutyric acid is a fragment, is the true active ingredient of Factor I solutions.

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¹ Florey, E., *Arch. Int. Physiol.*, **62**, 33 (1954); *Canad. J. Biochem. Physiol.*, **34**, 669 (1956).

² Florey, E., and McLennan, H., *J. Physiol.*, **129**, 384 (1955).

³ Florey, E., and McLennan, H., *J. Physiol.*, **130**, 446 (1955).

⁴ Bazemore, A., Elliott, K. A. C., and Florey, E., *Nature*, **178**, 1052 (1956); *J. Neurochem.*, **1**, 334 (1957).

⁵ Edwards, C., and Kuffler, S. W., *Fed. Proc.*, **16**, 34 (1957). Elliott, K. A. C., and van Gelder, N. M., *Fed. Proc.*, **17**, 216 (1958).

⁶ Purpura, D. P., Girado, M., and Grundfest, H., *Science*, **125**, 1200 (1957). Iwama, K., and Jasper, H. H., *J. Physiol.*, **133**, 365 (1957). Marrazzi, A. S., Hart, E. R., and Rodriguez, J. M., *Science*, **127**, 284 (1958).

⁷ McLennan, H., *J. Physiol.*, **130**, 79 (1957).