

Substitution of Cysteine for Protohæmin as the 'X' Factor for Growth of *H. influenzae*

THE indispensability of iron in the form of protohæmin, the so-called 'X' factor, to the growth of *H. influenzae* has long been an accepted fact. Recently, the Lwoffs observed that the oxygen consumption of suspensions of this organism containing minimal amounts of hæmin is remarkably increased by the addition of small quantities of this compound. Accordingly, they concluded that the 'X' factor functions by supplying, to the cell, material from which it can synthesize a catalytic respiratory system comprising cytochrome *C* and possibly cytochrome oxidase, peroxidase and catalase, although no direct evidence was advanced for the latter¹. If there is a correlation between the necessity of the hæmin for growth and its ability to promote oxygen consumption, it should be possible to grow the influenza bacillus by replacing the iron complexes with any other system which is capable of inducing oxygen consumption. Since reversible oxidation-reduction systems of suitable potential can function as efficient respiratory catalysts², we set out to determine the effect of these systems on the growth of *H. influenzae*.

Those dye systems, within the potential range which has been demonstrated to be most effective in respiratory catalysis, failed to support growth when added to a basic medium to which adequate amounts of 'V' (yeast extract) were added. Moreover, they actually inhibited growth even in the presence of the minimal amounts of protohæmin found necessary to support serial transfer (10^{-8} gm./c.c.). This inhibition was traced to the accumulation of hydrogen peroxide resulting from the activity of the supplementary dye catalyst.

Since cysteine is effective in protecting cells from the inhibitory activity of peroxide³, it should thus protect the organism from the inhibitory action of the dyes. Experiments proved this to be the case. Furthermore, cysteine plus suitable dyes effectively replaced hæmin as a growth requirement, and this combination supported growth in successive serial transfers. Moreover, cysteine alone was found to be capable of permitting serial growth. Whereas in the absence of hæmin, the sulphhydryl compound was required for aerobic growth, its presence was unnecessary for anaerobic growth. This confirms the results of Anderson⁴ who found that only the 'V' factor is required under anaerobic conditions.

Since anaerobic growth can occur, it is evident that oxygen consumption is not essential for multiplication. Further, since respiratory catalysts (dyes) alone cannot support growth, it is obvious that the 'X' factor cannot be replaced by a system which functions solely as a respiratory mechanism. However, since cysteine alone can replace hæmin, and, in the latter's absence, is necessary for aerobic but not for anaerobic growth, it becomes probable that the sole necessary function of either of these substances is to provide the organism with a mechanism for protection against hydrogen peroxide, formed under aerobic conditions through the activity of a non-hæmin respiratory system. Thus, *H. influenzae*, in the absence of the 'X' factor, is physiologically similar to *E. coli* in the presence of cyanide and methylene blue⁵, since both organisms, under these conditions, possess mechanisms for the formation of peroxide but none for its destruction. The role of hæmin is to supply this missing mechanism in the form of catalase. These experiments do not deny

the role of hæmin in promoting respiration, but merely point out that such a function is unnecessary and incidental to its principal action.

The foregoing results will be presented in more detail elsewhere.

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¹ Lwoff, A., and Lwoff, M., *Ann. Inst. Pasteur*, **59**, 129 (1937).

² Barron, E. S. G., and Hoffman, L. A., *J. Gen. Physiol.*, **13**, 483 (1930).

³ Quastel, J. H., and Wooldridge, W. R., *Biochem. J.*, **23**, 115 (1929).

⁴ Anderson, L. R., *Amer. J. Hyg.*, **13**, 164 (1931).

⁵ Broh-Kahn, R. H., and Mirsky, I. A., *J. Bact.*, **35**, 455 (1938).

Composition of the Aerial Insect Fauna up to 300 ft.

IN a recent letter in NATURE (April 2) on the aerial drift of insects up to 2,000 ft., Hardy and Milne make reference to the intensive study of such drift at lower levels by flying 3-ft. diameter nets simultaneously (usually for 3 hours round about midday) at heights of 277 ft., 177 ft. and 10 ft., on each of three masts placed along a 5/6 mile frontage at the Beam Wireless Station, Tetney, North Lincolnshire. A brief note of the principal findings may be of interest.

Thirty-one series of collections were made from March until November during 1934 and 1935 yielding 2,896, 4,740 and 15,659 insects belonging to 166, 197 and 298 species at the top, middle and bottom heights respectively, a total of 23,295 insects of 421 species. Most were winged adults of small size; but a number of nymphs (Hemiptera) and true apterous forms (Collembola and Psocoptera), in addition to 134 spiders (20 species) and parts of plants (31 species) were taken. Species of seven genera made up more than 50 per cent of the numbers collected: *Brevicoryne brassicae* L. (cabbage aphid) (3,334 individuals), *Sciara* spp. (2,484), *Pterodella pedicularia* L. (2,357), *Oscinis* spp. (frit flies) (1,708), *Aphidius* spp. (1,522), *Leptocera* spp. (967), *Limothrips cerealium* Hal. and *denticornis* Hal. (corn thrips) (402).

The average 'density of insect population', defined as the number of insects in one million cubic feet of air, declined with height, so that 75 per cent of the total population was below 100 ft. Diptera was the most numerous order up to that height and Hemiptera above. Aphididæ were the dominant family at all heights. The average composition of the fauna at each height is shown in the accompanying diagram.

The distribution of population across the front was even although local aggregations occurred in nets, especially at ground-level, often owing to the close proximity of host plants upwind. The greatest numbers and variety occurred during the months of June and September, when insects are most active in breeding and dispersal.

The most favourable conditions for the maximum numbers in the air varied from family to family, but in general the greatest numbers of all insects occurred at relative humidities below 59 per cent, at surface wind velocities below 9 m.p.h. and at