

B₃) La révélation immunochimique des protéines (Figure 4a) et des lipoprotéines (Figure 4b) confirme la disparition des protéines de mobilité directement inférieure à celle de l'albumine et des fractions lipidoprotidiques.

Discussion-conclusion. Le premier groupe de résultats n'autorise guère d'interprétation directe. Plus intéressantes apparaissent les données fournies par le second groupe, car il n'est pas de contradiction formelle entre les modifications du sérum dévoilées par électrophorèse en cellule, et celles que traduisent les deux autres techniques. A côté d'une interaction lipoprotéines-chlorpromazine, il apparaît, dans les conditions décrites, une plus inattendue précipitation de globulines- α_1 .

Les doses qui provoquent ces modifications diffèrent largement des doses thérapeutiques. Mais, pour donner plus de poids à la notion de chlorpromazine (ou de phénothiazine) comme réactif d'intérêt biochimique, il faut ajouter de sérieux arguments: précisions sur les paramètres de l'interaction, nature des liaisons formées, transformations structurales, sélection dans la précipitation des différentes protéines, applications éventuelles, etc.

Ces problèmes théoriques et pratiques font l'objet d'études en cours.

Summary. In vitro, the mixture 'Chlorpromazine-human serum' gives modifications of the lipoproteins and the α -globulins. These modifications are studied by paper electrophoresis, Tiselius-electrophoresis, and immuno-electrophoresis. Under the experimental conditions described, the selective precipitation of the α_1 -globulins seems quite unexpected.

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A Protein Fraction in Locust Hemolymph Associated with the Moulting Cycle

A number of studies have shown that hemolymph proteins change during development in insects. Most of these studies have been made on holometabolous insects, especially on *Hyalophora cecropia*^{1,2}, on *Drosophila*³⁻⁵, and on *Culex*^{4,6}, while only a few have been made on hemimetabolous forms⁷⁻⁹. STEINHAEUER and STEPHEN⁷ noted changes in blood proteins during the development of *Periplaneta* using paper electrophoresis, while MISSELIJN, KARCHER, DE KEYSER, and VAN SANDE⁸ studied the hemolymph proteins of immature and mature hemipterans of the genus *Triatoma* by means of agar gels. More recently COLES⁹ has examined starch electropherograms of the hemolymph of fifth instar and adult *Rhodnius*. In the present investigation, which is concerned with the cyclical appearance of one particular protein band in the electrophoretic pattern of the hemolymph of the locust, *Locusta migratoria migratorioides* (Reiche and Fairm.), horizontal starch electrophoresis was used since this technique allows much greater resolution. This protein fraction has been found to be present in each of the instars during the development of the locust, appearing immediately prior to the moult and disappearing soon thereafter. No attempt will be made to discuss the overall pattern of proteins occurring in the hemolymph and their changes with development since these will be covered in a future publication.

Methods. Locusts from stock provided by the Anti Locust Research Council, were reared under the conditions described by HUNTER-JONES¹⁰. Hemolymph samples were obtained by allowing the cut limb sockets of the locust to drain directly on to 5 mm squares of Whatman's No. 3 filter paper which were then inserted into the gel. Zone electrophoresis of the hemolymph was carried out in starch gels¹¹, the hydrolysed starch having been obtained from the Connaught Medical Research Laboratories and prepared according to their directions. The semi-discontinuous buffer system used was that of POULIK¹² as modified by FERGUSON and WALLACE¹³. The

pH of the buffer was 8.5 and a voltage gradient of 10 V per cm was maintained across the gel for 3 h at a temperature of 5°C. The gel was sectioned horizontally into two equal portions each of which was stained for 3 min in a saturated solution of Naphthylene Black 10B in methanol-acetic acid-water¹¹.

Results. A total of 19 distinct hemolymph protein fractions has been demonstrated during the development of the locust, but the complete number has never been found to be present in any one developmental stage (e.g. in the early 3rd instar hemolymph, 11 bands occur). On the whole the band pattern is fairly constant throughout the locusts' development until such time as the animals become sexually mature¹⁴. The hemolymph band patterns for the period prior to and immediately after the second moult are shown in the accompanying Figure. Of particular interest is band 14 which is absent in the intermoult period but which appears a short time prior to the moult. It increases in staining intensity to reach a maximum at the time of moulting and thereafter decreases

¹ W. H. TELFER and C. M. WILLIAMS, J. gen. Physiol. 36, 389 (1953).

² H. LAUFER, Ann. N.Y. Acad. Sci. 89, 490 (1960).

³ C. WUNDERLY and H. GLOOR, Protoplasma 42, 273 (1953).

⁴ P. S. CHEN, Revue suisse Zool. 66, 280 (1959).

⁵ E. J. DUKE and E. M. PANTELOURIS, Comp. Biochem. Physiol. 10, 351 (1963).

⁶ V. ZAMEN and W. T. CHELLAPPAH, Expl. Parasit. 13, 108 (1963).

⁷ A. L. STEINHAEUER and W. P. STEPHEN, Ann. ent. Soc. Am. 52, 733 (1959).

⁸ G. MISSELIJN, D. KARCHER, F. DE KEYSER, and M. VAN SANDE, *Protides of the Biological Fluids*, Proc. of the 7th Colloquium, Bruges 1959 (Elsevier, Amsterdam 1958).

⁹ G. C. COLES, J. Insect Physiol. 11, 1317 (1965).

¹⁰ P. HUNTER-JONES, *Instructions for Rearing and Breeding Locusts in the Laboratory* (Anti Locust Research Centre, London 1956).

¹¹ O. SMITHIES, Biochem. J. 61, 629 (1955).

¹² M. D. POULIK, Nature, Lond. 180, 1477 (1957).

¹³ K. A. FERGUSON and A. L. C. WALLACE, Nature, Lond. 190, 632 (1961).

¹⁴ F. W. MCCORMICK and A. SCOTT, unpublished.

until after 12 h it is present only as a trace, and after 24 h is absent. This protein band is found to be strongly present in the late embryo and again in the first instar. Its behaviour in succeeding instars is similar to that shown in the Figure for the 3rd instar. It has not been found to be present in immature or mature adults.

None of the other bands have been found to show this cyclical behaviour; however, around the period of the moult, digestion of starch at the point of insertion of the sample suggests the occurrence of carbohydrases at this time.

Discussion. The only comparable study is that of STEINHAEUER and STEPHEN⁷ on *Periplaneta*. These workers using paper electrophoresis detected only three distinct bands, and they found them to be present in all stages of cockroach development. It is of interest to note that their band 2 was the most variable in its behaviour, showing distinct similarity with band 14 in the present investigation. Unlike band 14, however, which reached a peak of concentration at the time of the moult and dis-

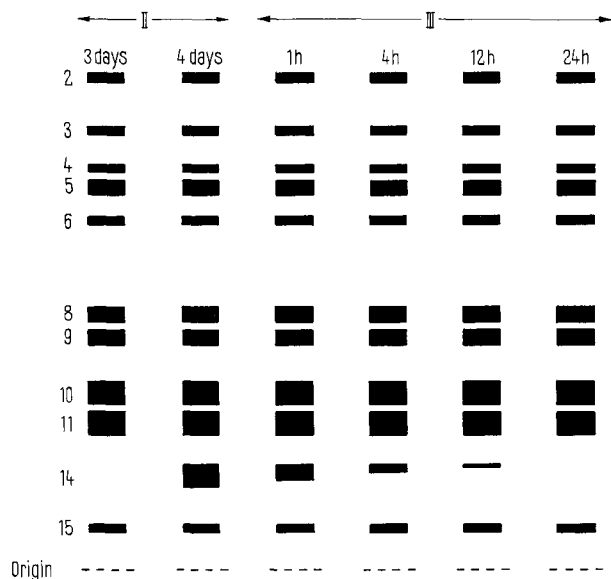
appeared soon after this, STEINHAEUER and STEPHEN⁷ record their band 2 as absent only for a short time in the intermoult period. Moreover, while band 14 was absent in all the adult locusts examined, STEINHAEUER and STEPHEN⁷ found band 2 continued to be present for 3–4 weeks in adult male cockroaches, and for some days in adult females. It would therefore appear probable that the band 2 of STEINHAEUER and STEPHEN⁷ corresponds to a number of the bands appearing on the starch electropherogram of which band 14 is but one. An electrophoretic study of the eluted band 2 of STEINHAEUER and STEPHEN⁷ would be of considerable interest. MISSELIJN et al.⁸, in an agar gel electrophoretic study of the hemolymph of three species of *Triatoma*, both mature and immature, record no differences with respect to age in the individuals which they examined. Similarly, COLES⁹ noted no large protein changes associated with moulting in *Rhodnius*. No record of a similar moulting protein fraction occurring in holometabolous insects has been found. The absence of other records is perhaps understandable, for it is only by regular sampling throughout the entire developmental stages of the insect that cyclical changes such as this become apparent.

It would appear probable that the band is intimately associated with moulting in some way, and further attempts are being made to investigate this. In this context, it is of interest to note the similarity in the cyclical behaviour of band 14 in the locust hemolymph and in the mitotic activity of the prothoracic gland¹⁵ in view of the suggestion by WILLIAMS¹⁶ that the secretion of the prothoracic gland may correspond to, or be associated with a protein fraction.

Résumé. Une étude électrophorétique de l'hémolymph aux divers stades de développement du criquet, *Locusta migratoria migratorioides*, révèle la présence d'une fraction protéique dont le comportement semble être lié au cycle de la mue.

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Diagrammatic representation of starch electropherograms of locust hemolymph samples made during late second and early third instars. The intensity of staining of the bands is represented by the relative thickness of the bands in the diagram.

¹⁵ K. U. CLARKE and P. LANGLEY, *Nature*, Lond. 194, 160 (1962).

¹⁶ C. M. WILLIAMS, *Fedn Proc. Am. Soc. exp. Biol.* 10, 564 (1951).

Growth of Some Chemoautotrophic Bacteria at Different Oxygen Tensions

In aerobic chemoautotrophic bacteria, molecular oxygen acts as a final electron acceptor in the oxidation of the inorganic substrates; the oxygen thereby being reduced to water. A maximum amount of energy is produced in these processes on which growth and other energy-consuming processes are dependent.

It is well known that most chemoautotrophic bacteria, despite their obvious aerobic nature, are difficult to culture on solid media although they might grow readily in

liquid cultures where the diffusion of oxygen is much slower. This discrepancy has sometimes been ascribed to a harmful effect of the agar or other gelling agents. Such an explanation seemed unlikely and an investigation was initiated to find out what effects oxygen might have on the growth and substrate oxidation in some chemoautotrophic bacteria, viz. *Nitrosocystis oceanus*, *Nitrosomonas europaea*, *Nitrobacter agilis*, and *Thiobacillus thiooxidans*.

The organisms were spread by means of a glass rod on the surface of mineral agar media using Oxoid Ion-agar no. 2 as solidifying agent. The substrates were 1.0% $\text{Na}_2\text{S}_2\text{O}_3$ (*T. thiooxidans*), 0.2% NH_4Cl (*N. oceanus*),