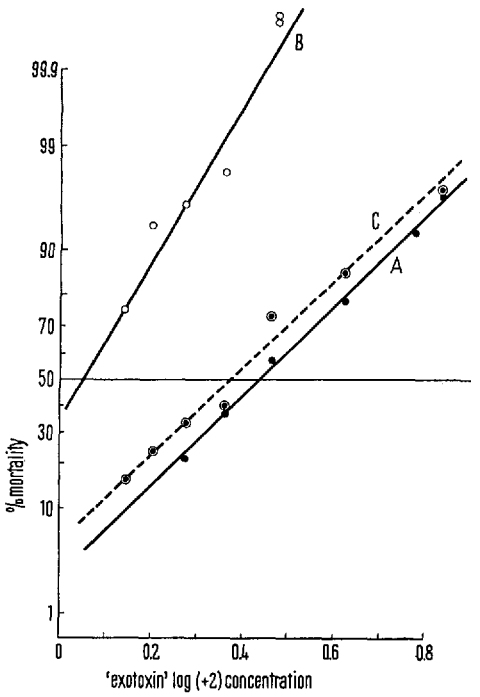
'exotoxin' in the corn-yeast-agar medium (curve A) and the synthetic medium (curve B). The medium lethal concentration (LC_{50}) is much lower in the yeast-free synthetic medium, and the slope of curve B is steeper than that of curve A. These differences in slope and LC_{50} are mainly due to the presence or absence of dry yeast in the media. This is demonstrated by curve C which represents results of tests with medium C, i.e. synthetic medium to which 2% of dry yeast was added. This addition reduces the slope of curve B to a value which is practically identical with that of curve A, and the LC_{50} is also very similar to that with the corn-yeast-agar medium.



Probit regression lines of mortality of *Drosophila* reared on 3 media with different concentrations of 'exotoxin'. (A) corn-yeast-agar medium; (B) synthetic medium C of SANG[2]; (C) SANG's medium plus 2% of dry yeast. Each point represents 100 test larvae. The line of the 50% mortality level indicates the different LC_{50} values at the points where it crosses the probit regression lines. Ordinate: instead of probits, % mortality is given by a probability distribution scale. Abscissa: log 100 times concentration of 'exotoxin' preparation in medium.

The results show that yeast reduces the toxic action of the 'exotoxin'. Bioassays on media that do not contain yeast are therefore more sensitive. As a consequence it must be postulated that bioassays for 'exotoxin' should either be made with yeast-free media, or with standardized media containing a defined proportion of yeast. A more detailed analysis of the yeast effect will be published elsewhere[3].

Zusammenfassung. Die toxische Wirkung des sogenannten «Exotoxins» von *Bacillus thuringiensis* auf *Drosophila* wurde geprüft: (A) in Mais-Trockenhefe-Agar-Medium, (B) synthetischem Medium C nach SANG[2] und (C) synthetischem Medium plus 2% Trockenhefe. In hefefreiem Medium ist die LC_{50} bedeutend niedriger und die Probitkurve viel steiler als in hefehaltigen Medien. Hefe reduziert also die toxische Wirkung des «Exotoxins» beträchtlich.

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Entomologisches Institut, Eidg. Technische Hochschule, Zürich (Switzerland), 7. Juni 1967.

[3] J.-M. PERRON and G. BENZ, J. Invertebrate Pathol., in print.

[4] Supported by Fellowship No. 1560 of the National Research Counsel of Canada. – Present address: Département de Biologie, Université Laval, Québec, Canada.

Separation of 'Soluble' Immunoprecipitating Antigens Originating from Rabies Virus Infected Cells by Chromatography on Ecteola Cellulose and by Gel Filtration

Rabies virus has been successfully purified by chromatography on Ecteola cellulose[1,2]. Preliminary experiments have revealed that most of the 'soluble' antigens can be eluted from Ecteola cellulose using solutions of lower ionic strength than are required to elute the whole virus[2]. The present communication will present in more detail the chromatographic technique of separating the 'soluble' antigens originating from the infected cells and will substantiate the feasibility of their purification by gel-filtration on Sephadex G-200.

The crude 'soluble' antigen preparation was obtained from infected tissue culture fluids by zinc acetate precipitation and subsequent removal of virus from the redissolved sediment through high speed centrifugation[3]. Prior to chromatography, this preparation was extensively dialyzed against 0.01 *M* *Tris*-(hydroxymethyl) aminoacetate buffer (TB) pH 7.0. Six ml of the antigen preparation was applied to a column (dia. 1.6 · 27 cm) of Ecteola cellulose (Serva, Heidelberg). For elution, a continuous gradient of increasing NaCl concentration (220 ml TB-220 ml 0.45 *M* NaCl in TB) was used. Fractions (5 ml) were collected and analyzed for protein[4] and precipitating antigen[5] using 0.75 ml aliquots. Fluorescein isothiocyanate-labeled antirabies γ-globulin (Baltimore Biological Labs.) mixed with non-infected tissue culture fluid material was used for the fluorescent precipitin test.

[1] J. B. THOMAS, A. S. RICKER, G. M. BAER and R. K. SIKES, Virology *25*, 271 (1965).

[2] A. R. NEURATH, T. J. WIKTOR, M. V. FERNANDES and H. KOPROWSKI, unpublished data. The preliminary experiments were performed at the Wistar Institute of Anatomy and Biology, Philadelphia, Pa. The presented results were obtained while A.R.N. worked at Wyeth Laboratories, Philadelphia, Pa. Rabies virus infected tissue culture material was obtained from the Wistar Institute.

[3] A. R. NEURATH, T. J. WIKTOR and H. KOPROWSKI, J. Bact. *92*, 102 (1966).

[4] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

[5] A. R. NEURATH, Z. Naturf. *20b*, 974 (1965).

The results presented in Figure 1 show that 5 antigens could be separated by chromatography on Ecteola cellulose.

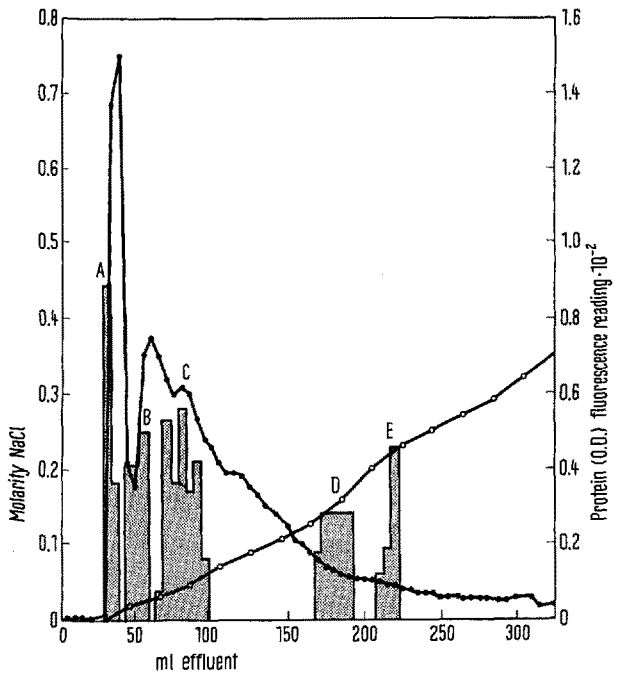

Fig. 1. Chromatography of 'soluble' antigens from rabies virus infected cells on Ecteola cellulose. —●— protein (O.D.), —o— molarity NaCl. Dashed areas: fluorescent precipitating antigen.

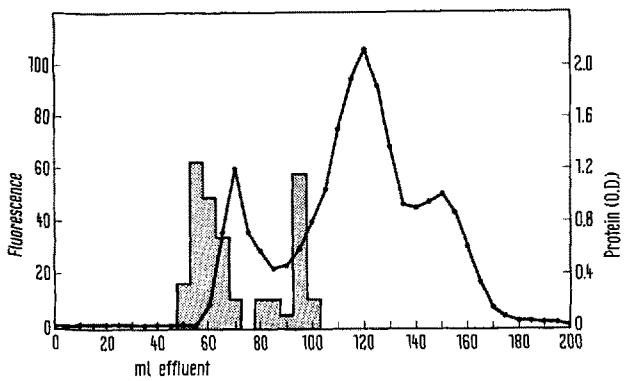

Fig. 2. Gel-filtration of 'soluble' antigens from rabies virus infected cells on Sephadex G-200. For symbols see Figure 1.

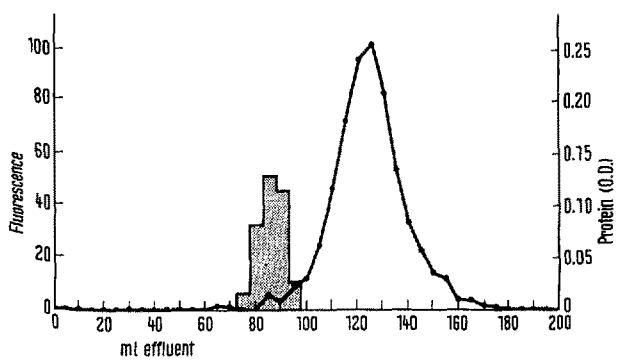

Fig. 3. Gel filtration of antigen(s) corresponding to peak A (Figure 1) on Sephadex G-200. For symbols see Figure 1.

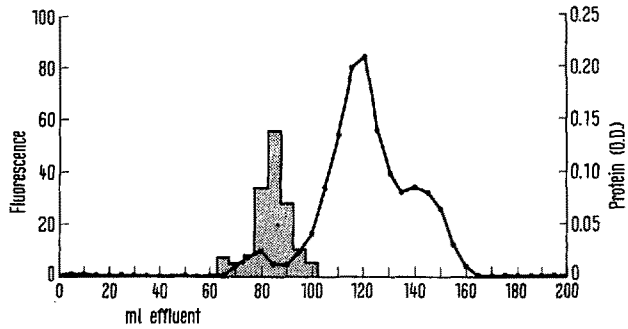

Fig. 4. Gel filtration of antigen(s) corresponding to peak B (Figure 1) on Sephadex G-200. For symbols see Figure 1.

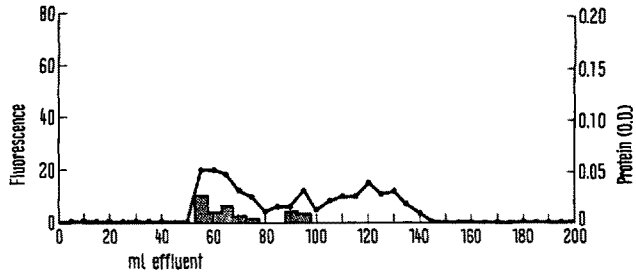

Fig. 5. Gel filtration of antigen(s) corresponding to peak E (Figure 1) on Sephadex G-200. For symbols see Figure 1.

The original antigen preparation (3 ml) and pooled fractions corresponding to each peak of precipitating antigen separated by chromatography on Ecteola cellulose were submitted to gel-filtration on columns (dia 3.4 · 38 cm) of Sephadex G-200 (Pharmacia, Uppsala). A 0.14 *M* solution of NaCl in TB was used for elution. Fractions were collected and analyzed as described for chromatography on Ecteola cellulose. With the original preparation, 2 classes of antigens could be separated from each other and from the bulk of tissue culture fluid proteins (Figure 2). Gel-filtration of antigens previously separated on Ecteola cellulose revealed that those eluted by solutions of consecutively increased ionic strength had relatively greater proportions of higher molecular weight antigens (Figures 3–5).

The present results, like those obtained with rabies-infected mouse brains using different experimental techniques[6], indicate the complexity of rabies virus 'soluble' antigens and offer a useful approach for elucidating their immunological relationship to the virion.

Zusammenfassung. Chromatographie an Ecteola-Cellulose ermöglicht die Trennung von 5 «löslichen» immunopräzipitierenden Antigenen aus einer mit Tollwut-Virus infizierten Zell-Kultur-Flüssigkeit. Gel-Filtration derselben an Sephadex G-200 führt zur Abtrennung von zwei Antigen-Klassen, die sich in ihrer Grösse unterscheiden. Die Kombination beider Verfahren ermöglicht eine weitgehende Reinigung der Antigene.

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20th March 1967.

[6] T. H. Mead, J. gen. Microbiol. *27*, 415 (1962).