## Isolation of Two Antigenic Fractions from Hydatic Fluid of *Echinococcus granulosus*

Hydatid disease caused by the *Echinococcus granulosus* is widely spread in Argentina. An important factor which interferes in the laboratory diagnosis of this disease is the lack of an antigenic preparation which does not give rise to false positive reactions, both serologically and in the intradermic test of CASONI. Another difficulty is the variability in the results obtained in different laboratories using the same method and serum from the same patient.

It is obvious that these problems are due to the different constituents of the cyst used as the source of antigens (hydatid fluid, scolex, germinative membrane) and also to differences in the composition of cyst harvested from a given species[1-6]. The variable antigenic activity and lack of specificity cannot be resolved with a simple chemical standardization of the crude antigenic material. A more reasonable approach is the purification and isolation of the antigen(s) concerned. It was recently reported that an antigenic fraction active in hemagglutination can be successfully prepared by column chromatography of hydatid fluid[7].

In this paper we report the isolation by column chromatography of 2 antigenic fractions employing hydatid fluid (HF) of ovine cyst as starting material. HF was chosen because previous work, confirmed in our laboratory, showed that it contains more antigenic activity than the scolex and the germinative membrane[8,9].

The first problem to be solved was the selection of a buffer system for column chromatography which would not result in loss of antigenicity. To this end ovine HF was concentrated by ultrafiltration 10–20 fold and divided into samples which were dialysed extensively against various buffers as indicated in Table I. The dialysed samples were centrifuged at 1200 *g* for 20 min.

Table I. Comparison of the effect of various buffer systems on the antigenicity of hydat difluid

| Buffer | Development of precipitate | Antigenicity of supernatant | |
|---|---|---|---|
| | | HA[a] | I.D.R.[b] |
| Phosphate 0.0175 pH 6.4 | +++ | 1/20 | ++ |
| Phosphate 0.0175 pH 7 | +++ | 1/20 | ++ |
| Phosphate 0.0175 pH 7.4 | +++ | 1/30 | ++ |
| *Tris*-HCl 0.01 pH 7.4 | 0 | 1/40 | +++ |

[a] Titers in passive hemagglutination (HA) using tanned sheep red cells and serum of a patient with hydatidosis. [b] Intradermo reaction at 15 min in a patient with hydatidosis employing 0.1 ml of test material. 0, negative; ++++, maximal response.

Table II. Antigenic capacity of active fractions obtained by column chromatography and of unseparated hydatid fluid

| Fraction | IDR | HA |
|---|---|---|
| IV | ++++ | 0 |
| VII | + | 1/32 |
| Total HF | ++ | 1/20 |

For explanations see legend to Table I.

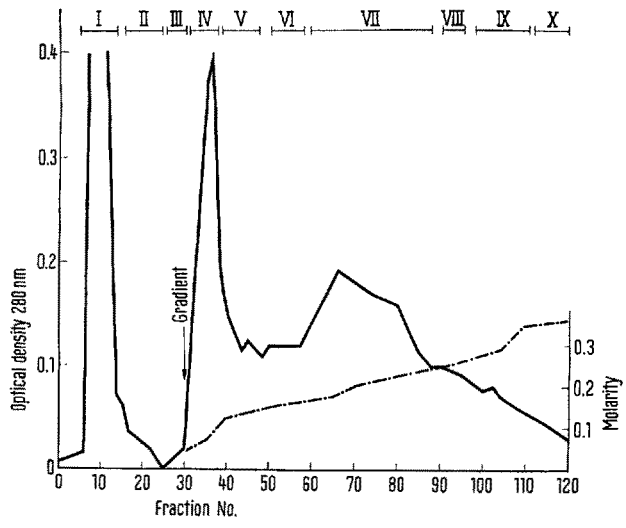


Fig. 1. Fractions of hydatid fluid obtained by DEAE-Sephadex G 50 column chromatography employing a molarity gradient elution system. Fractions were pooled as indicated, dialysed against 0.15 *M* sodium chloride and concentrated.

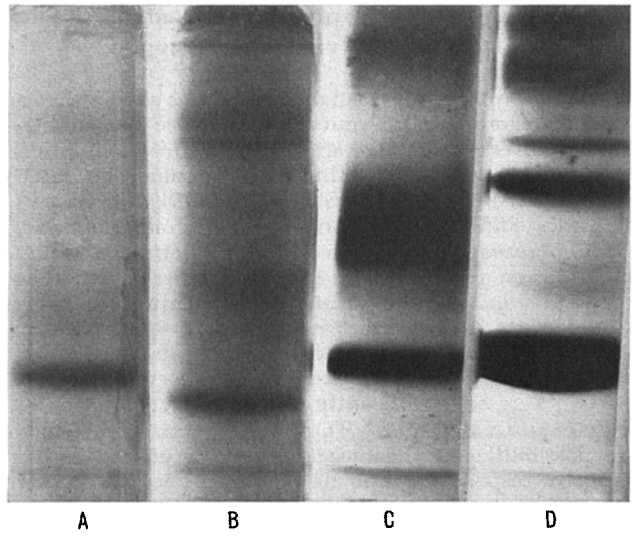


Fig. 2. Acrylamide gel electrophoresis of fraction VII (A), fraction IV (B), concentrated total hydatid fluid (C) and normal human serum (D).

[1] A. GRAÑA, Trop. Dis. Bull. *43*, 139 (1946).
[2] H. J. BENSTED and J. D. ATKINSON, Lancet *264*, 265 (1953).
[3] G. A. GARABEDIAN, R. M. MATOSSIAN and A. Y. DJANIAN, J. Immun. *78*, 269 (1957).
[4] L. NORMAN, E. H. SADUN and D. S. ALLAIN, Am. J. trop. Med. Hyg. *8*, 46 (1959).
[5] I. G. KAGAN, D. S. ALLAIN and L. NORMAN, Am. J. trop. Med. Hyg. *8*, 51 (1959).
[6] I. G. KAGAN and L. NORMAN, Am. J. trop. Med. Hyg. *12*, 346 (1963).
[7] L. NORMAN and I. G. KAGAN, J. Immun. *96*, 814 (1966).
[8] L. CASTAGNARI and A. TOLU, Policlinico (Sez. Medica) *71*, 395 (1964).
[9] M. N. HARIRI, C. W. SCHWABE and M. KOUSSA, Am. J. trop. Med. Hyg. *14*, 592 (1965).

The antigenicity of the supernatants was tested by the passive hemagglutination method (HA)[10] and by the CASONI intradermo reaction test (IDR)[11]. The results (Table I) indicated that the precipitate appearing in presence of phosphate buffer was accompanied by a diminution in the antigenic activity of HF.

Concentrated HF dialysed against *Tris*-HCl 0.01 *M*, pH 7.4 buffer was placed on a 20 · 1.5 cm column packed with DEAE-Sephadex A 50 medium, equilibrated with the *Tris*-HCl buffer. Elution was performed with a 0.01–0.4 *M* NaCl gradient. The chromatographic fractions were pooled as indicated in Figure 1, concentrated and tested for HA and CASONI reaction.

Maximal antigenic activity in the HA test was found in fraction VII and in the IDR in fraction IV as summarized in Table II. HF fraction IV and fraction VII were analysed by electrophoresis in polyacrilamide gel[12] and by inmuncelectrophoresis[13] using rabbit antisera against human serum, ovine serum, human IgG, and IgA. Gel electrophoresis revealed 4 components in fraction IV and only one component in fraction VII (Figure 2). The latter has a mobility similar to albumin.

By inmunoelectrophoresis it was shown that fraction IV contained IgA. Further characterization of the components present in the antigenic fractions is in progress.

*Resumen.* Utilizando columna cromatográfica con DEAE-Sephadex A-50 medium, pudimos aislar dos importantes fracciones antigénicas a partir de liquido hidático. Una (IV) con gran actividad en reacción intradermica y otra (VII), altamente purificada con gran actividad en hemaglutinación pasiva.

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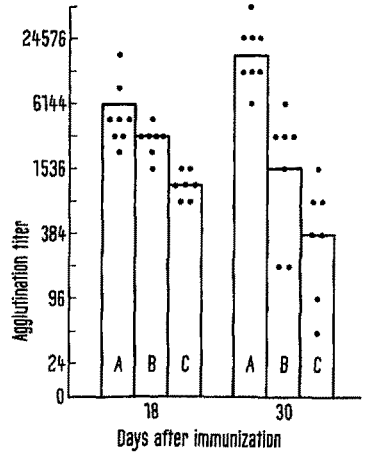
[10] J. EJDEN and A. LANARI, Medicina, B. Aires *21*, 165 (1961).
[11] T. CASONI, Folia clin. chim. microsc., Bologna *4*, 5 (1911).
[12] B. J. DAVIS, Ann. N.Y. Acad. Sci. *121*, 404 (1964).
[13] J. J. SCHEIDEGGER, Int. Archs Allergy appl. Immun. *7*, 103 (1955).
[14] Career investigator of Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

## Potentiation of the Immunosuppressive Effects of Cytoxan by Hyperbaric Oxygen

Previous work from this laboratory[1] has shown that a regimen of long-term intermittent hyperbaric oxygenation (HPO) exerts a protective effect on mice infected with a leukemogenic virus. Also, this HPO treatment has been demonstrated to cause a transient decrease in size of thymuses and spleens of otherwise normal animals[2]. These observations suggested that the effect of HPO might be on proliferation of lymphoid tissue and that hyperbaric oxygenation, alone, or in conjunction with other agents, might exert a depressing effect on the immune response. In subsequent experiments it was noted that circulating antibody titers to sheep red blood cells (SRBC) were not significantly affected by HPO in normal or cytoxan-treated mice[3]. These findings did not exclude the possibilities of demonstrable oxygen effects on the antibody response of animals immunized with different types of antigenic stimuli. The results of such a study employing bovine serum albumin (BSA) in complete Freund's adjuvant as the antigenic system are described in the present report.

The HPO regimen employed has been described in detail[1,2] and, briefly, was as follows. Five-week-old female Balb/c/jax mice were subjected to 97% oxygen in 3.4 atmospheres absolute pressure for 30 min periods, twice daily, 5 days per week. Temperature inside the chamber was maintained at 70 °F ± 3° by means of a thermostatically controlled water jacket surrounding the chamber. The hyperbaric oxygen regimen was started on the day of immunization, several hours after cytoxan injection, and was continued throughout the experiment. No discomfort or convulsive behavior was observed either during or following oxygen treatment. Mice were immunized by a single i.p. injection of 0.25 ml of a complete Freund's adjuvant (Difco Laboratories, Detroit) emulsion containing 1.25 mg of bovine serum albumin (Fraction V, crystalline, Pentex). Four hours after immunization, cytoxan (Cyclophosphamide, Meade-Johnson) was administered as a single i.p. injection of 80 mg/kg weight. Antibody titers were determined on two-fold serial dilutions of individual mouse sera using the tanned, antigen-coated erythrocyte method of STAVITSKY[4].

As can be seen in the Figure, the suppressive effects of cytoxan were significantly augmented by hyperbaric oxygen treatment, the greatest suppression appearing after 30 days, when control antibody levels were at a maximum. Hyperbaric oxygen treatment of immunized mice not receiving cytoxan resulted in titer values




Agglutinin responses to BSA in complete Freund's adjuvant, expressed as reciprocal titers, in Balb/c/jax mice. Each circle (●) represents serum from a single animal, while bar heights represent mean titers for each group. (A) immunized only, (B) immunized plus cytoxan, (C) immunized plus cytoxan plus HPO.

[1] B. LIBET and B. V. SIEGEL, Cancer Res. *22*, 737 (1962).
[2] B. V. SIEGEL, Aerospace Med. *38*, 399 (1967).
[3] B. V. SIEGEL, Life Sci. *6*, 1009 (1967).
[4] A. B. STAVITSKY, J. Immun. *72*, 360 (1954).