whether this selection eliminates cells genetically sensitive to the treatment or if the lost cells simply represent aging cells or cells already damaged by *Mycoplasma* infection.

If this second hypothesis is true, it could well explain why, after this treatment, the growth characteristics, the virus sensitivity, and the morphology of the surviving cells do not appear to deviate from described prototypes.

The results indicate that, when *Mycoplasma* isolation is performed in microaerophylic conditions, simple antibiotic treatment of infected cultures is not an effective cure. This cure appears to be effective only after hypotonic-antibiotic treatment.

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Circumoval Precipitins: Localization by Gel Filtration in Serum of Humans Infected with *S. mansoni.* (29736)

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The circumoval precipitin test(1) is based on the immunochemical reaction occurring between an antigen present in *Schistosoma mansoni* ova and a specific antibody present in the serum of the infected animal or human. The precise location of the antigen in the egg has not been clarified.

The antibody present in humans has been found to migrate in paper electrophoresis with the gamma globulin fraction. A positive circumoval reaction is given by the gamma globulin fraction separated upon fractionation of sera of infected patients by continuous flow paper electrophoresis and starch electrophoresis techniques(2,3). Its concentration in sera seems to be directly related to the severity of the infection(4). No information concerning the size of this antibody, however, was available. Since it was desirable to perform further physical chemical characterization of the antibody, this was attempted using the gel filtration technique which is based on the molecular sieve principle.

Materials and methods. Sephadex G-200, with a particle size of 40-120 μ and a water regain of 20 $\pm$ 2 g/g was obtained from Pharmacia Fine Chemicals, N. Y., and used in preparation of the molecular sieves or columns. These were prepared following the method described by Roskes and Thompson (5). Columns used had dimensions of 2.5 $\times$ 45.0 cm, a void volume of 65 ml, a gel bed of about 160 ml, and rate of flow of 18.0 ml per hour.

Sera obtained from 6 patients infected with the parasite were collected and the fractionation accomplished not later than a week after collection. All patients had marked chronic infections of *S. mansoni* as shown by a strongly positive (4+) circumoval precipitin test. Two ml samples of undiluted serum were applied to the columns and 1.7 ml fractions recovered with the aid of an automatic fraction collector.

Fractions showing the highest protein concentration in each peak were pooled and lyophilized. Circumoval tests were performed after dilution to 1 ml and dialysis for 18 hours against 0.15 N saline. The test was conducted essentially as described by Oliver-González(1). Ova used for the tests were recovered from livers or intestines of infected mice as described by Toro-Goyco *et al*(6). Protein concentration was determined using a Beckman DU Spectrophotometer and measuring the absorption at 280 mu. A single analytical ultracentrifuge run and 3 diffusion experiments were performed on the serum fraction containing the anti-egg antibody. A well-known Spinco Model L Ultracentrifuge was used for the ultracentrifuge experiment. Diffusion was performed using a bell-shaped porous diaphragm cell.

Results and discussion. The recovery of protein after elution through the column was from 98 to 100%. A standard pattern was obtained; 3 main peaks appeared as estimated by absorbancy measurements. The first peak, appearing with the void volume, contained the beta lipoproteins and macroglobulins; the second the 7 S globulins and the third peak contained the 4 S proteins (mainly albumin). The pattern difference from that of normal human sera was shown by a second peak (B) which is higher than the third peak (C). (See Fig. 1).

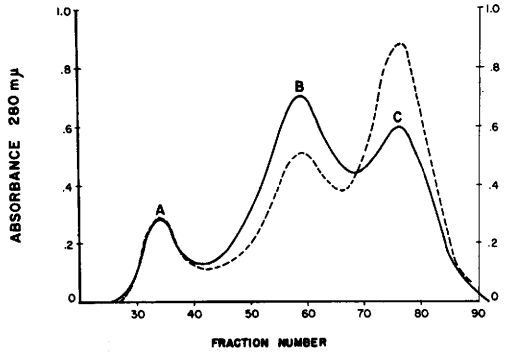

FIG. 1. Sephadex G-200 gel filtration pattern of serum. Broken line = normal serum; solid line = serum of individual showing a strongly positive circumoval reaction. Antibody is found in peak B.

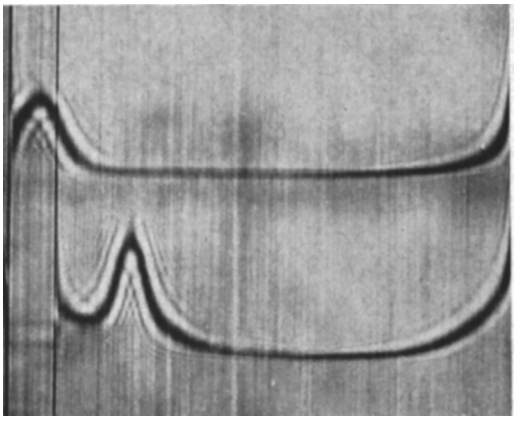

FIG. 2. Ultracentrifugal pattern. Lower tracing shows serum fraction containing the anti-egg antibody. Upper tracing shows enzyme preparation used to destroy the immunologic properties of the antibody. Direction of migration from left to right. Picture taken 24 min after centrifuge attained full speed.

In a series of tests performed with the fraction (peaks) from the sera of 6 different patients, only the B peak gave a positive circumoval reaction. Analytical ultracentrifugation of this fraction yielded a single moving component with a sedimentation coefficient $S_{20,\,w}$ of 6.6 S (Fig. 2). Proteins in this fraction have an average diffusion coefficient ($D_{20,\,w}$ of 3.0 $\pm$ 0.2 as determined by diffusion using a bell-shaped porous diaphragm cell.

Incubation of the positively reacting protein fraction with pinguinain* at 37°C for 2 hours prior to the performance of the circumoval test resulted in a negative circumoval reaction. One-tenth ml of the enzyme solution having a concentration of 10 mg/ml was added to the protein fraction containing

* Pinguinain is a very potent proteolytic enzyme isolated from the fruits of the spiny tropical plant *Bromelia pinguin* L. One-tenth ml of pinguinain solution containing 10 mg/ml was added to the protein fraction containing the anti-egg antibody. The homogeneity of the enzyme preparation used in this experiment is well illustrated by Fig. 2.

the anti-egg antibody. This result was observed upon incubation in 0.1 M acetate buffers (pH's 4.0 and 4.6). Incubation in 0.1 M phosphate buffers (pH's 6.6 and 7.3) showed the same result but required a longer time of hydrolysis. The pH of fractions incubated in acetate buffers was brought back to about 7.4 by addition of dilute NaOH prior to performance of the circumoval test. These results indicate that the anti-egg precipitin present in humans infected with *S. mansoni* is a gamma globulin with a sedimentation coefficient of 6.6 S; an approximate molecular weight calculated from sedimentation and diffusion data close to 200,000; and its physical chemical architecture and chemical integrity are needed to yield a positive circumoval reaction.

Summary. The gel filtration technique was used to fractionate samples of sera from 6 individuals infected with *S. mansoni*. The gel used, Sephadex G-200, allows the separation of serum into 3 fractions. The individuals studied showed a marked increase in the 7 S globulins. The specific antibody which was present in the serum of these individuals and which has been found to react with the *S. mansoni* ova yielding a positive circumoval precipitin test was found to be a normal size gamma globulin with a sedimentation coefficient of 6.6 S and a molecular weight close to 200,000. Partial enzymatic hydrolysis of this protein was found to alter its immunologic properties, rendering the circumoval test negative after incubation with reactive ova, indicating that the chemical integrity of this protein is needed to yield a positive circumoval reaction.

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Electrolyte and Water Exchanges in the Estrogen Treated Ovariectomized-Adrenalectomized Rat.* (29737)

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The reductions in fluid exchange and electrolyte excretion obtained in diethylstilbestrol (DES) treated ovariectomized rats has been attributed primarily to a decrease in food ingestion(1). However, a reduction in food intake cannot account for the depression of the urinary Na:K ratio observed, for pair-feeding, alone, did not affect the ratio. While this finding indicates a possible specific DES effect, direct or indirect adrenal cortical involvement was not excluded. Other investigators have found that electrolyte excretion in adrenalectomized rats is not altered by low or moderate doses of estrogens(2,3,4). Deming and Luetscher(2) obtained a sodium retaining effect only when the estradiol was administered in relatively large doses. In the experiments with adrenalectomized rats, the observation period was limited to 3 to 7 hours after estrogen administration. Others have obtained positive results in intact species(5,6,7,8,9,10) only after a more prolonged period of treatment(18-19 hours).

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