In contrast to these findings on fresh stool, Goldman and Brooke (1953) claim that trophozoites are to be found in faeces preserved with polyvinyl alcohol (P.V.A.), though it is not clear from their report whether *E. histolytica* is included in this category; and Brooke, Donaldson, and Brown (1954) recommend P.V.A. fixation for trophozoites in conjunction with formol-ether concentration for cysts and ova. We have not so far had much success with P.V.A. fixation, partly perhaps because stools sent to this laboratory are all relatively fresh (they are examined within two to three hours, unlike Goldman and Brooke's material) and partly because the incidence of trophozoites in our present material is low.

There appears to be no simple solution to the problem of finding *E. histolytica* trophozoites in chronic infections. Occasionally ulcers (with numerous trophozoites) are seen at sigmoidoscopy, though the parasites are not to be found in repeated stool examinations.

Summary

The value of the formol-ether method of concentrating faeces is emphasized as a diagnostic routine for intestinal parasites. A simplified technique is described, which takes only five minutes to perform.

All cysts and ova are concentrated without distortion. Faecal cysts were found nearly twice and ova three times as often as by direct searching.

Although the concentration procedure destroys free-form amoebae and cellular exudate, additional direct examinations of formed stools free of blood and mucus produced no new findings in this series of cases.

We are grateful to the physicians of the Hospital for Tropical Diseases, Sir Neil Hamilton Fairley, Sir George McRobert, and Professor A. W. Woodruff, for their co-operation which made this trial possible. We are also greatly indebted to the technicians who have conscientiously done so much of the work: Mr. A. V. H. Allen, Mr. C. Abrahams, Mr. E. R. Hesterman, and Miss L. Lowy.

REFERENCES

- Brooke, M. M., Donaldson, A. W., and Brown, E. (1954). *Amer. J. trop. Med. Hyg.*, **3**, 615.
 Faust, E. C., D'Antoni, J. S., Odom, V., Miller, M. J., Peres, C., Sawitz, W., Thomen, L. F., Tobie, J., and Walker, J. H. (1938). *Amer. J. trop. Med.*, **18**, 169.
 — Sawitz, W., Tobie, J., Odom, V., Peres, C., and Lincicome, D. R. (1939). *J. Parasit.*, **25**, 241.
 Goldman, M., and Brooke, M. M. (1953). *Publ. Hlth Rep. (Wash.)*, **68**, 703.
 Ritchie, L. S. (1948). *Bull. U.S. Army med. Dept.*, **8**, 326.
 — Pan, C., and Hunter, G. W. (1952). *J. Parasit.*, **38** (Suppl. to No. 4), p. 16.
 Wykoff, D. E., and Ritchie, L. S. (1952). *Ibid.*, **38** (Suppl. to No. 4), p. 15.

The Concentration of Antigen for the Complement-fixation Test for Toxoplasmosis

BY

S. C. MARSHALL AND R. PILLINGER

From the Bacteriology Department, Sheffield University

(RECEIVED FOR PUBLICATION JUNE 24, 1955)

The antigen commonly used in the complement-fixation test for toxoplasmosis is made from infected egg membranes (Sabin, 1949), and it is difficult to prepare it free of blood pigments. The antigen is stored at -20°C .

It was seen that on thawing the antigen the blood pigments were concentrated at the bottom of the container, and it was hoped that this separation might be used in making an effective antigen relatively free from blood pigments. This would be possible only if the antigen were concentrated at a different level from blood pigments.

Method

To determine whether or not this was possible, 31 ml. of antigen was prepared and stored at -20°C . in two parts, one of only 1 ml. and the other as a column of 30 ml. The smaller part was left as a sample of the original preparation. After 18 hours at -20°C . the sample and column were thawed slowly at room temperature. The column was carefully divided into upper and lower halves, each of which was then mixed before taking a 1 ml. sample of each. This left two portions of 14 ml. each which were again frozen in a column for 18 hours at -20°C . The thawing, dividing, sampling, and refreezing process was repeated four times in all. Finally, there were 31 samples, all of which had been frozen and thawed four times.

The samples were tested for haemoglobin colour, antigenic potency, specific gravity, and sodium chloride concentration as follows:

Index of Colour.—The samples were viewed against a white background and arranged in order from 1 to 31, varying from colourless to deep red.

Antigenic Potency.—Complement-fixation tests were set up with each sample in dilutions of 1 in 4, 1 in 8 . . . 1 in 256 against a dilution of positive pool human serum containing 4 units of antibody per unit volume and 1.5 M.H.D. of Richardson preserved guinea-pig complement. The haemolytic system (3% sheep cells: 5 M.H.D. amboceptor) was added after the tests had been standing for 18 hours in the refrigerator at 4°C . Readings were made after incubating in a 37°C . water-bath for 30 minutes.

Specific Gravity.—This was determined by comparing the weight of a standard volume of each sample against

TABLE
RELATIONSHIP OF 30 SAMPLES TO ORIGINAL TOXOPLASMA ANTIGEN

Index of colour ..	..	..	21*
Antigenic potency ..	..	..	100
Sodium chloride ..	..	..	100
Specific gravity ..	..	..	100

* With the exception of the index of colour, the results are expressed as a percentage of those obtained on the original specimen. They read in the same vertical order. † — = Insufficient material for test.

the weight of the same volume of distilled water. Fluid was delivered into a dry, marked glass container to avoid loss by "wash" caused by the use of a volumetric pipette. The same container was used throughout.

Sodium Chloride Concentration.—Titrations were made with a standard silver nitrate solution using potassium chromate as indicator.

Our results are recorded in the accompanying table.

Conclusions

Toxoplasma antigen can be concentrated by selective freezing and thawing.

As well as the antigen, other constituents of the membrane extract are concentrated to an equal extent by this method.

These results draw attention to the necessity for thoroughly mixing antigen which has been kept at -20°C . before using it for testing.

We wish to thank Professor C. P. Beattie for his encouragement and Dr. J. K. A. Beverley for much helpful advice.

REFERENCE

Sabin, A. B. (1949). *Pediatrics*, **4**, 443.