Since, however, albumin and globulin values obtained by electrophoresis are likely to become more widely used in clinical work, and since Majoor's method gives results said to be closer to those of electrophoresis than Howe's method, this alone would seem to be a good reason for employing Majoor's.

Summary

Howe's (using 22% sodium sulphate concentration) and Majoor's (using 26% sodium sulphate concentration) methods for differential plasma protein estimation were compared by means of parallel assays on a series of normal and pathological plasmas.

We found that there is little advantage in one method over the other, because the reproducibility of results is identical, the normal ranges are equal, and the separation of results into diagnostic patterns is only slightly better when Majoor's method is used. The arithmetic difference between the two albumin levels is relatively constant in the conditions studied, and therefore either albumin level is reasonably predictable from the other.

Since Majoor's method is said to give results closer to those of electrophoresis than Howe's, this, together with the slight advantage indicated by our own results, would seem to be a good reason for employing Majoor's method.

We wish to thank Miss Elizabeth Lahey, B.Sc., for carrying out some of the analyses, and Dr. W. I. Cranston for statistical advice.

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A.C.P. Broadsheets

The notice concerning the price of the broadsheets on page 354 of the previous issue has proved misleading. For the first 25 copies the price is 1s. each broadsheet, and thereafter 9d. for each broadsheet.

The Value of Formol-Ether Concentration of Faecal Cysts and Ova

BY

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It is recognized that protozoal cysts and helminth ova will be detected in stools more frequently if they have been concentrated before the search is made. Of the methods used for the recovery of both cysts and ova, the formol-ether technique (Ritchie, 1948) avoids the faults in the popular zinc sulphate centrifugal flotation method (Faust, D'Antoni, Odom, Miller, Peres, Sawitz, Thomen, Tobie, and Walker, 1938; Faust, Sawitz, Tobie, Odom, Peres, and Lincicome, 1939) that cysts are liable to distortion while some ova are too heavy to be brought to the surface. The formol-ether method has been evaluated (Wykoff and Ritchie, 1952) and compared favourably with the zinc sulphate method (Ritchie, Pan, and Hunter, 1952); the object of this paper is to draw attention to its value as a routine diagnostic measure and to describe a simplified procedure.

Method

Stools from 670 unselected patients referred to this hospital were examined directly and again after concentration and the findings compared. For the direct examination the whole of one microscope cover-slip was searched carefully for cysts and ova; a concentrated specimen was then searched in the same way by the same technician. A number of highly skilled technicians were employed, all of whom had received at least six months' whole-time training in the searching of stools, and who, in most cases, had had two or more years' experience.

The simplified formol-ether concentration is carried out as follows:

About 1 g. of faeces is thoroughly emulsified with about 7 ml. of 10% formol-saline, and strained through wire gauze (40 mesh per inch) into a centrifuge tube. Ether, 3 ml., is added and the mixture shaken vigorously for one minute. It is then centrifuged, accelerating slowly and gradually over a period of two minutes to a speed of 2,000 r.p.m., and then allowed to come to rest. The debris on the surface and at the interface between the two liquids is loosened from the wall of the tube with a stick and the supernatant is decanted, the last drop or two being allowed to run back. The upper part of the tube is wiped clear of fatty debris. The small deposit is shaken up and poured on to a slide. Some practice is required to obtain optimum results.

Results

The concentration achieved is of the order of 20 to 30 times. In our experience the results are as good as those given by the rather more complex technique described by Ritchie (1948) which makes use of a larger quantity of faeces, as good as the acid-ether method for Schistosome ova, and better than the zinc sulphate method for ova in general; for the concentration of cysts the formol-ether method has the advantage over others that there is a complete absence of distortion.

TABLE I

COMPARISON OF DIRECT EXAMINATION AND OF CONCENTRATION OF STOOLS IN 670 UNSELECTED PATIENTS

	Number of Cases Found	
	By Direct Search	After Formol-ether Concentration
Cysts		
<i>Chilomastix mesnili</i> ..	0	2
<i>Entamoeba coli</i> ..	38	63
<i>E. histolytica</i> (large race) ..	17	24
.. (small " ") ..	6	27
<i>Endolimax nana</i> ..	20	37
<i>Giardia lamblia</i> ..	25	32
<i>Iodamoeba bütschlii</i> ..	4	6
Total cysts ..	110	191
Ova		
Ascaris ..	4	7
Ancylostoma ..	2	15
Hymenolepis ..	0	2
Schistosoma ..	3	7
Taenia ..	4	5
Trichostrongylus ..	1	4
Trichuris ..	17	65
Total ova ..	31	105
Larvae		
Strongyloides ..	3	4

The results of the survey, given in Table I, indicate the general value of the technique. In all of the 15 groups more cases were detected in the concentrated than in the direct specimens; in not a single instance was any cyst or ovum found by direct examination which was not recovered also by concentration. (In one case, Schistosome ova were found directly in surface scrapings but not by concentration of the substance of the stool.) In addition to the ova included in this series, those of *Clonorchis sinensis*, *Heterophyes*, *Paragonimus*, and *Strongyloides stercoralis* have been concentrated at different times; the technique is effective for both fertile and unfertile ascaris ova. *Hymenolepis nana* seem to be the least satisfactory ova to concentrate, though two cases were picked up in this series.

In a series of 33 cases of untreated schistosomiasis (*S. mansoni*) in which activity was proved by the finding of live ova or by a positive complement-fixation test in the presence of dead eggs, two concentrated stools and multiple rectal snips were examined in all cases. Live ova were detected at least as frequently in stools (14 cases) as in rectal snips (11 cases), although the latter often revealed dead ova encapsulated in fibrous nests which were not passed in the faeces.

Amoebic Trophozoites.—Unfortunately, the formol-ether method suffers from the failing of most concentration procedures, that free-form amoebae and cellular exudate are destroyed; it is evidently necessary therefore to make direct examinations of many stools in addition to concentration. But about half the stools received for examination at this laboratory are well-formed specimens with no macroscopic trace of blood or mucus. A trial was made to determine whether direct microscopy in these cases would reveal cellular exudate or vegetative forms of *E. histolytica*.

For this purpose, all patients attending the hospital were divided into two groups. In 462 consecutive cases referred to one physician, one stool was examined both directly and after concentration in all cases; in those admitted to the wards a second specimen was dealt with similarly. Concurrently, in 471 cases referred to two other physicians soft or liquid stools or specimens in which blood, pus, or mucus was visible macroscopically were examined directly and after concentration, but well-formed stools were examined by concentration alone. (Selected cases were given multiple examinations which are not recorded.) The results are given in Table II.

TABLE II

CONCENTRATION OF STOOLS WITH AND WITHOUT DIRECT EXAMINATION OF FORMED SPECIMENS

	No. of Cases Examined	<i>E. histolytica</i> Cysts		<i>E. histolytica</i> Trophozoites		Cellular Exudate	
		No. of Cases	%	No. of Cases	%	No. of Cases	%
All specimens examined direct and after concentration							
1st specimen	462	29	6.3	3	0.6	10	2.2
2nd specimen	129	3	2.3	0		2	1.5
Formed specimens concentrated only. Rest direct and concentration							
1st specimen	471	38	8.1	1	0.2	12	2.5
2nd specimen	228	3	1.3	0		4	1.8

No distinction is made between large and small races of *E. histolytica*. The findings in the second specimens refer only to results not observed at the first examination.

It will be seen that the total incidence of *E. histolytica* is the same in the two groups (9.2% and 9.6%); the incidence of cellular exudate is not increased by microscopy of formed stool (3.7% against 4.3%). Unfortunately the incidence of trophozoites is too low to be significant; in the whole series of 933 patients only four cases were found, all of them from soft specimens in which blood or mucus was visible macroscopically. It would appear, therefore, to be unprofitable to search clean formed stools directly, at least in this country, unless perhaps one were looking for trophozoites of relatively non-pathogenic protozoa. This had been our impression from previous experience.

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.

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

BY

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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