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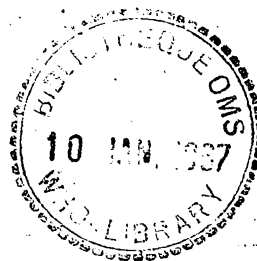
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(Avec résumé
en français)

THE USE OF HYPOTONIC SALINE TO INCREASE THE CONCENTRATION OF
PLASMODIUM FALCIPARUM GAMETOCYTES IN BLOOD SAMPLES

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INTRODUCTION

The defects of the standard 100-field examination of the thick blood film to detect asymptomatic, low-grade malaria infections, particularly among immune or semi-immune people, has been stressed by Dowling & Shute (1965), who also drew attention to the significance of this deficiency in the late stages of malaria eradication campaigns; when the detection of potential sources of infection is of paramount importance. Muirhead-Thomson (1954) showed that vector anopheline mosquitos were able to become infective after feeding on individuals whose blood was apparently negative for gametocytes by routine thick film examination, and Dowling & Shute, in their evaluation of their method, stated that the changes were about even that a competent microscopist, examining thick blood films, would record a zero gametocyte rate for the group under study, where a more intensive and prolonged search would show this to be as high as 20%. The true gametocyte rate of the group could possibly be even higher.

Worth (1954) demonstrated the possibility of concentrating malaria parasites by centrifuging, at high speed, samples of blood collected routinely in heparinized microcapillary tubes; but, despite the good results which he recorded with this method, the need for electricity to operate the centrifuge, and the difficulties

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encountered with the heparin anticoagulant, were considered by Dowling et al. (1966) to be factors which might lessen its value for routine field use. The introduction by Holmstedt (1965) of a portable, hand-operated, high-speed centrifuge dispensed with the need for electricity, but the rather complicated loading technique and the small number of specimens which this device could hold were thought to make it inconvenient for general field use in malaria practice.

Modifications to this centrifuge, by Dowling et al. (1966), simplified the loading and storage of the capillaries and greatly increased the number of specimens which could be spun at a time. Field trials using this redesigned centrifuge (the DRSM model), together with Sequestrene anticoagulant, which had been found to improve both the staining and the identification of parasites, resulted in a marked increase in the over-all positivity of the groups sampled; this increase being attributed to the greater numbers of Plasmodium falciparum trophozoites and P. malariae seen in the concentrate films. It was noted however that no species differential layering of parasites occurred in the centrifuged blood column and, also, that no increase in the numbers of P. falciparum gametocytes was found, although the general concentration factor achieved with other malaria parasites was between two and five times.

This paper describes a field technique by which gametocytes of P. falciparum are concentrated in centrifuged microcapillary blood samples following sedimentation in hypotonic saline. In addition a modified method of Giemsa staining is described which reduces the number of parasites lost from the thick blood films during their dehaemoglobinization in the aqueous stain.

METHOD

In initial experiments specimens of parasitized blood were sedimented through saline solutions of varying strengths, prior to centrifugation, to determine: (a) what degree of differential osmotic fragility, if any, exists between normal erythrocytes and those parasitized with different species and stages of human plasmodia; (b) whether or not any such difference could be used to separate, and so concentrate, the parasitized cells; (c) whether or not the osmotic effect of the hypotonic saline could be used to alter the specific gravity of the parasitized red

cells sufficiently for them to be separated, more easily, from normal red cells during centrifugation; and (d) what effect such saline immersion and centrifugation would have on the staining properties of the parasitized red cells.

Preliminary trials were carried out using suitable cases selected, by thick film screening, from five to nine year old schoolchildren, taking for each experiment: two thin and two thick blood films from finger-prick blood which were the controls, the duplicate thick and thin blood films being held in reserve in case of loss or damage to the originals, and one ml of blood by vene-puncture which was expelled into a sterile "Bijou" bottle containing 0.3 ml of sodium citrate-dextrose anticoagulant (see Annex).

Saline solutions. Using a stock 2.5% saline solution and distilled water a range of saline solutions was prepared in numbered test-tubes which were fitted with plastic sealing caps. This range extended from 0.3% up to 0.85% in steps of 0.05% and, in later experiments, was reduced to a series of 10 tubes (0.4-0.85%). Due to contamination with blood from capillaries and to the gradual growth of algae, fresh solutions were prepared for each experiment.

Sedimentation. In the laboratory the "Bijou" bottle was gently agitated to mix the blood and two thick and two thin films made direct from the bottle sample. These acted as anticoagulant controls, the duplicates again being held in reserve. Dry, serially numbered microcapillary tubes were then introduced into the bottle, one by one, and blood taken up to a predetermined point (see Annex), the bottle being gently agitated before each sampling. In this way a fairly constant volume of blood was ensured for the whole series. The outside of the capillary was wiped clean of excess blood and the tip inserted into the appropriate tube of saline, care being taken to see that the blood was kept at the end of the capillary at the moment of contact with the saline to avoid air locks.

Saline was drawn up until the tube was almost filled, a space of 3-4 mm being left to allow for the plasticine sealer. After sealing the bottom of the capillary this was then stood at an angle of 45° in a plasticine strip pressed to a glass sheet.

This angling of the capillaries served both to increase the rate of sedimentation and to facilitate the vertical separation of the lighter from the heavier red cells in the blood column. After 15 minutes the glass plate was reversed and stood upright on its edge, thus inverting the capillaries. Sedimentation was allowed to occur for a further 15 minutes after which, again, the capillaries were reinverted and allowed to sediment for a further 30 minutes. By this time almost all cells had settled into the lower half of the tube allowing it to be cut at the 50 mm mark for loading into the DRSM centrifuge.

During centrifugation maximum traction was avoided for fear of rupturing the osmotically-swollen parasitized cells and 100 pulls at moderate speed were used to pack down the cellular contents (see Annex). This gave a clear buffy coat. Tubes were removed and placed vertically in the plasticine strip and cut approximately 2 mm below the creamy platelet layer. A small drop of blood was taken on to a clean polished slide, the size of drop being controlled by tilting the slide to about 30-40° and lightly touching the cut end of the capillary against it. If this tilting was omitted the weight of the supernatant plasma/saline caused too large a drop of blood, often containing both blood and plasma, to be extruded, thereby reducing considerably the parasite concentration achieved.

Overnight desiccation of sub-buffy concentrate thick blood films was avoided for it was found that this made them powdery and much material was lost from them even before staining. (Since most of the experimental work described was carried out during the dry season in West Africa, this observation may not be valid for similar trials carried out under conditions of higher atmospheric humidity.) To avoid this all such films were kept in a slide box which was placed overnight in an empty desiccator dish on the bench at room temperature. This allowed them to dry naturally and prevented their destruction by ants.

Staining. The normal "control" films were stained by the standard Giemsa method. The concentrate films, and anticoagulant control films made directly from the treated venous blood, were stained with Giemsa solution made up in distilled water buffered to pH 7.8 (see Annex). This successfully overcame the pink discolouration

due possibly to the excess anticoagulant in the commercial microcapillaries, as reported by Dowling et al. (1966), and restored "normal" colour values. It was noted that the quality of Giemsa staining after saline immersion of films was enhanced, particularly with older stages of parasites.

RESULTS

Microscopic examination of all films was carried out using X 100 objective and X 7 eyepieces, 1000 oil immersion fields being examined for all thin films and 100 such fields for all thick films. To assess accurately the effect of the experimental method used, and particularly to obviate the effect of parasite loss during staining, only thin films were made from the concentrates and compared with the "normal" thin film controls. In experiment "A" (see Fig. 1) it was established that when capillaries were cut at the lower-middle level, following the method used by Dowling et al. (1966), maximum concentrations of P. falciparum trophozoites and P. malariae occurred at a saline strength of 0.55% (i.e. X 3.4 and 8.5 degrees of concentration respectively). In this same experiment similar tubes, cut at the upper-middle level, showed fewer P. malariae (X 3.1) but gave an increase in P. falciparum trophozoites (X 4.5) and showed a significant rise in the P. ovale count (X 8.0).

Unfortunately the sub-buffy levels were not explored at this stage, but it is distinctly possible, in view of the subsequent findings with P. falciparum gametocytes, that this level may yield even higher concentrations of P. ovale parasites, especially at slightly higher saline strengths. The application of this method to specimens of blood parasitized with P. vivax may permit higher concentrations to be obtained than those reported by Dowling et al. (1966) with P. cynomolgi.

In a later experiment with gametocytes of P. falciparum a thin film was made from the sub-buffy level of the spun column after sedimentation through 0.65% saline, and spread very rapidly (see Annex). This served to concentrate the white cells in a narrow strip along the film. Examination of this area showed 23 falciparum trophozoites and 191 gametocytes compared with six trophozoites and six gametocytes in the corresponding thin "control" film, i.e. concentration factors of X 3.8 and X 32 respectively. To confirm this finding a case with a lower P. falciparum

gametocytaemia was selected (three gametocytes per 100 thick film fields) and a series of 10 saline dilutions used. The normal thin film failed to show any gametocytes in 1000 fields, and on later re-examination, none in 5000 fields (exhaustive examination of the duplicate control thin film showed three gametocytes in 9000 fields).

The results of the concentrate series of thin films are shown in Fig. 2 (Experiment "B"). From this it can be seen that, at the optimum saline strength of 0.7%, 25 gametocytes in 1000 fields were recorded giving a minimum concentration factor of X 25.

A differential layering test using 0.65% saline (which had given the best results in a previous test) and the same blood sample revealed no gametocytes in the lower-middle, middle or upper-middle levels of the spun column, indicating that, under certain optimum conditions, gametocytes of P. falciparum can be differentially layered together with the white cells during centrifugation following saline sedimentation. Finally a number of plain 50 mm microcapillaries were filled with blood from the bottle sample and spun without sedimentation. Films were then made from the sub-buffy, middle and lower levels. The sub-buffy concentrate showed three gametocytes in 1000 fields, the middle layer film showed none and the lower layer film showed one in 1000 fields.

To test the ability to repeat this result with dry Sequestrenised microcapillary tubes, from finger-prick blood, the following experiment was carried out with a known falciparum gametocyte case. One thick blood film was taken as a control and one Sequestrene tube filled to the 40 mm mark, wiped and filled with 0.7% saline. Following sedimentation and centrifugation, as described, a thin film was made from the sub-buffy layer. The next morning both films were stained, the thick film with 3% Giemsa (pH 7.2) for 30 minutes and the concentrate thin film, after methanol fixation, by 10% Giemsa (pH 7.8) for 30 minutes. Examination of both films was confined to that part showing the highest density of white cells and revealed 23 gametocytes in 100 fields of the control thick film compared to 49 in 100 fields of the thin film; thus confirming that a concentration of between X 30 and X 40 was being achieved by this method with P. falciparum gametocytes.

Thick film problems. Thick films made from the upper levels of concentrated blood gave disappointingly poor results and showed obvious signs of "floating off" in the stain. To try to reduce this loss partial fixation of the thick films was attempted and, for the field trial, the following method of staining was adopted: duplicate thick films (marked "A") were also stained by this method, to act as stain controls.

Giemsa stain at 15% was made up in 10% methyl alcohol in distilled water and buffered to pH 7.8. Films were stained face down on a curved formica board, the stain being run gently underneath. After 30 minutes the films were very gently rinsed in a stream of distilled water and stood to drain and dry face down in the rack. In this way "floating off" of parts of the film was considerably reduced although the concentration of gametocytes attained (between X 6 and X 8) indicated that many of these parasites were still being lost; either as a result of floating off from the film or possibly by being ruptured during the staining process. The quality of staining of the concentrate thick films was considered excellent, with parasites standing out clearly against a clean white background and gametocytes and schizonts could be distinguished even when the white cells completely filled the field. It was found however that, when the capillary tubes were cut a little too high, the background was partially obscured with blood platelets and, because of this, the smaller ring-form parasites were difficult to distinguish. To reduce the numbers of both platelets and white cells in the final film the fact that gametocytes are not differentially layered during centrifugation, unless this is preceded by saline sedimentation, was utilized in the following manner.

Microcapillary samples of blood were spun at maximum traction with the DRSM centrifuge and then cut at the sub-buffy level, thus removing most of the white cells and platelets. By placing a dry, clean capillary against the end and tilting both tubes together the blood sample was transferred to the clean tube. Saline was then taken up and sedimentation and centrifugation applied. In this way the gametocytes were brought to the "sub-buffy" layer now comparatively free from white cells. This technique, involving tube-transfer and double spinning, was considered too time-consuming for routine field use and was not pursued further, although it succeeded in reducing the number of white cells per field without seriously affecting the parasite count.

The morphology and staining of the parasites in films made from other levels of the spun blood column were also found to be enhanced, although the compacting of the P. falciparum ring-forms might at first cause some confusion, as mentioned by Dowling et al. (1966). Pigment when present was always clear and unmistakable. It was noted also that no "rounded-up" forms of P. falciparum gametocytes occurred in concentrate films, their absence from both thick and thin concentrate blood films removing a possible source of confusion to the inexperienced microscopist.

FIELD TRIAL

For the first part of this trial 32 schoolchildren, between the ages of 14-17 years, were sampled. From each child two thick films were taken by finger prick, the duplicate series being numbered 1A-32A and used as controls for the modified staining technique. Care was taken to ensure that both blood drops and films were approximately the same size, and free from serum dilution by undue squeezing. The finger was then wiped with dry cotton-wool and a fresh drop of blood squeezed up. This was taken into a Sequestrenised capillary to the 45 mm mark, the tube wiped, filled with 0.7% saline and sealed at the bottom with plasticine.

On return to the laboratory inversion sedimentation was applied followed by centrifugation. Capillaries were cut 2 mm below the buffy coat and a small thick film spread with the drop taken on to the slide. Next the capillary was cut at the lower-middle level and a second thick film made on the same slide. All films were stained next morning, having been kept overnight at room temperature, and 100 oil immersion fields were examined from that part of each film showing the highest density of white cells.

Study of the results shown in Table 1 shows that:

- (a) the over-all positivity for P. falciparum gametocytes of the children sampled, in the "sub-buffy" films, was increased by 37.4%, i.e. from 9.4% (normal thick films) to 46.8% (concentrate thick films);
- (b) an increase in the positivity of this group (sub-buffy films) for microfilariae (Dipetalonema perstans) of 27.2%, i.e. from 6.2% to 34.4%;

(c) the over-all positivity of the group for malaria parasites was increased by 6.2%, i.e. from 78.2% to 84.4%.

In cases where the normal thick film showed slightly older rings of P. falciparum the concentrate thick film showed an improvement in the numbers of these forms. But, despite this and apart from the obvious improvement with gametocytes and microfilariae, the "sub-buffy" films seemed to show no advantage over the normal thick films. In nine cases they failed to detect P. malariae parasites found in the controls, but, on two occasions, they revealed infections with this species which were undetected in the normal control thick films.

If, however, the results of the first 50 fields examined, of the sub-buffy film, and the first 50 fields of the lower-middle layer film (on the same slide) are added together and compared with 100 fields of the normal thick film, the following facts emerge:

- (a) There was an over-all increase in the positivity of the group, for P. falciparum gametocytes, of 34.3% - i.e. from 9.4% to 43.7%.
- (b) There was an increase in the positivity of the group for P. malariae of 6.3% - i.e. from 40.6% to 46.9%.
- (c) The positivity of the group for microfilariae rose by 15.6% to 21.9%.

One interesting outcome of the trial was shown up by the results of the duplicate series of thick films, where the effect of the modified staining method alone produced an increase in falciparum gametocytes, over the normal control thick films, of 12.5%, raising the positivity of the group for this parasite from 9.4% to 21.9%. From Table 1 it can be seen that, although the average count per film of P. falciparum is only slightly increased (by 20%), that of P. malariae is increased by almost 50%. Three children shown as negative in the controls were found to be positive in the duplicate series. In one case, however, the control revealed a positive which was not recorded in the corresponding duplicate.

The lower-middle level concentrate films gave a poor result with falciparum gametocytes, as was expected, but both the P. falciparum ring-form and the P. malariae counts were noticeably raised; the average count per film of the latter

being increased by 300%. Two cases with P. malariae infections and one with a P. falciparum ring-form infection, all missed by the normal control thick films, were detected by concentrates from this layer. When the results of 50 fields of sub-buffy and lower-middle concentrate films are added together the average count per film with P. falciparum trophozoites is increased by just over 40%, while that of P. malariae is increased by 80%.

DISCUSSION

Among adults living in areas of tropical Africa, where malaria is endemic and where P. falciparum predominates, naturally acquired immunity tends to suppress the parasitaemia, often to a level which is submicroscopic to present methods of detection. In many such cases gametocytes continue to remain in the peripheral blood, often at a level too low for detection either by the standard thick film or by present field concentration methods. The ability to find these forms of the parasite, by which alone, in the presence of suitable vector anophelines, transmission is maintained, may have significance in the context of malaria eradication programmes and the technique described may also be of particular value in circumstances where a specific search is made for gametocytes of P. falciparum.

The filarial larvae detected were all of the same species (Dipetalonema perstans) and it is not known what effect, if any, this method might have in concentrating Mansonella ozzardi microfilariae or the larger, sheathed microfilariae of Wuchereria bancrofti, Brugia malayi and Loa loa.

Recent advances in field methods of concentrating malaria parasites have succeeded in increasing considerably the sensitivity of the thick blood film to detect low-grade malaria infections generally, but have been unable to demonstrate any improvement with P. falciparum gametocytes. By the method described such forms of the parasite are concentrated by 30-40 times in thin blood films, and between 6-8 times in thick films; this increase being attributed to the reduction in the specific gravity of P. falciparum gametocytes which occurs during their immersion in hypotonic saline and which causes them to rise in the blood column during centrifugation. In vitro haemagglutination of the parasitized red cells during their sedimentation through the electrolyte was not thought to be significantly enhanced, for

this would have been observed in the isotonic saline sample, and any differential osmotic fragility would be negligible at the saline strength (0.7%) giving maximal concentration of parasites.

Despite the improved retention of parasites in concentrate films, as a result of the modified Giemsa staining method used, many are still being lost during staining. Better methods of staining, which would allow a greater proportion of parasites to be retained in the thick film, would utilize more fully the concentration factor achieved by saline sedimentation and centrifugation. This would greatly improve the sensitivity of the thick blood film and would lower, still further, the threshold of detectable P. falciparum gametocytaemia.

The work described represents an attempt to study some of the factors involved in malaria parasite concentration only and the method adopted is not offered, or considered, as a substitute for existing techniques.

SUMMARY

A technique is described whereby Plasmodium falciparum gametocytes are concentrated for blood films in centrifuged microcapillary blood specimens following sedimentation through hypotonic saline. The application of this method, either in the field or laboratory, is simple and takes little longer than present concentration methods. The results given by a limited field trial show a marked increase in the positivity for P. falciparum gametocytaemia. The possible advantages of this method are discussed.

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RESUME

L'auteur décrit une technique permettant d'obtenir une concentration des gamétocytes de Plasmodium falciparum dans des échantillons sanguins centrifugés en tubes capillaires après sédimentation dans du soluté hypotonique. Les étalements sont préparés à partir du concentré obtenu. Appliquée sur le terrain ou au laboratoire, cette technique est simple et ne demande guère plus de temps que les méthodes de concentration actuellement en usage.

Un essai limité sur le terrain a permis de constater que la recherche des gamétocytes de P. falciparum dans les étalements épais donne avec cette méthode des résultats positifs cinq fois plus fréquents, l'augmentation étant moindre pour les autres formes de parasites du paludisme.

L'auteur expose en dernier lieu les avantages possibles de la nouvelle méthode.

TECHNICAL NOTES

1. Anticoagulant. The following formula was used:

Sodium citrate 2 g
Dextrose 3 g
Distilled water 120 ml

After filtering this was dispensed in 0.3 ml amounts into clean, dry "Bijou" bottles which were then "flash" sterilized in a pressure cooker by raising the pressure to 20 lb. (9.1 kg) and immediately allowing to cool slowly. This prevented caramelization of the dextrose and bacterial overgrowth was negligible, even after overnight incubation at 37°C.

In later experiments 10 cm x 0.1 cm (internal diameter) "Exogen" Sequestrene microcapillary tubes were used.

Vene-puncture technique was employed only during experimentation with known parasitized blood when comparatively large volumes (1-2 ml) were needed for the full saline dilution range. The field method utilized normal finger-prick blood samples.

2. Numbering of capillary tubes. This was done using an ink marker with a felt wick and was found to be quick and effective. Numbering was carried out against a template so that the first mark was at the same point on the tube, i.e. 45 mm from one end. Blood was taken up to this point during the tests, thus standardizing blood volumes used in any series.

3. Centrifuge. As mentioned in the text only moderate force was used with the DRSM centrifuge during these experiments. No clotting was experienced in any of the tubes and only one specimen, due to a faulty capillary, was lost during spinning. The addition of a thin circular polyvinyl sheet, following the recommendation of Dowling et al. (1966), obviated the need for taping the tubes in place and allowed far speedier removal to the vertical position after spinning. Although noticeably quieter the performance of the disc with this sheet attachment could not be assessed. The polyvinyl sheet was held in place by two strips of selotape, one on each side.

Annex

4. Buffering of staining solutions. The following formula was used for all concentrate and anticoagulated blood films:

Potassium dihydrogen phosphate	1.0 g
Disodium hydrogen phosphate	8.0 g
Distilled water	1 litre

5. Spreading of thin films from centrifuged blood. Difficulty was found initially in making good films from concentrated blood. With drops taken from the middle or lower levels of the spun column spreading very slowly produced good even films. The reverse was found to be necessary with samples taken from the upper-middle and sub-buffy levels of the centrifuged blood; good films being possible only when spread as rapidly as possible, immediately the spreader made contact with the blood drop.

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TABLE 1. SHOWING DETAILS OF PARASITE COUNTS PER 100 FIELDS (THICK FILM) - FIELD TRIAL

Serial Number	Normal thick				Normal thick "A"				Concentrate thick (sub-buffy)				Concentrate thick (lower-middle)				50 Fields of sub-buffy and 50 Fields of lower-middle			
	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria
1	11	-	-	-	12	-	-	-	10	-	2	-	18	-	2	-	18	-	3	-
2	110	1	5	-	173	2	4	-	320	7	-	-	164	-	3	-	236	4	3	-
3	47	2	3	-	42	2	3	-	54	19	-	-	104	-	11	-	65	10	4	-
4	173	-	4	-	239	-	6	-	234	2	-	1	256	1	6	-	227	1	4	1
5	1 140	-	4	-	1 370	1	5	-	1 170	5	3	-	1 380	-	8	-	1 270	2	6	-
6	-	-	-	-	-	1	-	-	-	6	-	-	-	-	-	-	-	4	-	-
7	1 060	-	2	-	1 400	-	2	1	2 370	-	-	1	860	-	5	-	1 620	-	3	-
8	-	-	-	-	2	-	-	-	-	-	-	-	5	-	-	-	2	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
10	3	-	-	-	1	-	-	-	13	-	-	-	1	-	-	-	7	-	-	-
11	7	-	-	-	14	-	-	-	37	-	-	2	18	-	-	-	21	-	-	1
12	3	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	2	-	-	-
13	17	-	3	-	25	-	2	-	28	2	2	1	42	-	8	-	30	2	2	-
14	1 120	-	2	-	1 610	-	2	-	1 640	-	3	1	2 430	-	11	-	2 040	-	10	-
15	124	-	3	-	107	-	8	-	53	-	-	-	213	-	7	-	132	-	2	-
16	-	-	-	-	1	-	-	-	2	3	-	-	-	-	-	-	1	-	2	-
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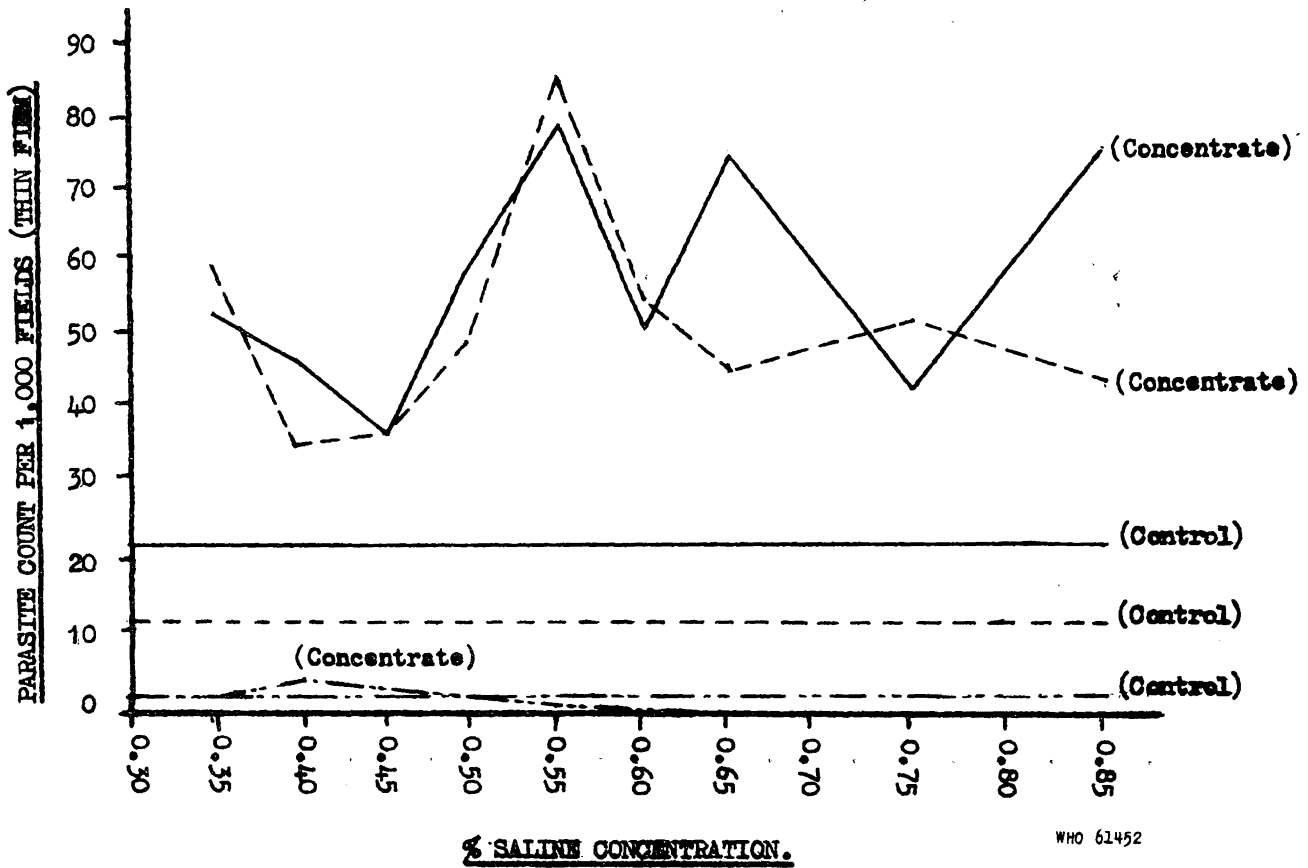
TABLE 1. SHOWING DETAILS OF PARASITE COUNTS PER 100
FIELDS (THICK FILM) - FIELD TRIAL (continued)

Serial number	Normal thick				Normal thick "A"				Concentrate thick (sub-buffy)				Concentrate thick (lower-middle)				50 Fields of sub-buffy and 50 Fields of lower-middle			
	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria	F	Fg	M	Micro-filaria
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	13	-	3	1	24	-	3	-	52	2	-	2	42	-	9	-	54	2	6	2
25	11	-	-	-	9	-	-	-	6	-	-	1	14	-	-	1	6	-	-	1
26	7	1	2	-	14	-	5	-	21	1	-	-	4	-	11	-	13	-	4	-
27	58	-	1	-	58	-	4	-	105	-	1	-	59	-	5	-	73	-	2	-
28	-	-	-	-	-	-	-	2	-	-	-	4	-	-	-	-	-	-	-	2
29	2	-	-	-	2	-	-	-	1	5	-	-	4	-	-	-	3	2	-	-
30	109	-	3	2	114	1	5	-	142	2	-	5	198	-	17	-	168	1	5	2
31	5	-	-	-	8	-	-	-	16	2	3	-	22	-	2	-	21	2	1	-
32	1	-	-	-	3	-	-	-	1	-	-	-	3	-	-	-	3	-	-	-

F = Plasmodium falciparum trophozoites.

Fg = P. falciparum gametocytes.

M = P. malariae parasites.



WHO 61452

LEGEND.

- = P. falciparum ring-forms.
- - - = P. malariae.
- · - · = P. ovale.

FIG. 1: SHOWING PARASITE COUNTS PER 1,000 FIELDS
OF THIN BLOOD FILMS MADE FROM LOWERMIDDLE
LEVEL OF CONCENTRATES IN EXPERIMENT "A".

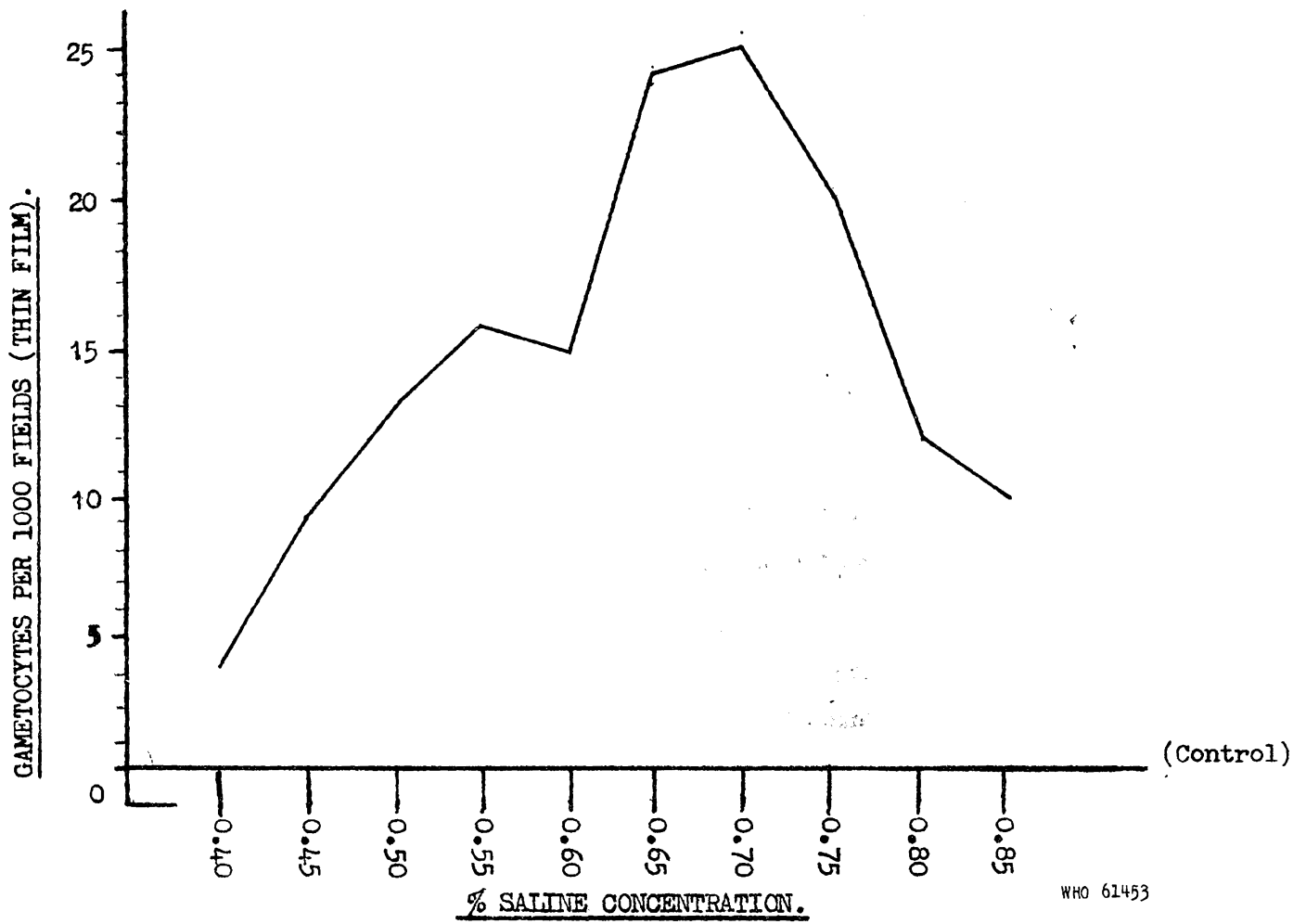


FIG. 2: SHOWING RESULTS OF GAMETOCYTE COUNTS PER 1000 FIELDS OF THIN BLOOD FILMS MADE FROM SUB-BUFFY LEVEL OF CONCENTRATES IN EXPERIMENT "B"

The purpose of the WHO/Mal series of documents is threefold:

- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

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