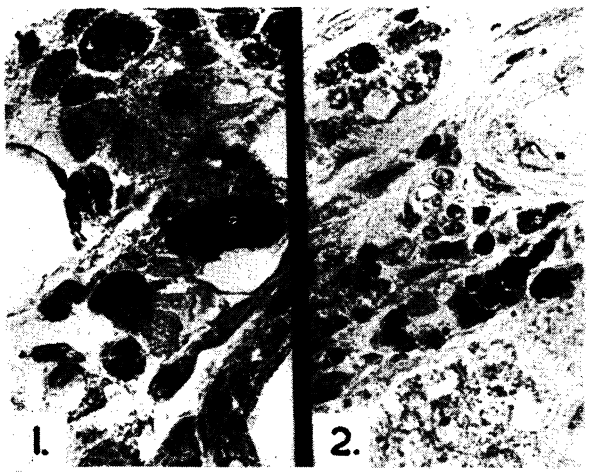

FIG. 1. Masses of ceroidlike pigment in traumatized mesenteric tissue of a rat. The sudanophilic material appears black in the photograph.

FIG. 2. Ceroid in the fibrous tissue in a section of cirrhotic liver of a choline-deficient rat. The sudanophilic pigment (black) lies in clumps which correspond to the distribution of cystic hemorrhages.

Both photomicrographs are of paraffin sections stained with Oil Red 0, hematoxylin, and Light Green. (× 650.)

manner resembled ceroid in other ways, for they were acid-fast, Prussian blue–negative, fluorescent,[3] and reacted positively to Mallory's hemofuchsin test; unstained they were orange-brown.

In a second experiment, 0.10 ml of heparin[4] was injected locally into a loose tag of fatty mesenteric tissue of each of 5 adult male rats of the Wistar strain subjected to laparotomy under ether anesthesia. A loosely tied ligature around the root of the mesenteric tag impeded venous return and systemic absorption of the anticoagulant. Fatty hematomata were produced by crushing the tissue between hemostats. The incisions were closed, penicillin was administered subcutaneously to prevent infection, and the animals were maintained on a stock diet for 10 days. Paraffin sections of the traumatized mesentery obtained at autopsy were examined by the methods used to identify ceroid. The morphology (Figs. 1, 2) and histochemistry of pigment found in the scar tissue differed in no essential from that of ceroid.

These results indicate that under certain conditions a mixture of lipids and some component of free red cells can produce ceroid or a closely related substance. Perhaps cells other than erythrocytes are capable of a similar reaction with certain lipids; this is under investigation. Preliminary experiments[5] with fat extracted from livers of choline-deficient rats have indicated that this lipid may react *in vitro* with erythrocytes to produce the same results obtained with cod liver oil. These observations may indicate means of further investigations concerning the nature not only of ceroid, but also of other lipochrome pigments.

[3] The fluorescence of ceroid in sections was first described by Popper *et al.* (7). The fluorescence of lightly colored particles of the substance produced *in vitro* above was light-brown; darker particles exhibited little fluorescence. The writer is indebted to Hans Popper, of the Hektoen Institute for Medical Research, of the Cook County Hospital, Chicago, who made the fluorescent examination for this report.

[4] Supplied in a solution of 1,000 u/ml in physiological saline by the Connaught Medical Research Laboratories, University of Toronto.

[5] These experiments and others of a similar nature have been conducted by W. G. B. Casselman, Banting and Best Department of Medical Research, University of Toronto, and will be published elsewhere.

References

1. LILLIE, R. D., DAFT, F. S., and SEBRELL, W. H., JR. *U. S. Pub. Health Repts.*, **56**, 1255 (1941).
2. HARTROFT, W. S. *Seminar on Metabolic Aspects of Liver Function.* Metabolic and Endocrinology Study Section of the National Institute of Health, USPHS, 15 (1948).
3. LILLIE, R. D., *et al. U. S. Pub. Health Repts.*, **57**, 502 (1942).
4. HARTROFT, W. S. *Anat. Record*, **106**, 61 (1950).
5. HARTROFT, W. S. and RIDOUT, J. H. (To be published.)
6. RIDOUT, J. H., *et al. Biochem. J.*, **40**, 494 (1946).
7. POPPER, H. GYÖRGY, P., and GOLDBLATT, H. *Arch. Path.*, **37**, 161 (1944).

An *in Vitro* Method of Screening Amebicidal Agents Using the Phillips Culture

William T. Bradner[1] and George W. Rawson[2]

Microbiological Laboratories, Ciba Pharmaceutical Products Company, Inc., Summit, New Jersey

The testing of potential amebicidal compounds by *in vitro* methods is unfortunately beset with several difficulties. The most important is the problem of determining whether an "active" compound is truly amebicidal or whether its action is only indirect and, presumably, due to the inhibition or destruction of necessary bacterial associates. This difficulty is somewhat lessened by the use of *Endamoeba histolytica* cultures with a single bacterial symbiont instead of the usual mixed bacterial flora. However, one encounters difficulties in maintaining consistently an abundant growth level in liquid media. The use of solid media has several inherent disadvantages, the most important of which are the adsorption of the drug being tested on the surface of the solid material and the protection of amebae enmeshed in the suspended solids.

The experiment described in this report, using the bacteria-free Phillips culture (*E. histolytica* strain F 22 with *Trypanosoma cruzi*) (*1, 2*), was initiated in the belief that the use of another protozoan as a symbiont for the ameba would yield a population that would be much easier to observe and control than bacteria. Thus it was hoped that the problem of direct drug action on the amebae would be solved and disadvantages of present *in vitro* screening methods would be reduced. Various known amebicidal and chemotherapeutic agents, including antibiotics, were chosen for the test, and a comparison was made with the Stone's-Locke's egg slant (SLES) culture (*3*) currently in use in this laboratory, and the Phillips culture.

[1] Present address: Biology Department, Lehigh University, Bethlehem, Pa.

[2] We wish to extend our appreciation to C. W. Rees, of the National Institutes of Health, for supplying us with the Phillips culture.

TABLE 1

AMEBICIDAL, TRYPANOCIDAL, AND BACTERICIDAL ACTIVITIES *in Vitro* OF SELECTED COMPOUNDS

Drug	(*A*) Phillips (amebae—*T. cruzi*)	(*B*) *T. cruzi* (alone)	(*C*) SLES (amebae-bacteria)	(*D*) Bacterial flora (collectively)	(*E*) Bacteria in SLES medium
Emetine hydrochloride	1: 32,000	Inactive	1: 16,000	Inactive	Inactive
Terramycin hydrochloride	1: 4,000	"	1: 1,000	1: 4,000	1: 2,000
Sulfanilamide	Inactive	"	Inactive	Inactive	Inactive
7-Iodo-5-sulphonic acid–8-hydroxyquinoline (Diodoquin)	1: 1,000	"	"	"	"
7-Iodo-5-chloro–8-hydroxyquinoline (Vioform)	1: 8,000	"	1: 4,000	"	"
p-Carbamino-phenyl-arsonic acid (Carbarsone)	1: 8,000	"	1: 4,000	1: 1,000	"
Penicillin G, sodium	Inactive	"	Inactive	1: 128,000	"
Streptomycin (calcium chloride complex)	"	"	"	Inactive	"
Aureomycin	1: 16,000	"	1: 8,000	1: 4,000	1: 2,000
Chloramphenicol (chloromycetin)	1: 2,000	"	Inactive	1: 16,000	Inactive
Bacitracin	1: 1,000	"	1: 1,000	1: 8,000	1: 2,000

Four tests were run simultaneously with each of the compounds listed in Table 1, using (*a*) the Phillips culture (*E. histolytica–T. cruzi*), (*b*) *T. cruzi* alone, (*c*) the SLES culture (*E. histolytica*–mixed bacterial flora), and (*d*) the four bacterial isolates derived from the SLES flora. Since a regular constituent of the Phillips culture is sodium thioglycollate solution (0.3% diluted 1: 6), it was used as a vehicle for the drug dilutions. One hundred mg of each compound was dissolved in 16.7 ml of sodium thioglycollate solution which, at the 1: 6 ratio used with other constituents of the Phillips culture, would give a final dilution of 1: 1,000. This constituted the highest concentration of the drugs tested, and further dilutions were made serially in multiples of two, covering a range from 1 : 1,000 to 1 : 128,000, inclusive.

A. The Phillips drug tubes were petrolatum-sealed and incubated 48 hr at 37° C, at which time they were examined through the side walls under the microscope (low power). As a check, a drop of thoroughly mixed fluid from each tube was also examined microscopically to determine what effect the test compound had on the amebae. Subcultures were subsequently made of all negative or doubtful positive dilutions.

B. The *T. cruzi* culture was tested separately by adding 0.2 ml of drug dilution to 1 ml of trypticase *T. cruzi* culture (*1*), thus giving the necessary 1: 6 dilution at each concentration. Following incubation for 48 hr at 22°–24° C, samples were examined microscopically for motile organisms. Negative tubes (those with no actively motile organisms) were subcultured into NIH diphasic blood agar medium (*4*).

C. One-ml amounts of pooled material from SLES flask cultures were similarly tested with 0.2 ml of each drug concentration and checked microscopically after 24 hr at 37° C. Subcultures covering the range of doubtfully positive tubes were made in SLES medium.

D. In a similar manner, each bacterial culture was tested, using 5 ml nutrient broth as a medium, inoculating with a loopful from a 24-hr broth culture, and adding 1 ml of each drug concentration. After 48 hr at 37° C the tubes were rated positive or negative for growth, and subcultures were made into nutrient broth.

The values shown in Table 1 represent end points which may be defined as the highest dilution of drug that completely inhibited the growth of the test organism as verified by subculture. In tests where growth occurred at the 1: 1,000 dilution, the drugs are considered inactive. Column E represents the drug activities against the bacterial flora within the SLES drug tubes as determined by observed motility. If motile organisms were present at the 1: 1,000 dilution the drug was likewise considered inactive.

In almost every test where amebicidal activity was noted, the Phillips culture was, on the average, about twice as sensitive as the SLES culture. The Phillips culture was never *less* sensitive.

There was no instance among the compounds tested where inhibition of the *T. cruzi* occurred, although in six cases (almost all of them antibiotics) inhibition of the bacterial flora occurred (column D). It is noteworthy that considerable variance is apparent between the antibacterial activity in the SLES culture and in broth (columns D and E). The presence of large amounts of solids is probably an important reason for the lowered activity in the former. However, inactivity may have been rated on the presence of only one bacterial species which in itself was incapable of supporting ameba growth. Obviously, judging only by the SLES cultures, it would be very difficult to distinguish direct amebicidal action from indirect action (inhibition of bacterial flora). It is to be expected, however, that compounds may be encountered that would inhibit *T. cruzi.*

In this experiment, all Phillips amebae cultures were observed both through the side walls of the tube directly (*5*) and by microscopic examination of the

contents. Sufficient correlation of the two methods was obtained to deem the drop examination unnecessary. The efficacy of this procedure is supported by the fact that all doubtful tubes (absence of motile trophozoites) were subcultured.

Though a retest of the active compounds failed to reveal any significant variation of end points, the final practicality of the technique as described above will necessarily depend upon more extensive comparison with available *in vitro* methods. On the basis of the results noted thus far, however, the Phillips culture would seem to provide a feasible means for *in vitro* amebicidal screening, reserving a bacteria-amoeba culture for a control check of active compounds.

References

1. PHILLIPS, B. P. *Science*, **111**, 8 (1950).
2. PHILLIPS, B. P., and REES, C. W. *Am. J. Trop. Med.*, **30**, 185 (1950).
3. BOECK, W. C., and DRBOHLAV, J. *Am. J. Hyg.*, **5**, 371 (1925).
4. JOHNSON, E. M. *J. Parasitol.*, **33**, 85 (1947).
5. SHAFFER, J. G., *et al. Am. J. Hyg.*, **47**, 345 (1948).

Changes in the Total Circulating Eosinophile Count in Cyclotron Workers

Campbell Moses and Madeline Platt

Addison H. Gibson Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Several workers (1–5) have reported eosinophilia in response to x-ray and radium exposure. In 1942 Warren (*6*) reported the blood findings in 4 cyclotron workers who were exposed while sanding a dee. These workers demonstrated an initial fall in white cell count followed by a gradual rise. Of the 3 differential counts reported in this group, 2 had eosinophilia of 4% and 5%.

This report is based upon observation of 3 workers who received an indeterminate exposure while sanding the copper dees of a cyclotron. This exposure consisted of approximately 3 hr on each of 2 successive days. At the end of this period an exposure of 2,400 mr/hour was recorded at a distance of 12 in. from the surface being sanded. Although no pulmonary symptoms were noted following this exposure, inhalation of radioactive dust probably occurred, at least to some degree.

Red and white cell counts, hemoglobins, and differential leukocyte counts at bimonthly intervals prior to this exposure failed to reveal any significant variations. Repetition of these procedures at weekly intervals after exposure revealed only a transient leukopenia, which promptly returned to normal. Determination of the total circulating eosinophiles was made by the technique of Randolph (*7*). All blood counts were taken between 10:00 A. M. and 12:00 noon, without control of the antecedent diet or fluid intake.

Fig. 1 records the variations in the total number of circulating eosinophiles in the exposed and nonexposed personnel. It is evident that workers A and B, who were exposed during the sanding operation, demonstrated a marked increase in the number of total circulating eosinophiles over the nonexposed personnel. Worker C, who apparently had as much exposure as A and B, did not demonstrate the marked eosinophilia shown by the others. It should be noted

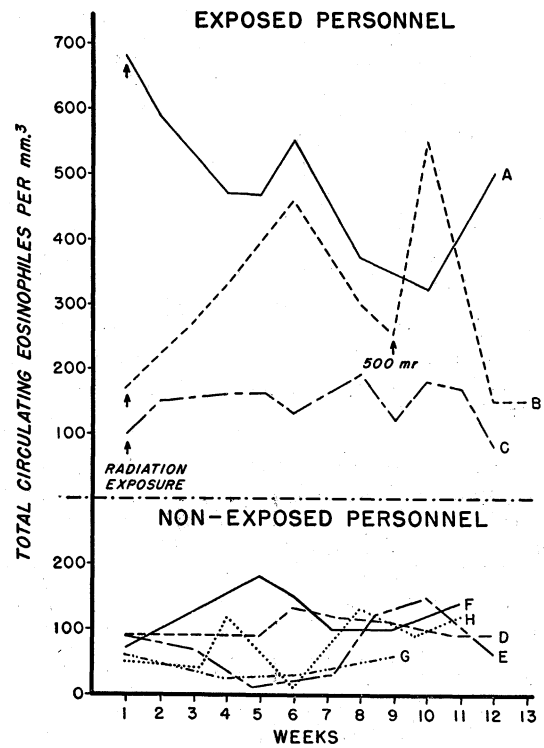

FIG. 1.

that worker A had a high eosinophile count immediately after exposure. Worker B evidenced a gradual increase and decrease in the total number of circulating eosinophiles until the ninth week, when in handling a hot target he received an estimated 500 mr of total-body irradiation. This was followed by a sharp rise in eosinophiles and an abrupt return to normal levels. Although total circulating eosinophile counts were not made prior to exposure, careful survey has failed to reveal any evidence of hypersensitivity or parasitic infestation, and all counts have been entirely normal in the 6 months since the last count recorded in Fig. 1.

It is suggested that the total circulating eosinophile count may be a useful indication of exposure to radiation in individuals employed in x-ray, cyclotron, and other laboratories with radiation hazards.

References

1. MINOT, G. R., and SPURLING, R. G. *Am. J. Med. Science*, **168**, 215 (1924).
2. PHILLIPS, G. W. *Lancet*, **2**, 745 (1933).
3. GOODFELLOW, D. R. *Acta Radiologica*, **17**, 1 (1936).
4. PETERSEN, W. F., and SAELHOF, C. C. *Am. J. Roentgenol.*, **8**, 175 (1921).
5. LAWRENCE, J. S., VALENTINE, N. N., and DOWDY, A. H. *Blood*, **3**, 593 (1948).
6. WARREN, S. *Radiology*, **39**, 194 (1942).
7. RANDOLPH, T. G. *J. Lab. Clin. Med.*, **34**, 1696 (1949).