this impaired esterification to be specific for DNA. A certain specificity does exist, however, in view of the more striking decreases in P^{32} incorporation into DNA than into RNA, with no change in phospholipid turnover, a finding which would not be anticipated if a diminished capacity for phosphorus esterification were the sole metabolic defect produced by radiation. Hence the action through which radiation alters the metabolism of DNA remains obscure.

It is conceivable that the important effects of radiation are directed principally toward cell surfaces, resulting in altered permeability characteristics leading to loss of diffusible cofactors. Such a concept, although capable of reconciling most of the data, is not readily amenable to experimentation in as complex an organism as the rat.

Summary. Negligible changes were observed in the activities of succinic dehydrogenase, malic dehydrogenase, cytochrome oxidase, and adenosine triphosphatase in a thymus homogenate prepared from a rat exposed to 800 r of gamma radiation; however, pronounced diminution both in concentration and in P^{32} incorporation of thymus DNA, and to a lesser extent RNA, were evident 3 hours after exposure of the rat, and became progressively greater during a 96-hour post-irradiation interval. The capacity of a thymus homogenate to esterify inorganic phosphate was appreciably diminished in the irradiated rat. It is suggested that the diminished respiration observed in slices of thymus from irradiated rats is not a primary radiation effect, since comparable changes do not occur in the activity of individual respiratory enzymes studied here.

1. Bloom, W., *Histopathology of Irradiation from External and Internal Sources,* McGraw-Hill Book Co., New York, 1948.

2. Barron, E. S. G., *Effects of X-Rays on Tissue Metabolism.* AECD-2316 (CH-3654), October 29, 1946.

3. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.,* 1943, v149, 217.

4. DuBois, K. P., and Potter, V. R., *J. Biol. Chem.,* 1943, v150, 185.

5. Potter, V. R., *J. Biol. Chem.,* 1946, v165, 311.

6. ———, *J. Biol. Chem.,* 1947, v169, 17.

7. Schneider, W. C., *J. Biol. Chem.,* 1948, v176, 259.

8. Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.,* 1945, v161, 83.

9. Schneider, W. C., *J. Biol. Chem.,* 1945, v161, 293.

10. Friedkin, M., and Lehninger, A. L., *J. Biol. Chem.,* 1949, v177, 775.

11. Rose, M. H., and Ely, J. O., *Am. J. Roentgenol.,* 1949, v62, 723.

12. Lavik, P. S., Harrington, H., and Buckaloo, G. W., *Fed. Proc.,* 1950, v9, 194.

13. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol.,* 1949, v32, 537.

14. Barron, E. S. G., and Dickman, S., *J. Gen. Physiol.,* 1949, v32, 595.

15. Barron, E. S. G., *Adv. in Enzymology,* 1943, v3, 149.

16. Potter, V. R., Lyle, G. G., and Schneider, W. C., *J. Biol. Chem.,* 1951, v190, 293.

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Cultivation of *Endamoeba Histolytica* in Embryonic Fluids.* (19593)

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Since the first cultivation of *Endamoeba histolytica* by Boeck and Drbohlav(1), the goal of parasitologists has been to maintain this protozoön free of association with other organisms. Thus far all attempts have failed, although within the last few years progress has been reported. Shaffer and co-workers(2-5) cultivated *E. histolytica* in a modified thiogly-

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collate medium in which bacterial growth was inhibited by the use of penicillin in the medium, while Phillips(6) was able to maintain cultures of *E. histolytica* in which *Trypanosoma cruzi* was the sole associated microorganism. The experiments herein reported were carried out to develop a transparent medium which would 1) provide dependable multiplication of amebae; 2) not require bacteria or other microbial associates, or if required, only in small numbers; 3) permit storage of the medium, as contrasted with the medium of Shaffer *et al.* which must be prepared just before use, and 4) provide large numbers of amebae sufficiently free of bacteria and particulate matter so that precisely controlled studies on the metabolism of these protozoa could be carried out. This medium was to be developed to be used as a research tool and not for laboratory diagnosis.

Materials and methods. Strains of amebae employed. 1) Clone of strain 22, isolated in 1947 from a human brain abscess and used experimentally since 1949 in our laboratory (7). The associated bacterial flora consisted of a species of *Corynebacterium* closely resembling *C. hoffmani* and a second diphtheroid which was considered to be a variant of the above or a member of the same genus obligately parasitic on the first species, since it could not be cultured separately either aërobically or in an atmosphere of phosphorus pentoxide(8). 2) *Strain NRS* grown with a mixed, undetermined bacterial flora. 3) *Strain U.C.* grown with a mixed, undetermined bacterial flora, obtained through the courtesy of Dr. William Balamuth, Northwestern University, Evanston, Ill. 4) *Strain 19t,* obtained by us from a human infection, freed of its bacterial associates and successfully cultured with organism *t*(9). 5) *Strain 103* grown with organism *t,* obtained through the courtesy of Dr. Charles W. Rees, National Institutes of Health, Bethesda, Md. In addition, amebae from other sources were occasionally employed. With the exception of strain 103 *t,* which was cultured routinely in a diphasic whole-egg medium, all strains were maintained in Balamuth's aqueous egg yolk infusion medium(10).

Preparation of media. Embryonic fluids were obtained from chicks, human beings, cows and dogs. For collection of chick fluids 9- to 13-day embryonating eggs were candled and drilled according to the method described by Beveridge and Burnet(11). After the shell was removed and the shell membrane teased away, amniotic and allantoic fluids were removed aseptically with a Pasteur pipet. Amniotic fluids were collected from human sources at cesarean section and by aspiration just before delivery, from one dog after 3 to 4 weeks of gestation and from another dog just before delivery, and from one cow after 3½ months and another cow after 5 months of gestation. Some of these fluids unavoidably contained traces of blood. After collection they were distributed aseptically in 3.8 ml amounts in 125 x 14 mm sterile test tubes.

TABLE I. Cultivation of *E. histolytica* in Embryonic Fluids from Different Sources.

Source of fluids	Amebic population 48 hr after inoculation	
	With traces of blood	Without blood
Human-amniotic, full term	+	—
Dog-amniotic, " "	—	—
Chick embryo-amniotic, >14 days	—	—
Dog-amniotic, 3 to 4 wk	++++	*
Cow-amniotic, 3½ mo	++++	++++
" " 5 mo	+++	++++
Chick embryo-amniotic, 9 to 13 days	++++	++++
Chick embryo-allantoic, 9 to 13 days	++++	*
Chick embryo-amniotic and allantoic mixed, 9 to 13 days	++++	*

—=no amebae seen; +=survival, no apparent multiplication; ++=survival, minimal multiplication; +++=Some multiplication; ++++=definite multiplication.
* Not tested.

Results. Cultivation in embryonic fluids from different sources. The amebae used in the preliminary study were from a clone of strain 22. For inoculation from aqueous egg-yolk infusion medium, 0.2 ml of 48-hour cultures was introduced into the various embryonic fluid media. Penicillin and streptomycin were added to make a final dilution of 1,000 units of each per ml of medium. After inoculation the tubes were sealed with petrolatum and incubated at 37°C for 48 hours.

The results (Table I) suggest that the species of animal from which the fluid was taken is not as important for survival and multiplication of the amebae as is the time of embryonic or fetal development at which fluids were collected.

Sterility tests for bacteria were made in each case at 48 hours as follows: An inoculum of 0.05 ml from each of the media was introduced into each of 3 tubes containing respectively 10 ml of brain-heart infusion, 10 ml of thioglycollate broth, and 10 ml of aqueous egg-yolk infusion medium(10), to which pennase had been added, and incubated for 10 days. In all of these media the bacteria of clone 22 have been shown to grow abundantly. Since amounts of the antibiotics sufficient to inhibit the growth of bacteria might be carried along with the inoculum into the subcultures, thus possibly giving a false impression of sterility, the inoculum was occasionally freed of the antibiotics in the following manner: The 48-hour-old amebic cultures were centrifugalized at 1500 r.p.m. for 10 minutes in a conical test tube; 0.05 ml of the sediment was then inoculated into another conical test tube containing 10 ml of sterile saline solution and centrifugalized again at 1500 r.p.m. for 10 minutes; 0.05 ml of this sediment was then inoculated into the 3 bacteriologic media and incubated at 37°C for 10 days. As a further safeguard, 0.05 ml of bacterial suspension from 48-hour clone 22 cultures on aqueous egg-yolk infusion medium was inoculated into each of the 3 bacteriologic media mentioned above, to which penicillin and streptomycin, in amounts equal to a final dilution respectively of 5, 10 and 50 units per ml, were added together with pennase. These doses are the equivalent of at least 2 to 10 times the amount carried over from the amebic cultures which were being tested for sterility. Bacteria always developed in the controls. Yet all amebic cultures in these initial experiments except those in cow's amniotic fluid obtained 5 months after beginning of gestation were negative for viable bacteria after 48 hours of incubation.

Maintenance of cultures. The ease of obtaining chick embryonic fluid plus the promising preliminary results suggested further investigation of these fluids. Attempts were made to maintain cultures of amebae of clone 22 by serial passages in amniotic-allantoic fluid mixtures obtained from 9-13-day-old chick embryos, but in repeated trials the amebae gradually decreased in numbers until after 2 or 3 transfers all cultures became negative for amebae. However, when bacteria from clone 22, either from aqueous egg-yolk infusion cultures or from bacterial cultures on nutrient agar slants, were added with antibiotics to these embryonic-fluid amebic cultures, the amebae could be maintained. Population counts using an inoculum of 2,000 to 3,000 amebae per tube indicated a 5- to 10-fold increase in numbers of amebae at the termination of a 48-hour culture period. In nearly 100 cultures tests for bacterial sterility after 48 hours were negative except for controls to which no antibiotics had been added. (Since the completion of these studies this medium has been experimentally employed in our laboratory by Major Lyman P. Frick, MSC., who has demonstrated a living bacterial population of approximately 200 organisms per ml in 48-hour cultures.) Seitz-filtrates of bacterial cultures failed to support amebic growth. Also an anaerobic environment induced by placing the cultures in an anaerobe jar failed to promote continued amebic growth in serial subcultures unless living bacteria were added at the time of each subculture. The technic for routine maintenance of cultures was as follows: 1. Amniotic and allantoic fluids from chick embryos were pooled and tubed in 3.8 ml quantities. 2. At the time of subculture 0.3 ml of a 48-hour bacterial culture, 0.1 ml of antibiotic mixture, and 0.3 ml inoculum from a 48-hour-old amebic culture were added making a total volume of 4.5 ml. 3. The tubes thus inoculated were sealed by pouring a one cm layer of sterile melted petrolatum directly onto the surface of the culture. 4. The cultures were incubated at 37°C for 48 hours, after which time they were subcultured again in like manner. Bacterial cultures of clone 22 flora were maintained routinely in aqueous egg-yolk infusion medium. The antibiotic mixture consisted of penicillin G and streptomycin sulfate to give a final concentration of 1,000

units of each per ml of culture. After harvesting from the chick embryo, the medium has been stored for one month at 4°C without diminution of its growth-promoting properties for amebae. Further studies revealed that blood in the small amount of approximately 0.01 ml of packed cells, which usually contaminated the fluids during harvest, promoted better protozoan growth than clear fluid. This amount was thereafter routinely incorporated in the medium at the time of harvest.

Cultivation of amebae from different sources. Approximately 0.3 ml of menstruum from a dysenteric dog passing numerous trophozoites in his stools and 0.3 ml from a child's stool containing scanty trophozoites were inoculated into the medium. Also 0.3 ml amounts each of amebic cultures of 5 strains with different monobacterial or multibacterial associates were inoculated into 10 culture tubes of chick embryonic fluids. While 5 of these tubes were left with their original floras only, the other 5 received 0.3 ml each of 48-hour-old culture of clone 22 bacteria, grown on aqueous egg-yolk infusion medium, before being sealed and incubated. The results 48 hours after inoculation indicate (Table II) that all of the strains of amebae to which the flora of clone 22 had been added grew in this medium, while only 2 of the strains tested grew solely with their own bacterial associates. Additional studies revealed that strains NRS and 103 could be maintained in this medium provided that they were supplemented at each subculture with 0.3 ml of clone 22 bacteria as indicated above.

TABLE II. Cultivation of *E. histolytica* from Different Sources in Chick Amniotic-Allantoic Fluids.

Sources of amebae	Amebic population 48 hr after inoculation: With original flora	Amebic population 48 hr after inoculation: With addition of Clone 22 flora
Clone 22	+++	+++
Dog's dysenteric menstrum	+++	*
Child's feces	++	*
Strain NRS	—	+
" 103 t	—	+++
" U.C.	+++	+++
" 19 t	—	+++

— = no amebae seen; + = survival, minimal multiplication; ++ = some multiplication; +++ = definite multiplication.
* Not tested.

Discussion and summary. 1. These experiments indicate that *Endamoeba histolytica* trophozoites can be cultured successfully in embryonic fluids. Amniotic and allantoic fluids from 9- to 13-day-old chick embryos have given the most consistent results. While growth of amebae occurred in aerobic as well as anaerobic environments, more dependable results were obtained under the latter condition. The fluids retained their ameba growth-supporting qualities after storage up to 30 days, but not after Seitz filtration. 2. The extremely low bacterial population has made large numbers of amebae available for physiologic studies. It is generally realized that bacterial interference must be minimized before metabolic studies of a fundamental nature can be undertaken.

1. Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v5, 371.
2. Shaffer, J. C., and Frye, W. W., *Am. J. Hyg.*, 1948, v47, 214.
3. Shaffer, J. C., Walton, J. C., and Frye, W. W., *Am. J. Hyg.*, 1948, v47, 222.
4. Shaffer, J. C., Ryden, F. W., and Frye, W. W., *Am. J. Hyg.*, 1948, v47, 345.
5. ———, *Am. J. Hyg.*, 1949, v49, 127.
6. Phillips, B. P., *Science*, 1950, v111, 8.
7. Faust, E. C., Sadun, E. H., Everritt, M. G., Bradin, J. L., Jr., and Lewis, R. A., *J. Parasitol.*, 1950, v36, 336.
8. Everritt, M. G., Sadun, E. H., and Carrera, G. M., *Exp. Parasitol.*, 1952, in press.
9. Faust, E. C., Lewis, R. A., Heilbrunn, I., Hickman, M. L. M., and Everitt, M. G., *Rev. Med. Mex.*, 1948, v28, 206.
10. Balamuth, W., *Am. J. Clin. Path.*, 1946, v16, 380.
11. Beveridge, W. I. B., and Burnet, F. M., *Med. Res. Council*, 1946, Rept. No. 256.

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