NaCl and 0.05% Na_2HPO_4, followed by 100-200 ml of 4% formaldehyde. Then both optic nerves (the anatomically well defined part from the bulb to the chiasma) and both optic tracts (anatomically as far as possible corresponding parts) were removed and weighed immediately. Then each of these 4 samples were pressed separately between 2 tin foils, the resulting layer being about 2 mg of nervous tissue per sq. cm. The tin foils, after being covered with a cellophane sheet of about 0.02 mm in thickness, were wrapped directly around the counter tube. In this way the rate of radioactivity was determined, and from the number of disintegrations per second the content of P^{32} per mg fresh nervous matter was calculated. In order to obtain a test of the sensitivity of the whole experimental procedure, we determined on the same rabbits the P^{32} content of the right and left dorsal roots S_2, taking anatomically corresponding pieces (from the ganglion to the cord) of about the same weight as the optic nerves. Working on the assumption that the P^{32} uptake herein is the same on both sides, we found a mean error of $\pm$ 4% of the methodical procedure as a whole.

The data are given in Table I. The relatively great differences in the absolute values of certain rabbits, which are otherwise under the same conditions, are sufficiently explained by the fact that the perfusing process apparently was not equally efficient in every case. This becomes clear by a comparison of the values of the intravascular fluid which remained in the circulatory system after the perfusing process.

Table I shows that the values for the total phosphorus metabolism of the optic nerves (and optic tracts) are practically the same whether the optic system is in a stage of activity or not. This seems to be true for the average of all of the experiments as well as for each of the 3 groups calculated separately. And since the bulk of all P-compounds of a nerve belong to the nerve sheet of its fibers, our data may support the conception, that the metabolism of the nerve sheet is not immediately altered by the activity of the axis cylinder. Furthermore Table I shows that the phosphorus metabolism of the optic nerves is about twice as high as that of the immediately adjacent optic tracts. This is in accord with the fact that there are also remarkable histological differences (Schindler),[7] and that the total metabolism (measured by the CO_2-production) of a nerve becomes greater approaching to the nerve cell body (Tashiro).[8]

Conclusion. We may therefore conclude that there is no difference between the phosphorus metabolism of these active and inactive nerves.

Summary. No difference was found by means of P^{32} in the phosphorus metabolism of stimulated and unstimulated nerves.

7 Schindler, E., *Z. f. Augenheilk.*, 1926, 15.

8 Tashiro, S., and Adams, H. S., *J. Biol. Chem.*, 1914, **18**, 329.

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Studies on the Culture of *Endamoeba histolytica.**

J. N. DELAMATER AND F. A. HALLMAN. (Introduced by John F. Kessel.)

From the Department of Bacteriology and Parasitology, School of Medicine, University of Southern California, Los Angeles.

Endamoeba histolytica has been grown in culture for many years.[1] Little is known, however, concerning the metabolic requirements of this organism or of the true functions of bacteria which so far appear to be necessary to the *in vitro* cultivation of this

* Supported by a grant from the Parke-Davis Company, Detroit.

1 Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, **5**, 371.

protozoan. Previously-used culture media are unusually complex. In order that the metabolic requirements of *E. histolytica* and the relationships of its associated bacteria could be more fully studied, our efforts have been directed to the development of a simple medium. This paper reports such a medium and the presence of a growth factor in human serum which is essential to the propagation of *E. histolytica* under the cultural conditions described herein.

Conditions and Method. The cultivation of *E. histolytica* entails consideration of numerous variables; the strain of ameba, the associated flora and the basic conditions of culture. These factors have been controlled in so far as possible.

1. Strain of *E. histolytica.* The organism used in these experiments was the NRS strain, originally obtained for use by this department from Dr. Henry E. Meleney. Used for comparative studies, was a strain obtained from Dr. Charles W. Rees of the National Institute of Health.

2. Bacterial flora. The bacterial flora present in our culture of the NRS strain includes a diphtheroid, *Staphylococcus aureus, Escherichia coli,* a gamma streptococcus, and a Gram-variable, branching bacillus which has not yet been identified. The only bacterium present in Rees' strain is the so-called "organism T."[2]

3. Culture media. Raw egg white was selected as a starting point following numerous attempts to grow amebae on various parts of fertile eggs. The constituents of egg white are largely known,[3] and it was believed that this substance, therefore, would constitute a suitable base for a medium. The basic medium used in this work is a buffered infusion of egg white to which has been added rice starch and either whole human serum or its protein-free filtrate.

(a) Egg white infusion medium: To egg white, collected in a sterile manner, is added sterile M/15 phosphate buffer, pH 6.8, in a ratio of 3 parts of buffer to one part of egg white. The mixture is allowed to infuse, with occasional shaking, at 4°C for at least 24 hours before decanting the supernate from any thick albuminous sediment which fails to dissolve.

(b) Rice starch. The rice starch used was obtained from Dr. Kessel's laboratory. It consists of husk-free starch granules approximately 1.5 μ in size. After sterilization by dry heat at 180°C, for one hour, it is added by loopfuls, as necessary, to each culture tube.

(c) Human serum. Pooled Wassermann-negative serum, obtained from the Serology Laboratory at the Los Angeles County Hospital, has been used in these experiments. It is Seitz-filtered and kept in sterile containers at 4°C until used.

(d) Protein-free fraction of human serum. At first, partially deproteinized serum was prepared by heating serum at 100°C for 20 minutes in a water bath. More recently, all protein has been removed by acidifying the serum to pH 5.2 and heating, as before. This procedure appears to remove all precipitable protein.

(e) Dialysate. The protein-free fraction of human serum was dialyzed with 2 volumes of 0.9% saline solution for a 24-hour period.

4. Assessment of growth. Accurate assessment of multiplication of amebae in culture is frequently very difficult. In testing whether or not a constituent of the medium was vital to propagation, results have been interpreted on a basis of presence or absence of amebae. This, in turn, required differentiation between "survival of the inoculum" and "multiplication." If amebae persist in cultures in fairly constant numbers through serial dilution of numerous subcultures, they must propagate. When they survive without propagation in serial dilution through subculture, we have observed that cultures become negative between the 2nd and 6th transplants. Continued presence of amebae beyond the 6th transplant has therefore been considered significant.

Description of Results. 1. Egg white infusion medium containing rice starch with

[2] Rees, C. W., and Reardon, L. V., *J. Parasit.*, Suppl., 1944, **30**, 10.

[3] McNally, E. H., and Denton, C. A., *Composition of Hen's Eggs,* A compilation Bureau of Animal Industry, U. S. D. A.

human serum added in dilution of 1:10 has supported growth of the NRS strain of *E. histolytica* since December 1946. Transplants have been made at 48-hour intervals.

2. Egg white infusion medium containing rice starch with partially deproteinized human serum added in a dilution of 1:10, has thus far supported growth of the NRS strain of *E. histolytica* through 42 transplants for 66 days.

3. Egg white infusion medium containing rice starch with completely deproteinized human serum added in a dilution of 1:10 has thus far supported growth of the NRS strain of *E. histolytica* for 38 days through 27 subcultures.

4. Egg white infusion medium containing rice starch with dialysate of the protein-free fraction of human serum added in a dilution of 1:5 has so far supported growth through 7 transplants for 10 days.

5. Egg white infusion medium containing serum or its protein-free fraction but *without rice starch* has failed to support growth of *E. histolytica* beyond the 3rd subculture. We have observed that there is a more rapid rise in the bacterial population in the absence of starch than when starch is present.[4] The cause for this may only be implied. Rice starch appears to suppress bacterial proliferation at least temporarily. In our hands, excessive growth of bacteria is detrimental to amebae in culture and amebae die out. If, however, bacterial multiplication is restricted by starch, as in these experiments, more viable amebae are found. This mechanism is being studied further.

Rice starch is likewise actively phagocytosed by *E. histolytica* and, as Boeck and Drhbolav[1] described, its presence enhances proliferation of amebae in culture.

6. Egg white infusion medium containing rice starch but without human serum or its protein-free fraction, uniformly fails to support growth of this strain of *E. histolytica*. However, growth of the accompanying bacterial flora is uninhibited.

These experiments demonstrate the presence of a growth-promoting substance in human serum necessary to the propagation of the NRS strain of *E. histolytica*, under these cultural conditions. This substance resists heating at 100°C for 4 hours and is dialyzable.

7. The partially deproteinized fraction of human serum diluted with an equal quantity of M/15 phosphate buffer with rice starch added constitutes, in itself, an excellent medium for the cultivation of *E. histolytica*. Growth has so far been supported through 35 subcultures for 50 days. Various dilutions of the protein-free fraction have also been tried. A 1:10 dilution also supports growth well. Analysis of the constituents remaining in this fraction is being made.

8. Growth of Rees' strain of *E. histolytica*, with organism "T," has likewise been tried in the protein-free human serum fraction diluted in an equal volume of M/15 phosphate buffer containing rice starch. No amebae survived beyond the third day. However, when the bacteria, accompanying our NRS strain, were added to Rees' strain, rapid proliferation occurred which has now been maintained for 23 days through 15 subcultures. It is interesting that under identical cultural conditions, Rees' strain and the NRS strain of *E. histolytica* behave in a similar manner in the presence of identical enzyme systems contributed by the bacteria present. This suggests that cell variation and culturability of *E. histolytica* may depend largely upon the bacteria present at the time of isolation. Detailed study of the biology of the accompanying bacteria may furnish more exact information concerning the physiologic requirements of *E. histolytica, in vivo* and *in vitro*.

Summary and Conclusions. 1. A heat-stable (100°C for 4 hours), dialyzable substance has been demonstrated in the protein-free fraction of human serum. This substance is essential to the growth of the NRS strain of *E. histolytica* in egg white buffer infusion medium containing starch. 2. Par-

[4] Balamuth, W., and Howard, B., *Am. J. Trop. Med.*, 1946, **26**, 771.

tially deproteinized filtrate of human serum diluted in an equal volume of M/15 buffer, with rice starch added, constitutes a simple medium for the propagation of *E. histolytica*. 3. Rice starch appears to act as a temporary inhibitor to bacterial multiplication in these cultures. Such suppression leads to more satisfactory growth of the amebae present. 4. Study of the biology of the bacteria in these cultures may give further information concerning the complex metabolic requirements of *E. histolytica*.

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Redistribution of Residual Blood Volume in Hemorrhagic Shock; Relation to Lethal Bleeding Volume.*

ROBERT C. FOREMAN.† (Introduced by Carl J. Wiggers.)

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

When unrestricted hemorrhage is permitted from a major artery bleeding proceeds until death eventuates; the total amount of blood that has flowed out is called the *bleeding volume*. When death occurs hemorrhage has either ceased or is reduced to a negligible ooze even though a considerable amount of blood obviously still remains in the body; this remainder is called the *residual volume*. It is known that bleeding volume of animals is less than normal in shock, an observation that has often been used as evidence that the total blood volume is reduced in shock. The object of this study was to determine the magnitude of the residual volume and its distribution in the body after hemorrhagic shock as compared to that of normal animals.

Methods. Dogs weighing 7.9 to 23.3 kg were anesthetized with morphine sulfate and sodium barbital. Both femoral arteries and a femoral vein were cannulated for the purposes of bleeding, reinfusion, and the continuous recording of mean arterial blood pressure with a mercury manometer. A tracheal cannula was inserted. For determining the bleeding volume in the control animals blood was allowed to flow freely from a femoral artery cannula at the rate of 2 cc/kg/minute until bleeding ceased and/or death ensued. These events were virtually simultaneous in all cases. For producing hemorrhagic shock the animals were bled so that mean arterial pressure was maintained at 50 mm Hg for 90 minutes and then at 30 mm Hg for 45 minutes more, after which all withdrawn blood (heparinized, warmed, and filtered) was reinfused. The bleeding volume of the shock animals was determined in this way: One hour after reinfusion, hemorrhage was begun at the rate of 2 cc/kg/minute and was continued in the same manner until death as for the control animals.

After death the residual volume was determined without delay. The femoral cannulae were tied off. The chest was opened by midline thoracotomy. With suitable precautions to prevent blood loss the inferior vena cava was sectioned about 1 cm distal to the right atrium. Two cannulae were inserted; one distally into the inferior vena cava and the other proximally toward the atrium and the superior vena cava. The atrium was clamped across so as to occlude the right atrio-ventricular orifices. Through this arrangement as much venous blood as possible was collected by the upper cannula from the region of the body drained by the superior vena cava and the azygos vein, while blood from regions drained by the inferior vena cava was collected from the lower cannula. The blood still remaining in these 2 territories was next washed out. A cannula inserted through the wall of the left

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† Research Scholar in Physiology.