resorptions and uterine index above 3.00. The animals fed the basal diet with added tocopherol gave a negative response, with resorptions and a low uterine index.

The remaining rats were kept an additional 10 days, then sacrificed and pooled samples of liver tissue and blood were obtained for vitamin A determinations. Blood from the soy bean lecithin-fed group of rats yielded vitamin A values more than 3 times as great as those of the other groups.

Discussion. The results shown in Chart I indicate that vitamin E is not the determining factor for the differences in growth. The animals fed tocopherol grew no better than those on the basal diet alone. Furthermore, the growth of both of these groups was inferior to that of rats given soybean lecithin. That the vitamin A metabolism is primarily involved is not only concluded from the experimental conditions but also by the appearance of the animals, showing a well defined vitamin A deficiency of the non-lecithin-fed controls regardless of the vitamin E intake. The rats fed lecithin showed no signs of vitamin A deficiency. The blood vitamin A findings are further confirmation of the observed differences in vitamin A metabolism.

The evaluation of vitamin E deficiency by Mason's formula shows that the unknown factor is essential for vitamin E metabolism also and that the rats, even after receiving adequate amounts of vitamin E, develop vitamin E deficiency if the unknown factor is absent. These results are in agreement with the findings of Patrick and Morgan.

Summary. Our earlier findings, that soy bean lecithin contains an unknown factor essential for the utilization of vitamin A, have been confirmed. It has been found that in the rat, as in the chick, vitamin E metabolism is also influenced by this factor.

14738

Simple, Standardized Culture Medium for Physiological Studies on *Entamoeba histolytica.**

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Since the successful cultivation by Boeck and Drbohlav[1] of *Entamoeba histolytica* in the presence of a bacterial flora, a valuable method has been available for the study *in vitro* of this important protozoan pathogen, especially in regard to diagnostic procedures. Other culture methods also have been developed.[2,3,4,etc.] These have in common the use of a solid nutrient slant and a fluid overlay containing an isotonic salt solution plus serum, or egg albumin, or other protein substances. There are several disadvantages in these procedures: (1) It is tedious to prepare culture tubes, especially in the case of egg-slants; (2) the fluid overlays must be sterilized by filtration owing to their coagulability upon heating; (3) the media become turbid owing to active growth of the bacterial flora, resulting in poorer visibility and limited life of the cultures; (4) the amebæ adhere to the slants and render quantitative counting difficult.

More recently Reardon and Rees[5] reported in a brief note that it was possible to omit the serum from the method of Boeck and

* Aided by a research grant from the Graduate School of Northwestern University.

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

2 Dobell, C., and Laidlaw, P., *Parasitol.*, 1926, **18**, 283.

3 Tanabe, N., and Chiba, E., *Acta med., Keijo*, 1928, **11**, 221.

4 Cleveland, L., and Collier, J., *Am. J. Hyg.*, 1930, **12**, 606.

5 Reardon, L., and Rees, C., *J. Parasitol.*, 1939, **25** (Suppl.), 1.

Drbohlav. Snyder and Meleney[6] have reported, without amplification, that they could replace previous methods of cultivation "by an entirely liquid medium prepared by infusing coagulated whole egg in 0.8% sodium chloride."

As part of a general program of one of us designed to study the cellular biology of *E. histolytica,* an attempt has been made to prepare a standardized culture medium which at the same time would avoid the above disadvantages and would be inexpensive and available in any experimental or diagnostic laboratory. Of the several leads followed, that first suggested by Snyder and Meleney proved best. The present paper reports the preparation of a standardized fluid culture medium from an infusion of coagulated egg yolk made up in a buffered salt solution.

It was necessary to consider a number of factors in infusing coagulated egg: the temperature of coagulation, the fineness of subdivision prior to infusing, the fractions of the egg employed, the "concentration" of egg substance employed, the medium in which the actual infusion occurs, etc. All these factors were investigated before the following basic procedure was established: To prepare 250 ml of culture medium, 2 eggs are hard-boiled for 15 minutes. Upon cooling, the egg whites are discarded and the yolks are crumbled in a beaker containing 125 ml of 0.8% sodium chloride solution. The mixture is boiled for 10 minutes, and after replacement of water of evaporation the infusion is filtered by suction and restored to 125 ml volume. The filtrate is autoclaved 20 minutes at 15 pounds pressure. Upon cooling, a slight precipitation of yolk settles, and is removed by simple filtration, after which 125 ml of M/15 phosphate buffer (pH 7.5) is added, making the total salt concentration M/30 phosphate solution in 0.4% sodium chloride. This final mixture is tubed in 5 ml amounts, autoclaved as before, and then is stored under refrigeration until use. Before introducing amebæ a bacterial loop of sterile rice starch is added to each tube. The sterile medium is almost completely transparent when prepared in this manner. It was found, on the other hand, that addition of buffer prior to the first autoclaving, or preparation of the infusion in distilled water, produced a densely turbid medium containing visible aggregates of egg protein.

Under these cultural conditions the growth of several strains of *E. histolytica* has proved uniformly good. Maximal growth is usually attained between the second and fourth days, while active, feeding amebæ persist on the average for more than nine days. The medium itself remains quite transparent throughout this period. The concentration of amebæ is less here, although not greatly so, than in the media of Boeck and Drbohlav or of Cleveland and Collier. This difference is due to the omission of additional nutrients (liver infusion, serum, etc.) and is more than compensated for in cyclical and cellular studies by the prolonged vigorous life of cultures.†

Modifications were sought in this basic medium to permit rapid acceleration of growth when required. The two most successful methods found were: (1) to double the concentration of egg yolks; and (2) to add Wilson liver concentrate powder 1-20‡ in concentration of 0.5%. The concentration of egg yolk cannot be increased indefinitely owing to the tendency of its colloidal particles to settle out of solution. This particular liver concentrate gave the best results of the several tested. Use of either modification, or both together, increased the population count to the highest

[6] Snyder, T., and Meleney, H., *J. Parasitol.*, 1943, **29**, 278.

† After submitting the present paper for publication, there has come to our attention the work of Rees, C., Bozicevich, J., Reardon, L., and Daft, F., *Am. J. Trop. Med.*, 1944, **24**, 189. In comparing media for their effects on the growth of *E. histolytica,* they reported that " . . . there were striking differences between the yields from whole egg, egg white, and egg yolk media, with the last two showing scanty growth." Since their whole egg and egg yolk media were coagula, the present report suggests that our egg yolk infusion may yield nutrients in a more available form than their egg yolk coagulum. This would explain the difference in relative effects on growth and would suggest further study of the nature of the biochemical factors involved.

‡ Obtained through the courtesy of Dr. David Klein, Wilson Laboratories, Chicago, Ill.

levels obtained with the media of other investigators.

The above modification, with both increased concentration of egg yolk and Wilson liver powder, is being tested in a Chicago hospital which cultures some 200 stools weekly. Comparative results indicate provisionally that with this culture medium: (1) there is a greater incidence of excystation of intestinal amebæ; (2) maximal growth of amebæ taken from fresh stools is *at least* as abundant as in other media; (3) the bacterial flora and *Blastocystis* do not grow as well in this medium.

Summary. The advantages claimed for the present culture medium for *E. histolytica* include the greater ease of preparation (and cleaning of used tubes), the prolonged life of cultures, its special suitability for studies on cellular biology, and its equal or superior adaptability for use in clinical diagnosis.

14739 P

The Anti-diamidine Activity of Sodium Nucleate.

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It has been shown recently[1,2] that aromatic diamidines which are active against protozoa *in vivo* and *in vitro* also possess antibacterial activity *in vitro* and are of value in wound therapy.

According to Fildes[3] the essential action of chemotherapeutic compounds lies in their interference with the functioning of vital cell metabolites. This interference may take the form of oxidation of the metabolites, molecular combination with it, or competition for the enzyme which acts on it.

Hawking and Smiles,[4] studying the distribution of stilbamidine in trypanosomes, found that this fluorescent substance tends to concentrate in the blepharoblast and in certain plasma granules. Adler and Tchernomoretz[5] noted that after treatment of certain babesia infections with stilbamidine, the first signs of degeneration occur in the nucleus of the parasites.

The present note describes an attempt to elucidate the mechanism of action of aromatic diamidines on bacteria *in vitro*.

The experiments were carried out with *B. coli* and *S. aureus* using two diamidines, stilbamidine (4:4-diamidino stilbene) and pentamidine (4:4-diamidino-diphenoxypentan). *B. coli* was grown on ammonium-phosphate, glucose, inorganic salts medium and *S. aureus* on the semi-synthetic medium of Knight.[6] The pH was 7.3-7.4, and incubation temperature 37°C. The standard inoculum was 0.1 ml of 1:10,000 dilution of a 24-h agar slant, in 5 ml of culture medium. Inhibition was defined by absence of growth after 48 hours. The sensitivity of *S. aureus* to both diamidines is 3-4 times as great as that of *B. coli*. Stilbamidine is less potent than pentamidine against either of the two test organisms.

The addition of peptone, meat extract, or marmite to the medium lessened or abolished the effect of the diamidines, the degree depending on the amount added. Serum (10%) did not show such an effect. Substances similar in structure to the diamidines: creatin,

[1] Fuller, A. T., *Biochem. J.*, 1942, **36**, 548.

[2] Thrower, W. R., Valentine, F. C. O., McIndoe, A. H., Tilley, A. R., Morley, G. H., Bentley, J. P. M., Kohn, F., Hall, M. H., and Gross, C. D., *Lancet*, 1943, **1**, 133.

[3] Fildes, R., *Lancet*, 1940, **1**, 955.

[4] Hawking, F., and Smiles, J., *Ann. Trop. Med.*, 1941, **35**, 45.

[5] Adler, S., and Tchernomoretz, J., *Ann. Trop. Med.*, 1941, **34**, 199.

[6] Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 966.