

4 days after inoculation, and dying upon the 8th day after inoculation, amebic ulcers were numerous throughout the colon, especially in the rectum, and motile trophozoites of *Endamoeba histolytica* were present in the lesions. In these very susceptible dogs the reaction appears very promptly and the invasion of the tissues by the amebae, with the production of very severe lesions, is surprisingly rapid and extensive.

The gradual disappearance of the positive reaction was noted in the dogs that recovered spontaneously or after being placed upon a liver diet which resulted in the disappearance of the symptoms of infection.

The results of the complement fixation test upon 42 dogs free from infection with *Endamoeba histolytica* used as controls were uniformly negative and at autopsy these animals did not show any evidence of amebic infection.

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Studies Upon the Filterable and Non-Filterable State in the Tunnicliff Coccus of Measles.

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This communication reports the results of tests upon the differences in filtrability of the Tunnicliff coccus of measles under *in vivo* and *in vitro* growth conditions. The question whether microorganisms ordinarily regarded as non-filtrable can become filtrable and vice versa, under special conditions of growth, is of more than academic interest. Kendall,¹ with the use of his "K" medium, claims that it is relatively easy to cause non-filtrable organisms to become filtrable and filtrable to become microscopically visible and non-filtrable.

Different environmental conditions cause some microorganisms to undergo rather marked changes in size and shape of the individual cell. Particularly is this true for certain parasitic varieties capable of cultivation *in vitro*, where they are distinctly larger and often differently shaped than they are *in vivo*. Adaptation to the artificial environment is the accepted explanation for the morphological variation. The reaction of the culture medium, quality of nutritives,

¹ Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**, No. 8.

absence of the living host influence and the osmotic pressure are undoubtedly responsible for this *in vitro* metamorphosis.

Experiments. Full-grown, healthy guinea pigs were intracardially injected with 1 cc. quantities of a heavy saline suspension from a 24-hour blood-agar growth of pure cultures of the Tunnicliff coccus of measles. The cultures used for animal inoculation were isolated in 1926 by Duval and Hibbard² directly from the blood of human measles upon a modified Noguchi plasma medium. The morphology was that of a Gram positive diplococcus corresponding in size to the ordinary pneumococcus.

Another series of animals was inoculated intracardially with 5 cc. of the Berkefeld "N" filtrate from 24-hour growth of the culture. From the filtrate tested for sterility upon a variety of media, no growth of any kind was obtained. Likewise, there were no filtrable forms present in the filtrate since the animals inoculated failed to react and nothing was recovered from their cultured blood.

Three days after inoculation the animals were sacrificed and the hearts' blood collected under sterile conditions for study. Parts of the collected guinea pig blood were filtered through the Berkefeld "N" filter and planted upon a variety of media including the "K" medium of Kendall. Parts of the unfiltered blood were likewise cultured upon similar media. Regularly, though with considerable difficulty and then only upon special media, was the coccus of measles recovered from the Berkefeld "N" filtered guinea pigs' blood. Growth in the coccal form was obtained earlier upon "K" medium of pH 8.4-7.2 than any other. Upon this medium the coccus culture was established in 2 to 3 days.

Comments. We believe that the results are further evidence that the Tunnicliff coccus of measles *in vivo* (man, guinea pig, rabbit, monkey) is extremely minute (virus form) and readily filtrable through the "N" Berkefeld filter. On the other hand, in adaptation to an *in vitro* environment it becomes the familiar coccal form which is not filtrable. Of further significance is the fact that in repeated passage of the culture through animals there is the same remarkable metamorphosis.

Experiments with culture filtrate prove conclusively that there is no separate filtrable virus in symbiosis or otherwise with the Tunnicliff coccus culture. Likewise, tests show that the culture *in vitro* does not contain a filtrable and non-filtrable form of the same micro-

² Hibbard, R. J., and Duval, C. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **23**, 853.

organism (Tunncliff coccus). In view of Hadley's³ claim that certain cultures of microorganisms possess simultaneously, filtrable and non-filtrable forms, it was considered a possibility in the case of the Tunncliff coccus but was unsupported by experimentation.

Special media have nothing to do with causing the Tunncliff coccus to become filtrable or reinducing it subsequently to become non-filtrable. The interchangeable metamorphosis is determined by natural environmental factors (living tissue) on the one, and by artificial growth conditions on the other hand.

Finally Tunncliff's coccus of measles is capable of existing in two states, filtrable (*in vivo*) and non-filtrable (*in vitro*).

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Eosin-Light Injury of Erythrocytes. 1. The Influence of Certain Ions.*

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To investigate the problem of photo sensitization in pellagrins, need has been felt for quantitative *in vitro* methods. The observations here described were made with the hope of aiding in the development of such methods.

As a sensitizing agent yellow eosin (National Aniline and Chemical Company) has been used. The light source was a nitrogen-filled tungsten filament lamp of 1500 watts, operated at 115 volts by alternating current. The suspension of erythrocytes was contained throughout exposure to light and measurement of degree of hemolysis in standard Pyrex test tubes of 16 mm. outside diameter with walls 1.5 mm. thick. Equal exposure of each tube was insured by placing the tubes in a device to be described in detail elsewhere. This apparatus moved tubes in a circle 30 cm. in diameter about the light source at the approximate center, while the tubes were immersed in a constant temperature waterbath at 37.5° C. and received light through plane windows of plate glass 3 mm. thick. The layer of tap water between the tube and the plate glass was 2 mm. where the tube's circumference came closest to the window. Adequate provision for aeration and maintenance of suspension was

³ Hadley, P., *J. Infect. Dis.*, 1927, **40**, 301.

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