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Studies on a Medium Yielding the Filtrable Phase of the Tubercle Bacillus.

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In recent studies[1, 2, 3] on a filtrable phase in a life cycle of the avian tubercle bacillus we have stated that a "modified" Kendall medium was employed, but that further study of it was under way in order to assess more clearly the environmental factors responsible for the phenomenon. This represents a progress report on that work.

Technique. Human brain is ground and extracted 3 times with 4 volumes of 95% alcohol, then once with 4 volumes of benzol. Extractions are conducted at 37°, with 2 days for each one with occasional stirring. The residue should be waxy in character, instead of fibrous as is the case for intestine. If this does not obtain, we cut the time of benzol extraction to ½, ⅓, or more. White, powdery residues have not proved as efficacious as amber, waxy ones. Two percent of the dried residue has been used with Tyrode's solution prepared according to the footnote in Kendall's paper,[4] but with the exception that sodium bicarbonate and glucose are omitted. After autoclaving, the reaction is not adjusted with sodium bicarbonate or any other alkali.

There were 2 reasons for employing brain. First, because the strains of tubercle bacilli (Avian S and Bovine III) with which we worked refused to grow in the intestinal K medium; and second, because we wished to take advantage of the possible nutritious influence of the lipoids whose cultural bearing we suspected from previous unpublished experience with this organism. Then the curious partition coefficients of some of the brain lipoids, the phosphatids, facilitated their retention in the dried residue. A suggestive indication of the lipoid function is the fact that if we extract all of them growth is doubtful, with the production of granules and dissociation changes negative. Moreover, when certain of these extracted

[1] Mellon, R. R., and Fisher, L. W., *J. Bact.*, 1932, **23**, 18.

[2] Mellon, R. R., and Fisher, L. W., *J. Inf. Dis.*, 1932, **51**, 117.

[3] Mellon, R. R., Fisher, L. W., and Richardson, Ruth D., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 80.

[4] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**, 8, 1.

lipoids are replaced in the medium, a definite affinity for them on the part of the growing organism has taken place.

But a second factor, apparently significant in its importance, is a reducing substance present in somewhat variable amounts in different brains, indicated by the partial reduction of methylene blue when the medium is let stand at 30° in the incubator for 3 to 5 days. There is thus effected a very definite change in its oxidation-reduction potential. The importance of this reducing substance is suggested by the fact that a human brain which contained no trace of it has consistently refused to grow the tubercle bacillus, even though it showed 5% of lipoids on extraction. A sheep's brain, containing a very small amount of the substance and whose lipoid content is yet undetermined, shows a small amount of growth; while a human brain containing enough to effect approximately a 50% reduction of methylene blue shows an excellent growth of the organism—avian tubercle bacilli, S form—when grown for 2 or 3 weeks at 30°C. This is the temperature at which most of the experiments were conducted. Present data, therefore, point to the rôle of 2 factors at least. Growth occurs in this medium diffusely or as a downy precipitate.

When peptone broth is employed it merely replaces the Tyrode's solution. A few such experiments were run with as high as 3% peptone for the purpose of testing Kendall's theory regarding its antagonistic effect in this type of dissociation. Such antagonistic effect was not observed. This, together with the negative results with the perfectly fat-free residue, leads one to suspect that the mechanism of growth and dissociation is connected with one or more of the chemical factors which we still have under investigation, rather than with unaltered(?) protein as Kendall believes.

Not only with intestine have we failed to get positive filtrates, but almost wholly with lung and liver K media. The relative constancy (25-30%) with which we succeeded with brain medium would seem promisingly to limit the mechanism to some chemical factors fairly specific perhaps, for brain. By the same token, no support is afforded a contamination hypothesis in explanation of the origin of these non-acid-fast forms. Moreover, their dissociation and isolation occur at times in the original, or the reinoculated, media[2] *without filtration;* and certain of such isolated cultures in their early generations are filterable through Berkefeld N candles that have never been in contact with K media. In other words, they conform to Hadley's G-types. Such stabilized non-acid-fast cultures (cocci and diphtheroids) have been transformed to typical

acid-fast strains of an extreme and rather unique R form of the avian bacillus as previously reported. More recently this R form has been dissociated to an avian S culture, serologically and in virulence essentially the same as the original. Thus the last step in the cycle is complete.

Summary. Human brain from which the lipoids have been partially extracted with alcohol and benzol has, in our hands, been successful in yielding the filtrable and non-acid-fast cyclostages of the avian S tubercle bacillus. This appears to be due to two chief factors: (1) the lipoids functioning possibly in a metabolic way; (2) a reducing substance which provides an oxidation-reduction potential of suitable range.

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Replacement of Gonadotropic Action of Pituitary in the Hypophysectomized Rat.

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We have pointed out that the administration of anterior-pituitary-like hormone (A.P.L.) to the hypophysectomized rat cannot prevent the degenerative changes in the ovary which invariably occur after the removal of the pituitary.[1] We also found that it is impossible to induce maturation of follicles and corpus luteum formation in the hypophysectomized rat with A.P.L. injections. In the absence of the hypophysis, A.P.L. leads only to the luteinization of thecal cells but it does not act upon the ovary as it would under normal conditions. The present experiments show us that it is possible to obtain both follicle maturation and corpus luteum formation in the hypophysectomized rat if certain pituitary extracts are administered simultaneously with A.P.L.

In the first series, 6 rats were hypophysectomized when 22 days old, and from that time they received 0.5 cc. of a 0.5% aqueous ammonia extract (1 cc. = 1/10 gm. of anterior lobe tissue), and 25-50 units of A.P.L. daily for 9 days. They all showed squamous vaginal smears on the fourth to the sixth day, and the histological appearance of their ovaries was the same as that of normal immature

[1] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.