Kolnitz[12] have shown that rat thyroids 400-1000% above the normal weight can be produced by a goitrogenic diet.

Since this work was completed, Remington has reported, at the April, 1937, meeting of the American Chemical Society, that neither saturated nor unsaturated fats, up to a level of 13% of the diet, have any measurable effect upon the size, dry matter, or iodine content of the thyroid gland. Our results are in accordance with his. Under the conditions of our experiments, neither the type nor the amount of fat in the diet had any demonstrable influence upon the weight or histology of the thyroid.

9380

Observations on the L-Organism of Klieneberger.

LOUIS DIENES AND GEOFFREY EDSALL.

From the Department of Pathology and Bacteriology, and the Anaphylaxis Laboratory, Massachusetts General Hospital, Boston.

The *streptobacillus moniliformis*, under various names, has been reported to occur in rats in pulmonary disease,[1,2,3] otitis media,[4] and as inhabitant of the nasopharynx;[5] in mice, as an agent of systemic disease;[5, 6, 7] in humans, in fevers following the bite of rats or other small animals,[8-11] or as an epidemic[12] or isolated[13] infection of variable transmission. A similar organism has been observed by Smith[14] in pneumonia of calves. All organisms described in the above reports are characterized morphologically by huge bulbous or

12 Levine, H., Remington, R. E., and von Kolnitz, H., *J. Nutrition*, 1933, **6**, 325.

1 Tunnicliff, R., *J. Inf. Dis.*, 1916, **19**, 767.

2 Jones, F. S., *J. Exp. Med.*, 1922, **35**, 361.

3 Klieneberger, E., and Steabben, D. B., *J. Hyg.*, 1937, **37**, 143.

4 Nelson, J. B., *J. Inf. Dis.*, 1930, **46**, 64.

5 Strangeways, W. I., *J. Path. and Bact.*, 1933, **37**, 45.

6 Levaditi, C., Selbie, R. F., and Schoen, R., *Ann. Inst. Pasteur*, 1932, **48**, 308.

7 Mackie, T. J., Van Rooyen, C. E., and Gilroy, E., *Br. J. Exp. Path.*, 1933, **14**, 132.

8 Schottmüller, H., *Derm. Woch.*, 1914, **58** (Supp.), 77.

9 Blake, F. G., *J. Exp. Med.*, 1916, **23**, 39.

10 Dick, G. F., and Tunnicliff, R., *J. Inf. Dis.*, 1917, **23**, 183.

11 Scharles, F. H., and Seastone, C. V., *New Eng. J. Med.*, 1934, **211**, 711.

12 Parker, F., Jr., and Hudson, N. P., *Am. J. Path.*, 1926, **2**, 351.

13 Levaditi, C., Nicolau, S., and Poincloux, P., *Compt. Rend. Acad. Sci.*, 1925, **130**, 1188.

14 Smith, T., *J. Exp. Med.*, 1918, **28**, 333.

club-like swellings, which appear early in a developing culture, and later make up a large part of it. These swollen forms gained great theoretical importance when Klieneberger[15] observed that they really represent an organism separate from the bacillus, which could be cultivated and grown alone, showing no reversion to bacillary forms in the 2 years of observation. Furthermore, the properties of this organism (designated "L" by Klieneberger) differ in many respects from those of bacteria and fungi, and resemble the curious organisms of *pleuropneumonia bovis* and of agalactia. The L-organism being very soft and fragile, its real form is difficult to ascertain. It is known, however, to be very pleomorphic, exhibiting large disc-like forms, small filter-passing granules, and filaments of greatly varying size. Thus we have an entirely new type of observation before us: The cultures of a bacillus causing disease both in animals and in man appear to consist of 2 organisms, one of which shows properties associating it with organisms usually classed with the filtrable viruses. Recently Klieneberger[3] has found that the pleuropneumonia-like organism is frequently found alone, without the bacillus, in the lung-abscesses of rats. In contrast, however, all observations indicate that the streptobacillus cannot be freed of the characteristic swollen forms of the L-organism.

In the course of some studies on rats undertaken in the Anaphylaxis Laboratory, the opportunity arose to make some observations upon the symbiont present in cultures of *Streptobacillus moniliformis*. These observations confirm Klieneberger's work and extend the knowledge of the pathogenicity of the L-organism. The middle ear of a rat showing typical "twisting" was opened, and the small amount of pus found therein was planted on a medium similar to that recommended by Klieneberger[15] (centrifuged boiled blood agar added to an equal volume of horse serum). In 2 to 3 days about half a dozen typical *Streptobacillus moniliformis* colonies developed on the plate. The remainder of the plate was densely covered with tiny colonies corresponding to those of Klieneberger's L-organism. Impression-preparations of the bacillary colonies always showed the bacteria clearly, but bacterial forms were never found in the first or subsequent subcultures of the L-organism. These have been maintained pure and viable by frequent transfer for 5 months to date (May, 1937), and have been submitted to several animal passages. The L-organism has preserved its peculiar character and has not reverted to a bacterial form.

Another strain of the organism was obtained, in association with

[15] Klieneberger, E., *J. Path. and Bact.*, 1935, **40**, 93.

various bacillary forms, from a gastric abscess in a rat. We examined also about 12 large and small abscesses in rats' lungs, and found repeatedly in the sticky pus the short filamentous forms seen first by Tunnicliff,[1] and identified later with the L-organism by Klieneberger.[3] But neither in solid nor liquid media, nor by mouse-inoculation could we obtain any growth of these organisms, although in 3 cases the streptobacillus grew out.

A strain of particular interest because of its source was obtained from an excised suppurating Bartholin's gland from a human patient. On ascitic agar the pus yielded a very dense growth of L-forms, without any accompanying bacteria. The patient was a laboratory worker coming in frequent contact with rats. The clinical description of this case will be reported elsewhere.[16]

Klieneberger published no data concerning the pathogenicity of her cultures. Our cultures possessed a marked pathogenicity for white mice. We injected white mice subcutaneously and intraabdominally with small pieces of agar containing a dense pure growth of tiny L-colonies, ground and suspended in saline. The mice died in from one to 10 days. Animals dying in approximately one to 3 days showed no pathologic changes except a very slight pleural exudate. Mice dying later showed abundant clear pleural exudate, and in some animals a thick subcutaneous edema developed over the whole ventral surface of the body. In animals injected intraäbdominally, more or less peritonitis and peritoneal exudate were also present, and some of these animals also developed subcutaneous edema. From the pleural, peritoneal and subcutaneous exudates the L-organism grew abundantly in pure culture, but no organisms were demonstrable in the exudate by direct microscopic examination, using either the dark field, or various staining methods. The heart's blood was sterile in all cases except one, dying within 24 hours, which yielded a few L-colonies. It is interesting to note that the disease produced by the pure L-organism differs in some respects from that ascribed to the streptobacillus in previous observations.[5, 6, 7, 17]

The pathogenicity of the L-organism for mice is not uniform. Our first strain when first tested killed 2 mice in 4 days. After 2 animal-passages death occurred only after 5, 6 and 10 days. Some fully grown white mice, previously employed in pneumococcal typing, showed no symptoms following injection of L-organism suspensions. The mice dying in 1 to 3 days, on the other hand, were

16 Dienes, L., and Parsons, L., to be published.

17 Van Rooyen, C. E., *J. Path. and Bact.*, 1936, **43**, 455.

half-grown animals raised in the laboratory. Twelve mice injected subcutaneously with large amounts of pus from the lung-abscesses of rats developed no signs of any infection. Thus it is possible that both the condition of the strain and of the animal influences the pathogenic action.

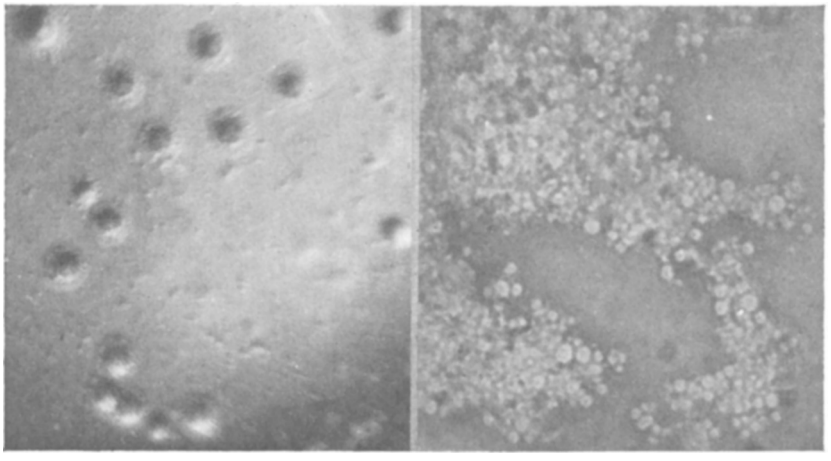

FIG. 1. FIG. 2.

Fig. 1 shows the colonies of L-organism. Original plate from pus found in middle ear of a rat. (About 50 ×.)

Fig. 2, impression preparation from similar colonies; dark background with mercurochrome (1000 ×).

Our observations concerning the morphology of the L-organism confirm those of Klieneberger. We have never seen bacillary or coccal forms in the L-type cultures. The L-form is distinguished morphologically from the accustomed forms of bacteria and fungi by its great fragility and pleomorphism. Our cultures in most instances seemed to consist only of disc-like forms of different sizes. But sometimes, both in our strains and in one furnished by Klieneberger, filaments were present in abundance. The connection between the streptobacillus and the L-organism is entirely obscure. The morphologic development of streptobacillary colonies rather suggests that the L-form, if it is not a derivative of the bacillus, is either a parasite or an internal symbiont thereon, and not simply an admixture to the culture. The large, swollen forms seem to arise by a direct transformation of the bacteria and at first there is no evidence for their independent multiplication. Their subsequent independent growth in the culture, however, shows that they are not the degenerative products of the bacteria, as suggested by Van Rooyen,[17] but are a definite viable form of organism.

Summary. In confirmation of Klieneberger's observation, the L-organism was cultivated from rats. It was also cultivated from a human suppurating lesion. The strains were morphologically and culturally similar to those of Klieneberger, and maintained their character during 2 to 5 months' observation. The L-organism exerts a definite pathogenic action on mice.

9381 P

Effects of Minute Amounts of Lead in the Diet of the Dog.

M. K. HORWITT* AND GEORGE R. COWGILL.

From the Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

The observation that 100 mg. of lead per kilo of diet did not cause any apparent change in the growth, blood and reproductive performance of the rat,[1] led to the extension of the study to another species, namely, the dog. Two litters of 3 puppies each were divided into 3 groups, so that one dog from each litter was in each group, and fed a diet containing 41.2% casein, 29.4% sucrose, 18.3% lard, 7.2% butter, 2.6% bone ash and 1.3% of Cowgill-Karr salt mixture.[2] Seventy grams of dried yeast and 10 gm. of cod liver oil were added to each kilo of diet. Measured amounts of lead acetate were mixed with the salts so that the increment of lead for each group was 0, 25 and 100 mg. of lead per kilo of diet, respectively. The puppies grew well on this ration. Litter I, born of a 12-kilo mongrel, gained an approximate average of 1500 gm. per month and litter II, born of a fox terrier, gained about 700 gm. per month. The added lead in the diets of groups 2 and 3 did not seem to affect the comparative growth of the dogs. One dog in group 3 was a bit smaller than its litter mates, but this animal was quite plump and its decreased size probably was due to the fact that it had more of a smaller breed in its genetic constitution.

Weekly examinations were made of the blood of each dog for hemoglobin content, erythrocyte count and basophilic granulations (stippling) when stained with Wright's blood stain.

---

* Lead Research Fellow.

1 Horwitt, M. K., and Cowgill, G. R. In process of publication, Reported at Meetings of the American Society of Biological Chemists at Memphis, April, 1937.

2 Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725.