with Bouin solution.[4] The coverslips were stained with Giemsa and differentiated with tapwater and ascitic fluid. The blocks were incubated between 25-30°C. Bacterial forms were never visible in the large bodies in the original cultures or immediately after transplantation. After a few hours of incubation, however, many large bodies appeared tightly filled with bacteria and their growth produced the round dense colonies. Bacteria developed more often in the large bodies transplanted from 48-hour-old cultures than from 24-hour cultures. The appearance of the culture and the large bodies filled with bacteria are illustrated in the photographs (Fig. 1).

The bacteria according to all probability were produced inside the large bodies by transformation of the contents of the large bodies. They did not grow in from the outside. We mentioned above that bacteria were never visible in the large bodies in the original cultures. They appeared after as short incubation as 1½ hours and were quite numerous after 5 hours. The bacterial multiplication did not start during this period. The large bodies were either completely filled with bacterial forms or contained none. One never saw only a few bacteria or bacteria in vacuoles as occasionally occurs in bacterial contamination of cultures of L1. The bacteria inside the large bodies are much smaller and thinner than the bacteria which later start to grow outside of them. Bacteria are never visible in the autolyzed large bodies which do not stain well. They are always in those which are deeply stained and well maintained. The growth of bacteria from the content of large bodies was observed in 2 other bacterial species, which makes it easier to accept that a similar process occurs in *Streptobacillus moniliformis*.

Apparently in all species the large bodies possess a double potentiality to develop either into regular bacteria or into pleuropneumonia-like (L) forms. The L forms under appropriate conditions return to regular bacterial morphology. They might represent intermediate forms in the development of the bacteria inside the large bodies. These forms in certain cases progress immediately into regular bacterial forms; while in others they are able to grow in this intermediate form.

[4] Klieneberger, E., and Smiles, T., *J. Hygiene*, 1942, **42**, 110.

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## A Toxic Factor Associated with the Agent of Lymphogranuloma Venereum.

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The production of a toxic factor during the development of the agent of lymphogranuloma venereum has been suspected for the following reasons: (A) The disease can occur in man in severe generalized form[1] with marked headache, chills, and other symptoms suggesting toxemia. (B) The cause of death of the chick embryo after yolk-sac infection is most readily explicable as due to a toxin, since the agent does not invade the embryo to any great extent[2] and disturbance of nutrition would appear unimportant since weight of embryos dead of the infection does not differ from that of uninfected embryos of the same age. (C) In mice, convulsive deaths occurring some 30 hours after intracerebral inoculation suggest toxemia and are not wholly accounted for by the lesions formed in the meninges. Bearing on this problem Gildermeister and Haagen

[1] Harrop, G. A., Rake, G., and Shaffer, M. F., *Trans. Am. Clin. and Clim. Assn.*, 1941, **56**, 154; *Trans. Assn. Am. Phys.*, 1941, **56**, 101.

[2] Rake, G., and Jones, H. P., *J. Exp. Med.*, 1942, **75**, 323.

TABLE I.
Toxic Factor in Yolk-Sac of Moribund Embryo.

| Dilution of "Toxin" | | | | | |
|---|---|---|---|---|---|
| | | | | *Sediment | 4, <15, <15, <15 |
| 1/5 | †5, | 5, | 6, | 6, | 7 |
| 1/10 | <18, | <18, | <18, | <18, | 23 |
| | | | | *Supernate | S, S, S, S |
| 1/20 | <18, | <18, | 21, | 24, | S |

Original (1/5) dilution in infected yolk. Further dilutions in normal yolk. 12 g mice used. All inoculations 0.5 ml intravenously.

* Original dilution centrifuged at 18,000 r.p.m. in cold for 1 hr. Sediment resuspended to original volume.

† 5 = died in 5 hr. S = Survived.

have demonstrated a "toxin" from rickettsia grown in the yolk-sac of the developing embryo.[3]

A toxic product has been demonstrated in the heavily infected yolk-sac of moribund embryos. Little is present in less heavily infected yolk-sacs, *i.e.*, those from embryos which would not die for another 12 or 24 hours, and it disappears after death if the eggs remain in the incubator or more slowly if at room temperature. Yolk-sacs are shaken in their own yolk and fluids to make a 1/5 or 1/10 suspension and further dilutions are prepared in broth, normal yolk, or normal serum (human, rabbit, or calf). Even under favorable conditions the toxic factor is not present in large amounts, and it has not been possible to demonstrate it in dilutions of infected yolk-sac higher than 1/40 or of infected yolk higher than 1/2 (Table I).

The lethal action can be demonstrated in 10 to 20 g mice following intravenous inoculation of 0.5 ml volumes. Death also occurs within 17 hours after intraperitoneal inoculation of 2 ml of a 1/5 suspension. After intravenous inoculation mice may die in 4 hours and most fatalities occur within 24 hours. Convulsions may precede death particularly when this is rapid. That death is due to a toxic product and not to infection is shown by the rapidity of death in many mice (mice infected intracerebrally with the agent of lymphogranuloma venereum, the most rapid method, very rarely die before 30 hours, and usually take 36 to 48 hours); by the fact that the mice either die acutely or become perfectly well while infected animals which survive show all degrees of chronic disease; and by the fact that microscopic examination of the organs reveals no evidences of infection. The tissues do, however, show the results of the "toxin." Focal lesions are found in the brain; small areas of edema and hemorrhage occur in the lung; and the kidneys on occasion show fibrin thrombi in the glomeruli and destruction of the tubular epithelium. Most constant and important, however, are numerous foci of necrosis in the liver which recall those seen in livers of mice dying from psittacosis.[4] It may be stated that it has recently proved possible to demonstrate a similar toxic factor connected with the agent of psittacosis. This "toxin" is more lethal, or is present in larger amounts, than is that of the agent of lymphogranuloma venereum.[5]

The toxic factor seems to be associated with the bodies of the agent. Thus when infected yolk-sacs shaken in infected yolk are first centrifuged at 1500 r.p.m. and the supernate from this then recentrifuged at 18,000 r.p.m. for 1 hour in the cold, the "toxin" is found in the resuspended final sediment of elementary bodies and not in the supernate from which 99% of the agent has been removed[6] (Table I). Similarly it is lacking in any filtrates in which the agent is held back by the filter. Sediment prepared in a similar way from normal yolk-sacs gives no evidence of toxicity.

Antibodies are produced against the toxic factor. Thus serum from rabbits immunized with suspensions of the agent will prevent the

[3] Gildermeister, E., and Haagen, E., *Deut. Med. Woch.*, 1940, **66**, 878.

[4] Rivers, T. M., Berry, G. P., and Rhoads, C. P., *J. Am. Med. Assn.*, 1930, **95**, 579.

[5] Jones, H., and Rake, G., to be published.

[6] Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

TABLE II.
Resistance of Mice Recovered from Sublethal Dose of Toxic Factor.

| Mice | Dilution of "toxin" | | | | | |
|---|---|---|---|---|---|---|
| Controls | 1/10 | <17, | <17, | 18, | 19, | <40 |
| | 1/20 | 22, | S, | S, | S, | S |
| | 1/40 | S, | S, | S, | S, | S |
| Recovered from sublethal dose 10 days before | 1/10 | <16, | 23, | S, | S, | S |
| | | S, | S, | S, | S | |

All inoculations 0.5 ml intravenously.
All mice weighed 15-16 g.

lethal action of the "toxin" when mixed with it and the mixtures allowed to stand for 1 to 2 hours at room temperature before inoculation, or when injected intravenously a few minutes preceding the dose of toxin. Moreover, mice which have recovered from one sublethal dose of the toxic factor received 10 to 14 days before are more resistant to reinoculation than are control animals. (Table II.)