exercise of a muscle that had been made ischemic, results in pain. Their work showed that inadequate blood supply to a working muscle causes pain. In my experiments the blood supply, save for the lowered oxygen content, was unaltered in composition, yet pain appeared during exercise. This shows that oxygen want is the the chief contributing cause of pain developing in an exercised muscle. It is, therefore, suggested that the cause of pain in muscular exercise is the incomplete oxidation of the products of muscle metabolism. The same process may be responsible for the pain of angina pectoris (Rothschild and Kissin[3]) and of intermittent claudication.

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Filter-Passing Bacteria in Polluted Water.

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References to epidemics of gastro-enteritis attributable to polluted drinking water are numerous in the literature. Usually these outbreaks are followed by cases of typhoid fever; occasionally they consist only of acute intestinal distress without the subsequent development of cases of typhoid fever. A series of such epidemics in the Ohio River valley was investigated recently by Veldee.[1] He stated, "There is no epidemiological evidence to show that the ailment was produced by a viable organism contained in the water supply, unless we are to assume the sudden appearance of some bacterium or virus whose presence was not indicated by the established methods of water analysis."

The possibility of the presence of some such microorganism in sewage-contaminated water was investigated. This report concerns the isolation and preliminary study of a form of viable organism from polluted river water, the filtrates of which were apparently sterile when tested by ordinary bacteriological methods.

A sample was taken from the Chicago River at a point where it was heavily polluted with domestic sewage. After filtration through

[3] Rothschild, M. A., and Kissin, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 577.

[1] Veldee, M. V., *Am. J. Pub. Health*, 1931, **21**, 1227.

paper, the water was filtered through a tested Berkefeld N filter and the filtrate collected aseptically. Cultures of this filtrate yielded no growth in solid or liquid media. Serial plating of the filtrate was begun, using the Hauduroy technique[2] as modified in this laboratory.[3] The method consists essentially of surface inoculation of litmus lactose agar plates, incubation for 48 hours, and inoculation of subsequent plates with the washings from the surface of the preceding plate. The inoculations are confined to a square area in the center of the plates, and the capillary pipettes and broth to be used in the operation are sterilized by autoclaving. Three series of plates were used; the first plate of each series was inoculated with a different amount of river water filtrate. The time of appearance of growth in each series of plates varied inversely as the amount of inoculum used on the first plate. For example definite signs of growth appeared in the 7th serial transfer of the set of plates which received 8 drops of the original filtrate. The set of plates derived from an original inoculation of 6 drops of filtrate first showed growth in the 8th serial transfer; and the set inoculated at the beginning with only 4 drops of filtrate did not show growth until the 9th serial transfer. Each of the 3 series of plates was handled in exactly the same manner, with the exception of the amount of filtrate used on the first plates. Aside from the time of appearance, the growth in all 3 series was apparently identical; this fact tends to rule out the probability that we were dealing with chance contaminants. That the appearance of visible growth was delayed in the series of plates receiving smaller inocula, also adds support to the conception that this growth was derived from the filtrate and not from extraneous sources.

The first sign of growth was a thin grayish film appearing over the inoculated area of the plates, and confined to this area. Under the low power of the microscope this was seen to be a confluent patch of growth without discernible structure. Smears showed small Gram negative rods with Gram positive granules scattered throughout, or single granules at each end of the rod. Transferring was continued as above to increase the growth. In the subsequent cultures the growth increased in amount, as evidenced by the thicker film, but was always confluent. In attempting to get isolated colony formation by streaking some of the material on plates by means of a wire, it was found that growth took place in only the more heavily inoculated areas and was confluent here also.

[2] Hauduroy, Paul, *C. R. de la Soc. de Biol.*, 1927, **97**, 1932.

[3] Ryan, V. M., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 899.

Growth in fluid media was slight; no fermentative reactions could be elicited in dextrose, mannitol, xylose, arabinose, saccharose, or maltose. Lactose broth became slightly acid in 10 days, without gas formation. The organisms were non-motile. Each of 2 rabbits were inoculated intravenously with 1 cc. of emulsion from the 13th serial plate; in one case the organisms were living, in the other, killed by heating at 65°C. for an hour. No effects were apparent in the animals, and the inoculations were repeated at intervals to produce agglutinating serum.

After 5 injections of 1 cc. each, the serum agglutinated the homologous organisms in a dilution of 1:640. The serum was also tested against various strains of typhoid, paratyphoid, colon, Shiga, and Flexner dysentery bacilli. No agglutination was noted except in the case of one strain of Flexner dysentery bacilli, which agglutinated in a serum dilution of 1:80. Absorption agglutination tests showed that the agglutinins for the Flexner strain could be completely removed without lowering the titer of the agglutinins for the river water organism.

This feebly growing, biochemically inert organism, occurring in a filterable form in polluted water, may have some sanitary significance, especially in view of the fact that it appears to be antigenically related to a known producer of dysentery. Repetition of these experiments, and further studies, including animal feeding tests are in progress.

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Skin Reactivity of Mothers and Infants to Gonococcus Vaccines.*

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The skin reactivity of the newborn and young infant to intradermal injections of specific and non-specific substances has been studied by many investigators. Their results have invariably indicated that the skin in early life responds poorly or not at all to substances that cause marked reactions in adults and older children. Thus the dermal response to antigens prepared from the diphtheria

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