RESEARCHES

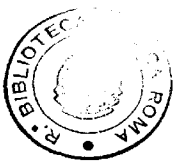
OF THE

LOOMIS LABORATORY.

MEDICAL DEPARTMENT

OF THE

UNIVERSITY OF THE CITY OF NEW YORK.



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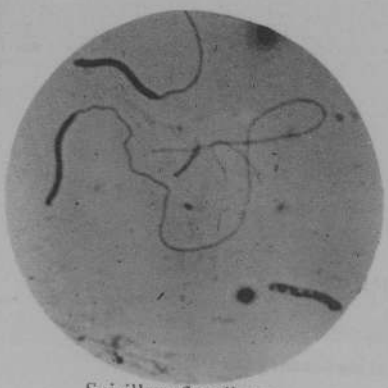
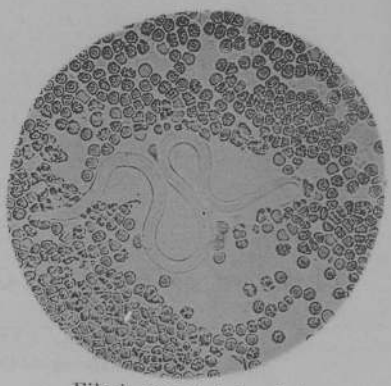
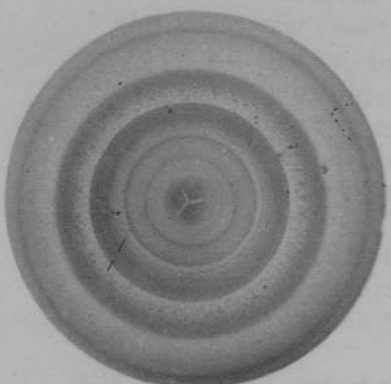


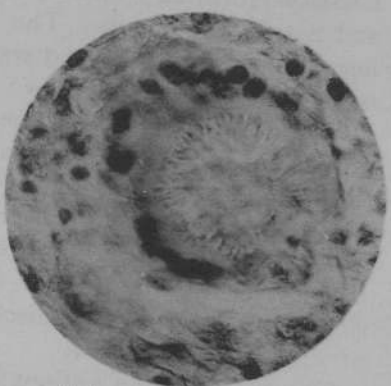
Spirillum flagellatum



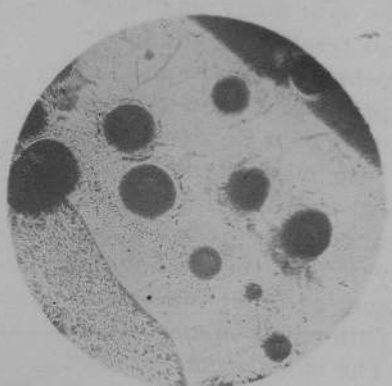
Filaria sanguinis hominis.



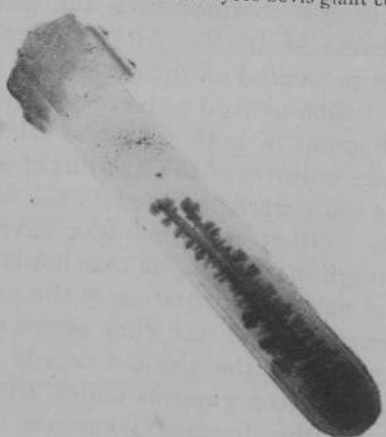
Bac. concentricum.



Actinomyces bovis giant cell.



Colonies of tubercle bacilli.



Pure culture of Lepra bacillus.

PURE CULTURES OF LEPRA BACILLUS.

J. M. BYRON, M.D.,
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HAVING had the opportunity, in the month of June last year, to obtain a few nodules of leprosy tissue from a patient at Charity Hospital, I made a series of inoculations in animals and artificial cultures. The fresh tissue was made into a fine emulsion in sterilized water, and inoculated subcutaneously into four animals, two guinea-pigs and two rabbits. One of the rabbits and one of the guinea-pigs were also inoculated in the anterior chamber of the eye. Neither of the animals contracted the disease, and in two the autopsy did not present any abnormality. One of the guinea-pigs, which is still living, presented after a few days a very large abscess at the point of inoculation, but the pus and the shreds of necrotic tissue discharged did not show the bacilli of leprosy.

Out of the same patient twelve tubes were inoculated with lymph obtained from the wound made, and kept in an incubator at 37° C. After three days ten out of the twelve tubes presented on the surface of the agar many small colonies, which proved to be of a bacillus resembling the tubercle bacillus in both form and microchemical reactions.

Pure cultures of the bacillus of leprosy, or at least claimed to be such, were obtained for the first time by Bordoni-Uffreduzzi. Other experimenters have also claimed this honor, although in not a single case has the disease been reproduced in animals by inoculation of the pure cultures. Such a fact, however, would not very seriously impair the pathogenic character of the germ towards man, as other well-established micro-organisms which are pathogenic in man do not transmit the disease to animals. The inoculations which I made in animals with the fresh tissue of a leper would tend

to prove that the bacillus of leprosy is not transmissible, at least to rabbits and guinea-pigs.

The bacilli of leprosy, as is well known, are abundantly found in the granulomatous new formations of leprosy, in the pus and detritus of leprosy ulcerations, etc. They have not been found in blood, saliva, urine, etc., of lepers, confining themselves only to the tissues which they invade. The bacilli appear as small, curved rods, about three μ in length and 0.2 μ wide, presenting the appearance of short chains of beads, strikingly resembling tubercle bacilli, from which it would be almost impossible to differentiate them, except by the characteristic reactions. In pure cultures the rods are rather thicker and many have their extremities club-shaped; probably these are only early involution forms of the rods when artificially cultivated.

To obtain a pure culture of leprosy bacillus the skin involved is washed first with soap and water, then with 1 : 1,000 solution of bichloride of mercury, next rinsed with alcohol, and dried with ether. A deep scarification is made, enough to reach the diseased tissue. The little wound is squeezed, and with a sterilized platinum loop a small drop of the lymph which oozes is transferred to the surface of an agar tube. The best formula that I know for preparing agar suited for this kind of culture is the following:

Ordinary bouillon, 100 parts; glycerin, 6 parts; rock candy, 0.5 part; agar, 1.5 parts. Boil until the agar is completely melted, neutralize, and filter. Separately dissolve four parts of gelatin in twenty parts of bouillon, filter, neutralize, and add to the filtered agar. The adding of the solution of gelatin after the preparation of the agar is to avoid its being transformed into paragelatin or non-coagulable gelatin, which happens if it be boiled for a very long time, as is necessary when preparing agar. This culture medium answers admirably not only for lepra bacillus, but also for tubercle bacillus, gonococci, and other germs which scarcely grow or do not grow at all on ordinary media, and, best of all, discards from laboratory use blood serum, which involves so many tedious manipulations to obtain it in a pure condition. The infected tubes are kept in an incubator at a temperature of 37° C. After thirty-six or forty-eight

hours, minute, star-like, grayish-white little dots appear on the surface of the agar. These little colonies spread slowly for two or three days, when the growth seems to stop. Then they are about the size of a small pin's head. From this point the growth is extremely slow, and it takes months before they are as large as in the tube of the photograph. In plate or dish cultures (Petri's) kept at 37° C. the colonies appear as round, irregular, whitish dots, which under a low power look like little, irregular heaps, darker in the centre, of a yellowish color on the outside, limited by wavy edges. The colonies grow both upon the surface and in the depth of the agar. Linear cultures obtained by passing an infected needle upon the surface of an agar tube are, I think, quite characteristic; but care should be taken that no condensation water collects in the tube, otherwise the true appearance of the culture is impaired. At the end of thirty-six or forty-eight hours a slight line of grayish-white color begins to appear along the track of the needle, and from here the growth continues to extend outward, producing after many weeks a beautiful fern-like appearance, almost transparent, of a grayish-white color, and about one-sixteenth of an inch wide. The culture is slightly prominent in the centre, from which point radiate little rib-like heaps that run to the edges. Old potatoes dipped in a solution of five per cent glycerin and one per cent of candy sugar are also a good, thriving ground for this germ, the only difficulty being in keeping the potatoes from drying and to select those having a neutral reaction. The growth is absolutely colorless, and cannot be detected except by preparing cover glasses from the vicinity of the needle track. Cultures of lepra bacilli in distilled sterilized water do not thrive, but if the water contains a small amount of organic matter the germs will multiply with relative rapidity. Those experiments I have not yet finished. Cultures in bouillon with the addition of glycerin and sugar grow luxuriantly during the first two weeks as small, whitish, glutinous flakes, which float in the clear medium and which afterwards subside to the bottom. The best temperature for the growth of the lepra bacillus seems to be that of the human body. The growth, though, even under the most favorable conditions, is exceedingly

slow. The resisting power of lepra bacilli to the action of the exterior surroundings is as great as, if not superior to, the tubercle bacillus. I have kept dry cultures of this germ at the temperature of 98° F. for six months, and when re-inoculated in fresh media a new and vigorous growth sprang up from them.

The bacilli, when stained, present under the microscope some oval spaces which do not take the dye, giving the appearance of a little chain of beads. Such spaces are considered by most bacteriologists as spores. Experiments on animals in order to produce this disease artificially have been performed by several observers, among them Damsch and Vossius, who state that they have been able to produce local nodules of leprosy, but not a general infection. The experiments which I have undertaken are yet far from completion. The exceedingly long period of incubation and the slow evolution of the disease forbid me from giving to-day any conclusions, which I am sure would be premature and without any practical value. Later on perhaps I may be able to publish further details as to the results of experimentation on animals.