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ORIGINAL ARTICLES.

BACILLUS ICTEROIDES AND BACILLUS X.

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In order that there may be no misapprehension upon the part of bacteriologists who are now engaged in researches relating to the etiology of yellow fever, I give below a brief extract from a paper which I shall send to the *Centralblatt für Bakteriologie* for publication.

EXTRACT.

As stated in my previous paper, my information relating to Sanarelli's bacillus, when this paper was written, was obtained from a translation in the *British Medical Journal*, of his "Address" given before the University of Montevideo, June 10, 1897. I have since read his paper in the *Annales* of the Pasteur Institut, and upon my return from Europe in September I passed through Paris, and through the courtesy of Dr. Roux obtained a culture of the "bacillus icteroides" which had recently been sent to the Pasteur Institut by Dr. Sanarelli. I now recognize the fact that there are certain cultural differences, some of which have been pointed out by Sanarelli in his paper in that journal. At present bacillus X is non-motile, while Sanarelli's bacillus is actively motile. But in my original cultures, as stated in my published report, bacillus X was motile. The presence of flagella may now be demonstrated by proper staining methods.

In judging of cultural differences now existing, it must be remembered that bacillus X has been cultivated in artificial media for eight years. In the comparative study now being made in the pathologic laboratory of the Army Medical Museum in this city, the peculiar seal-like colonies, to which Sanarelli attaches so much importance, have not been observed in cultures of bacillus X. Again, bacillus X causes gas production in lactose bouillon, while Sanarelli's bacillus does not.

In view of these facts complete identity in biologic characters can not be maintained.

The next question which arises is, whether the bacillus obtained by Sanarelli from yellow fever cadavers in Rio de Janeiro, and by me from yellow fever cadavers in Havana, are varieties of the same species. Comparative experiments are now being made under my direction with a view to determining this question.

In my opinion bacteriologists are often too much inclined to differentiate species upon slight cultural differences, such as the appearance of colonies, ferment action, etc. Sanarelli himself has shown us, in his paper published in the *Annales* of the Pasteur Institut, three varieties of the colon bacillus, which may be distinguished by differences in the colonies formed upon gelatin plates. Every experienced bacteriologist knows that varieties of all the best known species of bacteria exist and that these may differ as to their pathogenic power, the rapidity and character of their growth in various culture media, their power to produce pigment, the production of acid, of gas, etc.

If bacillus X and Sanarelli's bacillus icteroides are not even varieties of the same species, the question will remain whether one, or the other, or neither, is concerned in the etiology of yellow fever.

It is evident that neither has a claim worthy of serious consideration upon any other ground than that which relates to its pathogenic action. Sanarelli's claim is based chiefly upon his inoculation experiments upon the lower animals and upon man, and his results deserve the most careful consideration. Comparative experiments upon dogs, rabbits and guinea pigs are now being made, under my direction, with Sanarelli's bacillus and with my bacillus X. It is my intention to publish full details of these experiments hereafter. At present I must content myself with an account of some preliminary experiments which show that bacillus X has remarkable pathogenic properties when tested upon dogs by Sanarelli's method (injection into a vein). These experiments have been performed in the pathologic laboratory of the Army Medical Museum by Maj. Walter Reed, surgeon U. S. A., in charge, assisted by Dr. James Carroll, hospital steward U. S. A., and the record given below has been made for me by Dr. Reed.

Experiment 13.—July 23, 1897, 1:20 P. M. Young dog, No. 327, weight thirteen pounds, inoculated into ear vein with 5 c.c. of a twenty-four hour culture in glucose bouillon of X from blood of rabbit 314. A very active lively animal. At 1:35 P. M. appears very sick, lying down, does not respond to voice. At 2:05 P. M. vomits considerable quantity of partially digested food, followed by fluid stool with tenesmus. At 2:23 P. M. a small brown watery stool. This repeated at short intervals with much tenesmus. At 2:51 P. M. and 3:05 P. M. vomits with much effort a small quantity of grayish frothy fluid mixed with mucus; 3:30 P. M. vomits; 4 P. M. dog lies on its side with extremities rigidly extended. Temperature in rectum 104.1 F.; prior to injection, temperature 101 F. July 24, 9 A. M., temperature 102; refuses food or drink; lies on side with extremities extended; during the day has several thin dark fluid stools containing blood and mucus; 4 P. M. temperature 104; died at 6 P. M., twenty-eight and one-half hours after inoculation.

Autopsy.—Thorax: Thymus gland large, dark red; shows a number of small hemorrhages beneath surface. Mediastinal glands swollen, dark red in color. A small hemorrhage over right auricle, which is distended with blood. Left auricle empty. Right ventricle distended, left contracted. Hemorrhages beneath endocardium in latter. Valves normal. Myocardium, pale red. Numerous hemorrhagic areas beneath pleura over right lung. Both lungs, all lobes congested. Lower lobe right lung edematous. On cut section, reddish fluid exuded freely.

Abdomen: Numerous hemorrhages beneath peritoneal surface of duodenum.

Liver: Of a mottled pale and red color, the light color predominating.

Spleen: Enlarged, dark red, firm.

Kidneys: Enlarged, cortex swollen, pale.

Adrenals: Small, pale.

Stomach: Mucous membrane over greater curvature, of uniform dark red color. No erosions to be seen.

Intestines: Duodenum and upper part of jejunum contains considerable quantity of a fluid black tarry material. Mucous membrane of small intestine pale throughout. Peyer's patches not swollen.

From ileo-cecal valve to anus the longitudinal rugæ of mucous membrane are the seat of marked hemorrhage, which extends into submucosa. Some fluid blood in large intestine.

Bladder: Contracted, containing about 4 c.c. of albuminous urine. Cultures from blood, liver and spleen show numerous colonies of X. Kidney, urine and bile negative.

Microscopic examination of sections shows wide spread necrosis, with fatty degeneration of liver parenchyma and cloudy swelling of renal epithelium.

Experiment 35.—August 6, 1897. Dog No. 347. Weight ten pounds. Injected at 2:45 P. M., with 13 c.c. of a seventy-two hour culture in 2 per cent. lactose bouillon of X from rabbit No. 338. Animal much prostrated by injection; 3:05 P. M. vomits food with much retching; again vomits at 3:15 P. M., followed by watery stool containing mucus. Temperature 4 P. M. 96.4 F.; prior to injection, rectal temperature 101 F. Found dead at 8 o'clock following morning, less than eighteen hours.

Autopsy.—Thorax: Subendocardial hemorrhages in left ventricle. Sub-pleural hemorrhages, upper lobe, right lung. Hemorrhagic infarct lower lobe, same lung.

Abdomen: Liver pale, grayish color.

Spleen: Slightly swollen, dark red, soft.

Kidneys: Deeply congested.

Stomach: Contains about 200 c.c. of fluid blood. Mucous membrane throughout of a dark red color. Both small and large intestines contain much fluid blood. Mucous membrane of small intestine swollen and of a uniform dark raspberry red. Less injection of large intestine, though this is quite marked. Bladder contracted, empty.

Cultures positive from blood, liver, spleen and urine. Negative from bile and kidney.

Microscopic examination of sections of liver show cloudy swelling of cells with congestion of capillaries.

These experiments show that bacillus X injected into the circulation of dogs, produces symptoms and pathologic lesions similar to those produced by equal quantities of a culture of Sanarelli's bacillus. The persistent vomiting, intestinal hemorrhage, albuminous urine (dog 327) and profound changes in the liver cells are certainly very remarkable and give additional weight to the supposition made in my report that "it is possible that this bacillus is concerned in the etiology of yellow fever" (p. 272).

AN IMPROVED METHOD OF DETECTING CASTS IN THE URINE.

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As is well known, two methods are in use for obtaining the sediment from urine for the purpose of microscopic examination. The oldest and most frequently used is by natural subsidence. A quantity of the urine is placed in a cylindric or conical vessel and allowed to stand at rest for twelve or twenty-four hours, or even longer, when the sediment thrown down is removed by a pipette and a drop or more of it examined under the microscope. The other method is by the use of the centrifuge; the urine being placed in suitable tubes and subjected to rapid revolution the sediment is quickly thrown to the outer ends of the tubes, from which it may readily be removed by a pipette and placed under the microscope for examination.

Each of these methods has its advantages and each its disadvantages. The method by subsidence has the advantage of permitting a large amount of urine to be operated on, but it requires much time and the deposit produced, especially in urine from cases of interstitial nephritis, is apt to be very light and occupy a considerable space, so that the microscopic examination of two or three drops of it reveals the character of but a small fraction of the entire sediment, easily permitting casts and other elements to escape detection when they are sparingly present.

In quickness of application and concentration of sediment to a small bulk, easy of exhaustive examination under the microscope, the use of the centrifuge is greatly to be preferred, and on these accounts it has of late years come more and more into general use. It has, however, one serious drawback, particularly in searching for casts when sparingly present, and that is the small amount of urine from which the sediment is obtained. In most centrifuges the tubes for holding the urine are of not more than 15 c.c. capacity, and since in using them they are never completely filled the sediment obtained, even when two of the tubes are used, represents only from 20 to 25 c.c. of the fluid under examination. It is perfectly obvious that in looking, for example, for casts in a case of suspected cirrhosis of the kidney, negative results from comparatively so small an amount of urine would not be of great value; 200 or 300 c.c. of the urine might reveal the presence of casts while 30 c.c. might show none.

During the past two years a combined subsidence and centrifuge method has been devised and put in use by us which leaves little or nothing to be desired for ease and certainty in detecting casts. While of much value in examining for casts in any specimen of urine, it is particularly useful in those many and perplexing doubtful cases in which casts, if present at all, are very sparingly found and which, by either of the usual methods, can be detected, if at all, only after repeated and tedious microscopic examination.

Our method is practiced as follows: About 250 c.c. or more of the urine, as freshly voided as possible, are poured into a glass percolator, such as are used by druggists in making tinctures and fluid extracts. The cylindric or Oldberg form is to be preferred, as the sediment collects more completely at the bottom than when using the conical form. The opening at the bottom of the percolator is closed with a perforated rubber or cork stopper through which passes a piece of glass tubing about 4 centimeters long, and this is connected with a short piece of pure rubber tubing provided with a pinch cock. The entire apparatus, before pouring the urine in, should be washed thoroughly clean and sterilized by the application for a sufficient length of time of a suitable germicidal agent, such as a dilute solution of formaldehyde, followed by washing with recently boiled water. After pouring in the urine a gram or two of chloral hydrate, dissolved in a few cubic centimeters of warm distilled water, are added to retard decomposition; the percolator is now covered with a glass plate and set aside in a cool place for eighteen or twenty-four hours. At the end of this time the sediment of the urine will be found in the bottom of the percolator; if the urine is from a case of suspected interstitial nephritis, for which this method is particularly adapted, the deposit will usually be a light flocculent cloud, and sometimes instead of being at the very bottom of the percolator it will be found floating a few centimeters above it. By opening the pinch cock on the rubber tubing the sediment is drawn off and collected in one or two centrifuging tubes; in all cases the first cubic centi-