A similar analysis was made using an aliquot portion of the original alkaline solution instead of the barium carbonate precipitate. By this analysis 19.09 g. of volatile acids, calculated as CO_2, were found. Assuming that acetic acid is the volatile acid present in addition to CO_2, 3.09 g. of acetic acid would be present in the total volume of alkaline solution. This weight is equivalent to 0.10 per cent of the original casein.

Quantitative determinations of other products will not be possible until a later time.

This is a preliminary report.

[1] Johnson, T. B., and Daschavsky, P. G., *J. Am. Chem. Soc.*, 1919, xli, 1147.
[2] Johnson, T. B., and Daschavsky, P. G., *J. Biol. Chem.*, 1924, lxii, 197.

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Microbic Dissociation III. B. C. G. (Bacillus Calmette-Guérin).

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We shall describe the dissociation of a tubercle bacillus of low virulence, originally of bovine origin, which was isolated by Calmette in 1908, and passed through 230 subcultures on a special bile medium. It is claimed that at the time of isolation it produced lesions in rabbits, but after long cultivation it has lost its pathogenicity for small laboratory animals. The organism is designated as "B.C.G." (Bacillus Calmette-Guérin) and for the last five years has been extensively used for vaccinating children, with the object of attaining prophylactic immunization.

The cultures in our possession were obtained from three different sources. The first was received from Dr. Watson of Ottawa, Canada, and the second was brought from Paris by Dr. Lawrason Brown in the spring of 1926. After seeding this second culture on Sauton's fluid medium, growth and cultural differences were noticed in some of the flasks, and it was dissociated into two distinct types of colonies.

However, the study reported here on dissociation was carried out with the third culture, which came direct from the Pasteur Institute, sent to us by Professor Calmette. As soon as it was received it was suspended in salt solution and filtered as described previously,[1] and inoculated on the surface of gentian-violet medium plates. After a

period of 5 to 6 weeks' incubation, two distinct types of colonies were seen. One of the colonies predominated in large numbers. From one of the plates two colonies, having different characteristics, were removed and again cultivated. This procedure was repeated until dissociation was well established. It can be seen from the photographs that these two colonies have distinct cultural characteristics. Colony R is very waxy, the folds are very smooth and raised, extending from the center to the periphery. The periphery is clean cut, rounded, slightly raised and does not extend into the medium. This colony was very difficult to emulsify and suspensions prepared from it spontaneously agglutinated at 7.2 pH.

Colony S presents an entirely different picture. There are many small, irregular wrinkles which are not smooth and do not appear waxy. The periphery is very irregular and sometimes extends into the medium. This colony is very easily emulsified and the resulting suspension does not agglutinate spontaneously at 7.2 pH.

On Sauton's medium the R colony appeared as small islands surrounded by veil-like filaments. These small islands have a smooth appearance and can be easily lifted when an attempt is made to remove them from the fluid medium. On the other hand, the S colony occurs more in solid masses covering the whole surface of the fluid medium. In attempting to remove a small fraction from the surface growth, the large mass is lifted.

Biological Characteristics of the Two Colonies. Both produce a tuberculin, render inoculated guinea pigs skin hypersensitive to tuberculin (O.T.) and produce precipitins and complement fixing antibodies in rabbits following intravenous inoculation, using homologous, heterologous and Petroff's antigens.

Results of Inoculation in Animals Skin Test Negative to Tuberculin.

I. *Guinea Pig Inoculation.* (a) *Subcutaneous:* Colony R, in doses of 2 mg. dry weight (approximately 12 mg. moist weight) produces a localized nodule which is resorbed in 40 days. Colony S, in like dosage, produces a local lesion which breaks down. There is extension to the neighboring lymph nodes and one animal died of generalized tuberculosis as early as 34 days. The lesion is transmissible in series, producing generalized tuberculosis on subcutaneous inoculation in a second group of normal animals. One animal is still living after 70 days, with local lesion and palpable lymph nodes.

(b) *Left Ventricular:* Colony R. Doses of 1.25 to 2 mg. dry weight are well supported and the animals have lived as long as 150 days. One died of unknown cause in 30 days, and was free of

tuberculosis. Colony S, in similar dosage, produces generalized tuberculosis and the animals died in 17 to 26 days.

(c) *Intratesticular:* Colony R. Inoculation of 1.25 mg. dry weight produced locally typical tuberculous lesions which spread to the neighboring glands and spleen and persisted in animals killed in 51 and 75 days respectively. Colony S. The same dosage results in generalized tuberculosis and death in 75 days. (d) *Intraocular:* (Anterior Chamber or Vitreous) Colony R. A localized lesion was produced with 0.2 to 0.25 mg. limited to the inoculated eye. The animals are still living after 90 days. Colony S. 0.2 to 0.25 mg. produced death from generalized tuberculosis in 42 to 91 days. (e) *Intraspinal:* Colony R. One animal died in 20 days following 0.4 mg. The cause of death was undetermined. Colony S. A like dosage caused death in 9 to 19 days of meningitis.

II. *Rabbits.* (a) *Intraocular:* (Anterior Chamber) Colony R. 0.1 mg. produces a localized caseous lesion. The animals are still alive after 90 days. Colony S. Similar dosage produced localized caseous lesions. (b) *Left Ventricular:* Colony R. Dosages of 0.8 mg. produced no lesions in 3 months. Colony S. Two animals killed 87 days after a similar dosage showed multiple caseous tubercles in both kidneys and in one, tubercles in the lung and pericardium.

(c) *Intraspinal:* Following inoculation of Colony R and S respectively, the animals are still living after 90 days.

Attempts to Raise the Virulence of the Original Whole Undissociated Strain by Rapid Passage Through Guinea Pigs. Four series of guinea pigs skin tested negative to tuberculin were inoculated in the right testicle with 1.25 to 2.5 mg. dry weight. In 1 to 2 weeks the testicle was removed, ground up and reinjected into the right testicle of other normal guinea pigs. In only one series on the second passage have we succeeded in producing a generalized lymphatic tuberculosis which is transmissible in series on subcutaneous and intraperitoneal inoculation of other pigs. Rabbits inoculated intraperitoneally with this strain, which culturally resembles the S colony of the dissociated B.C.G. strain, develop localized caseous lesions which have so far persisted 80 days.

This is a preliminary report.

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[1] Petroff, S. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 632-634.

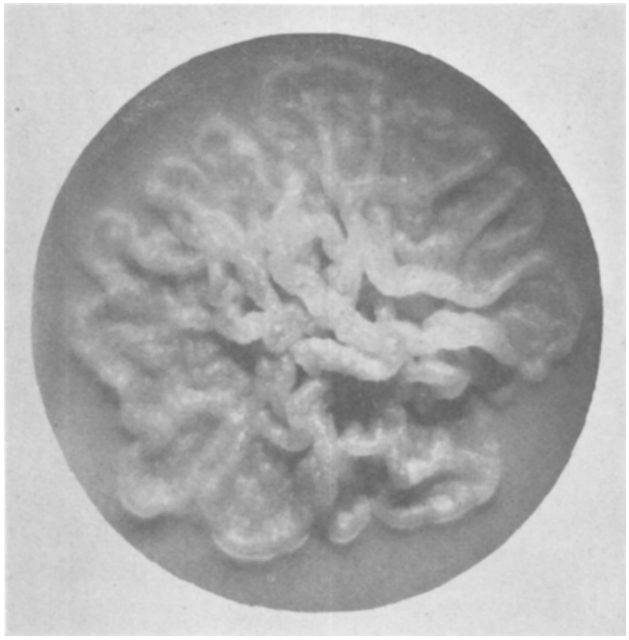

FIG. 1.
B.C.G., Colony R, on gentian-violet medium, 20 X magnification.

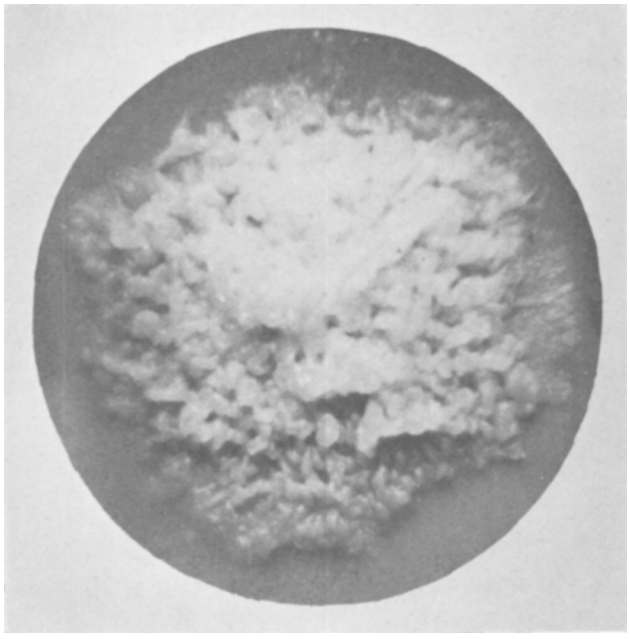

FIG. 2.
B.C.G., Colony S, on gentian-violet medium, 20 X magnification.