

children, with 18 children without record of having had measles, 17 gave positive reaction varying in size from 1 by 1.5 cm. to 2.5 by 5 cm. One child was negative, and on going over this child's record it was found that he had had measles. One child with a 3 by 2 reaction developed measles the day following the reading made 18 hours after the injection. Eight students with a history of measles were tested, and two gave a faint questionable reaction. One was subsequently retested with a dilution of 1 to 2 and gave a strongly positive reaction. Thirteen small children in the Charity Hospital were tested and 8 gave a positive and 5 a negative reaction. Three of the patients with a positive reaction subsequently developed measles. One of the children with a negative reaction was kept for 3 weeks in the measles ward without developing the disease. Fifteen convalescent measles cases gave a negative reaction. One child, just as rash was appearing, gave a positive reaction which two days later was negative; another a slight, faint erythmia 2 x 3 cm.

*Summary.* The toxic filtrate of a bouillon culture of organisms isolated by blood culture when injected intradermally into susceptible individuals causes a transient erythmia, whereas in persons who have had measles, the skin reaction is negative: Confirmatory proof that there is a specific, now isolated, organism of measles which produces a toxin that may be used as an index of immunity to the disease.

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<sup>1</sup> Tunnicliffe, R., *J. Inf. Dis.*, 1925, xxxviii, 193.

<sup>2</sup> Ferry, N. S., and Fisher, L. W., *J. Am. Med. Assn.*, 1926, lxxxvi, 932.

### 3442

#### Further Studies Upon the Etiology of Measles.

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In a previous communication<sup>1</sup> we reported having obtained in pure culture a hemophilic diplococcus from the unfiltered blood of human measles, which apparently was identical to the coccus isolated by Tunnicliff<sup>2</sup> and similar in some respects to the coccus described later by Corona.<sup>3</sup> These workers regard their respective isolation as the active exciting agent in measles. In our earlier report we reserved opinion regarding the possible causal relation that the coccus

might bear to measles, inasmuch as it was obtained from the unfiltered blood, and did not conform with our conception of the excitant of measles (filterable virus).

A recent epidemic of measles in New Orleans has afforded the opportunity to make further studies concerning the incidence of this coccus in the disease.

Blood was secured by veni-puncture from 6 children in the active eruptive stage of measles, and immediately defibrinated. Within 3 hours after the blood was collected 1 cc. portions of both filtered and unfiltered was cultured upon a variety of nutrient media. At the same time rabbits were intravenously inoculated with 1 cc. quantities of the filtered and unfiltered measles blood. The purpose of the animal injections was to serve as controls upon the infectiousness of the blood employed, and also to attempt the cultivation of the coccus in question from the blood of those reacting to the inoculation.

The special nutrient medium which was found most suitable for the initial growth of the coccus was semi-solid sheep serum agar, which had been prepared in tall sterile tubes, and the reaction adjusted to approximately 0.6 per cent acid to phenothalein. The culture medium was inoculated with the measles blood by means of a long fine capillary pipette. This was introduced to the bottom of the culture tube and the content slowly ejected from the pipette as it was withdrawn through the column of medium. First generation growths of the coccus were also attempted from the measles blood by spreading 1 cc. quantities of the inoculum over the slanted surface of human blood agar. This blood agar medium was at least 1 week old before it was used for cultivation purposes, as in our experience the freshly prepared blood medium is not suitable.

In the cultures prepared from the blood of all the cases of measles studied, a small Gram positive diplococcus was recovered in pure culture upon the special media above mentioned. The cultivated coccus completely corresponds in all 6 instances, and also to that of Tunncliff's and our own previous isolations. It is noteworthy that in no instance was there encountered in our cultures any micro-organism other than the coccus described.

As pointed out by Tunncliff, the primary culture of the coccus is obtained with considerable difficulty; however, once visible growth is established, the subplants grow readily and correspond in their general macroscopic appearance to a freshly isolated culture of *B. influenzae*. To obtain the first macroscopic colonization of the coccus we have found it necessary to incubate the primary culture

at 37° C. for 10 to 14 days. At the end of this period there is seen for the first time on microscopic examination of the stained preparation a few Gram positive diplococci and short chains of definite coccal involution forms. After the second or third transfer to fresh media of a culture at this stage of development, subsequent generations grow readily in visible form under aerobic conditions at 37° C.

It appears that this coccus from the blood of measles is not only filterable but apparently invisible for that period in which it is living in an *in vivo* environment. Aside from the question of its possible etiological relation to the disease, there is no doubt that the microorganism exists in a filterable form in the circulating blood during the eruptive stage of measles. Tests which we have carried out proved that cultures of this coccus growing well on blood agar medium are not filterable through the Berkefeldt N candles. No growth occurs upon media inoculated with large amounts of the culture filtrate. Likewise rabbits inoculated with quantities of culture-filtrate fail to react, while the animals injected with the culture react leucocytically and pyrexially. Furthermore, the coccus may be cultured from the filtered blood of reacting animals, but the same difficulty is experienced in obtaining the initial artificial growth as in the instance of the human measles blood.

It is of interest to note that the coccus grown anaerobically for several generations becomes quite small and will pass through the Berkefeldt N filter. We have further determined that the coccus can be recovered directly from the host by aerobic methods of cultivation. In our hands it has not been necessary, as claimed by Tunnicliff, to employ anaerobic methods in order to obtain the primary growth upon artificial media.

The fact that we have repeatedly obtained a culture of this microorganism in the non-filterable form from the Berkefeldt N filtered infected blood is of unusual interest, since it proves for this bacterium a biological capacity to modify its size in accordance with environmental conditions.

A filterable form of a living microorganismal agent occurs regularly in the circulating blood of measles cases during the eruptive stage, and is cultivable from the Berkefeldt N filtered blood upon special media where it assumes and retains in this environment a spherical or coccal shape, measuring on the average 0.5 micron in diameter. It would seem that the presumably invisible filter-passing form is a constant feature of the microorganism in an *in vivo* habitation, while the larger visible form (non-filterable) is its basic morphology in an *in vitro* environment.

The coccus, in culture, is a facultative aerobe, and decidedly positive to Gram's method of staining. It grows commonly in pairs and short chains whose individual cells are always spherical. Macroscopic colonization of the coccus under favorable growth conditions is comparable to that of the freshly isolated *B. influenzae*.

Cultures of the coccus injected into the rabbit induces after an incubation period of 4 to 5 days a pyrexia and leucopaenia. The coccus may be again recovered in culture from the filtered blood of the reacting animal.

The bouillon culture-filtrate of the coccus (after 5 to 7 days growth to 37° C.) produces in the skin of humans susceptible to measles a characteristic reaction, and no reaction in the convalescent or person immune to measles. This phase of the work has been done by Dr. J. Musser and is reported concurrently in this issue of the PROCEEDINGS.

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<sup>1</sup> Hibbard, R. J., and Duval, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1926, **xxiii**, 853-856.

<sup>2</sup> Tunnelliff, R., *J. Am. Med. Assn.*, 1917, **lxviii**, 1028.

<sup>3</sup> Caronia, *Pedicatia*, 1923, **xxxi**, 801.

### 3443

#### Experimental Production of Carcinoma-like Epithelial Metaplasia in Mammary Gland of the Dog.\*

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In a previous communication<sup>1</sup> the histological changes produced in the mammary gland of the rabbit by the inversion of nipple flaps were reported. Various proliferative changes in the inverted skin and glandular epithelium occasioned by the procedure were described. The defensive factors of the rabbit host to the continued growth of the stimulated and proliferating epithelium were noteworthy. Most of the nodules thus produced were eventually fibrosed or absorbed. Certain of the larger nodules, persisting now far more than a year, have been found to contain altered skin and its appendages which produce retention cystic masses.

As tumors of the mammary gland of the rabbit rarely occur it was concluded that even though the normal glandular epithelial cell

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\* Aided by a grant from the B. M. Harrod Cancer Research Fund.