boiled with the food. Although the yeast is dehydrated, it may contain some spores which might cause a slight fermentation. This difficulty, however, may easily be overcome by autoclaving the yeast for a short while before using. The plain dried brewers' yeast (procured from The Vitamin Food Company, New York) was found to be the most satisfactory.

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SPECIAL ARTICLES

HUMAN IMMUNIZATION WITH A DERMAL VACCINE CULTIVATED ON THE MEMBRANES OF CHICK EMBRYOS[1]

IN previous papers with A. M. Woodruff[2,3] we have reported the successful cultivation of vaccine virus on the chorio-allantoic membrane of chick embryos, following the method of Woodruff and Goodpasture in their study of fowl-pox[4]; and we have suggested that this method be applied to the preparation of anti-smallpox vaccine on a large scale. Recently these experiments have been successfully repeated with dermal strains of vaccine by Nauck and Paschen[5] and by Stevenson and Butler.[6]

With the purpose of determining the practicability of preparing and using vaccine cultivated on the chick membranes for human vaccination, we began 15 months ago culturing a dermal strain of vaccine derived from the laboratories of the New York City Board of Health. It was found that an infection free of bacteria could frequently be obtained on primary inoculation, but to insure bacterial sterility the infected membranes were ground and filtered through a Berkefeld N candle. The filtrate, having shown no growth on bacterial media, was centrifuged and a pure strain of vaccine was obtained by inoculating the sediment upon the exposed chorio-allantoic membranes of 10 and 12 day chick embryos. This strain has been propagated during the past fifteen months through eighty-five successive generations without mammalian passage, and apparently it has become stabilized.

The results of the work of the past year have convinced us of the practicability of preparing by this method a vaccine, free of bacteria, with a potency and durability that will insure a stable product over a sufficiently long period to be safe and reliable under field conditions. The vaccine may be preserved dry or glycerinated, and except for its cultivation it is prepared and utilized in the manner now employed for calf vaccine.

After determining that the chick vaccine shows no essential differences from calf vaccine in its pathogenicity for rabbits and monkeys, and that it induces, so far as we can determine by serological and crossed vaccination experiments, an equally substantial immunity in these animals, we made observations upon its effects in man.

Seventeen persons, ranging in age from 3 to 40 years, were chosen. They were judged to be non-immune from the fact that vaccination scars were absent. They were vaccinated over the deltoid muscle of the left arm by the scratch method in the following groups.

Seven persons were inoculated with a dermal strain of vaccine virus which had been carried on the chick membrane through six successive passages. This virus had been stored in the refrigerator at 0° C. over a period of five months, and was finally prepared by adding four parts of 50 per cent. glycerol to one part of ground material. Bacteriological tests proved this vaccine to be entirely free from contaminating micro-organisms. Tests on the rabbit proved it to be of reliable potency.

Seven persons were inoculated with a regular calf strain of vaccine virus prepared by E. Squibb and Sons. This was contained in capillary pipettes in the usual manner of vaccine virus prepared for routine vaccinations. These vaccinations were performed to serve as a control and for comparison with the reactions produced by the chick strain of vaccine.

Four persons were vaccinated with a dermal strain of vaccine virus which had been cultivated on the chick membrane through seventy-five successive passages. This vaccine had been stored in the refrigerator for a period of three months and was prepared in the same manner as mentioned above.

Of the seven persons vaccinated with the regular calf strain of virus, one failed to develop any reaction. One developed a typical vaccinoid reaction which reached its height on the sixth day and then rapidly subsided. The remaining five developed a typical vaccinia which reached the height of its reaction on the tenth to the twelfth day. These reactions were typical in every respect as to the appearance of the successive stages of the lesions and the

[1] Aided by grants from the Divisions of International Health and Medical Sciences of the Rockefeller Foundation.

[2] SCIENCE, 74: 1919, 371, 1931.

[3] *Amer. Jour. Path.*, 8: 271, 1932.

[4] *Amer. Jour. Path.*, 7: 209, 1931.

[5] *Zentrbl. f. Bakt., Parasitkd., u. Infkr.*, I Abt. Orig. 128, 171, 1933.

[6] *Lancet*, ccxxv, 228, 1933.

general symptoms of malaise, slight fever and adenitis.

Of the seven persons vaccinated with the vaccine virus carried through six successive passages on the chick membrane one responded with a typical vaccinoid reaction. The remaining six developed typical vaccinia lesions which passed through the successive stages in the usual manner. General symptoms also were typical of the disease.

The four persons vaccinated with the vaccine virus carried through seventy-five successive passages on the chick membrane all responded with positive takes and developed lesions and symptoms in every respect typical of vaccinia.

Daily observation and comparison of the lesions produced by the regular commercial vaccine and those produced by the vaccine cultivated on the chick membrane showed them to be quite comparable to each other throughout their entire course. The following differences were noted. The lesions developing from the virus cultivated on the chick embryo were slightly milder in their appearance. The different stages of the lesion were delayed about one day, as compared with those appearing from the regular calf vaccine. There was less induration of the surrounding subcutaneous tissues, and the involvement of the adjacent lymph nodes was not quite so extensive. In the pustular stage the lesions from the chick strain of vaccine did not contain as large an amount of pus. The crusts were thinner and more flaky and when separated did not leave so marked a depression as those from the calf strain of virus. On the whole the lesions from the chick strain of vaccine were less painful and caused less discomfort.

The lesions produced by the vaccine virus carried through seventy-five passages on the chick membrane were definitely milder throughout the first eight days of their course, but passed through the successive stages in a typical manner. They then rapidly increased in severity to reach their height on the tenth to the twelfth day when their appearance was quite comparable to the lesions produced by the regular calf strain of virus.

The scars left by all the vaccinations are quite comparable as to size, all averaging about 1 to 1.5 cm in diameter. They are all slightly depressed below the surrounding surface. Those produced by the calf strain of virus are slightly deeper. There is evidently less scar tissue formation in the lesions produced by the chick strain of vaccine, as they feel thinner and are less pitted and wrinkled.

The persons vaccinated with the calf strain of virus and the virus from the sixth passage on the chick were revaccinated nine weeks after the primary vaccinations by the scratch method. A control scarification was made in each case. Those vaccinated with the virus from the seventy-fifth passage were revaccinated in the same manner eight weeks after the primary vaccinations.

The persons vaccinated with the chick strain of virus were revaccinated with a calf strain of vaccine virus prepared by E. Squibb and Sons. Those vaccinated with the calf strain of virus were revaccinated with a strain of chick vaccine from the sixth passage of good potency as tested out on the rabbit.

Except in the case of one person who failed to respond to the primary vaccination typical immune reactions developed in all cases following the revaccination. These were characterized by a slight papule and reddening along the line of scarification which were present after twenty-four hours and reached their maximum intensity between twenty-four and forty-eight hours. By the end of seventy-two hours all reactions had subsided. Several had completely disappeared, while a few were still present as a small papule with very slight reddening along the scarification. Observations on the seventh day showed the lesions had subsided.

Some of the advantages which might be expected from the use of chick embryo vaccine over the calf virus are the ease with which it may be produced at any time fertile hen-eggs are available, absence of bacteria and other contaminating agents, and, if present indications are confirmed, the availability of a fixed strain of virus which does not require mammalian passage to maintain its virulence.

Additional studies are in progress intended further to simplify and improve the technique of chick vaccine production and to test the relative durability of its immunizing effect in man.

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THE EFFECT OF IRON ON THE ESTABLISHMENT OF THE OXIDATION-REDUCTION POTENTIAL OF ALLOXANTIN

ON considering the various instances where heavy metal salts act as catalysts in oxidation-reduction processes, the following observation seems to be of interest in which iron acts as a catalyst for the establishment of an oxidation-reduction potential.

Biilmann and Lund[1] have shown that an acidified solution of alloxantin establishes a definite potential at the blank platinum or gold electrode. The interpretation is based on the well-founded assumption that alloxantin in an aqueous solution is split into dialuric acid and alloxan and that these two substances behave as the components of a reversible oxidation-

[1] E. Biilmann and H. Lund, *Ann. Chim.* (9) 19: 137, 1923.