Chloroform in the Preparation of Vaccine.

Among a number of investigations in relation to the preparation of vaccine lymph, undertaken by the expert staff of the Local Government Board at the vaccine lymph laboratories, that by Dr. Alan Green (Suppl. Thirty-second Rep. Med. Off. Local Gov. Bd., London) on the action of chloroform in removing the extraneous micro-organisms from calf lymph, is of outstanding importance. Dr. Green has been working at this subject for some years, and the results, both as tested by bacteriological methods and by actual use of the vaccines prepared by his method, show the high efficiency and possible utility of the chloroform process. By his experiments he has shown that, as a routine measure, the removal of the extraneous micro-organisms can be effected in two ways—(1) By passing a current of air and chloroform vapour through the emulsion for from one and a half to two hours, and subsequently placing the tubes containing it, sealed so as to retain the chloroform already present, in the ice-chest for from eighteen to forty-two hours; or (2) by passing a current of air and chloroform vapour continuously through the vaccine emulsion for six hours. The vaccine emulsion is prepared by triturating 1 part by weight of vaccine pulp with, as a rule, 3 parts of sterile distilled water by means of a lymph-triturating machine or a mortar and pestle. The water is absolutely necessary in order that the chloroform may enter into solution. A current of sterile air is then passed through pure liquid chloroform, the air in its passage becoming charged with chloroform. The mixture of air and chloroform is next passed through the vaccine emulsion, and the water in the emulsion becomes saturated with the chloroform, this saturated chloroform water being harmless to the specific germ of vaccine, its potency being unimpaired, while the extraneous organisms are speedily eliminated. Contact with pure chloroform, or exposure to the vapour of pure chloroform, of vaccine pulp undiluted with water, markedly diminishes the potency of the lymph. Many of the vaccines treated by the chloroform method contained a large number of extraneous micro-organisms, the average number being 20,000 per platinum loopful. In one case the number was 700,000. Like glycerin, chloroform fails to kill spore-bearing forms.

Up to the issue of his report, forty-five vaccines had been prepared with chloroform and issued for general vaccinating purposes. Each vaccine was carefully tested before and after treatment by chloroform, the vaccines for the series being taken from calves in whom the vesiculation suggested that the potency of the vaccine was not of great stability. In these forty-five vaccines the average number of extraneous organisms present in the untreated emulsion was 14,000 per
platinum loopful. In every case these were eliminated, either after one to six hours of the chloroform process, or at the end of eighteen to forty-two hours subsequent storage, in the case of vaccines subjected to the passage of air and chloroform for only two hours. The vaccines were kept in the laboratory on an average for fifteen days after collection before being used. In all, 48,027 cases were vaccinated with the vaccines, with the following results:

| Number of Cases | Percentage of Success |
|-----------------|-----------------------|
|                 | Case. | Insertion. |
| Primary vaccinat | 35,873 | 97.8 | 91.2 |
| Revaccinations   | 12,154 | 97.1 | 91.9 |
| Total            | 48,027 | 97.6 | 91.3 |

These figures show a very high "case" and "insertion" success. Some of the chloroformed vaccines had control glycerinated portions. The pulp collected from the calf was divided into two portions, one being prepared by Dr. Green's process and issued after an average interval of fifteen days, the other being treated by the ordinary glycerin method and issued after an average interval of seven weeks after collection. The chloroformed lymphs showed a rather higher "case" and "insertion" success than the glycerinated lymphs.

Summarising his results, Dr. Green states that (1) crude calf lymph can be freed from extraneous germs in from one to six hours; (2) vaccine, in times of urgent need, as during epidemics, can be much more rapidly prepared; (3) the vaccines thus prepared give a high "case" and "insertion" success; (4) by the immediate use of vaccines of potency of short duration, economy of material and calves can be effected; (5) in hot climates, where the preservation of the potency of vaccine for any length of time is often difficult, it seems probable that the early elimination of extraneous organisms might be of considerable advantage. Mr. Power, the medical officer of the Board, is of opinion that "should our expectations of this chloroform process (whether alone or in conjunction with glycerin) be realised, those urgent demands which arise suddenly during smallpox epidemics may be in future satisfied within two weeks from the date of vaccination of the necessary calves, instead of, as now, not until lapse of double or treble that period."

**Bacterial Test for Pollution of Air.**

DR. MERVYN GORDON (Suppl. Thirty-second Rep. Med. Off. Local Gov. Bd., London) gives the result of a prolonged investigation carried out by him with a view to obtaining a means of determining bacteriologically, in the air of public rooms, etc., the pollution resulting from
the dissemination of saliva, etc., from the respiratory passages and mouth. In his present series of experiments he has directed his attention chiefly to an examination of saliva, and to experiments on the dissemination of saliva in the act of speaking. He bacteriologically examined the saliva from the mouths of twenty-five different persons to ascertain what micro-organisms were most abundantly present in saliva, and if any one organism could be regarded by its abundance and constant presence as characteristic of saliva. Such an organism he found in *Streptococcus brevis*, which, he states, is commonly present to the number of not less than 10,000,000 per cubic centimetre of saliva. This organism is readily recognisable by the fact that when grown in neutral red broth, the cherry-red colour of the medium is changed to canary yellow, showing in reflected light a green shimmer. Though this change can take place under aerobic conditions, it is more pronounced when cultivation is carried out under anaerobic conditions at 37°C. Minute traces of saliva produce the same change in neutral red broth under anaerobic conditions, owing to the presence and growth of the *S. brevis* contained in the saliva. For this reason neutral red broth "is, when incubated anaerobically for forty-eight hours at 37°C., a culture test whereby very minute droplets of saliva may be readily detected."

Dr. Gordon next proceeded to experiment as to the dissemination of droplets of saliva in the air of a room by means of loud speaking. He exposed, both in a large and in a small room, plates of neutral red broth at varying distances from the speaker, and proved by the subsequent incubation of the broth under anaerobic conditions, the presence in the air of particles of saliva at a distance of at least 40 ft. in front of, and 12 ft. behind, the speaker, who spoke for an hour. [These distances were the extreme points at which the air was tested.] Control plates exposed in the rooms for two hours previous to the commencement of the experiments demonstrated the absence of the *S. brevis* from the air. A streptococcus ("air streptococcus") was sometimes present in the air of the room before experiment, but it is easily distinguishable from the streptococci characteristic of saliva. Much seems to depend on the manner of speaking aloud. In one experiment, in which the reader, whose voice was distinctly heard 45 ft. away, did not exert the same energy as the others, no salivary streptococci were isolated from any of the plates, even from those quite near to the speaker. Two experiments were made to determine the aerial contamination associated with silence and ordinary quiet conversation, and "failed to show that droplets of saliva travel from the mouth into the air of the room during either of these acts."

Dr. Gordon considers that the streptococcus test for saliva "promises to be capable of application as an index of the possible access of morbific virus to air, in a sense similar to that in which the *B. coli* (etc.) test is an index of possible access of morbific matter to water," but points out that its value as a means of estimating pollution of air by breath-borne material, and as a test for efficiency of ventilation, is a matter for the future to determine.
Vegetables and the Spread of Infectious and Parasitic Diseases.

In a research carried out on the vegetables in common use in Padua, Dr. A. Rizzoli (Gior. d. r. Soc. Ital. d'ig., Milano, 1903, No. 1) has obtained results which, in the particular circumstances, are so far reassuring. The experiments were made in vegetables usually eaten raw, including lettuce, fennel, radish, celery, endive, and strawberries. These vegetables and strawberries had been grown in gardens manured with human excrement, and irrigated with sewage water. The vegetables were freshly cut, and were cleaned, washed, and prepared as for the table, the washing being done with ordinary water containing from fifty to one hundred micro-organisms per c.c. Of each vegetable so prepared 100 grms. was taken, placed in a sterilised flask containing 1000 c.c. of sterilised distilled water, and the contents of the flask were violently shaken up so as to wash off all the adhering organisms. The liquid was then allowed to settle in the flask, surrounded by ice, and was used for cultures, inoculation of animals, and for microscopical examination.

The washings from fresh strawberries (previously unwashed) gave 353,000 per c.c., and those from the prepared vegetables varied from 31,725 per c.c. in the case of fennel to 100,424 for endive. The vast majority of the colonies were those of saprophytic organisms, though streptococci, staphylococci, and B. coli communis were sometimes found. The B. typhosus was never found.

Of fifty rabbits inoculated with the sediment, six died, and their blood contained streptococci, staphylococci, and B. coli communis. Of twenty guinea-pigs inoculated, four died, and B. coli was found in their blood; while among forty mice inoculated there were only a few deaths, the cause of death being uncertain.

In the microscopical examination of the sediment, no eggs of cestodes were found, and on only one occasion the eggs of Ascaris lumbricoides. The eggs of earthworms and of insects were common, as were also protozoa, monads, and vorticellæ. Amœbæ were also sometimes present.