Conclusions. — Trypanosomes from the frog may be cultivated on blood-agar, but, in the authors' experience, with considerable difficulty.

From a frog infected with *Tr. rotatorium* a flagellate organism was cultivated, showing important points of difference from *Tr. rotatorium.* It is possible that, owing to the technical difficulties of the experiment, some other organism may have found its way into the tubes. This is improbable.

Undoubted trypanosomes developed in blood-agar prepared from a frog whose blood, during life, showed no trypanosomes. They resembled *Tr. rotatorium,* but were usually much smaller. As this blood-culture medium was inoculated with blood from another source containing *Drepanidium,* it nearly led to the conclusion that *Trypanosoma* might develop from *Drepanidium.* We have here an illustration of the ease with which mistakes may occur in the cultivation of hematozoa which are suspected of passing through cycles. Such a possibility had been pointed out in advance by Novy and MacNeal before this society [page 23 (87)].

There was no evidence from the experiments to show that development of *Drepanidium* can occur on blood-agar.

It is unlikely that material with which further studies may be made can be secured before next summer (1905). As trypanosomes are now exciting so much interest, and are being so widely studied, the authors deemed it best to report their results at this time, although the work is incomplete.

19 (65). **"Experimental measles": LUDVIG HEKTOEN.** (Presented by **EUGENE L. OPIE.**)

The search for the cause of an infectious disease like measles becomes greatly simplified when we learn how to secure the unknown "virus" in relatively pure form unmixed with common microbes. Various methods may now be applied to the investigation of the virus. The transmission of measles from mother to fetus would seem to point to the presence of the cause of the disease in the blood. In the twenty cases of fetal measles collected by Ballantyne, it seemed that the infection of mother and fetus must have been simultaneous, because the eruption in both corresponded in character. In order to learn something further as to the presence

in the blood of the cause of measles, inoculations of human beings would seem to be necessary; because, so far as we now know, this disease is probably not communicable to animals. Grünbaum's experiments with measles in the chimpanzee appear to have given negative results.

Critical review of the literature shows that almost without exception the recorded experiments in the inoculation of measles, for which positive results have been claimed, are without real significance. The claims that the experiments of Home, of Wachsel, of Speranza, of Katona, of McGirr, of Bufalini gave definitely positive results do not stand close scrutiny in the light of the evidence at hand: In many instances the rubeolous nature of the sickness, sometimes very mild, following the inoculation and regarded by the experimenters as measles, is not at all securely established, and in practically all cases the possibility of natural infection was not excluded. These experiments, practically all of which were undertaken with the idea of producing a modified form of the disease, consequently permit no conclusion as to the infectiousness of the blood or other substances in measles. If we accept Mayr's results as they are given by him it may be concluded that in measles, nasal mucus and cutaneous scrapings (containing blood, epithelial débris, and tissue juices) may contain the cause of measles at or near the height of the eruption.

In the following experiments the author tried to determine whether or not in measles at the height of the attack the blood contains the cause of the disease. In these experiments special care was taken to exclude natural infection.

1. The blood injected was taken from a boy of 9, who, in the later stages of desquamation after an uncomplicated attack of scarlet fever, developed a rather mild but typical attack of measles. The first symptoms of measles appeared after he had been free from fever for about two weeks. There was headache, coryza, cough, running of the eyes, and mild febrile symptoms. Three days later a papular eruption was noted, and on the fourth day a typical rubeolous rash was present that soon began to fade, and was followed by branny desquamation.

On the fourth day 4 c.c. of blood were withdrawn from a vein at the right elbow after carefully scrubbing the skin with soap and

water, followed with alcohol. Two flasks each containing 50 c.c. of ascites broth (peptone broth 2 parts, ascitic fluid heated to 55° C. for 45 minutes 1 part) were inoculated[1] at once with 1 c.c. and 3 c.c. of blood, respectively, and placed in the incubator at 37° C. for 24 hours. At the end of this time both flasks appeared sterile, the corpuscles having settled, the supernatant fluid being clear. Subcultures made at this time upon ascites-agar, glycerin-agar, and Löffler's serum, and kept under aërobic and anaërobic conditions remained sterile; and the flask of ascites broth containing 1 c.c. of blood remained permanently sterile.

Four cubic centimeters of the mixture of 50 c.c. of ascites broth and 3 c.c. of blood, which had been kept in the incubator at 36° C. for 24 hours, were injected under the skin of the chest of a healthy medical student aged 24, just finishing desquamation after an uncomplicated attack of scarlet fever, and who readily gave his consent to the experiment. This man was not in the same hospital as the boy furnishing the blood for injection, but had been for twenty-six days in a different institution, at that time as well as before and afterward entirely free from measles.[2] So far as could be learned, and careful inquiry was made, the man injected had not had any disease at all resembling measles except scarlet fever. At no time did any local symptoms appear at the site of the injection. On the thirteenth day after injection the temperature was 101° F.; in the evening it rose to 103° F. At 9 the following morning he was given a warm bath and immediately afterward a red, papular, blotchy eruption broke out on the forehead and spread quite rapidly to the face, neck and chest. Dr. James B. Herrick, who saw him at this time, felt no hesitency in making the diagnosis of measles. By 2 o'clock an unmistakably typical full-blown, rubeolous rash was present over the greater part of the body. The temperature remained above normal for two days, when it fell to normal about the same time that the eruption began

[1] In experiments 1 and 2 a few drops of blood were allowed to run out before inoculating the ascites broth, which was done without the needle of the syringe touching the culture fluid.

[2] In both experiments the injections were made by the author. At the time the injections were made he had not seen any cases of measles within 24 hours. When in the measles ward the usual precautions were used and, of course, similar precautions were followed when visiting the subjects of the experiments — clean long gowns, caps, clean hands, etc. Freshly autoclaved syringes were used for the injections.

to fade. An uneventful recovery followed without any complications whatsoever, the desquamation being branny. There was during the entire illness freedom from respiratory symptoms of all kinds. Even during the preëruptive period there were no special local symptoms (morbilli sine catarrho). The patient's subjective condition was not much changed, if at all, at any time during his illness. The appetite continued unimpaired.

2. In this case the blood was furnished by a well-developed Irish servant girl, 21 years old, who passed through an uncomplicated attack of typical measles. About 30 hours after the earliest appearance of the rash, which still was coming out upon the extremities, 10 c.c. of blood were withdrawn from a vein at the elbow and distributed equally among 4 flasks each containing 50 c.c. of broth and 25 c.c. of ascites fluid. These flasks all remained perfectly sterile so far as bacteria demonstrable by the usual methods were concerned.

After 24 hours at 37° C., 5 c.c. of the mixture of blood in ascites broth were injected subcutaneously in the back of M., aged 28, who had not had measles so far as he knew and who gave his consent to the experiment. This patient was also recovering from a mild attack of scarlet fever, and had been at the time of inoculation for twenty-four days the sole occupant of the isolation room of a general hospital in which at that time there were no other cases of measles. There were no local changes at the site of the injection. The temperature and general condition remained normal until the evening of the eleventh day, when the temperature rose to 99.8° F., and the next day a mild conjunctivitis already suspected a day or so previously became definitely apparent. On the thirteenth day there was some cough, the tonsils were bright red, and there was an increased amount of mucous in the throat. In the afternoon the temperature, which was rising, reached 103° F. During the next night a typical rubeolous eruption came out, the first spots being noticed on the nose, and then on the forehead, face, scalp, chest, back and abdomen. The rash consisted of pink macules and papules, which disappeared readily on pressure, being largest and brightest red over the face. The forehead was quite uniformly red. The patient was not seriously ill ; there was some loss of appetite, but he slept well during the night, having been somewhat restless the preceding night. Recovery was prompt.

Cultures of the blood on the thirteenth day (1 c.c. of blood in each of three flasks each containing 50 c.c. of broth and 25 c.c. of ascites fluid) remained permanently sterile.

Conclusions. — The results of these two experiments permit the conclusion that the virus of measles is present in the blood of patients with typical measles some time at least during the first thirty hours of the eruption; furthermore, that the virus retains its virulence for at least twenty-four hours when such blood is inoculated into ascites broth and kept at 37° C. This demonstration shows that it is not difficult to obtain the virus of measles unmixed with other microbes and in such form that it may be studied by various methods.

20 (66). **"The formation of the centrosome in enucleated egg-fragments": NAOHIDÉ YATSU.**

To test whether the centrosome is a permanent cell organ or not, Professor E. B. Wilson (1901) made an experiment on the sea urchin egg by treating, with a salt solution, enucleated egg fragments obtained by shaking. He observed that asters containing centriole and capable of division were produced in the enucleated fragments. He, therefore, came to the conclusion that at least some of the centrioles in the asters thus formed must have arisen *de novo*. Some writers criticized his results, saying that the formation of the centrioles in the enucleated fragments observed by him might have been due to the shaking-out of the nuclear fluid into the cytoplasm. Wilson, therefore, suggested that his experiment be carried out by the author in a somewhat different manner — instead of shaking, to cut eggs singly and to treat the nucleated and enucleated pieces separately. The author tried this experiment on the egg of *Cerebratulus* in the summers of 1903 and 1904. Strict precautions were taken to prevent accidental fertilization, everything used for the experiment being sterilized. Individual eggs were cut into nucleated fragments (*i. e.*, fragments containing the first maturation mitotic figure) and also enucleated fragments. The latter were kept for an hour in a solution of calcium chlorid. Then they were transferred to sterilized sea water. Asters were produced in almost all enucleated fragments thus treated. What is more striking, all the asters had centrioles which were identical with those found in the whole eggs subjected to the same treat-