THE PRODUCTION OF ANTIBODIES BY TISSUES LIVING OUTSIDE OF THE ORGANISM.*

By ALEXIS CARREL, M.D., and RAGNVALD INGEBRIGTSEN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATE 39.

INTRODUCTION.

The recent development of an efficient technique for large cultures has rendered possible the study of the functions of tissues living outside of the organism. In order to ascertain whether tissues cultivated *in vitro* retained the property of reacting against antigens by producing antibodies, we attempted to induce guinea pig bone marrow and lymph glands to become hemolytic for goat red blood corpuscles. We are very much indebted to Dr. Noguchi, whose advice enabled us to obtain immediately positive results in these experiments.

METHOD.

Guinea pig bone marrow and lymph glands were cultivated in guinea pig plasma in Gabritschewski boxes. Goat blood was selected as an antigen because it is only slightly, or not at all hemolysed by guinea pig serum. The cultures containing goat blood and their controls were incubated for five days. Then the presence of hemolysins was determined in the fluid exuded or extracted from the medium.

Preparation of the Cultures.—Blood was taken from the carotid artery of a guinea pig through an oiled cannula, and plasma was obtained by the ordinary technique.[1] The lymph glands of the neck were extirpated. Then the femurs were resected and the bone marrow was removed. After having been washed in Ringer's

* Received for publication, January 25, 1912.

[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

solution, the bone marrow and lymph glands were cut into very small fragments and suspended in Ringer's solution. They were spread on the cover of a Gabritschewski box, and covered by plasma. In one experiment, guinea pig serum and agar were substituted for plasma. Goat blood was taken aseptically and washed three times in Ringer's solution. Two drops of washed red blood corpuscles were generally added to a culture containing twenty drops of plasma and five or six drops of a suspension of tissues in Ringer's solution. A mixture of bone marrow and lymph gland, and, exceptionally, bone marrow alone, was used. Larger and smaller quantities of blood were also employed, but the results were less satisfactory. Together with every culture containing goat red blood corpuscles, a control culture without antigen was prepared. Other control cultures were made composed only of guinea pig plasma, goat blood, and bone marrow killed by heat. When more precise microscopical examination was needed, small hanging drop cultures in hollow slides were also studied.

The cultures were deposited in the incubator at a temperature of 39° C., and examined every day from the standpoint of growth of tissues, of phagocytosis, and of possible development of bacterial colonies. After four, and generally five days, the cultures were removed from the incubator.

Examination of the Results.—The Gabritscheswki boxes were opened and the plasmatic jelly was cut in small pieces and aspirated into a large pipette. The fluid that covered the bottom of the box was also taken. Fluid and medium containing the tissues were placed in glass tubes, frozen in ice and salt, brought back to room temperature, and centrifugated. The amount of fluid extracted from each culture was 0.5 or 0.75 of a cubic centimeter. The technique of Epstein and Ottenberg,[2] which permits testing the hemolytic power of very small amounts of serum, was followed. A 5 per cent. suspension of goat washed red blood corpuscles was used. Two parts of extract were mixed with one part of a suspension of blood corpuscles. The characters of the hemolysins were investigated by the ordinary methods.

[2] Ottenberg and Epstein, *Arch. Int. Med.*, 1909, iii, 467.

EXPERIMENTS.

Seventeen experiments were made. In several cases, complications occurred, such as premature death of the tissues, concentration of the plasma, bacterial infection, etc. These experiments were discarded, even if the results were only slightly modified. We shall describe only the experiments of which the technique was entirely satisfactory.

Experiment 1.—A culture was made, in a Gabritscheswki box, of guinea pig bone marrow and lymph gland in ten drops of guinea pig plasma and two drops of washed goat corpuscles.

The control culture contained the same amount of bone marrow and lymph gland in ten drops of guinea pig plasma, without goat blood. After four days in the incubator at 39° C., the cultures were still living and both were in the same condition. They were cut in small pieces, squeezed, frozen for ten minutes, and centrifugated. The fluid was tested against goat blood.

After two hours in the incubator (39° C.) and four hours in the ice box, there was slight hemolysis in the extract from the culture with goat blood, and no hemolysis in the control.

Experiment 2.—A culture was prepared of guinea pig bone marrow in a medium consisting of sixteen drops of guinea pig serum and four drops of 2 per cent. agar. Two drops of washed goat corpuscles were added.

The control culture contained no goat blood. After four days the cells of the cultures were still in good condition. On the morning of the fifth day in the incubator, the cultures were partially dying. They were cut in pieces, frozen, and centrifugated. The fluid was tested against goat corpuscles.

After two hours in the incubator (39° C.) and ten hours in the ice chest, the extract from the culture with goat blood showed marked hemolysis; the control showed no hemolysis.

Experiment 3.—1. A culture was prepared of guinea pig bone marrow and lymph gland in twenty drops of guinea pig plasma and two drops of goat red blood corpuscles.

2. A culture of bone marrow and lymph gland in plasma without goat blood.

3. Plasma and goat blood without tissue.

After five days the cultures were in good condition. There was no disintegration of the leucocytes. They were put in cold storage for two days and then cut in small pieces, frozen, centrifugated, and tested against goat blood. There was hemolysis in the extract from the culture with goat blood, and no hemolysis in the controls.

Then the rest of the tissue extract from the first culture (hemolysis +) was divided into two parts (I and II).

Part I was heated to 56° C. for half an hour and divided into two parts. To one of these (a), goat corpuscles were added. After two hours in the incubator and four hours in the ice chest, there was no hemolysis. The second part (b) was kept for later use.

Part II consisted of fresh extract placed with normal goat corpuscles for four hours in the ice chest and then centrifugated for one minute. To the centrifugated cells was added normal guinea pig serum (complement); after two hours in the incubator and eighteen hours in the ice chest, there was complete hemolysis.

The control contained normal goat corpuscles and complement. There was no hemolysis. The centrifugated fluid was divided into two parts (a′ and b′). The first part (II a′) was mixed with normal goat corpuscles. After two hours in the incubator and eighteen hours in the ice chest, there was slight hemolysis. To the second part (II b′) were added equal parts of heated extract (I b) and normal goat corpuscles. There was slight hemolysis.

Experiment 4.—1. A culture was made of guinea pig bone marrow and lymph gland in twenty drops of guinea pig plasma and two drops of goat blood.

2. Control, without goat blood.

After five days in the incubator, the cultures were in good condition. They were cut in small pieces, frozen, centrifugated, and tested against goat blood. The fluid extracted from the culture with goat blood showed complete hemolysis after six to seven minutes. In the control, traces of hemolysis occurred after two hours in the incubator and eighteen hours in the ice chest.

The extract of culture 1 (hemolysis +) was divided into two parts (I and II). Part I was heated to 56° C. for half an hour, and divided into three parts. To one of these (a), goat corpuscles were added. After two hours in the incubator and eighteen hours in the ice chest, a trace of hemolysis occurred. To the second (b) were added goat corpuscles and guinea pig serum (complement). Marked hemolysis occurred after two hours in the incubator, and eighteen hours in the ice chest. The third part (c) was kept for later use.

Part II consisted of fresh extract and was kept with normal goat corpuscles for eighteen hours in the ice chest. To the sedimented corpuscles was added normal guinea pig serum (complement). After two hours in the incubator and sixteen hours in the ice chest, there was marked hemolysis.

The control contained goat corpuscles with complement. After two hours in the incubator and sixteen hours in the ice chest, there was a trace of hemolysis.

The supernatant fluid was divided into two parts, a′ and b′. The first part (II a′) was mixed with normal goat corpuscles and placed for two hours in the incubator and eighteen hours in the ice chest. There was a trace of hemolysis.

To the second part (II b′) were added equal parts of heated extract (I c) and normal goat corpuscles. After two hours in the incubator and eighteen hours in the ice chest, there was marked hemolysis.

RESULTS.

The fluid of the cultures containing goat blood has acquired the power to hemolyse markedly goat red blood corpuscles, while the serum of the control cultures remained non-hemolytic. It is certain then that hemolysins appeared in the cultures under the influence of the antigen.

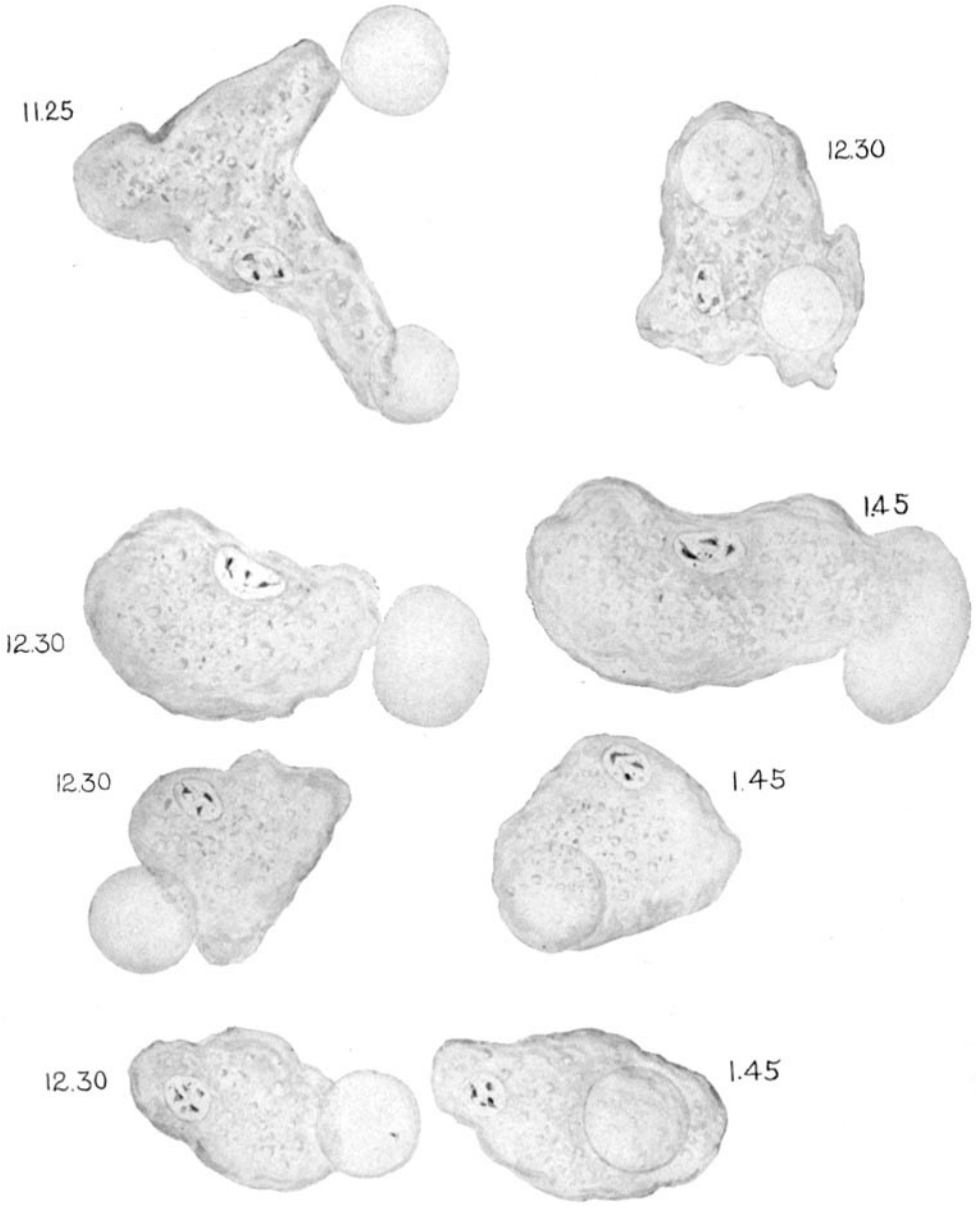

FIG. 1.

A four day old culture already contained hemolysin, but much less than the cultures five days old. The appearance of the hemolysins was preceded by phagocytosis of the goat red blood corpuscles by the guinea pig leucocytes. Examination of the small hanging drop cultures, prepared together with the cultures in Gabritscheswki boxes, showed that, on the second day of cultivation *in vitro,* leucocytes were still inactive against the red blood corpuscles, but that on the third day they phagocyted them rapidly. Camera lucida drawings of the successive phases of phagocytosis were taken (figure 1).

The nature of the hemolytic power of the cultures acquired by the serum was then investigated. This fluid hemolysed goat red blood corpuscles without the addition of complement. After having been heated at 56° C. for half an hour, it lost its hemolytic power. Then, by addition of complement, it regained the power to hemolyse goat red blood corpuscles.

Goat red blood corpuscles were placed for four hours at 0° C. in the fluid extracted from a five day old culture. They were then separated by centrifugation, and a little complement was added. They became hemolysed, while fresh goat blood corpuscles, to which guinea pig serum was added, remained unaltered. It was found also that the fluid, separated from the red corpuscles by centrifugation and mixed with goat fresh red corpuscles, had lost almost completely its hemolytic power. Therefore, it appeared that the hemolytic power acquired by the cultures was due to substances acting as natural hemolysins.

CONCLUSIONS.

Since guinea pig bone marrow and lymph gland cultivated for five days with goat blood generate substances that are hemolytic for goat red blood corpuscles, it can be concluded that tissues living outside of the organism react against an antigen by the production of an antibody.

EXPLANATION OF PLATE 39.

The drawing represents goat erythrocytes phagocyted by guinea pig leucocytes. The corresponding figures on the left and right sides were drawn at two different times on the third day.