

SUCCESSFUL TRANSPLANTATION OF A LEG IN ALBINO RATS WITH REESTAB- LISHMENT OF MUSCULAR CONTROL¹

SUCCESSFUL transplantation of legs has been accomplished in some lower forms of animals such as the salamander, and in 1907 Carrel² succeeded in transplanting a leg in a mammal, namely, the dog. He reported that the new leg healed normally, but no regeneration of nerves nor return of function of the muscles were reported.

In the experiments reported here, successful transplantation of entire legs was accomplished, and in addition, reestablishment of considerable muscular control of the new leg was obtained.

The right hind leg, including all the bones and muscles below the knee joint, was transplanted from one albino rat to the back of another rat. Animals from eight days to three weeks old were used. The sciatic nerve of the transplanted leg was united to the lateral branch of the sciatic nerve of the right hind leg of the host or animal receiving the new leg. Junction of the nerves was made by first fraying the two ends to be united and then making a side-by-side union.

The animal contributing its leg was parabiotically united to the rat which was to gain the new leg. This union was effected by making a partial amputation of the leg through the muscles above the knee joint, leaving the medial muscles and vessels undisturbed, thus allowing sufficient circulation to maintain the life of the leg. The cut surface of the muscle tissue was united to the soft tissues on the back of the host animals, and the two animals were kept united until the connective tissue and vascular union between the

leg and the back of its new host was well established. While the new circulatory connections were being made, the limb received its nutrition from its original owner by way of the medial half of the leg which had not been cut in the partial amputation.

Development of an adequate nutritional relationship between the leg and its new host was tested by placing a ligature between the leg and its original owner. The leg was then carefully watched for signs of impaired blood supply. If it remained warm and the natural pink color persisted for several hours, a satisfactory vascular supply was indicated. The leg was then completely amputated from its original owner. This second operation merely involved amputating the medial half of the leg.

After several weeks, slight motion was apparent in the new leg which was massaged and exercised daily. There was a gradual increase in the muscular response until the leg was able to lift a ten gram weight over one centimeter. A muscle contraction is evoked in the new fifth leg by stimulation of the animal's own right hind leg from which a branch of the sciatic nerve had been grafted to the nerve of the transplanted leg. Thus upon a stimulation of the rat's right hind leg, the animal attempts to move this leg from the source of irritation, but since the nerve of this leg has been grafted to the nerve of the transplanted leg both legs respond. The extra leg also moves when the animal walks, and there is ability to flex the toes.

In one of these animals, at the date of writing, it is three months since the first signs of movement in the transplanted leg were observed and there has been no loss of muscular activity.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE EXTRACTION OF LABILE BACTERIAL ANTIGEN BY DISRUPTION OF THE BACTERIAL CELLS AT LOW TEMPERATURE

ALTHOUGH a number of methods for disrupting bacterial cells have appeared in the literature, there is at present only one known method which will effectively break up the cells of the hemolytic streptococci, without destroying labile components. This method, described by Chambers and Flosdorf,¹ utilizes sonic vibration at ordinary temperatures. However, the sonic vibrator is not generally available on account of its high cost.

The growing interest in the labile antigens of bacteria, liberated on disruption, has led to the publica-

tion of the following method, which is simple and inexpensive.

Bacteria are grown in mass culture, centrifuged at high speed and then dried in the lyophile apparatus of Flosdorf and Mudd.² The lyophile process consists of drying the bacteria *in vacuo* from the frozen state. The dried bacteria are taken from the lyophile apparatus and placed directly into a mortar. Liquid air is then poured directly over the bacteria and allowed to evaporate. This cools the mortar. Another portion of liquid air is then poured over the bacteria, freezing them to a temperature of about -180° C. The bacteria become very brittle and can be easily disrupted with a few turns of the pestle. Resistant bacteria, like the streptococcus, require two or three applications of liquid air with subsequent grinding. About

¹ A preliminary report.

² A. Carrel, *Jour. Am. Med. Assn.*, 51: 1662, 1908.

¹ Leslie A. Chambers and Earl W. Flosdorf, *Proc. Soc. Exp. Biol. and Med.*, 34: 631-636, 1936.

² Earl W. Flosdorf and Stuart Mudd, *Jour. Immunol.*, 29: 389, 1935.