SUCCESSFUL TRANSPLANTATION OF A LEG IN ALBINO RATS WITH REESTABLISHMENT OF MUSCULAR CONTROL[1]

SUCCESSFUL transplantation of legs has been accomplished in some lower forms of animals such as the salamander, and in 1907 Carrel[2] 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 publication 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. Asn.*, 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.

500 cc of liquid air are required to grind one gram of streptococci. Approximately 85 per cent. of the bacteria are broken up by this process. After the bacteria and the mortar come to a temperature above 0° C., water or saline can be added in sufficient quantity to dissolve the contents of the bacterial cells, and the few whole bacteria can be removed by means of a filter.

It is to be noted that the bacteria are subjected to a freezing temperature during most of the process, thus preventing destruction of any labile material present. This method has been used in obtaining the extremely labile antigen of the hemolytic streptococcus by Mudd, Czarnetzky, Pettit and Lackman.[3]

Wool, cotton, defibrinated silk, rubber and many other substances can be ground to a very fine powder by the use of this method.

As the concentration of oxygen increases with the evaporation of nitrogen from the liquid air, it is very important to use only small quantities of material at a time, and to avoid the presence of fats or other easily oxidizable substances. It is not advisable to use liquid air without previous knowledge of its characteristics, as explosions can occur when care is not used.

After completion of our experiments it was brought to our attention that Macfayden and Rowland[4] were able to disrupt the cells of the typhoid bacillus by grinding them in a conical mortar chilled by the use of liquid air externally. They used moist bacteria in the form of a paste, but were only able to disrupt about 10 per cent. of the cells. Although their method was published in 1903, it seems to have been generally overlooked, as the interest in soluble bacterial antigens was not so intense at that time.

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SODIUM ETHYL XANTHATE AS A PLANT POISON

SODIUM ethyl xanthate, the sodium salt of ethyl xanthic acid, has a pronounced toxic effect on the aerial parts of herbaceous plants. Applications of the salt in water solution at the rate of one pound per square rod were found to be effective. Sodium ethyl xanthate is very soluble in water and does not have an appreciable corrosive effect on spray equipment. In all tests made so far there has been no indication that its use creates a fire hazard.

The alkali metal ethyl xanthates are produced by the reaction of carbon bisulfide with a mixture of alcohol and caustic alkali. The reactions may be represented by the following equations:

$$(1)\ C_2H_5OH + NaOH \longrightarrow C_2H_5ONa + H_2O$$
$$(2)\ C_2H_5ONa + CS_2 \longrightarrow OC_2H_5CS_2Na.$$

Ethyl xanthates are unstable in water solution. They hydrolyze autocatalytically to form principally sulfides and thiocarbonates. It may be that these decomposition products are the cause of the toxicity of sodium ethyl xanthate. While the xanthates of other metals have not been investigated, they very likely are also toxic to plants.

The above results were obtained in cooperative investigations with the Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

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PHYSIOLOGICAL APPARATUS

BEARING in mind the recent article by Marsh[1] on the continuous renewal of culture solutions, the writer rather questions the simplicity of construction and practicability of the apparatus. Conclusions from laboratory experiments should be based upon results from a large number of cultures in a series which have been maintained for a period of time to insure safe analyses. As I have previously pointed out,[2] individual nutrient reservoirs are to be avoided, since they cause a serious loss of time and labor when used for complicated and extended investigations. Secondly, open nutrient systems of the type cited are hardly practical in most laboratories, due to contamination of the solutions by algae, etc.

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PARSONS, W. VA.

[1] R. P. Marsh, SCIENCE, 84: 163–164, 1936.
[2] Robert E. Wean, SCIENCE, 82: 336, 1935.

[3] Stuart Mudd, E. J. Czarnetzky, Horace Pettit and David Lackman, *Jour. Bact.*, 31: 571, 1936.
[4] Allan Macfayden und Sydney Rowland, *Centr. Bakt.*, Orig., 34: 765, 1903.

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