water-in-oil emulsions as a means of prolonging the effect of various therapeutic agents, but this method has received little, if any, attention.

It was found recently[4] that the intramuscular injection of water-in-oil emulsions of penicillin results in prolongation of penicillin effect as compared with similar amounts of penicillin injected in aqueous solution by the same route. It was also shown that a single simultaneous injection of 100,000 Oxford units of penicillin in water-in-oil emulsion and 50,000 units in aqueous solution cured all of 40 patients with acute gonococcal infection so treated.

Previous methods of preparing water-in-oil emulsions, although simple procedures in the bacteriological laboratory,[2] can not readily be carried out at the clinic or bedside, making it necessary to prepare the emulsions in advance. There are disadvantages to aqueous solutions, whether emulsified or not, regarding potency and stability, since they may be affected adversely by storage and shipping. The technic to be described overcomes these difficulties. In addition it insures the sterility of the preparation, since the procedure is carried out in closed, sterile containers.

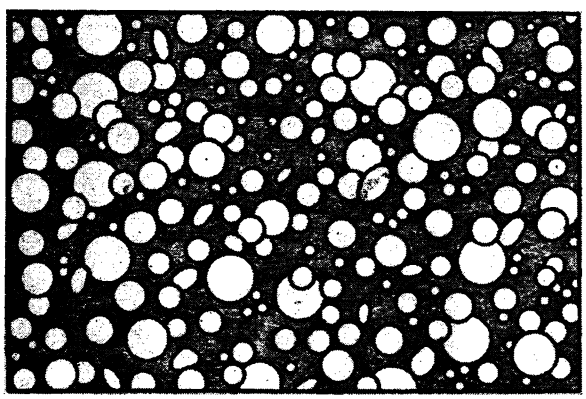

FIG. 1. Schematic drawing of a water-in-oil emulsion of aqueous penicillin solution in peanut oil. The penicillin is contained in the water droplets.

Water-in-oil emulsions of penicillin can be made as follows: One and four tenths ml of sterile 0.85 per cent. salt solution are drawn into a sterile syringe and ejected into a vial containing 100,000 Oxford units of dry penicillin. To the penicillin solution 3.1 ml of an autoclaved mixture containing 11 parts of a lanolin-like substance[5] and 20 parts peanut oil are added by means of a sterile syringe and needle of 17 gauge. With the needle still inserted through the rubber cap of the vial, the mixture can be readily emulsified by repeated withdrawals and ejections. As soon as the mixture assumes a uniformly creamy, slightly viscid consistency, satisfactory emulsification has occurred and it is ready for injection. Examination of such an emulsion under the microscope shows aqueous droplets of microscopic size in an oily menstruum (Fig. 1).

Water-soluble drugs and biologic products, as well as aqueous suspensions of particulate matter, such as bacteria or viruses, can be emulsified by this technique.

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INTRACARDIAC TRANSPLANTATION IN THE URODELE

TRANSPLANTS are sometimes used in determining the extent to which embryonic structures are capable of independent development. Such transplants must not only be provided with adequate nourishment, but they must be effectively isolated from environmental factors which might alter their intrinsic developmental pattern.

In the transplantation sites most commonly used for this purpose the implanted material is exposed to the possible influence of a number of extrinsic factors. The nature and extent of this influence in a given case is not easily determined. Moreover, it is difficult to make a direct comparison between one transplant and others of a similar series because of slight variations in the composition and proximity of the neighboring tissues. Under these conditions the developmental capacity of the implanted tissue is difficult to determine.

It has been found that these experimental conditions can be improved considerably by using the heart cavity as the transplantation site. The method was developed for work with *Ambystoma maculatum,* to which species the following account and comments apply.

The host animals are chosen at a stage when the heart is beating but before the actual circulation of blood has commenced. For this species embryos of Harrison's stage 35 are used. The animals at this stage are not very active. It is therefore unnecessary to use an anesthetic except when it is needed at the same time to quiet an older donor.

The operations are performed under a dissecting microscope after arranging the donor and host in the usual way. The transplantation site is prepared by making a transverse incision through the ventral body

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[3] C. B. Strauch, *Jour. Am. Med. Asn.*, 92: 1177, 1929.

[4] A. Cohn, B. Kornblith, I. Greenstein, K. J. Thomson and J. Freund. Experiments to be published.

[5] The lanolin-like substance used is sold under the trade name of Falba (Manufactured by Pfaultz and Bauer, Inc., New York, N. Y.); other satisfactory emulsifying agents, producing water-in-oil emulsions, may be used.

wall just anterior to the liver, thus exposing the heart. This incision is then carried through into the sinus venosus. Since the circulation is not yet fully established the resulting hemorrhage soon subsides. The tissue to be implanted, having been previously secured and cached in a depression beside the prospective host, is then inserted into the open heart cavity. The incision closes rapidly and the retention of the implant is assured. The host can then be transferred to the container in which it is to be reared. After an appropriate interval the animal is sacrificed and the graft is recovered by sectioning the heart region.

The development of a number of embryonic tissues has been studied by this method, and it is felt that the encouraging results which have been obtained are in a large measure due to the adaptability of the transplantation site. The heart cavity is easily accessible and can accommodate fairly large pieces of tissue. Operations can therefore be performed rapidly and with little mechanical injury to the donor tissue. Since the incision closes rapidly it is possible to transfer the host without waiting for the implant itself to become fixed. The operation apparently causes no permanent damage to the host, and the mortality is negligible.

The blood plasma undoubtedly serves well to nourish the implant until vascularization has taken place. Thus a favorable environment is provided for the tissue during this critical period.

That a permanent attachment takes place very soon is indicated by the fact that the implants are not carried forward with the onset of circulation but can be recovered very near to their original site. When older hosts are used the implants often are carried as emboli which obstruct the blood supply to one or more gills, but this does not occur when tissues are implanted into the host before the onset of circulation.

The grafts survive in a very high percentage of cases. This indicates that physiologic conditions for the transplant are quickly restored and maintained in the heterotopic site.

Only the tissues of the blood vascular system are included in the environment. These factors are subject to little variation and probably act in a purely nutritive capacity. Thus a near approach to the controlled environment of the culture method is afforded.

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A MODIFICATION OF THE UREASE TEST FOR PROTEUS

THE ability of Proteus species to decompose urea[1] is considered an important identifying characteristic by Bergey,[2] and has recently been recommended by Ferguson *et al.*[3] as a routine differential test in enteric bacteriology.

In the test as employed by the above workers, inoculation of suspected organisms into a urea medium is followed by a twenty-four-hour incubation at 37° C. before results are obtained. Since the test is dependent upon the presence of the enzyme urease it seemed apparent that if a large amount of inoculum were employed in a small amount of urea medium decomposition could be quickly determined.

The medium used is that of Ferguson and Hook. It contains 2 per cent. urea (Baker's C.P.), 0.1 per cent. KH_2PO_4, 0.1 per cent. K_2HPO_4, 0.5 per cent. NaCl and 1.0 per cent. C_2H_5OH in distilled H_2O. The pH is adjusted to 7.0. A sufficient quantity of 0.2 per cent. aqueous Phenol Red is added to give a definite color. The medium is sterilized by Seitz filtration. Sterilization is not necessary, however, if the medium is stored in the refrigerator or made up in small amounts for relatively quick utilization.

In order to run the test a heavy inoculum scraped from an initial Kligler Iron slant or other solid medium is suspended in 0.1 ml of urea medium. The suspension is incubated at 37° C. and observed at five-minute intervals for a period of thirty minutes. A positive reaction, as indicated by a definite change of the indicator to pink, will usually occur in the first ten to fifteen minutes.

The test as described was run against a series of known Shigella, Proteus and Salmonella strains. During a five-month period the test has been routinely used in this laboratory[4] with biochemical and serological checks. No false positives have been observed.

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[2] "Bergey's Manual of Determinative Bacteriology," 1939, 5th edition, Williams and Wilkins Co., Baltimore, Md.

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