other detailed information will be published as soon as conditions permit.[6]

ALVIN W. HOFER
NEW YORK STATE AGRICULTURAL EXPERIMENTAL STATION,
GENEVA

OSCAR W. RICHARDS
RESEARCH DIVISION,
SPENCER LENS COMPANY,
BUFFALO, N. Y.

EFFECT OF ALLOXAN DIABETES ON THE GROWING RAT

THE purpose of this present investigation was to study alloxan diabetes in the immature animal and to observe the effect of early insulin deprivation on somatic growth. The effect of alloxan in destroying the β-cells of the Langerhans islets[1-5] provides a convenient method of producing diabetes experimentally. It is difficult to ascertain to what extent this metabolic disturbance affects growth in man, since most juvenile diabetics have already attained an excessive height for their age at the time the diabetes becomes apparent.

The animals used for this experiment were 26-day-old Sprague Dawley rats on a diet of Wayne Fox Chow Blox. They were divided into four groups, each consisting of four males and four females. One group was not injected and served as control for the other three groups, which were injected subcutaneously with a 1 per cent. solution of alloxan monohydrate in doses of 25, 30 and 40 mg per gm of body weight, respectively. Within half an hour after injection, alloxan appeared in the urine. It was accompanied by a fall in pH and by the red color which alloxan produces in the presence of amino acids. To control the hypoglycemia[6] which develops after the initial hyperglycemia, the rats were given a 5 per cent. glucose solution to drink for the first day after injection. Insulin was at no time administered to these animals. The alloxan treated rats except one of the females injected with the lowest dose developed diabetes. Glycosuria was more severe in the group with the largest dose and the diabetes persisted in this group, although it disappeared in several of the animals with smaller doses. All the animals in this experiment were lively after the first day and survived until sacrificed at 61 days of age or at some later date.

The effect of alloxan diabetes in stunting the growth of the rat is apparent from the figures in Table 1.

TABLE 1

Dose of alloxan mg/100 g	Sex	Body weight in grams at the age of: 26 days	35 days	42 days	48 days	55 days	61 days
Control	F	46	74	104	121	144	158 (144–168)*
25	F	45	64	94	112	131	144 (136–153)
30	F	45	67	98	115	124	149 (142–155)
40	F	47	57	70	78	95	106 (55–138)
Control	M	46	74	103	126	163	191 (180–213)
25	M	50	72	100	116	143	164 (131–192)
30	M	46	74	109	124	147	179 (169–195)
40	M	47	56	64	70	76	87 (58–111)

* Figures in parenthesis indicate range of values.

Certain other differences were observed in the diabetic rats. One out of eight animals, 0/8, and 3/8 of the 25 mg, 30 mg and 40 mg groups respectively developed cataracts of the lenses.[7] The dwarfed animals had distended abdomens, and at autopsy the stomach and intestines of these animals measured longer, relative to their body weight, than those of the controls. Some of these rats also showed infantile primary and secondary sex organs.

Summary: Severe alloxan diabetes dwarfed the immature rat.

ANNETTE CHESLER
R. TISLOWITZ
BIOLOGY LABORATORY,
SCHERING CORPORATION,
BLOOMFIELD, N. J.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE, RAPID TECHNIC OF PREPARING WATER-IN-OIL EMULSIONS OF PENICILLIN, DRUGS AND BIOLOGICS[1]

WATER-IN-OIL emulsions have proved of value in experimental immunization procedures[2] and may become applicable to the immunization of man and domestic animals. Strauch[3] described the use of

[6] The authors wish to acknowledge helpful suggestions in regard to this work by H. J. Conn and Mary A. Darrow; the senior author wishes to express his gratitude to A. H. Bennett for the use of experimental equipment.

[1] H. R. Jacobs, *Proc. Soc. Exp. Biol. and Med.*, 37: 407, 1937.

[2] J. S. Dunn, H. L. Sheehan and N. G. B. McLetchie, *Lancet*, 244: 484, 1943.

[3] M. G. Goldner and G. Gomori, *Endocrinology*, 33: 297, 1943.

[4] J. H. Ridout, A. W. Ham and G. A. Wrenshall, SCIENCE, 100: 57, 1944.

[5] E. Thorogood, *Feder. Proc.*, 3: 48, 1944.

[6] C. C. Bailey and O. T. Bailey, *Jour. Am. Med. Asn.*, 122: 1165, 1943.

[7] C. C. Bailey, O. T. Bailey and R. S. Leech, *New Eng. Jour. Med.*, 230: 533, 1944.

[1] From the Public Health Research Institute of the City of New York, Inc.

[2] (a) J. Freund and K. McDermott, *Proc. Soc. Exp. Biol. and Med.*, 49: 548, 1942. (b) J. Freund and M. V. Bonanto, *Jour. Immunol.*, 48: 325, 1944. (c) M. W. Chase, *Proc. Soc. Exp. Biol. and Med.*, 52: 238, 1943. (d) L. M. Kopeloff and N. Kopeloff, *Fed. Proc.* (*Am.*

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.

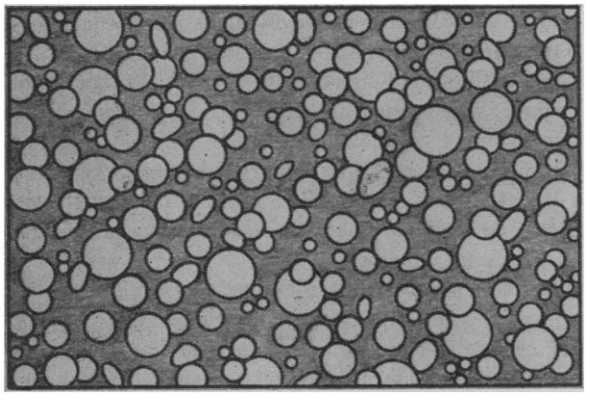

FIG. 1. Sehematic drawing of a water-in-oil emulsion of aqueous penicillin solution in peanut oil. The penicillin is contained in the water droplets.

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.

JULES FREUND
K. J. THOMSON

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

Soc. Exp. Biol.) 2: 99, 1943. (e) W. F. Friedewald, SCIENCE, 99: 45, 1943, and *Jour. Exp. Med.*, 80: 477, 1944.

[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.