treated prior to alloxan induced diabetes, tolbutamide has an effect different from insulin.

The present findings may be due to a toxic effect and not related to pancreatic function; however daily doses in rats as high as 500 mg/kg of tolbutamide for 9 months have shown no histological changes in the internal organs except the pancreas(10).

Summary. 1) Starvation or glucose plus tolbutamide potentiates the diabetogenic activity of alloxan with increased mortality. 2) Glucose alone or glucose plus insulin protects against alloxan diabetes. 3) Different results obtained with insulin or tolbutamide cannot be explained on the basis of liver glycogen content, blood glucose, or theoretical status of pancreatic activity at time of alloxan injection.

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Transfer of Acquired Immunological Tolerance of Skin Homografts in Mice Joined in Parabiosis.* (25268)

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Acquired immunological tolerance has been produced in several species of animals(1-4). As originally defined, the phenomenon is produced by exposing animals to antigens during period of immunological immaturity. Immunological tolerance to subsequent tissue or organ homotransplantation has usually been achieved by injection of the prospective recipient with viable hematopoietic cells from the prospective donor. In most instances spleen cells have been used, injected either subcutaneously or intraperitoneally during embryonic life or intravenously in the immediate neonatal period. Hasek's technic(4) of embryonal parabiosis has also been effective in production of immunological tolerance. Tissues successfully homotransplanted following establishment of immunological tolerance have been skin in mice(1,2,5), skin in fowl(6), skin in rats(3,7), skin in dogs(8), ovaries(9), adrenals(10) and hypophyseal glands in mice (11). Moreover, mice have been made tolerant to homologous adenocarcinoma by subcutaneous injection of tumor cells in the neonatal period or by injection of viable homologous spleen cells from the prospective donor strain prior to birth, followed later by graft of the tumor from the donor strain (12). To date, then, all examples of true immunological tolerance have been achieved by exposure to donor strain antigen prior to delivery or in the immediate neonatal period. Recently, however, we observed that weanling Z(C3H) mice, injected with viable spleen cells taken from (Z x Ce) F_1 hybrid mice, became tolerant and accepted skin grafts from (Z x Ce) F_1 hybrid donors(13). Similarly, female mice of the A and C57 Bl (subline 1) strains were made tolerant of male skin isografts, either by intravenous injection of male spleen cells in older animals or by establishing a parabiotic union of adult females with isologous males(14). Finally, using the technic of parabiotic union, it has also been demonstrated that adult mice growing homolo-

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gous mammary adenocarcinomas following homotransplantation of tumor immediately after birth appeared to transfer their tolerant state to normal isologous mice of the same strain(unpbl). In the latter experiment, the previously unconditioned parabiont lost its ability to reject the homologous tumor and, like its partner which had been made tolerant by injection of tumor in the neonatal period, permitted progressive growth of the tumor homotransplant. On the basis of these observations, experiments have been performed to ascertain whether or not tolerance of homologous skin grafts, induced in mice by neonatal injection of spleen cells, can be transferred by parabiosis to normal, unconditioned adult animals of the same strain. This communication reports experiments in which acquired immunological tolerance of Ce skin was apparently transferred from tolerant Z(C3H) mice to previously unconditioned Z(C3H) mice by placing the latter in parabiosis with the former.

Material and methods. Highly inbred mice of the Z† and Ce strains were used. Tolerance was induced in mice of the Z strain by intravenous administration during neonatal period of viable spleen cells taken from adult donors of the Ce strain. The technic for intravenous injection in newborn mice and for preparation of spleen cells has been described (5). Each Z newborn received from 3 to 5 million viable Ce spleen cells. Cell viability was determined by the Schrek method(15). Injected mice were used approximately 2 months after weaning. In one set of experiments (Group I), pretreated Z mice received a full-thickness skin homograft taken from adult Ce donors, followed one month later by establishment of parabiosis with normal, non-treated isologous mice. Parabiotic pairs were kept together for 15 days, at which time the normal partner of each pair received a full-thickness skin graft taken from a Ce donor. In another group, (Group II) adult Z mice, which also had been treated at birth with Ce spleen cells but had not been tested for tolerance of Ce skin, were joined in parabiosis with normal, non-treated isologous animals. In this group, after both animals had been in parabiosis for 15 days, the union was surgically discontinued, and 8 days later both members received skin grafts taken from Ce homologous donors. Finally, a group of controls consisting of normal, non-treated mice of the Z stock, joined in parabiosis with other normal mice of the same strain, were studied. After 30 days union, each member was grafted with a piece of skin taken from Ce donors. In most instances, mice were kept under observation for at least 3 months following skin grafts, and in some groups the follow-up period was extended to one year. Parabiosis was of the celomic type(16,17), briefly described as follows: After nembutal anesthesia, both animals were placed parallel to each other, and lateral incisions were made including skin and muscle extending from base of ear to proximal insertion of the femur. To achieve confluence of celomic cavities, the incision was extended into the peritoneal cavity from lower edge of last rib to the iliac crest. The peritonea of the 2 animals were then sutured together with surgical 5-0 catgut. Both skin and muscular layers were joined with skin clips. No bandages or dressings of any kind were used. Parabiotic pairs were housed individually in plastic cages with free access to Purina Fox Chow and tap water.

Results are summarized in Table I. In the first experiment, (Group I in the Table) the previously unconditioned Z parabionts failed to reject the Ce skin homotransplant in a high percentage of cases (Fig. 1).

In the second experiment, (Group II), both animals conditioned at birth by intravenous injection of viable Ce spleen cells and those conditioned only by having been in parabiosis with the pretreated animals, tolerated the homologous skin graft in a high percentage of cases.

In contradistinction to these results were those obtained in the control mice, (Group III). In this experiment, consisting of 12 adult, normal Z mice placed in parabiosis with 12 additional normal Z mice all rejected the Ce skin homotransplant at the usual time for rejection of skin grafts (Fig. 2).

† Z is used to simplify designation of C3H mice originally obtained in 1956 from Dr. John J. Bittner's laboratory.

TABLE I. Transfer of Acquired Immunological Tolerance of Skin Homografts by Parabiosis.

Group	Parabiosis	Days in parabiosis*	No. of pretreated parabionts accepting Ce skin grafts†	No. of non-pretreated parabionts accepting Ce skin grafts†
I	C3H tolerant of Ce skin × normal C3H	15	11/11 (100%)	8/11 (73%)‡
II	C3H pretreated with Ce spleen × normal C3H	15	14/17 (82%)§	10/14 (71%)§
III	Normal C3H × normal C3H	30		0/24 (0%)

* Time in parabiosis prior to transplantation.
† Numerator indicates No. of successful skin grafts and denominator No. grafts attempted.
‡ Transplantation of Ce skin performed while animals were still maintained in parabiosis.
§ Ce skin graft was performed 8 days after separation of parabiotic union.

Discussion. These results demonstrate that failure to reject Ce skin homotransplants is a regular characteristic of Z strain mice which have been placed in parabiosis with Z mice previously made tolerant of Ce tissues by injection of Ce spleen cells at birth. In all instances where successful homotransplantation has been recorded, the graft was maintained in excellent condition during entire period of observation ranging from 3 months to one year. Similar results were obtained when grafting of Ce skin was carried out in the "normal" parabiont 8 days following discontinuation of a parabiotic union with a tolerant mouse which had lasted 15 days. These observations appear to indicate that integrity of the parabiotic union is not essential to success of subsequent homotransplantation. These findings support and extend our previous observations, indicating that failure to reject homologous adenocarcinoma can be transferred to otherwise resistant isologous mice by placing them in parabiosis with an animal tolerant to the tumor.

It is difficult to explain these findings in the light of our present understanding of transplantation immunity and immunological tolerance. Although other bases might be operating, it seems likely that the parabiotic union in some way permitted transfer of the tolerant state from animals in which tolerance has been produced in the conventional manner to the normal partner. This possible interpretation is supported by 2 additional observations. The first is(18) that tolerant mice possess in their spleens all antigens capable upon injection into newborn isologous animals of conferring tolerance to the original donor strain. Since tolerance transferred in this manner persists indefinitely, it may be presumed that antigens involved are present as intact cells derived from the original donor.

Secondly, we observed that in certain well defined and perhaps limited systems, tolerance need not be produced in neonates but may be achieved in much older animals, either by intravenous injection of spleen cells or by establishment of a parabiotic union(13,14). It seems of real significance that in experiments herein reported failure of the transplantation reaction, and very likely immunological tolerance, have also been produced by presumed exposure to foreign antigen in animals well beyond the neonatal period. It seems possible that during the period of parabiotic union between Z mice tolerant of Ce tissue and normal Z mice a continuous transfer of Ce antigens has occurred, and that the dosage obtained and perhaps the constant presence of antigens involved have been sufficient to abrogate the immunological resistance of the normal Z strain parabiont to the homologous Ce skin homotransplant. It is significant that the apparent immunological unresponsiveness seems to be permanent and consequently indistinguishable from that induced by injection of Ce spleen cells into Z mice during the neonatal period(5). The best possible explanation for these observations appears to be that intact Ce cells have been transferred across the parabiotic union from the tolerant animal to its partner, thus inducing tolerance in the previously normal host. If this be the case, it is difficult to understand why intravenous injection of Ce spleen cells in adult Z recipients does not produce tolerance but rather im-

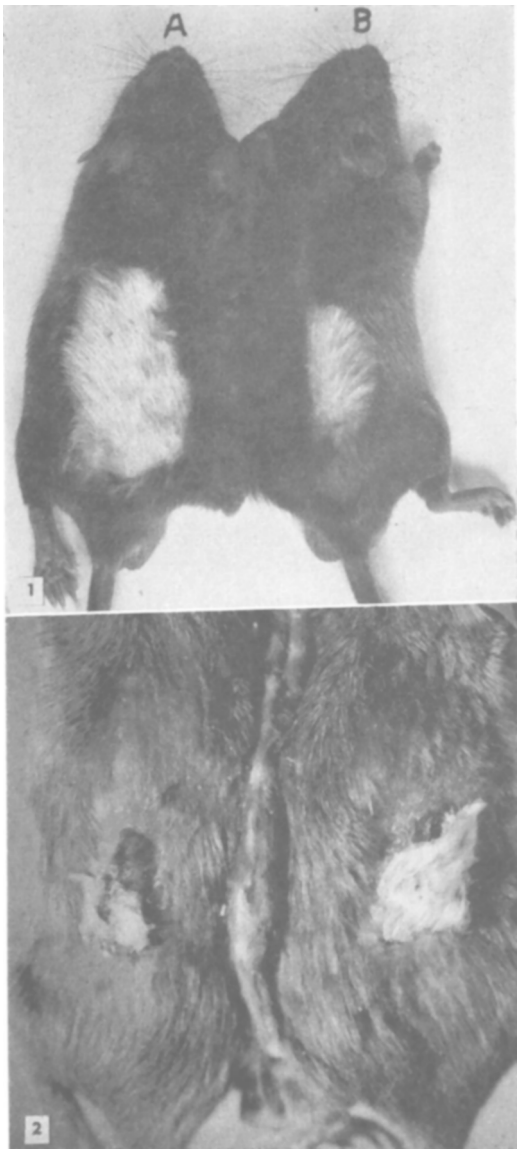

FIG. 1. Transfer of tolerance of Ce homologous skin graft in Z (C3H) mice joined in parabiosis. In mouse A tolerance was induced by neonatal inj. of Ce spleen cells. Mouse B, non-treated at birth shows tolerance of Ce skin after being in parabiosis with mouse A for 15 days.

FIG. 2. Parabiosis between 2 normal Z (C3H) mice during 30 days and subsequently grafted with Ce homologous skin. Note sloughing of Ce graft in both members of pair.

munity. Perhaps, as might be inferred from Egdahl and Hume's(19) experience with cross circulation in dogs and Kamrin's(20) experience with parabiosis in rats, the parabiotic state permits maintenance of a homotransplant of multipotent Ce cells for a sufficient period to permit them to induce a tolerant state which could not be induced by a single intravenous injection of spleen cells at this age. Whatever the mechanism responsible for this phenomenon might be, the observations reported herein provoke additional reflection on possible relationships between immunological tolerance, immunological paralysis(21), and other forms of specific immunological unresponsiveness(22-25).

Experiments to ascertain the essential duration of the parabiotic union, attempts to extend these observations to other inbred strains of mice, and transfer studies to determine whether Ce cells are, indeed, present in spleens of adult animals made tolerant in this manner, are in progress.

Summary. 1. Normal, adult Z(C3H) mice, connected in parabiotic union with Z(C3H) mice tolerant of Ce tissue, fail to reject homografts of Ce skin. 2. Lack of immunological responsiveness to Ce tissues persists following disruption of parabiotic union. 3. The possibility that failure of transplantation reaction is due to transfer of immunological tolerance to previously unconditioned adult recipient is presented. 4. The implications of these observations are discussed.

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Effect of Thyroxine, Thyrotrophic and Somatotrophic Hormones on Skin of Dwarf Mice.* (25269)

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It is well known that thyroid hormone affects the composition of connective tissue. Histological studies have shown that the metachromatic mucopolysaccharide-containing ground substance decreases and mast cells decrease in size and granularity under the influence of thyroxine(1,2). The thyrotrophic pituitary hormone (TSH) exerts, besides stimulating the thyroid gland, a direct action on tissues which has been demonstrated in thyroidectomized animals(3,4,5). Injection of TSH into thyroidectomized guinea pigs is followed by mobilization of fat from normal depots. This tissue fat is replaced by a mucinous substance characterized by a content of acid mucopolysaccharides. The fat mobilizing principle is inhibited by thyroxine(6). Like TSH, somatotrophin (STH) has a direct effect on connective tissue. Various experiments have shown that STH stimulates fibroblast proliferation and formation of collagen (7). In the present work the effect of thyroxine, TSH and STH on number of mast cells and concentrations of hexosamine and hydroxyproline in the skin of dwarf mice has been studied. Hexosamine is an important constituent of acid mucopolysaccharides in the ground substance of connective tissue and was taken as a measure of concentration of these compounds. As collagen is the only protein in connective tissue which contains hydroxyproline, this amino acid was taken as an expression of amount of collagen.

Material and methods. Adult dwarf mice and their normal siblings were used. The dwarf mice are born with hypoplastic pituitary glands(8) which seem devoid of acidophil cells producing STH and basophil cells producing TSH(9). Dwarf mice are the only laboratory animals known to have pituitary (secondary) myxedema (with no or very little TSH and STH production). They were selected for the experiment because of this abnormality. In the first experiment, dwarf mice were divided into 5 groups with 5-16 animals in each group (no normal mice) (Fig. 1). The animals were injected intraperitoneally daily for 10 days. Group I: thyroxine (Roche) 0.1 ml (0.1%), II: TSH (Armour) 0.017 USP unit in 0.1 ml sterile water, III: STH (Armour) 0.5 mg in 0.1 ml sterile water, IV: Phyol‡ (Alfred Benzon) = STH 0.5 mg in 0.1 ml sterile water, V: physiological saline, 0.1 ml. On the 11th day the animals

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