particles, the observed difference between the sonicated group and all other vaccinated animals was greater than the theoretical (X^2 = 6.14; theoretical X^2 1 df (.95) = 3.84). Similarly, comparison of those mice challenged with 10^4 particles, showed the difference between those vaccinated with sonicated material and with the other types of vaccine, to have X^2 of 5.2, again greater than the theoretical, with the same confidence limits. Although complete protection was not attained, there can be little doubt that at least some degree of resistance was demonstrated. Whether or not this resistance was related to the presence of circulating antibody is being investigated.

Our conclusions therefore differ from those of Hurd and Drake(4) and of Winner(5) who, however, not only employed a different species of experimental animal but also possibly challenged with excessive doses inasmuch as both vaccinated and non-vaccinated animals died in less than a week.

Summary. Mice were vaccinated by repeated subcutaneous inoculations of living, merthiolate-killed, or sonically-ruptured cells of *C. albicans* and were then challenged intravenously with 10^4 or 10^5 viable particles of a homologous strain. Although not all of the vaccinated mice survived challenge, a significant degree of resistance was conferred since fewer deaths occurred among vaccinated animals.

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Runt Disease in Adult Tolerant Mice Induced by Intravenous Injection of Immunologically Competent Cells.* (26406)

CARLOS MARTINEZ,† J. M. SMITH AND ROBERT A. GOOD‡
Departments of Physiology and Pediatrics, University of Minnesota, Medical School, Minneapolis

It is now well established that mice, rats, chicks, and other species, injected at birth or prior to birth with immunologically competent lymphoid cells from adult animals, often fail to grow, and succumb within a few weeks. This syndrome, which has been variously called runt disease, homologous disease, immunological disease, or wasting disease, has been found to be the result of an immune reaction elicited in the transferred, immunologically competent, adult cells by the antigens of the host. A similar phenomenon has been produced in adult F_1 hybrid mice injected with mature parent cells and in mice receiving a lethal dose of x-rays followed by administration of bone marrow cells taken from unrelated donors (For review see 1.) Finally, Casterman(2) recently described a similar type of syndrome in adult mice treated with large numbers of homologous lymphoid cells. In all of these situations, the clinical and gross pathologic findings have been similar, consisting of initial enlargement of the spleen, failure to grow, development of diarrhea, and terminating, often with infections, in a state characterized by extreme atrophy of the lymphoid tissues, especially the spleen and lymph nodes.

According to Billingham(1), the ability of the injected cells to induce this disease has 3 bases: 1) maturity and immunological competence of the inoculated cells; 2) presence in the host of transplantation antigens not present in the grafted cells; and 3) accep-

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† Am. Cancer Soc. Professor of Physiology.

‡ American Legion Memorial Heart Research Professor of Pediatrics.

TABLE I. Homologous Disease in Tolerant Mice Induced by Intravenous Injection of Spleen Cells.

Group	No. of mice	Mean body wt, g		Mean spleen wt, mg	Spleen wt, mg/100 g body wt
		Initial	Final		
Z normal	8	22.40	26.70	156 ± 21	584
Z tolerant of A*	8	25.05	27.00	169 ± 18	629
Z tolerant of A inj. with A cells†	14	22.40	21.90	273 ± 39	1246

* Tolerance was induced by intrav. inj. of A cells at birth.
† 20 million A spleen cells once a week for three weeks.

tance of the transplanted cells by the recipient without destructive reaction against these cells.

In the experiments herein described, attempts were made to induce immunological "runt disease" in adult mice tolerant of another homologous strain. The essential manipulation was injection of large numbers of immunologically competent cells derived from donors of the same strain as those used to induce tolerance at an earlier date. Specifically, an effort was made to produce runt disease in adult Z§ mice, previously made tolerant by injection of A strain spleen cells at birth, by further administration during adult life of large numbers of immunologically competent spleen cells from A strain donors. This paper reports the production of severe immunological disease using this technic.

Method. Mice of the Z and A strains were used. Z strain mice were injected intravenously at birth with a viable spleen cell suspension taken from adult donors of the A strain. Spleen cell suspensions were prepared by the method described previously (3) and the injection performed as recommended by Billingham and Brent(4). Approximately 40 days following spleen injection, treated animals received a full-thickness abdominal skin homograft taken from adult donors of the A strain. The technic of skin grafting was the same as that described earlier(3). After a period of observation ranging from 2 to 5 months, mice showing tolerance of A tissue, as revealed by successful establishment of the homologous skin graft, became the experimental group. These mice were perfectly well, without overt signs of homologous disease. They were then subjected to 3 weekly intravenous injections of approximately 20 million viable spleen cells from adult, male, A strain donors. Body weights were determined before the first injection and at weekly intervals thereafter. At the end of the fourth week, the animals were sacrificed, the spleen removed and weighed in a torsion balance. Spleen and lymph nodes were then fixed in 10% formaldehyde and embedded in paraffin, and histological sections were prepared and stained with hematoxylin and eosin in the usual manner.

Two groups of control animals, consisting of Z mice made tolerant of A tissue but not treated further, and normal, untreated mice of the same strain, were also weighed at weekly intervals and sacrificed at the same time as the group of experimental mice. Histological studies of spleen and lymph nodes were also carried out in the control mice.

Results. The results are summarized in Table I. The normal, untreated Z strain mice gained an average of almost 4 g in body weight during the 4 weeks of experiment. In this group the mean absolute weight of the spleen at the end of experiment was 156 ± 21 mg. The control group of Z mice previously made tolerant of A tissue gained almost 2 g in the same period, and mean weight of the spleens was 169 ± 18 mg. The difference between average spleen weights in these 2 control groups is not statistically significant. Finally, the experimental group of Z strain mice, tolerant of A strain tissue and injected with additional spleen cells from strain A donors, showed loss of body weight

§ Z is used to designate mice of C3H strain originally obtained from Dr. J. J. Bittner's mouse colony.

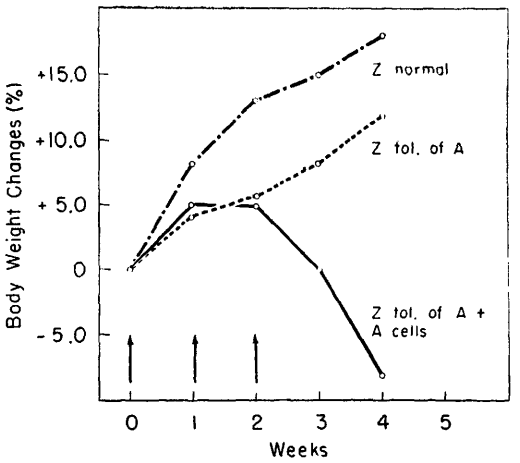

FIG. 1. Changes in body wt observed in normal Z strain mice, Z tolerant of A tissue and Z tolerant of A tissue inj. with mature competent A strain spleen cells. Arrows indicate time at which injections were performed in the latter group.

and a marked increase in weight of spleen. Spleens of the animals of this group weighed on the average 273 ± 39 mg. The difference between mean spleen weight of this group and that of either the normal or tolerant control groups is significant at the 5% level.

Fig. 1 illustrates changes in body weight observed during the experiment in the 3 groups of mice involved. While both groups of controls, *i.e.*, normal Z strain mice and Z mice tolerant of A tissue, gained in body weight during 4 weeks of observation, the experimental group of tolerant mice injected with additional mature spleen cells showed an initial increase during the first week, followed by a leveling-off after the second injection, and a marked decrease in weight beginning about the time of the third injection of homologous spleen cells.

Fig. 2 illustrates pathologic changes observed in the spleens of representative animals of each group sacrificed at the end of experiment. The lymphoid follicles in the spleens of the normal mice, as well as those in the tolerant, non-runted animals, are well preserved. In contrast, sections from tolerant animals which had subsequently been treated with large numbers of adult spleen cells from strain A donors, showed a complete disorganization of the splenic tissue, with less of the normal architecture, apparent necrotizing changes, and complete disappearance of lymphoid follicles. Similar atrophic changes were also observed in the inguinal and axillary lymph nodes removed from the runted animals, although the alter-

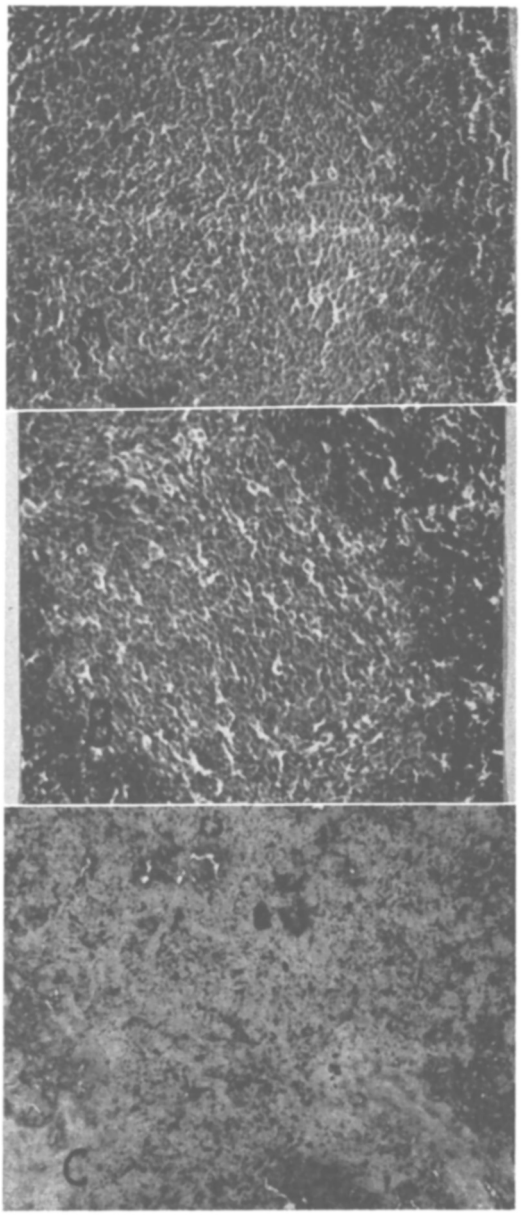

FIG. 2. Photomicrographs of spleen sections taken from normal and experimental animals. Hematoxylin-Eosin. (A) Normal Z mouse; (B) Z tolerant of A tissue; (C) Z tolerant of A tissue inj. with immunologically competent A strain cells. Magnification 154 ×.

ations observed were less profound than those seen in the spleens of these mice.

Discussion. The results of these experiments demonstrate that it is possible to induce runt disease in adult Z mice if these animals have previously been made tolerant of A tissue homografts. The effective procedure was simply injection of an additional complement of homologous cells. In these animals, the tolerant state had been induced at birth by a single injection of approximately 4 million mature, A strain spleen cells per mouse. This number of homologous spleen cells was sufficient to produce tolerance but apparently insufficient to produce runt disease in the newborn animals in this donor-host combination. When these animals had reached 2 to 5 months of age, runt disease was reproducibly produced by giving the tolerant recipients 3 weekly injections of spleen cells from the strain of mice to which they possessed tolerance.

The syndrome observed appeared to be similar to the "graft versus host" reaction obtained in: 1) newborn animals injected with homologous lymphoid cells; 2) F_1 hybrid mice receiving inoculation of mature lymphoid cells from donors of either parental strain; and 3) mice rescued from lethal irradiation by an inoculation of homologous bone marrow cells. Many mice treated in this way died of their homologous disease. This lethality as well as the pathologic findings, particularly atrophy of the lymphoid tissues and loss of body weight, are indications that the underlying mechanisms might be similar to that observed in newborn or adult animals accepting homologous lymphoid cells. Animals made tolerant by injection of homologous cells at birth are incapable of reacting against these cells and are, therefore, defenseless when invaded by immunologically competent cells having the full capacity of reaction against the host antigens.

It is interesting to note that Z strain mice tolerant of A tissue which are not given further treatment do not show any striking clinical or pathologic disease. In this donor-host combination, immunological runt disease is indeed induced in many Z animals injected with adult A cells at birth. However, the animals surviving this pretreatment grow and develop in a normal fashion, and at the time studied have lymphoid tissue of normal structure. Dosage of spleen cells given at birth is doubtless an important factor in the outcome of these animals. Their clinical and histologic well-being may be explained by assuming that the cells injected during the neonatal period have themselves gradually become tolerant of the antigens of the host and hence are incapable of reacting against these antigens, by a mechanism such as the exhaustive sensitization of the grafted cells, proposed by Simonsen(5) or the development of tolerance of adult cells by some other mechanism. Certainly the development of tolerance by adult cells is implied in our previous studies with tolerance induced in adult mice by parabiosis or intravenous injection (6,7).

When these doubly tolerant animals are then treated with an additional complement of homologous, immunologically competent cells, these new cells, if provided in sufficiently large numbers, attack the host's tissues, particularly the lymphoid tissues, and accomplish the destruction of the recipient. These observations might then be interpreted as evidence in favor of development of tolerance or exhaustion of the adult cells injected in the neonatal period.

Further, the large number of immunologically competent homologous cells might be able to cope with the antigens available in the tolerant recipient and only experience what Simonsen termed productive sensitization. Since productive sensitivity of the grafted cells is considered by Simonsen to be a precondition of exhaustion, one wonders whether the larger number of cells used to produce runt disease in our experiment would cause runting in tolerant animals if given in small divided dosages during a longer period of time. Experiments to answer this question are in progress.

Perhaps the greatest significance of these observations, besides their theoretical interest, is that they provide a model for production of immunological-homologous disease in

adult animals in which it will be possible to study the impact of the "graft versus host" reaction in absence of such complicating factors as neonatal immunological incompetence, neonatal failure of gamma globulin and protein synthesis, rapid growth and development, shifting blood and tissue volume, or of the devastating general effects of irradiation. Investigations of the effect of this "graft versus host" reaction on protein synthesis, immunological competence of the host, and the effect of prior immunological commitment of administered cells on capacity to develop the reaction, may now be effectively investigated.

Summary. 1. Runt disease in adult mice of the Z (C3H) strain made previously tolerant of A tissue by antigenic exposure at birth was obtained by injecting these animals intravenously with large numbers of immunological competent spleen cells taken from A strain donors. 2. The main pathological findings in these animals were: a) marked body weight loss; b) Splenomegally and c) Atrophy of lymphoid tissue followed by death.

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Anaerobic Conversion of Acetate to Squalene by Rat Skin.* (26407)

J. L. GAYLOR (Introduced by C. A. Baumann)

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, and Graduate School of Nutrition, Cornell University, Ithaca, N. Y.

When rodent skin is incubated with labeled acetate *in vitro,* sterol synthesis is substantial (1) and much of the label is found in sterols other than cholesterol(2). The relative activities of these sterols cannot however, be taken as a measure of precursor-product relationships since skin is so complex anatomically, and rate of penetration of acetate into different parts of the skin probably varies. Grinding of the skin has thus far yielded inactive preparations. Another approach to the problem might be possible if an active sterol precursor could be established within the tissue without the sterols themselves becoming labeled. The present studies describe conditions for accumulation of labeled squalene in rat skin.

Methods. In each experiment, one gram of rat skin was cut into pieces of 10 sq mm and incubated for 3 hours at 37°C in 6 ml of saline-phosphate buffer which contained 2.0 μc/0.4 μM of acetate-1-C^{14}. The buffer (pH 7.4) was prepared by mixing equal volumes of isotonic saline and 0.1 M sodium phosphate solutions. Potassium chloride and magnesium sulfate were added to final concentrations of 0.0125 M and 0.0026 M respectively. Nitrogen was bubbled into the solution for 10 minutes; all incubations were carried out under nitrogen. At the end of incubation period, the pieces of skin were filtered from the medium and each gram was saponified for 4 hours under nitrogen in 10 ml of 50% aqueous ethanol (v/v) which contained 20% of KOH (w/v) and 7.5% of pyrogallic acid (w/v). The unsaponified fraction was ex-

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