C^{14}-valine incorporation into the protein fraction of both tissues. However, H^3-uridine incorporation into RNA was inhibited only in brain tissue. TCAP caused changes in the nucleic acid metabolism of frog ganglia in general quite similar to those produced by DNP and azide. However, TCAP did inhibit adenine incorporation into RNA whereas DNP and azide did not.

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Studies on Homologous Disease Using Germfree Mice.* (31214)

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Intravenous injection of newborn mice with spleen cells from an adult mouse of a different strain can induce very regularly a complex syndrome called homologous or runt disease (1,2,3,4). The disease is fatal whenever the cell inoculum is large.

Does this graft *versus* host (G.V.H.) reaction develop if the animals are maintained free of infectious agents, as provided by germfree methodology? In other words, is the disease in conventional animals the effect of the G.V.H. reaction alone, or is it the result of a combination of the G.V.H. reaction plus some enhancing effect of infection, the G.V.H. reaction alone being relatively benign? In the first hypothesis, axenic mice should react like conventional mice; in the second case, axenic mice should show only the basic G.V.H. reaction.

The second hypothesis seemed plausible because certain microorganisms can induce runting but not complete homologous disease in newborn mice: strain Copenhagen of *Salmonella typhimurium*(5), polyoma virus(6,7, 8), lymphocytic choriomeningitis virus(9), reovirus(10), thymic agent(11). We also know that neonatal injection of a large dose of hydrocortisone(12,13) or neonatal thymectomy(14,15,16) will induce symptoms of runting in conventional but not in germ free mice. However, contrary to the infectious hypothesis of the homologous disease, it is known that runting can be induced by spleen cells which are free of infectious agents injected by intraperitoneal(16) or intravenous (17) routes.

Axenic animals have an immunologic status different from that of the conventional counterpart. It is underdeveloped or dormant: the lymph nodes have few germinal zones and the serum globulin levels are far below that of the conventional animal. The immunogenic mechanism of the axenic animal is functional-

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TABLE I

Group	I	II	III	IV	V
Donor	Conventional	Axenic	Axenic	Axenic	Axenic
Recipient	"	"	Conventional	Conventional	Conventional
Multiplicity and quality of cells transformed	1×	1×	1×	2×	1×, vaccinated
Control					
Survivors/Total	10/14	18/23	11/13	9/11	8/11
Treated animals					
Survivors/Total	0/34	0/40	0/35	0/41	1/48
Death occurring less than 24 hr after injection	5	10	7	9	4
Death from homologous diseases	29	30	28	32	43
Mean time survival of animals dead of homologous disease (days)	10	12, 5	13, 3	10, 4	10, 7

ly intact. If this is a significant point, then the role of cell-donor and of cell-recipient must be identified.

Materials and methods. The recipient mice were newborn C3H/jax. Some were 2, 3, or 4 days old when injected, but the majority were less than 24 hours old. The cell donors were adult C57/Bl mice. Five groups of animals were compared:

Group I: donors and recipients were propagated and maintained under conventional conditions.

Group II: axenic donors and recipients were propagated and maintained in transparent, flexible film isolators.

Group III: the donors were axenic and the recipients were conventional.

Group IV: the donors were axenic and the recipients were conventional, as for Group III, except that the number of cells in the inoculum was doubled.

Group V: axenic donors had been immunized against typhoid-paratyphoid organism at 15 to 35 days before being used. Recipient mice were conventional.

All the animals were maintained in the same type of cage. The parents of all recipients and donors were fed heat-sterilized food.

Treatment. Newborn C3H/jax less than 24 hours old were injected *via* the orbital branch of the anterior facial vein with 5×10^6 spleen cells suspended in 0.05 ml of heparinized Tyrode solution, except for those of Group IV which received 10^7 cells. Some baby mice in Groups I and II were inoculated with the same number of cells on the third or fourth day after birth.

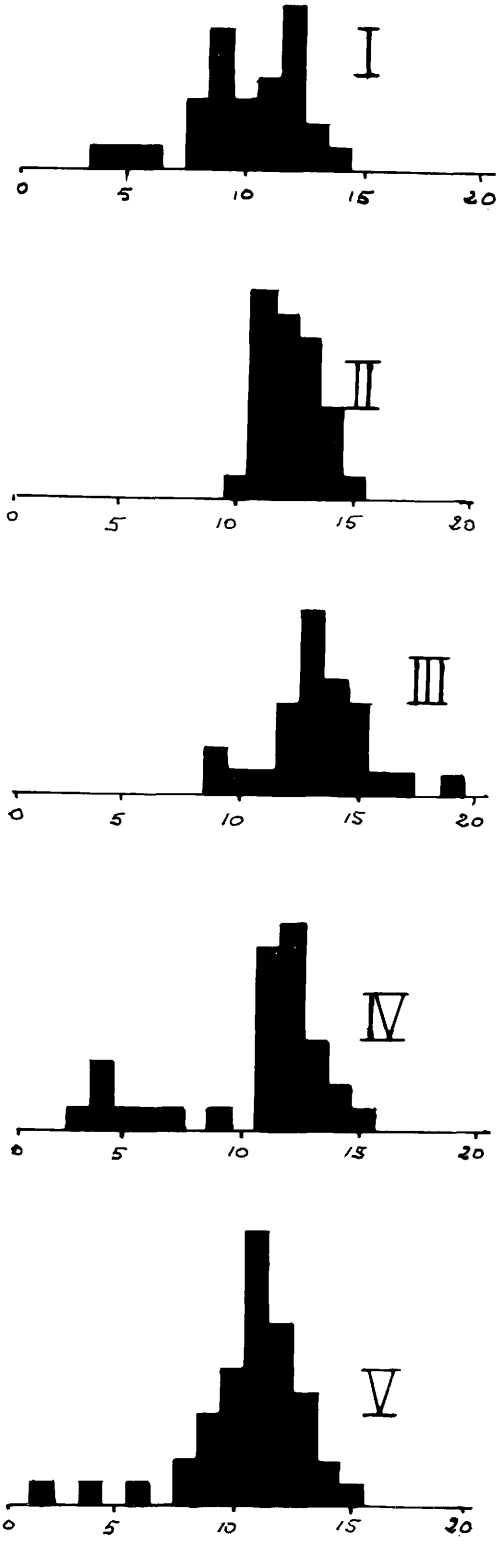
In each litter some untreated mice represented controls. The animal colonies were examined for bacterial sterility at weekly intervals.

Donors of Group V were immunized by 2 intraperitoneal injections of formalized typhoid-paratyphoid vaccine (Pasteur Institute) which was subjected to a further heat-sterilization of 121°C for 25 minutes. Each inoculum contained 10^7 bacterial cells.

As a control for latent infection, some axenic recipients were inoculated with spleen cells from an isologous 10-day-old mouse suffering from acute runting to determine if a transmissible agent was associated with this syndrome.

Some of the newborn mice in Group II were given one subcutaneous injection of 0.35 mg of hydrocortisone acetate, in an attempt to elicit a latent infectious agent. Sera from adults of that isolator unit were tested for reovirus antibodies. Furthermore, a crude extract of axenic adult mouse brain was inoculated intracerebrally into adult guinea pigs to detect latent lymphocytic choriomeningitis virus.

Results. Mortality. Fig. 1 and Table I summarize the mortality among the experimental animals. All of the treated animals of the 5 groups died. Those which died within 24 hours after injection of allogeneic cells,



did not have time to develop typical homologous disease. All of those which survived more than 24 hours after inoculation developed typical(1) homologous disease (except one which survived in Group V). There was a mean survival time of 10 days in the group of the conventional mice, 12.5 in the axenic group, 13.5 days in the conventional animals injected with cells derived from germ free donors, 10.4 days in Group IV which received 10^7 cells and 10.7 days in Group V in which the donors were immunized.

Groups II and III are significantly different ($p<0.001$) from Groups I, IV, V. However, there are no significant differences among mean survival times of Groups I, IV and V.

Clinicopathological study. The evolution of homologous disease was similar to that described by previous authors. The evolution of clinical disease was identical for the animals of the 5 different groups. Between the onset of symptoms and death, the time varied from 2 to 4 days. The clinical and pathological phenomena have been described in detail by others(1,2,3,4).

In this work every autopsied animal had hepatomegaly. The liver was pale and spotted with whitish, subcapsular foci which were distributed over the surface but were more numerous near the anterior edge. These foci as shown by histology were necrotic infarcts. In contrast to observations of others(1,4), there was no splenomegaly; but there was uniformly a terminal splenic atrophy with partial or complete disappearance of follicular structure, and a diffuse infiltration of splenic parenchyma by young lymphoid cells or by groups of epithelioid cells located near blood vessels.

The disease did not develop when mice over 48 hours old were inoculated with the standard dose of cells (5×10^6). Also homologous disease could not be transmitted with spleen cells from such mice to isologous recipients.

Discussion. "Germ free animals" may not be free of latent virus. Mouse leukemia vi-

FIG. 1. Age at death of animals from homologous disease (in days).

ruses are transmitted vertically from the mother to the ovum or to the fetus during gestation and they are responsible for leukemia in germ free mice. Systematic controls by electron microscopy have revealed viral particles in the thymus of germ free mice of 7 strains(18,19). However, our animals were maintained in a bacteriologically sterile environment during the entire experiment, and conventional viruses have not been demonstrated in them at this laboratory or elsewhere(18,20). We may therefore exclude conventional infection as the cause of death of young mice which are manifesting G.V.H. reaction. This may be so unless there exists an unknown ubiquitous infectious agent which is exclusively responsible for homologous disease and which acts only by the intraperitoneal or intravenous routes and which is innocuous, even in animals which have been thymectomized or in which lymphoid atrophy has been induced by hydrocortisone.

The syndrome which was described by others(5,6,7,8,9,10,11) as "runt disease" involves infection and is very different from the immunologic dyserasia called homologous disease, as found in these experiments and those of certain others(1,2,3,4).

Failure to induce homologous disease in baby mice more than 48 hours old (Groups I and II), with the standard dose of cells, suggests that the immunologic tolerance declines in the same time-pattern in germ free and in conventional mice, in spite of the spontaneous lymphoid hypoplasia of the germ-free mouse.

The differences of mean survival time among the groups is significant: Groups I and III have identical recipient animals but different donors and survival times; Groups II and III have different recipients but identical donors and survival times. So the mean time of survival seems to be independent of the status of the recipient but related to the axenic or conventional status of the donors. The fact that the experiment necessarily does not include transfer of cells from conventional donors to axenic recipients cannot alter the above conclusion.

The quality of the donor spleen is a significant factor; with the same number of cells, axenic mouse spleen is less quickly effective in inducing runt than the cells from the conventional spleen. The inoculum was modified in Groups IV and V, either by doubling the number of cells or by previously immunizing the donors. One may thus state that there are at least 3 factors which can modify the effectiveness of the induction: 1) quantity of cells, 2) previous immunization, and 3) certain infectious agents.

Since the population of spleen cells in the inoculum is very heterogenous, it is difficult to determine the type(s) of cells which are responsible for G.V.H. reaction. Among immunologically competent cells small lymphocytes seem to be active. Some differences in the spleens of axenic and of conventional animals were described. Thorbecke(21) has reported a smaller number of plasma cells and of secondary follicles in germ-free than in conventional mice.

Increasing the number of injected cells, and thereby the absolute number of effective cells produces the same results as the injection of cells derived from an immunized donor or from a conventional one.

Despite the short survival time of Group V, it is still not known whether immunization causes an increase of specific cells or a qualitative modification of existing cells. Siskind and Thomas(22) have studied the induction of homologous disease with different doses of splenic cells and showed that the incidence of disease decreased with smaller doses. The same authors failed to induce the disease with cells which have been lysed by freezing and thawing and by cell homogenates. Both these experiments indicate that intact, living cells in sufficient dosage are necessary for the regular induction of the disease.

The present studies have shown that homologous disease produced in germ free animals or with the aid of germ free donor cells is in all essentials identical to the classical homologous disease. On the other hand, all the attempts to induce runt disease by a variety of infectious agents have resulted in syndromes which vary among themselves and which are different from typical homologous disease(1,2,3,4).

Therefore, one may conclude that runting is not identical with homologous disease and that the latter is not caused by an infectious agent.

Summary. Studies of homologous disease in newborn mice using axenic and conventional animals (a) provide evidence that for the same dosage of cells, populations of spleen cells have different effects according to their origin. They are more efficient if donors are conventional or immunized axenic, and less efficient if the donors are only axenic. The basis for these differences has not been determined. (b) Provide no evidence that superinfection plays a role in the G.V.H. reaction in this short time system; (c) definitely separate homologous disease of newborn from similar syndromes induced by other investigators with experimental infectious agents.

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Effect of Pinealectomy and Melatonin on Feed Consumption and Thyroid Hormone Secretion Rate.*† (31215)

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Evidence that the pineal is an endocrine gland and secretes the hormone, melatonin, has been suggested by recent research(1). When rats were pinealectomized, it was shown that the ovaries hypertrophied similarly to the effect produced by constant light(2). It was then shown that constant light tended to depress the secretion of melatonin and darkness stimulated its production(3). It would thus appear that melatonin depressed the precocious development of the ovary, and light, by depressing melatonin secretion, stimulates the gonadotropic hormones and ovarian development.

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