Ca dependent by a fundamentally different factor(s) or similar factor(s) which are so tightly bound to the contractile proteins that they cannot be extracted. In addition it was concluded that the low tension generated by glycerinated uterine fibers was probably not due to breakdown and release of proteolytic enzymes from lysosomes since progesterone which supposedly inhibits lysosome breakdown had no effect on the maximum tension generated.

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Differential Mortality of Male and Female Germfree C3H Mice Introduced into a Conventional Colony.* (31664)

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A difference in mortality rates between germfree male and female C3Hf/Pi mice exposed to a conventional colony was noticed in this laboratory. This was at variance with published observations(1). A similar discrepancy existed with respect to the effect of age on survival.

Strains and species of germfree animals appear to differ in ability to respond to environmental contamination(2). Germfree mice are, however, capable of responding to antigenic stimulation(3,4). The purpose of this report is to document the effect of age and sex on the ability of one strain of germfree mice to survive exposure to a conventional colony.

Materials and methods. Male and female germfree C3Hf/Pi mice were maintained in aluminum, autoclavable isolators(5). They were then removed from the isolators at ages ranging from 12 to 498 days and transferred into our conventional mouse colony (an ordinary, barrier maintained colony; not derived from a germfree source). While in a germfree state the mice were fed a formula consisting of ground liver, bean meal, corn oil, powdered milk, wheat germ, salt, water, and supplemental vitamins. When removed they were placed on Old Guilford high-fat mouse breeder diet.

For analysis, males and females were divided into 3 groups by age; under 2 months, 2 to 6 months, and over 6 months of age. These age categories were established prior to their being removed from the germfree environment, the purpose being to test the hypothesis, derived from earlier subjective observations, that males died more readily than females, and that old mice died more readily than young ones.

The data were evaluated by the method of Pilgrim and Dowd(6). This method makes a continuous correction for death due to extraneous causes (*e.g.*, sacrifice for histological study). Animals dying of extraneous causes are utilized up to the time of death, and are then mathematically eliminated from subsequent consideration. The corrected data

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TABLE I. Raw Data on Survival of Germfree C3Hf/Pi Mice Exposed to a Conventional Colony.

			Days of exposure to conventional colony											
			0	5	10	15	20	25	30	35	40	45	50	55	60
2-8 weeks old	Alive at start of interval	♂	39	39	31	30	29	19	19	15	15	9	9	9	9
		♀	27	27	22	20	20	15	15	14	14	10	10	10	10
	Died during interval	♂	0	6	0	1	1	0	0	0	1	0	0	0	0
		♀	0	2	0	0	0	0	0	0	0	0	0	0	0
	Sacrificed during interval	♂	0	2	1	0	9	0	4	0	5	0	0	0	0
		♀	0	3	2	0	5	0	1	0	4	0	0	0	0
	% survival (corrected)	♂	100	84.6	84.6	81.9	79.1	79.1	79.1	79.1	73.9	73.9	73.9	73.9	73.9
		♀	100	92.6	92.6	92.6	92.6	92.6	92.6	92.6	92.6	92.6	92.6	92.6	92.6
2-6 months old	Alive at start of interval	♂	70	70	53	22	19	12	12	12	11	11	11	11	11
		♀	43	43	34	31	29	27	27	27	27	27	27	27	27
	Died during interval	♂	0	8	26	3	1	0	0	1	0	0	0	0	0
		♀	0	1	1	1	0	0	0	0	0	0	0	0	0
	Sacrificed during interval	♂	0	9	5	0	6	0	0	0	0	0	0	0	1
		♀	0	8	2	1	2	0	3	0	0	0	0	0	0
	% survival (corrected)	♂	100	88.6	45.3	39.1	37.0	37.0	37.0	33.9	33.9	33.9	33.9	33.9	33.9
		♀	100	97.7	94.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8
More than 6 months old	Alive at start of interval	♂	29	29	13	3	2	2	2	2	1	1	1	1	1
		♀	20	20	16	14	13	13	13	10	10	10	10	10	10
	Died during interval	♂	0	15	7	1	0	0	0	1	0	0	0	0	0
		♀	0	4	1	0	0	0	0	0	0	0	0	0	0
	Sacrificed during interval	♂	0	1	3	0	0	0	0	0	0	0	0	0	0
		♀	0	0	1	1	0	0	3	0	0	0	0	0	0
	% survival (corrected)	♂	100	48.3	22.3	17.9	17.9	17.9	17.9	8.9	8.9	8.9	8.9	8.9	8.9
		♀	100	80	75	75	75	75	75	75	75	75	75	75	75

are then plotted by the standard actuarial technique of graphing the logarithm of the percent survivors against time. When plotted in this manner the slope of any segment of the curve is the death rate; and the shape of the curve shows whether the mortality rate increases or decreases with age.

Results. The age of the germfree mice at time of exposure exerts a profound effect on their subsequent survival (Table I, Fig. 1 and 2). By the fifth day of exposure a change is readily apparent in the mortality rates of the 3 groups of male mice; mice older than 6 months of age dying most rapidly (Fig. 1). After the fifth day, the mortality rate of the male mice less than 2 months of age begins to decrease, and after approximately one month's exposure the mortality rate is almost zero. The male mice over 2 months of age continue to have high mortality rates for approximately 35 days, and then the rate drops to zero. When the mortality rates of all groups approach zero, 74% of the under 2 month group, 34% of the 2-6 month group, 9% of the group older than 6 months of age have survived. The first month of exposure appears to be critical in surviving the transition from a germfree to a conventional environment.

Female germfree mice are not as suscep-

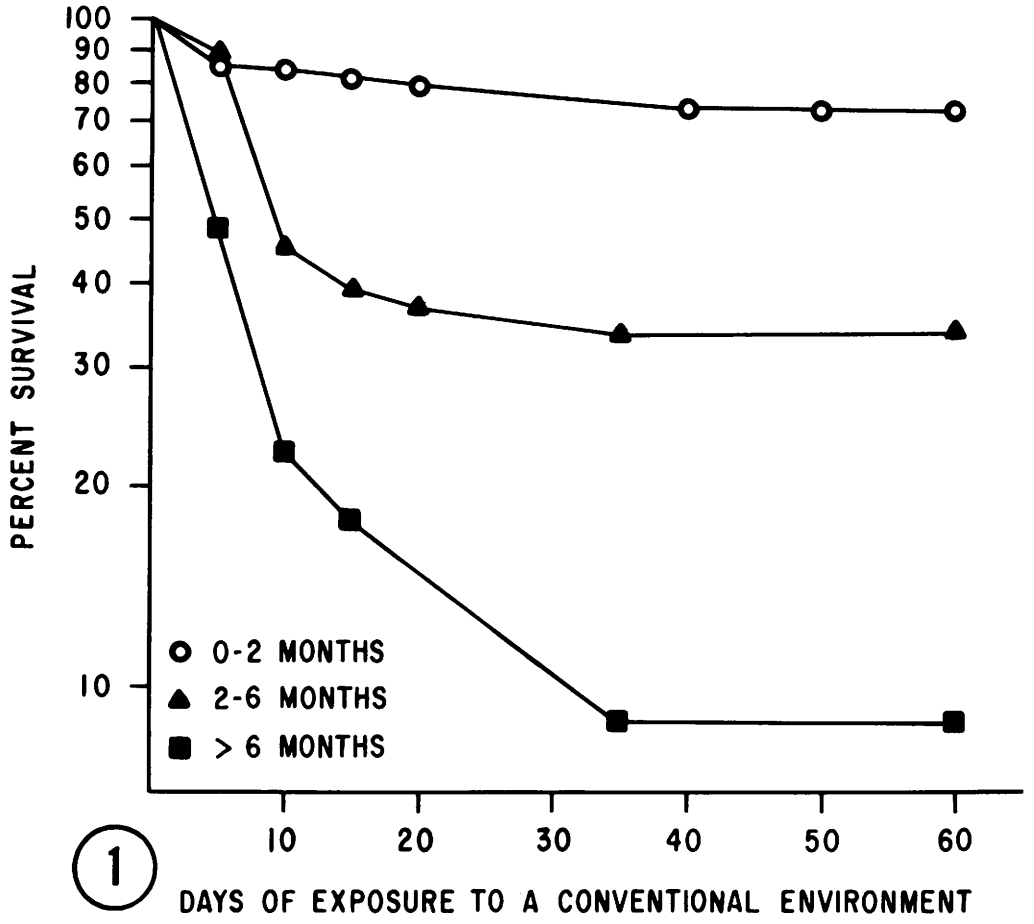

FIG. 1. Survival of 3 different age groups of germfree male C3Hf/Pi mice exposed to a conventional mouse colony (corrected).

tible to the effect of exposure as are males (Fig. 2). By the fifth day of exposure, the mortality rate of female mice less than 2 months old has approached zero. The mortality rate of female mice 2-6 months of age decreases until the 15th day, when it becomes zero. Again, the greatest mortality is experienced by the mice more than 6 months old; their mortality rate is high for 10 days and then approaches zero. At the end of the 10-day period 93% of these under 2 months of age, 92% of those 2-6 months old, and 75% of the group older than 6 months have survived.

Discussion. It has been reported that lack of microbial flora influences the ability of germfree animals to react to a conventional environment, but that they are capable of reacting against infection(2,7). Experimental susceptibility to endotoxins and virus has produced variable results(3,4,8-10), but it has been shown that the reticuloendothelial system is functional in its ability to phagocytize particulate matter, or to produce specific antibodies(3,4,11).

Administration of exogenous estrogens to conventional animals produced increased phagocytosis, decreased lymphocyte counts, and increased resistance to specific bacterial infections(12-15). Conventional C3H female mice have been shown to produce higher antibody titers more rapidly, and over a longer period of time than males(16).

C3Hf/Pi germfree male mice are more susceptible than females when transferred to a conventional colony. The cause of this differ-

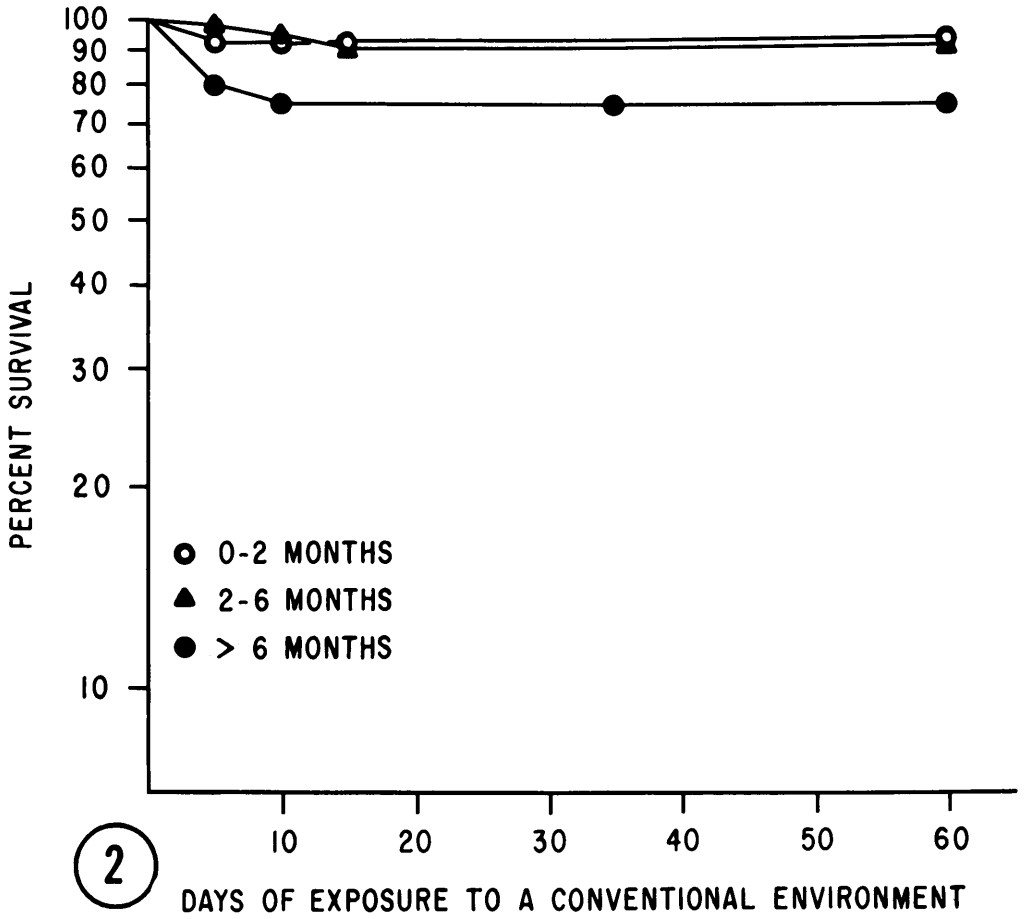

FIG. 2. Survival of 3 different age groups of germfree female C3Hf/Pi mice exposed to a conventional mouse colony (corrected).

ence in susceptibility, which seems to be dependent on both age and sex, is unknown. The intestinal submucosa becomes very edematous and ulcerated within 24 hours after mice are transferred into the conventional colony. This phenomenon may be related to the early death of these animals. Experiments in progress suggest that gonadectomy of male germfree C3H mice greater than 6 months of age will increase the probability of their surviving exposure to a conventional colony.

Summary. When germfree C3Hf/Pi mice are introduced into a conventional colony, there is a high death rate which is dependent on both age and sex. The death rate is higher in males than females, and higher in older animals than younger ones. The death rate remains high for approximately one month, following which it approaches that of conventional C3Hf/Pi mice.

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Subcellular Distribution of Histamine in Human Leucocytes.* (31665)

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Histamine is released from washed leucocytes of allergic individuals during incubation with specific antigen(1,2). We have shown (3) that combination of antigen with antibody on the washed sensitive cell is an important rate limiting step in the histamine release process. In addition, this phase of the reaction can be separated from the subsequent series of events leading to the appearance of histamine in the medium. The intracellular events initiated by the combination of added antigen and antibody bound to leucocytes is unknown. To investigate the nature of certain of these events, the intracellular distribution of histamine and of beta glucuronidase, a representative hydrolytic enzyme, was studied. Mobilization of these substances during specific immunologic histamine release and under nonspecific conditions was studied.

Materials and methods. Human leucocytes. Whole blood was obtained from a volunteer with a clinical history of rhinitis and asthma due to mold spores and with a marked immediate cutaneous wheel and erythema reactivity to mold antigen. Control blood samples were obtained from nonsensitive subjects with no cutaneous reactivity to any of a variety of antigens.

Antigen. Dry mold antigens (a mixture of alternaria, hormodendrum, helminthosporium, penicillium, monilia, aspergillus, rhizopus, chaetomium, fusarium and phoma) were extracted with buffer(4). This antigen extract resulted in release of histamine from leucocytes of mold sensitive individuals, but not of nonallergic controls.

Histamine release. Leucocyte preparations were obtained from whole blood by sedimentation and washing and the cellular histamine was released by incubation with antigen as previously described(1,2). The histamine was determined fluorometrically by the method of Shore *et al*(5). Tris ACM medium, used for suspension of leucocytes, is a salt solution containing Ca^{++}, Mg^{++} and human albumin and is buffered at pH 7.4 using Tris-hydroxymethylaminomethane.

Leucocyte fractions. Washed human leucocytes were disrupted and separated into subcellular fractions by the method of Hirschhorn and Weissmann(6). The fractions were designated as follows: *nuclear,* particulate matter sedimented at 2,000 *g* for 10 minutes; *granular,* sedimented at 27,000 *g* for 20 min-

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