administration. Since the half-life of alloxan at 37°C and pH of 7.4 is approximately one minute(15), if the alloxan inactivated MDH by combining with the essential -SH groups, the activity of this enzyme should have decreased in the period immediately after alloxan administration.

In contrast to the changes in MDH activity in the rat islet tissue following alloxan administration, the insulin content was unchanged at 24 hours. By 48 hours, however, the insulin content was less than 5% of the normal values(16). The observed decrease in MDH activity in the islet tissue may be a late manifestation of the death of the beta cell brought about by altered membrane permeability(4).

Our findings that MDH activity in the islet decreases after selective destruction of the beta cells suggested that the concentration of MDH in the beta cells of the islets is greater than that in the alpha cells.

Summary. Malic dehydrogenase activity was determined in the microdissected islets obtained from normal and alloxanized rats. There was no change in the enzyme activity up to 12 hours after alloxan administration. At 24 hours after alloxan, enzyme activity was decreased to about half the value found in the normal; at 72 hours it was decreased to one-third. By contrast, enzyme activity in the acinar tissue was not changed at these time periods, but was significantly decreased at 168 hours after alloxan administration. The malic dehydrogenase activity in the islet is about 3 times greater than that in the acinar pancreas; enzyme activity of the beta cell appears to be considerably greater than that in the alpha cell.

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Search for Virus in Human Malignancies. 3. Sex Segregation and Neoplasia in ICR/Ha Mice.* (28747)

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A previous report from this laboratory(1) described findings in a controlled study to recover viruses in ICR/Ha mice from a variety of human malignant tissues. These studies revealed a high incidence of spontaneous malignancies, especially mammary

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carcinoma, among non sex-segregated mice and definitive evidence for the lack of induction of neoplasia in mice given material of human malignant origin. These findings are in sharp contrast to those of Grace *et al*(2,3) who reported a high incidence of tumors (primarily mammary carcinoma) in ICR/Ha mice which received human malignant materials and a low incidence in control animals. It seemed important that an explanation for this apparent difference be found and clarified.

Our previous preliminary findings(1) recorded that sex segregation with consequent retention of virginity appeared to reduce the incidence of spontaneous mammary carcinoma in female ICR/Ha mice. Further work has clearly shown the striking effect of sex segregation on lowering spontaneous mammary tumor occurrence. The findings are presented and discussed in relation to the Grace *et al*(2,3) reports.

Materials and methods. Mice of the ICR/Ha strain were bred at random and maintained in these laboratories as previously described(1). All manipulations were the same except that at weaning at 21 days of age, 213 mice were sex-segregated and retained as such for the entire period of observation. A portion of the animals in the sex-segregated and in the non sex-segregated groups received extracts of human malignant materials as noted in Tables I and II. The animals included in the groups were at random with respect to sex. The proportion of males and females initially in each of the groups was nearly alike. The animals were palpated for presence of tumors at least once each week and, in addition, each cage was examined twice daily for dead animals to prevent cannibalism and to permit autopsy. Of importance, all but 8 of 94 segregated mice which died without palpable tumors during the first 550 days were autopsied and all organs which appeared abnormal at autopsy were examined histopathologically as previously described(1). Animals in the non-segregated group were sacrificed beginning at day 550, hence the data relating to a statistical comparison of incidence of tumors in the segregated and non-segregated groups are limited to this period. Data relating to histologic type of tumor in sex-segregated and non sex-segregated mice are included for the entire 650-day period of observation.

Results. Incidence of malignancy. Our previous studies(1) using non sex-segregated mice revealed no significant difference in incidence in malignancies between groups of ICR/Ha mice which received experimental human malignant materials or were held as comparable placebo or uninoculated control groups. In studies in which sex-segregated ICR/Ha mice were used, only 4 malignancies developed in the total of the inoculated, placebo and uninoculated control groups. Hence, for comparison of incidence of neoplasia in the sex-segregated and non sex-segregated groups, the animals were combined without regard to inoculation status. The findings are as shown in Table I. The results are expressed as cumulative rates of occurrence of malignancy at the time periods shown and adjusted for nonspecific deaths by standard life table procedures. Ninety-five per cent confidence limits for the difference between the 2 groups were calculated and a difference was considered significant statistically for a particular time period only if it exceeded the accompanying $\pm$ value.

The rates of death from non-neoplastic cause were roughly the same in the non sex-segregated and in the sex-segregated groups. By contrast, the rates for occurrence of neoplasia were many-fold higher among the non-segregated animals regardless of the time period analyzed. For instance, by day 400, 93 cases of neoplasia had developed among the 1318 survivors of the non-segregated mice (6.5%) whereas none of 158 surviving segregated animals had developed a neoplasia. Further, all but 2 of the 55 segregated animals which died during this period were autopsied and none showed evidence of malignancy on observation for gross pathology. Organs with abnormal appearance which were examined histopathologically were similarly negative.

Table II summarizes the occurrence of neoplasia in the uninoculated portion of the

TABLE I. Neoplasia in Sex-Segregated and in Non Sex-Segregated ICR/Ha Mice, Including Inoculated and Uninoculated Animals.

Segregation status and observation	Cumulative occurrence according to age, in days† 200	250	300	350	400	450	500	550
Non sex-segregated								
No. weaned	1931							
No. non-neoplastic deaths	384	415	466	525	613	680	755	815
Survivors	1547	1516	1465	1406	1318	1251	1176	1116
No. mammary carcinoma (%)	3 (.2)	13 (.8)	29 (1.9)	63 (4.3)	89 (6.2)	107 (7.6)	134 (9.9)	147 (11.1)
No. other neoplasms (%)	0	0	0	2 (.1)	4 (.3)	8 (.6)	23 (2)	46 (4.3)
Sex-segregated								
No. weaned	213							
No. non-neoplastic deaths	9	16	21	46	55	62	74	94
No. survivors	204	197	192	167	158	151	139	119
No. mammary carcinoma (%)	0	0	0	0	0	0	2 (1.4)	2 (1.4)
No. other neoplasms (%)	0	0	0	0	0	1 (.6)	1 (.6)	2 (1.4)
Analysis of differences between non sex-segregated and segregated groups*								
Mammary carcinoma	.2 ± .2%	.8 ± .5	1.9 ± .7	4.3 ± 1.1	6.2 ± 1.3	7.6 ± 1.4	8.5 ± 2.5	9.7 ± 2.6
Other neoplasms	0	0	0	.1 ± .2%	.3 ± .3	0 ± 1.4	1.4 ± 1.5	2.9 ± 2.4

* 95% confidence limits.

† All calculations are according to standard life table methods.

TABLE II. Neoplasia in Sex-Segregated and in Non Sex-Segregated ICR/Ha Mice, Including Uninoculated Animals Only.

Segregation status and observation	Cumulative occurrence according to age, in days† 200	250	300	350	400	450	500	550
Non-segregated								
No. weaned	616							
No. non-neoplastic deaths	63	75	95	119	137	153	187	212
No. survivors	553	541	521	497	479	463	429	404
No. mammary carcinoma (%)	0	3 (.6)	9 (1.7)	20 (3.8)	31 (6.1)	37 (7.4)	45 (9.2)	54 (11.4)
No. other neoplasms (%)	0	0	0	0	0	1 (.2)	2 (.5)	9 (2.4)
Sex-segregated								
No. weaned	95							
No. non-neoplastic deaths	1	2	6	26	29	32	35	44
No. survivors	94	93	89	69	66	63	60	51
No. mammary carcinoma (%)	0	0	0	0	0	0	1 (1.6)	1 (1.6)
No. other neoplasms (%)	0	0	0	0	0	0	0	0
Analysis of group differences*								
Mammary carcinoma	0	.6 ± .6%	1.7 ± 1.1	3.8 ± 1.7	6.1 ± 2.1	7.4 ± 2.3	7.6 ± 4.2	9.8 ± 4.4
Other neoplasms	0	0	0	0	0	.2 ± .5%	.5 ± .7	2.4 ± 1.6

* 95% confidence limits.

† All calculations are according to standard life table methods.

TABLE III. Neoplasia in Inoculated and Uninoculated ICR/Ha Mice According to Histologic Type, Sex, and Sex-Segregation Status (650-Day Observation Period).

	Neoplasia, by day 650							
	Non sex-segregated*				Sex-segregated*			
	No. of neoplasms		% of each histologic type among		No. of neoplasms		% of each histologic type among	
Histologic type	♂	♀	Total neoplasia	Total minus mammary carcinoma	♂	♀	Total neoplasia	Total minus mammary carcinoma
			%	%			%	%
Mammary carcinoma	3	152	72.0	—	0	2	15.4	—
Malignant lymphoma	10	20	13.9	49.0	3	4	53.8	63.6
Pulmonary carcinoma	5	2	3.2	11.5	0	2	15.4	18.2
Renal carcinoma	2	0	<1	3.3	1	0	7.7	9.1
Fibrosarcoma	0	0	0	0	1	0	7.7	9.1
Hepatoma	3	3	2.8	9.8	0	0	0	0
Cutaneous squamous cell ca.	2	3	2.3	8.2	0	0	0	0
Myeloid leukemia	2	2	1.9	6.6	0	0	0	0
Cavernous hemangiosarcoma	2	1	1.4	4.9	0	0	0	0
Adrenal medullary ca.	2	0	<1	3.4	0	0	0	0
Undifferent. sarcoma	0	1	<1	1.7	0	0	0	0
Adenoca., primary site unknown	1	0	<1	1.7	0	0	0	0
Totals	32	184			5	8		
Including mammary carcinoma		216				13		
Excluding " "		61				11		

* Initial composition of each group was at random with respect to sex; proportion of males to females within each group was approximately equal.

total group of animals included in Table I. Although the numbers were reduced substantially, these findings among uninoculated animals are similar to the total group and emphasize the greater importance of sex segregation status on development of malignancy than whether or not the animals were given materials of human malignant origin. The differences in incidence of neoplasia between non-segregated and segregated animals shown in Tables I and II were highly significant statistically.

As previously noted, the experiments with the non-segregated mice were terminated beginning on day 550 and continuing through day 650 during which time the animals were killed, autopsied and examined for neoplasia. All remaining sex-segregated animals were sacrificed on day 650. Fig. 1 presents the findings in the total group of animals for the full 650-day period. The importance of sex segregation on development of malignancy is dramatically illustrated.

Histopathology. Table III summarizes the histopathologic type for each malignancy

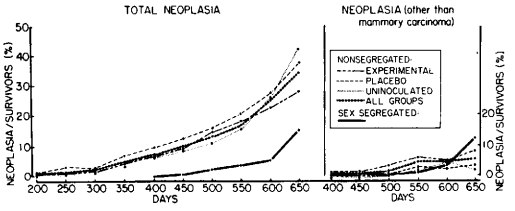

FIG. 1. Neoplasia in sex segregated and non-segregated mice of the ICR/Ha strain.

which occurred in the entire group of animals during the 650-day observation period. The majority of neoplasms were mammary carcinomas and the incidence was about 5 times as high among non-segregated as among sex-segregated animals. By contrast, the incidences of other malignancies were roughly the same in the 2 groups considering the sampling error and the sizes of the 2 populations. It is of interest that the second most frequent kind of neoplasm was malignant lymphoma.

Discussion. Spontaneous mouse mammary carcinoma appears to be caused by a virus which is carried by a variety of strains of mice. Induction of mammary carcinoma in

such carrier mice may result from the interplay of a variety of biological factors such as breeding and pregnancy with resultant hormonal alterations(4,5,6).

Our investigations to search for viruses in human malignant materials took cognizance of the influence of breeding pressure on induction of spontaneous mammary carcinoma (4) and experiments were so designed that identical conditions of breeding were maintained in both test and control groups. In the earlier work, there was no sex segregation and animals in the test and control groups were allowed to breed freely. The issue, however, were discarded shortly following birth. When it became obvious that mammary carcinoma was the predominant spontaneous type neoplasm which occurred in our studies with ICR/Ha mice, a group of mice were sex-segregated at weaning and retained as virgins. Some of these animals were injected with human malignant specimens and others were held as controls. It was clear that the injection of human neoplastic material did not influence the occurrence of malignancy. There was, however, a much smaller incidence of malignancy among the segregated animals. This was due to drastic reduction in occurrence of mammary carcinoma. There was no significant reduction in incidence of other neoplasia.

These studies serve to reemphasize the extreme importance of maintaining identical control groups with equal breeding pressure in studies to test the oncogenic quality of malignant materials injected into mice. Mice of the ICR/Ha are especially susceptible to spontaneous mammary carcinoma and for this reason are not optimal for studies of this sort. They were chosen for our purpose, however, mainly because of their freedom from polyoma virus infection which presents an even greater hazard to experimental investigations than does mammary carcinoma.

Our findings are in striking contrast to those of Grace *et al*(2,3) who reported a high incidence of mammary carcinoma in mice given human malignant tissue extracts, and relative freedom from malignancy in groups of poorly defined control animals. Analytical comparison of our data with those of Grace *et al*(2,3) is all but impossible. Although Grace recorded that some of the mouse litters were "sexed" at weaning and others were allowed to breed at will, the exact numbers of survivors in the sex-segregated and non-segregated groups at time of calculation of incidence of neoplasia were not stated. Thus, the precise ratios necessary for a definitive evaluation are lacking and it is possible that the experimental groups were heavily weighted in favor of non-segregated animals while the other groups were not. There is some suggestion for inequity as shown by a breakdown of Grace's data(2) which shows that 74 of the 85 neoplasia recorded were mammary carcinoma. Three of the 74 occurred in males, 10 in nonpregnant females, and 61 in pregnant females. This suggests that a large proportion of the animals were non sex-segregated.

The ICR/Ha mice used by Grace in his work are known to exhibit a high level of spontaneous mammary carcinoma. These mostly occur, however, after one year of age and the incidence is around 30-35% among breeders.† Two differences between the Grace work and our own are worthy of mention though it is difficult to envision how they could account for the contrast in results. First, ICR/Ha mice used by Grace were propagated at Roswell Park Memorial Institute by cousin to cousin mating whereas those used by us were bred at random. Second, carcinomas recorded by Grace were for animals less than one year of age. However, in our groups, at no time, going back to day 200, was the rate for neoplasia significantly higher in the experimental group than in the control group. Even though mammary carcinoma appeared as early as day 189, only 71 of all neoplasia noted occurred during the first 365 days of life (experimental, 38 animals, control 33). At no time was the rate of neoplasia significantly higher in the experimental group than in the control group. With these differences, it is possible to con-

† Personal communications, Dr. J. T. Grace, Jr. and Dr. T. Hauschka, Roswell Park Memorial Inst., Buffalo, N. Y.

clude definitively that no significant difference in incidence in neoplasia occurred among our test and control groups. Grace's data, by contrast, do not permit a definitive appraisal of the differences which were recorded, but leave them highly suspect of spontaneous mammary carcinoma influence.

Summary. Mice of the ICR/Ha strain were sex-segregated at 21 days of age and were followed for development of neoplasia for a period of 650 days in comparison with groups which were not sex-segregated. There was a high incidence of spontaneous neoplasia in the non-segregated mice and this was principally mammary carcinoma. Incidence of mammary carcinoma was drastically reduced by sex segregation but incidence of other malignancies of minor occurrence was not affected. Injection of extracts of human malignant materials apparently did not influence occurrence of malignancy either in non-segregated or in sex-segregated mice. These findings are discussed in relation to those of Grace *et al* who reported a high incidence of mammary carcinoma in ICR/Ha mice given human malignant tissue but not in control groups.

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Influence of Antioxidants on Myoglobin Concentrations in Vitamin E-Deficient Guinea Pig Skeletal Muscle.* (28748)

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The absence of an antioxidant, possibly vitamin E, is implicated in many of the symptoms of nutritional muscular dystrophy in animals(1). Both methylene blue (MB) and N, N′-diphenyl-p-phenylene-diamine (DPPD) have been used to protect experimental animals against the consequences of Vit. E deficiency. This role of MB, DPPD, and other antioxidants, was reviewed by Dam(2). Muscular dystrophy in calves is prevented by MB administration(3). DPPD given to lambs on a Vit. E-deficient diet delays the onset of dystrophy symptoms(4). Draper and Csallany(5) noted that DPPD brings about a rapid remission of gross dystrophic symptoms in rabbits maintained on a tocopherol-deficient diet from which all lipoid material is absent. Thus, this antioxidant possesses a curative activity with respect to muscular dystrophy in the rabbit which is independent of the presence of Vit. E in the diet. The augmented cholesterol in dystrophic rabbit muscle declines very slowly in response to DPPD treatment(6). In the guinea pig, DPPD only delays the appearance of dystrophic symptoms, and does not prevent a rise in muscle cholesterol or total lipid(7,8). Because of the differences between rabbit and guinea pig in regard to experimental results and interpretation, we sought to determine the effect of MB and DPPD supplementation on myoglobin concentration in skeletal muscles of guinea pigs fed a Vit. E-deficient diet. In these experimental animals, the myoglobin concentration is markedly in-

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