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In the last two Reports of Progress for the year ending December 25, 1978, three experiments (III, IV and V) were dealt with in part. The primary reason for this incomplete treatment was that we initiated experiments with a highly productive inbred mouse strain, BALB/cByJ. This decision produced some very exciting results but cramped our facilities to the extent that we were forced to alter original schedules for the completion of experiments. This delay also lead to our request for an extension of the contract without funds.

Experiment III has been described in detail, and statistical analyses of the dominant lethal results and the mutagenic effects on quantative traits have been described for the F_1 generation groups representing mutagenized spermatozoa and spermatogonia. In this final report we will complete the discussion of results on quantitative trait analysis for Experiment III for the F_2 generation groups representing both mutagenized spermatozoa and spermatogonia.

Experiment IV, representing a limited repetition of Experiment III, including only F_1 generation groups treated as spermatozoa, has been outlined previously. Statistical analyses of dominant lethal data and the results of quantitative trait analyses will be presented here.

Finally, Experiment V was outlined in the previous Report of Progress in respect to experimental design, numbers of parental animals and a schedule of matings up to but not including the establishment of F_1 crosses. Mating schedules for this experiment, dominant lethal data and the results of quantitative trait analyses will be added in the present report.

The three experiments to be discussed in this report are devoted primarily to the effects of a single mutagenic agent in altering the expression of quantitative traits in the inbred mouse strain, BALB/cByJ. In Experiment III, two other inbred mouse strains were also considered (DBA/2J and C3H/HeJ), but the numbers of young produced by these strains were so low as to provide results that must be confirmed to be useful. In all three experiments the mutagenic agent employed was triethylenemelamine (TEM). In order to maximize the number of animals in each group, only two doses (.1 and .2 mg TEM per kilogram of body weight) were employed in addition to a control group. In all these experiments the number of matings set in each dose group was adjusted to compensate for dominant lethal effects of treatment in the high dose groups in such a way that similar numbers of F_1 progeny would be produced in all treatment groups. Treatment effects were tested in spermatozoan and spermatogonial stages

of spermatogenesis in progeny of the F_1 and F_2 generations, except that in Experiment IV only F_1 generation spermatozoan treated young were produced.

In all three experiments, the quantitative traits studied to determine the effects of mutagenic treatment were: 1) time of development of the righting response, 2) body weight at weaning, 3) the defecation portion of the open field test, 4) tail length at seven weeks of age, 5) hematocrit at seven weeks, and 6) brain weight. The time at which brain weights were taken was variable depending upon the desirability of obtaining data which precluded the early sacrifice of an individual. However in Experiments IV and V brain weights were taken at approximately 15 weeks of age for males and approximately 19 weeks of age for females.

In addition to the continuously varing traits listed above, productivity traits were also studied to determine if treatment had been effective. These traits included: 1) percent dead implantation scars of total scars, 2) total implantation scars, 3) percent females born, 4) total young born, 5) percent females weaned and 6) total young weaned. Traits 1 and 2, which are based upon inspection of uterine scars in parental generation females, were analyzed for F_1 generation young only. Other productivity traits were studied in both F_1 and F_2 generations.

As has been general practice, females of crosses involved in spermatozoan treatment tests were isolated from each other ten days after the initiation of matings and checked daily for the presence of a litter through day 21 after the termination of matings. Young were sexed and marked by toe clipping for identification at birth. Starting on day 5, progeny were checked for the ability to right themselves and were checked daily until the trait appeared. All animals were weaned at four weeks of age, at which time body weight and sex were recorded, and individuals were permanently marked by coded ear punching. The defecation portion of the field test was conducted on mice at five weeks of age; tail length and hematocrit measures were taken for mice at approximately seven weeks of age. As indicated above, brain weights were taken at about 15 weeks of age for males and at about 19 weeks of age for females.

For all experiments, the mutagenic treatment consisted of intraperitoneal injection of triethylenemelamine carried in .25 cc Hank's balanced salt solution prepared immediately prior to injection. Control males received the same quantity of carrier only. Doses were based on the mean body weight of a random sample of the mice to be treated.

Results: Quantitative Traits, Experiment III. Results of analyses of data for both spermatozoan and spermatogonial treated groups of the F_1 generation were considered in the last Report of Progress. In this report, analyses of data with respect to the F_2 generation for spermatozoan and spermatogonial treated groups for strain BALB/cByJ will be discussed. Where meaningful comparisons can be made, the results of earlier analyses have been repeated.

Time of Development of the Righting Response. In the last Report of Progress no significant differences were reported among dose groups for this trait for either spermatozoan or spermatogonial series of the F_1 generation. However, in both cases it was noted that there was a roughly linear increase in the time of development of the righting response with increasing mutagen dose. In the F_2 generation of the spermatozoan treated group the same situation obtains, i.e., there is an increase in time of development of the righting response with increasing dose. Again the relationship is roughly linear and the differences are not significant ($p < .10$).

With respect to the spermatogonial treated series in the F_2 generation, differences among dose groups were not significant. Interestingly, the pattern is not consistent with that observed in other germ cell/generation groups. The face value differences among means involve a very slight increase in time of development of the righting response from the control to the low dose group followed by an appreciable decrease in time of development of righting response between the low dose and high dose group. As indicated earlier, "it may be concluded that righting response would not be a useful trait to employ if BALB/cByJ were the strain being used."

Body Weight at Weaning. In the most recent Report of Progress it was reported that when means were adjusted for litter size there were highly significant differences ($p < .005$) among the F_1 spermatogonial dose groups, but differences among dose group means in the F_1 generation spermatozoan treatment series were not significant. Table 1 is taken in part from this source but includes, in addition, data for the F_2 generation spermatozoan and spermatogonial treatment groups. As may be seen, significant differences were found among dose group means for both of these treatment groups, with the more impressive differences being found among the spermatogonial treatment means.

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Table 1. Effect of TEM dose on body weight at weaning of F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ in Experiment III. Males were treated at the spermatozoan or spermatogonial germ cell stages. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance. Sexes and replicates are combined for simplicity.

Strain	Stage	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
BALB	F_1 Spermatozoa	n.s.	18.00 (226)	17.83 (134)	17.67 (72)
BALB	F_2 Spermatozoa	$p < .05$	17.17 (641)	17.45 (270)	17.10 (98)
BALB	F_1 Spermatogonia	$p < .005$	16.49 (376)	16.87 (458)	17.06 (419)
BALB	F_2 Spermatogonia	$p < .01$	16.84 (462)	17.17 (466)	17.11 (509)

The results indicate that body weight may be a very useful character employing BALB/cByJ, with appreciable increases in body weight expected from relatively weak mutagenic doses in the F_2 generation only of the spermatozoan treatment group but in the F_1 and F_2 generation groups of spermatogonial treated groups. As has been pointed out previously, the fact that this approach detects differences in the spermatogonial

stages is particularly exciting in view of the difficulty of measurement of mutagenic effect generally for this stage. The biological significance of these results will be discussed at greater length later in this report.

Tail Length at Seven Weeks of Age. As stated in the last Report of Progress there were no significant differences among dose group means in the F_1 generation spermatozoan treatment group, but highly significant differences were found among dose groups in the F_1 generation spermatogonial treatment group (Table 2). The same pattern emerges in the F_2 generation groups in that the spermatozoan treatment groups show no significant differences, while the spermatogonial groups show significant differences that closely parallel those of the F_1 spermatogonial group in pattern. In each case there is an impressive increase in length of tail in the low dose groups with respect to the tail length mean in controls. This is followed by a slight, nonsignificant, reduction in tail length at the highest dose level in both the F_1 and F_2 spermatogonial groups. This parallels precisely the pattern for the same groups in body weight. The close correspondence between tail length effect and body weight effect has been observed in a number of other experiments in this series. Although it seems likely that body weight and tail length are associated and not independent, the two traits employed together would increase reliability of conclusion when they pointed in the same direction.

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Table 2. Effect of TEM dose on tail length at seven weeks of age of F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ in Experiment III. Males were treated at the spermatozoan or spermatogonial germ cell stages. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined.

Strain	Stage	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
BALB	F_1 Spermatozoa	n.s.	82.04 (222)	82.26 (128)	82.07 (70)
BALB	F_2 Spermatozoa	n.s.	80.88 (613)	80.92 (264)	80.66 (96)
BALB	F_1 Spermatogonia	$p < .001$	81.88 (366)	82.57 (441)	82.51 (406)
BALB	F_2 Spermatogonia	$p < .05$	79.60 (436)	79.96 (449)	79.88 (486)

Hematocrit at Seven Weeks of Age. In the most recent Report of Progress it was reported with respect to the F_1 generation test groups that differences among treatment group means were significant for neither spermatozoan nor spermatogonial treated series. However, in both cases factorial analyses of variance indicated that the observed differences did approach significance ($p \sim .10$). Analysis of F_2 generation groups revealed highly significant differences among dose group means in the spermatogonial series; differences among dose means in the spermatozoan series, however, were not significant (Table 3). The pattern observed in the F_2 spermatogonial series indicates very little difference between the low dose group and controls but a significant decrease in hematocrit in the high dose group. Inasmuch as other traits (body weight and tail length) of usefulness in detecting mutagenic effects have been of greatest importance in the detection of low dose effects, the hematocrit trait may be of value in detecting the effects of more highly mutagenic agents.

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Table 3. Effect of TEM dose on hematocrit at seven weeks of age of F_1 and F_2 progeny of treated male parents in inbred strain BALB/cBy in Experiment III. Males were treated at the spermatozoan or spermatogonial germ cell stages. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined.

Strain		Stage	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
BALB	F_1	Spermatozoa	$p \sim .11$(n.s.)	48.88 (226)	48.85 (133)	49.28 (72)
BALB	F_2	Spermatozoa	n.s.	48.78 (635)	48.65 (263)	48.90 (97)
BALB	F_1	Spermatogonia	$p \sim .08$(n.s.)	48.73 (365)	48.86 (437)	48.63 (410)
BALB	F_2	Spermatogonia	$p < .005$	48.85 (453)	48.82 (463)	48.51 (507)

The hematocrit analyses exhibit closely parallel response patterns of the F_1 and F_2 generation effects, both for the spermatogonial group and for the spermatozoan group. With respect to the spermatogonial groups the difference between means for the control and low dose group is quite modest in each case and is followed by a much more impressive decrease in hematocrit at the highest dose group. By contrast, in the spermatozoan groups, both the F_1 and F_2 generation means exhibit slight reductions from control to low dose groups followed by modest increases in hematocrit at the highest dose level.

Defecation Portion of the Open Field Test. In the last Report of Progress significant differences were found among dose group means only for the F_1 generation spermatogonial series. However, differences between means in the F_1 spermatozoan series were sufficiently great and the pattern of response among replicates and sexes sufficiently consistent as to suggest that the differences might well be real. In the F_2 generation results no significant differences were found among dose group means for either the spermatozoan or spermatogonial treatment series, but differences between means were appreciable leading to approaches to significance in both cases (Table 4).

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Table 4. Effect of TEM dose on defecation rate at five weeks of age in F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ in Experiment III. Males were treated at the spermatoaoan and spermatogonial germ cell stages. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined.

Strain		Stage	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
BALB	F_1	Spermatozoa	$p \sim .20$	4.53 (226)	4.18 (134)	3.92 (72)
BALB	F_2	Spermatozoa	$p \sim .19$	4.17 (641)	4.37 (270)	4.52 (98)
BALB	F_1	Spermatogonia	$p < .05$	4.03 (376)	4.36 (458)	4.38 (417)
BALB	F_2	Spermatogonia	$p \sim .24$	4.70 (462)	4.94 (466)	4.79 (509)

The dose response patterns that emerge are not consistent among the germ cell stage treatment/generation groups, but they are interesting and may well reflect real response patterns. In the F_1 generation spermatozoan groups, defecation rate decreases in a roughly linear fashion with increasing dose. By contrast, in the F_2 generation spermatozoan treatment group, defecation rate increases in a roughly linear fashion with increasing dose. With respect to spermatogonial series treatment, in the F_1 generation there is a pattern involving an increase in defecation rate among treated groups relative to controls, and the differences are significant. However the difference between the two treatment groups is negligible. Finally, in the F_2 generation spermatogonial series, there is a face value increase in defecation rate from the control group to the low dose group followed by a modest decrease.

Brain Weight at Fifteen to Nineteen Weeks of Age. In previous reports we have reported on brain weights at "Approximately Thirteen Weeks of Age". It turns out that this figure is, in practice, much closer to fifteen weeks of age for males and nineteen weeks of age for females. In the last Report of Progress, we provided the results of analysis of brain weights in the F_1 generation spermatogonial group where highly significant differences were found involving an increase in brain weight from control to low dose group followed by a modest decrease in brain weight at the higher dose. Mean values were not provided for the F_1 generation spermatozoan groups because differences among dose groups were not significant. Means for both groups are presented here in Table 5 in order to show the patterns involved.

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Table 5. Effect of TEM dose on brain weight at approximately thirteen weeks of age in F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ treated at the spermatozoan and spermatogonial germ cell stages. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined.

Strain	Stage	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
BALB	F_1 Spermatozoa	n.s.	482.11 (212)	481.84 (114)	476.64 (51)
BALB	F_2 Spermatozoa	$p \sim .01$	472.46 (630)	475.72 (266)	473.78 (94)
BALB	F_1 Spermatogonia	$p < .01$	473.04 (350)	476.14 (420)	474.85 (389)
BALB	F_2 Spermatogonia	$p < .001$	464.32 (459)	465.88 (464)	468.29 (506)

It can be seen that the F_1 spermatozoan pattern reflects a very modest drop in brain size from the control to the low dose group followed by a more impressive decrease in brain weight in the high dose group. For both of the F_2 generation groups, differences among dose groups proved to be highly significant, and the patterns are quite interesting. For the F_2 spermatozoan dose groups, there is an increase in brain weight from control to low dose group followed by a modest decrease in the high dose group, the mean brain weight of which is still appreciably greater than control. The relationship of F_2 to F_1 spermatozoan results for brain weight parallels very closely that for body weight in that in both, there are more or less linear decreases in weight (non-significant) with increasing mutagenic dose in F_1 generation results followed by significant increases from control to low dose group

in the F_2 generation results. The parallel continues to high dose groups where brain weight decreased (only slightly) while body weight decreased (appreciably). In the F_2 spermatogonial groups there is a modest increase from control to low dose group followed by an impressive increase to the high dose group. The numbers involved and the improbability that such differences could be due to chance alone leave little doubt as to the reality of these differences.

Results: Dominant Lethality, Experiment IV. Data obtained on dominant lethal parameters for the A and B replicates in Experiment IV are outlined in Table 6. These results parallel quite closely the results obtained in

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Table 6. Dominant lethality parameters with standard errors and sample size of Replicate A and B series BALB/cByJ females and their F_1 young in Experiment IV. Females belonging to the two experimental and a single negative control group were mated for a period of seven days immediately following treatment, thus represent effects of mutagenic agent upon spermatozoa.

	TEM dose, mg/kg of body weight		
	0	.10	.20
Replicate A			
Live Scars/ ♀	4.60 ± 1.12 (20)	3.04 ± .74 (25)	.97 ± .21 (70)
Dead Scars/ ♀	.35 ± .22 (20)	.80 ± .23 (25)	.46 ± .14 (70)
Total Scars/♀	4.95 ± 1.14 (20)	3.84 ± .87 (25)	1.43 ± .30 (70)
Total Live Births/♀	6.73 ± .85 (11)	3.14 ± .63 (14)	1.67 ± .36 (21)
Total Weaned/♀	6.27 ± .92 (11)	3.14 ± .63 (14)	1.43 ± .28 (21)
Percent of Dead Scars of Total	7.1	21.8	32.2
Percent Perinatal Losses	19.6	42.1	48.5
Replicate B			
Live Scars/♀	5.00 ± .94 (19)	2.54 ± .53 (28)	1.84 ± .35 (68)
Dead Scars/♀	.47 ± .19 (19)	.50 ± .17 (28)	.46 ± .11 (68)
Total Scars/♀	5.47 ± .97 (19)	3.04 ± .63 (28)	2.29 ± .41 (68)
Total Live Births/♀	4.87 ± .69 (15)	3.27 ± .40 (15)	2.86 ± .48 (28)
Total Weaned/♀	4.87 ± .69 (15)	3.27 ± .40 (15)	2.79 ± .48 (28)
Percent Dead Scars of Total	8.6	16.4	20.1
Percent Perinatal Losses	23.2	31.0	36.0

Experiment III and demonstrate quite clearly that administration of mutagenic agents was effective. Data on live scars, dead scars and total scars represent numbers per treated female. It can be seen that, as has been found in the past, number of live scars per female decreases more or less linearly with increasing dose as do total scars per female. Dead scars per female,

by contrast, tend to show an increase from control to the lower dose followed by a decrease. Statistical analyses of scar data were carried out by ANOVA; these were based upon numbers of pregnant females (i.e. having uterine scars) within each group. Analysis of total scars was carried out on data after square root transformation. As expected, differences among means due to dose were highly significant ($p < .001$). Differences between replicates were not significant nor were group x dose interactions. A factorial analysis of the percent dead scars of total scars, carried out after arc sine transformation, revealed that the differences among dose groups were significant ($p \sim .014$). Again, there were no significant differences between replicates, and the group x dose interaction was not significant. As has been found in the past, considering only females with scars for this analysis, increase in percent dead scars and in percent perinatal losses from control to both treated groups was impressive, but the difference between treated groups itself was not. It is generally felt that the number of zygotes lost continues to increase with higher doses, but that an increasing proportion of the losses at high mutagenic exposures are made up of pre-implantation losses which are not represented by uterine scars.

Results: Quantitative Traits, Experiment IV. The primary value of this repetition is the demonstration that, as was reported for Experiment III, spermatozoan treated F_1 generation young are not of particular usefulness in the detection of mutagenic activity. This is true with respect to all traits.

Time of Development of the Righting Response. As was the case in Experiment III, no significant differences due to dose were found. Contrary to what was found in Experiment III, there was a roughly linear decrease in the time of development of the righting response with increasing mutagen dose in Experiment IV.

Body Weight at Weaning. Paralleling the results of Experiment III, differences due to dose among F_1 progeny of spermatozoan treated groups were not significant. Parallel elements in the dose means are limited to a decrease in body weight below controls at the highest mutagenic dose in both experiments. However, the approaches to significance are so slight that nothing would be gained by combining the two experiments.

Tail Length at Seven Weeks of Age. The parallel with Experiment III continues with no significant differences among dose groups observed in the F_1 generation spermatozoan treated groups. There is an interesting parallel in that in both experiments, after adjustment for litter size, there is an increase in tail length from control to low dose group followed by a modest decrease. Again, however, approaches to significance in both experiments are so slight as to suggest coincidence in the parallel results.

Hematocrit at Seven Weeks of Age. Again, as was the case in Experiment III, hematocrit differences due to dose were not significant. There was observed an element of similarity in the two experiments in that very slight reductions were found from control to low dose group followed by

an increase of some magnitude in the high dose group. Again, it is estimated that statistical significance would not be achieved by combining the two experiments in a factorial analysis.

Defecation Portion of the Open Field Test. Differences among means approached significance more closely than in other traits in the series ($p \sim .16$), and differences among means in Experiment III also approached significance rather closely ($p \sim .11$). However, the patterns of distribution of means is not similar in the two experiments. There was reported a more or less linear decrease in defecation rate with increasing dose in Experiment III, whereas in Experiment IV there is an increase from controls to both dose groups, with the high dose group mean being slightly higher than the low dose group.

Brain Weight at Fifteen to Nineteen Weeks of Age. As with other traits in F_1 generation spermatozoan test groups, significant differences due to dose were not found in either Experiment III or Experiment IV. Similarity in pattern of mean distribution exists only in that brain weights of treated group progeny were reduced below control means. Differences among means were considerably less in Experiment IV than in Experiment III.

Experiment V: Methods. As outlined in detail in the Report of Progress for the period June 25, 1978 through December 25, 1978, mice of the parental generation were assigned to one of six replicates. The first three of these, carried out two weeks apart, involved mutagenized spermatozoa. These matings involved trios of females mated with each male. The remaining three replicates involved mutagenized spermatogonial cells, the mutagenic treatment having been administered over eight weeks prior to establishment of matings. A schedule of matings was included in the Report of Progress. All matings had been carried out at that time except those for the final replicate, F, which was established on January 8, 1979 instead of January 10, 1979 as planned. In this experiment, in order to maximize number of matings, males were selected from three different age groups and varied from thirteen to nineteen weeks at treatment. Care was taken to assure that all age groups were approximately equally represented in the control and different treatment groups.

A schedule of matings of F_1 generation crosses is provided in Table 7. Pairs and trios of females were mated with single males (in addition to pair matings) in order to use all available females. The numbers of females

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Table 7. Schedule of Matings of F_1 Generation Crosses in Experiment V.

Replicate	A	B	C	D	E	F
Matings Established	2/8/79	2/22/79	3/5/79	2/14/79	3/15/79	5/1/79
Matings Separated	2/22/79	3/8/79	3/19/79	3/28/79	3/29/79	5/15/79

and males involved in F_1 generation crosses are shown in Table 8. Crosses were established between individuals taken randomly within dose and replicate, except that sibling crosses were precluded.

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Table 8. Numbers of females and males involved in F_1 generation crosses in Experiment V. Where females available within a group outnumbered males, pairs or trios of females were mated with males as necessary to exploit all females. Otherwise pair matings were employed.

Replicate:	Spermatozoa Treated			Spermatogonia Treated		
	A	B	C	D	E	F
	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂
control	52/52	52/36	70/62	25/25	46/31	45/38
.1 mg/kg	81/74	85/85	73/73	63/52	51/38	57/23
.2 mg/kg	81/67	96/85	91/69	52/46	40/40	41/35

Results: Dominant Lethality, Experiment V. Three replicates (A, B & C) were employed in Experiment V examining the effects of mutagenic action upon spermatozoa. The data obtained on dominant lethal parameters for these three replicates are outlined in Table 9. In general, these results parallel quite closely those obtained in earlier experiments, particularly those in which strain BALB/cByJ mice have been employed. In Replicate B total scars, which usually decrease from control to the low level dose, actually showed a modest increase. However, the higher dose revealed a substantial reduction in total scars that is characteristic of the dominant lethal test. In Replicate C there was observed a drop in percent dead scars of total from the control to the low mutagen dose. However, dead scars are a low frequency phenomenon, and a modest change can produce a sizable effect upon percentage dead scars of total. Inasmuch as total scars show a very straightforward linear decrease with increasing dose as expected, it seems likely that the low percentage of dead scars in the low dose group is most simply attributable to chance variation. In general, it can be said that live scars and total scars tend to decrease with increasing dose. Dead scars, by contrast, tend to show an increase from control to low dose group. The change from low dose to high dose group is unpredictable. Statistical analysis of dead scars as a percentage of total scars were carried out by ANOVA following arc sine transformation. Differences among dose groups were found to be highly significant ($p \sim .002$). Differences among replicates also proved to be highly significant ($p < .001$), probably due to the aberrant figure recorded for Replicate C described above. Group X dose interactions were not significant. Inspection of the differences between dose group means revealed that the increase in percent dead scars of total was rather modest from control to low dose group, but considerably greater from low dose to high dose group. Analysis of total scars was carried out after square root transformation of data. Differences among dose groups proved to be highly significant ($p < .001$). There was found a clear linear trend of decreasing total scars with increasing mutagen dose in Replicates A and C. While the data of Replicate B were not sufficiently different to affect the statistical results, they probably were responsible for a significant dose x group interaction ($p \sim .024$). It may be recalled that differences

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Table 9. Dominant lethality parameters with standard errors and sample sizes of Replicate A, B and C series BALB/cByJ females and their F_1 young in Experiment V. Females belonging to the two experimental and a single negative control group were mated for a period of seven days immediately following treatment, thus represent effects of mutagenic agent upon spermatozoa.

	TEM dose, mg/kg of body weight		
	0	.10	.20
Replicate A			
Live Scars/♀	5.07 ± .94 (27)	4.15 ± .60 (59)	2.19 ± .30 (112)
Dead Scars/♀	.22 ± .15	.42 ± .18	.66 ± .13 (112)
Total Scars/♀	5.30 ± .97 (27)	4.58 ± .62 (59)	2.85 ± .34 (112)
Total Live Births/♀	7.40 ± .70 (15)	4.91 ± .51 (32)	2.76 ± .38 (55)
Total Weaned/♀	7.00 ± .68 (15)	4.84 ± .37 (32)	2.71 ± .30 (55)
Percent Dead Scars of Total	4.2	9.2	23.2
Percent Perinatal Losses	19.0	35.9	38.0
Replicate B			
Live Scars/♀	3.65 ± .81 (34)	3.64 ± .51 (59)	2.57 ± .33 (115)
Dead Scars/♀	.06 ± .04 (34)	.27 ± .09 (59)	.40 ± .04 (115)
Total Scars/♀	3.71 ± .81 (34)	3.92 ± .54 (59)	2.97 ± .36 (115)
Total Live Births/♀	6.25 ± .70 (16)	5.14 ± .47 (36)	3.92 ± .32 (51)
Total Weaned/♀	5.56 ± .55 (16)	4.83 ± .38 (36)	3.65 ± .28 (51)
Percent Dead Scars of Total	1.6	6.9	13.5
Percent Perinatal Losses	28.2	14.0	32.4
Replicate C			
Live Scars/♀	4.77 ± .74 (44)	3.69 ± .57 (59)	2.59 ± .35 (103)
Dead Scars/♀	.43 ± .15 (44)	.15 ± .05 (59)	.34 ± .09 (103)
Total Scars/♀	5.20 ± .75 (44)	3.85 ± .59 (59)	2.93 ± .37 (103)
Total Live Births/♀	5.81 ± .52 (26)	5.81 ± .49 (27)	3.73 ± .38 (44)
Total Weaned/♀	5.58 ± .45 (26)	5.70 ± .43 (27)	3.41 ± .33 (44)
Percent Dead Scars of Total	8.3	3.9	11.6
Percent Perinatal Losses	28.1	28.0	38.6

among means in total scars due to dose were highly significant in Experiment IV where dead scars decreased in linear fashion with increasing dose in both replicates. Differences among replicates were not significant. In spite of the irregularities present, the analyses of total scars and of

dead scars as a percent of total scars indicate clearly that the mutagenic agent was effective.

Results: Quantitative Traits, Experiment V. I. Spermatozoan Treatment, F_1 Progeny. The results of the analysis of quantitative traits in F_1 progeny of males in which germ cells were mutagenized as spermatozoa are of value for only a few traits. For these traits, differences among means are significant and often they parallel the results found in Experiments III & IV involving the same strain and mutagen doses. It should be born in mind that this experiment involves sample sizes 50% larger than the combined numbers of those involved in Experiments III & IV. In total, data were obtained from nearly 1300 young in the spermatozoan treatment group of Experiment V. Three of the traits involved (body weight at weaning, tail length at seven weeks and hematocrit at seven weeks) would be expected to be of usefulness in detecting mutagenic effects.

Time of Development of the Righting Response. As was found in Experiments III & IV no significant differences due to dose were found. Further, the pattern of distribution of dose means is quite different in Experiment III from the patterns of Experiments IV & V which are strongly parallel. In Experiment V with its relatively large sample size the difference among doses approaches significance ($p \sim .12$). For practical purposes, however, this would not appear to be a very useful trait in the ascertainment of mutagenic damage in F_1 generation young where spermatozoan treatment is involved.

Body Weight at Weaning. In Experiments III & IV differences among dose groups were not significant. However, with the much larger sample size of Experiment V, differences among dose groups are highly significant ($p \sim .005$) after adjustment for litter size (Table 10). There is also a highly significant difference between sexes and among replicates. However, none of the interactions between group, dose and sex is significant.

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Table 10. Effect of TEM dose on body weight at weaning of F_1 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III, IV and V. Males were treated at the spermatozoan germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance. Sexes and replicates are combined for simplicity.

Experiment	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	n.s.	18.00 (226)	17.83 (134)	17.67 (72)
IV	n.s.	18.18 (143)	18.27 (97)	18.13 (109)
V	$p < .005$	16.28 (317)	16.66 (488)	16.28 (490)

Inasmuch as there is no difference between means of the control and high dose groups after adjustment for litter size, it follows that the low dose mean is highly significantly greater than both the control and high dose means. The numbers are sufficiently large in this experiment to leave little doubt as to this result. Further, the results of Experiment IV show

the same pattern, that is an increase from control to low dose mean followed by a decrease to high dose mean. However, the differences among means in Experiment IV are of considerably lesser magnitude than are the differences among Experiment V means. In Experiment III there is a drop in body weight from low dose mean to high dose mean that is quite similar in magnitude to that of Experiment IV, but there is, contrary to the results of the other two experiments, a drop in mean body weight from control mean to low dose mean. As was indicated earlier the differences among means in Experiments III & IV did not approach significance. It is emphasized that the discussions above pertain to means adjusted for litter size by covariance. With respect to the raw data, there is a more or less linear increase in body size from control to low dose group followed by virtually no change to high dose group. The maintenance of such high body weight in the high dose group is probably largely attributable to the reduced mean litter sizes with increasing mutagen dose.

As was indicated above, from a purely practical viewpoint, to employ this trait as an indicator of mutagenic activity, experiments should be designed involving multiple replicates and based upon totals of 300 to 500 F_1 young in the control and each test group.

Tail Length at Seven Weeks of Age. As was pointed out above there were close parallels between the results of Experiments III & IV, although differences among dose group means were not significant. Experiment V exhibits the identical pattern and is associated with a highly significant difference ($p < .002$) among dose group means after adjustment for the effects of litter size by covariance (Table 11). Undoubtedly, the large sample sizes (300 to 500 in each group) and an increased magnitude in the difference between means contributed to the statistical significance.

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Table 11. Effect of TEM dose on tail length at seven weeks of age of F_1 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III, IV and V. Males were treated at the spermatozoan germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined for simplicity.

Experiment	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	n.s.	82.04 (222)	82.26 (128)	82.07 (70)
IV	n.s.	82.18 (129)	82.51 (95)	82.41 (103)
V	$p < .002$	81.12 (303)	81.75 (472)	81.09 (467)

There is usually a close correlation between body weight and tail length. This correlation is quite clear in Experiments IV & V. If body weights in Experiment III had been closely correlated with tail length (they were not), the parallelism found in Experiment IV & V with regard to body weight would have been even more strongly supported.

After adjustment for litter size, tail length was found to increase from the control to the low dose group, but to decrease to about the level of control in the high dose group; this is the same pattern that was found with respect to body weight at weaning in Experiments IV & V. Returning to tail length in Experiment V, there are highly significant differences among replicates and, as expected, highly significant differences between sexes. However, none of the interactions between replicate, dose and sex was significant.

From the existing evidence, this trait would appear to be most useful in the detection of mutagenic damage in F_1 progeny of males in which germ cells were mutagenized as spermatozoa. In combination with the body weight at weaning trait, reliability of the test would be increased. Nevertheless, substantial numbers (300 to 500) of F_1 young would be required to demonstrate effects with confidence.

Hematocrit at Seven Weeks of Age. The "element of similarity" in respect to hematocrit in Experiments III & IV referred to above are also present in Experiment V. That is to say, there is a reduction in hematocrit from control to low dose group followed by an increase of the same magnitude above the control in the high dose group (Table 12). Thus, the difference between low and high dose group means is about twice as great as the difference between the control and either dose group mean. While the differences among groups are highly significant ($p < .01$), the level of significance is due largely to the magnitude of the difference between treated group means. This trait would be of usefulness in detecting mutagenic damage, particularly in combination with the tail length and body weight traits. In addition to significant differences among replicates and between sexes, there were found highly significant replicate by sex ($p < .002$) and dose x sex ($p < .005$) interactions. Table 12 shows the dose group means after adjustment for litter size for the three experiments considered.

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Table 12. Effect of TEM dose on hematocrit at seven weeks of age of F_1 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III, IV and V. Males were treated at the spermatozoan germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined for simplicity.

Experiment	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	$p \sim .11$ (n.s.)	48.88 (226)	48.85 (133)	49.28 (72)
IV	n.s.	48.56 (129)	48.44 (96)	48.70 (104)
V	$p < .01$	47.95 (314)	47.74 (482)	48.13 (483)

Defecation Portion of the Open Field Test. This trait would appear for practical purposes to be of negligible value in the detection of mutagenic effects in F_1 generation progeny of males where mutagenic treatment is directed at spermatozoa. Although all three experiments exhibited differences among means that approached significance ($.1 < p < .2$) the patterns emerging are in striking contrast with one another. As indicated

above, Experiments III & IV were quite different from one another. The results of Experiment V add to the diversity by showing an increase from control to low dose rate like that in Experiment IV followed by a decrease from low to high dose rate comparable to that in Experiment III. It seems reasonable to suppose that some of the differences between means may well be real, but the factors producing the differences need not be those being tested.

Brain Weight at Fifteen to Nineteen Weeks of Age. As in the case of the previous trait, brain weight would not appear to be of practical value in the determination of mutagenic damage involving F_1 progeny of treated males where germ cells are mutagenized at the spermatozoan stage. As in the case of Experiments III & IV no significant differences were found among dose group means. While the patterns of dose group means was parallel in Experiments III & IV, with brain weights of treated groups generally falling below control means, this is reversed in Experiment V where brain weights of treated groups are greater than those of controls.

Results: Quantitative Traits, Experiment V. II. Spermatozoan Treatment, F_2 Progeny. Results obtained in the F_2 generation of spermatozoan treated germ cells are, from a purely practical viewpoint, even less useful than those in the F_1 generation where there were at least a few very exciting traits. There are some very interesting evidences of heritability implicit in some of the results to be discussed below, but the likelihood that this would be a good stage to employ in testing for mutagenicity is not impressive.

Time of Development of the Righting Response. As was found in Experiments III, IV and V in the F_1 generation and in Experiment III in F_2 generation results there were no significant differences among groups. It is worth pointing out a parallel between the results in Experiment III of F_1 and F_2 generation trends. In both, there was observed a more or less linear increase in the time of development of the righting response with increasing dose. Differences among means did not approach significance in the F_1 generation results but did so in the F_2 generation results ($p \sim .10$). It is also of interest that in the spermatogonial F_1 generation results in Experiment III, a similar parallelism exists. This will be discussed below. It should be borne in mind that male parents of the spermatozoan F_1 progeny were also parents of F_1 spermatogonial progeny. However, in one case it would have been the spermatozoan stage cells affected, but in the other spermatogonial cells would have been involved. The similarity of these test results carried out at quite different times is quite provocative.

Body Weight at Weaning. Differences among dose groups in Experiment V were not significant ($p \sim .22$) but they are quite interesting in that they parallel very closely the results of the F_2 generation spermatozoan test group in Experiment III where differences among means were significant ($p \sim .05$). In both groups there is an increase in body weight from control to low dose groups after adjustment for the effects of litter size by covariance. At the higher dose, the results of Experiments III & V are not closely parallel in that there is no difference between the low and

high dose group means in Experiment V, but there is a significant drop from low dose to high dose group mean in Experiment III. The inconsistency of response in high dose means will be seen again in our consideration of spermatogonial groups.

It may be recalled that in the F_1 spermatozoan test groups in Experiment III there was found a non-significant linear decrease in body weight means from control through the low dose to the high dose group that is at variance with other observations with respect to the effect of mutagenic dose on body weight at weaning. It is worth noting that this effect did not appear to be heritable, and it is of even greater interest that before adjustment for litter size there were highly significant increases in body weight in both sexes of both replicates from control to low dose group and to high dose group. Why the adjustments for litter size resulted in such a remarkable difference primarily in the control and high dose group means is difficult to guess, but these data will be examined in greater detail at a later date.

From a practical viewpoint, body weight at weaning in the F_2 generation of the spermatozoan treatment group would not be a good trait to employ in testing for mutagenic effect, but it is about as good as any.

Tail Length at Seven Weeks of Age. In Experiment V, differences among groups were not significant, nor were they significant in the comparable F_2 generation of the spermatozoan treatment group in Experiment III. The only parallel in the results of the two different experiments is that there was a decrease in mean tail length from control to high dose group mean in both experiments, and the decrease is of approximately the same magnitude in each experiment. In the low dose groups, the mean is slightly above control in Experiment III, appreciably below the control mean in Experiment V. It may be recalled that in the F_1 generation spermatozoan treatment groups in all three experiments (III, IV & V) there was an impressive increase from control to low dose group mean followed by a similarly impressive decrease to high dose group mean. However, differences among tail length means were not significant except in the case of Experiment V ($p < .002$). Thus, it might be said that the pattern in the F_2 generation spermatozoan treatment in Experiment III is reminiscent of the F_1 generation spermatozoan test group results except that the increase from control to low dose group mean is very slight, while the subsequent decrease is several times as great. In Experiment V, it is of interest that the usually observed strong correlation between body weight and tail length is not evident. As with the body weight trait, it may be said that tail length is not a good trait to test for mutagenicity in the spermatozoan treatment stage in the F_2 generation.

Hematocrit at Seven Weeks of Age. This trait is probably the most interesting in this particular germ cell stage test group because of the parallels that exist with the same germ cell stage in Experiment III and with Experiments III, IV & V in the F_1 generation spermatozoan treatment groups.

In Experiment V differences among groups approach significance ($p \sim .055$). The most important element in common in all five of the sets of results cited above is a drop from control to low dose group. In all experiments except V, there is a face value increase from low dose group to high dose group mean, not significant in Experiment III, F_2 spermatozoan test group, but approaching significance in Experiment III, F_1 spermatozoan test group ($p \sim .11$). In the F_1 generation spermatozoan test group in Experiment IV the increase was not significant, but it was highly significant in Experiment V ($p < .01$).

While these evidences go a long way toward establishing the reality of the differences dealt with, the numbers required to demonstrate a significant effect would not lead one to conclude that this is a useful trait for the ascertainment of mutagenic effect in an F_2 generation spermatozoan treated test group.

Defecation Portion of the Open Field Test. Differences among groups in Experiment V are not significant. From an academic viewpoint it is of interest that there is a face value increase from control to low dose group followed by a decrease from low to high dose group mean as was found in the F_1 generation spermatozoan test group in Experiment V. In the F_2 generation spermatozoan test group, Experiments III & V have in common only an increase from control to low dose group means. In general, it may be said that none of the spermatozoan groups tested for this test in either experiment has revealed significant differences among dose group means.

Brain Weight at Fifteen to Nineteen Weeks of Age. In Experiment V differences among means are not significant, and the distribution of means in Experiments III & V are in remarkable contrast to one another. In Experiment III differences among means proved to be highly significant based upon increases in both treated groups over the control mean (see Table 5). In the Experiment V results, there is an interesting parallel between F_2 spermatozoan and F_1 spermatozoan treatment groups in that there is a decrease from control values to both low and high dose group means, but in neither case were there significant differences among means.

It is clear that brain weight would not be a useful trait for the ascertainment of mutagenic damage in F_2 generation spermatozoan treated groups.

In summary, if it is desirable to ascertain with confidence the heritability of mutagenic effects by testing an F_2 generation, spermatozoan treatment would not be recommended employing BALB/cByJ as an experimental strain.

Results: Quantitative Traits, Experiment V. III. Spermatogonial Treatment, F_1 Progeny. In Experiment V, in spermatogonial treatment groups, significant differences due to dose were found with respect to every trait examined with the exception of brain weight. As indicated above in Experiment IV, there was no comparable treatment group. However, there were reported above results from a comparable group in Experiment III, and

comparisons will be made in the discussion below with respect to specific traits. It may be recalled that significant differences among dose groups were found in all except two traits in Experiment III. These were time of development of the righting response and hematocrit. With respect to three traits, significant differences among dose groups were found in both Experiments III & V, and the dose mean differences that are significant in both experiments are parallel. It is of particular interest that these parallel differences were found with respect to the control and low dose treatment group means. These three traits are: body weight at weaning, tail length at seven weeks and defecation portion of the open field test.

Time of Development of the Righting Response. In Experiment III with sample sizes exceeding 1200, no significant differences were observed among dose groups as reported above. In contrast, in Experiment V with less impressive sample sizes totaling some 750 young, highly significant differences were found among dose groups ($p < .01$) after adjustment for litter size by covariance. There was observed a linear decrease in the time of development (increase in rate) of the righting response with increase in mutagen dose. There were also highly significant differences ($p < .001$) among the three replicates and a significant group x dose interaction ($p < .05$). In Experiment III there were also highly significant differences ($p < .001$) between replicate means but no significant interactions or other complications.

It can only be concluded that factors other than those being tested may have major effect upon the time of development of the righting response. In general, it may be said that all of the three replicates in Experiment V exhibited greater mean righting response times than those observed in Experiment III. Of course, changes in food or water quality, or subtle environmental changes of which we are not aware could be responsible for the general differences between times of development of the righting response or the differences in response to mutagenic agent observed in the two experiments. It seems evident that until such factors can be identified and controlled, this trait will not be particularly reliable for the detection of mutagenic effects in F_1 generation young of males treated at the spermatogonial germ cell stage. An equally evident conclusion is that the time of development of the righting response may be affected by mutagenic treatment. Interestingly, the highly significant decrease in time of development of the righting response with increasing mutagen dose parallels closely the results of the F_1 generation spermatozoan test groups ($p \sim .12$), but not those of the F_2 spermatozoan groups.

Body Weight at Weaning. In Experiment III it may be recalled that highly significant differences among dose group means ($p < .001$) were found in this treatment stage after adjustment for litter size by covariance. These differences were based upon a roughly linear increase in body size with increasing mutagen dose. In Experiment V significant differences were also found among dose group means ($p < .025$) after adjustment for litter size (Table 13). This statistical significance is due primarily to an increase in mean from control to low dose group that parallels very closely the difference between control and low dose group in Experiment III.

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Table 13. Effect of TEM dose on body weight at weaning of F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III and V. Males were treated at the spermatogonial germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance. Sexes and replicates are combined for simplicity.

Experiment	Generation	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	F_1	$p < .001$	16.49 (376)	16.87 (458)	17.06 (419)
V	F_1	$p \sim .025$	15.53 (221)	16.03 (287)	15.81 (258)
III	F_2	$p < .01$	16.84 (462)	17.17 (466)	17.11 (509)
V	F_2	$p < .05$	15.78 (515)	15.86 (617)	15.56 (581)

In contrast to the situation in Experiment III, there is a decrease in body weight between the low and high dose group means which, however, is not significant. Similarly the difference between low and high dose means in Experiment III is not significant. Thus, the general conclusion that may be drawn from these two experiments, III and V, is that body weight at weaning, adjusted for litter size by covariance, increases with mutagenic treatment. However, the effect of doubling the mutagenic dose while not entirely clear, appears to be other than linear.

It seems likely that this trait, particularly in combination with that to be discussed next, tail length at seven weeks of age, could be quite useful in providing a very sensitive method for the detection of mutagenic damage in spermatogonia. This is particularly important when it is considered that very few mammalian tests are effective in the detection of spermatogonial damage. Since it is in spermatogonial cells that mutagenic damage may accumulate, the usefulness of this assay could be very great. Cytogenic techniques may also be employed to detect mutagenic damage in spermatogonial cells. Whether this assay is comparable in sensitivity to such techniques can only be determined by direct comparison, but if the effects we have measured are based upon polygenic variation as seems most probable, sensitivity of this assay could prove to be very great indeed.

Tail Length at Seven Weeks of Age. The parallel between the results of Experiments III & V are impressive. After adjustment for the effects of litter size by covariance, differences among means based upon factorial ANOVA were highly significant ($p < .005$) in Experiment III. In Experiment V, differences among means after comparable analysis were also highly significant, ($p < .001$). In both experiments there is a parallel increase in magnitude from control to low dose group mean followed by a decrease of considerably lesser magnitude in high dose group means (Table 14). The similarity in the distribution of tail length means and the means for body weight is quite clear, and this has been observed on a number of occasions in other experiments involving the same and other inbred strains. In this particular case, tail length seems to have provided a more reliable measure of mutagenic effect than body weight at weaning. However, the correlated results of both traits provide an indication of even greater reliability.

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Table 14. Effect of TEM dose on tail length at seven weeks of age of F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III and V. Males were treated at the spermatogonial germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined for simplicity.

Experiment	Generation	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	F_1	$p < .001$	81.88 (366)	82.57 (441)	82.51 (406)
V	F_1	$p < .005$	79.64 (217)	80.47 (262)	79.93 (238)
III	F_2	$p < .05$	79.60 (436)	79.96 (449)	78.89 (486)
V	F_2	n.s.	81.72 (500)	81.85 (583)	81.75 (535)

In both Experiments III and V, differences between the sexes with respect to tail length are, as usual, highly significant ($p < .001$) and differences between replicates (Experiment III) and among replicates (Experiment V) are significant. In both experiments there is an interesting indication of a dose x sex interaction, significant in Experiment III ($p \sim .02$), but only approaching significance in Experiment V ($p < .10$). As with body weight at weaning the effect of doubling the mutagenic dose is not to produce more of the effect of a single dose. It appears that tail length, adjusted for litter size by covariance, increases with mutagenic treatment. However, the effect of increasing mutagenic doses is other than linear and may actually result in a reversal of direction of effect. This has been observed many times with a number of traits, and it seems quite likely that it is real.

Hematocrit at Seven Weeks of Age. The similarities that exist between Experiments III & V with respect to hematocrit are not based upon differences that are significant. However, the observed increase in hematocrit from control to low dose group mean may very well be real. In Experiment III, differences among groups only approached significance ($p < .10$), but the greatest difference was that between the two dose group means. In Experiment V, there are highly significant differences among groups ($p < .001$), but both low and high dose group means are greater than the control group mean, and there appears to be a roughly linear increase in hematocrit with increasing dose (Table 15).

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Table 15. Effect of TEM dose on hematocrit at seven weeks of age of F_1 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III and V. Males were treated at the spermatogonial germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined for simplicity.

Experiment	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	$p < .10$ (n.s.)	48.73 (365)	48.86 (437)	48.63 (410)
V	$p < .001$	48.08 (224)	48.32 (285)	49.04 (256)

In view of the limited response, if real, of hematocrit to low mutagen doses and the variable response to high dose treatment in this germ cell stage, hematocrit would not seem to be a very likely prospect for the efficient ascertainment of mutagenic damage.

Defecation Portion of the Open Field Test. Next to the tail length and body weight traits, defecation rate exhibited the most interesting parallels of any in the spermatogonial F_1 generation results (Table 16). In both experiments (III and V) differences among means were significant ($.01 < p < .05$). Although there did not appear to be significant variation due to litter size, the factorial ANOVA was carried out after adjustment for whatever litter size effects existed. In both experiments there were highly significant differences between sexes. In Experiment III there was no significant difference between replicates, but in Experiment V differences among replicates were highly significant. In neither experiment were any interactions significant.

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Table 16. Effect of TEM dose on defecation rate at five weeks of age in F_1 and F_2 progeny of treated male parents in inbred strain BALB/cByJ in Experiments III and V. Males were treated at the spermatogonial germ cell stage. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined.

Experiment	Generation	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	F_1	$p < .05$	4.03 (376)	4.36 (458)	4.38 (417)
V	F_1	$p \sim .01$	4.10 (229)	4.38 (299)	3.84 (258)
III	F_2	n.s.	4.70 (462)	4.94 (466)	4.79 (509)
V	F_2	n.s.	3.74 (521)	3.83 (619)	3.74 (581)

As with the body weight and tail length traits, there are important parallels between the control and low dose group means. Also as with the body weight and tail length traits, the low dose and high dose group relationships are dissimilar. It is of interest that there is a highly significant drop from low dose group to high dose group in Experiment V which parallels the face value change of the body weight and tail length traits in the same experiment. In contrast, in Experiment III, the difference between low and high dose group means is not significant, and varies in the three traits from slight increases in fecal deposition rate and body weight to a slight decrease in tail length. In view of the close parallel of defecation rate, body weight and tail length traits, it is reasonable to suggest that all three traits employed together would provide an element of reliability of results that could be most useful.

Brain Weight at Fifteen to Nineteen Weeks of Age. This trait, which appeared to have interesting potential in work with other strains and in the same germ cell stage treatment group with BALB/cByJ in Experiment III, failed to show significant differences among means in Experiment V (Table 17). In contrast with the highly significant increase from control to

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Table 17. Effect of TEM dose on brain weight at approximately fifteen (males) to nineteen (females) weeks of age in F_1 progeny of male parents in inbred strain BALB/cByJ treated at the spermatogonial germ cell stage. Results of Experiments III and V are included. Sample sizes are given in parentheses. Means have been adjusted for the effects of litter size by covariance, and sexes and replicates are combined.

Experiment	Generation	Significance	0 mg/kg	0.1 mg/kg	0.2 mg/kg
III	F_1	$p < .01$	473.04 (350)	476.14 (420)	474.85 (389)
V	F_1	n.s.	457.05 (210)	455.66 (288)	455.68 (251)
III	F_2	$p < .001$	464.32 (459)	465.88 (464)	468.29 (506)
V	F_2	$p < .001$	442.22 (506)	444.87 (607)	447.69 (551)

low dose group mean in Experiment III, there was a face value decrease from control to low dose group mean in Experiment V. From these data it is clear that a consistent effect cannot be expected, and brain weight would not be a trait of choice employing strain BALB/cByJ in an F_1 generation test of spermatogonial damage.

Results: Quantitative Traits, Experiment V. IV. Spermatogonial Treatment, F_2 Progeny. In general, in spermatogonial test groups, the results of tests involving F_2 progeny are almost as useful and quite as exciting as those involving F_1 progeny. Tests involving spermatozoan test groups, either F_1 or F_2 generation, have been far less interesting. In these latter groups, except for three traits (body weight at weaning, tail length at seven weeks and hematocrit at seven weeks, and these only in the F_1 spermatozoan test groups), there have really been few traits that would appear to be of potential usefulness in the practical ascertainment of mutagenic damage. However, in striking contrast, most traits exhibited significant differences among dose groups in one or the other experiment in F_1 spermatogonial test groups, and three, body weight at weaning, tail length at seven weeks and defecation rate showed parallel effects in pattern in Experiments III and V, primarily with respect to differences between control and low mutagen dose groups. With respect to F_2 generation spermatogonial tests Experiment III results were discussed above. In experiment V, three traits stand out in exhibiting, as in Experiment III, significant differences among means and in having parallels with the mean distributions of Experiment III. These are, perhaps curiously, body weight at weaning, hematocrit at seven weeks and brain weight at fifteen to nineteen weeks of age. But, perhaps equally important, the defecation rate and tail length traits, which showed such impressive effects of mutagenic treatment in F_1 spermatogonial test groups, offer strong support for those conclusions by demonstrating very similar mean distribution patterns in F_2 results. The lack of significant differences seems largely a result of decreased magnitude of the differences between means. It will be attempted in this section to tie together meaningful comparisons not only of Experiments III and V, but also meaningful comparisons of F_1 and F_2 spermatogonial results.

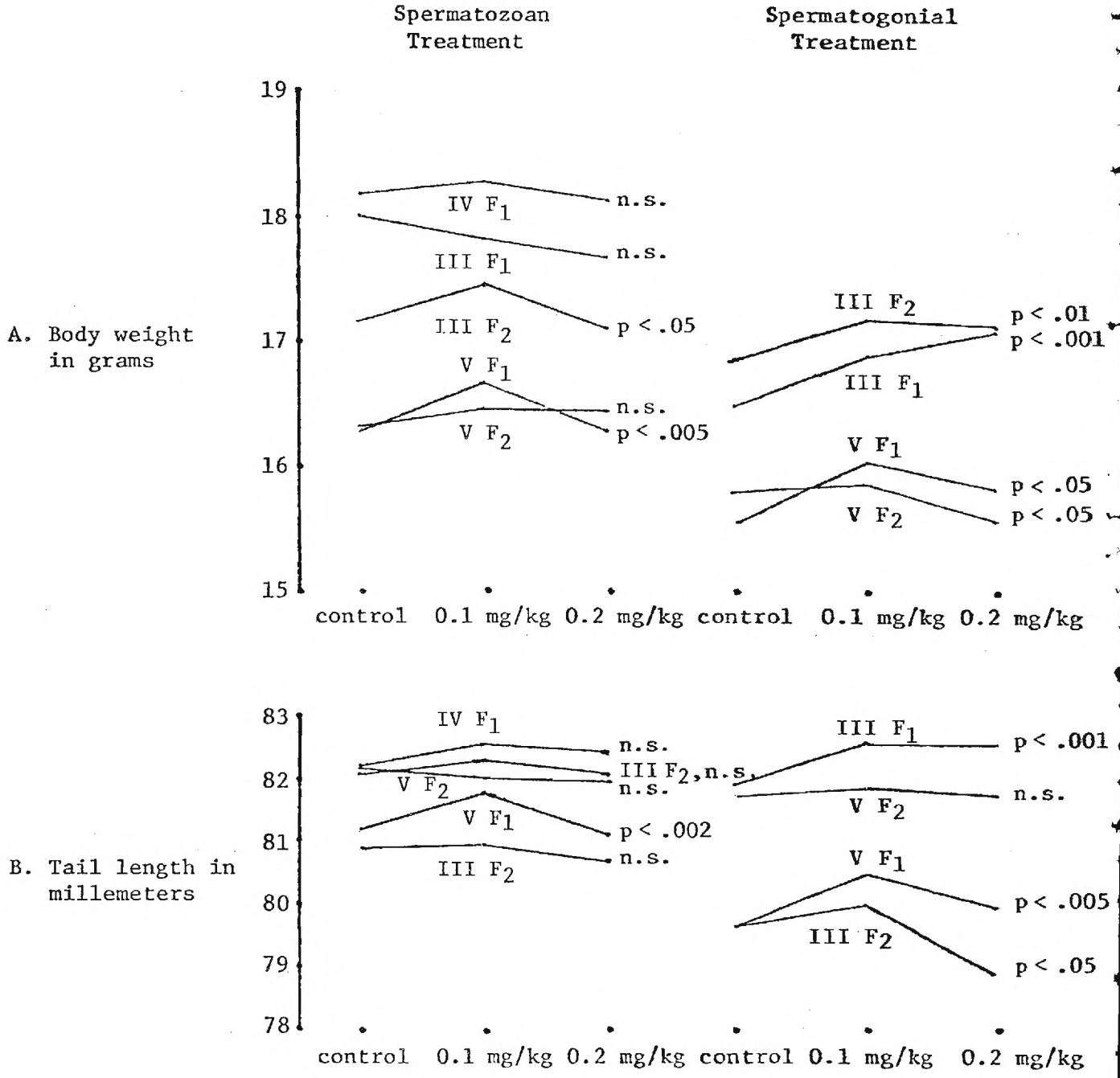
Time of Development of the Righting Response. In Experiment V, there were not found significant differences among means and, in fact, such differences were of lesser magnitude than in any other germ cell stage/generation group tested. It may be recalled that there were highly significant differences among means in the F_1 generation spermatogonial tests based upon a roughly linear decrease in time of the development (increase in developmental rate) of the righting response with increasing mutagen dose. There seems to be little doubt as to the reality of the differences found in that experiment, but either the responsible genetic variance was lost, or the appropriate environment to foster the development of the differences observed did not exist, when the F_2 generation experiments were conducted, or both. Further, F_1 spermatogonial test results from Experiment III, reported above, did not support the F_1 spermatogonial results of Experiment V, and the F_2 generation results in Experiment III only support those of Experiment V in exhibiting very modest differences among means and no particular pattern of response.

The righting response trait, at least insofar as strain BALB/cByJ is concerned, is not a very useful trait to employ in the ascertainment of mutagenic damage.

Body Weight at Weaning. In Experiment V differences among means were found to be significant as they were in Experiment III in spermatogonial, F_2 generation tests (Table 13). It will be recalled that there were also significant differences among means in both Experiments III & V in the F_1 generation spermatogonial test groups. However, the primary importance of the F_2 spermatogonial generation results does not lie in their usefulness for the ascertainment of genetic damage, but in the evidence they provide for heritability of effect. The F_1 generation spermatogonial stage tests would serve far better for detection of mutagenic effects. The mean distribution patterns seen in the F_2 tests are nicely derivable genetically from those found in the F_1 tests. There are increases in the F_2 generation tests from control to low dose group in both experiments. This difference is not significant in Experiment V, although it is in Experiment III. In the F_1 generation spermatogonial tests, the increase from control to low dose group was impressive and significant in both experiments. In Experiment V, F_1 generation results, there was evident an appreciable drop from low to high dose group, and this same trend is present in the F_2 spermatogonial results and the difference is significant. One interesting difference: the high dose group in Experiment V F_1 was appreciably above control, while in the F_2 results the same group is appreciably below control. However, there is a decrease from low dose mean to high dose mean in both F_1 and F_2 generation results. The relationship between the high dose means of Experiment III & V remains approximately the same in the F_1 and F_2 generation results (see Figure 1A).

These patterns just described have close similarities to mean distribution patterns for spermatozoan results in both the F_1 and F_2 generation with the notable exception that, in Experiment III of the F_1 generation, there was an apparent linear decrease in body weight with increasing mutagen dose. However, differences among means in this experiment were not significant.

Figure 1. Effect of triethylenemelamine dose on body weight at weaning and tail length at seven weeks in F_1 and F_2 generation progeny of treated males of inbred strain BALB/cByJ in Experiments III, IV and V. Effects of treatment on spermatozoa and spermatogonia are shown. Sexes and replicates are combined for simplicity. Probability figures shown are taken from factorial ANOVA results for each experiment and refer to significance of differences among means within an experiment.



Tail Length at Seven Weeks of Age. Differences among means due to dose in Experiment V, F_2 generation spermatogonial results were not significant, but the pattern of distribution of means is very similar to what it was in the F_1 generation spermatogonial results as well as to the results of Experiment III in both F_1 and F_2 generation spermatogonial tests. This pattern involves an increase from control to low dose group followed by a less pronounced decrease to the high dose group. In all spermatogonial tests except this, significant differences among tail length means were recorded. These results are important in that they provide evidence of heritability with the magnitude of the differences among means reduced in F_2 generation results. In the F_2 spermatogonial results of Experiment III, the mean distribution pattern is similar to that in Experiment V, and the magnitude of the differences among means are reduced, but the increase from control to low dose group is significant (Table 14).

Only one of the spermatozoan tests, that for the F_1 generation in Experiment V, resulted in significant differences among means, and these were impressively so. In this test, there was also found the generally observed increase from control to low dose group mean followed by a decrease to the high dose group mean. The distribution of means for all tests, spermatogonial and spermatozoan, are graphed for ease of visualization in Figure 1B.

Hematocrit at Seven Weeks of Age. In Experiment V, differences among dose group means were significant, with the significance itself due primarily to an increase from control to low dose group in the F_2 spermatogonial results. In F_1 generation spermatogonial results of the same experiment there were highly significant differences among means and an increase from control to low dose group comparable in magnitude to that in the F_2 results.

With respect to the response in the high dose group, results are quite dissimilar in the F_1 and F_2 generations. In the former, there was a highly significant increase from low to high dose group; in the F_2 generation results there was a decrease. As has been pointed out previously, high dose group results do appear to be erratic and unpredictable.

Elements of concordance between the results of Experiment III and V are present, but not very useful. In F_1 generation results of Experiment III there was found the same increase from control to low dose group that was found in Experiment V. However, unlike the results of Experiment V, there was a non-significant decrease from low dose to high dose group means. In F_2 generation results, by contrast, Experiments III and V are alike in exhibiting decreases from low to high dose group means. However, in Experiment III there was recorded a slight decrease from control to low dose group mean as opposed to the significant increase found in Experiment V.

Everything considered, while there may be elements of similarity based upon heritability, hematocrit, in strain BALB/cByJ, would not appear to be useful either in providing persuasive evidence of heritability of mutagen induced variance or, for practical purposes, in the ascertainment of genetic damage. Even so, the impressive levels of significance involved in certain differences do indicate that effects of mutagenic treatment exist, but they appear to be unpredictable.

Defecation Portion of the Open Field Test. The results of Experiment V in the F_2 generation of the spermatogonial test are quite comparable to those of Experiment III not only in that there are no significant differences between means, but also in the close parallel in distribution of means. In both experiments there is an increase from control to low dose mean followed by a decrease from low to high dose mean. As has been noted with respect to other traits, this is the same pattern found in F_1 generation spermatogonial results (with the exception of one data point) except that the differences between F_2 generation means are much reduced. Inasmuch as the differences among means in the F_1 results were significant in both experiments III and V, it is reasonable to suppose that the effects are heritable but with reduced degree of expression in the F_2 generation.

For purely practical purposes the defecation rate trait would not appear to be of usefulness in an F_2 generation spermatogonial test to ascertain mutagenic damage.

Brain Weight at Fifteen to Nineteen Weeks of Age. Results obtained with this trait are clearly the most useful of those obtained in the F_2 generation of the spermatogonial tests. As with the results of Experiment III, there are highly significant differences among means ($p < .001$), and the results of Experiment V parallel precisely those of Experiment III in that there is a linear increase from control to low dose group continuing on to high dose group. The magnitude of the increases in both experiments are quite comparable (Table 17).

It is concluded that brain weight can provide a very sensitive measure of mutations induced at multiple loci by a mutagenic agent in the F_2 generation progeny of treated males. It should be pointed out that results with F_1 young may have been distorted to an appreciable extent by the necessity of permitting females to rear their young to weaning before taking the brain weight of the females themselves. This introduced a variable that may be partioned out by a more detailed analysis. Most importantly, however, this factor does not intrude into the measurement of brain weight of the F_2 progeny because there was no intention to produce an F_3 generation and no need to distribute brain weight measurements over an excessively broad span of ages.

Discussion. The purpose of these experiments has to determine the usefulness of the cumulative effects of mutations induced by a powerful mutagenic agent, triethylenemelamine, in bringing about changes in means of a series of traits of a continuously varying nature and, therefore, likely determined by genes at multiple loci.

Dominant lethal effects have been studied in the F_1 generation of every experiment conducted primarily to assure that the mutagenic agent employed had been effective, and also to provide a standard by which any polygenic effects observed might be compared. Suffice it to say that in every experiment conducted, highly significant dominant lethal effects were observed, thus indicating that potent mutagenic agent had been successfully administered. These experiments have led to the confirmation of

a hitherto undescribed late dominant lethal effect, the results of which have been published (Favor, Soares and Crenshaw, Mutation Research, 54 (1978) 333-342). This dominant lethal effect was found to occur at about the time of parturition and to vary directly with increase in mutagen dose.

In the discussion of specific effects below, we have concentrated upon a single mouse inbred strain, BALB/cByJ, primarily because the last three experiments were conducted with this strain because of its very high productivity. Some of the other strains studied probably exhibited much greater mutagenic effects, but the poor productivity of the strains could make it considerably more expensive to conduct experiments of the size required to demonstrate mutagenic effect. In fact, it is not known that the economics of the situation would favor use of the BALB strain employed, but indications from preliminary studies made it seem most likely that this would be the case. Nevertheless, the other strains studied, Strain C3H/HeJ and DBA/2J, showed clear indications of response, some similar, some different, to the same mutation dose and employing the same traits.

One generalization may be stated that may be of some usefulness with respect to sample sizes required to demonstrate statistical significance in most groups in these studies. In general, in order to provide a base sufficient for useful statistical analysis of the data developed for the traits recommended below, on the order of 400 to 500 young will be required for each control and experimental group in an experiment. These numbers may be produced by combining two or three replicates, but the replicates should be carried out in reasonably close temporal succession.

Although it has not been specifically stated in the discussion of every trait in the report above, the statistical analyses carried out are always factorial analyses of variance (dose x replicate x sex). In every case, dose/group means have been adjusted by covariance for the effects of litter size, even if the effects of litter size could not be shown to be significant.

In view of the goal of these experiments, it has been far more important to demonstrate repeatable effects than to demonstrate rectilinear relationship between increasing mutagenic dose and degree of effect. An interesting fact that has emerged, that appears to be repeatable and that appears from a number of evidences to be heritable, is variation in the direction of response with the two different doses employed. While rectilinear response was observed in some traits, quite often traits were found to increase above control at the low dose level and to decrease back toward or beyond control level at the high dose. The opposite was also observed for other traits, that is to say decreased response occurred at the low dose level relative to control, followed by a return toward or even above control level at the high dose. It does not strain the imagination to develop hypotheses to account for this sort of response. For the body weight trait, for example, it is reasonable to suppose that a heterotic effect may obtain at low mutagen dose, or simply that allelic mutants for increased body size tend to be dominant over those for decreased body size. Either mechanism could account for increases in body size at low mutagen doses. However, it is clear that if enough mutations are induced, as by a doubled mutagenic

dose, the net effect on the development of the organism may be sufficiently detrimental as to lead to a general reduction in body size or failure to develop the same body size at the time weights are taken.

* * *

Turning to a specific consideration of the traits of importance in each generation of the two germ cell stages tested, we will consider first F_1 generation responses in those tests in which germ cells were treated as spermatozoa. It may be recalled that only three traits proved to be potentially useful in reflecting effects of the mutagenic agent. However, the economic significance of this stage is very great inasmuch as experiments can be run in a relatively short time, about six months. (F_1 spermatogonial tests would require an additional 2 months and F_2 spermatozoan tests require approximately a year.)

In Experiment V, with over 300 young involved in the control group and between 450 and 500 in each of the experimental groups, there were highly significant differences among dose groups with respect to both body weight and tail length. In both traits there was an increase from control to low dose group followed by a decrease to high dose group. With respect to both traits the low dose mean was highly significantly greater than either the control or the high dose mean. Accordingly, it is suggested that these two traits, in combination, would be quite useful in the detection of the effects of agents of relatively low mutagenicity. With respect to the same germ cell/generation test in Experiments III and IV, differences among means were not significant, in part because of relatively low numbers. (Both experiments combined included less than 1300 mice.) However, the distribution of means in both experiments follows the same pattern as that in Experiment V with respect to tail length, and the pattern of means in Experiment IV paralleled that in Experiment V with respect to body weight.

Hematocrit also proved to be useful in Experiment V as an indicator of mutagenic damage. In this case, there was a modest reduction (non-significant) from control to low dose treatment group followed by an appreciable increase (highly significant) between low and high dose group means. Although differences among means were not significant for the comparable germ cell stage/generation tests in Experiments III and IV, the pattern of distribution of means offers precise support for the results of Experiment V with its much larger numbers. It is our interpretation, that hematocrit might be particularly useful in the ascertainment of the effects of agents of relatively high mutagenicity. In every case the reduction from control to low dose was relatively modest, and the subsequent increase to high level dose mean somewhat greater.

We conclude that the traits body weight at weaning, tail length at seven weeks and hematocrit at seven weeks may prove to be quite useful in the detection of mutagenic effects over a broad range of dosages, employing the strain BALB/cByJ in adequate numbers, in an F_1 generation test involving mutagenized spermatozoa.

* * *

Results of the analysis of data on F_2 generation young in the spermatozoan treated series indicate that this is not a particularly useful stage for in the detection of mutagenic effects.

With respect to body weight, evidence of heritability of the increase from control to low dose group followed by a decrease to high dose group was found in Experiment III, and the differences among means were significant ($p < .05$), in spite of relatively modest numbers. (There were approximately 650 young in the control group, 270 in the low dose and 100 in the high dose group.) That numbers are not the problem, however, is indicated by the results of Experiment V in which about 750 young were tabulated each for control and high dose group and over 1000 for the low dose group. In this experiment, heritability of the F_1 generation effect of increase from control to low dose group was indicated, but there was no subsequent decrease to the high dose group as in the F_1 generation. None of these differences however were significant in Experiment V.

With respect to the hematocrit trait, heritability of effect was suggested by the results of both Experiments III and V in that there was a drop from control to low dose group paralleling that of the F_1 generation results. In Experiment V, with the very large numbers involved, this difference approached significance ($p \sim .055$). Also paralleling the F_1 results, there was an increase from low dose group to high dose group in Experiment III (not significant) but not in Experiment V.

In spite of the often inconclusive results of analyses of our data on brain weight, the impressive results to be discussed below with respect to spermatogonial effects suggests that prospects for this trait may be explored a bit further before being discarded as a trait suitable for spermatozoan testing. In the first place, there has been a problem of variation in time of acquisition of brain weight due, in females, to the necessity of permitting F_1 females to rear their young to weaning prior to taking brain weights. In males also, in F_1 generation results, brain weights have been postponed for some males while tests for evidence of translocations were carried out. More detailed analyses of our data will be carried out with appropriate partitioning of these variables as time permits. In our F_2 generation spermatozoan germ cell stage test, in Experiment III, there was a highly significant increase in brain weight from control to low dose group, paralleling a significant increase in body weight in the same experiment (there was also a non-significant decrease from low to high dose group, also paralleling a decrease in body weight). However, this mean distribution pattern received no support from the results of Experiment V in which very large numbers were involved. It is worth noting, however, that the F_2 generation spermatozoan results in Experiment V with respect to brain weight showed interesting similarities, suggesting heritability, to the F_1 generation spermatozoan results. Clearly, at this stage of development, brain weight would not appear to be a useful trait for the detection of mutagenic damage in spermatozoan test groups.

* * *

The results of both of the spermatogonial generation tests are exciting, and show considerable promise for use in the detection of mutagenic activity. On the one hand, it is of particular interest that mutations induced in spermatogonial cells are relatively easily detectable, because there are not many tests available in mammalian systems in which spermatogonial damage can be detected. The question as to why this might be so in our test when spermatozoan damage is not so easily detected is an interesting one. It is suggested that for most of the traits employed, it may be that mutations induced in spermatozoa may be so much more dramatic in their effect in both directions that erratic results eliminate the general trends which may be evident in spermatogonial damage, where far less dramatic mutations only may survive repair or elimination.

The F_1 generation spermatogonial germ cell stage results were far and away the most exciting and useful of any obtained. With respect to three traits, body weight at weaning, tail length at seven weeks and the defecation portion of the open field test, significant differences were found to exist among means in both Experiments III and V, and the dose mean differences that were significant in both experiments were parallel.

With respect to body weight at weaning, there were increases in body weight from control to low dose groups in both Experiments III and V, highly significant in the former, significant in the latter. In both experiments, differences between low and high dose groups are not significant. In Experiment III there was recorded a face value increase; in Experiment V, a decrease.

Closely paralleling these body weight patterns, there were highly significant increases in tail length in both Experiments III and V between the control and low dose groups. In both experiments there were decreases from low dose to high dose groups, but these differences were not significant. It is of particular interest that the numbers involved in this particular germ cell stage/generation test were relatively modest. In Experiment III, numbers ranged from about 370 in the control group to between 360 and 410 in the experimental test groups. In Experiment V, the numbers were even lower, ranging from about 220 in the control groups to between 240 to 260 in the experimental groups.

Employing the traits body weight at weaning and tail length at seven weeks in a spermatozoan and a spermatogonial, F_1 generation test, there exists in the approach under investigation here a powerful mechanism for the demonstration of mutagenic effects, particularly with relatively low potency mutagenic agents.

The defecation portion of the open field test also revealed significant increases from control to low dose group, paralleling the body weight and tail length traits, in both Experiments III and V. While the levels of significance in these experiments were not as impressive ($.01 < p < .05$) as in the body weight and tail length traits, incorporation of this trait would add an additional degree of confidence to an F_1 spermatogonial test.

The parallelism with the body weight and tail length traits extends to the relationship between low dose and high dose group means, but in an interestingly different way. In Experiment V with respect to all three traits, body weight, tail length and fecal rate, there was observed a decrease from low dose to high dose group. In Experiment III some similarity was found in that there is little difference between low and high dose group means for all three traits. In body weight there is a modest increase from low to high dose; in tail length there was a modest decrease from low to high, and in fecal rate there was very little difference.

* * *

As indicated above, the F_2 generation spermatogonial germ cell stage test was not nearly so impressive in the kinds of conclusions it provided as were the F_1 generation spermatogonial tests. This was true in spite of considerably greater numbers (450 to 600 in most test groups). However, some of the most interesting results with respect to heritability were developed in this group, and brain weight proved to be a remarkably exciting and useful trait in contrast to what it had been in other groups.

With respect to the brain weight trait, there proved to be a highly significant more or less linear increase in brain weight with increasing mutagen dose in both Experiments III and V, and the mean distribution patterns are quite similar in both experiments. It is probably not just coincidental that this was an F_2 generation test and one in which brain weights could be taken over a relatively short span of time in the life of the experimental animal. Everything considered, this germ cell stage/generation group would not be one of choice if the primary interest were the confident ascertainment of mutagenic damage. However, brain weight stands out as the best choice of traits if it were desirable, on other grounds, to test mutagenic effect in the F_2 generation of mutagen induced spermatogonial damage. It seems likely that spermatogonial mutagenesis generally results in fewer unrepaired mutations as well as mutations of a less dramatic and extensive nature than is the case in spermatozoan mutagenesis. It further seems likely that even this damage may be somewhat diluted by selection in transmission from the F_1 to the F_2 generation. Accordingly, it is concluded that very slight increases (with increases in mutagenic dose) in the frequency of mutations (themselves of very slight effect) results in an increase in brain weight in strain BALB/cByJ. It is reasonable to suppose that the reduction in brain weight size often observed (but not as a significant effect in our experiments) may be real and may result from an increase in the frequency of mutations generally or in the frequency of mutations having a generally deleterious effect upon brain development.

The hematocrit trait in F_2 spermatogonial results was of interest in that there were differences among means in both Experiments III and V (respectively $p < .005$ and $p < .05$). However, the parallel aspect of the mean distributions lies only in a reduction from low to high dose group. This difference is means is highly significant in Experiment III, but not in Experiment V. There was an impressive increase in Experiment V from control to low dose group but little difference between control and low dose group in Experiment III.

One other trait, body weight at weaning, revealed differences among means in both Experiments III and V. These were highly significant ($p < .01$) in Experiment III, based largely upon an increase from control to low dose group, and significant ($p < .05$) in Experiment V, with the significance based largely upon a decrease from low to high dose group mean. However, in both cases there is an increase from control to low dose group followed by a decrease from low to high dose group. The magnitude of the differences are such that this would not appear to be a highly reliable trait in the ascertainment of mutagenic damage in this germ cell/generation test group. Nevertheless, the pattern provides evidence for heritability of the increase from control to low dose group.

Also of interest in respect to heritability are the tail length and fecal rate traits, although significant differences among means are limited to the tail length trait in Experiment V. Of greater importance, the traits tail length and fecal rate in both Experiments III and V show patterns that parallel precisely the body weight trait in Experiments III and V, and all three reinforce a general heritability of the pattern found in the F_1 spermatogonial test results. More specifically, in both experiments, for all three traits, there is an increase from control to low dose group that is significant always in the F_1 spermatogonial test results but only half the time in the F_2 spermatogonial test results. The magnitude of the difference between control and low dose group means is uniformally lesser in the F_2 spermatogonial results than in F_1 spermatogonial results, as might be expected if the genetic variance were diluted as by natural selection in the passage from one to the next generation.

* * *

As a final note it is emphasized that we have concentrated in this report upon those traits in which highly significant differences among means were found in at least one test and where there was support of direction of change of means in other tests. For obvious reasons, consistency of change of direction as a mutagenic effect is important in the development of confidence in this approach. However, the pioneering nature of this effort is emphasized. When one is dealing with the numbers of organisms dealt with in this study, there is a high level of confidence that those differences between dose group means that are highly significant really do reflect real differences due at least in part to mutagenic effects induced. It is perfectly reasonable to suppose that these differences, based as they are upon different mutagenic events are real effects. Of course unnoticed environmental differences between replicates, even in our carefully controlled animal colony rooms, could lead to different effects based upon interaction of genotype and environment.

The nature of the statistical analyses employed (factorial analysis of variance considering the effects of variation in mutagenic dose, sex of organism and in temporally spaced replicates, in addition to a partitioning out of the effects of litter size by covariance) is a highly sophisticated tool. The use of traits that are known to vary easily with environmental effects as, for example, body weight, has been criticized. However, it is clear that the critics do not appreciate that the fundamental

nature of the analysis of variance technique is to compare variation within dose/sex/replicate group with that variation found to exist between groups. Our animals have been carefully coded so that the investigators and workers are not aware of the dose group to which a given individual belongs. Further, all dose groups are distributed randomly within the same colony rooms. They receive the same food, the same water and are handled by the same assistants. Clearly environmental factors may vary in time, and this is very likely a major source of the variation that has been recorded in this study between replicates. But environmental factors that are known to affect a given trait are absolutely the same, insofar as they can be controlled, for all animals within an experimental replicate. If our statistical analysis says the trait is a good one, it is a good one irrespective of philosophical opinion to the contrary. The proof of the pudding, in such case, clearly lies in the eating thereof.

We have emphasized the practical approach in these studies. It is felt that justification has been provided for the investigative employment and for further theoretical exploration of this general approach. These most promising tests would be based upon the traits outlined above in the F_1 generation of spermatogonial treatment and of spermatozoan treatment, with the greater reliability at this time resting with the former. At present, this approach appears to be more useful in the detection of very slight mutagenic damage than of great mutagenic damage, but further investigation, perhaps involving additional traits, may well reveal some that will be affected only by higher mutagenic doses. There were indications that some other inbred strains, for example C3H/HeJ, would bear further investigation. Although considerably less productive than the strain reported upon here, there were evidences that mutagenic events might be more strongly reflected for some traits in this strain than in the strain receiving primary attention.

Respectfully Submitted

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