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## Evidence of Prenatal Heterosis Relating to X-Ray Induced Congenital Anomalies.* (26292)

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Heterosis or hybrid vigor has been established for adult mice with respect to their tolerance of ionizing radiations (Rugh and Wolff(1). It has also been studied with respect to single organ (testis) reaction where it was found that the resistance of the gonad in the hybrid was more like a simple dominant relation than synergistic heterosis (Rugh, Funk and Wohlfromm(2). Since heterosis can be demonstrated at sexual maturity (2 months of age in the mouse) it seemed pertinent to determine whether it existed prior to birth, and at a time when ionizing radiations quite consistently caused resorptions and congenital anomalies. Thus, this study was designed to establish the resistance of the hybrid to irradiation effects caused by 200 r x-rays at the period of greatest neurogenesis, namely 8.5 days gestation.

*Materials and method.* The animals used were the CFl Swiss white stock from Carworth Farms, the C57 black/6 stock from Jackson Memorial Laboratory, Bar Harbor, Me., and their $F_1$ hybrids resulting from the cross between the CFl females and C57 males. This cross is easily made and results in highly viable offspring.

Matings were begun at 5 p.m. The males were removed at 9 a.m. and each exposed female was examined for presence of a vaginal plug. Those with such indications of mating were segregated and at 8.5 days the experimental animals were subjected to 200 r x-rays. The controls, of the same stage of pregnancy, were kept under identical conditions but were not irradiated. At 18.5 days gestation age each of the experimental and control females was killed by cervical dislocation, the uteri were immediately excised, and each fetus, or implantation site, was dissected out and examined. In this way, every successfully fertilized egg which reached the uterus and established an implantation site (at 4.5 days) could be included in the total counts. Each of some 2630 implantation sites in 302 pregnancies was counted as to whether it was normal, resorbed, dead, or showed anomalous development. The anomalies included stunting, exencephaly, anencephaly, protruding intestine, and prominent eye defects. The "normal" mice were so designated when, from all evidence, they appeared normal as to size, development, and absence of obvious anomaly. Mice at 18.5 days are essentially at the delivery stage of development (normally 20-21 days) but if allowed to reach delivery, the mother would generally kill and eat those which are abnormal. Thus, by studying the fetuses *in utero* we are able to present data on total irradiation effect.

The irradiation facilities used included a single x-ray tube at 48.5 cm distance to the position of the gravid uterus, the radiation interrupted by 0.28 mm Cu. and 0.50 mm Al. filters so that the output in air at the level of the 8.5 day embryos was 50 r/minute. Since the mouse is relatively small and the embryos are close to the body surface, extrapolation to rads is well within the maximum range of 3%. The total dose was 200 r in 4 minutes.

*Experimental data.* Table I presents an adequate number of pregnancies and implantations for statistical significance. Although

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TABLE I.

| | Cross (mice) | Preg-nancies | Implanta-tions | Normal | Resorptions | Dead | Anomalies |
|---|---|---|---|---|---|---|---|
| Controls—no irradiation | CF1 × CF1 | 61 | 630 | 591 (94) | 37 ( 5.7) | 2 (0.3) | 0 |
| | CF1 × C57 | 39 | 348 | 325 (93) | 31 ( 9 ) | 1 (0.5) | 1 ( 0.5) |
| | C57 × C57 | 33 | 253 | 203 (80) | 31 (12 ) | 9 (3.5) | 10 ( 4 ) |
| X-rays: 200 r @ 8.5 days | CF1 × CF1 | 74 | 704 | 270 (38) | 274 (39) | 33 (4) | 127 (18) |
| | CF1 × C57 | 58 | 315 | 137 (44) | 96 (30) | 22 (7) | 51 (17) |
| | C57 × C57 | 37 | 380 | 18 ( 5) | 334 (90) | 20 (5) | 10 ( 2) |

Percentage of total implantations given in parentheses.

the C57 stock does not mate readily and the general fertility is low, among the successful matings there were 253 control and 380 x-rayed implantations available for this analysis.

Among the controls (with no irradiation) one would expect a higher rate of resorptions and anomalies among the C57 stock than among the CF1 stock, but note that among the hybrids there was an intermediate level of resorptions (9%) between controls (5.7%) and pure C57 stock (14%). Anomalies appeared much more frequently among the C57 mice. These included eye anomalies which apparently occur in 8-10% of all mice from this stock (Dickie(3)). Thus, among the normal (control) mice of pure or hybrid crosses the C57 strain showed a higher level of intra-uterine resorptions and anomalies than did either the hybrids (which included the C57 genes) or the pure CF1 stock.

The incidence of anomalies in the CF1 strain has been reported elsewhere (Rugh and Grupp(4,5,6)), but to the figures are added those from more recent observations. These do not alter the percentage values, but increase the totals involved. The normal fetuses among the hybrids are higher by 6% (44% over 38%) than among the pure CF1, and far higher than among the pure C57 (5%), thus a slight indication of heterosis. This is further substantiated by the lower incidence of resorptions among the hybrids, 30% as contrasted with 39% for the CF1 and 90% for the C57 stock. The incidence of anomalies among the hybrids and the CF1 strains is about the same, and somewhat higher than among the C57 mice simply because most of these implantations resulted in resorptions (90%). Thus, there appears to be clear cut evidence that resistance to 200 r x-rays at 8.5 days gestation is somewhat better in the hybrids than in either of the parental stocks, both with respect to percentage normals and percentage resorptions. Heterosis, therefore, extends back to the 8.5 day stage, at least with respect to the damaging effect of ionizing x-rays.

*Discussion.* Heterosis is generally measured by overall survival, longevity, productivity, etc. whether or not altered by some traumatic experience, but always compared with the parental stocks. There is no evidence that this hybrid vigor extends to further generations, but it seems to be a rather general rule for all extensive heterozygotic conditions. The heterozygote is therefore not a blending of certain potentialities, but may be actually a synergistic effect resulting in a more viable generation. If one crosses these 2 strains and tests their adult hybrid offspring against the same age pure strains, the hybrids show a much greater resistance to the lethal effects of ionizing radiations than does either parental strain. There is a considerable difference between the 2 pure strains, but neither is as radioresistant as their hybrid offspring. This resistance must be due to composite effects, certainly more than one because irradiation death is never simple. Irradiation death may be due to hemorrhage, to epithelial destruction, to edema and excretory malfunction, to secondary infection, to extensive and prolonged anemia, etc. But some of these causes must be ruled out in the embryo or fetus because they could not (and do not) exist. Nevertheless, there appears to be evidence herein presented that the hybrid between the CF1 and

the C57 strains, even at 8.5 days gestation, seems to withstand the trauma of ionizing radiations better than either of the parental strains, as measured by subsequent embryonic development.

This study points up one more factor that may be overlooked, namely that heterosis shifts percentages toward a higher survival, but this includes a higher level of anomalies than among the very sensitive C57 strain. Thus, one sees here an example of heterozygosity aiding the survival of anomalies.

One might raise the question of the effect of reversing the hybrid cross, mating the CFl males with C57 females. Genetically there should be no difference, but it must be admitted that any cytoplasmic effects on the very early development might be involved. However, since the exposure to x-rays occurred long after implantation (which occurs at 4.5 days when the germ layers are forming) it can be assumed that any differences relating to the origin of the cytoplasm would have disappeared. Possibly heterotic influences are present at the moment of fertilization, only to be manifest later when tests are possible. Certainly they appear to be working at 8.5 days so that the more complicated investigations of earlier stages seem indicated.

*Summary and conclusions.* 1. Embryonic mice at 8.5 days gestation from pure C57 and CFl strains, and their hybrids, were x-irradiated to 200 r to determine whether prenatal heterosis was manifest. Criteria were the number of normal, resorbed, dead, or anomalous mice seen at 18.5 days in the hybrids as compared with the pure lines. 2. The hybrids at 8.5 days exhibited some degree of heterosis based upon the higher percentage of normal fetuses, and the lower percentage of resorptions when comparisons are made with the pure strain parental stocks. 3. For heterosis to be manifest, the hybrids had to overcome the severely deleterious effect of the presence of C57 genes as indicated by the 90% resorptions among this parental stock and only 30% in the hybrids, while the CFl showed 39%. Thus, there is evidence of more than mere dominance of the more resistant strain genes, hence heterosis at least by the time of organogenesis. 4. The heterosis for the embryo results in better survival, hence better survival also of the x-irradiation-produced congenital anomalies.

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## Excretion of Urinary N-$C^{14}$-Methyl Morphine and Pulmonary $C^{14}O_2$ in the Monkey and Dog after Subcutaneous Injection of the Labelled Drug.* (26293)

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The application of radioisotopic tracer technics has provided much useful information on the biological disposition of the potent analgesic agents. March and Elliot(5), Elliot *et al.*(4), and Achor and Geiling(1) have studied the physiological disposition of $C^{14}$-labelled morphine in man, in rats and in mice, respectively. The present report of-

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