Resistance of A/Jax Mouse Embryos with Spontaneous Congenital Cleft Lip to the Lethal Effect of 6-Amino-nicotinamide*

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A common feature of inbred lines of animals is the tendency for a minority of the animals in the strain to be born with congenital malformations of a frequency and type that are characteristic for the given strain (Grüneberg, 1952). It is clear that the differences between strains in the numbers and types of malformations they manifest are genetic in origin, but it is not clear why some animals within a litter are born defective while their genetically indistinguishable litter-mates are normal.

The congenital cleft lip that occurs in the highly inbred A/Jax mouse strain is a case in point. In litters observed just before term about 14% of the embryos have a cleft lip, usually with an associated cleft palate. More or less tenuous associations have been observed between cleft lip frequency and maternal age (Reed, 1936; Davidson, 1963), litter size (Davidson), and uterine position (Trasler, 1960), but these will not account for the fact that in a representative A/Jax litter one or two animals have a cleft lip and the others are normal. One postulated explanation is that the increased homozygosity resulting from inbreeding makes the embryo developmentally unstable (Lerner, 1954). If so, it should be easy to increase the frequency of cleft lip in the A/Jax strain by exposing the mother to various teratogenic agents during pregnancy, but experiments with a number of teratogens have not supported this hypothesis (D. G. Trasler, personal communication). In the present paper an increase in the frequency of cleft lip in A/Jax embryos at term following maternal treatment with 6-amino-nicotinamide is shown to result not from the production of cleft lip by the treatment but from the resistance of embryos with 'spontaneous' cleft lip to a dose of the teratogen that kills most of their normal litter-mates.

During a study of the teratogenic effects of the nicotinamide analogue 6-amino-nicotinamide (6AN) in the mouse (Pinsky and Fraser, 1959, 1960; Goldstein, Pinsky, and Fraser, 1963), the nature and frequency of defects in the offspring following treatment of the mother during pregnancy were found to depend on, among other things, the genotypes of mother and foetus and the stage of gestation at which the drug was given. The period of exposure of the embryo to the effects of the drug could be precisely timed by injecting nicotinamide two hours after the 6AN, in a quantity sufficient to prevent the teratogenic effects of the analogue when given concurrently (Pinsky and Fraser, 1960). When this treatment was applied to A/Jax mice on day $10\frac{1}{2}$ of gestation, a large proportion of the embryos were resorbed. Most of the survivors had cleft lip, but it was not clear whether these defects had been induced by the treatment, or whether the embryos that were destined to have 'spontaneous' cleft lip had survived the treatment that killed their normal litter-mates. A series of experiments was, therefore, carried out to elucidate this question.

Materials and Methods

A/Jax female mice were maintained on Purina Lab Chow and water, with milk, bread, and lettuce added once a week. Males were placed in cages with nulliparous females overnight; the females with vaginal plugs the next morning (day 0) were weighed and left until the day of treatment, when an injection of 6AN was given at about 2 p.m., followed two hours later by an injection of nicotinamide. On day 18 the animals were killed and the number and position in the uterus of normal and cleft lip embryos and resorption sites were recorded. A resorption site was defined as any remnant of an implanted embryo, ranging from a barely

TABLE

FREQUENCY OF CLEFT LIP (CL) FOLLOWING TREATMENT WITH 6AN FOLLOWED TWO HOURS LATER BY NICOTINAMIDE (NIC)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Dose of 6AN (mg./kg.)		19	19	19	38 (I.P.)	19	14·25 (I.P.)	9·5
Dose of NIC (mg./kg.)		7·3	7·3	7·3	14·6 (I.P.)	14·6	5·5 (I.P.)	7·3
Day of treatment	Control	10½	9½	11½	11½	10½	10½	10½
No. of litters	32	10	10	10	10	5	5	5
No. of implantations	288	82	92	82	116	54	56	51
% resorptions	19·8	86·6	40·2	39·0	82·8	79·6	85·7	39·2
Total embryos at term	231	11	55	50	20	11	9	31
No. of embryos with CL	29	9	9	6	3	5	4	5
% CL of implantations	10·1	11·0	9·8	7·3	2·6	9·3	7·1	9·8
% CL in term embryos	12·6	81·8	16·4	12·0	15·0	45·5	44·4	16·1
Expected % CL in term embryos	12·5	74·5	(16·7)	(16·4)	(58·0)	49·1	62·2	16·5

recognizable nubbin of tissue to a formed, but more or less macerated, dead embryo.

Results

The Table presents the results.

Column (1) shows that in the untreated controls there were 288 implantation sites. Of these, 19·8% were resorbed, and 12·6% of the young at term had cleft lip. The value for cleft lip embryos calculated as a proportion of all implantation sites is somewhat lower than this (10·1%), since the total is increased by the resorptions, which cannot be classified for cleft lip. Column (2) shows the results following treatment on day 10½ with a 'standard' intramuscular injection of 6AN (19 mg./kg.) followed two hours later by nicotinamide (7·3 mg. kg.). Most of the embryos were resorbed (86·6%), and 9 of the 11 survivors had cleft lip (81·8%). According to the control values, this is about the number (8·2%) expected from 82 implantation sites without treatment, which suggests the possibility that the treatment spared the embryos with cleft lip, rather than inducing cleft lip. The bottom row of the Table represents the expected proportion of cleft lip embryos at term, on the assumption (based on the control value) that 10% of all implantations were potential cleft lip embryos and that all of them survived the treatment.

Columns (3) and (4) show that the resorption frequencies following a standard treatment on day 9½ or 11½ are much lower (40% and 39%, respectively) than the day 10½ value. There is no significant indication of a preferential survival of cleft lip embryos, but at this level of resorption the excess due to a sparing effect would be small anyway. Column (5) shows that, following a double dose of 6AN (intraperitoneally) on day 11½, the excess of cleft lip embryos at term does not occur, even though the resorption frequency is high, so that the postulated differential resistance of cleft lip embryos seen at day 10½ is no longer present at day 11½.

The subsequent columns represent various treatments on day 10½. It can be seen that whatever the resorption frequency, the number of cleft lip embryos is about that which is expected if all the cleft lip embryos that would have occurred without treatment had survived (bottom row of Table). Thus in a series in which the dose of nicotinamide was doubled, to see if a single dose was counteracting the analogue (Column (6)), there were 54 implantations, of which 80% had resorbed. One would have expected 5 cleft lip embryos without treatment (10% of 54) and in fact 5 were observed.

Column (7) shows the results of a series in which the doses of 6AN and nicotinamide were reduced by one-quarter and given intraperitoneally to see if this route of administration would reduce inter-litter variation. Again there is an excess of cleft lip embryos among the survivors. Column (8) shows that when the 6AN dose is reduced to half the resorption frequency falls, and the excess of cleft lip embryos among viable young disappears. Since the teratogenic dose range is generally somewhat lower than the embryo-lethal dose (Murphy, 1960), one would still expect an excess of cleft lip embryos if the treatment were inducing cleft lip. However, this does not seem to be the case.

Thus the data strongly support the hypothesis that an A/Jax embryo destined to have a cleft lip is, on day 10½, relatively resistant to a dose of 6AN that will kill most of its normal litter-mates. At this stage formation of the lip has not yet commenced.

Maternal treatment with a folic acid antagonist on day 10½ also increases the proportion of cleft lip embryos at term, suggesting that the postulated differential resistance of the cleft lip embryo is not limited to the effects of 6AN (D. G. Trasler,

personal communication). Experiments are in progress to define more precisely the period during which the preferential resistance exists, and the manner by which the treatment kills the normal embryos.

Summary

About 15% of the embryos of the inbred A/Jax mouse strain are born with a congenital cleft lip. When pregnant A/Jax mice are treated on day 10½ of gestation with a dose of 6-amino-nicotinamide that kills a high proportion of the embryos, most of the survivors have a congenital cleft of the lip. Evidence is presented to show that these are the embryos with spontaneously occurring cleft lip, which at this stage are more resistant to the treatment than their normal litter-mates. On day 10½ the lip has not yet begun to form. Thus there is a physiological difference between A/Jax embryos destined to have a congenital cleft lip and their normal litter-mates before there is any detectable anatomical difference.

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