GENETIC AND ENVIRONMENTAL FACTORS IN THE TERATOGENIC EFFECTS OF BORIC ACID ON CHICKEN EMBRYOS¹

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THERE is much variation in the extent to which hereditary and environmental agencies contribute to the origin of developmental malformations. At one end of the range we may postulate the existence of defects that arise in the presence of a single gene, the expression of which is independent of the residual genotype as well as of the environment. Such cases, if indeed on further study they are found to exist at all, are certainly rare, the great majority of mutants with teratological end-effects, whether they are dominant or recessive, being subject to genic modification. At the other extreme, and equally uncertain as a reality, one can envisage defects of purely environmental origin and for which variations in genetic resistance do not exist. In between these hypothetical limits, we find abnormalities produced by the interaction, in every conceivable combination, of environmental and hereditary forces.

We have shown recently (LANDAUER 1952) that an interesting syndrome of malformations can be produced by injecting, at the end of four days of development, boric acid into the yolk sac of chicken eggs. The resulting defects are, of course, environmental in nature par excellence. In such a situation it requires perhaps particular justification to study the participation of genetic factors in the manner and degree of response produced by the teratogenic substance. Because we believe that particular interest does, in fact, inhere in the nature of response differences to boric acid treatment, we have made observations on several breeds of fowl and on crosses between some of them. The results of these experiments are to be reported here.

MATERIALS AND METHODS

The stocks used for our experiments were White Leghorns (dominant white), White Minorcas (recessive white), Black Minorcas (solid black plumage) and Silver Gray Dorkings. As is commonly true, some of our White Minorca stock was heterozygous for dominant white. In addition to these "pure" stocks, we used eggs (F_1 embryos) from the following matings: reciprocal crosses between White and Black Minorcas, reciprocal crosses between White Minorcas and White Leghorns, and a cross of Black Minorca ♀♀ × White Leghorn ♂.

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All eggs were incubated in the same forced-draft incubator. After 96 hours of incubation, they received an injection into the yolk sac of 0.05 cc 5% boric acid (2.5 mg) dissolved in saline. Our records are based on all embryos and chicks surviving the 13th day of incubation. For the sake of greater conciseness, the descriptive records of malformations have been summarized into larger groups. Thus, the group of "minor beak defects" includes all cases of cross-beak, shortened maxilla, shortened mandible or parrot-beak; as "complex beak defects" we classified all cases in which a shortened mandible (sometimes in combination with twisting of the upper beak) was combined with cleft palate and/or facial coloboma (both at times unilateral); the heading of "tarsometatarsus defects" includes cases of shortening of the bone with or without bending; finally, under "toe defects" we have summarized all abnormalities of the toes ranging from a shortening of the fourth toe—the mildest expression of abnormality—to complete absence of all toes.

In order to provide a basis for evaluation of the incubation conditions and the incidence of sporadic malformations, we give in table 1 data for untreated control eggs. These came from the same matings and were incubated during the same period and in the same machine as were the experimental eggs. It can be seen that embryonic mortality was low and hatchability of the eggs excellent in all matings, except that of the Silver Gray Dorking fowl. In these latter, the higher mortality during incubation was presumably due to a certain measure of inbreeding during several preceding generations. The incidence of sporadic malformations was also highest among the Dorking embryos (2.3%); it was quite low in all other matings (0 to 1.2%).

TABLE 1
*Embryonic mortality and incidence of malformations in untreated eggs
of the matings used for experiments.*

Mating	Number of fertile eggs	Mortality during incubation in %		Hatched	Malformations among survivors of 13th day
		To end of 2nd week	14-22 days		
				%	%
Black Minorca	376	5.6	9.1	85.4	0
White Minorca	220	3.2	4.5	92.3	0.5
Black Minorca ♀♀ × White Minorca ♂	335	5.4	6.3	88.4	0.6
White Minorca ♀♀ × Black Minorca ♂	249	2.4	7.6	90.0	0.8
White Minorca ♀♀ × White Leghorn ♂	266	4.1	6.0	89.8	1.2
White Leghorn ♀♀ × White Minorca ♂	353	2.5	5.1	92.4	0.3
Black Minorca ♀♀ × White Leghorn ♂	305	3.9	7.6	88.5	0.3
Silver Gray Dorking	307	14.0	19.2	66.8	2.3

The effects of boric-acid treatment at 96 hours of incubation on embryos from these matings are shown in table 2. These data are the results for the whole experimental period, viz. for eggs put into the incubator from February 12 to May 20, 1952.

Seasonal trends

In order to determine if conditions changing with the season had any influence on the response of embryos to boric acid, the data from each of the different breeds and crosses were divided into two groups for comparison, those from settings between February 12 and April 1 representing the early part of the season, those from settings between April 15 and May 20 forming the results for the later part of spring.

Analysis of these data revealed some very striking seasonal differences in response to boric acid. This was particularly true for the data from Black Minorcas. The incidence of complex beak defects in Black Minorca embryos was 57.6 percent during the early part of the season, but only 32.1 percent in

TABLE 2

Embryo mortality and incidence of malformations after injection of 2.5 mg boric acid at 96 hours of incubation. Summary of results for whole season.

	B.M. W.M.		B.M.♀♀ × W.M.♂♂		W.M.♀♀ × W.L.♂♂		W.L.♀♀ × W.M.♂♂		B.M.♀♀ × W.L.♂♂		S.G.D.	W.L.
Mortality and hatching in percent of number treated; curled toe paralysis in percent of hatched chicks; malformations in percent of survivors of 13th day.												
Number treated	656	414	569	494	533	667	556	372	345			
Mortality from 96 hours to end of 2nd week %	22.3	15.0	19.3	23.9	19.1	21.3	16.9	36.6	38.0			
Mortality 14-22 days %	62.2	65.5	65.2	53.4	61.9	58.3	56.3	62.6	49.3			
Hatched %	15.5	19.6	15.5	22.7	18.9	20.4	26.8	0.8	12.8			
Curled toe paralysis (among hatched) %	43.2	44.4	45.5	34.8	35.6	50.7	43.0		36.4			
Number surviving 13th day	510	352	459	376	431	525	462	238	214			
Simple beak defects %	22.9	14.8	28.5	33.5	26.5	11.6	24.5	2.5	10.3			
Complex beak defects %	43.9	53.7	40.3	18.4	46.9	54.3	38.7	91.2	50.0			
Tibiotarsus bent %	4.3	1.1	2.6	1.3	4.2	1.0	1.5	0.8	3.7			
Tarsometatarsus defects %	29.6	23.9	15.3	16.8	19.0	19.2	13.4	50.8	23.4			
Toe defects %	14.7	11.4	10.9	6.9	10.0	9.9	14.5	22.7	20.6			
Normal %	24.7	21.6	26.6	35.4	21.3	22.9	28.6	4.2	28.5			

Breed designations: B.M. = Black Minorca, W.M. = White Minorca, W.L. = White Leghorn, S.G.D. = Silver Gray Dorking.

late spring ($\chi^2 = 33.41$; $P < .0001$). The reverse was true for the single beak defects, the incidence having been 13.6 percent early, but 31.0 percent late (difference = $17.4 \pm 3.57\%$). As with the complex beak defects, malformations of the tarsometatarsus declined among the Black Minorca embryos with advancement of the season; during early spring the incidence was 45.3 percent, but only 16.1 percent during late spring ($\chi^2 = 52.08$; $P < .0001$).

The experiments with White Minorca embryos yielded a similar, though less pronounced, seasonal dependence of the complex beak abnormalities. They declined from 63.4 to 47.7 percent ($\chi^2 = 8.32$; $P < .01$). The simple beak defects rose in incidence, but the difference was not significant. There was no significant seasonal change in incidence of tarsometatarsus defects.

No clear seasonal trends were discernible among White Leghorn embryos for any of the boric-acid-induced malformations. The various crosses between breeds and the Silver Gray Dorkings all showed a seasonal decline of complex beak defects and a rise in the simple beak abnormalities, but none of the differences were significant.

Intra-breed heterogeneity

For a given breed or cross and within a limited span of time the incidence of particular malformations, produced by boric-acid treatment, remains relatively constant. We were interested to determine if this comparative constancy of response was brought about by uniformity of behavior of the progenies from different mothers or if it existed in spite of heterogeneity between them. The question was tested on the results with embryos of Black and White Minorca fowl. The analysis was based on eggs put into the incubator between February 12 and March 18, and was directed toward the question whether heterogeneity

TABLE 3

Individual records of Black Minorca females in regard to response of their progenies to the injection of 2.5 mg boric acid at 96 hours of incubation.

Mother's number	Total progeny	Free of malformations	Beak defects of any kind	Tarsometatarsus defects
1498	9	2	5	3
1500	7	3	4	2
01	6	1	5	3
02	10	2	8	5
03	7	1	6	4
04	10	3	7	4
05	10	7	3	1
06	6	1	5	4
07	11	0	11	11
08	14	3	11	5
09	7	2	5	4
10	11	1	10	9
11	11	6	5	4
12	5	0	5	4
13	10	1	9	8
14	13	4	8	6
15	12	2	10	9
16	4	0	3	4

between mothers existed in the following three features: (1) freedom from all malformations; (2) abnormalities of the beak (any kind); (3) defects of the tarsometatarsus. The relevant data for Black Minorcas are shown in table 3.

Tests for heterogeneity of these data gave, with 17 degrees of freedom, the following results: freedom from malformations $\chi^2 = 29.21$; P about 0.025; occurrence of beak defects $\chi^2 = 28.71$, P about 0.025; presence of malformations of the tarsometatarsus $\chi^2 = 38.59$, $P < 0.01$. Since some of the progenies were very small, tests for heterogeneity were repeated by limiting the analysis to the ten hens that had had progenies of at least ten embryos. The results thus obtained show an even higher level of significance (all $P < .01$). It is clear, therefore, that there were significant differences between Black Minorca mothers in regard to the response of their progenies to boric-acid treatment.

A similar analysis of our data for White Minorca progenies, but with the size of the total sample considerably smaller than that from Black Minorcas, brought evidence for highly significant heterogeneity in the production of tarsometatarsus malformations ($\chi^2 = 32.92$; $Df = 12$; $P < 0.01$). The White Minorca progenies showed no significant heterogeneity, however, as far as beak defects or freedom from all malformations were concerned.

Breed response differences

It is the most outstanding feature of our data that the Silver Gray Dorking embryos responded to boric-acid treatment with a much higher incidence of malformations than any of the other breeds and stocks tested. A comparison with the data for White Leghorns showed that complex beak defects and malformations of the tarsometatarsus were produced with much higher frequency in the Silver Gray Dorking stock, and this was true during the early as well as the late part of the experimental season. Abnormalities of the toes were in the Dorking stock significantly more common during early spring, but during the later part of the season the differences were not significant, though the trend was in the same direction.

The Silver Gray Dorkings differed from the Black Minorcas in a manner very similar to that just described in comparison to the data for White Leghorns, except that during the early part of the season the differences between the two stocks were not significant in incidence of malformations of tarsometatarsus and toes, but became highly significant during the second half of the season. The data for Silver Gray Dorking and Black Minorca embryos are given in table 4. A comparison of the results for Dorking embryos with those obtained from White Minorca stock and from the various crosses yielded differences of a similar nature, and these need not be discussed in detail.

It might be asked if the greater response of the Dorking embryos to boric acid was the consequence of a heightened "debility" of the stock on account of inbreeding. There had, however, been only a minor degree of inbreeding and, what is even more to the point, Dorking embryos did by no means give uniformly greater response to all teratogenic substances. The effect of insulin on the extremities, for instance, did not differ between Dorking and White

TABLE 4

Differences, in response to boric acid treatment, between Black Minorca and Silver Gray Dorking embryos. The size of the samples on which the percentages are based is shown in parentheses.

Malformations	Black Minorca %	Silver Gray Dorking %	Significance	
			χ^2	P
<i>Complex beak defects</i>				
Early	57.6 (236)	92.2 (141)	50.74	<.0001
Late	32.1 (274)	89.7 (97)	78.10	<.0001
Whole season	43.9 (510)	91.2 (238)	149.82	<.0001
<i>Tarsometatarsus defects</i>				
Early	45.3 (236)	53.2 (141)	2.16	.14
Late	16.1 (274)	47.4 (97)	38.48	.0001
Whole season	29.6 (510)	50.8 (238)	31.70	.0001
<i>Toe defects</i>				
Early	17.4 (236)	18.4 (141)	0.078	about .80
Late	12.4 (274)	28.9 (97)	13.97	<.001
Whole season	14.7 (510)	22.7 (238)	7.30	<.01

Leghorn (table 7) or Minorca embryos. The most reasonable explanation of the dissimilarities in response to boric acid would, therefore, seem to be that we are dealing with real stock or breed differences.

Boric-acid treatment did not lead to significant differences between Black Minorca and White Leghorn embryos in the incidence of (complex or single) beak defects. This was true in early and late spring. The same applies to Black versus White Minorcas, except that during late spring the White Minorca embryos showed a significantly higher incidence of complex beak defects ($\chi^2 = 12.01$; $P < .001$). This situation is explained by the fact that a sharp seasonal decline of complex beak defects (and an increase in simple beak abnormalities) occurred in Black Minorcas, but that only relatively slight changes of the same kind took place in White Minorcas.

The frequency of tarsometatarsus abnormalities in response to boric-acid treatment was significantly higher in Black Minorcas than in White Leghorns during the early part of spring ($\chi^2 = 57.70$; $P < .0001$) and for the whole season ($\chi^2 = 32.39$; $P < .0001$), but (because of the seasonal decline in incidence among Black Minorcas) these differences disappeared during late spring. A very similar situation is revealed by a comparison of the data for Black and White Minorcas, namely, a great excess among Black Minorcas of tarsometatarsus defects during early spring ($\chi^2 = 19.28$; $P < .0001$), with a reversal of the situation in late spring (because of a seasonal decline in response among the Black Minorcas, but not the White Minorcas), resulting in an actually higher incidence among the White Minorca embryos at this time ($\chi^2 = 5.80$; $P < .02$). The data for toe defects in Black and White Minorca embryos showed trends of incidence similar to those for tarsometatarsus abnormalities, but the differences were not statistically significant.

During the early part of the season boric-acid treatment produced complex beak defects with much higher frequency in White Minorca than in White

Leghorn embryos ($\chi^2 = 17.73$; $P < .0001$), but the two breeds did not show significant response differences in any other respect.

The effect of outcrossing Black and White Minorcas to White Leghorns on response to boric-acid treatment

In comparing the responses to boric acid of Black and White Minorca embryos, respectively, with those of White Leghorn embryos, some interesting differences have been reported above. The principal dissimilarities between the two Minorca breeds on the one hand and White Leghorns on the other, were found in the fact that during early spring Black Minorca embryos gave a much higher incidence of tarsometatarsus abnormalities than did White Leghorn

TABLE 5

The incidence of complex beak and of tarsometatarsus defects, produced by boric acid during early and late spring, in Black and White Minorca embryos and in F_1 embryos of Black Minorca ♀♀ × White Leghorn ♂ and White Minorca ♀♀ × White Leghorn ♂. The size of the samples on which the percentages of beak and tarsometatarsus defects are based is shown in parentheses.

Mating	Complex beak defects Incidence in %		Significance of differ- ences in early spring	Tarsometatarsus defects Incidence in %		Significance of differ- ences in early spring
	Early spring	Late spring		Early spring	Late spring	
B.M.	57.6 (236)	32.1 (274)	$\chi^2 = 9.88$ $P < .002$	45.3	16.1	$\chi^2 = 35.40$ $P = .0001$
B.M. ♀♀ × W.L. ♂	42.3 (189)	36.3 (273)		18.0	10.3	
W.M.	63.4 (134)	47.7 (218)	$\chi^2 = .61$ $P \text{ abt. } .42$	22.4	24.8	$\chi^2 = .001$ $P \text{ abt. } 1.0$
W.M. ♀♀ × W.L. ♂	58.9 (151)	40.4 (280)		22.5	17.1	

Breed designations: B.M. = Black Minorca, W.M. = White Minorca, W.L. = White Leghorn.

embryos, whereas White Minorca embryos responded with a higher incidence of complex beak defects than did White Leghorn embryos. In other respects, embryos of the two Minorca breeds did not significantly differ from White Leghorns.

Some of the results obtained with F_1 embryos of Black Minorca ♀♀ × White Leghorn ♂ and of White Minorca ♀♀ × White Leghorn ♂, as compared with the maternal Minorca breeds, are shown in table 5. During the early part of spring, outcrossing of Black Minorca females to a White Leghorn male led to a great reduction in incidence of complex beak and of tarsometatarsus defects. Outcrossing White Minorca females to a White Leghorn male, on the other hand, if compared with the White Minorca data, did not result in significant changes of response to boric acid. During late spring neither of the two outcrosses differed significantly from the maternal breeds in incidence of complex

beak defects, and both showed a slight, but probably significant lowering in the frequency of tarsometatarsus abnormalities (the χ^2 being 4.66 and 4.33, respectively, with a P in either case of about .03).

Plumage color and response to boric acid

According to preliminary reports by BERNIER (1948-1950), chickens with black plumage have higher riboflavin requirements than white-feathered ones. Since our earlier observations (LANDAUER 1952) had shown that malformations induced by boric acid are related to disturbances of riboflavin utilization, it seemed of interest to determine if differences in response to boric acid, comparable to those found by BERNIER, exist between embryos which are dissimilar in their genotype for down color or between embryos from (or eggs laid by) mothers with dissimilar plumage color.

The fact that the most extreme responses to boric acid were shown by embryos from the two colored breeds used in our tests, namely, Silver Gray

TABLE 6

Data comparing the response to boric acid of phenotypically white embryos segregating in reciprocal crosses of Black and White Minorca fowl. Incidence of curled toe paralysis in percent of hatched chicks; incidence of other traits in percent of survivors of 13th day of incubation. The size of the samples on which the percentages are based is shown in parentheses.

	White embryos and chicks		Significance of difference	
	19 white mothers	20 black mothers	χ^2	P
Curled toe paralysis (among hatched) %	6.7 (90)	48.5 (33)	28.77	<.0001
Free of malformations %	40.7 (268)	28.9 (204)	6.97	<.01
Complex beak defects %	17.2 (268)	38.2 (204)	26.54	<.0001
Toe defects %	3.4 (268)	11.5 (204)	9.28	<.01

Dorkings and Black Minorcas, is suggestive rather than compelling evidence; for, we might deal with breed differences that are determined by genetic factors unrelated to pigmentation. It was, therefore, necessary to search for other evidence with a bearing on our problem. Such evidence was provided by our data from reciprocal crosses between Black and White Minorca fowl in which, as stated earlier, segregation of embryos with black and with white down occurred. In order to minimize the chances of the results being influenced by the sire, we used in each of the two pens three different cocks in rotation during the first and again during the second part of the hatching season. There was no suggestion in the data that the results had been influenced by the use of different sires.

Table 6 gives data for all phenotypically white embryos and chicks produced by mothers with either white or black plumage. It can be seen that considerably fewer white chicks from black than from white mothers were entirely free of malformations. Furthermore, it is evident that in white chicks from black mothers, as compared with those from white mothers, boric-acid treatment was

responsible for a much higher incidence of curled toe paralysis, complex beak defects and malformations of the toes. All these differences are of high statistical significance. Similarly, it was found that among black embryos and chicks from white mothers the incidence of complex beak defects was 18.8 percent, whereas for black chicks from black mothers the corresponding figure was 42.3 percent (χ^2 for difference = 16.81, with $P < .0001$).

Another comparison may be made by contrasting the responses of the embryos according to their phenotypical down coloration, but without reference to their mothers' plumage color. In thus combining the data for the two reciprocal matings between Black and White Minorca fowl, it is found that more white than black embryos and chicks were free of malformations ($\chi^2 = 12.07$; $P < .001$) and that major beak defects were probably, during the early part of spring, significantly more common among black embryos and chicks than among the white ones ($\chi^2 = 4.34$; $P = .035$). There were no significant differences between the two groups in the incidence of abnormalities of tarsometatarsus and toes. On the whole, it is clear that the mother's plumage color is a much more potent factor in determining the embryo's susceptibility and teratogenic response to boric acid than is its own prospective down color. No significant response differences occurred in reciprocal crosses which did not involve dissimilarity of plumage color (White Minorca $\times$ White Leghorn).

DISCUSSION

It has been reported on earlier occasions that in the experimental production of malformations the outcome is influenced by genetic factors as well as by conditions of the environment. Intra-breed differences in teratogenic response to mechanical shaking and to the injection of insulin are known to exist among White Leghorn hens (LANDAUER and BAUMANN 1943; LANDAUER and BLISS 1943). Dissimilarities in teratogenic reaction to selenium-containing feed and to injection of insulin were observed between different breeds and stocks (LANDAUER 1940, 1948). In the case of insulin-induced rumpleness reciprocal crosses between breeds with greatly differing responses have shown that maternal influences play an important role in determining susceptibility of the progeny (LANDAUER 1948). The incidence of beak defects and micromelia after insulin treatment was found to decline from early to late spring (LANDAUER and RHODES 1952). KALTER and FRASER (1952) have similarly reported recently that in mice the *in utero* production of cleft palate by treatment of the pregnant mothers with cortisone is influenced by genetic and maternal factors, the incidence of the malformation differing between strains and a genetically influenced maternal-fetal relationship contributing in some manner to the production of cleft palate by cortisone.

These findings should not really be an occasion for great surprise. Since the maintenance of life against the manifold adversities of the internal and external environment is the mainspring of evolution, the existence of a great variety of protective mechanisms should be expected, and the presence of some of these will only be revealed under particular conditions of stress. The really intriguing

problems, about which we know very little, relate to the physiological processes that bring about varying degrees of teratological response and to the manner in which these processes act either upon the organism as a whole or on special parts. It is in this field that our present observations seem to contribute some information.

In considering the implications of our results, the following facts should be taken into account: (1) Earlier observations render it likely that boric acid produces its teratological effects by an interference with riboflavin-containing enzymes (LANDAUER 1952). (2) Within and between breeds differences in riboflavin requirements or in response to riboflavin supplements are known to exist (DAVIS, NORRIS and HEUSER 1938; LAMOREUX and HUTT 1948; LERNER and BIRD 1948). (3) The occurrence of seasonal trends in the chemical composition of eggs has been demonstrated (CSONKA 1950). (4) Black chickens apparently have higher riboflavin requirements than white ones (BERNIER 1948-50). (5) An increase in the riboflavin concentration of eggs has been observed during late winter and early spring (JACKSON *et al.* 1946).

Our present observations suggest not only that the teratogenic effectiveness of boric acid differs within and between breeds, but also that some of these variations are accounted for by physiological processes associated with plumage pigmentation. Pigment formation is not yet under way in the embryos during the developmental stages in which boric acid has teratogenic effects. (If, however, there is competition for an essential substrate or coenzyme between the affected organs and the melanoblasts, evidence of it should during later stages be found in down pigmentation of colored embryos. That this is, indeed, the case, will be shown in another publication, LANDAUER 1953.) The clearest indication for a relationship between plumage pigmentation and the effects of boric acid came from reciprocal crosses between Black and White Minorca fowl. Since the white parents were heterozygous for recessive and dominant white, segregation for black and white down occurred among the progeny of these crosses, and it could be shown that the prospectively white (and also the black) embryos from *black* mothers were more susceptible to the teratogenic action of boric acid than those from white mothers.

This "maternal" effect of plumage color on susceptibility of the embryos is presumably related to chemical composition of the eggs. Taking into consideration the facts reviewed previously, it seems plausible to suspect that differences in riboflavin content of the eggs laid by colored and white hens influence the extent to which embryos are able to withstand the action of boric acid. It is also noteworthy that the seasonal trend (from early to late spring) toward a lower incidence of malformations (after boric-acid treatment) was most evident in the progenies of mothers with pigmented plumage, especially the Black Minorcas. No similar trend was discernible in White Leghorn stock, and in White Minorcas the changes were much less marked than in Black Minorcas. It may be assumed that, as in other respects, with advancing spring (higher ambient temperatures) the maternal organism of chickens can better afford to spare critical compounds (in the present instance presumably ribo-

flavin) for incorporation into their eggs, and it is not surprising that hens with pigmented plumage are more influenced by these environmental changes than are white hens.

The difference between colored and white breeds in the seasonal decline of malformations after boric-acid treatment is especially marked in the case of the tarsometatarsus. In this instance, the trend exists only among the progenies of colored fowl. Additional evidence exists in our data for the conclusion that the teratological responses of the tarsometatarsus (and probably of the toes as well) have features that differentiate them from those of the facial skeleton.

TABLE 7

Comparative data for the teratological responses of White Leghorn and Silver Gray Dorking embryos to treatment with boric acid and insulin. The facial defects include all abnormalities of beak and palate; malformations of the extremities are represented by tarsometatarsus defects after boric acid, by micromelia after insulin.

Treatment	N	White Leghorn		N	Silver Gray Dorking	
		Facial defects %	Abnormal extremities %		Facial defects %	Abnormal extremities %
Boric acid						
2.5 mg 96 hrs.	214	60.3 $\pm$ 3.42	23.4 $\pm$ 2.97	238	93.7 $\pm$ 1.57	50.8 $\pm$ 3.23
Insulin						
2 U 96 hrs.	118	16.1 $\pm$ 3.35	29.7 $\pm$ 4.18	65	29.2 $\pm$ 5.64	18.5 $\pm$ 4.82
Insulin						
5 U 96 hrs.	79	43.0 $\pm$ 5.54	79.7 $\pm$ 4.47	60	58.3 $\pm$ 6.37	71.7 $\pm$ 5.80
Insulin						
2 U 120 hrs.	180	13.9 $\pm$ 2.58	46.7 $\pm$ 3.72	58	36.2 $\pm$ 6.20	51.7 $\pm$ 6.45
Insulin						
5 U 120 hrs.	76	50.0 $\pm$ 5.72	93.4 $\pm$ 2.85	45	64.4 $\pm$ 7.17	93.3 $\pm$ 3.74

Intra-breed heterogeneity between treated progenies of different mothers, for instance, is more marked for defects of the tarsometatarsus than for abnormalities of the beak. It is not clear at present whether these dissimilarities of response are merely an expression of quantitative differences in susceptibility of the respective tissues or whether they are consequences of qualitative diversity in the physiological functions of the affected parts.

It is known, however, from earlier studies (LANDAUER and RHODES 1952) that local specificities of reaction to teratogenic substances do, in fact, exist. Additional evidence for the same conclusion may here find its place. Table 7 contrasts the reactions to boric acid and insulin of White Leghorn and Silver Gray Dorking embryos, as expressed by incidence of facial defects and of abnormalities of the extremities. Several facts emerge clearly from a scrutiny of

these data and their comparison with facts already set forth in the present report: (1) that, as was pointed out at an earlier occasion, the responses to boric acid and insulin differed quantitatively (as well as qualitatively) between the two regions, the facial parts showing a higher incidence of malformations than the extremities after boric acid treatment, but the reverse holding after insulin injection; (2) that Silver Gray Dorking embryos reacted in both body regions to boric acid with a much higher incidence of abnormalities than did White Leghorn embryos; (3) that, in contrast to boric acid, the incidence of abnormal extremities brought about by insulin treatment did not differ significantly between the two breeds, whereas the frequency of facial defects produced by insulin was, as in the case of boric acid, consistently higher in Dorking as compared with Leghorn embryos. It follows from these observations that various parts of embryos may give dissimilar quantitative responses to one and the same or to different teratogenic substances, and it is these dissimilarities, as well as the total incidence of all types of defects, that may become exaggerated in the presence of maternal plumage pigmentation. There is little doubt that we are dealing with competition phenomena and it seems likely that agencies associated with maternal (and to a lesser degree embryonic) pigmentation serve to exacerbate the situation.

SUMMARY

A comparative study was made concerning the teratogenic effects of boric acid, injected into the yolk sac of 96-hour embryos belonging to several different breeds of fowl and crosses between them. Eggs of the following stocks were used: Silver Gray Dorking, White Leghorn, Black and White Minorca, reciprocal crosses between the two Minorca stocks and between White Leghorn and White Minorca, as well as from Black Minorca ♀♀ × White Leghorn ♂. The majority of White Minorcas were heterozygous for dominant white plumage. The principal results were as follows:

1. A distinct seasonal trend in teratological response to boric acid was found among the embryos of some stocks, the incidence of induced malformations declining from early to late spring. This trend was most marked in the Black Minorca data.
2. Significant heterogeneity in response to boric acid was shown to exist between progenies of different mothers belonging to one and the same breed.
3. Highly significant differences in response to boric acid treatment were found between breeds. The two colored breeds used in our tests, namely, Silver Gray Dorkings and Black Minorcas, gave a higher incidence of malformations than did the white breeds.
4. Outcrossing of Black Minorca females to a White Leghorn male resulted in a great lowering in incidence of complex beak and of tarsometatarsus defects among the F_1 embryos. The same did not hold for outcrosses of White Minorca females to a White Leghorn male.
5. Plumage color of the mother had a definite effect on response of her progeny to boric acid. In segregating progenies it was found that white em-

bryos from black mothers showed more malformations than white embryos from white mothers. The phenotypical down color of the embryos themselves has an effect of the same nature, but to a much lesser degree.

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