FURTHER STUDIES ON THE GENETICS OF ABNORMALITIES APPEARING IN THE DESCENDANTS OF X-RAYED MICE

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In 1923 the senior writer and BAGG described the occurrence of abnormalities of the eyes and limbs of mice descended from animals which had been treated with X-rays. It was shown that these abnormalities were inherited. The object of the present communication is to present data on the type of inheritance involved and to describe certain of the results of selection upon the development of the character.

NATURE OF THE ABNORMALITY

BAGG and the senior writer (1924) gave a preliminary description of the eye abnormality. It was found that one or both eyes might be affected. In 227 defective eyes the following grades of abnormality were listed:

Slight atrophy of eyelids	12
Marked but not complete atrophy of eyelids	114
Eyelids completely missing	101
Eyes slightly atrophic, no corneal opacity	45
Eyes slightly atrophic, marked corneal opacity	36
Eyes markedly atrophic, no corneal opacity	146

The anatomical changes associated with the eye abnormalities include various degrees of atrophy of the optic tract culminating in its complete disappearance. The skull may be asymmetrical, being twisted toward the side of the head containing the abnormal eye. The diameter of the orbit is sometimes reduced.

The limbs show many different types of abnormality. Of these the commonest is a club-foot condition. The digits are often flexed and fused. The bones of the metatarsus and phalanges may be appreciably shortened. The digital muscles may also be much atrophied.

As the number of animals recorded increased, varying degrees of polydactyly were also encountered and described. BEAN (1929) listed certain of these types and pointed out that a single foot could show multiple abnormalities. She cited one example in which the basic characteristics of polydactyly, brachydactyly, and syndactyly were all present. The most

consistent defect found by her was the reduction, by fusion, of the number of carpals or tarsals. BAGG (1929) in examining a large number of abnormal limbs lists the following numbers of various manifestations of the character.

1. Club feet with dorsal flexion and syndactylism	291
2. Club feet with palmar flexion and syndactylism	9
3. Club feet with marked distortion of entire foot	6
4. Syndactylism without abnormal flexion	9
5. Hypodactylism	27
6. Congenital amputation	16
7. Polydactylism	93

The next step in investigating the true nature of the character was to trace its appearance in embryonic development. This was begun by BAGG who published in 1924 figures showing that there are present, during the latter part of pregnancy, in embryos destined to become abnormal, extensive hemorrhagic lesions. These lesions have an etiological relationship to the structural abnormality of the eye. The lesions are late in pregnancy full of extravasated blood. BAGG concludes that there is apparently "a relation between the presence of a defective blood vascular system in the embryos and the later arrested development of the abnormal organs." He indicates that the presence of hemorrhagic lesions on the feet of certain embryos may mean that abnormalities of the limbs also have a similar origin. In 1929 the same investigator described the origin of the foot abnormality as follows:

"Between the twelfth and thirteenth days of prenatal life there occurs a localized arrest in development of a part of the foetal foot which is associated apparently with a perivascular lymph stasis almost always on the dorsum of the foot. This condition results in the formation of a well-defined bleb, or blister-like area, in the subcutaneous tissue of the foot which is filled with a clear amber-colored fluid. The next step . . . is the escape of blood into the bleb and, in consequence, the formation of a well-defined hemorrhagic lesion."

Our knowledge of the origin of the abnormality has been greatly increased by the recent work of BONNEVIE (1931). She confirmed the results of BAGG with older embryos (15–25 mm) but finds that the conditions described by him are a secondary stage in the development of the anomalies. She states: "In the younger embryos (9–11 mm) clear blebs are found also on the median dorsal part of the embryo, especially across the shoulder

region as well as on the hind part of the body. At the head clear blebs are at this stage often found resting on the upper jaw."

"Still earlier embryos (7–9 mm) have no blebs at all on the distal part of their feet while on the back of their body the blebs may be very large. Two embryos were found with blebs covering their whole back and others in which the blebs of the shoulder region were prolonged in the direction of one of the fore feet. The blebs of the hind part of the back are often seen to be continued on the broad root of the embryonic tail, and in somewhat older embryos (9–11 mm of length), isolated blebs are very often found along the dorsal side of the tail."

She continues by pointing out that the gradual change in the localization of the blebs seems to indicate a mechanical displacement of fluid, first appearing at some place in the median dorsal line of young embryos. The amniotic membrane may help by pressure in this process of displacement. Blood lesions never arise at points where the blebs are passing by but only where they are arrested. Bleeding probably occurs from the underlying capillaries. The shoulder blebs move down into the limbs causing lesions there. The discrepancy between lesions of the right and left front feet noted by BAGG (1929) and MURRAY (1929) is probably accounted for by a mechanical factor—the curling up of the embryo in a spiral. The direction of the spiral is in the great majority of cases right sided. This would give a greater opportunity for left front foot abnormalities.

In the unselected material tabulated by BAGG and by MURRAY the total incidence of abnormalities of the left front foot was 772 and of the right front foot 464. BONNEVIE believes that the existence of more frequent abnormalities in the front limbs as compared with the hind limbs is due to the escape and disappearance during ontogeny of a number of posterior blebs via the tail.

The origin of the clear fluid which fills the blebs is of great interest. As far as BONNEVIE'S work extends the origin of the fluid seems to be cerebrospinal and not lymphatic as suggested by BAGG. BONNEVIE adopts as a working hypothesis the conclusion that the fluid accumulates inside the medullar tube causing swelling of the myencephalon region. From this center the blebs are distributed, some being resorbed while others remain to cause anomalies. The investigation is being continued.

In view of the nature of the abnormality as shown by these morphological studies the earlier genetic symbol *h* (hemorrhagic lesion) used by the writer (1924) to describe it had probably better be replaced by M^{BL} for normal and m^{bl} (myencephalic blebs) for the abnormal types.

THE GENETICS OF THE ABNORMALITY

In 1923 soon after the appearance of the abnormality the writer and BAGG reported that it followed the general genetic behavior of a Mendelian recessive. There were, however, produced from matings of abnormal×abnormal 374 abnormals and 73 normals. This means that 16.3 percent of these mice, supposedly genetic abnormals $m^{bl}m^{bl}$, were normal in appearance. In 1924 the numbers had been increased to 594 abnormals and 132 normals, a percentage of 18.1 normal overlaps. A number of these were bred together and gave a total of 106 abnormal, 42 normal young. In this case the percentage of "normal overlaps" had been raised to 28.3.

During the past eight years selection of various sub-strains of the abnormality have been carried on by the writer.

Two main sub-strains, one in the direction of increased proportion of eye abnormalities, the other in the direction of increased proportion of normal overlaps, have been developed. The former of these is designated as Line "700," the latter as Line "9000A." To the results recorded by the writer (1931) additions have been made which now may be tabulated as follows:

TABLE 1

LINE 9000A				LINE 700					
GENERATION OF SELECTION	NORMAL	ABNORMAL	PERCENT ABNORMAL	GENERATION OF SELECTION	NORMAL	ABNORMAL EYE	PERCENT ABNORMAL EYE	ABNORMAL FEET	PERCENT ABNORMAL FEET
1	44	0	0.0	1	10	74	88.0	1	1.2
2	40	3	7.0	2	8	97	92.3	0	0.0
3	62	1	1.6	3	6	104	94.5	0	0.0
4	160	2	1.3	4	8	86	91.2	0	0.0
5	184	0	0.0	5	7	44	86.2	0	0.0
6	112	0	0.0	6	3	23	88.4	0	0.0
7	131	0	0.0	7	3	49	94.2	0	0.0
8	71	0	0.0	8	7	48	87.2	1	1.9
9	80	0	0.0						
Total	884	6	0.7	Total	52	525	90.9	2	0.4

From table 1 it is easily seen that the two strains have been separated by selection so that for the last five generations in Line 9000A there have been no abnormals while in Line 700 the percentage of abnormals over eight generations is 90.19. At the same time Line 700 has practically been freed from the foot abnormality (see table 1).

TABLE 2

LINE 9000B				LINE 9000C			
GENERATION OF SELECTION	NORMAL	ABNORMAL LIMBS	PERCENT ABNORMAL LIMBS	GENERATION OF SELECTION	NORMAL	ABNORMAL LIMBS	PERCENT ABNORMAL LIMBS
1	8	29	78.3	1	6	27	81.8
2	3	43	93.4	2	5	39	88.6
3	21	108	83.7	3	6	62	91.1
4	7	76	91.5	4	9	84	90.3
5	0	55	100.0	5	1	48	97.9
6	2	81	97.5	6	2	41	95.3
7	0	48	100.0				
Total	41	440	91.4	Total	29	301	91.2

TABLE 3

LINE 9000B

GENERATION OF SELECTION	ABNORMAL FRONT ONLY		ABNORMAL FRONT AND HIND		ABNORMAL HIND ONLY	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
1	15	57.6	9	34.6	2	7.6
2	23	53.4	17	39.5	3	6.9
3	43	39.8	55	50.9	10	9.2
4	34	44.7	36	47.3	6	7.8
5	28	50.9	25	45.4	2	3.6
6	44	54.3	36	44.4	1	1.8
7	25	52.0	22	45.8	1	2.0
Total	212	48.5	200	45.7	25	5.7

LINE 9000C

GENERATION OF SELECTION	ABNORMAL FRONT ONLY		ABNORMAL FRONT AND HIND		ABNORMAL HIND ONLY	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
1	5	17.2	15	51.7	9	31.0
2	5	12.8	20	51.2	14	48.2
3	12	19.3	32	51.6	18	29.0
4	25	29.7	38	45.2	21	25.0
5	15	30.0	21	42.0	14	28.0
6	13	31.7	20	48.7	8	19.5
Total	75	24.5	146	47.8	84	27.5

If Line 9000A is analyzed as to the nature of the abnormality it is found that of the 6 abnormals, 4 had abnormal feet and 5 had abnormal eyes. In other words, 99.4 percent had normal eyes and 99.5 percent normal feet.

Lines 9000B and 9000C are additional sub-strains in which selection has been carried on for increase in incidence of foot abnormalities. They are closely related in origin to Line 9000A.

The incidence of limb abnormalities in these lines in succeeding generations of selection is given in table 2.

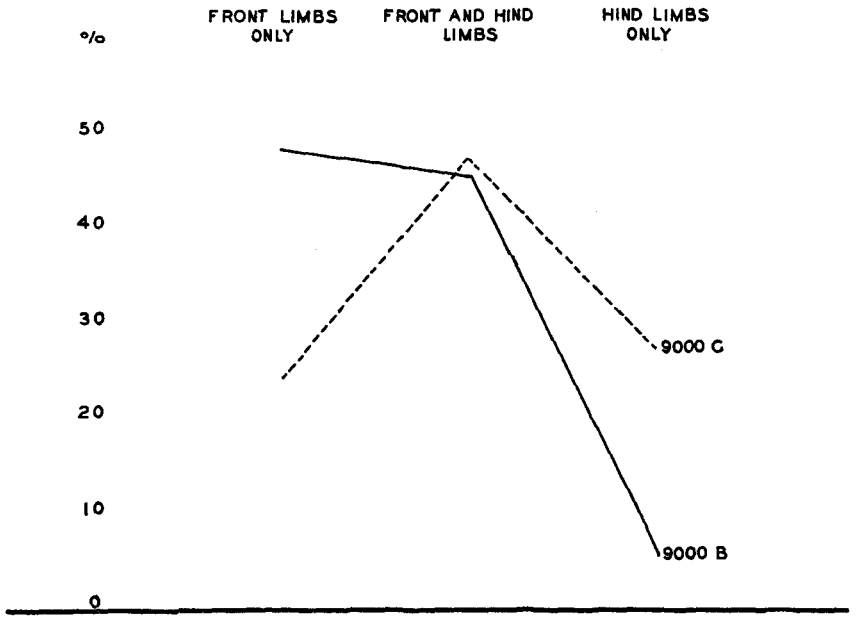

FIGURE 1.

The incidence of the "abnormal limb" character may therefore be greatly increased by selective breeding. In Line 9000B selection has been toward abnormalities of the front feet; in Line 9000C it has been in the direction of abnormality of the hind feet. Table 2 shows that while progress in separating the two locations of foot abnormalities has been slow there has been a distinct increase in the percentage of animals with abnormalities in the front or hind feet or in both. Localization is therefore difficult but increase in the percentage of total foot abnormalities has been successful. BAGG in 1929 reports the incidence of foot abnormalities in his stock in 432 animals out of 5280. He says that the incidence of foot abnormalities can be "greatly increased" by selection. MURRAY (1929), using the basic stock from which the writer took his material for Selection Lines 9000B and

9000C, reports that out of 1922 animals 356 or 18.5 percent had abnormal feet and 1212 or 63 percent abnormal eyes.

That there has been established some sort of difference between Line 9000B selected for front limb abnormalities and Line 9000C selected for hind limb abnormalities is shown by table 3.

In Line 9000B the results of seven selection generations give 48.5 percent of the foot abnormalities in the front limbs only. In Line 9000C the percentage for six selection generations is 24.5. On the other hand Line 9000C gives 27.5 percent with abnormalities in the hind feet only while Line 9000B has only 5.7 percent. There is then evidence (graphically shown in figure 1) of some progress in the direction of selection. The fact that the sixth selection generation of Line 9000C makes the poorest showing is not encouraging but may simply mean a chance deviation due to small numbers.

The occurrence of eye abnormalities in Lines 9000B and 9000C is also a matter of interest. Table 4 gives the results for the two groups.

TABLE 4

Line 9000B

GENERATION OF SELECTION	RIGHT EYE ABNORMAL	LEFT EYE ABNORMAL	BOTH EYES ABNORMAL	TOTAL ABNORMAL	NORMAL	PERCENT ABNORMAL
1	6	10	10	31*	6	83.7
2	17	20	5	42	4	91.3
3	51	49	17	117	10	92.1
4	42	27	6	75	8	90.3
5	22	24	3	49	5	90.7
6	22	38	14	74	9	89.1
7	22	20	2	44	4	91.6
Total	182	188	57	432	46	90.3

* There were five abnormal-eyed individuals of this generation in which the location of the eye abnormality was not recorded.

Line 9000C

GENERATION OF SELECTION	RIGHT EYE ABNORMAL	LEFT EYE ABNORMAL	BOTH EYES ABNORMAL	TOTAL ABNORMAL	NORMAL	PERCENT ABNORMAL
1	6	9	10	25	8	75.7
2	16	15	11	42	2	95.4
3	23	25	10	58	10	85.2
4	34	39	7	80	13	86.0
5	23	14	2	39	10	79.5
6	9	13	7	29	14	67.4
Total	111	115	47	273	57	82.7

The high degree to which the incidence and type of eye abnormality in strains 9000B and 9000C have paralleled those in Line 700 is further shown by the distribution of the abnormality. This is as follows (table 5).

TABLE 5

	RIGHT EYE ABNORMAL	PERCENT	LEFT EYE ABNORMAL	PERCENT	BOTH EYES ABNORMAL	PERCENT
Line 700	250	44.8	241	43.2	66	12.0
Line 9000B	182	42.6	188	44.0	57	13.4
Line 9000C	111	40.6	115	42.1	47	17.3

From the close correspondence of the figures in the three strains it is evident that the production and distribution of eye abnormalities is proceeding along the same general line in each case and is the result of the same morphogenetic process.

The distinctly lower percentage of abnormal eyes in Line 9000C, especially in the sixth generation, suggests evidence in support of the idea that gradually we may be getting rid of the abnormality at the anterior end of the body in that line. Further selection will, however, have to be carried on before this fact can be established.

The facts clearly evident are:

1. It is possible to obtain a line (700) high in eye abnormalities, 90.9 percent, and practically free from visible foot abnormalities, 0.4 percent.
2. A line (9000B) high in anterior limb abnormalities, 91.4 percent, is also high in eye abnormalities, 90.3 percent.
3. A line (9000C) relatively higher in posterior limb abnormalities is lower in eye abnormalities (82.7 percent total; 67.4 percent for the sixth selection generation).
4. A line (9000A) can be selected which is low in both eye (0.56 percent) and limb (0.45 percent) abnormalities.

These results show that progress has been made in isolating different degrees of the abnormality in different genetic sub-strains. The greatest degree of abnormality is found in Lines 9000B and 9000C. As the degree of abnormality is lessened the eyes seem to be the last portion of the body from which it disappears.

There is an interesting analogy with piebald spotting in this respect. In mice one of the best examples of persistent localized spotting is found in the white-faced race described by DUNN (1925). Definite persistence of a white forehead characterizes this strain. In out-crosses there is expressed a strong genetic tendency for the spotting to reappear on the forehead.

Localization therefore seems to be strongly established as is that of the eye abnormality above considered.

OUT-CROSS EXPERIMENT

After selection had clearly established in Line 700 a strain high in eye abnormalities it was thought that an out-cross to determine the nature of the inheritance of the abnormality would be desirable.

As a normal parent MURRAY'S strain of LITTLE dilute browns was chosen. Reciprocal crosses were made. A total of 143 F_1 animals, all normal, was obtained.

The two F_2 generations gave a total of 1206 young shown in table 6.

TABLE 6

CLASS OF PROGENY	F_2 dB_r ♀ / ABNORMAL ALBINO ♂	F_2 ABNORMAL ALBINO ♀ / dB_r ♂	TOTAL	EXPECTED 9: 3: 3: 1 UNCORRECTED
Colored normal	382	398	780	678.3
Colored abnormal	79	70	149	226.1
Albino normal	110	113	223	226.1
Albino abnormal	21	33	54	75.4
Total	592	614	1206	

Since inbred abnormals of Line 700 gave 9.1 percent normal overlaps (see table 1) it seemed entirely proper to correct for that figure and to consider that the actual number of abnormals obtained represented 90.9 percent of those which were actually genetically abnormal. Table 7 shows the data after this correction has been made.

TABLE 7

CLASS OF PROGENY	F_2 dB_r ♀ / ABNORMAL ALBINO ♂	F_2 ABNORMAL ALBINO ♀ / dB_r ♂	TOTAL CORRECTED	EXPECTED 9: 3: 3: 1 UNCORRECTED
Colored normal	377	392	769	678.3
Colored abnormal	86	76	162	226.1
Albino normal	108	110	218	226.1
Albino abnormal	23	36	59	75.4

It is clear that there is still a marked deficiency in the number of abnormals observed when compared with expectation. The simplest way to explain this is by hypothesizing differential mortality during embryonic life. The ratios given in tables 6 and 7 were based on counts made at birth.

In order to study the ratio *in utero* 33 pregnant F_1 females were killed. An examination of the embryos showed the following classes: colored normal, 186; colored abnormal, 34; albino normal, 48; albino abnormal, 16; *dead and disintegrating*, 25.

The presence of the twenty-five disintegrating embryos gives the probable clue as to what has happened to a considerable number of abnormal embryos. In most of the disintegrating embryos the process had proceeded so far that classification according to abnormal or normal was impossible. All those that could be identified however were abnormal. It is therefore reasonable to suppose that the group of degenerating embryos is in reality an abnormal class which should be added to the other recorded abnormal groups. In the data derived from litters examined *in utero* there were observed 234 normal and 50 abnormal young. The expected Mendelian proportions are 223 normal and 71 abnormal. If the twenty-five degenerating embryos are added to the abnormal group the numbers become 234 normal and 75 abnormal. A further correction of 9.1 percent of the non-degenerated abnormals recorded is also to be made. This would amount to approximately 4 animals. This would produce a final corrected result of 230 normal 79 abnormal. The expectation is 231 normal and 77 abnormal. The degree of resemblance between corrected observation and the Mendelian expectation is strikingly close.

If we next consider again the F_2 data to which reference has already been made, we shall have to apply to the original data the same two corrections as have already been utilized for the litters recorded *in utero*. The results of doing this are as follows (table 8):

TABLE 8

	NORMAL	ABNORMAL
Observed data uncorrected	1003	203
Expected on 3:1 ratio	904.5	301.5
After correction for "normal overlaps"	984.5	221.5
After correction for embryonic mortality	984	323
Expected on basis of correction	980	327

In applying the correction for embryonic mortality the *in utero* litters have been used as a standard. These showed clearly that for every two abnormal embryos which gave promise of reaching birth in an identifiable condition, there was one that degenerated. The birth ratio of abnormals may then be taken as representing approximately two-thirds of the number

actually formed. The difference may well be accounted for by differential mortality.

The question naturally arises as to whether the high embryonic mortality found in descendants of the Line 700 out-cross also exists in the Lines 9000A and 9000B which are related to Line 700. The opportunity has not yet presented itself to study large numbers of pregnant females. Those studied, however, show no embryonic mortality in Line 9000A and a considerable degree of mortality in *utero* in Line 9000B. Since the latter line is genetically homozygous for the abnormality it follows that certain abnormal individuals die before birth even when no competition with normal embryos is involved. Variation within the genetically abnormal embryos may be considered as constitutional in nature. Competition between normal and abnormal embryos *in utero* is environmental. The two types of factors are operative in producing the high embryonic mortality recorded in the F_2 generation of the out-cross.

LINKAGE TESTS

It is also interesting to determine whether or not any evidence exists for linkage of the abnormality with the genes for albinism, *c*, agouti, *A*, black, *B*, and intensity, *D*, with which it was introduced into the cross.

In order to determine this point the data for the inheritance of each of these pairs of allelomorphs must be tabulated separately.

The situation as regards dilution is as follows. The abnormal individuals are $DDm^{bl}m^{bl}$ in constitution; the dilute brown normals are $ddM^{BL}M^{BL}$. The F_1 generation is therefore $Dm^{bl}dM^{BL}$. In F_2 the intense normal DM^{BL} and dilute abnormal dm^{bl} types are crossover classes, while the intense abnormal Dm^{bl} and dilute normal dM^{BL} are linkage classes. The actual numbers obtained after correction for "normal overlaps" and embryonic mortality are as follows.

	Crossover Normal intense	*Linkage Normal dilute*	*Linkage Abnormal intense*	*Crossover Abnormal dilute*
Observed	516	162	124	67
Expected	486	162	162	54

Of the two linkage groups one is slightly lower than expected; the other is equal to expectation. Both crossover groups are higher. There is certainly no evidence of linkage.

The black gene is introduced along with the abnormality, the brown with its normal allelomorph. The cross is therefore $BBm^{bl}m^{bl} \times bbM^{BL}M^{BL}$. The F_1 generation is $Bm^{bl}bM^{BL}$ in formula. The F_2 corrected figures and expectation follow.

	Observed	*Expected*	
Black normal (crossover)	525	486	Higher
Brown normal (linkage)	161	162	Equal
Black abnormal (linkage)	128	162	Lower
Brown abnormal (crossover)	61	54	Higher

Again the crossover classes are higher than those showing linkage.

Agouti is absent from certain of the F_1 animals so the total numbers will be smaller than in the preceding crosses.

	Observed	*Expected*	
Agouti normal (crossover)	302	297	Higher
Non-agouti normal (linkage)	111	99	Higher
Agouti abnormal (linkage)	84	99	Lower
Non-agouti abnormal (crossover)	25	33	Lower

One linkage class is higher than expectation, one lower. One crossover class is also higher and one lower than their expected quota.

Color and albinism are easily recorded at birth so that the numbers will be higher than in any of the preceding crosses. The cross was made as follows: Colored normal $CCM^{BL}M^{BL}$ × albino abnormal $ccm^{bl}m^{bl}$. F_1 is therefore $CM^{BL}cm^{bl}$.

The F_2 corrected results and expectation follow.

	Observed	*Expected*	
Colored normal (linkage)	767	720	Higher
Colored abnormal (crossover)	212	240	Lower
Albino normal (crossover)	218	240	Lower
Albino abnormal (linkage)	86	80	Higher

Both linkage classes are higher than expectation and both crossover classes are lower.

When a χ^2 test is applied to these figures the probabilities are a little more than 3 chances out of 100 that random sampling will explain the results. The deviation of the four classes however shows that the ultimate recessive, or $ccm^{bl}m^{bl}$ group, departs very slightly from expectation. If a clear-cut case of linkage was involved this class should have been as much in excess as is the $CCM^{BL}M^{BL}$ group. Such is not the case. The final test of linkage must therefore await further experimentation.

OCCURRENCE OF FOOT ABNORMALITIES IN F_2 MICE

Among the 203 F_2 abnormals recorded at birth there were seven with abnormal feet. This is a percentage of 97.0 normals and 3.0 abnormals. It is doubtful whether this is significantly different from the record of the pure "700" stock. In this case there were 99.6 percent normals and 0.4 percent

abnormals. Whether there is a minor difference of some real significance between them cannot be determined from the numbers obtained. It is however certain that the increase in foot abnormalities is in no way comparable to the results which would have been obtained did the incidence of foot abnormalities depend upon a large number of modifying genes present in normal stocks but eliminated by selection from Line 700.

It will be remembered that CASTLE obtained a release from the result of selection of modifiers when he crossed his selected hooded rats with wild stock. No such result is here obtained in the out-cross with normal. This suggests that the reduction in foot abnormalities seen in Line 700 has been accomplished by some sort of change in the abnormal gene m^{bl} or by perhaps a single modifier which both Line 700 and, by chance, the dilute brown normal strain used possess.

The relation of the eye abnormality to that of the limbs will require further study. It is clearly a complex matter involving not only genetic but morphogenetic analysis. The isolation of a line ("700") in which the incidence of limb abnormalities is reduced to a minimum (0.4 percent) is important. The fact that up to now an increase in limb abnormalities by selection has been accompanied by an increase in eye abnormalities suggests that limb abnormalities are a secondary step in the process of bleb formation. As far as the data presented here are concerned limb abnormalities seem to be the result of an excess or overflow of bleb-forming activity which extends beyond the degree of that process necessary to form abnormal eyes. Further selection on the hind limb-abnormal Line 9000C may show that bleb formation influencing the posterior limbs but not the anterior can be separated off as a distinct morphogenetic and genetic process. At present however the results of selection have not reached that point.

CONCLUSIONS

1. The abnormality under consideration in its earliest recognized form consists of a swelling of the myencephalon in embryos 7–9 mm in length. This swelling gives rise to a number of blebs full of colorless fluid (BONNEVIE 1931). It is given the symbol m^{bl} (myencephalic blebs).

2. In its later stages the abnormality consists in deformities of eyes and limbs which follow arrests in development caused by local blood-filled blebs which distort and disturb normal symmetrical development by pressure (BAGG 1929).

3. The abnormality is inherited as a Mendelian recessive. In a line ("700") selected for high incidence of abnormal eyes there is 9.1 percent

overlap with normal. Limb abnormalities in this line occur in 0.4 percent.

4. By selective breeding of the descendants of normal overlaps, a line (9000A) has been established in which the incidence of abnormals has been reduced to 0.7 percent.

5. By selective breeding the incidence of limb abnormalities has been increased in two lines, 9000B (91.42 percent) and 9000C (91.2 percent).

6. Simultaneously these two lines have shown a distinct increase in the incidence of eye abnormalities until they have reached a point which compares favorably with line 700 (90.9 percent) selected for that purpose, Line 9000B (90.3 percent), and Line 9000C (82.7 percent). The distribution of eye abnormalities in these lines is practically identical with that seen in Line 700, the high eye-selection line.

7. When abnormal albino animals from Line 700 are crossed with normal colored mice from the MURRAY derivative of the LITTLE dilute brown (db_r) stock, the F_1 is all normal. The F_2 shows a 9:3:3:1 ratio with a significant deficiency of abnormal mice.

8. Besides the correction for normal overlap, 9.1 percent, studies of embryonic ratios *in utero* show that approximately 8 percent of the embryos degenerate *in utero*. Whenever identification of these has been possible, they have proved to be abnormals. The numbers obtained make a correction appear necessary. The incidence of degenerating embryos is sufficiently high to amount to one-half of the living abnormal embryos.

9. When corrections of the numbers for these two modifying agents are made the ratios obtained fit the Mendelian expectation closely.

10. When the separate factors are studied to determine possible linkage relations with the abnormal gene m^{bl} they all show clearly random segregation with the exception of the *Cc* pair of allelomorphs. In this case there is evidence of linkage which however must be tested by a backcross generation before being considered conclusive.

11. The low incidence of foot abnormalities in F_2 mice indicates that the abnormal-eyed parent line ("700") has not been developed by the gradual selection of many modifiers but by some other genetic process.

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