THE PHENOTYPIC MODIFICATION OF HEREDITARY POLYDACTYLISM OF FOWL BY SELECTION AND BY INSULIN*

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IT IS known from earlier investigations that hereditary polydactylism of fowl has a variable phenotype. The present report deals with two separate attempts to modify experimentally the expression of this mutation. In the first part we shall relate the results of matings which were made in order to test the effect of selection on several distinct phenotypes of polydactylism. In the second part a description will be given of the effects which the injection of insulin, at various stages of development, has on the expression of this mutation.

SELECTION EXPERIMENTS

The polydactylous or five-toed condition, which is typical of such breeds as Houdans, Dorkings and Silkies, is transmitted as a dominant trait (BATESON 1902; BATESON and PUNNETT 1905; HURST 1905; DUNN and JULL 1927; PUNNETT and PEASE 1929; WARREN 1944). All authors agree that the genetic transmission of polydactylism shows irregularities which have not been explained satisfactorily. There is no reason to believe that these breeds differ genetically in regard to polydactylism; in fact, what little is known about the history of Houdan and Dorking fowl suggests common ancestry for the polydactylous trait.

Within the five-toed breeds inheritance and morphological expression of the polydactylous condition show a high degree of regularity, although occasional individuals have an aberrant type of toe development or lack the extra toes altogether. But whenever representatives of the polydactylous breeds are crossed with normal-toed birds the resulting F_2 and further crossbred progenies tend to give irregular segregation ratios and show a great array of morphological variants of the polydactylous condition. Those variants include polyphalangism, that is the presence of a lengthened in place of a duplicated first toe, and heterodactylism, that is the presence of an extra toe on one but not on the other foot or a greater amount of duplication on one side as compared with the other. Throughout this report the term heterodactylism is limited to the association of one polydactylous or polyphalangeal and one normal foot. In order to avoid clumsy or misleading terminology we shall (if linguistically improperly) designate as "sinistral" heterodactylism presence of a hyperdactylous condition on the left, but not on the right, foot and as "dextral" heterodactylism the reverse condition.

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It was the purpose of our selection experiments to determine whether or not some of the special forms of expression of polydactylism are determined or influenced by specific genetic factors. The foundation material for our matings was obtained from DR. D. C. WARREN. It was a crossbred stock in which the polydactylous condition had originated from Silkie Bantam and Houdan fowl. Crosses of these chickens to White Leghorns furnished the material for the matings which will be discussed below. In addition, we have data from Dorking fowl matings.

The variation in expression of polydactylism in crossbred matings is far too great to allow of any but a somewhat arbitrary classification. We have used the following rules. All cases in which both feet showed more than four toes were called bilaterally hyperdactylous. Also included in this group were those birds in which one foot was polydactylous and the other polyphalangeal. Among the bilaterally polyphalangeal progenies we counted, in addition to uncomplicated cases, those in which one or both of the lengthened first toes had either slight lateral outgrowths or terminal duplications which did not involve more than the distal-most phalanx. Finally, the heterodactylous groups include all animals which had unilateral polydactylism, whether it was of the polyphalangeal type or a duplication *sensu stricto*. Our data consist of descriptive records from hatched chicks and embryos which had died during the last four days of incubation.

A few general observations are in order before we turn our attention to the results of selection for specific types of polydactylism. In the first place, our data give some information about the occurrence of segregation ratios with an excess of non-polydactylous chicks. In matings in which all parents were homozygous for polydactylism (matings 7-1942, 5-1943, 5-1944, 27-1945 in table 1; 4-1941, 4-1942, 4-1943 and 4-1944 in table 2) 12 non-polydactylous chicks or 0.5 percent were found among a total of 2440 chicks. Among 610 Dorking chicks (table 9, controls) the incidence of non-polydactylous progeny amounted to 1.3 percent. Matings in which all parents were heterozygous for polydactylism and had a *bilaterally polydactylous* or *bilaterally polyphalangeal* phenotype (matings 33-1939, 31-1941, 27-1942, 27-1943, 27-1944, 22-1941 in table 1 and 19-1939 in table 2) gave a segregation of 2382 polydactylous and 846 non-polydactylous chicks with an expectation of 2421 and 807, respectively. The deviation from a 3:1 expectation is not significant ($\chi^2 = 2.513$). In matings of heterozygous parents which were *heterodactylous* (data of tables 3 and 4), on the other hand, the progenies were composed of 2394 polydactylous and 1633 non-polydactylous chicks, where the expectation for a 3:1 ratio was 3020 and 1007, respectively. The deficiency in polydactylous offspring is statistically highly significant ($\chi^2 = 518.9$). This shows that heterozygous parents with a less pronounced phenotypic expression of polydactylism have a higher percentage of non-polydactylous progeny (normal overlaps) than those with a more extreme phenotype. It follows that the two groups must differ genetically.

Our data also demonstrate that heterodactylism occurs only as a rare exception in animals which are homozygous for polydactylism. The four matings of homozygous and bilaterally polydactylous parents listed in table 1 (matings

7-1942, 5-1943, 5-1944 and 27-1945) produced no heterodactylous chicks among 723 offspring. Another mating, in which all parents had six toes on each foot (table 8, controls), produced no heterodactylous chicks in a progeny of 273. Matings of homozygous, bilaterally polyphalangeal birds (4-1941, 4-1942, 4-1943 and 4-1944 in table 2) yielded 0.16 percent heterodactylism among a total of 1717 chicks, and a pen of Dorking fowl (table 9, controls) had 3.1 percent heterodactylous offspring in a progeny of 610 chicks. In contrast to these results, we found that those two matings of heterozygous and bilaterally polydactylous parents (33-1939 and 22-1941), in which little selection for any one particular type of expression of polydactylism had taken place, gave 13.0 percent heterodactylism among 794 hyperdactylous chicks. It can be concluded, as was suggested previously by BOND (1920), that as a rule heterodactylism is a heterozygous form of expression of polydactylism, and that, similar to the non-polydactylous overlaps, it occurs only rarely in homozygous polydactylous chicks.

PUNNETT and PEASE (1929) observed in their (rather small) material that matings which produced no chicks with dextral heterodactylism had fewer non-polydactylous offspring than occurred among progenies containing individuals with dextral heterodactylism. Such an association, if real, would be of considerable interest. We, therefore, tabulated our data from matings of heterozygous parents according to the incidence of dextral heterodactylism. The following frequencies of non-polydactylous progeny were found in the four groups.

Incidence of dextral heterodactylism in percent of all hyperdactylous	Total number of chicks	Non-polydactylous %
0	791	41.8±1.75
0.1–2.0	3932	32.6±0.74
2.1–4.0	912	31.7±1.55
>4.0	1688	44.1±1.25

Obviously there i no consistent trend. The difference in incidence of non-polydactylous chicks between progenies without cases of dextral heterodactylism and those in which more than four percent of the hyperdactylous chicks were dextrally heterodactylous was not significant. We conclude, therefore, that such differences as exist are spurious in nature.

The first of our selection lines was established for the purpose of breeding for a high incidence of the bilaterally five-toed condition. The birds for further matings were chosen each year from the progenies of those parents which had given the highest incidence of this condition. The relative frequency of bilaterally polydactylous chicks rose from an initial 82.5 to about 94 percent of all hyperdactylous offspring. These percentages include all forms of bilateral hyperdactylism, except bilateral polyphalangism. The effect of selection is not impressive, though undoubtedly real. At the end of this series of experiments all birds still were heterozygous for the polydactylism gene. This is explained by the subsequent observation that in our material homozygosity for poly-

dactylism was associated with the presence of more than five toes on each foot.

In 1941 a new line was established in which we selected for the occurrence of six toes on each foot (lower part of table 1). It can be seen that the incidence of animals with more than five toes on each foot rapidly rose from an initial 6.6 percent to 75.6 percent in the last generation. All animals of the last four generations were homozygous for polydactylism; bilateral polyphalangism was

TABLE 1

Selection for bilateral expression of polydactylism. The upper part of the table shows results of selection for five toes on each foot; the lower part gives the data of selection for the presence of more than five toes on each foot. Figures in parenthesis give incidence of chicks with more than five toes in percent of all polydactylous.

MATING AND YEAR	TOTAL PROGENY	TOTAL POLYDACTYLOUS	IN PERCENT OF ALL POLYDACTYLOUS			
			BILATERAL POLYDACTYLISM	BILATERAL POLYPHALANGISM	SINISTRAL HETERODACTYLISM	DEXTRAL HETERODACTYLISM
33-1939	685	492	82.5	7.3	8.3	1.8
28-1940	133	120	82.5	1.7	13.3	2.5
31-1941	614	473	94.3	2.1	3.2	0.4
27-1942	360	275	93.8	5.8	0.4	0
27-1943	314	259	88.8	10.4	0.8	0
27-1944	440	322	94.1	3.4	2.2	0.3
22-1941	416	302	82.5 (6.6)	0	14.2	3.3
7-1942	155	154	99.4 (31.2)	0.6	0	0
5-1943	215	214	100 (55.6)	0	0	0
5-1944	218	218	96.3 (72.0)	3.7	0	0
27-1945	135	135	94.8 (75.6)	5.2	0	0

rare, but the form of expression of bilateral hyperdactylism remained very variable.

The results of selection for bilateral polyphalangism are shown in table 2. Beginning with 1940 all animals in our matings were homozygous for hyperdactylism. The incidence of bilateral polyphalangism rose from an initial 68.3 percent (of all hyperdactylous) to 88.6 percent at the end of the experiment. This increase was accomplished by the virtual disappearance of heterodactylism and by a gradual decline in the frequency of bilaterally polydactylous animals. It must be added, however, that entirely uncomplicated bilateral polyphalangism was, in our material, more commonly found in matings of

heterozygous than of homozygous animals. As the parents became homozygous for hyperdactylism one or both first toes, in a high percentage of the polyphalangous progeny, showed duplication of the toe nail or of the first phalanx or small lateral outgrowths in varying positions. The lengthening of the first toe continued to exist, but was at the same time complicated by a trend toward duplication. Polyphalangism without duplication tends to be limited to heterozygotes. This accounts for our failure to establish a line which breeds true for uncomplicated bilateral polyphalangism. In an outcross of a homozygous, bilaterally polyphalangeal male to Leghorn females (table 6, mating 19-1940) the incidence of bilateral polyphalangism amounted to only 5.1 percent com-

TABLE 2

Results of selection for bilateral expression of polyphalangism.

MATING AND YEAR	TOTAL PROGENY	TOTAL POLYDACTYLOUS	IN PERCENT OF ALL POLYDACTYLOUS			
			BILATERAL POLYDACTYLISM	BILATERAL POLYPHALANGISM	SINISTRAL HETERODACTYLISM	DEXTRAL HETERODACTYLISM
19-1939	399	259	25.5	68.3	5.0	1.2
19-1940	294	279	28.7	69.2	1.4	0.7
4-1941	496	488	20.9	77.0	1.8	0.2
4-1942	394	394	31.7	66.8	1.5	0
4-1943	494	492	11.8	87.0	0.8	0.4
4-1944	333	333	9.6	88.6	1.2	0.6

pared with 69.2 percent when the same cock was bred to homozygous, bilaterally polyphalangeal females.

WARREN (1944) concluded from his data that polyphalangism is "a factor which behaves as an autosomal dominant with some unknown factors causing variability in dominance." Our observations cannot be reconciled with this interpretation. It seems likely, both from Warren's and our own results, that the modifying situation which produces the polyphalangous type of hyperdactylism has a relatively simple genetic basis, but the mode of transmission must differ considerably between stocks.

The results of selection for sinistral heterodactylism are reproduced in table 3. The matings consisted throughout of birds which were heterozygous for polydactylism since, as was stated previously, heterodactylism is as a rule a heterozygous expression of the mutation. In the course of seven generations of selection the incidence of sinistral heterodactylism rose from 12.6 percent to 33.1 percent of the hyperdactylous progenies. The difference equals 20.5 ± 4.06 percent. This highly significant increase in sinistral heterodactylism occurred entirely or almost entirely at the expense of bilateral polyphalangism which disappeared nearly completely from the stock. The results seem to show clearly that the modifying agencies which are responsible for bilateral polyphalangism and for sinistral heterodactylism are incompatible. The incidence of bilateral polydactylism was not affected, nor did selection have any notice-

able effect on the frequency with which dextral heterodactylism occurred. It should be noted that in three matings of heterodactylous and polyphalangeal males with sinistral expression of the hyperdactylous condition to Leghorn females (matings 31-1940, 16-1942 and 32-1938 in table 6) the incidence of sinistral heterodactylism remained relatively high in the resulting progenies (29.0, 13.3 and 28.9 percent, respectively). This may indicate some degree of dominance of the modifying factors which produce heterodactylism, but is

TABLE 3

Results of selection for sinistral heterodactylism.

MATING AND YEAR	TOTAL PROGENY	TOTAL POLYDACTYLOUS	IN PERCENT OF ALL POLYDACTYLOUS			
			BILATERAL POLYDACTYLISM	BILATERAL POLYPHALANGISM	SINISTRAL HETERODACTYLISM	DEXTRAL HETERODACTYLISM
27-1938	185	119	64.7	21.8	12.6	0.8
31-1939	683	436	66.1	14.7	17.9	1.4
31-1940	117	63	69.8	4.8	25.4	0
15-1941	203	121	68.6	2.5	25.6	3.3
16-1942	293	200	72.5	3.0	21.5	3.0
3-1943	428	250	72.0	1.2	25.6	1.2
3-1944	498	299	65.6	0.7	33.1	0.7

TABLE 4

Results of selection for dextral heterodactylism.

MATING AND YEAR	TOTAL PROGENY	TOTAL POLYDACTYLOUS	IN PERCENT OF ALL POLYDACTYLOUS			
			BILATERAL POLYDACTYLISM	BILATERAL POLYPHALANGISM	SINISTRAL HETERODACTYLISM	DEXTRAL HETERODACTYLISM
20-1941	243	129	63.6	3.9	24.0	8.5
8-1942	334	122	69.7	1.6	23.8	4.9
24-1943	265	134	68.7	5.2	18.7	7.5
24-1944	364	243	72.0	2.5	16.0	9.5
1-1945	149	100	66.0	2.0	17.0	15.0
1-1946	265	178	56.7	12.9	19.1	11.2

more likely a result of the presence of these modifying factors in our (outbred) polydactylous as well as the Leghorn stock.

The data from our line selected for dextral heterodactylism are presented in table 4. It can be seen that selection did not produce a pronounced or consistent change in the incidence of dextral heterodactylism, although there appears to be some trend toward a higher frequency of this type of hyperdactylism. A comparison of the first three and the last three generations of selection gives a difference in dextral heterodactylism of 4.01 ± 1.90 percent in favor of the later generations. This is on the border line of statistical signifi-

cance. A comparison of the data in table 4 with those of tables 3 and 6 shows, however, that every one of the matings of heterozygous and dextrally heterodactylous parents produced a higher incidence of dextrally heterodactylous offspring than any of the matings of heterozygous parents with hyperdactylism other than dextral heterodactylism. If the results for all sinistrally heterodacty-

TABLE 5

Comparison of over-all incidence of dextral heterodactylism, in percent of all polydactylous, in the lines selected for sinistral and dextral heterodactylism, respectively.

SELECTION FOR	TOTAL POLY-DACTYLOUS	INCIDENCE OF DEXTRAL HETERO-DACTYLISM %	DIFFERENCE IN %
Sinistral heterodactylism	1488	1.5±0.31	8.1±1.01
Dextral heterodactylism	938	9.6±0.96	

TABLE 6

Results of outcrosses.

TYPE OF CROSS	MATING AND YEAR	TOTAL PROGENY	TOTAL POLYDACTY-LOUS	IN PERCENT OF ALL POLYDACTYLUS			
				BILATERAL POLYDACTY-LISM	BILATERAL POLY-PHALANGISM	SINISTRAL HETERO-DACTYLISM	DEXTRAL HETERO-DACTYLISM
Leghorn×bilaterally polydactylous	28-1940	106	90	82.2	0	14.4	3.3
Leghorn×bilaterally polyphalangeal	19-1940	161	157	84.7	5.1	5.7	4.5
Leghorn×sinistrally heterodactylous	31-1940	194	69	62.3	1.4	29.0	1.2
	16-1942	221	105	86.7	0	13.3	0
Leghorn×sinistrally polyphalangeal	32-1938	532	194	53.1	16.5	28.9	1.5
Leghorn×dextrally heterodactylous	11-1940	352	169	81.1	1.2	15.4	2.4
	20-1941	488	232	83.6	5.6	8.2	2.6

lous (table 3) and all dextrally heterodactylous (table 4) parents are compared with reference to the frequency of dextral heterodactylism among the progenies a difference of 8.1±1.01 percent is found in favor of the matings of dextrally heterodactylous parents (table 5). This difference is highly significant. It cannot be doubted, therefore, that genetic factors influence the occurrence of dextral heterodactylism.

THE EFFECT OF INSULIN

Our experiments with insulin were designed to study the effect of this hormone on the expression of polydactylism. Iletin (Insulin, Lilly) was injected into eggs at 24-hour intervals between 24 and 144 hours of incubation. The details

of technique were the same as described earlier (LANDAUER 1945). Eggs from three different matings were used as follows: (a), Leghorn pullets mated to cocks of Silkie-Houdan-Leghorn crossbred origin which were homozygous for polydactylism and had six toes on each foot; (b), *inter se* matings of homozygous polydactylous fowl of Silkie-Houdan-Leghorn crossbred origin, all of which were bilaterally six-toed; (c), *inter se* matings of Silver Gray Dorking fowl. For each of the developmental test stages and the three sources of origin we had groups of eggs injected with two and five units of insulin and untreated

TABLE 7

Insulin experiments with eggs from matings of Leghorn pullets and homozygous polydactylous, bilaterally six-toed cocks of Silkie-Houdan-Leghorn crossbred origin.

TREATMENT	TIME OF INJECTION	N	BILATERAL POLYDACTYLISM %	NOT BILATERALLY POLYDACTYLOUS %	HETERODACTYLISM %	BILATERAL POLYPHALANGISM %	UNI- OR BILATERAL ECTRODACTYLISM %	NORMAL %
2 units insulin	24	73	68.5	31.5	8.2	19.2	1.4	2.7
	48	78	79.5	20.5	2.6	15.4	1.3	1.3
	72	100	46.0	54.0	8.0	40.0	0	6.0
	96	95	43.2	56.8	8.4	34.7	0	13.7
	120	74	81.1	18.9	2.7	16.2	0	0
	144	106	91.5	8.5	0.9	7.5	0	0
5 units insulin	24	67	77.6	22.4	4.5	16.4	0	1.5
	48	102	66.7	33.3	2.9	28.4	1.0	1.0
	72	102	52.0	48.0	2.9	35.3	1.0	8.8
	96	101	35.6	64.4	9.9	37.6	0	16.8
	120	77	81.8	18.2	0	15.6	0	2.6
	144	113	85.0	15.0	0.9	14.2	0	0
Untreated controls for stated periods of injection	24	130	83.1	16.9	0.8	16.2	0	0
	48	162	87.0	13.0	0.6	9.3	0	3.1
	72	119	85.7	14.3	0.8	13.4	0	0
	96	114	90.4	9.6	0	9.6	0	0
	120	78	84.6	15.4	0	15.4	0	0
	144	110	82.7	17.3	1.8	15.5	0	0

controls. The results of these experiments are reproduced in tables 7 to 9.

In the absence of experimental interference all three types of matings gave progenies with a high incidence of bilateral polydactylism. Among the F_1 offspring of Leghorn and homozygous polydactylous fowl 83.1 to 90.4 percent were bilaterally polydactylous (mean of aberrant types 14.3 ± 1.31 percent); 83.3 to 95.3 percent bilaterally polydactylous progeny were found among the offspring of *inter se* matings of homozygous polydactylous chickens of crossbred origin (mean of aberrant types 13.9 ± 2.11 percent), and for the Dorking progenies the corresponding figures were 93.3 to 100 percent (mean of aberrant types 4.6 ± 0.84 percent).

The over-all effect of insulin is shown by the totals of all chicks which were not bilaterally polydactylous. It can be seen that with all three stocks and with both dosages of insulin the maximum deviations from the normal incidence of bilateral polydactylism occurred at 72 and 96 hours. At the 120 and

144 hour test periods insulin did no longer change the expression of polydactylism. It is evident, on the other hand, that even at the earliest developmental stages which were exposed to insulin, 24 and 48 hours, the treatment produced an appreciable effect.

It is true for all three stocks that the two concentrations of insulin (2 and 5 units) did not result in consistently different results. This holds for the totals

TABLE 8

Insulin experiments with eggs from inter se matings of homozygous polydactylous, bilaterally six-toed fowl of Silkie-Houdan-Leghorn crossbred origin.

TREATMENT	TIME OF INJECTION	N	BILATERAL POLYDACTYLISM %	NOT BILATERALLY POLYDACTYLOUS %	HETERODACTYLISM %	BILATERAL POLYPHALANGISM %	UNI- OR BILATERAL ECTRODACTYLISM %	NORMAL %
2 units insulin	24	22	59.1	40.9	0	40.9	0	0
	48	18	66.7	33.3	0	27.8	5.6	0
	72	21	38.1	61.9	0	61.9	0	0
	96	23	39.1	60.9	4.3	52.2	0	4.3
	120	17	88.2	11.8	0	11.8	0	0
	144	43	93.0	7.0	0	7.0	0	0
5 units insulin	24	13	61.5	38.5	0	38.5	0	0
	48	16	56.3	43.8	0	43.8	0	0
	72	19	26.3	73.7	0	63.2	0	10.5
	96	16	37.5	62.5	0	43.8	0	18.8
	120	17	88.2	11.8	0	11.8	0	0
	144	37	94.6	5.4	2.7	2.7	0	0
Untreated controls for stated periods of injection	24	38	84.2	15.8	0	15.8	0	0
	48	47	85.1	14.9	0	14.9	0	0
	72	60	85.0	15.0	0	15.0	0	0
	96	43	95.3	4.7	0	4.7	0	0
	120	30	83.3	16.7	0	16.7	0	0
	144	55	83.6	16.4	0	14.5	0	1.8

of all aberrations from bilateral polydactylism as well as for the various individual types of expression. Furthermore, there is no consistent difference in the effects obtained at 72 and 96 hours. For statistical analysis it, therefore, seemed justified to combine within each of the three stocks all data obtained for two and five units of insulin at both 72 and 96 hours of incubation.

In regard to the extent of the insulin effect at the period of maximum action (72 and 96 hours) the following differences were found between the treated (2 and 5 units insulin) and control groups in percent of progeny which was not bilaterally polydactylous:

F_1 (Leghorn×outbred homozygous polydactylous)	43.8 ± 3.27 percent
Outbred homozygous polydactylous *inter se*	53.9 ± 6.14 percent
Dorking	20.9 ± 3.13 percent

It is evident that insulin produced in all three stocks a very striking and highly significant departure from the normal incidence of bilateral polydactylism. It can be seen, furthermore, that these insulin-produced phenotypic shifts were similar in extent in the heterozygous and homozygous progenies of

Silkie-Houdan outbred ancestry, but that in the Dorking stock the response was much less extreme.

We have already pointed out that even in the earliest stages which we tested insulin had some effect on the expression of polydactylism. The combined data for the 24 and 48 hour stages and the two dosages gave the following differ-

TABLE 9

Insulin experiments with eggs from Dorking fowl.

TREATMENT	TIME OF INJECTION	N	BILATERAL POLYDACTYLISM %	NOT BILATERALLY POLYDACTYLOUS %	HETERODACTYLISM %	BILATERAL POLYPHALANGISM %	UNI- OR BILATERAL ECTRODACTYLISM %	NORMAL %
2 units insulin	24	48	85.4	14.6	4.2	0	0	10.4
	48	69	89.9	10.1	1.4	0	2.9	5.8
	72	80	78.8	21.3	5.0	1.3	0	15.0
	96	65	70.8	29.2	12.3	1.5	0	15.4
	120	58	94.8	5.2	0	1.7	0	3.4
	144	55	96.4	3.6	3.6	0	0	0
5 units insulin	24	56	82.1	17.9	8.9	0	0	8.9
	48	72	88.9	11.1	5.6	0	0	5.6
	72	69	76.8	23.2	13.0	0	0	10.1
	96	61	67.2	32.8	8.2	0	0	24.6
	120	45	100	0	0	0	0	0
	144	55	92.7	7.3	3.6	1.8	0	1.8
Untreated controls for stated periods of injection	24	90	96.7	3.3	2.2	0	0	1.1
	48	136	93.4	6.6	5.9	0	0	0.7
	72	117	94.9	5.1	3.4	0.9	0	0.9
	96	104	93.3	6.7	2.9	0	0	3.8
	120	84	96.4	3.6	2.4	0	0	1.2
	144	79	100	0	0	0	0	0

ences between treated and control groups in incidence of chicks which were not bilaterally polydactylous:

F_1 (Leghorn×outbred homozygous polydactylous)	12.8±3.26 percent
Outbred homozygous polydactylous *inter se*	23.8±6.99 percent
Dorking	5.3±1.49 percent

The differences are significant for all three types of matings. In these early stages, as in the later ones which were discussed above, the Dorkings gave a much less pronounced response than the stocks of outbred origin.

At 120 and 144 hours of development the insulin effect has disappeared. A comparison within the treated groups (2 and 5 units insulin) of the values for the maximum response periods (72 and 96 hours) and the late stages (120 and 144 hours) gave the following differences in frequency of non-bilaterally polydactylous offspring:

F_1 (Leghorn×outbred homozygous polydactylous)	41.2±3.08 percent
Outbred homozygous polydactylous *inter se*	56.7±5.90 percent
Dorking	22.0±3.01 percent

These values are very similar to those given above for the differences between

treated and control groups at the period of maximum response, indicating the disappearance of the insulin effect in the late stages.

An examination of the data in tables 7 to 9 shows interesting differences between the three stocks in regard to the specific types of deviations from bilateral polydactylism which are produced by insulin. Among the untreated progenies from the outbred stock, whether hetero- or homozygous, bilateral polyphalangism is the most common aberration, and bilateral polyphalangism is also by far the most frequent aberrant phenotype in the insulin-treated material. In the Dorking stock, on the other hand, bilateral polyphalangism is very rare. It remains similarly rare after the injection of insulin. Heterodactylism was absent from the control data of outbred homozygous polydactylous origin and very rare in the insulin-treated material. Heterodactylism did occur among the control chicks of the outbred heterozygous polydactylous and the Dorking stocks and insulin significantly increased its incidence. Normal, that is non-polydactylous, chicks were more common among Dorking progenies than in the two other stocks and the same was true for the insulin data. It should be observed, however, that heterodactylism was found more

TABLE 10

Differences between insulin-treated (72 and 96 hours; 2 and 5 units) and control groups in incidence of various phenotypic expressions of polydactylism.

PHENOTYPE	F_1 (LEGHORN×OUTBRED HOMOZYGOUS POLYDACTYLOUS)	OUTBRED HOMOZYGOUS POLYDACTYLOUS *inter se*	DORKING
Heterodactylism	6.9±1.37	1.3±1.31	6.8±2.14
Bilateral polyphalangism	25.3±3.20	45.0±6.33	0.7±0.56
Normal	11.1±1.59	7.6±2.98	13.3±2.52

commonly than the normal condition among the controls of the outbred heterozygous polydactylous and Dorking chicks, but that in the insulin-treated material these positions were reversed, that is, the incidence of normal chicks in both instances was more exaggerated than that of heterodactylous ones. All these conclusions are illustrated by the data of table 10.

Attention must finally be called to the fact that in a number of instances ectrodactylism was observed in the insulin-treated material. All these cases occurred after the injection of insulin between 24 and 72 hours of development. For the three stocks and the two concentrations of insulin we had 1025 chicks from eggs which had been injected between 24 and 72 hours with an incidence of 0.68±0.26 percent of ectrodactylism. No cases of ectrodactylism were found among 899 chicks from control eggs incubated during the same periods. It is important to observe, however, that ectrodactylism is more likely to occur in polydactylous than in other stocks. Between 1938 and 1946 we found 8 cases of ectrodactylism (0.069±0.024 percent) among 11,654 chicks from matings of polydactylous fowl. No ectrodactylous chicks occurred among 1760 chicks obtained during the same period from non-polydactylous parents which

had been extracted from matings of heterozygous polydactylous fowls, nor were any ectrodactylous chicks met with among large numbers of Leghorn chicks hatched in the course of the same years.

DISCUSSION

The high degree of variability in the expression of polydactylism of fowl has been noted by all observers who have worked with this trait. All attempts to fit the phenotypes into a small number of distinct morphological groups do violence to the actual situation. A series of imperceptible steps links the four-toed with the six-toed condition and the five-toed phenotype with hyperphalangism. The same may even be said in regard to heterodactylism and bilateral hyperdactylism (plate I). Any attempt to study specific variants of hyperdactylism must, therefore, deal with trends in one or another direction of phenotypic expression and must delimit the end-points of these series from intermediate forms.

The data of earlier investigators have already given some indication that hereditary factors influence the specific expression of polydactylism. This is particularly true for the observations of WARREN (1944) on polyphalangism and several modes of expression of bilateral polydactylism. The data of the present report lead to the following conclusions:

1. Heterodactylism is generally a heterozygous expression of polydactylism, but occurs among homozygotes as a rare variant.
2. The incidence of sinistral heterodactylism can be raised by selection.
3. Dextral heterodactylism, which in the absence of selection is much less common than the opposite form of asymmetry, shows also less response to selection. However, *inter se* matings of parents with dextral heterodactylism produce significantly more progeny with heterodactylism of this type than any other kind of mating.
4. Bilateral polyphalangism responds readily to selection. Uncomplicated bilateral polyphalangism occurs chiefly, perhaps exclusively, among heterozygous animals. In homozygous polyphalangeal chicks the lengthened first toe generally, if not always, shows some indication of duplication.
5. The incidence of bilaterally five-toed and bilaterally six-toed chicks can readily be increased by selection. The presence of five toes on both feet was a heterozygous expression in our material of outbred origin, but was the rule among homozygous pure-bred Dorking fowl. Homozygous birds of our outbred stock had, almost without exception, more than five toes on one or both feet.
6. Our data, as those of earlier observers, show that non-polydactylous chicks are, on rare occasions, found among the progenies of homozygous polydactylous fowl. Heterozygous polydactylous parents with a low degree of ex-

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PLATE I.—Hyperdactylous feet, selected to illustrate the transgressive nature of variation of this trait. Top row: transition from four-toed to five-toed condition; middle row: stages leading from the five-toed to the six-toed phenotype; bottom row: steps connecting hyperphalangism with the five-toed expression of polydactylism.

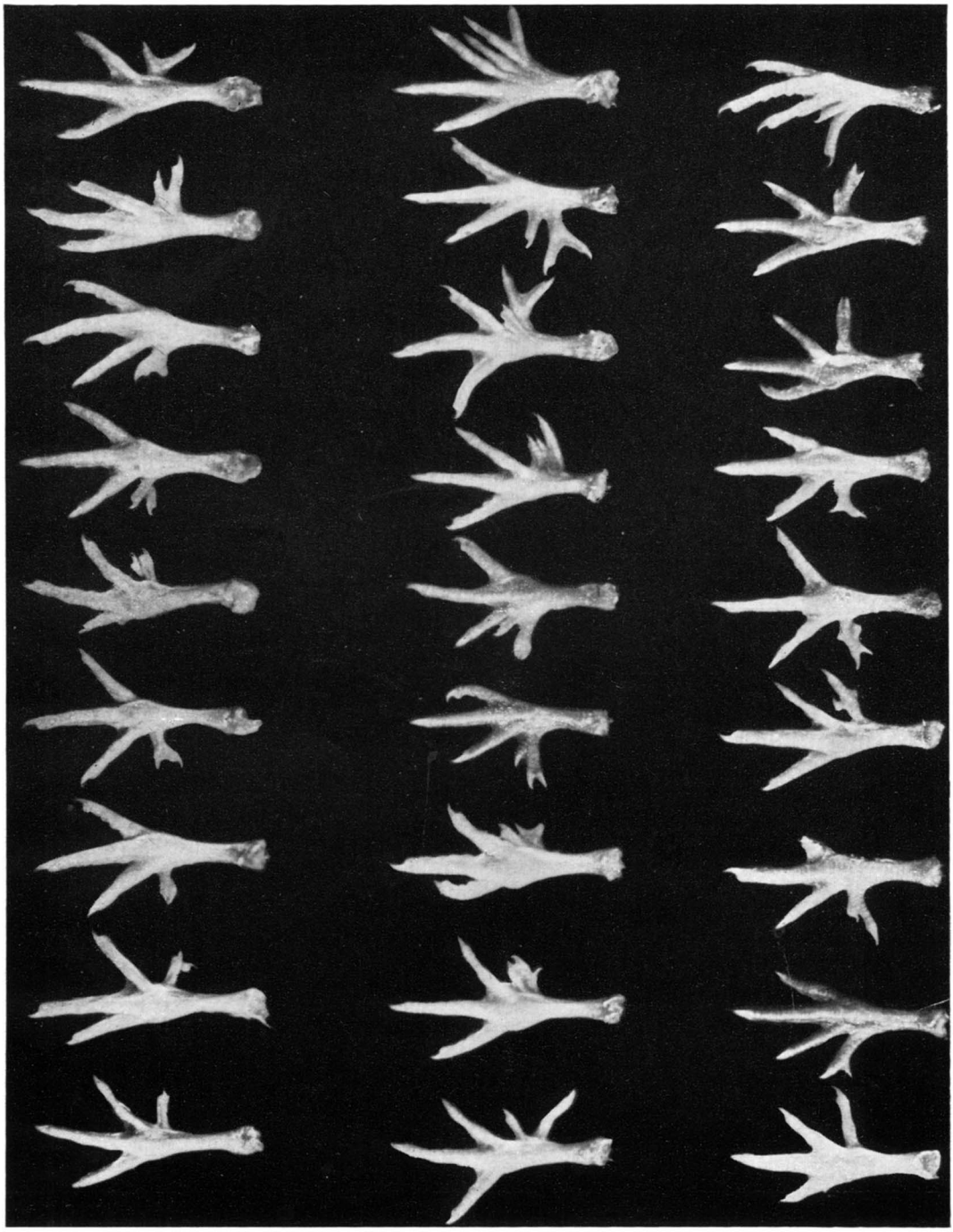

PLATE I. See opposite page for description.

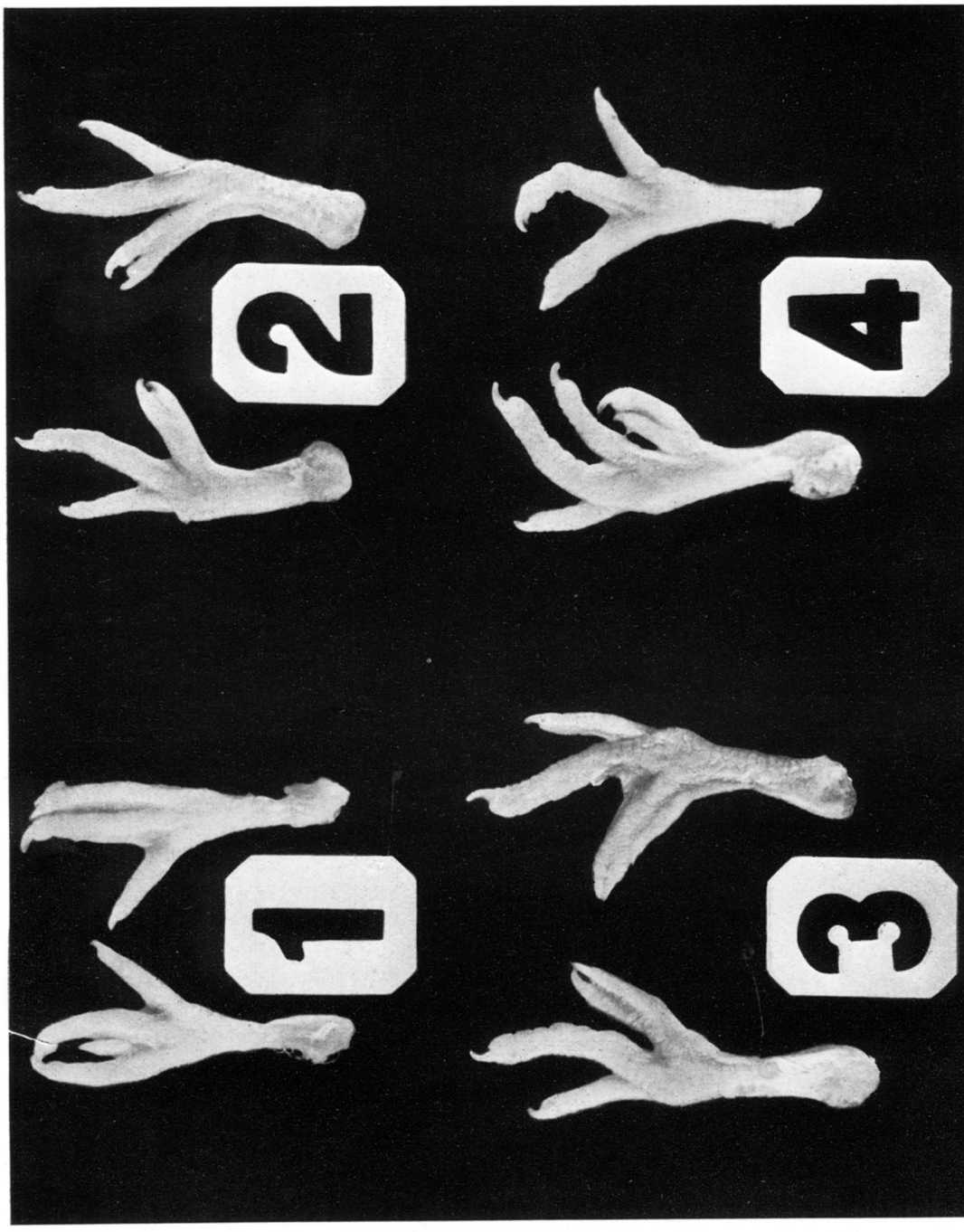

PLATE II. See opposite page for description.

pression of polydactylism (heterodactylism) produce significantly more non-polydactylous offspring than heterozygous parents with a higher degree of gene expression (bilateral polydactylism or bilateral polyphalangism).

These observations demonstrate beyond doubt that all the different forms of expression of polydactylism are influenced by hereditary factors. It is equally clear that none of the modifications are brought about by simple genetic changes. The transgressive nature of the variations points to the conclusion that the various forms of expression of polydactylism arise on the basis of developmental processes which can easily be disturbed and pushed into one of several directions. It seems likely, furthermore, that these processes are of a quantitative nature.

One of the most interesting aspects of polydactylism is the occurrence of unilateral gene expression, that is heterodactylism. All observers agree that sinistral heterodactylism is much more common than the opposite form (BOND 1920, 1925–1926; PUNNETT and PEASE 1929; VAINIKAINEN 1935; WARREN 1944; data of the present report), although it was shown by PUNNETT and PEASE that this trend may be reversed in exceptional matings. It was already observed by BOND and is confirmed by our own observations that the same kind of asymmetry is found in cases of unequal expression of bilateral hyperdactylism, that is an excess on the left side is much more common than the opposite situation. Furthermore, this generalization also holds for expression of polydactylism in the upper extremities. From our Silkie-Houdan-outbred stock a line has been produced by selection in which supernumerary digits are prominently developed in the wings (unpublished). Such well-developed supernumerary fingers occur only in birds which are homozygous for polydactylism. Sinistral heterodactylism of the wings is much more common than the dextral form of expression.

It is of considerable interest that conditions of asymmetry similar to those in fowl have been found in guinea pigs. It is always true that among the cases of asymmetrical expression extra toes occur more often on the left than on the right side (CASTLE 1906; PICTET 1932; KRÖNING and ENGELMANN 1934; WRIGHT 1934). Further, similar to the situation in poultry, KRÖNING and ENGELMANN observed that among cases of asymmetrical bilateral polydactylism in guinea pigs more pronounced sinistral development occurred with higher frequency than the opposite type. They also found that bilaterally

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PLATE II.—Examples of ectrodactylism from insulin-treated embryos. 1. First toe hyperphalangous on both feet; second toe double nail, third toe of left foot rudimentary, fourth toe of right foot absent, small toe rudiment with nail attached to tip of third toe, syndactyly of second and third toe. 2. Both feet show lack of first and partial duplication of second toe. 3. Left foot partial duplication, right foot incomplete triplication of second toe; first toe missing on both feet (on right foot the lateral component of the fused toe mass may represent the first toe). 4. Left foot five-toed; on right foot first toe missing, second toe partially duplicated.

polydactylous animals of their family with the highest incidence of sinistral heterodactylism commonly showed more digit development on the left side. Attempts by CASTLE to increase by selection the asymmetry of the two sides of the body with reference to the extra toe were unsuccessful.

BOND (1932), speculating on the causes of heterodactylism, suggested the occurrence of unequal distribution of gene material during early developmental stages. He said: "the fact that an old established racial character is, for the most part, distributed *equally* to the two halves of the organism, while a newly introduced, (such as an extra toe), is often distributed *unequally*, suggests the idea that the new factor, or gene, fuses with difficulty with the older established complex. In other words that the old and new factors tend to segregate during the process of cell division which underlies the bilateral development of the embryo. The result being that asymmetrical distribution of the extra toe character occurs on the two sides of the body." And again: "It looks indeed as if a condition of unstable equilibrium has been set up in the genetic machinery which controls embryological development, by the introduction of a foreign element or character into an old established racial genetic complex, and that this disturbance of equilibrium is in some way associated with the abnormal distribution of the character in question, to the two sides of the body."

The occurrence of such a bilateral gene segregation is unlikely on general grounds. Breeding data show that the ovary (left side of body) is not involved in such a segregation. The preferential expression of polydactylism on the left side, among cases of heterodactylism, would remain unexplained. The fact that the left body side tends to show the higher degree of polydactylous development in bilateral, but unequally expressed, hyperdactylism precludes the assumption of an unequal distribution of the main gene for polydactylism, and it would seem unreasonable to invoke different explanations for unilateral and for asymmetrical bilateral polydactylism. Finally, the fact that heterodactylism of the fingers is common in birds which are homozygous polydactylous would seem to dispose of any explanation based on unequal gene distribution to the two body sides.

The only other attempt to provide an explanation for the developmental mechanism of heterodactylism has been made by DAHLBERG (1929, 1943 a, b). His hypothesis is based on the assumption that symmetrically distributed genes may produce a reversibly asymmetrical organization of the soma. DAHLBERG refers to such conditions as "genotypical asymmetries" and believes that their explanation is likely to be as follows:

1. "That a particular gene may lead to unequal distribution of some cytoplasmic inclusion at a determinative cell division which occurs in the plane of lateral symmetry."

2. "That it is a matter of chance whether excess or deficiency of the cytoplasmic constituent is concentrated on one side or other of the partition formed at the determinative cell division."

3. "That the exhibition or suppression of what would otherwise be a sym-

metrical character is limited by this excess or deficiency in the descendants of cells from the determinative division."

In applying these ideas to heterodactylism of fowl DAHLBERG starts with the entirely unjustified assumption that "among descendants of heterozygotes which have the fifth toe on the left side, asymmetrical offspring with the fifth toe on the right side appear to turn up as often as the parental type," and that "orientation of the character is not fixed by the process of transmission." As has been pointed out earlier, all observers are agreed that (in the absence of specific selection) the incidence of sinistral heterodactylism is far in excess of cases of dextral heterodactylism. Our own observations furnish definite proof for the existence of genetic factors which favor the appearance of one or the other type of heterodactylism. One would have to make very complex and improbable assumptions to fit DAHLBERG'S hypothesis to the following established facts: (a) the left-right incidence of heterodactylism is not a chance event, but is influenced by the residual heredity of the embryo; (b) heterodactylism occurs, if rarely, in homozygous individuals and the incidence in homozygotes can be greatly increased by experimental manipulation (see below); (c) heterodactylism of the upper extremities may co-exist with symmetrical bilateral gene expression in the toes of individuals which are homozygous for polydactylism.

Two other possibilities of explanation, envisioned but rejected by DAHLBERG, viz., factors extrinsic to the embryo and chromosomal abnormalities (such as elimination or non disjunction of chromosomes), are equally in conflict with factual observations and do not need to be discussed here.

We believe that all variations in the expression of polydactylism of fowl can be understood on the basis of known differentials in the internal environment of the embryo. K. E. VON BAER (1828) first observed that during the third and fourth days of development (the period of origin of the limb buds) the whole right half of the body grows at a more rapid rate than the left side. This was confirmed by subsequent investigations and BRANDT (1913) called particular attention to the fact that the limb buds are included in this lateral asymmetry of growth. There is, however, reason to believe that the situation is much more complex than these observations would suggest. OLSEN and BYERLY (1935) found, by counting mitoses on the left and right side of the neural tube, that in embryos of eleven to sixteen somites the left side showed a higher rate of growth, but that the reverse, that is higher mitotic activity on the right side, obtained in somewhat later stages. This change from left to right in preferential growth intensity coincides with turning of the embryo on to its left side. It cannot be doubted that such differential growth rates, whether they are lateral or regional, are an important expression of morphogenetic events. They deserve intense quantitative study.

An expression of these lateral differences in growth intensity was found by RAWLES (1936) in a comparative study of the fate (in chorioallantoic transplants) of pieces from the head-process stage of chick blastoderms, that is, at a time when growth intensity is higher on the left side. She observed that in

comparison with grafts from the median region of the embryo those of lateral origin had a reduced developmental capacity (in respect to growth and differentiation), and that this reduction was consistently greater for pieces from the right side than for those from the left. It is evident that growth activity at the time of explantation played an important role in determining the fate of the explant. Similar observations have been made in amphibian embryos. For a general discussion of the bearing of early activity gradients on the origin of lateral asymmetry and inversion the reader is referred to HUXLEY and DEBEER (1934, chapter IV) and PERNKOPF (1937).

We suggest that these lateral asymmetries of embryonic growth, their reversal in the case of heterotaxia and their more detailed and as yet unknown local variations provide the complex mechanism by which the particular type of expression of polydactylism or its complete suppression is mediated. One would assume that such lateral or local growth differentials are influenced by multiple genetic factors and this is, in fact, supported by the results of our selection experiments. If the results of selection in one or another direction of the phenotypic manifestations of polydactylism are obtained by the accumulation of factors for certain types of specific growth patterns, one would assume the emergence of antagonistic relationships between some forms of expression. Such incompatibility was demonstrated for bilateral polyphalangism and sinistral heterodactylism.

We believe that an interpretation of this kind is strongly supported by the nature of those external agencies which have a modifying effect on polydactylism. STURKIE (1943) and WARREN (1944) observed that the expression of polydactylism can be suppressed or shifted to different phenotypes by exposing the developing embryos to low temperatures. This procedure was most effective during the third day of incubation and only slightly so by the fifth day. The data of STURKIE indicate that these results are brought about by developmental modifications of a quantitative nature. GABRIEL (1946) has shown that growth retardation of the whole limb anlage of homozygous polydactylous embryos, produced by treatment with colchicine, leads in a high percentage of cases to suppression of the supernumerary digits. Finally, there are the effects of insulin as related in this report. The period during which polydactylism can be modified by insulin, the time of its maximum action, and the phenotypic results are very similar to those obtained with low temperatures. It seems reasonable to assume that the three agencies, viz., low temperature, colchicine and insulin, produce their effects in the same way, viz., by depressing growth. It is likely that the growth retardations are differential and vary with conditions of local growth intensity. We do not know the causes which produce lateral asymmetries or local variations of growth rate in normal development, nor do we have any information about the specific biochemical events which may be affected by experimental conditions and thereby result in the assumed growth retardations. We do not imply that *every* agent which has a general retarding effect on growth will thereby produce the modifications to which we have referred. It is, in fact, quite possible that such retardations, in order

to be effective, must be mediated through specific channels which are concerned with particular aspects of growth.

Lateral asymmetries in the expression of hereditary traits, similar to those occurring in polydactylism of fowl, are found among widely different forms of higher vertebrates. We have already referred to the parallel observations for polydactylism of guinea pigs. Another instance of very similar nature is that of congenital absence of the palmaris longus muscle in man. The condition is hereditary (THOMPSON, MCBATTS and DANFORTH 1921). In cases of unilateral suppression the muscle is more often absent on the left than on the right side. Parents with unilateral absence may produce children with lack of the muscle on the same, the opposite, or both sides; parents with bilateral lack of the muscle may have children with unilateral defect. DANFORTH (1924) believes that "it is difficult to escape the conclusion that in some way the sides of the body, *per se*, exert an influence in determining whether or not a factor represented in the germ plasm will succeed in producing its full effect." With this inference we are in full accord.

Another case of interest is that of foot abnormalities found among the descendants of X-rayed mice. These abnormalities generally show a greater incidence on the left than on the right side of the body, but this asymmetry of expression could be overcome by selection (LITTLE 1931). We believe that the wide distribution of asymmetrical phenotypic manifestation of traits, with similar features of hereditary transmission, is a reflection of the equally wide distribution of lateral growth asymmetries in the embryonic development of the amniotes. Modifications of these growth rates may even account for the effect which nutrition and maternal age appear to have on phenotypic expression of polydactylism in guinea pigs (WRIGHT 1934; KRÖNING and ENGELMANN 1934).

The hereditary basis of embryonic growth differentials is presumably complex and if, in addition, these growth rates are subject to modification by external agencies, such as temperature or nutrition, it becomes more readily understandable that, as is the case in fowl, the factors which are responsible for asymmetrical phenotypes may be derived from non-polydactylous ancestors and transmitted by animals with bilateral expression of the gene.

It must not be concluded from the foregoing discussion that all abnormalities of the extremities which occur unilaterally as well as bilaterally should, among the unilateral variants, have a higher sinistral incidence. This is notably not true. An example of the opposite type which has a direct bearing on our discussion is that of "diplopodia," a recessive lethal mutation of fowl discovered by TAYLOR and GUNNS (1947). Embryos which are homozygous for the diplopod gene show partial doubling of the metatarsals and digits of the feet, the metacarpals and digits of the wings, shortening of the long bones and of the maxilla. There are generally six toes on each foot, but asymmetrical expression occurs. TAYLOR and GUNNS reported, and this is confirmed by observations of our own, that among diplopod embryos with asymmetrical toe duplication the greater abnormality is, in about five out of six embryos, found on the right

foot. Heterodactylism with unilateral duplication does not occur in this stock, but asymmetrical manifestation of the gene is of the opposite type from that found in ordinary polydactylism. It seems likely that such dissimilarities between mutant stocks are brought about by differences in the time at which the gene takes effect and by the relation of the determinative periods to the changing pattern of relative growth in various parts and regions of the embryo. Specifically, we suggest that in heterozygous polydactylous as well as homozygous diplopod embryos that side of the embryo is more resistant to the gene effect which at the determinative period shows more intense growth activity. It should be possible to test the correctness of these assumptions.

A few remarks should be made in regard to the modifying effect of insulin on polydactylism of fowl. The time of maximum effectiveness (72 to 96 hours of development) coincides with the time of origin and early organization of the limb buds. The fact that no dosage difference was produced by the injection of two and five units of insulin suggests that the maximum result is brought about by the lesser amount of the hormone. The results of our experiments, in comparison with the controls, indicate that insulin tends to push development into channels for which the embryos of a particular stock are prepared by their genotype.

It is of some interest to note that the insulin-induced phenotypic shift was similar in type and extent in the heterozygous and homogygous embryos of *outbred* origin and that the effect in these two stocks was much greater than that found for the *pure-bred* Dorkings. Differences of the same kind, though at much lower levels, exist between the *untreated* outbred and pure-bred stocks. While it is unquestionably true that certain forms of phenotypic expression of polydactylism or its complete suppression occur predominantly among heterozygotes, it is also evident that these effects are more likely to take place after the introduction of modifiers by outcrosses. The data of all investigators indicate that the frequency of occurrence and nature of these modifiers varies from stock to stock; they appear to be rare among Jungle fowl (BOND 1932; FISHER 1935).

It is obvious, of course, that in producing relatively true-breeding polydactylous races breeders have resorted to a great deal of selection. It has been suggested that the effect of this selection was to accumulate in the polydactylous stocks "dominance factors" which enhance the full expression of an originally semi-dominant mutation (FISHER 1930, 1935; HUTCHINSON 1931). The best that can be said for this hypothesis is that none of the available evidence provides factual support for it.[1] The results of crossbreeding suggest that factors which interfere with the "standard" expression of polydactylous breeds, viz., the bilaterally five-toed condition, are widely distributed among non-polydactylous stocks. These are factors which may lead to partial or complete suppression or to an exaggeration of the "standard" condition. They have

[1] It may be remarked in passing that the general hypothesis of dominance factors, as it applies to poultry, would merely shift the question of why so many mutants of fowl, compared with other animals, are dominant to that why *partial* dominance is unusually frequent.

presumably been removed, to a large extent but not entirely, in the production of polydactylous breeds. In non-polydactylous stocks these factors may provide added assurance for normal growth and development of the limb fields. This would explain their occurrence in all non-polydactylous stocks which have been tested. All results of crossbreeding experiments indicate that those factors which tend to exaggerate the polydactylous condition are much rarer in non-polydactylous stocks than those which have a partial or complete suppressing effect.

WARREN (1941) described a new type of polydactylism of fowl, known as "duplicate," which arose by mutation in a flock of White Leghorn fowl. The gene for duplicate appears to be an allele of that for polydactylism. The heterozygous expression of the duplicate mutant tends to be more extreme than that of ordinary polydactylism. The feet frequently have six toes and the wings show striking finger duplications. The homozygous condition appears to be semi-lethal. We have produced birds of very similar appearance, with digit duplications in both extremities, by selection from outcrossed stock of Silkie-Houdan origin. The modifying factors which favor expression of polydactylism in the wings were presumably derived from the normal stock. It seems reasonable to us, therefore, to assume that the original phenotype of polydactylism was similar to that of duplicate and that in the process of standardization breeders removed by selection all those genetic factors which either tended to suppress or partially inhibit bilateral and regular expression (five toes) in the feet or which promoted excessive, and often crippling and unsightly duplication of toes as well as fingers.

We believe that the developmental nature of polydactylism is somewhat elucidated by the occurrence in polydactylous stocks of ectrodactylism (enhanced by insulin treatment) and of malformations of the radius. Some cases of ectrodactylism are illustrated in plate II. These are from insulin experiments, but similar specimens are found, if more rarely, in untreated embryos. The first and fourth toe are most commonly absent in ectrodactylism. Particular attention is called to cases with duplication of the second toe, partial fusion of the second and third toe and rudimentary formation of the third toe (plate II). These instances certainly give weight to BOND'S (1926) statement that the factor for polydactylism "may influence all the digits."

A variety of abnormalities of the radius were observed in "duplicate"fowl and among polydactylous chickens from a family in which we had selected for the expression of hyperdactylism in the hand. They will be described in detail elsewhere. Suffice it to say here that these malformations range from complete or partial absence of the radius to its partial or even complete duplication.

Our observations on ectrodactylism and on radius abnormalities indicate that the effect of the gene is not merely one on the hallux, though it may usually be limited to it, nor that it is one which is solely responsible for splitting and excessive development of the first toe. GRÖNBERG (1894) suggested that polydactylism of fowl may represent an incomplete duplication of the whole foot. BRAUS (1908), on the basis of the material of KAUFMANN-WOLF (1908),

has demonstrated, convincingly I think, from the mirror-image symmetry relations of at least certain cases of polydactylism that they are instances of abortive duplication of the whole foot. Similar observations by GABRIEL (1946) led him to think in terms of an abortive duplication of the limb field. We believe, however, that such explanations are too narrow in view of the evidence from ectrodactylism and abnormalities of the radius. We believe the evidence, taken as a whole, indicates that the gene for polydactylism is responsible for an interference with the proper organization of the limb field. Factors of residual heredity, probably acting through lateral and local differential growth rates, and the presence of one or two genes for polydactylism, presumably decide whether this disturbance of the limb field will lead to ectrodactylism, complete or unilateral suppression of polydactylism, hyperphalangism, the bilateral presence of five toes, excessive forms of digit duplication in one or both pairs of extremities, and such abnormalities as lack or duplication of the radius. It may be supposed similarly that external agencies, such as low temperature, colchicine and insulin, also produce their suppressing or modifying effects (or, in the case of colchicine, even occasional duplication) by upsetting the proper organization of the limb field. The same conclusion applies presumably to the occasional appearance of polydactylism in non-polydactylous embryos as a result of insulin treatment (LANDAUER 1947). The developmental nature of these "disturbances" of the limb field remains to be discovered.

The concept of "modifying" genes, as it has been developed in the preceding pages, reveals the integrative interaction between the mutant gene and the specific developmental potentialities of the embryonic field in which the gene takes effect.

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SUMMARY

Observations on polydactylous fowl of outbred and pure-bred origin led to the following conclusions.

1. Heterodactylism was usually a heterozygous expression of polydactylism, but occurred as a rare variant among homozygotes. In unselected stocks sinistral heterodactylism was much more common than the unilateral dextral expression.

2. The incidence of sinistral heterodactylism could be raised by selection.

3. Dextral heterodactylism gave less response to selection than the sinistral form, but *inter se* matings of parents with dextral heterodactylism produced significantly more progeny of their own kind than any other type of mating.

4. Bilateral polyphalangism responded readily to selection, but uncomplicated forms of this variant were usually, perhaps always, heterozygous. In homozygotes polyphalangism tended to show some degree of duplication of the first toe.

5. The incidence of bilaterally five-toed and bilaterally six-toed chicks could be increased by selection. In our outbred stock the bilaterally five-toed condition was a heterozygous phenotype, homozygotes having more than five toes on each foot, whereas in pure-bred Dorkings the five-toed condition is the standard expression of homozygotes.

6. Non-polydactylous chicks occurred as rare variants in homozygous polydactylous stock. Among the progenies of heterozygous fowl the incidence of exceptional non-polydactylous chicks varied with the phenotype of the parents, matings of heterodactylous chickens producing significantly more non-polydactylous offspring than matings of bilaterally hyperdactylous animals.

7. Chicks with ectrodactylism are a rare but significant feature of polydactylous stocks.

8. The injection of insulin led to phenotypic modifications of polydactylism. The period of maximum response was between 72 and 96 hours of incubation. Insulin treatment may lead to an increased incidence of heterodactylism or hyperphalangism or the complete suppression of polydactylism; it also raised the frequency with which ectrodactylism occurred. The particular type of phenotypic response to insulin varied with the tendency of different stocks to produce one or another kind of aberrant phenotype. In embryos of outbred stocks, whether heterozygous or homozygous, insulin produced a much greater shift in phenotypic expression than in those with the standardized genotype of polydactylous breeds.

9. Hyperdactylism of the upper extremity was only found in animals which were homozygous for polydactylism. As is true for the toes, sinistral heterodactylism of the fingers was much more common than dextral heterodactylism. Heterodactylism as well as homodactylism of the fingers were only found in animals with bilateral hyperdactylism of the toes.

10. In "duplicate" and polydactylous stocks hyperdactylism of the hand is sometimes associated with abnormalities of the radius, ranging from entire lack to complete duplication.

11. It appears that in the production of five-toed breeds factors which have an inhibiting or suppressing and others which have an exaggerating effect on phenotypic expression of polydactylism have been removed by selection.

12. It is believed that the gene for polydactylism produces a disturbance in the organization of the limb field and that the phenotypic variations of expression are mediated by (naturally occurring or experimentally induced) lateral asymmetries of or local differentials in growth intensity of the regions concerned.

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