A MUTATION OF THE GUINEA PIG, TENDING TO RESTORE THE PENTADACTYL FOOT WHEN HETEROZYGOUS, PRODUCING A MONSTROSITY WHEN HOMOZYGOUS

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INTRODUCTION

It has been shown in the preceding papers (WRIGHT, 1934b, c) that the rather frequent occurrence of an atavistic little toe in guinea pigs depends on whether the combination effects of multiple genetic and environmental factors cross a certain threshold, and that perfect development depends on the crossing of a second threshold. Among the genetic differences studied, none could be ascribed to unit factors of major importance. The environmental factors included ones which acted alike on all progeny of a mating, others which were merely common to litter-mates (notably age of mother), others which were individual in incidence, and finally ones which were responsible for development on one hind foot rather than the other.

PENTADACTYLY IN THE GUINEA PIG

The diversity of factors indicated above must be further increased by the conditions found among the descendants of a particular polydactylous animal which appeared in a normal stock. This animal ♂ 1816 (born in May 1928) had vestigial little toes, consisting of minute balls of flesh with small nails, attached by mere threads to the feet. In addition there was a similar vestige in the position of a left big toe and equally vestigial structures in the position of thumbs on the fore feet. This was the first indication of atavistic digits, other than little toes, encountered in this colony of guinea pigs. None had been noted in the large colony of the U. S. Bureau of Animal Industry, to which the Chicago colony traced exclusively, as far back as 1915.

The thumb and big toe are in general much less common in guinea pigs than the little toe. DETLEFSEN (1914) described a sterile male, 1/8 blood *Cavia rufescens*, 7/8 blood guinea pig, as having 5 well-developed functional toes on the left fore foot and also on the left hind foot. He suggested that this may have been a reversion to the ancestral pentadactylous condition brought about by recombining factors. The anomaly never recurred in his experiments nor in my own experience with later generations of the same hybrid stock. STOCKARD (1930) in the course of extensive experiments with animals showing the little toe, found one with thumbs on

both fore feet but never found any with big toes. I have found no other record of these digits in guinea pigs in the literature. Since the birth of ♂ 1816, however, an additional case of a thumb (associated with a little toe) has occurred in an animal unrelated to ♂ 1816. This animal came from a stock (F) in which the little toe was common. Five of her 7 young showed little toes, but none showed the thumb nor has it reappeared among her numerous descendants of later generations.

ANCESTRY OF ORIGINAL PENTADACTYL

Male 1816 came from a somewhat inbred group. His sire (♂ 30899) mated with a full sister, both normal-toed, had produced 9 young, all normal. Mated next with one of his daughters, 13 young, all normal, were produced. Mated third with one of these young (daughter and granddaughter) he produced 17 young, one of which was ♂ 1816. One born in an earlier litter had vestigial little toes, 7 others born earlier were recorded as normal and the remaining 8, born subsequently were all normal. The condition of the hind feet had been a matter of routine record, but that of the fore feet was recorded only after the birth of ♂ 1816 and so must be considered somewhat uncertain in earlier animals. Two matings from the original pair produced 7 young, all normal. A mating of ♂ 30900 (full brother of ♂ 30899) to a female only remotely related but not known to inherit polydactyly of any sort, produced 8 normal young. Matings tracing exclusively to this pair produced 85 young, all normal. A mating between a son of ♂ 30899 and a daughter of ♂ 30900 produced only 19 normals. Altogether this group of matings, descended from two full brothers, a full sister and one other female produced 141 normals, excluding the mating which produced ♂ 1816, and his descendants. The partially dominant gene, which will be shown to be responsible for the new digits of the latter group probably traces to a mutation in the germ tract of the mother of ♂ 1816.

EVIDENCE FOR UNIT FACTORS

Male 1816 was mated with two normal daughters of ♂ 30900 one of which had already produced 14 normals, and the other 15 normals from matings with their sire. With ♂ 1816, one produced 3 polydactyls: 4 normals; the other 3 polydactyls: 11 normals. Five of these 6 polydactyls had thumbs. Clearly the presence of thumbs is transmitted in this case. The descendants of these two matings have been maintained for several generations without outcross (strain I). Table 1 shows the total results through November 1933. In this table N stands for normal and P for polydactyl of any sort. About two-thirds of those in strain I had both thumb and little toe represented, the rest having only one or the other.

Normal×normal should produce only normals if polydactyly is dominant. Actually, there were 8 polydactyls among 118 young. However, these 8 polydactyls were not distributed at random among the progeny of the 8 normal males and 18 normal females which produced this group. Seven of them came from one male (himself from polydactyl×polydactyl) mated with two females (from normal×normal). These two matings produced 7N:6P and 6N:1P respectively. The only other polydactyl from N×N came from a mating between brother and sister (from P×P). They produced 2N:1P. These 3 matings thus produced a total of 15N:8P, not significantly different from the ratio produced by matings N×P, sug-

TABLE 1

Results of all matings in strain I.

DAM	SIRE	NORMAL			POLYDACTYL			MONSTER (41digits)	TOTAL
		♀	♂	?	♀	♂	?		
N	N	51	59	—	2	6	—	—	118
N	P	58	51	1	29	39	—	—	178
P	N	5	8	—	8	6	—	—	27
P	P	39	30	—	42	47	1	1	160

gesting that they may belong in the same category genetically. The hypothesis of a dominant mutation, which usually produces polydactyly (ranging from 1 to 6 atavistic digits) but occasionally insufficient to cause crossing of the threshold from normality, is in harmony with the deficiency of polydactyls from N×P and the production of polydactyls by a few matings of N×N.

To prove segregation of such a major factor, it is necessary to show important differences between the progenies of normals and polydactyls of identical ancestry. Table 2 includes only those parents which came from matings of N×P. No distinction of sex is made since table 1 gives indication of no relation to sex.

TABLE 2

Matings between animals from a single sort of mating, N×P.

PARENTS	N	P	MONSTER	TOTAL
N(NP)×N(NP)	61	0	0	61
N(NP)×P(NP)	44	23	0	67
P(NP)×P(NP)	42	52	1	95

Clearly most normals and polydactyls of strain I from the same parents are genetically very different. Compare this result with that described in the preceding paper (WRIGHT 1934c) for a cross between normal strain 2 and the true breeding polydactyl strain D (little toes perfect, but no

thumbs or big toes). In this case F_1 was uniformly normal, F_2 showed a fair approach to a ratio of 3N:1P and the backcross of F_1 to strain D gave a fair approach to 1N:1P, all suggesting segregation of a single recessive factor for this polydactyly. Yet the breeding test of the normals and polydactyls from the backcross generation proved that they were scarcely distinguishable genetically. Mated with strain D, the normals gave only 23 percent normals, while the polydactyls (supposedly recessive) gave 16 percent normals. For this and other reasons, it was concluded that strains 2 and D differ by multiple factors, of which no one is of major importance. Conversely, it must be concluded that in strain I there is a single major factor, even though some overlap must be postulated.

LETHAL EFFECT IN HOMOZYGOTES

Table 3 shows the results of the matings, distinguishing the normals (N′) which were shown to transmit polydactyly from the others (N) and distinguishing polydactyls according as they came from matings N×P (including N×N′) or P×P.

TABLE 3

Tabulation of matings by source of polydactyl parents.

PARENTS	N	P	TOTAL	PARENTS	N	P	MONSTER	TOTAL
N×N	95	0	95	P(NP)×P(NP)	42	52	1	95
N×P(NP)	80	47	127	P(NP)×P(PP)	8	8	—	16
N×N′(PP)	15	8	23	P(PP)×P(PP)	19	30	—	49
N×P(PP)	43	35	78					

These matings include ones which were made in hope of obtaining homozygous polydactyls. There were 11 polydactyl males and 20 polydactyl females from P×P which were tested. The results (including some duplication because of matings P(PP)×P(PP)) were as follows. All but one of the males produced normals (53N:52P) and were thus probably heterozygous. The one exception was inadequately tested (2 young). Fifteen of the 20 females had normal young (36N:39P) and the other 5 had only from 1 to 3 young each (10 altogether). There is an indication here that homozygotes are not appearing in the proportions expected, if at all.

The ratio of normals to polydactyls from matings between polydactyls (69N:90P) is obviously more easily accounted for as a deviation from a 1:2 ratio than from a 1:3 ratio, that is, more easily on the hypothesis that the gene for polydactyly is lethal when homozygous. One of these matings produced a monster of unusual type. All of its legs were short and distorted. Each foot had from 8 to 11 webbed digits, a total of 39 (or 41 if allowance is made for two double nails). In addition, it had a protruding

brain and microphthalmia. We shall consider later evidence from cross-breeding experiments which indicate that this is the homozygous type, usually absorbed before birth, but occasionally reaching birth.

The following table shows the sizes of litters produced by matings of various sorts (N×N′ included with N×P).

TABLE 4

Size of litter from different types of mating in strain I.

PARENTS	SIZE OF LITTER						
	1	2	3	4	5	TOTAL	AVERAGE
N×N	6	15	9	8	—	38	2.50
N×P	12	28	24	13	2	79	2.56
P×N	2	3	5	1	—	11	2.45
P×P	39	31	13	4	1	88	1.83

The size of litter from P×P averages much less than from the other types of matings. The difference is clearly significant, being about 5 times its standard error. As all come from the same somewhat inbred stock and were born during the same period in the same laboratory, the difference gives strong evidence of antenatal depletion in those litters which might contain homozygotes.

DESCRIPTION OF HETEROZYGOTES IN STRAIN I

The symbol *Px* ("pollex") has been assigned the dominant gene which distinguishes the polydactyls from the normals in strain I. As shown above, it is necessary to assume that some animals of constitution *Pxpx* are normal phenotypically. The proportion of these may be estimated from the matings between polydactyls (all assumed to be *Pxpx*). As these produced 69N:90P it is necessary to transfer 16N's to the P group to give an exact 1:2 ratio. This gives 15 percent (=16/106) as the estimate of the proportion of *Pxpx* which are phenotypically normal. Again the matings of N×P and N×N′ produced a total of 138N:90P. Treating these as all *Pxpx*×*pxpx*, it is necessary to transfer 24N's to the P group to give an exact 1:1 ratio. This is more likely to be an underestimate than an overestimate since some of these matings may be *Pxpx*×*Pxpx* and moreover additional N×N matings (with only N young to date) may really be *Pxpx*×*pxpx*. As it stands, it indicates 21 percent (=24/114) as the proportion of *Pxpx* phenotypically normal. Combining these two estimates we get 18 percent (=40/220) as the estimate.

Table 5 shows the frequency of thumb on each forefoot and of little toes and big toes on each hind foot in animals considered to be *Pxpx* in this strain. This includes the 180 polydactyls descended from ♂ 1816 and 40±

normals estimated as above. The symbol OO means absence on both left and right sides, TO presence on left side only, OT presence on right side only and TT presence on both sides.

TABLE 5

Frequency of atavistic digits in Pxpx of strain I.

	OO	TO	OT	TT	ANIMALS	FEET WITH EXTRA DIGITS	
						NUMBER	PERCENT
Thumb	58±	18	7	137	220	299	68.0
Little toe	83±	13	9	115	220	252	57.3
Big toe	216±	2	0	2	220	6	1.4

The thumb is the most constant indicator of gene *Px* (73.6 percent of the animals, 68.0 percent of the forefeet). The little toe is nearly as characteristic (62.3 percent of the animals, 57.3 percent of the hind feet). The big toe is uncommon (1.8 percent of the animals, 1.4 percent of the hind feet). There appears to be a slightly greater tendency for left digits to return than right ones.

We have assumed that thumb, little toe and big toe are indicators of the same gene in spite of the fact that the little toe of this strain has exactly the same appearance as that found in many other strains in which it is certainly not an indicator of gene *Px*. But in strain I there is a strong correlation in occurrence with the thumb as indicated in the following table. The symbols for the feet in table 6 are written from left to right. On the hind feet the little toes are on the outside and the big toes on the inside.

TABLE 6

Correlation in occurrence of atavistic digits in animals of constitution Pxpx in strain I.

		HIND FEET						
		OO OO	TO OO	OO OT	TO OT	TT OT	TT TT	TOTAL
Fore	OO	40±	3	2	13	0	0	58±
	TO	9	0	0	9	0	0	18
Feet	OT	3	0	1	3	0	0	7
	TT	31	10	6	86	2	2	137
Total		83±	13	9	111	2	2	220±

Little toes were present in only 18 animals lacking both thumbs and these all came from matings known to transmit *Px*. Thumbs were present in 43 animals lacking both little toes. The big toes have appeared only in the presence of both thumbs and both little toes.

The appearance of the atavistic digits is shown in figure 2 in comparison with normal feet shown in figure 1.

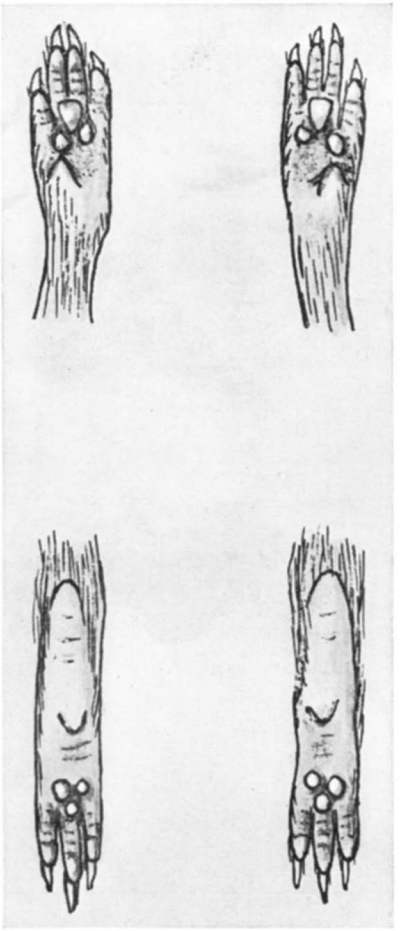

FIGURE 1.—Forefeet (above) and hind feet (below) of normal guinea pig. Note absence of thumbs, little toes and big toes.

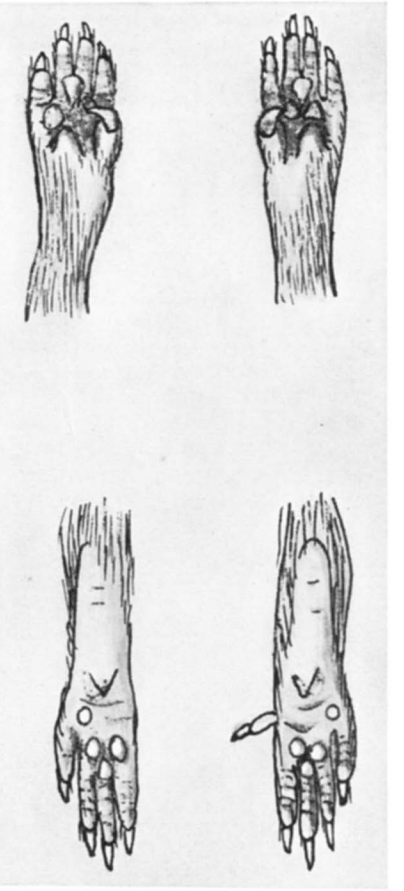

FIGURE 2.—Forefeet (above) and hind feet (below) of typical heterozygote (*Pxpx*). Note presence of well-developed thumbs and little toes, and of vestigial right big toe.

All types of polydactyls in strain I transmit about the same heredity. Table 7 is a comparison of the results of matings with normals.

No animals with little toes only happen to have been mated with normals. The multiple-toed monster, interpreted as a homozygote (*PxPx*) came from a mating of a male with one very weak little toe as the only extra digit, and a female with thumbs but no extra toes on the hind feet. Apparently both types were *Pxpx*.

TABLE 7

Progeny of Pxpx of various types, mated with normals.

PARENTS		OFFSPRING				
Pxpx	*pxpx*	NORMAL	POLLEX ONLY	LITTLE TOE ONLY	POLLEX LITTLE TOE	POLLEX HALLUX LITTLE TOE
Pollex, hallux, little toe	Normal	19	2	2	6	0
Pollex, little toe	Normal	56	5	3	19	2
Pollex only	Normal	48	16	3	24	0
Normal	Normal	15	3	0	5	0
Total		138	26	8	54	2

CROSSES BETWEEN STRAINS I AND D

Both normals and polydactyls of strain I have been mated with strain D, which for years had bred true to perfect development of the little toes. The origin of the latter was described by CASTLE (1906). As shown in the preceding paper (WRIGHT 1934c) crosses between this strain and various normal strains have given results which indicate multiple factors, no dominance, no lethal effects. The animals used in the present experiments were not all pure-blood, ranging up from 7/8 blood and averaging about 15/16 blood but all used had perfect little toes and came from parents of the same sort.

It seemed possible at first that even normals of strain I might contain latent factors which would cause the pollex to appear on increasing the polydactylous tendency by mating with strain D. This, however, turned out not to be the case. Both reciprocal crosses of *normals* from I with polydactyls from D, produced polydactyls as well as normals but the polydactyly was limited to the little toes. Neither did any other type of polydactyly appear in F_2 from either type of F_1 or in two successive backcrosses of F_1 to normals from I. Altogether 249 young were bred in this group of matings, none of which showed the pollex. This confirms the view that normality in strain I ordinarily depends on a recessive factor. It is, however, something of an accident that none of the normals used were concealed dominants (*Pxpx*). Table 8, matings 1 to 7 show the results classified by sex. Clearly there is no relation to sex.

Matings of *polydactyls* from I with the polydactyls from D gave a very different result (matings 8, table 8). The pollex was present in 28 of the 58 young. All of these showed little toes and two of them had the big toe on one or both sides. The remaining 30 were about equally divided between normals and animals with little toes. These 30 are much like the entire group from the matings of normal (I) $\times$ polydactyl (D). Evidently the results are in harmony with the hypothesis that the polydactyls of I are

Pxpx, if the pollex (but *not* in this case the little toe) be assumed to be an index for presence of *Px* in animals whose polydactyl tendency has been increased by infusion of 50 percent blood of strain D. It must be supposed that the factors peculiar to D increase the tendency toward development of the pollex (although it never appears in D), as well as of the little toe.

TABLE 8

Results of all matings between strains I and D and among the descendants. Parents classified by extra digits (M_1=pollex, P_1=hallux, P_5=little toe) and source. Young of undetermined sex in parentheses.

	DAM	SIRE	NORMAL (N)		LITTLE TOE (P_5)		POLLEX LITTLE TOE (M_1P_5)		POLLEX LITTLE TOE HALLUX ($M_1P_5P_1$)		MONSTER ABOUT 40 DIGITS	
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1a	P_5 (strain D)	N (strain I)	1	2	3	6						
1b	N (strain I)	P_5 (strain D)	13	9	20	19						
2	P_5 (from 1)	P_5 (from 1)	12	6	13	18						
3	N (from 1)	P_5 (from 1)	3	2	0	0						
4	N (from 1)	N (from 1)	14	15	5	4						
5	P_5 (from 1)	P_5 (from 2, 5)	2	3	9	11						
6a	P_5 (from 1)	N (strain I)	1	2	4	1						
6b	N (strain I)	P_5 (from 1)	8	5	3	0						
7a	P_5 (from 6a)	N (strain I)	17	12	0	0						
7b	N (strain I)	P_5 (from 6a)	2	3	0	1						
8	P_5 (strain D)	M_1P_5 (strain I)	10	4	9	7	17	9	0	2		
9	P_5 (from 8)	P_5 (from 8)	3	6	13	9						
10	N (from 8)	N (from 8)	4	1	3	4						
11	P_5 (from 9)	P_5 (from 9)	0	1	8	10						
12a	M_1P_5 (from 8)	P_5 (strain D)	0	1	4	3	0	1	5	1		
12b	P_5 (strain D)	M_1P_5 (from 8)	1	1	1	2	2	2	0	0		
13a	M_1P_5 (from 8)	N (strain I)	1	0	0	0	0	1	0	0		
13b	N (strain I)	M_1P_5 (from 8)	1	0	0	0	0	0	0	0		
14	M_1P_5 (from 8)	M_1P_5 (from 8)	4	5	9	8	18	18	5	2	3	0
15	M_1P_5 (from 8)	$M_1P_5P_1$ (from 8)	0	0	1	1	0	0	0	1	0	0
16	M_1P_5 (misc.)	M_1P_5 (misc.)	5	3	5	3	13	12	1	4	1	(3)
17	$M_1P_5P_1$ (misc.)	M_1P_5 (misc.)	0	1(1)	2	1	4	5(2)	3	4	0	(2)

The hypothesis of a definite gene *Px* was tested by matings between the different kinds of F_1's (from $Pxpx \times pxpx$). Matings between normal F_1's produced 5 normals: 7 with little toes as the only extra digits (matings 10, table 8). Matings between animals with perfect little toes but no other extra digits (matings 9, table 8) gave only a slightly different result, 9 normals: 22 with little toes. On the other hand, F_2 from F_1's with pollex

as well as little toe (matings 14 and 15, table 8) gave 44 with pollex (and little toes, including 8 with hallux as well) in addition to 9 normals and 19 with the little toes as the only extra digits. There were also 3 monsters of the same general type as described in strain I, interpreted as homozygotes. There is evidently a clear-cut genetic differentiation of F_1's with and without the pollex (in contrast with the lack of any clear-cut difference between those with and without little toe (in the group without pollex). Matings of "pollex"×"pollex" in later generations produced 21 without pollex (10 normals, 11 with little toe) to 48 with pollex (of which 12 had one or both big toes), and 6 of the multiple-toed monsters. Altogether "pollex"×"pollex" produced 49 without and 92 with this digit and 9 monsters in this group of crossbreds. This is in good agreement with the hypothesis that the pollex is a reliable indicator of *Pxpx* in the presence of the residual heredity of this cross. The 9 monsters are of course much in defect for *PxPx* but as will be seen there is reason to believe that most of these die early in development. Four of these 9 were in fact examined as foetuses.

A further test is furnished by small backcross progenies (matings 12–13, table 8). Matings between F_1 with thumb and strain D gave 13 without to 11 with thumb. More than half of the latter had big toes also. The tendency to develop pollex and hallux, far from being blended out, is evidently increased by backcrossing to strain D.

The results of the whole set of matings from D×I are summarized in table 9 using pollex (M_1) as the sole criterion for *Pxpx* among the offspring.

TABLE 9

Condensed summary of matings between I and D.

	pxpx	*Pxpx*	*PxPx*
pxpx×*pxpx*	311	0	0
Pxpx×*pxpx*	45	40	0
Pxpx×*Pxpx*	49	92	9

OTHER OUTCROSSES

A small number of matings have been made between "pollex" animals from F_2 of I×D and normals from a strain (A), which has never produced polydactyls of any sort. The young were 16 normals and 15 with thumbs (of which only 5 also had little toes). This is close enough to a 1:1 ratio but it is remarkable that 10 of the 15 called *Pxpx* are of a type which did not appear at all among 132 *Pxpx* of the preceding group of crossbreds (I×D). Evidently strain A is much farther from the threshold for little toe than are the normals of strain I although with more favorable residual heredity for the pollex. On backcrossing such animals with the thumb as the only extra digit to animals 7/8 blood D, there appeared 3 normals and 4 poly-

dactyls with both thumbs and little toes. The increased blood of D has here restored the tendency toward development of little toes. Incidentally, these animals are of interest as only 1/8 blood of strain I, giving further evidence that *Px* is a true unit factor, capable of withstanding indefinitely continued attempts at dilution.

A mating of other "pollex" animals (tracing to I×D) to yet another wholly normal strain (B) gave 7 normals, 5 with little toes but no thumbs and 3 with thumb and little toes. This further illustrates the difference in the residual heredity of different normal stocks.

THE HOMOZYGOTES

In the course of these experiments 10 monsters have appeared which have been interpreted as homozygotes *PxPx*. Six of these, while found dead, were evidently alive up to birth. They were of approximately the same size as their litter-mates. The other four were small foetuses taken from females which had died.

All of these monsters were of essentially the same peculiar type (figure 3): short legs, feet of approximately double the normal width, a large number of small similar digits, exhibiting externally little more than nails, hind legs rotated so that the soles are toward the belly, bulging forehead, with brain protruding in four cases, microphthalmia, nostrils connected with mouth by symmetrical clefts (in seven cases). One had 44 nails $\left(\frac{11,12}{12,9}\right)$ two had 41 $\left(\frac{11,9}{10,11}\right)$ $\left(\frac{11,11}{10,9}\right)$. In three, one or more of the feet had been partially eaten when found $\left(\frac{10,—}{8,8}\right)$, $\left(\frac{11,—}{9,—}\right)$, $\left(\frac{10,10}{—,—}\right)$. The other three were small foetuses, dead for some time, in which it could merely be determined that there were at least seven lobes on the limb buds.

Post-mortem examinations were made of 6 pregnant "pollex" females which had been mated with "pollex" males. There were found 2 foetuses without thumbs (*pxpx*), 11 with thumbs (*Pxpx*) and the 4 monsters (*PxPx*) referred to above. The proportion of monsters is approximately that expected in contrast with the great deficiency in litters recorded at birth. The reason for such deficiency was apparent. One of the litters contained three heterozygotes nearly at term (average weight 40 grams) and two small foetuses which had evidently been dead for some time but which were obviously of the monstrous type. These measured only 10 and 13 mm respectively in crown-rump length. Their placentas were about 10 mm in diameter in contrast with 20–24 mm for the large foetuses. In another case there were three heterozygotes near term and a small monster (15 mm long) on a placenta less than half the diameter of those of the large

foetuses. In the third of the litters containing a monster, there were one normal, two with thumbs and little toes, and one monster (41 digits) much

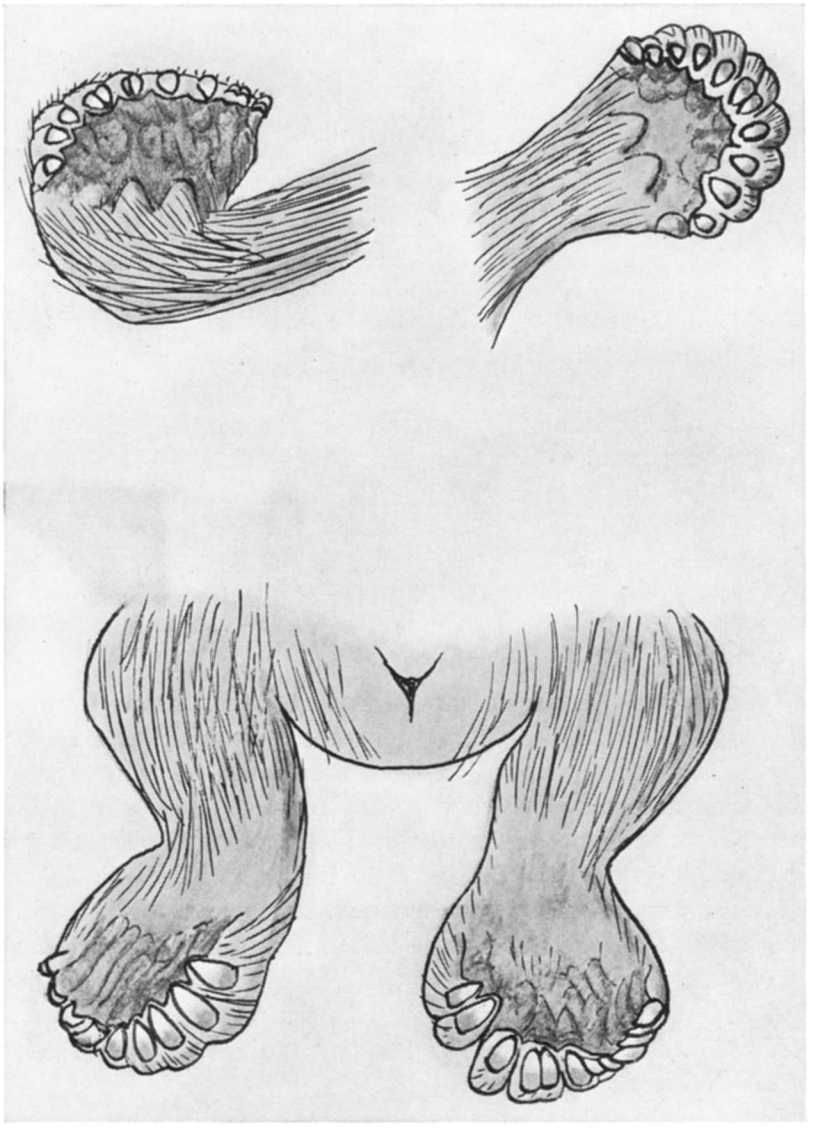

FIGURE 3.—Forefeet (above) and hind feet (below) of homozygote (*PxPx*). Note shortness and rotation of hind legs as well as multiple digits.

larger than those in the preceding litters. These foetuses were all of about the same size (11, 12, 10, 10 grams).

It is probable that most of the homozygotes die at a critical moment

when they are 10–15 mm long, while the few which survive this moment grow at a normal rate to birth.

Comparison with known types of mammalian monsters must await detailed anatomical study.

VIGOR OF HETEROZYGOTES

The contrast between the heterozygotes and homozygotes is great. The former are typically vigorous animals with no suggestion of malformation, seeming to differ from normals only in a more or less complete reversion to the pentadactyl foot. The latter are also polydactyls but of a type which one would consider unrelated if not due to the same factor. Their polydactyly appears to be merely a symptom of a generalized disturbance of development. It has seemed worth while to scrutinize the records of the heterozygotes for possible indications of abnormal tendencies.

In strain I, four animals, one normal and three polydactyl, showed ventral flexion of one or both of the forefeet. One of these was reared and lived for severaly ears, siring many young. He always walked on the knuckles of his left forefoot. In the crossbred group, there were also four animals (all with thumbs) with ventral flexion of one or both forefeet and one animal (also with thumbs) with slight torsion of the hind feet. Two of these were raised. These types of defect are found sporadically in other stocks in which gene *Px* is absent. It is probable, however, that in these cases gene *Px* was a factor.

One microphthalmic animal (other than the monsters) was born in the crossbred group. As this did not have thumbs it probably lacked gene *Px*. Microphthalmia occurs sporadically in most stocks of guinea pigs and probably had no special significance in this case.

Two animals were born in the crossbred lot, each of which had a vestige of a sixth digit on the right forefoot near the base of the fifth digit. One of these had strong thumbs, little toes and big toes, 21 digits in all. The other had strong thumbs and little toes but no big toes. In these two heterozygotes, there was a tendency to go beyond the atavistic pentadactyl foot but the character of the digits, apart from the sixth, was exactly like that of ordinary heterozygotes and not at all like that in the homozygotes.

The mortality records have been tabulated to see whether the heterozygotes are as vigorous as normals. Tables 10 and 11 show the results in strain I and in the crosses with D, respectively. In strain I, the polydactyls show less mortality at birth than the normals, and only slightly more between birth and weaning. In the crossbred lot, the polydactyls show a considerably higher mortality at both times.

In interpreting the significance of the differences it must be borne in

mind that there is a strong tendency for litter-mates to suffer the same fate. The standard errors based on individuals must be increased about 25 percent on this account. Even so, *pxpx* appears to be definitely superior to *Pxpx* in prenatal mortality among the crossbreds. Perhaps *Pxpx* has no appreciable effect in strain I in which its action is not complicated by that of similar factors, but combination with the effects of the modifiers from strain D leads to definite injurious effects.

TABLE 10

Mortality percentages of normals and polydactyls from different types of matings in strain I.

PARENTS	NORMAL (LARGELY *pxpx*)				POLYDACTYLS (*Pxpx*)			
	NUMBER	PERCENT BORN ALIVE	PERCENT RAISED OF BORN ALIVE	PERCENT RAISED	NUMBER	PERCENT BORN ALIVE	PERCENT RAISED OF BORN ALIVE	PERCENT RAISED
N×N	88	85.2	88.0	75.0	—	—	—	—
N×P, (N′)	139	72.7	80.2	58.3	86	86.0	78.4	67.4
P×P	68	79.4	81.5	64.7	90	86.7	78.2	67.8
Total	295	78.0	83.0	64.7	176	86.4	78.3	67.6

TABLE 11

Mortality percentages of thumbless and thumbed young from different types of matings in the group o matings derived from crosses between strains I and D.

PARENTS		NO THUMB (*pxpx*)				THUMB (*Pxpx*)			
		NUMBER	PERCENT BORN ALIVE	PERCENT RAISED OF BORN ALIVE	PERCENT RAISED	NUMBER	PERCENT BORN ALIVE	PERCENT RAISED OF BORN ALIVE	PERCENT RAISED
pxpx	*pxpx*	304	86.5	81.7	70.7	—	—	—	—
pxpx	*Pxpx*	45	91.1	95.1	86.7	42	85.7	80.5	69.0
Pxpx	*Pxpx*	47	87.2	80.5	70.2	85	67.1	77.2	51.8
Total		396	87.1	83.2	72.5	127	73.2	78.5	57.5

Another indication of physiological effect of *Px* was sought in the birth weights of animals born alive. The averages in four comparisons of *Pxpx* with *pxpx* from similar matings are shown below. The litter sizes were also averaged in order to apply corrections for the very important effect of litter size on birth weight. (It may be seen that individuals from *Pxpx* by *Pxpx* were born in larger litters than those from *Pxpx* by *pxpx* in the ID group, contrary to expectation if most homozygotes are absorbed before birth. But this cross-breeding experiment is too heterogeneous in the breeding of the dams to make possible a fair comparison between groups of matings.) Corrections are based on an average regression coefficient of

−13.5 grams per unit increase in size of litter found by averaging the values from the 8 populations.

TABLE 12

Comparison of birth weights of normals and polydactyls in 4 groups of matings.

STRAIN	PARENTS	TYPE	NUMBER	SIZE OF LITTER	MEAN BIRTH WEIGHT	CORRECTED BIRTH WEIGHT	DIFFERENCE
I	*Pxpx* · *pxpx*	Normal	94	2.83	76.2	80.7	
		Poly.	71	2.79	86.2	86.2	+5.5
	Pxpx · *Pxpx*	Normal	35	2.29	81.9	79.0	
		Poly.	56	1.88	95.6	87.1	+8.1
ID	*Pxpx* · *pxpx*	No thumb	42	2.31	96.4	93.8	
		Thumb	27	2.48	98.2	98.0	+4.2
	Pxpx · *Pxpx*	No thumb	38	2.79	83.7	87.6	
		Thumb	44	3.11	84.7	93.0	+5.4

The unexpected result is brought out that in all four comparisons of segregants, *Pxpx* is heavier than *pxpx*. The probability that the mean difference of the four paired comparisons (5.8 grams) can be due to random sampling is found to be .007 by Student's table ($t = 6.8$, 3 degrees of freedom).

Another (but not independent) test of this matter was made by tabulating the differences between *pxpx* and *Pxpx* in litters in which both occurred, averaging where more than one of a kind was found in a given litter. In strain I, the average of 62 differences was 4.7 with standard error of 1.4. In 39 litters of crossbreds, the average difference was 4.5 ± 1.4. These include stillborn young. There is no important change in the result on limiting to litters containing only liveborn young. There seems to be no reasonable doubt that the heterozygotes have a slightly higher prenatal growth rate than normals.

Tabulation of 30 day weights in strain I on the other hand give no clear indication of a difference.

TABLE 13

Comparison of weights at 30 days of normals and polydactyls of strain I.

	NUMBER	SIZE OF LITTER	BIRTH WEIGHT	WEIGHT AT 30 DAYS	CORRECTED WEIGHT AT 30 DAYS
Normal	85	2.63	80.5	240.6	244.2
Polydactyl	75	2.53	84.9	242.3	243.0

Tabulations of adult weights also gave no indication of appreciable differences. The effect seems to be limited to prenatal growth.

MODIFIERS PRESENT IN STRAIN I

In the preceding paper on polydactyly of the guinea pig, it was shown that the results of crosses among a number of inbred strains could be interpreted by assigning each strain a certain position on a linear scale, relative to thresholds for any development and for perfect development of the little toe. A certain amount of variability (S.D. = .80) where 1.00 is the distance between thresholds, had to be assigned even to completely homozygous strains, because of non-genetic factors. The position of the strains is shown in the upper part of figure 4.

We may now attempt to assign the normals of strain I to this scale. There is no reason to believe that these normals ever reach the threshold for polydactyly. The following table shows the distribution of *pxpx* individuals (thumb lacking) in F_1 and F_2 of crosses to strain D and of 2 successive backcrosses of F_1 to strain I.

TABLE 15

Percentage of 3-toed, poor 4-toed and good 4-toed among pxpx animals (no thumbs) from animals tracing to crosses of strains I and D.

	PARENTS		PERCENTAGE			TOTAL NUMBER
			3-TOE	POOR 4-TOE	GOOD 4-TOE	
F_1	Strain (I)	Good 4-toe	37.9	28.1	34.0	103
F_2	Good 4-toe (F_1)	Good 4-toe (F_1)	33.7	27.5	38.8	80
	3-toe (F_1)	3-toe (F_1)	70.9	7.3	21.8	55
	Pxpx (F_1)	*Pxpx* (F_1)	32.1	35.7	32.1	28
Total F_2			46.0	22.1	31.9	163
BX	Good 4-toe (F_1)	3-toe (I)	66.7	20.8	12.5	24
2BX	Poor 4-toe (BX)	3-toe (I)	97.1	2.9	0	35

There was something of a patroclinous tendency in the reciprocal crosses making up F_1 (not shown above) but not one that could be relied upon. There were indeed marked differences in the progenies of different I animals which suggested genetic heterogeneity of strain I itself. This is confirmed by the results in F_2 in which it is shown that F_1 individuals produced decidedly different progenies depending on whether they were good 4-toed or 3-toed. This was not the case in crosses previously described between strains D and 13, the latter of which was much more closely inbred than strain I. Moreover, the variability of F_1 is much greater than that found for genetically homogeneous stocks. Its standard deviation, assuming a normal distribution, cut by the two thresholds, comes out 1.39 (on a scale on which the thresholds are a unit distance apart, in contrast with

.75 for the inbred family 35. The mean on this scale is at 0.43 above the lower threshold, which is considerably higher than the value (−.56) for F_1 from 13×D. This indicates a location of I closer to the threshold than that of strain 13. No great accuracy is possible, however, because of the genetic heterogeneity clearly present in I. The F_1 population came from 17 animals of strain I which were not necessarily a good sample.

The total F_2 shows a variability (S.D. = 1.76) considerably greater than that of F_1, as expected. Its mean at +.18 differs but little from that of F_1. The results of the backcrosses of F_1 to strain I show that the type of polydactyly derived from D must depend on multiple factors. The polydactyl tendency is rapidly bred out in spite of selection. This agrees with the results of all other crosses of strain D with 3-toed stocks.

As the present experiments also involve the 3-toed strain A it will be well to locate it on the same scale. This somewhat inbred strain (maintained for about 10 years on a small scale as dominant in most known factors) has been mated with strain D. The results in F_1 and a backcross to D were as follows:

TABLE 15

Results of crosses of strain A with D.

	PARENTS	PERCENTAGE			TOTAL NUMBER
		3-TOE	POOR 4 TOE	GOOD 4-TOE	
F_1	3-toe (A)×4-toe (D)	89.0	11.0	0	82
BX	3-toe (F_1)×4-toe (D)	44.6	35.4	20.0	359

Clearly strain A is farther from the threshold for polydactyly than I or even 13, but not as far below as strain 2 which produced no polydactyls among 146 F_1's from the cross with D. The backcross results are, however, rather close to those obtained from (2×D)×D.

Estimate of the locations of the normals of strain I, and of F_1(I×D) and of the two successive backcrosses to I are shown below in figure 4, in the middle line. Strain A is inserted in its position on the top line of this figure.

GENE PX AS A MODIFIER OF THE LITTLE TOE

So far we have attempted to locate on this scale only animals of constitution *pxpx*. But gene *Px* may be treated as one of the group which tend to restore the little toe. Using 18 percent as the estimate of the percentage of *Pxpx* which are wholly normal in strain I, there were 37.7 percent 3-toed on the hind feet, 50 percent poor 4-toed and 12.3 percent good 4-toed. Calculation gives a mean of +.22 (above the lower threshold) and a standard deviation of 0.69. This is slightly less even than the value

(0.75) found for the inbred strain 35, derived from one mating in the 22nd generation of brother-sister mating. But we have already seen that strain I is undoubtedly heterozygous in the minor factors for development of the little toe. Theoretically its standard deviation should be greater than that of $F_1(I \times D)$ that is, greater than 1.39. Yet it is significantly smaller. However, it is certain that the effects of gene *Px* cannot

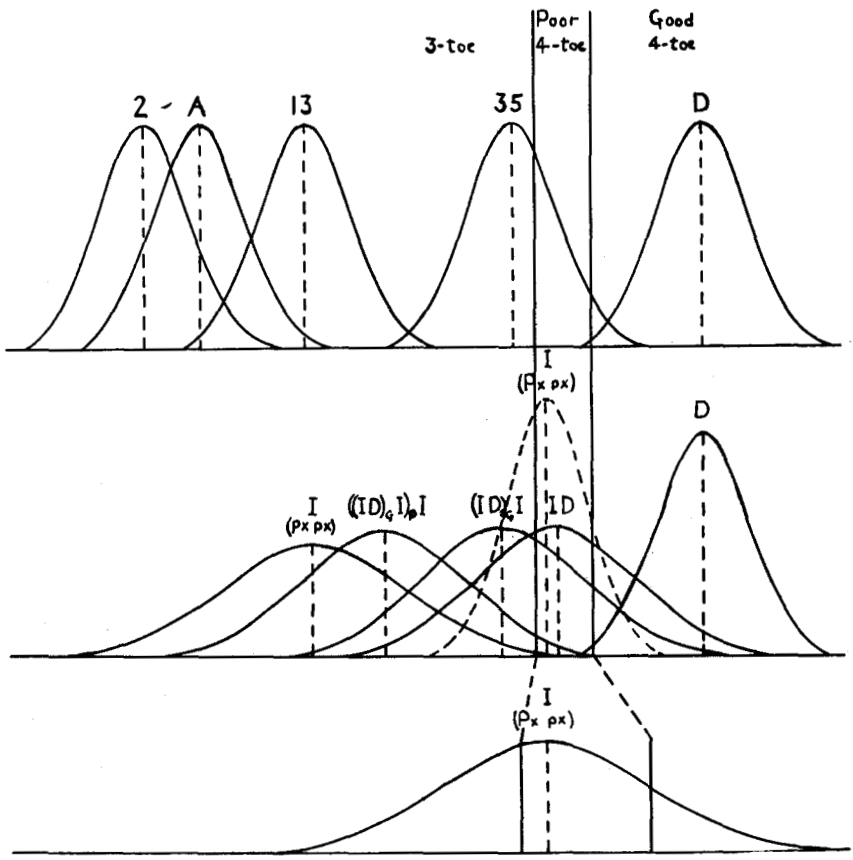

FIGURE 4.—Top line: Estimated distribution of factor complexes in 5 inbred strains of guinea pigs relative to thresholds for any and for perfect development of little toe. These estimates (except in the case of strain 35) are based on the proportions of 3-toed, poor 4-toed and good 4-toed in crosses.

Middle line: Estimated distribution of *pxpx* from strain I, based on that of F_1 (ID) and of two successive backcrosses of the latter to I. The position of *Pxpx* from I is shown in broken lines. Its small scatter is inconsistent with the wide scatter assigned I (*pxpx*) unless the thresholds are farther apart at the time of gene effect in this case (as illustrated on the bottom line).

be compared in all respects with those of the multiple factors from strains 35 and D. In the latter strains there is no recognizable tendency toward development of pollex and hallux, even with perfect development of the little toe. It is possible that the separation of the thresholds is greater in

the process affected by *Px* than that by the multiple factors. If this is the case, we are using a variable yard stick, in using the separation of the thresholds as the unit and the low variability of *Pxpx* from I may be only apparent. This idea is expressed in the two representations of *Pxpx* from I on the middle and bottom lines respectively of figure 4.

The processes affected by *Px* and by the multiple factors of D are nevertheless closely related since the latter also have effects in *Pxpx*, not only on the little toe but also on the pollex and hallux. The distribution of the digits in *Pxpx* from various sources is compared in tables 16 to 19. With the exception of two animals in pure I (and the foundation male No. 1816) the hallux has been found only with perfect development of both little toes. "Good" in the case of hallux means a full-sized, firmly attached, big toe on both hind feet. The criterion was somewhat less rigorous in the case of the pollex. It is assumed that none of the thumbless animals in groups other than pure I are *Pxpx*. There may be occasional exceptions.

Comparison of these distributions makes it apparent that strains D and A contain modifiers which affect the development of the thumb similarly in *Pxpx* but modifiers which act in opposite directions on the digits of the hind foot in *Pxpx*. Figure 5 is an attempt to represent the relations between these stocks on a 2-dimensional scheme. The thresholds for the lost digits are represented by curved lines, those for thumb and little toe intersecting. The thresholds for perfect development are indicated by broken lines approximately parallel to the thresholds for any development. Thresholds for the abnormalities of *PxPx* are represented below. The relations of these are, of course, quite uncertain. The strains are represented by circles intended to represent approximately the ranges within which the abnormal developmental processes are likely to fall. It will be seen that the lines can be arranged so that the modifiers have the same effects in *Pxpx* as in *pxpx*.

POLYDACTYLY AND OTOCEPHALY

It is interesting to compare the genetics of polydactyly with that of otocephaly of the guinea pig (WRIGHT and EATON 1923, WRIGHT 1934a). At first sight, these appear to be variations of utterly different character. Ordinary polydactyly involves morphological variations of a type which gives no appearance of abnormality to the animals and seems to be a mere reversal of an evolutionary trend. Otocephaly is an extreme type of monstrosity with defective mandible (agnathia) in the least defective grades, cyclopic eye in higher grades and loss of nearly the entire head (aprosopus) in extreme cases. Yet one of the factors of polydactyly, when homozygous, also produces a monstrosity. In both cases, it is clear that

TABLE 16

Occurrence of atavistic digits in 220 Pxpx from strain I.

		DIGITS OF HIND FEET					
		0	LITTLE TOE		HALLUX (LITTLE TOE GOOD)		TOTAL
			POOR	GOOD	POOR	GOOD	
	0	18.2	7.3	0.9	0	0	26.4
Thumbs	Poor	13.6	21.8	5.5	0.5	0	41.4
	Good	5.9	20.0	5.0	1.4*	0	32.3
	Total	37.7	49.1	11.4	1.9	0	100.1

* Includes 2 animals (0.9 percent) in which little toes were poor.

TABLE 17

Occurrence of atavistic digits in 120 Pxpx from F_1, F_2 and F_3 ($I \times D$).

		DIGITS OF HIND FEET					
		0	LITTLE TOE		HALLUX (LITTLE TOE GOOD)		TOTAL
			POOR	GOOD	POOR	GOOD	
	0	0	0	0	0	0	0
Thumbs	Poor	0	5.0	23.3	0.8	0	29.2
	Good	0	9.2	44.2*	10.0	7.5	70.8
	Total	0	14.2	67.5	10.8	7.5	100.0

* Includes one with vestigial 6th digit on right hand.

TABLE 18

Occurrence of atavistic digits in 11 Pxpx from $(1 \times D) \times D$.

		DIGITS OF HIND FEET					
		0	LITTLE TOE		HALLUX (LITTLE TOE GOOD)		TOTAL
			POOR	GOOD	POOR	GOOD	
	0	0	0	0	0	0	0
Thumbs	Poor	0	0	18.2	18.2	0	36.4
	Good	0	0	27.3	9.1	27.3*	63.6
	Total	0	0	45.4	27.3	27.3	100.0

* Includes one with vestigial 6th digit on right hand.

TABLE 19

Occurrence of atavistic digits in 15 Pxpx from $(I \times D) \times A$.

		EXTRA DIGITS OF HIND FEET					
		0	LITTLE TOE		HALLUX (LITTLE TOE GOOD)		TOTAL
			POOR	GOOD	POOR	GOOD	
	0	0	0	0	0	0	0
Thumbs	Poor	33.3	0	0	0	0	33.3
	Good	33.3	33.3	0	0	0	66.7
	Total	66.7	33.3	0	0	0	100.0

thresholds are involved. In both cases, the genetic results require the assumption of multiple factors, but in each case one factor of relatively great importance has been isolated. In both cases, moreover, a considerable role is played by non-genetic factors.

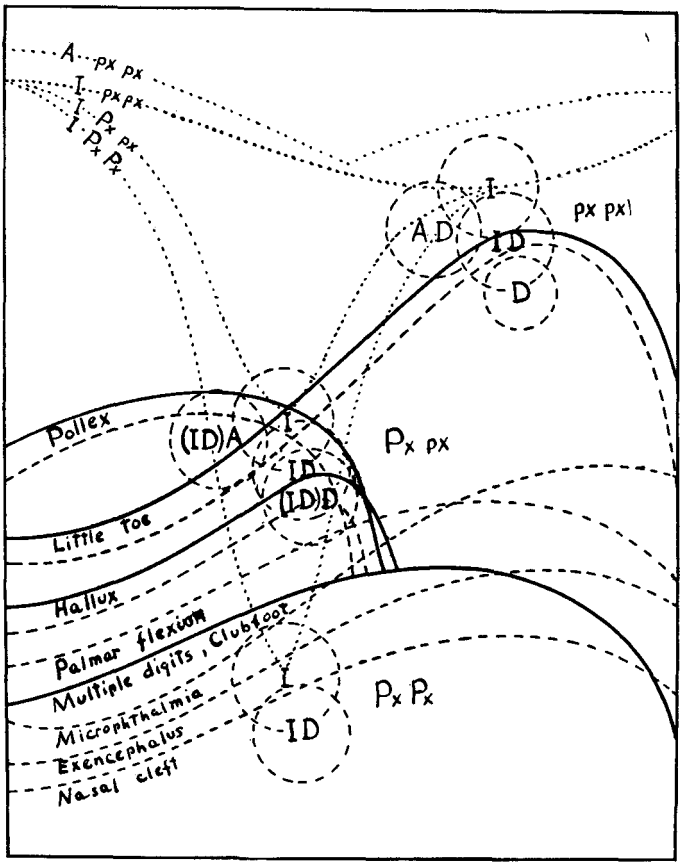

FIGURE 5.—Diagram representing the range of maximum effect (circles) of various gene complexes, relative to thresholds for the indicated abnormalities. The horizontal axis may be considered to represent time in development while depression below the top line represents degree of inhibitory process.

There are certain differences. In the case of polydactyly the non-genetic factors are largely common to litter-mates, which is definitely not the case with otocephaly. In the case of polydactyly, the gene *Px* produces sufficiently great effects to yield good Mendelian ratios under certain conditions, while the most important gene found affecting otocephaly (Ot_2) merely changes the percentage incidence from 5 percent to 23 percent.

It has long been known that otocephaly can be induced artificially by

a great variety of agents. CHILD (1911) suggested that this type of monster depends on a general inhibition to which the most active region of the primary metabolic gradient is most susceptible. STOCKARD (1921) accepted this general viewpoint and stressed the *moment* of incidence of inhibition as the factor determining the specific type of monster. In accordance with this theory, it was suggested (WRIGHT and EATON 1923) that the pertinent genes in the case of otocephaly of the guinea pig act in a fashion similar to environmental inhibiting agents—the specificity of their effects depending on the moment of development at which these came into play throughout the body. Specifically it was shown then, and in more detail later, (WRIGHT and WAGNER 1934) that all of the diverse manifestations could be traced to inhibitions of the anterior medullary plate, previously recognized by CHILD as corresponding to the most active and susceptible region of the primary gradient. (According to the work of SPEMANN, this is secondary to the underlying mesoderm or "organizer" and the results of MANGOLD and of ADELMANN [1934] indicate that in cyclopia the primary inhibition may be here. This, however, does not essentially alter the type of interpretation.) It was suggested that a large part of gene action on normal morphological variation may be of this sort, non-specific, except in the time and place at which it is called into play. It seems likely this type of interpretation can be put on the factors, genetic and non-genetic, affecting the number of digits of the guinea pig.

At first sight, it may seem most probable that in such a stock as D, we are concerned merely with the fixation under selection of a combination of ancient genes which had become nearly but not completely lost in the course of evolution. This becomes less probable on recalling that this evolutionary history has been common to all genera of the family Caviidae. Presumably the cavies lost their thumbs, big toes and little toes millions of years ago. In any event, it is highly improbable that *Px*, producing a gross monstrosity when homozygous, can be an ancestral gene.

The evolutionary process quite certainly did not consist in the dropping out of genes specifically determining the above digits. The basic heredity for perfect development of all of them is probably latent in all guinea pigs and other cavies. On present views, this heredity would be expected to be so involved in the development of the feet in general and indeed of the body as a whole that its dropping out would be incompatible with life. It is more likely that in the course of evolution this heredity has merely become overlaid by new heredity suppressing these particular parts of the feet. While some of the genes fixed in strain D may be the ancient alleles of such suppressors, they may also be new ones with general inhibitory effects acting at such a moment that they tend to suppress the effect of the digit suppressors and thus to restore the ancestral pentadactyl foot.

The effects of unfavorable environmental factors, such as immaturity of the mother, and conditions in winter and spring, in restoring the little toe(in stra in 35, WRIGHT 1934b) fit into this conception. With factor *Px* there is the complication that general growth rate seems actually to be stimulated in the heterozygote, along with the restoration of the lost digits but in the homozygote the effect seems clearly that of a general inhibition at such a moment that the limb buds and optic vesicles, maxillary processes and other parts of the head are affected injuriously. On this view the two dimensions of figure 5 may be interpreted as corresponding to time in development and degree of inhibition respectively. A somewhat similar 2-dimensional scheme was found to make possible a classification of the various types of head abnormality allied to otocephaly. The present scheme is, of course, assumed to apply at a later period of development.

SUMMARY

Guinea pigs (like all wild Caviids) normally have 4 toes on the front feet and 3 on the hind feet. Atavistic little toes are, however, rather common and stocks have been developed which breed true to their perfect development. These differ from normals by multiple factors. Atavistic thumbs have previously been recorded, to my knowledge, in only two animals, one of which had in addition, the only previously recorded big toe.

A guinea pig with vestiges of atavistic little toes, thumbs and one big toe, appeared in a normal stock (I). Breeding experiments within this stock indicated a semidominant mutation (*Px*). Among heterozygotes, 54 percent had both thumb and little toe represented (on one or both sides). These include 2 percent which also had one or both big toes. Twenty percent had thumbs but not little toes, 8 percent had little toes but not thumbs and about 18 percent were normal.

Crosses with a stock (D) characterized by well-developed little toes (due to multiple minor factors instead of *Px*) brought practically 100 percent representation of little toes and thumbs in the heterozygotes (*Pxpx*) and increased the incidence of big toes to 18 percent in the half-bloods and 55 percent in three-quarter-bloods. Crosses with a certain normal stock (A) increased the tendency to development of the thumb but reduced that of little and big toes. A 2-dimensional scheme is necessary to represent the effects of modifiers from different sources.

The heterozygotes are slightly heavier (about 7 percent) than the normals born in the same litters but have some tendency to ventral flexion of the feet.

The homozygotes usually die at an early stage of development. Those

which reach birth die immediately thereafter. The legs are short, the soles of the hind feet face the belly. All of the feet are of about double the normal width and have from 7 to 12 short undifferentiated digits each. There is always a bulging forehead, with occasionally protruding brain. All are microphthalmic. In several the nostrils have been connected with the mouth by clefts. The same gene which restores atavistic digits in heterozygotes produces a generalized monstrosity in homozygotes.

A comparison is made between the genetic situations back of polydactyly and otocephaly.

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