INHERITANCE IN GUINEA PIGS OF THE SUSCEPTIBILITY TO SKIN SENSITIZATION WITH SIMPLE CHEMICAL COMPOUNDS

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In studies on skin hypersensitivity induced by simple substances, it has frequently been noticed by various workers that an identical treatment would lead to different degrees of sensitiveness within groups of guinea pigs even when kept under uniform conditions of diet and housing (*cf.* 1, 2); similar observations have been made in experimental sensitization of human beings with simple chemical compounds, for instance by Sulzberger and coworkers (3, 4; *cf.* 5, 6). The question arose whether the variations observed in the animals bespeak hereditary properties, as one might well surmise, apart from other factors, but thus far there has been no definite proof. For the investigation of this matter we bred guinea pigs chosen for high (and low) susceptibility to experimental sensitization and studied susceptibility in the progeny; earlier results were reported briefly (7). The substance selected for sensitization was 2:4 dinitrochlorobenzene, which had proved to be a very suitable compound for experiments on drug allergy of the contact dermatitis type (1, 8). The experiments were devised to exclude the influence of feeding and seasonal factors (*cf.* 9, 10), which are therefore outside of the scope of this paper.

A particular influence of hereditary constitution is well known in certain forms of human allergy (hay fever, asthma)[1] (Cooke and Vander Veer (12), Coca and Cooke (13), Spain and Cooke (14)). Apparently this has not so far been induced experimentally in human beings in contradistinction to drug allergy in which with certain incitants sensitization is successful in almost every case (3, 15; *cf.* 16).

Various investigations have been conducted with laboratory animals which demonstrate inheritance of such qualities as the capacity to produce antibodies, a predisposition to anaphylactic effects, and resistance to infection. Inbred families of guinea pigs were studied by Lewis *et al.* and found to differ as regards ability to engender antibodies (17) and in resistance to tuberculous infection (18). Furthermore Lurie (19, 20) has found

[1] A genetic analysis is offered in a paper by Wiener *et al.* (11).

in inbred strains of rabbits marked differences towards infection by tubercle bacilli, and he was able to demonstrate several somatic characteristics associated with resistance or susceptibility. Well known are the extensive studies by Webster (21, 22), which have resulted in the establishment of strains of mice of high and low susceptibilities to bacterial infections, and to certain viruses.

An interesting report dealing with individual differences among guinea pigs in the amount of diphtheria toxin necessary for antitoxic immunization has been presented by Prigge (23): while the ratio of immunizing dose between the extremes among guinea pigs purchased for the Frankfort Institute had the surprisingly high value of 1:32,000, the individual differences with inbred strains, on the other hand, were less pronounced, being only 1:25 for one of these.

EXPERIMENTAL

Selection of Parents.—Male and female albino guinea pigs were sensitized and tested with 2:4 dinitrochlorobenzene[2] as described below, in order to obtain high and low reactors with which to set up breeding colonies for study of the inheritance of the susceptibility to sensitization in the progeny. The animals for starting both the colonies originated chiefly from two sources, one being the breeding room at the Institute, with a few added later from a third stock. There were several complicating factors in the choice of initial breeders. A selection among low reactor animals is limited because of the scarcity in common stocks of albino guinea pigs refractory to sensitization with dinitrochlorobenzene, a potent sensitizer (24); this is discussed further on. Then female guinea pigs exhibit in general a lower level of reactivity than males, making uncertain the estimation of female reactors of the lowest grade. Finally, a rather long sensitization procedure may succeed in disguising mediocrity by raising the sensitivity to an apparently acceptable level. Largely for these reasons it was found necessary, in addition to selecting the individuals on the basis of their own attained sensitization, to retain them in the breeding colony only after a trial mating and determination of the sensitization level of the offspring. Examples are given in Fig. 1. (The offspring of unsuitable breeders were likewise excluded.) This procedure once established, it proved feasible to erect colonies in which reactivities of the offspring were usually predictable (see Figs. 2 to 4).

To start the high reactor colony, 3 males (selected from 25 sensitized guinea pigs) and 6 females (chosen from 20) were employed; on the basis of the progeny test one male and one female were accepted. The data for this selection are shown in part in Fig. 1, *A*, *B*, *C*. Among the six litters resulting when 3 females (Nos. 481, 5, 8) were mated in turn with the same 2 males (Nos. 343 and 34), there was considerable variability as regards capacity for sensitization, ranging all the way from resistant to highly susceptible litters. The choice of Nos. 34 and 8 (Fig. 1, *C*) as initial breeders appeared expedient. Their 3 sons (the mother being no longer available) were bred with a new selection of females (8 picked from 63 sensitized individuals of which 5 were retained after an initial trial mating, and 2, themselves high reactors, transferred from the low reactor colony). By this means and through suitable matings involving 19 females and 21 males produced in the colony (in part backcrosses of offspring to parent and brother-sister inbreeding),

[2] Some animals used in the first matings had been sensitized with 2:4:6 trinitrochlorobenzene (picryl chloride) instead.

there were secured and tested 112 descendants representing 4 generations, after which pen-inbreeding using tested males was introduced. Some of the matings with less closely related males were made during the several months necessary for the maturing and testing of particular litters.

For the low reactor colony similar methods were followed (*cf.* Fig. 1, *D* and *E*), but consideration was given to the result of the longer course of injections in the progeny as

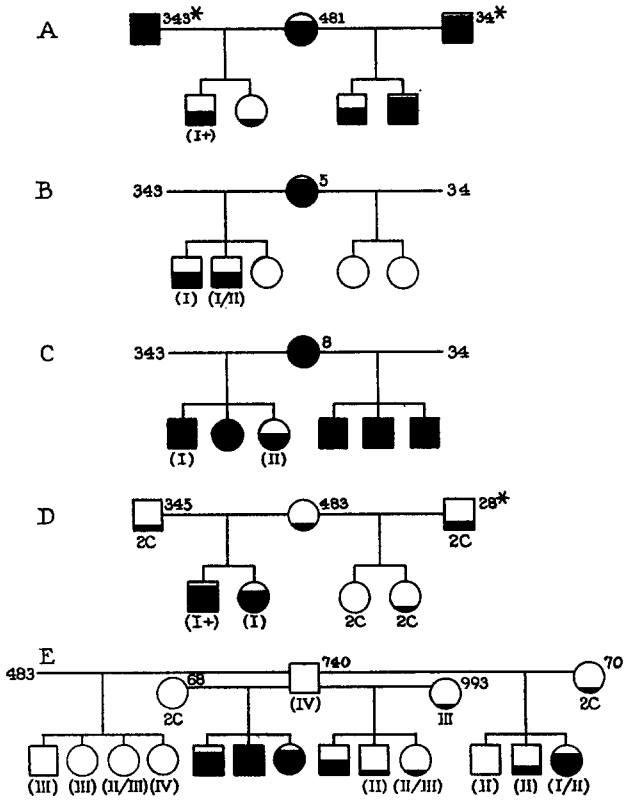

FIG. 1. Typical selection for breeding stock among sensitized animals by means of progeny test. Of the parents, those marked with an asterisk were sensitized with picryl chloride instead of dinitrochlorobenzene. All offspring were tested with the latter substance.

The degree of skin reactivity is indicated by the extent of shading, described on pages 714–715; circles and squares designate females and males respectively. The symbols show the level of sensitivity after the brief course of treatment (except that *2C* signifies test after 14 injections); the sensitivity levels after the second course, when determined, is indicated by Roman numerals.

Experiences with high reacting animals are given in *A*, *B*, and *C*, while trial matings among the low reactors are shown in *D* and *E*.

well, exemplified by the choice of Nos. 483 and 28 (Fig. 1, *D*) and the rejection of Nos. 345 ♂, 68 ♀, and 70 ♀. 5 males (picked from 52) and 18 females (chosen from 83) were introduced; 3 of the males and 10 of the females were kept after trial *via* the progeny test. From these there have been 110 descendants, representing 4 family generations, produced by the initial matings and by the breeding of 17 female and 10 male offspring.

Maintenance of the Colonies.—The animals were kept uniformly on a "dry diet," receiving hay, oats, and liberal amounts of chopped cabbage daily. Not more than 4 sows were housed with one buck; the females were removed to individual cages in the 2nd month of pregnancy, and offspring were segregated by sexes at an age of about 4 weeks. The guinea pigs remained in good health, without intercurrent infections.[3] Incidentally animals in the colony, skin-tested with Moen's antigen to detect carriers of streptococci causing epizootic lymphadenitis (25), were found not to react.

Sensitization Procedures.—Testing of the progeny was commenced when the animals weighed between 340 and 400 gm., at the most 500 gm.; the females were not mated before the final test results were obtained. Adequate numbers of male and female offspring from both the high and low reactor colonies were assembled for each sensitizing course, thus ensuring comparative testing, and cancelling possible seasonal or other variations due to external influences on the selection of animals for breeding.

For sensitizing, injections of 1/400 mg. dinitrochlorobenzene in 0.1 cc. saline were made into the skin of the back; solutions of the proper concentration were prepared freshly each time by diluting in saline an alcoholic 0.3 per cent solution of the recrystallized commercial preparation. It was soon learned that fewer injections of the incitant were necessary to sensitize animals of the "high reactor" class. All progeny therefore were first given 4 injections, twice weekly for 2 weeks, and were tested 2 or 3 weeks after the last injection: this first, brief course assisted the establishment of the "good" colony by indicating the superior reactors. Next the animals received a further course of 10 daily injections, and were tested again 3 weeks after the final injection. This longer treatment served principally to detect animals of intermediate capacity for sensitization and to exclude them from the low reactor colony;[4] at times the reactivity was found to have declined somewhat following the longer treatment (7 out of 66 animals in the susceptible colony, 13 out of 88 in the resistant colony).

For testing the sensitivity, 1 drop of a 1 per cent solution of 2:4 dinitrochlorobenzene in olive oil was spread on the belly, after clipping the hair, over an area of 8 to 10 sq. cm.; fresh sites were used for each test and normal animals were included as controls. The reactions were examined on the next day, following use of a depilatory 2 or 3 hours before. All males in an experiment, and likewise the females separately, were put together and then sorted out comparatively into four primary classes of reactors (negative up to high reactors) and the ratings were recorded without knowledge of their origins. The intensity of the reactions and the assigned grades were as follows: pink, commonly slightly elevated (I); pale pink (II); faint pink (III); negative or at most a minimal reaction (IV). Intergrades, as I/II, also were definitely recognizable. The symbols in Figs. 1 to 4 indicate the gradings by the relative amount of shading, I being shown as

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[3] An exception was the loss of 261 ♂, 262 ♀, in the more resistant colony.

[4] Likewise Webster (21, 22) originally used different doses of living bacteria in the establishment of his susceptible and resistant mice.

entirely black, IV as white, II/III as half black, and so on. A special symbol (see Figs. 2, 4) is assigned to unusually superior reactors which may be known as I+, namely those showing bright pink, often somewhat swollen, test sites. Readings after the first and second courses are indicated by an arrow, *e.g.*, II → I.

Since information was desired about the responses of the guinea pigs in the colonies to a second type of incitant, some of the lots following the terminal dinitrochlorobenzene testing were sensitized to poison ivy (26, 27; *cf.* 28), using poison ivy extract (Lederle),[5] a 13 per cent solution in acetone of extractives from *Rhus toxicodendron radicans*. To effect sensitization, 1 drop of a 1:5 dilution in alcohol was allowed to fall on the lumbar region of the back and was spread with a glass rod over an area about 15 mm. in diameter. On the 4th day the ivy was removed by cotton pledgets soaked in acetone. The test for cutaneous sensitivity, described in detail elsewhere (28), was made between the 10th and 14th days, single drops of dilutions in alcohol being applied to various areas of the skin. The reactions were recorded at 24 and 48 hours, and ratings assigned according to the lowest effective dilution and the intensity of the reactions. (These methods were used also in some breeding experiments undertaken with respect to susceptibility towards ivy sensitization.)

As regards the selection for high reacting animals, the responses of the progeny to sensitization with dinitrochlorobenzene are presented in Fig. 2[6] and are set forth summarily in Table I. The symbols indicate the skin reactivities developed by the "brief" course of 4 injections, with only 0.01 mg. in all of the incitant. It may be remarked at the outset that skin tests on some of the young made within 10 days after birth or when 2 to 3 months old were negative, proving that we were not here dealing with direct acquisition of hypersensitivity[7] from the sensitized mothers; furthermore, the onset of skin sensitivity to the successive injections has not been different in these animals from that observed with bought guinea pigs. Of the males born in the colony, 68 per cent became reactors of grade I following the brief course, and by further treatment with the incitant the proportion of animals in grade I was increased to some extent, *e.g.* from 66.6 to 78 per cent in the 36 so treated (Table I).

[5] This was supplied through the courtesy of Dr. Arthur F. Coca.

[6] For graphical reasons, there have been omitted from Fig. 2 the following crosses: 387 ♂ × 378 ♀, giving a daughter (II → I); the latter × father 387, giving one I + son, and one son and one daughter of grade I → I +; 681 ♂ × 197 ♀, producing 556 ♂ (I) and a daughter of grade II; and 556 ♂ × mother 197, giving a daughter of grade III → II; 387 ♂ × 197 ♀, producing a daughter (I/II → I). There were two instances of fertilization due to error; in the case of 94 ♀, impregnation was known to have occurred in a cage containing 3 males, all of grade I.

[7] This is also evidenced by the general correlation between the degrees of hypersensitivity induced by dinitrochlorobenzene and poison ivy extract, discussed further on, which was seen in animals whose ancestors had not been treated with ivy extract.

In detail, among these the retest after the second course gave the following result: with 13 there was no change in classification; slight changes, as I/II → I, occurred with 14; there was a substantial increase in the grading of 6; and with 3 the early high sensitivity (I) had declined to II, II, and III respectively.

Of the 45 females, 42.2 per cent were of grade I after the short treatment, and following another 10 injections in 30 of the animals there was a pronounced general rise in sensitivity, the proportion in grade I increasing from 36.6 to 73.3 per cent, and in effect obliterating the sex difference.

TABLE I

Sensitivity Responses of Progeny in the Susceptible and the Low Reactor (More Resistant) Colonies

Grade of response	Susceptible colony				Low reactor colony			
	Animals given both courses		Brief course only	Long course only	Animals given both courses		Brief course only	Long course only
	Brief course	Long course			Brief course	Long course		
	Males							
I+	5	7	6			1		
I	19	21	15	1	2	6		
I/II	3	5	2		1	5		
II	3	2	4		2	3		1
II/III	4				4	11	3	
III	2	1	1		9	6		1
III/IV					7	2		
IV			2		16	7	7	
	Females							
I+	1	8	4			6		
I	10	14	4		1	5		
I/II	2	2	2		2	1		
II	6	2	2		2	8		
II/III	3	2	1		5	4	2	
III	4		1		9	8	3	
III/IV	1		1		9	4	1	2
IV	3	2			19	11	1	1

Due to limited options, probably connected with the lower susceptibility of females, it proved necessary to use as breeders a number of females which were not especially superior after the brief course: while 18 out of the 21 bucks were early grade I reactors, this was true of only 14 out of 27 females. It may be supposed, and inspection of the applicable data would suggest, that an adequate choice among females would have improved the status of the colony. For this purpose, the mothers and their offspring are assigned to three classes in the appended tabulation on the basis of response to the brief sensitization course, uniformity among the fathers as regards early high sensitization appearing to permit an approximation of this sort.

Early rating of sows	No. of sows	Classification of offspring following brief course		
		I	I/II	II and below
I	12	25 (9 I+)	2	7
I/II	6	17 (2 I+)	3	11
II and below	8	15 (2 I+)	2	12

It appears that nearly three-fourths (73.5 per cent) of the offspring of grade I mothers are themselves of grade I, as compared with about 55 and 52 per cent of the offspring of mothers of the two lower categories respectively. And likewise suggestive, perhaps, is the proportion of animals exhibiting unusually brilliant reactions (designated as I +) born to the females of early high *versus* those of intermediate reactivity.

The genetic evidence of Fig. 2, by itself, suggests forcefully the segregation of factors influencing susceptibility to sensitization. Apart from the animals in class I, there is a variety of lesser grades, a common type being II/III → I or I/II. Familial tendencies in respect to this "delayed" sensitization are probably shown by 393 ♀ (IV → I), its sister 392 (III/IV → I) and 2 daughters of the latter by different males, 681 ♂ and 253 ♂, the ratings in question being II/III → I and III → I + respectively, and in the extreme case by 304 ♀ (IV → I+) and its three offspring by the grade I reactor 44 ♂ (see page 722). 2 animals given the full 14 injections responded slightly or not at all (268 ♀, III → IV; 370 ♀, IV → IV).

The following instances may be cited in which an inhomogeneous condition in the parents was not revealed fully at least by their phenotype testing but by segregation in the progeny. The mating of son and daughter from the backcross of 387 ♂ with the latter's mother, these 4 all being of early grade I, gave 2 daughters with unlike reactions, one of grade I +, the other II/III. Sharp contrasts among the offspring occurred also from the brother-sister cross 363 × 362 (*cf.* 360 ♀ born to 681 ♂ × 388 ♀). Again, from the backcross of 464 ♀ with its father 3 offspring were raised, one male a III, the other brother and sister each of grade I; thereupon a mating of the high reactor brother (255) with its sister gave rise to a son of grade II/III and 2 grade I females, while a backcross to its mother produced 2 males and a female which were II, IV, and I respectively. Then matings of 97 (I) with its mother 378 (II) gave 5 offspring which fell into several types, IV → IV (♀), II/III → I (♂), II → I (2 ♀ ♀), I → I (♂). A similar case of one offspring conspicuously resistant to sensitization in contrast to its brothers and sisters is afforded by the 5 progeny from the brother-sister mating 312 (I+) × 314 (I +).

In two families, probably because of small numbers, only grade I reactors were encountered (44 ♂ × 152 ♀, 46 ♂ × daughter 380).

Surveying the results obtained with the strain of high susceptibility, it has been possible to secure a marked improvement over usual guinea pig stocks. After the second generation, the regularity of the response to

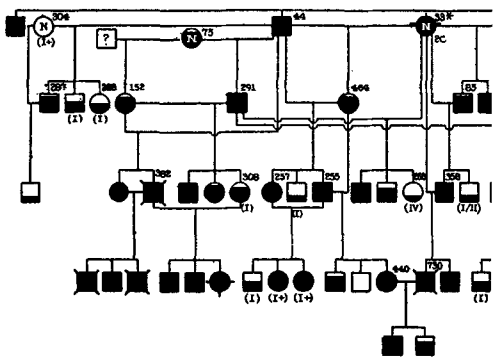


Fig. 2.
Susceptible colony
(Brief sensitization course)

(The special symbols ■ and ● are used for animals of grade I+)

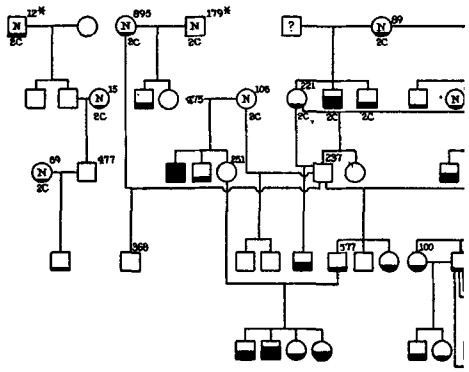


Fig. 3.
Guinea pigs with low susceptibility
(Brief sensitization course)

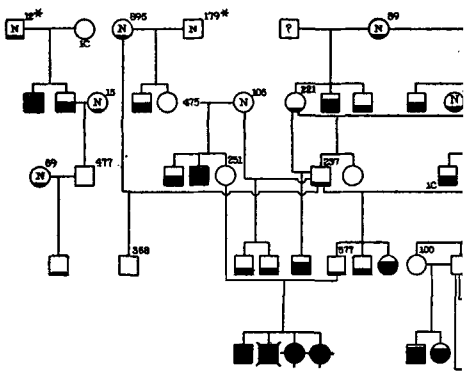


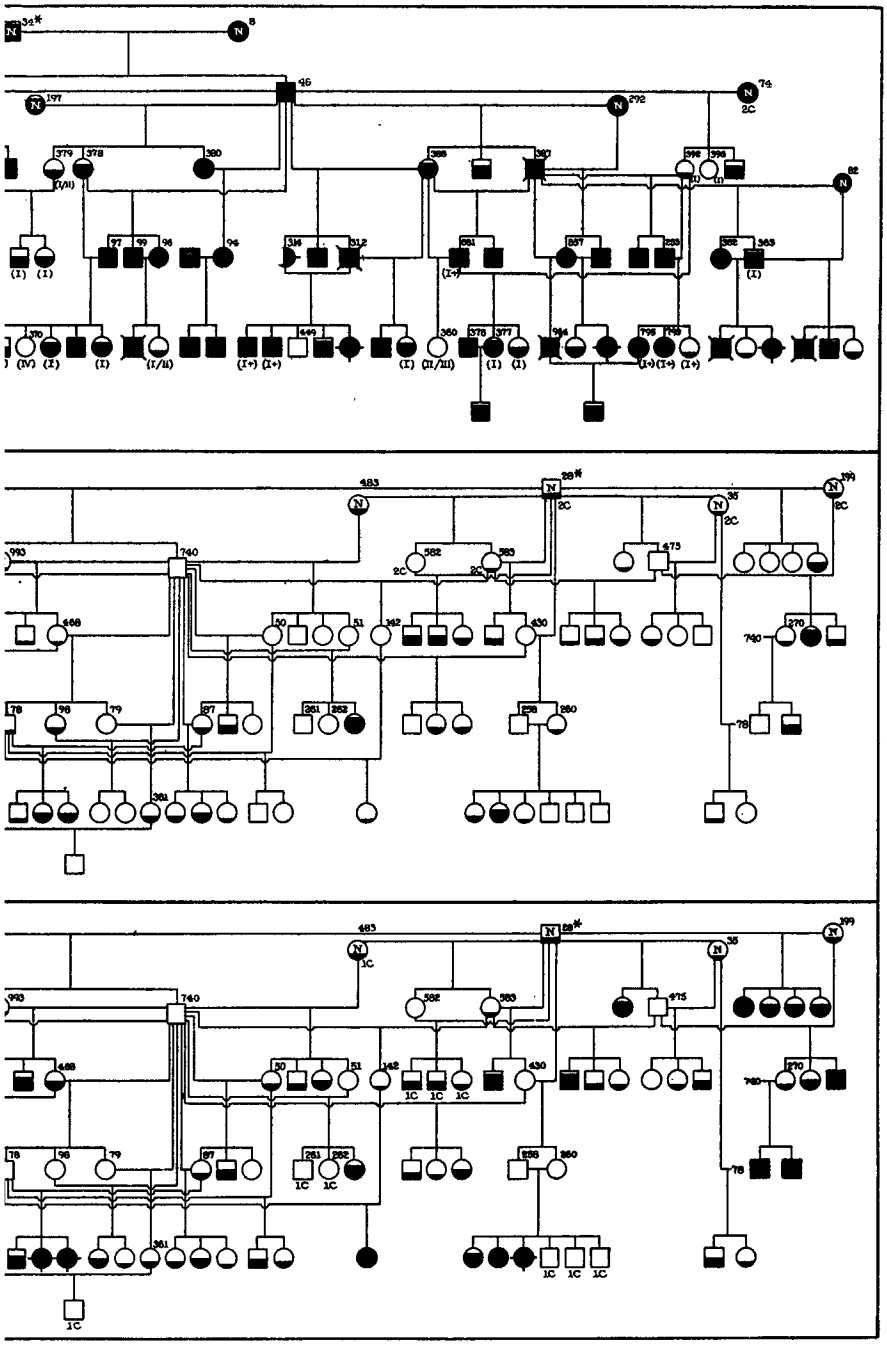
Fig. 4.
Guinea pigs with low susceptibility
(Both sensitization courses)

FIGS. 2 to 4. The levels of sensitivity are indicated by relative extent of shading (l those entirely black (grade I, superior reactors); in addition, the special devices noted respectively.

The symbols show the degree of sensitivity attained by the stated procedure, e courses of injections respectively. In Fig. 2 the grade after the second course, where

An identification number without accompanying symbol indicates that the animal i

N = animals introduced into the colonies; some of these (*) had been sensitized wit



pages 714–715), varying from symbols without shading (grade IV, resistant animals) to above are used to designate particularly brilliant reactors among the males and females

xcept that if data are wanting 1*C* and 2*C* signify the result after the first or second ver significant, is given in Roman numeral subscripts.
n question appears elsewhere in the figure, usually in the same horizontal line.
th picryl chloride.

experimental sensitization was not considerably increased by such selective matings as were practicable, the lack of a sufficient number of highly susceptible females probably contributing to this. But it would appear that the occurrence of animals exhibiting especially brilliant reactions (I+), often after only the brief course of injections, is increasing in frequency with continued selection of parents (Table II). Among the females of the fourth generation, for instance, 10 out of 23 were superior reactors at some time during the sensitization, as compared with 2 out of 12 in the third generation. Again, in preliminary tests, titrations by applying to the skin decreasing concentrations of the incitant have indicated that, whereas animals from unselected stocks sensitized by comparable procedure and picked as good reactors seldom reacted to a 1/25 per cent solution in olive oil, definite

TABLE II

Occurrence of Animals of Especially High Reactivity (Grade I+)

The number of I+ animals is shown first, followed by the total number submitted to the given sensitization procedure, *i.e.*, one or two courses.

Generation	Males		Females	
	I+ after brief course	I+ developed by 2d course	I+ after brief course	I+ developed by 2d course
F_2	1/8	0/4	0/9	0/4
F_3	2/20	1/18	1/12	1/10
F_4	8/28	3/11	4/23	6/14

reactions to this concentration have been observed with about two-thirds of the limited numbers of high reactor progeny tested.

It may be mentioned that also by another mode of sensitization, namely repeated applications to the skin of alcoholic solutions of the incitant, animals produced in the high reactor colony have shown themselves superior to other stocks.

In sharp contrast to the high reactor colony are the results with the strain bred for low susceptibility. The reactivity after the brief course of injections is shown in Fig. 3[8] and the effects seen after the second course are given separately as Fig. 4. The evident disparity between Figs. 2 and 3 is not extinguished even by the longer treatment, although the latter nearly always brought about an increase in reactivity. From the data in Table I, 41 per cent of the offspring raised in the colony belonged in grade IV after the short course, and 3 out of 4 (77.1 per cent) were grade III and below,

[8] The data in Figs. 3 and 4 do not show some lines which were abandoned in the third generation because of too few offspring.

that is, worthless for most experimental work in drug allergy; there were no significant sex differences. When further injections of the incitant were given, only 17 per cent of the males and 23.4 per cent of the females were of grade IV, although 36.6 and 49 per cent respectively were still not higher than grade III. It should be added that, while a few guinea pigs in average albino stocks appear to be refractory after 10 to 15 daily injections of 2:4 dinitrochlorobenzene, it is highly doubtful whether an absolute resistance will be encountered in any guinea pig under more intensive treatment, for instance repeated applications of oil or alcohol solutions to the skin.[9] The lowest category used in the charts, grade IV, does not signify entire resistance, for some trifling hyperreactivity to the daily injections was noted in nearly all these animals during the second course; however, in the final contact test after the rest period the animals could not be distinguished surely from those of the normal controls which showed some slight skin irritation to the chemical.

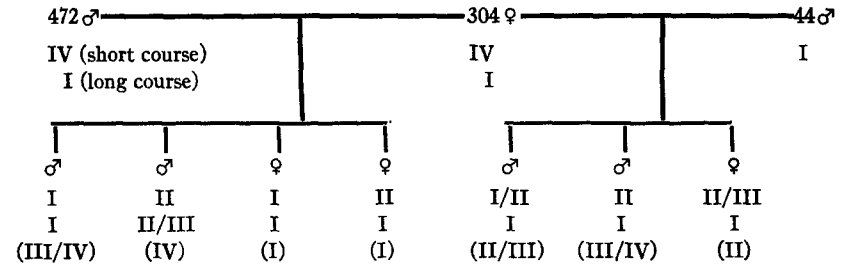
The greater difficulty in breeding for low susceptibility to sensitization was evident in the earliest attempts. Contributing complications in the selection of the breeding stock were mentioned above (page 712); probably as a consequence there was less initial homogeneity than among the high reactor stock. As regards improvement of the colony, a choice of "continuing" low reactors among the progeny, particularly brothers and sisters, was quite limited. Of the 7 male offspring suitable for breeding, 2 had given slight reactions after the brief course, while among 11 females attempts to mate 4 were fruitless, the breeding of 2 others was scarcely productive, and the remaining 5 included one which had been early grade III and 2 that were early grade II/III.

Since the tendency for resistant animals to produce susceptible offspring is much greater than the reverse, it is probable genetically that the chief factors contributing to resistance are dominant over those favoring susceptibility to experimental sensitization. A few instances of "early resistant" animals from the mating of susceptibles (Fig. 2, *e.g.* 449 ♂, and Fig. 1, *B*) appear, however, to be out of line with a simple presumption that "resistance is dominant over susceptibility." Indeed, the data otherwise suggest that a plurality of factors controls susceptibility and resistance, in particular because of the occurrence of a rather large number of patterns, instead of a few, in the acquired levels of sensitivity. Other than this, if

[9] Of some 234 albino guinea pigs sensitized in this manner, by only 5 to 7 daily applications, not more than 4 appeared to be refractory. This would then be analogous to the situation with *Primula* dermatitis (16), which is acquired by nearly every individual upon intensive treatment with extractives of the plant, although under natural conditions of contact marked individual differences in the capacity for sensitization are evident.

one considers only the following broad types: early susceptible, delayed susceptible (as III → I), and continuing resistant (IV → IV), it is not probable that a single pair of allelomorphs is adequate to explain the sensitization behavior of the progeny, *e.g.* in the line: father 28 × 583 ♀, 28 × 430 ♀, brother 258 × 260 ♀ (Figs. 3, 4). The intricacy is further to be seen from the various matings of 740 ♂ and of 78 ♂ (and from the discrepancies, mentioned below, between the sensitivities caused by use of two incitants). It will be noted in this colony that throughout the breeding of guinea pigs selected for their relatively high resistance, we did not meet with any positive instance of a "homozygous" animal as demonstrable by the testing of its progeny (although this well could be a consequence of the limited combinations of parents employed).

Of interest in connection with the genetic situation are the results of mating two delayed reactors of unusual behavior, and the female also with an early susceptible male: the gradings after the brief and second courses are given, and below, within parentheses, the sensitivity developed to poison ivy following treatment of the progeny with the latter.



Crosses involving members of the susceptible and resistant colonies were not undertaken since the latter group was not sufficiently uniform.

The essential difference between the two colonies and the evident segregations in the offspring indicate a genetic basis for susceptibility to drug allergy in the guinea pig, albeit a complicated one, and this is supported by the finding (after our breeding experiments were well along) of distinctly different levels of susceptibility among the albino stocks offered by various breeders. Among these instances may be mentioned one stock characterized by a low to moderate susceptibility to sensitization by intracutaneous injections of dinitrochlorobenzene and another which was distinctly inferior to other animals in our experience as regards skin sensitization to picryl chloride following intraperitoneal injection of this incitant in conjunction with dead tubercle bacilli (29).

We also conducted analogous breeding experiments with animals chosen

on the basis of high and low sensitivities developed to poison ivy (see page 715) instead of to dinitrochlorobenzene. The number of progeny examined was not large, but the results tended to show that there is an hereditary basis for susceptibility in this case also.

The question arose whether the difference between the two colonies is actually one of sensitization capacity or simply a lower or higher resistance to the primary toxicity of the incitant (upon which acquired sensitivities, possibly not unlike in degree, would be superimposed). For this purpose, 4 males and 4 females from each colony were assembled and, prior to the regular sensitization course, the skin reactions (primary toxicity) to different concentrations of dinitrochlorobenzene (1.5 to 0.75 per cent in alcohol, 10 to 1 per cent in olive oil) were determined. The brief intracutaneous sensitization course was then given, and after a rest period of 2 weeks the animals were tested with a 1 per cent solution in olive oil (which is seldom irritating and which had not sufficed to differentiate any of these same animals before sensitization).

There was, in fact, a difference between members of the two colonies, particularly among the females, with respect to the primary toxicity of dinitrochlorobenzene, skin irritations being produced in members of the high reactor colony by about one-half to one-third the concentration giving rise to the same degree of irritation in the low reactor colony. The significance of this as an explanation of the difference between the two colonies apparently can be discounted, however, because there was no consistent parallelism in the individual cases between primary toxicity and the level of hypersensitivity attained by treatment with the incitant. For instance, among the males from the high reactor colony the one exhibiting the greatest primary toxicity happened to be a poor reactor, and among the females from the low reactor colony the one least irritated by the incitant developed the highest sensitivity of the group. Another potent argument for actual differences in degrees of specific sensitivity is the frequent occurrence in the susceptible group of reactions to 1/25 per cent solutions of the incitant in olive oil, mentioned above, as compared with the poor responses to the 1 per cent test solution among members of the low reactor colony, a ratio much greater than the one between the concentrations producing irritation in non-sensitized animals of the two colonies. Nevertheless, this somatic difference between members of the two colonies may have a bearing on the multiplicity of sensitization patterns observed.

With a number of guinea pigs (100 progeny from the susceptible and resistant colonies, 37 offspring of animals selected according to their response to poison ivy extract, and 27 other guinea pigs), sensitization courses were

given successively with the two incitants to compare the respective responses. There was, in fact, commonly a parallelism between the degrees of sensitivity acquired to the two types of incitant, as stated for human beings by Wedroff and Dolgoff (5), yet discrepancies have appeared: these have been chiefly in the direction of a dinitrochlorobenzene sensitivity higher than that towards ivy, but there were at least 20 clear instances of the reverse.

Experiments of this sort should be extended, perhaps with the breeding of animals having unequal sensitivities to two incitants, and using methods of sensitization chosen to allow simultaneous testing with the respective chemicals.

The relationship observed will depend obviously upon the intensity of treatment with each sensitizer. For instance, the correlation was higher if the response to the ivy extract was compared with the dinitrochlorobenzene brief course. The experiences with 46 animals from the susceptible colony and 54 from the more resistant colony may be cited: after the brief course, 52 per cent of the offspring showed close agreement between the responses to the two incitants (*e.g.*, variations of not greater order than II *versus* I/II, IV *versus* III/IV, etc.), and after further dinitrochlorobenzene treatment in the second course 42 per cent were still in close correspondence.

Other data bearing on discrepancies may be cited.[10] 2 males from the DNCl (dinitrochlorobenzene) stock which had scarcely responded to picryl chloride after a course of 13 intracutaneous injections of this substance were then given the brief course of DNCl injections; both became definitely sensitive to the second incitant (I/II and III respectively), and the discrepancy was confirmed upon retesting with picryl chloride and dinitrochlorobenzene simultaneously. Again, in one experiment 20 guinea pigs from common stocks were sensitized concurrently with both ivy and dinitrochlorobenzene, the latter being here used as a 5 per cent solution in olive oil applied to the skin daily for 4 days, and were tested with the two substances at the same time. The sensitization levels with the two incitants corresponded in 15 animals; of the others, one was moderately sensitive to DNCl but quite high with ivy while two others showed the opposite relationship, another was high towards DNCl and very weak towards ivy, and still another exhibited rather good DNCl and low-to-moderate ivy responses.

COMMENT

The establishment by controlled breeding of two colonies of guinea pigs which differ significantly in the degree of sensitivity attained following the same sensitizing procedure demonstrates the existence of variations of an hereditary nature in the capacity for sensitization. The difference between the two colonies was striking in that the one strain gave in the great majority

[10] Compare the ivy and dinitrochlorobenzene responses in the tabulation on page 722.

of cases uniformly intense reactions after a brief course of intracutaneous injections with a total of 0.01 mg. substance, while the other responded almost regularly to an even greater number of injections with only a low grade sensitivity; however, these animals were not entirely refractory. In the latter group the uniformity was much less pronounced than among the good reactors, that is to say the offspring of poor reactors not seldom proved to be unequal, some individuals in a litter frequently exhibiting stronger effects than either parent. It might well be, however, that continuation of selective inbreeding would eventually lead to a more uniform strain of poorly reacting animals. It may be remarked that also Webster in the studies referred to above had greater difficulty in establishing a strain of mice with high resistance to infection with mouse typhoid than a highly susceptible strain.

Suggestive evidence for inherited differences as regards drug sensitization comes forth from experiences with guinea pig strains procured from different breeders. It appeared that guinea pigs (albinos) from some sources responded so poorly to sensitization that they were unsuitable for our experimental purposes, even after being kept for some time under our regimen.

The experiments were not carried far enough, and the situation is as yet too complicated, to offer a genetic analysis. Also it was not feasible to undertake repeated matings between parents of different types to obtain information about the ratios in the offspring. Several features, particularly the fact that the sensitivities do not fall into a few sharply discrete grades but show continuous transitions, would appear to contravene a supposition of a single pair of genetic factors.

Breeding experiments with parents selected for their reactivity to poison ivy, although made only on a small scale, tend to show that here again the degree of susceptibility is hereditary and that good and poor colonies can be established. This raises the point whether animals of different susceptibilities to sensitization with one simple substance will show the same behavior towards a different compound, that is, whether one deals with a susceptibility that is general or one varying to some extent according to the substances tested. From experiments in which animals were sensitized in succession to dinitrochlorobenzene and poison ivy, it would seem that there is roughly an accordance between the sensitivities developed to the two compounds (*cf.* 5) but there were several instances of discrepancy one way or the other. If further experience were confirmatory, this likewise would affirm the complexity of the hereditary basis underlying drug allergy. Observations indicating a degree of specificity in experimental sensitization of human beings have recently been reported (30).

It may be added that the possession of guinea pigs highly and uniformly susceptible to sensitization should be of value for experimental work in this field.

SUMMARY

It has proved possible to set up lines of guinea pigs of significantly different susceptibilities towards a compound of simple structure, namely 2:4 dinitrochlorobenzene. This provides direct evidence that the type of sensitization under discussion is influenced by heredity.

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