DEGREES OF SUSCEPTIBILITY TO DIPHTHERIA TOXIN AMONG GUINEA-PIGS. TRANSMISSION FROM PARENTS TO OFFSPRING.¹

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The success which has followed the important work of Ehrlich in establishing and maintaining an anti-toxin unit for diphtheria serum has greatly lightened the work of those whose duty it is to see that the anti-toxin placed in the hands of the practicing physician has certain definitely known toxin-neutralizing powers. In the Institute for Experimental Therapy, under the direction of Professor Ehrlich, carefully standardized toxin and anti-toxin are evaporated in vacuo and kept in a system of hermetically sealed glass bulbs, from which the air has been exhausted and in which some hygroscopic substance like phosphorus pentoxid maintains the serum in a dried state. These bulbs are kept in a dark, cold place. Once in two months a bulb is opened, the serum dissolved in a fluid made up of glycerine and ten per cent salt solution or some similar mixture, and distributed by the Institute to all parts of the globe. This solution is used for two months and then discarded for the fresh solution.

Having been the first to use this standard in this country, at first experimentally, then regularly, the writer takes this opportunity to testify from an observation for more than six years to the uniformity of this standard, so far as this uniformity can be objectively estimated from other fairly constant standards.

The most uncertain element, and yet one upon which the accuracy of the test depends no less than upon the maintenance of the toxin and anti-toxin standards, is the relative susceptibility of the guinea-pig to the diphtheria toxin. The unit toxin has been universally accepted as the minimum dose

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of culture fluid free from diphtheria bacilli which will kill a
guinea-pig, weighing approximately two hundred and fifty
grams, when injected under the skin. Death usually ensues
between the third and the fourth day. When the dose is
larger than the minimum fatal dose, death takes place more
rapidly, and it is possible within certain limits to roughly esti-
mate, from the period elapsing between injection and death,
the number of minimum fatal doses injected, entire and
fractional.

Guinea-pigs do not respond with mathematical accuracy
to the minimum fatal dose. In any series treated in the same
way irregularities may appear, and an examination of the
tables and protocols of published experiments shows that
irregularities are the rule rather than the exception. In such
a series in which irregularities occur, the investigator may
throw out all records of tests indicating greater than average
resistance, or he may average them together. In special cases
his judgment may incline to accept the testimony of the more
resistant animals, and thus the personal equation may enter.

For nearly ten years guinea-pigs have been bred under the
author's direction for testing diphtheria toxin and anti-toxin.
A careful record has been kept of the descent of the animals
bred and the source of those purchased from outside. The
use of such records may seem problematical, but like many
other data they occasionally subserve important uses, as will
be shown later. They also act as a control upon the breed-
ing animals, and enable us to promptly eliminate useless
individuals.

The irregular demands of the laboratory for guinea-pigs
were usually greater than the supply, and a considerable
number from outside sources have been used from the start.
It was noticed almost from the first that these guinea-pigs
were less resistant to diphtheria toxin than our own. But the
susceptibility varied from source to source. Those from cer-
tain sources possessed a maximum susceptibility. After
observing this for a number of years I was inclined to credit
it to insufficient food and to unsatisfactory environment. As
long as this difference could be gauged and as long as it
occurred regularly, it did not greatly interfere with the test. I usually allowed for it in the L + dose of toxin, the latter being about five to ten per cent less for animals from certain sources.

Assuming individual variability as a fact, and also that the food and care would have some influence in modifying susceptibility, we see nothing in these data which is specially noteworthy.

During the past year, however, irregularities occurred in my tests which were so conspicuous that they could no longer be disregarded. A careful inquiry, extending over many months, finally enabled me to account for these variations in susceptibility as a family inheritance, and to show that practically all the individuals of a litter possess the same degree of susceptibility or resistance, and that several litters of the same mother are the same in this respect. In the following pages are given some of the data taken from the records of routine tests.1

Guinea-pig No. 2944.

April 17, 1904. Nos. 3211–3213 born to-day (male, 2794).
May 18, 1904. No. 3211 (weight 273 grams) receives .007 cubic centimeter toxin subcutaneously. No effect.2
May 28, 1904. Another pig (257 grams) receives .0075 cubic centimeter of the same toxin. Dead in two days, three hours.
No. 3212 figures in the following test:
May 18. 0.18 cubic centimeter toxin plus one anti-toxic unit fatal to 249-gram pig in one and one-half days +. 0.185 cubic centimeter toxin plus one unit fatal to 253-gram pig in two and one-half days — .0.185 cubic centimeter toxin plus one unit has no effect on pig No. 3212 (255 grams). 0.19 cubic centimeter toxin plus one unit fatal to 260-gram pig in one day +.
No. 3213 figures in the following test which is not so satisfactory as the preceding, but indicates marked resistance:
200-unit test with 275-gram pig = death in one day, four hours. Injection in part into abdomen as omentum was hemorrhagic.
300-unit test with No. 3213 (272 grams), no effect.

1 These records are necessarily meager because the number of guinea-pigs in a litter is small, and each animal can be tested but once. Unless this test is so made as to involve the minimum fatal dose, it is apt to be useless for any estimation of susceptibility or resistance.

2 Examination on the third day.
These three pigs of one litter resisted without any local signs what was more than a minimum fatal dose.

Aug. 31, 1904. Second litter of four guinea-pigs born (male, No. 2794). Of these only one was used in such a way as to serve in our statistics. It resisted without any lesion the L + dose.

Oct. 4, 1904. Third litter of guinea-pigs (3420–3423) born (male, 2573). The above tests had not been made with any special reference to family immunity, as my attention had not yet been directed to this point by any conclusive data. In using this third litter, however, I arranged the tests so as to bring out any immunity:

Nov. 12, 1904. A 251-gram pig receives .007 cubic centimeter subcutaneously. Dies in two and one-half days +.

No. 3423 (253 grams) receives .007 cubic centimeter of same toxin. No effect.

November 15. No. 3422 (266 grams) receives .008 cubic centimeter of the same toxin. No effect.

Nos. 3420 and 3421 figured in the following test for determining the L + dose of a new toxin: 0.22 cubic centimeter plus one anti-toxic unit produced transient edema; 0.23 cubic centimeter, transient edema and fatal paralysis; 0.26 cubic centimeter death in four and one-half days; 0.27 cubic centimeter death in three days, seventeen hours; 0.24 cubic centimeter plus one unit has no effect on No. 3421 (253 grams); 0.26 cubic centimeter plus one unit has no effect on No. 3420 (271 grams).

Dec. 9, 1904. Fourth litter (Nos. 3524–3526) born (male, 2573). Two of these figure in the following test; the third remains unused.

Jan. 16, 1905. 0.28 cubic centimeter toxin plus one anti-toxic unit fatal to a 263-gram pig in two and one-half days +.

The same fatal to a 250-gram pig in three days and four hours.

0.32 cubic centimeter toxin plus one unit has no effect on No. 3525 (weight, 260 grams) nor upon No. 3526 (weight, 264 grams). Both had gained in weight on the fourth day.

No other pigs of similar insusceptibility have come under my observation, but others of a certain resistance have been traced, of which the following is a good example:

Guinea-pig, No. 3189.

Aug. 13, 1904. Litter of two (3336, 3337) born (male, 2832).

November 22. 275-unit test of a serum produces transient induration in a 273-gram pig.

350-unit test of same serum has no effect on No. 3336 (290 grams).

No. 3337 was used in a test in which relative resistance was not determinable.

Oct. 18, 1904. Four young born (Nos. 3448–9, 3484–5) (male, 344)

1 Not numbered until removed from mother. Some grow faster than others, hence the gap in these numbers.
The L + dose of a toxin having been determined as 0.26 cubic centimeter, the two following tests indicate resistance:

Dec. 1, 1904. 0.28 cubic centimeter toxin plus one anti-toxic unit produces large swelling in No. 3448 (258 grams). 0.30 cubic centimeter toxin plus one unit produces large swelling in No. 3485 (268 grams).

Dec. 7, 1904. 300-unit test of a serum is fatal to a 252-gram pig in two and three-fourths days. 350-unit test of same serum is fatal to 251-gram pig in one and one-half days. 425-unit test fatal to 250-gram pig in one and one-half days. 475-unit test produces large swelling in No. 3449 (269 grams) and death in four days.

In this test the serum would be valued at about 275 units in accordance with the first pig, and about 450 in accordance with the last.

December 1. 250-unit test fatal to 265-gram pig in two days. 350-unit test produces large induration and sloughing in No. 3484 (248 grams).

The resistance in this litter is much lower than in the offspring of No. 2944, yet it is sufficient to cause overvaluation of serum by about fifty per cent in units.

I have also tabulated the tests made with three litters of another breeding pig (No. 2898). In the offspring of this pig increased resistance is shown throughout, but it is less than in the offspring of No. 3189. I quote only the tests which are apprehended at once by those not familiar with the various constants.

**Guinea-pig No. 2898.**


November 16. .008 cubic centimeter of a certain toxin fatal to a 254-gram pig in two and one-half days.

December 2. .009 cubic centimeter of the same toxin filtered has no effect on No. 3023.

March 5, 1904. Nos. 3183–6 born (male, 2833).

April 26. 0.185 cubic centimeter toxin plus one unit is fatal to a 260-gram pig in one and one-half days. 0.19 cubic centimeter toxin plus one unit produces ulcer only in No. 3184. 680-unit test of a serum is fatal to No. 3185 (248 grams) in three and one-half days. 525-unit test of same serum fatal to a 252-gram pig in one day.

In endeavoring to trace the source of this transmitted immunity several questions at once present themselves:

2. The influence of the male.
3. The influence of selection in breeding.

The data available for an answer to the first question, the influence of any treatment with toxins or anti-toxins, are quite sufficient to rule this out. Through a fortunate combination
of circumstances two guinea-pigs come within the scope of this question which came from the same outside source at the same time, and were treated together, first with the same toxin-anti-toxin mixture, then with cultures of \textit{B. coli} by feeding. Between four and five months after the toxin-anti-toxin they were transferred to breeding pens. One (No. 2944, described above) gives birth to the most resistant guinea-pigs, the other to those of normal susceptibility. In the examination of the records of other females which have given birth to litters of normal susceptibility any influence of previous treatment is not in evidence.

The influence of the males upon the immunity of the offspring cannot be definitely stated at present, since there are no records of the toxin resistance of the males used. Most of them were obtained from dealers furnishing very susceptible pigs. The litters which I have described as above average resistance are descended from five different males, and it would appear, unless there is some fortuitous combination of circumstances here, that the immunity in these three instances was transmitted by the mother. Experiments are now under way to test more precisely the influence of the male.

The third question, the influence of artificial selection in breeding, is probably of most significance. In the extensive use of guinea-pigs for the testing of diphtheria toxin and anti-toxin the factor — susceptibility to toxin — figures exclusively. The tendency is to make these tests in such a way as to involve the smallest number of animals and yet obtain definite results. This can only be obtained by using amounts of toxin, or of the toxin-anti-toxin mixture, which represent as nearly as possible the minimum fatal dose. Guinea-pigs having a slightly higher than the normal or average resistance are likely to survive, and subsequently to be used for breeding. The most susceptible will have the least chance to survive. A selection of this kind, based on relative toxin immunity going on for a number of years, must eventually lead to considerable diversity.

In the breeding of guinea-pigs the writer has, at frequent intervals, introduced animals from outside more to prevent
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close inbreeding than to avoid variation in toxin resistance. In one instance of some degree of toxin immunity described above (No. 3189), the genealogy of the female could be traced through six generations of females, starting in 1895. The males were, however, almost wholly from outside sources. In two other instances of normal susceptibility the descent of the animals was traced through two generations from guinea-pigs obtained elsewhere. It is significant that the one highly immune guinea-pig which called my attention forcibly to this matter came from a dealer supplying, as a rule, very susceptible animals.

A number of important considerations of both scientific and practical bearing are involved in this subject. Of these I shall only point out a few at present, leaving the rest until more accurate data shall have been obtained by breeding with males of known susceptibility.

The importance of its scientific bearing is shown in the controversy between Ehrlich on the one hand and Arrhenius and Madsen on the other, concerning the nature of diphtheria toxin and its neutralization by anti-toxin. Unless the factor — guinea-pig — is better controlled and made more constant, neutralization experiments cannot very well lead to mathematically accurate formulæ. In the practice of testing anti-toxins both inaccuracy and cost are increased by variations in the susceptibility of guinea-pigs. It is evident that as the individuals of the same litter grow up together, and are used at the same time, or in close succession, a few relatively resistant litters used consecutively may cause considerable fluctuation in the standards.

A more careful selection of guinea-pigs as to relative toxin immunity should diminish the number of guinea-pigs needed in the standardizing of anti-toxins. At present irregularities in the results of tests are covered either by using larger numbers in the same test, or by repeating it. Both entail much skilled labor and hence expense. They are essentially unscientific, as they largely depend upon the element of chance.
In view of the increasing importance of an accurate standardization of commercial serums, and in view of the necessity of maintaining a uniform anti-toxic unit from year to year, it becomes necessary to breed with greater care, and to gauge the resistance of the offspring before placing too great reliance upon the tests made from day to day. In fact, the data here presented make it imperative for those endeavoring to maintain a standard to breed their animals with great care and selection. Fortunately the indications are that the offspring of the same female possess the same susceptibility, and should this prove true over relatively long periods of time no great difficulty will be encountered in acquiring and maintaining a homogeneous supply.

With the more precise determination of toxin susceptibility as a character transmitted from the parents to the young, the relative influence of food, temperature, air, light, etc., becomes quite uncertain, and only experiments in breeding can aid us in measuring this influence. Finally there remains to be determined for guinea-pigs a certain average susceptibility which is probably best accomplished with the use of dried and relatively stable toxin. The use of the expression "normal susceptibility" in the preceding pages is of course merely provisional, and employed to indicate the susceptibility of the majority of animals as observed over long periods of time.