

COMPARATIVE STATISTICS OF ANTITOXIN HORSES.
A STUDY OF THE RECORDS OF ONE HUNDRED
HORSES IMMUNIZED TO DIPHTHERIA TOXIN,
WITH COMPOSITE OF CURVES.

BY WILLIAM R. HUBBERT, M.D., DETROIT, MICH.

Although there is no method known by which we are able to select high-test horses in advance of the tedious immunizing process, statistics show, nevertheless, that some advance has been made. Up to 1896 no records are available. From 1897 to 1899 the records of a few horses show that 80 % tested below 300 units and 10 % above 500 units.

During the next period, 1900 to 1902, of 54 horses injected, 64 % tested below 300 and 25 % over 500 units.

The table gives the data of the last one hundred consecutive horses partially or completely immunized in the production of antitoxin, and includes twenty horses which either died or were sold before a test drawing of blood could be made. None of these last horses was able to attain to the maximum amount of toxin injection as compared with producing animals. Several of them were sold within two months of the date of purchase. With these omitted, a better showing could be made. The table does not include the earlier animals prior to Horse 56, many of which, like Horse 41, were high-test animals. As will be seen from the table, a horse attains its maximum antitoxic value within from two to ten months from the beginning of treatment, the average being about four months. It may continue to yield serum of marketable value for a few months longer (5.1 months is the average), when the decline begins, usually never to rise again. Some notable exceptions to this rule have been made. If the serum of a new horse does not test 300 units by the end of the fourth month after the beginning of treatment, it may be regarded as nearly certain

that the animal is not a suitable one for the work. A horse is usually at his best during the fourth month of immunization. I do not consider that the data given of the average length of time horses continue to produce antitoxic serums as stated in the table fairly represent the facts. In many of the averages given the horses are still under the treatment, and probably some of them will continue to yield potent antitoxin for some months or years to come.

Atkinson¹ has found from a study of the records of the horses of the Board of Health of New York City that there seems to be a maximal antitoxic limit of each horse beyond which the animal cannot be forced, no matter how much toxin is injected, and I am able to confirm this finding.

Horses attaining a high antitoxic strength exhibit a longer period of usefulness than do lower testing horses, but usually at the expense of a much lower unit value, as will be seen by reference to the table.

The amount of toxin necessary to maintain a horse at its normal antitoxic capacity varies from 60,000 to 90,000 fatal guinea-pig doses per injection, the horses being injected usually two to three times per week.

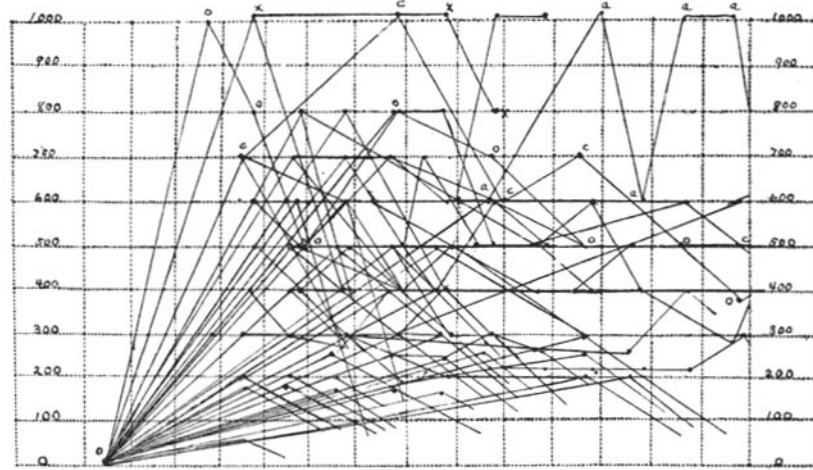
The results of the record have been attained by what are known as low-test toxins, namely, toxins ranging in lethal dose from $\frac{1}{200}$ to $\frac{1}{300}$ cc. per 250 grams of guinea-pig.

In the composite figure the antitoxic type curves of the last one hundred horses immunized to diphtheria toxin are given together with the curve of Horse 41. For the purpose of comparison all curves are represented as starting at the same time. Duplicate curves are not given. Each space from left to right represents a month of treatment and the upward curve indicates the number of antitoxic units present at any particular time.

Each dot in the curve represents a test point. The dots in the ascending limb of the curve show that at the time the tests were made the animal had reached a point at which it was able to withstand the injection of maximal amounts (300 to 700 cc.) of toxin as compared with immune horses. It will be noticed that

¹ *Journal of Medical Research*, 1903, iv, 173.

in nearly every case a decline in antitoxic strength began at this point, although the injections were continued without intermission. In a few cases the antitoxic strength increased beyond this point. The amount of toxin was rarely increased, and no attempts were made to force the animals, since it had been noticed that horses gave longer periods of service when the injections were not pushed beyond certain limits. Moreover, they



rarely died during treatment. The point at which the curves terminate indicates the time at which the horses were of no further use.

The striking feature of the composite is the uniformity with which the curves descend after having attained the maximum of antitoxic strength, and that the descent takes place despite a continuation of the immunization process. It is also noteworthy that the decline from maximum strength occurs with about the same rapidity with which the original gain in maximum activity was made. Almost without exception every rise to the maximum shows its corresponding decline, and, indeed, with almost the same inclination with which the ascension has been made. In the great majority of curves, the angle of declination equals the angle of ascension. The decline is continued until the animal has reached its normal antitoxic value, which in the case

of a high-test animal is usually high enough for therapeutic uses, and is about one half its maximum antitoxic value. A horse that takes a year to reach 700 units remains as long in service as does a horse that reaches 1000 units within three months. Both are good types. After having attained a given height, nearly all horses almost at once drop to a lower antitoxic level. It is very rarely that any of these horses ever again reach the antitoxic height from which it has dropped. A horse that takes a year to reach 200 units is of no practical value.

In the immunization of Horse 41² (*oaaa*), a period of ten months elapsed before the horse attained its maximum antitoxic strength. During the first eight months the antitoxin was stored up very slowly, but in the two following months the process was more rapid. The immunization was begun in July, 1901, and has been continued up to the present time—November, 1904—a period of three and a half years. The serum of Horse 41 has touched the thousand-unit mark six different times during that period. By increasing the amount of toxin 10,000 guinea-pig fatal doses more than the average, it was always possible to increase the antitoxic content of the serum of this horse from three to four hundred units per cubic centimetre without any additional inconvenience to the animal.

The curve of Horse 113 (*occc*) is interesting. This horse rose during three months to 700 units with ease, and it was with some difficulty that during the next three months it was forced beyond this point. Probably as a result of this the antitoxin curve came rapidly down with hardly an appreciable reascension until it nearly touched the 300-unit mark, when a slight rise was again manifested.

Horse 114 (*oxxx*) is still more interesting. It was immunized within a period of sixty days from the time of purchase, and attained a maximum strength of 1000 units per cubic centimetre, when it descended as rapidly as it had ascended originally until the 400-unit mark was reached. At this point the descent was checked in its downward course and the blood has remained at that strength until the present.

² Not included in the table.

Horse 164 (*xxxx*) was immunized in ninety days, and barring the usual midsummer drop, still continues to yield a 1000-unit serum, although occasionally dropping to 900 units or even lower at times.

A glance at the darkened portion of the composite clearly shows how short the period of usefulness of an antitoxin horse really is. It is at best on the average but a few months, though now and then a horse may last two or three years. In this respect the statistics of the table and the composite agree identically.

The number of guinea-pig doses given in the table represent averages, and are of no great importance as compared with individual cases. One horse (89) required as little as 521,500 fatal doses to develop 600 units, while another horse (84) required the enormous amount of 4,320,400 such doses before it reached the same number of units. The average amounts of toxin injected have been slightly more than given in Atkinson's figures.³

Horse 114, which developed 1000 units per cubic centimetre within two months from the beginning of treatment, was able to withstand without any great difficulty from 600 to 700 cc. of toxin injected three to four times per week.

The length of time required to develop antitoxin of maximal strength has averaged slightly longer with me than with Atkinson. I think the difference arises from my not having forced the horses. This fact may also have had something to do with the greater percentage of high-test horses given in my table, although the average length of time the horses continued to produce active serums is slightly in favor of Atkinson's results.

It has been found that it takes a longer time to immunize low-test animals than high-test ones; a 300-unit horse requires on an average 6.2 months to develop its maximal antitoxic strength, while horses capable of developing 1000 units average only 3.6 months, thus, nearly one half as long a time.

By reference to the table it will be seen that the statistics of the last fifty horses are far better than those given for the entire one hundred horses. This is mainly due to the more perfect

³ Loc. cit.

TABLE OF ONE HUNDRED CONSECUTIVE DIPHTHERIA HORSES.*

Horses producing diphtheria antitoxic serum. Last 100 consecutive diphtheria horses used (not all were tested).	Below 300 units per cc.	Above 300 units per cc.	Above 400 units per cc.	Above 500 units per cc.	Above 600 units per cc.	Above 700 units per cc.	Above 800 units per cc.	Above 1000 units per cc.
Number of horses producing diphtheria antitoxic serum (maximum strength).....	47	11	6	14	6	9	4	3
Average of guinea-pig fatal doses given to produce maximum antitoxin strength.....	—	1,158,327	1,011,033	1,321,560	1,568,366	1,414,313	1,385,120	1,526,666
Average period of injection developing maximum strength..	4.4 months	6.2 months	5.1 months	5 months	6.1 months	6.5 months	4.2 months	3.6 months
Average length of time horses continued to produce antitoxin testing as per table....	—	7.1 months	5.2 months	4 months	3.9 months	3.7 months	3.7 months	9 months

Per cent. of horses producing serum below 300 units per cc. = 47
 " " " " " above 300 " " " = 53
 " " " " " " 400 " " " = 42
 " " " " " " 500 " " " = 36
 " " " " " " 600 " " " = 22
 " " " " " " 700 " " " = 16
 " " " " " " 800 " " " = 7
 " " " " " " 1000 " " " = 3

* If the statistics of the last 50 consecutive diphtheria horses be taken, the results are far better than those given for 100 horses, and are as follows:

— 300 units 20 horses = 40 %
 + 300 " 30 " = 60 %
 + 400 " 24 " = 48 %
 + 500 " 22 " = 44 %
 + 600 " 12 " = 24 %
 + 700 " 10 " = 20 %
 + 800 " 5 " = 10 %
 + 1000 " 3 " = 6 %

methods used with the last horses. Since its foundation, to date, the laboratory has immunized upwards of 150 horses.

CONCLUSIONS.

1. Better results in the production of diphtheria antitoxin can be obtained with greater experience in the selection of the most suitable type of horses to be used. Young animals are usually to be preferred. Over one-half of all such horses can be made to yield 300-unit serum, while a third will yield 500-unit serum.

2. High-test horses require a shorter time to immunize and will yield a potent serum for a longer period than will low-test horses.

3. The period of usefulness of an antitoxin horse is short, and on an average endures only a few months.

4. A horse having attained a maximal antitoxic height begins to suffer a decline in antitoxin, which is usually as rapid as the ascent has been, and is unaffected by subsequent injections of diphtheria toxin.