SCIENTIFIC SECTION

FLUCTUATIONS IN THE RESISTANCE OF RATS TO NEOARSPHENAMINE AS OBSERVED IN ROUTINE TOXICITY TESTS OVER A PERIOD OF FOUR YEARS.*

BY A. E. JURIST AND W. G. CHRISTIANSEN.

Toxicity determinations on different samples of neoarsphenamine have been recorded in the literature many times and some papers have appeared describing toxicity studies on this substance. For example, Durham, Gaddum and Marchal (1) have applied the "characteristic" curve or integrated frequency method of biological assay to neoarsphenamine, and Morrell and Chapman (2) found considerable variations in the resistance of individual rats in one rat colony. It is proposed to discuss this question here from a somewhat different standpoint.

In the course of the routine manufacture of neoarsphenamine each lot is tested for toxicity. In these tests the Maximum Tolerated Dose of each lot is not determined but the test is of such a character as to determine whether or not the lot is satisfactory at a definite dosage. The requirements of the National Institute of Health are that 60% or more of the test rats must survive at a dosage of 240 mg. per Kg. of body weight, using rats weighing approximately 100 Gm. each. With rare exceptions neoarsphenamine will pass such a test easily. The routine tests carried out in this laboratory are run at two dosage levels, which for the purpose of this paper will be referred to as A mg./Kg. and B mg./Kg.[1] A large number of tests run during a period of years have shown certain variations in the number of rats surviving at these doses and the extent of this variation will become evident from the discussion which follows.

EXPERIMENTAL.

The rats used in these tests have been obtained from different sources so that the variations noted are little affected by this factor. Five rats, each of approximately 100-Gm. body weight, have been used in each of 963 tests, making a total of 4815 rats. Of this number 3220 rats were injected at dosage level A and 1595 at level B. The results have been grouped on the basis of monthly averages and cover a period of four years at dosage level A and three years at level B. Also, since the toxic manifestations of neoarsphenamine are noted chiefly in the kidneys of the rats surviving on the sixth day after injection, the results of the gross examination of the kidneys of the surviving rats[2] has been included. The extent of the kidney damage has been rated on a percentage basis using the following scale.

Undamaged	100%
Slight damage	75%
Definite damage	50%
Marked damage	30%
Marked damage with congestion	20%

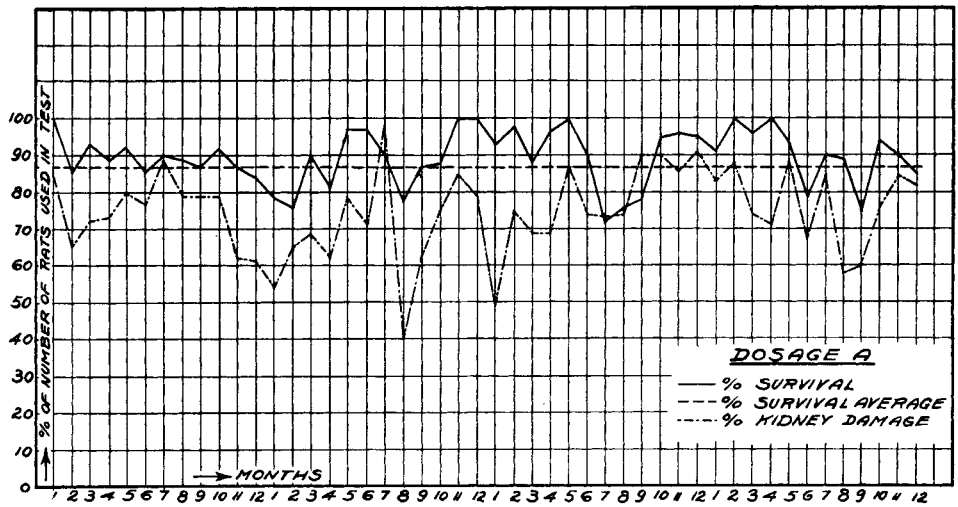
* Scientific Section, A. PH. A., Washington meeting, 1934.

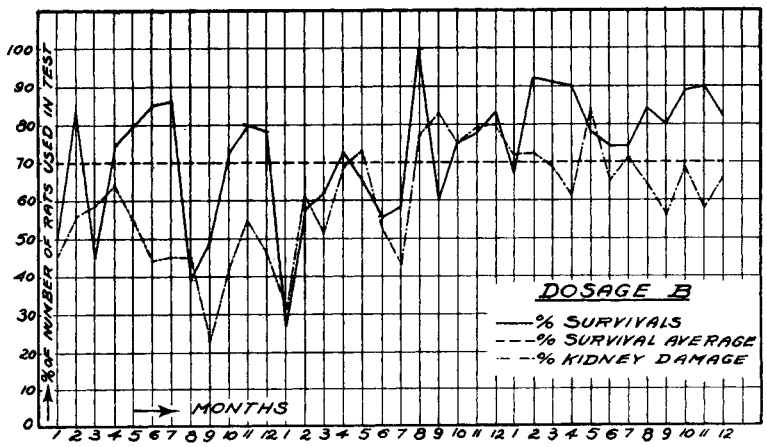
[1] The exact figures would contribute nothing to the discussion presented herein; they are of course considerably higher than 240 mg./Kg.

[2] The rats are killed and examined at the end of the test period.

The results of these tests have been summarized in the attached curves. The same scale is used in each case for the percentage of rats surviving and for the extent of the kidney damage noted in these rats. The years are divided into twelve months each, numbered in order from one to twelve. The dotted line drawn through the survival curve represents the average number of survivors in all

Fluctuations in Resistance of Rats to Neoarsphenamine as Observed in Routine Toxicity Tests of Dosages A and B over a Period of Four Years.





the tests included in each curve; this average in each case is drawn from the gross totals, not by averaging the monthly percentages. Separate sets of curves are given for the tests run at A mg. per Kg. and for those run at B mg. per Kg.

DISCUSSION.

The results obtained with the dosage of A mg. per Kg. will be taken up first. Out of a total of 3220 rats 2806 or 87% survived the test period. Out of a total of

48 months the percentage of survivors fell below 87% only 13 times. These drops below the average occurred in:

January of the 2nd year	July of the 3rd year
February of the 1st and 2nd years	August of the 2nd and 3rd years
April of the 2nd year	September of the 3rd and 4th years
June of the 1st and 4th years	December of the 1st and 4th years

Therefore the fall below the normal average took place seven times during the months from June to September, these being the hottest of the year, and five times during the months from December to March, these being the coldest of the year. These results, on the whole, appear to indicate that the resistance of rats to neoarsphenamine is independent of the season of the year during which the test is made, although the hotter weather may, to some slight extent, lower the resistance of the rats at times. This agrees with the conclusion of Morrell and Chapman (2) that while the susceptibility of a rat colony seems to vary from time to time this variation is not related to the season of the year.

At the same time an examination of the curves for kidney damage in the surviving rats shows that the percentage varies quite closely with the percentage of rats surviving. However, as would be expected, the per cent of kidney damage is greater than the percentage of deaths. This is to be anticipated because of the high dosage level. In only three instances is the per cent of kidney damage less than the percentage of deaths.

The results obtained at the other and higher dosage of B mg. per Kg. are not as uniform as those obtained at A mg. per Kg. Out of 1595 rats injected 1124 or 70% survived; but in 12 of the 36 months the percentage of survivors was below the average for the entire period. These drops below the normal occurred in:

January of all 3 years	June of the 2nd year
February of the 2nd year	July of the 2nd year
March of the 1st and 2nd years	August of the 1st year
May of the 2nd year	September of the 1st and 2nd years

In these the average falls below normal five times during the months from June to September, and six times during the months from December to March. This confirms the conclusion that variations in rat resistance are apparently independent of the season of the year. It may, however, be significant that at both dosage levels the percentage of survivors is below normal only twice during the months of mild weather, namely, October, November, April and May. The variations in the percentage of survivals, especially at the lower dosage, are not large enough, however, to justify the conclusion that this is really a seasonal variation.

The fluctuations in the survival curve are much greater at B mg. per Kg. than at A mg. per Kg. due, of course, to the fact that the dosage is much nearer the Maximum Tolerated Dose. Here again the curve for kidney damage parallels the survival curve closely; the extent of damage is greater than the percentage of deaths except in 9 of the 36 months. There are greater variations in the relation between the percentage of survivals and the extent of kidney damage at B mg. per Kg. than at A mg. per Kg. due to the fact that the dosage B is materially larger than A.

It is interesting to compare the yearly survival percentages at the two dosage levels.

	Per Cent Survivals.			
	A Mg./Kg.		B Mg./Kg.	
	Average.	Limits.	Average.	Limits.
First year	89.2	84–100	..	....
Second year	85.8	76–100	65.4	39–86
Third year	86.5	72–100	64.1	27–100
Fourth year	86.7	79–100	80.9	67–92

These figures show that even when neoarsphenamine gives a high percentage of survivals at a dosage level at which deaths occur, the use of still higher doses enables one to demonstrate differences in toxicity in batches which give practically identical results at the lower dose. Thus, the testing at A mg./Kg. during the second, third and fourth years shows that at this dose about 13% of the rats died and the fluctuations were all of about the same order whereas the testing at the higher dose showed a much lower per cent of deaths for the fourth year than for the second and third.

The toxicity tests were carried out in the Biological Laboratories of E. R. Squibb and Sons, at New Brunswick, N. J.

REFERENCES.

(1) Durham, Gaddum and Marchal, Spec. Report, Ser. No. 128 (1929), Med. Res. Council, London, England.

(2) Morrell and Chapman, *J. Pharmacol.*, 48 (1933), 391.

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FREE ALKALI IN GLASS.*

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It is obvious that glass bottles and ampuls of high soluble alkali content are unfit for use in finely adjusted pharmaceutical and chemical solutions.

The following experiment demonstrates the possible reactions resulting from glass of high soluble alkali content: A benzoic acid solution in an ordinary glass bottle is heated sufficiently to volatilize traces of benzoic acid in the neck of the bottle. The sealed bottle is permitted to stand several months and upon testing, the traces of volatilized benzoic acid will be converted to the sodium salt.

In the days before the advent of facilities to measure alkali and acids in minute amounts by the potential hydrogen apparatus, the soluble alkali in glass was determined by heating accurately measured amounts of $N/20$ HCl in the glass on test. Titrating the excess of $N/20$ HCl with $N/20$ NaOH (Phenolphthalein T.S.) and calculating the free alkali in the glass from the amount of $N/20$ HCl reacting with the free sodium in the glass.

For the past seven years we have used the following method for determining the amount of free alkali in glass:

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* Scientific Section, A. PH. A., Washington meeting, 1934.