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COMPARISON OF THE TOXICITY OF NEOARSPHENAMINE TO DIFFERENT STRAINS OF RATS.*

BY WILLIAM L. SAMPSON AND ALBERT R. LATVEN.[1]

That individual variation with a group of test animals plays an important rôle in the determination of the toxicity of neoarsphenamine has long been recognized. Voegtlin, in his many papers on the arsphenamines, repeatedly states that "due to the great individual difference in the susceptibility of animals it is difficult to obtain a sharply defined M. L. D." Hooper, Kolls and Wright (1) in their paper on the influence of diet on the toxicity of arsphenamines direct attention to the considerable variation in individual animals to arsphenamine poisoning. Heyle, Hart and Payne (2) have shown that the M. L. D. of a single lot of neoarsphenamine varied from 320 mg. per Kg. to 440 mg. per Kg. when determined on different groups of animals from their colony. Recently Morrell and Chapman (3) in their work on the determination of the characteristic toxicity curve of neoarsphenamine for rats have found that the L. D. 50 of a single lot of neoarsphenamine when tested at intervals over a period of eight months on rats from a carefully controlled colony varied from 380 mg. per Kg. to 500 mg. per Kg.

Except for a note in a report by Hart and Payne (4), in which they mention a difference of more than 100 mg. in the M. L. D. of a single lot of neoarsphenamine as determined in two different laboratories, there appears no direct evidence of a consistent difference in the tolerance to neoarsphenamine of rats from different colonies. Inasmuch as the official method for the determination of the toxicity of neoarsphenamine as recommended by the National Institute of Health, U. S. Public Health Service (5) calls for the use of healthy albino rats without specification as to strain, it occurred to us that an investigation of albino rats from different sources might prove of interest.

Young male albino rats from three different breeding colonies, hereafter referred to as colony I, colony II and colony III, were obtained for this investigation. We elected to use only male rats to eliminate any variation due to sex. Upon arrival in the laboratory the young animals were placed on an adequate diet similar to that recommended in the official method and were maintained on this diet for one week to minimize the possible influence of sudden change of diet on the resistance of the rat. Only obviously healthy animals weighing from 90 Gm. to 110 Gm. were used in the test.

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Samples of neoarsphenamine from four different sources were purchased on the open market. Several ampuls of each lot number were obtained. Four per cent solutions of the neoarsphenamine in cooled freshly distilled water were prepared in glass-stoppered cylinders with only the minimum amount of gentle agitation necessary to assure thorough mixing. This solution was slowly injected into the saphenous vein of the test animal. A fresh solution of the drug was used for each group of 5 to 6 animals injected. In this way use of solutions more than thirty minutes old was avoided. While we are fully aware of the precautions for the preparation and preservation of neoarsphenamine as recommended by Morrell and Chapman (6), we decided to use the above procedure because we believed that it would conform more closely with the procedures in general use in other laboratories performing this particular test.

A total of 710 animals from the various colonies were used in this study. Twenty animals were injected at each dose level except in a few cases where limitation of sample permitted the use of but ten or fifteen animals.

From the results of these injections dose mortality curves for the various neoarsphenamines were constructed. Figure 1, typical of these curves, represents the results obtained for Sample C, using rats from each of the three colonies. The slopes of these curves in their middle range are in fairly good agreement with one another and illustrate quite well the difference in resistance of rats from the different colonies.

Table I gives the L. D. 50 of the various neoarsphenamines as determined with rats from each of the three colonies. Inspection of this table shows that in every case animals from colony I tolerate much larger doses of the drug than do animals from the other two colonies. Similarly it is apparent that animals from colony II have in general, a slightly greater resistance than do those from colony III. Furthermore, it is evident that the order to relative non-toxicity of the four samples remains the same for each of the three colonies. In this connection it is interesting to note that it requires but 75 per cent as much of Sample B as of Sample F to cause 50 per cent mortality in animals from colony I, 62 per cent as much to produce a similar toxicity in animals from colony II, and 68 per cent as much to effect the same results in animals from colony III. Sample C is 74 per cent as toxic as Sample B for animals for colony I, 68 per cent as toxic for animals from colony II, and 77 per cent as toxic with animals from colony III. The agreement in the relative toxicity of Samples A and B is not quite so good, being 76 per cent for colony I, 88 per cent for colony II and 96 per cent for colony III.

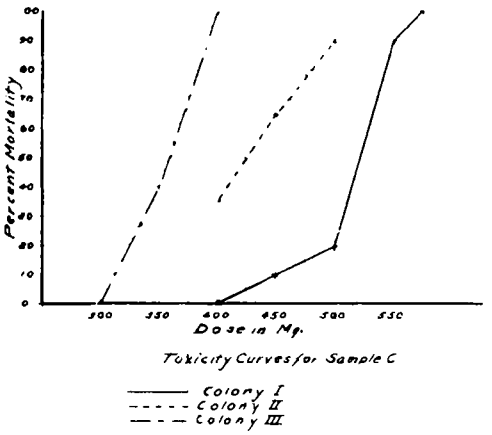

Fig. 1.

TABLE I.

Sample.	Colony I, L. D. 50.*	Colony II, L. D. 50.*	Colony III, L. D. 50.*
A	500 mg.	325 mg.	290 mg.
B	385 mg.	287 mg.	280 mg.
C	520 mg.	425 mg.	360 mg.
F	510 mg.	460 mg.	410 mg.

*L. D. 50 doses of various neoarsphenamines for rats from different colonies.

The results of this experiment offer evidence of a definite and consistent variation in the resistance to poisoning by neoarsphenamine of rats from different colonies. Investigating in an entirely different field Cleghorn and co-workers (7) have reported that there is a definite difference in the survival time following adrenalectomy of rats from different strains. On the other hand, Durham, Gaddum

and Marchal (8) state that while they have no actual data concerning the extent to which sensitiveness to the salvarsan group may differ with different strains of mice, "It can only be said that there is no evidence as yet of wide permanent difference in this response between stocks used in different European laboratories."

It is apparent from the foregoing data that it is not possible to make a significant comparison of toxicity among samples of neoarsphenamine when rats from different strains are used. It is equally apparent that the relative non-toxicity of several samples of neoarsphenamine may be quite accurately determined if one strain of animals is used in making the test. Hence, the determination of the toxicity of neoarsphenamine, like most biological assays, is without significance unless a physical standard of reference is used to standardize the resistance of animals from a single colony.

SUMMARY.

1. There is a definite and constant difference in the tolerance to neoarsphenamine of albino rats from different colonies.

2. The relative non-toxicity of several samples of neoarsphenamine remains constant when tested on animals from any single colony.

3. In order to standardize the resistance of animals from different colonies, a physical standard of reference, supplied by some central authority, should be used for comparison in every assay of neoarsphenamine.

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A STUDY OF SOLUTION OF MAGNESIUM CITRATE.*

BY ARTHUR OSOL AND LINWOOD F. TICE.[1]

The purpose of the investigation reported in this paper was to study solution of magnesium citrate U. S. P. XI to definitely establish whether the reduction in citric acid content from 35 to 33 Gm. per bottle constituted a desirable change and assured a stable product.

It has been claimed that 33 Gm. of citric acid provided a sufficient amount to permit the preparation of a stable solution of magnesium citrate, *i. e.*, one which upon standing would not be subject to precipitation. Arny and Schaefer (1) have reported that in their opinion "the increase in citric acid content of the 350-cc. bottle of solution of magnesium citrate from the 33 Gm. of the U. S. P. IX to the 35 Gm. of the U. S. P. X was inadvisable and unnecessary."

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