## Toxicity Tests for Novarsenobenzene.

ALTHOUGH the arsenobenzenes of therapeutic value are, theoretically, chemical individuals, different batches of the same compound may vary both in toxicity and therapeutic efficiency, so that biological tests on each batch are essential before clinical use. Two separate tests are required, since toxicity and therapeutic efficiency are not proportional: in fact, they may vary inversely, and a toxic sample may have only a negligible therapeutic effect. The tests are usually carried out on mice. In the therapeutic test the animals are first infected with a trypanosome and the curative effect of a small dose of the drug under test observed; in the test for toxicity, larger doses are given and the mortality determined, the dosage being such that some proportion, but not all, of the animals injected die within some specified period after the administration. The toxicity tests in use for novarsenobenzene and neosalvarsan have been exhaustively examined by Durham, Gaddum, and Marchal:[1] their conclusions are of interest both from the point of view of toxicity tests in general, and also from the fact that they have led to the formulation of a simple and trustworthy test for this compound.

The test required is not one for the determination of the potency of the sample in terms of the standard, but one ensuring that its toxicity does not exceed that of the standard by more than a specified amount, and the investigation was directed to settling the limit of permissible toxicity and prescribing a simple test which would exclude the majority of samples exceeding this limit whilst passing the majority of those lying within it. The standard preparations were obtained from Prof. Kolle, of Frankfurt, who distributed them on behalf of the Health Organisation of the League of Nations.

The first aim was to obtain a curve relating death-rate to dose injected: this 'characteristic' curve has the S-shape common to toxicity curves in general. To eliminate possible variations in sensitiveness of different batches of mice injected on different days, the range of doses was given to animals of each batch each day, the experiment being continued for several days until a sufficient number of animals had been injected with each dose. Throughout the investigation, however, no evidence of a day or seasonal variation in sensitiveness was obtained. The curve is steeper, that is, small variations in dosage cause greater variations in mortality, when animals of a uniform stock are used, and especially when they are of much the same weight: greater accuracy is obtained with fewer animals of uniform sensitiveness than with a much larger number of a mixed stock. The curve was steepest with doses between 0·4 mgm. and 0·5 mgm. per gm. body weight, the mortality being observed over a period of three days after the injection. Investigation of the relationship between weight and sensitiveness showed that mice of 13-15 gm. weight are about 25 per cent more resistant than those of 18-20 gm. or 24-26 gm. Females of 18 gm. in weight or more are 8-10 per cent more susceptible than males, but there is little difference between the sexes in the case of the smaller animals: the males, however, are more uniform in their reaction. It appears from the results obtained that sex can be neglected, but that, if possible, animals of similar body weight should be used: if the limits of variation in weight are within 2 gm., the dose can be expressed in mgm. per mouse.

[1] Medical Research Council. Special Report Series, No. 128: Reports on Biological Standards. 2: Toxicity Tests for Novarsenobenzene (Neosalvarsan). By Florence M. Durham, J. H. Gaddum, and J. E. Marchal. Pp. 40. (London: H.M. Stationery Office, 1929.) 9d. net.

Having obtained the characteristic curve with the use of a large number of mice, it is possible to calculate the probability that not more than a certain number of a small number injected, for example, 5, 10, or 30, will die for different values of the true mortality, and hence the probability that the true toxicity of a given sample will be disclosed when only a small number of animals is used. In the specific case under investigation the upper limit of permissible toxicity has been fixed at 20 per cent above standard, so that the question to be settled is the probability of a sample within this limit passing and of one exceeding this limit being rejected, when the test is carried out with the use of only a small number of animals.

The tests at present in use have been examined with the aid of these calculations: the British is not sharply discriminative and allows an undue proportion of toxic samples to pass; the German gives a sharper discrimination but still passes many toxic samples; the Japanese and American are even less discriminative than the British, and fail to exclude many samples exceeding the limit of 20 per cent greater toxicity than standard.

As a result of the investigation an improvement in the test is proposed, to give greater discrimination and to exclude more of the toxic samples. The drug is dissolved in water, freshly redistilled with a condenser of hard glass, at 2 per cent strength, and the fresh solution injected into the tail vein of the animal. The mice used are fasted overnight, weighed, and then given food, the injection being made an hour or so later. The test is carried out in three stages. In the first, 10 animals, weighing 18-20 gm., are taken and into each is injected 7·6 mgm. of the novarsenobenzene: if not more than 2 die, that is, 20 per cent mortality, the sample is passed forthwith: among those passing would be samples with a toxicity less than that of the standard and about 61 per cent of those with a toxicity equal to that of the standard. The others are injected in the same dose into a further 10 mice, and the total mortality on the 20 animals so far used observed: if this is not more than 40 per cent, the sample passes: a further 38 per cent of the samples of standard toxicity should pass at this stage. Further, if more than 15 of the animals have been killed, the sample can be rejected without further test, as exceeding the limit of permissible toxicity. The remaining samples are injected into a further 10 animals: those which have killed not more than 15 of the total of 30 injected are passed, whilst those causing a higher death-rate are rejected. Hence the final demand of the test is for the survival of at least 50 per cent of 30 mice on a dose of 0·4 mgm. per gm. The test passes all samples of toxicity up to that of the standard, and fails 0·4 per cent of those of toxicity 10 per cent above that of standard, 59 per cent of those of toxicity 20 per cent above, and 99·6 per cent of those of toxicity 30 per cent above. If this test is considered to be too severe, 0·38 mgm. per gm. or a total dose of 7·2 mgm. can be used, when 94 per cent of samples of toxicity 20 per cent above that of standard would pass, but only 11 per cent of those of toxicity 30 per cent above.

The actual dosage used may require adjustment according to the sensitiveness of the stock of mice employed: the standard enables this sensitiveness to be tested from time to time and also allows of comparable results being obtained in different laboratories with different conditions of diet, temperature, etc. Samples of the drug properly dried and kept at low temperatures remain stable over several years, and show no increase in toxicity after this time.