pleted by Raper's method gave 0.80, 0.82, 0.775, 0.78, 0.74, 0.81,[1] 0.80,[1] 0.78, 0.81%.

SUMMARY.

Small quantities of sulphur (1 mg.) in organic substances can be determined by the fusion method, by weighing barium sulphate as accurately as with the benzidine method, using Benedict's reagent. For lipins the fusion method is preferable because the chance of loss by puffing is eliminated.

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THE BIOLOGICAL TESTING OF SALVARSAN AND ITS DERIVATIVES.*

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In the armamentarium of the modern physician certain compounds of arsenic have a very important position. This position has been gained by the clinical and experimental evidence which has accumulated during the past eighteen years since the announcement of the discovery of Salvarsan by Ehrlich. While compounds of arsenic were used prior to this and hundreds have been synthesized and tried in the treatment of disease since then, none have equalled Salvarsan and Neosalvarsan for use in the treatment of syphilis.

The properties which the physician seeks are purity, complete solubility, relative non-toxicity and therapeutic efficiency. These properties are controlled by the manufacturer in his laboratories before the product is offered to the physician. For this purpose he maintains a staff of chemists and biologists, the first to conduct the chemical and physical tests, the second to test the product for its toxicological and therapeutic properties by the modern scientific methods which have been developed for these purposes. These methods are the result of the investigations of workers in Government and private laboratories both in this country and abroad. The early work of Ehrlich and Hata in Germany has been followed by that of Dale in England, Danysz in France and Voegtlin and others in the United States. This laboratory has carried on such biological tests since the advent of Salvarsan as an American made product. It is this type of studies that has given Salvarsan and Neosalvarsan uniform reliability without sacrificing therapeutic activity. Another factor contributing to the uniformity of these products is their manufacture on a large factory scale rather than a number of small lots such as are made in smaller laboratories.

In order that a compound shall have value in the treatment of a disease it is necessary that it be tolerated by the animal body in a much larger amount than is required to kill the micro-organism which is the causative factor in this disease. It is obvious that if quinine were tolerated by the plasmodium of malaria in larger amounts than can be borne by man, quinine would never have been used in the treatment of this infection. So, in the field of organic arsenicals, those compounds are discarded which have a curative dose approaching the tolerated

[1] 0.1 Gm. samples.

* From the Laboratory of Biological Testing, H. A. Metz Laboratories, Inc., 644 Pacific St., Brooklyn, N. Y.

dose or lethal dose. It is the function of the biologist to determine, in searching for new drugs, as nearly as he may the range between these doses; as a control test on the established specifics, to determine that the product does not depart from the standards established for a satisfactory preparation intended for the treatment of human disease.

The standards for the control of the manufacture and testing of Salvarsan and its derivatives have been promulgated by the U. S. Public Health Service under Act of Congress, 1902, and in addition to the tests made by the manufacturer, samples of all lots made for sale are submitted to the Hygienic Laboratory for additional tests, both chemical and biological.

In this country, the biological tests, both for toxicity and therapeutic value, are made upon white rats. In England, Canada, and Germany, white mice are used by many workers. It is probable that the uniformity of the animals used is of more importance than the species chosen. The test for toxicity of the product, or for the maximum dose of the product which is tolerated by the test animals, consists in observing, for a certain period, animals which have been injected with definite amounts of the drug. The period of survival is noted. Autopsies are made and the condition of the organs noted in case of death. The survivors are killed at the end of the test period and examined. The test period for Salvarsan is 48 hours; for Neosalvarsan, 7 days. Various factors are of importance in attaining standard conditions for these tests and these will now be described briefly.

As has been pointed out by Lake and others, the care of the animal is of fundamental importance. The rats must be free from disease, must have an adequate diet, plenty of clean water and must be kept under favorable conditions of temperature and ventilation in clean cages. These conditions can be secured by the proper selection of the articles of diet to include the necessary proteins, carbohydrates, mineral constituents and vitamines; a competent and trustworthy assistant completes the requirements which one should have before attempting to test these products.

The rats are kept on a standard, dry food diet with plenty of clean water for at least a week before use. Weights on the animals are taken at the beginning and end of the period of observation. Those which have shown a normal rate of growth are selected as fit for use. Since the preparations are given on a weight basis, the animals are deprived of food for twelve to eighteen hours before weights are taken. In order to obtain a further idea of the effect of the drug, weights are taken at the end of the period of observation following the injection of the drug.

The drugs are given in an aqueous solution which is injected into the saphenous vein, *at a definite rate.* As has been shown by Lake, too rapid injection increases the apparent toxicity of Salvarsan. Myers points out that the rate of injection has an effect on the size of the protein-salvarsan aggregate formed in the blood stream and so affects the toxicity directly. Skilled assistance and gentle handling of the animals during the injection contribute to the constancy of results.

The *preparation* of the solution before injection is of *extreme importance.* The *concentration, temperature, effect of shaking, age of solution,* and, in the case of Salvarsan, *the amount of alkali* added *are all factors in determining the toxicity of a given product.* A concentration of 2% is used in testing Salvarsan and Silver

Salvarsan; one of 4% for Neosalvarsan and Neosilver Salvarsan. The solutions are made in freshly distilled water at room temperature, *i.e.*, 20–25° C. While some of the arsphenamines on the market require hot water for solution, it has been found that the original German Salvarsan and Salvarsan made in the United States according to the method of Ehrlich become much more toxic if dissolved in hot water. Furthermore, the latter are easily soluble in cold water. It is important that the Salvarsan be fully alkalinized so that the di-sodium salt is injected. This solution will have a slightly higher alkalinity than the blood. It is less toxic than either the solution of the hydrochloride or the solution of the mono-sodium salt of Salvarsan. Calibrated 1 cc syringes are used for making the injections.

At present the volume of solution prepared is 20 cc. *This is allowed to stand 15 minutes* before the first rat is injected. Three doses of the drug are injected in three rats each or in nine rats altogether. The doses are spaced at intervals of 20%, *e. g.*, 160, 200, and 240 mg. per Kg. for Salvarsan and that amount is considered the maximum tolerated dose which allows the survival of two out of three of the animals. Under the official regulations, 60% of at least 5 rats are required to tolerate 100 mg. per Kg. In the event that all die or all survive, it is necessary to decrease or increase the range of dosage to determine the maximum tolerated dose.

The second biological test on a product is that to determine its parasiticidal value. It is not practicable to determine this value directly upon the organism of syphilis, the *Treponema pallidum*, either in a test-tube or in the body of an animal. Fortunately, resort may be had to indirect methods which have been shown to be most valuable in estimating the relative value of an arsenical in the treatment of protozoal infections. Dale, in a series of experiments on Neosalvarsan, has shown that those preparations which are of high trypanocidal value in the laboratory are also, in general, most effective in clearing up the lesions of syphilis and eliminating the *Treponema pallidum* from the patients examined in his clinics; in other words, clinical results are paralleled in and may be predicted from the results of the experimental laboratory. Voegtlin, at the Hygienic Laboratory, has described a rapid and quite satisfactory method for the determination of the trypanicidal value of drugs of the Salvarsan type. The organism used is *Trypanosoma equiperdum*, the causative factor in a disease of horses known as "la dourine" or horse syphilis. It is non-infective towards man but is very virulent towards rats and mice, thus eliminating the possibility of spontaneous recovery of these animals. It is propagated by transferring a few drops of blood from an infected animal to the peritoneal cavity or blood stream of a fresh animal. In the absence of treatment the rat will certainly die in from three to five days after the appearance of organisms in the blood stream.

The test is conducted by inoculating a series of animals with the blood of a infected rat. The blood is so diluted that each animal receives from 50 million to 70 million living organisms. Each animal should show 100,000 to 250,000 organisms per cu. mm. twenty-four hours after inoculation. At present the tests at this laboratory are made by injecting three animals per dose and using three different doses at intervals of $33^1/_3$%. The animals which show 1,000 or less organisms per cu. mm. twenty-four hours after treatment are considered as

having received the minimum effective dose (M. E. D.) or over. That dose is the M. E. D. which is effective in reducing the organisms to 1,000 or less per cu. mm. in two of the three rats injected therewith.

It has been found that the virulence of the organism is subject to many variables and for this reason it is highly desirable to inject part of the rats on a given infection with a control preparation. By this means the accuracy and value of the method are considerably increased. For the past two years this laboratory has used a preparation of Salvarsan as a control.

It should be noted that the M. E. D. is not the dose which gives an absolute cure but is merely the dose which clears the blood stream of organisms for a short period. Voegtlin and Smith have shown it to be a function of the curative dose, therefore, it is permissible to use it as a means of measuring the relative parasiticidal value of different compounds and to assume that a product having a high M. E. D. as compared to the standard product (Salvarsan) will also have a relatively high curative effect in the treatment of disease. In using this method it is possible to get results in forty-eight hours after inoculating the animals with organisms whereas in the determination of the curative or sterilizing dose of earlier investigators at least sixty days' observation was necessary in order to be sure that no relapses would occur.

The method of injection of the drug in this test is that described for the toxicity test, using more dilute solutions since the M. E. D. is but a fraction of the tolerated dose. At present a uniform volume of 50 cc is used in order to allow a greater accuracy in weighing the drug.

Twenty-four hours after treatment a drop of blood is taken from the tail of each animal and a smear made. Those specimens showing organisms are noted and a count made to determine the number of parasites per cu. mm. This information is used to determine the M. E. D. as described above. The counts are made by diluting the blood with a carbol-fuchsin staining fluid and counting in the ordinary chamber used for counting blood cells.

Recent years have seen much progress in the application of quantitative methods to the biological testing of drugs. The improved methods of evaluating pharmaceutical arsenicals have had a two-fold aim in this laboratory: first, to give to the physician a Salvarsan and a Neosalvarsan of as low toxicity as is compatible with a high therapeutic activity thereby maintaining the most favorable chemotherapeutic ratio or index; second, to ascertain for the medical profession the toxicity and parasiticidal values of such new compounds as are synthesized by chemists for use in the treatment of disease.

COÖPERATION IN PHARMACEUTICAL RESEARCH.*

BY H. V. ARNY.

Two years since it was my privilege to present a paper before this association under the title "Research and the Cash Register" wherein it was pointed out that the publication of high grade research was of direct personal benefit to every mem-

* Read at the 53rd annual convention of the New Jersey Pharmaceutical Association, held at Spring Lake, June 12–15, 1923.