

gummy substances, such as serum, dextrose, etc., are used to encourage the adhesion of the virus to the point. The glycerin may first be largely extracted from the ripened glycerinated virus by pressing it between blotting papers.

All vaccine virus is tested according to modern bacteriologic methods for streptococci, tetanus spores and other virulent bacteria. These tests include animal inoculations. The tests made by the manufacturer must be satisfactory before the virus is placed on the market and permanent records are required of each lot. Special tests are made to determine the absence of foot-and-mouth or tetanus infection.

The calves are kept in quarantine under observation for seven days before being vaccinated. Only healthy calves free from diseases of the skin are used for this purpose. The calves are killed or otherwise rendered insensible to pain before the virus is removed. The practice of renting calves for the purpose of propagating vaccine virus is no longer countenanced by the federal regulations. The animals must be autopsied as soon as practicable after the removal of the virus in order to determine the presence of lesions indicating other infections than cowpox (vaccinia). The federal regulations further require that the vaccine virus taken from an animal showing indications of complicating infections must be destroyed.

All establishments manufacturing vaccine virus for use in interstate traffic are required to operate under government supervision, which has been described in my article on "The Federal Control of Serums, Vaccines, Etc." (see p. 249, this issue).

Reports received by the Surgeon-General from health officers and others all over the country indicate that since the operation of the law the vaccine virus has been much more satisfactory than before.

WHY VACCINE VIRUS SHOULD BE IN THE PHARMACOPEIA

Vaccine virus was the first and it is the oldest and best specific preventive known. It is a drug in the broadest sense of that term, and as such is handled by every pharmacist. One of the first advantages in admitting vaccine virus into the Pharmacopeia would be to establish for it an official and legal name. This would help avoid much confusion now existing on account of the bacterial vaccines and other substances called "vaccines" used in the prevention and cure of disease. For almost a hundred years vaccination was a specific term limited to the introduction of the virus of vaccinia into the skin for the prevention of smallpox. In recent years the term "vaccination" has been used in a generic sense to include the introduction of many different substances, in many different ways and for many different purposes. To establish a definite and accurate nomenclature is one of the important functions of the Pharmacopeia.

Furthermore, a certain amount of confusion would be avoided and increased definiteness would be given to the various forms in which vaccine virus is marketed by adopting such titles as "virus vaccinium glycerinatum" and "virus vaccinium siccum," etc.

To include vaccine virus in the Pharmacopeia would be one of the best means of calling the attention of all pharmacists to the fact that it must be kept in a cool, dry place, etc. Much of the vaccine virus on the market is inert because not properly handled in the trade. Hence, concise, authoritative directions in the Pharmacopeia would have an educational value and would help prevent smallpox and save life.

The objection that vaccine virus is an indefinite substance, the "active principle" of which is not known, is no longer valid, for the Pharmacopeia contains many such substances, including the ferments, against which similar objection holds.

The objection that vaccine virus can not be "assayed" by the average druggist also lacks force when we recall that the potency and purity of vaccine virus in interstate traffic is cared for by the federal government under the law of July 1, 1902, which relieves the pharmacist of this responsibility. Further, other substances, such as serum antidiathericum, the testing of which requires special training and special laboratories, have been admitted into the Pharmacopeia.

The Pharmacopeia should briefly state the essential requirements of the law above mentioned concerning false labeling or marking of any package or container of vaccine virus; requiring further that each package of vaccine virus must be plainly marked with the proper name of the article, the name, address and license number of the manufacturer, and the date beyond which the contents can not be expected, beyond reasonable doubt, to give specific results. Such information in the hands of every druggist will serve an educational purpose and also help the federal authorities in the rigid enforcement of the law.

The Pharmacopeia should briefly state the method of preparing vaccine virus and specify the difference between glycerinated preparations and dry points and the other forms found on the market. As a precedent, it might be stated that the Belgian Pharmacopeia (third edition, 1906, page 194) has introduced vaccine virus under the title of "vaccinum," and the Swiss Pharmacopeia (fourth edition, 1907, page 512) has introduced vaccine virus as "vaccinum," synonyms: "Kuhpockenimpfstoff," "vaccine," "vaccin jennérien," "vaccino jennèriano."

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ANTIDIPHTHERITIC SERUM AND ANTIDIPHTHERITIC GLOBULIN SOLUTIONS*

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Until recently the only means we had of giving diphtheria antitoxin was in the whole serum of the horse in which it had originated. The accumulated knowledge obtained in the investigations of a number of workers allowed the development of a practical method for eliminating a portion of the non-antitoxic serum substances while retaining the antitoxin. Because of this, besides the whole serum, we have at present on the American market two globulin preparations containing diphtheria antitoxin. As these are rapidly displacing the whole serum, I will give a brief description of them.

GLOBULIN PREPARATIONS

The Gibson process of purification and concentration is based on the fact that antitoxin is associated with the globulins soluble in saturated sodium chlorid solution. This purification appeared to be considerable, as

* Read in the Section on Pharmacology and Therapeutics of the American Medical Association, at the Sixtieth Annual Session, held at Atlantic City, June, 1909.

in the average normal horse serum the proteins are distributed as follows: albumin, 40 per cent.; globulin soluble in saturated sodium chlorid solution, 42 per cent.; globulin insoluble in saturated sodium chlorid solution, 18 per cent. Thus it would appear as though about 58 per cent. of non-antitoxic protein was eliminated.

Investigation carried on by Banzhaf and Gibson on the distribution of the proteins in immunized horses brought out the fact that, while the total proteins of the horses were increased during the progress of immunization, the so-called non-antitoxic proteins, the albumins and globulins insoluble in saturated sodium chlorid were greatly diminished. The globulins carrying the antitoxins were greatly increased. This work showed that in the average antitoxic horse serum the proteins were distributed as follows: albumin, 12 per cent.; globulin carrying the antitoxin, 78 per cent.; globulin insoluble in saturated sodium chlorid, 10 per cent. This brought out the fact that the Gibson process was eliminating in some preparations only about 22 per cent. of the total proteins.

About a year ago Banzhaf experimented with antitoxic serums heated at different temperatures and periods of time, and found changes taking place in the proteins which allowed further purification of antitoxin; thus, if antitoxic serums of 600 units per cubic centimeter containing albumin 12 per cent., globulin carrying the antitoxin 78 per cent., globulin non-antitoxic 10 per cent., were heated for from twelve to fifteen hours at a temperature of 57 C., a rearrangement of the proteins would take place, so that albumin would now be 9 per cent., globulin and all the antitoxin 50 per cent., and globulin non-antitoxic 41 per cent., showing an elimination of 50 per cent. non-antitoxic proteins. The loss of antitoxin on heating for this period of time and temperature is from 5 to 7 per cent. The units per gram of coagulable protein for this serum would be 9,446. With the Gibson process using the same serum, we have 11,456 units per gram protein, while with the heating method we have 17,060 units per gram.

For routine work at the Research Laboratories we do not try to concentrate more than to give an antitoxic potency of five or six times the original serum, as we do not want the total solids to be more than 16 to 18 per cent. The treatment of the antitoxic serum or of the globulin solution to fractional precipitation with different concentrations of ammonium sulphate allows of obtaining globulin preparations of somewhat greater degrees of antitoxic potency for each gram of protein. This method is often employed to obtain a fair preparation from a serum of low potency or, again, to obtain a very high potency serum.

The physician should keep in mind that the producer can concentrate the antitoxin either by eliminating the non-antitoxic proteids of the serum or by lessening the percentage of water holding the proteids in solution. The latter expedient, if carried too far, is a detriment.

ANTIDIPHTHERITIC HORSE SERUM

The blood serum from different horses varies not only in antitoxic potency, but also in its liability to produce disagreeable after-effects. Although theoretically it would be possible to test out the bleedings from each horse and to exclude that producing most serum effects, yet practically this would be so expensive as to be prohibitive.

It has been found that long standing and heating to 57 C. have some effect in lessening the after-effects.

The improvement is so slight that most American producers have used unheated serum and have not stored their serum for this purpose.

With the present U. S. government insuring the unit strength and sterility of the serum, it is safe to say that the antidiphtheritic serum supplied by the different producers is practically identical, if of equal antitoxic strength. The elegance and convenience of the container is the main difference. The different lots of serum of the same manufacturer will vary in liability to produce rashes, and this, together with the idiosyncrasy of the patient, causes some physicians to approve and others to condemn the preparations of the same manufacturers.

COMPARISON OF GLOBULIN AND WHOLE SERUMS

The effects of the globulin preparations have to be considered from two standpoints: Have they the curative substances of the whole serum, and, if so, have they any advantages except in concentration over the whole serum? I have most carefully watched the results following the injection of the whole serum and of the Gibson and the Banzhaf modifications. These tests were carried out with preparations made from portions of the same lot of serum. The rashes and after-effects were undoubtedly much less after the Gibson injections than after the whole serum and somewhat less after the injections of the Banzhaf modification than after that of Gibson. Curiously enough, only certain types of rashes are eliminated. The urticarial reactions still frequently follow.

As is well known, certain French and Viennese investigators have asserted that the curative value of antidiphtheritic serum was only partly in the antitoxin, and even that the antitoxin was the least important part. These assertions were based partly on supposed clinical results, but chiefly on the results of animal experiments in which guinea-pigs with multiple fatal doses of living diphtheria bacilli were treated with serums of low and high potency. It appeared from their results that the amount of serum rather than of antitoxin units was of saving value.

These experiments were repeated by us in a very extensive series of tests and later there was a second independent test under Ehrlich's direction. The results were exactly the reverse of those earlier reported. The serums used in Vienna were fortunately obtained by Ehrlich, and he was astonished to find that they had been exceedingly inaccurately tested. When the serums were arranged as to their true unit value the results obtained by Krams were found to reverse what he supposed and really to strengthen the conclusion that the antitoxin was practically the only curative substance in serum. These results probably apply equally to tetanus antitoxin.

Professor Calmette, when over here last September, promised me to test a number of French serums as well as a number of American samples. He stated that if he found any that proved to have other important curative substances than antitoxin he would send them to me for control testing. He has not as yet sent any.

I am sure I am safe in asserting that so far as animal tests can be depended on (and I believe they can be) the globulin preparations contain all the important curative substances of the whole antidiphtheritic serum.

THE ACCURACY OF STATEMENT IN THE TRADE CIRCULARS

I have looked over the circulars accompanying the preparations sent out by different American producers. There are extremely few statements to which exception

can be taken. Two might be mentioned. One is that the globulin solution contains nothing but antitoxic globulins. We do not know what antitoxin is, but we believe that the globulin solution still contains much non-antitoxic substance, and we certainly hope to remove still more of the non-antitoxic globulins. The other is the claim that the special precipitating agent used gives a different final result. It seems to me that it is very creditable to the honesty and intelligence of the producers that they are so fair and sensible in their claims for their special preparations.

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THE COMMERCIAL PREPARATIONS OF TETANUS ANTITOXIN *

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Antitetanic serum is the serum of certain animals, usually that of horses, immunized to the toxins of the tetanus bacillus. It is marketed in both the liquid and dry forms. Some manufacturers prepare an antitetanic

by Behring (2), the French method of Roux, and (3) the Italian method after Tizzoni.

The European methods are admitted, even by their users, to be unsatisfactory and, in the main, not accurate; in addition, they are complicated and difficult to use. The American unit commends itself for its simplicity, directness, and accuracy. I am informed that it may be adopted officially by the Belgian government.

There is at the present time no standard for serums for veterinary use. We examined, in the Hygienic Laboratory, several different makes of veterinary tetanus antitoxin and found most of them to contain less than 25 units per cubic centimeter, while the minimum strength of the serums for human use now on the market—over which there is federal control—is at least 150 units per cubic centimeter, and much of it considerably stronger.

The need of uniformity in measuring the strength of tetanus antitoxin has long been felt and is very evident from the accompanying table,² from which it will be seen that before the promulgation of the American standard the tetanus antitoxin on the market varied extravagantly in the unit strength claimed.

As will be seen from the table, most of the foreign serums were far below the American in potency.

TABLE SHOWING THE DIFFERENCES IN THE METHODS OF TESTING TETANUS ANTITOXIN BEFORE THE ADOPTION OF THE AMERICAN UNIT

Name of Manufacturer.	Labeled.	Contents c.c.	Units claimed according to special methods of standardiza- tion, per c.c.	Units accord- ing to the American standard, per c.c.
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).....	One immunizing dose (No. 2929 F.)		0.5	434
New York City department of health (submitted by Dr. W. H. Park for comparison).....	1,000,000 immunizing units (No. 2121).	..	700	166
H. K. Mulford Co. (bought on open market).....	Tetanus Antitoxin, 5 fach. Normal, Prüfungs dose=1/500. *	10	100,000	77
Farbenfabrik vorm. Meister Lucius & Bruning, Hoechst, a/M (submitted for comparative tests).	60,000,000 units.	10	6,000,000	333
H. K. Mulford Co. (No. 9971 submitted for tests).		..	60,000	90
Parke, Davis & Co.		700
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).....		..	75	769
Pasteur Institute, Paris (10 c.c. fluid).....	[Unit value not stated.]	10	Not stated.	40
Pasteur Institute, Paris.....	[Unit value not stated.]	10	Not stated.	40
Tizzoni (No. 2912, Dry).....	[Unit value not stated.]	10	Not stated.	83.3†
Pasteur Institute (No. II, 7).....	[Unit value not stated.]	10	Not stated.	66
Institut Bactériologique de Lyon et du Sud-Est...	[Unit value not stated.]	10	Not stated	‡

* Dissolved in 26 c.c.

† This equals 833 units per gram of the dry serum.

‡ Less than 50.

globulin; this contains a solution of the globulins of the blood, which are soluble in sodium chlorid solution, together with the antitoxin.

All tetanus antitoxin sold in interstate commerce in the United States must conform to the official standard adopted by the United States Public Health and Marine-Hospital Service, which was officially promulgated Oct. 25, 1907.¹ This unit may be defined as follows: "The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea-pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the United States Public Health and Marine Hospital Service."

Besides the American unit above alluded to, there are three other units for measuring the strength of tetanus antitoxin. They are (1) the German method described

Tetanus antitoxin is used both as a prophylactic and as a curative agent in tetanus. The dried and powdered serum has been used as a dusting-powder for wounds. Used as a prophylactic, the dose is 1,500 units; as a curative it should be given in doses of 3,000 to 20,000 units, repeated during the course of the illness.

Tetanus antitoxin is made in the following laboratories: Parke, Davis & Co., Detroit, Mich. (license No. 1); H. K. Mulford Co., Philadelphia, Pa. (license No. 2); Lederle Antitoxin Laboratories, New York, N. Y. (license No. 17); New York State Department of Health, Albany, N. Y., and the Department of Health, city of New York.

The liquid serum is marketed either in syringes ready for use or in glass vials. Each syringe of tetanus antitoxin made by the American producers contains from 1,500 to 5,000 units without regard to the volume of the serum; the unit value per cubic centimeter varies from 150 to 500 or 600 units.

Many of the foreign serums, as is the case with most of the foreign diphtheria antitoxins, are labeled to con-

* Read in the Section on Pharmacology and Therapeutics of the American Medical Association, at the Sixtieth Annual Session, held at Atlantic City, June, 1909.

1. Rosenau, M. J., and Anderson, John F.: The Standardization of Tetanus Antitoxin (an American Unit Established under Authority of the Act of July 1, 1902), Bull. 43, Hyg. Lab., U. S. P. H. and M.-H. S., Washington, 1908.

2. Bull. 43, Hyg. Lab., U. S. P. H. and M.-H. S., p. 11.