THE ACTION OF CERTAIN CHEMICAL AGENTS ON THE STERILITY AND ACTIVITY OF TISSUE EXTRACTS.*

BY WYLY M. BILLING.

In the past we have had several methods open to use for the sterilization of organic extracts. Heat is most commonly used and is generally effective, as regards the sterilization of the extract but frequently reduces the potency of the extract by denaturization. In part this difficulty has been overcome by pasteurization at the hydrogen ion concentration at which the extract shows the greatest stability. For those extracts that are exceedingly thermo labile the candle filter is generally used. Even here a study of the hydrogen ion concentration has proved of value. Dudley, in 1923 (1), has shown that the newly discovered insulin, the sterilization of which has always been attended with some loss, may be filtered without detectable loss by first rendering the solution alkaline to p_H 7.5.

When Mills, in 1921 (2), succeeded in isolating what he considers the natural coagulating agent of tissue, that is, tissue fibrinogen, and showed that, when freed from toxic albumins and foreign tissue, it could be safely depended on for the control of hemorrhage by internal administration, it became necessary to devise some method for the sterilization of the final suspension before any use could be made of the discovery.

Tissue fibrinogen because of its chemical nature defied the customary methods of sterilization. In the first place, even the heat necessary for tyndallization was found to so denaturize it as to cause a great decrease in its activity. In its active form it is a finely divided suspension and for this reason a candle filter could not be used. Changing the hydrogen ion concentration from the isoelectric point results in a complete solution which can be berkfelded but this also destroys the activity.

With these points in mind we undertook a study of the effect of chemical agents in sterilizing the product and also upon its activity.

It was necessary at the outset to use only those chemical agents which would not, in the quantity employed, be toxic to the host and it was hoped indeed to find one that would not even have an irritating effect on injection.

The method employed was, in general, to add the agent under examination in a definite quantity to a solution of tissue fibrinogen of known potency and thereafter determine the presence of microörganisms by culturing out on agar-agar plates which were incubated 48 hours at 37° C. Parallel determinations of the potency were also made.

The potency of the coagulant is most easily and accurately ascertained by measuring the effect of a known quantity of the tissue fibrinogen in accelerating the clotting time of citrated horse plasma which has been recalcified.

Five-hundredths of 1 per cent. of sodium citrate is added to freshly drawn horse blood to prevent coagulation. This is then freed from corpuscles by centrifuging. One cc. of this citrated plasma is placed in a 10-cc. test-tube. This is put into a water-bath, maintained at a temperature of 40° C. until the contents of the tube reach that temperature. Five-hundredths of one cc. of the coagulant under examination is now added and mixed thoroughly. The quantity of 1% solution of calcium chloride required for coagulation (the quantity required for this

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particular plasma having been previously determined) is then added, mixed thoroughly and the test-tube replaced in the water-bath. The time elapsing between the addition of the calcium chloride solution and the formation of a solid clot (as recorded by a stop watch) is noted as the clotting time of this sample.

Comparison of the clotting time cannot be made except when the same lot of plasma is used and used on the same day, for the age of the plasma affects the results. Therefore to compare the relative potency of different lots of coagulant it is necessary to ascertain the normal clotting time of each lot of plasma used and to use this normal clotting time as a factor in establishing the degree of potency.

Below are given the results, in minutes, observed with one specimen of tissue fibrinogen when tested on plasma on the first, twelfth and fortieth days:

	1st day.	12th day.	40th day.
Clotting time with tissue extract	0.61	0.70	0.83
Normal clotting time	3.25	3.79	4.40

By dividing the smaller figure by the larger we get what I have termed the "clotting quotient" which furnishes directly comparative figures. In these examples the clotting quotient for the first day is 0.1877, for the twelfth day 0.1844, and for the fortieth day 0.1886. This shows that there was no material change in the efficacy of the coagulant during the forty days although the elapsed time in each operation differed. The "clotting quotients" ascertained before and after the addition of preservatives are comparable in the same way. In reading the results it must be borne in mind of course that the smaller the fraction the greater the activity of the coagulant.

EFFECT OF ADDITION OF CHEMICAL ANTISEPTICS.

Tricresol.—Tricresol because of its prominence in this field of work was first studied.

Solutions containing 0.3% tricresol were placed in a refrigerator at 34–40° F. immediately after the addition of the tricresol. Subsequent culturing showed that the solutions had not been sterilized as they still contained spore bearing, rod-shaped bacilli. When incubated for several days immediately after the addition of tricresol a sterile solution resulted. Probably the difference of action is due to the fact that the tricresol acts on the fully developed organisms but not on the spores. However, the activity of the extract was greatly reduced.

Clotting quotient before adding tricresol = 0.090
Clotting quotient 4 days later = 0.122
Clotting quotient 7 days later = 0.180
This shows a loss of 50% activity in about one week.

Acriflavine.—A series of tests with acriflavine in dilutions of 1: 1000, 1: 2000, 1: 5000, 1: 10,000, 1: 20,000 and 1: 40,000, both in neutral and slightly alkaline (p_H = 9.0) solutions of tissue fibrinogen were made. In none of these was sterility obtained and the potency diminished in proportion to the amount of acriflavine present. Also those made slightly alkaline depreciated in potency more rapidly than those left neutral.

Sodium Nitrate, Sodium Nitrite, Sodium Benzoate and Borax.—Similar experiments were tried with these salts. All prevented putrefaction for a considerable time but in no case was complete sterility obtained. In moderate strength, 1: 1000,

the effect on the activity was very slight but in more concentrated solution the activity was cut down in proportion to the quantity of chemical added.

Chlorine Compounds.—With the chlorine compounds, as chloramine T., chloretone and chloroform, it was found that complete sterility was obtained only with chloramine T. and then only in a concentration greater than 1:500. At this concentration the activity was decreased 60% in a week's time. With the chloroform and chloretone the loss of activity was not so great but they were not capable of sterilizing the solution.

Mercury chrome 220 and chinosol were tried with similar unsuccessful results.

After these results chemical sterilization seemed out of the question and two attempts along physical lines were made.

Freezing.—The extract in ampuls was subjected to a temperature of −45° C. to −50° C. for 10 minutes on 3 consecutive days. The activity was in no way impaired by this procedure but the spore-bearing organisms were not killed.

Pressure.—An old method of sterilizing by CO_2 under pressure was also tried. The extract was subjected to a pressure in an autoclave of 400 lbs. per square inch for 24 hours. This did not impair the activity of the extract nor did it sterilize it as was shown by subsequent cultures.

Mercuric Chloride.—Mills (3) furnished a method about this time (1922) which later gave very satisfactory results. The method, although no single step is new, is novel in the combination of steps.

It consists of the sterilization of the extract with bichloride of mercury. This is known to be an active sterilizing agent. Even anthrax spores are readily destroyed by it in a comparatively short time.

However, the mercuric chloride is toxic to the higher forms of life and so must be completely removed before the solution can be safely injected subcutaneously.

To accomplish this end the finished extract is placed in a dialyzer and mercuric chloride solution added to 1:500 concentration. This is allowed to stand for 4 hours, care being taken that the solution comes in contact with all parts of the interior of the dialyzer. Twelve per cent. of sterile sodium chloride is then added. This serves to dissolve the mercury protein precipitate with the formation of a soluble sodium proteinate and re-formation of mercuric chloride. After allowing to stand for several hours to complete the solution it is placed in a dialyzer against water to which strips of bright copper are added. As the mercuric salt passes through the dialyzer it is replaced from solution by the copper, thus rendering the process irreversible. Inasmuch as the sodium chloride passes out along with the mercuric salt it quite often happens that the protein again combines with any mercuric salt which may remain in the dialyzer. In such case it is necessary to resalt to about 12% and repeat the dialyzing process against water and copper. Where it is desired to use the resultant extract for subcutaneous injection it is advisable to finally dialyze against a known volume of water which will give a 0.9% solution of salt when equilibrium has been reached. The removal of the mercury is quantitative, for after destroying the organic matter there will be less than one milligram deposited on a gold electrode from a 25-cc. sample.

The resulting extract is in every way as active as it was before sterilization. No diminution in the acceleration of the clotting time of plasma could be detected and complete sterility is effected in every case.

While we have used this method of sterilization only in tissue fibrinogen it offers very interesting possibilities in the ever-growing field of protein preparations for injection because we are able by it to obtain a sterile product without permanently affecting its nature.

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BIOCHEMICAL LABORATORIES
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ABSTRACT OF DISCUSSION.

The author was asked if the mercuric bichloride left in the solution would be sufficient to sterilize the product. The author replied that this was impossible for the concentration of mercuric chloride in the finished product was only 1 in 25,000 while 1 to 800 is a minimum quantity to affect the sterilization of anthrax bacillus.

The author was further asked whether the method had been applied to vaccines. In answer thereto he said that it offered a very interesting possibility for the sterilization of vaccines without heat, but he had not completed experimentation and, therefore, was not in position to give a definite answer.

PNEUMOCOCCUS ANTIBODY SOLUTION.*

With Special Reference to Its Nature, Preparation, Administration and Effect.

BY PAUL S. PITTENGER.

Antibody solutions are a new classification of biologic products just beginning to come into use.

Their importance cannot be overestimated as clinical data (1, 2, 3) already indicate that Pneumococcus Antibody Solution will no doubt prove to be the greatest discovery in the biologic field since the discovery of Diphtheria Antitoxin.

Immediately following the discovery of Diphtheria Antitoxin and publication of clinical data showing the remarkable reduction in mortality as a result of its use, pharmacists were called upon by physician and layman alike for all kinds of information in reference to its nature, preparation, administration and effect.

Clinical data have already been published by competent clinicians (1) showing that the lobar pneumonia mortality has been reduced to one-half the usual rate in cases receiving early treatment with Pneumococcus Antibody Solution.

It is to be expected, therefore, that we as pharmacists will within the near future be called upon to supply this new product and give information in reference to it.

In view of the fact that few physicians or pharmacists are as yet familiar with this latest classification of biologic products, I thought that a paper on *Antibody Solutions* would be acceptable at this time.

Pneumococcus Antibody Solution is the first of these solutions to be successfully produced on a manufacturing scale. I will therefore confine my remarks to a description of this product.

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