SERIES D. Titrations in the three previous series show concordant results for uniform material and for the same methods. The results of titration in H_2O or NaCl solution and in presence of $AgNO_3$ seemed worthy of further experimentation.

1 Cc. NaOH V. S. = 0.02462267 Gm. NaOH
= 0.06034740 Gm. H_3PO_4 in presence of H_2O or NaCl solution
= 0.02011580 Gm. H_3PO_4 in presence of $AgNO_3$
= 0.08741942 Gm. Na_2HPO_4 in presence of $AgNO_3$

1 Cc. HNO_3 V. S. = 0.03141450 Gm. HNO_3
= 0.07080973 Gm. Na_2HPO_4

Na_2HPO_4 (dried at 110–115° C.).

1.0718 Gm. with 20 Cc. H_2O and two drops modified indicator required 15.2 Cc. HNO_3 V. S. = 100.42%

0.8008 Gm. with 50 Cc. H_2O and 4 drops modified indicator required 11.35 Cc. HNO_3 V. S. = 100.37%

After adding HNO_3 V. S. to total 15 Cc. 60 Cc. $AgNO_3$ (5%) were added and titrated with NaOH V. S. to brown coloration in supernatant liquid; 21.3 Cc. NaOH V. S. required less 12.15 Cc. for the 15 Cc. HNO_3 V. S. gives 9.15 Cc. NaOH V. S. = 99.63%

0.903 Gm. with 20 Cc. H_2O and 85 Cc. $AgNO_3$ (5%) required 10.3 NaOH V. S. = 99.71%

0.4522 Gm. with 50 Cc. NaCl (5%) and 2 drops modified indicator required 6.3 Cc. HNO_3 V. S. = 98.65%

1.3688 Gm. with 100 Cc. NaCl (10%) and 4 drops modified indicator required 19 Cc. HNO_3 V. S. = 98.29%

H_3PO_4. 15.705 Gm. (*d*) per 100 Cc. 10 Cc. used for each of the following:

with 100 Cc. H_2O and 4 drops modified indicator required 21.90 Cc. NaOH V. S. = 84.17%

with 100 Cc. NaCl (5%) and 4 drops modified indicator required 22.10 Cc. NaOH V. S. = 84.92%

with 100 Cc. NaCl (10%) and 4 drops modified indicator required 22.35 Cc. NaOH V. S. = 85.88%

with 150 Cc. $AgNO_3$ (5%), no indicator, required 67.2 Cc. NaOH V. S. = 85.44%

In some other experiments an effort was made to replace the silver nitrate by the cheaper lead nitrate but the results were so discordant that they are not recorded; it had been hoped that the greater insolubility of the lead phosphate would make this an ideal method but the precipitation of the indigo-carmine and the absence of a colored precipitate with excess of NaOH made one entirely dependent upon change in color of the methyl-orange.

From the results in Series D it seems likely that, with the aid of this modified indicator and in presence of sodium chloride, a method will be found for directly titrating phosphoric acid and sodium phosphate; the necessary quantity of sodium chloride will be found to differ for these two substances.

CHEMICAL LABORATORY,
PHILADELPHIA COLLEGE OF PHARMACY AND SCIENCE,
JULY 1921.

THE BIOLOGIC STANDARDIZATION OF LOCAL ANAESTHETICS.*

With reference to the Effects of Sterilization on Solutions of Cocaine and Procaine.

BY PAUL S. PITTENGER.

One of the most important duties of the Control Department of a large pharmaceutical manufacturing house is to carefully investigate all complaints received. This is especially true in cases where the physician claims the preparation does not

* Read before Scientific Section A. Ph. A., New Orleans meeting, 1921.

possess the required activity. Such complaints at times make it necessary to check the chemical assay with biologic tests, even though the chemical test is supposed to give a true index to the therapeutic value of the drug or preparation.

About a year ago we received a complaint that a certain lot of Procaine tablets were inert. The chemical analysis showed the stated amount of Procaine present. In order to check the chemical assay, we gave a dog a subcutaneous injection of a solution of the tablets. After 5 minutes, the site of injection was touched with a red-hot wire. The dog showed no signs of pain or irritation, thus proving that the solution produced complete local anaesthesia, and that the complaint was unfounded.

This test suggested the possibility of devising a biologic method for the quantitative valuation of local anaesthetics. We therefore carried out a series of experiments in order to determine the sensitiveness of such a test when employing the following technique.

Method. Closely clip the hair from about 1 sq. in. of a dog's back, inject the solution with an accurately graduated hypodermic syringe in such a way that the *point* of the needle is in the *center* of the spot from which the hair was clipped. After 5 minutes, touch the center of the spot with a red-hot wire loop and note whether the animal shows any signs of pain or irritation.

The solution injected should be of such strength that the volume injected is approximately 1 Cc.

The wire loop should be about 3 mm. in diameter and made from about 20 gauge wire.

The following results were obtained with Cocaine and Procaine:

Expt. No. 1.

		COCAINE.	
Weight.	Dilution.	Dog.	Results.
0.004	1 Cc.	Dog No. 1	Sensitive
0.005	1 Cc.	Dog No. 2	Sensitive
0.005	1 Cc.	Dog No. 3	Sensitive
*0.006	1 Cc.	Dog No. 4	Not sensitive
*0.006	1 Cc.	Dog No. 1	Not sensitive
0.0065	1 Cc.	Dog No. 2	Not sensitive
0.007	1 Cc.	Dog No. 3	Not sensitive
0.008	1 Cc.	Dog No. 4	Not sensitive

These results show that the minimum amount of Cocaine that will produce complete local anaesthesia of a limited area is 0.006 Gm., also that the method is sensitive to a variation of 1 mg. of Cocaine or 0.1 Cc. of a 0.6% solution.

Expt. No. 2.

		PROCAINE.	
Weight.	Dilution.	Dog.	Results.
0.05	1 Cc.	Dog No. 5	Sensitive
0.06	1 Cc.	Dog No. 6	Sensitive
0.065	1 Cc.	Dog No. 7	Sensitive
0.065	1 Cc.	Dog No. 8	Sensitive
*0.07	1 Cc.	Dog No. 5	Not sensitive
*0.07	1 Cc.	Dog No. 6	Not sensitive
0.075	1 Cc.	Dog No. 7	Not sensitive
0.08	1 Cc.	Dog No. 8	Not sensitive

You will note from the above experiment that the minimum amount of Procaine that will produce complete local anaesthesia of a limited area is 0.07 Gm. and that the method is sufficiently sensitive to note variations in the dose of only 7%.

This method is, therefore, satisfactory for the quantitative determination of the activity of the local anaesthetics.

These results also show that Cocaine is approximately eleven times as active as Procaine.

Having received several requests from salesmen for information as to the effect of sterilization on solutions of Cocaine and the satisfactory results obtained by the foregoing experiments, led to investigation along this line.

I find it is the opinion of a great many physicians and statements have frequently appeared in the literature, to the effect that sterilization destroys a large portion of the activity of Cocaine solutions, and that solutions of this substance rapidly decompose on standing.

For example—Sollman[1] states that one of the disadvantages of Cocaine is the

"Instability of solutions"—"Contrary to older statements they are not materially decomposed by boiling."—"Long continued boiling decomposes Cocaine into benzoyl-ecgonin and methyl alcohol. It was therefore believed that solutions could not be sterilized by boiling. In fact, however, the decomposition on boiling half an hour is insignificant (Merck, 1907; Holbrook, 1912); an even longer boiling only decreases the activity, for the decomposition products are merely inactive and not toxic."

T. Baumeister[2] reports on various cases of inflammation of the eyes produced by non-sterile Cocaine solutions. He therefore advises never to keep the Cocaine solutions for any length of time and to use freshly prepared solutions which should be boiled for three minutes immediately before use. He further claims that sterilizing at 58° does not render Cocaine solutions sterile, and that by prolonged heating at a higher temperature the solutions are decomposed.

Ebert,[2] however, claims that Cocaine solution can be sterilized without danger in a current of steam, and that any decomposition of the solution which might take place is not produced by the high temperature, but by the alkalinity of the glass in which the heating is carried out.

When alkali-free glass is used, a sterilization for three-fourths to one hour in a current of steam does not affect Cocaine solutions in the least.

Max Nymann and R. Bjorksten[3] describe a method which they state is particularly adapted for controlling the degree of decomposition of sterilized solutions of Cocaine.

John E. Virden[4] states

"That solutions of Cocaine have their anesthetic properties injured by boiling seems to be a belief rather generally accepted by the medical profession, and is so taught in the Medical Schools, Schools of Pharmacy and Nurses Training Schools.

"Some writers on Ophthalmology and Ophthalmic Surgery give us no definite information on this point, some tell us that boiling is injurious while others usually guardedly say that they have boiled these solutions to render them sterile.

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[1] Sollman, "Manual of Pharmacology," 1917, p. 261.

[2] *Pharm. Ztg.*, through *Pharm. Weekblad*, 54 (1917), 647.

[3] *Phar. Zentralb.*, 52 (1911), No. 4, 71, 74.

[4] *Amer. Jour. of Surg.*, N. Y., 1915, p. 288.

"Having read many of the indefinite and conflicting statements of these authors, and knowing that all my friends were afraid of injuring their Cocaine solutions by boiling, I determined to satisfy myself on this point, although it had been my practice for several years to boil these solutions before using them on any serious surgical operation.

"The solution used in these tests was composed of Cocaine Muriate of 4% strength. They were not boiled in a water-bath but over an Argand gas flame in my office."

This is followed by a series of case reports in which each of a series of solutions was boiled for periods ranging from 3 to 15 minutes each time before solution was used, the evaporation being made up with sterile distilled water.

The summary of the experiments follows:

"Solutions No. 1 and No. 2 were boiled on 4 occasions for periods of 3 minutes, making a total boiling of 12 minutes.

"Solutions No. 3 and No. 4 were boiled for periods of 3 minutes each, making a total boiling of 24 minutes.

"Solution No. 5 was boiled for periods of 15, 12 and 15 minutes, making a total boiling of 42 minutes.

"In no case have I failed to secure the desired anaesthesia with a small amount of the solution. Also there have been no undesirable effects such as irritation of the cornea or conjunctiva, and the healing of surgical wounds has not been interfered with in any way.

"Hence I am fully convinced that frequent or even prolonged boiling of solution Cocaine Muriate does not injure or destroy their anaesthetic value, nor does it make them more dangerous to the tissues to which they must be applied in ophthalmic surgery."

All of the foregoing statements, however, are based upon chemical investigations, except those of Virden which are based on clinical observations. The fact that a 4% solution, after several boilings, still produced local anaesthesia, does not, however, prove that the boiling did not partially reduce the activity of the solution. A 4% solution may contain twice the actual amount of Cocaine required to produce the necessary anaesthesia for the operations mentioned. In such a case, reductions of 25% or 40% in activity would not be apparent.

I therefore decided to check the effect of aging and the various forms of sterilization upon solutions of Cocaine and Procaine, by *quantitatively measuring any variations in activity* by the previously described method. Accordingly, a series of experiments were started as follows:

First.—Prepare a solution of Cocaine and one of Procaine of such strength that 1 Cc. will contain the *minimum* amount of salt that will produce local anaesthesia in 5 minutes when tested by the foregoing method, reserving a sample of each of the salts used.

Cocaine 0.6% solution
Procaine 7.0% solution
} based

on experiments Nos. 1 and 2.

Second.—Divide each solution into four portions, fill each portion into ampuls as follows:

(A)—Fill into sterile ampuls without sterilizing or Berkfeldting.

(B)—Add 0.3% Three Cresols, Berkfeldt and fill into sterile ampuls.

(C)—Fill into ampuls and Arnold sterilize on three consecutive days.

(D)—Fill into ampuls and autoclave for 5 min. at 115° C.

Third.—Test each lot to determine effect of sterilization.

Fourth.—Test each lot every three months to determine rate of deterioration on standing.

Fifth.—At the end of six months make fresh solutions of the reserve salts and test to determine whether or not the salt deteriorates with age.

The results obtained to date follow:

Expts. Nos. 3 and 4.

COCAINE SOLUTION 0.6 %.

Filled into sterile ampuls without sterilizing or Berkfeldting.

Results.

Dose.	Animal.	Expt. No. 3. Immediately after filling.	Expt. No. 4. 3 mos. after filling.
0.7	Dog No. 1	Sensitive	Sensitive
0.8	Dog No. 2	Sensitive	Sensitive
0.9	Dog No. 3	Sensitive	Very slightly sensitive
0.9	Dog No. 4	Not sensitive	Not sensitive
*1.0	Dog No. 5	Not sensitive	Not sensitive
*1.0	Dog No. 6	Not sensitive	Not sensitive
1.1	Dog No. 7	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 3 and 4.

Expts. Nos. 5 and 6.

PROCAINE SOLUTION 7 %.

Filled into sterile ampuls without sterilizing or Berfeldting.

Results.

Dose.	Animal.	Expt. No. 5 Immediately after filling.	Expt. No. 6. 3 mos. after filling.
0.8	Dog No. 8	Sensitive	Sensitive
0.9	Dog No. 9	Slightly sensitive	Sensitive
0.9	Dog No. 10	Slightly sensitive	Sensitive
*1.0	Dog No. 11	Not sensitive	Not sensitive
*1.0	Dog No. 12	Not sensitive	Not sensitive
1.1	Dog No. 13	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 5 and 6.

Expts. Nos. 7 and 8.

COCAINE SOLUTION 0.6 %.

0.3% Three Cresols, Berkfeldted and filled into sterile ampuls.

Results.

Dose.	Animal.	Expt. No. 7. Immediately after filling.	Expt. No. 8. 3 mos. after filling.
0.8	Dog No. 1	Sensitive	Sensitive
0.9	Dog No. 2	Very slightly sensitive	Sensitive
0.9	Dog No. 3	Sensitive	Slightly sensitive
*1.0	Dog No. 4	Not sensitive	Not sensitive
*1.0	Dog No. 5	Not sensitive	Not sensitive
1.1	Dog No. 6	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 7 and 8.

Expts. Nos. 9 and 10.

PROCAINE SOLUTION 7 %.

0.3% Three Cresols, Berkfeldted and filled into sterile ampuls.

Results.

Dose.	Animal.	Expt. No. 9. Immediately after filling.	Expt. No. 10. 3 mos. after filling.
0.8	Dog No. 7	Sensitive	Sensitive
0.9	Dog No. 8	Sensitive	Sensitive
0.9	Dog No. 9	Sensitive	Sensitive
*1.0	Dog No. 10	Not sensitive	Not sensitive
*1.0	Dog No. 11	Not sensitive	Not sensitive
1.1	Dog No. 12	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 9 and 10.

Expts. Nos. 11 and 12.

COCAINE SOLUTION 0.6 %.

Filled into ampuls and Arnold sterilized on 3 consecutive days.

Results.

Dose.	Animal.	Expt. No. 11. Immediately after filling.	Expt. No. 12. 3 mos. after filling.
0.8	Dog No. 13	Sensitive	Sensitive
0.9	Dog No. 14	Sensitive	Sensitive
0.9	Dog No. 15	Sensitive	Slightly sensitive
*1.0	Dog No. 16	Not sensitive	Not sensitive
*1.0	Dog No. 17	Not sensitive	Not sensitive
1.1	Dog No. 18	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. in both experiments Nos. 11 and 12.

Expts. Nos. 13 and 14.

PROCAINE SOLUTION 7 %.

Filled into ampuls and Arnold sterilized on 3 consecutive days.

Results.

Dose.	Animal.	Expt. No. 13. Immediately after sterilizing.	Expt. No. 14. 3 mos. after sterilizing.
0.8	Dog No. 19	Sensitive	Sensitive
0.9	Dog No. 20	Sensitive	Sensitive
0.9	Dog No. 21	Sensitive	Sensitive
*1.0	Dog No. 22	Not sensitive	Not sensitive
*1.0	Dog No. 23	Not sensitive	Not sensitive
1.1	Dog No. 24	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 13 and 14.

Expts. Nos. 15 and 16.

COCAINE SOLUTION 0.6 %.

Filled into ampuls and autoclaved for 15 min. at 115° C.

Results.

Dose.	Animal.	Expt. No. 15. Immediately after sterilizing.	Expt. No. 16. 3 mos. after sterilizing.
0.8	Dog No. 13	Sensitive	Sensitive
0.9	Dog No. 14	Slightly sensitive	Sensitive
0.9	Dog No. 15	Not sensitive	Sensitive
*1.0	Dog No. 16	Not sensitive	Not sensitive
*1.0	Dog No. 17	Not sensitive	Not sensitive
1.1	Dog No. 18	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 15 and 16.

Expts. Nos. 17 and 18.

PROCAINE SOLUTION 7%.

Filled into ampuls, autoclaved for 15 min. at 115° C.

Results.

Dose.	Animal.	Expt. No. 17. Immediately after sterilizing.	Expt. No. 18. 3 mos. after sterilizing.
0.8	Dog No. 19	Sensitive	Sensitive
0.9	Dog No. 20	Slightly sensitive	Slightly sensitive
0.9	Dog No. 21	Sensitive	Sensitive
*1.0	Dog No. 22	Not sensitive	Not sensitive
*1.0	Dog No. 23	Not sensitive	Not sensitive
1.1	Dog No. 24	Not sensitive	Not sensitive

Minimum amount required to produce complete local anaesthesia is 1.0 Cc. for both experiments Nos. 17 and 18.

It will be noted from the results of experiments Nos. 1 to 18, inclusive, that the amount of solution required to produce complete local anaesthesia was, in all cases, the same. These results would tend to prove, therefore, that the forms of sterilization described above *do not affect the activity of solutions of Cocaine or Procaine.*

It also should be noted that the unsterilized solutions, the solutions sterilized without heat, and the solutions sterilized with heat all showed exactly the same activity after 3 months as immediately after being prepared.

We will continue our experiments by testing samples from each lot of solution every 3 months, and fresh solutions of the reserve salts every 6 months. The final results of this series of experiments will then be presented in a subsequent paper.

Finally, the author wishes to acknowledge his indebtedness to Mr. Arnold Quici for most of the laboratory work in connection with this paper.

PHARMACODYNAMIC LABORATORY,
H. K. MULFORD COMPANY.

MICROBIOLOGY IN THE TWO-YEAR COURSE IN PHARMACY.*

BY E. E. STANFORD.

The writer has experienced some doubts as to the wisdom of preparing a paper under this title. If the following remarks be embalmed in type, it is, perhaps, conceivable that they may be read by the pharmaceutical educator of no distant date in somewhat the same spirit in which the economic zoölogist of to-day might pursue the theories of some medieval writer regarding the domestication, training and usefulness of the dodo. Nevertheless, if the most of us, the present writer included, be compelled by necessity to engage, so to speak, in the training of a present-day dodo, we must, for conscience's sake, be concerned in turning him out as respectable and efficient a fowl as his generic limitations permit, though the result thereof may indeed be to perpetuate the species longer than many of us would desire. Future developments must come from present seed, and only in so far as we discharge the responsibilities of to-day worthily may we hope to accelerate the evolution of our "bird" into one with the wings of a baccalaureate degree to elevate him to an equal plane with practitioners of our sister professions.

It is perhaps no wonder that microbiology, or to use the commoner less inclusive term, bacteriology, as a most recent addition to our sciences, has not long claimed a definite place in pharmaceutical education. No pioneer in the matter, certainly, the writer introduced the subject in his curriculum only two years ago, and there are still institutions where, for one reason or another, the study has not obtained a place. In this brief time, however, he has formed certain fairly definite notions as to the scope and place of bacteriology in the brief course in pharmacy. In the hope, therefore, of provoking some thought or discussion which may not be wholly without benefit, he ventures on record at this time, although, unfortunately, he cannot present in person to profit by any discussion which may arise.

The vital need to the modern pharmacist of some knowledge at least of the phenomena of microbiology in relation to human life can hardly be gainsaid. Three major objections to the inclusion in the short course of a separate study of the subject are sometimes cited: lack of time, lack of equipment, and lack of personnel

* Read before Section on Education and Legislation A. Ph. A., New Orleans meeting, 1921.