THE PREPARATION OF AUTOGENOUS VACCINES BY THE RETAIL PHARMACIST.

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The advances in pathology and bacteriology during the past 20 years have to some extent revolutionized the art and science of medicine. The recognition of the bacterial origin of many diseases and the careful study of histo-pathological and physiologic-pathological changes wrought by these specific microorganisms and the products of their activity, the toxins, has naturally led the thinking practitioner of medicine from the purely empirical employment of drugs to the adoption of such remedial agents which appear to be "physiologically" indicated. "The physicians first and principal duty is to cure disease and to alleviate suffering." In this endeavor he has from time immemorial studied, experimented and tried, only too often to find disappointment instead of success crowning his work.

I shall not attempt to enter here into the whys and wherefores, the advantages and disadvantages of vaccine therapy. We are confronted with the fact that vaccines, autogenous and heterogenous, are coming more into use, displacing and replacing much of the old time prescription work. It therefore behooves the pharmacist to either fall in line with the procession and supply the legitimate demand for vaccines or see his business dwindle day by day, part, the part that never belonged to him, going to the department stores and corporation drug stores, the other part, professionally and legitimately his, going to the "manufacturing chemists," who were enterprising enough to recognize early the trend toward vaccine therapy and to meet or even anticipate this demand. "But," I hear the pharmacist say, "it takes a good deal of money and considerable experience to start a vaccine laboratory, and I have neither." Now, if you thought that a new $2000 soda water apparatus would add to the profits of your business and you had neither the money nor the experience to run one, what would you do? I suppose you would borrow the money and hire an experienced soda man to run it. The same principle applied to your vaccine laboratory will work out just as well. To begin with, many things needed in a laboratory should be found in every well regulated pharmacy. For the balance of the equipment about $500 judiciously distributed will complete your outfit. As to the man, there are any number of young physicians, recently graduated, having a good hospital training in bacteriological work, who would be only too glad to spend part of their time in a well equipped laboratory for a moderate compensation. And a little study on your part during some of the time devoted to stock quotations, to racing charts and to base-ball scores, will soon enable you to grasp the fundamental principles of this, relatively new, branch of therapeutics and ere long you will be interested enough to want to know more about it.

Historical.—Vaccines as therapeutic agents are by no means new. It is only the recognition of its rationale that is new.

From time immemorial attempts have been made to cure disease by the causative agent of that disease. "A hair of the dog is good for the bite" is an old saw. Pliny suggests that the natural immunity of the Psylli against snake venom is probably due to their habit of drinking water from wells inhabited by venomous snakes. Other tribes were in the habit of producing what we now would call "actively acquired immunity" by cutaneous applications of small doses of snake venom. And a most drastic example of vaccination as a prophylactic measure is of course Jenner's application of cow virus against smallpox.

It may be of some little interest to follow up the history of smallpox vaccination for some time before Jenner's discovery, or rather rediscovery.

Arabian physicians were acquainted with the nature and treatment of smallpox. They probably were the first to whom it occurred to produce the disease by inoculation. Avicenna, of Bokhara, is credited with the discovery. It was carried by the Tartars and Chinese to Surat, Bengal and China.

Circassia: Dr. De la Motraye saw the operation of vaccination performed on a girl in 1711.

Constantinople: Dr. Kennedy reports seeing it done in 1715.

Dr. Timoni also saw it there in 1715, and Dr. Pylarini in 1716.

Cassem Aga, Ambassador in England from Tripoli, describes it in 1728.

India: Howell describes inoculation against smallpox in 1767. In Hindostan inoculation was performed by a particular tribe of Brahmins, who were delegated annually for this service.

China: D'Enrecolles speaks of it as being practiced there in 1718, but meeting with a good deal of opposition.

France: Dr. Boyer mentions it under the year 1717.

Spain: Here it was introduced in 1729, but not practiced until 1772, when it was used by Dr. Miguel Gorman.

Italy: According to Monsieur de la Condamine inoculation had been secretly practiced by the people of Naples from time immemorial. During the outbreak in Rome, in 1754, inoculation was publicly introduced by Peverini.

Germany and Austria: Inoculation was first performed in Hannover in 1724. Mr. Maitland operated on H. R. H. Prince Frederick and afterward on eight children of a Baron.

Holland: Inoculation introduced by Dr. Tronchin, who first operated on one of his own sons.

Denmark, Sweden and Switzerland: Inoculation is mentioned in the years 1754, 1754, 1751, respectively.

Russia: Operation performed in 1768 by Dimsdale.

America: In 1721 Dr. Zabdiel Boylston inoculated 244 people.

Scotland: In 1726 operation performed by Mr. Maitland.

Ireland: In 1723 first operation performed at Dublin by Dr. Bryan Robinson.

England: In 1717, yielding to the persuasion of Lady Mary Wortly Montagu, the operation was performed by Mr. Maitland on an old Greek woman.

Jenner's attention was first attracted to inoculation in 1776. Nothing further

was heard of this until 1788. His first paper on the subject appeared in 1797. In 1796 the first successful vaccination was performed by Jenner on James Phillip.

Rationale of Vaccine Therapy.—The rationale of vaccine therapy briefly stated is this: When the pathogenic microorganisms invade the animal system there ensues a war between invader and invaded. The body mechanism endeavors to ward off the attack first by "non-specific" agents of defense, that is agents opposed alike to *all* pathogenic bacterial infections, and secondly by the production of *specific* agents of defense—that is—agents directed against that particular species of microorganism which is the individual disturbing factor at that particular time. As this is not to be a treatise on the specific action of vaccines, but rather a description of the methods of preparation of such vaccines, I will content myself by stating that these *specific* agents of defense are called antibodies, immune bodies, etc. Now, broadly speaking, according to whether these antibodies are present in sufficient numbers to overcome the invading disease-producing bacteria or not the patient will either recover or succumb. Let me impress this point: Nature *always* produces *some* antibodies in every bacterial infection—it is merely a question of producing *enough*. By the introduction of vaccine into the peripheral tissues these are stimulated to produce the *specific* antibodies and send them to the aid of the antibodies already being produced at the seat of lesion—in a case of existing infection—or, when used as a prophylactic measure, such as vaccination against smallpox or typhoid fever, we stimulate the tissues by carefully measured doses of the bacterial toxin to the production of *specific* antibodies, which, stored within the animal body, are ready, for a greater or lesser period of time after vaccination, to meet that particular invading enemy and destroy him—or rather—make the animal non-susceptible to the action of that particular microorganism or its toxin. This then, in brief, is the principle of vaccination; an artificially but actively acquired immunity against a specific microorganism or its toxin.

Classification of Vaccines.—Having given a brief outline of the history and rationale of vaccine therapy, we will next consider the classification of vaccines. Vaccines are divided into two great classes: The heterogenous and the autogenous vaccines. In the heterogenous the bacteria used for the preparation of the vaccine are obtained from outside of the body of the patient—that is—some other animal, of the same or different species, is the source of supply and the vaccine is made in advance of the arising need and held ready for therapeutic or prophylactic use—stock vaccines. In the case of the autogenous vaccines the microorganism is recovered from the patient, either from some accessible focus of infection or from the circulating blood, and is then used for the preparation of the vaccine for that particular patient, so that the very same strain of bacteria which causes the disease is used for the making of the vaccine to combat that disease.

Obtaining the Specimen.—When we consider that (1) the skin and mucous membranes are always the seat of numerous microorganisms, and (2) frequently pathological lesions are due to a mixed infection, it becomes at once apparent that the obtaining and isolating of the causative microorganism is of prime importance. I shall therefore mention a few fundamental rules whose observance

in taking the specimen for the making of autogenous vaccines are the "sine qua non" of vaccine making.

1. Take the specimen yourself or have a well trained assistant from your laboratory attend to that. Many physicians, with the best intentions in the world, will fail in the observance of what, to them, seems a minor detail, with the result of contamination of specimen.

2. Observe the highest degree of asepsis in all your work—prepare as carefully for the taking of the specimen as the most careful surgeon prepares for a major operation.

3. Do not use an antiseptic on the skin or over the surface of the lesion from which you are about to gather your material—in the case of a surface infection, such as acne, carbuncle, etc., but wash the place with plenty of sterile water and gather your material from the depth of the lesion.

4. If a blood culture is to be made cut down on the vein, after the dry skin has been painted with tincture of iodine, and give yourself a liberal field to work in.

5. Do not fail in all cases to make a few primary smears, on clean glass slides or cover glasses direct from the lesion.

6. Having obtained your specimen do not delay any longer than absolutely necessary in preparing the vaccine.

After collecting the specimen and staining the primary smear, determine whether the infection is caused by a single species or whether it is a mixed infection. Even though the primary smear shows the presence of only one species a plate culture should always be made. In the case of mixed infection the numerical relation of the various microorganisms should be ascertained.

Having obtained the offending microrganism or microorganisms in pure culture the actual preparation of the vaccine begins.

Utensils Needed.—A good surgeon always lays out every instrument which may be required in the course of the operation and prepares for any emergency which might arise. We, too, will proceed to collect all the pharaphernalia needed for the making of our vaccine. Hence the following articles should be on hand:

1. Twenty-four-hour pure culture of the microorganisms.
2. Sterile agar slants (two).
3. Sterile pipettes, graduated 10 cc.; 5 cc.; 1 cc. (six each).
4. Normal salt solution in 250 cc. flasks (two).
5. Sterile test tubes (6x⅝) thick walled, soft glass (six).
6. Water bath held at 60° C. and water bath for melting agar.
7. Blast lamp.
8. Special Bunsen burner with "sparflamme."
9. Glass knife, spool of adhesive plaster 1", Tr. Iodine 25 cc.
10. Sterile capillary pipettes in glass tube.
11. Watch crystals (6).
12. Glass dish with 0.25 percent Lysol.
13. Sheet lead with wire attached.
14. Green soap, cotton, alcohol.
15. Smearing slides (6), plain slides (6).
16. Wright stain, glass pencil, staining rack, distilled water.

17. Tubes of 1 percent Dextrose agar (2).
18. Sterile vaccine bottles, plugged and capped with paper (6).
19. Rubber caps for same.
20. Small casserole, small funnel.
21. Trikresol 25 cc.
22. Pair of heavy forceps.
23. Melted paraffin.
24. Counting eye piece, microscope, immersion oil, etc.

How to Make Some of These Things.—Capillary pipettes: Take a piece of No. 4 glass tubing about 5″ long; heat the centre over the blast flame to redness, wait till it cools off a little, then with slight rotary motion pull the two ends apart, and cut in the middle. This will give two pipettes.

Smearing slides: Take an ordinary glass slide, mark with glass knife on one edge and break off along line.

Counting eye-piece: Take the eye-piece of your microscope, unscrew top lense, cut out a round piece of carboard to fit inside of eye-piece; in this cardboard cut out a square piece, and lay two hairs across this square opening. These may be fastened with a drop of balsam.

Making the Vaccine.—Everything needed being on hand, we proceed with the making of the vaccine.

1. Put about 5 cc. of sterile normal salt solution into a sterile test tube (6x⅝).
2. Start water bath to be held at 60° C.
3. Make a thick emulsion of the microorganism to be used in the salt sol. (step 1), rubbing small quantities against the sides of the tube until thoroughly emulsified before mixing with the main bulk of the salt solution in the tube.
4. Hermetically seal this tube of emulsion in the blast flame.
5. After cooling, shake the emulsion vigorously for at least 15 minutes.
6. Cut and break off the end of the sealed tube over 0.25 percent Lysol sol.
7. With a sterile capillary pipette enter the tube and withdraw a small quantity of the emulsion and deposit it on a watch crystal.
8. Put the capillary tube in Lysol sol.
9. Cover the watch crystal with another watch crystal, to prevent evaporation.
10. Reseal the tube containing the emulsion.
11. When cool, wrap the tube in sheet lead to which a wire has been attached, and sink in the water bath held at 60° C. Keep there for 1 hour.
12. Put 1 or 2 cc. NaCl sol. in another watch crystal.
13. Mark 5 capillary pipettes about 1 inch from the small end and fit with rubber nipple.
14. Prick cleansed finger tip and allow a small drop of blood to accumulate.
15. With a marked capillary pipette suck up 1 unit (up to mark), admit a bubble of air.
16. In same pipette suck up one unit of salt sol., admit a bubble of air.
17. Suck up, in same pipette, one unit of emulsion from watch crystal.
18. Mix these on a clean slide by alternately sucking up and expelling, avoiding air bubbles.

19. Deposit a drop of this mixture on 2 clean glass slides and smear with smearing slide, same as ordinary blood smear.

20. Repeat 15, 16, 17, using 2 units of salt sol. instead of 1, and proceed as in 18 and 19.

21. When dry, stain these films, marked for identification, with Wright's stain.

22. Limit the ocular field by cross hair preparation (counting eye-piece).

23. Examine stained smears by high power, and if satisfactory—

24. Examine under oil immersion.

25. Count the bacteria and the red corpuscles in successive fields until 500 red blood cells have been counted.

26. Determine the number of bacteria per cc. by the following equation: R. B. C. : Bacteria :: 5000 million : X.

27. Decide upon the dose per cc. and the total quantity of vaccine to be supplied, and from this determine the quantity of undiluted emulsion to be put into the dispensing bottle (vaccine bottle).

28. After the one hour has elapsed (step 11), cut tube and pipette off the quantity desired, which put into dispensing bottle.

29. With a sterile pipette remove about 1/10 cc. of emulsion, which put into a tube of melted dextrose agar, held at about 45° C. Make a shake culture.

30. Re-seal tube.

31. Add the necessary quantity sterile salt sol. to the emulsion in the dispensing bottle, thoroughly mix.

32. Remove about 1/10 cc. from bottle and put into melted dextrose agar (29).

33. Mark tube step 29 "emulsion," and tube step 32 "bottle."

34. Put into the completed vaccine 0.25 percent trikresol.

35. Put sterile rubber cap on dispensing bottle.

36. Paraffin and label.

This, gentlemen, completes the making of the vaccine, excepting the delivering and collecting of fee.

Again I want to impress upon you the necessity of extreme cleanliness and the need of using nothing but sterile appliances. Furthermore, the vaccine should not be released until 24 hours incubation of the tubes (steps 29 and 32) prove the absence of living micro-organisms.

I trust that I have succeeded in demonstrating to you that the making of autogenous vaccines is not as difficult a proposition as would appear at first glance and that the average pharmacist, with a little training, can prepare these vaccines successfully to the benefit of the patient, the satisfaction of the physician, the enrichment of himself and the credit of his profession.

THE MANUFACTURE AND ASSAY OF HYPOPHOSPHOROUS ACID.

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This acid was introduced into the United States Pharmacopoeia of 1890, and retained in the eighth revision, largely because of its value as a preservative of pharmaceutical preparations containing iodides, which are liable to decomposition by exposure to light and air.