

(b) Proceed as directed under (a) above until chloroform is evaporated. At this stage dissolve the residue in about 10 mils of alcohol, dilute with water to 25 mils and titrate with  $\frac{N}{10}$  sodium hydroxide solution, using phenolphthalein as indicator. 1 mil of  $\frac{N}{10}$  sodium hydroxide is the equivalent of 0.016 Gm. of sodium salicylate.

(c) Ignite a given weight of the material, extract the residue with water and titrate with  $\frac{N}{10}$  sulphuric acid, using methyl red as indicator. 1 mil of  $\frac{N}{10}$  sulphuric acid is the equivalent of 0.016 Gm. of sodium salicylate.

*Comments.*—Acetanilide was substituted for antipyrin in two cases.

#### PRESCRIPTION G.

Phenacetin and salol, each 60 grains, to be mixed and made into one dozen powders.

Each powder should contain 5 grains each of phenacetin and salol.

#### METHODS OF ANALYSIS.

These methods are given in an article recently published in the *J. Ind. and Eng. Chem.*, 7, 681, 1915, by W. O. Emery, G. C. Spencer and C. C. LeFevre.

*Comments.*—The compounding was well done.

#### SUMMARY.

1. The results in this article show that there is considerable room for improvement in the quality of the drugs examined.

2. An examination of the mixtures procured on prescriptions shows that carelessness in compounding obtained in some cases and actual substitution in a few others.

3. A few of the methods of analysis used are new, some an adaptation of well-known procedures and others are the common ones in use.

BUREAU OF CHEMISTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE.

## TUBERCULINS.\*

BY L. K. DARBAKER.

Barnard and Baron claim that pronounced reactions are more easily obtained with patients in the incipient stages; moderate reactions occur with advanced lesions, and that the absence of a reaction in a clinically advanced patient heralds a speedy death.

*The Ophthalmic or Calmette Reaction.*—Calmette placed a drop of a 10 percent solution of tuberculin in the eye. This was claimed, in tuberculous patients, to be followed by a more or less severe conjunctivitis, while in the non-tuberculous patients a slight reddening only occurred. The reaction appears in three to ten hours and is highest in six to twelve hours, disappearing in twenty-four to

\* Concluded from p. 627, July issue.

thirty-six hours. A secondary test is sometimes made, when the first test is negative, by placing the tuberculin in the uninoculated eye. This test at best was dangerous and was nearly always followed by serious after-effects. It is now seldom used. Patients in advanced stages of the disease frequently did not give the reaction.

*Percutaneous or Moro Test.*—A piece of ointment about the size of a pea, composed of Koch's "Old Tuberculin" 5 mils and lanolin 5 grammes, is rubbed over an area of about 8 mm. for one-half minute, permitting the ointment to remain on the skin and be absorbed. When the skin in twenty-four to forty-eight hours shows no reaction and no itching, the reaction is said to be "negative;" if within two to ten hours distinct red papules of 1 to 2 mm. in diameter appear, disappearing in a few days, accompanied with itching, the reaction is said to be "Weak Reaction," and if within the first twenty-four hours, 100 or more papules of about 3 mm. in size appear, the surrounding skin becoming red, accompanied with slight itching and lasting unchanged for several days, then gradually fading out, the reaction is said to be "Moderately Strong." If within a few hours after applying the ointment, 100 or more papules of 5 to 8 mm. in size appear on an inflamed background, some papules erupting, and the papules not being confined to the point of inoculation but extending to the immediate surrounding area, accompanied by itching, and in a few days the papules drying up and becoming scaly, all disappearing in two weeks except a brownish pigmentation, the reaction is said to be "Strongly Positive" or "Strong Positive."

*The Subcutaneous Test.*—"Old Tuberculin" is injected in doses of from 1 to 10 milligrammes. Koch stated that 10 milligrammes could be administered to a healthy patient without producing characteristic reaction symptoms, and used three doses of 1, 5, and 10 milligrammes, injected every three days. A positive reaction gives rise of temperature, pain in head and back, heavy feeling in limbs and general lassitude. The temperature rise is usually in 3 to 16 hours but sometimes only after several days, and returns to normal in 24 to 48 hours. This reaction in many cases is followed by sloughing at the injection site.

*Intracutaneous Test.*—Consists of injecting "Old Tuberculin" intradermally, which is followed by a nodular area of inflammation in positive cases.

All tuberculin tests exhibit the following reactions to some extent:

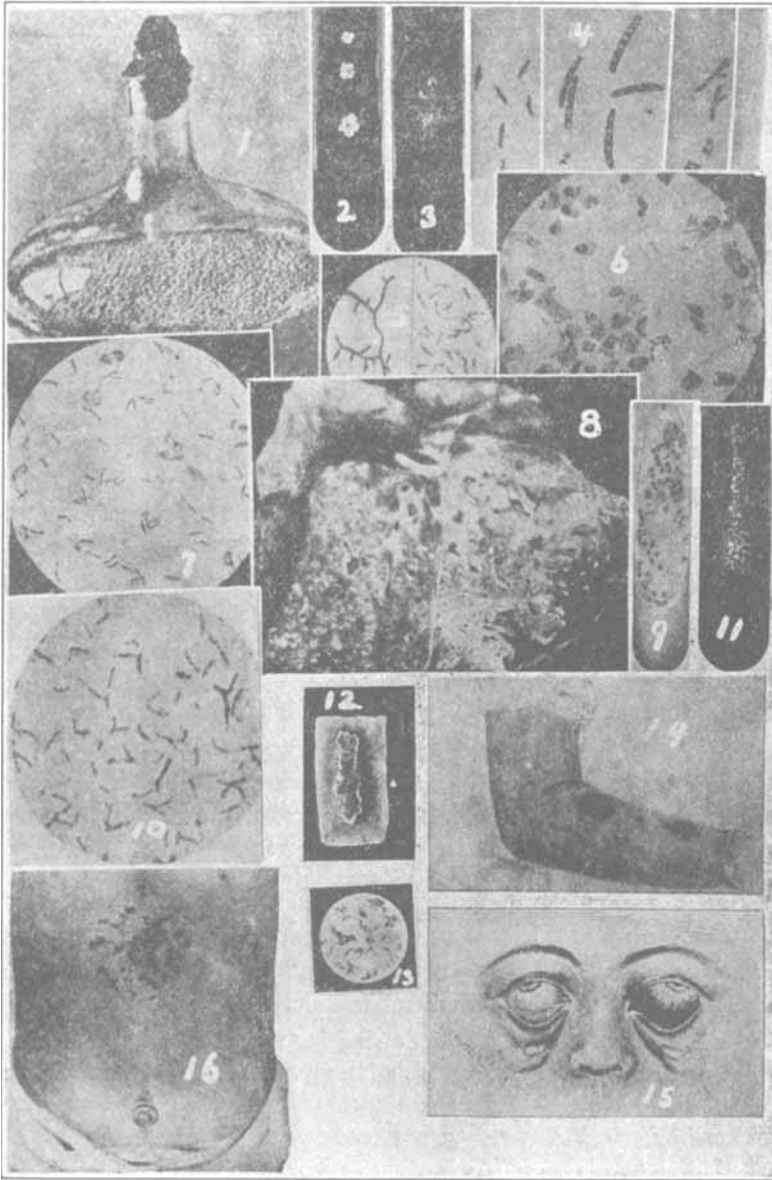
First, general reaction.—Elevation of temperature, headache, malaise.

Second, local reaction.—Increasing local symptoms such as increasing the amount of sputum.

Third, stick reactions.—Inflammation at injection site.

Many tests are better, safer, and convey no danger to the patient, as the Wasserman Modified Tuberculosis Test, Abderhalden Serum Test, and the testing on animals. Dr. Leteve states "All tuberculin tests are dangerous to use on man, although valuable for cattle."

*Tuberculin Therapy.*—Tuberculin is not suited for patients in a febrile condition, or in whom destruction of tissue is advanced. The curative value of tuberculins depends on the local inflammatory reactions which they cause around the tuberculous foci. Tuberculins, if used, must be administered in such very small doses as to cause the smallest local reactions possible. The treatment begins



1. Broth Culture of the Human Type. Copied from McFarland's Book on Pathogenic Bacteria. 2-3. Human Type Organism. Copied from Saunder's Medical Hand Atlas. 2. 14 days' growth. 3. 40 days' growth. 4. Various Morphological Forms of the Human Type. Copied from McFarland's Pathogenic Bacteria. 5. Copied from Saunder's Medical Hand Atlas. 6. Tubercle Organisms in Urine. From a Tuberculous Bladder. Copied from Klopstock and Kowarsky. 7. Tubercle Organisms in Sputum. 8. Tuberculous Kidney, showing Tubercles. The dark spots indicate the tubercles which have been torn down and excreted. Copied from McFarland's Pathogenic Bacteria. 9-10. Avian Tuberculosis Organism. Copied from More's Principles of Bacteriology. 11-12. Piscicocolla Type Organism. Copied from Saunder's Medical Hand Atlas. 11. 30 days' growth. 12. 14 days' growth on potato. 13. The Piscicocolla or Fish Type Organism. 14. Von Pirquet Reaction. The middle scarification is the control. Copied from a set of lantern slides on Antitoxins and Vaccination, donated to the writer by Alexander & Company. 15. Calmette Ophthalmic Reaction. Copied from a set of lantern slides on Antitoxins and Vaccinations donated the writer by Alexander & Co. 16. Percutaneous or Moro Test. Copied from a set of lantern slides on Antitoxins and Vaccines, donated the writer by Alexander & Co.

with "B. E.," "B. F." or "T. R.," for pulmonary cases, a dose of 0.000,000,01 (1/100,000,000) Mg.; for glandular or bone infection, a dose of 0.000,000,1 Mg. is given, these doses being gradually increased every week or two for a long period of time, extending over several years.

Much smaller doses must be given in febrile cases. The best results seem to be obtained by giving very small doses at long intervals, and the cure is said to be recognized when the patient has permanently lost the power to react to tuberculin.

Probably the therapeutic value of tuberculins depends upon the formation of antibodies following the injections. These antibodies are produced throughout the whole animal body, while the antibodies produced by the disease are produced only by the tissues directly involved.

The opsonic index has been used as a guide to the dosage, but it is a question as to whether the condition of the patient is a guide to the amount of the injections.

Sahli states "Tuberculin treatment is not an active immunization, pushed to its farthest limits, but rather the essence of the treatment is to stimulate the natural functions of the body."

Voorsanger, in an article in the *Interstate Medical Journal*, sums up the tuberculin question as follows:

"The periods of tuberculin are: First, the first users of tuberculins gave the preparations in excessive doses. This was followed by disastrous results. Second, tuberculin then was generally discarded. Third, he hopes that through careful animal experimentation a true specific for tuberculosis may be found."

Tuberculin over three months' old must not be used. The greater the dilutions of tuberculins, the sooner they become useless.

"We must remember that tuberculins are toxins and not antitoxins, that any product of the tubercle organism, either in whole or in part, is a toxin, and that when administering a toxin into the human body, we wish to obtain an overproduction of antibodies, so as to raise the natural defence already in the body, to stimulate them and to gain as great a production as possible.

"The use of tuberculins made from the bovine type for the treatment of tuberculosis caused by the human type and the use of tuberculins made from the human type organisms when the disease is caused by the bovine type is common. The bovine type organism produces a greater number of antibodies.

"We do not establish an immunity to tuberculosis, but to tuberculins. We are still experimenting; we are not dealing with a specific; we are striving for the extermination of one of the world's greatest scourges. It matters little how this is accomplished. Up to the present day, tuberculins will not cure alone, but with proper care and surroundings, tuberculins may aid and hasten some cures.

#### SUMMARY.

Cultures of the tubercle organisms are grown in a peptone beef-broth, containing 5 percent glycerin, for 4 weeks, killed by heat, and yield:

I. Bazillen Emulsion, or "B. E." Preserved with 20 percent glycerin. The Bazillen Emulsion upon filtration yields:

II. The filtrate which contains all the broth-soluble constituents.

A. "T. O." or Koch's Original Tuberculin. Prepared by grinding the cultures in a mortar, washing, centrifuging and filtering. Five percent glycerin is added as a preservative.

B. "B. T." or Koch's Old Tuberculin. Prepared by evaporating the cultures, which have been killed by heating at 100° C. for one hour, to 1/10 of the original volume in vacuum, filtering, and preserving the filtrate by adding 50 percent glycerin.

C. "B. F." or Denny's Original Filtrate or Broth Filtrate. Prepared by filtering the cultures through paper and then through porcelain, and preserving the filtrate with 0.4 percent tricresol.

III. The precipitate or residue remaining after filtration, and containing the bacterial organisms:

A. "T. P." or Tuberculin precipitate. The residue remaining after filtration, standardized so that 1 mil contains 5 Mg. solids.

B. "T. P. P." or Purified Tuberculin Precipitate. The residue washed and precipitated with alcohol.

C. "T. R." or Koch's Tuberculin Residuum. Prepared by grinding the dried residue in a ball mill until by microscopical examination shows no whole organisms. The process requires from two to four months. Twenty percent glycerin is added as a preservative. It is then standardized so that 1 mil contains 1 Mg. of solids.

D. "T. C." or Bovine Tuberculin. The residue remaining after the filtration of cultures of the bovine organisms.

The "T. O." is used in the following tuberculin tests:

1. Subcutaneous Test. 0.1 to 10 Mg. injected under the skin.
2. Intracutaneous Test.
3. Cutaneous or Von Pirquet Test. By scarification.
4. Ophthalmic or Calmette's Test. Dropped in eye, 10 percent solutions. ("T. P. P." is also used in this test.)

Also used in suppositories; 1 Mg. or more in each.

The "T. R." is used in the Percutaneous or Moro Ointment Test. Using "T. R." or "B. E." with 5 grammes of lanolin.

All the tuberculin preparations must be tested physiologically and also for sterility.

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