It should be noted that in all cases the excessive variations are represented by deficiencies, for example, —92, —82, —72%, etc.

Comments.—The directions should be followed to the letter and the determination should be carried out as rapidly as is consistent with careful manipulation. On no consideration should the morphine be allowed to remain in the alkaline salt solution for any considerable time, over $^1/_4$ to $^1/_2$ hour, as loss of morphine due to oxidation occurs.

It has been found to be imperative to keep the proper ratio of NH_4OH to ammonium salts in the solution to be extracted, hence the specific directions in regard to the addition of acid and ammonia.

It is also imperative in the final shake-out with chloroform to shake immediately after making alkaline, for while freshly precipitated morphine is readily extracted by the solvent used, if allowed to stand it becomes crystalline and its extraction becomes very difficult.

The procedure for estimating the morphine is somewhat involved on account of the small amount and the other ingredients contained therein. Considerable practice is also required to obtained accurate results. Experienced workers, however, obtain fairly concordant results.

The method for estimating alcohol differs in several particulars from the procedure given by Thorpe and Holmes,[15] or as modified by E. Richter[16] or A. Reuss.[17] Experience shows that in many cases a preliminary distillation of the original material before salting out and extracting with petroleum ether facilitates shaking-out and distillation and gives better results.

(*To be continued.*)

TUBERCULINS.*

BY L. K. DARBAKER.

"Tuberculosis was, without doubt, recognized and described by the early writers of medicine, but at that time it was known only under the general name 'consumption'—a name that to the present day is still in common use. This name was given the disease from the fact that patients dying from it have certain symptoms, such as loss of weight, good appetite, morning cough, night-sweats, and, although in apparent good health, going down slowly and easily to certain death—each sufferer always hopeful to the latest minute of life. Upon opening the bodies of these patients nodules or tubercles were found in the various affected parts; hence this name.

"Tuberculosis is a simple infection, caused by the *Tubercle streptothrix*, and is rarely fatal. The body, in protecting itself, forms a wall around the invading organism; hence the tubercle."

"Consumption is a complex infection in which the various streptococci and staphylococci are associated with the tubercle organism. The streptococci and

[15] *J. Chem. Soc. Trs.*, 83, Pt. I, 314, 1903.

[16] *Pharm. Ztg.*, 59, 430, 1914.

[17] *Pharm. Zentrh.*, 56, 61, 1915.

* Read before Pittsburgh Branch, A. Ph. A.

staphylococci attack and tear down by liquefaction the walled-up tubercles, and this is the material coughed up by the pulmonary consumptive. It is a consuming disease which, unless properly treated in time, always causes death."

In 1843 Klemke and in 1865 Villemain demonstrated the infectiousness of tuberculosis by animal experimentation, these results being later substantiated by Klebs, Chauveau, Baumgarten and Cohnheim. Baumgarten saw the first tubercle organism in sections of tubercular tissue from which the tissue had been dissolved by potassium hydroxide. About the same time, in 1882, Koch, by a special staining method, demonstrated the presence of the tubercle organism in all tubercular lesions, and confirmed the previous experiments in infecting animals. Later he obtained the tubercle organism in pure cultures.

The tubercle organism is an obligate aerobe, in the form of a non-flagellated rod, often slightly curved and exhibiting a number of spherical, oval or elongated clear spaces, which at one time were thought to be spores, but it has since been proven that spore reproduction of this organism is unknown. Many theories have been advanced as to the cause of these spaces, among which are the following: Vacuoles, or a form of degeneration, and a reserve form of nutritious material. Doctor Leteve, an authority on this subject, states, "When the organism shows the spaces there are two theories, first, the organism is sick, or is attacked; this is why we find this condition in the organism in old cultures and in sputum; second, the organism is in full health and grows more rapidly. Personally, I believe it is caused by the breaking down of the organism."

The organism shows many morphological variations under different conditions, and is classed as a streptothrix, although some still place it in the true bacteria class of the schizomycetes, and others, as being between the streptothrix—a *hypomycetes*—and true bacteria. However, it is evident that the organism is of a higher form than that of true bacteria.

The organism contains about 90 percent water, $2^1/_2$ percent fatty, wax-like coating and $7^1/_2$ percent protoplasm and cellulose. It is on account of the resistance of this fatty, wax-like coating on the outer wall to the ordinary stains that a special stain containing a mordant, as phenol, must be used, and if the staining process is not hastened by the aid of heat, it requires ten hours or longer for the penetration of the stain into the organism. But after the organism is once stained it resists giving off the stain to such an extent that it will not be decolorized with a 3 percent HCl in alcohol, or a 15 percent H_2SO_4 solution. Organisms having this property are called "acid fast" and include the tubercle organism, *bacillus leprae, smegma bacillus*, and others obtained from butter, hay and dung. About forty varieties belonging to this group have been described.

The tubercle organism grows in media containing 2 to 6 percent glycerin at a temperature of 36 to 40° C. The best medium consists of glycerin 5 percent, sodium chloride 5 percent, peptone 2 percent, guinea pig meat broth 1000 mils. The growth appears as a creeping, dry, whitish membrane, and when grown in a liquid medium for some time the whitish membrane sinks to the bottom.

The tubercle organism is resistant to desiccation and in a dry state will live for about three months, but if exposed to sunlight it will be destroyed in a few hours, and in diffused daylight in five to seven days. It is also destroyed in 24 hours in a mixture of equal parts of sputum and 5 percent phenol, or by one hour's exposure to Röntgen rays. The organism is destroyed by moist heat at 55° C. in five hours, at 60° C. in one hour and at 95° C. in ten minutes, but if the organism is imbedded in sputum, it requires at least five minutes' boiling before destruction occurs. In some cases when the organism is grown on potato, putrid sputum, etc., or passed through a number of cultures, its virulence is decreased; in other cases the virulence is not altered.

Koch claimed that the bovine type organism could not infect man, and this was the general belief previous to 1901, when Smith and others conclusively proved that, although the tuberculosis of cattle and that of man were caused by different organisms, yet the type of one could and would infect the other. It is now known that practically all the human tuberculosis of the abdominal cavity, intestines, bones, skin, and glands is an infection caused by the bovine type organism.

The pulmonary type of infection is the most common. It is caused by the human type organism and is acquired by the inhalation of the organisms in dried sputum. Tuberculosis is not inherited, but as the babe is in constant association with the tuberculous mother, who will throw out in one day as many as 7,200,000,000 organisms, the organisms being thrown by the coughing five or more feet, the babe having weak lungs readily acquires the disease, the weakened condition of the lungs furnishing a fertile field for the organism growth.

The most susceptible age is from one to three years. From the third to the fifteenth year of a child's life the greater part of the day is spent in the open air, but after the fifteenth year it is not in the open air so much and from that age to middle life the disease shows a great increase in the number of cases.

A successful recovery from the disease produces some immunity but not sufficient to prevent a second infection.

In practically all autopsies the tubercles or healed lesions are found, showing that all or nearly all persons have at some time had a tuberculosis infection.

Smith, in the *Journal of the American Medical Association,* January 8, 1916, states, "10 percent of all deaths in children under the age of 15 years are due to tuberculosis. Since 8 percent of the deaths are caused by the bovine type, acquired by drinking milk obtained from tubercular cows, all milk for human consumption should be pasteurized." The tubercle organism may lay dormant and harmless in the body tissues for an indefinite period. Von Behring believed that nearly all cases of tuberculosis had their start in infancy.

Ten years ago the field was almost barren of organizations fighting the great white plague, but to-day there are in the United States 12,000 local tuberculosis organizations, 575 hospitals and sanatoriums, 540 special dispensaries, 1000 dispensary physicians, 4000 nurses, 400 open air schools. Over $100,000,000 has been spent in fighting the disease.

All animals are prone to tuberculosis. The hog, goat, lion and tiger in their natural elements are the most resistant.

There are many types of organisms causing the disease:

Human type, previously described.

Bovine type, infecting cattle and man. This type differs from the human type by usually being of the pulmonary nodular type, although it may become general (miliary). The nodules grow to a large size and the organisms show signs of branching.

Avian type, common to birds and chickens, appearing as yellowish white nodules in the intestines and liver. The nodules vary in size from that of a pea to the size of a walnut. The growth is moist and mucous-like, while that of the human type is brittle, warty and dry. The organism is club shaped and more easily cultivated than that of the human type; it also grows at a higher temperature, 40 to 50° C. This type is less pathogenic to guinea pigs but more so for rabbits.

Piscum type, the organism causing tuberculosis in fish.

Pseudo types of many varieties have been found in various animals, as mice and sheep, but time does not permit going into details.

Killed cultures of the human organism, when given subcutaneously, may produce necrosis, abscesses and a subnormal temperature. When given to small animals intravenously, it causes a rapid emaciation and death in from a few days to a few weeks. Guinea pigs and rabbits are very susceptible. By beginning with very small doses, the animal may become habituated to the toxin intoxication and eventually withstand large doses. This is also true of the various toxic substances as tuberculins.

The protein and alkaline extracts of the organism cause abscesses when given subcutaneously. The toxin is a complex substance, containing among other things a fever-producing body, also found in the tuberculins, which are toxic substances produced by the organisms. This fever-producing body is the cause of the rise in temperature when tuberculins are injected. It is 100 times as toxic for tubercular animals as for the healthy. There is also present a temperature-reducing body, called "toxalbumin," which is destroyed by heat at 100° C. Another body has been isolated which will cause fatal convulsions when injected into animals. These bodies show their greatest effect when injected into the brain, and this should be the method of standardization of tuberculins.

In 1881, Koch announced an agent to be used for the specific diagnosis of tuberculosis, giving this substance the name of "Tuberculin." This preparation consisted of cultures of the human type organism, grown in a peptone broth containing 5 percent glycerin, for four weeks. At the end of four weeks' growth the cultures were killed by heating at 100° C. for one hour, evaporating this killed fluid culture to 1/10 its original volume in vacuum at a low temperature, removing the bacterial cells by filtration and adding 50 percent glycerin as a preservative. This preparation is known as "Old Tuberculin," or "O. T."

The "Original Tuberculin" differs from the "Old Tuberculin" by not being heated and evaporated and by having 0.5 percent phenol added as a preservative. The active toxic substances may be precipitated out by the addition of 66 percent alcohol.

The next advance in tuberculins was the "T. A." or "Alkaline Tuberculin," which was made by extracting cultures with 10 percent sodium hydroxide solution. Its diagnostic value was said to exceed that of the former tuberculins on account of the longer duration of the reaction. However, in view of its containing undissolved cells, which caused the formation of abscesses at the site of injection, it rapidly fell into disuse.

For the purpose of immunization, Koch, in 1897, prepared a fluid, which contained all the organism constituents and at the same time was readily absorbed with but little abscess formation. Dried masses of the organism, filtered from the cultures, were ground in an agate mortar and after being suspended in distilled water, were centrifuged. The emulsion consisted of two layers; the upper opalescent, whitish liquid was designated as "Tuberculin Obers" or "T. O." After removing this fluid, which contained the water-soluble constituents, the residue containing the water-insoluble constituents was dried, ground in mortars, suspended in water and centrifuged; this process being repeated until no sediment remained. It was then preserved with 20 percent glycerin. This preparation was called "Tuberculin Rest" or "Tuberculin Residue" or "T. R." and consisted of an emulsion containing the minute fragments of the organism cells, which were readily absorbed and caused few abscesses. It has also been given the name "Koch's New Tuberculin."

Koch later devised another preparation for the active immunization, and also for performing the agglutination tests. This preparation consisted of the dried and ground organisms emulsified with 20 percent glycerin and water, and was designated as "neutuberculin" or "Bazillen Emulsion" or "B. E."

A short time later Denny's "Bouillon Filtrate" or "B. F." was produced. This was composed of the unheated filtrate from a broth culture, filtered first through paper and then through porcelain, and contained all the soluble products of the organism as it grows in a broth medium.

Many analogous preparations have been made by the various investigators, among which the most important are: "Tuberculocidin" of Klebs; Tuberculins of Schweinitz and Dorset; those of the Dennys; "Tubercle Toxins" of Maragliano, in which the antitoxic sera were used; the "Oxytuberculin" of Herschfelder; the "T. D." and the "T. D. R." of von Behring; "Tuberculoplasm" of Büchner, and many others.

The true toxin of the organism has been claimed to have been obtained by Marmoreth, by growing the young virulent cultures on a very complicated medium, and he denies that the tuberculin represents the true organism toxin.

"Bovine Tuberculin" is manufactured from the tubercle organisms of the bovine type and is claimed by some to be of equal and even of superior value to that made from the human type, and also that it will cause less reaction.

"Sensitized Tuberculin" or "Sero-Tuberculin" consists of a Bazillen Emulsion sensitized with anti-tuberculin horse serum—that is, the virulent cultures are mixed with the immune serum obtained from the horse which has been immunized (??) against the organism. The cultures are permitted to stand in the immune serum for 24 hours, then the organisms are washed free from the serum and repeatedly centrifuged with saline solution, mixed with physiological saline solution, standardized, killed by sterilization with heat and a small amount of preservative added. It is claimed that his preparation overcomes the infiltrations at the site of injection.

Standardization.—Tuberculin can not be accurately standardized. On account of the great susceptibility of tuberculous animals to tuberculin, the standardization is estimated on the quantity required to kill such animals. From 0.5 to 1 mil of tuberculin when injected into a healthy guinea pig will cause no local or general reaction, but if 0.1 to 0.15 mil be injected into a tubercular pig, death results in from 24 to 48 hours. Lingelsheim recommends intracerebral injections into healthy pigs. On account of the extreme toxicity of tuberculin when injected into the central nervous system, this method requires only 1/180 as much tuberculin to cause death as the subcutaneous or intraperitoneal injections.

Behring bases the value of tuberculins on their toxicity for healthy pigs and expressed 1 mil (1000 M.) or 1 gramme of the toxin as fatal for each 1000 grammes of pig tissue; his "T. D." having a value of 1,250 M. and the" T. D. R." of 12,500 M.

The most reliable method is that of the Frankfort Royal Institute of Experimental Therapy, which is: Two series of pigs are injected with pure cultures of the tubercle organism and then are injected with decreasing doses of tuberculin. In one series a standard preparation of tuberculin is used, in the other series the tuberculin to be tested is injected. If the minimum fatal dose of the sample is the same as the standard, it is of official strength, but if stronger, dilutions are made, if weaker it is concentrated by evaporation.

Koch's "T. R." represents 2 milligrammes of solids to the mil; the "B. E." 5 milligrammes of solids to the mil. Many American manufacturers use a preparation of which one mil represents one milligramme.

All tuberculin injections are dangerous and the writer has heard several physicians state that "undoubtedly they had helped many patients to the great beyond, by the administration of tuberculins."

For diagnostic purposes the following technic is pursued: First, being assured that the patient has no continuous fever by noting the temperature every two hours for several days, 1 milligramme of tuberculin is injected subcutaneously; for very young or very weak patients only 0.05 to 0.1 milligramme is used. Many authorities never use more than 0.1 milligramme in any case as the initial dose. If no rise in temperature is produced by this amount, in the course of a few days a second injection of a slightly larger amount is given; usually an interval of three days elapsing between doses. Koch used as much as 10 milligrammes before concluding that the reaction was negative. Lowenstein recommends that the cumulative action of three to four very small doses at intervals of three days is of more value, and this view is now generally accepted.

Many theories have been advanced as to the cause of the reactions but as yet none have been accepted. In view of Naegelis finding that 97 percent of autopsies yield tuberculosis lesions, active or healed, the value of tuberculin reactions would seem to be relative only. It is thought by some that tuberculin produces an inflammatory reaction around the tuberculous areas, which may cause the dissemination of the organisms, but Tradeau, Baldwin and Kinghorn in animal experimentation found such was not the case.

The "Cutaneous or von Pirquet Reaction" depends on the increased capacity of the skin in tuberculous patients to react to tuberculin. The ventral surface of the forearm is cleansed with ether or soap and water, two drops of tuberculin are placed on the cleansed area at points about 10 cm. apart. The skin beneath the tuberculin is then scarified as for ordinary smallpox vaccination and several strands of cotton are placed across this area to prevent the spreading of the tuberculin. A third scarification is made about equi-distant from the others but no tuberculin used; this is for the control.

The Traumatic reaction occurs in a few minutes. All the points of scarification show a small papule surrounded by a soft red areole, which disappears in a few hours. A slightly raised red spot about the size of a pin head remains; this becomes covered with a crust, which is succeeded by a pigmentation and a gradual return to normal in a week or so. Negative reactions show the same conditions as the control site; the swelling lasting about twenty-four hours only and the areola being less than 5 mm. in size. The positive reaction has an incubation period lasting from three to twenty-four hours, usually less than twenty-four hours. Those developing later than twenty-four hours, von Pirquet calls "Torpid Reactions." Such reactions occur more frequently in children and clinically unsuspected cases.

The development of the inflamatory reaction begins usually with a slightly raised areola, reddening, spreading from the point of scarification and increasing rapidly in diameter and height. The papule diameter varies, averaging 1 Cm. Small vesicles sometimes form on the papule. The color differs from the normal skin by being a deep red. Very pale papules may develop in cases of fatal tuberculosis, and when this occurs the reaction is designated as "Cachectic Reaction."

The maximum development is reached usually in forty-eight hours after which time the swelling is reduced, the red color changing to violet, then to a yellow and finally becoming brown. The swelling appears in five to eight days and the pigmentation in a few weeks. Observations are best made forty-eight hours after vaccination. Usually in eight to fourteen hours a progressive rise in temperature of 2 to 5° is noted; receding after two to six hours duration.

Sometimes when a negative reaction is obtained, a second injection produces a positive reaction, and if this occurs, the site of the original injection reddens.

The reaction is very delicate and since most adults have healed tubercles, the reaction as an indicator of active lesions is of value only in very young children.[1]

(To be continued.)

PHARMACOLOGICAL EQUIVALENTS AND THERAPEUTIC UNITS, A PROPOSED REFORM IN PRESCRIBING.

The author proposes a method for prescribing, which is devised to relegate the whole of the responsibility of correct dosage to the pharmacist. It is suggested that the prescriber should not trouble about the doses of the drugs to be given. When prescribing, he should merely indicate a certain number of appropriate doses, leaving the amount to be given in each case entirely to the compounder. It is argued that the former has no time to learn and remember doses; whereas the latter has always his books at hand to which he can refer. To carry out this scheme, the agreed normal dose for twenty-four hours either in weight or volume, for an adult, of any preparation, is designated the "pharmacological equivalent," and represented by the letters, E. P. One-tenth part of this is to be known as the "therapeutic unit," and represented by the figures U. T. Under this scheme a prescription would be written thus: Pyramidon, 5 U. T.; Phenacetin, 3 U. T.; exalgin, 2 U. T. For 5 cachets to be taken in twenty-four hours. Twenty-five such to be sent. When excipients and liquid vehicles are prescribed, these are to be left entirely to the pharmacist, thus: Potassium bromide, sodium bromide, ammonium bromide, of each, 4 U. T.; distilled water, syrup of orange, of each, q. s. Three tablespoonfuls a day. Send sufficient for ten days.—Dr. Y. Delarge (*L'Union Pharm.*, 1917, 58, 113; through *Pharmaceutical Journal*).

[1] Ricket's "Infection and Immunity."