three or four times the volume of the former. This will remove the remainder of the sempervirine as the hydrochloride, together with some other alkaloidal material, also any remaining gelsemic acid. Distil off the greater part of the $CHCl_3$ and shake the residue with several portions of distilled water. This will transfer the hydrochlorides of the alkaloids to the aqueous liquid. Mix the combined aqueous extracts with some sand and evaporate at a low temperature. Extract the residue with acetone. Sempervirine hydrochloride is left behind with the sand from which it may be extracted with alcohol.

The acetone solution may be further treated as above.

D.—The acid liquid which has been extracted with benzol and $CHCl_3$ is made just neutral with NaOH and evaporated to dryness at a low temperature. The residue is treated with alcohol in small amounts which leaves behind NaCl and gelsemine hydrochloride. In solution we have a small amount of gelsemine hydrochloride together with gelseminine hydrochloride and any other alcohol soluble hydrochlorides. This alcoholic solution is evaporated to a small volume and allowed to stand for some time when the major portion of the gelsemine hydrochloride will separate out. Filter, mix the filtrate with sand and evaporate at a low temperature. Extract the residue with acetone. The so-called gelseminine hydrochloride goes into solution while a small amount of alkaloidal material remains in the sand from which it may be extracted with alcohol.

Physiological Test: Thus far no complete pharmacological data has been obtained concerning this alkaloid. It may be stated, however, that 1 cc. of the solution of the hydrochloride containing 0.001 gm. of the salt killed a guinea pig weighing 90 gms. in 48 hours. A mouse weighing about 20 gms. with the same dose died in the same time, the salt having no *immediate* toxic effect.

NOTE: For the crude material containing the mixed alkaloids we are indebted to Messrs. Eli Lilly and Company and for crude and authentic material in working up former papers we are indebted to J. U. Lloyd, Parke, Davis and Company and H. K. Mulford Company. For their generous supply of material we desire to express our thanks.

Toxicity of Sempervirine: A report from the pharmacological laboratory of Parke, Davis & Co., gives the toxicity of Sempervirine as Minimum Lethal Dose = 0.00014 gm. per gram weight of frog. For this report the writer is indebted to Dr. E. M. Houghton.

LEUCOCYTIC EXTRACT—ITS PREPARATION AND USES.*

ARTHUR R. MEINHARD.

It is now a well established fact that one of the chief forms of protection of the animal body to bacterial invasion is through the action of certain of the white blood cells or the so-called phagocytic cells. These cells, which are easily able to wander through the walls of the blood vessels, are drawn by positive chemotactic action to the infected area and act by ingesting the trouble causing bacteria, destroying them and neutralizing their poisons. It is the purpose of this paper to dwell especially upon the nature of these poison neutralizing sub-

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stances in the leucocytes, methods whereby they can be obtained from the lower animals for use in human infections, and their use in medicine.

Bacteria may be divided into two general classes, according to their poison producing powers; first, those which secrete soluble toxins of which the tetanus and diphtheria bacilli are examples, and second, those that do not secrete soluble toxins in which the poison or endotoxin is more or less fixed to the bacterial body. Examples of these are the pneumococcus, meningococcus, and typhoid bacillus.

Curative sera are easily obtained for the first of these two classes of bacteria by the injection into animals of graduated amounts of toxin which results in the formation in the blood of the immunized animal of an anti-toxin. In this way are procured tetanus and diphtheria anti-toxin.

The production of an anti-toxin for this second group of bacteria has not met with any success. The injection of these endo-toxins into an animal results not in the production of an anti-toxin but in the production of bactericidal and bacteriolytic substances.

Immune sera have been prepared for the treatment of streptococcus and pneumococcus infections, but the action of these sera is probably due to the bactericidal powers of the serum and to an increase of immue bodies, the so-called opsonins of Wright or the bacteriotropins which may or may not be the same immune body. These bodies attach themselves to the invading bacteria and render them more susceptible to the action of the leucocytes, and not to anti-toxins in the serum. The use of these sera has not met with as great success as has the use of anti-toxins.

As mentioned above, one method of action of the leucocytes on the bacterial body is through the preparation of the bacteria by the opsonins for ingestion by the leucocytes. The amount of this immune body in the serum controls the extent to which the bacteria are taken up by the leucocytes, destroyed, and their contained poisons neutralized by the ferment termed by Petterson[1] endolysin.

Hiss[2] however speaks of another form of leucocytic action upon bacteria which is independent of the amount of opsonin in the serum. There is a gradual depression of the phagocytic atcion of the leucocytes up to the height of the infection, and then an increase in action as the infection terminates. It was with this theory in view that Hiss[3] made the following statement: "In many diseases we are dealing probably with an immunity a large part of whose mechanism is individually cellular, not only in the sense of phagocystosis and digestion but in the neutralization of poisons given rise to by the disintegration of the bacteria, a mechanism in which the protecting cells must intervene and unaided by bodies in the plasma, neutralize within themselves the poisonous products of the invading microörganisms."

With this hypothesis in view Hiss[4] and later Hiss and Zinsser[5] undertook a series of experiments to see if they could obtain suitable extracts of leucocytes containing these neutralizing bodies in solution for the treatment of various infections, and to study the action of these leucocytic extracts upon different bacteria. Theoretically, such extracts if containing this poison neutralizing substace or endolysin in solution, should when injected into the bodies of human beings or other animals rapidly diffuse through the blood stream and neutralize the toxins or other poisonous substances, in this way com-

ing to the aid of and relieving the leucocytes for the time being and allowing them to form new endolysins within themselves.

The method finally used by Hiss after much experiment for obtaining leucocytic extracts was as follows and is much the same method as is in use today. Rabbits of about 2000 grammes weight were tied down, the thoracic region shaved and sterilized and ten cubic centimeters of aleuronat mixture injected into each pleural cavity, between the intercostal spaces, care being taken to avoid puncturing the lungs. The aleuronat mixture was prepared by dissolving 3% starch and 5% aleuronat in cold meat infusion broth then boiling over a free flame for five minutes and finally filling into large test tubes and sterilizing in an autoclave. At the end of 18 to 24 hours the injected animals were killed, the pleural cavities opened, and the contained cloudy fluid consisting of serum, leucocytes and some erythrocytes, put into sterile centrifuge tubes and centrifuged at high speed until the supernatant serum was clear and then this serum was poured off. The leucocytes were then extracted in distilled water in an incubator for six or eight hours, then the extract examined bacteriologically for sterility and if sterile was used.

The first experiments of Hiss were on rabbits injected with large doses of staphylococcus. That the strain used was virulent was shown by the death in every case of the animals used as controls, while the other animals which were given small doses of leucocytic extract at intervals of one to five hours after the injetcion of the bacteria either completely recovered or lived for much longer period of time than did the control animals.

Later, further experiments were carried on by Hiss and Zinsser[6] on rabbits, using pneumococcus, typhoid bacilli, and meningococcus. Here the results were about the same as mentioned above, and further, some of the animals recovered when leucocytic extract was not given until 24 hours after a dose of bacteria had been given, large enough to kill the control animals. In these experiments was shown beautifully the resulting drop in temperature after an injection of leucocytic extract showing a neutralization of the poisons.

In view of these successful experiments it was thought advisable to try leucocytic extract upon human beings. It was first tried on 24 people suffering from meningococcus infection. Most of these cases were of the severe type or came under treatment late in the disease when the infection had brought about a state of grave toxæmia, and great emaciation. Two of these cases left the hospital before treatment was fully established, fourteen were discharged as cured and eight died, which calculated in percentages gives 63.6% cured and 36.4% fatal. The injections of leucocytic extract resulted in an improvement of the symptoms depending upon the central nervous system, and vomiting, delirium, stupor and hyperæsthesia were either greatly diminished or disappeared after one or two injections of from five to twenty-five cc. of the extract. There was also a marked reduction in the temperatures.

Seven cases of lobar pneumonia were also treated with the extract. Here there was a recovery of 100% of the cases treated. Here also there was the characteristic drop in temperature, and a change for the better in the subjective symptoms.

Later eleven cases of staphylococcus infection were treated by the same authors[7] with splendid results. Eight of these were cases of chronic furun-

culosis which had lasted in spite of surgical and dietetic treatment for periods of from a few months to some years. In all but one of these cases there was a complete cure made, while in three acute cases treated the same results followed.

Floyd and Lucas[8] reported the use of leucocytic extract in forty cases of pneumonia with a mortality of 12% while in a series of twenty-five cases not treated with leucocytic extract the mortality was more than doubled.

Lambert[9] treated a number of cases, most of them being erysipelas, with results which in his opinion warranted further trials.

Meinhard[10] in experiments upon thirty-nine rabbits and using four times the dose of pneumococcus necessary to kill these animals showed a mortality for the control animals not treated with leucocytic extract of nearly 100%, with recovery of almost 100% for treated animals, although some of these animals did not receive the extract until nearly forty-eight hours after the initial injection of pneumococcus. In these experiments was noted the typical fall of temperature and increase of appetite following the injections of the extract. A small amount of preservative was used for the first time in these experiments and shown to be of no harm to the potency of the extract. A further improvement was also made in the extract by adding a strong solution of sodium chloride to the extracted leucocytes, enough of this salt solution being added to make the extract of physiological salt solution strength. This was done as it had been shown that when the watery solution of the extract was injected it was very painful, while injections of physiological salt solution were not.

Reynolds[11] reporting a series of nine cases of pneumonia with 100% recoveries remarks: "A complete description of these cases represents a severe form of pneumonia running a full course with moderate temperature curve, scarcely noticeable delirium, comparative freedom from toxic effects on the kidneys, and terminating by crisis at the usual time."

Besides these cases reported leucocytic extract has been used with great success on the Pacific Coast for the treatment of lobar pneumonia but as yet many of these cases have not been reported.

Reviewing these cases it is easy to see that leucocytic extract is of use in neutralizing bacterial poisons in the animal body as evidenced by the fall in temperature, general lessening of toxic symptoms and decrease of central nervous system symptoms. It also in neutralizing these poisons gives the leucocytes a chance to recuperate and form new endolysins.

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