type. Partially immunized animals killed by an overdose of organisms have been found to exhibit intense hyperemia of the peritoneum and abdominal wall, with serosanguinous fluid in the abdominal cavity.

Normal sera from horses, sheep, rabbits and chickens have been titrated against pneumococcus toxin and have ordinarily been found to exhibit very little power, either to prevent cutaneous reactions in rabbits or lung changes in mice. The same is true of regular antipneumococcus sera or antibody solution, and such immune sera as antistreptococcus serum, scarlet fever antitoxin and diphtheria antitoxin.

Further work is in progress. A detailed account of some of our experiments will be published soon.

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On a specific pneumococcus antitoxin.

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Specific pneumococcus antitoxins have been produced in this laboratory by various procedures, including those of Dochez, Larson, and the procedure developed in this laboratory by one of the authors (Olson), using a specific pneumococcus toxin.

The Larson procedure, which consists of injecting whole culture, appropriately attenuated, by means of a highly purified castor oil soap, has been extensively employed by us, using principally rabbits and sheep. Recently somewhat better yields of antitoxin have been obtained by injecting subcutaneously into rabbits, sheep or horses, progressively increasing doses of the sterile pneumococcus toxin.

The same pathological lesions were observed in the larger animals after the injection of successive large doses of toxin, as had previously been noted in smaller experimental animals and reported in a previous paper.

The highest concentration of antitoxin has been secured by starting with a very small amount of toxin and gradually increasing the size of the dose.

The procedure now adopted in testing each lot of antitoxin before using it experimentally on cases of human pneumonia, are as follows:

(1) *The rabbit Skin Test.* A series of dilutions of the antitoxin are mixed in equal proportions with a given concentration of the toxin, such that 0.1 cc. of the mixture contains twenty-five skin test doses of the toxin. The solutions in question are then incubated for a period of one to two hours and a considerable series of skin tests are carried out on a series of rabbits, using 0.1 cc. doses of the solutions in question. In this manner the amount of serum required to neutralize twenty-five skin test doses of toxin is determined. The law of multiple proportions appears to hold approximately for the neutralization of double or quadruple the number of skin test doses of a given toxin by a given antitoxin. We have adopted as a provisional temporary unit the amount of antitoxin required to neutralize one million skin test doses.

(2) *The Mouse Lung Test.* A similar series of dilutions of antitoxin are mixed in equal volumes with a fixed amount of toxin, so that 0.5 cc. of the mixture will contain approximately five thousand skin test doses of toxin. The mixtures in question are incubated for a period of one and one-half to two hours, and 0.5 cc. doses injected intraperitoneally into mice, a large number of duplicate tests being put on at each concentration. The test animals in question, with a series of controls which have received the toxin antitoxin, are killed at the end of 24 hours by means of chloroform vapor, if they have not already died as a result of the the toxin without antitoxin, are killed at the end of 24 hours by means of chloroform vapor, if they have not already died as a result of the toxin injection. A gross and if necessary a histologic comparison of the lungs are made, and from a statistical consideration of the data the approximate point at which the toxin is neutralized by the antitoxin is determined.

In comparing sera of varying antitoxic concentration, it is found that the variation between the skin test results is far less pronounced than that between the mouse lung results, and from a comparison of the data in question with such clinical results as are available to date, we are inclined to believe that the lung test will ultimately afford a more accurate index of the probable therapeutic value of the preparation than does the skin test.

(3) *Pharmacological Respiratory Tests.* A series of dilu-

tions of antitoxin are mixed in equal volumes with a fixed amount of toxin and after incubation for a period of 24 hours the solutions in question are injected intravenously into dogs. The determination of the point at which the antitoxin fails to inhibit the characteristic respiratory depression exerted by the toxin may afford a rough index of antitoxin value.

(4) Dr. H. M. Powell has obtained a flacculation reaction between the toxin and antitoxin which may afford an additional means of standardization.

(5) The antitoxic sera are tested as regards their effect on the temperatures of a series of rabbits with a view to affording some possible information regarding the presence or absence of chill-producing substances.

The pneumococcus toxins and antitoxins do not appear to be type specific. After carrying out a series of preliminary experiments on rabbits, a group of 25 sheep were immunized as follows: Five were given toxin from a Type 1 organism, five from a Type 2, five from a Type 3, five from a so-called Type 4, and five from a mixture of toxin derived from all four types.

After a suitable period of immunization the animals were bled and the individual groups were tested separately against all four toxins. While the antigenic value of the toxins produced from the cultures employed varied somewhat, and there was consequently a variation in the titre of antitoxin produced, it was found that with any one antitoxin its power to neutralize a given number of skin test doses of any one of the toxins employed was approximately equal.

On account of the difficulty invariably associated with the administration of large amounts of unconcentrated serum to cases of human pneumonia, an attempt has been made to purify and concentrate the pneumococcus antitoxin in the Chemical Division of the Lilly Research Laboratories. George B. Walden and Jasper P. Scott, working with one of the authors (Clowes), have succeeded in purifying the pneumococcus antitoxin to the extent of removing up to 99.9 per cent of the associated serum proteins, without appreciable loss of antitoxin when tested by the methods outlined above.

The antitoxin may consequently be concentrated from one to two thousandfold if so desired, but in practice it appears preferable to employ a twenty to one hundredfold concentration, which in its purest form gives a water-white, limpid, non-viscous solu-

tion. This purified antitoxin may be boiled for half an hour without suffering any appreciable loss of potency.

When injected intravenously in cases of human pneumonia the highly purified solutions do not appear to cause serum sickness. The chemical constitution of this highly purified pneumococcus antitoxin is being investigated at the present time in the Lilly Research Laboratories.

Experiments on the use of both unconcentrated and concentrated pneumococcus antitoxin in cases of human pneumonia have been in progress for a considerable period of time. The results obtained to date appear to justify further clinical experiments on a larger scale.

This conclusion is also supported by the interesting observation that more recently prepared antitoxic sera derived from animals which are now attaining a higher measure of immunity, exhibit strikingly increased protective effects when tested by the mouse lung method.

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Experiments on the development of the ear of amblystoma punctatum.

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A series of experiments was performed during the years 1922-1925 on embryos of *Amblystoma punctatum.* Two main lines of investigation were carried out, namely, experiments to determine the limits of the regenerative capacity of the tissue surrounding the normal ear region, and experiments regarding the nature of the developing ear itself.

The first group involved extirpation of ectoderm in the ear region of embryos at different stages of development. The size of the pieces removed was 0.2 mm., 0.3 mm., 0.4 mm., 0.5 mm., and 0.6 mm., respectively. It was found that complete regeneration generally followed when the operation was performed on stages earlier than that in which invagination of the ectoderm had occurred. Regeneration was completely checked only by the re-