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Anisocoria after bilateral sympathicotomy.

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The fundamental mechanistic factors in the Schafer phenomenon consisted of pseudo- and true paradoxical phenomena induced in part by the sympathetic section and in part by lesion of the nociceptive paths at the first operation. The mechanistic factor in the second operation hinges upon the "shock" incidental to the general surgical procedure rather than the second sympathetic section. The Schafer phenomenon can be duplicated by operations in other parts of the body, *e. g.*, by section of one sciatic followed some days later by the section of the other sciatic. The most interesting feature of the Schafer phenomenon is the shortening of the period of incubation for true paradoxical dilatation phenomena. This is induced by the shock of the second operation which causes suspension of function in the decentralized neurones situated within, and distal to, the superior cerivcal ganglion.

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Studies on purification of antibodies. II.

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This paper is the continuation of one delivered before this Society[1] describing the metal precipitation and purification of typhoid agglutinating extract. The extract is prepared by sensitizing typhoid bacilli with antityphoid horse serum, washing the

[1] Ottenberg, R., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 14.

complex with saline to remove horse serum and subsequently dissociating agglutinin from bacteria by means of weak alkali.

In that paper it was stated that a M/200 final concentration of $CuCl_2$ was used to precipitate agglutinin from the extract. We have found that at the proper hydrogen ion concentration, much smaller amounts of copper will precipitate all the agglutinin. The limiting concentration of copper seems to vary with different extracts, and with the same extract at varying hydrogen ion concentrations. For instance, an extract agglutinating in a dilution of 1:640 was precipitated with copper chloride. When 1.0 cc. N/40 HCl was added to 10 cc. of extract before the addition of copper, the final concentration of M/4400 $CuCl_2$ was sufficient to bring down all of the agglutinin in the precipitate, while final concentrations of M/6600 and M/8800 brought down half the agglutinin and a final concentration of M/17600 did not bring down any.

When only 0.75 cc. N/40 HCl was added to 10 cc. of extract before addition of copper chloride, a final concentration of M/4400 brought down no agglutinin in the small amount of precipitate that formed and a final concentration of M/2500 of copper chloride was necessary to recover all the agglutinin.

In purifying the extracts by copper precipitation an orienting experiment is first performed. Portions of 10 cc. of extract are placed in centrifuge tubes and varying quantities of N/10 HCl added. To each tube is added 1 cc. of M/400 copper chloride solution and water to bring the total volume to 12 cc. Within certain limits of acidity a cloudy precipitate forms in the tubes. The tubes are allowed to stand in the icebox for 24 hours, when the precipitate has generally flocculated and fallen to the bottom and sloping sides of the tube. The tubes are centrifuged to pack the precipitate and the supernatant liquid is then poured off. At each end of the range of precipitation the liquid does not become entirely clear even on repeated centrifuging.

The precipitate is then dissolved in a volume of N/200 HCl equal to the original volume of extract taken. The pH of the supernatant is determined and both supernatant and dissolved precipitate examined for agglutinating power (24 hour incubation, room temperature).

It is found that the tubes at the end of the series do not have all of the agglutinin in the precipitate, but some is demonstrable in the supernatant fluid. The supernatant fluids from the tubes in

the middle of the series have some agglutinin present, generally agglutinating in a dilution of 1:40 and 1:80 and rarely as high as 1:160. The range of pH in the tubes showing precipitates is from about 4.6 to 7.2 with optimum precipitation at about 6.4 as has been found before. It is also noteworthy that in almost every instance the dissolved precipitate shows a definite increase in agglutinating value over the original extract, notwithstanding the fact that there is also some agglutinin recovered in the supernatant liquid. For example, the copper precipitate of an extract agglutinating in 1:800 dilution when dissolved in N/200 HCl was found to agglutinate at 1:900 and sometimes 1:1000, and in addition the supernatant fluid agglutinated at 1:40 or 1:80. This phenomenon may be due to either or both of two causes, (1) the removal of extraneous bacterial matter which interferes with agglutination by combining with agglutinin, or (2) the influence of copper on agglutination (note—copper chloride alone in M/500 solution does not agglutinate). We have excluded the possibility of acid agglutination by hydrogen ion determination of the test mixtures; the buffer effect of the bouillon as well as the great dilution of the acid alone are enough to explain this.

Using the tube showing most agglutinin in the precipitate with least volume of precipitate as a guide to optimal proportions, a large quantity of the extract is precipitated by adding a corresponding amount of acid and copper chloride solution. The mixture is allowed to stand in the icebox 24 hours and then centrifuged to separate the flocculent precipitate. The precipitate is then dissolved in a volume of N/200 HCl equal to the volume of extract used. The supernatant and dissolved precipitate are analyzed chemically and examined for agglutinating power.

Ten cubic centimeter portions of the dissolved precipitate are then treated in the same manner as the original extract with the difference that the addition of copper chloride is now omitted.

In this second precipitation there is a slight narrowing of the reaction zone, precipitation occurring between about pH 5.0 and 7.0 with a maximum again at about pH 6.4.

A large volume of the dissolved first precipitate is now treated with acid to bring it to the desired pH, allowed to stand 24 hours in the icebox and centrifuged. This second precipitate is then

treated in the same manner as the extract and first precipitate and a third precipitate obtained, which is in turn dissolved in N/200 HCl.

By following the above technique we have been able to prepare a solution of typhoid agglutinin which we believe to be purer than any hitherto prepared. The dissolved third precipitate agglutinated completely in 1:900 dilution, with partial agglutination up to 1:1200. It had a nitrogen content of .00042 gm. per 100 cc. (determined by the Kjeldahl method on 100 cc. sample). The agglutinating power was diminished about 22 times, while the nitrogen content was diminished 3450 times. Thus the nitrogen was diminished 156 times as much as the agglutinin. The dissolved third precipitate, when examined by the usual tests gave the following results:

	Original serum	Serum 1/3500[1]	Original extract	Third precipitate
Titre	*++ 12800 + 25600	++ 4 + 8	++ 800 + 1100	++ 900 + 1200
Nitrogen	1.45 gm. per 100 cc.	0.00041 gm. 100 cc. calculated	0.0031 100 cc.	0.00042 gm. 100 cc.
Millon	+	+	+	—
Ninhydrin	+	—	—	—
Biuret	+	+	+	
Xanthoprotein	+	+	+	?
Kuttner reaction[2]	+	+	+	—
Adamkiewicz	+	—	—	—
Sulfur		—	—	—
Phosphorus				trace

* ++ indicates complete and + incomplete agglutination.

The dissolved third precipitate and the original extract were tested for specificity. They did not agglutinate *B. paratyphoid* A or B, *B. dysenteriæ* (Flexner and Shiga types), *B. coli communis* in dilutions of 1:10 or higher. Copper chloride alone agglutinated in M/330 concentration but not in M/500.

[1] This dilution was chosen because it represents the same nitrogen content as the third precipitate.

[2] Kuttner, T., PROC. SOC. EXP. BIOL. AND MED., 1918, XV, 91. This is a color reaction for NH_3 and free amino groups.

The amount of copper added to the extract in the first precipitation was 0.01816 gm. per liter. The first supernatant fluid had a copper content of 0.0146 gm. per liter. Thus the precipitate had in it 0.00356 gm., representing one liter of extract. Fifty cubic centimeters of the dissolved third precipitate showed no detectable copper when the organic matter was destroyed by nitric acid, the material evaporated to dryness, taken up in concentrated hydrochloric and again evaporated to dryness, taken up in very dilute hydrochloric acid and examined by the ferrocyanide method.

The above tests would seem to preclude agglutinin being a protein, or even a protein hydrolysis product. The small quantity of nitrogen may be in agglutinin, or may be due to a small amount of nucleic acid present as an impurity from the bacteria.

Since it is known that the method of extraction, namely weak alkali, will extract nucleic acid from bacteria, and since the nucleic acids are precipitable by copper, the nitrogen present may well be in the form of nitrogenous base as an impurity derived from the bacteria. If so, possibly the agglutinin is represented by carbohydrate. On the other hand, the carbohydrate itself may also be derived from the bacterial nucleic acid. In this case the chemical factors representing the agglutinin may be so minute in amount as to escape detection entirely. Analyses now in progress may throw some light on this question.

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The isolation of a crystalline substance (M. P. 223°C) having the properties of "bios."

(Preliminary report).

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A crystalline substance melting sharply at 223° C. has been isolated by the use of differential adsorbents from autolyzed yeast which in very small amounts (0.005 mg. per cc. of Ful-