color with orcinol, hydrochloric acid and copper sulfate, but it gave very little or no crystallized osazone with phenylhydrazine. Upon further heating of the acid solution, black products are formed, the solution yielding a crystallized osazone.

If the insoluble part resulting from the treatment with hydrochloric acid is heated further with new portions of acid, more reducing sugar is formed. The final solutions gave with orcinol and copper sulfate a green color instead of the purple. These solutions were dextrotatory and gave a crystalline phenylosazone. The insoluble material resulting from complete cleavage contained higher fatty acids and a base resembling sphingosine.

From beef kidneys, not containing heterogenetic antigen, only an insignificant quantity was obtained of a material similar in its solubility to the above. In a second experiment, such a product was not obtained.

Aside from the fraction described others were found which were similarly active, were water soluble, but gave a weak orcinol-copper test.

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Further studies on the production of staphylococcus aureus toxin.

JULIA T. PARKER, J. GARDNER HOPKINS and ANNE GUNTHER.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

In a previous paper[1] we showed that certain strains of *Staphylococcus aureus* produce an exotoxin which causes necrosis when injected intradermally into rabbits. With the methods used at that time only 8 out of 118 strains from pathogenic sources produced a toxin of sufficient strength to be demonstrable by this method. Recently, however, by two improvements in method, it has been possible to obtain the toxin from 13 out of 14 strains. These changes were: first, the growth of the staphylococcus culture in an atmosphere containing 10 per cent carbon dioxide and,

[1] Parker, Julia T., *J. Exp. Med.*, 1924, xl, 761.

second, the use of "Proteose peptone"* instead of Witte peptone in the broth media.

For cultivation in the presence of CO_2, the method of Cohen and Fleming[2] was used. The stronger toxin production in this atmosphere may be due to the fact that the pH of the broth cultures does not rise above 7.6 even after 8 days growth. When grown under the usual atmospheric conditions, even after 3 or 4 days growth, the staphylococcus cultures have a pH of 8 or over.

There appeared to be some variation in different strains, but a strong toxin was produced most regularly when "proteose peptone" was used.

In the production of other bacterial toxins it was found the amount produced by the staphylococcus varied greatly when different commercial brands of peptone were used in the medium.

The table is given to show the amount of toxin which seven different strains of *staphylococcus aureus* produced on media prepared in exactly the same way, except for the presence of 6 different peptones. All of the cultures were grown in the same jar with 10 per cent carbon dioxide at 37° for 3 days.

PEPTONES.

Strain No.	W	P	Proteose	C	F	B
169	0	0	+++	++	0	0
170	0	±	++	+	±	0
174	±		++++	++	+	++
175	±		+	++		+
176	0		++	+++		0
177	++		+++	+		+++
180	+++		+++	+++		

The toxins were tested in the usual way; namely, 0.1 cc. of a sterile Berkefeld filtrate of each was inoculated intradermally into rabbits. 0 indicates no reaction; ±, red reaction 2 by 2 cm. lasting at least three days; +, yellow center 2 by 2 cm. surrounded by erythema; ++, yellow center 3 by 3 cm. surrounded by erythema; +++, yellow center 3 by 3 to 4 by 4 cm.; ++++, yellow center 4 by 4 cm. to 6 by 6 cm.

For the convenience of those who may wish to make staphylo-

* "Proteose Peptone" was obtained from the Digestive Ferments Co., Detroit, Michigan.

[2] Cohen and Fleming, *J. Infect. Dis.*, 1918, xxiii, 337.

coccus toxin, the method we have used for preparation of the medium is given in detail.

Two pounds of chopped veal is placed in one liter of distilled water and left in the ice box over night. The infusion is then strained through cheese cloth, boiled, filtered and the reaction adjusted to pH 7.4. After autoclaving, the infusion is inoculated with *B. coli* and incubated for 18 hours. The broth is then sterilized in the Arnold, filtered, the volume made up to one liter, and 80 grams of "proteose peptone" (Digestive Ferments Co.) and 10 grams of NaCl are added. The whole is boiled in a double boiler for 40 minutes, filtered, the volume brought to one liter and the pH adjusted to 7.4, one liter of phosphate buffer [N/15 ($Na_2H\ PO_4$ and $KH_2\ PO_4$)] pH 7.4 is added, the media is distributed in Ehlermeyer flasks and autoclaved.

On this media in the presence of 8 to 10 per cent CO_2, the strongest toxin is obtained after 6 to 8 days' growth of the organism.

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The occurrence of scarlet fever without a rash during epidemic.

FRANKLIN A. STEVENS

[*From the Department of Medicine of the College of Physicians and Surgeons of Columbia University and the Presbyterian Hospital, New York.*]

During the winter of 1924-25 an epidemic of scarlet fever and acute streptococcus pharyngitis occurred among the nurses at Presbyterian Hospital, New York City. Early in the epidemic the entire nursing staff was tested for susceptibility to scarlet fever by means of intracutaneous toxin injections. Following this series of skin tests cultures were obtained from all throats showing an angina. Practically all of the cases of pharyngitis and tonsilitis showed hemolytic streptococci. The strains of hemolytic streptococci recovered from these cases were tested for agglutination with scarlatinal immune sera and with sera pre-