of procaine HCl with 20 mg per kg of either barbital gave an incidence of 91% convulsions in 60 cases, instead of the expected 84% found for procaine alone. However, if these same amounts of these barbitals were injected half an hour earlier than the procaine, no convulsions appeared at all. The barbitals therefore seem to have no *local* action in inhibiting local anesthetic convulsions.

Full details of these experiments will be published later. Meanwhile, it may be concluded that $CaCl_2$ tends to inhibit local anesthetic convulsions when administered in the same solution by altering permeability, as indicated by the fact that the addition of $MgCl_2$ to the solution counteracts this anticonvulsant action. The anticonvulsant effect of calcium salts seems to be proportional to the extent of ionization. Epinephrine and potassium ions tend to inhibit local anesthetic convulsions when administered in local anesthetic solutions by local constricting effects. Barbiturates inhibit local anesthetic convulsions *only* by central depressant action.

(The writer wishes to thank Dr. H. Wastl for her painstaking work on the animal experiments mentioned, and Mr. Arthur S. Jensen for his conductivity measurements of organic calcium salts.)

10970 P

Production of Pyrogen in Sera by Bacteria.

CO TUI AND M. H. SCHRIFT.

From the Laboratory of Experimental Surgery, New York University College of Medicine.

The occurrence of fever and chills is a relatively frequent phenomenon following intravenous serum therapy. Among the many writers calling attention to this reaction, it suffices only to mention Bullowa[1] who speaks of its high incidence in the use of refined and unrefined pneumococcus sera. The subject has been a fruitful field of research and speculation. The cause of this reaction has been variously attributed to hemolysis, to the use of anti-coagulants, to the presence of toxic fractions of proteins, and to bacterial contamination.

Felton[2] has shown the reactive principle to be in the acid precipitate of the water-insoluble protein of the sera, and intimates that it may

[1] Bullowa, J. G. M., *Management of the Pneumonias*, Oxford Press, 1937, p. 317.

[2] Felton, L. D., and Kauffman, G., *J. Infect. Dis.*, 1931, **49**, 335.

be of bacterial origin—"for at no time has it been possible to concentrate serum without some bacterial growth." Sabin and Wallace[3] have demonstrated that anti-coagulants, Berkefeld filtration, dialysis and moderate heating have no effect in removing the reactive factors from whole blood or sera.

In our previous work on pyrogen we have shown the similarity of the symptomatology following the intravenous injection of reactive infusion-fluids,[4] reactive inulin,[5] reactive acacia,[6] and the cell-free fluid of typhoid vaccine.[7] The symptomatology in the main consists of a rise in temperature, leucopenia, and in severe cases, chills and gastro-intestinal disturbances.

The present work deals with the production of pyrogen (the chill- and fever-producing factor) by inoculating normal horse sera with bacteria and incubating them for variable periods of time. Dogs were used for these experiments. It may here be mentioned that dogs were first used by Sabin and Wallace[3] in their work on the chill-producing substances in therapeutic sera. We have found dogs quite satisfactory in our work on inulin, water and acacia, and prefer them to rabbits which were used by Seibert[8] in her work on the pyrogen in distilled water. The phenomenon of leucopenia which

TABLE I.
Pneumococcal Serum—Type I (N. Y. C. Dept. of Health).*
Dog No. 34—10 kg. January 18, 1937.

Time	Temp. °F	W.B.C.†	Remarks
11:10 A.M.	101.2		
11:15	101.2	9,600	10 cc intravenously
11:45	103.6		depressed
12:00	103.6	6,500	
12:30 P.M.	103.4		shivering
1:00 P.M.	101.8		vomitus
1:30	101.8		
2:00	102.0		depressed
2:30	102.0		
3:30	101.8		
4:30	101.6		

* We are grateful to Drs. Muckenfuss and Falk of the New York City Department of Health for supplying us with material and for their interest and encouragement throughout the course of this work on pyrogen.

† W.B.C. = white-blood-cell count.

3 Sabin, A. R., and Wallace, G. B., *J. Exp. Med.*, March 1, 1931.

4 Co Tui, McCloskey, Schrift and Yates, PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 297; *J. A. M. A.*, 1937, **109**, 250; *Annals Surg.*, 1937, **106**, 1089.

5 Co Tui, Schrift, McCloskey and Yates, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 227.

6 Co Tui, Schrift and Ruggiero, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 533.

7 Co Tui, Benaglia, Ruggiero and Yates, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 272.

8 Seibert, F. B., *Am. J. Physiol.*, 1923, **67**, 90.

TABLE II.
Bacterial Production of Pyrogen in Normal Horse Serum.*

Organism	Incubation period	Wt of dog, kg	Vol. inj., cc	Changes in temp., °F	Changes in W.B.C., × 1000	Symptoms
Sterile control	48 hr	12	25	102.4-102.4	18. -17.3	none
Proteus vulgaris	48 "	16	10	102.4-106.4	7.8- 0.8	shivering, vomitus, diarrhea; depressed
B. coli	48 "	16	10	102.2-106.0	15.2- 1.9	shivering, vomitus, diarrhea
Staph. aureus	48 "	12	25	101.4-103.4	15.8- 3.3	vomitus, shivering
Water organism "S," genus unidentified	72 "	12	10	100.4-102.8	14.0- 2.9	shivering
" " "C," " "	72 "	13	10	100.8-102.2	15.0- 5.5	none
B. subtilis	72 "	12	25	101.4-102.0	12.7- 7.0	"

* Horse serum was kindly supplied by Sharp and Dohme of Philadelphia, Pa.

accompanies this pyrogenic reaction seems to be more sensitive in dogs and affords an added check.

Table I shows the symptomatology following the injection of a known "reactive" serum. Table II represents experiments in which normal sera were inoculated with various microörganisms from culture slants, and, after incubation, and filtration through Berkefeld "W" candles, were then injected intravenously into dogs. It is significant to note that none of the sera after incubation showed perceptible turbidity, but that subcultures made from all revealed viable organisms.

It will be seen in the control experiment in Table II that the administration intravenously of 25 cc of a normal horse serum incubated at 37°C for 48 hours caused neither a change in temperature nor in blood count in the control experiment. However, the administration of from 10 to 25 cc of this same serum inoculated with bacteria gave "reactions" consisting of different degrees of pyrexia and leucopenia, the more profound cases being accompanied by vomiting and diarrhea. *Proteus vulgaris* (Exp. 2), *B. coli* (Exp. 3), *and Staphylococcus aureus* (Exp. 4) all caused severe reactions. The 2 water-organisms caused mild reactions and *B. subtilis* gave a slight or questionable reaction, with a definite leucopenia but a doubtful pyrexia. In this connection it is interesting to note that in a work to be embodied in a future report, we have found *B. subtilis* to be productive of a great deal of pyrogen in infusion-broth media.

Conclusion. The growth of some common bacteria in "normal non-reactive sera" causes them to produce a pyrogenic symptom-complex when injected intravenously into dogs. This symptom-complex consists of fever, leucopenia, and in severe cases chills and gastro-intestinal disturbances, and is indistinguishable from that produced by therapeutic reactive sera, reactive acacia, reactive inulin, or reactive infusion-fluids.

On the basis of these experiments it is suggested that the chill- and fever-producing substance in sera may be pyrogen, caused either by direct bacterial growth or by pyrogen already present in chemicals used in the purification and concentration of therapeutic sera.