thoven's "Saitenphonograph".[2] The 3rd heart sound appears in the records of all of the few subjects examined. So far we have had opportunity to obtain a record from only one case showing a heart murmur.

4048

Extracellular Production of Toxin by Clostridium botulinum.

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It is well known that bacterial free filtrates containing the toxin of *Clostridium botulinum* increase in toxicity when mixed with certain substances such as normal blood serum and non-specific antitoxins. The observed increases have, as a rule, been relatively small (2 to 10 fold) and the observations have been variously interpreted by the several investigators. The possibility that the toxin might be produced extracellularly by the action of the enzymes of the organism on a suitable substrate has not been seriously entertained by the more prominent workers in this field; on the contrary, the belief is generally held that the toxin is produced only intracellularly.

From our own work with *Clostridium botulinum* (Type A) we have been led to the conclusion that the toxin is produced entirely intracellularly, or nearly so, in some media, while it may be produced both intracellularly and extracellularly in certain other media.

If a bacterial free filtrate of the botulinum organism is mixed with sterile skimmed milk and the mixture incubated at 37° C. until proteolysis ensues, a material increase in toxicity takes place. This is illustrated by the example given in Table I. In this experiment 1 part of the culture filtrate was mixed with 4 parts of sterile skimmed milk and the mixture incubated at 37° C. for 4 days. As a control, some of the same filtrate was incubated alone at the same temperature for the same period of time. After incubation, series of guinea pigs were inoculated with, (1) the incubated filtrate mixed with 4 parts of sterile physiological salt solution, (2) the incubated filtrate mixed with 4 parts of sterile skimmed milk within an hour previous to inoculation, and (3) the incubated filtrate-milk mixture referred to previously.

[2] Einthoven and Hoogerwerf, *Pfluger's Archiv.*, 1924, cciv, 275.

TABLE I.
Action of Botulinum Filtrate upon Milk—4 to 1 Mixture.

cc. given	1	1/10	1/100	1/1000	1/5000
NaCl Solution and Filtrate (Mixed after incubation)	+	—	—	—	—
Milk and Filtrate (Mixed after incubation)	+	+	—	—	—
Milk and Filtrate Mixed (Incubated 4 days at 37° C.)	+	+	+	+	—

This result has been obtained again and again and we feel that these results together with certain other observations lend support to the view that the toxin may be produced extracellularly by the action of the enzymes of the organism on certain protein substrates. Similar results have been obtained with purified casein solutions in place of skimmed milk, and likewise when the filtrate is incubated with a suspension of sterile yeast cells. On the other hand, we have not succeeded in demonstrating a comparable increase in toxin when the filtrate is allowed to act upon peptone, ash-free gelatin, extract of fresh beef (in concentrations used in bacteriological media), and trypsin digested milk. While the trypsin digested milk yielded a greater increase in toxicity than the other substances which are listed as giving negative results, the increase was not comparable to that obtained from normal skimmed milk. This is suggestive.

That the increased toxicity obtained by the action of botulinum filtrate upon skimmed milk is due to an increase in the specific toxin is indicated by the fact that this toxicity was completely counteracted by a type A antitoxin procured from a biological supply house.

4049

Chronic Barbital Poisoning in the Rabbit.

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Clinically chronic barbital poisoning has been observed repeatedly. On the other hand no observations upon the effects of repeated injections of this drug upon animals seem to have been reported. The desirability of comparing similarities of symptoms in man and in laboratory animals is obvious.