

4. By analysis of the data from an entirely different angle the same general conclusion is apparent. The total number of generations attained before the F_1 generation appeared, plus those from the F_1 to the origin of the F_2 , and so on to the F_5 generation, amounts to date to from 450 to 550 generations, according to the series followed. The first line reached 234 generations without conjugation, therefore by conjugation it thus far has been possible more than to double the number of fissions obtainable without conjugation.

In brief, all the data thus far secured indicate that in this pedigree culture, under the conditions of the experiment, the survival value of conjugation in the majority of cases is marked—the exconjugant lines exhibiting a higher division rate and out-living the non-conjugant lines.

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The precipitation of botulinus toxin with alcohol.

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While attempting to purify botulinus toxin by precipitation with ethyl alcohol, we found that the alcohol causes destruction of this toxin. Even a weak solution (20–30 per cent.) of ethyl alcohol is capable of quickly destroying many thousand lethal doses of botulinus toxin in vitro. This destruction takes only five to ten minutes if the mixture is kept at 37° C. On the other hand the toxins of tetanus and diphtheria are much more resistant to the destructive effects of alcohol.

It had been observed in several outbreaks of botulism that those who had partaken freely of alcoholic beverages while eating the incriminated food were not severely affected or remained entirely well, whereas others eating as much or even less of the same food were severely and sometimes fatally poisoned.

We assumed that this protection from botulinus poisoning when alcoholic beverages are partaken might be due to the direct destructive action of the ethyl alcohol on the toxin in the stomach. The problem was approached experimentally. Two series of three guinea pigs each of equal weight received each *per os* enough botulinus toxin to kill them within 24 to 48 hours. Each guinea pig of one of the series received 6 c.c. of 30 per cent. alcohol *per os* immediately following the toxin. The guinea pigs of the first series died within the usual time, whereas the animals which received the alcohol survived and are apparently normal two weeks after the experiment.

The effect of alcohol must be ascribed solely to its direct destructive action upon botulinus toxin. This action is quite unlike etherization¹ which delays the rate of absorption of botulinus toxin. That alcohol destroys botulinus toxin only by direct action is shown by the following experiments: Guinea pigs receiving botulinus toxin by the mouth die within the usual incubation period observed in normal controls despite the administration of large amounts of alcohol subcutaneously. The same fatal results are seen when the toxin is given intraperitoneally and the alcohol administered by the mouth.

We are at present studying the question of the effect of stomach contents as well as quantitative and time relation necessary to assure the destructive action of alcohol upon toxin in the stomach. We are also studying the question of the mechanism of this destructive action of alcohol with the view of finding satisfactory substitutes for it.

(This work is a part of the investigation of food poisoning, conducted under the Direction of Dr. M. J. Rosenau, professor of preventive medicine and hygiene, Harvard Medical School. The investigations were made under the auspices of the Advisory Committee of the National Research Council on the Toxicity of Preserved Foods, and under a grant to Harvard University from the National Canners Association.)

¹ J. Bronfenbrenner and H. Weiss, *PROC. SOC. EXP. BIOL. AND MED.*, 1921, xxiii, 253.