## Presence of a Labile Toxin in Yolk-Sac Cultures of Rickettsia

GIROUD[1] reported the presence of a specific toxic substance in suspensions of Rickettsia-infected organs which produced dermal lesions in rabbits. Gildenmeister and Haagen[2] found that yolk-sac cultures of Rickettsia are toxic for mice when injected intraperitoneally. We have been studying the nature of the toxin present in yolk-sac cultures of Rickettsia and summarize below some of the observations noted thus far.

Yolk-sacs of infected eggs were removed on the fourth day after inoculation, placed in buffered broth (one part nutrient broth and one part phosphate buffer *p*H 7·4), in the proportion of 1 part sac to 10 parts fluid and shaken in a mechanical shaker for 60 minutes. This process is sufficient to break up the infected cells, the rest of the sac membrane remaining intact, and yields a suspension relatively free from tissue cells and debris. The suspension is then decanted, centrifuged lightly to remove gross debris, and an opalescent fluid rich in Rickettsia obtained.

This suspension of Rickettsia when injected intradermally into rabbits gives on the second or, more usually, third day an indurated, inflamed nodule with a central necrotic area. If the suspension is centrifuged in an angle centrifuge at 4,500–5,000 revolutions for about two hours the supernatant fluid, now practically free of Rickettsia, still gives a typical skin reaction in rabbits even when diluted twenty to forty times. The injection of this supernatant fluid intraperitoneally into mice or rats is followed regularly, on the third to the fifth day, by a considerable enlargement of the spleen and liver without any Rickettsia being found even when murine strains are used. The sedimented Rickettsia resuspended in an equal volume of phosphate buffer (*p*H 7·4) and injected in the same manner give a similar but stronger reaction in rabbits, and in mice and rats Rickettsia can readily be seen in the enlarged spleen. This suspension of Rickettsia still produces a reaction in dilutions of 1 : 80, that is, it is two to four times as toxic as the supernatant fluid. If this suspension of Rickettsial organisms is frozen and thawed seven or eight times, the same reactions are obtained, although no live Rickettsia can be demonstrated either by yolk-sac cultures or by animal inoculations.

It appears, therefore, that the rabbit skin lesion and the splenic enlargement in mice and rats are caused by a toxic substance present in the supernatant fluid freed from Rickettsia, as well as in the organisms themselves. This toxin is present in cultures of both human (louse-borne) and murine (flea-borne) strains of Rickettsia.

This toxic substance, whether in the supernatant fluid or in the organisms, was found to be extremely labile. It was completely inactivated by heating for half an hour at 56–60° C., largely so at 50° C., but not at all at 40° C. The original culture suspensions diluted with an equal volume of distilled water, and kept at 37° C., lost their toxicity partly after two days and completely after three. A reduction in toxic strength occurred also after seven days in the ice box (10–12° C.).

Shaking with ether (Squibb U.S.P. for anæsthesia) for half an hour completely inactivated the toxin both in the supernatant fluid and in the Rickettsial suspension. After complete removal of the ether, no toxic effect resulted from injecting any of the fractions obtained by this treatment.

Rapid freezing and thawing of culture suspensions, seven or eight times, apparently killed the organisms, but the toxic strength of the supernatant fluid obtained after centrifugation in the angle centrifuge was enhanced. Similar treatment of the supernatant fluid, free of Rickettsia, resulted in a reduction of the toxicity. It appears that repeated freezing and thawing is to some extent injurious to the toxin, but that if the treatment is carried out when Rickettsia are present, the injury to the toxin is compensated by fresh toxin liberated from the organisms.

It seems, therefore, that the Rickettsial strains studied contain near the cell surface a toxic substance which is readily liberated into the medium in which they grow or are suspended. This toxin is highly labile, being destroyed at 56° C. in half an hour and at 37° C. in three days. From the point of view of vaccine preparation its inactivation by simple, rather brief, treatment with ether is of particular interest.

Our work has not yet proceeded far enough to indicate whether the toxins produced by the murine and human strains are antigenically distinct. Both produce the same reactions in rabbits, mice and rats, and both are equally labile.

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[1] Giroud, P., *C.R. Soc. Biol., Paris*, **127**, 864 (1938).
[2] Gildenmeister, E., and Haagen, E., *Deut. Med. Woch.*, **66**, 979 (1940).

## A New Synthesis of Xanthine

BY the action of alkaline potassium hypobromite on phthalamide, Hoogewerff and van Dorp[1] obtained 2 : 4-dihydroxyquinazoline. Various applications of this intramolecular Hofmann reaction have since been reported; for example, when applied to succinamide, maleamide and pyrazine-2 : 3-dicarboxyamide, the reaction leads to dihydro-uracil[2], uracil[3] and lumazine[4] respectively. We have found that the reaction is applicable to *glyoxaline-4 : 5-dicarboxyamide* (I), which on treatment with alkaline hypobromite solution gives xanthine (II).

The ready availability of the diamide (I) makes this method an attractive synthetic route to xanthine and substituted xanthines. The reaction appears to be of a general nature, and its application to *1-methyl-*