A New Antibacterial Substance from *Inoloma traganum* (Inolomin)

After examination of a great number of representatives of different families of *Hymenomycetes*, an antibacterially effective substance was found in the receptacles of *Inoloma traganum* (g. *Cortinarius, Ochrosporeæ, Agariceæ*). We discovered it for the first time in August, 1946, in the fungi collected in the coniferous forests of the Giant's Mountains (near Velká Úpa) and still later in many other localities. Thus the substance seems to be a permanent component of the fungi of this species and independent of their physical environment. The young violet fungi whose head is still spherical, contain far more of the substance than old fungi with a flat head, in which the substance several times was not found at all. Its quantity is about the same in the foot and head.

The first extracts were prepared by crushing the fresh fungi with distilled water in the ratio 1:5 by means of a glass crusher, the mash was centrifuged and, the liquid passed through a paper filter. We obtained a pale violet colloid solution. Its effectiveness was tested by the suppression of the growth of an aerial micrococcus on meat-pepton agar. (On an evenly inoculated plate we placed a ring of filter paper soaked with the solution and the whole was then incubated. The effectiveness was determined by the width of the sterile zone from the border of the filter paper, in mm). These watery extracts had an effectiveness of 6–10 mm.

After filtration with Seitz EK filter we obtained a clear yellowish solution whose effectiveness was unchanged. The extract may also be concentrated by evaporation at a p_H of 5–7 at 100°C.

After saturating the watery extract with ammonium sulphate the colloids are precipitated and can then be separated easily by filtration through paper. The clear yellowish filtrate does not lose its effectiveness. To the filtrate we add animal charcoal (5 g to 1 l), stir it by shaking, and after half an hour filter it. The colouring matters and impurities were adsorbed so that the filtrate is clear, hyaline and its effectiveness was not sensibly lowered. (When washing out the dried animal charcoal with ethanol we obtain after filtration and evaporation a yellowish brown wax-like substance insoluble in a phosphate buffer p_H 7, which on the surface rolls itself up into spheres. Traces of the effective substance are, however, mixed in this substance and are easily dissolved in the buffer). To the filtrate we add a certain quantity of carborafine (30 g to 1 l), stir it from time to time and filter it after two hours. The still moist carborafine with the adsorbed effective substance we extract for one hour at 37°C with concentrated ethanol (600 cc to 30 g). After filtration we evaporated the filtrate at a temperature lower than 65°C (preferably in a vacuum). We obtain a yellow glassy substance which quickly becomes moist in the air, and which is easily soluble in water and in a phosphate buffer of p_H 7. The feebly yellowish 10% solution shows a prevention of the aerial micrococcus in a zone of 12–14 mm. The sterile Seitz-filtered solution does not lose its effectiveness after standing a few days at laboratory temperature. Upon autoclaving at 1½ atm. it turns dark brown and loses its effectiveness after 40 minutes. We believe that this concentration contains still more than one half of impurities. Until the substance is better chemically defined we shall call it *Inolomin* after the fungus *Inoloma traganum*.

The preparation affects only certain micrococci and pseudodiphtheria. As we ascertained in some thirty cultures of aerial micrococci, the substance affects only certain forms which do not ferment saccharose, levulose, glucose and starch. Further it affects certain "dry pseudodiphtheriæ". So far no other species of microbes have been found which are sensitive to *Inolomin*, and we believe that just because of its specific effect on certain micrococci and pseudodiphtheriæ it might have a considerable theoretical importance in the study of the analogy of the metabolism and systematic position of the two genera mentioned.

Toxicity: A 10% solution of our concentrate is for white mice entirely non-toxic. The mice (about 16–20 g) bear an intravenous injection of 1 cc of the solution without any symptoms.

We demonstrated the occurrence of a similar antibacterial substance also in some other species of the genus *Cortinarius*. We cannot say as yet whether they are identical with our *Inolomin*.

The present communication has to be considered only as an introduction to the study of the various and very interesting antibacterial substances of the higher fungi of our country.

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Zusammenfassung

Im Pilz *Inoloma traganum* wurde ein neuer antibakterieller Stoff gefunden. Diese Substanz wirkt nur auf einige Arten von Mikrokokken und Pseudodiphtheriebazillen. Das neue Antibiotikum wurde aus einer Wasserlösung durch Adsorption an aktive Kohle konzentriert, mit konzentriertem Äthanol eluiert und durch Verdampfen der Lösung gewonnen. Für eine Maus ist der Stoff bei intravenöser Applikation nicht toxisch. Er könnte theoretisch beim Studium der systematischen Einordnung von Mikrokokken und Pseudodiphtheriebazillen bedeutsam sein.

Influence de l'énervation sur la composition des extraits protidiques des muscles striés

Des modifications protidiques des muscles énervés ont déjà antérieurement été signalées. En négligeant les résultats déjà anciens de STEYRER[1], obtenus avec des méthodes qui n'ont plus qu'un intérêt historique, les modifications connues[2] portent exclusivement sur la myosine de WEBER-EDSALL (moindre solubilité, avec formation de précipités particulièrement denses; difficulté d'obtenir des fils à partir de cette myosine, faible biréfringence des fils obtenus, faible activité ATPasique).

On sait que si l'on extrait de la pulpe musculaire par une solution de force ionique qui ne dépasse pas μ: 0,15, à un p_H de 7,20 environ, on fait passer en solution la plupart des constituants protidiques solubles du muscle sauf ceux du groupe II[3] (myosines α, β et γ).

La fig. 1 *A* représente un tracé électrophorétique de muscle normal extrait dans de semblables conditions: on y reconnaît une bande principale, la plus lente (*I*), correspondant aux myogènes (groupe I de JACOB), la

[1] A. STEYRER, Beitr. Chem. Physiol. Path. *4*, 234 (1903).

[2] E. FISCHER et V. W. RAMSEY, Amer. J. Physiol. *145*, 571 (1946); Arch. Phys. Therapy *25*, 709 (1944); Feder. Proc. *4*, 21 (1945). – E. FISCHER, E. HUF, V. W. RAMSEY et C. R. RYLAND, Feder. Proc. *6*, 1 (1947).

[3] J. JACOB, Bioch. J. *41*, 83 (1947).