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CRYSTALLINE HORSE LIVER CATALASE

ONE of us (O. D. F.) observed that after dialysis for three weeks near the isoelectric point, a solution of horse liver catalase prepared by the method of Agner¹ deposited a small amount of active crystalline material. Not enough of this precipitate was obtained for accurate analysis.

Later, by modifying the method for preparing crystalline beef liver catalase² we obtained crystalline horse liver catalase in good yield. The crystals are in the form of very fine needles. After preliminary purification, including fractional precipitation with dioxane, crystallization was induced by slow addition of ammonium sulfate to the properly buffered catalase in a solution containing 3.0 per cent. of dioxane. Catalase crystals have been obtained also from preparations made by Agner's directions up to the point where the material is adsorbed by passing it through a column of calcium phosphate.

A sample of once-recrystallized horse liver catalase has been found to have a *Kat. f.* of 50 to 55 thousand, which is of the order of magnitude of that of the best preparation of Agner. We do not think that the material is yet entirely purified, since the content of iron is 0.2 per cent. instead of 0.09, which would correspond to the hemin iron. It is possible that our material was contaminated with a small amount of a protein high in iron which was observed during the preparation of the catalase crystals, and which is probably identical with the ferritin of Laufberger.³

The percentage of hemin, determined by colorimetry and by analysis for hemin iron, is about 0.9, which agrees with the claim of Stern and Wyckoff⁴ that horse liver catalase contains about 0.1 per cent. of hematin.

Treatment of the once-recrystallized horse liver catalase with acetone and HCl split off the hemin which dissolved in the acetone. Evaporation of the acetone caused the hemin to precipitate, leaving a small part of the non-hemin iron in the supernatant liquid. Most of the non-hemin iron was in the protein residue.

Our analysis of the sample of once-recrystallized

horse liver catalase showed that the copper content was practically negligible, contrary to the finding of Agner that the copper content of horse liver catalase is high enough to be of possible significance.

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INFECTION OF CHICKS AND CHICK EMBRYOS WITH RABIES¹

IT is the purpose of this communication to present a preliminary report of observations made on chicks and chick embryos inoculated with rabies virus. These investigations were undertaken in order to study the lesions produced in this species and also to determine the changes which might take place in the virus following its adaptation to this unnatural host.

Rabid dog brain passed once through mice was the source of the virus used in this work. Histologically, this dog brain and the mouse-passage brain showed typical Negri bodies. The inoculum consisted of 0.03 cc of 10 per cent. brain emulsion.

Intracerebral inoculation of day-old chicks was followed by signs of rabies after 19 days on the first passage, and 4 passages did not reduce this period appreciably. There was considerable variation in the incubation period and also in the duration of the disease; some animals died within 2 days after the first signs of the disease, while others showed definite paralysis for 2 weeks before death. All these chicks went through a stage of excitement before the onset of flaccid paralysis.

At autopsy no gross lesions were demonstrable. Microscopic examination of brains and cords revealed many Negri bodies, some quite small, others huge. Non-specific changes in the form of acute necrosis of ganglion cells and massive perivascular accumulations of lymphocytes and large mononuclear phagocytes were present throughout the central nervous system.

Portions of brain from the 4th intracerebral passage in chicks were ground and emulsified and 0.03 cc were inoculated intracerebrally into 13-day-old chick embryos. The eggs were opened and the embryos were inoculated according to the technique which had been developed in this laboratory. After 4 days one embryo was sacrificed and its brain was inoculated into the brains of other embryos; subsequent passages have been made every 6 or 7 days, and the virus is now in its twelfth generation.

We have not allowed any chick embryos inoculated by the intracerebral route to hatch. Several embryos inoculated by this route have died between the sixth

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¹ K. Agner, *Biochem. Jour.*, 32: 1702, 1938.

² J. B. Sumner and A. L. Dounce, *Jour. Biol. Chem.*, 121: 417, 1937.

³ V. Laufberger, *Bull. Soc. Chim. Biol.*, 19: 1575, 1937.

⁴ K. G. Stern and R. W. G. Wyckoff, *Jour. Biol. Chem.*, 124: 573, 1938.