

*2<sup>e</sup> expérience*

Culture par agrandissement. Souche Ar. 3. Liquide de culture à double concentration de sels nutritifs. Concentration de la cholestérine: 2 par 1000. Volume final = 4900 cm<sup>3</sup>. Durée de l'expérience: 18 jours. Substance totale recueillie = 9,071 g. Portion cétonique = 1,239 g (rend. 13,64%). Le produit recristallisé de l'alcool méthylique-acétone (3:2) donne p. f. = 80-81°C qui n'est pas abaissé par le mélange avec le  $\Delta^4$  cholesténone.

*3<sup>e</sup> expérience*

Culture par agrandissement. Souche Ar. 3. Liquide de culture à double concentration de sels nutritifs. Concentration de la cholestérine: 0,625 par 1000. Volume final = 4000 cm<sup>3</sup>. Durée de l'expérience: 1 mois. Substance totale recueillie = 2,141 g. Portion cétonique = 0,236 g (rend. 11,02%). Le produit recristallisé de l'alcool méthylique-acétone (3:2) donne p. f. = 80°C en mélange.

Il résulte de ces observations que le pouvoir oxydant des microorganismes appartenant au genre *Flavobacterium* a réellement une extension remarquable, puisqu'il agit même sur des substances qui, comme le cholestérol, présentent une grande résistance aux attaques des microorganismes et des enzymes. Il est donc tout à fait justifié de poursuivre et d'approfondir les expériences entreprises avec ce bacille.

CARLO ARNAUDI et CESARINA COLLA

Institut de microbiologie générale, agricole et technique de l'Université de Milan, le 23 septembre 1948.

*Summary*

Continuing his experiences upon the oxidative activities of organisms belonging to genus *Flavobacterium*, the author reports the conditions in which *Flavobacterium maris* effects the oxidation of cholesterol to cholestenone. This transformation is obtained within 20 days with yields which vary from 11 to 13%.

### On the Hemagglutinins of Beans and of Influenza Virus

We have extracted the hemagglutinin contained in the seeds of *Papilionaceæ*<sup>1</sup> in the form of a substance soluble in organic solvents, and have applied essentially the same method to the extraction of the hemagglutinin of influenza virus<sup>2</sup>. The tests were made by SALK'S method<sup>3</sup>.

The vegetable hemagglutinin was extracted by soaking and grinding commercial dried beans in water, precipitating at  $p_H$  5.0 with an equal volume of ethanol, and treating the precipitate with iso-propanol. After thorough stirring, the mixture was centrifuged, and the resulting water-clear supernatant found to have hemagglutinating activity in high titer. The solution could be dried and the active material redissolved in organic solvents without loss.

With influenza virus the method was as follows: To 50 ml of infected allantoic fluid was added 1.25 ml of *N* acetic acid, which lowered the  $p_H$  to 5.0. To this was

<sup>1</sup> K. LANDSTEINER and H. RAUBITSCHEK, *Centralbl. Bakt. Orig.* 45, 660 (1908). - O. WIENHAUS, *Biochem. Z.* 18, 228 (1909). - E. C. SCHNEIDER, *J. Biol. Chem.* 11, 47 (1912). - M. EISLER and L. PORTHEIM, *Z. Immunitäts.* 47, 59 (1926). - K. LANDSTEINER, *The specificity of serological reactions*, rev. ed. (Cambridge, Harvard University Press, 1945), p. 5.

<sup>2</sup> G. K. HIRST, *Science* 94, 22 (1941); *idem.* *J. Exp. Med.* 75, 49 (1942). - L. McCLELLAND and R. HARE, *Canad. Pub. Health J.* 32, 530 (1941). - G. K. HIRST, *J. Exp. Med.* 76, 195 (1942).

<sup>3</sup> J. E. SALK, *J. Immunol.* 49, 87 (1944).

then added 50 ml of ethanol and the mixture was allowed to stand some thirty minutes until clear flocculation occurred. The precipitate was collected by centrifugation, the supernatant discarded, and the tubes allowed to drain. The precipitate was then treated with 5 ml of iso-propanol. The water-clear supernatant, obtained after stirring and centrifuging, contained the hemagglutinin. As with bean hemagglutinin, the solution could be dried and the active material redissolved in organic solvents. The method was equally successful with virus purified by ultracentrifugation, whereas normal allantoic fluid yielded a hardly visible precipitate and no agglutinin.

The apparent yield of the procedure was in general much higher than would have been expected from the original, suggesting that red cells were much more susceptible to the agglutinin in finely dispersed suspension than to the same substance attached to particles (whether virus or ground seeds), so that the actual percentage of hemagglutinin removed remained unknown. Both bean and virus purified hemagglutinins proved extremely sensitive to traces of normal serum. Normal, as well as group A immune ferret serum, in dilution of 1:20,000, still caused appreciable inhibition of group A virus extracted hemagglutinin.

The purified hemagglutinins could be removed from solution by adsorption on red cells, but the amount of red cells needed was considerably larger than that required for the adsorption of virus under the same conditions. Red cells from which virus had been eluted, and which had become, as shown by HIRST, unable to adsorb virus, gave only suggestive evidence of being less able to adsorb a small amount of extract. Cells agglutinated by an excess of virus extract could be returned to their native state simply by heating to 45°C for a few minutes.

These facts show that the hemagglutinins of *Papilionaceæ* and of influenza virus are not proteins, and suggest that the two substances are of similar nature. According to BURNET and collaborators<sup>1</sup> the hemagglutinins of vaccinia and of ectromelia virus are phospholipidic complexes.

J. BOURDILLON

From the Division of Laboratories and Research, New York State Department of Health, Albany, November 30, 1948.

*Résumé*

On a obtenu l'hémagglutinine des semences de papilionacées et celle du virus de l'influenza en solution dans les solvants organiques, par essentiellement la même méthode d'extraction. Ces deux substances semblent être apparentées.

<sup>1</sup> F. M. BURNET, *Nature* 158, 119 (1946). - F. M. BURNET and W. C. BOAKE, *J. Immunol.* 53, 1 (1946). - F. M. BURNET and J. D. STONE, *Australian J. Exp. Biol.* 24, 1 (1946). - J. D. STONE, *Australian J. Exp. Biol.* 24, 191 (1946); 24, 197 (1946).

### Un nuovo metodo specifico di determinazione della attività antianemico pernicioso

L'unica prova specifica e decisiva dell'azione anti-perniciosa di una sostanza è data dalla sua capacità di avviare l'emopoiesi megaloblastica verso la normoblastosi; manca però sino ad oggi un metodo fondato su tale capacità; che dia perciò garanzie di specificità, se si escludono le prove sull'ammalato di anemia pernicioso.