(II) and hydroxylamine thus provides a simple and quicker means for the reconstitution of functional hemocyanin.

[4] Present address: National Research Laboratory, C.S.I.R., Jamshedpur 7, India.

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Zusammenfassung. Es wird eine neue und einfache Methode zur Rekonstituierung von Hemocyanin (Apohemocyanin) bei *Cancer pagurus* aus kupferfreiem Hemocyanin beschrieben.

H. S. MAHANTI[4] and R. WITTERS[5]

Laboratory of Biochemistry II, University of Louvain (Belgium), 12 October 1972.

A Simple Procedure for Detecting Proteins Synthesized in Organ Cultures

At present the study of in-vitro protein synthesis by isolated cells, tissues and organs is widely used for many purposes (e.g., molecular events implicated, effects of drugs, control of hormones). On the other hand, isolation and detection of synthesized proteins generally require complicated methods.

In the course of research on the production of serum proteins by liver explants in vitro, we have developed an immunological procedure for demonstrating the synthesized proteins which involves the utilization as a support medium for immunological analysis of the same nutrient medium used as a nutrient[1]. In such a way the proteins synthesized in the organ cultures, if released, are adsorbed into surrounding medium and so are further detectable by means of immunodiffusion, avoiding their isolation and purification and taking advantage of high sensibility and specificity of antibody-antigen reaction.

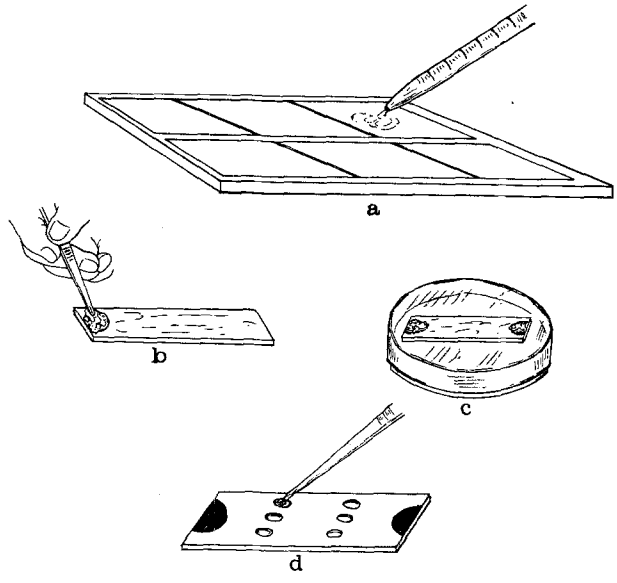

Fig. 1. Some steps of the detection procedure are indicated: a) filling the slides with nutrient medium; b) placing the liver pieces upon the solid media; c) maintaining slides inside petri dishes; d) applying the antiserum into the wells.

To a sterile solution of gelose 1% in Gey fluid kept at 40 °C calf serum and Tyrode's containing sodium merthiolate (0.5 mg/ml; S.I.C., Milano) were added in the following ratios 10:5:1. Sterilyzed glass slides (25 mm × 75 mm; Gelman Instr. Co., Michigan) were covered by means of a 10 ml pipette to obtain a uniform layer of solution (about 4 ml/slide) (Figure 1a).

Livers, removed from 14-day-old chick embryos, were cut and several pieces (each about 1 mm × 1 mm) were placed upon solid media at the extremities of the slides (Figure 1b) and kept in culture in sealed Petri dishes (Figure 1c). At different periods of incubation, the slides were removed, liver explants taken out and processed for histological examination. Wells were then stamped on the slides using a gel punch and the antiserum (anti total chicken serum; Sycco, N.J.) was applied into the wells (Figure 1d).

Immunodiffusion was performed according to Ouchterlony technique[2]. Histological examination demonstrated that under our conditions liver explants underwent a good morphogenesis as previously described[1]. Immunological investigations showed the precipitation lines to be referred to serum proteins (Figure 2).

Riassunto. Viene descritta una tecnica per evidenziare proteine sintetizzate in vitro in espianti d'organo. Tale tecnica è basata sull'impiego del terreno nutrizio solido come substrato per la immunodiffusione.

P. LOCCI, A. CARUSO, M. A. BODO and P. CARINCI[3]

Istituto di Istologia ed Embriologia generale, Via del Giochetto, I-06100 Perugia (Italy), 20 February 1973.

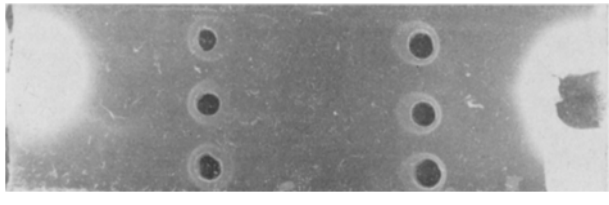

Fig. 2. Outcherlony tests. The precipitation lines are due to reaction between the anti-serum anti total chicken serum protein applied into wells and the serum proteins synthesized by 8 day cultured liver explants and diffused into solid medium.

[1] M. A. BODO and P. CARINCI, Experientia *28*, 1397 (1972).

[2] O. OUCHTERLONY, *Gel diffusion technique in Immunological methods* (Ed. J. F. ACKROYD, Blackwell, Oxford 1964).

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