reaction, but as well detect any ferritin that is present due to pinocytosis or non-specific phagocytosis.

The electrostatic action of copper grids generally tends to attract ferritin molecules non-specifically; therefore it is not desirable to use the direct application of the immune ferritin conjugate on the grid as unreacted ferritin is present in the background. Although some degree of specificity can be determined, in critical evaluations the unreacted ferritin can give rise to erroneous interpretations of antigen-antibody reactions at the subcellular level. Fragments of antigens separated from encapsulated organisms can also present these results. Therefore direct application of the conjugate on copper grids should generally be used for screening procedures, while for critical evaluations thorough washing and embedding should be used.

Immune electron microscopy presents a method of detecting antigens at the submicroscopic level as well as providing a method for the study of pathogenesis at the subcellular level. When used with caution, adequate controls, and a realization of the problems of immunology and technique involved, it will no doubt provide information which has been heretofore only conjecture. As new and more sensitive immunologic techniques are evolved, advancements must be made to balance sensitivity against specificity. An equal effort should be made to provide immune sera with higher degrees of specificity, taking into consideration the immune response desired and the sensitivity of the immune technique involved. The 'pitfalls' presented should not discourage the use of ferritin conjugates in the detection of immune reactions, but only point out some problems involved. As advancements are made in electron microscopy and immunology, there is no doubt that a more effective method of conjugation will become available which will provide a more critical evaluation of the detection of antigen-antibody reactions at the subcellular level.

Zusammenfassung. Es werden die Fehlerquellen diskutiert, die bei der Untersuchung von Antigenen auftreten, wenn zu deren elektronenmikroskopischer Darstellung Antikörper, markiert mit Ferritin, verwendet werden. Durch geeignete Wahl der Fixierung können einige dieser Schwierigkeiten beseitigt werden.

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Cytodifferentiation Induced by Ribonucleoproteins

In previous research[1], we were able to demonstrate that ribonucleoproteins (RNP), extracted from different chicken organs following NIU's[2] method, induce changes in the development of the chick embryo rudiments or of the tissue cultures. Recently, we succeeded in improving these results. Our new findings will be presented in this paper[3].

RNP were extracted from heart (RNP_H) and liver (RNP_L) of adult chicken (often white Leghorn cock). The solution in Gey liquid of these RNP was prepared at a concentration corresponding to an absorption of 5.0 at 260 mμ in the Beckman spectrophotometer (1 cm cell) (i.e. diluting 1:10, the absorption was 0.5). This was the concentration used. Instead, only Gey fluid (Holtfreter fluid when the RNP was from frog, see below) was used in the controls. Shaken egg albumen was coagulated in hot water, and a piece 4 × 2 × 1 mm in size was then dried on filter paper under sterile conditions, imbibed with the Gey fluid or with the RNP solution. The imbibed pieces of coagulated albumen were grafted into the chorioallantois of a chick embryo of 8 days old. The shell was closed again with a coverslip and was opened after 5 days. The chorioallantois with the grafted albumen was then fixed in Bouin fluid, imbedded into paraffin and finally sectioned. The experimental procedure is summarized in Figure 1.

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[3] This investigation was supported by a grant from the Consiglio Nazionale delle Ricerche.

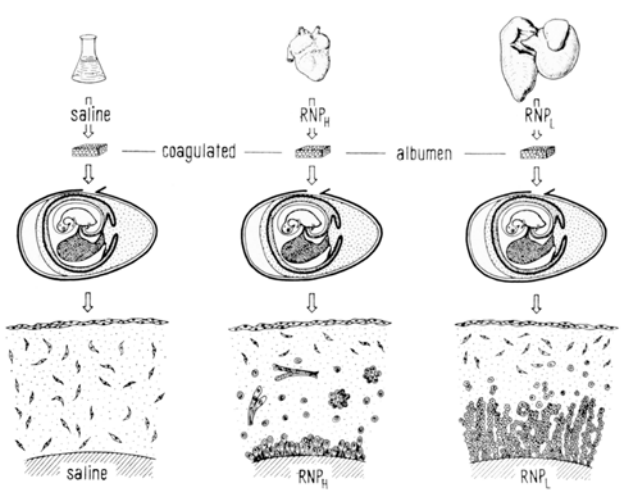

Fig. 1. Experimental procedure.

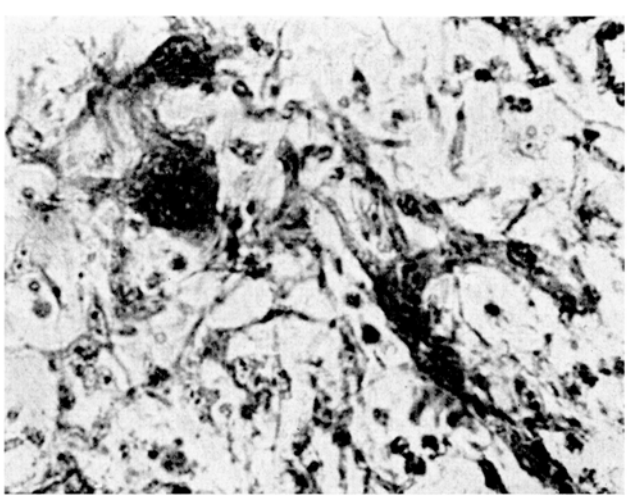

Fig. 2. Muscular elements induced by heart ribonucleoprotein (× 790).

The graft of egg albumen imbibed with saline (Gey solution or Holtfreter solution) does not induce any important change in chorioallantoic membrane, while the graft of egg albumen containing RNP_H induces the appearance of many flask-shaped or elliptical cells with intensely stainable cytoplasm and some multinucleate muscular fibers with fibrillar cytoplasm (Figure 2). We may therefore conclude that RNP extracted from heart can induce the formation of muscular fibers. The graft of egg albumen with RNP_L can induce the transformation of the epithelial cells adherent to the grafted albumen into characteristic tissue made up by polygonal cells piled up into parallel cords; some of these cells may be free in the mesenchymatic tissue (Figure 3). Consequently we may conclude that RNP extracted from liver induces the formation of liver cells.

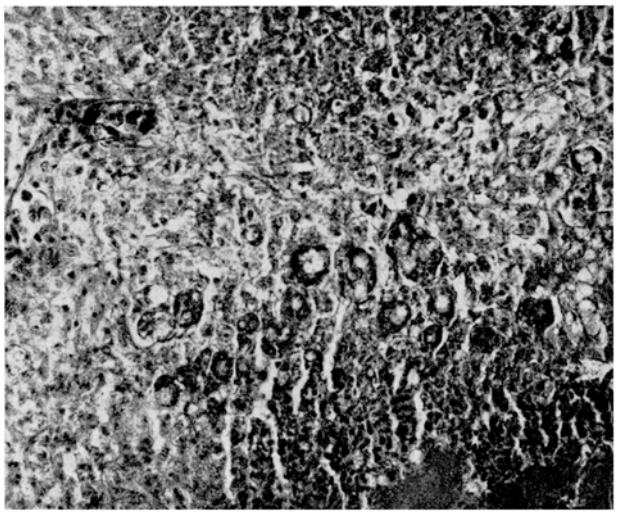

Fig. 3. Glandular structures induced by liver ribonucleoprotein. The graft is at the bottom of the picture (× 274).

We have studied also the action of RNP extracted from frog (*Rana esculenta*) liver and we have been able to show that this RNP is able to induce, in the chorioallantois of chick embryo, the same kind of tissue induced by the RNP extracted from the chicken liver.

After having imbibed a piece of albumen with chicken RNP, we heated it in order to denaturate the RNP, putting the whole thing in a test tube in boiling water for 10 min before implanting it in the chorioallantois. The amount of induced tissues was enormously reduced and only few or no cells were transformed.

We may therefore conclude: some thermolable substances which are able to induce organospecific cell differentiation, are present in our RNP preparations. Our RNP preparations are therefore organospecific: i.e. the heart RNP induces muscular structures; the liver RNP induces glandular tissue. The organospecificity is more effective than the speciesspecificity: i.e. frog liver RNP can induce in the chick embryo the same tissue induced by the chicken RNP_L[4].

Riassunto. Preparati di RNP di cuore o di fegato di pollo impiantati (in albume coagulato) su membrana corioallantoidea (Figura 1) inducono, in questa, formazione rispettivamente di fibre muscolari (Figura 2) e di tessuto ghiandolare (Figura 3). Il principio attivo viene distrutto con cottura a bagno maria. RNP di fegato di rana è in grado di indurre nella membrana corioallantoidea di pollo tessuto ghiandolare identico a quello indotto da RNP estratto dal fegato di pollo.

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Istituto di Zoologia, Università statale, Milano (Italy), May 12, 1961.

[4] After we sent this paper to the Editor, we found by the same method that: RNP extracted from frog heart induces the same structures induced by RNP from chick heart; RNP extracted from chick skeletal muscles induces the formation of a tissue different from that induced by RNP from heart, because the nuclei are peripherical in the so-formed structures; RNP extracted from chick kidney induces the formation of tubular structures and of some other structures tentatively interpreted as glomeruli.

The Heterogeneity of Serum Glutamic-oxalacetic Transaminase of Certain Mammalian Species

Several enzymes have proved to be heterogeneous when analysed by electrophoresis or chromatography[1]. These molecular forms (isozymes) of an enzyme, being similar in enzymic activity, exhibit characteristic patterns of distribution in each organ of an organism. During a study on esterases and transaminases in blood plasma (serum) of pigs with hepatic lesions[2], the electrophoretic pattern of certain sera from pigs with high glutamic-oxalacetic transaminase (GOT) activity was characterized by two fractions with GOT activity. Meanwhile, FLEISHER et al.[3] reported two fractions with GOT activity in crude extracts of heart and liver (man, dog, pig); after completion of the present work, the same authors[4] announced their observation of two GOT-active fractions in serum of dogs following acute injury of the liver. In addition, JUNGNER[5] reported briefly that, depending on the pH used on electrophoresis, GOT activity is detected in two or three fractions of human serum. The heterogeneity of serum GOT of certain other mammalian species will be briefly reported.

Blood samples were taken from individuals with known high transaminase activity. GOT and GPT (glutamic-pyruvic transaminase) were determined according to a method described by KARMEN et al.[6] as modified by ORDELL[7]. Transaminase activity was assayed at 340 mμ with a Beckman B spectrophotometer and expressed as $\Delta A \times 10^3$/min/ml serum. The activity of fractionated serum after electrophoresis was similarly expressed per ml fraction.

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