Länge des DNS-Moleküls noch nicht beantwortet werden kann. Aus diesen Beobachtungen, zusammen mit früheren und anderen noch nicht mitgeteilten, ergibt sich, dass die Osmiumfixierung noch nicht das DNS-Kolloid oder das DNS-Plasma «fixiert» im Sinne einer Verfestigung, sondern gewissermassen *die Weiche stellt* für die Vorgänge, die später bei der Entwässerung stattfinden. Es wurde von KELLENBERGER[16] die Forderung aufgestellt, dass das DNS-Kolloid so vorzubehandeln sei, dass bei der Dehydratisierung Vernetzungen entstehen. Dadurch geliert das Kolloid zuerst, was zur Folge hat, dass die nachfolgende Koagulation, beziehungsweise das Aneinanderlagern der Fadenmoleküle auf ein Minimum beschränkt werden kann.

Zusätzliche Einzelheiten dieser Arbeiten und nachfolgende Diskussionen der Resultate werden an anderer Stelle mitgeteilt werden[17].

Summary. The behaviour of pure isolated DNA towards the conditions of fixation and dehydration – prior to ultrathin sectioning – has been studied and compared with the behaviour of the DNA-plasm of the bacterial nucleus. It is found that those conditions which produce coarse coagulation of the free DNA also produce electron opaque bodies of variable aspect inside the bacterial nucleus. The coagulation figures obtained in both situations are very similar. This is a further direct evidence in favour of the view that the bacterial nucleus is an assembly of fine DNA-containing fibrilla in a highly hydrated plasma.

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[17] Vorliegende Arbeit wurde mit der Unterstützung des Schweizerischen Nationalfonds durchgeführt. HANNA RUHE und SOPHIA ANNEN sei für wertvolle Mitarbeit gedankt. E. KELLENBERGER danke ich für Einführung in Arbeitsgebiet und Problemstellung sowie wertvolle Diskussionen.

'Pitfalls' in Immune Electron Microscopy

Immune electron microscopy[1] is an extension of immunofluorescence[2] at the subcellular level. Not only have many of the problems of immunofluorescence been inherited, but it has become apparent that other problems exist. Some of the problems encountered are presented. It is hoped that other problems can be predicted early in the development of the technique, to provide a more orderly and realistic approach than was encountered in immunofluorescence.

Methods and Results. Fixation. Unfixed and 10% formalin-fixed chorioallantoic membrane and normal mouse liver, kidney, heart, and spleen were treated with an immune *Staphylococcus aureus* ferritin conjugate[1] for 1 h; the treated material was then thoroughly washed and the unfixed material fixed in 10% formalin. The specimens were embedded in paraffin and 4μ-sections stained with Gomori's iron stain[3]. As demonstrated by the iron stain, the tissues fixed prior to treatment with the immune conjugate exhibited a marked diminution of ferritin as compared with the tissues fixed subsequent to treatment with the conjugate.

Treatment on Copper Grids. Bacterial, viral, and mycotic agents were fixed in 10% formalin and drops placed on formvar and carbon covered grids[4]. The grids were air-dried, specific immune conjugate applied, then washed several times in deionized water. Electron microscopy revealed that even the most thorough washing failed to remove all of the ferritin conjugate not bound by the immune reaction.

Non-specific Reactions. Horse spleen ferritin[5] was diluted with phosphate buffered saline, pH 7.2, to contain 10 mg/ml of protein and conjugated with fluorescein isothiocyanate[6]. 0.5 ml of the ferritin fluorescein conjugate was injected intraperitoneally into mature mice. Sections of liver, embedded in paraffin, revealed that the ferritin-fluorescein conjugate was phagocytized by the Kupffer cells non-specifically (i.e. in regard to immune reactions). Observations were made using an ultraviolet light source and filters previously outlined[6].

Discussion. The proper use of fixatives is of great importance in the detection of antigens by immune electron microscopy. Penetration of antibody into cellular material is dependent upon adequate fixation. Correlation can be made with immunofluorescence; specific fluorescence is generally observed in the periphery of unfixed cells, while in fixed preparations the immune reaction can be observed in the periphery of the nuclear membrane. These observations are consistent with those of HIRAMOTO et al.[7].

Fixation prior to treatment with the ferritin conjugate prevents pinocytosis, resulting in a marked diminution of the unreacted conjugate as compared with material fixed after treatment with the conjugate. Formalin generally fulfills the criteria as an excellent fixative for this technique. It does not tend to alter the antigenic components, is germicidal for most pathogens, and preserves the cellular architecture. Fixatives generally used in electron microscopy are potent oxidants, and thorough screening should be made to determine their effect on the antigenic system to be used. This does not preclude the use of these oxidants to enhance the architecture if applied subsequent to the immune reaction.

In the detection of sites of toxin activity and *in vivo* experiments, the use of adequate controls cannot be too greatly stressed. Ferritin can be observed under the electron microscope in normal cellular material (HAMPTON[8], KUFF and DALTON[9], and BESSIS and BRETON-GORIUS[10]) as well as ferritin conjugate which has been non-specifically phagocytized by the reticulo-endothelial system. Therefore, in correlation with nonimmune conjugate controls, histochemical methods, fluorescein-labeled ferritin and electron microscopy should be utilized. These will not only provide a control of the immune

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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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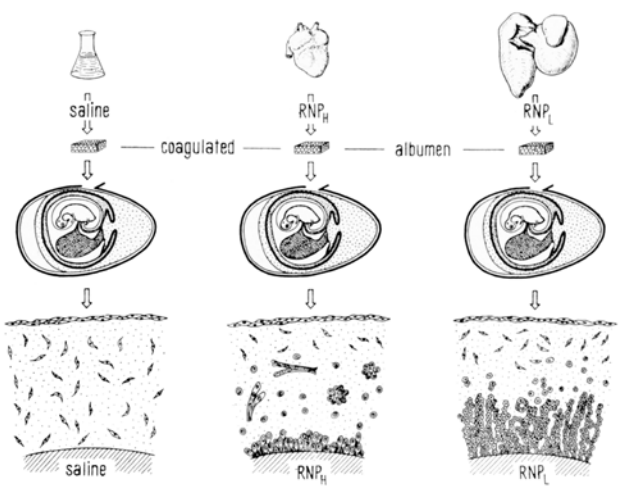

Fig. 1. Experimental procedure.

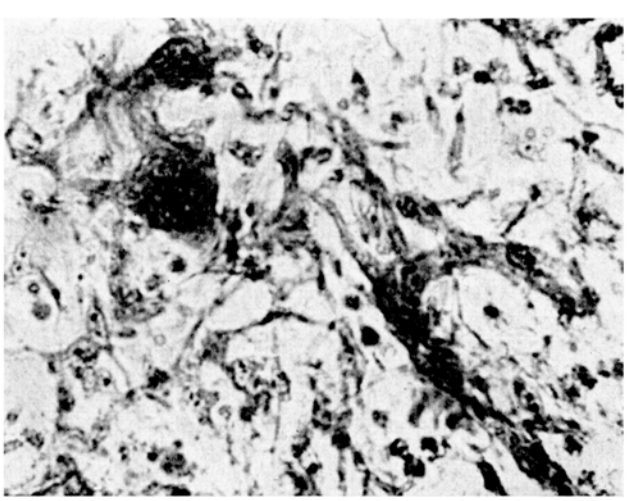

Fig. 2. Muscular elements induced by heart ribonucleoprotein (× 790).