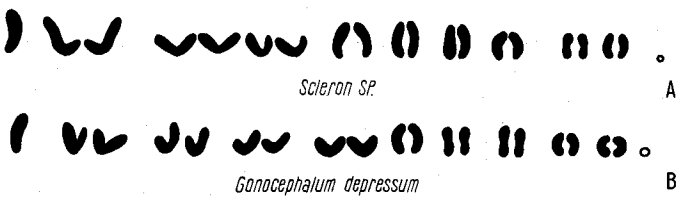

Fig. 2. A and B. Karyotype. The chromosomes are arranged according to their lengths.

chromosomes can be easily identified, as they stand out distinctly from the autosomes.

As described by SAXENA et al.[3], the presence or absence of various amino acid compounds in the whole insect and in different tissues is indicated in the Table II.

Discussion. The characteristics of organisms for animal taxonomic studies are morphological, ecological, cytological and geographical. Since morphology and physiology of organisms are determined by biochemical processes, in recent years biochemical characteristics have been taken into consideration[4]. These characteristics help to determine the systematic position of the organisms[5]. The present investigation deals with the similarities and differences in qualitative (presence/absence) distribution of the amino acids in the whole animal and in certain tissues of the animal.

The chromosome study of the two species shows that both of them possess the basic diploid number 20, characteristic of Coleoptera[6]. But they show a difference in the number of mediocentric and acrocentric chromosomes. They also show qualitative differences in the free amino acid pattern. The present cytological and biochemical investigations support the differences found in morphological and ecological characteristics.

Résumé. Le nombre des chromosomes diploïdes est de 20 dans les deux espèces de Coléoptères Ténébrionïdes *Gonocephalum depressum* et *Scleron* sp. Il y a 18 autosomes et 2 chromosomes sexuels. Ils s'observent dans les acides amino libres. Dasns le *Scleron* sp., il n'a en a que 14. La systématique de ces espèces est discutée sur la base de leurs caractères biochimiques.

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Ultrastructural Localization of Newcastle Disease Virus Surface Antigen in Infected HeLa Cells as Revealed by an Enzyme-Labelled Antibody Method

Previous studies have shown that both protein components of paramyxoviruses, the S and V antigens, are synthesized in the cytoplasm[1] and the viruses mature at the cell surface[2,3]. The S antigen has been reported to be incorporated in the nucleocapsid filaments, and the V antigen (surface antigen) in the spikes of budding particles and virions[4]. But the actual ultrastructural localization of the V antigen in infected cells before virus budding takes place has not yet been elucidated. The enzyme-labelled antibody method has recently been successfully employed for the ultrastructural localization of various cellular antigens[5]. Few papers have dealt with the application of this method in detection of viral antigens[6,7]. The purpose of this experiment was to identify the location of the Newcastle disease virus (NDV)-V antigen in HeLa cells at the early stage of infection by applying this method.

Materials and methods. Indirect enzyme-labelled antibody method was employed in this experiment. Specific antibody against V antigen was prepared by the dissociation of virus-antibody complex (HAI: 1/320)[8]. Conjugation of horseradish peroxidase (BEHRINGER und SÖHNE, Mannheim) to anti-rabbit IgG goat antibody was done with glutaraldehyde according to the method of AVRAMEAS[9]. Before use, all the antisera were absorbed with human liver powder and HeLa cell powder. Normal rabbit serum and antisera against Sendai virus (HVJ) served as control sera.

HeLa cells were infected with NDV (Miyadera strain) at a multiplicity of 10 p.f.u. per cell. Uninfected HeLa cells were used as controls. NDV-infected HeLa cells were fixed in a mixture of 0.5% glutaraldehyde and 3% paraformaldehyde in 0.05*M* phosphate buffer, pH 7.4 for 20 to 30 min at 4°C at 3, 4, 5, 7, 9, 15, and 19 h after infection. After overnight washing in several changes of PBS, the cells were incubated with specific antibody directed against NDV-V antigen for 24 h with gentle agitation, followed by washing PBS and treatment with peroxidase-labelled anti-rabbit IgG goat antibody for a further 24 h at 4°C. After washing thoroughly in several changes of PBS, the cells were refixed with phosphate buffered 2%

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glutaraldehyde for 10 min, and stained in GRAHAM and KARNOVSKY's medium[10] for peroxidase activity. Then, the cells were postfixed with OsO_4, dehydrated in graded ethanol and embedded in Epon. The control experiments included the use of normal rabbit IgG or anti-HVJ rabbit sera instead of anti-NDV-V antibody, and of uninfected HeLa cells treated with anti-NDV-V antibody followed by peroxidase-labelled anti-rabbit IgG goat antibody. Some of the fixed cells were stained with GRAHAM and KARNOVSKY's medium to detect endogeneous peroxidase activity without prior exposure to the conjugate.

Results. The reaction products of the cytochemical peroxidase procedure could be recognized in electron micrographs as granular masses of high electron density, indicating the localization of the antibody-antigen complex. As the electron opacity of ribosomes and glycogen particles obscured the presence of the reaction products in thin sections stained with uranium and lead, the present results were obtained with unstained sections.

Figure 1 shows part of cytoplasm of a HeLa cell 4 h after NDV-infections, in which the reaction products are

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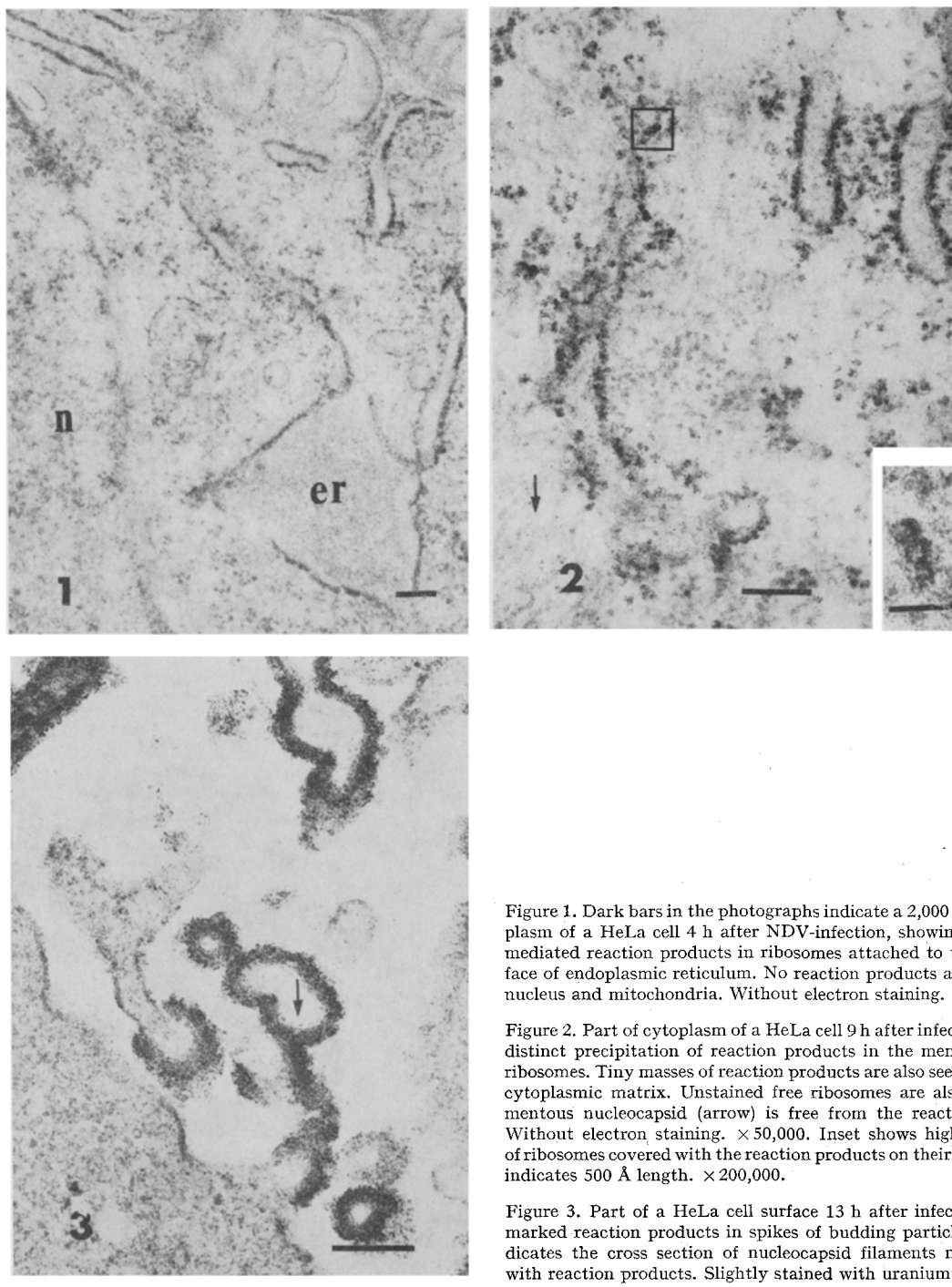

Figure 1. Dark bars in the photographs indicate a 2,000 Å scale. Cytoplasm of a HeLa cell 4 h after NDV-infection, showing peroxidase-mediated reaction products in ribosomes attached to the outer surface of endoplasmic reticulum. No reaction products are seen in the nucleus and mitochondria. Without electron staining. × 30,000.

Figure 2. Part of cytoplasm of a HeLa cell 9 h after infection, showing distinct precipitation of reaction products in the membrane-bound ribosomes. Tiny masses of reaction products are also seen freely in the cytoplasmic matrix. Unstained free ribosomes are also seen. Filamentous nucleocapsid (arrow) is free from the reaction products. Without electron staining. × 50,000. Inset shows high power view of ribosomes covered with the reaction products on their surface. Scale indicates 500 Å length. × 200,000.

Figure 3. Part of a HeLa cell surface 13 h after infection, showing marked reaction products in spikes of budding particles. Arrow indicates the cross section of nucleocapsid filaments not associated with reaction products. Slightly stained with uranium. × 60,000.

observed as fine granular precipitates associated with ribosomes lining on the outer surface of endoplasmic reticulum in the vicinity of the nucleus. In the cisternae of the endoplasmic reticulum, nucleoplasm, mitochondria and free ribosomes, no reaction products are observed. As shown in Figure 2, by 9 h after infection the stained ribosomes increase in number. As seen in an inset of Figure 2, the surface of ribosomes thus stained is actually covered with reaction products. Beside those seen in the membrane-bound ribosomes, tiny masses of reaction products are dispersed among the cell organelles in the cytoplasmic matrix at this stage. No reaction products are associated with cytoplasmic nucleocapsid filaments. Figure 3 shows part of an infected HeLa cell surface at 13 h after infection. The reaction products are mostly restricted in the spikes on the outer surface of budding particles. By accumulation of the reaction products, the layer of spikes increases in width to ca. 300–400 Å. Both the unit membranes beaneth the spikes and the tubules of nucleocapsid are not stained. In control experiments, no reaction products were observed anywhere in the cells. The specificity of the antigen-antibody reaction was further supported by the absence of the reaction products in the tubular nucleocapsid both in the cytoplasm and the budding particles.

Discussion. The NDV-V antigen consists of two major components, hemagglutinin and neuraminidase[11]. Recently, IINUMA et al. found biochemically that the synthesis of both the components was initiated by 4 h and reached maximum by 9 h after infection in HeLa cells[12]. Judging from their results, the present findings may be interpreted as indicating that the initial sites of synthesis of the components are in the membrane-bound ribosomes. Furthermore, the antigen thus synthesized may be released from the bound ribosomes into the cytoplasmic matrix, not into the cisternae of the endoplasmic reticulum, in contraction to the present concept of secretory protein synthesis[13]. Why the initial synthesis does not involve the free polysomes is a further problem.

Zusammenfassung. Nach indirekter, peroxidasekonjugierter Antikörpermethode wurden elektronenmikroskopisch mit Newcastle-Disease-Virus infizierte HeLa-Zellen geprüft, und in den frühen Infektionsstadien an den Ribosomen des endoplasmatischen Retikulums solcher Zellen und in den späten Infektionsstadien an den «Spikes» des spriessenden Viruskörperchens das Antigen der Virusoberflächen gezeigt.

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STUDIORUM PROGRESSUS

The Measurement of Transketolase Activity in Heart Preparations

Our studies, directed at quantitating the role of the pentose phosphate pathway of glucose metabolism during the interval of developing myocardial hypertrophy in the rat and the rabbit, have necessitated the examination of changes in the activity of each of the enzymes of the pentose phophate pathway. In particular our attention has been directed to the role of transketolase (EC 2.2.1.1) where according to the experimental results of MEERSON et al.[1], a significant increase (60%) in the activity of this enzyme occurred in rabbit heart following the development of myocardial hypertrophy, experimentally induced by stenosis of the aorta. It was implied[1] that the increase in transketolase activity reflected the activation of the pentose phosphate pathway reactions to provide ribose 5-phosphate for the increased nucleic acid metabolism associated with the enhanced protein synthesis in hypertrophy. The method used[1] for the measurement of transketolase activity was that of BRUNS et al.[2] where sedoheptulose 7-phosphate (see Equation 1) was determined colourimetrically after a 1 h incubation of ribose 5-phosphate with the tissue extract.

Equation 1: xylulose 5–P + ribose 5–P $\rightleftharpoons$ sedoheptulose 7–P + glyceraldehyde 3–P

We have found that the colourimetric method for the measurement of transketolase[2] in heart extracts was unsatisfactory for this purpose. The principal objections to the application of the method[2] are a) it is not specific for sedoheptulose 7-phosphate while ribose 5-phosphate and other pentose phosphates contribute to the colour yield and thus may act to overestimate sedoheptulose 7-phosphate and therefore the activity of transketolase. b) It has to be assumed that in the tissue preparation being assayed the enzymes ribulose 5-phosphate 3-epimerase (EC 5.1.3.1) and ribose 5-phosphate isomerase (EC 5.3.1.6) are present and of sufficient activity so that they are not rate limiting for the reaction of Equation 1 to proceed. c) Finally it is essential that transaldolase (EC 2.2.1.2) or other enzymes which will react with sedoheptulose 7-phosphate are absent from the reaction mixture, since the presence of these enzymes would lead to low estimates of transketolase activity.

RACKER[3] has outlined 3 assays for transketolase which may be used with highly purified enzyme preparations,

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