araldite-epon, contrast with uranyle acetate at 5% and with lead citrate according to Reynold), one can now have demonstrative pictures regarding the participation of the nucleolus in the initial processes of yolk formation. The Figure shows that from the nucleolus 'gemmae' or 'daughter-nucleoli' separate. These transfer themselves into the nuclear membrane. Both the surface of the nucleolus, completely irregular because of various offshoots, and its internal part because of the cavities of various forms and sizes, appear to indicate a notable activity both in the production of material and in its emission. This is especially clear at point *f* (in the insert *F*) where there is a picture of the separation of one of these 'gemmae' from the nucleolar surface. This picture has a strange resemblance to that which concerns the separation of certain forms of virus from the plasmatic membrane. At the level of the nuclear membrane, on the side of the nucleus, the 'gemmae' seem to retain their initial density; while, on the side of the cytoplasm (admitting that extrusion really takes place), they appear to have a lesser density. This is fairly clear in point *e* (in the insert *E*). In the cytoplasm there are also present various globules at the beginning of their formation. One of these (*v*, in the insert *V*) has 3 other small globules adherring to it almost as if they would come together into a larger one. They have the peculiarity of having the same ultra-structural appearance and the same density as the other globules close to the nuclear membrane. It cannot, however, be excluded that between the small globules and the larger ones there exists only a simple adhesion without this being the beginning of a fusion of the respective masses.

These new observations seem to give plausibility to the hypothesis, advanced a long time ago[1], that there is a close relationship between the production of the ribonucleoproteic material by the nucleolus and yolk production, in the sense that the former would emit, under the form of 'gemmae', the same material. The 'gemmae', after having overcome the nuclear membrane, would aggregate together, even after transformation, into yolk globules.

Application of the 'Low-Temperature Plasma Ashing Method for Biological Tissues' to Studies in the Field of Virology

K. Umemoto

Kyoto College of Pharmacy, Yamashina Misasagi, Higashiyama-ku, Kyoto 607 (Japan), 11 September 1975.

Summary. It was found that the behavior of a virus in host plant is reflected in the pattern of crystalline inorganic components of the host plant by the technique of the 'low-temperature plasma ashing method for biological tissues.'

Detailed examinations were made on the pattern of calcium oxalate crystals in the leaves of plants by comparing the pattern in the leaves affected by a virus with that in healthy leaves of the same kind, and by changing the combination of the virus and host plants. The viruses used were cucumber mosaic virus (CMV), cymbidium mosaic virus (CyMV), dendrobium mosaic virus (DeMV), and coltsfoot mosaic virus, and host plants were *Nicotiana tabacum* Linn., *N. rustica* Linn., *Dendrobium moniliforme* (Linn.) Sw., and *Beta vulgaris* var. *cicla*. Analysis of crystal pattern was made by the 'low-temperature plasma ashing method for biological tissues'[1,2] that the author had devised, with which biological tissues are completely ashed at low temperature by very reactive oxygen stream excited in a high-frequency electromagnetic field under a low pressure, preserving mineral microstructures precisely identical with the original tissue matrices.

When virus was limited to one kind and host plant varied: The cucumber mosaic virus was used, with *N. tabacum* Linn. and *N. rustica* Linn. as the host plants. These plants contain both crystal sand colony and crystal sand.

1. *N. tabacum* Linn. The main crystals distributed in healthy leaves are approximately globular crystal sand colonies (Figure A) but those in virally affected leaves are crystal sand colonies of a considerably complex shape, as shown in Figure A'.

2. *N. rustica* Linn. Crystal sand colonies are few in healthy leaves (Figure B) but are present in a considerable number in the affected leaves (Figure B'). Density of crystal sand distribution was the same as that in *N. tabacum* Linn., being higher in affected leaves than in healthy leaves.

When host plant was limited to one kind and viruses changed: The host plant used was *D. moniliforme* (Linn.) Sw., which contains both bundles of calcium oxalate raphides and crystal sand, and the viruses used were CyMV and DeMV.

1. CyMV. Bundles of raphides measuring 180–200 μm in length are found in healthy leaves (Figure C) and such crystals become 2–4 times longer in the affected leaves (Figure C'). The number of bundles of raphides tends to increase in affected leaves. This tendency becomes more marked in the distribution density of crystal sand.

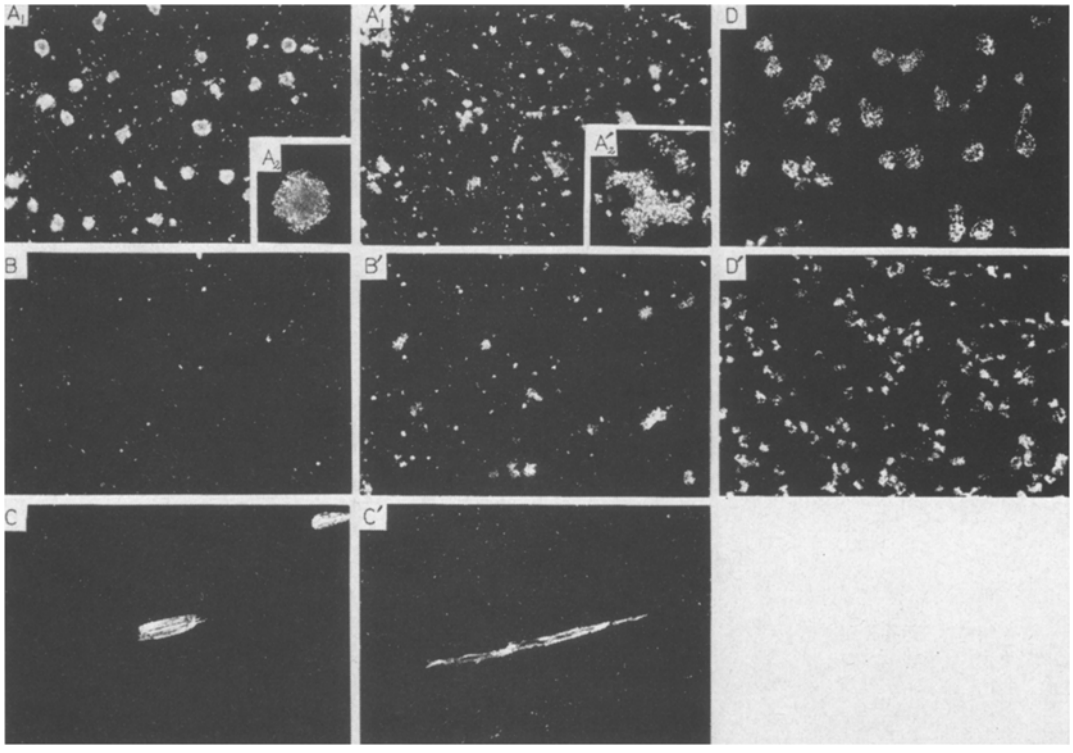
2. DeMV. Infection with virus had hardly any affect on the length of bundles of raphides in the leaves and on their distribution density, but the number of crystal sand tended to increase in affected leaves.

B. vulgaris var. *cicla*, which contains crystal sand colonies, was used as the host plant and its leaves were infected with coltsfoot mosaic virus. In this case, crystal sand colony was generally smaller than those in healthy leaves (Figure D), and the size of colonies in the affected leaves (Figure D') varied considerably, with shape becoming more complex. Distribution of crystal sand colonies tended to be higher in affected leaves.

It is quite clear from the foregoing results that the behavior of viruses in the host plant is reflected in the pattern of crystalline inorganic components in the host plant. In addition, the following conclusion may be drawn from the results of these observations: 1. The morphological principle of the crystals contained in the plants used in the present series of experiments remains unchanged even when the plant itself undergoes marked biochemical and morphological alterations. This fact can be applied to other plants with a considerably high probability. 2. When a plant possesses a pattern of

[1] K. Umemoto, Chem. pharm. Bull., Tokyo *23*, 1383 (1975); Yakugaku Zasshi (J. pharm. Soc. Jap.) *94*, 1627 (1974).

[2] K. Umemoto, Chem. pharm. Bull., Tokyo *19*, 217 (1971); Yaku gaku Zasshi (J. pharm. Soc. Jap.) *91*, 828 (1971).



Pattern of calcium oxalate crystals. *Nicotiana tabacum* Linn., A_1, healthy leaf; A_1', affected leaf; ×100. *N. tabacum* Linn., A_2, healthy leaf; A_2', affected leaf; ×400. *Nicotiana rustica* Linn., B, healthy leaf; B′, affected leaf; ×100. *Dendrobium*, C, healthy leaf; C′, affected leaf; ×100. *Beta vulgaris* var. *cicla*, D, healthy leaf; D′, affected leaf; ×100.

crystalline inorganic components markedly different from that in a healthy plant of the same kind, it is safe to conclude that at least some abnormality is present in the metabolic system of substances in that plant. 3. The pattern of crystalline inorganic components in the host plant can become an important indicator for probing the behavior of a virus in the host plant. Consequently, the pattern analysis of crystalline inorganic components may be expected to make a considerable contribution in the future for the elucidation of the proliferation mechanism of viruses and formation mechanism of crystalline inorganic components.

Poly(A) Associated RNA from Mitochondria and Microsomes of Rat Brain

A. CUPELLO[1] and G. ROSADINI

Centro di studio per la Neurofisiologia Cerebrale, Consiglio Nazionale delle Ricerche, Ospedale S. Martino, Genova (Italy), 19 January 1976.

Summary. Rat brain mitochondria contain a significant proportion of poly(A) associated RNA which is higher than that found in microsomes from the same source. When steady state poly(A) RNA of brain mitochondria was analyzed by microelectrophoresis, it displayed a characteristic separation pattern with a large amount of 'free' poly(A).

Most of eukaryotic messenger RNA's appear to be associated with a polyadenylate stretch at their 3′ end[2–5]. Whereas the function of this poly(A) 'tail' is not completely understood, it gives an important tool for mRNA isolation. In fact, poly(A) associated RNA may be isolated binding it specifically, in condition of high ionic strength, to Millipore filters[6], olygo(dT)-cellulose[7] or poly(U)-sepharose[8].

Recently the poly(A) associated fraction of RNA from rat brain polysomes was shown to be very active in an in vitro protein synthesis system[9]. On the other hand, microsomal poly(A) RNA isolated from the brain of myelinating rats can direct the synthesis of rat myelin encephalitogenic protein[10]. These results indicate that also the brain polysomal poly(A) associated RNA has the properties of messenger RNA.

Poly(A) associated RNA was detected also in mitochondria of HeLa cells[11,12] and thoroughly analyzed for sedimentation and electrophoretic characteristics[13]. As far as we are aware, no study on poly(A) RNA in brain mitochondria is at present available. Thus we studied poly(A)-associated RNA binding to Millipore filters in mitochondria of rat brain with regard to its amount and electrophoretic characteristics. We studied as well for comparison