

significant alteration. Only occasionally the membrane at one end of the mitochondria showed illdefined 'smeared' appearances. The electron-dense intramitochondrial bodies were of varied length and were sometimes disposed alongside the mitochondria axis. Their width was about 300 Å; they were located in the matrix and not connected with the mitochondrial cristae which were frequently parallel to them. The ultrastructure of the dense bodies demonstrate a certain periodicity of about 85–90 Å (Figure 2).

In general the intramitochondrial bodies described in the literature are varied in appearance. They are bound to the matrix or to the mitochondrial cristae<sup>6</sup>. Bodies located in the matrix, are described by many authors to be most frequently in liver or kidney cells<sup>1–3,9</sup>.

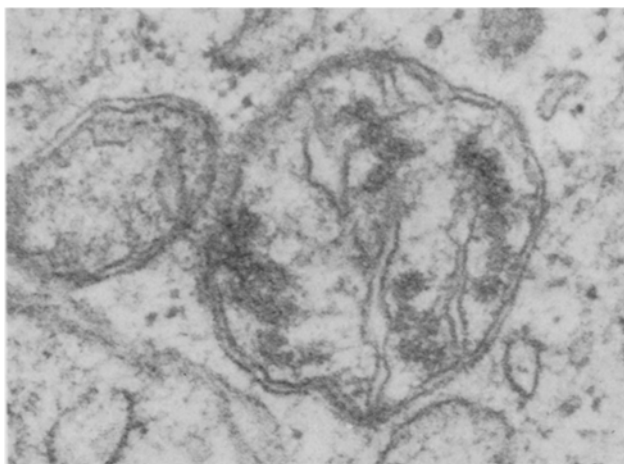


Fig. 1. Mitochondria containing several cross cut intramitochondrial bodies.  $\times 82,000$ .

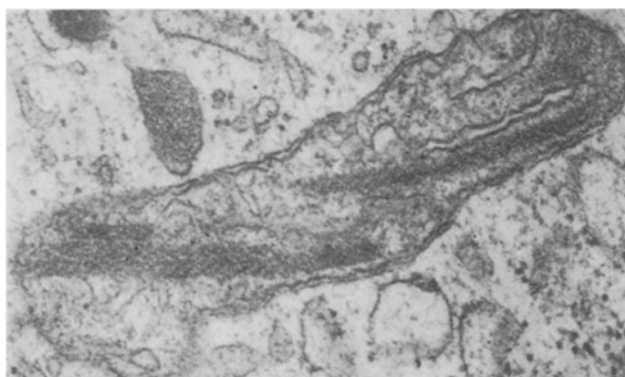


Fig. 2. Intramitochondrial body longitudinally cut. Its ultrastructure shows a certain periodicity.  $\times 60,000$ .

What is the origin and the function of these structures? After NASS and NASS<sup>14</sup>, the fine filaments depicted by them in the matrix of mitochondria in chicken embryos, represent DNA. After other authors, the filament bodies originate from the cristae as a result of disturbance of the phospholipid metabolism most frequently under the influence of some noxious effect<sup>3,4,15,16</sup>, or due to the peculiarities of breeding animals under laboratory conditions<sup>9</sup>.

The intramitochondrial bodies in the suprarenal cortex and in the testes are considered in relation to their possible participation in hormones formation<sup>6,17</sup>. After SVOBODA<sup>15</sup> they are an expression of degenerative changes or a manifestation of cell death in normal conditions in normal organisms<sup>4</sup>.

The appearance of intramitochondrial bodies in pancreatic B-cells is, in our opinion, related to stimulation – in this case by glybenclamide. It is possibly an expression of activating the enzyme systems in mitochondria for providing the cell with the necessary energy.

**Résumé.** Description des corps intramitochondriaux se trouvant dans les cellules B du pancréas du rat blanc stimulées par le glybenclamide (HB 419). Dans quelques uns de ces corps, une périodicité fut observée. L'apparition des corps intramitochondriaux serait liée à l'activation de certains groupes enzymatiques.

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## Location of the Avian Tumor Virus Group Specific Antigen in the BAI Strain A Virus Associated Myeloblast Cell

The group specific (GS) antigen of the avian sarcoma and leukosis viruses has been found in the soluble fraction of the cells infected with any of the avian tumor viruses<sup>1</sup>. However, none of the studies done so far have shown avian tumor virus GS antigen associated with any sub-cellular fractions, although electron microscope<sup>2</sup> and

fluorescent<sup>3</sup> antibody studies have demonstrated virus elaboration at the cell surface. In this study, using BAI strain A virus associated avian myeloblasts, and a technique for isolation of intact cell membranes, we have demonstrated that GS antigen is in fact associated with cell membrane fraction.

Myeloblasts collected from leukemic chickens<sup>4</sup>, and washed twice with medium 199 containing 50% chicken serum, were stored at  $-20^{\circ}\text{C}$  until needed. The cells were disrupted by 2 methods, one by breakage for 30 sec in a wig-L-Bug instrument, as described before<sup>5</sup> except the homogenizing medium used was 0.01 M phosphate buffered saline (PBS), pH 7.4, and the other by homogenization with a glass pestle. For the latter, 1 ml of frozen and thawed myeloblasts diluted to 10 ml with PBS were broken with 10 pestle strokes.

Cell membrane plus nucleus fractions were obtained from both types of preparations. Disruption with the wig-L-Bug yielded a layer of membranes of characteristic morphology, but only traces of the structures were seen in the phase contrast microscope after pestle breakage. The membrane-nucleus pellets were treated with sodium dodecyl sulphate (SDS) in 0.05% concentration. Mitochondria were sedimented at  $10,000 \times g$  for 10 min from the supernate of membrane-nucleus pellet. The resulting supernate was then centrifuged for 2 h at  $100,000 \times g$  to yield the microsome fraction, and the soluble fraction was the supernate after sedimentation of the microsomes. Nuclei without membranes were prepared from myeloblasts either by the wig-L-Bug or glass-pestle, isolated in a discontinuous sucrose gradient<sup>6</sup>, washed with PBS and treated with SDS, as were the nucleus-membrane fractions.

Microtechnique of Sever, as already described<sup>6</sup>, was used for measuring complement fixation (CF) values. The CF titre is expressed as the reciprocal of the highest antigen dilution giving 100% fixarion. Serum pool obtained from hamsters bearing transplanted S-R tumors<sup>7</sup> used in this study had a CF titre of 64 or more and did not react with normal chick tissue. The serum was inactivated at  $56^{\circ}\text{C}$  for 30 min.

The results revealed a marked difference in antigen distribution between the fractions obtained by pestle homogenization and those derived by wig-L-Bug breakage (Table). In the latter, most of the antigen was associated with the fraction containing membranes, in contrast to the pestle preparations in which the antigen was primarily in the soluble fraction and in much greater concentration than in the soluble fraction obtained with the wig-L-Bug. Variable and relatively small amounts of antigen were associated with the mitochondrial and microsome fractions. The preparations of nuclei showed

no trace of antigen. The data indicate that the antigen is primarily concentrated in the cell membrane which can be isolated in quantity from wig-L-Bug preparations but of which only traces remained after pestle homogenization. In this latter case, the antigen was extracted from the destroyed membranes and appeared in the soluble fraction.

Electron microscopic<sup>2</sup> studies have not shown many virus particles in the myeloblast cell cytoplasm, although there was virus elaboration by budding at the cell surface. Fluorescent<sup>3</sup> antibody studies with GS hamster serum have demonstrated the GS antigen inside the viral particle at the cell surface in the cells actively elaborating the virus. Biochemical studies done with the virus infected chick fibroblasts also involve trypsin released cell surface structures as the site of virus synthesis<sup>8</sup>. In this study, when intact myeloblast cell membranes were prepared and tested for GS antigen against hamster serum, most of the whole cell homogenate activity was found in the membranes whereas the cell soluble fraction in the same preparation showed a correspondingly decreased titre. Since in all homogenization procedures cell membranes are destroyed, it seems very plausible that some membrane antigen is released into the soluble fraction during homogenization.

The finding that the viral antigen is associated with cell membrane may be of importance for the understanding of the site of the synthesis of this virus in the myeloblast host cell. The present evidence, coupled with the fluorescent antibody and electron microscope studies, strongly suggests that the BAI strain A virus is synthesized in association with the cell membrane of the infected cell.

*Zusammenfassung.* Zellfraktionen von Myeloblasten leukämischer Hühner wurden auf das Vorliegen des für die Hühnerleukoseviren gruppenspezifischen Antigens untersucht. Das Antigen fand sich vorwiegend in der Fraktion der Zellmembranen.

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Percent of the total whole cell homogenate CF activity in different cell fractions obtained from wig-L-Bug and pestle homogenization

| Cell fractions   | Wig-L-Bug | Pestle homogenization |
|------------------|-----------|-----------------------|
| Membranes        | 62.5      | 3.7                   |
| Mitochondria     | 12.5      | 7.4                   |
| Microsomes       | 6.3       | 14.8                  |
| Soluble fraction | 18.7      | 74.1                  |

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<sup>9</sup> This work was done while the author was at Duke University Medical Centre, Durham, (N.C., USA).

## The Influence of Farnesenic Acid Ethyl Ester on the Differentiation of *Kaloterme flavicollis* Fabr. (Isoptera) Soldiers

LÜSCHER'S<sup>1</sup> experiments demonstrated the role of the corpora allata in the differentiation of *Kaloterme flavicollis*; moreover<sup>2,3</sup> it was found that the juvenile hormone and several analogous substances, when administered in various ways, induce differentiation of soldiers and intercastes both in *K. flavicollis* and in *Reticulitermes*

*lucifugus*. But while the fact itself is now a certainty, observations are lacking on the relationship between dose and effect, as well as on the side effects of treatment.

We treated *K. flavicollis* pseudergates with different doses of a crude preparation of farnesenic acid ethyl ester (FAEE)<sup>4</sup>. The preparation was dissolved in acetone,