es sich nur um sehr geringe Mengen (< 0,05% i.T.) handeln. – Auch in *Equisetum arvense* fanden wir eine geringe Maltosemenge, die hier ebenfalls keine Bedeutung als Speicherkohlenhydrat haben dürfte.

Durch autoradiographische Untersuchungen konnte KANDLER[13] zeigen, dass Maltose in höheren Pflanzen weit verbreitet ist. Dabei handelt es sich aber häufig um Mengen, die papierchromatographisch nicht fassbar sind und damit speicherungsphysiologisch keine Rolle spielen, wie auch neue Untersuchungen von KANDLER[14] gezeigt haben[15].

Summary. Some older results on the occurrence of maltose in higher plants have been verified; semiquantitative estimations were made. Also maltose is detected in *Aconitum lycoctonum* and in *Equisetum arvense*.

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Botanisches Institut der Technischen Hochschule Stuttgart (Deutschland), 15. Oktober 1965.

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[15] Der Deutschen Forschungsgemeinschaft danken wir für Sachbeihilfen.

The Demonstration of Lysosomes in the Bovine Adrenal Medulla

Recent work has demonstrated the presence of six acid hydrolases in homogenates of bovine adrenal medulla[1–3]. By the use of centrifugation on a sucrose density gradient it was found that these enzymes were concentrated in a fraction distinct from both the chromaffin granules and the mitochondria. The enzymes studied (acid phosphatase, acid DNase, acid RNase, β-glucuronidase, cathepsin and arylsulphatase) are characteristic of cell organelles defined as lysosomes by DE DUVE[4,5]. Several studies of the acid phosphatase content of adrenal medullary cells have been reported[6,7], but no definite intracellular localization of this enzyme has been described; COUPLAND[7] suggested that it may be associated with the occurrence of lysosomes.

Although the results quoted suggested very strongly that lysosomes were present in chromaffin tissue[2,3], it seemed desirable to confirm this by applying histochemical and electron-microscopic techniques to tissue sections, using acid phosphatase as a marker enzyme. Such methods would make it possible to determine the precise morphological location of the enzyme.

Methods. Small pieces of bovine adrenal medulla, obtained from the slaughterhouse, were fixed without delay for 3 h at 4 °C in a 3% solution of glutaraldehyde buffered with cacodylate to pH 7.4. The fixative was washed out with the same buffer overnight (4 °C) and frozen sections were prepared for histochemistry: sections were 10 μ thick for optical microscopy and 30 μ thick for subsequent electron microscopy. The sections were incubated at 37 °C for 15, 30 and 60 min in a fresh β-glycerophosphate medium (pH 5.0) prepared as described by GOMORI[8]. After incubation the sections were washed for 1 min in 1% acetic acid, in order to remove any non-specific adsorption of lead ions.

Sections intended for optical microscopy were treated with a dilute solution of yellow ammonium sulphide to demonstrate the reaction product; sections to be used for electron microscopy were treated for 1 h in a 1% solution of osmium tetroxide at room temperature before dehydrating in a graded series of alcohols, embedding in Araldite and preparing 800 Å thick sections for examination. Some of these sections were examined without further treatment, but others were stained with Reynolds' lead citrate for 8 min in order to increase their contrast. Control incubations were carried out in the absence of substrate and also in the Gomori medium plus 0.01 *M* sodium fluoride.

Results. With the light microscope acid phosphatase was found to be present in the bovine adrenal medulla, but rather long incubation periods were required. After incubation for 30 min the reaction product was localized to a few granules scattered throughout the cytoplasm of the chromaffin cells (Figure 1). After 60 min there was only a slight increase in the intensity of the reaction. There was no reaction in either type of control. These results confirm those of COUPLAND[7].

Electron-dense bodies, about 0.6 μ in diameter and bounded by a membrane, were seen on electron-microscopic examination of fresh tissue that had been fixed immediately in glutaraldehyde followed by osmium. These dense bodies were much less frequent than chromaffin granules, only one or two being seen in any section of a cell (Figure 2). These organelles had a variable electron density; such a polymorphic appearance is consistent with the morphological concept of the lysosome[5].

Electron microscopy was also used for sections that had been incubated in the Gomori medium, since it has been shown that the acid phosphatase test is specific and capable of application at the level of resolution afforded by the electron microscope[9,10]. When this procedure was

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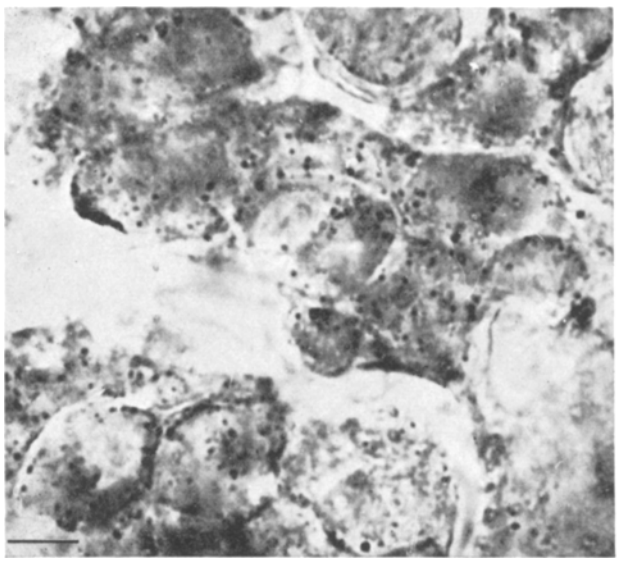

Fig. 1. Photomicrograph showing the granular localization of acid phosphatase in the cells of the adrenal medulla. The scale indicates 10 μ.

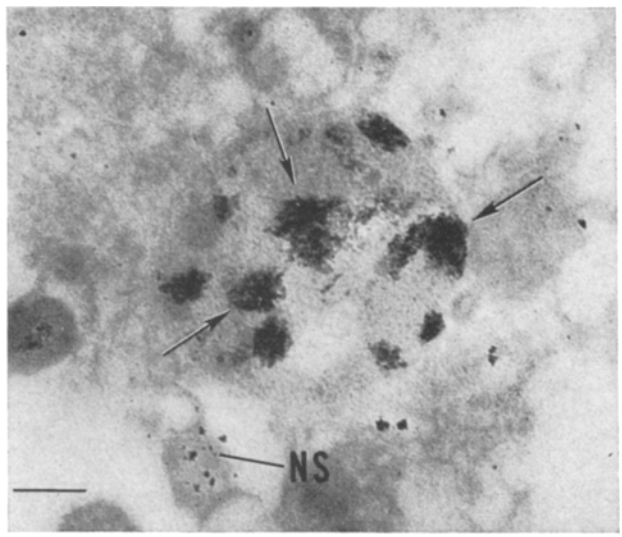

Fig. 3. Electron micrograph of a tissue section demonstrating acid phosphatase. Large granular deposits of lead phosphate (arrowed) are present in the lysosome. Occasional non-specific (NS) deposits of lead also occur. This section was not stained (cf. *Figure 4*). The scale indicates 0.1 μ.

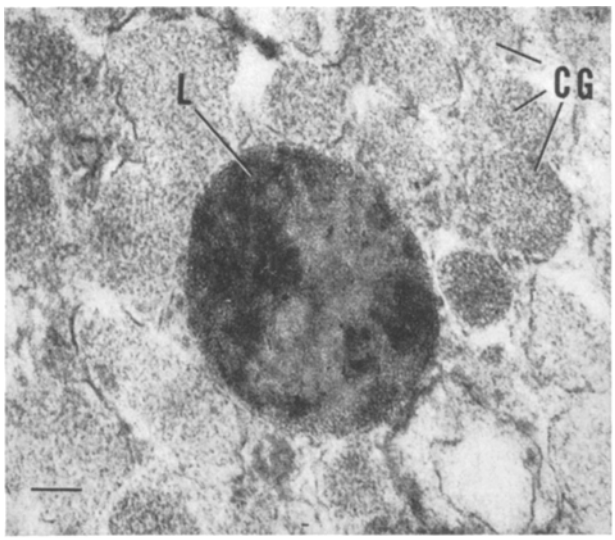

Fig. 2. Electron micrograph of part of adrenal medulla cell. Note the lysosome (L) surrounded by smaller chromaffin granules (CG). The scale indicates 0.1 μ.

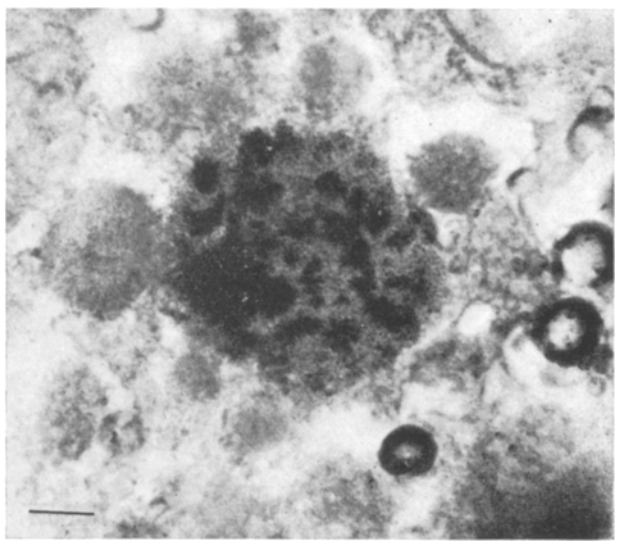

Fig. 4. Electron micrograph of a tissue section incubated for acid phosphatase, and further stained to increase the contrast. The reaction product in the lysosome is clearly visible.

applied to chromaffin cells (Figures 3 and 4) electron-dense deposits of lead phosphate were found in the polymorphic bodies described above. The polymorphic bodies were entirely free from lead deposit in either type of control.

Some slight electron-dense deposits were seen in other parts of the cell with both experimental and control incubated tissues. These deposits were associated with myelin figures (which were *only* seen after incubation) or with membranous elements in the cytoplasm. It seems likely that these deposits represent non-specific adsorption of lead ions.

Histochemistry thus demonstrates that the polymorphic bodies seen with the electron microscope contain acid phosphatase, and therefore fulfil both the morphological and one of the biochemical criteria of lysosomes. No reaction product was seen in chromaffin granules under the conditions of our experiments, a finding which differs from that of HILLARP and FALCK[11] in their early

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biochemical studies. COUPLAND[12] suggested that electron-dense bodies in rat adrenal medulla (which morphologically resemble those seen in our micrographs) were lysosomes. Our biochemical work and histochemical studies with the electron microscope confirm the interpretation that in the adrenal medulla there are three distinct cell organelles: mitochondria, chromaffin granules and lysosomes.

Zusammenfassung. Die Gomori-Reaktion für saure Phosphatase wurde an Nebennierenmarkschnitten von Rindern durchgeführt. Ihre elektronenmikroskopische Untersuchung ergab die Lokalisierung der Enzymaktivität in den Lysosomen. Chromaffine Granula und Mitochondrien zeigten keine Aktivität.

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University Departments of Human Anatomy and Pharmacology, Oxford (England), November 1, 1965.

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Changes of Chromosome Number in Cells of *Drosophila melanogaster* Cultured in vitro

It is well known from investigations on mammalian chromosomes that the karyotype of diploid cells cultured in vitro tends to become heteroploid as the culture ages (HSU and MOORHEAD[1,2]). The occurrence of heteroploidy may be related to the other changes in the growing cells as they become adapted to the artificial conditions of the culture. Obviously, such variability in chromosome number is a serious limitation to genetical analysis of cultured cells, because stability of the karyotype is a prerequisite for such studies (DE CARLI, MAIO, NUZZO, and BENERECETTI[3]).

Two hypotheses have been proposed to explain the changes of chromosome number that occur in cultured cells (WESTWOOD and TITMUSS[4], RUDDLE, BERMAN, and STULBERG[5]): (a) heteroploidy may result from non-disjunctions and other mitotic abnormalities, or (b) heteroploidy may be a secondary phenomenon, consisting of losses of individual chromosomes following a primary occurrence of polyploidy.

Since it is difficult in mammalian cells to identify all chromosome pairs unambiguously, no definite choice between these alternatives can be made as yet.

Cells of *Drosophila melanogaster* could be favourable material for studying variations in karyotype, since the identification of each of the 4 chromosome pairs is easy. Although attempts to obtain dividing cells of *Drosophila* in vitro have been largely unsuccessful for many years, there is now an excellent technique for culturing embryonic cells which continue to grow and divide at a high rate for long periods of time (HORIKAWA and FOX[6]). The data on variations of chromosome number of cultured cells of this insect, however, are still scanty.

The technique used in this study was that of HORIKAWA and FOX[6]. Eggs of the Varese wild strain laid over a period of 6 h were used for obtaining the embryonic cells. These were placed in H-5 medium supplemented with 10% new-born calf serum, and cultured at 30 °C. The chromosome analyses were made on squashes prepared 12, 18, 21, 24, 48, 72, 96 and 120 h after the cultures were begun. The cells from each culture were pretreated in an hypotonic solution of 1% sodium citrate for 10 min, then stained in aceto-lactic orcein (OSTER and BALABAN[7]) for 25–30 min. Chromosome counts were made on metaphases; only those which could be drawn unambiguously have been considered.

One to four cultures were made for each time interval, and because of the homogeneity of results from replicates the data obtained have been pooled (Table). In any case the number of cells analysed is more important than the number of cultures, since each culture contains cells derived from 2000–3000 eggs. In the first analysis (12 h), some heteroploid cells (11.9%) are already present in the culture. The frequency of heteroploid cells increases to 29.7% at 18 h, but no tetraploid cells were found. This value remains nearly constant for 48 h. During the interval between 48 and 72 h, the percentage of heteroploidy increases to 78.5% and then remains practically stable until 120 h (Figure 1). Polyploid cells were virtually ab-

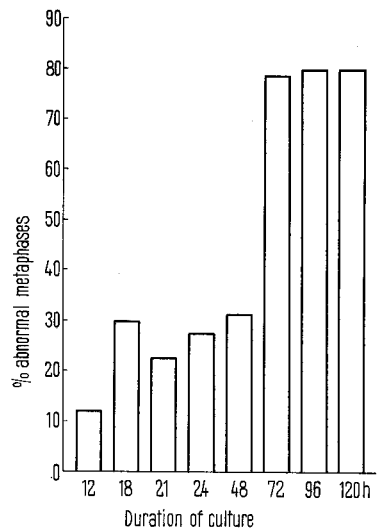

Fig. 1. Changes in percentage of abnormal metaphases of embryonic cultured cells at different stages of culture.

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