As was mentioned, the starch gel electrophoresis of both F2a and F3 histones shows the presence of at least two electrophoretic components in each of the fractions. The described dialysis against *n*-propanol and ethanol can fractionate the F2a histones into two components: the $F2a_I$ which is electrophoretically faster and the $F2a_{II}$ with slightly lower electrophoretic mobility in starch gel (Figure 2). These two fractions differ significantly in their content of several amino acids (e.g. arginine, lysine, alanine, glycine, etc.). The electrophoretically slower and still composite $F2a_{II}$ histone has, in addition, a higher ratio of leucine to isoleucine (Table).

The NH_2 terminal amino acid of the F3 histones is alanine (95%); proline, alanine glycine, and lysine were found in various amounts in the F2a fraction. In general, the recovery of DNP amino acids was very low in the F2a histones, indicating the inaccessibility of the NH_2 terminal for dinitrophenylation[3]. The same was found for the two subfractions $F2a_I$ and $F2a_{II}$.

The discovery of a lipid-bound histone fraction is not entirely new. BAKAY et al.[9] mentioned the presence of a small amount of lipid-like material in nucleohistone, but no composition was reported. A lipid material persistently appeared in the X-ray diffraction patterns of native calf thymus nucleohistones and could not be removed by purification of the nucleohistone by dissolving in water and precipitating with 0.14 *M* NaCl (the 60 Å spot). This indicates that the lipid is firmly attached to nucleohistone[10], and WILKINS suggested the possible presence of sphingomyelins in nucleohistones[11]. The phosphate groups of such lipids could easily combine with the seryl residues found in the 'lipo' histone (Table). WILKINS, on the basis of X-ray diffraction, estimated the amount of sphingomyelin-like lipid in calf thymus nucleohistone as 3%. This value is very close to the observed yield of the 'lipo' histone described in this paper. The amount of this material varies significantly in nucleohistones of different origin, with liver being about the richest source of such protein[12].

Zusammenfassung. Die argininreiche Fraktion des Kalbsthymushistons (Fraktion 2a) wurde in zwei Komponenten zerlegt. Zusammensetzung und elektrophoretische Trennung der Komponenten ($F2a_I$ und $F2a_{II}$), ebenso die Eigenschaften eines neuen, lipidgebundenen, serinreichen Histons wurden beschrieben.

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Phase Contrast Microscope Observations on the Pancreas of Fasting Rats

The morphological and histochemical changes observed in the pancreas of rats kept under conditions of prolonged fasting have been reported in previous papers[1,2]. They can be summed up as severe cytoplasmic damage, nuclear lesions, swelling and progressive disappearance of the mitochondria, together with a sharp reduction in the DNA and RNA content.

It has been previously indicated, however, that these changes are not accompanied by a notable reduction in the zymogen granules, which are present even after prolonged fasting (150–160 h). All these results have been obtained using classic histological and histochemical methods, such as Heidenhain's technique with iron-hematoxilin and Brachet's with methyl green-pyronine. The present study has been undertaken to try to resolve the problem of the persistence of the zymogen granules after 160 h fast.

Ultra-thin sections of rat pancreas were subjected to phase contrast microscopy, some of the specimens being previously subjected to silver impregnation.

Materials and methods. Twenty albino rats (Sprague-Dawley strain) weighing about 200–250 g, were kept under conditions of complete fasting, apart from ad libitum availability of water, for 160 h.

Another ten rats were kept on a standard diet as control. The animals were killed by decapitation, and small pieces of the pancreas were fixed immediately in 2% osmium tetroxide buffered at pH 7.2–7.4 (after Palade), dehydrated with ethyl alcohol and embedded in butyl-methyl-metacrilate. Sections of 1000–2000 Å thickness were cut, using a Porter-Blum microtome equipped with a glass knife. The sections, distended with chloroform vapours, were placed on cover-slips and observed in phase contrast under a Leitz Ortholux microscope equipped with objectives for high contrast. Some specimens were subjected to silver impregnation, according to JONES' technique[3] as modified by MARINOZZI[4], and following precautions reported elsewhere[5].

Results and discussion. The use of this technique confirms some data already provided by morphological and histochemical studies and also gives some new information.

Nuclear damage is visible, with optically empty nuclei. The nucleoli are often missing or very much reduced in

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size (Figure 2). This is in agreement with data obtained after staining with methyl-green pyronine and Feulgen's reaction. The zymogen granules appear less numerous than in the control specimens, but they are always present in spite of the evident cytoplasmic shrinkage and histochemical modifications mentioned above (Figure 2).

A new question is raised, however, by the behaviour of the centro-acinar cells. In previous experiments these cells have not been taken into consideration for technical reasons, but they are clearly visible under phase contrast. With this technique one can observe, in the centro-acinar cells of the normal pancreas, the ergastoplasm in the form of fine filaments or laminae which occupy most of the cytoplasm (Figure 1). After 160 h fast these cells are visible with shrunken cytoplasm and with the ergastoplasm arranged concentrically in an optically denser form and made up of thicker laminae (Figures 2 and 3). On the other hand, the nuclear and nucleolar damage in these cells seems to be less pronounced than in the exocrine cells. The nuclei still have a certain chromatin content and the nucleoli, although smaller in volume, are still present. A characteristic of these nucleoli is their optical density.

Regarding nuclear and nucleolar damage to the exocrine cells, the results of the present study confirm those previously obtained using other techniques. It is further confirmed that the zymogen granules of the exocrine cells do not completely disappear, but remain in reasonable numbers, despite the notable cellular damage. The study also indicates ergastoplasmic damage to the centro-acinar cells, accompanied by cytoplasmic shrinkage. The feature of the concentric arrangement of the ergastoplasm of the centro-acinar cells brings to mind those observed by EKOLM et al.[6,7] in the ergastoplasm of exocrine cells taken from the pancreas of animals subjected to ethionine or to obstruction of the excretory duct[8].

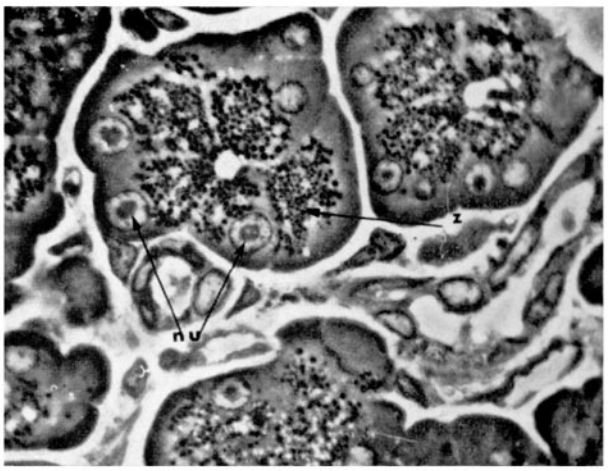

Fig. 1. Pancreas of rat. Pancreatic acinus containing numerous zymogen granules (Z). Sizeable nucleoli (nu) and finely divided nuclear chromatin are visible. Phase contrast, after silver impregnation. About 1000 ×.

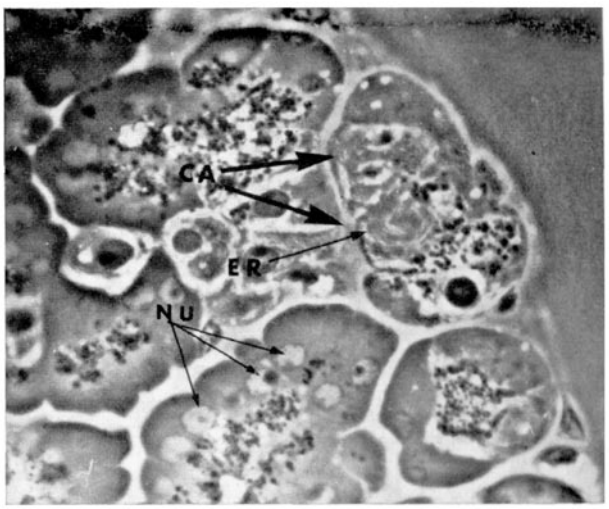

Fig. 2. Pancreas after 160 h fast. Shrunken cells with optically empty nuclei and small nucleoli (NU). Note a group of centro-acinar cells (CA) with very dense ergastoplasm arranged concentrically (ER). Phase contrast after silver impregnation. About 1000 ×.

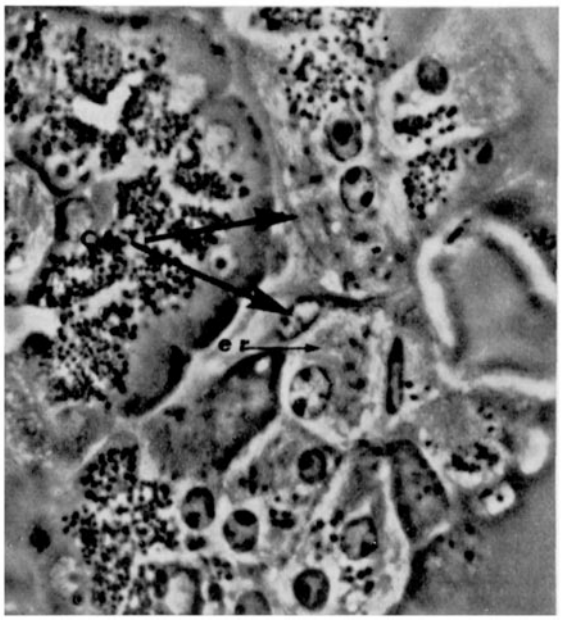

Fig. 3. Pancreas after 160 h fast. Group of centro-acinar cells (CA) with characteristic concentric arrangement of the ergastoplasm (er). The nucleoli of these cells are better conserved than those of the exocrine cells. Phase contrast after silver impregnation. About 1000 ×.

Riassunto. L'osservazione di sezioni ultrasottili di pancreas esocrino di ratti sottoposti a digiuno conferma i risultati delle precedenti ricerche e ne fornisce di nuovi sopratutto nei riguardi delle cellule centro-acinose.

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