secretion of 2 dogs in each of these groups was stimulated by 3 U/kg b.w. of secretin (Boots) injected into the femoral vein. HRP or carbon particles were infused intraductally when the effect of secretin had worn off. Two other dogs served as controls. Specimens from pancreatic tissues were fixed 30 min after intraductal infusions.

All the specimens from rat and dog pancreas were immersed into a +4 °C solution of 2% formaldehyde – 2.5% glutaraldehyde in 0.13 *M* sodium cacodylate-HCl buffer (pH 7.2). To demonstrate carbon particles, the fixed specimens were dehydrated and embedded in plastic (Durcupan ACM). Thin sections for electron microscopy were stained with uranylacetate and lead citrate. To demonstrate HRP, the fixed specimens were washed in 0.13 *M* cacodylate buffer, then cut into 50 µm slices and preincubated in the same buffer but containing 50 mg % 3, 3′-diaminobenzidine tetrahydrochloride (DAB) for 30 min. The slices were then incubated in the buffer containing 50 mg % DAB and 0.01% H_2O_2 for 30 min and fixed in 2% OsO_4 solution. All the specimens were dehydrated using ethanol and embedded in Durcupan ACM. Unstained ultrathin sections were examined.

Results. After the intravenous injection of HRP, peroxidase activity was occasionally seen in the pinocytotic vesicles of the acinar cells. A reaction product was, however, present in the interstitial space surrounding acini and acinar cells. The intercellular junctions of adjacent acinar cells obstructed the interstitial passage of HRP into the lumen (Figure 1). Carbon particles, however, infused by retrograde way entered secretin-stimulated acinar and centro-acinar cells. Despite the low infusion pressure, the luminal surface membranes were ruptured (Figure 2). By this way the intraductally infused HRP certainly entered the interstitial spaces around the acini and acinar cells (Figures 3 and 4).

Discussion. The crucial point in the pathogenesis of acute pancreatitis is: By which pathways can the digestive enzymes escape the excretory duct system and reach the interstitium in bulk? On the basis of histological observations it has been supposed that, due to the increased pressure, carbon particles or dyes injected intraductally may enter the interstitium through the acinar cells[10], intercellular gaps[10,11] or through intact cells bordering the isthmic segments of the excretory ductules[12]. The passage of different markers infused intraductally had been studied, and it was shown that carbon particles may enter seemingly intact acinar cells[13], thorotrast may reach the interstitium through spaces between acinar cells[14,15], without rupture of the excretory duct system, or intravenously given HRP can reach the lumen occasionally through some ductular cell junctions[6], because the interstitial spaces between acinar cells and most of spaces between centroacinar cells are occluded by zonulae occludentes[7,8].

The results presented here support the idea that enzymes may reach the gland interstitium even at low pressure through the ruptured acinar and centroacinar cells and possibly some interductular cell junctions[6]. However, another problem arises: Why is a rich ingested meal so rarely followed by pancreatic autodigestion though the pressure during digestion rises to the same or even to higher values[16] than we used in this experiment, resulting in the rupture of some acinar and centro-acinar cells and escape of ductal HRP or carbon particles into the interstitial spaces?

[10] A. R. RICH and G. L. DUFF, Bull. Johns Hopkins Hosp. *58*, 212 (1936).
[11] A. DUPREZ, S. GODART, R. PLATTERBORSE and J. M. DUPONT, in *Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas* (Eds K. HEINKEL and H. SCHÖN; Schattauer Verlag, Stuttgart 1964), p. 207–213.
[12] W. DOERR, Verh. dt. Ges. Path. *37*, 292 (1954).
[13] H. L. NUDELMAN, B. L. MUNGER and H. R. BERNARD, J. Am. Med. Ass. *192*, 387 (1965).
[14] Y. EDLUND, R. EKHOLM and T. ZELANDER, Acta chir. scand. *125*, 529 (1963).
[15] R. L. WALDRON, S. A. LUSE, H. E. WOLLOWICK and W. B. SEAMAN, Am. J. Roentg. *111*, 695 (1971).
[16] J. T. WHITE, R. G. ELMSLIE and D. F. MAGEE, Surgery Gynec. Obstet. 118, 1043 (1964).

Pyridine as an Unmasking Reagent for Lipoprotein Complexes in the Nervous System of Protein Deficient Squirrel Monkeys

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Summary. Acid hematin test with pyridine and Sudan black B controls was employed on selected areas of the brains of 115, 140 days fetuses, neonates and adult squirrel monkeys maintained on low and high protein diet. Our histochemical findings indicate that the reduction of phospholipids in the low protein fetuses and neonates is related to myelination, whereas in the adults, most of the lipids are bound to proteins and/or cerebrosides to form complexes, as revealed by the unmasking action of pyridine.

Preparations from a total of twenty-six animals were studied. A colony of 64 healthy female squirrel monkeys (*Saimiri sciureus*) were maintained in outdor cages for the purpose of breeding. Out of the 59 animals that became pregnant 35 were maintained on a diet low in protein content (4% and 8% protein calories) and 24 animals were fed a diet similar in composition, but high in protein content (25% protein calories) starting from 35 days after conception. Fetuses from animals maintained on low and high protein diets were removed at 115 days and 140 days after gestation. The neonates delivered at full term (175–178 days) and the young adults (3 years of age) were also used. The various histochemical tests employed for lipids are given in the table. The Baker's acid hematin test followed by its pyridine extraction control gave the most interesting results in the course of lipid formation during the period of brain development. In the 115 days and 140 days old fetuses (born to mothers given low and high protein diets), the acid hematin test gives moderate activity which persists more or less unchanged after pyridine extraction. At this stage some diffuse staining of the neuropil is also observed before pyridine extraction, which, as ALMEIDA and PEARSE[2] have stated, may indicate temporary storage of sphingolipid, to be used later in the process of myelination. By the time the animals are born at full term, the acid hematin staining with

Histochemical reactions of lipids in the selected areas of the nervous system of squirrel monkeys[a]

Techniques	115 days fetuses		140 days fetuses		Neonates		Adults	
	HP	LP	HP	LP	HP	LP	HP	LP
Sudan black B (Room temperature)	++ to +++	++ to +++	++ to +++	++ to +++	++ to +++	++ to +++	++ to +++	++ to +++
Sudan black B (60 °C)	++ to +++	++ to +++	++ to +++	++ to +++	++ to ++++	++ to ++++	++ to +++	++ to +++
Sudan black B (after pyridine extraction)	++	++	++	++	+++ to ++++	+++ to ++++	+++	+++
Acid hematin	++	++	++	++	++	+	+ to ++	+ to ++
Acid hematin (after pyridine extraction)	++	++	++	++	+++ to ++++	+++ to ++++	+++	+++

[a] The areas studied in detail include cervical spinal cord, medulla oblongata, thalamus, capsula interna and tractus opticus.
+ = Weak reaction; ++ = moderate reaction; +++ = strong reaction; ++++ = intense reaction.

the Baker's method is reduced, in the animals born to mothers maintained on a low protein diet during gestation. In the animals born to mothers maintained on high protein diets, the acid hematin staining remained unchanged. This is especially true in the medulla oblongata and the fiber system in the thalamus, capsula interna, tractus opticus, etc. In the white matter of spinal cord the improvement in acid hematin staining after pyridine extraction is not so dramatic as in the diencephalon area. This is because the white matter in the spinal cord is richer in lipids (including phospholipids) than the brain white matter, on account of a more abundant sheath supply in the former. When the sections of both low and high protein animals are extracted with pyridine at room temperature and at 60 °C for a period of 16 h and 24 h, respectively, and stained with acid hematin, the staining is significantly enhanced (Figures 1 and 2). In the adult animals maintained on low protein diets, the acid hematin is weak to moderate but becomes prominent after pyridine extraction. It appears that as the myelination proceeds, the amount of phospholipids demonstrable by the acid hematin method, accompanied by its pyridine extraction control, greatly decreases. It is possible that after myelination is completed, most of the lipids are more or less bound with other constituents such as proteins and/or cerebrosides, unlike those in the fetuses and neonates.

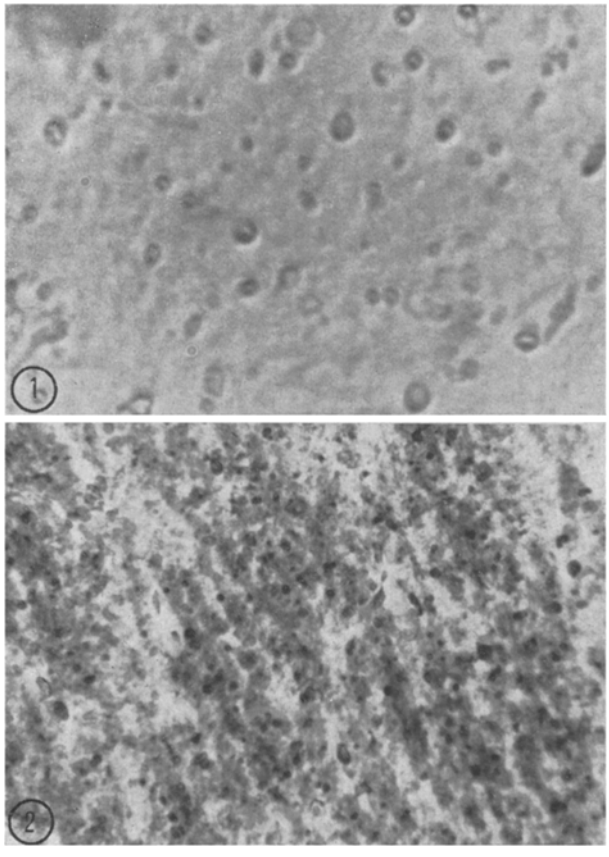

Fig. 1 and 2. Tractus opticus from squirrel monkey brain stained with acid hematin (Fig. 1) and acid hematin after pyridine extraction (Fig. 2). Note the sharp increase in staining after extraction with pyridine for 16 h at room temperature, followed by 24 h treatment at 60 °C.

In the ongoing studies the combined acid hematin and pyridine method was always controlled by Sudan black B staining after extraction.

Since the acid hematin test is performed on formaldehyde fixed tissues, it is possible that during formalin treatment some of the phospholipids stainable with acid hematin test are lost, and hence somewhat lesser quantities of phospholipids are demonstrable with the acid hematin method in the low protein animals. It is also probable that the phospholipids in the animals which have suffered a certain degree of dietary protein deficiency are more labile than those in animals fed high protein diets. The early losses may be of cephalins to be followed by other types of phospholipids[3]. In the myelin sheathes, the phospholipids present seem to be phosphatidyl serine, plasmalogens, sphingophopholipids and a little lecithin, but in most part the cholesterols and cerebrosides predominate, and they may become better stained after pyridine extraction in addition to complexes of lipoprotein nature, because it is known that acid hematin stains, in addition to phospholipids, proteins such as hemoglobin, fibrinogen, collagen, caseinogen and mucin, as well as nucleoproteins. Some of them in the bound form require pyridine extraction to become visible or stained with regular lipid stains.

It is believed that animals on low protein diet have either less amounts of phospholipids, or else they are of labile nature and predominate in lipoprotein complexes, cerebrosides, etc., which are unmasked after pyridine treatment and can thus be stained by acid hematin.

[1] Acknowledgments. This work was supported by U.S. PHS grants RR-00165 HD-06087 from National Institute of Health.

[2] D. F. Almeida and A. G. E. Pearse, J. Neurochem. *3*, 132 (1958).

[3] A. G. E. Pearse, *Histochemistry, Theoretical and Applied* (The Williams and Wilkins Company, Baltimore I. 1968).