Experimental Hylaline Droplets in the Rat Adrenal Cortex
Immunohistochemical and Enzyme Histochemical Studies

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Summary. Hyaline droplets and protein-containing vacuoles were produced in adrenocortical cells of female albino rats by methylandrostenediol administration. The lesions were shown to be invariably negative with the method for alkaline phosphatase, but they showed a varying degree of positive staining with the reactions for acid phosphatase and nonspecific esterases. The denser and more regular “droplets” tended to exhibit a more regular and stronger enzymatic activity, whereas the lesions of the “vacuole” type containing a less dense, presumably watery material; those regarded as “younger” were only faintly, if ever, positive with the methods for acid hydrolases.

With the immunohistochemical method the authors were able to demonstrate the presence of homologous plasma proteins within the lesions. Most of the droplets and vacuoles were shown to contain albumin, and some of them could be demonstrated to contain also globulin and fibrin in varying amounts. The simultaneous use of different antibodies labelled with contrasting fluorescent dyes as fluorescein isothiocyanate and lissamine rhodamine B 200, disclosed that most droplets were composed of a mixture of plasma proteins. With that method, it was possible to visualise inhomogeneities in the composition of droplets which largely corresponded to those described previously in preparations stained with conventional histological techniques as well as with some histochemical methods.
The authors conclude that this type of intracellular "hyaline" deposit represents a rather common reaction of adrenocortical cells and that it is comparable to the protein absorption droplets, phagosomes and their subsequent developmental stages known in other organs, thus being related to the pinocytotic-lysosomal system of the adrenocortical cell.

The problem of hyaline droplet formation within the adrenocortical cells has been discussed in considerable detail in several previous papers of ours devoted, in part, to hyaline droplet formation within the adrenal gland of human beings and, in part, to experimental hyaline droplet production in the rat (Motlík and Janoušková, 1960, 1963).

From the morphological point of view, we have stressed the differences of hyaline droplets in size and shape, as well as differences in their affinity to certain stains. Some "droplets" were shown to be of a rather irregular shape, more-or-less resembling "lakes" of hyaline material, but such formations were shown to exhibit gradual transitions to ordinary rounded intracytoplasmic hyaline bodies. The same is true of the so-called "protein-containing vacuoles" (PCV), intracytoplasmic vacuoles containing mostly irregular clots of hyaline material which was frequently shown to assume the shape of crescents closely adherent to the walls of the vacuoles (confront with the "crescent-shaped bodies" of Selye; Selye and Salgado) and to show, too, gradual transitions to true hyaline droplets of conventional morphological appearance.

The review of the literature as well as the findings of ours have led us to the preliminary conclusion that adrenocortical HD represent a lesion of variegated etiology and pathogenesis. However, a large proportion of them were believed to represent a pathobiotic lesion presumably associated with an intake of some proteinaceous material into the cell rather than representing a process of secretion or discharge. Furthermore, some of the morphological as well as histochemical findings indicated that the material ingested could have originated from the blood plasma and that the adrenocortical HD formation could be possibly regarded as secondary to some phenomena accompanying increased adrenocortical stimulation, particularly increased capillary permeability rather than related to the cellular secretory activity as such. The following investigations were undertaken to check this presumption.

**Material and Methods**

The experiment was carried out with the aim to demonstrate the presence of some enzymes and of plasma proteins within the experimental adrenocortical lesions of rats subjected to methylandrostenediol (MAD) or MAD+somatotropin (STH) administration, known to produce these lesions regularly in sufficient amounts (Motlík and Janoušková, see also for further literature).

Twenty-four female albino rats of the Wistar strain (Experiment V·66) whose average weight was about 130 g at the beginning of the experiment were used. The experiment lasted for 60 days. The rats were divided into groups consisting of 4 to 5 animals each. The controls (Group I, 5 animals) received no treatment. The experimental animals (Groups II—IV, 14 animals) were subjected to a left-sided nephrectomy performed by a semi-sterile technique. Since the other day after the operation, the animals were given 1 per cent saline solution for drinking in stead of water. Since the third day after the operation, the animals received daily subcutaneous injections of 10 mg of MAD (Spofa, 0.2 ml of microcrystalline suspension) the hyaline droplet producing potency of which had been verified by our previous experiments. Its efficiency was checked during the whole course of the experiment by weighing the animals. The last group of experimental animals (Group V, 5 animals) differed from the MAD-groups in that the former received approximately 6 E. U. of STH per animal per day as a subcutaneous injection of 0.2 ml of Somatotropin Spofa known to aggravate the MAD-