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"Hyaline droplet" formation and some other adrenocortical changes following methylandrostenediol treatment in the rat

By

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With 12 Figures in the Text

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In our previous paper (MOTLÍK and JANOUŠKOVÁ), we described the morphology of hyaline droplet formation in the cells of the adrenal cortex and medulla in human postmortem material. On the basis of our histological and some histochemical findings, we suggested that the hyaline droplet formation in the adrenal cortex was probably due to intracellular accumulation of protein material of various origin, the relation of this process to the secretory activity of adrenocortical (or medullary) cells being only indirect, if any. Some findings of ours, especially that of fibrillary material with morphologic and tinctorial properties of fibrin in some droplets, were indicative of the possibility that the intracellular protein material could have been derived from the blood plasma. In order to study the morphogenesis of the mentioned lesion, and to support some of our previous findings and views, we made an attempt at the experimental production of hyaline droplets in rat adrenals. For this purpose, we decided to make use of methylandrostenediol (MAD), the effect of which, as far as hyaline droplet production is concerned, is generally known, its administration giving standard and reproducible results (SALGADO and SELYE, SELYE and SALGADO, SKELTON). The great amount of "hyaline" or "colloid" droplets usually produced by MAD treatment according to the reports of various authors, seemed to appear very useful for the purpose of their histochemical analysis.

Material and Methods

For this group of experiments, Wistar albino rats of our own strain were used, descending from the inbred Wistar rat strain of the State farm at Konárovice (Czechoslovakia). The rats were fed on the Larsen diet with occasional carrot supplement before as well as during the whole course of the experiments.

Experiment 1. 32 female albino rats of the mentioned strain were used for this experiment. They were of approximately equal age, their initial body weight being 162 g (128 to 185 g). The animals were divided into 8 groups as indicated in Table 1. Groups I to IV received 17α-methylandrostene-Δ_5-3β,17β-diol (Methylandrostendiol Spofa) as aqueous microcrystalline suspension by sterile injection technique. The drug was administered subcutaneously in a single daily dose of 10 mg per rat. Groups V to VIII served as controls. All groups of animals were offered 1% saline solution to drink ad libitum. The animals were killed by decapitation in light ether narcosis. They were autopsied immediately, and various tissues removed for histological examination. The adrenals were fixed in BAKER's calcium formalin solution.

Experiment 2. 36 female albino rats of the mentioned strain were used in this experiment. They were of equal age and their average initial body weight was 126 g (103—143 g). The animals were divided into 7 groups as indicated in Table 2. Methylandrostendiol Spofa was

administered in the same manner as in Experiment 1. Somatotrophic hormone (Somatotropin Spofa, STH) was administered subcutaneously in a single daily dose of 4 Evans Units (corresponding approximately to 8 mg of the hormone, according to the producent's information). Rats belonging to group I did not receive any treatment, and they were offered tap water to drink ad lib. Other groups were offered 1% saline solution ad libitum. One week before the beginning of the experiment, animals belonging to groups V, VI and VII were unilaterally nephrectomized. This experiment lasted for 8 weeks. The animals were killed by decapitation following a head blow. Their further management was identical with that described under the heading of Experiment 1, with the only difference in that their adrenals were fixed in several fixatives, as in chilled neutral (non-buffered) 10% formalin, Baker's formalin-calcium, weak Bouin's solution prepared according to Baker's prescription, and in chilled acetone for enzyme reactions. The results of the latter have not been included in this paper, as they are dealt with separately.

Table 1. *The arrangement of Experiment 1*

Experimental group No.	Number of animals	MAD treatment	Saline solution	Duration of experiment (in weeks)
I	4	+	+	2
II	4	+	+	3
III	4	+	+	5
IV	4	+	+	9
V	4	0	+	2
VI	4	0	+	3
VII	4	0	+	5
VIII	4	0	+	9

Table 2. *The arrangement of Experiment 2*

Experimental group No.	Number of animals	Saline solution	MAD	STH	Unilateral nephrectomy
I	4	0	0	0	0
II	4	+	0	0	0
III	6	+	+	0	0
IV	6	+	+	+	0
V	4	+	0	0	+
VI	6	+	+	0	+
VII	6	+	+	+	+

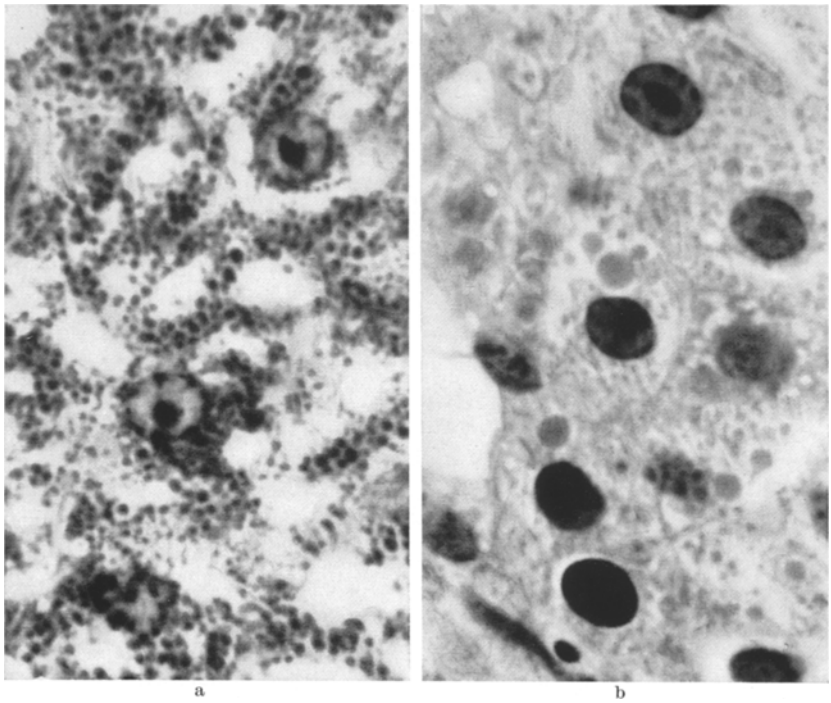
The staining methods and histochemical reactions used in both experiments were identical. Masson's blue trichrome stain, Mallory's phosphotungstic acid haematoxylin (PTAH), and the periodic acid — Schiff method (PAS) combined with modified Gömöri reticulin impregnation technique were used as histologic methods. Following histochemical reactions were used to obtain some information about the chemical composition of the lesions observed: PAS, PAS following salivary amylase treatment, coupled tetrazonium reaction (CTR), and the reaction for tryptophane according to Adams. Lipids were demonstrated in frozen sections by Sudan III. Some selected adrenals were stained for mitochondria by Altmann's method, for phospholipoid substances by Luxol Fast Blue (LFB) according to Klüver and Barrera, for SH- and SS-groups by the DDD method of Barrnett and Seligman, and for nucleic acids by Einarsson's gallocyanine method. All histochemical methods were performed according to Pearse, and Müller et al.

Results

In this report, only changes believed to bear some relation to hyaline droplet (HD) formation are dealt with. Others, as changes in fat distribution, have been omitted.

1. Droplet-like formations in normal rat adrenals. Using Masson's trichrome stain, we were able to demonstrate fine, strongly *fuchsinophilic granules* in the fascicular, reticular and juxtamedullary zones of formalin-fixed adrenals of control animals (Fig. 1). These granules were usually oval or rounded, rarely exceeding 1 micron in diameter. They were regarded identical with mitochondria, as they stained strongly with PTAH, took bright red colour with Altmann's

method and stained deep green-blue by LFB, especially after dichromate containing fixatives. The CTR gave a slightly positive result. Moderate amounts of these fine granules were found in fine cytoplasmic cords separating fat globules in the cells of zona fasciculata (Fig. 1 a), being more numerous in the "light" cells of the reticular zone, and filling up the cytoplasm of the "dark" cells of the same zone almost entirely. These granules were found to be most numerous in the bigger cells of the juxtamedullary zone, there being especially well defined and closely packed (Fig. 1 b).

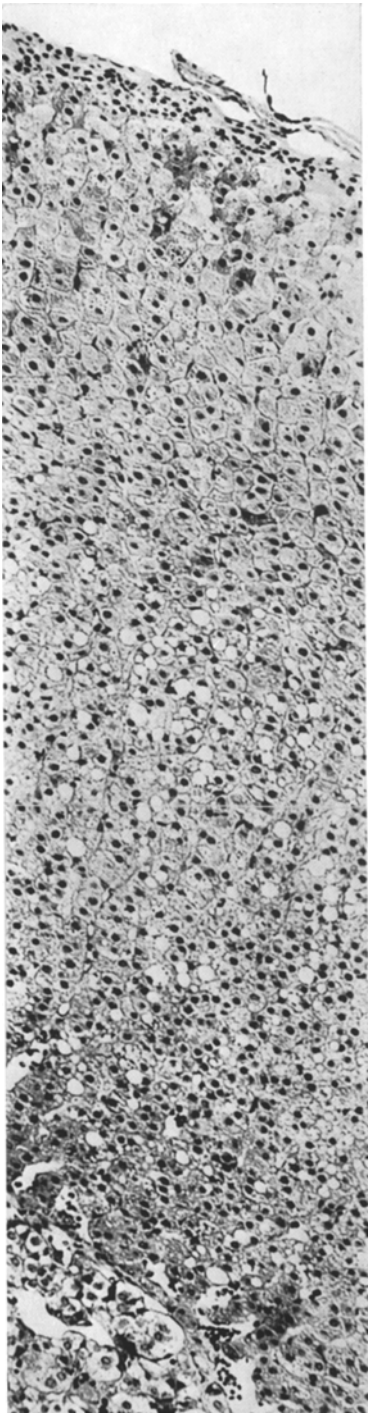


a b

Fig. 1 a and b. Control rat adrenal. a Zona fasciculata, showing numerous tiny granules (mitochondria) of relatively uniform size in the cytoplasmic cords separating fat vacuoles. PTAH stain, 1960 × (reduced to $^{9}/_{10}$). b Juxtramedullary zone with numerous mitochondria and some globules with gradual transitions between the both. MASSON'S trichrome stain, 1960 × (reduced to $^{9}/_{10}$)

Not infrequently, bigger *globular corpuscles* of homogeneous ("hyaline") appearance could be demonstrated among the fine granules (Fig. 1 b), their diameter rarely exceeding 2,5 to 3 micra with the upper limit of about 4 micra.

Apart from their size, these globules differed from the small granules (mitochondria) in that they were demonstrable not only after dichromate and formalin fixatives, but even following BOUIN'S fluid, which is generally known to cause mitochondrial destruction. Nevertheless, their boundaries were somewhat less clearly defined following BOUIN'S fluid in comparison to formalin or dichromate fixatives. The staining properties of the globules differed slightly from those of the small granules, some globules staining less intensively with the PTAH method and, on the other hand, showing occasionally weak PAS-positivity resistant to salivary amylase digestion. The bigger globules rarely exhibited weak Sudan positivity, the small granules showing constant, though very weak Sudan positivity.



All other reactions yielded identical results in both mitochondria and the globules. Some of these globules fulfil generally accepted morphologic criteria for HD (Fig. 1b). Numerous gradual transitions were noted between the globules and small granules. Only few droplet-like globules occured normally in one cell, and the number of cells containing them varied considerably. The habitual occurence of these droplet-like globules in adrenocortical cells of young normal female rats has been confirmed by examination of a large series of animals including adrenals of control rats from other experiments, following the use of several fixatives.

2. Some changes in the cytoplasm of adrenocortical cells following MAD administration. *a) Changes affecting intracytoplasmic granules* (Figs. 3, 4) occured as early as after 2 weeks duration of MAD administration (shortest period followed). They were inconspicuous at first, but were rather well marked after 3 and 5 weeks. These changes mainly consisted of small PTAH-positive granules enlargement and of elongation of some of them. These changes, accompanied by cell enlargement, affected the juxtamedullary and reticular zone first. Later on, especially during the fifth week, similar changes were noted in the zona fasciculata. At the same time, an increase of the number of bigger granules or globules was noted. Some of them resembled HD, their diameter reaching 4—4,5 micra. These "droplet-like" globules seemed to be in every respect identical with those occuring normally, including their tinctorial and histochemical properties. Some cells contained a great many of small, uniform granules, in addition to a number of bigger, somewhat irregular and sometimes even rod-shaped corpuscles, the latter being the only visible contents of the cytoplasm of other cells. Some of the globules were

Fig. 2. Adrenal cortex following MAD administration (Experiment 2, Group III), general view. Note atrophy of glomerulosa, lipid depletion of the outer cortical layers and predominance of optically empty vacuoles in the inner half of the cortex. In the outer layers, numerous PCV are shown as black dots surrounded by a clear "halo". Composite figure, Masson's trichrome stain, magn. approx. 200 × (reduced to $^4/_5$)

surrounded by an optically empty space, as if they were lying in vacuoles. Changes similar to those described above, occured in the outer fasciculata after prolonged MAD or MAD+STH treatment, i.e. in group IV of the first experiment, and in groups III, IV, VI and VII of the second experiment.

b) Protein containing vacuoles (PCV) (Figs. 2—6) occured approximately at the same time as did the changes of intracytoplasmic granules. We were able

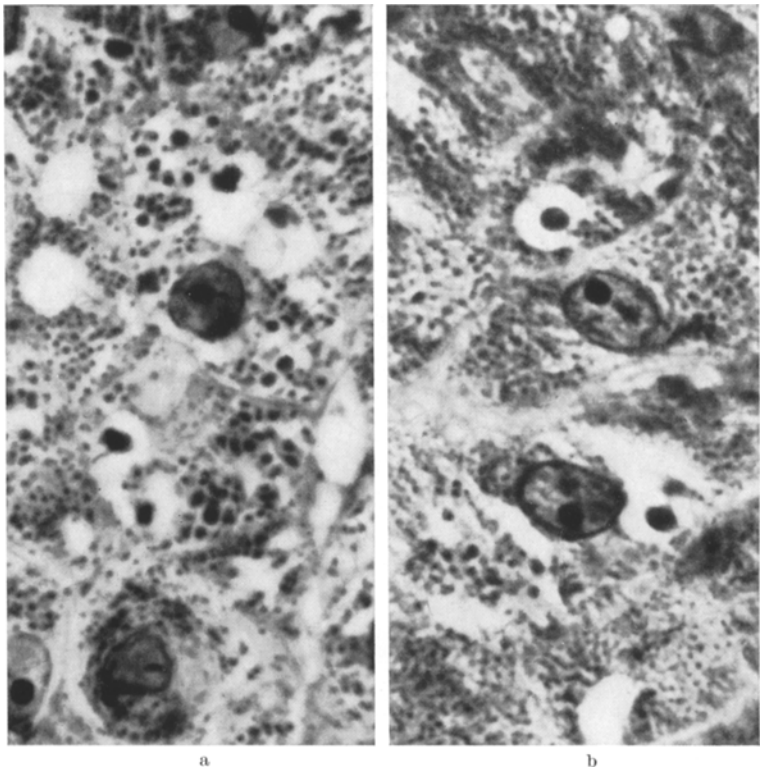


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Fig. 3. Animal from group III, Experiment 2. Mitochondrial changes, some globular corpuscles and PCV in the cytoplasm of zona fasciculata cells. Note deep indentations of nuclear surfaces at the bottom of the figure. PTAH stain, 1790 × (reduced to $^{19}/_{20}$). b Animal from group III, Experiment 2. Droplet-like globules inside of cytoplasmic vacuoles of zona fasciculata. PTAH stain, 1960 × (reduced to $^{19}/_{20}$)

to observe them as early as during the second week of our first experiment. PCV presented themselves as vacuoles with either sharp or somewhat ill-defined borders, and with polymorph contents, this being characterized by strong CTR positivity, by weak, occasionally moderate positivity of the tryptophane reaction, usually strong LFB positivity, and by PAS negativity in most cases (Fig. 5b). For the most part, PCV were completely Sudan negative. This could be demonstrated most clearly in groups I and II of Experiment 1, as the adrenal cortices in these groups were extremely poor in sudanophilic material. In the majority of cases, the vacuole contents stained vivid red by MASSON's trichrome stain, and deep blue or blue-black by the PTAH method. Rarely, the vacuole contents stained bluish when MASSON's stain was used, and took a pale yellow or pale

orange colour with the PTAH method. Sometimes, a fine inner structure of PCV contents could be disclosed by PTAH staining (Fig. 4), CTR or LFB (Fig. 6b). For the most part, the vacuole contents were amorphous, hyaline, having the character of an irregular clot lying loosely inside the vacuole, either in its middle or near its edge. Occasionally, the vacuole contents used to acquire the shape of a crescent, resembling Selye's "crescent shaped bodies" (Selye and Salgado) (Fig. 5a). In some "clots" inside the vacuoles, granules were found, corresponding to those described above, and belonging usually to the middle-sized or large variety

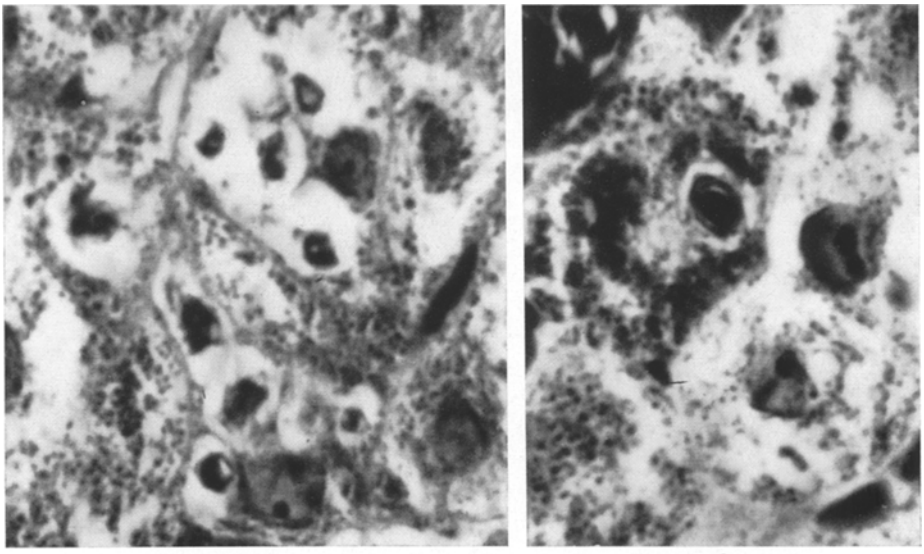

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Fig. 4. a Animal from group IV, Experiment 2. Outer fasciculata. PCV with polymorphous contents; note fibrillary edges of the contents, and its ill-defined irregular stratification or somewhat whorly structure. PTAH stain, 1790 × (reduced to $^{19}/_{20}$). b Zona fasciculata with numerous mitochondria in the cytoplasm, some of which are elongated, a small protein containing vacuole (bottom right), and an onion-like stratified body (center). PTAH stain, 1790 × (reduced to $^{19}/_{20}$)

(Fig. 6b). Sometimes, a clump of 3 or 5 granules used to constitute the only visible contents of the vacuole. Rod-shaped corpuscles were rarely found in these clumps. As indicated above, some vacuole contents acquired a peculiar whorly structure or ill-defined irregular concentric stratification (Fig. 4). Other vacuoles contained bigger, more dense formations, with smoother contours, resembling HD (Figs. 3b, 4a). Furthermore, irregular filamentous substance could be found in some PCV. Mutual transitions between all lesions described were suggestive of the idea that all of them were, in fact, merely various morphologic manifestations of a single process.

PCV were found in adrenocortical cells of all animals given MAD, occuring most frequently in the juxtamedullary and reticular zone after 2 weeks duration of MAD treatment, and in the zona reticularis and inner fasciculata after 3 to 5 weeks. After 9 weeks of MAD administration, a considerable decrease of the number of PCV was noted in the inner cortical layers, while a considerable amount of PCV were demonstrated in the peripheral layers of the cortex (Fig. 2). During the fifth week of the experiment, scarce, optically empty vacuoles occured in the

inner layers of the adrenal cortices of MAD treated rats (paraffin sections), being much more frequent after 9 weeks of MAD administration, and governing the microscopic appearance of the inner half or two thirds of the adrenal cortices of treated rats (Figs. 2, 6a, 7). On the contrary, PCV gradually disappeared from the inner cortical layers. In frozen sections, the majority of "empty" vacuoles was shown to contain lipoid material. Nevertheless, in some of them sudanophilic contents could not be demonstrated. Amylase resistant PAS-positivity of the PCV contents was observed infrequently, especially in groups III and IV of the

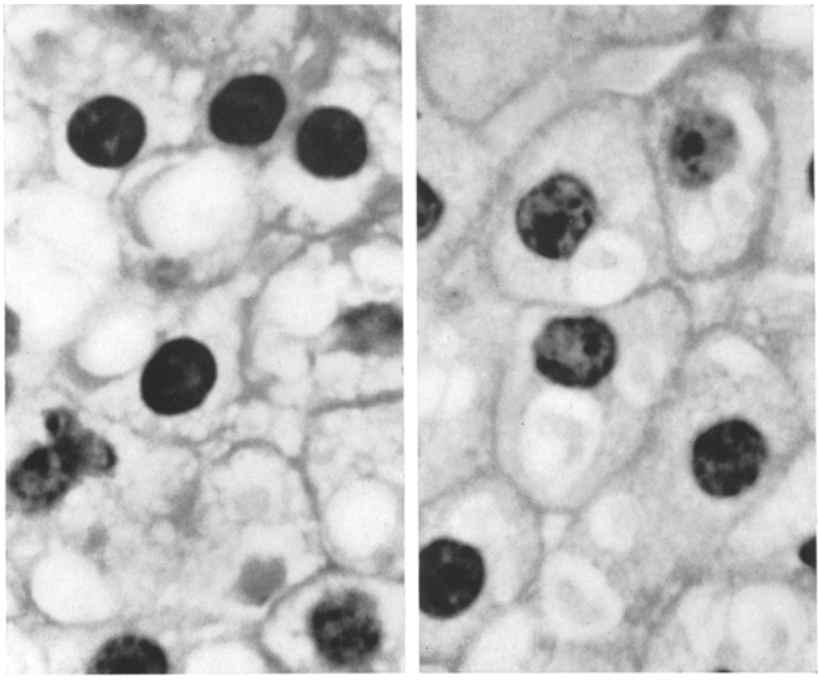

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Fig. 5a and b. Adrenals from two different animals from group III, Experiment 1. PCV with predominantly PAS positive (a) and negative (b) contents. In (a) some (but not all) vacuole contents attain a shape of a crescent. In a cell at the bottom of (a), there are two vacuoles, one with PAS positive, the other with PAS negative contens. Note "distinct cell boundaries" in both cases (for explanation see text). Haematoxylin-PAS following amylase treatment, 1960 × (reduced to $^9/_{10}$)

second experiment. Massive occurence of PAS positive PCV contents as observed in one case of the second group and one case of the third group of our first experiment has been considered exceptional (Fig. 5a).

c) Hyaline droplets (HD) and similar lesions (Figs. 7—10) were produced in groups III and IV of Experiment 1 in moderate amounts only, although systemic effects of MAD as thymus atrophy and renal hyperplasia were well pronounced. In Experiment 2, we were able to produce HD in satisfactory amounts, their incidence being almost massive in some instances. We were able to demonstrate these droplets in adrenocortical cells of rats belonging to groups III, IV, VI and VII, with their maximum occurence in group IV and VII. Unilateral nephrectomy did not seem to have any significant influence on this type of HD production. STH administration, on the contrary, seemed to favour strongly HD formation.

Only quantitative differences were noted between HD (and related lesions) following MAD administration and those following combined MAD+STH treatment. The distribution of HD was fairly irregular. Often their focal accumulation could be observed, while other cortical areas were completely devoid of

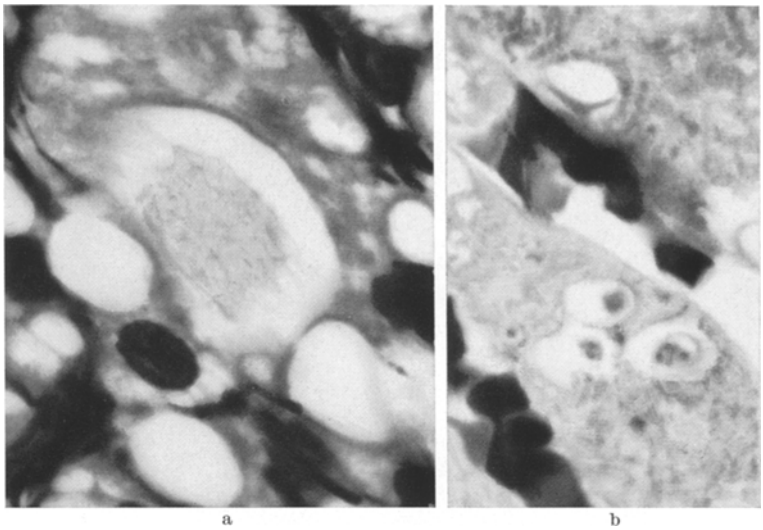

Fig. 6. a Another type of PCV with slightly filamentous contents in an animal from group IV, Experiment 2. Note numerous optically empty vacuoles. Modified Gömöri reticulin impregnation + Masson's trichrome stain, 1790 × (reduced to $^{9}/_{10}$). b Animal from Experiment 2, group III. Note several PCV, some of which attain a shape of a crescent (top), others contain several intensely staining granules or globules (lower half of fig.). LFB, no nuclear counterstain, 1790 × (reduced to $^{9}/_{10}$)

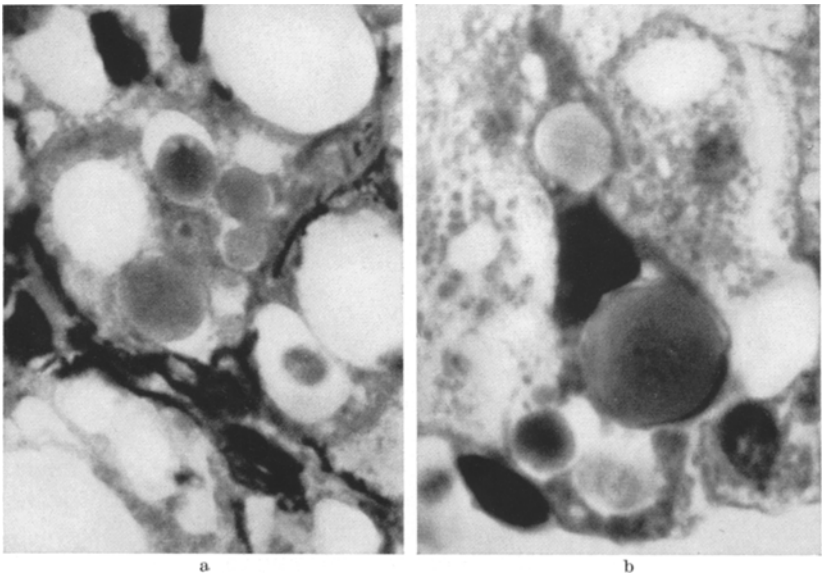

Fig. 7a and b. Hyaline droplets of various animals (Experiment 2). In (a) there is an optically empty vacuole besides several typical HD in one cell. Modified Gömöri reticulin impreg. + Masson's trichrome stain, 1790 ×. In (b) there are signs of cellular regression with nuclear pycnosis. Note uneven staining of the droplets. The light ones were light blue, the darker were red in the original preparation. Masson's trichrome stain, 1790 × (reduced to $^{19}/_{20}$)

their presence, or while they contained only small amounts of HD. In spite of these irregularities, HD seemed to occur most frequently in the inner and outer zona fasciculata, their distribution depending by far more on that of other lesions, as PCV and optically empty vacuoles, than on the anatomical cortical zonation. HD were found most regularly at the borderline between the vacuolized and not vacuolized layer of the cortex, their number usually decreasing both outwards and inwards. HD occured exceptionally in other cortical areas, but were never found in the zona glomerulosa, which was usually atrophic under given experimental

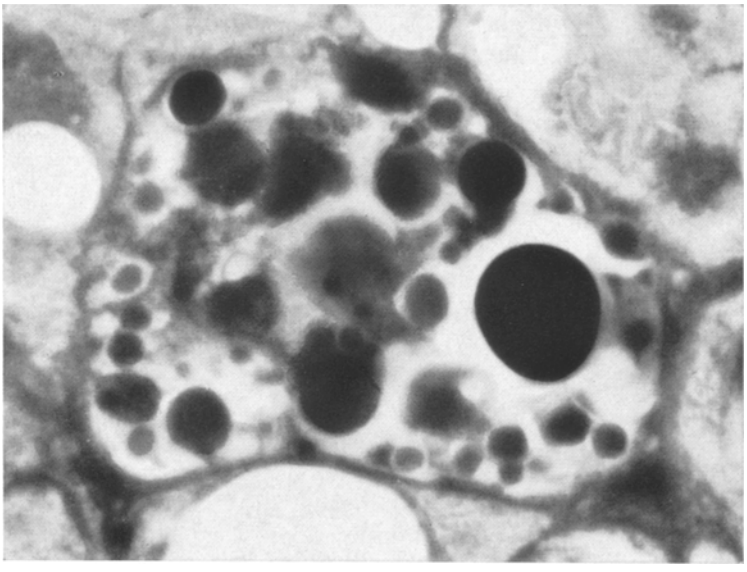

Fig. 8. An adrenocortical cell stuffed with hyaline droplets. Nucleus (out of focus) in the center of the cell. Note "distinct cell boundaries". CTR, 1960 × (reduced to $^{19}/_{20}$)

conditions (Fig. 2). The increase of HD number during the late phase of our experiments seemed to be connected with an increase of the number of optically empty vacuoles and with a decrease of PCV.

The morphologic appearance of HD produced in our experiments did not differ from that described by other authors in haematoxylin and eosin preparations [e.g. SKELTON, SELYE and SALGADO, SALGADO and SELYE (1), (2), CRANE et al.]. With MASSON's stain, some droplets stained red, while others stained blue (Fig. 7 b). Sometimes, various parts of a single droplet stained in a different manner. Most usually, the central areas of these droplets stained red, while their peripheries stained blue. In some, red stained areas were scattered through blue stained droplets. There were no relations between the droplet size and their staining properties. Not infrequently, several droplets of different staining affinities were found in a single cell (Fig. 7 b). Similar inhomogeneity was occasionally seen in PTAH preparations (Fig. 10). The droplets stained either deep violet blue or pale yellow, some of them taking both colours in the manner described above. In some bigger droplets, or in some intracellular "hyaline" deposits of non-droplet nature, we were able to demonstrate filamentous structures staining deep blue, being very conspicuous if the rest if the deposit stained yellow or orange.

These fibers used to cross one another in an extremely irregular fashion, sometimes becoming condensed, so that they seemed to lose their independence (Fig. 10). In addition to spherical "hyaline" or "colloid" droplets, we were able to demonstrate irregular intracytoplasmic deposits of similar "hyaline" material, especially in adrenocortical cells of animals belonging to groups III, IV, VI and VII of Experiment 2 (Fig. 9, 10a, b). The staining properties of these deposits did not differ from those of HD. Fibers seemed to occur somewhat more often in the irregular deposits than in typical HD. The simultaneous occurrence of both HD and these polymorphous formations in close topical relations, occasionally even in a single cell (Fig. 9), as well as their identical stainability, is indicative of their close relationship.

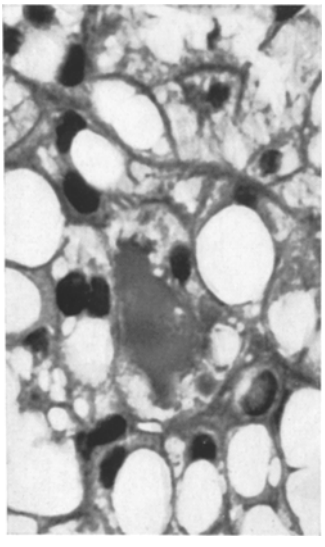

Fig. 9. Animal from group VIII, Experiment 2. Inner cortex. Note many optically empty vacuoles. In the center of the field, there is an irregular PAS positive "hyaline" mass in a cell, which, in addition, contains a small hyaline droplet near its lower border. Haematoxylin-PAS, 785 ×

We were unable to disclose any considerable histochemical difference between typical HD and other similar deposits. The PAS reaction yielded strongly positive results in some droplets and irregular deposits (Figs. 5a, 9), so that both became very conspicuous even at low magnification. Nevertheless, not all of them did show the same degree of positivity, and we were able to find faintly stained, and even PAS negative droplets and deposits. No inhomogeneity except some vacuolation could be disclosed by this reaction in the droplets. The CTR gave positive results in all droplets, though its intensity showed some variations (Fig. 8). The coloration of the droplets as well as of other deposits was rather deep, so that only rarely fibers could be seen inside, if they stained more intensively than the non-fibrillar mass. The reaction for tryptophane gave weakly positive to strongly positive results. With this reaction, we were unable to observe completely negative deposits. Differences were noted among various droplets, as far as the degree of positivity was concerned. With this method, some inhomogeneity could be shown in some droplets, comparable to that observed, for example, with the PTAH method or the Masson's trichrome stain. The fibers observed in PTAH preparations gave a strongly positive reaction for tryptophane, while the amorphous rests of the deposits used to stain less deeply. Some droplets stained by the LFB, while others were demonstrated to be negative with this stain. No inhomogeneity could be disclosed by this method in typical HD. We were never able to observe sudan positive material in HD. Einarsson's reaction for RNA was always negative, too.

d) Regressive changes following MAD administration (Figs. 2, 7b, 11). During long lasting MAD or MAD+STH administration, we were able to observe an increase in the number of the so-called dark cortical cells, characterized by cell body collapse, concave cell boundaries, granular, intensively acidophilic cytoplasma and pycnotic nuclei. On close examination, these cells were shown to

contain granules, similar in every respect to those observed in other adrenocortical cells; some of the dark cells contained bigger granules, and rarely HD of various size (Fig. 7 b). We were not able to observe further desintegration of these elements,

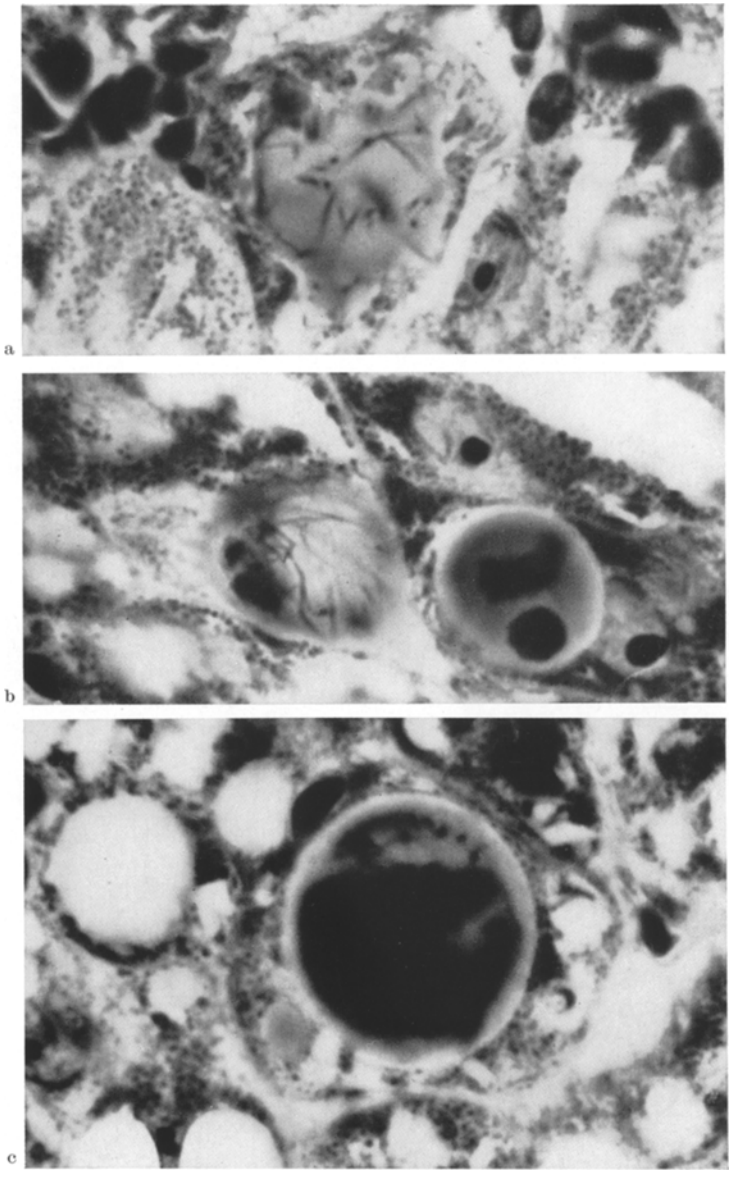

Fig. 10 a—c. Various examples of irregular intracellular deposits (a), and droplets (b), containing fibrillary structures, which sometimes become clumped (b, c). PTAH stain, 1960 × (reduced to $^{16}/_{20}$)

though some relation to the small granulated cells described below could not be excluded with certainty. In adrenal cortices of animals receiving MAD or MAD + STH, especially of those belonging to groups III and IV of Experiment 1, we were able to find small cells with indistinct boundaries, with many acidophilic, PAS

positive granules, measuring 0,5—2 micra, scattered through their light cytoplasm (Fig. 11). Their nuclei were usually small and pycnotic. These cells seemed to represent degenerating elements. Sometimes, their bodies became adapted to the pressure of neighbouring cells, thus acquiring an oblong shape, so that their granules seemed to lie in the intercellular spaces. These cells used to occur frequently in the inner zona fasciculata, being most numerous at the boundary between the vacuolized and not vacuolized cortical layer. Nuclear pycnosis, indicative of cell damage, was found in a certain amount of cells containing inclusions under

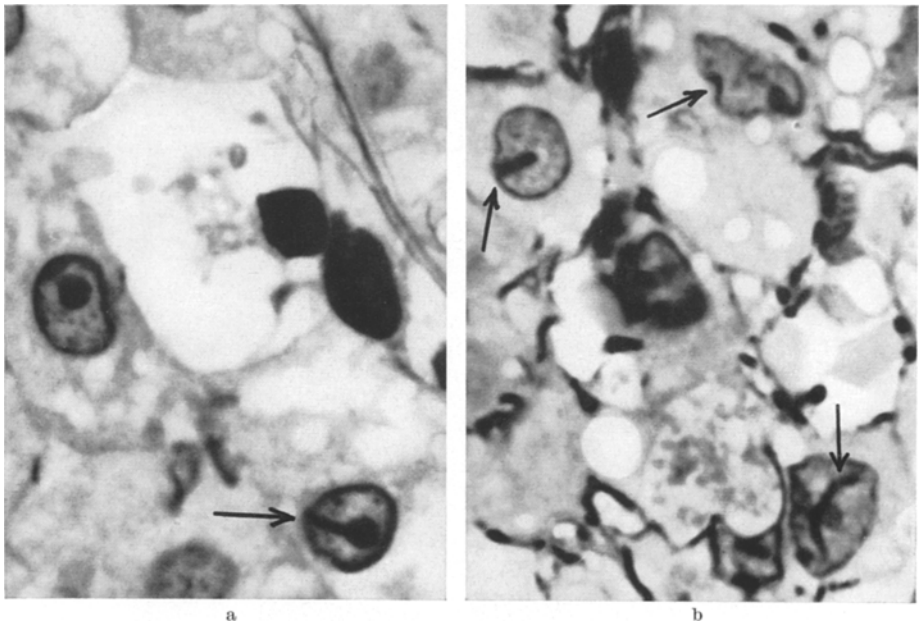

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Fig. 11 a and b. Small cells with PAS-positive granules in the cytoplasm showing regressive changes, in the cortex of animals from group IV, Experiment 1. Note deep indentations of the surfaces of some nuclei (arrows). Compare to Fig. 3a. Modif. Gömöri reticulin impreg. + haematoxylin + PAS, 1960 × (reduced to $^{19}/_{20}$)

consideration (Figs. 7 b, 11 a). On the other hand, the majority of cells containing some deposits did not display any nuclear change of regressive character (Figs. 3 to 5, 7, 10). According to their morphologic appearance, undisturbed vitality of these cells had to be assumed. On the contrary, some regressive changes were found in adrenocortical cells containing neither HD nor deposits of other types described. From these facts it is obvious, that no clear relationship could be found between regressive changes of adrenocortical cells and HD formation or deposition of similar material.

3. Some other interesting findings in the adrenals following MAD or MAD + STH treatment. *a) Haemorrhage* of various extent was observed in some animals belonging to groups IV and VII of Experiment 2. In a single case (Group IV) it was recognized macroscopically. Initial changes of this type presented themselves usually as focal diapedesis of erythrocytes, without severe damage to the surrounding tissue. Occasionally, blood corpuscles were found inside the adrenocortical cells, filling up the "empty" vacuoles. In more advanced cases, more

extensive interstitial haemorrhage was found, associated with both cytological and structural damage. With the exception of mechanical disruption, no changes were noted in reticular fibers. No arteriolar or other vascular changes were found in the adrenals as well as in other organs so far examined. In areas of haemorrhage, plasmatic extravasations were observed not infrequently. They were not necessarily topically related to haemorrhagic foci, and used to occur even if haemorrhagic phenomena were not present at all.

b) Cell contours. In groups III and IV of Experiment 1 and in groups III, IV, VI and VII of Experiment 2, conspicuous bluish cell contours were regularly

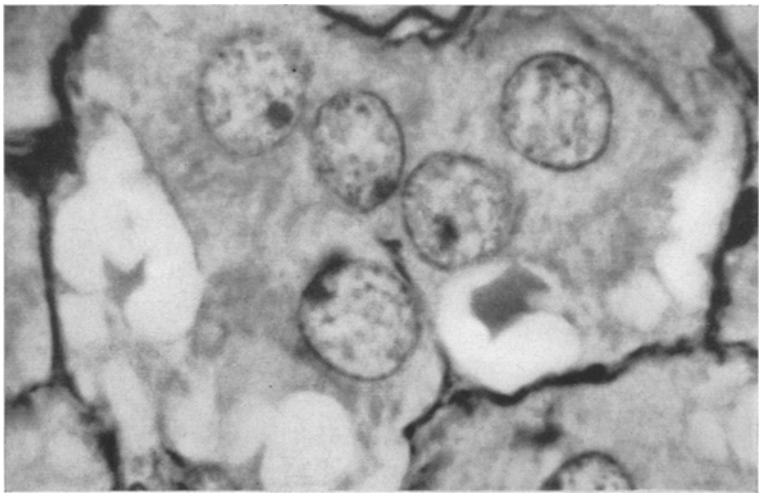

Fig. 12. Animal from group IV, Experiment 2. Optically empty and protein containing vacuoles with faintly PAS-positive contents in adrenal *medullary* cells. Modified Gömöri reticulin impregnation + haematoxylin + PAS, 1960 × (reduced to $^{19}/_{20}$)

observed in preparations stained with MASSON's trichrome. This finding was most frequent in the inner zona fasciculata, occuring to a lesser degree in the outer zona fasciculata and zona reticularis. In preparations stained by the PAS method (Fig. 5, 9), the cells were separated by a narrow PAS positive band, not always surrounding the entire cell circumference. The neighbouring cell membranes usually could not be differentiated from this band. A similar picture could be observed in CTR preparations (Fig. 8). On the other hand, no such band was demonstrable in LFB and gallocyanin preparations. With the latter method, we were able to differentiate easily neighbouring cell membranes, which were always found to be smooth and fine.

c) Changes in adrenal medullary cells (Fig. 12). Optically empty vacuoles were found in adrenal medullary cells of almost all experimental groups receiving MAD or MAD+STH, with the exception of the first group of Experiment 1. They were very numerous in some cases. Vacuoles containing clotted material of predominantly protein nature were found exceptionally after 2 or 3 weeks of MAD treatment (Experiment 1), but were regularly seen in other groups of animals receiving MAD or MAD+STH. From the morphological and histochemical point of view, these vacuoles did not differ significantly from the PCV observed in the

adrenal cortices of the same animals. Their contents was somewhat less polymorphous in comparison to that of cortical PCV, being usually homogeneous. Both optically empty vacuoles and PCV tended to be localized at the vascular poles of medullary cells. Exceptionally, these vacuoles were observed following saline solution administration of long duration, without any additional hormonal treatment. Apart from a small number of fine granules, obviously identical with mitochondria, no other intracytoplasmic deposits or inclusions were found in medullary cells.

Discussion

Normally occuring cytoplasmic granules and globules. In accordance with the views of Uotila and Kar, as well as of others (for ref. see Bachmann), the small fuchsinophilic granules are regarded here as identical with mitochondria. This view is believed to be supported by their morphological, tinctorial and histochemical properties, by their distribution, as well as by some recent electron microscopical investigations (Lever, Yamori et al., Fujita, Zelander). Especially the latter seem to admit no doubt as far as their identity is concerned. Nevertheless, several authors do not accept it (Ehrenbrand, for further ref. see Bachmann).

The problem of the globules found in the innermost layers of normal adrenal rat cortices seems to be much more difficult. The relation of the globules to mitochondria seems to be evident, as almost all staining and histochemical reactions used gave identical results in both. Most probably, some of the globules represent giant mitochondria. Nevertheless, some of the biggest globules exceed by far the upper limit of mitochondrial size as observed under normal conditions in rat adrenocortical cells (Lever, Yamori et al., Fujita, Schwarz et al., Miller). Slight tinctorial and histochemical differences from the ordinary properties of mitochondria, as observed in some globules, admit several explanations of their origin, the most probable of these being some mitochondrial change, some sort of mitochondrial degeneration or formation of so-called chondriospheres, as described in adrenal cortical cells of normal Syrian hamsters (De Robertis et al.), or protein storage in mitochondria. Although the idea of mitochondrial storing ability has not been generally accepted as yet (Altmann), recent papers of Zollinger an his co-workers, and especially those of Rouiller and of Gansler et al., seem to have unequivocally proved the possibility of protein storage in mitochondria and its significance for HD formation.

In this connection, we wish to call the reader's attention to the fuchsinophilic globules occuring in the zona glomerulosa, and adjacent layers of zona fasciculata of normal bovine adrenals, described by several authors (e.g. Schultz et al., there further ref.) as "fuchsinophilic", "azocarmine" or "giant" granules. These granules, resembling most closely the globules described in the innermost cortical layers of normal rat adrenals, were shown to represent independent corpuscles discernible by mitochondrial stains even after they had been isolated by differential centrifugation, and to be equipped with high contents of oxidative enzymes, especially of succinico-dehydrogenase (Schultz et al.). These findings indicate the close relationship of these granules or globules to mitochondria, and seem to support our views concerning the globules of rat adrenals.

On the other hand, these globules possibly could represent intracytoplasmic deposits topically independent of mitochondria, the material they are composed of being either a cell product or a cell "ingest" in the broadest sense, stored in the cytoplasm more or less in accordance with the ideas of ALTMANN, i.e. by cytoplasmic seggregation topically independent of mitochondria. SPARGO et al. were able to observe droplet-like protein deposits of similar nature in epithelia of renal papillary ducts, and of renal pelves of hypokaliaemic rats, demonstrating their independence from mitochondria by the electron microscope and their serum protein contents by immunohistochemical methods.

The real nature of the droplet-like globules of the normal rat adrenocortical cells remains obscure, though their relation to mitochondria seems to be very probable. Further histochemical and especially immunohistochemical and electron microscopic investigations are highly desirable, as only these methods are believed to be able to contribute significantly to the resolution of this problem.

Changes of intracytoplasmic granules in experimental animals. The changes of mitochondria observed in adrenocortical cells of our experimental animals fit some of ALTMANN's descriptions of qualitative changes of the chondriome. The globules occuring in increasing amounts under experimental conditions, could not be distinguished from those occuring spontaneously in adrenals of intact rats. These globules, as well as those occuring normally, are possibly derived from altered mitochondria, either by accumulation of some material inside mitochondrial bodies or by some sort of mitochondrial clumping. The finding of elongated or even rod-shaped, curved mitochondria adhering to the surfaces of some bigger rounded bodies (Figs. 3a, 4b), seems to support the latter idea of the origin of the globules under normal as well as under experimental conditions. The occurence of an optically empty zone surrounding the globules may be either regarded as result of droplet retraction due to processing the tissue, especially dehydration, or as evidence of droplet incorporation into vacuoles originating in the cytoplasm without necessary relation to cytoplasmic granules. Most probably, both possibilities take place, the latter usually being observed only under experimental conditions. The most advanced changes of this type represent morphologic transitions to PCV.

Protein containing vacuoles (PCV). PCV similar to those observed in our experiments are frequently found in the cytoplasm of cells of various organs, being most often reported in liver cells under various pathological and experimental conditions in man and in animals, as, for example, in intoxications, partial hepatectomy, hypoxia etc. (for ref. see ALTMANN). PCV of various types were reported to occur in the adrenal cortex by several authors [ALTMANN, LIEBEGOTT (1), (2), MOTLÍK and JANOUŠKOVÁ], and were regarded as signs of adrenocortical hyperactivity by LIEBEGOTT.

As far as the PCV occuring in our experiments are concerned, neither their origin, nor their significance are fully understood. The relatively frequent findings of intact or altered mitochondria showing some clumping and sometimes forming irregular masses staining with mitochondrial stains, the frequent occurence of the bigger globules in the vacuole contents, as well as its occasional deep LFB positivity may be regarded as suggesting the admixture of cytoplasmic components to the vacuole contens. On the other hand, some PCV seemed to contain neither

mitochondria nor materials derived from them. Some of them showed moderate to strong PAS positivity and tryptophane reaction positivity of their contents, indicating the presence of some material, not present in adrenocortical cells under normal conditions, and being possibly of blood plasma origin. Only slight quantitative differences were noted histochemically between the PCV contents and the blood plasma in neighbouring vessels.

Some of the bodies showing onion-like stratification (Fig. 4) resemble closely those described by Altmann et al. in hepatic cells following chronic thioacetamide poisoning. These bodies were thought to represent a lesion derived from the RNA-free variety of the endoplasmic reticulum. We do not accept Altmann's explanation for the lesions observed in our experiments in spite of their striking similarity to those of Altmann. On the contrary, these structures are felt to be related to the PCV, with predominance of the cytoplasmic (including mitochondrial) component in their contens.

Although some informations concerning the composition of the PCV had been gathered, enabling us to follow some steps of their formal genesis, their causal genesis and the mechanisms involved in the process of their formation remain unknown. The possibility of simultaneous participation of several factors, including disturbances of cellular and vascular permeability, partial necrosis of the cytoplasm, and a primary mitochondrial lesion has to be taken into account.

The majority of cells containing PCV did not display any signs of destruction or degeneration, but some of them, rarely, showed some nuclear pycnosis and cell body collapse. On the contrary, many affected cells had well preserved nuclei with prominent nucleoli, sometimes with deep nuclear surface indentations (Figs. 3a, 11).

The contents of PCV may be transformed into HD of typical appearance. Nevertheless, not all of the PCV do change into HD, as very few droplets were formed in comparison to the original number of PCV. As the decrease of the PCV could not be attributed to cell damage and to disappearance of the affected cells, the PCV had to be regarded reversible. The mechanism by which adrenocortical cells get rid of PCV, as well as the possible relation of PCV to the optically empty vacuoles remain unknown as yet.

Hyaline droplets (HD). HD were observed by several authors under various experimental conditions in the adrenal cortices of various animals [for ref. see Loustalot, Salgado and Selye (1), (2), Selye and Salgado, Skelton (1), (2), Motlík and Janoušková, Legait, Stutinsky).

Experimentally produced HD have been described as homogeneous, strongly acidophilic and PAS-positive rounded or spherical bodies. Stutinsky, using Masson's trichrome and azan stains, was the only author to notice some tinctorial inhomogeneity of the droplets, and Crane et al. were the first authors to describe some lipid-free vacuolation of adrenocortical cells of the zona fasciculata of MAD treated rats. With the exception of the paper of Selye and Salgado, no further description of other cytoplasmic changes connected with adrenocortical HD formation was found in the literature. The formal morphogenesis as well as the histochemical composition of experimentally produced HD have not been studied so far.

We were able to demonstrate that the idea of constant strong PAS positivity of HD was wrong, as some PAS negative droplets were observed in our experiments. As mentioned above, PAS positive material in the PCV or in the droplets is most likely to originate from the blood plasma. PAS positive and PAS negative droplets did not seem to represent fundamentally different lesions. PAS positive material could either have been present in HD from their very beginning in amounts not demonstrable by our histochemical methods, becoming visible after the droplet mass had been condensed, or could have occured in the deposits if the permeability increase had been big enough to allow PAS positive components of blood plasma to penetrate the capillary wall and the cell membrane. Similarly, positive tryptophane reaction seems to indicate the presence of some protein of extracellular origin, most probably derived from blood plasma. The presence of some material derived from the blood plasma has been proved by the finding of fibrillary material inside the droplets, which stained positively for fibrin by conventional stains as PTAH and gave a strongly positive reaction for tryptophane and the CTR. Such material may be regarded as fibrin with fair certainty (confront with PEARSE, ALTMANN). Evidence for the participation of a plasmatic component in the droplets lends valid support to the presumption that altered permeability played a significant role in HD formation. In fact, lesions attributable to increased capillary permeability were observed in human adrenals under conditions leading to HD formation (MOTLÍK and JANOUŠKOVÁ). The findings of intercellular hyaline material deposition following MAD administration [SKELTON (1)] and in regenerating adrenocortical tissue of enucleated adrenals in rats [SKELTON (2)], are felt to fall into this category. The resemblance of adrenocortical cells of MAD treated rats to plant cells as noted by SALGADO and SELYE (2), attributed by these authors to cell membrane thickening, is felt to be most probably due to accumulation of some PAS positive and CTR positive material in the intercellular space, as the cellular membranes were shown to remain thin under these experimental conditions for the most part. Furthermore, plasmatic extravasates of considerable size were noted being connected with haemorrhagic phenomena in most advanced cases. The latter may be regarded as evidence of vascular permeability disturbance of an extreme degree of severity, being by no means the result of arterial damage in our experiments. The existence or, perhaps, easy formation of reversible (?) multiple direct communications between the capillary lumina and the so-called subendothelial space observed by the electron microscope (LEVER, LUFT et al., YAMORI et al.) seems to offer a suitable ultrastructural basis for our observations.

Most authors regard the droplets as evidence of enhanced adrenocortical function or stimulation, or as direct morphologic manifestation of hormonal secretion (LIEBEGOTT, SELYE and STONE, and others). Most often, their relation to mineralocorticoid production in the adrenals has been suggested [SALGADO and SELYE (1), (2)]. Only rarely, the relation of the HD to hormone secretion was unequivocally rejected (LOUSTALOT) and other explanations as "degeneration" or "absorption of some material from damaged vessels" have been brought forward [SKELTON (1), (2)]. Other investigators made no attempts at more detailed analysis or explanation of the finding of HD in their material (LEGAIT et al., STUTINSKY). On the basis of our findings, HD cannot be regarded as secretory

granules, but the possibility of their indirect relation to the secretory process has to be admitted. The occurence of HD in cases of experimental hypertension (Rather) seems to depend more likely on increased vascular permeability accompanying experimentally induced hypertensive states, than on the raised blood pressure itself.

In conclusion, we wish to stress the fact that all the changes described seem to be related one to the other, as numerous morphological transitional forms could be observed. The morphological appearance, chemical composition and fate of the deposits observed seem to depend on several factors as on the degree of vascular and cellular permeability, on the rapidity of fluid inflow into the cell, on cell vitality, as well as on several others. It is probable that some decrease of cell vitality prevents the protein deposits from being removed, while, on the other hand, intact cells seem to be capable of getting rid of them.

No fundamental differences were noted by comparison of experimentally produced hyaline droplets and related lesions to hyaline droplets and other deposits observed in human adrenals (Motlík and Janoušková). This similarity, as well as the occurrence of similar lesions in adrenal medullary cells, both in humans and in our experimental animals, seem to support our view that these changes represent a non-specific process of pathobiotic nature.

Summary

In normal rat adrenals, droplet-like hyaline globules occur in the juxtamedullary and reticular zones. Following methylandrostenediol treatment, mitochondrial changes, droplet-like "globules" formation, "protein-containing vacuoles" of various types and hyaline droplets were observed. The material constituting the contents of the "protein-containing vacuoles" as well as that of hyaline droplets is believed to be derived in part from the blood plasma, in part from the cytoplasm. The findings presented here are believed to support the view that the hyaline droplets and related changes represent non-specific lesions of pathobiotic nature. The hyaline droplets in adrenocortical cells do not represent "secretory granules" in Liebegotts or Selyes sense, though their indirect relationship to the secretory process cannot be excluded.

Über die Bildung von „hyalinen Tropfen" und weitere Veränderungen der Nebennierenrinde als Folge einer Methylandrostenediol-Behandlung bei der Ratte

Zusammenfassung

Schon normalerweise finden sich tropfenartige hyaline Kügelchen in der reticulären und juxtamedullären Zone der Nebennierenrinde von Ratten. Nach der Methylandrostenediolverabreichung wurden Veränderungen an Mitochondrien, Bildung tröpfchenartiger Kügelchen, „eiweißhaltige Vacuolen" und hyaline Tropfen beobachtet. Das Material des Inhaltes der eiweißhaltigen Vakuolen sowie das der hyalinen Tropfen wird als teilweise vom Blutplasma, teilweise vom Cytoplasma stammend angesehen. Die Befunde unterstützen die Deutung der hyalinen Tropfen und der mit ihnen verwandten Gebilde als unspezifischer Veränderungen

pathobiotischer Natur. Hyaline Tropfen in den Nebennierenrindenzellen stellen also doch wohl nicht „Sekrettröpfchen" im Sinne LIEBEGOTTS oder SELYES dar, ihr indirekter Zusammenhang mit dem sekretorischen Prozeß kann jedoch nicht ausgeschlossen werden.

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