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Effects of Aminoglutethimide (Elipten® Ciba), a Steroid Biosynthesis Blocking Agent, on Adrenal Glands in Cushing's Syndrome

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Summary. Seven adrenal glands removed from 4 patients suffering from a central-type Cushing's syndrome after various periods of aminoglutethimide (AGL) treatment were subjected to conventional morphological and histochemical, particularly enzyme histochemical, studies and to biochemical incubation studies.

The adrenal glands of the AGL-treated patients showed marked lipoid adrenocortical hyperplasia with an excessive cholesterol or cholesterol ester accumulation leading to cholesterol crystallization and focal dystrophic and necrotic changes in the adrenocortical periphery. Histochemically, the foci of adrenocortical tissue so affected showed an increase in acid phosphatase reaction and a severe reduction of dehydrogenase activity. In chronically treated cases, the adrenal cortex showed some reduction in weight and the necrotic foci were replaced by cicatricose tissue.

The biochemical findings confirmed the blockade of cholesterol side chain cleavage, but the biosynthesis of corticosteroids from pregnenolone was unaffected.

Aminoglutethimide (Elipten® Ciba, further AGL) originally introduced as an anticonvulsant, has been early recognized to possess various side effects, the most important of which were those pertaining to the endocrine system (for reviews see Hughes and Burley, 1970; Motlík *et al.*, 1972). Of these, the most interesting one appeared to be that concerning the adrenal cortex. Following a long-term AGL administration, Camacho *et al.* (1967) observed adrenocortical insufficiency in human beings, persisting even after the AGL-treatment had been discontinued. The mechanism of AGL-induced adrenocortical insufficiency has been later elucidated by demonstrating that AGL was capable of blocking the adrenocortical C_{20}–C_{22} lyase, which prevents cholesterol from entering the steroid biosynthetic pathway. Owing to its steroid biosynthesis blocking properties, the drug has been used as an adrenostatic in human medicine with considerable success (cf. e.g. Smilo *et al.*, 1967; Horký *et al.*, 1968; Marek *et al.*, 1973).

However, morphological findings in the human adrenal glands following AGL administration have been scarcely mentioned until recently. Camacho *et al.* (1967) examined adrenal glands of children who had been receiving AGL for various,

usually rather long periods prior to death. They have been reported to exhibit hyperplasia of the adrenal cortices with intracytoplasmic storage of lipids, which has been regarded as being in accordance with the theoretical postulates. Givens *et al.* (1968, 1970) examined the adrenals of two females suffering from breast cancer and, as far as we are aware of, these authors were the first ones to report also on the morphology of adrenal glands in one case of AGL-treated central-type Cushing's syndrome. The findings in these three cases were essentially similar in that the adrenal glands showed high degrees of cortical hyperplasia associated with intracytoplasmic lipid accumulation and, in one of the cases, with the disappearance of a discernible zona glomerulosa. The authors claimed that this could be correlated with a reduction in aldosterone production.

In view of these facts, it seemed to be reasonable to present our findings, biochemical, morphological and histochemical, as obtained in a small group of patients suffering from the central-type, i.e. nontumorous Cushing's syndrome, who had been treated with AGL for various periods prior to adrenalectomy.

Material and Method

The present study has been based on four patients suffering from the so-called secondary or central-type Cushing's syndrome, the important pre-treatment clinical data of whom have been listed in Table 1, and the relevant biochemical data of whom have been summarized in Table 2.

All these patients received peroral aminoglutethimide (Elipten® Ciba) in doses varying between 1 and 2 g for various periods of time and had a two-stage adrenalectomy (Table 1). Save for the left adrenal gland of case D, all the adrenals were subjected to a thorough morphological and histochemical examination.

Table 1. Clinical features and scheme of treatment of the patients with central-type Cushing's syndrome

Patient	Sex and age	Duration of illness (yrs)	Clinical symptoms	AGL treatment prior to first adrenalectomy	First adrenalectomy	AGL treatment prior to second adrenalectomy	Second adrenalectomy
A (K. L.)	♀, 27	1	complete[a]	1.5 g daily 4 weeks	right (A I)	none 12 days	left (A II)
B (B. I.)	♀, 27	2	complete[a]	none	right (B I)	2 g daily 2 weeks	left (B II)
C (P. J.)	♀, 33	4	complete[a]	2 g daily 5 months	right (C I)	none 4.5 months	left (C II)
D (D. J.)	♂, 15	3	incomplete[b]	none	left[c]	1 g daily 6 weeks	right (D)

[a] Complete clinical symptoms included: truncal obesity, cervical fat pad, moon face, striae, plethora, hypertension, hirsutism, ankle oedema, muscular weakness, osteoporosis, prediabetes and amenorrhoea.

[b] Truncal obesity, cervical fat pad, moon face, plethora, striae, slight osteoporosis and prediabetes.

[c] Carried out 1 year prior to present admission, material not avialable for study.

Table 2. Examinations of steroids in the patients with Cushing's syndrome

Patient	A (K.L.)	B (B.I.)	C (P.J.)	D (D.J.)
Cortisol secretion rate, mg/24 hrs	61	43	73	52
Plasma cortisol, μg/100 ml				
at 8 a.m.	34	27	27	19
at 8 p.m.	38	23	29	23
Urinary cortisol, μg/24 hrs	387	132	570	270
Urinary 17-hydroxycorticoids, mg/24 hrs	33	28	25	24
(range)	(26–47)	(22–37)	(18–29)	(17–33)
Urinary 17-ketosteroids, mg/24 hrs	35	38	26	26
(range)	(25–47)	(28–47)	(21–30)	(19–30)
Corticotrophin and Metopirone tests	positivie	positive	positive	positive
Suppression by Dexamethasone	partial at 16 mg	partial at 8 mg	partial at 8 mg	partial at 8 mg

In all the patients, a two-stage adrenalectomy was carried out in the usual way under combined inhalation anaesthesia. After removal, the adrenals were immediately trimmed from fat, weighed and cut in parallel transverse slices approximately 2 mm thick, and examined macroscopically. Alternating slices were used for conventional histology, histochemistry, electron microscopy and biochemistry.

For biochemical studies, minced adrenal tissue (100±2 mg) was suspended in 5 ml of Krebs-Ringer phosphate buffer with glucose (20 mM/l) and incubated with 1 μC of 4-^{14}C-cholesterol or 7α-^{3}H pregnenolone at 37° C under oxygen in a Dubnoff shaker for 60 min. After incubation, 100 μg of unlabelled cortisol and corticosterone were added and the mixture pre-extracted with light petroleum. Thereafter, corticosterone was extracted from the aqueous phase with tetrachlormethane and cortisol with ethyl acetate. The dry residues were chromatographed on Whatman 1 paper to constant specific activities of the following solvent system: 1) toluene-propylene glycol, 2) benzene : methanol : water, 100:50:50 (Bush B_5), 3) benzene : cyclohexane : methanol : water, 100:100:100:50. Cortisol and corticosterone were visualized in the paper chromatograms by the means of UV light at 254 mμ. The steroids were extracted with ethanol and measured by selective light absorption at 240 mμ. The radioactivity of all samples was determined in SLT mixture (Spolana, Neratovice) using an ABAC 40 SL Intertechnik GUE liquid scintillation counter.

Alternately, adrenal slices were incubated simultaneously with 4-^{14}C-cholesterol and 7α-^{3}H-pregnenolone, 1 μCi each, and the incubation mixtures were processed as described elsewhere (Šulcová and Stárka, 1971). The ratio of corticoids and adrenal androgens was determined (Table 3).

Table 3. The ^{14}C:^{3}H ratio in steroids isolated after incubation of adrenal slices with 4-^{14}C-cholesterol and 7-^{3}H-pregnenolone (1 μCi each)

Patient	Treatment[a]	Cortisol	Cortico-sterone	Dehydro-epiandro-sterone	Andro-stenedione
K.L.	none (A II)	0.15	0.14	0.17	0.16
	AGL treatment (A I)	0.03	0.02	0.07	0.09
B. I.	none (B I)	0.16	0.14	0.16	0.17
	AGL treatment (B II)	0.03	0.02	0.07	0.06

[a] Cf. Table 1.

The tissues destined for morphological studies were, in part, fixed in neutral formalin or in neutralized Baker's fixative. Some tissue slices were quick-frozen on metal cryostat blocks in petroleum ether chilled with a mixture of acetone and dry ice, wrapped in a polyethylene foil and kept on dry ice or in a refrigerator at −22° C until further processing. For the enzyme studies, they were sectioned on a Pearse-Slee cryostat, usually not later than 3 days after operation.

The fixed adrenocortical tissue blocks were either embedded into paraffin (some of them after thorough washing and using the rapid vacuum technique for enzyme studies), or, after a 6–8 hours' period of fixation, the tissues were washed thoroughly in several changes of cold distilled water and sectioned on a freezing microtome for enzyme and lipid studies.

The conventional staining methods used included haematoxilin and eosin, Masson's trichrome stains, occasionally combined with a modified Gomori silver impregnation technique for reticulin fibres, the PAS method and, occasionally, Heidenhain's ferric haematoxylin and Mallory's phosphotungstic acid haematoxylin. Lipids were demonstrated with the Sudan stains as well as by the OTAN method combined with lipid extractions as suggested by Elleder and Lojda (1968).

A number of enzymes were demonstrated in the paraffin-embedded, frozen as well as in cryostat sections. The enzymes demonstrated in paraffin sections were the alkaline and acid phosphatase and nonspecific esterases, α-naphtyl esterase and naphtol-AS-esterase. The same enzymes, plus several substrate-specific phosphatases (IDP, TPP, GTP, ATP), lactate dehydrogenase and the NADH and NADPH tetrazolium reductases (diaphorases) were demonstrated in frozen sections. A number of other dehydrogenases were demonstrated in cryostat sections, namely the 3 β-hydroxysteroid dehydrogenase, isopropanol dehydrogenase (Hardonk, 1965), glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NAD- and NADP-linked isocitrate dehydrogenase, malate dehydrogenase and succinate dehydrogenase. The methods used for the demonstration of enzymes listed were those recommenced by Pearse (1960, 1968) modified, in some cases, by Lojda (1970).

Results

a) Biochemical Findings

AGL-treatment resulted in a marked impairment of the *in vitro* conversion of 4-^{14}C-cholesterol to cortisol and corticosterone by adrenal slices as shown in Fig. 1 summarizing the data obtained from patients K.L. and B.I. (cf. Tables 1–3). In contrast, the conversion of 7α-^{3}H-Δ_5-pregnenolone remained essentially unaffected. The results are in agreement with those of Dexter *et al.* (1967) who demonstrated that, in the rat, AGL inhibits adrenal steroid biosynthesis by interfering with the conversion of cholesterol to pregnenolone.

Similar conclusions can be derived from the experiments with the simultaneous incubation of ^{3}H-pregnenolone and ^{14}C-cholesterol both to corticosteroids and C_{19}-steroids. The latter, however, were not affected to such a degree as cortisol and corticosterone (Table 3).

b) Morphological and Histochemical Findings

The adrenal glands available for study could be divided into three groups, namely a) untreated cases: one case of the present series plus 52 adrenal glands obtained from patients suffering from the central type Cushing's syndrome who had been subjected to bilateral adrenalectomy, serving as controls for the purpose of the present study (see footnote to Table 4), b) adrenal glands from patients treated with AGL for various periods of time until the moment of operation and c) adrenal glands of patients treated with AGL for various periods of time, in whom, however, AGL administration had been discontinued at various intervals

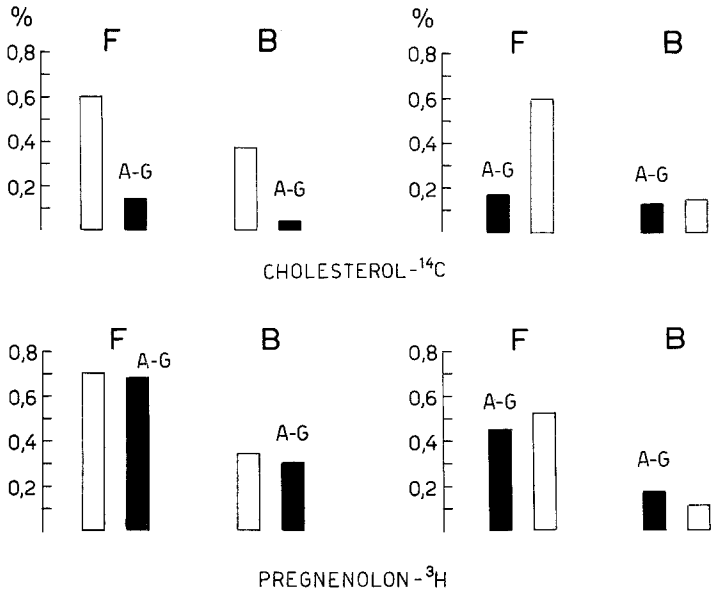

Fig. 1. Effect of AGL treatment on the *in vitro* conversion of cholesterol-4-^{14}C and Δ^5-pregnenolone-7α-^{3}H, respectively, to cortisol (*F*) and corticosterone (*B*), by adrenal slices obtained from patients A (left) and B (right). White columns = no treatment; black columns = AGL treatment prior to operation. For details on treatment see Table 1

Table 4. Morphological findings in adrenal cortices of AGL treated cases of central-type Cushing's syndrome as related to duration of treatment

Case	AGL treatment	Interval between cessation of AGL treatment and operation	Morphological findings			
			adrenal weight (g)	lipoid hyperplasia	regressive changes of acrenocort. cells	scarring
BI	0	0	9	(+)	0	0
BII	2 weeks	0	17.5	++++	+++	0
AI	4 weeks	0	13.5	+++	+++	0
D	6 weeks	0	10.3	+++	+++	0
CI	5 months	0	13.4	++	++	0
AII	4 weeks	12 days	11	++	++	0
CII	5 months	4.5 month	9	+	(+)	++

Bioptic cases of central-type Cushing's syndrome (control group)

Number of cases (sex)	Duration of illness	Weight of first adrenal (mean)	Interval between operations	Weight of second adrenal (mean)
26 (13 ♂ + 13 ♀)	0.5–10 years	5.2–11.2 g (7.49 g)	2 wks to 11 mos	5.0–14.0 g (8.15 g)

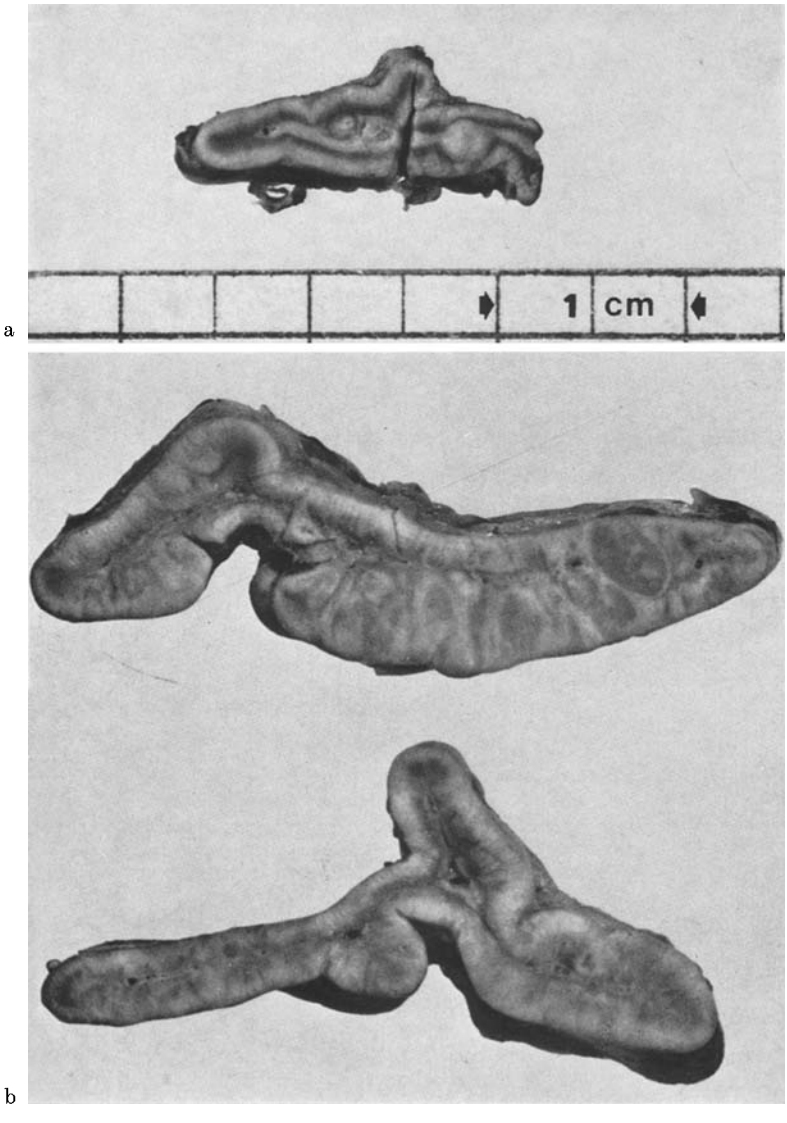

Fig. 2. a Case BI (top) and b BII (bottom). Difference between gross appearance of cross-sectioned adrenal prior to (BI) and following (BII) AGL treatment of two weeks duration (confront with Table 4)

prior to operation. According to the duration of AGL administration with or without interruption of AGL treatment before operation, the individual glands could be put in a sequential series as shown in Table 4, together with the organ weights and the essential microscopical findings.

Grossly, the adrenal glands of AGL-treated individuals could be invariably denoted as severely hyperplastic, the degree of hyperplasia exceeding the range

usual in untreated cases of the central-type Cushing's syndrome, which is obvious from the confrontation of the cases included in part 1 and part 2, respectively, of Table 4. In one case the right adrenal (BI) had been removed prior to AGL treatment and the left one (BII) following a previous AGL administration of two weeks' duration (Fig. 2a, b). The difference between the two was almost 100% of the original weight. In none of the cases of the control series of central-type Cushing's syndrome where both glands were removed successively (at intervals ranging between 2 weeks and 11 months) a similar difference could be observed. Hence the increase in weight in the AGL-treated cases is most likely to be attributed to the influence of the drug. A slight to marked regression in adrenal size could be noted after prolonged periods of withdrawal of AGL prior to operation (Table 4).

On cross section, the cortex appeared to be markedly widened. It usually showed a mottled appearance with intimately intermingling reddish-brown and yellow areas, with a prevalence of ochre yellow colour indicating the presence of lipid. In most of the specimens (BII, D, AI, CI, AII) there was a conspicuous narrow yellow band in the subcapsular region. Small, usually ill-defined intracortical micronodules were observed in some cases, but these might have been present before treatment. It should be mentioned in advance that these micronodules of adrenocortical tissue underwent identical changes attributable to AGL treatment as the remaining adrenal cortex of the respective cases.

Microscopically, the changes observed in the adrenal cortex of the patient treated with AGL for 14 days (BII) mainly consisted of adrenocortical hyperplasia resulting in a marked widening of the adrenal cortex with a slightly indicated micronodule formation and adrenocortical cell hypertrophy. Most of the hypertrophied cells showed a marked lipid accumulation, thus acquiring the appearance of hypertrophic spongiocytes. Their cytoplasm was finely spumoid, but in some cells it tended to become coarsely vacuolated. These changes occurred predominantly in the outer half of the cortex, affecting both the zona glomerulosa and the external fasciculata. At this early stage the two zones remained distinguishable from one another. In addition, there occurred dystrophic changes of adrenocortical cells presenting as extremely fine cytoplasmic vacuolation with lipid accumulation and occasional formation of small, needle-shaped or rhombic crystals within the cytoplasm believed to be cholesterol or cholesterol esters according to their morphology and lipid solubility. On occassion, such cells acquired an almost homogeneous, "vitreous" appearance in conventional preparations (Figs. 3 and 4). The nuclei of such cells tended to become pycnotic. Such regressive changes supposedly terminate in cell necrosis (cytolysis).

Histochemically, the altered cells were characterized by an increase in acid hydrolases, particularly acid phosphatase (Figs. 5 and 6) and by a depression or even loss of activity of virtually all the dehydrogenases studied (Figs. 7 and 8). No other histochemical changes were observed.

The above alterations were most frequent in the peripheral third of the adrenal cortex (Figs. 3 and 5), usually beginning at a certain distance from the capsule, so that a subcapsular band of lipid-rich adrenal cortex remained free from the dystrophic changes. Tiny irregular patches of altered cells occurred in the central fasciculata, too (Fig. 5). However, they were far less frequent and

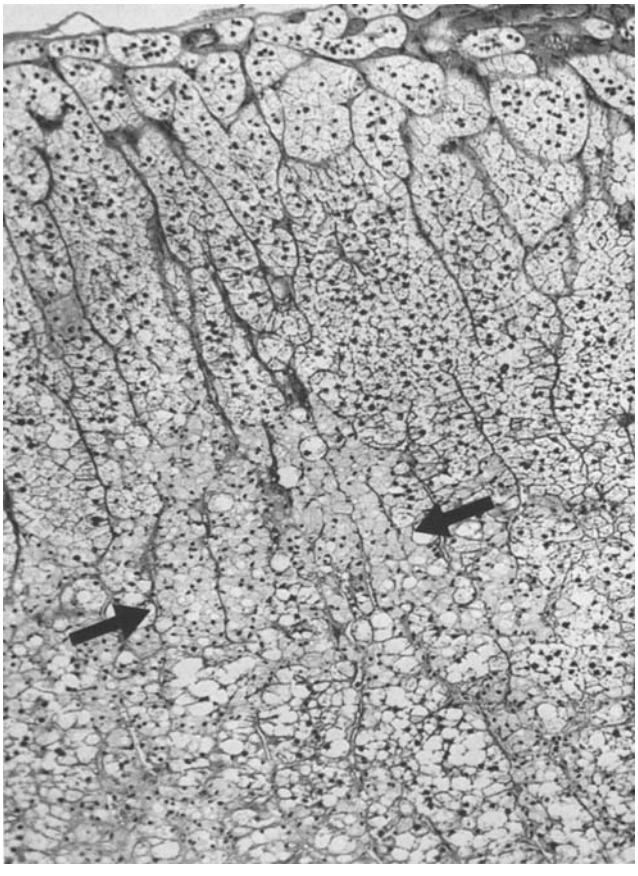

Fig. 3. Case BII. Outer half of adrenal cortex showing "lipoid" hyperplasia with coarse vacuolation of the cytoplasm of some adrenocortical cells (bottom). Note the circumscribed focus of dystrophic cells with homogenization of cytoplasm (arrows). Haematoxylin and eosin, magnif. ×130

their distribution appeared to be less regular. The lesions were not observed in the deepest adrenocortical layers, where the main findings included cell activation and hypertrophy without any considerable lipid accumulation (the so-called active-type hyperplasia). Occassionally, the foci of adrenocortical cell degeneration were accompanied by slight round-cell cellulization comprising lymphocytes, plasmacytes and histiocytic cells. The last ones exceptionally turned into phagocytes incorporating cell debris, presumably originating from destroyed adrenocortical cells. None of the dystrophic lesions and only negligible focal round-cell infiltrations, a milder degree of hyperplasia and cell hypertrophy associated with a very low degree of fatty infiltration were observed in the contralateral adrenal gland (BI) removed from the same patient prior to AGL administration.

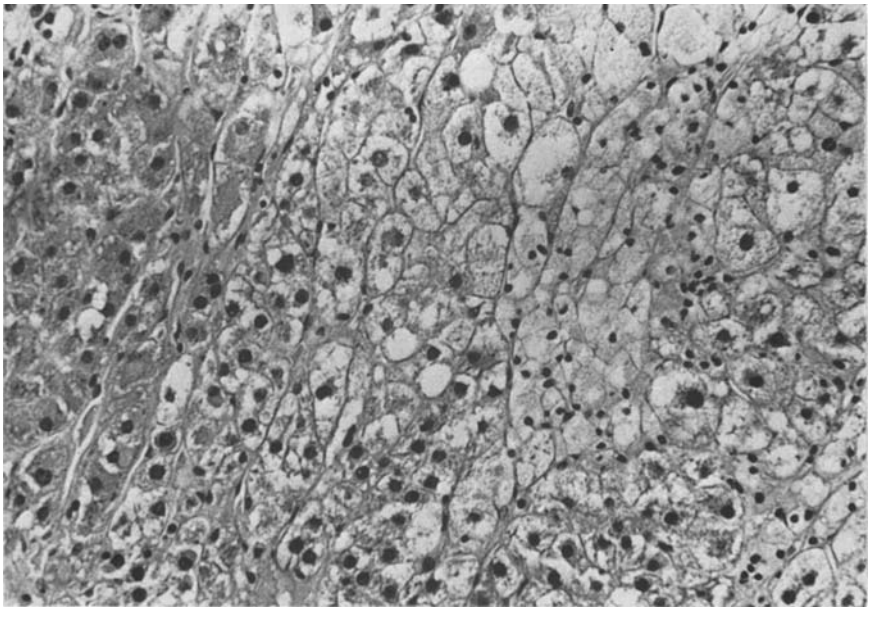

Fig. 4. Case AII. Area of adrenal cortex showing hyperplasia with presence of groups of compact cells (left) and a cluster of dystrophic adrenocortical cells showing nuclear pycnosis and homogenization of cytoplasm (right half of field). Haematoxylin and eosin, magnif. ×210

The adrenal cortices of the remaining cases (D, AI, CI, AII) were very similar to that of BII. In all of them there was a high degree of lipoid hyperplasia associated with the dystrophic changes of adrenocortical cells as described above. In cases of chronic AGL administration, there was an increasing tendency towards a loss of adrenocortical zonation, affecting not only the inner cortical areas but also the subcapsular region. After 6 months of AGL administration (CI) as well as after an 8 weeks' treatment followed by a period of 12 days without treatment prior to operation (AII), there was a milder degree of lipoid hyperplasia and the dystrophic cellular changes were less frequent and less extensive. No crystal formation was found in CI and AII, but otherwise the morphological and histochemical pattern was identical with that described in BII.

No dystrophic cellular changes were found in the adrenal gland removed at 4.5 months after the interruption of chronic AGL treatment (CII). In contrast, this gland contained multiple tiny irregular foci of fibrosis which could be correlated with the previous areas of degeneration or necrobiosis as regards their shape, extent and localization.

All the cases subjected to AGL treatment occasionally developed cortical micronodules as well as intra- or extracapsular proliferations of adrenocortical cells. Since however, such formations are frequently found in adrenal glands of untreated Cushing's syndrome, they cannot be regarded as a result of drug administration. Irrespective of whether they were well or ill defined, the micronodules as well as the intra- and extracapsular proliferations of adrenocortical

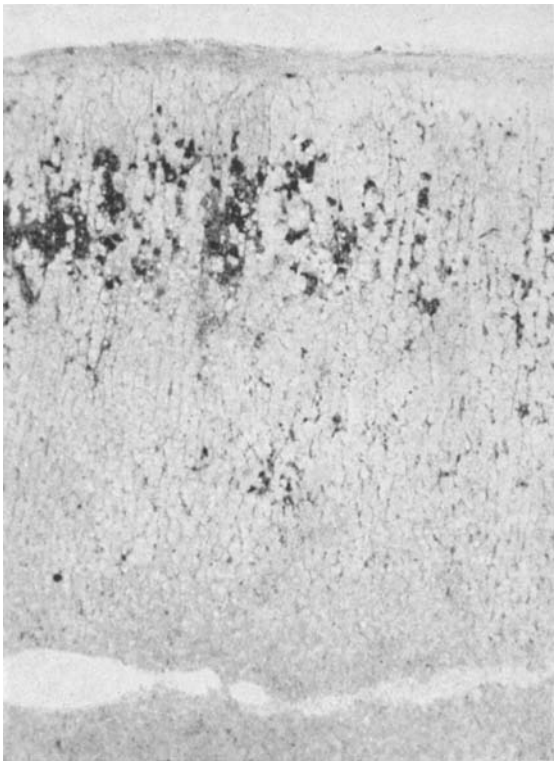

Fig. 5. Case BII. Areas of adrenocortical cell dystrophy as visualized by the acid phosphatase method. Note distribution of lesion (dark) occupying, in particular, the outer third of the adrenal cortex. Paraffin section, azo-coupling method for acid phosphatase, Light Green counterstain. Magnif. approx. ×40

cells displayed essentially the same degenerative and necrobiotic changes as the remaining cortex. This indicates that both the micronodules and the intra- or extracapsular proliferations react to AGL administration in the same way as the cortex proper.

Discussion

The main adrenocortical lesion in AGL-treated patients suffering from central-type Cushing's syndrome can be denoted as lipoid hyperplasia. Its pathogenesis seems to be quite clear today owing to the theoretical studies of Kahnt and Neher (1966), Fishman *et al.* (1967), Dexter *et al.* (1967) and Cohen (1968) who demonstrated that the blocking action of AGL upon the C_{20}–C_{22} lyase effective in the cholesterol side chain cleavage was primarily responsible for the adrenocortical changes. Under such conditions, the negative feedback mechanism results in an increase in ACTH-secretion leading, as usual, to the typical diffuse adrenocortical hyperplasia. The accumulation of lipids which may attain such a degree as to result in the development of lipid (cholesterol or cholesterol ester) crystals is another consequence of the AGL-induced block of steroid biosynthesis, reflecting the storage of non-utilized natural steroid precursors. It is interesting to note

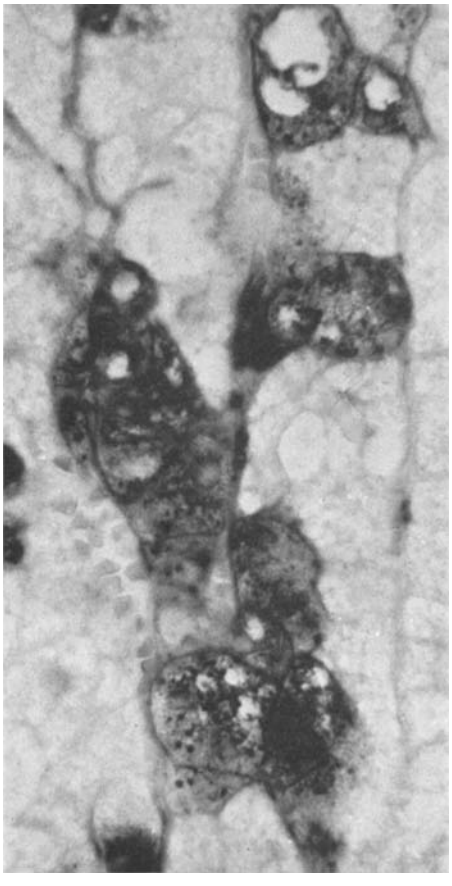

Fig. 6. Case CI. Area of adrenocortical cell dystrophy with an increase in acid phosphatase activity in the cytoplasm of dystrophic cells. Note predominantly corpuscular enzyme activity. Acid phosphatase technique as in Fig. 5, magnif. ×535

that the whole mechanism resulting in lipoid hyperplasia also operates in individuals suffering from the secondary-type Cushing's syndrome, where the adrenal glands already show signs of adrenocortical stimulation including hyperplasia. This has been documented by comparing the glands BI and BII, the former removed from a patient with an untreated Cushing's syndrome, the latter from the same patient after a short period of AGL treatment. The difference in weight as well as in the microscopical pattern of the two glands exceeds the range of differences observed in the series of patients with the central-type Cushing's syndrome treated by a two-stage total adrenalectomy, as observed previously.

The AGL-treated adrenal glands of the present series show some obliteration of adrenocortical zonation. In all the cases the inner zones (fasciculata and reticularis) were shown to fuse, whereas some loss of zonation in the cortical periphery accompanied by the absence of a morphologically discernible zona glomerulosa could be noted in the chronically treated cases only. It must be realized,

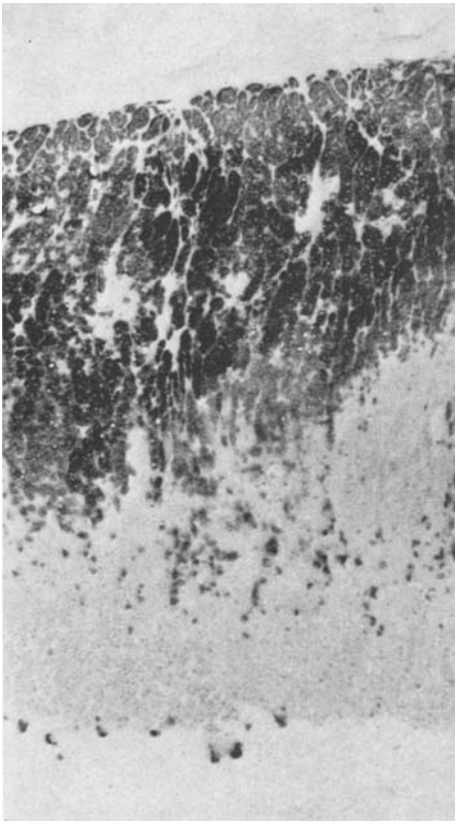

Fig. 7. Case CI. Focal adrenocortical cell dystrophy presenting as negative, "moth-eaten" areas in the cortical periphery showing a strong enzyme activity. Confront the localization of the lesions with that shown in Fig. 5. Method for 3 β-hydroxysteroid dehydrogenase, DHEA as substrate, magnif. approx. $\times 40$

however, that a definite loss of zonation occurs in all marked secondary adrenocortical hyperplasias and is also frequently seen in the secondary-type Cushing's syndrome. Therefore it is hardly possible to draw any conclusions concerning changes in the secretion of adrenocortical hormones in AGL-treated cases from the disturbances in adrenocortical zonation as claimed, e.g., by Givens *et al.* (1970).

Of the natural human adrenocortical disorders the one most closely resembling the AGL-induced adrenocortical changes is the so-called congenital lipoid adrenocortical hyperplasia of Siebenmann (1957). This disease resembles the state induced by AGL treatment not only morphologically, but also pathogenetically. Even in congenital adrenocortical lipoid hyperplasia there seems to be a block affecting the early stages of steroid biosynthesis resulting, on the one hand, in the accumulation of cholesterol (or its esters) and, on the other hand, in a depression of steroid biosynthesis with an impairment of the adrenocortical-pituitary feedback mechanism leading to an increase in ACTH release and a more-or-less diffuse

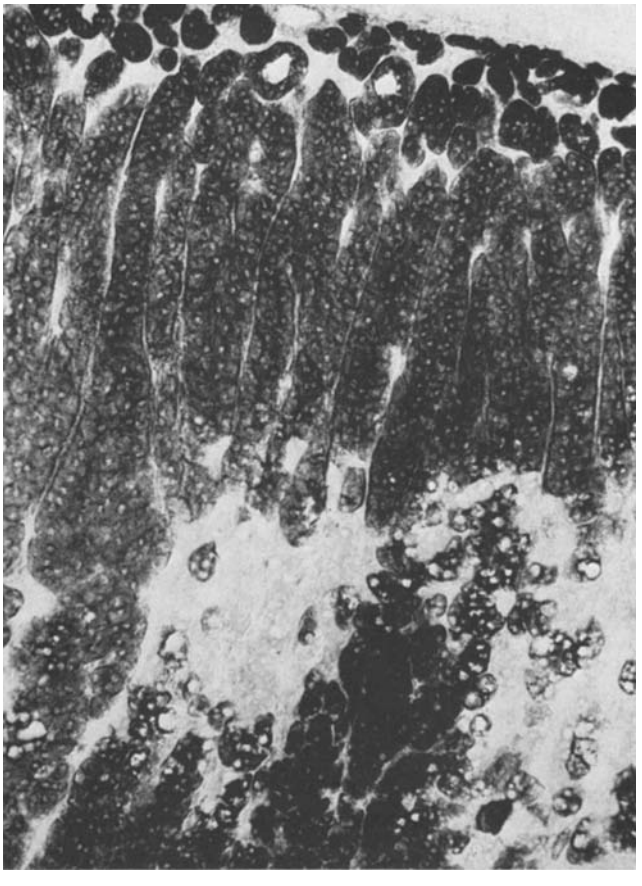

Fig. 8. Case BII. Areas of cell dystrophy showing reduction or virtual absence of enzyme activity. Method for glucose-6-phosphate dehydrogenase, magnif. approx. ×130

adrenocortical hyperplasia. In contrast to the AGL-induced changes as presented in this paper, those of congenital lipoid adrenocortical hyperplasia are usually more advanced and more uniform. The degree of adrenocortical changes may depend on the duration and the completeness of the block. It should be borne in mind that the blocking action of AGL is a competitive one and can be overcome by increased ACTH levels as shown by the relatively lower adrenal weights of the chronically treated cases. In contrast, it is questionable whether such a compensation is possible in the case of Prader-Siebenmann's disease. The relative lack of uniformity as observed in our AGL-induced changes may depend on the appearance of the adrenal cortex prior to AGL administration, and it should be recalled that in many cases of the secondary-type Cushing's syndrome the adrenal cortex shows a rather polymorph pattern. The effect of AGL upon adrenocortical cells possibly depends on their functional state, and it must be realized that the functional state (and, consequently, the morphology) of adrenocortical cells in the diffuse or diffuse-micronodular hyperplasia of the secondary-type Cushing's

syndrome may vary considerably in various areas of the hyperplastic adrenal cortex (Hradec and Motlík, 1970).

The human adrenocortical changes following AGL administration closely resemble those induced in various animals, especially in rats, by the same drug (Stárka and Motlík, 1971; Motlík and Stárka, 1972; Motlík *et al.*, 1972, see also for further references). Even in animals the AGL-induced adrenocortical lesions could be designated as lipoid hyperplasia, and cholesterol plus cholesterol esters were shown to be responsible for the lipid accumulation. In addition, they included dystrophic cellular lesions, necrobiosis or even necrosis of adrenocortical cells, inflammatory cell aggregates occurring in association with adrenocortical cell damage, and, later, absorption of the necrotic areas of adrenocortical tissue, condensation of their fibrovascular stroma resulting in the formation of a fibrous medullary capsule and associated with regeneration of adrenocortical tissue in the subcapsular and the peripheral region of the cortex.

Similar lesions could be produced by amphenone (Hertz *et al.*, 1955; Kracht, 1961), Δ^4-cholestene-3-one (Kracht, 1961; Frederickson *et al.*, 1958) and, recently, by aniline (Kovács *et al.*, 1971). All these lesions obviously share an essentially common pathogenesis though there seem to be some differences concerning the actual nature and position of the block. Thus, e.g. a long-term administration of amphenone (Kracht, 1961) and aminoglutethimide (Motlík and Stárka, 1972; Motlík *et al.*, 1972) led to a regression of the originally extreme adrenocortical lipoid hyperplasia.

The dystrophic and, sometimes, even the necrobiotic changes of adrenocortical cells may result in necrosis as shown by the morphological and histochemical findings presented here. In particular, the increase in acid phosphatase and the depression of the activity of all the dehydrogenases observed appeared to be a good and reliable marker of the regressive changes. There is some uncertainty as regards the mechanism proper of the cell death. First, the lethal effect of AGL may become realized through mitochondrial damage as demonstrated in animal experiments by Racela *et al.* (1969) and Marek *et al.* (1970a, b). No difference was found, however, in the reduction of mitochondrial and extramitochondrial dehydrogenases in the cases studied here. A finding of a preferential reduction in mitochondrial enzyme activities, which might have indicated a role of mitochondrial damage in the process of cell necrobiosis due to AGL could have possibly occurred at an earlier stage of AGL treatment. The other possible lethal factor might have been cholesterol overload as such which, in some cases, is really excessive. A combination of the two factors mentioned cannot be ruled out either, and perhaps other mechanisms leading to cell death should be also taken into account.

The fate of the necrobiotic and necrotic cells cannot be always traced with certainty. In some cases, nuclear pycnosis and a gradual loss of stainable basophilic substance indicates cytolysis. Occasionally, cell debris can be found in aggregates of round inflammatory cells, which obviously represent a mesenchymal reaction to the adrenocortical cell death, but whose significance is not fully understood. The presence of histiocytoid cells may represent an immediate reaction to the presence of cell depris, but the accumulation of the so-called immunocompetent cells is rather unclear. It should be mentioned that similar inflammatory cell aggregates also occur in adrenal glands from untreated cases of Cushing's

syndrome as well as in the majority of adrenal glands obtained from adults subjected to routine autopsies (cf. Motlík, 1964). Their almost stable relation to areas of adrenocortical hyperplasia has led to the suspicion that the occurrence of such foci might represent one of the morphological manifestations of regression of the hyperplastic cortex. Much larger aggregates of the same type have been found in the adrenal glands of our experimental animals (Motlík and Stárka, 1972; Motlík *et al.*, 1972).

The question whether the inflammatory cell aggregates represent a manifestation of an autoimmune reaction (remember the dense infiltrations in cases of autoimmune adrenalitis, experimental as well as spontaneous) cannot be solved on the basis of the present material. It should be stressed that the infiltrations undergo gradual cicatrization resulting in irregular foci of cicatricial connective tissue within the cortex as observed in case CII. Such scars represent a permanent adrenocortical lesion which, if very extensive, might have a bearing upon adrenocortical function.

The biochemical findings confirmed the blockade of cholesterol side chain cleavage by ACL treatment. The biosynthesis of corticosteroids from pregnenolone as a direct precursor of C_{21}-steroids in the adrenal was unaffected. The results suggest that the compensatory mechanisms operate besides ACTH stimulation in the AGL-influenced adrenal, as shown by the divergent values of radioactivity incorporation into corticosteroids and C_{19}-steroids.

In conclusion, it should be stressed that AGL administration may produce severe alterations in human adrenals even with normal therapeutic doses of 1–2 g pro die. If extensive enough, the necrobiotic and necrotic lesions might become functionally manifest and might presumably cause adrenocortical insufficiency eventually persisting after discontinuation of AGL administration. May be that such severe lesions occur in exceptionally sensitive individuals only, because such reports are rather rare in the literature.

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