Radioactivity of kidneys

| Group | | Right | Left |
|---|---|---|---|
| I | Control | 1.430/min | 1.574/min |
| | Control | 1.569/min | 1.594/min |
| II | R. renal vein ligation | 17.193/min | 2.947/min |
| | R. renal vein ligation | 11.534/min | 6.097/min |
| III | L. renal vein ligation | 2.003/min | 17.662/min |
| | L. renal vein ligation | Died | |
| IV | R. ureter ligation | 2.137/min | 2.080/min |
| | R. ureter ligation | Died | |
| V | L. ureter ligation | 8.425/min | 8.700/min |
| | L. ureter ligation | 4.227/min | 4.786/min |

sumably facilitates glomerular deposition of immune complexes. Immune complexes localize in areas of greatest turbulence. The capillaries of the glomerular tuft located between 2 arterioles are subject to 4 times higher blood pressures than other capillary beds, being unopposed by any pressure from surrounding tissues since the glomerular tuft lies within Bowman's space. The blood flow to the kidney represents a large percentage of the cardiac output. Decrease in renal arterial pressure as in coarctation of the aorta or increase in pressure in Bowman's space as in hydronephrosis, has reportedly been associated with inhibition of deposition of circulating immune complexes and inhibition of deposition of circulating immune complexes and arteritis. Conversly, hypertension has been suggested to increase the severity of these lesions due to the increase pressure in glomerular capillaries. However, evidence for an active process of deposition of immune complexes related to vasoactive amines, and several mediators of inflammation has been well documented[10].

It has been suggested by GERMUTH et al.[6] that the high hydrostatic pressure within the arterial system and glomerular capillary bed may be responsible for the unique susceptibility of these tissues to the penetration and localization of pathogenic soluble immune complexes and the development of glomerulonephritis. If this hypothesis were correct, reduction in renal hydrostatic pressure produced by constricting the main renal artery or interference with renal filtration produced by ligation of a ureter should inhibit the glomerular localization of complexes and should reduce or abolish the development of glomerulonephritis. Our study demonstrated that renal vein ligation is associated with increased deposition of immune complexes, probably due to increased hydrostatic pressure on the glomerular capillaries; however, ureteral ligation failed to produce significant differences in immune complex deposition between the two kidneys.

The use of radiolabelled antigen is a sensitive method of quantitating localization of immune complexes. Factors other than renal capillary hypertension should be considered in analyzing the reasons for preferential deposition of immune complexes in the renal glomerular capillaries.

## Subcellular Steroid Distribution in the Rat Adrenal Cortex[1]

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*Summary.* The subcellular distribution of main steroids (pregnenolone, progesterone, corticosterone) and of cholesterol – this last after correction according to exchanges occuring during differential centrifugation – is presented in the rat adrenal cortex. The distribution patterns are not necessarily the same as established according to the localization of hydroxylating enzymes.

The present accepted metabolic pathway of steroidogenesis in the mammalian adrenal cortex[2] suggests a continuous movement of the steroid molecule between different intracellular compartments. This scheme was established according to the subcellular localisation of different hydroxylating enzymes. Apparently there is little information concerning the corticosurrenal subcellular distribution of the different steroids. However it is as important in this metabolic scheme as the different enzymatic systems.

The purpose of the present paper is to establish the subcellular distribution of 3 important steroids (pregnenolone, progesterone and corticosterone) and of cholesterol in the rat adrenal cortex. These results are compared with the subcellular distribution of the organelles as defined morphologically, morphometrically and chemically after differential centrifugation.

HOLZBAUER[3] was the first to approach this problem. We agree with her conclusions, but nevertheless obtained some different results. This may be due to mainly technical differences: we distinguished an intermediate (IM) fraction between mitochondrial (MITO) and microsomal (Micr) fraction; the absence of high esterified cholesterol level in the postmicrosomal supernatant (Snt) indicates good liposomal (Lip) recovery; systematical morphological and morphometric control of subcellular fractions was made. Lastly we give results obtained only from rats, the cholesterol content and metabolism being different in adrenals which contain large amounts of esterified cholesterol (rat, guinea-pig, etc.) in comparison with those containing exclusively free cholesterol (beef, ewe, etc.)[4].

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The details concerning the techniques used were exposed in our previous publications[5–8].

In this study, 30 young male albino rats (SD strain) were killed by neck dislocation between 09.00 and 10.00 h for each experiment. The adrenal glands were rapidly cut into two, and the cortex isolated from the medulla and the capsule. The tissue was homogenized in 1 ml/100 mg tissue of 0.25 *M* sucrose, buffered with *tris* HCl at pH 7.4 in a motor-driven teflon-glass homogenizer (model Potter-Elvjehem, clearance 0.1 mm) either for 80 sec with 24 passages (standard method) or for 40 sec with 8 passages by hand. Cholesterol was extracted with ethanol/acetone 1:1 v/v, and steroids with methylene chloride. After this last extraction method, pregnenolone and corticosterone were separated by bi-dimensional thin-layer chromatography in the mixture methylene-chloride/methanol 98:2 v/v[9] at first, and in hexane/ethyl-acetate 3:1 v/v in the second run.

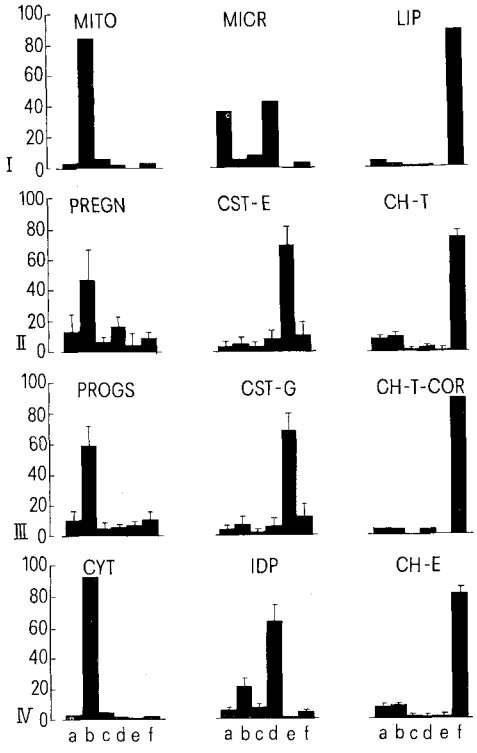


Fig. 1. Distribution patterns in the rat adrenal cortex subcellular fractions, obtained after differential centrifugation, visualized as histogramms. Values are expressed in % of the total amount of a given organelle in the intact organ (Line I) or in % of the total quantity of the product determined in the homogenate. (Lines II to IV).

MITO, corpuscular distribution of mitonchondria as subscellular organelle in the different fractions after separation by differential centrifugation; MICR, idem for microsomes; LIP, idem for liposomes. PREGN, pregnenolone-; PROGS, progesterone-; CST-E, corticosterone-distribution after standard homogenization; CST-G, idem after less energetic homogenization; CH-T, distribution of total cholesterol without correction; CH-T-COR, idem but values corrected according to the redistribution which occurs during the whole preparation procedure; CH-E, distribution of esterified cholesterol (without correction); CYT, distribution of cytochrome-c: $O_2$ oxydoreductase; IDP, distribution of the inosine-diphosphatase. Number of experiments: 6 for line I; 9 for cholesterol determinations; 5 for corticosterone assays and 4 for the other dosages. Absolute values, expressed in units per 100 mg tissue wet weight: total cholesterol, 4.6 ($\pm$ 1.4) mg; esterified cholesterol, 4.02 ($\pm$ 1.2) mg; pregnenolone 6.1 $\pm$ 2.5 $\mu$g; progesterone, 1.0 $\pm$ 0.50 $\mu$g; corticosterone, 1.0 $\pm$ 0.40 $\mu$g; IDP, 5.35 mgP/h; cytochrome, 2.4 $\mu M$/h. The fraction 'cell debris and nuclei' (DN) was obtained after centrifugation at 1,000 *g* for 2$\times$1 min; the mitochondria (MITO) at 8,000 *g* for 4 min.; an intermediate fraction (IM) – containing small mitochondria and microsome aggreagtes – at 18,000 *g* for 3 min. The remaining material was subdivised after spinning for 90 min at 100,000 *g* in the microsomal fraction (Micr), a postmicrosomal supernatant (Snt) and on the top of the last, a compact liposomal fraction (Lip) For each column of histograms: a, DN; b, MITO; c, IM; d, Micr; e, Snt and f, LIP.

Cholesterol (free and total) was determined according to Sperry and Webb[10]; pregnenolone, according to the colorimetric method of Munson[11] adapted to pregnenolone by Koritz[12]. Then progesterone was determined by radioimmunoassay by using a commercial biokit-assay (Biolab 003/NK-2). Finally, the assay of corticosterone was realized by fluorometry adapted from 3 different methods[13–15].

All the results are shown in Figure 1. The recovery of the main subcellular organelles (MITO, Micr, and Lip) is demonstrated on the first line. On the 4th line, the distribution of enzymatic markers can be seen (for MITO and Micr) and of the esterified cholesterol (EC), which can be considered as a true chemical liposome marker[4,5]. The concordance of the patterns on the 1th and the 4th lines assures the biochemical integrity of the 3 organelles studied. Total cholesterol distribution pattern is established before (CH–T) and after (CH–T–COR) correction, these last results taking into account the contamination due to the cholesterol redistribution which occurs during centrifugation. These last results suggest, for a well determined steroid, that the existence of a given concentration peak is true even before correction, but that the exact amount of the distribution cannot be evaluated before correction, above all in the fractions which contain less than 10% of this product.

Among the steroids studied without correction, pregnenolone shows an unusual distribution pattern with a mitochondrial peak which is lower than the morphological or marker peak of the same organelle, and with high SD values. This pattern may correspond either to a relatively high redistribution rate (but the Snt activity is not very pronounced) or, rather, to a bimodal distribution with the presence of a second peak in microsomes. Nevertheless, correction according to Allen[16] gives a proportion of 80% of pregnenolone in the mitochondria. Progesterone distribution pattern presents a clear mitochondrial maximum and general behaviour similar to the

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mitochondrial marker, confirming the results of HOLZBAUER.

Two distribution patterns for corticosterone are presented. After both kinds of homogenization, the distribution pattern is identical with the same high concentration of the steroid in the postmicrosomal supernatant. 3 explanations are possible: 1. Corticosterone is present in the cytosol and during centrifugation reaches the Snt compartment with weak secondary absorption on several subcellular components. 2. This molecule stays in the cell inside the tubules forming the endoplasmic reticulum (Er) and is released in the Snt when the transformation in microsomal vesicles occurs. 3. The binding of the corticosterone on any organelle is extremely weak and the release in the homogenization medium is instantaneous after the beginning of this procedure.

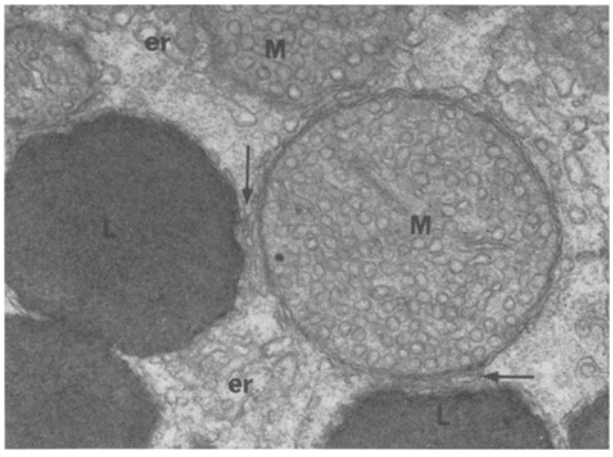


Fig. 2. Rat adrenal cortex. External fasciculata zone. Liposomes (L), mitochondria (M) and tubular elements of the endoplasmic reticulum (er). Arrows: er-cisternae between mitochondria and liposomes, in close contact with both organelles. All required enzyme-systems for the steroid pathway, the main steroids and cholesterol are present in this reduced space.
$OsO_4$-$H_2O$-fixative, reduced dehydration technique, lead-citrate stain[4]. × 30,400.

Lastly, the present steroid distribution pattern is not in agreement with the distribution suggested by the hydroxylating system; although both localization-scheme must not necessarely be identical. Enzyme systems fixed on (or in) organelle-membranes could enter into contact and react with the nearest steroid molecules which are either in the cytosol surrounding the organelles, or even fixed on another near-lying membrane system.

As can been seen in Figure 2, in the adrenocortical cells of the rat, there is a close relation between liposomes, mitochondria and endoplasmic reticulum, with a very important contact interface especially between the Er and the other organelles. This morphological fact, joined to the results of the steroid localization study reported here, suggest a partially new conception to resolve the problem of correlation between morphology and function in the adrenal cortex.

## Biosynthesis of α- and β-Ecdysone by the Crayfish *Orconectes limosus* in vivo and by its Y-Organs in vitro

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*19 January 1976.*

*Summary.* α- and β-ecdysone were synthesized from labelled cholesterol by premolt crayfish in vivo and by their Y-organs in vitro.

Molting in crustaceans – as in insects – is controlled by ecdysones (for review see[1]). From ablation-implantation experiments[2,3] it seems evident that in brachyuran crabs the sites of ecdysone biosynthesis are the paired Y-organs which were first described by GABE[4]. This assumption is confirmed by recent findings[5] which demonstrate a selective uptake of injected radioactive cholesterol, a biochemical precursor of ecdysones in insects, into the Y-organs of the crab *Hemigrapsus nudus* and by the demonstration[6] of α-ecdysone production by in vitro cultured Y-organs of *Pachygrapsus crassipes*.

However, reports on the Y-organs of Macrura are conflicting. Several distinctly different structures of macrurans have been described as 'Y-organs' but no evidence of their functional role as molting glands was given by ablation-implantation experiments or metabolic studies[7–10] (for reviews see[11,12]). In a recent report from our laboratory[13], the location and cyclic histological alterations during the intermolt cycle of a glandular structure of the crayfish *Orconectes limosus* were described. Preliminary extirpation experiments indicate the possible role of this organ as the molting gland of *Orconectes*.

The ability of putative Y-organs to synthesize ecdysones from cholesterol in vitro can be taken as an indication for their functional role as molting glands. We therefore investigated a) the ability of intact premolt crayfish to synthesize ecdysones in vivo as was recently demonstrated for α-ecdysone synthesis in molting lobsters[14],

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