95% ethanol → column chromatography on Dowex 1 × 2 → colorimetric determination of hexuronic acid (carbazole) and/or neutral sugar (anthrone) contents of each fraction. The different peaks were pooled and their values expressed in percentages of total carbazole material eluted from the column. Details of the method employed have been reported elsewhere[7].

Results and conclusions. The Table gives the results of AMP fractionations in urines of normal children of different ages and adults. Two changes of AMP patterns seem to be age dependent: with increasing age, the percentage of carbazole positive material in the heparitin sulphate (HS) fraction decreases, while the carbazole positive material in the chondroitin sulphate B (CSB) fraction increases. These changes are significant at the level of $p < 0.01$ between group I (infants) and group V (adults). The percentages of carbazole positive material in the chondroitin sulphate A (and/or C) (CSA) fraction remains almost the same throughout life.

Fractionation of acid mucopolysaccharides in urine of normal individuals

Groups of healthy persons	Age (years)	Carbazole positive material eluted from Dowex 1 × 2 column		
		HS-fr.[a] (%)	CSA-fr.[b] (%)	CSB fr.[c] (%)
I infants n = 7	1–2/12	40.7 ± 4.4	48.1 ± 5.1	11.3 ± 3.9
II small children n = 7	3–8	31.8 ± 7.0	57.5 ± 6.5	10.9 ± 2.7
III preadolescents n = 7	9–12	31.6 ± 7.1	57.7 ± 6.3	9.7 ± 2.3
IV adolescents n = 7	13–17	24.9 ± 3.8	58.9 ± 6.1	16.2 ± 3.6
V adults n = 9	22–64	28.2 ± 5.4	54.0 ± 4.3	19.1 ± 4.8

[a] HS-fr. = heparitin sulphate-fraction; [b] CSA-fr. = chondroitin sulphate A-fraction (contains also chondroitin sulphate C); [c] CSB-fr. = chondroitin sulphate B-fraction.

During the first 4 decades of life, the proportions of keratosulphate in human cartilage increase to a level of about 55% of total AMP, while the contents of chondroitin sulphate C (CSC) decline slightly (MATHEWS and GLAGOV[3]). In human aorta the total AMP contents increase during life on account of rising CSB and HS deposition. Hyaluronic acid (HA) and CSC decline during the same period (KAPLAN and MEYER[4]; BUDDECKE[5]).

Skin of pig embryos contains less CSB and more HA than skin from adult animals. In the latter LOEWI and MEYER[8] found a sixfold increase of CSB and a decrease of HA from 78% in embryonic to 30% in adult pig skin.

Our findings of an age dependent decrease of HS excretion in urine cannot be correlated with the reported changes of AMP patterns in various tissues; however there may be some as yet unknown connection between the increasing urinary CSB excretion and the rising deposition of CSB in skin as age advances[9,10].

Zusammenfassung. Die sauren Mucopolysaccharide im Harn von 37 Normalpersonen verschiedenen Alters (Säuglinge bis Erwachsene) wurden säulenchromatographisch fraktioniert und einzeln bestimmt.

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The Effect of Steroid Treatment on Ovarian Dehydrogenases in the Rat. Histochemical Study

Ovarian dehydrogenases directly or indirectly related to steroid pathways have been histochemically investigated in the rat, in physiological and in numerous experimental conditions[1–11].

In the present study ovarian 3β-hydroxysteroid dehydrogenase (3β-HSD), glucose-6-phosphate dehydrogenase (G-6-PD) and 20α-hydroxysteroid dehydrogenase (20α-HSD) of rats treated with progesterone and/or estradiol and with testosterone have been histochemically studied.

Eight groups, each of 5 Sprague–Dawley female rats weighing 200–250 g, were daily injected s.c. with the following steroids suspended in a standard medium: progesterone 1 mg (P_1) or 3 mg (P_3); testosterone 30 μg (T_{30}) or 300 μg (T_{300}); estradiol-benzoate 1 μg (E_1) or 15 μg (E_{15}); progesterone 3 mg + estradiol-benzoate 1 μg (P + E). Controls received 0.2 ml daily of medium alone.

Vaginal smears were checked daily at 10.00 and the regularity of the estrus cycle was assessed for 15 days before the experiment. All treatments started at metestrus-diestrus phase. The animals were sacrificed by decapitation after 20 days of treatment.

Cryostatic sections of the ovaries were prepared as previously described[11] and were then incubated for the demonstration of the 3β-HSD[4], G-6-PD[12] and 20α-HSD[1]. For each group of animals the number of corpora lutea (C.L.) positive to the different reactions and the number of follicles with 3β-HSD activity in the granulosa cells were established and the amount of diformazan deposition in thecal and interstitial cells was scored in a blind study on a 0–2 plus scale.

In ovarian sections from control animals, intense 3β-HSD and G-6-PD occurred in the follicular thecal cells, in granulosa cells of maturing follicles, in the interstitial tissue and in all C.L. 20α-HSD activity was demonstrated exclusively in involuting C.L. In rats sacrificed at met-

estrus and diestrus, the newly-formed 3β-HSD positive C.L. totally lack 20α-HSD activity and show a weak G-6-PD activity.

P_1 Group. Estrus occurred in all the animals, but the number of C.L. was less than in the controls, with a higher percentage of C.L. with 20α-HSD activity. The number of follicles with 3β-HSD activity in granulosa cells (maturing follicles) remained similar to that of the controls, while thecal 3β-HSD and G-6-PD activities appeared reduced. No variations in interstitial enzymatic pattern were observed.

P_3 Group. Estrus did not occur in this group. Therefore ovaries contained a markedly decreased number of small C.L. (Figure 1) all demonstrating 20α-HSD activity (Figure 2). The average number of maturing follicles remained at normal values. Furthermore no differences appeared in enzymatic pattern of thecal cells in comparison with P_1 group, while interstitial cells exhibited G-6-PD activity weaker than the controls.

T_{30} Group. Estrus occurred in this group and the findings did not differ from P_1 and E_1 treated animals.

T_{300} Group. Since estrus did not occur, a reduction was noticed of the number of C.L. all of them demonstrating 20α-HSD activity. Moreover no variations were observed in the number of maturing follicles. The thecal cells were devoid of enzymatic activities while interstitial cells retained 3β-HSD activity only.

E_1 Group. Estrus occurred in this group and the results did not differ significantly from P_1 and T_{30} groups. In addition no changes were detected in enzymatic activities of the thecal cells.

E_{15} Group. Since no cycle occurred, the average number of C.L. was markedly low (Figure 3). C.L. with 20α-HSD activity were very few and small (Figure 4). The number of maturing follicles remained practically unchanged as compared to controls. Enzymatic activities disappeared from thecal cells, while interstitium retained only weak 3β-HSD activity.

P + E Group. No cycle occurred also in this group and therefore the number of C.L. was markedly low with 50% of them demonstrating 20α-HSD activity. Unlike the E_{15} treated group, here maturing follicles had almost completely disappeared. Moreover thecal cells were losing enzymatic activities, while interstitial cells retained only 3β-HSD activity.

It is well known that the follicles with 3β-HSD activity in granulosa cells are preovulatory[5]. In this connection it is remarkable that their number in normally cycling rats approaches the number of ova shed by 1 ovary in the estrus, i.e. 4–6, as well as the number of the newly-formed C.L. (Table). The demonstration of the 20α-HSD activity in C.L. at proestrus phase may reveal the onset of involuting processes in the most recently-formed ones[1,5,11]. Moreover, although specific changes during the cycle could not be detected, the findings of the 3β-HSD and G-6-PD activities in thecal and interstitial cells indicate that they are involved in steroid production.

As shown in the Table P_1, P_3, T_{30}, T_{300}, E_1 and E_{15} treatments did not inhibit the follicle maturation. The presence of normally maturing follicles under progesterone treatment or during luteal phase has been observed in a range of mammals[13–15] together with a continuous secretion of

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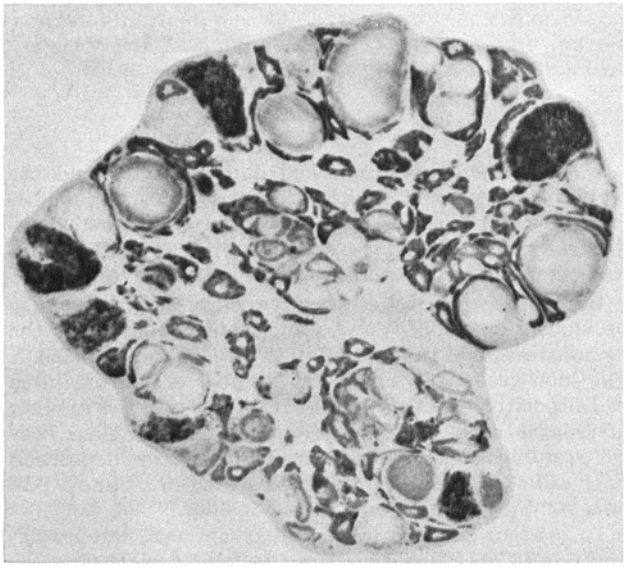

Fig. 1. Rat treated with progesterone 3 mg (P_3). Few and small C.L. 3β-HSD positive. Marked 3β-HSD activity in thecal and interstitial cells. × 25.

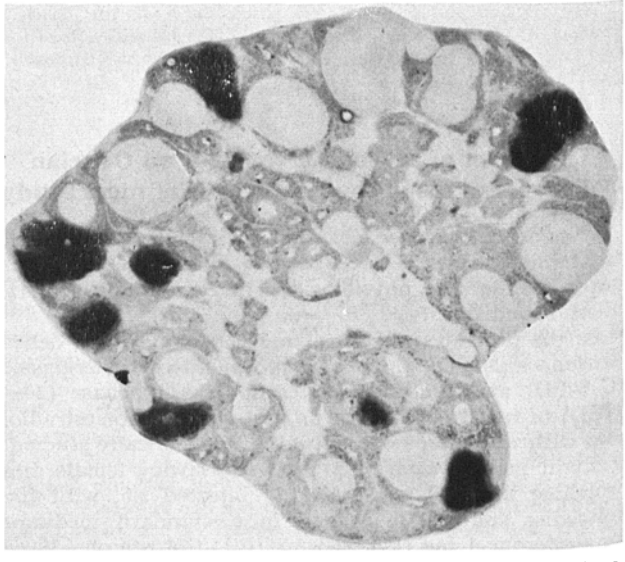

Fig. 2. Section contiguous to that of Figure 1. All the C.L. show 20α-HSD activity. × 25.

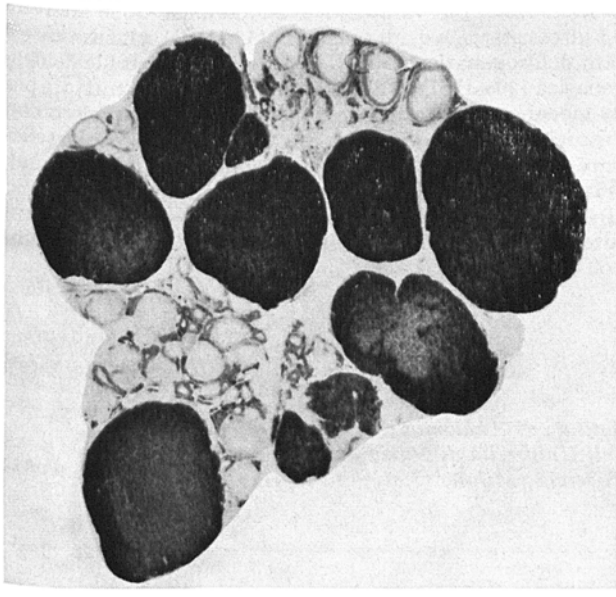

Fig. 3. Rat treated with estradiol 15 μg (E_{15}). The 3β-HSD activity is marked in C.L. and weak in interstitial cells. × 25.

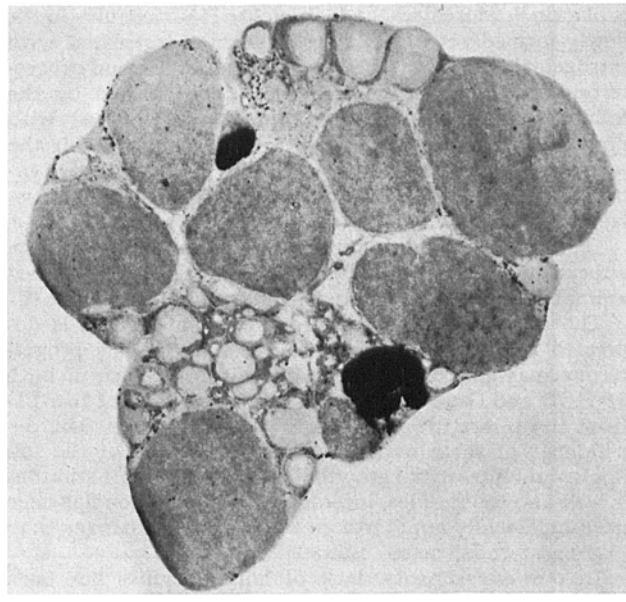

Fig. 4. Section contiguous to that of Figure 3. Most of the C.L. are 20α-HSD negative. Only few and small ones show 20α-HSD activity. × 25.

Effect of various treatments on enzymatic activities observed in corpora lutea (C.L.), follicular structures and interstitial cells of rat ovary. (Average value of 5 different animals)

Treatment	Follicles with granulosa cells 3β-HSD positive	Corpora lutea			Diformazan deposition			
		3β-HSD positive	20α-HSD negative	positive	Theca cells 3β-HSD	G-6-PD	Interstitial cells 3β-HSD	G-6-PD
Controls	5.1	34.5	6.4	28.1	2	2	2	2
Progesterone mg 1	5.3	25.2	3.3	21.9	1	1	2	2
Progesterone mg 3	5.0	12.5	0	12.5	1	1	2	1
Testosterone μg 30	4.9	44.0	6.5	37.5	1	1	2	2
Testosterone μg 300	6.6	13.1	0	13.1	0	0	2	0
Estradiol μg 1	5.5	23.7	2.6	21.1	2	2	2	2
Estradiol μg 15	6.5	10.9	5.4	5.5	0	0	1	0
Progesterone mg 3 + estradiol μg 1	0.3	8.5	3.6	4.9	0	0	1	0

estrogens[16]. These data have been explained by the fact that progesterone does not inhibit the tonic release of LH[16]. On the other hand P + E is the unique treatment able to prevent the onset of the 3β-HSD activity in the granulosa cells. These results are in agreement with other previously described observations of a depressed follicular growth after the treatment with progesterone and estradiol[17], and are due to the inhibitory effect on LH secretion exerted by the associated steroids[18].

P_3, T_{300}, E_{15} and P + E treatments prevent the ovulation and, therefore, the total number of C.L. is greatly reduced (about $^1/_3$) in treated animals as compared to controls. However, whereas in P_3 and T_{300} groups all C.L. exhibited 20α-HSD activity, in E_{15} and P + E treated animals the number of 20α-HSD negative C.L. strictly approaches that normally appearing at every estrus cycle. These results allowed us to conclude that whilst P_3 and T_{300} treatments did not prevent the onset of 20α-HSD activity in the younger C.L. present at the beginning of the experiment, E_{15} and P + E treatments inhibit the appearance of this enzymatic activity in the set of C.L. formed just before the treatment, but did not affect the involution of the older C.L. which showed 20α-HSD activity at the beginning of the experiment.

Our histochemical results are in agreement with the recently reported[19] morphological evidence of the maintenance of functional C.L. along the estrogen treatment, and might find a stimulating, though incomplete, interpretation in the ROTCHILD's explanation of luteolysis[20]; in fact, the appearance of the 20α-HSD activity in the C.L. of P_3 treated animals may be due to the tonic release of LH, which in rats is luteolytic even in the presence of

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prolactin[21]. Moreover the lack of 20α-HSD activity in the newly-formed set of C.L. observed in rats treated with estrogen alone (E_{15}) as well as with the association progesterone and estrogen (P + E) may be explained by the following facts: (a) estrogen alone, or combined with progesterone, more than progesterone alone, inhibit the secretion and/or the release of LH[18,22]; (b) estrogen explains a luteotropic action through the release of prolactin[14].

On the other hand, however, we could observe the appearance of the 20α-HSD activity in C.L. of T_{300} treated animals although testosterone inhibits the LH release[23].

The fact that the LH secretion and/or release is depressed by estrogen to a greater extent than by progesterone may be confirmed by the disappearance of both 3β-HSD and G-6-PD from the thecal cells and of G-6-PD from the interstitium in the E_{15} treated group. The dependence of these ovarian activities[3,8,9] and of the follicular and interstitial growth[24] on gonadotrophic stimulus is well known. Besides, interstitial cell inhibition has been morphologically noted to a greater extent in estrogen than in progesterone treated animals[17].

In our experiments, lack of both enzymes has been noted in the thecal cells of P + E and T_{300} treated animals. Moreover, in interstitial cells, G-6-PD activity was reduced or disappeared more frequently than the 3β-HSD activity. In this connection a direct correlation between the level of G-6-PD and the rate of steroidogenesis in the ovary has been noted[3].

Riassunto. La valutazione istochimica delle attività 3β-idrossisteroide deidrogenasica (3β-HSD), glucoso-6-fosfato deidrogenasica (G-6-PD) e 20α-idrossisteroide deidrogenasica (20α-HSD) a livello dell'ovaio di ratte trattate per 20 giorni con progesterone, testosterone, estradiolo o con l'associazione progesterone-estradiolo, permette di rilevare che, a dosi inibenti l'ovulazione, solo negli animali trattati con estradiolo o con progesterone ed estradiolo si inibisce la comparsa dell'attività 20α-HSD nei corpi lutei (C.L.) e si riduce quella 3β-HSD e G-6-PD nei follicoli e nella interstiziale.

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The Carbohydrate Metabolism Accompanying Intoxication by Aluminium Salts in the Rat

The effect of aluminium on mammal organism is not yet fully understood. It was found that intoxication with aluminium salts is accompanied by changes in metabolism of phosphorus compounds[1,2]. The common cause of serious disturbances is probably the formation of insoluble aluminium phosphate in the intestinal tract. This leads to the enhanced excretion of phosphorus from the organism and its negative balance. It was also found that, after peroral application of aluminium salts, the incorporation of intragastrically applied ^{32}P into the blood, liver, brain, kidney, spleen, muscle tissue and femur was decreased. Similarly the excretion of ^{32}P in the urine was lowered, while its excretion by the faeces was enhanced[3]. In the same paper it was confirmed that, during intoxication with aluminium salts, the levels of ATP decrease, while the levels of ADP and AMP increase.

It can therefore be supposed that application of increased doses of aluminium salts will influence the metabolism of carbohydrates. The findings of inhibition of glucose absorption in intestinal tract with aluminium salts[4] seems to confirm this view. We therefore studied some parameters of glycide metabolism in rats after the application of increased doses of aluminium chloride.

Two groups of white male rats, strain Wistar, weighing 175 ± 10 g were given the basal Larsen diet and water ad libitum, the control group received no aluminium, but the experimental group were given 200 mg aluminium/kg body weight incorporated into normal diet daily.

The Larsen diet consisted of: 622.6 g wheaten flower, 108.8 g dried milk, 163.3 g caseine, 32.7 g dried trefoil, 16.45 g calcium carbonate, 47.2 g margarine, 7.0 g fish liver oil, 2.4 g sodium chloride and tracer elements added.

Aluminium was added to the diet in the form of aluminium chloride. The extent of aluminium absorbed is about 10%. The experiment lasted 18 days. The weight of the rats under aluminium application decreased significantly ($P < 0.001$) compared with the control group, but neither the groups appeared sick. The last portion of diet and also of aluminium was given 24 h before decapitation of the animals.

The blood glucose was estimated according to SOMOGYI[5], liver and muscle glycogen according to GOOD[6], pyruvic acid in blood and liver following FRIEDEMANN and HAUGEN[7], lactic acid in blood, liver and muscle according to BARKER and SUMMERSON[8] and coenzyme A levels in liver according to HANDSCHUMACHER et al[9].

The results are summarized in the Table. The most pronounced change was the decrease of glycogen concen-

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