Content of the Renin-Like Enzyme in Pregnant and Non-Pregnant Rabbit Uterus

Previous studies of the content of renin-like enzyme in pregnant rabbit uterus have yielded different results. GROSS, et al.[1], estimated that pregnant uterus contains about 20% of the renin concentration (activity/g of tissue) of kidney cortex. FERRIS, GORDEN and MULROW[2] found the renin concentration of pregnant uterus to be the same or slightly higher than that of whole kidney. BING and FAARUP[3] found that some pregnant uteri have renin concentrations 9–10 times greater than that of kidney. These studies differed in methods of extraction and assay of enzyme, and their results cannot be compared. Recently we developed accurate means of assaying renin-like enzymes in aqueous extracts of kidney and uterus[4] and have used this method to determine renin content of pregnant and non-pregnant uterus.

New Zealand White and New Zealand Grey rabbits, 4 to 48 months of age, were used as tissue donors. Uteri were collected from 8 pregnant rabbits, 25–29 days after mating, and from 14 non-pregnant rabbits. 9 of the non-pregnant rabbits underwent bilateral nephrectomy and were exsanguinated 48 h later. Uteri were collected after exsanguination. The kidneys of 4 rabbits were extracted as pairs. Tissues were frozen in a solid CO_2-acetone slurry, thawed at room temperature and then put through one more freeze-thaw cycle. Each tissue was minced in a meat grinder. Water was added to give 2 ml/g of mince. The suspension was stirred for 10 min and then centrifuged at 2,000 rpm at 4 °C for 15 min. The supernatant was saved. The precipitate was resuspended in the original volume of water and then stirred and centrifuged as before. The second supernatant was added to the first. The aqueous extract of each tissue was diluted with 0.1 *M* sodium phosphate buffer, pH 6.0, to give a final protein concentration of 2–4 mg/ml, and the final solution was heated at 56 °C for 30 min, thus inactivating 'angiotensinase' enzymes of kidney and uterus without affecting renin activity[4]. Renin activity was assayed as described previously[4–7].

Extraction of the renin-like enzyme of uterus was easier than extraction of renin from kidney. In one experiment kidney and uterus were put through 2 freeze-thaw cycles, and then minced in a meat grinder. Each mince was extracted 3 times with water and each supernatant was tested for renin-like activity. The mince was resuspended in water a fourth time and the suspension was frozen, thawed and then homogenized in a Waring blender for 1 min. Its supernatant was assayed (Figures 1 and 2). Most of the renin-like activity of uterus was released into the first water extract. However the second extract of kidney contained as much renin as the first. Thus our standard extraction procedure allowed recovery of $>80\%$ of uterine enzyme and about 67% of renin from kidney.

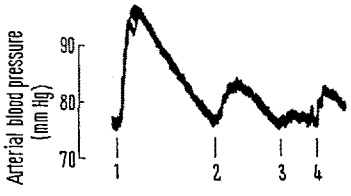

Fig. 1. Extraction of renin-like enzyme from the uterus. Minced uterus was extracted 4 times with water (see text for full details). Renin-like activity of each extract was compared by 'direct' assay. Numbers refer to extract number. All extracts were diluted 4-fold with water. The volume of each injection was 0.05 ml.

Renin-like activity/g (see Tables I, II and III) of pregnant uterus was 16 times greater than that of normal non-pregnant uterus and 9.5 times greater than that of non-pregnant uterus collected 2 days after bilateral nephrectomy. These differences are significant, $p < 0.001$ and $p < 0.01$, respectively (Student's *t*-test). There was a tendency for non-pregnant uterus collected after nephrectomy to contain more renin/g than the normal non-pregnant uterus, but the difference was not significant. There was a stronger tendency for the uterus taken after nephrectomy to contain more renin/uterus than the normal ($0.05 < p < 0.1$) suggesting that renin/g might be related to uterine weight. By combining pregnant and non-pregnant groups there was a strong correlation of uterine weight with renin/g, $r_s = 0.778$, $p < 0.01$.

Mean renin concentration of kidney was 7,540 IU/g, and renin activity/kidney was 94,000 IU. Renin activity/pregnant uterus was 114 times that of normal non-pregnant uterus ($p < 0.001$) and almost 38 times greater than that of non-pregnant uterus collected after nephrectomy ($p < 0.001$). Pregnant uterus contained about 24 times

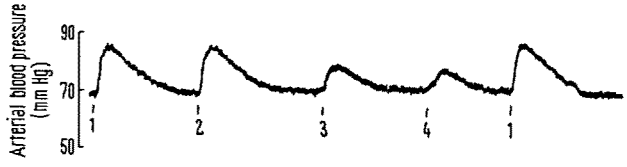

Fig. 2. Extraction of renin from the kidney. Minced kidney was extracted as described in the text. Other details are similar to those of Figure 2.

Table I. Renin activity of non-pregnant rabbit uterus

No.	Rabbit	Uterine wt. (g)	Renin concentration (IU/g)	Total renin (IU/uterus)
1	C	7.2	1,680	12,096
2	R1	6.8	6,240	42,432
3	85	11.2	2,080	23,296
4	80	3.0	6,620	19,860
5	89	1.2	960	1,152
	Sum	29.4	17,580	98,836
	Mean	5.88	3,516	19,767
	± S.D.	± 3.91	± 2,694	±15,261

Uterine extracts were prepared and their renin activities determined as described in the text.

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Table II. Renin activity of non-pregnant uterus, 48 h after Nephrectomy

No.	Rabbit	Uterine wt. (g)	Renin concentration (IU/g)	Total renin (IU/uterus)
1	R32	9.6	9,890	94,944
2	R30	16.0	7,440	119,040
3	R13	6.7	1,532	10,264
4	R12	6.6	4,228	27,905
5	B3	8.0	4,228	33,824
6	B2	4.4	9,886	43,498
7	A1	5.8	1,824	10,579
8	A	5.8	3,646	21,147
9	R31	14.2	12,664	179,829
	Sum	77.1	55,348	541,030
	Mean	8.57	6,150	60,114
	± S.D.	± 4.01	± 3,445	± 58,608

48 h after bilateral nephrectomy, rabbits were exsanguinated. Their uteri were collected and prepared as described in the text.

Table III. Renin activity of pregnant rabbit uterus

No.	Rabbit	Uterine wt. (g)	Renin concentration (IU/g)	Total renin (IU/uterus)
1	R60	38.6	37,440	1,445,184
2	R15	32.8	124,780	4,092,784
3	R2	25.0	66,160	1,654,000
4	R6	44.3	97,000	4,297,100
5	R50	47.4	38,800	1,839,120
6	ER	51.1	33,820	1,728,202
7	70	44.2	36,280	1,603,576
8	75	40.6	33,580	1,363,348
	Sum	324.0	467,860	18,023,314
	Mean	40.5	58,483	2,252,914
	± S.D.	±8.4	± 34,825	± 1,209,200

Uteri were collected 25–29 days after mating.

more renin than that of one kidney ($p < 0.01$). The concentration of renin-like enzyme of pregnant uterus was almost 8 times that of kidney($p < 0.01$).

Our results agree with those of BING and FAARUP[3], in which renin of simple aqueous extracts was measured by 'direct' assay. They presented evidence that results of their 'direct' assay were correlated with results of an 'indirect' assay. However, the 'indirect' assay used was not shown to be reproducible, and 'direct' assays are imprecise even when carefully controlled. Thus we compared results of our 'indirect' assay with those of a 'direct' assay. We found that one bioassay preparation may differ from another by 6-fold in terms of the dose of renin required for threshold response. Further, we found that a good bioassay preparation may vary in sensitivity by 3-fold over the course of 4–6 h. To lessen the magnitude of these problems, we performed all 'direct' assays in the shortest possible time using the same bioassay preparation. Unknowns were compared to our standard, a partially-purified renin prepared from kidney[5,6], a 'direct' renin unit being defined as the amount of renin contained in 0.25 ml of the standard renin solution. One-tenth of this amount raised mean arterial blood pressure by10–15 mmHg. Results are shown in Figure 3. Although agreement was not perfect, the correlation of 'direct' and 'indirect' assays was significant, $r = 0.962$, $p < 0.01$.

Our estimate of renin-like activity in pregnant uterus is several-fold higher than the estimates of GROSS et al.[1] and FERRIS, GORDEN and MULROW[2]. It may be significant that they measured renin after partial-purification procedures involving, in the first study, acidification (pH 2.8), and, in the second study, acidification (pH 1.6) and ammonium sulfate precipitation steps. Although neither of these studies presented evidence of the reproducibility of their fractionation procedures, the possibility exists that the uterine enzyme differs from renin of kidney and requires different means of purification[8]. On the other hand, renin occurs in at least four forms[9]. One form can be converted to another by mild acidification (pH 5), a point that could have biased the results of earlier studies. Recently we carried out parallel purifications of rabbit uterine renin and kidney renin to specific activities >1400 times that of the starting material[4]. We were unable to distinguish one enzyme from the other in terms of behavior on DEAE-cellulose and dextran gel (Sephadex G-100) chromatography or on starch gel electrophoresis. Nonetheless our study does not provide definitive evidence for deciding the question of whether renal renin and uterine renin are identical. However, we have shown that the specificity of reaction of the uterine enzyme with the tetradecapepide renin substrate and native renin substrate is identical to that of renin itself[10,11]. Thus if the uterine enzyme enters the circulation, as is indicated by in vitro perfusion studies[11], its physiological effects should be much the same as that of renin[12].

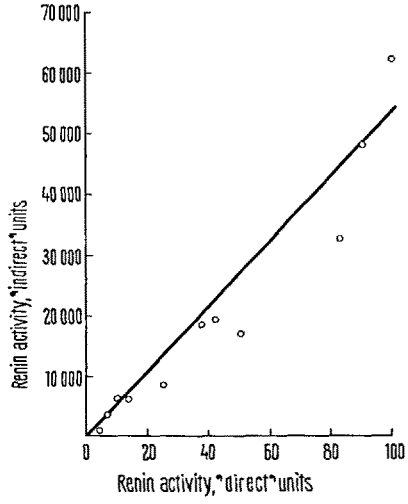

Fig. 3. Correlation of results of 'direct' and 'indirect' renin' assays. Extracts of 11 rabbit uteri (pregnant and non-pregnant) were assayed as described in the text. ○, experimental values. Solid line shows the calculated regression curve.

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Résumé. Chez les lapins gris et blancs de la Nouvelle-Zélande, la concentration d'une enzyme semblable à la rénine est 16 fois plus grande dans l'utérus gravide presque à terme que dans l'utérus non-gravide et 9.5 fois plus grande que dans l'utérus après néphrectomie bilatérale. En tenant compte du fort accroissement de l'utérus gravide, le contenu total de l'enzyme en question est 114 fois supérieur à celui de l'utérus non-gravide et 24 fois plus grand que celui du rein.

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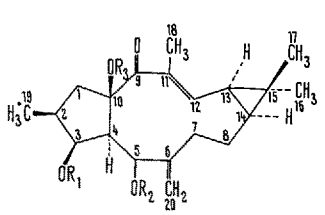
Further New Diterpene Esters from the Irritant and Cocarcinogenic Seed Oil and Latex of the Caper Spurge (*Euphorbia lathyris* L.)

From the hydrophilic neutral fraction of the seed oil of the caper spurge (*Euphorbia lathyris* L.) by multistage Craig distribution, the crystalline esters L_1, L_2 and L_3 and the resinous esters L_4 and L_5[1,2] have been isolated. L_1 and L_2 were identified as esters of the new macrocyclic diterpenes 6,20-epoxy-[3,4] and 7-hydroxy-lathyrol[5], respectively, and L_4 and L_5 as esters of the new tetracyclic diterpene ingenol[6,7]. Recently the structure of L_3 was clarified and further resinous (L_6, L_7) and crystalline (L_8) diterpene esters were isolated from the irritant and cocarcinogenic seed oil of the caper spurge. Also, a comparative investigation of the irritant latex of this species was undertaken.

Ester L_3, $C_{31}H_{38}O_7$ (MS), m.p. 156–158 °C is the diacetate-benzoate I of a new diterpene alcohol $C_{20}H_{30}O_4$[1,2]. I shows the following spectral data: UV (MeOH): λ_{max} = 229, 275 nm, ε = 16400, 15300; IR (KBr): 1730, 1705 (CO), 1640, 1613 (C = C-CO), 897 (C = CH_2), 705 cm^{-1} (C_6H_5). The NMR-spectrum ($CDCl_3$) indicates presence of two acetyl groups (δ = 2,23, 1,85; 2×3 H, S); the diamagnetic shift of the latter signal may be understood by the neighbourhood of the benzoyl group, the signal of which appears at' δ = 7,3–8,1 ppm (M). Further NMR data of I: H-12: 6,53, DD ($J_{12,13}$ = 11 cps, $J_{12,18}$ = 1–2 cps); H-5: 6,2, D ($J_{4,5}$ = 10 cps); H-3: 5,81, T ($J_{2,3} = J_{3,4}$ = 3,5 cps); H_2-20: 5,0, S, 4,78, S; H-1a: 3,6, DD ($J_{1a,1b}$ = 14 cps, $J_{1a,2}$ = 8,6 cps); H-4: 2,92, DD $J_{3,4}$ = 3,5 cps, $J_{4,5}$ = 10 cps); H-2: 2,3, M; H_3-18: 1,76, D ($J_{12,18}$ = 1–2 cps); H-13: 1,4, M; H_3-16, H_3-17: 1,22, S; H_3-19: 0,98 ppm, D ($J_{2,19}$ = 6,5 cps). The NMR data of the triester L_3 correspond to those of L_2, a tetraester of 7-hydroxy-lathyrol[5], with the exception that a signal of a geminal ester proton in position 7 is apparent. Thus the new diterpene alcohol is the parent of 6,20-epoxy- and 7-hydroxy-lathyrol, respectively, and therefore called lathyrol (II). Because of the close relationship of II with 7-hydroxy-lathyrol, *trans*-configuration of $\Delta^{8,9}$ and the absolute configuration as determined for the latter by X-ray diffraction analysis[5] may be adopted also for II. The positions of the three acyl groups in I remain to be determined. The saturated hydrocarbon corresponding to lathyrol (II) is proposed to be called lathyran.



I: R_1-R_3 = 2 $COCH_3$, 1 COC_6H_5
II: $R_1 = R_2 = R_3$ = H
III: $R_1 = R_2$ = $COCH_3$, R_3 = H
IV: R_1-R_3 = 2 $COCH_3$, 1 COC_5H_4N

By hydrolysis of I (0,5 m KOH in methanol) lathyrol (II), $C_{20}H_{30}O_4$ (MS), m.p. 168–169 °C is obtained. It is acetylated with Ac_2O/py to yield lathyrol-3,5-diacetate III, m.p. 134–136 °C, NMR ($CDCl_3$): H-3: 5,55, T; H-5: 5,87, D; OH-10: 2,9–3,4 ppm (broad).

L_6 (resinous, MS: parent ion *m/e* = 548) is a mono-ester of ingenol[7] with the highly unsaturated $\Delta^{2,4,6,8,10}$-penta-en-tetradecanoic acid. Transesterification of L_6 (1% $NaOCH_3$ in methanol) yields ingenol and the methyl ester $C_{13}H_{17}COOCH_3$ (MS) which, on hydrogenation with Pd/C, leads to the methyl ester of tetradecanoic acid identified by gas-liquid chromatography and mass spectrum.

Ester L_7 (resinous, MS: parent ion *m/e* = 580) was not further investigated because of lack of material. According to its UV-spectrum (MeOH) (λ_{max} = 278 nm, ε_{max} = 11600), a structural relationship of its parent alcohol to lathyrol (II) is indicated.

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