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Renin and Inactive Renin in Human Amnion at Term Pregnancy (41298)

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Abstract. Immunofluorescent analysis using antiserum to human kidney renin revealed the presence of renin in epithelial cells from human normal term amnion. Enzymatic analysis showed that both active and inactive renin are present. Renin activity was increased by either trypsin or acid treatment. The results suggest that epithelial cells of the amnion may be a source of active and inactive renin known to be present in amniotic fluid. The origin of the renin in the amniotic cells remains to be determined. It may function in physiological or pathological states as a regulator of membrane permeability, placental vascular control, or uterine contractility.

Renin activity was demonstrated in human amniotic fluid in 1964 (1) and in fetal membranes in 1968 (2). As a possible source of renin, the chorion laeve has attracted the most attention because it contained the highest concentration of renin in fetal and maternal tissues and because chorionic cells had the most consistent ability to synthesize renin when cultured *in vitro* (2, 3). However, exactly where the chorionic renin came from was not determined. By using immunohistology we have shown that the renin is localized in the trophoblastic cells of the chorion (4).

Although others had found that the renin concentration in the amnion was about 28% of that in the chorion (2), the relatively lower levels of renin in the amnion could have been explained by inclusion in the analyzed tissue of cells which are also present in chorion. But in view of our results localizing renin in trophoblast cells, this seems unlikely. The presence of renin in amnion might also be explained by extracellular contamination. Since the amnion contains several layers of noncellular material with only a single layer of cells (5), determination of concentration by wet weight might be misleading with respect to cellular content of renin. Furthermore, the earlier studies utilized acid treatment of the tissues in the assay of renin and this procedure is now known to activate an inactive species of renin (6). Accordingly, we have investigated the localization of renin and inactive renin in human amnion using both biochemical methods and immunocytochemistry with a specific antiserum which reacts with human kidney renin and inactive renin (7).

Materials and Methods. Standard indirect immunofluorescent analysis was performed as previously described (4, 8). In addition, biochemical analysis for renin and inactive renin was carried out on extracts of amnion. Amniotic membranes from normal-term placentas were frozen, thawed, and homogenized with 10 vol of Triton X-100 (0.1%). After centrifugation at 78,000g_{min}, the supernatant was assayed for renin and inactive renin by measurement of angiotensin I generated from sheep substrate (6). Inactive renin was indicated by the increment in activity that appeared upon pretreatment with trypsin (6). Renin was also activated by acid dialysis (9).

Results. The amnion showed strongly positive immunofluorescence in the epithelial cells indicating the presence of renin-like material (Fig. 1A). Control experiments using nonimmune rabbit serum at the same dilution showed only minimal background fluorescence (Fig. 1B). Positive fluorescence was distributed throughout the cytoplasm of the epithelial cells. Assay of extracts of amnion showed the presence of both free renin and inactive renin (trypsin activated). Most of the renin was in the in-

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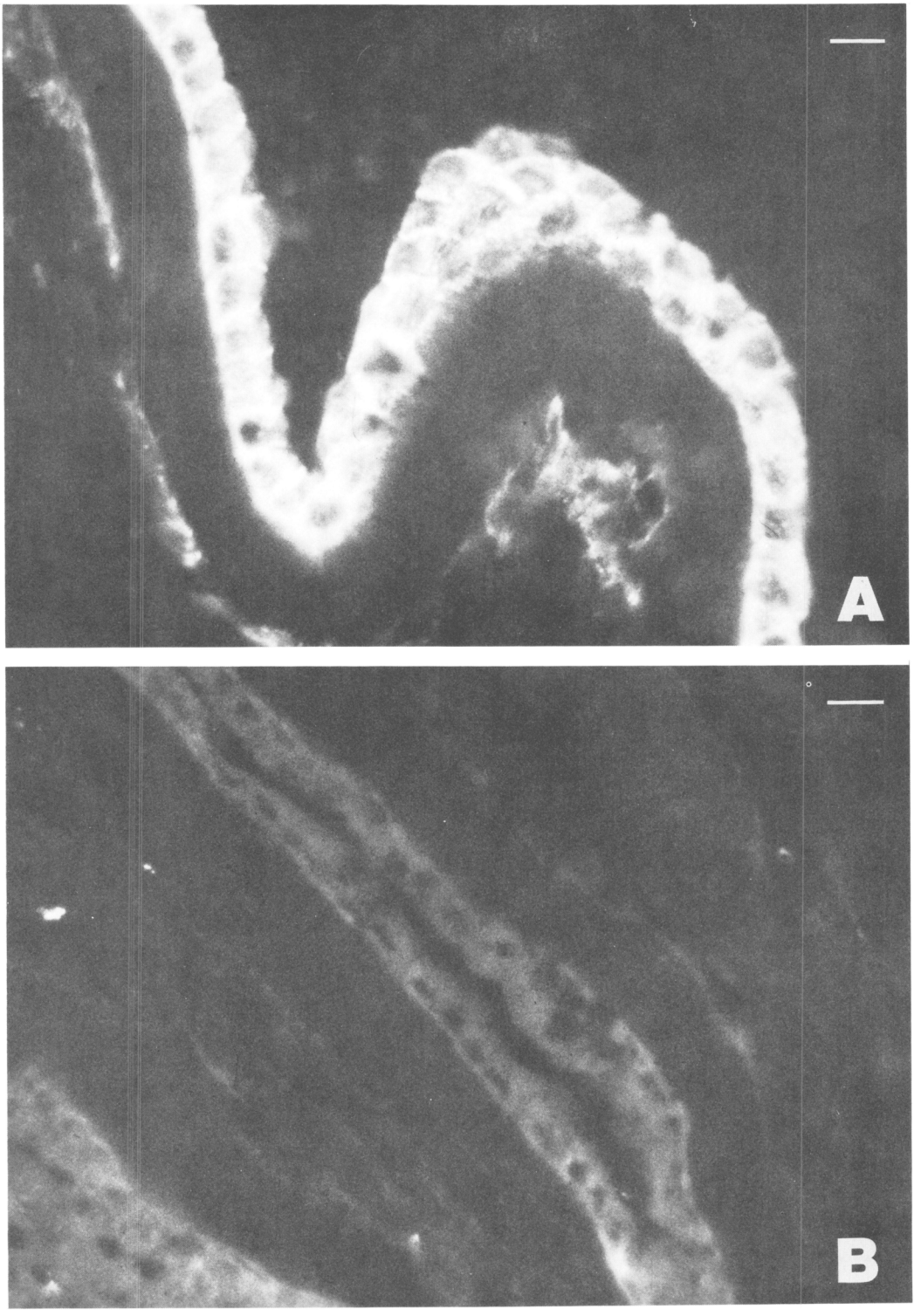

FIG. 1. Immunofluorescent demonstration of renin in human amnion. Unfixed cryostat sections (8–10 μm) of term amnion were stained by standard indirect immunofluorescent technique (8). (A) Positive fluorescence in epithelial cells revealed by treatment with rabbit anti-kidney renin (1:40). (B) Background fluorescence in control sections treated with normal rabbit serum (1:20). Sections were preincubated with human IgG (10 mg/ml) to prevent nonspecific interaction. Calibration line—20 μm.

TABLE 1. RENIN IN EXTRACTS OF HUMAN TERM AMNION

Sample	Renin (ng/hr/g)[a]		
	Free	Trypsin-treated[b]	Acid-treated[c]
1	175	1098	793
2	171	1339	696
3	577	1468	1483
4	583	1396	1561
5	449	1386	809
6	439	1030	669
7	247	1376	1030
Mean	377	1299*	1006*
SE	68	63	141

[a] Extracts were incubated for 30 min at 37°C with nephrectomized sheep substrate and then radioimmunoassayed for angiotensin (6).
[b] Preincubated with 10 μg/ml at 25°C for 30 min.
[c] Dialyzed to pH 3.3 and then to pH 7.5.
*$P < 0.001$ compared to free.

active form: total renin = 1299 ng/hr/g; free renin = 377 ng/hr/g (Table I). This value for total renin (active plus inactive) is similar to the value for renin reported previously using acid pretreatment, 1240 ng/hr/g (2). Acid treatment of the amniotic extracts also caused activation of renin (1006 ng/hr/g) (Table I). Thus the previously reported value for renin in the amnion (2) represented the sum of free plus inactive renin.

Discussion. Our results confirm earlier findings that human amnion contains renin-like activity, demonstrate that this activity is cross-reactive with antiserum to pure kidney renin, and show that the bulk of the activity is in an inactive form as is the renin in amniotic fluid (10). Moreover, the results demonstrate that renin is present in the epithelial cells and therefore does not represent contamination from chorionic tissue. One source of the high level of renin and inactive renin in amniotic fluid has been considered to be the cells in the chorion laeve described by Skinner *et al.* (2). Clearly the epithelial cells of the amnion, which are in direct contact with the amniotic fluid, could be another source. It should be noted that initial attempts to demonstrate synthesis of renin by cultured amnion were negative (3). An understanding of the possible synthesis, storage, and release of amniotic renin, as well as any processing of inactive renin to renin, remains for future investigation. Another interesting area for future research is an investigation of the physiological or pathological role of renin released from the fetal membranes. In view of the importance of the renin–angiotensin system for water and electrolyte control in the kidney, it would seem appropriate to look for effects on fluid and electrolyte transport in the fetal membranes in addition to the possible vascular effects of angiotensin in the placental unit. A role of the renin–angiotensin system in parturition also merits attention (11, 12).

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