Table 1. Effect of solution pH on the number of single cells released by collagenase treatment of a metastasizing lymphosarcoma

	pH of collagenase solution (cells released per g tumour ($\times 10^6$))			
	4.5	5.3	6.3	7.4
I	66	75	32	14
II	43	31	17	8
III	54	51	33	12
IV	71	95	40	18
V	50	46	20	10
VI	48	61	24	9
Mean	55	60	28	12

Each experiment in tables 1 and 2 is equivalent to 1 animal. Cell counts represent the mean of triplicate determinations.

Table 2. Effect of solution pH on the viability of single cells released by collagenase treatment of a metastasizing lymphosarcoma

	pH of collagenase solution (percentage viability)			
	4.5	5.3	6.3	7.4
I	52	60	64	78
II	44	64	65	65
III	54	54	65	62
IV	50	37	48	71
V	41	59	47	62
VI	43	54	51	53
Mean	47	55	57	65

Cell viabilities represent the mean of triplicate determinations.

zymes[8], and also that tumours contain high levels of acid proteases[9]. In addition, once collagenase has cleaved a molecule of collagen into 2 pieces, the fragments become more susceptible to digestion by nonspecific proteases[10]. Obviously, collagenase itself is still important in the digestion process because in the absence of the enzyme, insufficient numbers of cells were released to account for the data; however, it is highly likely that these nonspecific proteases are responsible for the effects observed with the reducing environmental pH. A similar process to this could be operating on a more limited scale in vivo in the primary tumour, thus, liberating the cells necessary for metastasis.

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Experimental formation of podocytes in the parietal layer of the Bowman's capsule[1]

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Summary. A metaplasic transformation of the parietal layer of the Bowman's capsule into podocytes is described in glomerular cysts induced by postnatal injection of methylprednisolone acetate to rabbits. Both the anomalous location of podocytes and their utility for the study of the biology of these cells are discussed.

Several experimental models of polycystic kidney induced by chemicals have been developed[2]. The one induced by the administration of adrenal corticosteroids to the newborn rabbit[3] has a particular interest owing to the similarity of the cysts to those of the human disease. Tubular as well as glomerular cysts are observed in the corticosteroid-induced polycystic kidney[4]. Both types of cysts are due to an alteration of the development of the subcapsular metanephrogenic zone[4,5]. Since the last structure has a postnatal morphogenesis, the study of the corticosteroid-induced polycystic kidney could contribute to the understanding of the processes that control the development and differentiation of the nephron. In this paper, we report the presence of podocytes in the parietal layer of Bowman's capsule of the glomerular cysts.

Materials and methods. The data are based on the analysis of 80 kidneys obtained of rabbits between 7 and 75 days old injected i.m. once with methylprednisolone acetate (20 mg/kg) within the 1st 24 h after birth[4].

After ether anaesthesia, the animals were fixed by perfusion through the aorta with 3% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.3. Kidneys fragments were obtained and immersed in fresh cold fixative for an additional 4-h period, transferred to buffer solution and postfixed in 1% osmium tetroxide for 2 h.

For light and electron transmission microscopy (TEM), the specimens were stained in block with uranyl acetate, dehydrated in acetone and propilene oxide, and embedded in araldite. Semithin sections were stained with 0.1% toluidine blue in 1% sodium borate solution. Ultrathin sections were stained with lead citrate[6], and observed with a Philips EM 201. For scanning electron microscopy (SEM), the kidneys fragments were dehydrated in acetone, dried by the critical point method[7], sputtering coated with gold and observed with a Philips SEM-501.

Results and discussion. Glomerular cysts could already be observed at 10 days after injection, but they grow up slowly and only reached a notable size 35–45 days after birth[4]. The cysts showed a large dilatation of the capsular space of Bowman and an atrophic glomerulus (figure 1); 25 days after injection, the parietal layer of all the glomerular cysts were completely occupied by stellate cells with morphological characters of podocytes (figure 2). These podocytes showed interdigitating foot processes separated by filtration slits which were lined up on the inner surface of a continuous basal lamina (BL). Contiguous foot processes always

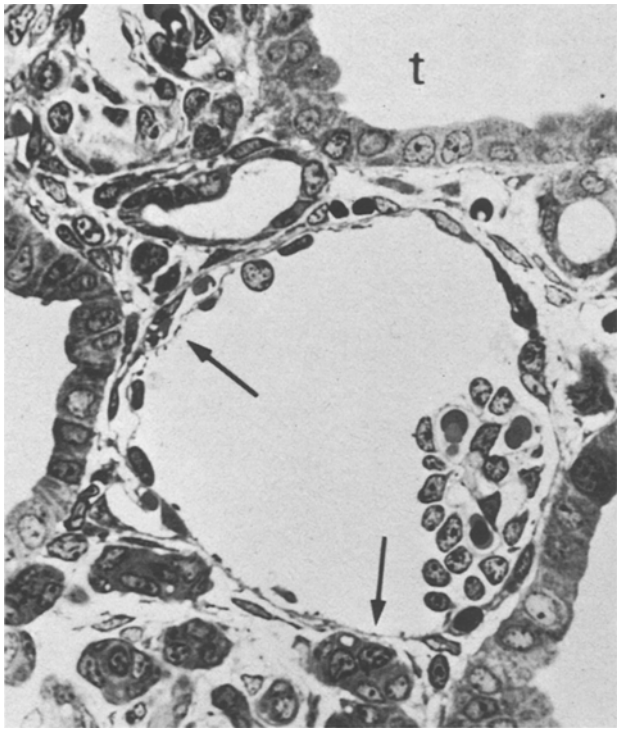

Fig. 1. Semithin section of a glomerular cyst of a 30 days treated animal. The parietal layer is formed by podocytes whose pediceles can be clearly distinguished (arrows). Note the presence of tubular cysts (t). Toluidine blue. × 400.

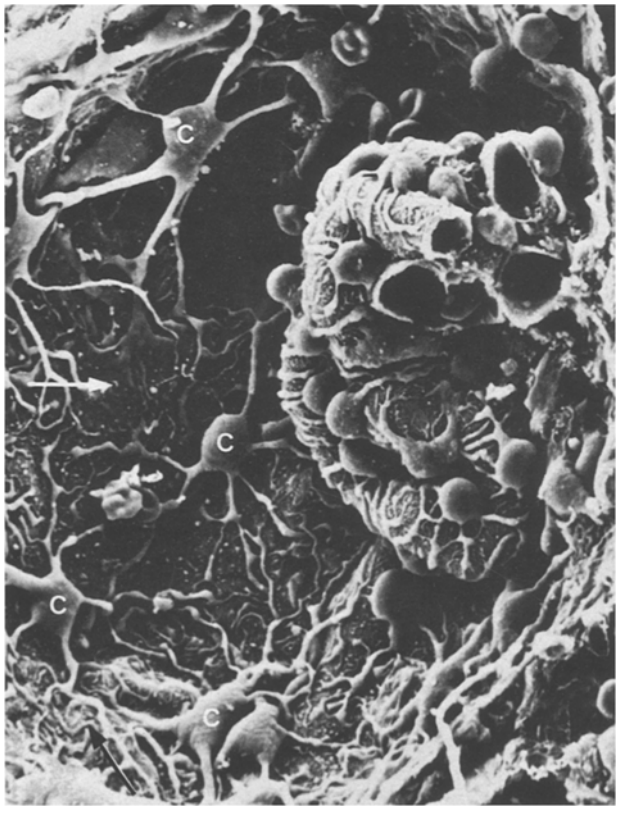

Fig. 2. Panoramic view of a fractured glomerular cyst. 50 days treated animal. The parietal layer is fully occupied by podocytes. Cell bodies (c) send long branching major processes. Numerous interdigitating pediceles can be observed (arrows). SEM. × 1000.

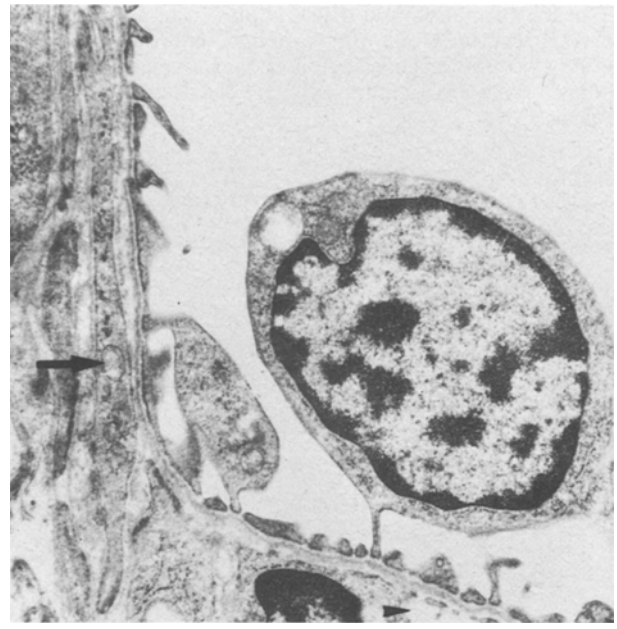

Fig. 3. Detailed view of the parietal layer of a glomerular cyst. 60 days treated animal. Numerous pediceles aligned upon the inner face of a BL can be observed. BL is closely associated by the outer face with a fenestrated capillary (arrowhead) and with a fibroblast-like cell (arrow). TEM. × 15,000.

originated from different cells, as observed in normal conditions[8]. BL consisted of a central dense layer and 2 thinner lamina rara layers (figure 3) like the glomerular basement membrane, although the pattern of macromolecular organization and aggregation characteristics of the last structure[9] could not be shown by the methods employed here. BL was most often in contact with long prolongations of fibroblast-like cells (figure 3) or with the basal pole of the tubular cells (figure 1). However, sometimes BL contacted also with capillaries that ran between the glomerular cysts and the surrounding tubules (figure 1). Immature forms of podocytes were observed in the parietal layer of the Bowman's capsule in glomerular cysts not fully developed, and podocytes showing degenerative changes were found in large glomerular cysts of 70 days old rabbits.

Although cells morphologically similar to those of the proximal convoluted tubule had been observed in the parietal layer of the Bowman's capsule[10-12], the presence of podocytes in this layer had never been reported. This anomalous location might be due to a progressive incorporation of the visceral layer into the parietal one, owing to the distension of the Bowman's space. However, at least 2 findings are inconsistent with this mechanical interpretation: a) The intracyst pressure should not increase since the cyst remains communicated with the urinifere tubuli[4], and b) the podocytes covered the whole inner surface of the cysts and different stages of differentiation of these cells were observed. It seems more likely that the normal cells become transformed into podocytes by a metaplasic process. The formation of podocytes, in places unrelated to capillaries observed here, is in disagreement with the suggestion[13] that capillaries are essential structures for podocyte differentiation. Recent evidence[14-16] suggests that the presence of polyanionic sialic acid surface coat in the podocytes may be required for the differentiation and maintenance of the normal foot process. On this basis, the metaplasia of the parietal layer of the Bowman's capsule could be attributed to an effect of the injected corticoids

upon the formation and distribution of the polyanions on the cell surface. Some observations[17] reporting alterations of the glycoproteins of the glomerular basement membrane induced by methylprednisolone, are consistent with the last hypothesis.

The production of podocytes in anomalous location reported here, might be useful for future experimental studies of some unresolved questions on the biology of the podocytes, such as their possible participation in the formation and renovation of the glomerular basement membrane.

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Epidermal growth factor stimulates proliferation of rat hepatoma cells producing α-fetoprotein

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Summary. Epidermal growth factor stimulated both [^{3}H]thymidine uptake and proliferation of rat AH66 hepatoma cells. However, the increase in cell number was not accompanied by a proportional increase in the levels of α-fetoprotein of the culture media. The effects of EGF on the cell proliferation were antagonized by N^6,$O^{2'}$-dibutyryl cAMP.

Epidermal growth factor (EGF) is a polypeptide with a molecular weight of about 6000, which has been isolated from the submaxillary gland of male mice[1], and recently from human urine[2]. EGF is capable of stimulating DNA synthesis and proliferation in epidermal cells[3] and various fibroblastic cells[4,5]. It has also been reported to stimulate the secretion of hCG by human choriocarcinoma cells, and is suggested to be a trophic modulator of hormonal secretion[6]. There seems, however, to be no report up to now about the EGF action on tumor cells producing onco-fetal proteins. We studied the effect of EGF on the transplantable rat hepatoma cells secreting α-fetoprotein (AFP)[7]. The observation suggests a role for EGF for the 1st time as a modulator of proliferation and AFP production in hepatoma cells.

Materials and methods. EGF was purified from the submaxillary glands of male mice by the method of Savage et al.[1]. Rat AH66 hepatoma cells (5×10^4) were cultured in plastic culture dishes (25 mm, Falcon) containing 2 ml of Ham's F12 medium supplemented with 0.2% fetal calf serum. Various concentrations of EGF, bovine insulin (Sigma) and/or N^6,$O^{2'}$-dibutyryl cyclic AMP(Bt_2cAMP, Sigma) were added into the low serum medium and the cells were cultured for varying times at 37 °C in a 5% CO_2-95% air mixture. Cells were counted with a hemocytometer, and the AFP concentration of the culture media was determined by radioimmunoassay[8]. To measure [^{3}H]thymidine uptake, 2 μCi of [^{3}H]thymidine (5 Ci/mmole, Radiochemical Center, Amersham, England) was added to 2 ml of culture media (final, 1 μCi/ml) on the 3rd day, and the culture was continued for further 6 h. The amount of [^{3}H]thymidine incorporated into the acid-precipitate of cell lysate was measured as described previously[9].

Results. The proliferation of AH66 cells cultured in Ham's F12 medium + 0.2% fetal calf serum was almost negligible. The addition of more than 0.5 ng/ml of EGF into the low serum medium caused a significant increase in [^{3}H]thymidine incorporation and cell proliferation after 3 days (figure). The presence of more than 5 ng/ml of EGF resulted in maximum stimulation of cell proliferation (figure). The manifestation of the EGF effect appeared to require a relatively long period of culture, since regardless of the presence of EGF no significant increase in the cell number was detected after 24 h in culture. On the contrary, the amount of AFP in the culture medium did not increase in proportion to the increase in cell number (figure). AFP synthesized and secreted into the medium during the 3 days of culture of AH66 cells with 5 ng/ml of EGF was rather smaller (107 ± 11 ng/ml) than that of the control (153 ± 18 ng/ml). From these data it was suggested that the amount of AFP produced by AH66 cells was smaller in those cells cultured with EGF. Similar effects on cell proliferation and AFP production were observed when the low serum medium was supplemented with insulin (50 ng/ml). Bt_2cAMP (2 mM), which inhibited the prolifera-

Effects of EGF, insulin and Bt_2cAMP on the proliferation and AFP production of AH66 hepatoma cells in culture

Treatment	Cells	AFP	
	$\times10^{-4}$/dish	ng/dish	ng/10^5 cells
None	7.3 ± 0.76	184 ± 35	250 ± 29
EGF	22.8 ± 2.8	174 ± 12	77.2 ± 12
Insulin	18.4 ± 3.8	201 ± 19	112 ± 28
EGF + insulin	17.6 ± 3.0	146 ± 14	83.8 ± 5.9
Bt_2cAMP	6.5 ± 1.7	300 ± 112	451 ± 89
EGF + Bt_2cAMP	11.3 ± 1.2	353 ± 30	312 ± 42.5

5×10^4 of AH66 cells were cultured for 3 days with EGF (10 ng/ml), insulin (50 ng/ml) and/or Bt_2cAMP (2 mM). The number of cells and the amount of AFP in the media were determined as described in materials and methods. Each point is the mean ± SD of triplicates.