activities of the offspring[9]. Similar findings have been reported by others[14]. X-irradiation of pregnant mice on gestational days 11, 12 and 13, with 1.05 Gy each time, also caused marked and significant changes in postnatal brain weights, protein content, acetylcholinesterase and Na,K-ATPase activities of all offspring (table). Increased mortality, however, was observed only with females and within 48 h after birth[2]. The enzyme activities and protein contents of the brains of irradiated females did not, even on the day of birth and the 2nd postnatal day, differ from those of the corresponding males.

Thus, these biochemical data do not help to explain the conspicuous mortality of the female offspring caused by this particular X-ray dose. It may be concluded that the investigation of postnatal brain acetylcholinesterase and Na,K-ATPase activities is suitable for the detection of more general radiation damage, but not the specialized damage being studied in this and previous investigations[2,5]. At present we are examining X-chromosome-linked enzyme activities in the brains of mice prenatally X-irradiated with 3×1.05 Gy.

1 We gratefully acknowledge the assistance of Miss C. Gutmann.

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Increased release of tumour cells by collagenase at acid pH: A possible mechanism for metastasis

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1 March 1979

Summary. The ability of collagenase to disaggregate a solid metastasizing lymphosarcoma has been shown to considerably increase with reducing environmental pH. It is suggested that this effect may be operating in vivo to release cells from a primary tumour.

The detachment of cells from primary tumours is an important, but poorly understood, step in the metastatic cascade[2]. As a result of a chance observation, it became apparent that the efficiency with which collagenase could disaggregate a solid metastasizing lymphosarcoma increased with the lowering of the pH of the enzyme solution used. In view of the fact that intra-tumour pH is frequently found to be on the acid side[3], and that some evidence already exists to implicate collagenase in the release of primary cells into the circulation[4], it was thought that this effect could have significant bearing on this part of the metastatic process. This communication reports these findings.

Materials and methods. Phosphate buffer saline (PBS) solutions at pH 4.5, 5.3, 6.3 and 7.4 were prepared. Primary lymphosarcomas were removed from Syrian hamsters 18/20 days after implantation, and the excised tissue was roughly chopped, taking care to discard any necrotic on fibrous material. 4 aliquots of approximately 1 g were set up in glass universal bottles, and each aliquot was washed twice with 10 ml of its appropriate PBS by allowing the pieces of tumour to settle out and discarding the supernatant. Cell suspensions were prepared by stirring the chopped tissue for 1 h at 37 °C in 5 ml PBS (pH 4.5, 5.3, 6.3, or 7.4) containing 0.2 mg/ml collagenase (type II, Sigma). A stirring action was achieved using a glass-coated metal bar (15×2 mm) in the universal, placed on a magnetic stirring-base (Gallenkamp; setting 3). After treatment, any undisaggregated pieces were allowed to settle out, the supernatant was carefully decanted off, and 15 ml PBS (pH = 7.4) was added to the remaining pieces. The whole was resuspended and allowed to settle out again. The supernatant of the latter procedure was pooled with the first supernatant and the single cells pelleted by centrifiguration. Preliminary experiments had shown that this type of treatment was sufficient to remove over 95% of the single cells. The cell pellet was resuspended in 10–20 ml PBS (pH = 7.4) cell number was determined using an improved Neubauer counting chamber and cell viability was determined using a Trypan Blue method previously described[6].

Results and discussion. Lowering the pH of the collagenase solution considerable increased the yield of single cells for all the tumour preparations investigated (table 1). Statistical analysis of the data on a paired basis (Wilcoxon) indicated that the yield using PBS, pH = 6.3, was significantly greater ($p < 0.025$) than that using PBS, pH = 7.4. Furthermore, the yield using PBS, pH = 5.3, was significantly greater ($p < 0.025$) than that using PBS, pH = 6.3. There was no significant difference in the yields at pH 4.5 and 5.3. The pH of the PBS only had a significant effect ($p < 0.025$) on cell viability (table 2), if suspensions prepared using PBS pH 4.5 and 7.4 were compared.

It is unlikely that our results can be explained by an increase in the activity of the particular collagenase preparation we used because it has been shown that this type of preparation has optimum activity between pH 7 and 9[7]. What seems more feasible is that this change is brought about by the effect of the lowered pH on the tumour. It is well known that at acidic pH collagen becomes more susceptible to digestion by nonspecific collagenolytic en-

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