

Characteristics of Cell Cultures Derived from Renal Glomeruli¹ (36977)

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The renal glomerulus appears as a relatively simple structure composed of three cell types; capillary endothelium, epithelial cells overlying the capillaries and mesangial or intercapillary cells. By virtue of receiving 20–25% of cardiac output glomeruli are well situated for filtration but morphological simplicity belies their capacity for functions beyond this essentially passive role in urine production. It is known that glomeruli are endowed with a biochemistry rich enough to support more demanding though still undescribed activities. For example, high levels of glycolytic and oxidative enzymes have been demonstrated in isolated preparations (1–4), and it has long been established that nephrotoxic antigen is localized almost exclusively to the glomerulus (5–7). Moreover, during rejection of dog kidney allografts the glomerulus was observed to be the earliest site of biochemical changes in studies comparing isolated preparations of glomeruli and tubules (4).

In conjunction with continuing studies of basic features of transplant rejection we developed a culture system for isolated renal glomeruli several years ago and have successfully cultured glomeruli derived from mouse, guinea pig, hamster, rabbit, cat, dog, goat and calf. In addition to describing our procedure and characteristics of the cultures, we include representative results from studies of enzyme modulations occurring in culture.

As an example of the promising potential for the glomerular culture system we have recently reported on the production of erythropoietin by long-term cultures of goat glomeruli (8).

Methods. Isolation of glomeruli. A rela-

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tively pure preparation of glomeruli is derived from kidney cortex by a method deviating only slightly from one described more than 20 yr ago (6). The procedure is as follows: observing sterile precautions throughout, three stainless steel screens of varying pore size are placed in series, starting with a No. 60 mesh (246 μm pore), an intervening No. 100 mesh (147 μm pore) and a final No. 200 mesh (74 μm pore). The kidney cortex, in portions roughly two centimeters square, is forced by spatula through the initial screen during continuous and generous rinsing with isotonic saline. Large aggregates of tubules and debris are trapped on the underlying No. 100 screen while fragments of single tubules and intact glomeruli pass to the lowermost fine screen. Further rinsing of the final screen washes most tubules through, leaving almost pure glomeruli. (Depending on species, age, and history of the donor, the average glomerular diameter may be such that the No. 100 screen retains the greatest yield of pure glomeruli). Progress of the purification process is followed microscopically by periodic examination of aliquots. When continued rinsing fails to achieve discernible increase in purity, the preparation is transferred to a tube of saline and centrifuged for 3–5 min at 500–1000g or simply allowed to settle by gravity. The saline is aspirated and the glomeruli are diluted with 20–25 vol of a medium such as Parkers 199 or Eagle's MEM.

Culturing procedure. An amount of glomerular suspension just adequate to cover the growth surface of each culture vessel is added to pyrex or polystyrene dishes or flasks (for example, about 0.8 ml in the case of a 25 ml Falcon flask). The culture vessels are then incubated at 38° in an atmosphere of

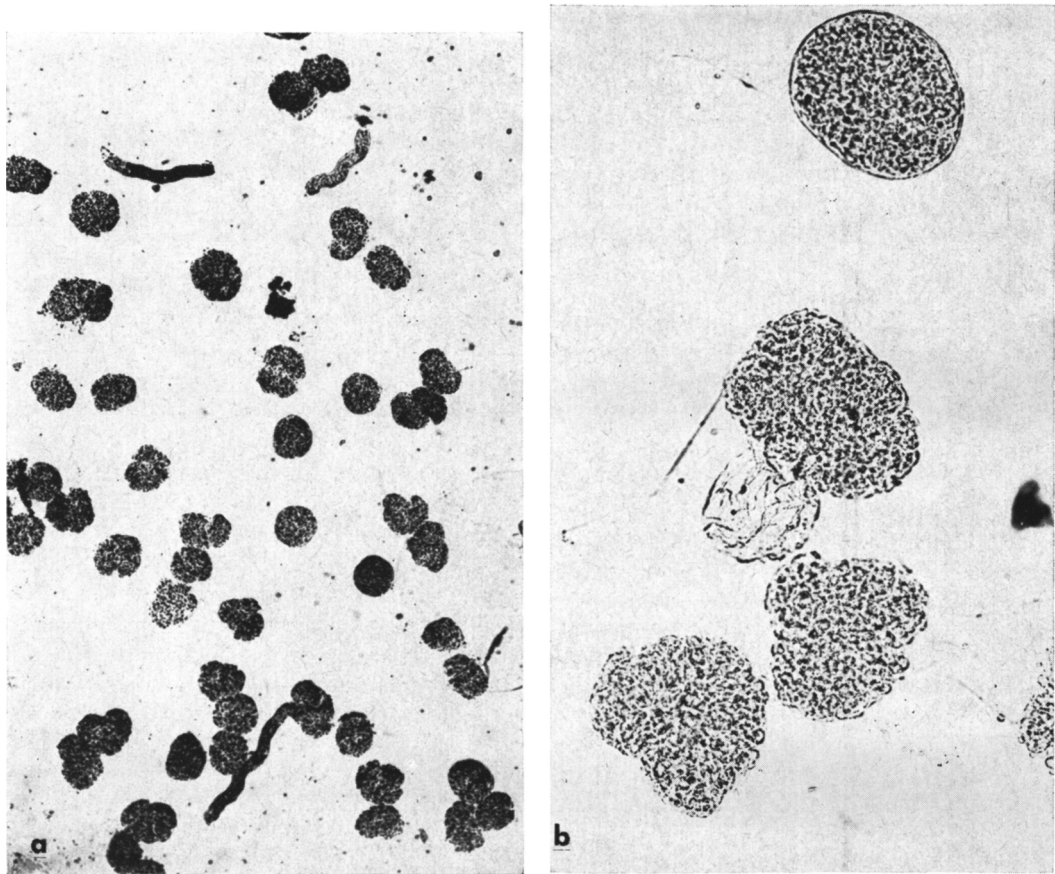
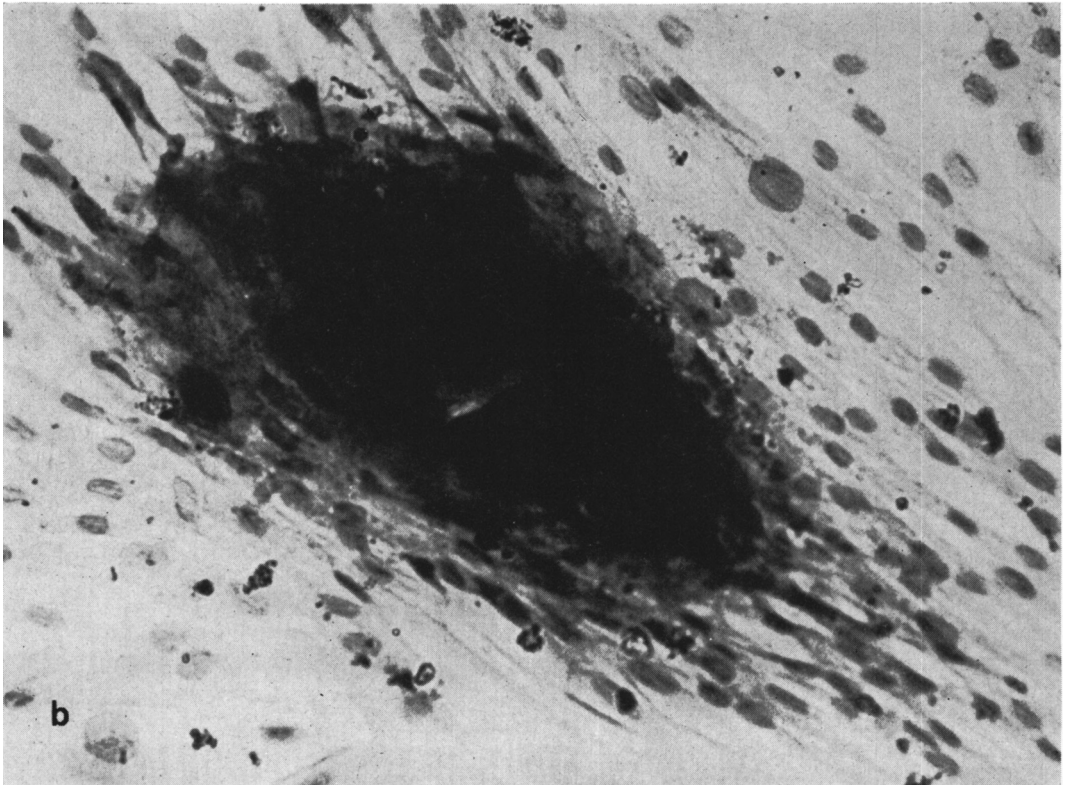
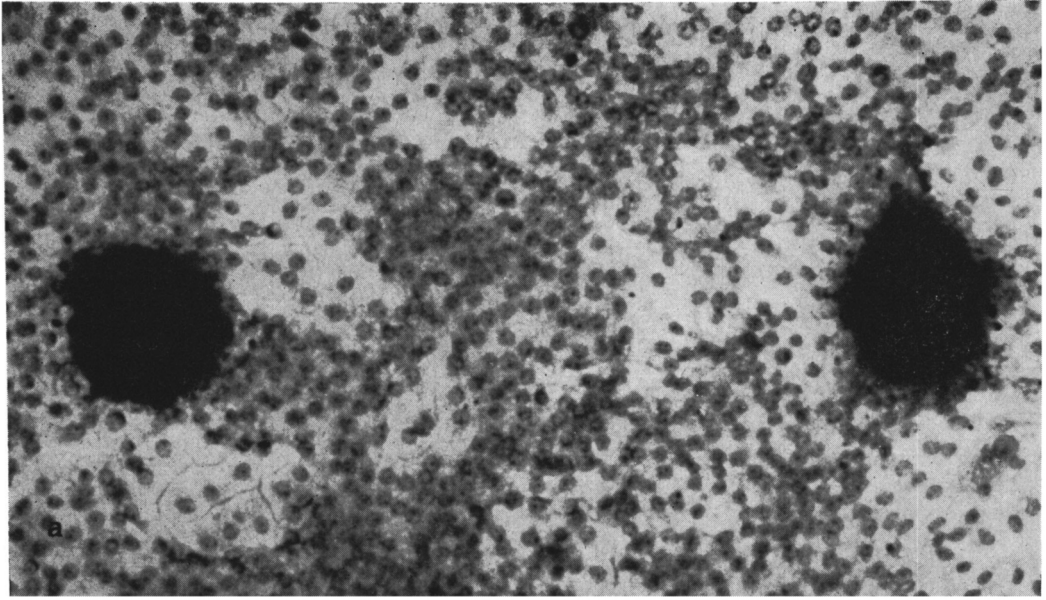


FIG. 1. Fresh preparation of renal glomeruli (dog): (a) 30 \times ; (b) approx. 60 \times .

95% air, 5% CO₂ for a period of 30–60 min during which time numerous glomeruli attach to the surface. It is important that this brief incubation be done in synthetic medium only, since serum appears to inhibit glomerular attachment. Following preliminary incubation, the medium is poured off and complete serum-containing growth medium is added gently so as not to disturb attached glomeruli. Trials with isotonic saline as the preliminary incubation medium showed that although glomerular attachment occurred, later cellular outgrowth was severely limited. Thus, a complex mixture like 199 appears necessary during the attachment reaction if significant cell growth is to follow. A satisfactory growth medium is 70–80% medium 199, and 20–30% calf serum, in addition to penicillin and streptomycin in amounts routinely used in cell culture. We have had

success with other media, including Eagle's MEM and McCoy's 5A Modified, and varying types and concentrations of serum, including horse and fetal calf in concentrations as low as 12% in primary cultures. Renewal of medium after 5 or 6 days usually produces a noticeable increase in proliferation. Incubation for approximately 8–21 days under the conditions listed above results in cellular monolayers arising from most of the attached glomeruli. Preparations derived from embryonic or very young kidneys usually proliferate more rapidly than those derived from adult tissue, sometimes yielding complete monolayers in 5 days.

Harvesting procedure. Cells and glomeruli are scraped from the growth surface with a plastic spatula or detached with trypsin. Glomeruli can then be separated from the cell monolayer by repeated pipetting with a



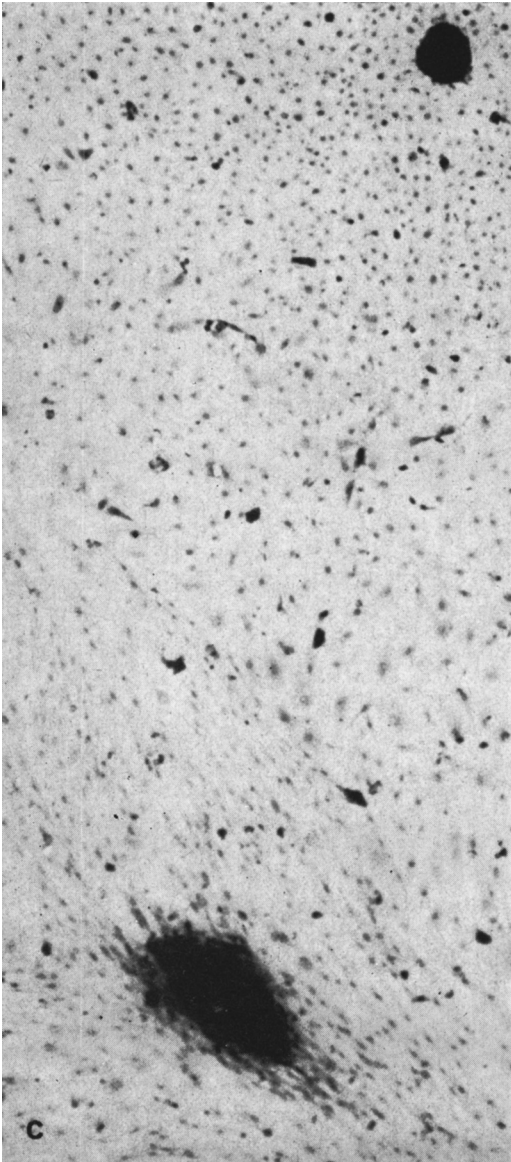


FIG. 2. Renal glomerular cultures (dog). 11 days in culture. Hematoxylin and eosin. (a) Two darkly staining encapsulated glomeruli and cellular outgrowth from each; approx $85\times$; (b) Decapsulated glomerulus and fibroblast-like outgrowth; approx $400\times$; (c) Epithelial and fibroblast-like cell populations arising from encapsulated and decapsulated glomeruli respectively; approx $50\times$.

5 ml serological pipette. Cell sheets are further disaggregated into a single-cell suspension by continued pipetting. Glomeruli are

allowed to sediment and the suspended cells then are aspirated. Alternatively, individual glomeruli can be picked off the growth surface with a Pasteur pipette, leaving the monolayer intact and available for separate harvest. Thus, one may deal separately with the cultured glomeruli and the cells to which they gave rise for purposes of subculture or biochemical analysis.

Morphology and growth characteristics. Initial glomerular preparation. The glomeruli are relatively uncontaminated with tubular fragments but total purity is rarely achieved and is unnecessary for culture purposes since the tubules do not give rise to cellular outgrowth and usually are eliminated completely during the first change of growth medium. Figure 1a is a low-power view of a typical field in a sample of the fresh preparation. Tubules are scarce. The glomerular capsule has been removed in many instances, resulting in a cauliflower-like appearance. A higher power view illustrating both encapsulated and unencapsulated forms is shown in Fig. 1b. Different cell populations arise from each type of glomerulus and are described below.

Glomeruli and cells in culture. Rarely is there evidence of cell growth before 3 days incubation. Contrary to the relatively rapid proliferation occurring in primary cultures of trypsinized suspensions of whole kidney cortex, the rate in glomerular cultures is slow. By 6 to 9 days, distinct populations are visible, consisting of a central glomerulus and a ring of cells originating from it. At this stage the appearance of the culture vessel is patchy, *i.e.*, islands consisting of a glomerulus and its cellular outgrowth separated by clear areas. No growth occurs in the absence of a glomerular focus.

Of the two distinct cell types which arise, an epithelial-like population is the more abundant. It is always derived from encapsulated glomeruli and is characterized by relatively small, tightly packed cells containing a relatively small spherical or ovoid nucleus. Figure 2a is a low power view of a population stained on the dish in which it grew. Two darkly stained glomeruli are visible in the midst of their cellular outgrowth.

Less abundant is a fibroblast-like popula-

TABLE I. Mitotic Index in Cultures of Dog Renal Glomeruli at 11 Days.

Total cells in colony*	Mitotic figures	% Mitotic figures	Total cells in colony	Mitotic figures	% Mitotic figures
750	103	14.0	550	101	18.0
328	7	2.1	800	76	9.5
812	27	3.3	268	9	3.4
138	24	17.0	532	12	2.2
245	18	7.4	504	18	3.6

* Epithelial colonies.

tion which is invariably associated with unencapsulated glomeruli. In these instances the glomeruli are spread over a large area of the growth surface. Figure 2b shows a decapsulated glomerulus and its typical cellular outgrowth while Fig. 2c illustrates adjacent populations of each type in the same culture. Strict segregation with no overlap is a prominent feature of the outgrowth and usually persists for the lifetime of cultures of canine origin. However, this apparent contact inhibition is not operative in long-term cultures of goat glomeruli in which we routinely observe overgrowth and stacking of

epithelial cells with time.

Mitotic index was determined in a number of cultures and found to vary widely from one glomerular colony to the next. In a typical count done on a single petri dish in which 10 individual epithelial colonies associated with encapsulated glomeruli were counted separately, the percentage of mitotic figures, as indicated in Table I, range from a low of 2.1% to a high of 18%. The range may reflect the tendency for outgrowth from individual glomeruli to be initiated at varying intervals in culture, from as early as 3 days to as late as 9 to 10 days.

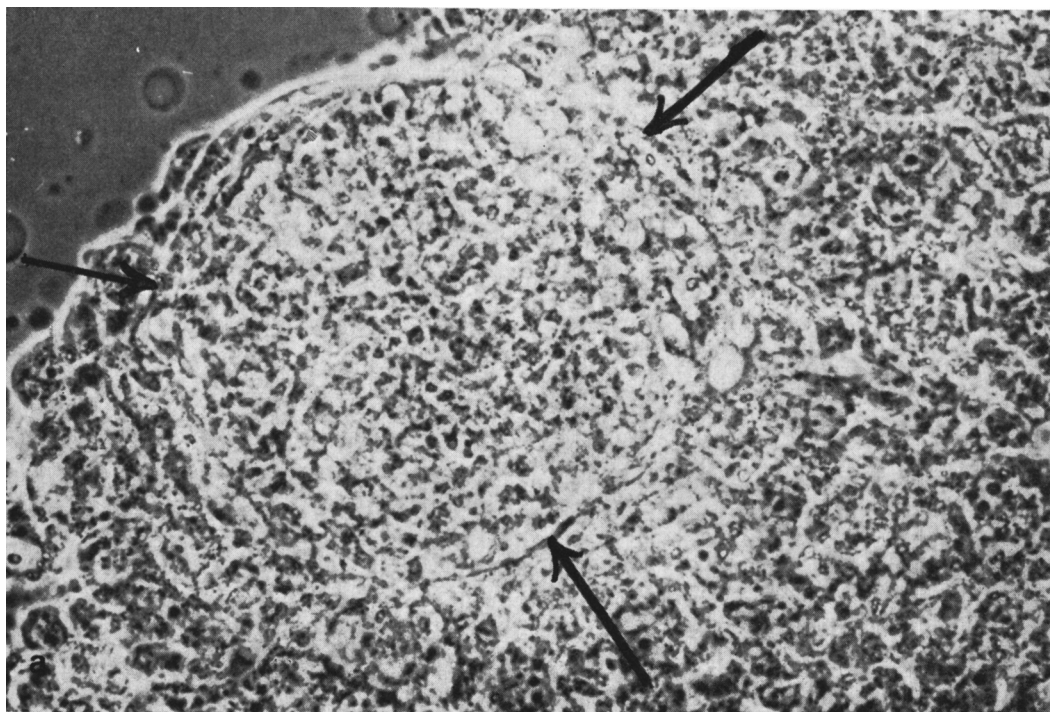


FIG. 3a and b. Phase contrast (approx 140X). Glomerulus and cells harvested as described.

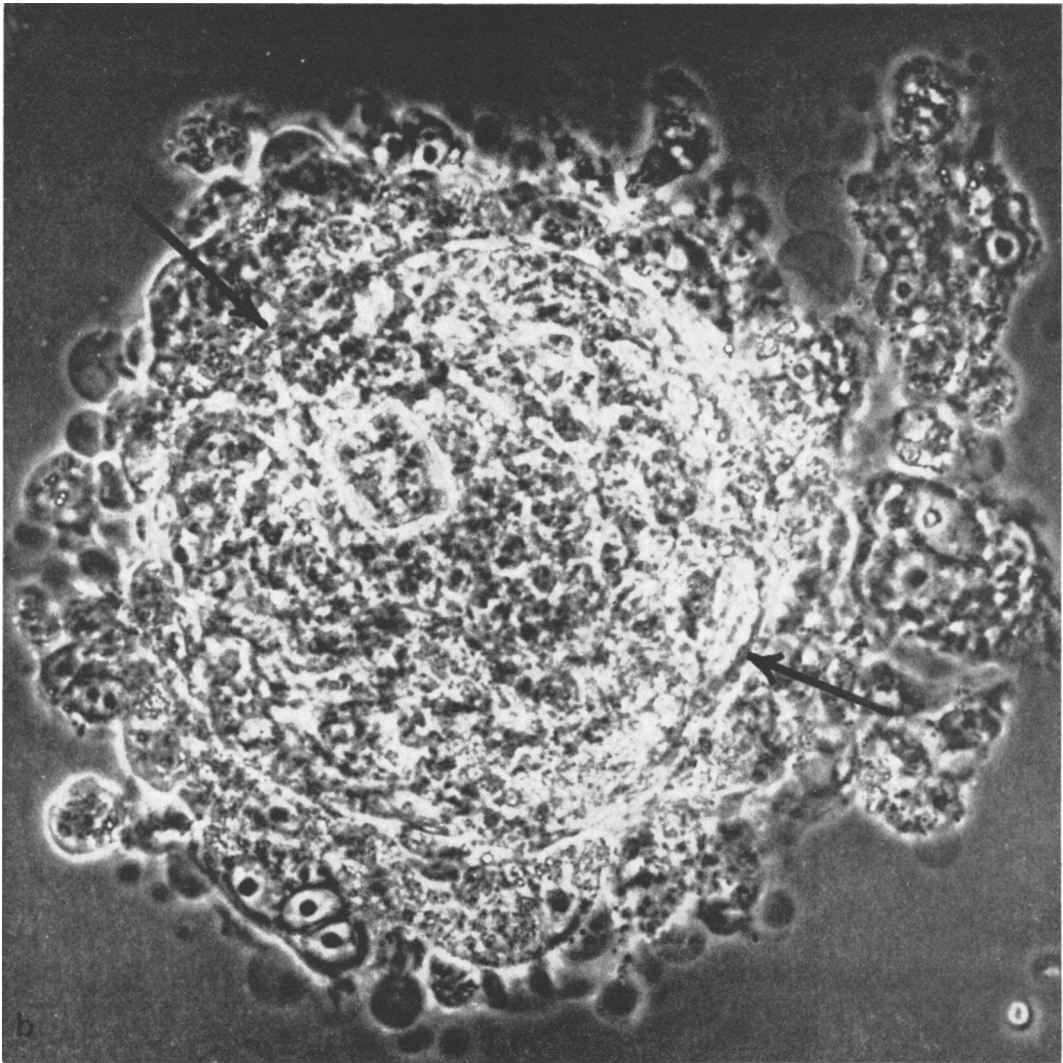


FIG. 3b. A more advanced state of separation. Arrows denote glomerular capsule.

Enzyme profile. Biochemical characterization of the culture system is possible by utilizing the harvesting procedure described. Figure 3 shows two stages in the separation of the cultured glomerulus from the cell monolayer to which it gave rise. Repeated pipetting strips the cells from the parent glomerulus thus providing pure fractions of each.

Three enzyme systems involved in different aspects of metabolism were chosen for assay to determine the direction and degree of modulation in epithelial cultures. An indicator of hexose monophosphate shunt ac-

tivity, glucose-6-phosphate dehydrogenase and two purine-metabolizing enzymes, adenosine deaminase and nucleoside phosphorylase were compared in the original glomerular preparation and glomeruli and cells harvested after 9–15 culture days. Figure 4 indicates a reduction of specific activity of the systems involved in purine metabolism in both intact glomeruli and cell outgrowth following culture. Shunt activity on the other hand, was increased significantly beyond that seen in the original glomerular preparation.

The altered enzyme profile might be linked to changes in regulatory factors or equally

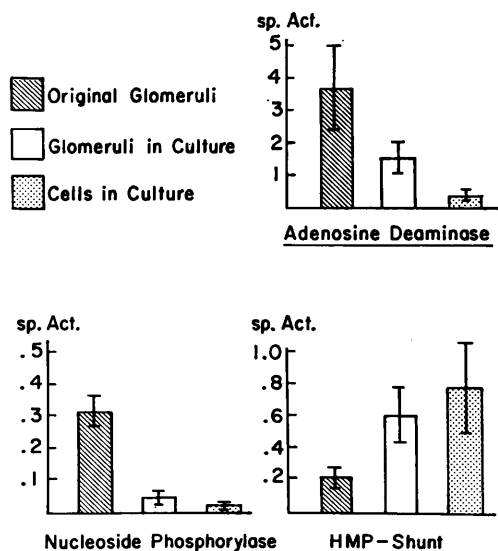


FIG. 4. Enzyme profile of glomerular cultures. Specific activity expressed as follows: adenosine deaminase: Δ OD at 265 nm/mg protein/30 min; nucleoside phosphorylase: Δ OD at 292.5 nm/mg protein/10 min; HMP-shunt: Δ OD at 340 nm/mg protein/10 min. Assays as described in Refs. (11, 12). Mean values and standard errors shown. Dog glomerular cultures 9–15 day, epithelial monolayers.

possibly to selective survival in culture of one cell type with essentially unchanged regulation and display of enzyme complement. Since the glomerular cell type giving rise to the monolayer has yet to be determined, a decision between modulation and selection is not possible from the results described for the cultured cells.

Discussion. Two reports appearing recently deal with glomeruli in culture. In the one (9), individual glomeruli in Rose chambers were studied with time-lapse cinematography. Contractile cells were a prominent feature of these cultures and encapsulated glomeruli not only failed to show outgrowth but were stated to consist mostly of pyknotic cells when

fixed and stained. Obviously the method, results and potential applications are unrelated to those described here. The other report (10) deals with a system more closely resembling ours and is valuable especially for the electron microscopy shown.

We have had enough experience with the glomerular culture system as utilized in ongoing studies of renal transplantation and the physiology of erythropoietin production (8) to feel that it may be profitably employed in a wide variety of research areas.

Summary. A method is described for culturing renal glomeruli. Resultant cell monolayers are epithelial or fibroblast-like, depending upon their origin from either encapsulated or decapsulated glomeruli, respectively. The system appears to have potential usefulness in several research areas.

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