SOME OBSERVATIONS OF THE METABOLIC ACTIVITY OF GLOMERULI

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Disease of the renal glomerulus or disturbance of glomerular function is the principal feature of most diffuse diseases of the kidney. Despite this, knowledge of the metabolic activity of the glomerulus has not kept pace with the growth of similar knowledge about the various tubular segments of the nephron. The preponderance of glomerular lesions in most diffuse renal diseases and the nearly exclusive glomerular involvement in the early stages of acute glomerulonephritis, membranous glomerulonephritis and similar disorders raises the question whether some attribute or attributes of glomerular metabolism render it uniquely susceptible to injury.

Most studies have concentrated on ultrastructural, immunopathologic or histochemical alterations in the glomeruli in various diseases. Studies showing accumulation of gamma globulins within the glomerulus have been reported in a number of renal diseases. In some instances, these accumulations appear to represent specific antibodies to some component of the basement membrane of the glomerulus, while in others this material has been identified as complement containing antigen-antibody complexes usually located adjacent to the basement membrane.[1, 2] The mechanism whereby accumulation of these substances leads to diffuse damage or alteration in glomerular permeability remains unclarified. It seems unlikely that the disruptive effect upon glomerular function is a purely mechanical one resulting from obstruction of the filtering surface of the glomerulus since this is inconsistent with the observed changes in glomerular function.[3]

Studies of glomerular permeability in many of these disorders suggest that some diffuse structural alteration underlies the disturbed function.[3, 4] The great rapidity with which the nephrotic syndrome may develop in a previously normal kidney and the rapid return to normal function occasionally observed under the influence of steroid therapy

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are well established. Such rapid change in the functional integrity of the glomerulus is consistent with a high order of metabolic activity. This seems quite likely to be the case if alterations in permeability exhibited in the nephrotic syndrome result from structural alteration in the glomerular basement membrane since rapid repair or replacement of this portion of the glomerulus necessarily implies great metabolic activity.

The studies reported here were undertaken in search of a means to assess the metabolic properties and behavior of the glomerulus. The findings thus far lend some substance to the foregoing conjectures.[5]

Methods

A modification of the Krakower-Greenspon technique for the isolation of glomeruli was used in these studies. Kidneys from freshly killed rats were chilled, minced and homogenized in a loose-fitting Tenbroeck homogenizer and then washed successively through three stainless-steel screen meshes (mesh sizes were 80 x 80, 150 x 150 and 200 x 200 strands per square inch). The glomeruli were trapped on the second and third screens and could be washed free of contaminating tubular fragments. The isolation procedure usually required fifteen to twenty minutes for completion and yielded 10,000 to 15,000 glomeruli from each kidney. (Figure 1).

Oxygen consumption was measured in a Warburg manometric apparatus or alternately by means of a small lucite respirometer fitted with an oxygen electrode. Measurements were made in a Krebs-Ringer-Phosphate solution with glucose or other substrate added to the incubation medium. Protein was measured by Lowry's technique and oxygen consumption expressed as μl/mg protein/hr.

Metabolic activity of these isolated glomeruli was further characterized by measurement of the incorporation rate of C^{14} labeled amino acids into perchloric acid precipitable protein of the glomerular cells and comparison with similar data from cortical tubular cells. The incubation medium used for these studies was Krebs-Ringer-Phosphate and unlabeled amino acids in approximately the proportions found in the kidney, omitting only lysine. C^{14} lysine was added to this medium and glucose was added to a final concentration of 50 mg per cent. The glomerular preparation was incubated in this medium for a 3 hour period. Following incubation, the radioactivity found in the perchloric acid precipitable protein was measured. A similar technique was used to obtain data on incorporation of H^3-cytidine into ribonucleic acid. In some experiments the glomerular basement membrane was isolated following incubation and the radioactivity found in the basement membrane was compared with the quantity of radioactivity found in other protein components.[6]

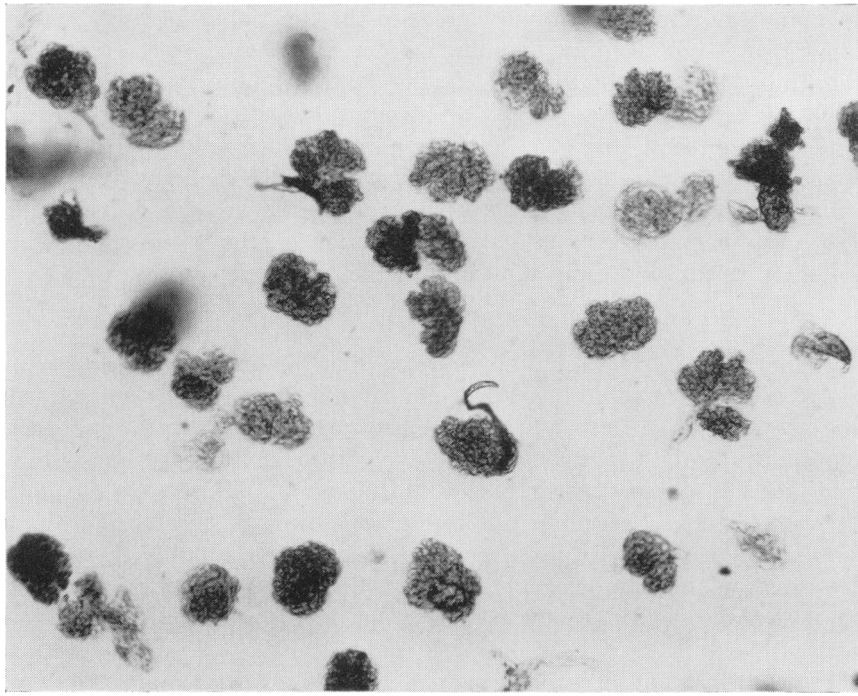

FIG. 1. Photomicrograph of preparation of isolated glomeruli (× 75).

Similar data on oxygen consumption and amino acid incorporation were obtained on isolated intact kidneys as a means of assessing the degree to which mechanical trauma associated with the glomerular isolation may have been responsible for some of the observed differences in behavior between the glomeruli and tubules. A pulsatile pump was used to perfuse the kidneys with oxygenated blood containing either C^{14} lysine or H^3 cytidine. Following perfusion, glomeruli were isolated from the kidneys and incorporation of the labels into the protein and ribonucleic acid of the glomeruli and tubules was measured.

OXYGEN CONSUMPTION

The high rate of oxygen utilization exhibited by the intact kidney has been well documented. Studies relating oxygen consumption and sodium transport have identified a linear correlation between these two phenomena that is maintained over a five fold range.[7] This finding plus the related observation that a number of inhibitors of electron transport block *in vivo* sodium reabsorption provided the basis for the inference,

made by several investigators, that the major portion of oxygen consumption by the kidney yielded energy to support sodium transport. Thus, it seems plausible to expect that portions of the kidney not involved in sodium transport may exhibit much lower oxygen consumption rates. Contrary to the expected difference, the isolated glomeruli exhibited a rate of oxygen consumption indistinguishable from that of the cortical tubular cells for all substrates tested except succinate (Table I).

Since some tissues such as the liver exhibit a lower rate of metabolic activity when studied by means of slice incubations as compared with perfusion of the intact organ, the possibility that the similar rates of oxygen consumption noted in these studies was due to an abnormally low rate for the tubular cells was considered. This was excluded by measuring oxygen consumption in the intact perfused kidney. These perfusion studies yielded an oxygen consumption of 212 ± 7 (Std. error of mean) microliters O_2/mg protein per hour. This value is quite similar to the value obtained with the cortical cell suspension using succinate as substrate.

Two inferences were drawn from these studies. First, it was apparent that the glomeruli exhibited a rapid metabolic rate as judged by oxygen consumption. For all the substrates examined except succinate, rates of oxygen consumption were indistinguishable when the glomeruli were compared with cortical cells. Secondly, the nearly identical oxygen utilization rates found in the perfused kidney and in the cortical cell suspension indicates that valid comparison can be drawn between the glomeruli and suspensions of cortical tubular cells in respect of metabolic activity.

These observations fail to provide any clues to the most important question: What useful purpose does this high rate of glomerular oxygen consumption serve? Since the glomerulus has no known transport functions analogous to those of the tubules, this metabolic activity must meet other needs. One possibility that merited exploration was a high rate of turnover of the structural components of the glomerulus.

TABLE I

Glomerular Oxygen Consumption Substrate Effects

Substrate	Oxygen Consumption μl/mg prot./hr	
	Glomeruli	Tubular Cells
Glucose	87.4 ± 26 (S.E.M.)	61.3 ± 13
α-ketoglutarate	64 ± 13	60 ± 8
Pyruvate	43 ± 11	44 ± 5
Succinate	125 ± 12	277 ± 13

Protein Synthesis in the Glomerulus

Incorporation of C^{14} labeled amino acids into perchloric-acid precipitable protein of the glomerulus was studied as an index of the turnover rate of the protein components of the glomerulus.[5] Incubation of the glomeruli with Krebs-Ringer-Phosphate medium containing C^{14} lysine resulted in the incorporation of C^{14} lysine into protein at a much more rapid rate than did corresponding studies on the cortical cells (Table II). The time course of one such experiment is shown in Fig. 2.

TABLE II

Incorporation of Labeled Precursors into Protein and Ribonucleic Acid

Labeled Precursor	C^{14} Lysine in Protein cpm/mg. Prot.	H^3 Cytidine in RNA cpm/μg RNA
Glomeruli	4786 ± 663 (S.E.M.)	65.5 ± 11
Cortical Cells	540 ± 101	11.4 ± 1.2
"P" Value	< .001	< .001

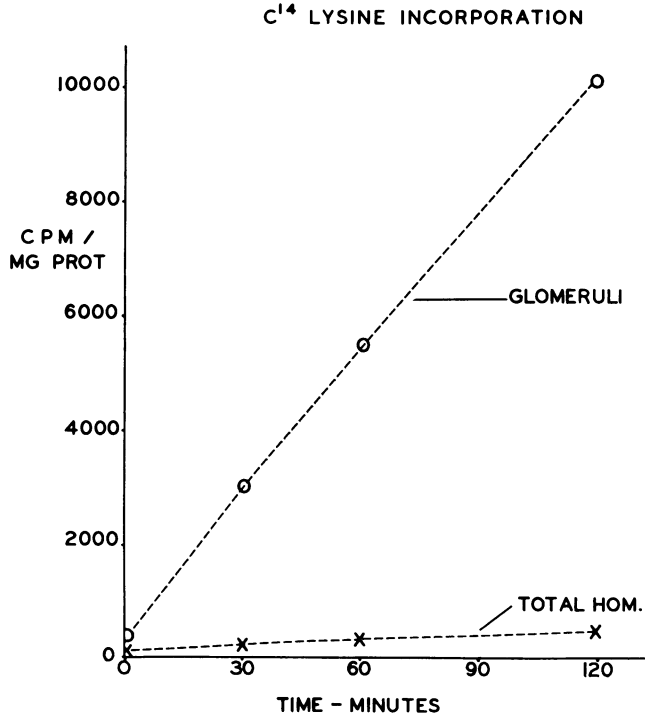

Fig. 2. Time course of incorporation of C^{14} lysine into glomeruli and tubular cells identifying persisting difference between rates of glomeruli and tubular cells.

This difference in the rate of incorporation was maintained for periods up to four hours and at the end of this time the quantity of radioactivity per milligram of protein was 8 to 10 times greater in the glomeruli than in the other cortical cells. Puromycin, a known inhibitor of protein synthesis, produced approximately 90% inhibition of the C^{14} lysine incorporation under these conditions.

An attempt was made to localize the site at which this incorporation was occurring by fractionating the soluble protein and the basement membrane of glomerulus. This was accomplished by exposing the glomeruli to 60% trichloroacetic acid following incubation with the radioactively labeled material. This treatment solubilizes all of the proteins except the basement membrane.[6] A separation was accomplished by centrifugation and radioactivity measured in the soluble protein and in the glomerular basement membrane after solubilization in alkali. The results of these studies are shown in Fig. 3. The quantity of radioactive label per mg of glomerular basement membrane was significantly greater than that observed in the other proteins of the glomerulus. Thus these studies would appear to indicate that under the conditions existing in these experiments the glomeruli exhibit very considerable metabolic activity. Their oxygen consumption rate is approximately the same as that observed in the cortical cells and they

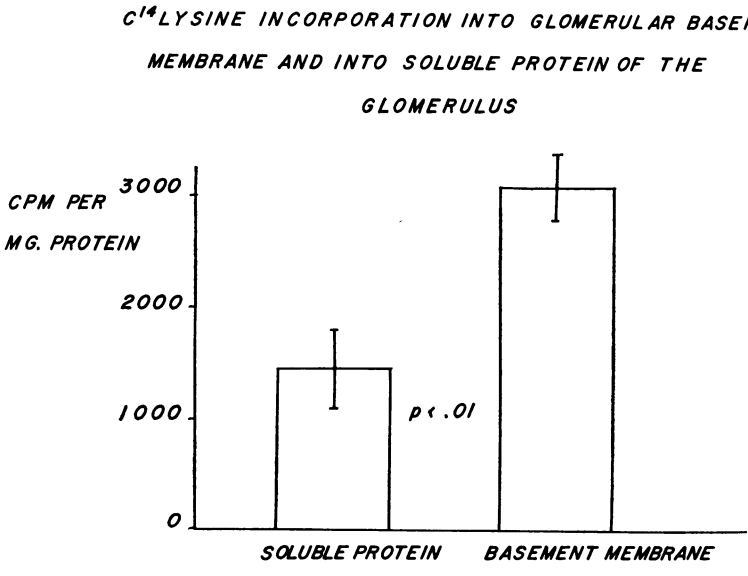

FIG. 3. Incorporation of C^{14} lysine into glomerular basement membrane. After three hours of incubation with medium containing C^{14} lysine, there is approximately twice as much C^{14} in the basement membrane as in other protein of the glomerulus.

incorporate labeled amino acids into the cellular protein of the glomerulus at a much more rapid rate than occurs in the tubular cells. This increased incorporation presumably reflects an increased turnover of cellular proteins and these studies indicate that a major part of this rapid protein turnover is occurring within the basement membrane.

Previously reported studies failed to yield evidence that this disparity between glomeruli and tubules was due to greater damage sustained by the tubules during preparation of the tissue for incubation.[5] However, to obtain further evidence excluding the possibility that this difference was artifactual, the kidney was exposed to the labeled amino acid in situ during a period of perfusion. This system permitted measurement of the incorporation of C^{14} labeled lysine into the perchloric acid precipitable proteins of the kidney. Following perfusions for periods up to 2 hours the kidneys were removed, the glomeruli isolated in the usual fashion and the incorporation of labeled lysine measured. These results are seen in Table III. As with the *in vitro* studies, there was a significantly greater incorporation of lysine into the glomeruli. Both the oxygen consumption studies and the C^{14} lysine incorporation studies in this preparation corroborated the findings by the *in vitro* studies. The data indicate the consistently more rapid protein uptake within the glomeruli, a finding which presumably reflects an increased protein turnover in these structures.

Since it has been clearly established that much of the protein synthetic activity within the cell is under the control of the ribonucleic acid of the cell, additional support for the apparent finding of increased protein synthetic activity within the glomeruli was sought by measuring the rate of tritiated cytidine incorporation into ribonucleic acid (RNA) within the glomeruli and tubular cells. In vitro incubations with H^3-labeled cytidine revealed findings that were qualitatively similar to the findings with C^{14} lysine (Table II). These observations were also repeated in studies on the isolated perfused kidneys. Results were similar (Table III). Thus all the data obtained in these studies provide evidence to support the proposition that the glomeruli are indeed meta-

TABLE III

Incorporation of Labeled Precursor into Protein and RNA of Perfused Kidney

Precursor	C^{14} Lysine in Protein cpm/mg. Prot.	H^3 Cytidine in RNA cpm/μg RNA
Glomeruli	2059 ± 399 (S.E.M.)	16.8 ± 3.3
Cortical Cells	648 ± 80	5.3 ± 1.6
"P" Value Paired "T" Test	< .0005	< .0005

bolically quite active and that they maintain a much higher rate of turnover of endogenous protein than is found in the tubular cells. Since the basement membrane appears to contain the greatest quantity of radioactivity, much of this protein turnover must represent repair or replacement of the basement membrane.

Comment

These studies identify the glomerulus as the seat of great metabolic activity, judged both by an oxygen consumption rate that is as high as that of the tubular cells for most substrates and by a high rate of protein synthesis. The rapid rate of incorporation of C^{14} lysine into the protein of the glomerulus implies that there is a high turnover rate of at least some of the proteins of the glomerular structures. Since the major portion of the radioactivity was found in the basement membrane of the glomerulus, it seems likely that much of this metabolic activity serves to maintain a rapid turnover rate of the glomerular basement membrane. If these findings are correctly interpreted, then the integrity of the basement membrane is dependent upon the ability of the kidney to maintain this membrane in a state of constant repair. Although recent information contributed by Spiro[8] and Misra and Kalant[9] on the composition of the basement membrane establish that it is composed of protein, lipids and glycopeptides, no information is yet available about the chemistry of this membrane formation or about its turnover rate.

The paradox of increased permeability to macro-molecules associated with increased thickness of the basement membrane may also be construed as evidence that there is some associated structural change in the membrane beyond simple mechanical blockage from deposited proteins such as antigen-antibody complexes. An understanding of the basis for this permeability change requires more complete knowledge of mode of membrane synthesis and the means whereby repairs are effected. Information bearing on this question at present has been provided almost exclusively by studies of the ultra structure of the kidney. Kurtz and Feldman identified new basement membrane formation occurring beneath the epithelial cells on the outer or uriniferous side of the basement membrane.[10] Other workers,[11, 12] studying material from human disease, have interpreted electron micrographs as showing deposits of new basement-membrane-like material largely on the endothelial side and predominantly in the stalk or mesangial region. It thus appears that there is histologic evidence for participation of all three cell types in basement membrane formation.

The studies presented here provide additional evidence of a different sort in support of active basement membrane formation in the normal glomerulus. No information is available from the present studies that identifies the cell type responsible for this activity, but the higher rate of labeled amino acids into the basement membrane indicates that this function represents a major part of the metabolic activity of the glomerulus.

In view of the well documented changes in the structural and functional characteristics of the basement membrane in the glomerulonephritides and other diseases involving the glomerulus, the present studies would appear to offer a possible explanation for the susceptibility to injury exhibited by this important structural element of the glomerulus. The incorporation studies provide support for a high rate of turnover of the basement membrane, a process which must be dependent upon the integrity of the cellular elements of the glomerulus. Metabolic derangements or immunologic injury to these cells may thus be expected to interfere with their function of maintenance of repair of the basement membrane.

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