Variation of Thickness of Glomerular Basement Membrane in Various Experimental Circumstances

Since introduction of electron microscopy into the field of renal pathohistology, precise changes of the thickness of the glomerular basement membrane (GBM) have been discussed by many investigators. There have been, however, only a few basic studies on GBM of the kidney in a variety of non-pathological circumstances. Thus, several factors which may influence the thickness of GBM have been investigated.

Materials and methods. Adult male albino rats of Wister-King A strain were used throughout the experiments. The experiments were designed as indicated in Table I. Experiment I (18 rats) was to evaluate the variation of GBM thickness according to the methods of sacrifice of animals. Similarly, experiment II (18 rats) was made for the evaluation of the hemodynamic conditions which may cause the changes in GBM thickness; experiment III (6 rats) for the evaluation in compensatory hypertrophy; and experiment IV (6 rats) for the evaluation of the changes due to ischemic glomerular lesion.

All rat kidneys were examined by light and electron microscopy. A quantitative measurement of the thickness of GBM was made at the periphery of the glomerular loops[1] from electron micrographs. 200 measurements of GBM were made in the glomeruli of each rat. Also the mean and the standard deviation (S.D.) were calculated from each group (600 measurements in 3 rat kidneys).

Results. The mean GBM thickness and the frequency distribution curve of each experiment group were shown in Table II, Figures 1 and 2.

Experiment I: Light microscopically, no significant change was noted in the glomeruli of 3 groups of rat kidneys. But the glomerular capillary lumens of 'Ether' and 'Pentobarbital' were apparently patent, whereas those of 'Decapitation' were relatively reduced in their space, giving an impression of compressed glomerular tuft. The frequency distribution curve of GBM in these

Table I

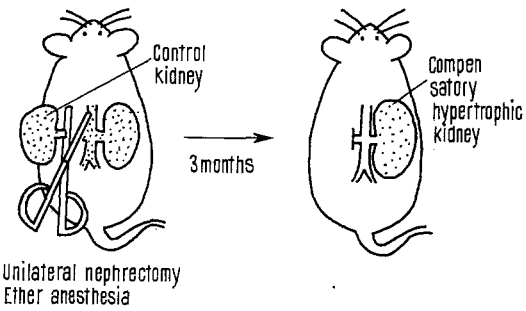
Experiment I

	No. of rats	Method of sacrifice
Ether	6 (3)	Ether anesthesia
Pentobarbital	6 (3)	10 mg/100 body wt. Pentobarbital (i.p. injection)
Decapitation	6 (3)	Decapitation and exanguination

Experiment II

	No. of rats	Experimental condition
Water deprivation	6 (3)	Water deprivation for 3 days
Water diuresis	6 (3)	Feeding only 20% sucrose solution for 6 days
Saline perfusion	6 (3)	Saline perfusion with high pressure through renal artery in situ

Experiment III



Experiment IV

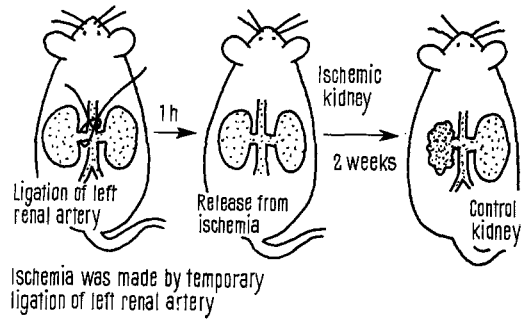


Table II

Experiment I

Method of sacrifice	Mean thickness of GBM S.D. Å	T value Decapitation	Pentobarbital
Ether	1892 ± 402	Diff. = 142 Å T = 6.34	Diff. = 71 Å T = 3.43
Pentobarbital	1963 ± 371	Diff. = 71 Å T = 3.31	
Decapitation	2034 ± 372		

Experiment II

Method of treatment	Mean thickness of GBM S.D. Å	T value Saline perfusion	Water diuresis
Water deprivation	1822 ± 375	Diff. = 126 Å T = 5.83	Diff. = 61 Å T = 2.77
Water diuresis	1883 ± 389	Diff. = 187 Å T = 8.30	
Saline perfusion	1696 ± 129		

Experiment III

	Wt. of kidney average (g)	Mean thickness of GBM S.D. Å	Control vs. Hypertrophic kidney
Control kidney	0.78	1885 ± 375	
Compensatory hypertrophic kidney	1.63	1990 ± 560	Diff. = 105 Å T = 3.5

Experiment IV

	Wt. of kidney average (g)	Mean thickness of GBM S.D. Å	[a] Excluding damaged glomeruli
Control kidney	1.44	1981 ± 457	[b] Including damaged glomeruli
Ischemic kidney	1.30	1965 ± 565[a] 2237 ± 564[b]	

$T > 2.56$ was significant at $P = 0.01$.

groups had a single peak. The shape of the curve in 'Decapitation' was relatively flatter than the other two.

Experiment II: With the light microscopic observations, there was no obvious difference between 'Diuresis' and 'Deprivation' in the glomerular lumen. However, glomerular lumens of 'Perfusion' were dilated, and also the number of the loops of capillary were reduced. The frequency distribution curve of GBM in this experiment II showed a single peak. The curve of 'Perfusion' had a large shoulder at thinner side, and the curve of 'Diuresis' had a small shoulder at thicker side.

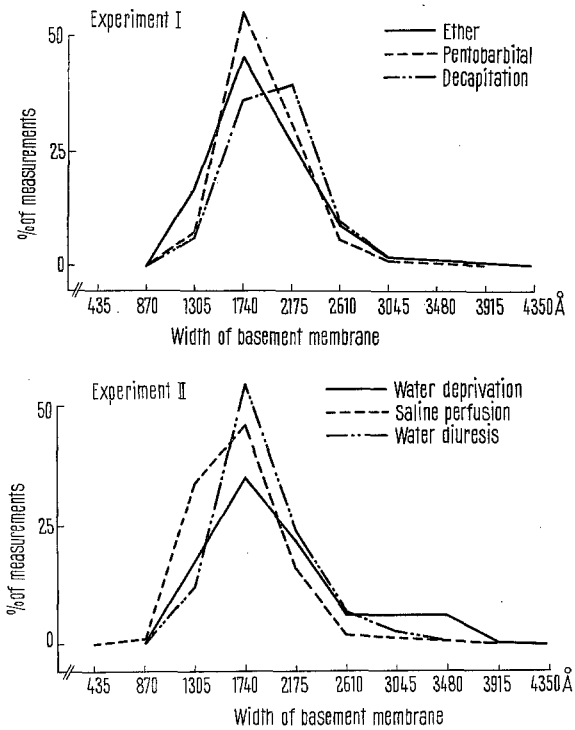


Fig. 1. Distribution curves of the thickness of basement membrane.

Experiment III: Light microscopically, the size of the glomerulus of the hypertrophic kidney was increased, the capillary lumens, however, were not dilated in comparison with the control kidney. The frequency distribution curves of these kidney glomeruli showed a single peak, but the curve in the hypertrophic kidney was flatter than the control one.

Experiment IV: Grossly, ischemic kidney was reduced in size and the subcapsular surface was rough. Light microscopically, majority of the glomeruli of the ischemic kidney were unchanged, but the glomeruli of affected areas showed varying degrees of scarring, and their basement membrane were wrinkled. There was no marked difference between the ischemic and the control kidney in respect to the patency of the glomerular capillary lumens, when damaged (scarred) glomeruli were excluded. The frequency distribution curves of GBM in the control kidney and ischemic kidney excluding damaged glomerular loops showed a single peak, but the curve of GBM in the ischemic kidney including damaged glomerular loops showed 2 peaks.

Discussion. The significance of the thickness of GBM in many pathological conditions has attracted many researchers' interest, and a large number of studies have been published on this subject. However, the results obtained by quantitative methods in the study of glomerular basement membrane thickness in the same nature of the disease (e.g. diabetic glomerulosclerosis) have been contradictory in spite of extensive studies. One reason may be the fact that the thickness of GBM in the human can vary considerably even in normal persons[2]. Most investigators, however, have not been much concerned with this fact. On the other hand, there is no available data concerning to the factor responsible for the variation of the thickness of the GBM in various functional state of capillaries, although KIMMELSTIEL[3] suggested that expansion and contraction of the capillary lumen may be one of the factors of this variation.

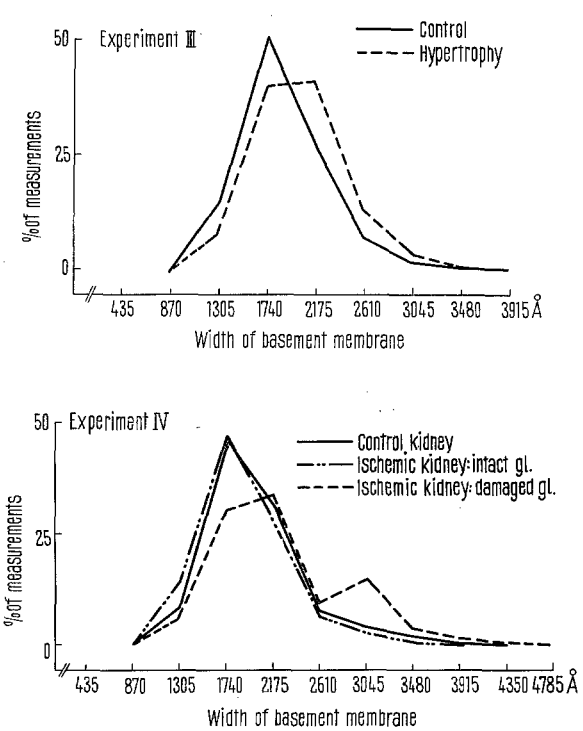


Fig. 2. Distribution curves of the thickness of basement membrane.

According to our observation, the mean thickness and the shape of frequency distribution curve of the glomerular basement membrane varied with the condition of the kidney. For example, *expansion* or *contraction* of capillary lumens gives rise to *thin* or *thick* basement membrane. Thus, these changes of the capillary lumens must be one of the important factors responsible for the variation of the thickness. And these results agree essentially with KIMMELSTIEL's prediction, and also are similar to the observation in the wall of small arteries during vasoconstriction and vasodilatation[4,5]. These results, however, not concur with SIPERSTEIN's[6] observation that the basement membrane width is not a function of the size of vessel, although his conclusion was not drawn from the functional state of the capillaries and also not from glomerular capillary.

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5 R. L. VAN CITTERS, B. M. WAGNE and R. F. RUSHMER, Circulation Res. *10*, 668 (1963).
6 R. H. U. SIPERSTEIN, J. clin. Invest. *47*, 1937 (1968).

It is interesting that, even when the mean thickness of the GBM in different conditions are very close, their frequency distribution curves are not always similar in shape and peak. These factors may cause a misleading impression on the thickness of the glomerular basement membrane when a conclusion is made from a small number of measurements. Therefore, it must be stressed that a frequency distribution curve of the thickness of the GBM based on large number of measurements is essential for the evaluation of the thickness.

Conclusion. Several factors responsible for the variation of the thickness of glomerular basement membrane of the rat kidney in various non-pathological circumstances are investigated, and following results are obtained.

(1) The mean thickness and the shape of frequency distribution curve in the glomerular basement membrane vary with condition of the kidney. (2) The expansion and the contraction of the capillary lumens can be one of the important factors responsible for the variation in the thickness in the non-pathological subject. (3) Even when the mean thickness of the glomerular basement membrane in different conditions are close, their frequency distribution curve are not always similar in shape and peak. (4) A compensatory hypertrophy may not produce gross change in width of the glomerular basement membrane in comparison with that of controls. (5) Affected (scarred) glomerular loops in the ischemic kidney show thick irregular basement membrane, whereas unaffected glomerular loops in such kidneys do not show thickening of the basement membrane.

Zusammenfassung. Mehrere Faktoren wurden untersucht, die verantwortlich sind für die Dickenvariabilität der Glomerulisbasalmembran der Rattenniere unter verschiedenen, nicht pathologischen Bedingungen. Durchschnittsdicke und Frequenzverbreitungskurve der Basalmembran ändern sich in Abhängigkeit vom Nierenzustand.

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Effect of 17 β-Estradiol on Early Cleavage Patterns in the Embryo of *Fucus distichus*

At 12 °C early development in *Fucus distichus* L. Powell (= *Fucus gardneri* Silva) is characterized by an oogamous fertilization, with the immediate formation of a fertilization membrane. A gelatinous sheath develops around the zygote over the next 6–8 h, followed by a dramatic polarizing response in the formation of a rhizoidal protuberance within 12 h. The rhizoid is fully formed by 24 h at which time the first cleavage plate is formed at right angles to the polar rhizoidal axis. The resulting cells are now presumed to be different from each other in that the rhizoidal cell gives rise to the holdfast of the mature plant, while the other cell gives rise to the thallus. Various environmental factors directly affect rhizoidal formation in *Fucus*[1]. However, the rhizoidal or polar axis once established preconditions the subsequent 3-dimensional distribution of embryonic mass which is characterized by an orderly displacement of cells. Orderly cell displacement can be viewed as a regulating mechanism for cutting off the regions of intracellular differentiation initiated by the zygote and preconditioned by the polarizing action. These 2 factors then, polarization and a predetermined cell displacement, seemingly are essential conditions for normal development in *Fucus* as in many other organisms.

In *Fucus distichus* it had been observed that young embryos with atypical cleavage patterns during the first 4–6 cell divisions were able to develop into normal embryos within a few weeks[2]. As a consequence, an attempt was made to induce this effect in young embryos in culture through the administration of mitotic poisons; namely, 17 β-estradiol and di-ethyl-stilbestrol, since these hormones were used successfully in inducing atypical cleavages in developing sea urchin embryos and other animal tissues[3]. The objective was to determine whether the heretofore described orderly displacement of cells around the embryonic polar axis was essential to continued embryo growth and survival. Previously, spindles had been rotated in *Fucus* using light as a stimulus. However, the resulting cells were symmetrically divided and the ultimate fate of the embryos was not reported[4].

Uniform cultures of zygotes 1 h old were obtained by following the methods for mass discharge of gametes and timing of fertilization in this monecious alga.

Percentage of population with rhizoids

Hormone concentration in ng/ml	After 24 h	After 4 days	After 5 days
12	40.0	68	98.0
37	34.7	65	97.5
111	24.0	69	97.0
333	17.0	60	91.0
1000	00.0	00	00.0

The relationship of di-ethyl-stilbestrol to the growth rate of young *Fucus* embryos. The growth rate, as evidenced by that percentage of the population which has well-formed rhizoids within a 24 h period, is retarded by an increasing concentration of hormone in a straight-line relationship. By 4 days about 70% of the embryos in all concentrations (except 1000 ng/ml) had polarized and by the 5th day it was close to 100%. Thus, the greater part of an embryo population is affected by a concentration of 333 ng/ml of hormone and growth lags by about 5 days.

The Table shows the relationship of varying concentrations of stilbestrol to the early growth rate of *Fucus*. Di-ethyl-stilbestrol has exactly the same effect as 17 β-estradiol except at lower concentrations. The delay in growth closely parallels the increase in concentration up to 1000 ng/ml of the hormone in seawater. At this, the highest concentration level tested, all growth ceases and the embryos do not survive. At 333 ng/ml rhizoid formation is delayed up to 5 days for the greater majority of embryos. At the end of this time, however, most of the embryos have well-formed rhizoids but some of the zygotes remain apolar. Subsequently, these cells undergo the first cleavage rather uniformly. However, the cells formed are markedly unequal in size, the plane of the spindle is quite randomly oriented and a wide variety of 2-celled embryos results (Figure). The second and subsequent cleavages are also disoriented. This effect continues with diminishing consistency for several cell

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[4] C. M. Child, *Patterns and Problems of Development* (Chicago University Press, Chicago, Illinois 1941).