SOME ASPECTS OF KIDNEY DEVELOPMENT

FROM LATE FOETAL LIFE TO PUBERTY

by

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CONTENTS

INTRODUCTION AND HISTORICAL SURVEY

The work to be described in this thesis is an attempt to establish what are the normal histological appearances of the human kidney during its development from late foetal life to puberty.

In a thesis presented for the degree of M.D. at Edinburgh University in 1900, Herring provided an extensive survey of the literature of renal development up to that date. Bowman had published his work on the development of the renal glomerulus and it was generally accepted that Bowman's capsule was an expansion of the end of the renal tubule, which was either pierced or invaginated by a vascular tuft composed of afferent artery, capillary loops and efferent artery. It had been accepted that this vascular tuft was covered by a layer of epithelium, in some respects similar to that lining Bowman's capsule, that changed in appearance during the development of the glomerulus. In the field of embryology, there was disagreement about the origin of the metanephric nephron. Some workers believed the secretory portion of the nephron to be continuous from the beginning with its collecting tubule, while others held that the secretory portions arose separately in the renal cortex, joining and becoming continuous with their collecting tubules later. It was to the elucidation of this point that much of Herring's work was directed and he came to the conclusion that the secretory portion did in fact develop separately in a condensation of foetal connective tissue round the ampullary growing end of the collecting tubule and that the lumen of the secretory tubule is not at first continuous with that of the collecting tubule.

About this time, a number of workers were studying the detailed structure of the human nephron by means of the microdissection and measurement of individual nephrons and by the making of models by injection and wax plate reconstruction techniques. In 1903-4 Stoerk published an extensive and detailed descriptive account of the microscopic structure of the adult human kidney, illustrated by many plaster and wax-cast models, but he does not provide any quantitative information. In 1909, Peter published work in which he had estimated the diameter and length of different sections of the microdissected nephron from adult human kidneys, and in 1911, Huber published measurements of the tubules of three microdissected human nephrons.

These are all detailed morphological descriptions of individual nephrons, but other workers were attempting to form an "over-all" picture of kidney structure and development. In 1914, Waschetko had estimated the number of glomeruli per microscopic field in the kidneys of six albino rats aged from one day to five weeks. He concluded that in this animal the number of glomeruli does not increase with post natal growth, which is due to the increase in size and diameter of the glomeruli and tubules already present. In 1917, Kittelson published a study of the post natal growth of the kidney of the albino rat in which he measured the increase in kidney weight and volume relative to body weight and age, the increase in volume of the cortex and medulla relative to each other and to the kidney volume, the increase in the volume of the renal cortex and medulla separately related to body weight, the total number of renal corpuscles per cubic ml. of cortex at different ages, the diameter and calculated volume of renal corpuscles at different ages and the ratio of the total volume of the renal corpuscles to that of the renal cortex and to the total kidney volume at different ages. These measurements were made on the kidneys of seven albino rats. In 1925, Arataki repeated and extended Kittelson's work on the albino rat. He measured the kidney volume, enumerated the glomeruli, measured the diameter and volume of the glomeruli and the diameter of the convoluted tubules. He also related the weight and size of the kidneys to increase in body weight and length. Arataki concluded that, in the rat, new glomeruli can be formed and become fully mature in seven days during the early post-natal period and he suggests that if the human kidney behaves in a similar way, the number of glomeruli present could be expected to increase up to the age of 8.3 years.

Toldt, Külz, Hauch and Felix made determinations of the relation of cortex to medulla in the human kidney at different ages.

Hauch and Felix report a rapid relative growth of the cortex in man up to seven years. Külz measured the diameters of glomeruli at the periphery and centre of the cortex at different ages in man.

In 1923 Campos published a paper on the pathological changes in the kidney in congenital syphilis in which he used a series of normal controls comprising 43 infants of whom 20 were premature and 23 full term. In this control group he found that all the kidneys of premature infants of 5 to 8 or 9 months gestation showed a subcapsular zone of metanephrogenic tissue whereas all the kidneys of full term infants showed no metanephrogenic zone. He quotes Aschoff as saying that the nephrogenic zone is present in the subcapsular layer until 3-6 months after birth.

In 1928 Vimtrup published his work on the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals. His series included two children, one aged 1 year and one aged 3 years, and three adults. He concluded that there is a direct relationship between the weight of a kidney and the number of glomeruli in it. Kunkel in 1930 published a study on the number and size of glomeruli in the kidneys of different mammals and Rytand in 1937-38 published work on the number and size of mammalian glomeruli related to kidney weight, but both these workers were concerned only with adult kidneys.

In 1940, Gruenwald and Popper published a paper on the histogenesis and physiology of the renal glomerulus in early post natal life. In the section of this work that is concerned with histological examinations they describe a survey of the kidneys of 39 embryos, foetuses and children up to 22 months of age. Their object was to re-examine the development of Bowman's capsule with particular reference to the development of the visceral layer of epithelium. Only the most advanced glomeruli near the cortico medullary boundary were examined and these were classified in four stages of maturity according to the appearance of the epithelium of Bowman's capsule and the epithelial covering of the capillary tuft. Their earliest stage is not the S-shaped proglomerulus, but represents a slightly later stage of development when Bowman's capsule and the capillary tuft are present in recognisable, though rudimentary, form. Nor does their latest stage (4) represent a fully mature glomerulus of adult type, since they describe it as showing "almost completely expanded capillary loops in the glomerular tuft with epithelium still present in small islands at the apices of the tufts". They found all their four stages to be present in embryonic life but after birth they noted a considerable increase in the number of the most mature glomeruli, the expansion of the capillary loops apparently proceding rapidly. It was not stated whether any glomeruli of fully adult type were found in the foetal material examined. These authors emphasise the point that there is no gradual flattening of the tuft epithelium, but, rather, fragmentation due to rapid expansion of the tuft which outgrows its covering. In this study, development as far as stage 4 had been reached by all juxta-medullary glomeruli within the second year of life, but great individual variation was found in the actual time taken. There was no evidence that birth had any effect on this process.

In 1943, Potter and Thierstein published a survey of the kidneys of 1000 foetuses and infants, designed to discover whether the histological appearance of the kidney could be taken as an indication of the general maturity of the child. The criterion they used was the presence or absence of the nephrogenic zone beneath the capsule and this was studied in relation to body weight, body length, gestation age at birth, and the survival time of premature infants. They concluded that the production of new nephrons ceases when the total potential number have been produced, regardless of whether the foetus is still in utero or birth has already taken place. They found that the presence of the nephrogenic zone is related to foetal size (body weight and body length) and that maturity as far as the production of new nephrons is concerned, is almost always attained during the time that the foetus or infant weighs between 2,100 gm and 2,500 gm. and measures from 46 to 48.9 cms. They do, however report nine cases out of their 1000 in which it seemed probable that kidney development had depended on the duration of intrauterine life rather than on body size.

In 1949, in a paper on the retardation of the post natal development of kidneys in persons with early cerebral lesions, Roosen-Runge describes the kidneys of a group of twenty normal control cases.

Of these twenty cases, eight are children aged 1 week to 2 years, and twelve are aged 12 to 45 years. The maturity of these kidneys was assessed by their relative weight (i.e. actual weight divided by expected weight for body size), by the state of differentiation of the cells covering the capillary tuft, the diameter of the glomeruli, the differences in diameter between the peripheral and central glomeruli, the size of the glomerulus-free zone beneath the capsule and the diameter of the renal tubules. In this group of normal controls, no cuboidal epithelium was found over the glomerular tuft beyond the age of 14 months and they state that this stage is normally reached by the age of two years.

Though a great amount of work has been published on renal growth and development, much of it has been done on animals other than man, Most of the earliest work is concerned with detailed morphology rather than with the general developmental pattern of the kidney, while the children's kidneys included in the later series are either too few to give statistically reliable results or belong to limited age groups. It has not, therefore, been possible to form from the literature so far published any clear impression of the changing histological pattern of the renal cortex throughout childhood. A description of this changing pattern is the object of the work to be presented in this thesis.

MATERIAL

The kidneys were obtained from post mortem examinations carried out by Dr. J. L. Emery and his staff at the Children's Hospital, Sheffield, and by courtesy of Dr. A. J. N. Warrack, at the City General and Nether Edge Hospitals, Sheffield.

Records and material from 1,000 cases were available, and from these 235 were selected for the survey. Their ages ranged from 26 weeks gestation to 13½ years.

The kidneys had been fixed in 10% formol saline and the sections cut from paraffin blocks. These routine sections were stained with Masson's trichrome, but in the survey, fresh sections stained with haematoxylin and eosin were used.

SELECTION OF CASES.

Selection was made initially by using the post mortem record numbers of the cases in conjunction with tables of random numbers but after about 100 cases had been selected in this way it was found that the natural incidence of mortality had weighted the series very heavily in favour of infants under 6 months of age. In order to obtain a balanced series it was necessary to select cases aged six months or more, and this was done by taking them in chronological order of death from the laboratory register. This method gave sufficient randomness in the sample, and provided roughly even numbers of cases above and below six months of age.

Only normal kidneys were used, the routine sections from each case being examined, and any showing recognised pathological change, or related to any congenital abnormality of the renal tract were discarded.

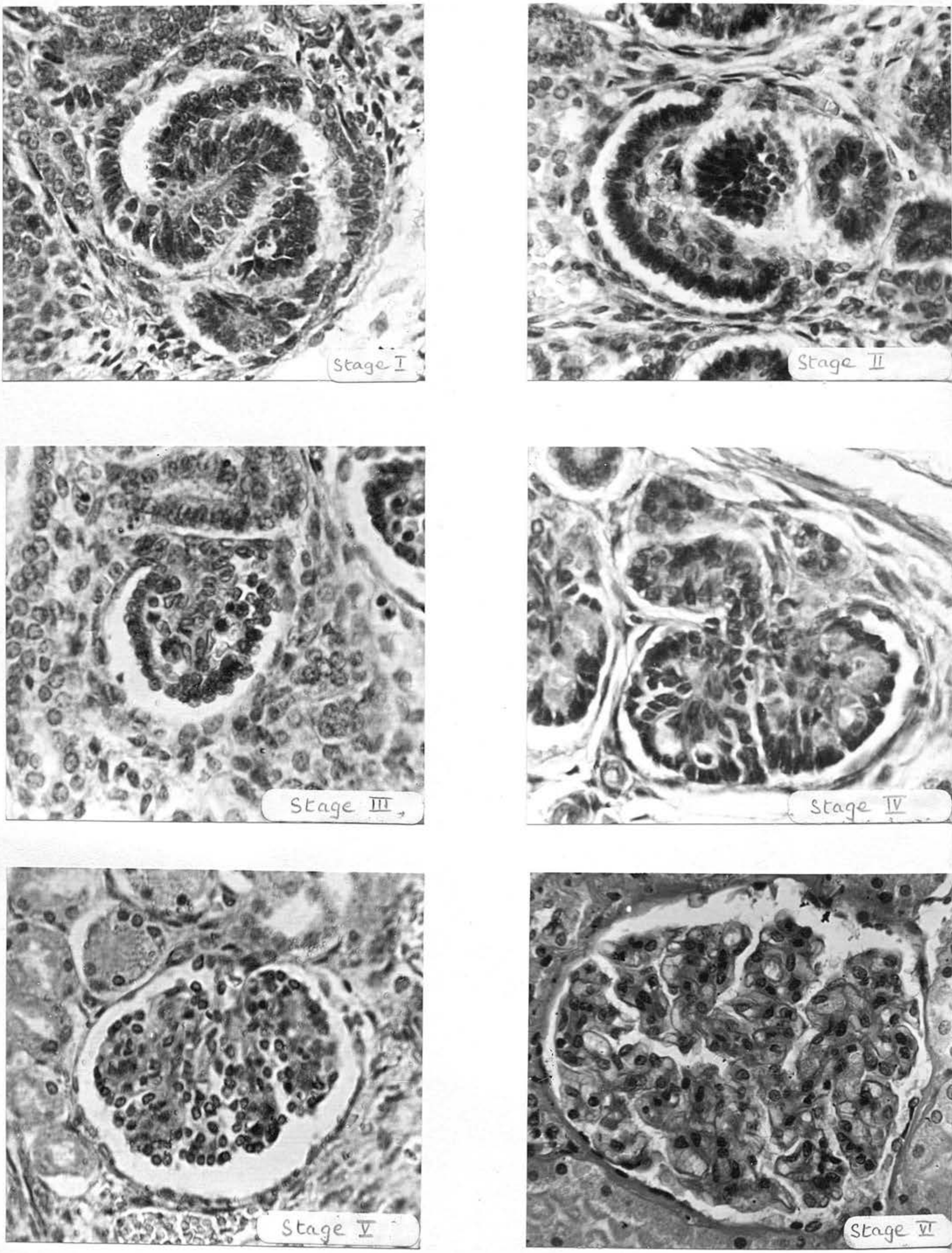
PART I. THE HISTOLOGICAL DEVELOPMENT OF RENAL GLOMERULI

METHOD

Glomeruli were classified in six stages of maturity and counts were made of the number at each stage throughout the depth of the cortex. The sections used showed the columnar arrangement of the glomeruli clearly and at least ten columns from each case were counted and classified. Most routine sections were unsuitable for this purpose, and special kidney blocks had to be cut in such a plane that the sections from them included a complete papilla in longitudinal section with the corresponding cortex showing unbroken medullary rays running out to the periphery. One column was regarded as the area lying between two adjacent complete medullary rays and extending from the medulla to the capsule. The taking of these special blocks was extremely time consuming, therefore a series of "slide" counts was made on routine sections that were seen to include the full depth of the cortex. In making these slide counts the cortex was traversed radially from capsule to medulla and then, the slide having been moved along, from medulla to capsule. This process of examining adjacent strips of the full depth of the cortex was continued until at least 100 glomeruli had been

FIGURE I

EXAMPLES OF THE SIX STAGES OF GLOMERULAR DEVELOPMENT



counted and classified.

The criteria for classification were as follows:

Stage 1: The "S" shaped proglomerulus (in fact a pronephron).

Stage II: The glomerular tuft is recognisable but is mushroom shaped and possesses no true vascular pole. Bowman's capsule is crescentic in cross section and is lined by columnar epithelium.

Stage III: The glomerular tuft now has a vascular pole but it is not yet lobulated, and it is covered by a continuous layer of epithelium.

Stage IV: The glomerular tuft is now lobulated. Its epithelial covering is still continuous, and dips down into the clefts between the lobules.

Stage V: The epithelial covering of the capillary tuft is fragmented, being represented by groups of cells in broken pallisade.

Stage VI: The epithelial covering of the capillary tuft has practically disappeared and is represented by only a few cells scattered singly over its surface.

Photographs of typical examples of these six stages of development are shown in Figure I.

In addition to these developmental stages, two more categories were needed; one for abnormal, involuting or scarred forms, and one for unclassifiable glomeruli, i.e. those in which there was present no more than an empty Bowman's capsule or a small group of cells from the surface of a capillary tuft.

REPRODUCIBILITY TEST

PURPOSE

Though the criteria of classification of the glomeruli were clearly defined and simple, it soon became obvious that there were borderline cases in which doubt was felt about the correct placing in one of two adjacent categories. The difficulty was most obvious when the decision lay between stage V and stage VI, and it was important to know what effect this had on the reproducibility of the results. The application of criteria involved a subjective judgement in which independent observers might differ from each other and in which a single observer might not show consistency. Also, two methods of counting had been tried and it was necessary to know whether or not they gave comparable results.

It was to investigate the extent to which these factors might affect results that the reproducibility test was designed by Dr. Jowett of the Department of Statistics in the University of Sheffield.

METHOD

Choice of Cases

The cases selected for the reproducibility test all contained high proportions of stage V and stage VI glomeruli because the greatest conscious difficulty in classification had been found in the decision between stage V and stage VI. From a scatter diagram in which the percentage of stage VI glomeruli was plotted against age, four age groups between 6 months and 6 years were selected. From each of these a case showing a high, a high medium, a low medium, and a low stage six count was taken and these sixteen cases were the ones used in the reproducibility test.

METHOD OF COUNTING

From each case three kidney sections were chosen and named.

Section A was a routine one, not cut from a special survey block, in which the cortical renal columns were not complete. This section was used for slide counts as already described.

Section B was one cut from a special block, showing complete renal columns. This section was used for column counts and also for slide counts. The column counts were carried out each time on the same two marked renal columns.

Section C was similar to section B but was used only for column counts of two marked renal columns.

Thus four types of count were done on each of the sixteen cases.

The slide count on Section A was named count S.

The slide count on section B was named count S^1

The column count on section B was named count C

The column count on section C was named count C^1

The sixteen cases were named alphabetically from A-P and were taken for counting each day in alphabetical order. One of the four types of count was carried out on each case, each day, and the method of counting to be used was determined by a latin square drawn in such a way that each type of count was preceded and followed by each of the other types in turn. One cycle of counts was completed in four days, and it was then repeated so that the whole reproducibility test was completed in eight consecutive days.

Each day's sixteen counts were done at a single session and the results were recorded in such a way that the previous day's count was not seen.

The test was carried out by two independent observers, and neither set of results was seen until both were complete.

RESULTS

The results were analysed to show each observer's individual reproducibility and the comparability of slide and column counting and of the results of the two observers. They were also examined for any effect of training or fatigue.

It was found that the series of cases used provided percentages of glomerular stages other than stage V too small for statistical analysis, therefore only the stage V results are presented here. This is the most critical group because the decision between stage V and stage VI was the most difficult of all to make. It therefore seems fair to assume that the reproducibility of the counts of the other stages is at least as good as that of the stage V counts.

The Reproducibility of Individual Observer's Results

Each observer's slide counts and column counts were considered separately. For each of the sixteen cases there were four slide and four column count results. Each of these sets of four results was averaged and its standard deviation and coefficient of variation calculated. This gave four sets of 16 coefficients of variation, one for slide counting and one for column counting, from each observer. In order to obtain a general impression of each observer's reproducibility in slide counting and in column counting the arithmetic mean and standard deviation of each of these sets

TABLE I

REPRODUCIBILITY OF COUNTS OF

STAGE V GLOMERULI.

OBSERVER	Coefficients of Variation (15 cases)			
	COLUMN COUNTS		SLIDE COUNTS	
	Mean	S.D.	Mean	S.D.
1	11.6	7.6	13.7	21.2
2	28.7	21.3	43.0	25.5

of coefficients of variation were calculated, and are presented in Table I.

Observer I, in column counting shows a mean coefficient of variation of 11.6 with standard deviation 7.6 and in slide counting a mean coefficient of variation of 13.7 with standard deviation 21.2. The very high standard deviation in the slide counts is due to three cases with coefficients of variation of 29.6, 80.3, and 43.8; the other coefficients of variation all fall within the range 2.0 - 9.1.

Observer 2, in column counting shows a mean coefficient of variation of 28.7, with standard deviation 21.3 and in slide counting a mean coefficient of variation of 43.0 with standard deviation 25.5.

T A B L E   II.

COMPARISON OF RESULTS FROM THE TWO METHODS OF COUNTING.

OBSERVER	% DIFFERENCE	
	Mean	S.D.
1	7.2	7.6
2	12.9	8.9

The Comparability of Results from two methods of Counting.

The two observers' results were considered separately. In each of the sixteen cases, the mean column count result was compared with the mean slide count result and the difference found by subtraction. Half this difference was then expressed as a percentage of the average of these two means. This gave sixteen percentage differences for each observer. The mean and standard deviation of these differences were calculated and are presented in table II.

Observer I shows a mean percentage difference of 7.2 with standard deviation 7.6.

Observer II shows a mean percentage difference of 12.9 with standard deviation 8.9.

T A B L E III

COMPARISON OF RESULTS FROM

THE TWO INDEPENDENT OBSERVERS.

METHOD OF COUNTING	% DIFFERENCE	
	Mean	S.D.
COLUMN	24.3	11.7
SLIDE	32.7	13.5

The Comparability of Results from two independent Observers.

The slide counts and column counts were taken separately. The mean percentage difference between the two observers' results were calculated as described above and they are presented in Table III.

In column counting the two observers show a mean percentage difference of 24.3 with standard deviation 11.7.

In slide counting they show a mean percentage difference of 32.7 with standard deviation 13.5.

Table IV

EFFECT OF ORDER OF COUNTING ON

COEFFICIENTS OF VARIATION.

CASE GROUPS	OBSERVER 1		OBSERVER 2	
	Average coefficients of variation		Average coefficients of variation	
	Slide Count	Column Count	Slide Count	Column Count
1 A-D	4.2	8.6	24.0	16.2
2 E-H	10.4	8.3	36.0	22.9
3 I-L	25.0	23.0	57.3	36.5
4 M-P	16.0	12.8	58.4	41.2

The Effect of the Order of Counting.

The cases were divided into four groups according to the order of counting and the average coefficients of variation in each of these groups was found. The results are presented in Table IV.

Observer 1 shows a sharp increase in variation between the first and second halves of the day's series of counts, but there is no steady rise.

Observer 2, on the other hand, does show a steady increase in variation in both types of counting as the series proceeds.

DISCUSSION

The best reproducibility is shown by Observer 1 when carrying out column counts. Though the standard deviation of these results indicates a wide scatter, it is the lowest in the series. This observer's slide count results, though showing a mean coefficient of variation not much higher than that of the column counts, have a very high standard deviation, for the reason already given. They must therefore be regarded as less reliable than the column counts.

Observer 2 shows a very great variation in the results of both types of counting but that of the slide counts is almost twice as great as that of the column counts.

Comparison of the Two Methods of Counting.

Observer 1 shows slightly more consistency than Observer 2. Between the two methods of counting, Observer 1 shows a slightly better reproducibility than between individual counts. This is probably because the comparison was between averages of four instead of between individual counts.

There was no constant direction in which the results differed. The higher result was as often in column as in slide counting.

These comparisons seem to indicate that provided a sufficient number of counts is made, it does not greatly matter which method is used.

Comparison of the Results of Independent Observers.

There is no comparability between the results of the two observers. The reason for this may lie in the effect of training. Observer 1, who had spent some months in examining sections of kidneys and had worked out the method of classification used in the survey, showed very much better reproducibility than Observer 2, who used the classification for the first time in carrying out the reproducibility test. On the other hand, Observer 2 had very much more general histological experience than Observer 1, and it seems likely that while Observer 1 was able to apply the criteria of classification with more consistency, Observer 2's general experience of histological material caused various factors not included in the criteria, such as tissue shrinkage, to be taken into account.

The Effect of the Order of Counting.

The reproducibility test was designed to eliminate any effect of the order of carrying out the different types of count, but the plan necessitated the completion of each day's series of counts at one session. Both observers reported considerable fatigue towards the end of the session and it seemed important to look for any effect this may have had on the results. It seemed likely that fatigue would result in greater variability of results in the cases counted towards the end of the day's series. The sections used varied considerably in quality, but the poorer ones were fairly evenly distributed.

The greater variation in results of both Observers in the later part of the series seems to indicate that fatigue does reduce consistency of judgement seriously.

The Application of the Reproducibility Test to the Main Survey.

The main survey was carried out by Observer 1 only. The criteria for classification of glomeruli were the same as in the reproducibility test, but in some important ways the two studies are not comparable.

In the main survey the sections used were on the whole, of considerably better quality than those available for the reproducibility test, because of a better technique of taking blocks, and more uniform staining. Column counting was carried out on at least ten columns instead of on two. The element of fatigue is not so serious because there was no fixed series of counts to be completed at a single session. In the main survey, about six to eight counts were completed each day.

No strict comparison is therefore possible between the results of the main survey and those of the reproducibility test, but the ways in which the two differ are to the advantage of the main survey. It seems fair to assume that the results of the main survey are at least as consistent as those of the reproducibility test.

CONCLUSIONS.

The results of the reproducibility test cast doubt on the reliability of quantitative conclusions drawn from histological surveys in which a continuous spectrum of change has been classified in discrete categories. It has, however, indicated certain factors which influence the results and has suggested that the following conditions should be observed.

1) Criteria should be as simple and as clearly defined as possible and classification should be into groups as large and as few as the enquiry will permit.

2) An observer should undergo a period of training in the application of the criteria until sufficient consistency of judgement is achieved.

3) Standard examples of the criteria should be kept before the observer for continual reference in order to avoid any unconscious alteration of criteria as the work proceeds.

4) Histological preparations used should be of a uniformly high standard.

5) A session at work should not be continued to the point of fatigue.

6) The largest number of observations possible should be made.

In the reproducibility test just described, not all these safeguards had been applied, but it appears that Observer 1 showed a variation in results of the order of 10 - 15%.

THE METHOD OF CALCULATION OF RESULTS IN THE MAIN SURVEY

Each case in the survey had a separate working sheet on which the results of the counts were recorded, and the number of glomeruli in the eight categories was expressed as a percentage of the total count. At the time of counting, only the post mortem number of the case was known, but after the count had been completed the age, post mortem body weight, crown-rump length and total kidney weight, the cause of death and duration of illness were obtained from the hospital post mortem records and recorded on the working sheet. In children of less than two months, the age from conception, i.e. gestation time plus post natal age at death was used. The weight and crown-rump length of the older infants and children was not always known.

The results were analysed according to the age, the combined weight of the two kidneys, the post mortem body weight and crown-rump length of the child. The whole of this information was not available in every case but those in which it was incomplete were used as far as they could be. The result of this is that the four analysis groups do not comprise exactly the same cases but the disadvantages of this were thought to be outweighed by the advantages of analysing as large a series of results as possible and this view was strengthened when the degree of individual variation became evident.

Within each analysis group the cases were subdivided by convenient divisions of the appropriate growth factor and for each subgroup the average percentages of the eight glomerular categories were calculated. In the analysis by age, twenty-two subgroups were made originally but the number of cases in some of them was too small and, also, it was seen that over several groups there was no appreciable change in the glomerular pattern. A number of the subgroups were therefore combined to form a total of twelve larger subgroups.

RESULTS

The Analysis by Age

There are two hundred cases in this group and the results are presented in Table V and graphically in Fig. II.

Stage I glomeruli account for 9% of the total in the premature infant of 24-27 weeks gestation but the number shows a rapid fall as "full term" is approached and from the 36th week of pregnancy less than 1% are at this stage. Stage II, however, though showing an early fall in percentage similar to that of stage I appears to remain, in very small numbers, during the first two years of life in some cases. Immature glomeruli represented by stages III, IV and V represent over 80% of the total during the first year of life, but in the second year there is a steep fall in their numbers to about half the total and this figure is maintained until the 6th year. Between 6 years and 12 years there is another steady fall until in the age group 12 to 15 years no immature glomeruli are seen.

Stage six, the fully adult form is present in small numbers in the kidneys of foetuses from the 24th week, but shows only a very slight increase in numbers until the second year of life when it comes to represent between 40 and 50% of the total. This proportion is maintained until the 6th year when there is again a steady increase in numbers of Stage VI until at 12 years all the glomeruli are of adult form.

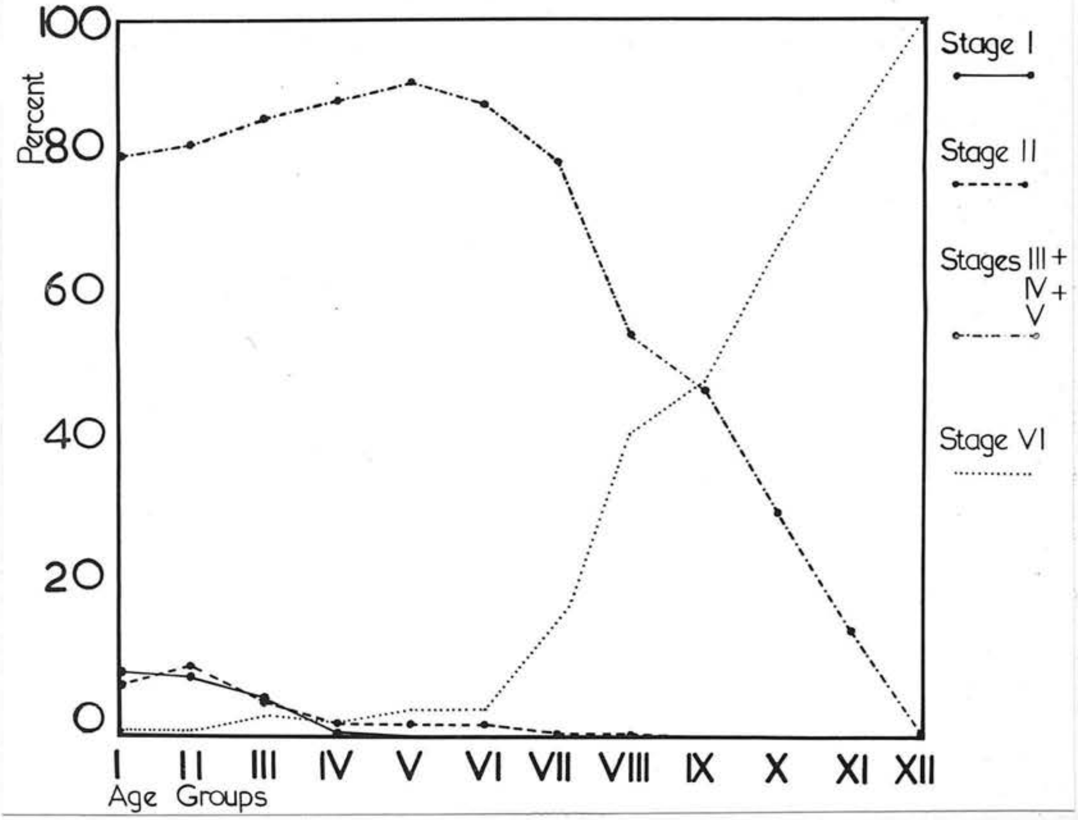
T A B L E V

THE AVERAGE PERCENTAGE OF GLOMERULI AT SIX STAGES OF MATURITY IN TWELVE AGE GROUPS.

AGE		24 ū 28 wk.	28 ū 32 wk	32 ū 36 wk	36 ū 40 wk	40 ū 44 wk	44 wks ū 6 mth	6 ū 12 mths.	1 ū 2 yrs.	2 ū 6 yrs.	6 ū 9 yrs.	9 ū 12 yrs.	12 ū 15 yrs.
% GLOMERULAR STAGES.	I	9.0	8.7	4.8	0.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	II	7.8	10.0	4.0	2.2	2.2	1.9	0.6	0.0	0.0	0.0	0.0	0.0
	III IV V	81.0	82.6	85.8	88.5	90.9	88.3	80.0	56.1	48.9	31.5	14.9	0.0
	VI	0.8	0.8	3.0	2.2	4.3	3.9	15.7	42.1	49.7	68.4	84.9	100.0
Glomeruli Unclassified	%	1.3	3.4	2.5	6.4	3.3	6.0	3.4	1.8	2.2	0.0	0.3	0.0
Number of cases.		4	18	12	21	29	23	28	22	25	8	7	3
AGE GROUP		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII

FIGURE II

CHART SHOWING THE PERCENTAGE OF GLOMERULI AT THE SIX STAGES OF MATURITY AGAINST AGE IN TWELVE AGE GROUPS

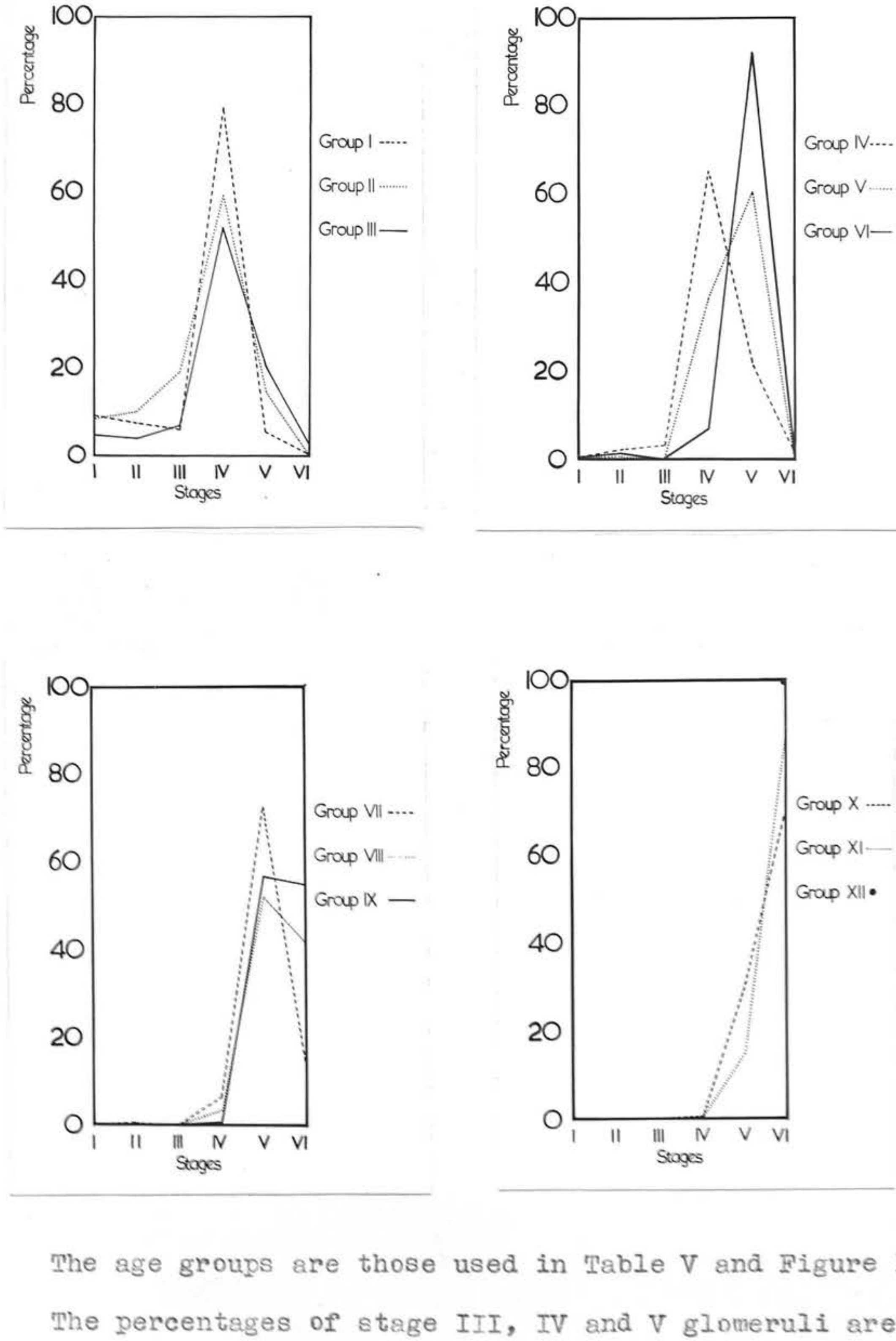


The age groups are those used in Table V.

The percentages of stages III, IV and V are combined in a single curve.

FIGURE III

PROFILE GRAPHS SHOWING THE PERCENTAGES OF GLOMERULI AT THE SIX STAGES OF MATURITY PRESENT IN TWELVE AGE GROUPS



The age groups are those used in Table V and Figure II. The percentages of stage III, IV and V glomeruli are shown separately.

The profile graphs (Fig. III a-d) give an impression of the general pattern of glomerular maturity in the twelve age groups and are simply a different method of plotting the figures given in table V. Here, the three stages of fully formed, but immature, glomeruli are shown separately. It will be seen from these profile graphs that while stage I is present in appreciable numbers the great majority of glomeruli are at stage IV. By the time stage I has disappeared, the majority are at stage V. From 6 months to two years, stage V and stage VI are present in almost equal proportions, but with stage V predominating, but after 6 years there is a rapid maturation, stage VI becoming more and more numerous until at 12 years all are of this type.

Table VI and Fig. IV show the case incidence of the glomerular stages in the twelve age groups. In only one case out of 32 was Stage I present after the 40th week from conception and in only one case out of 29 was Stage II present after the end of the first year. From six months onwards there are apparently some cases that show no immature glomeruli, but from 9 years onwards they are present in a minority of cases, while the three cases of 12 - 15 years showed nothing but fully mature glomeruli.

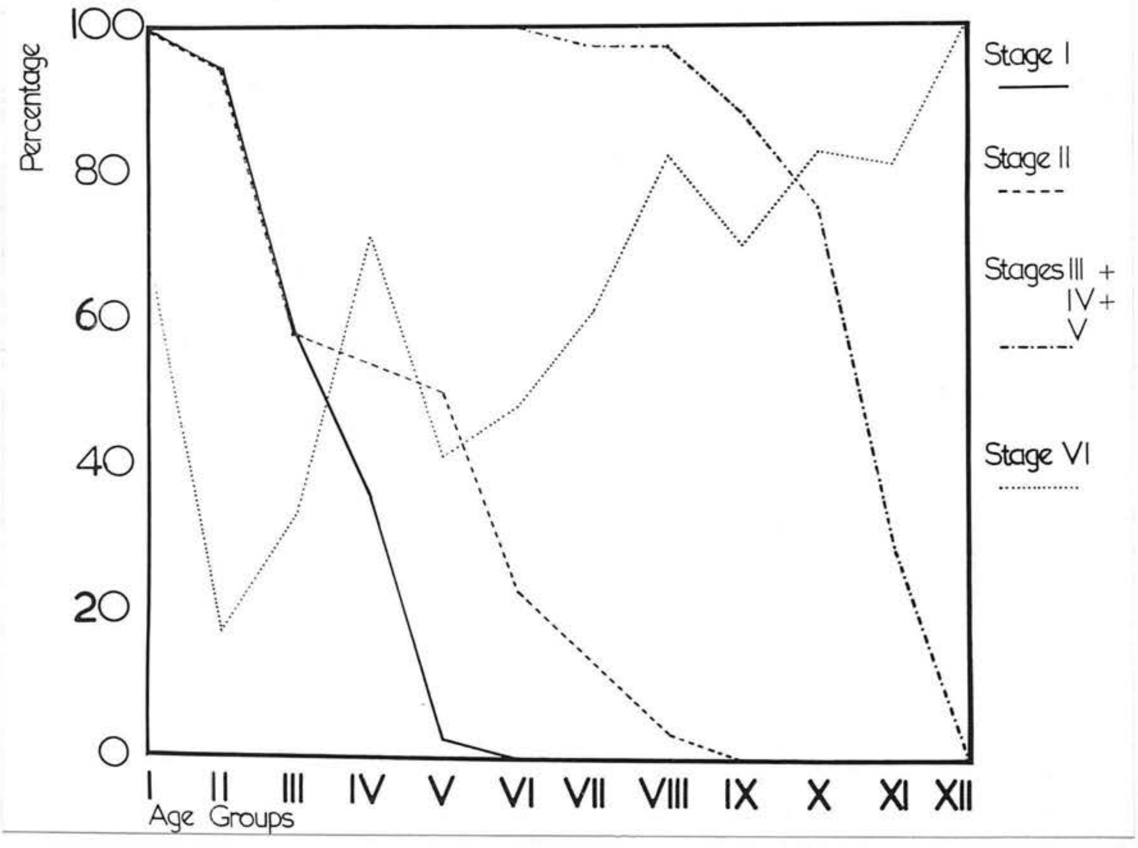
T A B L E VI

THE CASE INCIDENCE OF GLOMERULAR DEVELOPMENTAL STAGES IN TWELVE AGE GROUPS.

CASE GROUPS			CASES							
AGE	GROUP No.	TOTAL CASES	STAGE I GLOMERULI PRESENT		STAGE II GLOMERULI PRESENT		STAGES III, IV & V. GLOMERULI PRESENT		STAGE VI GLOMERULI PRESENT	
			NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Under 28 wks.	I	3	3	100	3	100	3	100	2	66
28 ū 32 wks.	II	17	16	94	16	94	17	100	3	17
32 ū 36 wks.	III	12	7	58	7	58	12	100	4	33
36 ū 40 wks.	IV	14	5	36	7	50	14	100	10	71
40 ū 44 wks.	V	32	1	3	9	28	32	100	13	41
44 wks ū 6 mth.	VI	21	0	0	7	33	21	100	10	48
6 ū 12 months	VII	31	0	0	3	9.6	30	97	19	61
1 yr ū 2 yr.	VIII	29	0	0	1	3.4	23	79	24	82
2 yr ū 6 yr.	IX	17	0	0	0	0	15	88	12	70
6 yr ū 9 yr.	X	8	0	0	0	0	6	75	7	87.5
9 yr ū 12 yr.	XI	7	0	0	0	0	2	29	6	86
12 yrs & over	XII	3	0	0	0	0	0	0	3	100

FIGURE IV

CHART SHOWING THE CASE INCIDENCE OF GLOMERULI AT THE SIX STAGES OF MATURITY IN TWELVE AGE GROUPS



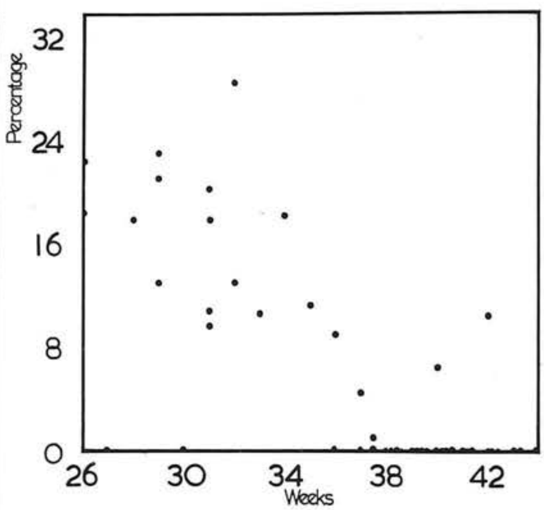
The age groups are those used in Tables V and VI and in Figures II and III.

The percentages of cases showing stages III, IV and V are combined in a single curve.

FIGURE V

SCATTER DIAGRAMS SHOWING THE COMBINED PERCENTAGE OF STAGE I AND STAGE II GLOMERULI AGAINST THE AGE FROM CONCEPTION

a) Liveborn Infants



b) Stillborn Infants

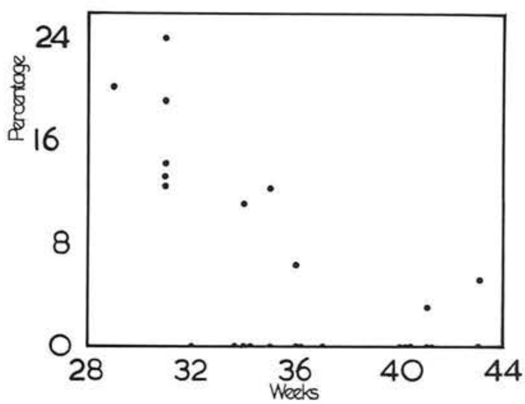
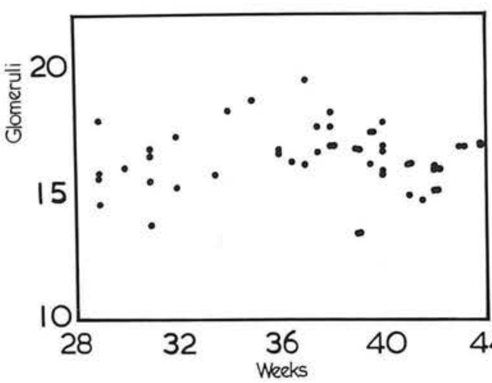


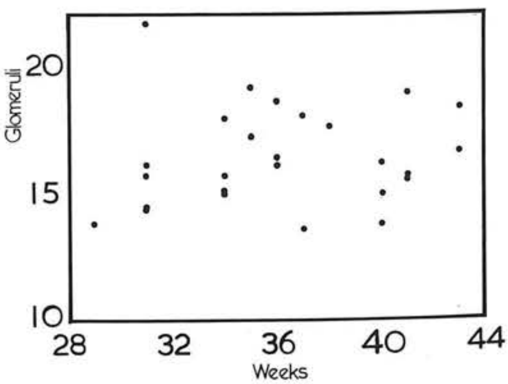
FIGURE VI

SCATTER DIAGRAM SHOWING THE AVERAGE NUMBER OF GLOMERULI PER COLUMN AGAINST THE AGE FROM CONCEPTION

a) Liveborn Infants



b) Stillborn Infants



In order to obtain some impression of the effect of birth on the production of new glomeruli the percentages of Stage I and stage II were plotted against age from conception in liveborn and stillborn infants on two separate scatter diagrams. These were then compared to see if any difference in the pattern of the graphs was obvious. They are shown in fig. V a& b. It will be seen that the patterns are strikingly similar. On two more scatter diagrams the average total number of glomeruli per renal column in the sections was plotted against age from conception in live-born and stillborn infants. Again the two diagrams (Fig. VI) show strikingly similar patterns. They both appear to reach their maximum about the 36th week of gestation and the actual numbers of glomeruli in the columns are similar.

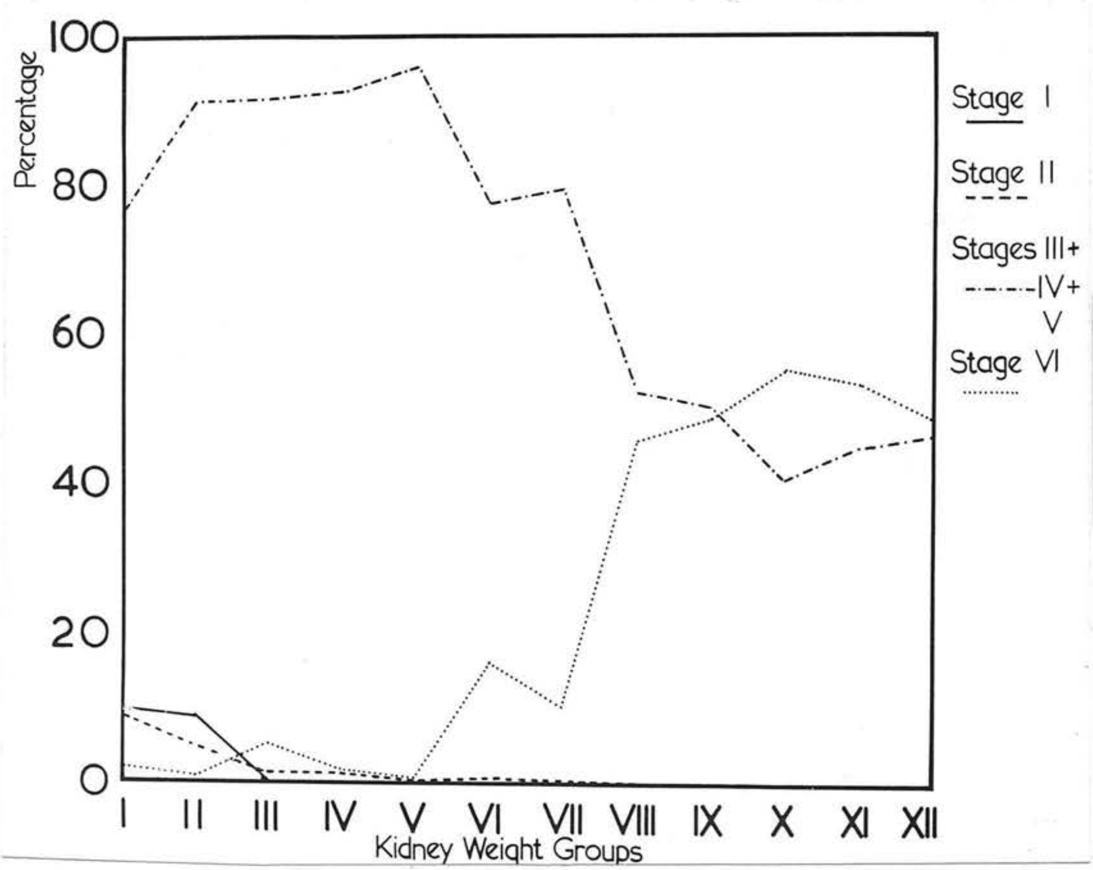
T A B L E VII

THE AVERAGE PERCENTAGE OF GLOMERULI AT SIX STAGES OF MATURITY IN TWELVE KIDNEY WEIGHT GROUPS.

KIDNEY Wt. (G)		Under 10	10 ū 20	20 ū 30	30 ū 40	40 ū 50	50 ū 60	60 ū 70	70 ū 80	80 ū 90	90 ū 100	100 ū 150	150 ū 200
% GLOMERULAR STAGES	I	9.9	4.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	II	9.3	4.6	1.6	1.5	0.0	1.1	0.73	0.0	0.0	0.0	0.4	0.0
	III	15.6	8.8	2.0	0.6	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IV	54.5	61.2	60.8	59.1	27.6	9.9	3.0	0.7	0.6	1.5	0.9	0.0
	V	10.6	18.1	23.5	27.0	67.9	67.1	80.5	54.5	43.1	50.7	56.9	51.0
	III IV V	77.2	86.0	86.4	92.4	95.4	77.9	84.9	52.2	50.3	40.8	44.2	51.0
	VI	2.0	1.0	5.4	1.8	0.5	16.4	10.4	45.4	49.3	55.4	53.8	49.0
Unclassified		1.3	4.2	6.1	4.3	4.1	5.4	4.1	2.0	0.4	3.7	1.5	0.0
KIDNEY Wt. GROUP		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
NUMBER OF CASES		10	24	50	33	11	14	10	5	6	9	20	4

FIGURE VII

CHART SHOWING THE PERCENTAGE OF GLOMERULI AT THE SIX STAGES OF MATURITY AGAINST KIDNEY WEIGHT IN TWELVE KIDNEY WEIGHT GROUPS

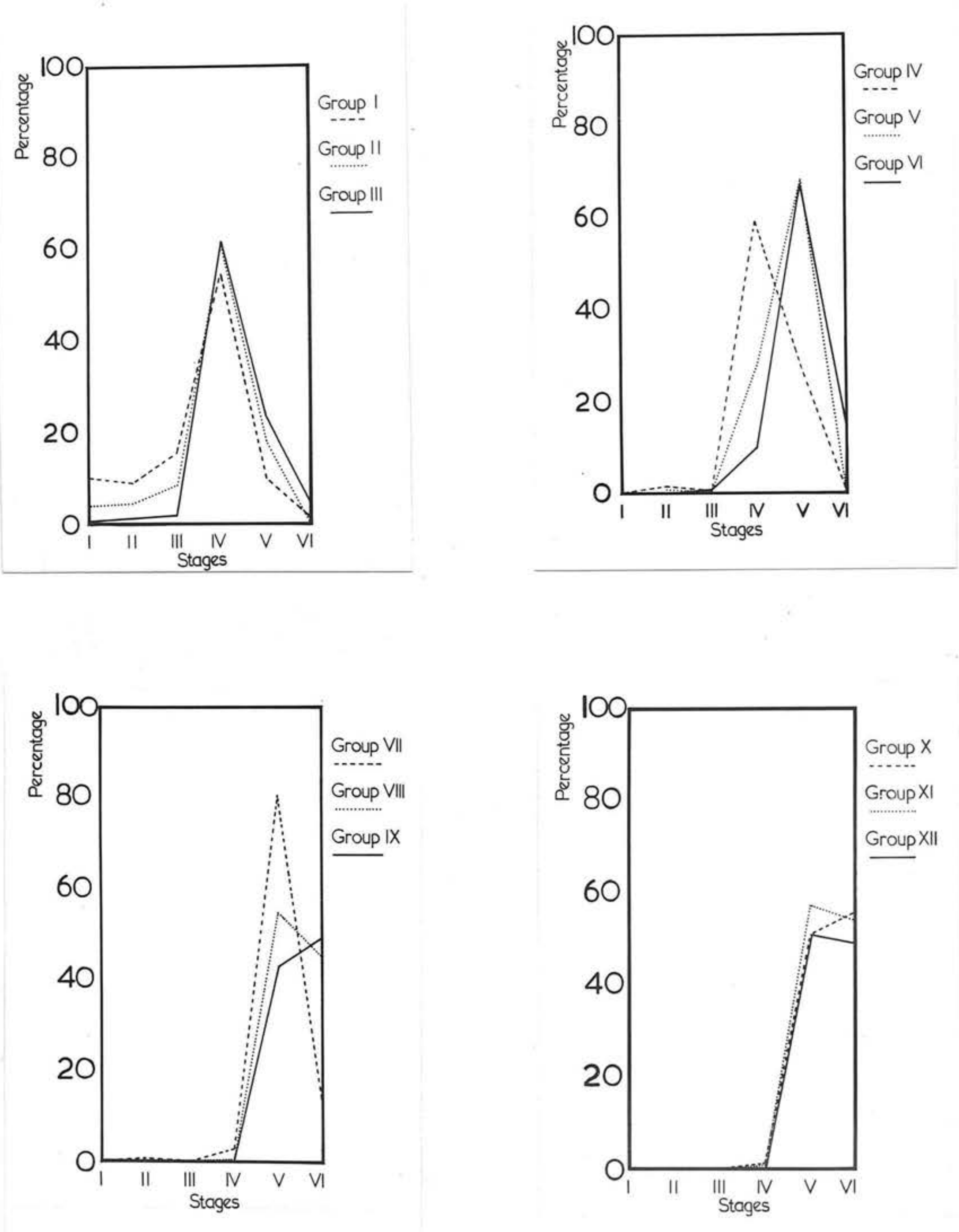


The kidney weight groups are those used in Table VII.

The percentages of maturity stages III, IV and V have been combined in a single curve.

FIGURE VIII

PROFILE GRAPHS SHOWING THE PERCENTAGES OF GLOMERULI AT THE SIX STAGES OF MATURITY PRESENT IN TWELVE KIDNEY WEIGHT GROUPS



The kidney weight groups are those used in Table VII and in Figure VII.

The percentages of maturity stages III, IV and V are shown separately.

In kidneys with a combined weight of less than 10 Gm stages I and II account for about 10% of the total glomeruli. There is then a rapid decrease in the percentage of these two stages and stage I has disappeared from kidneys whose combined weight is 30 Gm or more. Stage II continues to be present in very small numbers until a combined kidney weight of 50 Gm is reached. In the kidneys weighing less than 50 Gm, between 80 and 100% of the glomeruli are at stages III, IV and V, but from 50 Gm there is a marked fall in the percentage of these stages until at 90 to 100 Gm they represent only about 50% of the total. This fall in stages III, IV and V is mirrored by a rise in the percentage of stage VI. In the groups with a kidney weight of 100 - 200 Gm there are roughly equal numbers of fully mature and immature glomeruli.

The profile graphs (Fig. VIII a-d) show that after the disappearance of stage III glomeruli, the majority are at stage V and that it is not until after the disappearance of stage IV that 50% or more of the glomeruli are fully mature.

The case incidence of the different glomerular stages is shown against kidney weight in Table VIII and Fig. IX. Only 80% of the smallest kidneys in the series showed stages I and II. There were no stage I glomeruli in cases with combined kidney weights of more than 40 Gm. All the cases with a combined kidney weight of less than 50 Gm show stages III, IV and V. At least 75% of cases with a combined kidney weight of 50 Gm and more show these stages. Between a third and half of the cases with kidneys weighing less than 70 Gm show stage VI and about three quarters of the cases with kidney weight greater than 70 Gm show fully mature glomeruli.

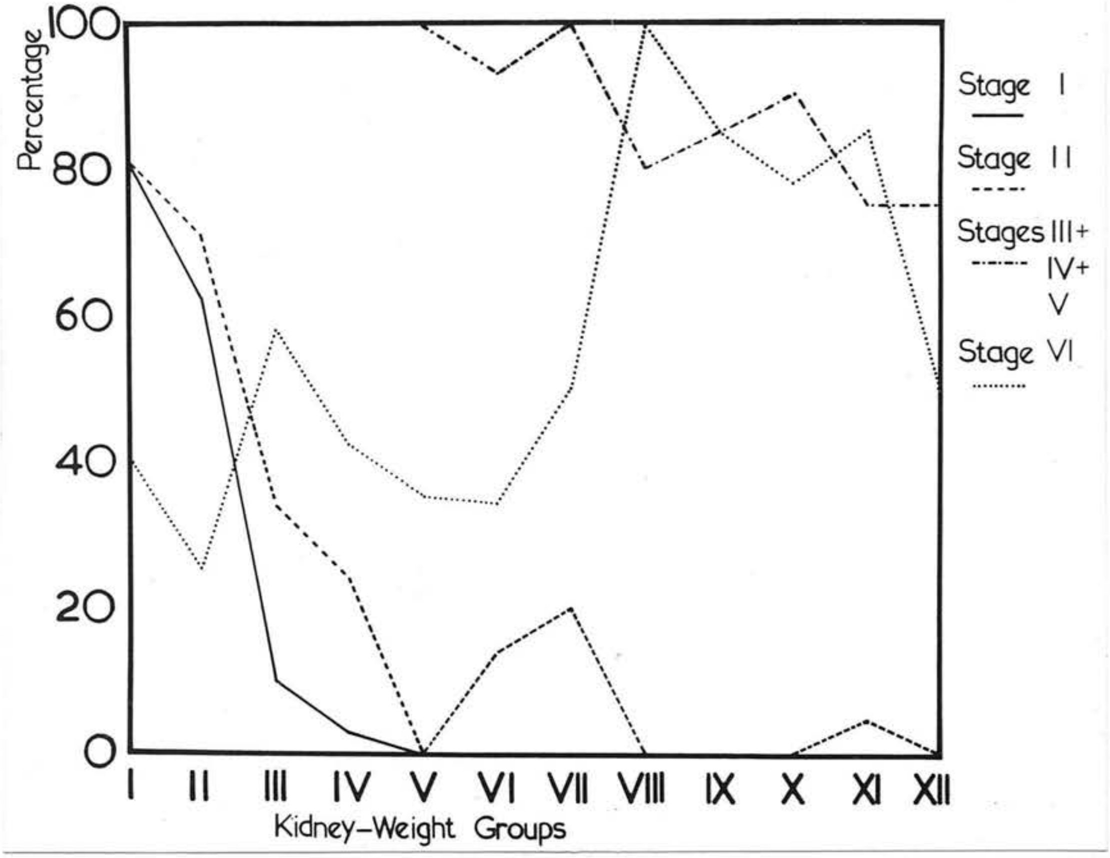
T A B L E VIII

THE CASE INCIDENCE OF GLOMERULAR DEVELOPMENTAL STAGES IN TWELVE KIDNEY WEIGHT GROUPS

CASE GROUPS			CASES							
KIDNEY WEIGHT(G)	GROUP No.	TOTAL CASES	Showing STAGE I Glomeruli		Showing STAGE II Glomeruli		Showing STAGES III IV & V Glomeruli		Showing STAGE VI Glomeruli	
			NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Under 10	I	10	8	80	8	80	10	100	4	40
10 ū 20	II	24	15	62.5	17	70.9	24	100	6	25
20 ū 30	III	50	5	10	17	34	50	100	29	58
30 ū 40	IV	33	1	3	8	24	33	100	14	42.4
40 ū 50	V	11	0	0	0	0	11	100	4	36.4
50 ū 60	VI	14	0	0	2	14.3	13	92.9	5	35.7
60 ū 70	VII	10	0	0	2	20	10	100	5	50
70 ū 80	VIII	5	0	0	0	0	4	80	5	100
80 ū 90	IX	6	0	0	0	0	5	83.3	5	83.3
90 ū 100	X	9	0	0	0	0	8	88.9	7	77.8
100 ū 150	XI	20	0	0	1	5	15	75	17	85
150 ū 200	XII	4	0	0	0	0	3	75	2	50

FIGURE IX

CHART SHOWING THE CASE INCIDENCE OF GLOMERULI AT THE SIX STAGES OF MATURITY IN TWELVE KIDNEY WEIGHT GROUPS



The kidney weight groups are those used in tables VII and VIII.

The percentages of cases showing maturity stages III, IV and V have been combined in a single curve.

The Analysis by Body Weight

There are 51 cases in this analysis and the results are presented in Table IX and in Fig. X.

In infants weighing between 500 and 1000 Gm. stages I and II account for 8 - 10% of the glomeruli, but by the time the body weight has reached 2000 - 2,500 Gm they account for less than 1%. Throughout the period of this graph, the very great majority are at stage IV. In infants with a body weight of less than 3,500 Gm. the percentage of stage VI varies from 0 - 1.5, but in infants weighing 3,500 - 4,000 Gm there has been a sharp increase in stage VI to 8.5%.

T A B L E IX

THE AVERAGE PERCENTAGE OF GLOMERULI AT SIX STAGES OF MATURITY IN SEVEN BODY WEIGHT GROUPS.

BODY WEIGHT AT POST-MORTEM (G)		500 ū 1000	1000 ū 1500	1500 ū 2000	2000 ū 2500	2500 ū 3000	3000 ū 3500	3500 ū 4000
glomerular stages %	I	10.6	5.5	3.6	0.7	0.1	0.4	0
	II	7.9	9.4	4.3	0.6	2.0	0.5	1.8
	III	5.6	8.4	12.9	1.6	1.5	1.4	-
	IV	54.8	55.4	55.6	65.5	74.1	65.6	-
	V	17.6	25.4	21.3	28.2	22.5	30.8	-
	III IV V	75.4	80.9	89.9	93.9	88.9	97.5	84.9
	VI	1.5	0.7	0.0	1.2	1.4	0.3	8.5
	U.	4.6	3.5	2.3	3.6	6.7	1.3	4.8
Number of Cases in Group.		3	7	9	9	9	11	3
GROUP NUMBER		I	II	III	IV	V	VI	VII

FIGURE X

CHART SHOWING THE PERCENTAGE OF GLOMERULI AT THE SIX STAGES OF MATURITY AGAINST BODY WEIGHT

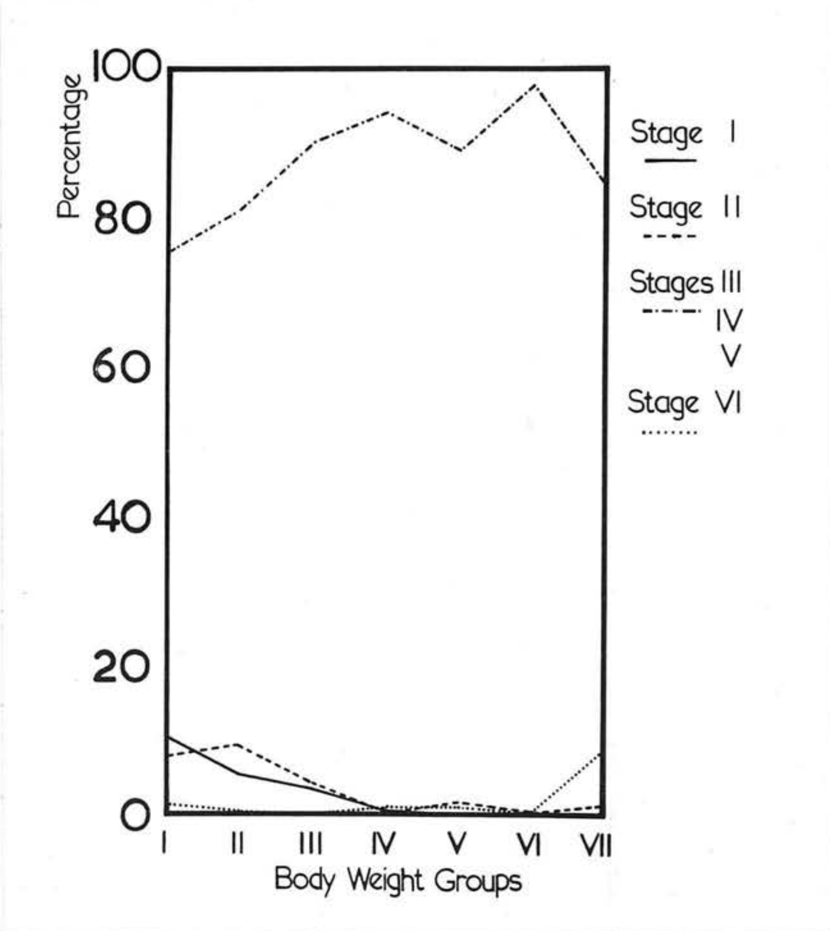
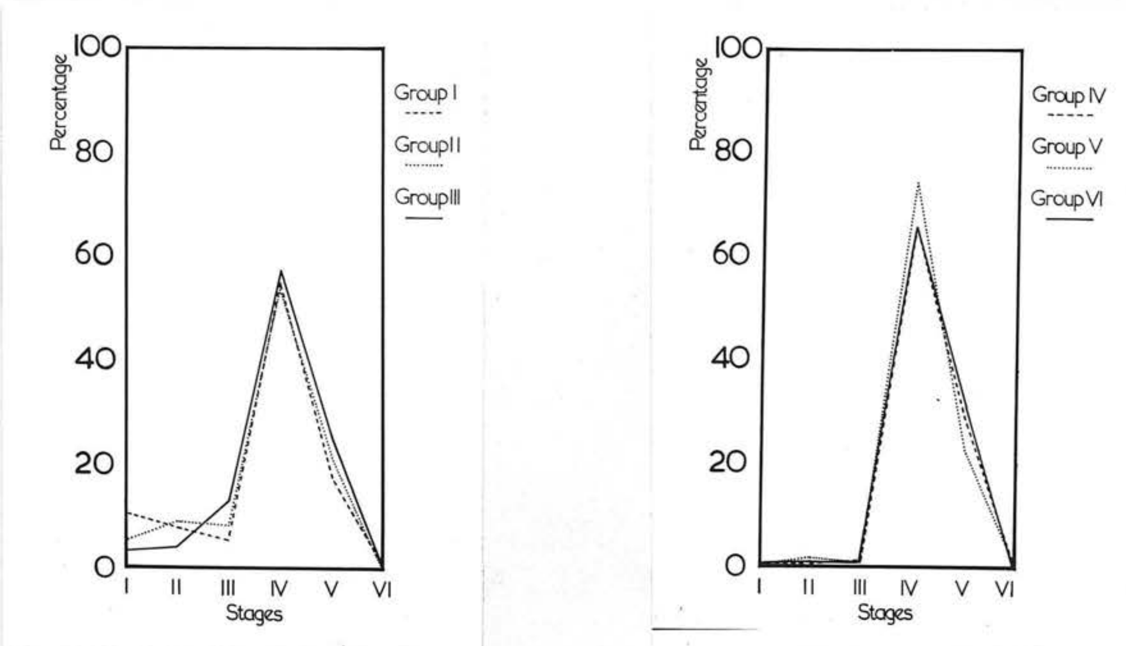


FIGURE XI

PROFILE GRAPHS SHOWING THE PERCENTAGE OF GLOMERULI AT THE SIX STAGES OF MATURITY PRESENT IN SIX BODY WEIGHT GROUPS



The body weight groups are those used in table IX

Figures XI a&b are profile graphs as in the other analyses.

Table X and Fig. XII give the case incidence of the different glomerular stages in the seven body weight groups. All the cases showed stage III, IV and V. All the smallest babies showed stages I and II but from 2,000 Gm onwards the majority did not. Stage I had disappeared altogether from infants weighing 3,500 - 4,000 Gm, but from 2,000 Gm onwards the proportion showing stage II varied from 18 - 42%. Throughout the graph the percentage of cases showing stage VI was very variable and seemed to bear no relation to the percentages of the other stages present.

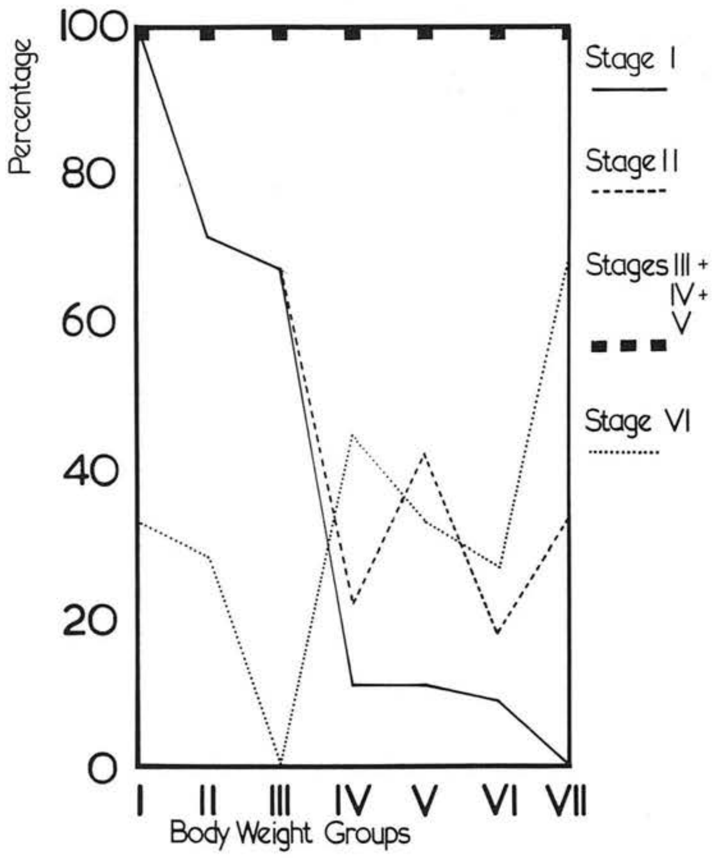
TABLE X.

THE CASE INCIDENCE OF GLOMERULAR DEVELOPMENTAL STAGES IN SEVEN BODY WEIGHT GROUPS.

CASE GROUPS			CASES							
BODY WEIGHT (G)	GROUP No.	TOTAL CASES	STAGE I GLOMERULI PRESENT		STAGE II GLOMERULI PRESENT.		STAGE III, IV & V GLOMERULI PRESENT.		STAGE VI GLOMERULI PRESENT	
			NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
500 ū 1,000	I	3	3	100	3	100	3	100	1	33
1,000 ū 1,500	II	7	5	71.4	5	71.4	7	100	2	28.6
1,500 ū 2,000	III	9	6	66.7	6	66.7	9	100	0	0
2,000 ū 2,500	IV	9	1	11.1	2	22.2	9	100	4	44.4
2,500 ū 3,000	V	9	1	11.1	4	44.4	9	100	3	33.3
3,000 ū 3,500	VI	11	1	9.1	2	18.2	11	100	3	27.3
3,500 ū 4,000	VII	3	0	0	1	33.0	3	100	2	66.7

FIGURE XII

CHART SHOWING THE CASE INCIDENCE OF GLOMERULI AT THE SIX STAGES OF MATURITY IN SEVEN BODY WEIGHT GROUPS



The body weight groups are those used in Tables IX and X

The percentages of cases showing stages III, IV and V are combined in a single curve.

Analysis by Crown-Rump Length

There are 90 cases in this analysis and the results are presented in table XI and in figure XIII.

In crown-rump length group I (20 - 25 cm) stages I and II account for 8% of the total. In group II there has been a slight rise in the percentage of stage II and a slight fall in stage I, presumably because stage I glomeruli have matured to stage II and there has not been an equivalent production of new proglomeruli. There is a steep fall of both stage I and stage II between groups II and III and from 35 cm onwards Stages I and II account for less than 1% of the total. Throughout the period of this graph, the very great majority of glomeruli are immature. Stage VI varies in percentage between less than 1% and 3.3% until in group V there is a sharp rise to 8%.

T A B L E XI

THE PERCENTAGE OF GLOMERULI AT SIX STAGES OF MATURITY IN FIVE CROWN-RUMP LENGTH GROUPS.

CROWN-RUMP LENGTH (cm)	Glom. Stages	20 ū 25	25 ū 30	30 ū 35	35 ū 40	40 ū 45
Average % present	I	7.9	7.5	0.8	0.5	0.5
	II	8.1	9.5	1.6	0.8	0.3
	III	9.5	6.5	4.6	1.1	2.2
	IV	62.6	54.9	71.4	57.3	38.1
	V	15.3	23.5	20.4	30.5	55.4
	III IV V	80.5	78.4	89.1	94.9	83.4
	VI	1.4	0.8	3.3	0.6	10.3
	U	2.0	3.9	5.2	1.2	3.7
NUMBER OF CASES IN GROUP.		5	15	31	29	10
GROUP NUMBER		I	II	III	IV	V

FIGURE XIII

CHART SHOWING THE PERCENTAGE OF GLOMERULI AT SIX STAGES OF MATURITY AGAINST CROWN-RUMP LENGTH.

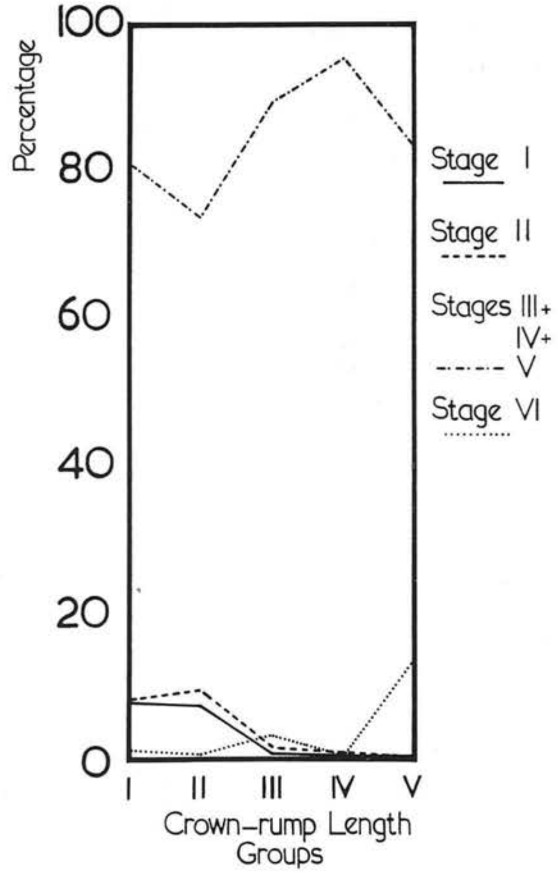
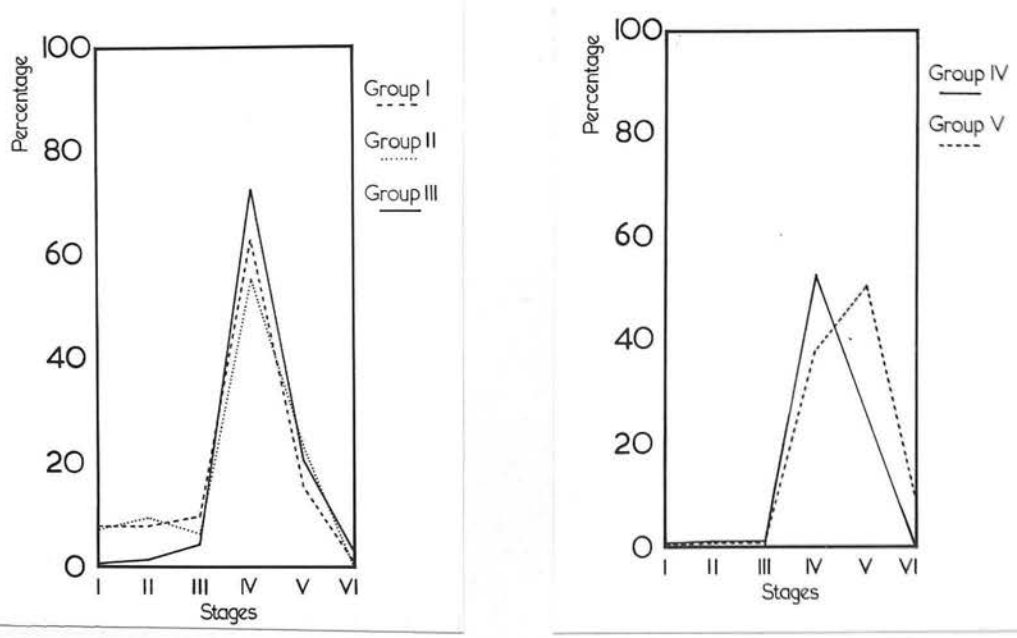


FIGURE XIV

PROFILE GRAPHS SHOWING THE PERCENTAGE OF GLOMERULI AT THE SIX STAGES OF MATURITY IN FIVE CROWN-RUMP LENGTHS



The crown-rump length groups are those used in Table XI.

The profile graphs (Fig. XIV a&b) show that in infants of crown-rump length less than 40 cm the majority of glomeruli are at stage IV. There is then a shift to the right and in the group with a crown-rump length of between 40 and 50 cm the majority are at stage V.

Table XII and Figure XV show the case incidence of the different developmental stages in the five crown-rump length groups. Stages III, IV and V are present in all the cases. 80% of the shortest infants show stages I and II and these two stages are still present in 20 - 30% of the longest infants.

The percentage showing stage VI varies throughout the series between about 15 and 50 percent.

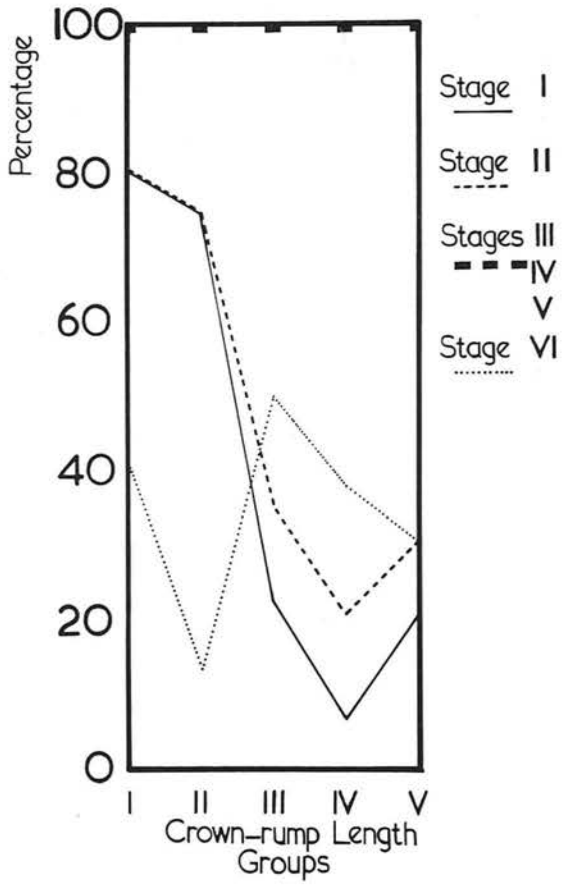
TABLE XII.

THE CASE INCIDENCE OF GLOMERULAR DEVELOPMENTAL STAGES IN FIVE CROWN-RUMP LENGTH GROUPS.

CASE GROUPS			CASES							
CROWN-RUMP LENGTH (cm)	GROUP No.	TOTAL CASES	STAGE I Glomeruli present		STAGE II Glomeruli present		STAGES III, IV & V Glomeruli present		STAGE VI Glomeruli present	
			NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
20 ū 25	I	5	4	80	4	80	5	100	2	40
25 ū 30	II	15	11	73.3	11	73.3	15	100	2	13.3
30 ū 35	III	31	7	22.6	11	35.5	31	100	15	50
35 ū 40	IV	29	2	6.9	6	20.7	29	100	11	37.9
40 ū 45	V	10	2	20	3	30	9	99	2	20

FIGURE XV

CHART SHOWING THE CASE INCIDENCE OF GLOMERULI AT THE SIX STAGES OF MATURITY IN FIVE CROWN-RUMP LENGTH GROUPS.



The crown-rump length groups are those used in Tables XI and XII.

The percentages of cases showing stages III, IV and V are combined in a single curve.

DISCUSSION OF RESULTS

The four analyses show similar patterns of glomerular development and the minor differences between them can be accounted for by the re-grouping of cases for analysis against each maturity factor.

In the four analyses 80 - 100% of the most immature kidneys showed stages I and II present and these accounted for about 10% of the total. Stage I appears to show a better correlation with maturity than does stage II which was found surprisingly late in some cases. It may be that these persistent stage II glomeruli are abnormal and should be ignored in any assessment of general kidney maturity.

Stage III has a disappearance curve that follows that of stage I closely and though stage III persists rather longer than stage I it should probably be classed with stages I and II as a proglomerular form. Some glomeruli never pass through stage III, lobulating before the vascular pole has been formed, so that the presence or absence of this stage is not a reliable guide to kidney maturity.

The fully formed immature glomeruli (stages IV and V) persist in the majority of cases until the child is nine years old and in some until twelve years though they are not the most numerous type in the kidney after the age of 6 years.

The fully mature (Stage VI) glomeruli are present in very small numbers in perhaps about half the cases under six months of age but after this age there is a fairly steady increase both in the percentage of cases showing this stage and in the proportion of the total glomerular count represented by it until stage VI is the only one found in the three cases aged 12 years and more.

The analyses by crown-rump length and post mortem body weight include only very young children because these measurements had not usually been made at the post mortem of the older children. The age was the piece of information most usually available so that most of the older cases appear in this analysis. The kidney weight too was known in most cases, but again it is the oldest children in whom it is lacking.

CONCLUSIONS

Inspection of tables and graphs of the analysis by age suggests that there are three phases of glomerular maturation and one resting phase in which little change in the glomerular pattern occurs.

Phase I can be called the nephrogenic phase in which new glomeruli are being formed and stage I glomeruli are present in the cortex. This phase is apparently over in the majority of cases by the 36th week from conception, or by the time the kidneys weigh 30 Gm and the child weighs 2,500 Gm, or has a crown-rump length of 35 cms, but in a very few cases it may persist until sometime between the 44th and 48th week from conception. An exactly similar "tail" is present in the stage I curve in the other three analyses, so that this occasional persistence of stage I cannot be a false finding due to faulty calculation of the gestation time.

Phase II - The nephrogenic zone has disappeared and there is maturation of all the glomeruli with increasing proportions of the stage V and VI and decreasing proportions of stage IV. This phase apparently continues until stages V and VI glomeruli each account for about 50% of the total. This appears to be at about two years of age or when the combined kidney weight is between 70 and 80 Gm.

Resting Phase - In this phase, the glomerular developmental pattern achieved during the second year apparently persists with little change until the child is about six years old.

Phase III - This is the phase of final glomerular maturation in which the stage V glomeruli develop into stage VI. In the majority of cases this phase is complete by the 9th year but in about 30% it is not completed until sometime between nine and twelve years of age, after which time all the glomeruli in the kidney are of fully mature adult form.

PART II. AN ESTIMATION OF THE CHANGES IN THE RELATIVE MASS OF GLOMERULAR AND CONVOLUTED TUBULAR TISSUE IN THE GROWING KIDNEY

INTRODUCTION.

The changing pattern seen in the renal cortex during growth is due to changing proportions of glomerular and tubular tissue as well as to the changing appearance of the glomeruli. In the younger kidneys this is demonstrated by the increasing distance between the most peripheral glomeruli and the capsule, and in all by the progressively widening separation of glomeruli from each other by tubular tissue.

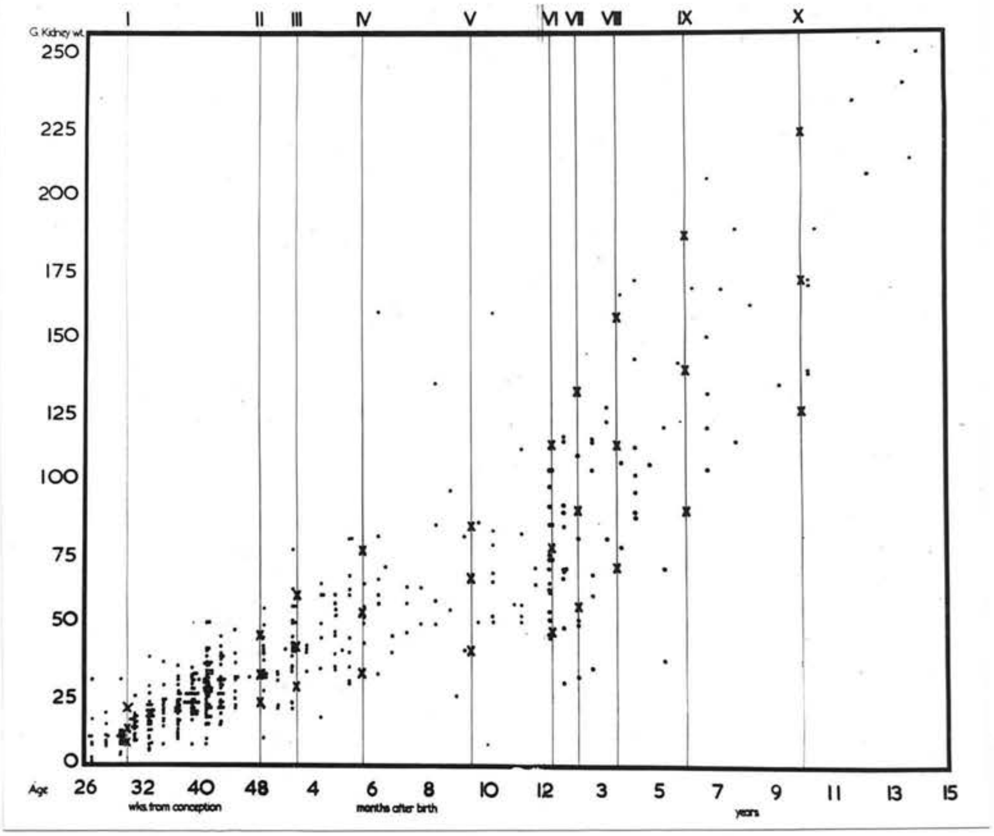
Quantitative estimations of the relative growth of different parts of the kidney have already been made by a number of workers, but it has not been possible to obtain any clear information about the changing proportions of glomerular and tubular tissue in the renal cortex during growth. The investigation of this aspect of kidney development was undertaken.

MATERIAL

The same material was used in this as in Part I of the investigation.

<u>FIGURE XVI</u>

SCATTER DIAGRAM SHOWING THE COMBINED WEIGHT OF RIGHT AND LEFT KIDNEYS AGAINST AGE



The vertical lines numbered with Roman numerals indicate the age groups from which cases were selected.

The crosses represent the 10th, 50th and 90th percentile points of kidney weight in these age groups.

METHOD

The selection of cases

The nature of the method made the use of a very large series of cases impracticable but the wide individual variation found in other aspects of kidney development (reported in Part I of this thesis) showed the importance of obtaining a representative series if any valid conclusions were to be drawn from a small number of cases. The method of selection was adopted on the advice of Dr. J. H. Jowett of the Department of Statistics in the University of Sheffield, who undertook the statistical work involved in it.

The weights of the kidneys were plotted against age on a scatter diagram (Fig XVI). The age from conception, calculated from the mother's last menstrual period, was used for all infants who died less than two months after birth, but in those surviving two months or more the post natal age was used. The scatter diagram was then transferred onto a logarithmic age scale and the cases grouped according to age, with about 20 cases in each group. The mean kidney weight and the 10th and 90th percentile weights in each group were determined and the three curves drawn and smoothed

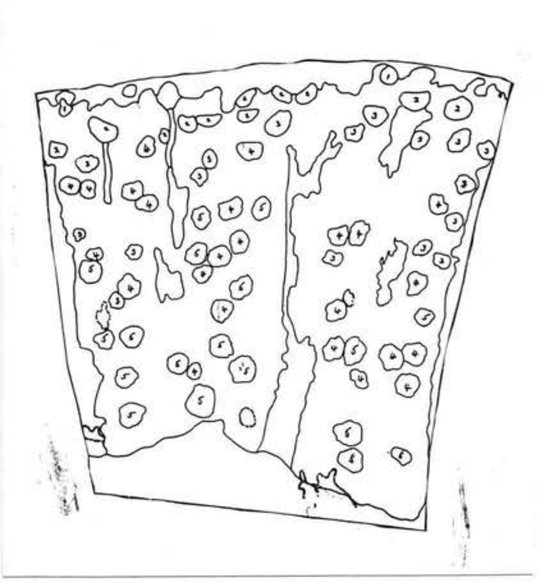
Smoothed values of the three measures were read off the graph at ten different ages selected in such a way that on the logarithmic age scale they were equally spaced. These thirty points were then plotted on the original scatter diagram and the case nearest to each of them was selected for investigation. In age group VII only one case was found to be suitable on technical grounds for use in the survey.

The Making of Tracings

By inspection under the microscope of a section from each kidney, a suitable area of cortex showing three adjacent renal columns separated by clear medullary rays was selected and marked. This area was then projected onto a sheet of white paper at a magnification of x 140 and the outlines of the renal capsule, glomeruli, blood vessels and medullary rays were traced. Each glomerulus was given a maturity grading according to its histological appearance using the criteria described in part I of this thesis, and the appropriate number was written within each glomerular outline. A contact photographic print was made of each completed tracing and a representative series of these is shown in figure XVII.

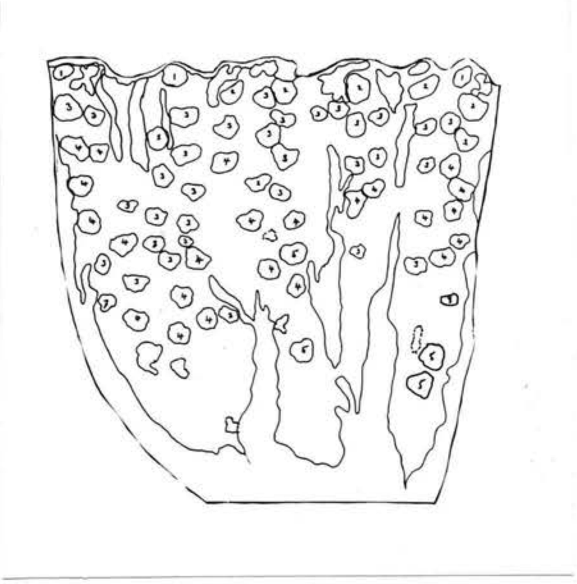
FIGURE XVII

EXAMPLES OF TRACINGS OF THE RENAL CORTEX



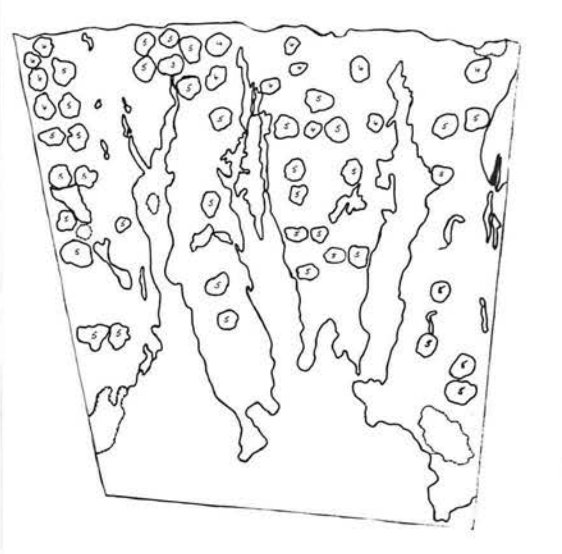
Age 26 weeks gestation

Kidney weight 7.7 gm.



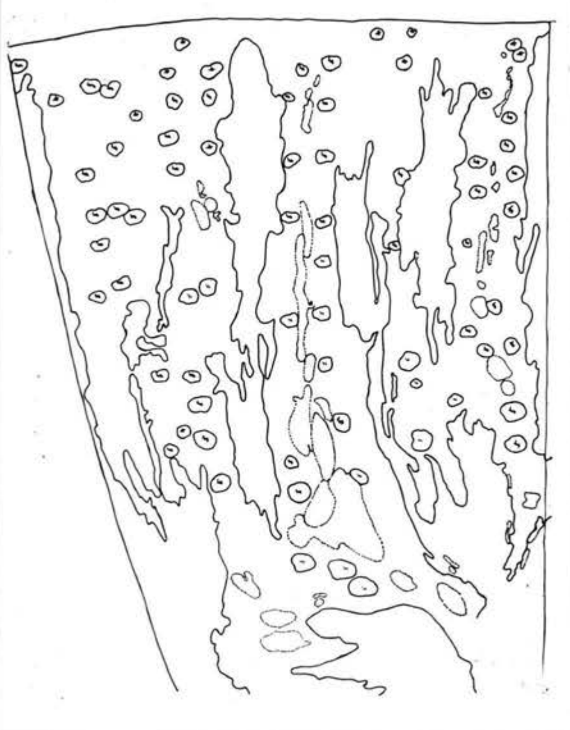
Age 35 weeks gestation

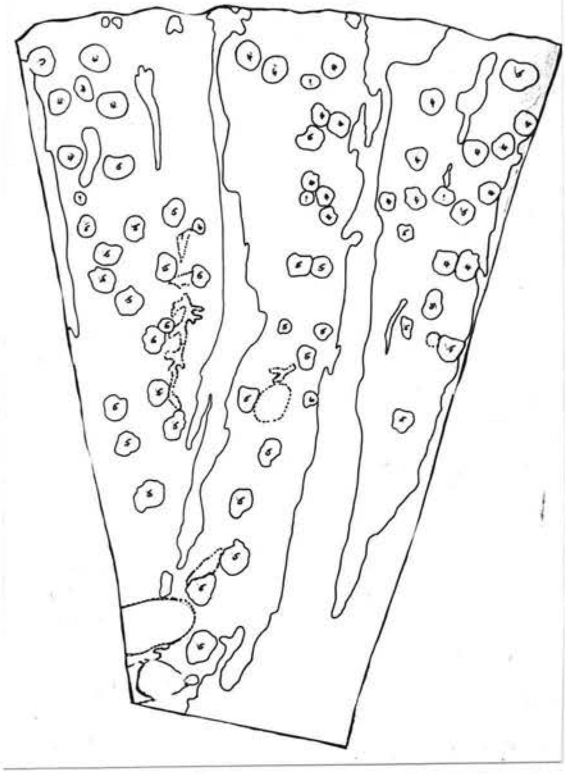
Kidney weight 28 gm.



Age 26 weeks gestation

Kidney weight 21 gm.

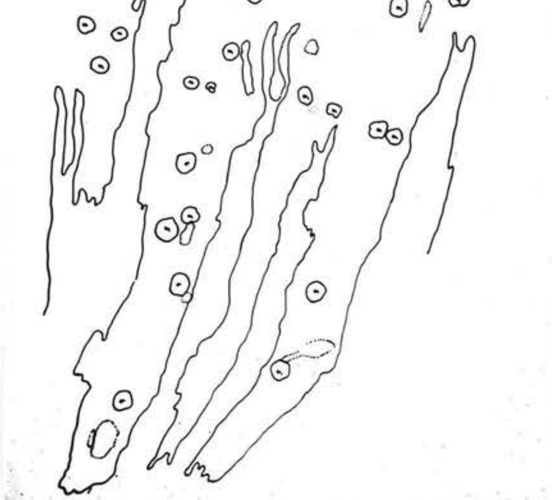




Age 10 months

Kidney weight 78 gm.

Age 40 weeks gestation.



Age 10 years.
Kidney weight 172 gm.

Weighing

From each contact print, the outlines of the renal capsule, medullary rays, blood vessels and glomeruli were cut out, the remainder of the tracing then representing the convoluted tubules. Three standard squares of 1 sq.mm. projected at magnification x 140 were cut from widely separated parts of each print, and these, the pieces representing glomeruli, and the convoluted tubules were weighed on a laboratory balance. Glomeruli of different maturity were weighed separately and counted. The three standard squares weighed separately were found to be equal to the nearest 10 milligrams, therefore the other weights were corrected to the nearest 0.01 gm.

The corrected weights from each tracing were used as measures of the area occupied by glomeruli and convoluted tubules in the corresponding microscopic section. These areas were assumed to be directly proportional to the mass of glomeruli and convoluted tubules in the kidney, and were used in relating these quantities to each other. It was not possible to include loops of Henle in the tubular measurement by this method, because of uncertainty in distinguishing them from collecting tubules in the medullary rays.

RESULTS

The results of the weighings are presented in Tables XIII and XIV. In Table XIII the total glomerular weight in each case is shown and in Table XIV the average values for each kidney weight group (with the exception of group VII) have been calculated. The mass of glomeruli relative to convoluted tubules is expressed as a percentage in each kidney weight group and presented graphically in Figures XVIII and XIX.

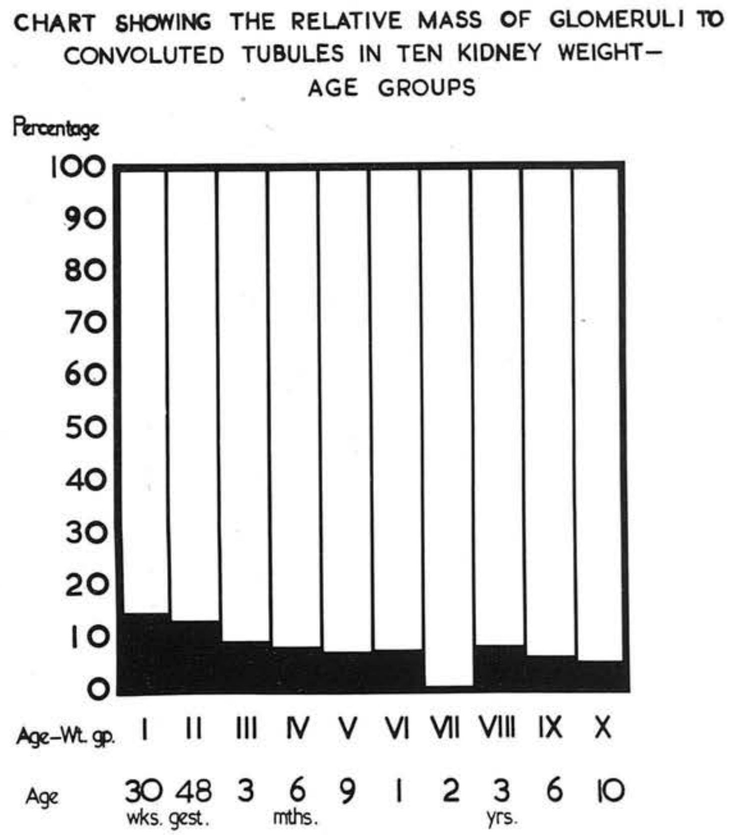
T A B L E XIII

CASE No.	Group	Age	Kidney Weight(G)	Glomerular Weight(G)	Convoluted Tubule Weight (G)
1299	I L	31-32 wks	8.8	0.17	1.60
778	I M	30 wks.	12.5	0.59	3.46
1558	I U	32-33 wks	21.0	0.68	3.45
936	II L	11 wks.	21.0	0.25	2.91
568	II M	49 wks	35.0	0.38	4.40
1528	II U	45 wks	47.5	1.75	8.39
1007	III L	3 months	22.0	0.59	6.83
1423	III M	3 months	40.0	1.98	22.94
1610	III U	4 months	50.0	2.22	17.66
405	IV L	6 months	34.0	0.84	8.63
1074	IV M	5½ months	54.0	0.73	6.25
915	IV U	5¾ months	64.0	0.41	7.77
1599	V L	9 months	37.5	0.74	6.76
1382	V M	10 months	53.0	0.79	15.02
1453	V U	10 months	78.0	0.96	12.54
1732	VI L	18 months	54.0	0.71	4.49
1533	VI M	21 months	70.0	2.06	27.70
1215	VI U	16 months	105.0	0.45	6.56
992	VII L	2 yr.3 mth	50.0	0.91	10.95
	VII M				
	VII U				
1445	VIII L	3yr.6 mth	80.0	1.47	12.91
1573	VIII M	4yr.5 mth	113.0	1.80	21.85
1797	VIII U	4 yr.6mth	162.0	1.68	21.07
1456	IX L	6yr.6mths	105.0	1.64	19.72
532	IX M	5yr.2mths	161.0	0.85	14.59
1147	IX U	7yr.10mth	190.0	0.52	10.79
1377	X L	9 years	135.0	1.75	25.49
1449	X M	10 years	172.0	0.76	17.98
1181	X U	13yr.6mth	215.0	1.52	28.93

TABLE XIV

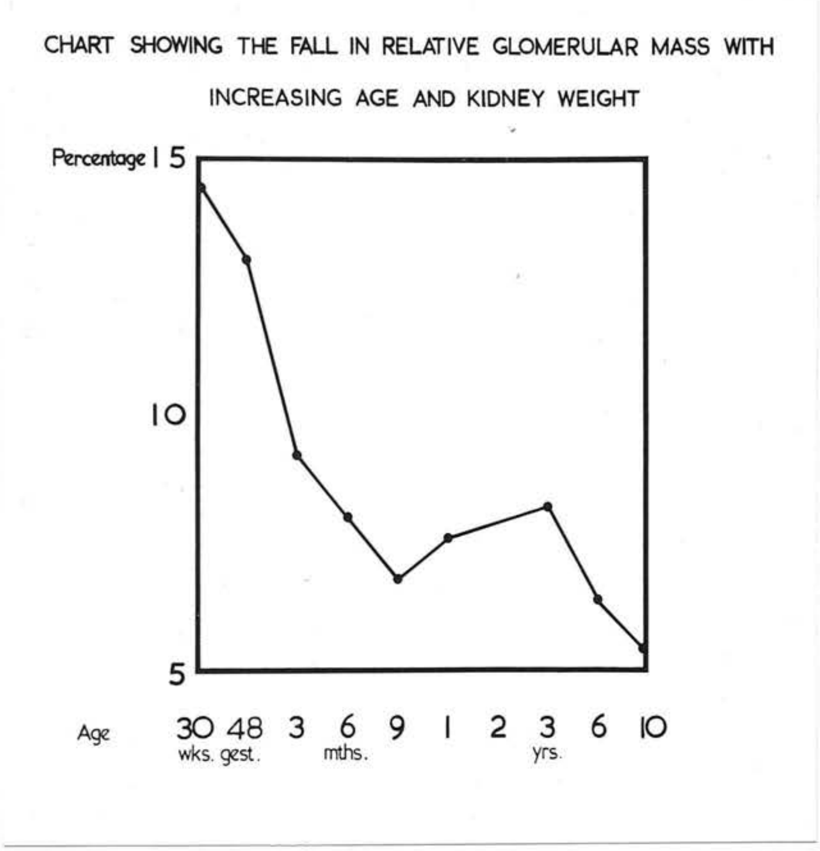
GROUP	Total Glom.wt.(G)	Total Convoluted Tubule weight (G)	Total Glom.wt.& Total Sec.Tub.wt.	% Glom.wt.	% Convoluted Tubule Wt.
I	1.44	8.51	9.95	14.5	85.5
II	2.38	15.70	18.08	13.0	87.0
III	4.79	47.43	52.22	9.2	90.8
IV	1.98	22.65	24.63	8.0	92.0
V	2.49	34.32	36.81	6.8	93.2
VI	3.22	38.75	41.97	7.6	92.4
VII	-	-	-	-	-
VIII	4.95	55.83	60.78	8.1	91.9
IX	3.01	45.10	48.11	6.2	93.8
X	4.03	72.40	76.43	5.2	94.8

FIGURE XVIII



The age-weight groups numbered I to X are those shown by vertical lines in figure XVI.

<u>FIGURE XIX</u>



The points on the graph correspond with the kidney weight-age groups shown in Figures XVI and XVII.

Table XV shows the number and weight of glomeruli at three different stages of maturity in each case. In each kidney weight group the average weight of 100 glomeruli at each of these stages has been calculated and the weights converted to areas by means of the standard 1 mm. squares. They are presented graphically in figure XX.

FIGURE XX.

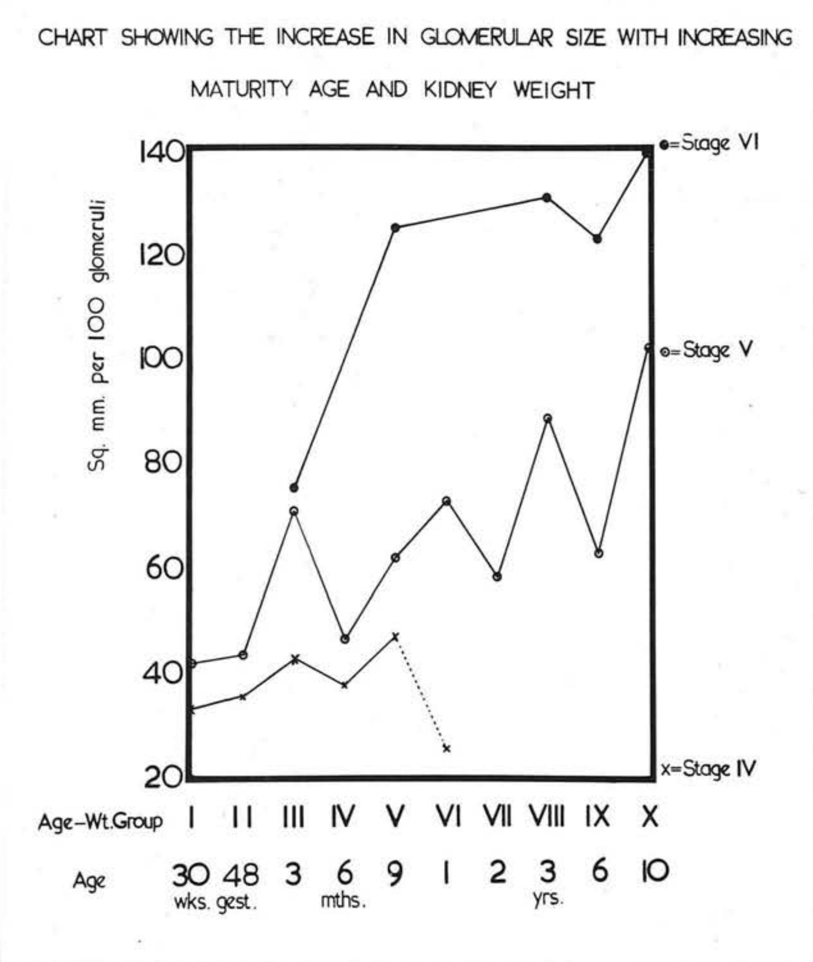


CHART SHOWING THE INCREASE IN GLOMERULAR SIZE WITH INCREASING
MATURITY AGE AND KIDNEY WEIGHT
Sq. mm. per 100 glomeruli
140
120
100
80
60
40
20
●= Stage VI
⊙= Stage V
x= Stage IV
Age-Wt. Group
I II III IV V VI VII VIII IX X
Age
30 48 3 6 9 1 2 3 6 10
wks. gest.
mths.
yrs.

DISCUSSION

The ratio of glomerular to convoluted tubular mass in kidney weight group 1 is 14.5% and in group V it is 6.8%. In the age period from 30 weeks gestation to the 10th post natal month there is therefore a fall in the ratio to less than half its initial value. Thereafter the downward trend of the graph is interrupted by a rise which is difficult to interpret. There is no evidence of accelerated glomerular growth during this period but three other possible explanations remain: - 1) that tubular growth is markedly slowed or halted; 2) that tubular growth continues but is confined to the loops of Henle which were not included in the measurement; 3) that in a large series of cases this portion of the graph would have shown a flattening of the curve rather than a rise. There is no evidence to indicate which of these possible explanations is the correct one, but the third seems the most likely. The age period represented by groups V to VIII is one in which the pattern of glomerular maturity changes little, as postulated in part I of this thesis, and it may be that other aspects of kidney development are similarly little altered during this time. Between groups IX and X there is another steep fall, making a striking contrast between the glomerular mass of 14.5% in group I and 5.2% in group X.

In the correlation of glomerular size with age, kidney weight and glomerular maturity (Fig. XX), the smallness of the series has resulted in very irregular curves. Nevertheless, each shows a quite definite upward trend. It seems fair to ignore point VI on the stage IV curve because it represents only four glomeruli, present in two cases. In each kidney weight-age group, increased glomerular maturity is associated with increased size, and there is no exception to this. Further, if the three curves are smoothed with the eye and trend lines drawn, it will be seen that the largest glomeruli in stage IV correspond roughly in size with the smallest in stage V and that the largest in Stage V correspond roughly with the smallest in Stage VI, while growth in Stage VI continues throughout the period of the graph. This suggests strongly that the histological changes seen in the glomerular tuft epithelium during the maturation process are directly related to increasing glomerular size, and may be no more than a simple result of it.

The method of selection of cases for this series excluded those whose kidney weight fell outside the

10th and 90th percentile range. This was done deliberately, for the reason already stated, but the apparent degree of individual variation within the group has been diminished. An impression of the scatter of kidney weight at any age can be gained from Figure XVI, and the variation possible between the histological pictures of different kidneys of similar ages is illustrated in Figure XVII, where the younger and lighter kidney has a picture of histological maturity in advance of the older and heavier one.

CONCLUSIONS

The investigation has shown that between the 30th week of intrauterine life and 10 years of age, increasing kidney weight is accompanied by an increase both in the convoluted tubular mass and in the size of the glomeruli, but the growth of convoluted tubules is relatively greater than that of glomeruli. This results in a fall in ratio of the glomerular to the convoluted tubular mass of 9.1% between the 30th week of gestation and 10 years of age, but there is a period from 10 months to 4 years when the ratio probably alters little and is somewhere between 8.6% and 6.2%.

The investigation has also shown that the histological changes seen in the epithelium of the maturing glomerular tuft are directly related to increasing glomerular size, and may be a simple result of it.

The true individual variation in weight and histological pattern between kidneys of similar age is greater than appears in this investigation. This is the result of the method of selection of cases which was designed to provide a small representative series from which extreme cases were excluded.

PART III. THE MICRODISSECTION OF IMMATURE NEPHRONS

In order to gain a more detailed impression of the growth of the nephron, and in particular of the renal tubule, a series of microdissections of immature kidneys was made.

MATERIAL AND METHODS

The amount of foetal connective tissue present in the kidneys of the most immature infants made satisfactory maceration of fixed kidneys a difficult problem. It was therefore decided to work with fresh material only.

The kidneys were used as soon as possible after removal from the body at autopsy, and this was always within an hour or two. The right and left kidneys were weighed separately and if both kidneys and the renal tract appeared normal to the naked eye, one was fixed in the routine way in formol-saline, while the other was perfused with concentrated hydrochloric acid, three parts, to one part of distilled water at a pressure of 10 lb per square inch, by means of a polythene cannula tied into the renal artery. When the whole of the surface of the kidney had changed colour, perfusion was considered complete and the kidney was allowed to stand for an hour in acid similar to that used for the perfusion. The capsule was then stripped off and the whole kidney divided easily into its natural lobules. No further division of the kidney was made at this point and the separated lobules were allowed to stand in the acid for as long as necessary to achieve a degree of maceration suitable for micro-dissection. This time proved to be variable and was a matter of trial and error for each kidney. If the kidney was not required for dissection immediately, it was preserved after maceration in a solution of equal parts of 1% formol saline and 1% chloral hydrate.

The dissection was carried out through a stereoscopic microscope at a magnification of x 50, by means of simple mounted needles. The method of mounting was to remove the nephron from the dish by means of a clean pipette and to transfer it into a drop of glycerine in a hollow ground slide. The nephron was then arranged in its correct shape, by means of the mounted needles under the microscope and a cover slip put on. The preparation was sealed with glycel. This did not make a permanent preparation but preserved the nephron in good shape and position until it could be photographed.

The aim was to dissect out and mount a series of nephrons from each kidney representing the least mature and most mature, i.e. the subcapsular and juxta-medullary generations of nephrons from each kidney, with a representative series between them. Technical difficulties resulted in failure to dissect out, or failure to mount such a complete series from a single kidney. In Fig. XXI a, photographs of a series of nephrons from three immature kidneys are shown.

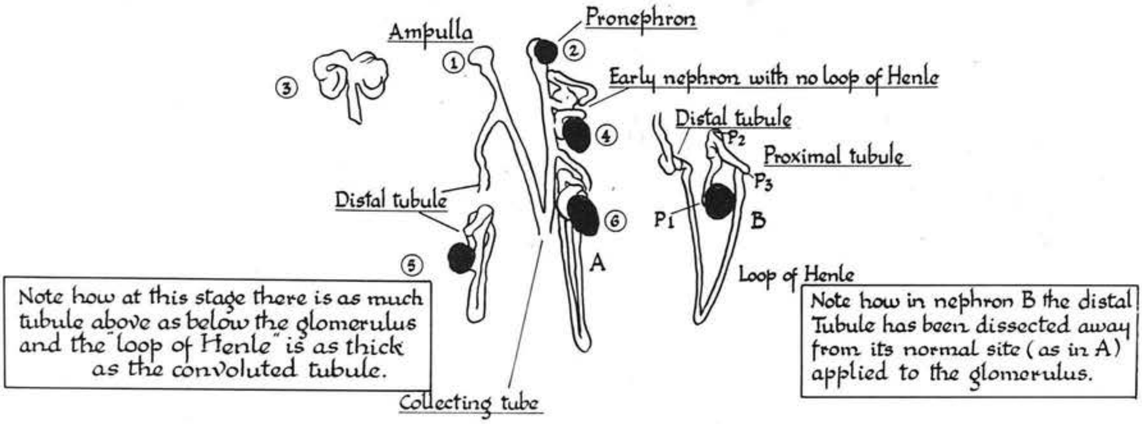
Figure XXIb is a labelled key to the photographed nephrons.

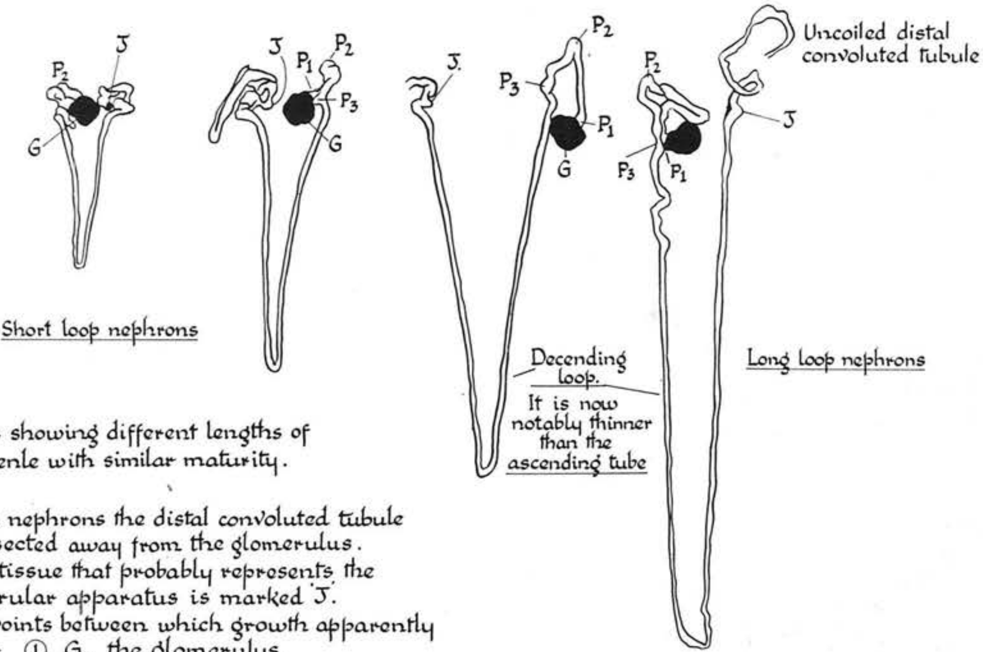
FIGURE XXIa

PHOTOGRAPHS OF A SERIES OF NEPHRONS DISSECTED FROM THREE IMMATURE KIDNEYS.

FIGURE XXIb

KEY TO FIGURE XXIa





Four nephrons showing different lengths of the loop of Henle with similar maturity.

In all of these nephrons the distal convoluted tubule has been dissected away from the glomerulus. The mass of tissue that probably represents the juxta-glomerular apparatus is marked J. Other fixed points between which growth apparently occurs are :— ① G, the glomerulus. and ② P_1. P_2 P_3, the primary bends in the proximal convoluted tubule.

In the course of dissecting these and many other immature nephrons the impression was gained that growth of the proximal convoluted tubule takes place between certain fixed points. The first of these is the glomerulus and the others, marked P_1, P_2 and P_3 in Figure XXIb represent the primary bends of the proximal convoluted tubule. The growth of the loop of Henle appears to begin after the three primary bends have been formed and at first there is no apparent difference in calibre between it and the convoluted tubules not between its descending and ascending parts. The distal convoluted tubule does not appear to have such a fixed pattern of growth as the proximal convolution but its junction with the ascending limb of the loop of Henle is invariably marked by a highly refractile spot (marked I on the key) in the region of which the tubule is moulded round the glomerulus and is very firmly adherent to it.

GENERAL CONCLUSIONS.

The impression that it is very difficult to obtain objectivity and consistency in observations on histological material was confirmed by the reproducibility test to which the method of classification of renal glomeruli was subjected. This test showed a variable and sometimes high degree of inconsistency in individual judgements of glomerular maturity. The inconsistency was most marked when the test was carried out by an observer who had had no previous training in applying the criteria, but it was also increased by fatigue. The average results of groups of counts showed better reproducibility than the results of single counts. It appeared that, as would be expected, better reproducibility was obtained with good histological preparations than with poor ones. It apparently made no significant difference to the results whether the counts were made by the "column" or the "slide" method.

It was concluded that the method of classification and counting of renal glomeruli used in the test could give a general picture of glomerular maturation but was not sufficiently objective to give reliable detailed quantitative information.

It appears that maturation of renal glomeruli takes place in three phases with a period of rest preceding the final maturation. The first phase is one of nephrogenesis which in most cases is over by the thirty-sixth week from conception and is not affected by the incident of birth when this occurs prematurely. During the second phase all glomeruli mature gradually until about half are of adult form and the rest are at the stage preceding full maturity. This phase is over in most cases by the end of the first year of post natal life. There then follows the resing phase, which lasts until about the end of the fifth year, in which there is little or no change in the pattern of glomerular maturity. Phase three, that of final maturation during which the remaining immature glomeruli reach adult form, is completed in most cases by the age of nine, though in about a third of this series it lasted until nine to twelve years of age.

A study of the changes in the relative glomerular and secretory tubular mass in the renal cortex indicated that, as development proceeds from the thirtieth week from conception to the tenth year of post-natal life there is a marked fall in the proportion of the cortex occupied by glomeruli and a corresponding rise in the proportion occupied by secretory tubules. In the most immature kidneys the ratio of glomerular mass to secretory tubular mass was 14.5% but by the tenth year it had fallen to 5.4%. This fall in relative glomerular mass was apparently arrested between the ages of ten months and four years, which corresponds closely with the resting phase of glomerular maturation.

It appeared that the changes in the glomerular tuft epithelium used as criteria of glomerular maturity were directly related to glomerular size suggesting that these changes may be a simple result of expansion of the capillary tuft.

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