SOME ASPECTS OF COMPENSATORY HYPERPLASIA OF THE KIDNEY

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THE mechanisms controlling tissue growth and regeneration are largely unknown, although disturbances of these may well play an important part in many diseases. One of the basic problems in any study of these processes is to estimate the rate at which new cells are formed under different conditions. In organs with only diploid cells, the rate of incorporation of radioactive materials into DNA will give a general index, but when the organ contains cells of several histological types, the most informative method is still mitotic counts. A fair amount is known about the later stages of the enlargement of the remaining kidney after unilateral nephrectomy, but the early changes and the changes in the mitotic rate of various cell types have not been examined in any detail. As the kidney may have certain advantages as an experimental model for the study of regeneration, the normal mitotic rate in the kidney and the changes during hyperplasia have been examined with special reference to the first 3 days after unilateral nephrectomy. At the same time, changes in the basement membranes and in stainable fat were sought, as these are early and prominent in the regenerating liver (Aterman, 1952 ; Stowell, 1948).

METHODS

Male albino rats of an inbred strain of Wistar origin maintained on a diet of MRC rat cubes and weighing 112–180 g. were used. Under ether anaesthesia and with aseptic precautions the right kidney was exposed through a short incision in the loin. The renal pedicle was ligated, sparing the adrenal, and the kidney removed : the incision was then closed in 2 layers with cotton sutures. Sham operations in which the kidney was exposed, handled but not removed were performed in other animals. Most operations were performed at 2–4 p.m., and groups of animals were killed after 8, 16, 24, 32, 40, 48, 56, 64 and 72 hr. and after 4, 6, 8 12, 16 and 20 days. To investigate diurnal variations, other groups of animals were operated upon at 10–12 p.m., and at 6–8 a.m. The remaining kidney was removed immediately after death, bisected transversely at the level of the hilum, and fixed in mercuric chloride-formalin and in 10 per cent formalin : transverse paraffin and frozen sections were prepared from the level of the hilum. Frozen sections were stained with Sudan IV, and paraffin sections with haematoxylin-eosin and with PAS-haematoxylin.

For counting mitoses, the kidneys were zoned in the manner described by McFarlane (1941). Peter's sub-division (Peter, 1908) is much less convenient in the rat. By McFarlane's method the kidney is divided into (1) the outer cortical zone, containing most of the glomeruli and the convoluted portions of the proximal and distal tubules, (2) the inner cortical zone, merging into the former, containing few glomeruli, and being mainly composed of the straight portions of the proximal tubules, the ascending broad limbs of the loops of Henle, and collecting tubules, (3) the outer medullary zone, containing mainly the ascending limbs, thin loops and collecting ducts, and (4) the inner medullary zone containing the narrow limbs of the loops of some nephrons, and the larger collecting ducts only. These zones were represented to a varying extent in the sections which were rarely exactly median. To allow for this, a

small area of the high power field, measuring 111 × 67 μ, was delineated by an eyepiece graticule and the number of mitoses of each cell type counted in 150 of these areas in each zone. If more than one section was needed, they were spaced at least 30 μ apart, to avoid counting the same mitosis twice. Prophases before the dissolution of the nuclear membrane, and late telophases were excluded.

RESULTS

In otherwise normal rat kidneys lymphocytic infiltration is common, and spontaneous hydronephrosis is occasionally found : any kidney showing hydronephrosis or more than a very occasional small group of lymphocytes microscopically was discarded, and the experiment repeated.

Nearly all the mitoses in the normal kidney and during hyperplasia occur in the proximal tubules, the collecting tubules, the ascending limbs or the distal tubules. The latter 2 respond differently during hyperplasia and have therefore been classed separately. No significant difference could be found between the right and left kidneys of the 18 sham operated animals, and in view of this, the results of some other experiments which were identical except that the left kidney rather than the right was removed have been included.

Normal mitotic rate and diurnal variation

Mitoses were counted in 3 groups of 40 kidneys removed at 6–8 a.m., 2–4 p.m., and 10–12 p.m. When the results were plotted as frequency histograms, it was obvious that the distributions were not normal, most being strongly positively skewed ; Fig. 1 shows the histograms for the total numbers of mitoses of the 4 main cell types in kidneys removed at 2–4 p.m. This means that the standard deviations will not give reliable frequency limits and to show changes in the mitotic rate, the best indices for the control of population would seem to be the mean and some estimate of the upper limit. In Table I the means and the values below which 95 per cent of all normal readings fall are given for the total counts, and for the counts for each zone and cell type.

In 18 mitoses, just under 3 per cent of the total, the cell type could not be determined. Mitoses were very rarely seen outside the renal tubules : only 4 connective tissue mitoses were seen in the 40 kidneys removed at 2–4 p.m., 3 in those removed at 10–12 p.m., and 2 at 6–8 a.m. Three other mitoses were seen, 2 in thin loops, and 1 in the visceral epithelium of a glomerulus.

There is an obvious and uniform diurnal variation, the counts at 2–4 p.m. being approximately double those at 6–8 a.m., when the rate was lowest for almost all the classes shown in Table I.

TABLE I

	2–4 p.m.		10–12 p.m.		6–8 a.m.	
	Mean	95 per cent upper limit	Mean	95 per cent upper limit	Mean	95 per cent upper limit
Total counts	7·6	18	4·2	9	3·6	8
Outer cortex	2·9	8	1·7	6	1·1	4
Inner cortex	2·1	6	1·1	4	1·0	4
Outer medulla	2·6	8	1·3	5	1·5	5
Inner medulla	0·1	2	0·02	1	0·0	1
Proximal tubules	4·0	10	2·1	7	1·6	6
Distal tubules	0·6	3	0·4	3	0·3	3
Ascending limbs	2·2	7	1·1	4	1·3	4
Collecting ducts	0·4	3	0·3	3	0·2	2

Compensatory hyperplasia

The mitotic counts during hyperplasia in the animals operated upon at 2–4 p.m. are given for the 4 predominant cell types in Figs. 2 and 3. The dotted lines show the levels below which 95 per cent of the counts in normal animals fall, the levels usually being different at different times of day. The means for the various groups are joined by the continuous line. The results for the different zones are apparently

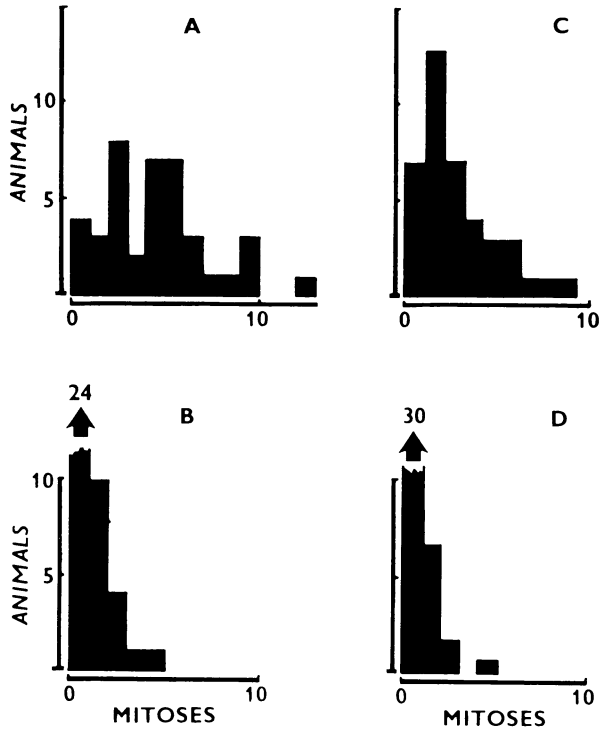

FIG. 1.—Frequency histograms for mitotic counts in kidneys removed at 2–4 p.m. Ordinates: number of animals; abscissae: mitotic counts. (A) Proximal tubules, (B) Distal tubules, (C) Ascending limbs, (D) Collecting ducts.

largely determined by the predominant cell type and so are not given in detail. The means for the different zones are shown in Fig. 4.

The sham-operated animals show no significant increase in mitotic activity at any time. In the operated animals the proximal tubules show some increase in mitotic activity at 24 hr. followed by a rapid climb to a maximum at 40 hr. and an equally sharp fall to normal levels by 56 hr.; there is a second smaller rise at 3 days continuing until at least 12 days. The distal tubules have a single, less well marked increase in activity at 32–40 hr.: the ascending limbs have an increased rate at 40 hr., but the largest rise occurs in a second later peak at 3 days. The collecting ducts show a similar but less well marked pattern with peaks at 40 hr. and 4 days. As in the normal kidney, mitoses were almost confined to the tubules. Two mitoses were seen in the visceral epithelium of glomeruli; a single connective tissue

mitosis was seen in 4 of the 12 kidneys removed at 48 hr. after operation, and in one of the 15 removed after 24 hr.: there were three in one of the 8 removed at 3 days. These counts are too low to more than suggest a possible connective tissue response around 48 hr. The cell type of 17 mitoses (1·3 per cent of the total) could not be decided.

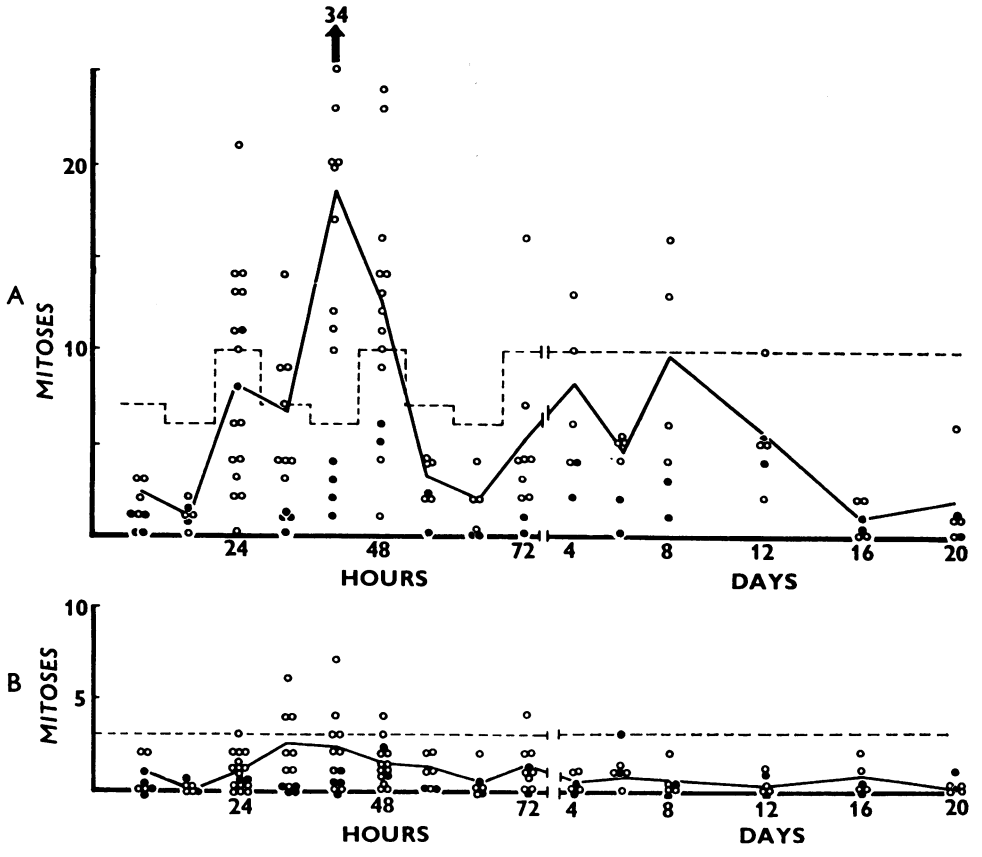

FIG. 2.—Total mitotic counts of the different cell types. Ordinates: number of mitoses; abscissae: time after operation. The time scale differs before and after the break in the abscissae.

● Sham operated animals.
○ Unilaterally nephrectomized animals.
(A) Proximal tubules, (B) Distal tubules.

The outer cortex showed the greatest mitotic activity, whereas only 10 mitoses were seen in the inner medulla throughout the whole course of hypertrophy. The pattern of the mitotic response, though not the absolute level seemed to be determined by the predominant cell type. Although both the collecting ducts and the the ascending limbs lay largely in the outer medulla, and showed a similar biphasic response, in the inner cortex the proximal tubules had the characteristic single peak at 40 hr., whereas the ascending limbs in the same zone showed a biphasic response with a maximum at three days.

Mitotic counts were made per unit area rather than per 1000 cells. To find the approximate rate as a proportion of cells present the number of cells of each type were counted in every 10th field of the 150 fields examined in each zone, in 15 control animals and in 2 from each of the post-operative groups. There was no significant variation with time of day or with time after operation. The average total number of cells examined in each section was 16, 290 (s.d. 1029), comprising

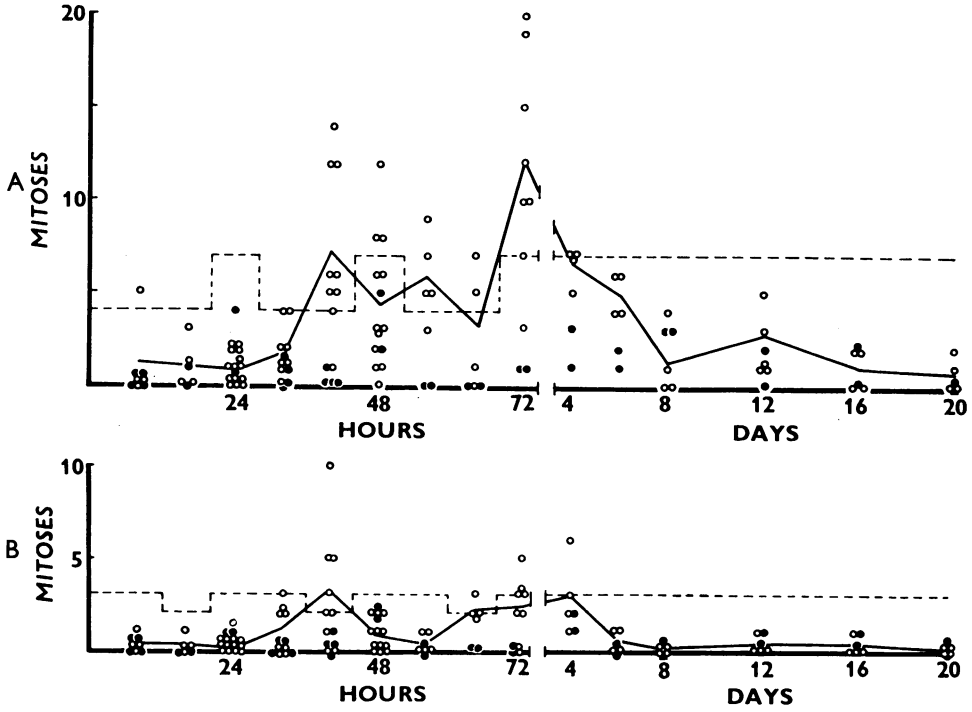

FIG. 3.—Total mitotic counts of the different cell types. Ordinates: number of mitoses; abscissae: time after operation. The time scale differs before and after the break in the abscissae.

● Sham operated animals.
○ Unilaterally nephrectomized animals.
(A) Ascending limbs, (B) Collecting ducts.

6400 proximal tubule, 1270 distal tubule, 4660 ascending limb and 3960 collecting duct cells (1290 of the latter if the mitotically inactive inner medullary zone containing the thin loops and the largest collecting ducts only is excluded). From these figures the approximate rate per 1000 cells can be calculated. and the results for the 4 main cell types are shown in Fig. 5. The highest rate in unoperated animals was 0·61/1000 in the proximal tubule cells at 2–4 p.m., and the highest rate during hyperplasia, 4·38/1000, was reached in the proximal tubule cells of the outer cortex at 40 hr. It can be seen from Fig. 5, that if the inner medulla is excluded, the absolute mitotic rates of the various cell types reach comparable levels, in contrast to the considerable differences observed for the mitotic counts per unit area.

Effect of the diurnal rhythm

The shape of the mitotic response differs when operations are carried out at different times of day. Similar experiments were performed (over the first 48 hr.) starting at 10–12 p.m. or 6–8 a.m. The average total counts, with the results of the experiment starting at 2–4 p.m. for comparison, are shown in Fig. 6 with the number of animals in each group in brackets over the appropriate point. The 6–8 a.m. experiment has an earlier less well marked peak than the 10–12 p.m.

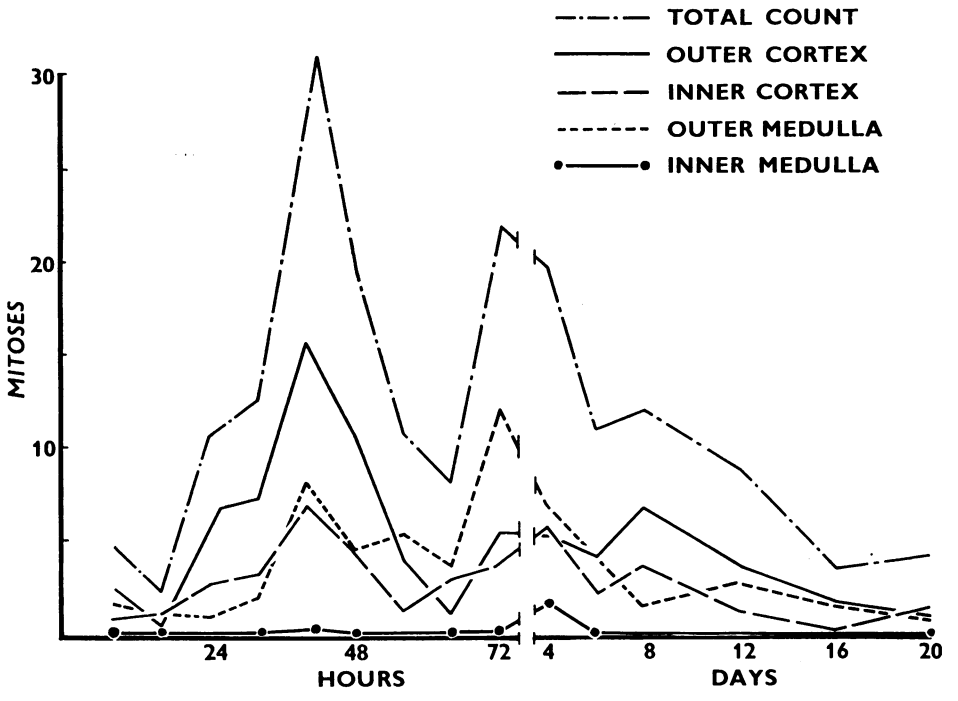

FIG. 4.—Average mitotic counts per standard area against time for the 4 zones separately and for all zones together.

experiment, which has a significant rise only at 40 hr.; the 2–4 p.m. results have an intermediate form. These differences are too great to be accounted for by chance sampling errors. When the variances of the groups are compared by Snedecor's F, and the means by the " t " test where appropriate, there is a significant difference ($P = < 0{\cdot}05$) between at least 2 of the group means at all times from 24–48 hr., and at 32 hr. the chance probability of the differences is less than 0·01 in each case.

Distribution of mitoses

During counting, mitoses often appeared to occur closer together than would be expected purely by chance. To examine the distribution, several sections, spaced more than 30μ apart, were taken from 8 kidneys with high mitotic counts, and 600 fields counted in the outer cortical zones of each. Nearly all the mitoses

occurred in the proximal tubules, and these and the distal tubule mitoses were examined separately. The number of fields containing 0, 1, and 2 or more mitoses was determined, and compared with the number expected in each of these classes from a purely random (Poissonian) distribution. The results for the proximal tubules are shown in Table II. As the " expected " value in the 2 or more class was often very low, the results in several animals have had to be pooled to apply significance tests, but it is obvious that clumping is not very marked if in fact it exists : this is confirmed by a χ^2 test which gives a chance probability of these

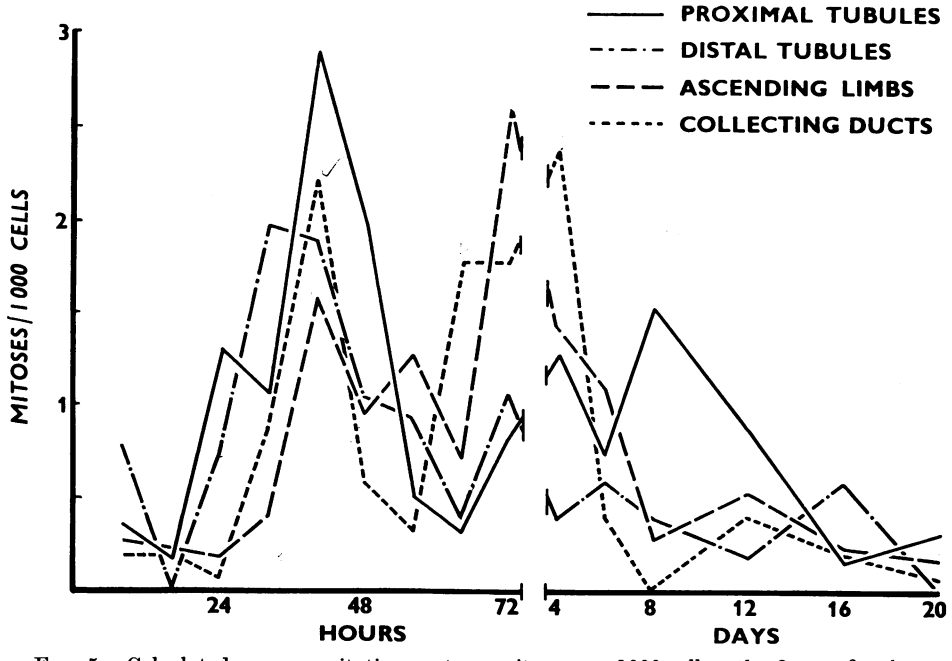

FIG. 5.—Calculated average mitotic counts as mitoses per 1000 cells : the figures for the collecting duct cells exclude the cells of the inner medulla.

results occurring with a purely random distribution of $> 0{\cdot}1$. The distal tubular counts were unhelpful, as more than one mitosis was seen only once in the 4800 fields examined.

Basement membrane changes

Paraffin sections (5 μ) of kidneys removed at operation and of those removed at the end of the experiment were stained by the P.A.S. method and lightly counterstained with haematoxylin. The basement membranes of the tubules and glomeruli stood out clearly, but no change could be detected at any interval after nephrectomy.

Changes in stainable fat.

Frozen sections of the kidneys were stained with Sudan IV. No significant amounts of fat were seen in the cortex or outer medulla before or after operation.

TABLE II

		Number of mitoses/field				
		0	1	2	3	>3
A 552	Observed	556	42	2	0	0
	Expected	555·8	42·6		1·6	
A 532	Observed	524	72	4	0	0
	Expected	525·2	70·0		4·8	
A 542	Observed	569	26	5	0	0
	Expected	565·0	33·9		1·2	
A 562	Observed	559	40	1	0	0
	Expected	559·5	39·2		1·3	
A 312	Observed	532	59	9	0	0
	Expected	527·9	67·7		4·4	
A 362	Observed	521	72	6	1	0
	Expected	519·0	75·3		5·7	
A 332	Observed	518	73	7	1	1
	Expected	512·9	80·4		6·7	
A 822	Observed	521	70	8	1	0
	Expected	517·4	76·7		5·9	

Total $\chi^2 = 6{\cdot}83$. 4 d.f. $p > 0{\cdot}1$.

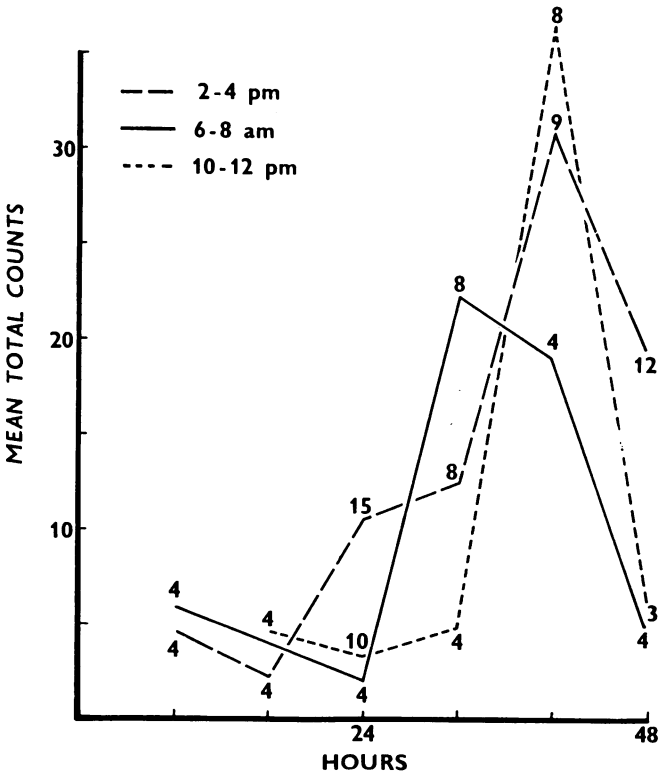

FIG. 6.—Mean total mitotic counts over the first 2 days in experiment started at 2–4 p.m., 10–12 p.m., or 6–8 a.m.

In the inner medulla, tiny sudanophilic droplets could be seen. These did not appear to lie within the cytoplasm of the cells of the collecting ducts or of the thin loops, but lay within the stellate connective tissue cells between these. The droplets ranged from the barely visible up to about 2 μ and were usually most easily visible around the nucleus. This distribution agrees with that previously given by Rice and Jackson (1934), although they noticed occasional droplets also in cortical tubules and in the epithelium of the thin loops. There was no detectable change in the size, number, or distribution of the droplets at any time after operation.

DISCUSSION

Assessment of changes in mitotic rate is difficult as the range of normal mitotic activity is fairly wide, and occasional inexplicably high values are found. The frequency distribution is often strongly positively skewed. This is unfortunate, as this means that to use the standard deviation to establish chance probabilities assuming a normal distribution as is customarily done could give misleading results with small samples, and makes any simple mathematical estimate of significance difficult : control groups have to be large before the importance of a variation in the mean can be assessed. The diurnal variation is marked ; and the results are in general agreement with those of Blumenfeld (1943), who also found a diurnal rhythm in the mitotic counts of the renal cortex with a maximum at 2–4 p.m., though he placed the minimum at 10–12 p.m. rather than 6–8 a.m.

There have been only a few previous quantitative studies of the mitotic rate during compensatory hyperplasia. Carnot and May (1938) counted the number of mitoses in the different zones of the regenerating kidney after giving colchicine, but as they used very few rats at rather large time intervals their results are difficult to assess. Dustin and Zylbersac (1939) also using colchicine did not give numerical results, but stated that there was a marked response at 24 hr., the mitotic response being mostly in the proximal tubules between the first and fourth days, on the fourth day in the glomerulus, and on the seventh day in the thick limbs of the loops of Henle and in the distal tubules : none were seen in the thin loops or in the collecting ducts. These results are very different from those presented here ; the only apparent difference in technique lies in their use of colchicine. Although this is usually considered merely to block mitoses in metaphase, this is by no means certain, and in some circumstances it appears to have a stimulating action as well (Eigsti and Dustin, 1955) : certainly, when compared with normal mitotic counts in tubular regeneration in the kidney, it can produce very anomalous results (Bartman, 1960). Rollason (1949) counted the mitoses in the various zones of the remaining kidney of 14 day old rats 1, 2, 3, 6, 8, 14, 16, 22 and 56 days after nephrectomy with appropriate controls. There was a significant rise in all zones on the second day only. The reports of Ogawa and Sinclair (1958) and Frank (1960) are based on few animals and do not describe the responses of the various cell types ; the former note that there appears to be a later response in the outer medulla.

The mitotic activity found in these experiments is by no means as brisk as that of the regenerating liver, where after a 68 per cent hepatectomy the mitotic rate may reach 4 per cent at 24 hr. (Brues and Marble, 1937), compared with at most 0·43 per cent in the kidney (after some 50 per cent of the renal tissue has been removed). However, though small in absolute terms, the rise is still well

marked ; at 40 hr. the mitotic rate in the proximal tubules has a mean value 11·5 times the normal mean, and three times the 95 per cent normal upper limit. Individual values were very variable, but all were above the normal range.

The most striking thing about the responses of the various cell types is the rapidity with which the mitotic rates change during the first few days. The peaks can easily be missed ; 8 hr. before and 8 hr. after the 40 hr. peak in the proximal tubules the rate was 30–60 per cent lower, and individual values at these times frequently fell within the normal range. The controlling mechanisms are unknown, though for the regenerating liver attempts have been made to fit the pattern of response to mathematical relationships implying that the rate at which new cells are formed is inversely proportional to the tissue deficit (Brues and Marble, 1937) : even if this is true, it is complicated by the marked diurnal variation in the mitotic rate since discovered in the regenerating liver (Jaffe, 1954). Certainly the rapid fall in the mitotic rate of the proximal tubule cells from the 40 hr. peak to effectively normal levels at 64 hr. is entirely disproportionate to changes in the deficit of renal tissue during this period. The total number of new cells formed in excess of normal growth can be calculated if it is assumed that the normal duration of a mitosis is about 40 min.—a reasonable assumption in mammals—and that the mitotic rate is constant from 4 hr. before until 4 hr. after each experimental point. By 68 hr., the number of excess proximal tubule cells formed on these assumptions is only 4·3 per cent, which is only a small fraction of the 40–70 per cent increase expected eventually from the weight gains of the kidney during compensatory hyperplasia (Addis and Lew, 1940 ; MacKay, Addis and MacKay, 1938) and this low figure is supported by the finding that most of the increase in the DNA content of the regenerating kidney does not start until several days after operation (Mandel, Mandel and Jacob, 1950 ; Miyada and Kurnick, 1960). This suggests that the changes in mitotic rate must be determined by some other mechanism than a simple function of the tissue deficit. The mitotic rates found during the later part of the experiment can only be regarded as approximate. The kidney increases in weight until at least 20 days, and there is probably a continuing formation of new cells during the whole period. However, the increases in rate need only be very small. If a mitosis takes about 40 min. to complete, a 70 per cent hyperplasia could be produced in this time by a steady increase in rate of only 1 mitosis/1000 cells, an increase which would require a very large number of animals to define accurately.

The patterns of response obtained when experiments are started at different times of day differ quite markedly, though less than the extreme variations found by Jaffe (1954) in the liver during regeneration. It was not possible to explain the differences between these patterns on various assumptions about the nature of the diurnal changes. These differences as well as the difference in the control figures at different times of day, are important in comparing the results of different experiments. The exact form of the diurnal rhythm and mitotic response may well be strain-specific, and like the relation between kidney weight and body weight would need to be determined for each colony. This is supported by the slightly different results for the diurnal rhythm found by Blumenfeld (1943) and the more marked later response during hyperplasia noted incidentally by other workers (Robertis and Poch, 1947 ; Sulkin, 1949).

In the liver, the sinusoidal basement membrane shows a most interesting series of changes during regeneration starting as early as 3–6 hr., and it has been

suggested that these bear some functional relationship to the onset of mitosis (Aterman, 1952): however, as the basement membranes of the renal glomeruli and tubules are not entirely analogous to those of the hepatic sinusoids, no conclusions can be drawn from the absence of similar changes in the kidney.

The absence of changes in the stainable fat also differs from the marked early accumulation in the regenerating liver (Stowell, 1948): the functional significance of this accumulation is unknown, but the present results suggest that it is more likely to be a reflection of the special relationship of the liver to fat metabolism than a general characteristic of regenerating tissues.

SUMMARY

A survey was made of the mitotic activity of the various cell types and zones in the normal kidney of the albino rat, and in the kidney undergoing compensatory hyperplasia. There is a well-marked diurnal rhythm in the normal kidney. During hyperplasia there was a sharp peak of mitotic activity in the proximal tubules 40 hr. after operation with smaller increases also maximum at that time in the distal tubules. The ascending limbs and collecting ducts showed 2 peaked responses with maxima at 40 hr. and at 3 and 4 days respectively. No definitely significant increase in the number of connective tissue mitoses was found at any time. The mitotic activity was greatest in the outer cortex; mitoses were extremely rare in the inner medulla. No changes could be found in the basement membranes of the tubules or in the distribution of stainable fat.

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REFERENCES

ADDIS, T. AND LEW, W.—(1940) *J. exp. Med.*, **71**, 325.
ATERMAN, K.—(1952) *Arch. Path. (Lab. Med.)*, **53**, 209.
BARTMAN, J.—(1960) *Rev. belge Path.*, **27**, 219.
BLUMENFELD, C. M.—(1943) *Arch. Path. (Lab. Med.)*, **33**, 770.
BRUES, A. M. AND MARBLE, B. B.—(1937) *J. exp. Med.*, **65**, 15.
CARNOT, P. AND MAY, R. M.—(1938) *C.R. Soc. Biol., Paris*, **128**, 641.
DUSTIN, A. P. AND ZYLBERSZAC, S.—(1939) *Bull. Acad. Méd. Belge.*, **4**, 315.
EIGSTI, O. J. AND DUSTIN, P., Jr.—(1955) 'Colchicine in Agriculture, Biology and Chemistry'. Iowa State College Press.
FRANK, G.—(1960) *Arch. Biol.*, **71**, 489.
JAFFE, J. J.—(1954) *Anat. Rec.*, **120**, 935.
MCFARLANE, D.—(1941) *J. Path. Bact.*, **52**, 17.
MACKAY, LOIS L., ADDIS, T. AND MACKAY, E. M.—(1938) *J. exp. Med.*, **67**, 515.
MANDEL, P, MANDEL, LILA AND JACOB, M.—(1950) *C.R. Acad. Sci. Paris*, **230**, 786.
MIYADA, D. S. AND KURNICK, N. B.—(1960) *Fed. Proc.*, **19**, 208.
OGAWA, K. AND SINCLAIR, J. G.—(1958) *Texas Rep. Biol. Med.*, **16**, 215.
PETER, K.—(1908) 'Untersuchungen über Bau und Entwickelung der Niere'. Jena (Fischer).
RICE, H. G. AND JACKSON, C. M.—(1934) *Anat. Rec.*, **59**, 135.
ROBERTIS, E. P. AND POCH, G. F.—(1947) *Rev. Soc. Argent. Biol.*, **23**, 34.
ROLLASON, H. D.—(1949) *Anat. Rec.*, **104**, 263.
STOWELL, R. E.—(1948) *Arch. Path. (Lab. Med.)*, **46**, 164.
SULKIN, N.M.—(1949) *Anat. Rec.*, **105**, 95.