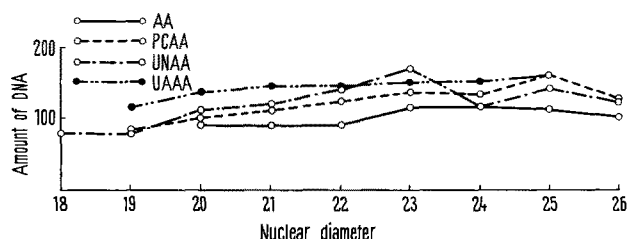


DNA content of the rat kidney nuclei fixed in the 4 different fixatives

No. of nuclei	Fixative	DNA content	Difference between means	t-value	P
40	AA	102.45 \pm 3.50 (A)	A v. B = 13.71	2.51	< 0.025
43	PCAA	116.16 \pm 4.06 (B)	A v. C = 22.59	3.22	< 0.005
42	UNAA	125.04 \pm 5.98 (C)	A v. D = 39.23	6.51	< 0.001
41	UAAA	141.68 \pm 4.88 (D)	B v. C = 8.88	1.23	N.S.
			B v. D = 25.52	4.03	< 0.001
			C v. D = 16.64	2.15	< 0.005



The relation of the amount of DNA and nuclear diameter in tissues fixed by the different fixatives.

was also accelerated in the uranium and platinum containing fixatives as compared with that in acetic acid-alcohol preserved tissue. The mean DNA content in tissues fixed by the 4 different fixatives were statistically analysed to obtain information regarding the relative efficiency of the different fixatives for staining by the Feulgen procedure. The relevant data are presented in the Table and the Figure. From the Table it is evident that yield of DNA values is best in tissues fixed in acetic acid-alcohol containing 1% uranyl acetate (UAAA). The

DNA values, however, remain unaltered in the tissues fixed either in acetic acid-alcohol containing 1% uranyl nitrate (UNAA) or platinum chloride (PCAA). The yield is least in tissues fixed in acetic acid-alcohol (AA). These results thus indicate that the nucleoprotein complex of the cell nuclei is perhaps better precipitated in tissues fixed in acetic acid-alcohol fortified with metallic compounds such as uranyl acetate, uranyl nitrate or platinum chloride, as compared with tissue fixed in acetic acid-alcohol³.

Zusammenfassung. Zugabe von Platinchlorid oder Uranylverbindungen zu Eisessig-Alkohol erhöht den DNS-Wert des fixierten Gewebes beträchtlich.

M. K. DUTT

Department of Zoology of the University, Delhi 7 (India), 1 May 1968.

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Changes in the Numbers of Deoxyribonucleic Acid Synthesizing Nuclei in the Adrenal Cortex Following Unilateral Nephrectomy in the Rat

The changes that occur in the remaining kidney following unilateral nephrectomy have been extensively studied¹⁻³ but little is known about its effect on other organs. During the course of an investigation of the changes in the numbers of deoxyribonucleic acid (DNA) synthesizing nuclei in the rat kidney in compensatory renal hyperplasia⁴ we found that the weight of the adrenal glands was increased following unilateral nephrectomy. We decided to establish if this weight increase was accompanied by any increase in the number of DNA synthesizing nuclei in the various zones of the adrenal cortex.

Materials and methods. The animals used were albino male rats of an inbred strain with an initial body weight of about 200 g, which were maintained on a commercial pellet diet and tap water. Right nephrectomy was performed through a lumbar incision under ether anaesthesia with care to avoid injury to the right adrenal or its blood supply. Antibiotics were not given and post-operative infection did not occur. The rats were killed in groups of 6 or 12 animals at periods ranging from 12 h to 6 weeks after the operation. 4 groups of rats were used as controls, consisting of 2 groups of normal and 2 of sham-nephrec-

tomized animals. The 2 groups of normal rats had body weights of about 200 and 260 g. The 200 g group was used for comparison with the nephrectomized and sham-nephrectomized animals killed during the first week after operation, and the 260 g group for comparison with those killed after a longer interval. The 2 groups of sham-nephrectomized rats were killed at 2 days and 1 week after operation, which consisted of pushing the right kidney through a lumbar incision and then replacing it within the abdominal cavity.

Tritiated thymidine was used to label nuclei synthesizing DNA. It was given as an i.p. injection at a dose level of 0.7 μ Ci/g of final body weight. The labelled thymidine was injected at 10.00 and the rats killed at 14.00 to

¹ G. E. G. WILLIAMS, Br. J. exp. Path. 42, 386 (1961).

² H. A. JOHNSON and J. M. V. ROMAN, Am. J. Path. 49, 1 (1966).

³ G. THRELFALL, D. M. TAYLOR and A. T. BUCK, Am. J. Path. 50, 1 (1967).

⁴ H. P. R. BURY, W. A. J. CRANE and L. P. DUTTA, Br. J. Urol. 37, 201 (1965).

Effect of right nephrectomy on the numbers of DNA synthesizing nuclei in the left adrenal cortex

Operation performed	Time after operation	No. of rats	Final body weight (g)	Left adrenal weight, mg/100 g body weight	Labelling indices, No. of labelled nuclei/100 nuclei	
					glomerulosa	outer fasciculata
None		16	197 \pm 2.0	10.8 \pm 0.5	0.60 \pm 0.06	0.81 \pm 0.13
		6	257 \pm 5.0	9.2 \pm 0.4	0.57 \pm 0.09	0.65 \pm 0.11
Sham nephrectomy	2 days	6	211 \pm 4.9	10.8 \pm 0.7	1.03 \pm 0.18 ^b	1.92 \pm 0.22 ^a
	1 week	6	218 \pm 1.7	11.5 \pm 0.4	0.55 \pm 0.14	0.77 \pm 0.16
Right nephrectomy	12 h	6	213 \pm 2.6	11.5 \pm 0.5	0.50 \pm 0.06	0.55 \pm 0.14
	1 day	6	195 \pm 3.5	11.2 \pm 0.9	1.23 \pm 0.31 ^b	1.83 \pm 0.65
	2 days	11	194 \pm 3.8	13.4 \pm 0.7 ^a	2.40 \pm 0.47 ^a	2.42 \pm 0.34 ^a
	3 days	12	203 \pm 2.8	10.9 \pm 0.3	0.99 \pm 0.10 ^a	1.38 \pm 0.12 ^a
	4 days	12	200 \pm 3.4	14.5 \pm 0.8 ^a	0.73 \pm 0.17	1.87 \pm 0.22 ^a
	1 week	11	219 \pm 3.1	11.6 \pm 0.5	0.67 \pm 0.08	1.12 \pm 0.11 ^b
	2 weeks	12	238 \pm 4.3	11.7 \pm 0.5 ^a	0.53 \pm 0.10	1.00 \pm 0.13 ^b
	3 weeks	11	245 \pm 6.5	11.9 \pm 1.1 ^b	0.72 \pm 0.12	1.15 \pm 0.20 ^b
	4½ weeks	5	277 \pm 12.0	10.6 \pm 0.5 ^b	0.52 \pm 0.15	0.50 \pm 0.13
	6 weeks	6	286 \pm 11.3	8.7 \pm 0.8	0.47 \pm 0.12	0.53 \pm 0.10

Results are expressed as means \pm standard errors. ^a $P < 0.01$, ^b $P < 0.05$.

exclude diurnal variations in DNA synthesis and mitotic rhythm⁵. Autoradiographs of the left adrenal were prepared with Kodak AR 10 film, as previously described⁶, and the % of labelled nuclei (labelling index) was determined in the zona glomerulosa, the outer and inner parts of the fasciculata and in the zona reticularis. The labelling index in each case was based on a count of 2000 nuclei per zone. Counts of the glomerulosa and reticularis were made on the full thickness of the zones. Zona fasciculata was measured on the outer and inner layers of the zone, each layer being 8–10 cells thick. Labelled fibroblasts and vascular endothelial cells were excluded as far as possible from the counts.

Results. (See Table.) The adrenal weights, measured in relation to final body weight of both groups of sham-operated animals, do not significantly differ from those of the control rats. In the uninephrectomized animals the adrenals are significantly heavier at 5 of the time intervals studied, though in the rats killed 6 weeks after operation their weight is within normal limits suggesting that the increase may be only temporary.

The sham-nephrectomized rats show a significant increase in the glomerulosa and outer fasciculata labelling indices at 2 days, but at 1 week the counts have returned to normal.

The uninephrectomized rats show significant increases in the glomerulosa labelling index at 1, 2 and 3 days. The greatest increase is at 2 days when the labelling index is about 4 times that of the control rats and about twice that of the sham-operated animals. The outer fasciculata labelling index of the uninephrectomized rats is raised for a much longer period. It is significantly increased in the groups killed between 2 days and 3 weeks after operation and has returned to normal levels by 4½ weeks. The greatest increase is at 2 days when the labelling index is about 3 times that of the control rats and is slightly greater than that of the sham operated animals.

The labelling indices of the inner fasciculata and reticularis of both sham-operated and uninephrectomized groups did not significantly differ from those of the control rats and are not included in the Table. In the 200 and 260 g control groups the labelling indices of the inner fasciculata were 0.19 and 0.12 and of the reticularis 0.33 and 0.23 respectively.

Discussion. WRETE⁷ found that in mice killed 1 month after unilateral nephrectomy the hypophysis and thyroid were significantly heavier than those of controls and that the adrenals showed no difference in wet weight but probably some decrease in dry weight. Our results show an increase in adrenal weight in the rat following uninephrectomy, though this increase may be only temporary. We further show that this increase in weight is accompanied by an increase in the number of DNA synthesizing nuclei in the glomerulosa and the outer fasciculata.

A sham-nephrectomy also leads to an increase in DNA synthesizing nuclei but in this case the magnitude of the increase in the glomerulosa is much less and in the outer fasciculata its duration is very much shorter. BRENNER⁸ found that when mice were stressed by a s.c. injection of carbon tetrachloride there was an increase in the number of DNA synthesizing nuclei in the glomerulosa and outer fasciculata which reached a maximum on the third or fourth day and had returned to normal levels by 1 week. These results correspond fairly closely to our findings following the stress of sham-nephrectomy in the rat.

The increased duration of the response seen after uninephrectomy in the outer fasciculata and possible the increased magnitude of the response in the glomerulosa make it likely that an additional factor, possibly homeostatic readjustment to the nephrectomy, is superimposed upon the initial stress effect of the operation.

Zusammenfassung. Histoautoradiographisch wird mit ³H-Thymidin der Nebennierenrinde gezeigt, dass nach unilateraler Nephrektomie bei der Ratte die Thymidineinbaurate in der Nebenniere (zona glomerulosa und zona fasciculata) ansteigt.

H. P. R. BURY and W. A. J. CRANE

Department of Pathology, University of Sheffield (England), 3 April 1968.

⁵ T. E. HUNT and E. A. HUNT, *Anat. Rec.* 149, 387 (1964).

⁶ W. A. J. CRANE and L. P. DUTTA, *J. Path. Bact.* 86, 83 (1963).

⁷ M. WRETE, *Acta anat.* 2, 81 (1946).

⁸ R. M. BRENNER, *Am. J. Anat.* 112, 81 (1963).