THE INFLUENCE OF HYPOTENSIVE DRUGS ON RENAL STRUCTURE IN EXPERIMENTAL HYPERTENSION,

(with particular reference to the relationship of hydrallazine to the experimental reproduction of disseminated lupus erythematosus).

VOLUME II

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CHAPTER 6

Experimental Series V.

Introduction.

In previous experiments it was shown that DCA in large doses profoundly modified renal structure, and that the severity and extent of the changes produced in this way varied according to the route by which the DCA was given and according to the rapidity of its absorption. Later, the action of hydrallazine on the kidneys of animals subjected to the DCA hypertensive regime was studied, and the evolution of focal hemiglomerular necrosis demonstrated under certain specific conditions.

In investigating the precise mechanism by which these focal glomerular lesions were brought about stress was laid on the possible influence of certain incidental effects of hydrallazine, such as its convulsive action, and on the possible role of anaesthesia in promoting a glomerulotoxic action. (See experiments III(a) and III(b), pages 224, 226). It was further suggested that the influence of DCA in causing hypertension might be the crucial factor in precipitating glomerular lesions - although in fact the most striking lesion of malignant hypertension, fibrinoid necrosis, was prevented by the very administration of

hydrallazine which precipitated glomerular necroses. This suggestion was investigated by subjecting animals to another procedure known to cause hypertension , namely the application of unilateral renal artery clamps, and injecting them in turn with progressively larger doses of hydrallazine (experiment IV, page 232)

The same argument suggested that the role of DCA in the evolution of glomerulonecrosis might be more clearly defined by substituting steroids with similar actions in comparable experiments. In this way, it was thought that it might prove possible to determine the importance of the action of DCA on electrolyte balance, in a manner similar to the use of "renal" hypertension in experiment IV to define the importance of an action on the blood pressure mechanism.

With this aim in mind, it was decided to examine the effect of hydrallazine on the kidneys of animals subjected to the action of other steroids with certain properties similar to those of DCA. For this purpose 9 α-fluorocortisol and cortisone were chosen. Each of these was known to influence body electrolyte regulation. It is known that cortisone may cause hypertension in certain circumstances in experimental animals, while 9 α-fluorocortisol

is noted for the potency of its action on sodium excretion.

Experiment V (a)

(Animal groups 19, 20, 34)

The experiment was performed in two parts. In the first part (animal group 19) 9 α-fluorocortisol was given in increasing doses to a group of rats maintained on 1% sodium chloride solution following unilateral nephrectomy. The animals were given salt in place of drinking water. A further group of animals (group 20) was subjected to unilateral nephrectomy, given salt to drink, and injected daily with 9 α-fluorocortisol: in addition these animals were treated by the daily injection of increasing amounts of hydrallazine.

In the second part a further group of animals was given daily injections of 9 α-fluorocortisol and, later, injections of increasing amounts of hydrallazine, but were not subjected to previous unilateral nephrectomy and were not given salt.

Group 19

Materials:

Four male and four female albino Wistar rats of a

laboratory bred strain were used. They weighed 90 - 120 g. They were maintained in a manner which did not differ significantly from that used in the case of the animals of earlier experiments.

9 α-fluorocortisol was used in the form of a 0.025% oily suspension. Later an aqueous suspension with tragacanth was found more simple to handle.

Methods:

Unilateral (left) nephrectomy was performed by the standard procedure (Appendix 2, Volume II).

Histological methods employed at the close of the experiment did not differ from thos of earlier experiments.

Procedure:

Following left nephrectomy, 1% sodium chloride solution was substituted for drinking water and daily injections of 0.1 ml. 9 α-fluorocortisol started (0.025 mg.) by the intramuscular route. Blood pressures and weights were recorded weekly, or as often as was possible. The amounts of 9 α-fluorocortisol was gradually increased, and at the end of the experiment (after 5 weeks) the dose had

risen to 0.2 mg. At this time the animals were killed by bleeding under ether anaesthesia, and tissue collected for histological examination.

Group 20.

Materials:

Four male and four female albino Wistar rats of a laboratory bred strain were used, weighing 30 - 60 g. They were maintained in the same way as those of the preceding group.

9 α-fluorocortisol was used in the form of a 0.025% aqueous suspension.

Hydrallazine was used as a 2% solution, each sterile ampoule containing 20 mg. in 1 ml.

Methods:

Unilateral (left) nephrectomy was performed by the standard procedure (Appendix 2, Volume II).

Serum electrolytes were measured by flame photometer.

Blood pressures were recorded by tail

plethysmograph (Appendix 4, Volume II).

Histological methods used at the end of the experiment were identical with those used in earlier experiments.

Procedure:

Following left nephrectomy, 1% sodium chloride was substituted for the drinking water and daily intramuscular injections of 0.1 ml. of 0.025% suspension of 9 α-fluorocortisol were started. The amount given was similar to that used in the preceding group of animals and rose to 0.2 mg. daily at the close of the experiment. Beginning one week after the operation, increasing amounts of hydrallazine were given by single daily intramuscular injection, starting with 0.1 ml. (2 mg.) and increasing gradually to 4 mg. daily after five weeks. The five animals surviving for this time were then killed by bleeding under ether anaesthesia and the principal organs subjected to histological examination.

Group 34

Material:

Four male and four female albino Wistar rats of

of a laboratory bred strain, weight 80 - 120 g., were used. They were maintained in circumstances which did not differ significantly from those of animals in previous groups.

9 α-fluorocortisol was used in the form of a 0.025% aqueous suspension.

Hydrallazine was used as a 2% sterile solution.

Methods:

Blood pressures were recorded by tail plethysmograph (Appendix 4, volume II).

Histological methods used were identical with those of previous experiments.

Serum electrolytes were recorded by flame photometer.

Procedure:

Injections of increasing amounts of 9 α-fluorocortisol were given daily by the intramuscular route, beginning with an amount of 0.1 ml. (0.025 mg.) and increasing more slowly than in the previous experiments to a dose of 0.3 ml. (mg. 0.075) after three weeks. At the same time

hydrallazine in increasing amounts was also given daily intramuscularly, starting with ml. 0.05 (mg. 1) and rising after three weeks to mg. 6. At the end of this time the animals were killed and the organs subjected to histological examination.

Results:

Group 19

Blood pressure: (Text Fig. 26, Table 82)

No significant change in blood pressure levels was detected in either male or female group.

Weight: (Text. Fig. 27, Table 83)

For the first four weeks of the experiment the weight of both male and female groups remained almost constant, neither decreasing nor showing the normal age increase. After this time a rapid decline in weight began and continued until the time of death.

Terminal measurements: (Table 84)

Heart weight:

The mean heart weight was 0.54 g., and the mean

Text Figure 26.

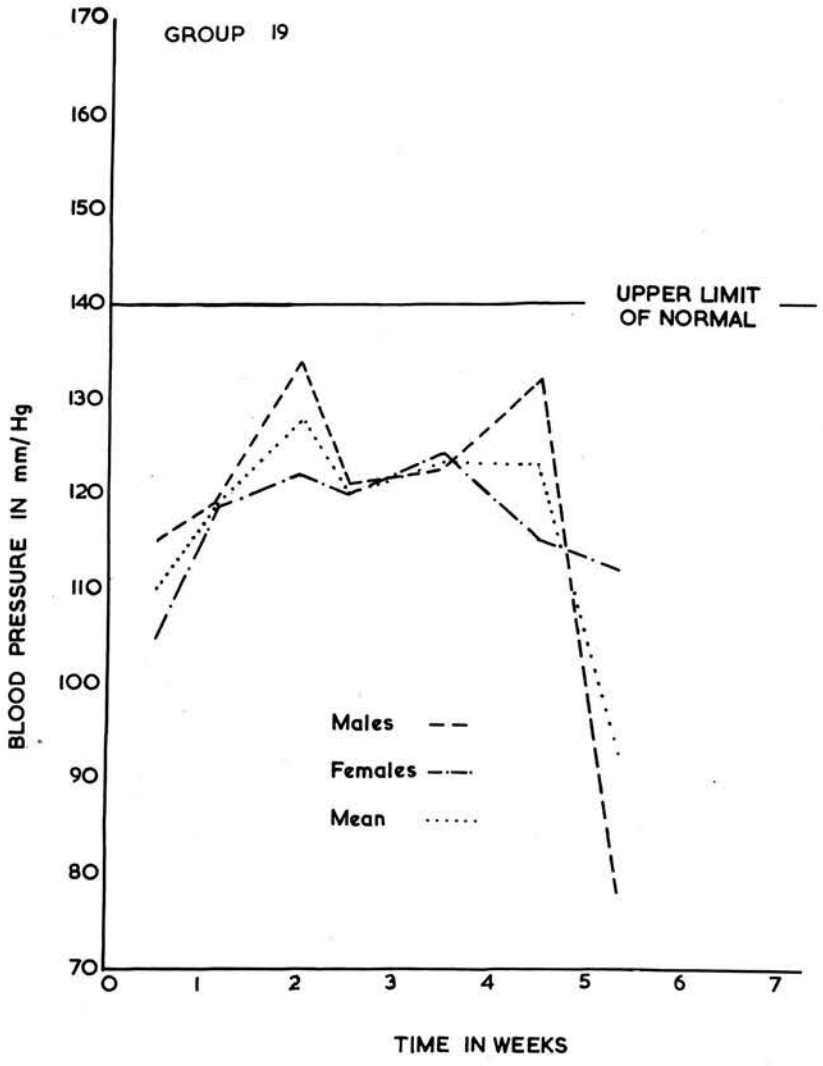


TABLE 82.

Experiment V(a). Animal Group 19.

BLOOD PRESSURES.

Rat	Date 26.10.57	30.10.57	4.11.57	7.11.57	14.11.57	21.11.57	27.11.57
Male							
1	122	128	124	120	127	155	62
2	132	94	127	126	115	110	82
3	92	134	138	118	128	132	91
Mean	115	119	134	121	123	132	78
Female							
1	112	147	119	123	126	105	146
2	78	96	119	126	138	155	82
3	129	118	130	124	114	98	
4	101	115	119	121	118	103	
Mean	105	119	122	123	124	115	114
Grand Mean	110	119	128	122	123	123	93

Text Figure 27.

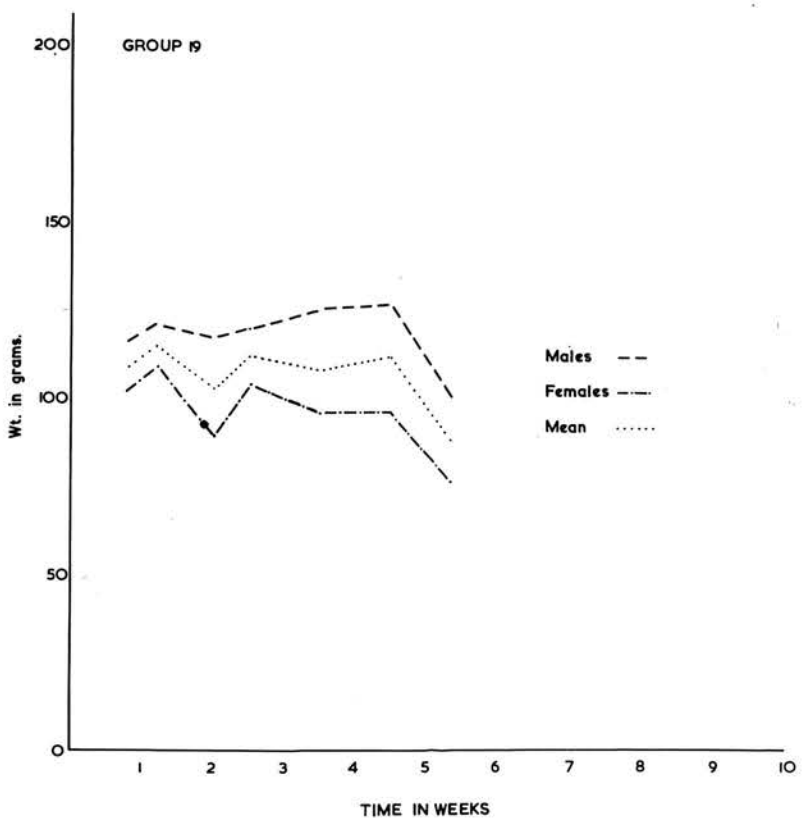


TABLE 83.

Experiment V(a). Animal Group 19.

WEIGHTS.

Rat	Date 26.10.57	30.10.57	4.11.57	7.11.57	14.11.57	21.11.57	27.11.57
Male							
1	95	105	95	100	120	150	85
2	145	145	145	145	145	112	95
3	108	112	110	115	110	118	120
Mean	116	121	117	120	125	127	100
Female							
1	112	145	90	95	90	87	83
2	85	115	110	110	105	108	83
3	122	85	75	125	110	109	65
4	90	94	85	85	80	80	
Mean	102	109	90	104	96	96	77
Grand Mean	109	115	108	112	108	112	88

TABLE 84.

Experiment V(a). Animal Group 19.

TERMINAL MEASUREMENTS.

Males	Weight g.	R.kidney g.	Heart g.	Serum Sodium mg./100 ml.	Serum Sodium m.Eq./l	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
1	-	-	-	-	-	-	-
2	95	0.76	0.53	716	311.3	17.2	4.41
3	85	0.70	0.54	540	234.8	26.8	6.88
4	120	0.98	0.60	550	239.2	21.5	5.51
Mean *	100	0.81	0.56	602	262	21.8	5.6
Females							
1	-	-	-	-	-	-	-
2	83	0.61	0.57	520	226.0	17.0	4.36
3	83	0.68	0.46	-	-	-	-
4	65	0.66	0.53	500	217.4	20.0	5.12
Mean *	77	0.65	0.52	510	222.0	18.5	4.74
Grand Mean *	88	0.73	0.54	536	239.7	20.1	5.17

* Mean of survivors of experiment.

heart/body weight ratio 0.61). The difference between males and females was not significant.

Kidney weight:

The mean weight of the kidneys at the end of the experiment was 0.73 g. and the mean kidney/body weight ratio 0.83%. Again the difference between the sexes was not significant.

Serum sodium:

Extremely severe disturbances in serum sodium levels were found. The mean serum sodium after five weeks treatment was 239.7 m.Eq./l (normal 135 - 145 m.Eq./l.). In the case of one male animal the level reached 311.3 m. Eq./l., and largely as a result of this single observation the mean male serum sodium was considerably higher than the female.

Serum potassium:

With the exception of a single male animal the levels of serum potassium fell within the normal range (mean 5.17 m.Eq./l.; normal 3.5 - 5.5 m.Eq./l.).

Histological changes:

Surprisingly few glomerular changes were found. It was occasionally possible to detect slight enlargement of the tufts, but the severity of this change was minimal. Tubular changes were largely restricted to the slight to moderate dilatation of cortical collecting tubules, and the intratubular protrusion of both cytoplasm and nuclei of the epithelial cells of both proximal convoluted and of collecting tubules, some of the cells of the latter also showing vacuolation.

There was no ballooning of glomerular cells, no glomerular necroses, and no vascular changes.

Group 20

Blood pressure: (Text Fig. 28, Table 87)

The mean blood pressure level recorded five hours after the daily injection of hydrallazine was considerably lower than in control group 19. Males and females did not differ significantly.

Weight: (Text Fig. 29, Table 88)

As with group 19, there was a constant initial rise in weight, of equal degree in both sexes, coming to an end

TABLE 85.

Experiment V(a). Animal Group 19.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR AND VASCULAR CHANGES	Rat Males 2	3	4	Females 1 (Autolysis)	2	3	4 (Autolysis)
Endothelial cell proliferation	-	-	-	-	-	+	-
Epithelial cell proliferation	-	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-	-
Crescent formation	-	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-	-
Endothelial cell swelling	(+)	(+)	(+)	-	-	-	-
Enlargement of tufts	(+)	(+)	(+)	-	(+)	+	-
"Ballooning" of cells	(+)	-	-	-	-	-	-
Segmental tuft necrosis (acute)	-	-	-	-	-	-	-
" " (subacute)	-	-	-	-	-	-	-
" " (fibrosed)	-	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-
"Explosive" lesions	-	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-	-	-	-	-	-

TABLE 86.

Experiment V(a). Animal Group 19.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males 2	3	Rat 4	1	Females 2	3	4
Dilatation of				(Autolysis)			(Autolysis)
a. prox.conv. tubules	-	-	-	-	-		-
b. thin Henle limb	-	-	-	-	-		-
c. thick Henle limb	-	-	-	-	-		-
d. dist.conv. tubules	-	-	-	-	-		-
e. collecting tubules	+	+	(+)	-	-	+	-
Cytoplasmic protrusion tubular cells.	++ prox.conv. & coll. tubules	++ prox.conv. & coll. tubules	++ prox.conv. & coll. tubules	-	++ prox.conv. & coll. tubules	++ coll.	(+) Conv.& coll. tubules
Nuclear protrusion of tubular cells.	"	"	"	-	"	"	"
Vacuolation of epithelium of collecting tubules	(+)	(+)	(+)	-	-	-	-
Proteinuria	-	-	-	-	(+)	-	-
Hyaline casts	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-	-

Text Figure 28.

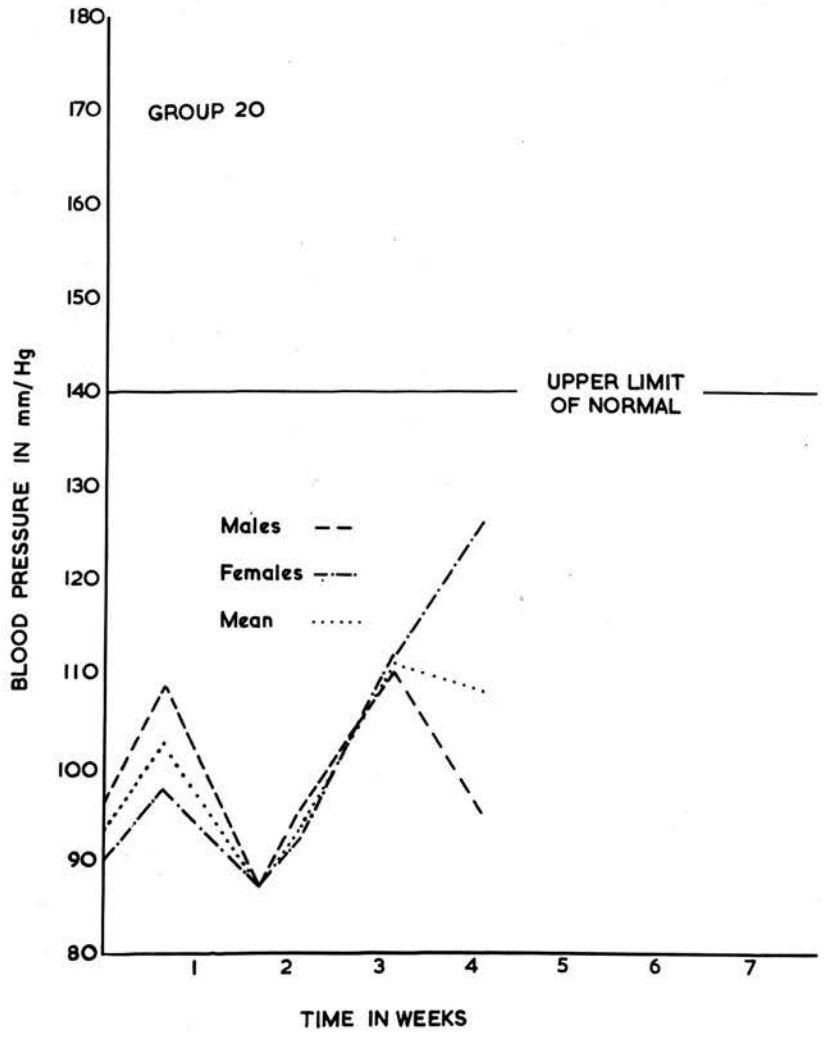


TABLE 87.

Experiment V(a). Animal Group 20.

BLOOD PRESSURES in mm./Hg.

Rat	Date 26.10.57	30.10.57	4.11.57	7.11.57	14.11.57	21.11.57	29.11.57
Male							
1	108	99	84	81	109	117	
2	92	96	94	91	111	93	
3	96	112	85	99	109	83	
4	88	129	84	96	116	89	
Mean	96	109	87	92	111	95	
Female							
1	74	89	85	89	106	123	
2	100	114	81	100	114	125	
3	96	90	88	105	117	129	
4	92	94	94	87			
Mean	90	97	87	95	112	126	
Grand Mean	93	103	87	93	112	108	

Text Figure 29.

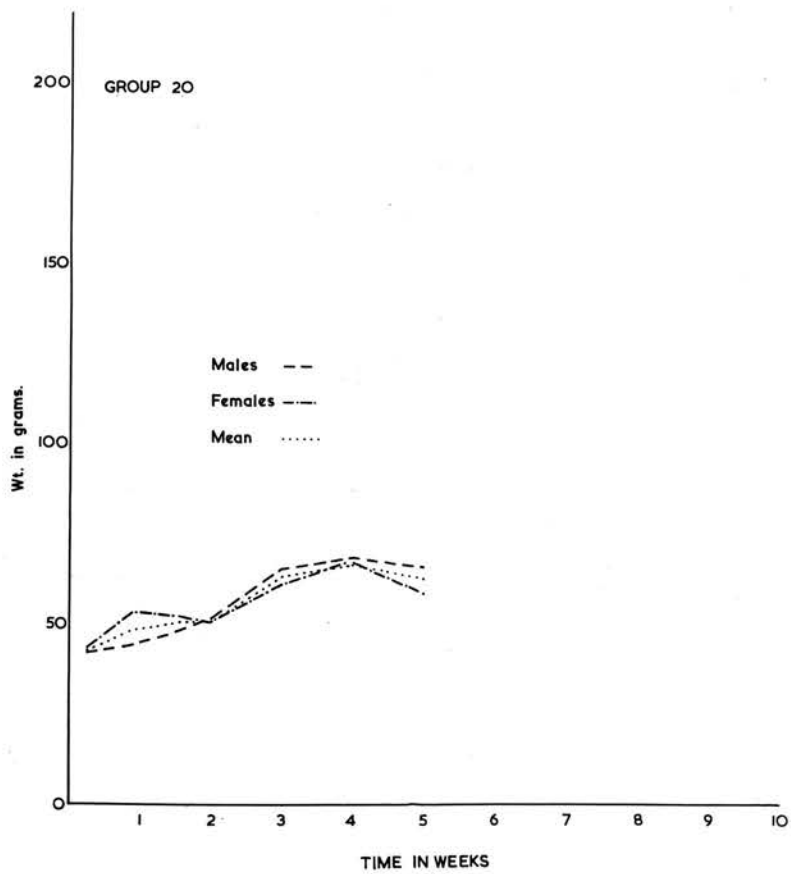


TABLE 88.

Experiment V(a). Animal Group 20.

WEIGHTS IN GRAMS.

Rat	Date 26.10.57	30.10.57	4.11.57	7.11.57	14.11.57	21.11.57	28.11.57
Male							
1	45	54	50	50	70	75	60
2	43	55	55	45	65	68	70
3	42	50	48	55	50	57	
4	44	55	50	55	60	71	
Mean	43	53	52	53	61	67	65
Female							
1	40	45	50	50	70	65	55
2	43	47	45	45	60	75	60
3	44	42	48	55	65	63	63
4	47	43	50	50			
Mean	43	44	48	50	65	68	59
Grand Mean	43	48	50	50	63	67	62

TABLE 89.

Experiment V(a). Animal Group 20.

TERMINAL MEASUREMENTS.

Males	Weight	R.kidney	Heart	Serum sodium mg./100 ml.	Serum sodium m.Eq./l.	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	60	0.72	0.75	370	160.8	13.6	3.49
4	70	0.88	0.58	355	154.3	15.4	3.95
Mean*	65	0.80	0.66	362	157.0	14.5	3.72
Females							
1	-	-	-	-	-	-	-
2	55	0.67	0.57	516	224.4	13.6	3.49
3	60	0.64	0.45	460	200.0	14.5	3.75
4	63	0.74	0.57	590	256.6	18.9	4.83
Mean*	59	0.67	0.53	522	227.0	15.3	4.02
Grand Mean *	62	0.72	0.58	458	199.0	14.9	3.90

* Mean of survivors of experiment.

after four weeks and being succeeded by a fall which continued to the end of the experiment.

Terminal measurements (Table 89)

Heart weight:

The mean heart weight at the close of the experiment was 0.58 g., the heart/body weight ratio being 0.94%. The difference between the sexes was not significant.

Kidney weight:

The mean kidney weight at the close of the experiment was 0.72 g., the kidney/body weight ratio being 1.16%. The difference between the sexes was not significant.

Serum Sodium:

The mean serum sodium level at the end of the experiment was 199.0 m.Eq./l., the measurements for the female survivors being significantly higher than for the male. (Normal 135 - 145 m.Eq./l.)

Serum potassium:

The mean serum potassium level (3.9 m.Eq./l.)

fell within the normal range(3.5 - 5.5 m.Eq./l.). The female levels were slightly higher than the male.

Histological changes: (Tables 90, 91)

Glomerular changes were slight and little different from those of group 19. There was no evidence of tuft necrosis. Patchy dilatation of the cortical collecting tubules was seen, accompanied in some instances by the protrusion of cytoplasm and nuclei of both proximal convolutions and of collecting tubules into the tubular lumina. Occasional vacuolation of medullary collecting tubule cells was recognised.

No vascular changes were seen.

Group 34

Blood pressure: (Table 92)

Small numbers of measurements only were made, but no evidence was obtained that the blood pressure was influenced by treatment. Hydrallazine appeared effectively to suppress any tendency for the pressure to rise. There was no sex difference in the response.

TABLE 90.

Experiment V(a). Animal Group 20.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Males		Females		
	3	4	2	3	4
Endothelial cell proliferation	-	(+)	-	-	-
Epithelial cell proliferation	-	-	-	-	-
Capsular thickening	-	-	-	-	-
Capsular adhesions	-	-	-	-	-
Tubularisation	-	-	-	-	-
Endothelial cell swelling	-	-	-	-	-
Enlargement of tufts	-	(+)	-	-	-
Segmental tuft necrosis acute	-	-	-	-	-
" " " subacute	-	-	-	-	-
" " " fibrosed	-	-	-	-	-
Transudative capillary change	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-
"Explosive" lesions	-	-	-	-	-
Fibrinoid arteriolar nedrosis	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	- Crowding of glomeruli	-	-	-

TABLE 91.

Experiment V(a). Animal Group 20.

Extent and severity of renal changes.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males			Females	
	3	4	2	3	4
Dilatation of					
a. prox.conv. tubules	-	-	-	-	(+)
b. dist.conv. tubules	-	-	-	-	-
c. thin Henle limb	-	-	-	-	-
d. thick Henle limb	-	-	-	-	-
e. collecting tubules	-	(+)	+	(+)	+
Protrusion of tubular cytoplasm	+ prox.conv.& collecting tubules	-	(+)	+ collecting tubules	(+) coll. tubules prox.conv.tubules
Protrusion of nuclei	(+) prox.conv.& collecting tubules	-	(+)	+ collecting tubules	(+) collecting tubules
Vacuolation of collecting tubular epithelium	+	(+)	(+)	(+)	(+)
Proteinuria	-	-	-	-	-
Hyaline casts	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-
Necrosis	-	-	-	-	-
Regeneration	-	-	-	-	-

TABLE 92.

Experiment V(a). Animal Group 34.

BLOOD PRESSURES in mm./Hg.

Males	19.2.58	24.2.58
1	90	
2	94	105
3	70	86
4	65	94
Mean	76	95
Females		
1	72	79
2	82	98
3	78	96
4	90	110
Mean	81	97
Grand Mean	78	96

TABLE 93.

Experiment V(a). Animal Group 34.

WEIGHTS in grams.

Males	19.2.58	24.2.58
1	180	165
2	160	150
3	148	155
4	155	170
Mean	166 g.	163 g.
Females		
1	125	135
2	120	125
3	115	134
4	135	142
Mean	124	134
Grand Mean	145	149

Weight: (Table 93)

No significant weight gain was detected during a small series of observations, and no evidence of a significant sex difference was seen.

Terminal measurements (Table 94)

Heart weight:

The mean heart weight at the close of the experiment was 0.99 g., the heart/body weight ratio being 0.7). There was no sex difference between these observations.

Kidney weight:

The mean kidney weights at the close of the experiment were respectively 0.89 g. and 0.84 g. for the left and right kidneys. The kidney/body weight ratios were 0.63% and 0.59%, and the sexes did not differ significantly in this respect.

Serum sodium:

The mean serum sodium level had risen (159 m.Eq./l.) and was outside the range of normality (135 - 145 m. Eq./l). The mean level for females, as in group 20, was higher than for males.

TABLE 94.

Experiment V(a). Animal Group 34.

TERMINAL MEASUREMENTS.

Males	Weight g.	Heart g.	Kidneys g.		Serum Sodium mg./100 ml.	Serum Sodium m.Eq./l.	Serum Potassium. mg./100 ml.	Serum Potassium. m.Eq./l.
1	154	1.20	1.07	1.01	368	160.0	16.4	4.21
2	156	0.87	0.99	0.93	360	156.5	17.9	4.59
3	154	0.90	1.01	0.85	332	144.3	19.2	4.91
4	169	1.40	0.92	0.91	360	156.5	20.5	5.25
Mean	158 g.	1.09g.	0.99g.	0.92g.	355	154.3	18.5	4.74
Females								
1	120	0.93	0.75	0.77	365	158.7	15.9	4.08
2	122	0.82	0.75	0.72	400	174.0	16.4	4.21
3	130	0.85	0.81	0.82	376	163.4	14.6	3.75
4	125	1.00	0.87	0.75	368	160.0	17.4	4.46
Mean	124 g.	0.9g	0.79g.	0.76g.	377	164.0	16.1	4.12
Grand Mean	141 g.	0.99g.	0.89g.	0.84g.	366	159.1	17.3	4.43

Serum potassium:

The mean serum potassium level at the close of the experiment (4.43 m.Eq./l.) fell within the normal range, (3.5 - 5.5 m.Eq./l.), and there was no significant sex difference.

Histology: (Tables 95, 96)

Histological changes were conspicuously absent. There was no evidence of glomerular enlargement, or of glomerular necrosis and the renal tubules were apparently intact. No vascular lesions were found.

Discussion:

The results of these experiments showed first that 9 α-fluorocortisol did not cause renal changes similar to those resulting from overdosage with DCA although the influence on sodium balance, reflected indirectly by alteration in terminal estimations of sodium, was very considerably greater; and, second, that treatment of these animals with increasingly large doses of hydrallazine did not result in renal lesions similar to those which follow the administration of hydrallazine to animals with DCA overdosage.

TABLE 95.

Experiment V(a). Animal Group 34.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Males 1	2	3	4	Females 1	2	3	4
Endothelial cell proliferation	-	-	-	-	-	-	-	-
Epithelial cell proliferation	-	-	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-	-	-
Crescent formation	-	-	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-	-	-
Enlargement of tufts	-	-	-	-	-	-	-	-
"Ballooning" of tuft cells	-	-	-	-	-	-	-	-
Segmental tuft necrosis (acute)	-	-	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-	-
Explosive lesions	-	-	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication.	-	-	-	-	-	occasional interstitial aggregates of chronic inflammatory cells	-	-

TABLE 96.

Experiment V(a). Animal Group 34.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males				Females			
	1	2	3	4	1	2	3	4
Dilatation of								
a. prox.conv.tubules	-	-	-	-	-	-	-	-
b. thin Henle limb	-	-	-	-	-	-	-	-
c. wide Henle limb	-	-	-	-	-	-	-	-
d. dist.conv.tubules	-	-	-	-	-	-	-	-
e. collecting tubules	-	-	-	-	-	-	-	-
Cytoplasmic protrusion of tubular cells	-	-	-	-	-	-	-	-
Nuclear protrusion of tubular cells	-	-	-	-	-	-	-	-
Vacuolation of collecting tubule cells	-	-	-	-	-	-	-	-
Proteinuria	-	-	-	-	-	-	-	-
Hyaline casts	-	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-	-	-

In group 19, 9 α-fluorocortisol appeared to suppress normal body growth, while causing sodium retention. Nevertheless the retention of sodium was not reflected in an increase of systemic blood pressure, nor in the production of oedema. A slight progressive increase in body weight was noted, but this was ultimately succeeded by an abrupt terminal loss of weight. Accompanying the slight overall weight change, and the absence of influence on blood pressure, were minimal organ weight changes. In no instance did the measurements suggest unusual hypertrophy of heart, or hyperplasia of kidney, findings which accord with the microscopic analysis.

Irrespective of the ultimate chemical explanation of the mode of action of 9 α-fluorocortisol, the evidence derived from this experiment indicated that this steroid was much more potent than DCA in influencing sodium retention (at least as far as this is reflected in alterations in serum sodium levels) and much less potent in its influence both on renal structure and on blood pressure. In this context, the apparent discrepancy between the severity of the renal changes caused by DCA and those caused by 9 α-fluorocortisol is noteworthy in view of the importance attached by many authors to the relationship between the salt retention caused by DCA and its effect both on renal

structure and on blood pressure levels. There is some evidence that this difference may be limited to certain species (Selye and Hall, 1943).

The evidence obtained from the earlier experiments suggested that the role of hydrallazine in precipitating glomerular necrosis was perhaps related to the mode of action of slowly absorbed DCA. It was therefore anticipated that 9 α-fluorocortisol and hydrallazine would influence renal structure. The observations made with group 19 suggested that this relationship is not a close one (as far as renal structure and sodium balance in the rat are concerned) and the discovery (group 20) that hydrallazine did not react in 9 α-fluorocortisol overdosage as it did with excess DCA was not altogether surprising.

In analysing the results obtained in group 34, two factors were thought to be particularly important. The first of these was the shorter period of treatment; the second was the lower dose of 9 α-fluorocortisol given, chosen because it was thought that the rapid terminal weight loss in groups 19 and 20, and indeed the failure to gain weight appreciably during the experiment, were the consequences of an excessively high dose.

The results confirmed that the lower dose did not

affect the renal response, but it should also be remembered that unilateral nephrectomy had not preceded treatment, a choice of procedure made at the same time as the decision to reduce the dose of 9 α-fluorocortisol and for the same reason.

Summary:

(1) To investigate the role of DCA in the causation of the focal glomerular lesions which accompany prolonged use of hydrallazine, other steroids with certain similar general properties were selected for comparison.

(2) The first, 9 α-fluorocortisol, which has an intense sodium retaining action, was used to examine the importance of the salt-retaining action of DCA in the pathogenesis of the lesions precipitated by hydrallazine.

(3) It was shown that 9 α-fluorocortisol did not cause glomerular, tubular or vascular lesions resembling those caused by DCA in spite of its confirmed and intense action on sodium metabolism.

(4) The influence of increasingly large doses of hydrallazine on the kidneys of animals given 9 α-fluorocortisol was investigated. It was shown that focal glomerular lesions were not produced. The experiment involved the study of two dosage schedules of 9 α-fluorocortisol neither

of which predisposed to the glomerular-damaging action of hydrallazine.

Experiment V (b)

(Animal groups 17, 18)

In the introduction to experimental series V reasons were given for studying the relationship between the nature of the steroid and the modifying effect of hydrallazine. It was suggested that the glomerular necroses precipitated by hydrallazine in the kidneys of uninephrectomised animals given excess DCA and salt might not be specifically the effect of DCA, but might be the result of an incidental disturbance, e.g. on electrolyte metabolism or on blood pressure. To investigate this suggestion, the effects of 9 α fluorocortisol were studied (experiment V(a), page 4). In the present experiment, and the two which followed it, the role of another steroid, cortisone, was investigated in the same way.

Material:

3 male and 4 female albino rats of a laboratory bred strain were used in each of groups 17 and 18. They weighed 100 - 120 g. and were maintained in circumstances which did not differ significantly from those used in earlier experiments.

Cortisone was used in the form of a water-miscible suspension of the acetate each 10 ml. sterile bottle containing 25 mg.

Hydrallazine was used as a 2% aqueous solution.

Methods:

Bilateral adrenalectomy was performed through lumbar incisions. At the same time the left kidney was removed by the standard technique (Appendix 2, Volume II).

Blood pressures were recorded by tail plethysmograph (Appendix 4, Volume II).

Histological methods used in the examination of material obtained at the close of the experiment did not differ significantly from those used previously.

Procedure:

The left kidney and left adrenal were removed through a left lumbar incision and the right adrenal through a right lumbar incision, under ether anaesthesia. Following operation, the animals were injected with cortisone 2.5 mg. intramuscularly at once and each day thereafter. 1% sodium chloride was substituted for the drinking fluid.

Beginning one week after operation the animals of group 18 were injected daily with increasing amounts of hydrallazine by the intramuscular route. The amount given rose gradually from mg. 3 at the beginning of the experiment to mg. 5.5 at the time of death one month later. No larger dose could be used owing to the extreme sensitivity of the cortisone-maintained, bilaterally-adrenalectomised animal to the action of hypotensive drugs; and in fact this experiment had to be terminated somewhat sooner than planned owing to rapid weight loss and a high spontaneous mortality rate.

At the end of the four weeks period of treatment the animals were killed by bleeding under ether anaesthesia, and the blood so obtained used for the estimation of serum sodium and potassium levels. Tissue was collected from the principal organs and submitted to histological examination.

Results:

Group 17

Blood pressure: (Text. Fig. 30, Table 97)

A transient apparent rise in mean blood pressure during the early part of the experiment was succeeded by a period during which the mean pressure fluctuated but did not

Text Figure 30.

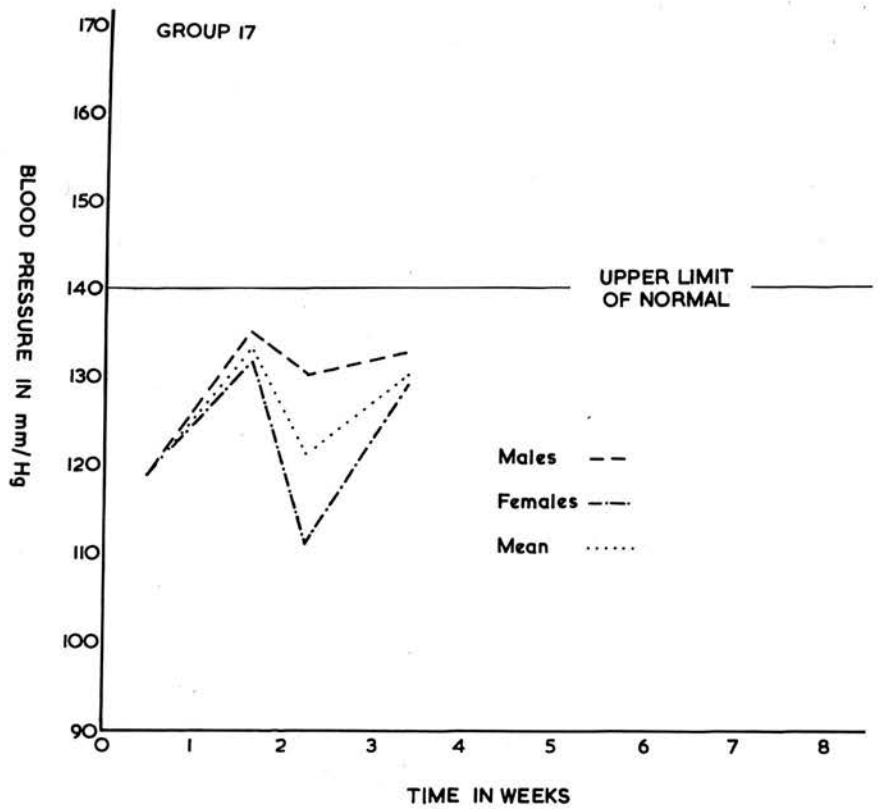


TABLE 97.

Experiment V(b). Animal Group 17.

BLOOD PRESSURES IN mm./Hg.

Rat	Date 8.11.57	16.11.57	20.11.57	25.11.57
Male				
1	121	131	110	116
2	116	139	151	149
3				
4				
Mean	119	135	130	133
Female				
1		128	123	118
2		137	95	139
3		130	114	130
4				
Mean		132	111	129
Grand Mean	119	133	121	130

rise significantly. There was no sex difference in this response.

Weight: (Text Fig. 31, Table 98)

A steady decline in weight occurred throughout the experiment. The rate and severity of the decline was similar for males and females.

Terminal measurements: (Table 99)

Heart weight:

The mean heart weight at the close of the experiment was 0.61 g., and the heart/body weight ratio 0.70%. The ratio was higher in males (0.80%) than in females (0.69%).

Kidney weight:

The mean kidney weight at the close of the experiment was 0.89 g., and the kidney/body weight ratio 1.01%. The ratio for males (1.14%) was higher than that for females (0.93%).

Serum sodium:

The mean terminal serum sodium was 148.7 m.Eq./l.,

Text Figure 31.

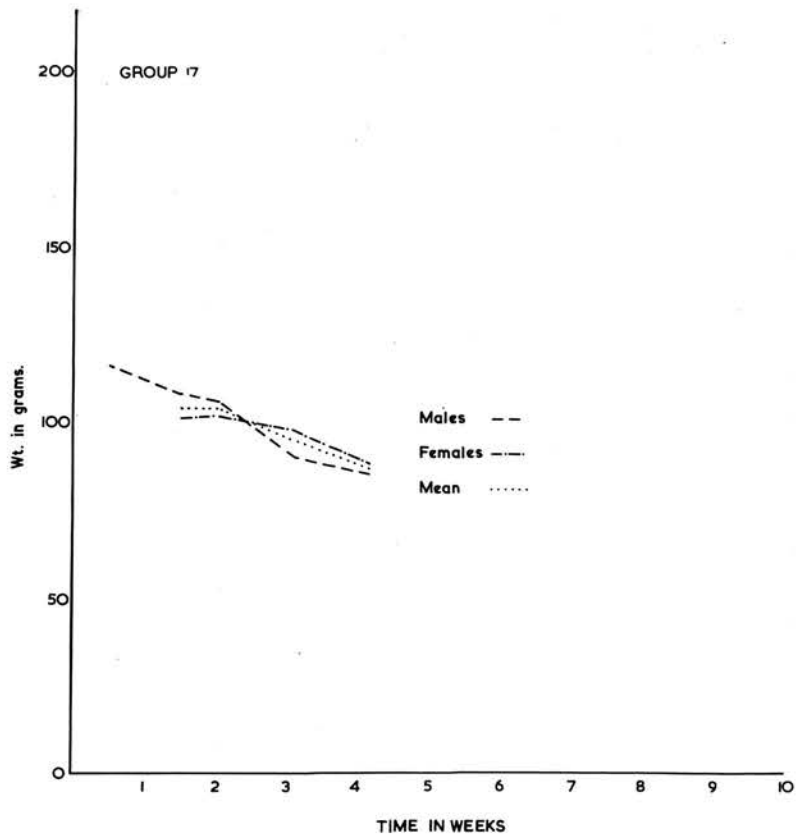


TABLE 98.

Experiment V(b). Animal Group 17.

WEIGHTS in grams.

Rat	8.11.57	16.11.57	20.11.57	28.11.57	6.12.57
Male					
1	125	112	102	80	75
2	120	105	110	100	98
3					
4					
Mean	117	108	106	90	86
Female					
1		106	98	110	110
2		102	115	85	85
3		95	94	90	69
4					
Mean		101	102	98	88
Grand Mean	117	104	104	95	87

TABLE 99.

Experiment V(b). Animal Group 17.

TERMINAL MEASUREMENTS.

Males	Weight g.	R.kidney g.	Heart g.	Serum sodium mg./100 ml.	Serum sodium m.Eq./l.	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
1 (11.11.57)	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	75	0.87	0.63	346	150.8	19.8	5.06
4	98	1.11	0.76	320	139.1	24.6	6.32
Mean *	86	0.99	0.69	333	144.4	22.2	5.69
Females							
1	-	-	-	-	-	-	-
2	85	0.84	0.65	340	147.8	17.8	4.56
3	110	0.83	0.54	330	143.4	20.5	5.25
4	69	0.79	0.46	374	162.5	18.8	4.82
Mean *	88	0.82	0.55	348	151.2	19.0	4.88
Grand Mean *	87	0.89	0.61	342	148.7	20.3	5.2

* Survivors only.

a figure just without the normal range (135 - 145 m.Eq./l.). The female level was slightly higher than the male.

Serum potassium:

The mean terminal potassium level was 5.2 m.Eq./l., a figure within the normal range 3.5 - 5.5 m.Eq./l. The figure for females was considerably lower than that for males, and indeed the male mean (5.69 m.Eq./l) was just outside the normal range.

Histological results: (Tables 100, 101)

Glomerular changes were extremely slight: in one tuft only was there hyaline droplet change; the remainder did not differ significantly from normal. Occasional collecting tubules were slightly dilated and sometimes both cytoplasm and nuclei of proximal convoluted tubules could be seen protruding within the tubular lumina. The blood vessels were intact.

Group 18.

Blood pressure: (Text Fig. 32, Table 102)

There was little difference between the blood

TABLE 100

Experiment V(b). Animal Group 17.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Rat Males 3	Males 4	Females 2	Females 3	Females 4
Endothelial cell proliferation	-	-	-	-	-
Epithelial cell proliferation	-	-	-	-	-
Capsular thickening	-	-	-	-	-
Capsular adhesions	-	-	-	-	-
Crescent formation	-	-	-	-	-
Tubularisation	-	-	-	-	-
Endothelial cell swelling	-	-	-	-	-
Ballooning of tuft cells	-	-	-	-	-
Enlargement of tufts	-	-	-	-	-
Segmental tuft necrosis(acute)	-	-	-	-	-
" " " (subacute)	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-
Transudative capillary change	-	-	-	-	-
Hyaline droplet change	-	-	-	-	(+)
Explosive lesions	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-	-	-	-

TABLE 101.

Experiment V(b). Animal Group 17.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Rat Males 3	Males 4	Females 2	Females 3	Females 4
Dilatation of					
a. prox.conv.tubules	-	-	-	-	-
b. thin Henle limb	-	-	-	-	-
c. thick Henle limb	-	-	-	-	-
d. dist.conv.tubules	-	-	-	-	-
e. collecting tubules	-	-	(+)	(+)	(+)
Cytoplasmic protrusion of tubular cells	+ Occasional prox.tubules	+ Occasional prox.conv.	(+) v. occasional prox. conv. tubules	-	(+)
Nuclear protrusion of tubular cells.	+ Occasional prox.tubules	+ Occasional prox.conv.	-	-	-
Proteinuria	(+)	-	-	-	+
Hyaline casts	-	-	-	-	(+)
Hyaline droplet change	- Eosinophilic material in some prox.tubules	-	-	-	(+)
Necrosis	-	-	-	-	-
Regeneration	- Some interstitial protein-containing fluid	-	- Some interstitial protein-containing fluid	- Some interstitial protein-containing fluid	- Some interstitial protein-containing fluid

Text Figure 32.

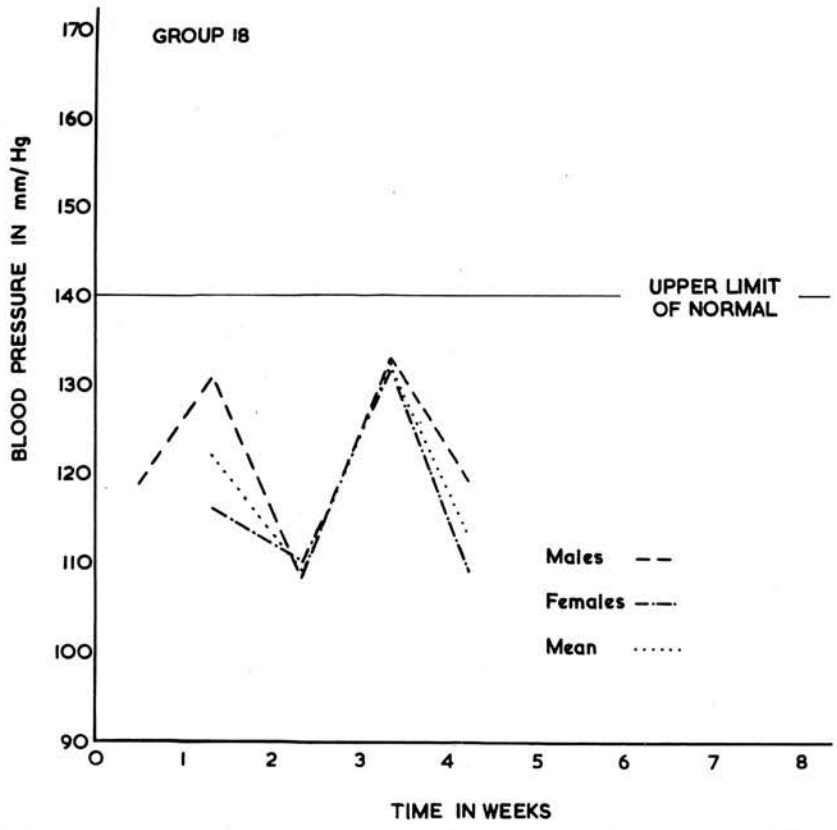


TABLE 102.

Experiment V(b). Animal Group 18.

BLOOD PRESSURE IN mm./Hg.

Rat	Date 8.11.57	13.11.57	20.11.57	28.11.57	4.12.57
Male					
1	123	128	109	127	119
2	113	142	107	148	119
3	124	126	109	125	119
4					
Mean	119	131	108	133	119
Female					
1		115	84	124	112
2		106	126	133	110
3		118	112	143	115
4		125	119	127	99
Mean		116	110	132	109
Grand Mean	119	122	109	132	113

pressure response seen in this animal group and that of group 17. No significant rise occurred, and the levels for males and females did not differ significantly.

Weight: (Text Fig. 33, Table 103)

The mean weight, in spite of some fluctuation among the individuals, remained almost constant throughout the experiment. The progressive weight loss observed in group 17 was not seen, although there was no evidence of the normal rise with age.

Terminal measurements: (Table 104)

Heart weight:

At the close of the experiment the mean heart weight was 0.73 g., giving a mean heart/body weight ratio of 0.62%. The ratio was higher for males (0.78%) than for females (0.52%), but the initial body weights for the two sexes at the beginning of the experiment were different.

Kidney weight:

The mean kidney weight at the close of the experiment was 0.86 g., giving a kidney/body weight ratio of 0.74%. The value for males (1.0%) was significantly higher

Text Figure 33.

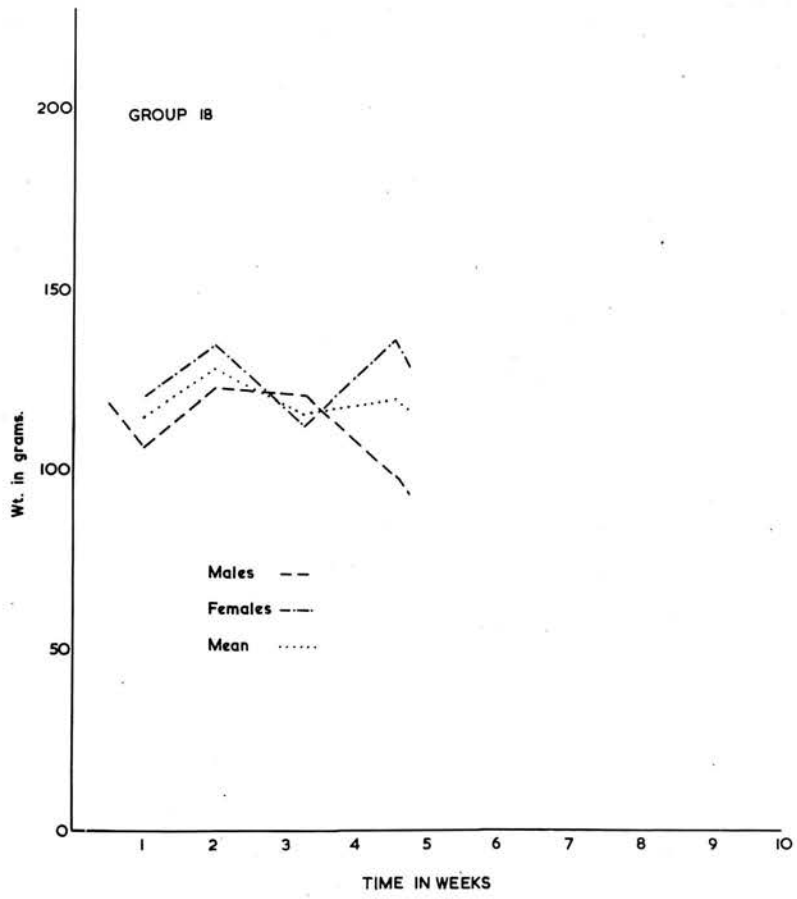


TABLE 103

Experiment V(b). Animal Group 18.

WEIGHT IN GRAMS.

Rat	Date 8.11.57	13.11.57	20.11.57	25.11.57	4.12.57
Male					
1	95	95	113	120	110
2	120	110	127	115	95
3	125	115	128	125	85
4					
Mean	117	106	123	120	97
Female					
1		125	102	125	175
2		105	133	95	145
3		120	145	115	105
4		130	155	114	120
Mean		124	134	112	136
Grand Mean	117	114	128	116	119

TABLE 104.

Experiment V(b). Animal Group 18.

TERMINAL MEASUREMENTS.

Males	Weight g.	R.kidney g.	Heart g.	Serum sodium mg./100 ml.	Serum sodium m.Eq./l.	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
1	-	-	-	-	-	-	-
2	106	0.96	0.75	346	150.4	19.6	5.01
3	80	0.90	0.70	330	143.4	18.9	4.85
Mean *	93	0.93	0.72	338	146.9	19.2	4.92
Females							
1	165	0.99	0.95	352	153.0	14.2	3.65
2	145	0.81	0.72	352	153.0	16.6	4.26
3	90	0.75	0.59	360	156.5	21.2	5.43
4	115	0.78	0.66	352	153.0	18.9	4.85
Mean *	129	0.83	0.73	354	153.3	19.8	4.55
Grand Mean *	117	0.86	0.73	349	151.5	18.2	4.67

* Means of survivors

than the value for females (0.65%)

Serum sodium:

At the end of the experiment the mean serum sodium was 151.5 m.Eq./l., just outside the normal range (135- 145 m.Eq./l.). The difference between males and females was slight.

Serum potassium:

The mean terminal potassium level was 4.67 m.Eq./l. being within the normal range (3.5 - 5.5 m.Eq./l.). The difference between males and females was not significant.

Histological changes: (Tables 105, 106)

The glomeruli were unchanged and did not differ significantly from normal. In particular, there were no glomerular necroses. Tubular changes were slight, and widespread, with minimal dilatation of the collecting tubules, and occasionally of the convoluted tubules, and very slight, occasional intratubular protrusion of both cytoplasm and nuclei of the proximal convoluted tubules. Occasionally protein was present within the collecting tubules.

TABLE 105.

Experiment V(b). Animal Group 18.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Males 1	2	4	Females 1	2	3
Endothelial cell proliferation	-	-	-	-	-	-
Epithelial cell proliferation	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-
Endothelial cell swelling	-	-	-	-	-	-
Enlargement of tufts	-	-	-	-	-	-
Segmental tuft necrosis(acute)	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-
Explosive lesion	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-	-	-	-	-

TABLE 106.

Experiment V(b). Animal Group 18.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Females 1	2	4	Males 1	2	3
Dilatation of						
a. prox.conv.tubules	(+)	(+)	(+)	-	-	-
b. dist.conv.tubules	(+)	(+)	-	-	-	-
c. thin Henle limb	-	-	-	-	-	-
d. thick Henle limb	-	-	-	-	-	-
e. collecting tubules	(+)	(+)	(+)	-	(+)	-
Cytoplasmic protrusion of tubule cells	(+) (convoluted tubules)	(+) prox.conv. tubules	(+) prox.conv. tubules	(+) prox.conv. tubules	(+)	
Nuclear protrusion of tubule cells	(+) (convoluted tubules)	(+) prox.conv. tubules	(+)	(+)	(+)	-
Proteinuria	-	-	-	-	(+)	(+)
Hyaline casts	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-

Discussion:

Although previous observers have shown that cortisone, with or without added salt, causes systolic hypertension in adrenalectomised animals, the present experiment did not confirm this observation. The reason for this may be that one kidney was also removed. Alternatively, and perhaps more likely, is the suggestion that the animals were too small: the severe systemic disturbance caused by bilateral adrenalectomy and unilateral nephrectomy may have masked the blood pressure rise. A third explanation for the failure of the blood pressure to rise significantly could have been an excessive or an inadequate dose of cortisone. In this connection all that can be said is that the dose used was the same as that recommended by previous workers (Knowlton and Loeb, 1957; Ledingham, 1954).

Irrespective of blood pressure changes, the renal histological response was conspicuously slight, and no significant glomerular lesions were seen. Tubular changes were of doubtful significance. This confirms previous observations (Knowlton and Loeb, 1957). With the minimal renal morphological effects were changes in serum sodium and potassium which could not be accepted as significant although occasional individual measurements exceeded the normal range. The heart and kidney weights were not

increased when expressed in terms of the body weight at death.

The addition to this regime of increasing doses of hydrallazine exerted a certain ameliorating influence on these responses. Thus the mean body weight remained almost constant instead of falling, while the mean blood pressure level of group 18 was considerably lower than of group 17. Organ weights, in terms of organ/body weight ratios, did not differ significantly from normal, while the levels of serum sodium and of serum potassium measured at death fell almost entirely within the limits of normal.

Again, the influence of the added hydrallazine on renal structure was slight. No focal glomerular necroses were seen, and there was minimal alteration in tubular structure.

In the interpretation of these results it must be remembered that rapid deterioration in the condition of the animals, and in particular an extreme sensitivity to the hypotensive action of hydrallazine, prevented the administration of doses of this drug as large as were used in earlier experiments. For the same reason it was not possible to continue their treatment as long as with other experimental groups. With these provisos, and with the

realisation that it was not possible to substantiate the action of cortisone on the blood pressure in (control) group 17, it may be stated that hydrallazine does not precipitate focal glomerular necroses in cortisone-treated, bilaterally adrenalectomised rats maintained on sodium chloride following unilateral nephrectomy.

Experiment V (c)

(Animal groups 27, 28)

In the previous experiment the influence of hydrallazine was studied on the kidneys of animals previously subjected to unilateral nephrectomy and bilateral adrenalectomy; the animals were maintained on daily injections of cortisone with salt to drink. The conclusion was reached that hydrallazine did not affect glomerular structure in these circumstances.

It was felt that an extension of this investigation would be desirable because of the uncertain role of unilateral nephrectomy in prejudicing the development of hypertension in animals given cortisone. At the same time it was decided to omit added salt from the drinking water because of the observations of other workers that the hypertension due to cortisone evolves slightly more rapidly when a sodium chloride solution is not substituted for drinking water.

The aim of this experiment therefore was to determine the influence on renal structure of increasing doses of hydrallazine given to animals previously subjected to

bilateral adrenalectomy and maintained on daily injections of cortisone acetate.

Group 27

Material:

Four male and four female rats of a laboratory bred Wistar strain were maintained in circumstances which did not differ from those of animals in the previous experiments.

Cortisone was used in the form of a sterile suspension of the acetate, each 1 ml. containing 25 mg.

Methods:

Bilateral adrenalectomy was performed through lumbar incisions, under ether anaesthesia.

Blood pressures were measured by tail plethysmograph under light ether anaesthesia (Appendix 4, Volume II).

Histological methods employed did not differ from those of earlier experiments.

Procedure:

Bilateral adrenalectomy was performed and the animals maintained on daily intramuscular injections of 2.5 mg. of cortisone acetate. Treatment continued for six weeks; at the end of this time the animals were killed by bleeding under ether anaesthesia and the tissues submitted to histological examination.

Results:

Blood pressures: (Text Fig. 34, Table 107)

Evidence of a rise in blood pressure was obtained but the rise was not sustained and at no time reached the upper limit of normality. Males and females responded in a similar manner.

Weight: (Text Fig. 35, Table 108)

By contrast with the animals of group 17 (experiment V (b), page 39) there was a steady rise in the mean weight from 62 g. after one week to 79 g. at the close of the experiment. The male animals increased in weight considerably more than the smaller females, the respective gains being 24 g. and 4 g.

Text Figure 34.

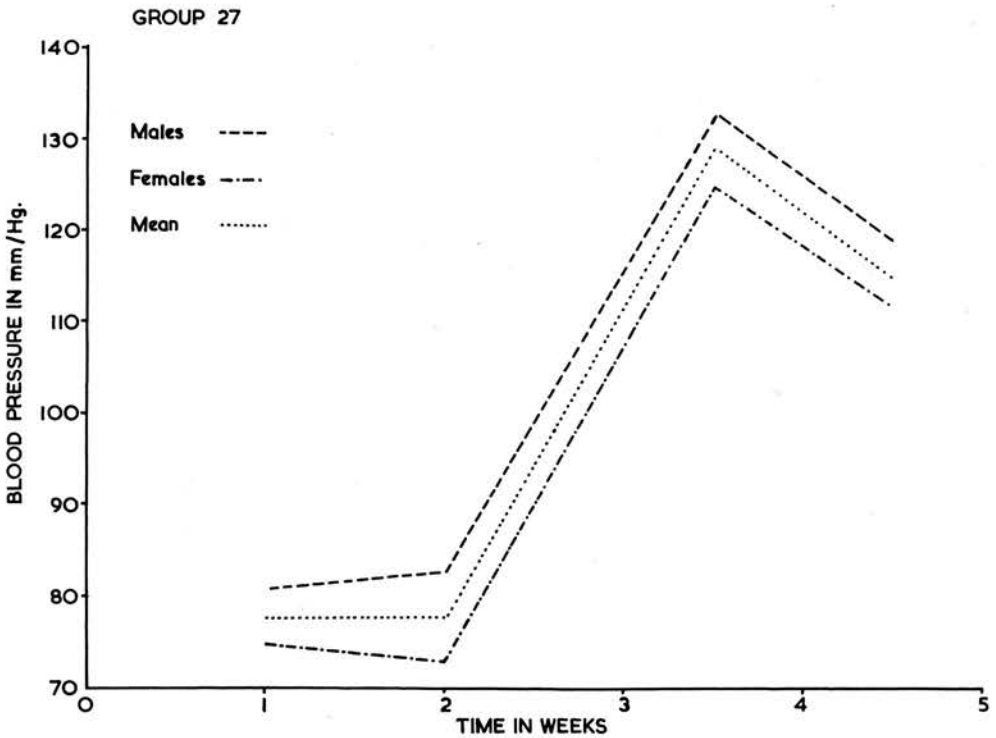


TABLE 107.

Experiment V(c) Animal Group 27.

BLOOD PRESSURES in mm./Hg.

Rat	6.1.58	13.1.58	21.1.58	28.1.58
Male				
1	66	91	148	78
2	88	77	126	128
3	99	75	132	98
4	74	89	126	-
Mean	82	83	133	119
Female				
1	59	59	142	112
2	81	74	118	110
3	79	81	106	115
4	81	79	134	112
Mean	75	73	125	112
Grand Mean	78	78	129	115

Text Figure 35.

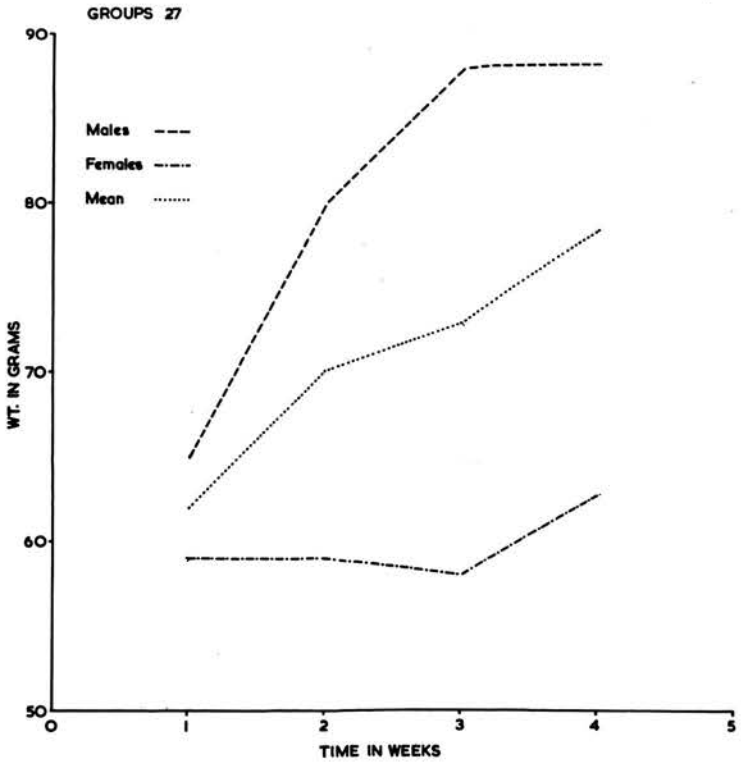


TABLE 108.

Experiment V(c) Animal Group 27.

WEIGHTS IN GRAMS

Rat	6.1.58	13.1.58	21.1.58	25.1.58
Male				
1	45	82	52	78
2	70	63	122	128
3	80	85	93	98
4	65	90	87	52
Mean	65	80	88	89
Female				
1	60	62	55	75
2	55	57	75	65
3	55	63	58	67
4	65	54	55	
Mean	59	59	58	63
Grand Mean	62	70	73	79

Terminal measurements: (Table 109)

Heart weight:

The mean heart weight at the close of the experiment was 0.62 g., and the heart/body weight ratio 0.58%. The difference between males and females was slight, the male ratio being 0.50%, the female 0.68%.

Kidney weight:

The mean kidney weights at the close of the experiment were 0.45 g. and 0.53 g., and the kidney/body weight ratios 0.45% and 0.49%. The weight ratios for the male and female animals were respectively 0.37% and 0.63%. As with the heart, therefore, the kidney/body weight ratios are considerably higher in the females than in the male, bearing in mind that smaller numbers of male animals survived to the end of the experiment.

Serum sodium:

The mean serum sodium level at the close of the experiment was 144.6 m.Eq./l., a figure within the normal range of 135 - 145 m.Eq./l. Only a single male estimation was available but this did not differ significantly from the female.

TABLE 109.

Experiment V(c) Animal Group 27.

TERMINAL VALUES

(Group begun 29.12.57)

Rat	Weight g.	Kidneys g.		Heart g.	Serum Sodium mg./100 ml.	Serum Sodium m.Eq./l	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l
Male								
1 (1.2.58)	52	0.51	0.60	0.52				
2 (5.2.58)	62	0.36	-	0.34				
3 (13.2.58)	168	0.61	0.64	0.75	330	143.4	19.4	4.96
4 (")	145	0.53	0.58	0.82	Insufficient			
Mean *	156	0.57	0.61	0.78				
Female								
1 (28.1.58)	-	-	-	-	-	-	-	-
2 (13.2.58)	73	0.45	0.48	0.55	334	145.1	22.7**	5.86**
3 (")	77	0.39	0.46	0.48	330	143.4	19.2	4.91
4 (")	75	0.42	0.47	0.57	343	149.1	40.0**	10.3**
Mean*	75	0.41	0.47	0.51	336	145.9	19.2	4.9
Grand Mean*	107	0.48	0.53	0.63	334	144.6	19.3	4.93

* Survivors only

** Slightly haemolysed

Serum potassium:

The mean level of serum potassium at the close of the experiment was 4.93 m.Eq./l., a figure within the normal range (3.5 - 5.5 m.Eq./l.). The male and female results again did not differ significantly, although only a single male estimation was available.

Histological changes: (Tables 110, 111)

The glomeruli were virtually normal. Occasionally it was possible to detect slight glomerular enlargement but this was inconstant and of doubtful significance. Tubular changes were also inconstant, patchy, and minimal. In one animal widespread renal infection had destroyed all recognisable structures. In the remaining animals it was noticeable that the brush borders of the proximal convoluted tubules were unusually prominent.

In none was there evidence of glomerular necrosis or of vascular damage.

Group 28

Material:

Four male and four female rats of a laboratory bred

TABLE 110.

Experiment V(c). Animal Group 27.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Male 2	Male 3	Male 4	Female 1	Female 2	Female 3	Female 4
Endothelial cell proliferation	-	-	-	-	-	-	-
Epithelial cell proliferation	-	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-	-
Crescent formation	-	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-	-
Enlargement of tufts	Occasional glomeruli are slightly enlarged						
Ballooning of tuft cells	-	-	-	-	-	-	-
Segmental tuft necrosis (acute)	-	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-	-
Arterial hypoplasia	-	-	-	-	-	-	-

TABLE III.

Experiment V(c). Animal Group 27.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males 2	3	4	Females 1	2	3	4
Dilatation of			Extensive infection destroying renal structures				
a. prox.conv.tubules	-	-	-	-	-	-	-
b. thin Henle limb	-	-	-	-	-	-	-
c. wide Henle limb	-	-	-	-	-	-	-
d. distal conv.tubules	-	-	-	-	-	-	-
e. collecting tubules	-	-	-	-	-	-	-
Cytoplasmic protrusion of tubular cells	(+) prox.conv.	+ prox.conv.	- Brush borders unusually prominent	-	-	-	-
Nuclear protrusion of collecting cells.	(+) prox.conv.	+ prox.conv.	-	-	-	-	-
Vacuolation of collecting tubule cells	-	-	-	-	-	-	-
Proteinuria	-	-	-	-	-	-	-
Hyaline casts	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-
Necrosis							
Regeneration	Some tubular change due to fixation artefact.						

Wistar strain were used, and maintained in a manner which did not differ from that of previous experiments.

Cortisone was used in the form of a sterile suspension of the acetate, each 1 ml. containing 25 mg.

Hydrallazine was used as a 1% solution, each 1 ml. ampoule containing 20 mg.

Methods:

Bilateral adrenalectomy was performed through lumbar incisions under ether anaesthesia.

Blood pressures were recorded under light ether anaesthesia, by tail plethysmograph (Appendix 4, Volume II).

Procedure:

Bilateral adrenalectomy was performed and the animals maintained on daily intramuscular injections of 2.5 mg. of cortisone acetate. Four days after operation the daily intramuscular injection of increasing amounts of hydrallazine was begun, starting with 1 mg., daily but rising little after six weeks owing to the extraordinary sensitivity of adrenalectomised animals to the hypotensive action of hydrallazine. At the end of the six weeks period of

treatment the animals were killed by bleeding under ether anaesthesia, and the tissues submitted to histological examination.

Results:

Blood pressure: (Text Fig. 36, Table 112)

The hydrallazine effectively suppressed any tendency which may have existed for the blood pressure to rise, and the mean blood pressure during the four weeks of observation showed a slight overall fall. There was no difference between the male and female response.

Weight: (Text Fig. 37, Table 113)

Throughout the four weeks of the experiment the mean weight rose steadily from 52 g. to 65 g. Male and female weights rose in parallel, although male animals were consistently heavier.

Terminal measurements: (Table 114)

Heart weight:

The mean heart weight at the end of the experiment was 0.66 g., and the heart/body weight ratio 1.01%. The

Text Figure 36.

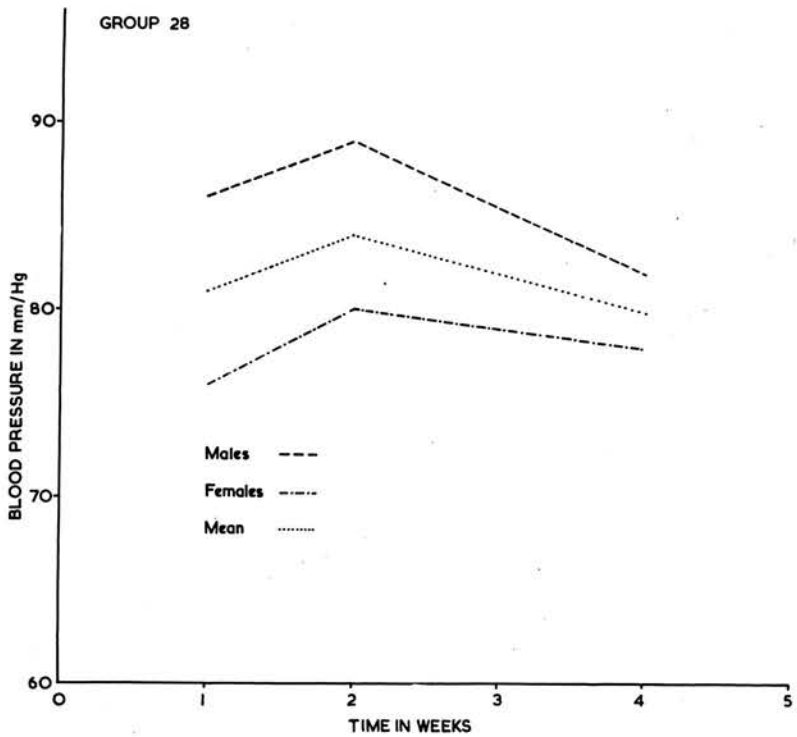


TABLE 112.

Experiment V(c) Animal Group 28.

BLOOD PRESSURES in mm./Hg.

Rat	6.1.58	13.1.58	21.1.58	26.1.58
Male				
1	84	98		78
2	92	87		82
3	82	83		87
4				
Mean	86	89		82
Female				
1	70	79		88
2	80	84		72
3	78	78		68
4				
Mean	76	80		78
Grand Mean	81	84		80

Text Figure 37.

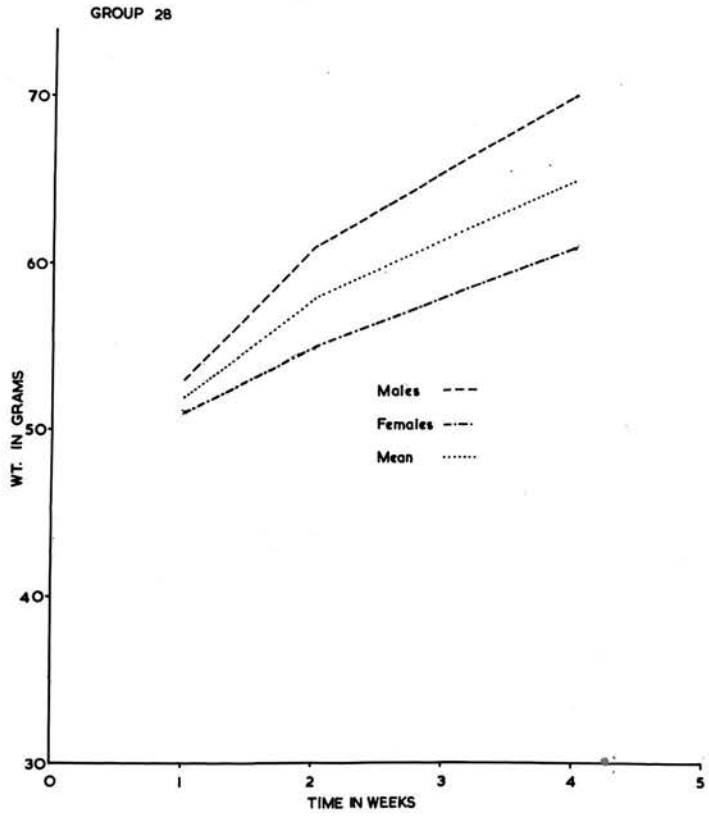


TABLE 113 .

Experiment V(c) Animal Group 28.

WEIGHTS IN GRAMS

RAT	6.1.58	13.1.58	27.1.58
Male			
1	55	58	50
2	60	75	80
3	45	50	80
4			
Mean	53	61	70
Female			
1	50	54	50
2	55	58	64
3	50	52	70
4	(45)		
Mean	52	55	61
Grand Mean	52	58	65

difference between males (1.09%) and females (0.96%) was slight.

Kidney weight:

The mean kidney weights at the end of the experiment were 0.73 g. and 0.75 g., and the respective kidney/body weight ratios 1.12% and 1.16%. The mean male ratios were higher than the female (1.24% and 1.3% against 1.01% and 1.01%) but these differences were of doubtful significance owing to the small number of observations.

Serum sodium:

The mean serum sodium level at the close of the experiment was 171.3 m.Eq./l., considerably above the upper limit of the normal range (135 - 145 m.Eq./l.). This rise was almost entirely due to the much greater rise in mean male values (184.5 m.Eq./l.) than in female (145.1 m.Eq./l.), but only a single female measurement was available for comparison.

Serum potassium:

Only small numbers of measurements were available for comparison but the mean was considerably raised (6.78

TABLE 114.

Experiment V(c) Animal Group 28.

TERMINAL VALUES

Rat	Weight g.	Kidneys g.	g.	Heart g.	Serum Sodium mg./100 ml.	m.Eq./l	Serum Potassium mg./100 ml.	m.Eq./l
Male								
1 (2.1.58)	-	-	-	-	-	-	-	-
2 (26.1.58)	50	-	-	-	-	-	-	-
3 (27.1.58)	80	0.77	0.89	0.57	400	174.0	24.5	6.30
4 (")	80	0.78	0.75	0.89	450	195.0	30.0	7.70
Mean *	63	0.78	0.82	0.69	425	184.5	27.2	7.0
Female								
1 (7.1.58)	-	0.35	0.42	0.25	-	-	-	-
2 (11.1.58)	50	0.35	-	0.21	-	-	-	-
3 (24.1.58)	64	0.45	0.44	0.43	-	-	-	-
4 (27.1.58)	70	0.91	0.95	0.85	334	145.1	24.7	6.35
Mean *	67	0.57	0.69	0.64	334	145.1	24.7	6.35
Grand Mean *	65	0.65	0.76	0.68	395	171.0	26.2	6.78

* Survivors of 26 days only included.

m.Eq./l.) and well outside the upper limit of normal (3.5 - 4.5 m.Eq./l.)

Histological changes: (Tables 115, 116)

Histological changes were slight. No glomerular lesions were seen, and the tubules showed only slight, occasional cytoplasmic protrusion within their lumina. The brush borders were again unusually prominent.

Discussion:

Bilateral adrenalectomy with tap water to drink, and cortisone by daily injection did not influence the renal structural response to hydrallazine. Other reactions were observed, e.g. the weight rose steadily in group 28 whereas in all previous groups (17, 18 and 27) there was either a constant fall, or no significant change. The group treated with cortisone only (group 27) differed only slightly from that given cortisone and hydrallazine; it cannot be concluded that hydrallazine was responsible for the precipitation of any significant renal structural change.

As in group 18 (and its control group 17) no evidence was obtained to show that cortisone sensitized the kidney to the action of hydrallazine. In this way its reaction

TABLE 115.

Experiment V(c). Animal Group 28.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Male 3	4	1	Female 2	3	4
					Autolysis	
Endothelial cell proliferation	-	-	-	-	-	-
Epithelial cell proliferation	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-
Crescent formation	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-
Enlargement of tufts	-	-	-	-	-	-
Ballooning of tuft cells	-	-	-	-	-	-
Segmental tuft necrosis (acute)	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-	-	-	-	-

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TABLE 116.

Experiment V(c). Animal Group 28.

TUBULAR CHANGES	Males		Females			
	3	4	1	2	3	4
				Autolysis		
Dilatation of						
a. prox.conv.tubules	-	-	-	-	-	-
b. thin Henle limbs	-	-	-	-	-	-
c. wide Henle limbs	-	-	-	-	-	-
d. dist.conv.tubules	-	-	-	-	-	-
e. collecting tubules	-	-	-	-	-	-
Cytoplasmic protrusion of tubular cells	Occasional very slight cytoplasmic protrusion					
Nuclear protrusion of tubular cells	Brush borders unusually prominent					
Vacuolation of collecting tubule cells	-	-	-	-	-	-
Proteinuria	-	-	-	-	-	-
Hyaline casts	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-

resembled that of 9 α fluorocortisol. Minor and inconstant electrolyte disturbances were found, but the ultimate effect on renal structure was slight.

Experiment V (d)

(Animal group 33)

In experiments V(b) and V(c) it was impossible to confirm the observation that adrenalectomised rats respond to daily cortisone injections by the development of sytolic hypertension. Under these circumstances, the demonstration that large doses of hydrallazine did not cause focal glomerular necrosis was clearly open to the criticism that it did not provide a valid comparison with animals given DCA, in terms of the blood pressure response. Before concluding that hydrallazine and cortisone do not cause significant glomerular damage it was decided to study the influence of hydrallazine on animals given cortisone but not subjected to adrenalectomy.

The aim of this experiment therefore was to determine whether hydrallazine would cause focal glomerular necrosis in animals given excess cortisone.

Material:

4 male and 4 female albino Wistar rats of a laboratory bred strain were used. They were maintained under circumstances which did not differ from those of previous

experiments.

Cortisone was used in the form of a suspension of the acetate, each 1 ml. containing 25 mg.

Methods:

Blood pressures were recorded by tail plethysmograph (Appendix 4, Volume II).

Serum electrolytes were measured by flame photometer.

Histological methods were similar to those used in previous experiments.

Procedure:

Injections of 2.5 mg. cortisone were given by daily intramuscular injection. Increasing amounts of hydrallazine were also administered intramuscularly, starting four days after the cortisone and continuing for a period of nearly three weeks. The amount of hydrallazine given rose from 1 mg. daily at the start of the experiment to 6 mg. daily at its termination; the cortisone treated animals being very much less sensitive to the hypotensive action of hydrallazine than those previously subjected to bilateral adrenalectomy (group 28, experiment V(c)) or bilateral

adrenalectomy with unilateral nephrectomy (group 18, experiment V(b)).

After three weeks the animals were killed by bleeding under ether anaesthesia; the tissues were submitted to histological examination, and the blood used for the estimation of serum sodium and potassium.

Results:

Terminal measurements: (Table 117)

At the time of death the animals' mean weight was 128 g., the females being significantly smaller than the males. The mean heart weight was 0.84 g., and the heart/body weight ratio 0.66%. The mean kidney weights were 0.85 g., and 0.84 g., and the respective kidney/body weight ratios 0.66% and 0.66%.

The mean serum sodium level was 148.4 m.Eq./l., a figure just outside the normal range (135 - 145 m.Eq./l.). The mean serum potassium level (4.96 m.Eq./l.) lay within the normal range (3.5 - 5.5 m.Eq./l.).

Histological changes: (Tables 118, 119)

The structure of the kidneys was virtually normal.

TABLE 117.

Experiment V(d). Animal Group 33.

TERMINAL MEASUREMENTS.

Males	Weight g.	Heart g.	Kidneys g.	Serum Sodium mg./100 ml.	Serum Sodium m.Eq./l.	Serum Potassium. mg./100 ml.	Serum Potassium. m.Eq./l.
1 (18.2.58)	85	0.60	0.85 0.74	-	-	-	-
2 (27.2.58)	143	0.9	0.97 1.10	345	150.0	17.7	4.54
3 "	154	0.92	0.92 1.10	340	147.8	20.5	5.25
4 "	152	1.19	1.00 1.07	340	147.8	22.3	5.76
Mean *	149	0.91	0.93 1.00	341	148.8	20.2	5.18
Females							
1 (18.2.58)	90	0.65	0.75 0.68	-	-	-	-
2 (27.2.58)	120	0.77	0.76 0.70	348	150.3	16.4	4.21
3 "	88	0.72	0.70 0.60	336	146.0	20.5	5.25
4 "	115	0.92	0.90 0.80	340	147.8	18.7	4.80
Mean *	108	0.77	0.78 0.69	341	148.0	18.5	4.75
Grand Mean *	128	0.84	0.85 0.84	341	148.4	19.4	4.96

TABLE 118.

Experiment V(d). Animal Group 33.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Males 2	3	4	Females 1	2	3	4
Endothelial cell proliferation	-	-	-	-	-	-	-
Epithelial cell proliferation	-	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-	-
Enlargement of tufts	-	-	-	-	-	-	-
Ballooning of tuft cells	-	-	-	-	-	-	-
Segmental tuft necrosis (acute)	-	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-
"Explosive" lesions	-	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication.	-	-	-	-	-	-	-

TABLE 119.

Experiment V(d). Animal Group 33.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males			Females			
	2	3	4	1	2	3	4
Dilatation of							
a. prox.conv.tubules	-	-	-	-	-	-	-
b. thin Henle limb	-	-	-	-	-	-	-
c. wide Henle limb	-	-	-	-	-	-	-
d. dist.conv.tubules	-	-	-	-	-	-	-
e. collecting tubules	-	-	-	-	-	-	-
Cytoplasmic protrusion of tubular cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Nuclear protrusion of tubular cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Vacuolation of collecting tubular cells	-	-	-	-	-	-	-
Proteinuria	-	-	-	-	-	-	-
Hyaline casts	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-	-

There was minimal intratubular protrusion of both cytoplasm and nuclei in the proximal convoluted tubules, but these changes were so slight as to be of doubtful significance. No focal glomerular necroses were seen and the blood vessels were intact.

Discussion:

The results of this experiment serve to lend emphasis to those of the previous two groups, and confirm that the action of hydrallazine on the kidneys of animals given cortisone is distinct from the action of hydrallazine on the kidneys of animals given DCA. The conclusion is reached that neither bilateral adrenalectomy nor unilateral nephrectomy influences the response. Weight is lent to the argument that the focal glomerular necroses found in animal groups 7, 10, 14 and 16 are determined specifically by the action of DCA and greatly accelerated by the intermittent administration of hydrallazine. There is no evidence to suggest that other steroids are effective in predisposing the kidney to these changes in spite of similar actions on sodium excretion or on the blood pressure. There is thus indirect evidence to show that sodium retention per se is not the property of DCA which leads the glomerulus to respond so acutely to the action of hydrallazine. This is

difficult to explain since it has already been shown that only those groups receiving salt to drink develop glomerular necroses. The influence of sodium appears to be indirect, perhaps exerted through its effect on the blood pressure regulating mechanism of the adrenal cortex.

CHAPTER 7

Experimental Series VI

In the development of focal glomerular necrosis hydrallazine appeared to act by expediting the evolution of an uncommon response to the action of DCA. Salt, with or without unilateral nephrectomy, was necessary for this development. It was shown that similar lesions were not produced by steroids possessing either the salt retaining action (9 α-fluorocortisol) of DCA or an effect on blood pressure (cortisone). In earlier experiments it was further demonstrated that the effect of hydrallazine on glomerular structure was not due to the occasional production of convulsions, or to the use of ether anaesthesia for injections, but was influenced by the frequency of injection.

To explain the importance of intermittent action in causing glomerulonecrosis an alteration of blood pressure regulation was suggested. Hydrallazine is known to have a prolonged action when given in large doses (Smirk, 1957; Tripod, 1956), and the omission of a single daily injection each week might be expected to lead to episodic relaxation in the control of hypertension. This relaxation, it was thought, might cause a sufficient intermittent rise in blood pressure to induce focal glomerular necrosis. Similar lesions have been described in both severe experimental

and in malignant type human hypertension, in both of which, however, the glomerular lesions have invariably been accompanied by fibrinoid arteriolar necrosis. Nevertheless, Pickering (1955) has emphasised that severe fluctuations in blood pressure do not always cause fibrinoid arteriolar necrosis: phaeochromocytoma, for example, is rarely accompanied by this change.

It appeared to the author that additional information might therefore be obtained about the mechanism causing the focal glomerular necrosis of hydrallazine intoxication by comparing its action with those of other hypotensive drugs. For this purpose pentolinium and reserpine were selected and a third drug, compound 14179, became available by chance. By administering these agents intermittently it was thought that the effect on blood pressure regulation in animals subjected to the DCA hypertensive regime might be expected to resemble that exerted by hydrallazine. The difference in rate of absorption and of duration of action of these other hypotensive drugs suggested that an intermittent effect would be produced if they were given once daily. In view of their more rapid action, a total period of treatment of four weeks was expected to be adequate.

The present experiment, therefore, was planned to test

the hypothesis that discontinuous hydrallazine administration stimulates the appearance of focal glomerular necrosis in animals subjected to the DCA hypertensive regime by allowing periodic relaxation of blood pressure control, and thus of intermittent episodes of hypertension.

Experiment VI (a)

(Animal group 26)

Material:

Male and female albino Wistar rats of a laboratory bred strain were maintained in conditions which did not differ significantly from those used in earlier experiments.

DCA was used in the form of a 1.25% aqueous suspension with tragacanth.

Pentolinium tartrate (Ansolysen) was used as a 1% aqueous solution, diluted from stock and stored at + 4° C.

Methods:

Unilateral (left) nephrectomy was performed by the standard procedure (Appendix 2, Volume II).

Blood pressures were recorded by tail plethysmograph (Appendix 4, Volume II).

Histological methods used were identical with those employed in previous experiments.

Procedure:

Following left nephrectomy, 1% sodium chloride solution was substituted for the drinking water and daily intramuscular injections of the DCA suspension begun: 3.1 mg. was injected daily.

Beginning 7 days after the operation, 0.05 ml. of the 0.05% solution of pentolinium tartrate (0.025 mg.) was injected once daily intramuscularly. After 4 weeks the dose which was progressively raised had reached 1 ml.

At the conclusion of this period of treatment the animals were killed by bleeding under ether anaesthesia and blood submitted for estimation of serum sodium and potassium.

Results:

Blood pressure: (Text Fig. 38, Table 120)

The blood pressure, measured as in previous experiments 5 - 6 hours after injection, showed evidence of a progressive rise during the 4 weeks of observation. Nevertheless, the rise was slight by comparison with a control groups (group 25, experiment I(f) and did not exceed the

Text Figure 38.

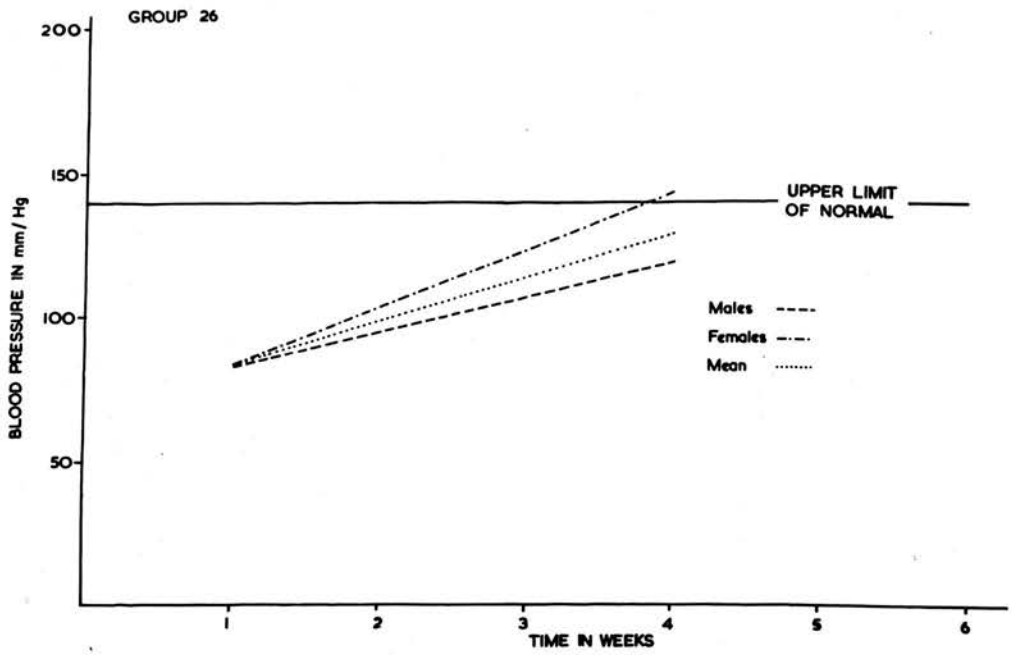


TABLE 120.

Experiment VI(a). Animal Group 26.

BLOOD PRESSURE READINGS.

Rat	7.1.58	28.1.58
Male		
1	81	130
2	85	72
3	83	120
4	81	150
Mean	82	118
Female		
1	95	136
2	75	146
3	73	148
4	88	
Mean	83	143
Grand Mean	82	129

Text Figure 39.

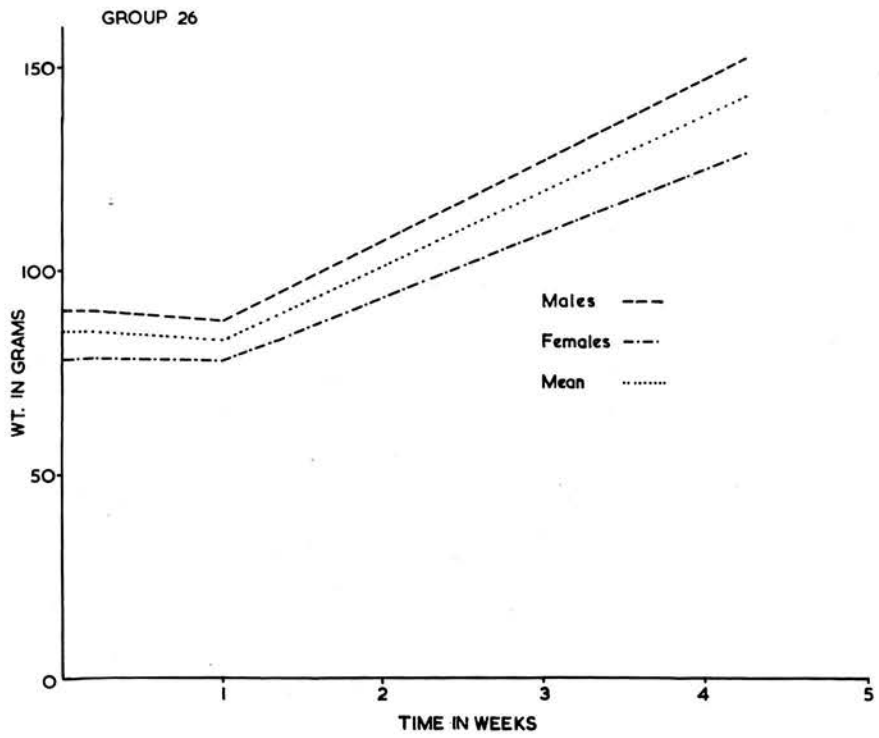


TABLE 121.

Experiment VI(a). Animal Group 26.

WEIGHTS.

Rat	29.12.57 g.	7.1.58 g.	28.1.58 g.
Males			
1	100	105	180
2	90	80	123
3	86	85	130
4	85	80	170
Mean	90	88	151
Females			
1	72	85	145
2	68	90	112
3	95	70	125
4	82	65	
Mean	79	78	127
Grand Mean	85	83	141

upper limit of normal. The male and female responses were not significantly different.

Weight: (Text Fig. 39, Table 121)

The mean weight rose steadily during the experiment from an initial level of 84 g. to a value of 140 g. afer 4 weeks. The males and females responded similarly and in parallel.

Terminal measurements: (Table 122)

Heart weight:

The mean heart weight at the close of the experiment was 1.17 g., and the mean heart/body weight ratio 0.90%. The value for females (1.1%) was considerably higher than for males (0.76%) but this must be interpreted in the light of the slightly different survival times.

Kidney weight:

The mean kidney weight at the close of the experiment was 1.56 g., and the mean kidney/body weight ratio 1.28%. The difference in ratios between male (1.13%) and female (1.37%) was only slightly less than the difference

TABLE 122.

Experiment VI(a). Animal Group 26.

TERMINAL VALUES (begun 28.12.57)

Rat	Weight g.	R.kidney g.	Heart g.	Serum Sodium mg./100 ml.	Serum Sodium m.Eq./l.	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
Male							
1 (20.1.58)	68	1.05	0.90	-	-	-	-
2 (4.2.58)	115	1.30	0.85	-	-	-	-
3 (4.2.58)	115	1.35	0.75	-	-	-	-
4 (6.2.58)	156	1.70	1.35	350	152.1	16.1	4.14
Mean *	129	1.45	0.98	350	152.1	16.1	4.14
Female							
1 (6.2.58)	132	1.55	1.15	368	160.0	18.6 ***	4.77
2 (")	112	1.75	1.45	341	148.2	19.7 ***	5.04
3 (")	142	1.95	1.50	336	146.0	17.1 ***	4.39
4 (")	94	1.35	1.18	377	163.8	21.7 ***	5.56
Mean *	120	1.65	1.32	355	154.0	19.3	4.94
Grand Mean *	122	1.56	1.17	354	153.0	18.8	4.78

* Survivors only

*** Slightly haemolysed.

in the heart ratios.

Serum sodium:

The mean serum sodium at the end of the experiment was 153.0 m.Eq./l., a figure outside the normal range of 135 - 145 m.Eq./l. The difference between males and females was slight, but only a single male observation was available for analysis.

Serum potassium:

The mean serum potassium level at the close of the experiment was 4.78 m.Eq./l., a figure within the normal range (3.5 - 5.5 m.Eq./l.). Again, the difference between males and females was not significant.

Histological results: (Tables 123, 124)

Glomerular changes were slight. There was generalised enlargement of the tufts. By comparison with control group 25, experiment I(f), the effects were strikingly modified. No fibrinoid or other vascular lesions were seen, and it appeared that the hypertensive effects of DCA had been

TABLE 123.

Experiment VI(a). Animal Group 26.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	1	2	4	1	2	3	4
Endothelial cell proliferation	(+)	(+)	Autolysis	+	(+)	(+)	+
Epithelial cell proliferation	-	-		-	-	-	-
Capsular thickening	-	-		-	-	-	-
Capsular adhesions	-	-		-	-	-	-
Tubularisation	-	-		-	-	-	-
Enlargement of tufts	(+)	(+)		+	(+)	(+)	+
Ballooning of tuft cells	(+)	-		(+)	-	-	+
Segmental tuft necrosis (acute)	-	-		-	-	-	-
" " " (subacute)	-	-		-	-	-	-
" " " (fibrosed)	-	-		-	-	-	-
Transudative capillarychange	-	-		-	-	-	(+)
Hyaline droplet change	-	-		(+)	-	-	-
Fibrinoid arterial necrosis	-	-		-	-	-	(+)
Fibrinoid arteriolar necrosis	-	-		-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-		-	-	-	-

TABLE 124.

Experiment VI(a). Animal Group 26.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Male 1	Male 2	Male 4	Female 1	Female 2	Female 3	Female 4
Dilatation of			Autolysis				
a. prox.conv.tubules	-	-		-	-	-	-
b. thin Henle limb	-	-		-	-	-	-
c. wide Henle limb	-	-		-	-	-	-
d. dist.conv.tubules	-	(+)		-	-	-	-
e. collecting tubules	(+)	(+)		+	(+)	(+)	(+)
Cytoplasmic protrusion in tubular cells	-	(+) conv.&coll.	+ conv.&coll.	-	-	(+) prox.conv. & coll.	+ conv &coll
Nuclear protrusion in tubular cells	-	(+) conv.&coll.	+ conv.&coll.	-	-	(+) conv.&coll.	+ conv & coll
Vacuolation of collecting tubule cells	-	-		-	-	-	-
Proteinuria	-	-		+	(+)	-	(+)
Hyaline casts	-	-		-	-	-	-
Hyaline droplet change	-	-		-	-	-	-
Necrosis	-	-		-	-	-	-
Regeneration	-	-		-	-	-	-

almost completely annulled. By contrast, electrolyte disturbances, including the enlargement of tufts and cell ballooning, were still present and were accompanied in some instances by hyaline droplet change or by the transudation of protein-containing fluid into the subcapsular space of the glomerulus.

No focal glomerular necroses were seen. There was widespread slight to moderate dilatation of collecting tubules, and minimal intratubular protrusion of both cytoplasm and nuclei of both the convoluted and collecting tubules. The tubular changes as a whole were of similar character and degree to those seen in the corresponding group (group 25, experiment I(f)) not treated with pentolinium. There was occasional proteinuria.

Discussion:

In intermittent doses which maintained the mean blood pressure level within the limits of normal*, pentolinium was shown to cause conspicuous amelioration of the effects of DCA on renal structure. Fibrinoid necrotic lesions in renal arterioles were almost entirely prevented, while the severity of the glomerular lesions was reduced. Glomeruli

*see group 7, experiment II(d), page 156.

undergoing the explosive lesion which accompanies severe DCA overdosage were not seen, and the changes were mainly those of glomerular enlargement with only moderate cellular ballooning.

However, in none of the kidneys examined were focal glomerular necroses observed.

It may be concluded that intermittent administration of pentolinium tartrate by once daily intramuscular injection maintains the mean blood pressure within normal limits and modifies the lesions caused by excess DCA. The lesions in the kidney associated with severe hypertension are prevented, while those which the author believes are the result of electrolyte and fluid disturbance are not influenced.

Nevertheless, intermittent pentolinium did not reproduce the glomerulonecrotic action of hydrallazine. In earlier experiments hemiglomerular necroses were found most frequently when DCA was given by slow subcutaneous absorption. In the present experiment DCA was given by intermittent daily injection and it is possible that this modified the response to pentolinium. Nevertheless, clear evidence was provided that the single daily injection of pentolinium suppressed the most severe hypertensive lesions caused by DCA and maintained the mean blood pressure within

the limits of normal. The absence of focal hemiglomerular necroses cannot therefore be attributed to an inadequate influence on the DCA hypertensive mechanism, and the experiment provides evidence confirming that the stimulus to glomerulonecrosis occurring during treatment with hydrallazine is a property not necessarily shared by other drugs which influence blood pressure in DCA hypertension.

Experiment VI (b)

(Animal Group 35)

The observations made in the previous experiment were continued and expanded in the present experiment in which reserpine was used as a hypotensive agent in place of pentolinium. The object of the experiment was to determine whether hypotensive agents other than pentolinium would prove effective in inducing focal glomerular necroses of a kind precipitated by hydrallazine.

Material:

4 male and 4 female albino Wistar rats of a laboratory bred strain were maintained in circumstances which did not differ from those of previous experiments. The animals weighed 90 - 115 g.

DCA was used in the form of a 1.25% aqueous suspension.

Reserpine was used as a 1 in 25 aqueous solution of the proprietary preparation "Serpasil", each 1 ml. of the ultimate dilution containing 0.04 mg. of reserpine.

Methods:

Unilateral nephrectomy was performed by the standard method (Appendix 2, Volume II).

Blood pressure was recorded by tail plethysmograph (Appendix 4, Volume II).

Histological methods used were the same as those employed in previous experiments.

Procedure:

Left nephrectomy was performed and daily injections started of 3.1 mg. DCA, by the intramuscular route. 1% sodium chloride solution was substituted for the drinking water, and 4 days later daily intramuscular injections of 0.1 ml. (0.004 mg.) of the diluted reserpine were begun. Injections were continued for 4 weeks in amounts increasing gradually from 0.1 ml. (0.004 mg.) daily to 1 ml. (0.04 mg.) daily. At the end of this time the animals were killed by bleeding under ether anaesthesia and the tissues submitted to histological examination. Blood collected at necropsy was used for the estimation of serum sodium and potassium.

Results:

Blood pressure: (Text Fig. 40, Table 125)

The mean blood pressure rose slightly during the period of observation but at no time exceeded the upper limit of normal. There was little difference between the response of the male and of the female groups.

Weight: (Text Fig. 41, Table 126)

The animals of both sexes gained weight more rapidly during the early part of the experiment than in the latter.

Terminal measurements (Table 127)

Heart weight:

At the close of the experiment the mean weight of the hearts was found to be 1.45 g., giving a mean heart/body weight ratio of 0.97%. The corresponding figures for male and for female groups were respectively 1.54 g. and 1.0%, and 1.36 g. and 0.94%.

Text Figure 40.

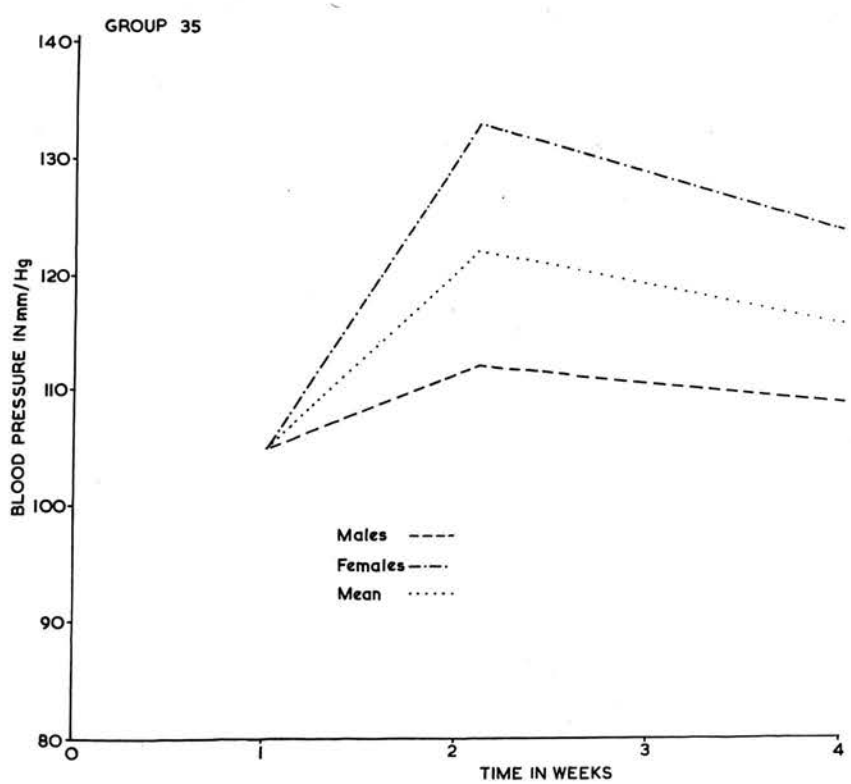


TABLE 125.

Experiment VI(b) Animal Group 35.

BLOOD PRESSURES in mm./Hg.

Rat	17.2.58	25.2.58	10.3.58
Males			
1	122	96	141
2	90	118	94
3	94	98	109
4	114	136	90
Mean	105	112	109
Females			
1	104	108	111
2	108	138	119
3	102	146	127
4	108	142	141
Mean	105	133	124
Grand Mean	105	122	116

Text Figure 41.

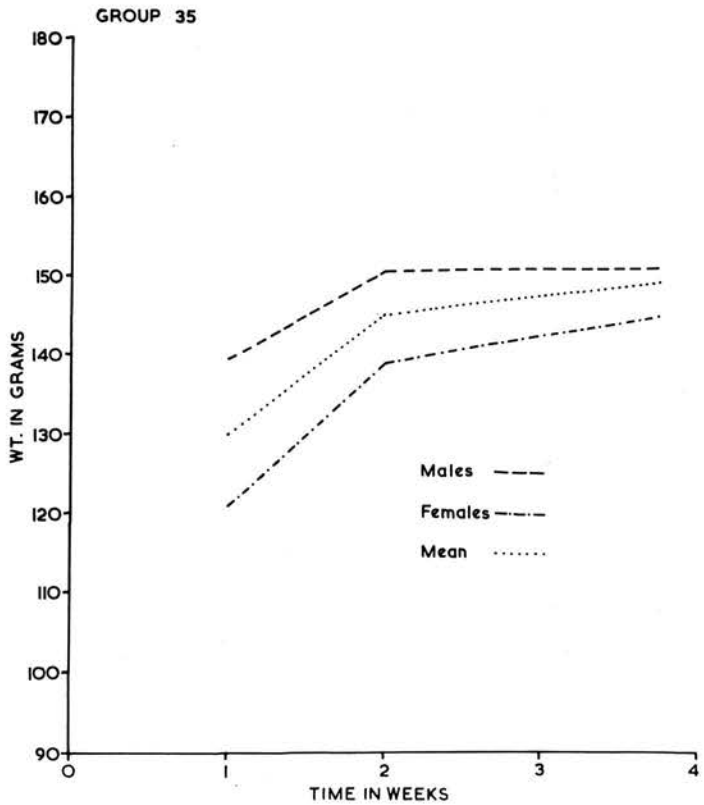


TABLE 126.

Experiment VI(b) Animal Group 35.

WEIGHTS IN GRAMS

Rat	17.2.58	25.2.58	10.3.58
Males			
1	127	125	90
2	155	175	175
3	162	168	130
4	115	135	120
Mean	139	151	154
Females			
1	125	140	175
2	117	145	135
3	132	130	150
4	117	135	125
Mean	122 g.	138 g.	146 g.
Grand Mean	130 g.	145 g.	150 g.

TABLE 127.

Experiment VI(b) Animal Group 35.

TERMINAL MEASUREMENTS

Rat	Weight (in grams)	Heart Weight (in grams)	Kidney Weight (in grams)	Serum Sodium mg./100 ml.	Serum Sodium mEq./l.	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
Male							
1	190	1.90	1.40	357[*]	155.1	19.7	4.04
2[*]	175	1.50	1.94	345	150.0	17.7	4.54
3[*]	130	0.94	1.50	345	150.0	17.9	4.59
4	120	1.84	1.92	370	160.8	16.0	4.11
Mean	154 g.	1.54 g.	1.69 g.	354	153.9	17.8	4.07
Females							
1	175	1.70	1.31	366	159.1	16.2	4.16
2[≠]	135	1.20	1.50	357	155.1	13.0	3.34
3[*]	150	0.91	1.80	366	159.1	16.2	4.16
4	125	1.64	1.43	379	164.7[H]	(34.0)	(8.73)
Mean	146 g.	1.36 g.	1.51 g.	367	159.5	15.1	3.88
Grand Mean	150 g.	1.45 g.	1.60 g.	360	156.7	16.7	4.13

[*] Evidence of generalised pyaemic abdominal infection with liver involvement.
≠ Pericardial oedema. [H] Specimen haemolysed.

Kidney weight:

The mean kidney weight was found to be 1.60 g., and the mean kidney/body weight ratio 1.06%. The mean male kidney weight was 1.69 g., with a kidney/body weight ratio of 1.1%; the mean female kidney weight was 1.51 g., with a kidney/body weight ratio of 1.03%.

Serum sodium:

The mean serum sodium at the close of the experiment was 156.7 m.Eq./l., a figure considerably higher than the upper limit of the normal range (135 - 145 m.Eq./l.). There was no significant sex difference.

Serum potassium:

The mean terminal serum potassium was 4.13 m.Eq./l. a figure within the normal range of 3.5 - 5.5 m.Eq./l.

Histological changes: (Tables 128, 129)

Under the conditions of the present experiment, reserpine prevents the development of severe DCA renal lesions. Reserpine does not prevent the appearance of collecting tubular dilatation, intratubular cytoplasmic

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TABLE 128.

Experiment VI(b). Animal Group 35.

GLOMERULAR CHANGES	Males 1	2	3	4	Females 1	2	3	4
Endothelial cell proliferation	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Epithelial cell proliferation	-	-	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-	-	-
Endothelial cell swelling	-	-	-	-	-	-	-	-
Enlargement of tufts	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Segmental tuft necrosis (acute)	-	-	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-	-	-
Hyaline droplet change	-	(+)	-	-	-	-	-	-
"Explosive" lesions	-	-	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-	-	-	-	-	-	-

TABLE 129.

Experiment VI(b) Animal Group 35.

KEY: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males				Females			
	1	2	3	4	1	2	3	4
Dilatation of								
a. prox. conv. tubules	-	-	-	-	-	-	-	-
b. thin Henle limb	-	-	-	-	-	-	-	-
c. wide Henle limb	-	-	-	-	-	-	-	-
d. dist. conv. tubules	-	-	-	-	-	-	-	-
e. collecting tubules	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Protrusion of tubular cell cytoplasm	Occasional Pronounced in collecting tubules				Occasional, but severe in collecting tubules.			
Protrusion of tubular cell nuclei	Occasional				Occasional.			
Vacuolation of collecting tubule cells	-	-	-	-	-	-	-	-
Proteinuria	-	-	(+)	-	-	-	-	-
Hyaline casts	-	-	-	-	-	-	-	-
Hyaline droplet change	-	-	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-	-	-
	Many small interstitial protein aggregates							

protrusion, and glomerular enlargement, all of which are manifestations of DCA administration. Many small, interstitial protein aggregates are present but neither vascular fibrinoid change nor focal glomerular lesions are seen.

Discussion:

The severe effects of DCA overdosage are prevented by reserpine, as they are by hydrallazine and by pentolinium. Like the latter, however, and unlike the former, the prevention of these severe DCA effects, thought by the author to be the manifestation of the hypertensive fraction of DCA overdosage, is not accompanied by the appearance of focal hemiglomerular necroses.

This result lends weight to the suggestion that the cause of the hemiglomerular segmental necrosis seen in rats given excess DCA is not simply a blood pressure response, but is determined by some factor, perhaps an immunity response, specific to hydrallazine and not shared by other hypotensive drugs which may nevertheless be equally effective in controlling blood pressure changes.

Experiment VI (c)

(Animal Group 31)

Through the courtesy of Ciba Laboratories Ltd. it was possible to obtain a small quantity of a new hypotensive drug, Compound 14179; related chemically to hydrallazine, it had not been submitted to clinical trial but its hypotensive activity had been studied in pharmacological experiments. It was decided to administer compound 14179 to rats treated with DCA to determine whether it would influence renal structure in the same way as hydrallazine or whether the effect on the kidney would resemble more closely those of pentolinium or reserpine.

Material:

4 male and 4 female albino rats of the laboratory Wistar strain were maintained in circumstances which did not differ materially from those of animals in earlier experiments.

DCA was used in the form of a 1.25% aqueous suspension.

Compound 14179 was used as an aqueous solution each 2 ml. sterile ampoule containing 20 mg.

Methods:

Unilateral nephrectomy was performed by the standard procedure. (Appendix 2, Volume II).

Blood pressures were recorded by tail plethysmograph under light ether anaesthesia. (Appendix 4, Volume II)

Histological methods employed at the close of the experiment did not differ from those used in earlier work.

Procedure:

Left nephrectomy was performed, and 1% sodium chloride solution substituted for drinking water. The daily intramuscular injection of 3.1 mg. DCA was then begun. 5 days after the operation the daily intramuscular injection of increasing amounts of compound 14179 was started, beginning with 0.1 ml. (1 mg.) and rising after 4 weeks to 2.0 ml. (20 mg.). At the end of this time the animals were killed by bleeding under ether anaesthesia, and the tissues submitted to histological examination. Blood was used for the estimation of serum sodium and potassium.

Results:

Blood pressure: (Text Fig. 42, Table 130)

No significant rise in blood pressure occurred during the experimental period, and at the termination of the experiment the mean pressure was 101 mm.Hg. Males and females did not differ in response.

Weight: (Text Fig. 43, Table 131)

During the experiment a considerable rise in weight occurred in both female and male groups. As with the previous experimental group (group 35, experiment VI(c)), the rate of gain in weight appeared to decrease as the experiment progressed.

Terminal measurements: (Table 132)

Heart weight:

At the close of the experiment the mean heart weight was 1.41 g., with a mean heart/body weight ratio of 0.89%. The corresponding figures for the males were

Text Figure 42.

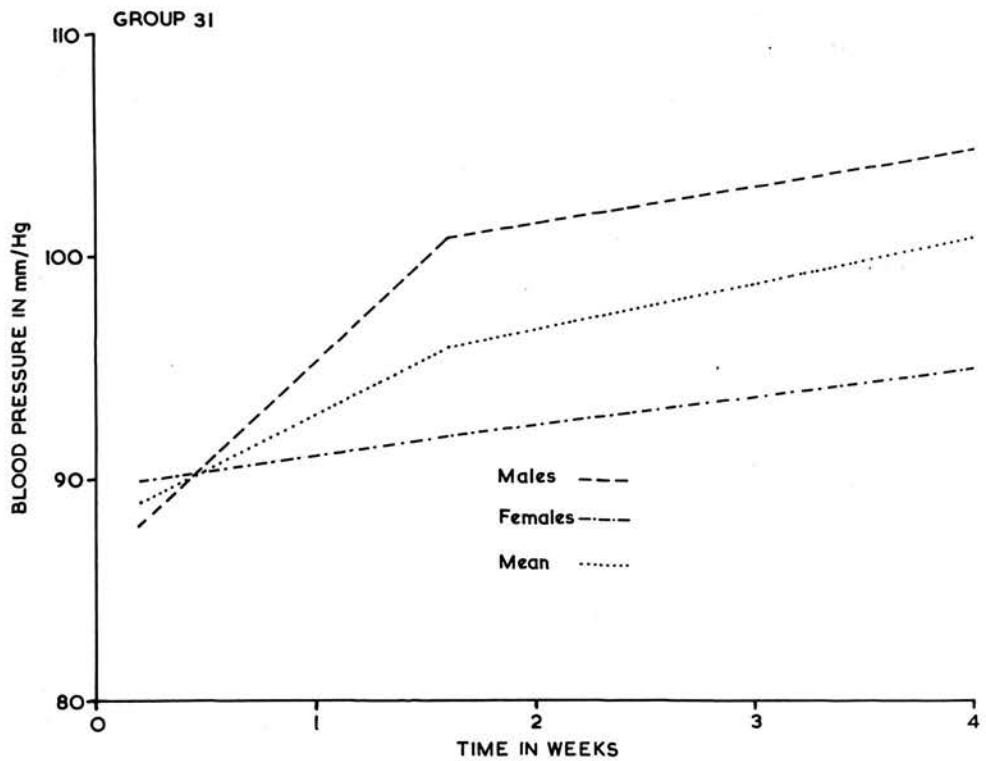


TABLE 130.

Experiment VI(c) Animal Group 31.

BLOOD PRESSURES in mm./Hg.

Rat	13.2.58	24.2.58	10.3.58
Males			
1	94	-	
2	72	104	109
3	82	102	99
4	106	98	107
Mean	88	101	105
Females			
1	94	85	95
2	78	90	80
3	92	94	99
4	96	100	105
Mean	90	92	95
Grand Mean	89	96	101

Text Figure 43.

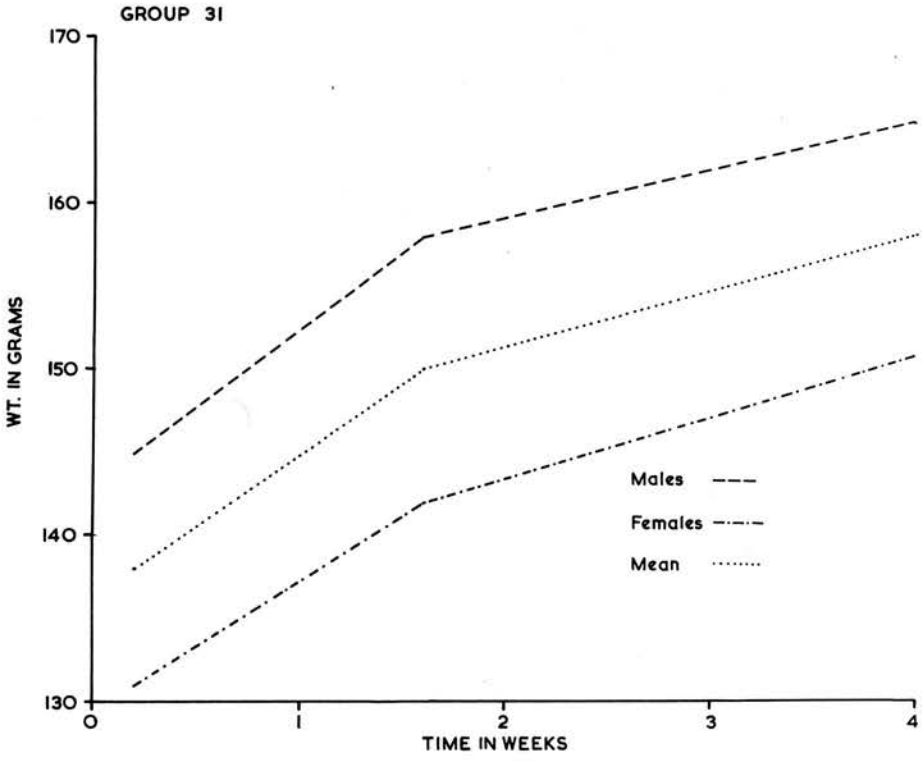


TABLE 131.

Experiment V.I(c) Animal Group 31.

WEIGHT IN GRAMS.

Rat	13.2.58	24.2.58	10.3.58
Males			
1	140	160	165
2	175	164	180
3	110	148	160
4	155	160	155
Mean	145	158	165
Females			
1	135	145	150
2	130	148	155
3	125	140	160
4	134	135	140
Mean	131	142	151
Grand Mean	138	150	158

1.30 g. and 0.79%, and for the females 1.51 g. and 1.0%: a result showing a considerably more pronounced hypertrophy in the female.

Kidney weight:

The mean kidney weight at the close of the experiment was 1.51 g., giving a mean kidney/body weight ratio of 0.96%. The corresponding figures for males were 1.61 g. and 0.92%, and for females 1.41 g. and 0.94%. These results did not, unlike the heart weight, show a sex difference.

Serum sodium:

At the close of the experiment the mean serum sodium was 152.5 m.Eq./l., a figure outside the normal range (135 - 145 m.Eq./l.). The level for males was 154.4 m.Eq./l., and for females 150.6 m.Eq./l., figures which are not significantly different.

Serum potassium:

The mean terminal level of serum potassium was 4.04 m.Eq./l. (within the normal range, 3.5 - 5.5 m.Eq./l.). The male group had a mean serum potassium (3.99 m.Eq./l.) slightly lower than the female, but these figures do not

TABLE 132.

Experiment VI(c) Animal Group 31.

TERMINAL MEASUREMENTS

Rat	Weight (in grams)	Heart Weight (in grams)	Kidney Weight (in grams)	Serum Sodium mg./100 ml.	Serum Sodium m.Eq./l.	Serum Potassium mg./100 ml.	Serum Potassium m.Eq./l.
Males							
1	165	1.10	1.50	357#	155.1	13.5	3.47
2	180	1.20	1.90	366	159.1	15.7	4.03
3	160	1.60	1.40	349	151.7	20.0	5.12
4*	155	1.30	1.80	349	151.7	13.0	3.34
Mean	165 g.	1.30 g.	1.61 g.	355	154.4	15.5	3.99
Females							
1*	150	1.50	1.70	336	146.0	14.5	3.73
2	155	1.90	1.20	349#	151.0	20.3	5.20
3	160	1.30	1.80	354	153.8	12.7	3.26
4	140	1.60	1.10	349#	151.7	16.4	4.21.
Mean	151 g.	1.51 g.	1.41 g.	347	150.6	15.9	4.10
Grand Mean	158 g.	1.41 g.	1.51 g.	351	152.5	15.7	4.04

* Evidence of generalised abdominal pyaemic infection with liver involvement.
# Slight haemolysis.

differ significantly.

Histological results: (Tables 133, 134)

Throughout the kidneys of all animals in this group were the characteristic early renal changes of DCA overdosage. These included enlargement of the glomerular tufts, dilatation of collecting tubules and intratubular cytoplasmic protrusion. By contrast, none of the advanced changes due to DCA were present.

Discussion:

Within the limitations imposed by the duration of this experiment, it appeared that daily injections of increasing amounts of compound 14179, while suppressing the rise of blood pressure caused by DCA, prevented the evolution of that moiety of the DCA renal lesion which is believed by the present author to be caused by the rising blood pressure.

However, in spite of this action on blood pressure, compound 14179 did not prejudice glomerular structure by causing hemiglomerular necrosis.

It is possible that the duration of the experiment may have determined this difference. Alternatively (and

TABLE 133.

Experiment VI(c). Animal Group 31.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

GLOMERULAR CHANGES	Males 1	2	3	4	Females 1	2	3	4
Endothelial cell proliferation	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Epithelial cell proliferation	-	-	-	-	-	-	-	-
Capsular thickening	-	-	-	-	-	-	-	-
Capsular adhesions	-	-	-	-	-	-	-	-
Tubularisation	-	-	-	-	-	-	-	-
Endothelial cell swelling	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Enlargement of tufts	(+)	(+)	+	+	(+)	+	(+)	+
Segmental tuft necrosis (acute)	Doubtful lesion	-	-	-	-	-	-	-
" " " (subacute)	-	-	-	-	-	-	-	-
" " " (fibrosed)	-	-	-	-	-	-	-	-
Transudative capillary change	-	-	-	-	-	-	-	-
Hyaline droplet change	(+)	-	(+)	-	-	(+)	-	-
"Explosive" lesions	-	-	-	-	-	-	-	-
Fibrinoid arterial necrosis	-	-	-	-	-	-	-	-
Fibrinoid arteriolar necrosis	-	-	-	-	-	-	-	-
Arterial intimal hyperplasia with elastic reduplication	-	-	-	-	-	-	-	-

TABLE 134.

Experiment VI(c). Animal Group 31.

Key: Slight (+)
Moderate +
Severe ++
Extreme +++

TUBULAR CHANGES	Males 1	2	3	4	Females 1	2	3	4
Dilatation of								
a. prox.conv.tubules	-	-	-	-	-	-	-	-
b. dist.conv.tubules	-	-	-	-	-	-	-	-
c. thin Henle limb	-	-	-	-	-	-	-	-
d. thick Henle limb	-	-	-	-	-	-	-	-
e. collecting tubules	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Protrusion of tubular cytoplasm	Occasional (particularly in collecting tubules)				Occasional (particularly in collecting tubules)			
Protrusion of tubular nuclei	Occasional				Occasional			
Vacuolation of collecting tubule cells	-	-	-	-	-	-	-	-
Proteinuria	-	-	-	(+)	-	(+)	-	-
Hyaline casts	-	-	-	-	-	-	-	-
Hyaline droplet change	(+)	(+)	-	(+)	-	(+)	-	-
Necrosis	-	-	-	-	-	-	-	-
Regeneration	-	-	-	-	-	-	-	-

perhaps more likely) from a comparison with other hypotensive drugs, is the suggestion that hemiglomerular necrosis in the kidneys of animals treated with DCA may be a manifestation of treatment specific for hydrallazine. This suggestion supports the idea that hydrallazine alone determines hemiglomerular necrosis under these conditions, perhaps by acting as a haptene group, but it is contrary to the theory that hemiglomerular necrosis is caused specifically by variation in blood pressure.

CHAPTER 8

GENERAL DISCUSSION

The experiments described in this thesis have demonstrated for the first time that the hypotensive drug hydrallazine may modify the morphological changes produced in the kidney by DCA, and precipitate widespread focal hemiglomerular necrosis. At the same time, and incidentally, the experiments provide the only detailed published study known to the author of the comparative influence of hypotensive drugs on the rat kidney in experimental hypertension.

No claim can be advanced to have reproduced the renal lesions of disseminated lupus erythematosus. Nevertheless, the data provide evidence for the production of changes which resemble those of disseminated lupus erythematosus more nearly than have those found in other experimental studies.

The morphology of the renal lesions caused by hydrallazine.

The majority of the renal changes found as the consequence of treatment with hydrallazine have taken the form

of necroses of part of the glomerular tuft. The remainder of the tuft has almost always been normal. The affected glomeruli have been found scattered throughout the cortex of the involved kidney, and investigation has provided no evidence that the lesions have involved nephrons of any of the three principal varieties (McFarlane, 1941); neither has evidence been found to suggest that glomeruli with a particular variety of blood supply (e.g. those of the juxta-medullary zones) are especially liable to involvement.

Depending to some extent on the method by which animals were killed, hemiglomerular necroses of this type have contained greater or smaller numbers of red blood cells. In some instances, where damage to the capillary walls in the involved segment appears to have been particularly intense, aneurysmal-like capillary dilatation has occurred. In almost all instances of this kind, however, the outline of the tuft, although displaced, has been intact, and the capillary basement membrane, defined by staining with the periodic acid-Schiff reaction, has been preserved. In many other cases the bulk of the involved segment has been occupied by fibrinous material staining red with Picro-Mallory, purple with phosphotungstic acid haematoxylin, bright pink with eosin and positively by the periodic acid-Schiff method. Red cells and fibrin have often been found lying

together among the remains of the damaged glomerular segment.

In every instance, the blood vessels of the involved kidney have been normal. None has shown fibrinoid necrosis. In this, the changes have been in striking contrast to those described in malignant hypertension (Wilson and Byrom, 1939) in which identical glomerular changes are always associated with fibrinoid arteriolar necrosis.

In the same way there has been no obvious relationship between the frequency of glomerular damage and the intensity of other, particularly tubular, lesions. No evidence has been found therefore to suggest that the lesion is one of the whole nephron. Occasionally, particularly when the glomerular lesion is large, part or much of the related tubules show hyaline droplet change. It is particularly noticeable, in those examples, that proteinaceous material lies within the tubular segments showing hyaline droplet change but not within the more proximal part of the same tubule or within the associated subcapsular glomerular space. The evidence suggests in these instances that partial insufficiency of blood flow through the glomerulus has led to localised tubular ischaemia, thus precipitating hyaline droplet "change", and that protein has either

escaped directly into the tubule or has failed to be reabsorbed by the normal tubular-protein reabsorption mechanism (evidence for the existence of which has been derived from isotope studies).

Many hemiglomerular necroses have been small. Allowing for difficulty in comparing glomeruli which have often been cut tangentially, it is still clear that the proportion of the tuft implicated in the damaging process varies considerably. Occasionally, the whole tuft appears to be necrotic. This, however, is exceptional, and may in some cases be explained by tangential sectioning.

In some kidneys it was possible to trace the evolution of the glomerular lesions through a stage of resolution to a point at which the involved segment was represented by a fibrous scar. In many examples, both segmental tuft replacement fibrosis and acute necrotic lesions were present in the same kidney. In some instances lesions of widely differing ages were present in adjacent glomeruli. Segments at

differing stages in the evolution of the necrotic process were however never observed in the same glomerulus.

The conclusion is possible that the influence causing the segmental necrosis is not only focal in activity but is episodic in character. Alternatively it could be maintained that a continuously acting necrotising influence was effective for example in only those glomeruli which for metabolic or vascular reasons had more or less of their normal blood supply than usual. Thus it is believed that many nephrons exist much of the time in a "resting" state, a fact accounting for the large renal functional reserve. As in the liver lobules the activity of the different glomeruli is probably determined by its blood supply, this in turn determining, in these animals, the distribution of the necrotic lesions.

Factors influencing the production of focal hemiglomerular necrosis.

I. Anaesthesia.

In the course of the experiments described in this thesis, attention was drawn to several factors, incidental to the experimental procedure, which might have determined the occurrence of glomerular lesions. In comparing the

animals of group 7, experiment II(d), for example, with those of the control group (group I(B), experiment I(c)) it was clear that an untoward difference existed in their mode of treatment. Thus, in group 7, daily hydrallazine injections were given under ether anaesthesia. The suggestion was advanced that the anaesthetic itself might either precipitate glomerular damage directly, or might facilitate its occurrence. This possibility was excluded when it was found that animals injected daily with an innocuous fluid, under similar anaesthesia, did not sustain glomerular damage.

2. Convulsions.

A second factor distinguishing the groups with widespread glomerular lesions from those in the control groups was the occasional occurrence, in the animals given large doses of hydrallazine, of major epileptiform convulsions, a reaction characteristic of hydrallazine overdosage in dogs or rats. It was thought that the occurrence of these convulsions might have caused the frequent glomeruli necroses by a non-specific action on the renal circulation, although previous evidence that such a reaction may cause renal damage is not known to the author. The effect of this mechanism was studied by observing the response of a

group of animals (group 32, experiment III(b)) to increasingly large doses of a convulsant drug, picrotoxin. It was shown that the occurrence of convulsions did not, by itself, cause focal glomerular necrosis. This finding confirmed sporadic observations made in earlier groups of animals of whom a significantly large number died from convulsions at an early stage of the experiment.

In considering the aetiology and pathogenesis of focal glomerular necrosis induced by hydrallazine, the influence of these extraneous factors may, I believe, be discounted.

3. The action of Hydrallazine.

The possibility that hydrallazine exerted a direct damaging influence on the glomeruli of rats was examined (group 3, experiment II(a) and discarded, an observation agreeing with all earlier observations on this topic made either in man or other mammals. The suggestion was also considered that unilateral nephrectomy, by lowering the renal reserve, or by throwing upon the remaining kidney the additional metabolic stress of compensatory hypertrophy, might predispose to the damaging action of hydrallazine. Hydrallazine was given to unilaterally nephrectomised rats in doses corresponding to those given to animals of the

groups which developed focal necroses (Group 2, experiment II(b)). No evidence of renal damage was discovered. In the same way it was thought that DCA itself might sensitize the kidneys of otherwise normal rats to the necrotising influence of hydrallazine. Another group of animals (group 11, experiment II(c)) were therefore treated with DCA and with hydrallazine, and it was found that the combination of the two drugs alone exerted no influence on the structure of the glomeruli.

The conclusion was now inescapable that hydrallazine exerts its damaging influence only upon animals previously treated by unilateral nephrectomy with excess DCA and salt.

4. Repetition of the Experiment: the Significance of Intermittent Injections.

Before proceeding to a study of possible modifications in the response to hydrallazine which might be caused by varying the dose, form, or route of administration of DCA, it was thought desirable to confirm this conclusion by repeating the experiment.

A further group of animals (group 15, experiment II(d)) was therefore treated in a manner thought to be identical with that adopted in the treatment of group 7 (experiment

II(d)). The results were disappointingly negative. It was then realised that the treatment of this group differed in two ways from that of the original. First, the injections were given without anaesthesia, and second, the animals were injected on each day of the week. Observation had already shown that anaesthesia did not precipitate glomerular necrosis. It was therefore concluded that not only must the dose of hydrallazine be large in order to exert an influence on the glomerulus, but its daily administration must be discontinuous.

It proved difficult to incorporate this observation into any satisfactory theory put forward to account for the action of hydrallazine on the rat glomerulus. Later in this discussion however it will be suggested that this intermittency of action permits an excessive fluctuation in blood pressure which may be held to account for the glomerular damage.

5. <u>The influence of the dose of DCA</u>.

To study the effect of the dose on the evolution of hydrallazine-induced glomerulo-necrosis, implants of varying amounts of DCA were used. A study of the action of these implants, with salt, in unilaterally nephrectomised animals

suggested that 200 mg. of DCA, in the slowly absorbed preparation used in these experiments, influenced blood pressure and renal structure only slightly more rapidly than did 100 mg. It was not surprising therefore to find in comparable groups given hydrallazine that the incidence and severity of glomerulonecrosis was no greater in the group given 200 mg. DCA than in the group given 100 mg.

6. Influence of the mode of administration of DCA.

To study the influence of the mode of administration of DCA on the nature of the response to hydrallazine, it was decided to compare the effect of daily injections of DCA in the form of an aqueous suspension with that of both slowly absorbed and rapidly absorbed implants. For reasons not related to the present discussion, and which are detailed in the description of the experiment itself (group 21, experiment I(e) and group 22, experiment II(f)), the study of the effect of rapidly absorbed implants proved unsuccessful.

Daily injections of DCA precipitated a rapid rise in blood pressure and severe renal lesions, changes which are discussed in detail later (page 149). When hydrallazine was given to animals treated in the same way (group 24,

experiment II(g)) several remarkable effects were observed. It was found that hydrallazine suppressed the mean blood pressure, and simultaneously prevented the more severe renal lesions known(from study of the control group) to result from the DCA regime itself. Hydrallazine however did not prevent, or indeed alter, the swelling and ballooning of the cells of the glomerular tuft, nor did it influence the severe and widespread tubular lesions which are common in DCA overdosage and which are thought to be the result of electrolyte disturbances. The kidneys of this group therefore showed widespread and almost uniform glomerular and tubular changes of the type attributable to the known alterations of sodium, potassium or water regulation, but were almost entirely free from fibrinoid lesions of arteries, arterioles or glomeruli. The conspicuous periglomerular or perivascular proliferation of swollen endothelial-like cells, described by Selye (1950) as nephrosclerosis, was prevented. By contrast, and due apparently to the absence of effect on the main tubular changes, the kidneys were not only no smaller than those of animals not given hydrallazine, but in fact had continued to enlarge at the abnormal rate. (Fig. 83, Volume III)

In none of the kidneys of animals treated in this way were focal glomerular necroses seen. It appeared,

therefore, that more rapidly absorbed DCA causing severe renal lesions did not predispose to hemiglomerular necrosis in the same way as had the earlier, slowly-absorbed DCA implants. At the same time it must be remembered that the animals of this group received injections of hydrallazine on each day of the period of study. The failure of focal glomerular lesions to develop may reasonably be attributed to the mode of administration of hydrallazine rather than to a different effect of DCA, the animals being comparable with those studied in group 15 (experiment II(d)).

7. The influence of sex and diet.

Throughout the later experiments each animal group was arranged to contain equal numbers of males and females. No significant difference was found between males and females, in terms of the response to hydrallazine. Occasional minor differences were found between male and female blood pressure, weight or electrolyte changes, but these were generally slight and have been mentioned in the course of the appropriate experiment.

All animals through the experiment were maintained on a standard diet, the composition of which is given in Appendix 1, volume II. No evidence was found at any time of choline deficiency. The renal cortical changes did not, in

any case, resemble those described by Moore (1957) in his study of choline deficiency.

Distinction of renal changes caused by hydrallazine from those due to DCA

The literature dealing with the influence of DCA on the rat kidney has been reviewed in the introduction to this thesis. The conclusion that previous accounts of the renal change caused by DCA were controversial led to the experiments described in Chapter 2 (experiment Series I). Particular care was taken to investigate the nature of the effects caused by DCA itself, so that valid comparison with those of hydrallazine would be possible.

The nature of the DCA lesion

(see also experiment I(f), page 108
(Figs.5-55, Volume III)

It was found possible to trace the evolution of the renal lesions caused by DCA, and to detect the influence on these evolving lesions of factors such as size of dose, mode of administration and solubility. (see experiment series I).

The earliest detectable evidence of DCA overdosage was an increase in glomerular size, with the presence in

the glomerular tufts of more P.A.S. positive material than is normally seen. Progressive enlargement of the tuft was accompanied by dilatation of the collecting tubules. Sometimes collecting tubular dilatation was extreme: it appeared quite independently of glomerular enlargement, with which however, it was often associated, and was sometimes rapid in onset. Although later in the experiments much protein-containing material lay within the dilated tubules, dilatation was often seen in the absence of proteinuria. The suggestion by Selye (1950) that obstruction of the collecting tubules by protein-containing casts caused tubular dilatation could not be substantiated. Changes in the proximal convoluted tubules, and sometimes in the distal convolutions, accompanied collecting tubule dilatation. Dilatation of the proximal convolutions was seldom more than of moderate degree, but tubular cells often showed bizarre alteration in size and shape: many were separated at their inner margins in a manner which could not be attributed to fixation artefact. Cytoplasmic processes often protruded into the tubular lumina, and in more severely affected tubules, the nuclei lay in or near the protruded portions of the tubular cells. Bizarre appearances were produced in this way and tangential sections through the tubules, longitudinally, revealed clusters of divided nuclei

lying apparently free within the lumina.

Changes of a broadly similar pattern were detected in the collecting tubular cells. Here, however, cytoplasmic swelling and tubular dilatation were pronounced. In the most severely affected collecting tubules, cells could be seen protruding like drumsticks into the lumen, while the arrangement of the remaining epithelial cells resembled those of the proximal convolutions.

The collecting tubular changes were in many respects identical with those described by Oliver and his colleagues (Oliver et al, 1957) in experimental potassium depletion. Their presence confirmed the suggestion of potassium loss obtained by terminal serum potassium estimations. In this way indrect evidence was gained of the activity of the administered DCA.

Later glomerular changes have already been described (experiment I(f), page 108, volume I) and their progression to glomerular disintegration, frequently accompanied by widespread fibrinoid arterial, arteriolar and glomerular change, has been discussed.

In the study of the lesions precipitated by hydrallazine it has already been shown that this drug prevented the

grosser, more acute forms of vascular damage caused by DCA. At the same time hydrallazine did not influence the electrolyte-type lesions which included glomerular ballooning and swelling, vacuolation or protrusion of tubular epithelial cells.

A comparison of DCA- and hydrallazine-induced glomerular lesions

A comparison of the nature of the lesions caused by DCA, with those caused by hydrallazine clearly reveals their complete independence of form and distribution. The characteristics of these two main types of change may now be summarised.

Hydrallazine-induced, hemi-glomerular necroses.	DCA induced glomerular lesions.
1. Focal	1. Diffuse
2. Involve usually half of tuft.	2. Involve whole of tuft
3. Never accompanied by vascular changes.	3. Often accompanied by fibrinoid vascular change in later stages.
4. Unaffected part of tuft normal.	4. Majority of tuft cells swollen and ballooned.

No reasonable doubt now exists therefore that the changes induced by hydrallazine are entirely distinct from those caused by DCA.

Two anomalous results call for comment at this point. It was noted in the examination of the kidneys of group 14 (experiment I(b)) and of group 16 (experiment I(c)) that rare, isolated, focal, hemiglomerular necroses were present. This finding is clearly incompatible with the theory that these lesions are only caused by the action of hydrallazine on the remaining kidneys of animals subjected to unilateral nephrectomy and given excess DCA and salt. The animals of neither of these groups received hydrallazine, and only one was subjected to uninephrectomy. It must be concluded that rare glomerular lesions identical with those precipitated by hydrallazine may be caused by the isolated renal action of DCA and salt, or by DCA, salt and uninephrectomy.

This conclusion suggests that the action of hydrallazine on the kidney may take the form of an acceleration of a tendency of DCA and salt to cause hemiglomerular necrosis. Again, the rare lesions found in groups 14 and 16 might be attributed to an alternative mechanism. It has been noted, for example, that hemiglomerular necrosis can occur in experimental malignant hypertension (although always in

association with vascular lesions) and it might be possible, although unlikely, for the isolated necroses found in groups 14 and 16 to be the result of such an alternative agency.

The cause of the glomerular lesions precipitated by hydrallazine.

The factors influencing the development of focal hemi-glomerular necrosis in animals given hydrallazine have been described; it is now necessary to attempt to explain their development.

Five analyses have been made:

(1) A comparison was made between the hydrallazine lesions and those of similar morphology found in human diseases.

(2) The lesions caused by hydrallazine were considered in relation to others found by other workers in alternative experimental procedures.

(3) A series of experiments (Series V) were undertaken to contrast the results of treatment with DCA with those of treatment with certain other steroids in relation to the effect of hydrallazine on the glomeruli. In this way it was hoped to determine whether the glomerular changes caused by hydrallazine were a specific result of earlier treatment

with DCA.

(4) A further series of experiments (Series VI) was performed with the aim of comparing the effects of other hypotensive drugs on renal structure in DCA hypertension with those caused by hydrallazine. The purpose of these experments was to elucidate the role of intermittent hypotension in precipitating focal glomerular necrosis.

(5) Finally, the specificity of the response to hydrallazine and DCA was studied in a group of animals made hypertensive by the application of renal arterial clips. In this way it was hoped to define in greater detail the precise role of DCA in the evolution of the glomerular lesions precipitated by hydrallazine.

(I) A comparison between the glomerular lesions precipitated by hydrallazine and those of similar morphology occurring in human diseases of known aetiology.

In a small number of human diseases renal lesions are found which are indistinguishable from those characteristic of the action of hydrallazine in the rat. In a further series of human diseases glomerular lesions are seen which bear a generic resemblance to those caused in the rat by hydrallazine, but which are clearly not identical.

(a) Human lesions closely resembling those precipitated by hydrallazine.

The human renal lesion which most closely resembles those induced by hydrallazine in the rat kidney is that of the so-called focal endocarditic glomerulonephritis, known alternatively as focal embolic glomerulonephritis. These glomerular lesions are found in between one half and nine-tenths of cases of subacute bacterial endocarditis. Believed for many years to be of embolic origin, the segmental tuft necrosis in this condition is now thought to be of allergic or hypersensitivity origin. The lesion originates as a more or less circular focus of fibrinoid, frequently limited to one or two segments of the glomerular tuft, and rarely involving the whole tuft. The acute, eosinophilic lesion undergoes organisation and forms a densely hyalinised, hard, segmental collagenous mass, whose outline and shape maintain those of the original lesion. The portion of tuft not involved is often entirely normal. The lesions may be few or many, but it is thought that in about 8% of cases the severity of the renal involvement is sufficient to cause renal failure.

Allen (1951) states that those focal lesions are practically pathognomonic (in the human) of subacute bacterial

endocarditis, but casts considerable doubt on their embolic nature, giving the following reasons: (1) The focal tuft necroses rarely contain bacteria; (2) They are never seen in acute bacterial endocarditis; (3) They are occasionally seen in the absence of subacute bacterial endocarditis, and (4) the likelihood of many small emboli landing in one organ and not in others appears slight. In addition, Allen points out, true septic emboli do not cause this type of lesion.

The close similarity in almost every respect between these lesions and those found as a result of treatment with hydrallazine suggests that a common factor may be concerned in their development. It is not easy to see what this factor could be. There is no evidence to suggest than an allergic or hyperimmune basis could be concerned in the development of the hydrallazine lesions, although, in the case of the human hydrallazine syndrome, it has been suggested that hydrallazine may combine with protein as a haptene, leading to an allergic disorder (Henn, Parkin, Hargraves and Odel, 1955). It appears more likely to the present author that the mechanism causing the development of glomerular lesions in the rat, which can perhaps be related to the regulation of blood pressure, may also account for the changes in endocarditic glomerulonephritis. This

This hypothesis however is outside the scope of the present thesis.

Focal glomerular damage morphologically similar to that caused by hydrallazine is sometimes observed in the kidneys of persons with disseminated lupus erythematosus. Clearly this resemblance is of the greatest significance in relation to the hypothesis on which these experiments were planned. It must at once be emphasised that focal glomerular necrosis is not a characteristic renal lesion in disseminated lupus erythematosus. Occasionally however focal necrosis is seen in the kidneys of persons with confirmed disseminated lupus. Attention is drawn to such a case by Muehrcke, Kark, Pirani and Pollak (1958) in their monograph. On page 122 of this analysis of the renal changes in disseminated lupus erythematosus they illustrate a segmental glomerular lesion in a human glomerulus from a confirmed case. They describe this lesion as "typical local necrosis with adhesions to Bowman's capsule". Within the necrotic area, however, haematoxylin bodies are stated to be present. These bodies cannot be distinguished in the black and white photograph which they show.

It appears that this occasional segmental lesion of disseminated lupus erythematosus can be distinguished from

the segmental lesion caused by hydrallazine by the absence in the latter of haematoxylin bodies. In spite of this distinction it is probably fair to comment that the effects of hydrallazine resemble those found in disseminated lupus erythematosus more nearly than any which have previously been identified in an experimental animal.

In polyarteritis nodosa (Davson, Ball and Platt, 1948) the vascular changes may involve glomerular tufts, leading to a focal inflammatory lesion of the kidney which sometimes resembles those seen in the kidneys of animals given excess DCA and hydrallazine. This glomerulitis of polyarteritis may be of a necrotising variety, in which case the appearance of the lesion may resemble that due to hydrallazine. Nevertheless, in polyarteritis a greater proportion of the tuft is involved, and the other renal vessels usually show evidence of fibrinoid necrosis. There is no reason therefore to associate the focal necroses of hydrallazine intoxication with the glomerulitis of polyarteritis nodosa. From this point of view a disorder of the immune mechanism, thought to play a part in the evolution of polyarteritis, cannot be invoked as an agency in the genesis of the hydrallazine lesion.

Malignant hypertension is sometimes associated with

the appearance of hemiglomerular necroses which resemble those seen in the kidney of the rat given DCA and hydrallazine. This is particularly the case in experimental malignant hypertension (Wilson and Byrom, 1939). Nevertheless, such changes in malignant hypertension are always associated with severe vascular lesions which frequently take the form of fibrinoid necrosis. This association provides a clear cut distinction from the hemiglomerular lesions found as a result of the treatment of uninephrectomised rats with slowly absorbed DCA, salt and hydrallazine; in these animals the development of vascular lesions is prevented.

Attention has recently been drawn by Dick (1957) to a condition which he calls glomerular lysis. In a patient dying with anuria following the treatment of a skin rash with B-dimethylaminoethyl benzhydryl ether hydrochloride he found almost entire destruction of the glomeruli of both kidneys. The condition is mentioned at this point because of the clear resemblance of some of the "lysed" glomeruli shown in his illustrations to those of advanced DCA overdosage in the rat. However, the lysed glomeruli do not resemble the hemiglomerular lesions seen as a result of hydrallazine treatment.

A further condition which calls for comparison with the present experimental lesions is the acute necrotizing glomerulonephritis described by Dunn and Montgomery (1941). This lesion is found in association with bilateral renal cortical necrosis, often with pregnancy toxaemia. In spite of the common factor of glomerulonecrosis, the hydrallazine-induced lesion is distinguished from the necrotising glomerulonephritis by its focal distribution, by the absence of vascular disease or of cortical necrosis, and by the frequent restriction of the lesion to one segment of the glomerular tuft.

(b) Human renal lesions of segmental glomerular type, but distinguishable from those precipitated by hydrallazine.

In a small number of distinctive conditions in the human, segmental glomerular lesions develop, often of distinctive character: they provide evidence of a negative nature about the segmental changes precipitated by hydrallazine.

The first of these is the apparently structureless Kimmelstiel-Wilson body found in the glomeruli of a proportion of cases of diabetes mellitus, and regarded by many

as pathognomonic of this disease (Lambie and MacFarlane, 1957). Occasionally, cavernous dilatation of capillaries occurs at the margin of such a body, and the whole lesion thus produced occupies a significant proportion of the glomerular tuft, and requires to be distinguished from focal, segmental glomerulonecrosis. The laminated argyrophilic appearance of the Kimmelstiel-Wilson body is usually characteristic.

Occasionally the focal distribution of amyloid deposits in affected glomeruli bear a superficial resemblance to focal necrosis with the presence of eosinophlic amorphous material. The distinction is usually possible without resorting to special stains. There is no obvious relationship between such focal amyloid deposits and the lesions found in the kidneys of the experimental animals in the present series.

(II) A comparison between the glomerular lesions precipitated by hydrallazine and those of similar morphology occurring in other experimental circumstances.

Glomerular lesions more or less similar to those caused by hydrallazine have been described previously as the result of a variety of experimental procedures. It seemed

possible that a review of these results might assist in the analysis of the mechanism by which the hydrallazine lesions are brought about.

(a) Uranium nitrate

Suzuki (1926) gave uranium nitrate to rabbits, and paid particular attention to the glomeruli. There was epithelial cell swelling in the tuft, and the intraglomerular proliferation of tissue. Hyaline droplet change of the tuft was found. Later tubular atrophy developed. Dake (1926) also injected rabbits subcutaneously with uranium nitrate - he found ceullular enlargement of the glomerular tufts, nuclear pyknosis and occasional rupture of the walls of capillaries.

In spite of a distant generic similarity between these experimental findings and those caused in the rat by hydrallazine, no conclusion could be reached as to whether a fundamental similarity existed between the effects of the two drugs. It seems doubtful whether this is in fact the case, and it would clearly be unwise to suggest an analogy between their respective glomerulotoxic actions.

(b) Mercuric chloride.

Oliver and Smith (1931) examined the effect of mercuric chloride, and of several other chemicals, on the kidney of the frog. Mercury particularly caused focal glomerular necrosis. Later, Oliver (1932) used the dual blood supply of the frog's kidney to study the mode of action of mercuric chloride. The glomeruli included foci of oedema, necrosis and nuclear degeneration.

Again there is no direct evidence to suggest that the mode of action of the two drugs is in any way the same, although morphologically these changes resemble in their formation and situation those found in glomeruli damaged by hydrallazine.

(c) Bacterial allergy.

Lukens and Longcope (1931) sensitized rabbits to haemolytic streptococci and studied their response. About half the animals developed glomerular changes, which included hyaline capillary thrombi, necrosis and exudation of polymorphonuclear neutrophils. They described the lesions as being focal in distribution; they occurred in the kidneys of both sensitized and intact animals, but were more common in the former, and resembled the so-called "embolic"

lesion (see page 156, Volume II).

Evidence exists therefore to show that a procedure known to sensitize animals to bacterial products may result in focal renal lesions closely resembling those of the hydrallazine reaction. The idea that hydrallazine could act as a haptene suggested that a form of allergy might be responsible for the evolution of the focal glomerular lesions which it causes, a suggestion that has been discussed earlier in this thesis (page 157, Volume II).

(d) Bacteria: Diphtheritic and Streptococcal lesions.

Clawson (1926) injected a live culture of Streptococcus viridans into the hearts of rabbits, but found lesions which took the form of infarcts. When streptococci agglutinated by a specific antiserum were injected in the same way, glomerular lesions were produced of the focal endocarditic type. The so-called "endocarditic" lesions were also studied by Baehr (1931).

Patrassi (1932) injected diphtheria toxin singly or repeatedly into rabbits and produced a focal toxic glomerulonephritis, while Pescatori (1930) described incidental renal lesions in animals injected intraarticularly with diphtheria bacilli.

Leiter (1924) and Gray (1928) also studied the focal

glomerular lesions caused by the repeated injection of diphtheria and streptococcal toxins.

It will thus be seen that a considerable volume of work suggests that focal glomerular lesions, often of the "endocarditic" type can be produced by the repetitive injection of animals with bacterial products or toxins. Most of this work has been carried out with rabbits and it is doubtful to what extent the conclusions are applicable to rats. For the immediate purpose, however, it serves to show that lesions morphologically resembling those precipitated by hydrallazine can be induced by procedures of a kind known to lead to an immune response. Again, therefore, indirect evidence is provided which suggests that an immunity mechanism may play some part in the renal response to hydrallazine.

(f) The effects of transient renal ischaemia.

In a personal communication to the author Sheehan (1957) stated that he had observed glomerular lesions resembling those produced in the rat by hydrallazine, in rabbits in which transient renal ischaemia had been produced by temporary constriction of the renal artery or aorta. Reports of work of this kind have been made by Scarff and

Keele (1943) and by Koletsky (1954). The former authors removed one rabbit kidney, and clamped the other renal artery for varying periods of time. They measured blood pressures and blood urea levels, and showed that during the first 2-4 days after clamping the proximal convoluted tubules underwent degeneration but the glomeruli remained intact. After 10 days, when a progressive rise in the blood urea occurred, the proximal convoluted tubular epithelium became progressively thinned. Later regeneration occurred.

Koletsky (1954) indicated that similar changes occur in the rat. A cycle of tubular necrosis, repair and atrophy follow temporary ischaemia, involving particularly the proximal convolutions. No evidence of glomerular damage was found under these circumstances.

It may be concluded that temporary interruption of the blood supply to the kidney does not always cause glomerular lesions of the focal type found in the present experiments. This confirms the observations made in experiment III(b) (group 32) in which sufficient picrotoxin to cause generalised convulsions (and thus possibly a transient disturbance of renal blood supply) did not affect glomerular structure.

(f) Effect of adrenalin and noradrenalin on renal structure.

In rabbits, complete or patchy cortical ischaemia may be caused by the intravenous injection of adrenalin or noradrenalin (Moses, 1952). This pressor effect is thought to be accentuated by previous treatment with DCA. It therefore seemed possible that the action of hydrallazine on glomerular structure might in some way be related to the ischaemia caused by noradrenalin. This however is clearly not the case, a finding which is in confirmation of the earlier work of Vallery-Radot, Albeaux-Fernet and Delamore (1932).

(g) The influence of renin on the kidneys of rats previously treated with DCA.

Earlier observations on the influence of repeated injections of renin into rats (Masson, Corcoran and Page, 1950) led to a study of the influence of previous treatment with DCA on the response to renin. The results were remarkable (Masson, Corcoran and Page, 1951) although the conclusion that the effects so produced resembled human eclampsia were clearly open to discussion (Masson, Corcoran and Page, 1952). Among the renal lesions produced in this way

are some which appear (in illustration) to resemble those caused by the action of hydrallazine on the rat kidney, and which take the form of tuft necrosis.

This is of particular interest in view of the claim by Renzi and Gaunt (1953) that the renal changes caused in this renin + DCA syndrome could be prevented by the administration of hydrallazine.

At first it appeared that these results conflicted with those obtained in the present experiment. Closer analysis revealed however that the results were not mutually exclusive. Thus, quite apart from the differing doses and mode of administration of hydrallazine in the two experiments, it was clear that Renzi and Gaunt had not allowed sufficiently for the alteration in the response to DCA brought about by hydrallazine. It appears to the present author that the results of Renzi and Gaunt could be interpreted as confirming the notable effect of hydrallazine on the renal changes in DCA overdosage, and that the prevention of the eclampsia-like syndrome was simply a reflection of this fundamental response.

(h) <u>The renal response to vasopressin</u>.

Byrom (1937) investigated the histological effects

produced by vasopressin. He showed that the changes were of three principal kinds (1) direct infarction, or ischaemic changes not quite amounting to necrosis; (2) damage to the blood vessels leading to increased permeability; and (3) medial necrosis of the constricted blood vessels when spasm due to vasopressin was maintained.

Clearly these changes, which do not include focal glomerular necrosis, are in no way similar to those caused by hydrallazine, and do not support the suggestion that a vasoconstrictor action such as that caused by vasopressin plays any part in the development of lesions caused by hydrallazine.

(i) Renal changes caused by 5-hydroxytryptamine.

Fiore-Donati and Erspamer (1957) have studied the changes produced in the rat kidney by 5-hydroxytryptamine. They have demonstrated that most of the changes found are those of renal cortical necrosis. There is no reason to suppose that the effects they describe are related to the focal action created by hydrallazine.

(j) Deficiency diseases: the structure of the kidney choline deficiency.

Comments in the literature occasionally draw attention to the occurrence of focal glomerular lesions caused by choline deficiency. Moore (1957), in a careful analysis of the response to choline deficiency, showed that the weanling rat kidney underwent three main changes. Initially, the tubules showed simple fatty changes. Later this progressed and necrosis of the proximal convoluted tubules occurred. Finally cortical necrosis developed. Moore analysed the glomerular damage occurring in this cortical necrosis and drew attention to the fact that although the majority of the glomeruli underwent complete infarction, occasionally only half the tuft was ischaemic.

The significance of this observation is the wide acceptance that these glomerular changes are due to ischaemia: it is not clear how choline could cause this change, but the demonstration that hemiglomerular necrosis may be one of the results lends weight to the suggestion that the hemiglomerular necroses caused by hydrallazine could be ischaemic in origin.

It is important to emphasise in this context, that hydrallazine-induced glomerular necrosis was not the result

of choline, or of other nutritional deficiency, in our experimental animals: the kidneys of untreated control groups were intact.

III. The relationship between the renal response to hydrallazine and the nature of the steroid used to promote hypertension or sodium retention.

Neither cortisone nor 9α -fluorocortisol was found to cause a significant rise in systolic blood pressure. It is difficult to account for this since other workers have confirmed that cortisone causes a rise in blood pressure in either adrenalectomised or normal animals given either salt or tap water to drink (Knowlton and Loeb, 1957).

The absence of this pressor effect in the animals of experimental Series V may be attributed to several possible alternative factors:

(1) The animals may have been too small. Personal observations suggest that the rat of less than 80 - 100 g. weight is excessively sensitive to bilateral adrenalectomy, and that subsequent cortisone in apparently adequate doses neither maintains life indefinitely nor leads to a rise in blood pressure. There is progressive weight loss due

(according to the work of others) to progressive water loss, and the hypertensive action of cortisone is concealed.

(2) Because of their relatively small size, too much cortisone may have been given. This is considered unlikely - the dose (2.5 mg. daily) used by other workers was adhered to, except in the case of the animals of groups 17 and 18.

(3) The cortisone used may have been inactive: the animals may in this way have died from adrenal cortical insufficiency; the absence of hypertension would have been an inevitable sequel. This explanation cannot be wholly true because the length of survival of all animals in this experimental series was significantly longer than could be the case in animals adrenalectomised but given no replacement therapy.

The failure of hypertension to accompany the use of either cortisone or 9 α-fluorocortisol in animal groups 17, 18, 19 and 20 may in part have been the consequence of the unilateral nephrectomy which was carried out before beginning treatment.

The response of the blood pressure to 9 α-fluorocortisol was surprising. An intense action on the control

of sodium excretion was demonstrated, the level of serum sodium at the time the animals were killed being found in some instances to be 50% or more above normal. By analogy with DCA a severe effect on blood pressure regulation was anticipated. This did not develop. Study of the animals' weights suggested that 9 α-fluorocortisol caused death by an action (perhaps similar to that of cortisone on water balance) involving progressive weight loss and a severe systemic metabolic disturbance, but not accompanied by hypertension.

In view of these results it was not altogether surprising to find that cortisone did not lead to any drastic change in renal morphology, thus confirming the work of previous observers (Knowlton and Loeb, 1957). With 9α-fluorocortisol the severe systemic disturbance in sodium regulation suggested that correspondingly advanced renal tubular changes would be found. This was not the case, and emphasised an important point, namely, that morphological changes in renal tubules are more likely to be proportional to total body or intracellular electrolyte levels than they are to vary with extracellular or particularly serum electrolyte concentrations. This was suggested by Knowlton and Loeb's demonstration that the degree of systemic cortisone-induced hypertension is proportional to total body potassium

and not to the level of serum potassium.

In the corresponding groups of animals given hydrallazine it was observed first, that focal glomerular necrosis did not occur, and second, that in some instances the hydrallazine appeared to alleviate the severe systemic disturbance caused by the steroids. The reason for this was not clear. An alteration in electrolyte regulation was suggested.

That focal glomerulonecrosis did not occur in the group given 9 α-fluorocortisol and hydrallazine suggested, but did not prove, that the influence of DCA on salt metabolism was not the property responsible for the predisposition to glomerulonecrosis. Nevertheless, a less simple relationship than this may probably be nearer the truth since earlier experiments (Series I) have already shown that DCA and salt alone may in rare instances cause focal glomerulonecrosis, but that DCA itself has no such effect.

(IV) <u>The influence of other hypotensive drugs on renal structure in steroid hypertension</u>.

It was thought that the action of hydrallazine on the glomeruli of uninephrectomised rats made hypertensive with DCA and salt might have been due to the direct action of

single daily doses of the drug on blood pressure. Although the experience of others in malignant hypertension suggested that in this condition glomerulonecroses were invariably associated with severe vascular damage (Wilson and Byrom, 1939) it was still thought that a fluctuating blood pressure could result in glomerular damage without necessarily causing fibrinoid arteriolar necrosis. Evidence of the mode of action of daily injections of hydrallazine was given in experiment II(g).

On this account experimental series VI was planned. In this series the actions of reserpine, pentolinium, and of compound 14179 were investigated. It was shown that neither pentolinium, nor reserpine nor compound 14179 led to focal glomerular necroses. This observation is open to the criticism that the circumstances optimal for the induction of glomerulonecrosis did not apply: thus DCA was given in a rapidly absorbed form, and daily administration of hydrallazine was not intermittent. Nevertheless, these experiments do provide some evidence that single daily injections of these hypotensive drugs, in doses sufficient to maintain the blood pressures at reasonably low mean levels, do not prejudice glomerular structure. Thus, less weight can be given to the theory that hydrallazine causes glomerulonecrosis purely by its hypotensive effect.

The experiments provided an effective demonstration that pentolinium, reserpine or compound 14179, in hypotensive doses, prevent the development of the more severe renal lesions caused by DCA. At the same time, the slighter glomerular and tubular changes caused by DCA were not influenced, thereby supporting the suggestion put forward by Gardner (1958) that only part of the renal glomerular damage caused by DCA can be attributed to its effect on blood pressure.

(v) <u>The influence of hydrallazine on the kidneys of animals made hypertensive by the constriction of one renal artery</u>.

The theory has been advanced that the action of hydrallazine on renal glomerular structure is conditioned by a specific property of DCA, and that in fact hydrallazine probably acts by precipitating an action exerted in rare instances by DCA itself. The suggestion was made that this action was related to variations in blood pressure, but experiments with other hypotensive drugs have not confirmed this. It was felt that strong evidence of the role of blood pressure fluctuations in causing focal hemiglomerular necroses would be provided if it were possible to demonstrate their production in the kidneys of animals

made hypertensive by a mechanism other than steroid overdosage. For this reason experimental series IV was undertaken.

The evidence obtained in these experiments suggested that the control of renal hypertension by single daily injections of hydrallazine did not precipitate focal hemiglomerular necroses. It is therefore concluded that the intermittent hypotensive action of hydrallazine will not, alone, cause focal glomerulonecrosis.

The role of hypotension in causing these renal lesions is therefore most doubtful. Although, because of slight dissimilarities between the experimental procedures, it cannot be stated categorically that intermittent hypotensive episodes play no part in precipitating glomerular damage there is at least sufficient evidence to render suspect such a mechanism.

GENERAL SUMMARY

(I) Changes resembling those of disseminated lupus erythematosus are occasionally produced in humans with hypertension treated for long periods with large amounts of

hydrallazine. The hypothesis was advanced that treatment of rats with experimental hypertension with correspondingly large amounts of hydrallazine might lead to the production of an experimental replica of disseminated lupus erythematosus. Attention was concentrated on the morphology of the kidney.

(II) To test this hypothesis it was decided to study hypertension brought about in the rat by giving large doses of deoxycortone acetate (DCA) and salt following unilateral nephrectomy. The first part of the work described in this thesis (experimental series I) was therefore concerned with defining the changes induced in the rat kidney by this regime. The evolution of a sequence of glomerular and tubular changes was demonstrated, and the influence of dose, and of rate and route of absorption were defined.

(III) Animals subjected to this hypertensive regime were then treated with increasingly large doses of hydrallazine, given by daily intramuscular injection. In the kidneys of the majority of the rats treated in this way following the subcutaneous implantation of slowly-absorbed DCA, focal hemiglomerular necroses were found in varying numbers. These glomerular necroses were present only in animals

given DCA in this particular form; they did not develop in rats injected with hydrallazine each day, but only in those given injections six days weekly. This intermittent effect was linked with the use of slowly absorbed DCA, and the absence of lesions from animals injected each day may be attributable to the simultaneous use of rapidly absorbed DCA in larger amounts.

The role of DCA in the evolution of these renal lesions was studied by using various forms of the steroid given by different routes. Only the slowly absorbed subcutaneous implants led to focal glomerular necroses.

(IV) The importance of the convulsions which occasionally followed the use of excessive amounts of hydrallazine, and of the ether anaesthesia used in some of the experiments, were studied. Neither convulsions by themselves, nor ether anaesthesia, led to glomerular damage.

(V) The pathogenesis of the glomerular lesions caused by hydrallazine was then studied. By giving hydrallazine to animals subjected to an alternative hypertensive regime (unilateral renal artery clipping) and by treating animals given excess DCA with alternative hypotensive drugs, evidence was obtained to show that the focal glomerular

necroses precipitated by hydrallazine were probably not simply due to variations in blood pressure.

(VI) In a further series of experiments the role of DCA in precipitating focal glomerular necrosis was examined by contrasting the influence of other steroids possessing certain common properties. Cortisone and 9 α-fluorocortisol did not predispose to the glomerular lesions precipitated by hydrallazine.

(VII) In an attempt to define their cause, the lesions produced by hydrallazine in animals given excess DCA and salt following unilateral nephrectomy were contrasted with those occurring in the human kidney. They were considered most closely to resemble the renal effects of subacute bacterial endocarditis (focal endocarditic glomerulonephritis) the glomerular lesions of which are now believed by many to be the result of an immunity disturbance.

It was shown that occasional similar lesions have been described in disseminated lupus erythematosus, but it was not felt justified to claim, on this basis, that the latter disease had been reproduced experimentally. Nevertheless, it was considered permissible to state that no lesions more closely resembling those of disseminated lupus

erythematosus had previously been produced in an experimental study.

(VIII) In the same way, the lesions precipitated by hydrallazine were compared with those found by other observers in a variety of different experimental procedures. From descriptions in the literature it became clear that the renal changes most closely resembling those precipitated by hydrallazine had occurred in experimental animals injected either with certain heavy metals (uranium or mercury) or with a number of bacterial extracts or toxins. Similar lesions had also been described in experimental malignant hypertension and in experimental choline deficiency but in each of these conditions the other renal changes were distinctive.

(IX) Hydrallazine may therefore be said to precipitate a highly individual form of glomerular lesion under highly specific experimental circumstances. The development of this lesion does not appear, from a series of ancillary experiments, to be the result of blood pressure variations caused by hydrallazine, but may be a form of hypersensitivity analagous to that found with other materials in a variety of animal experiments. DCA appears to play an

essential part in the evolution of focal glomerulonecrosis, a part which is not clearly related to a direct influence on sodium metabolism.

The relationship of this work to the aetiology of human disseminated lupus erythematosus is obviously not certain, but the experiments described have raised a considerable number of problems which must become the subject of future enquiry.

APPENDIX 1.

Composition of rat food.

Wheat By-products	20%
Ground oats	17½%
" maize	18¾%
" barley	8¾%
Meat and bone meal	8¾%
Fish meal	5%
Skim milk powder	7½%
Whey powder	6¼%
Unextracted dried yeast	1¼%
Molasses	5%
Salt	0.4%
Vit.A	5300 units/lb.
Vit.D_3	1320 units/lb.

APPENDIX 2.

Technique of nephrectomy in the rat.

Merthiolate was used as an antiseptic; it also served to dampen the hair, which was not shaved, and by its colour, to distinguish the animals which had been operated upon.

Under ether anaesthesia, an incision 1.5 - 2 cm. long, was made in the loin, over the point at which the kidney could be palpated between finger and thumb, and below the costal margin. The kidney was gently squeezed through the wound which, if of the correct size, allowed the kidney to be protruded in this way but prevented it from returning spontaneously. Mosquito forceps and an aneurysm needle were used to reflect the fat, fascia, and suprarenal from the kidney; mosquito forceps were applied to the renal pedicle, which was then ligated. The kidney was excised with a Bard-Parker scalpel, the muscle and skin closed with a single suture, and the skin cleaned with Merthiolate. The animal was returned to a warm cage.

Technique of DCA implantation.

Through a small skin incision in the posterior cervical region the implants were inserted subcutaneously, as far from the wound opening as possible. The wound

edges were carefully approximated and united by at least two layers of interrupted sutures: it was important to be certain that the implant could be extruded when the animal resumed its normal activity.

APPENDIX 3.

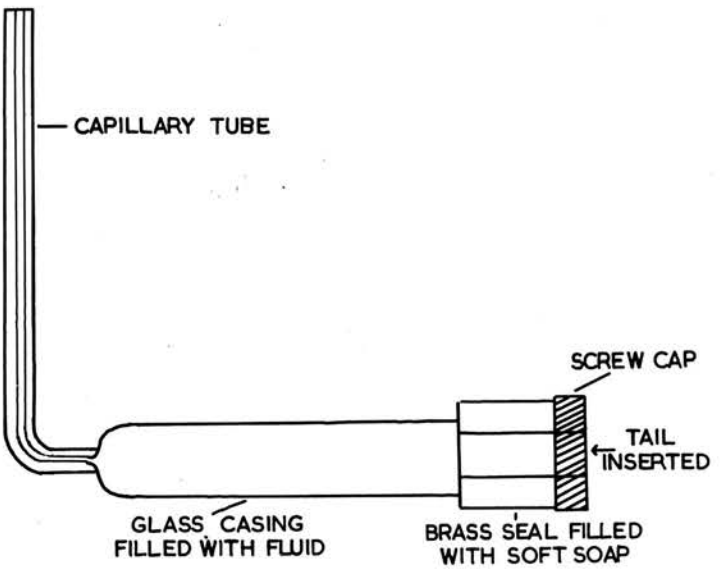
Apparatus and methods for recording blood pressure

1. Plethysmographic recording device (Fig. 44) (Byrom and Wilson, 1938).

This apparatus consisted of a portion of glass tubing, made from a suitable $\frac{5}{8}$" hard glass test tube, sealed at its base to a length of thick-walled capillary tubing, and fitted with a small side opening. The proximal end of the test tube was inserted into part of a rubber bung, which itself formed the base of a brass seal. The seal was made from a sawn-off microscope-objective box, and the space between the inserted test tube and the outer wall of the brass seal was filled with grease or soft soap: the end was closed by the screw cap of the brass objective box, which was fitted with a rubber washer and perforated in its centre to allow the tail of the animal to pass through.

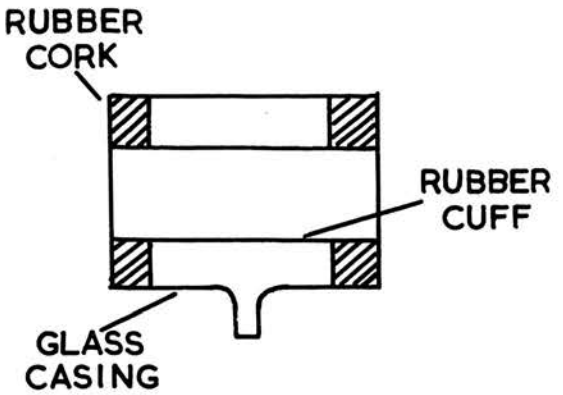
In conjunction with the plethysmograph, an occluding cuff (Fig. 45) of a type identical with those used in the optical recording apparatus, was used. The side piece of the glass cuff was attached to a sphygmomanometer of a standard medical type; when the pressure was raised by compressing the rubber manometer bulb, the inner rubber cuff gripped the rat tail. The

TEXT FIGURE 44.



PLETHYSMOGRAPH FOR RECORDING BLOOD PRESSURE OF RAT.

TEXT FIGURE 45.



RECORDING CUFF THROUGH WHICH TAIL OF RAT IS INSERTED FOR USE WITH OPTICAL OR PLETHYSMOGRAPHIC PRESSURE RECORDERS.

brass screw top of the seal could be tightened until the soft soap provided a water-tight seal around the tail.

2. Optical blood pressure recording device (Fig. 46) (Byrom, 1947).

The apparatus comprised:

(1) A source of light, conveniently a 6 V. car headlamp bulb, connected through a transformer to the main supply.

(2) A system of mirrors, enabling a long optical axis to be employed as a means of magnifying the small pulse pressure changes of the rat, and a recording screen.

(3) A manometric system, in which two cuffs similar to those described above, each attached to a mercury manometer, were used respectively to occlude the arterial pulsation.

The apparatus is represented diagrammatically in Figs. 45 and 46 . The mirror attached to the small rubber diaphragm contained inside the perspex box was situated 8 feet from the plane mirror. From the latter the light was reflected back onto a ground glass screen. To enable the pressures within and without the small diaphragm to be equilibrated, the air-tight perspex box was used, attached to a simple system of valves.

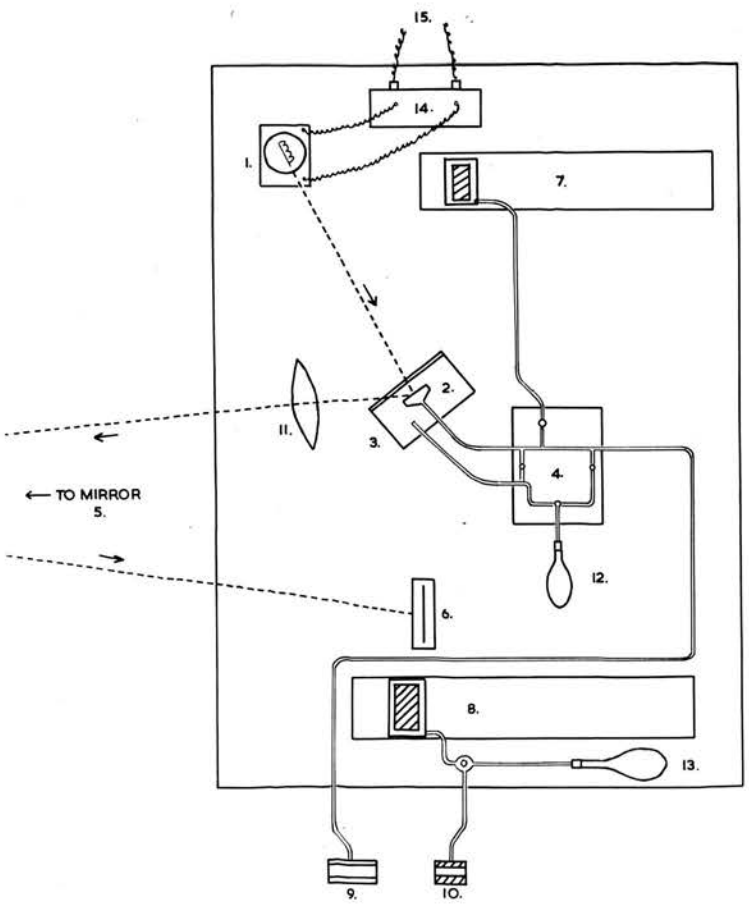
In using this apparatus it was desirable, but

not essential, to have the surrounding light diminished, and for the room to be quiet and free of movement - small oscillations of the floor were sufficient to cause the beam of light to move; this imposed a limitation on the use of this apparatus in a busy laboratory.

TEXT FIGURE 46.

OPTICAL PRESSURE RECORDING APPARATUS.

1. LIGHT SOURCE
2. DIAPHRAGM WITH ATTACHED MIRROR
3. AIR-TIGHT PERSPEX BOX
4. PRESSURE EQUILIBRATING DEVICE
5. PLANE MIRROR
6. RECORDING SCREEN
7. 'RECORDING' MANOMETER
8. 'OCCLUDING' MANOMETER
9. 'RECORDING' CUFF
10. 'OCCLUDING' CUFF
11. CONVEX MIRROR
12. } MANOMETER BULBS
13. }
14. 6v. TRANSFORMER
15. 200-250v. MAIN'S SUPPLY



APPENDIX 4.

Modified blood pressure recording device

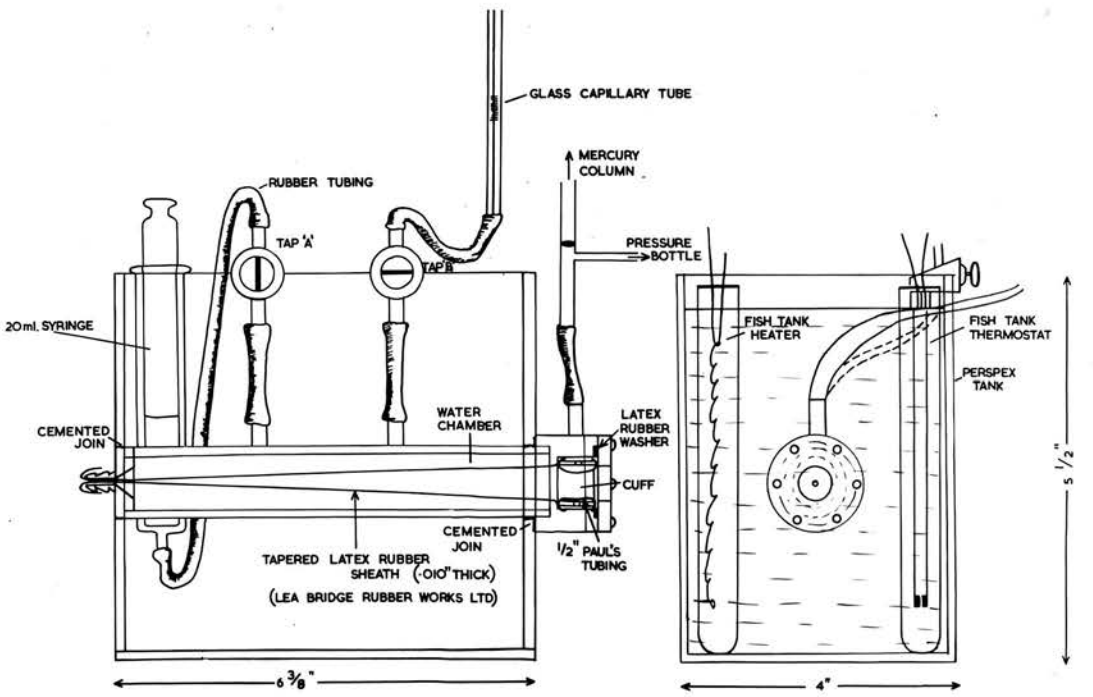
(by courtesy of Dr. M.A. Floyer).

This apparatus (shown in Text Figs. 47 and 48) was employed for much of the experiment as a means of recording blood pressure.

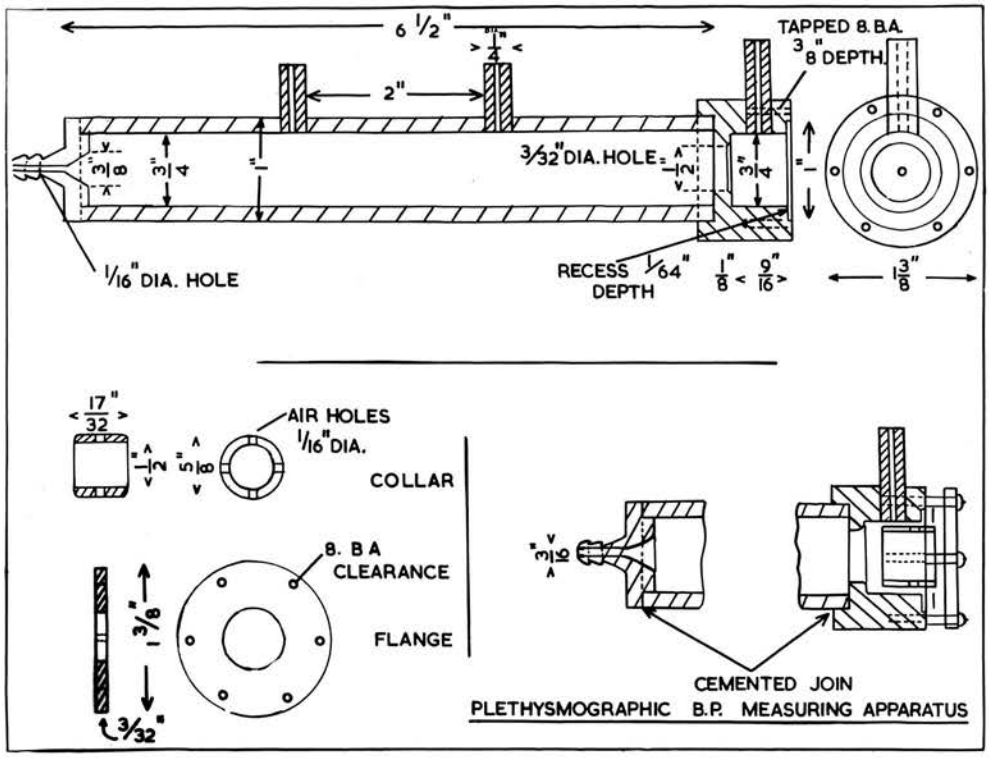
In principle it is a plethysmograph operating in almost identical manner to that described in Appendix 3. It is modified by the inclusion in the chamber which contains the tail, of the occluding and recording cuffs, and by the enclosure of the greater part of the apparatus in a thermostatically regulated water bath. A further water bath provides a platform on which the animal lies.

Light ether anaesthesia is used to quieten the animals. As with the other blood pressure recording devices illustrated here, the slight negative error caused by the use of anaesthesia was not thought to be significant from the point of view of the present experiments.

TEXT FIGURE 47.



TEXT FIGURE 48.



MATERIAL: PERSPEX.

APPENDIX 5.

Unless otherwise stated, the descriptions are based on the examination of sections stained by the Picro-Mallory method. In many instances sections from the same kidney were also stained by haematoxylin and eosin, Weigert's elastic stain, and periodic acid-Schiff.

GROUP 1.

Rat 1. L. renal clip 9.5.55.
Died (anaesthesia) 13.6.55.

At necropsy the silver wire was found around the left renal artery. The left kidney was pale but intact.

Microscopically there was generalised reduction in the number of renal tubules. The appearances were those of partial infarction. Many collecting tubules were dilated; some contained casts. There was a patchy increase in the amount of interstitial connective tissue, and occasional foci of round cells (mainly lymphocytes). Many glomeruli showed ischaemic changes; there was medial hypertrophy of the walls of the inter-:lobular arteries.

Limbs: The bones and joints were normal.

Heart: cartilaginous metaplasia of connective tissue at margin of attachment of valves.

Lungs: peribronchial lymphocytic hyperplasia.

Spleen: prominent follicular centres.

Liver: no significant abnormality.

Conclusion: Partial renal infarction was produced by the clip.

Rat 2. L. renal clip 9.5.55.
Killed 13.6.55.

The left kidney was slightly congested - the clamp placed around the left renal artery was not found. Adhesions were present at the site of right nephrectomy. Microscopically, the kidneys were well preserved, and showed no evidence of ischaemia. The glomerular tufts were normal, with thin capsules, and there were no arterial changes. The tubules were well preserved, and there was no interstitial fibrosis.

Liver, Limbs, Heart: no significant abnormality.

Spleen: prominent germinal follicular centres.

Lungs: peribronchial lymphocytic infiltration.

Conclusion: The application of the renal clip has caused no significant change in renal structure.

Rat 3. L. renal clip 3.8.55.
Died 5.8.55.

There was left renal infarction, with small amounts of perirenal blood. Microscopically, the renal changes did not differ significantly from those found in rat 3. The periadrenal fat was much congested, but the adrenal itself was intact.

Rat 5. L. renal clip 3.8.55.
Died 6.8.55.

The left renal arterial clip was found in situ. There was complete left renal infarction.

Rat 6. L. renal clip 3.8.55.
Died 10.8.55.

The lungs were intensely congested. The left renal arterial clip was in its correct position; the kidney was intact.

Rat 8. L. renal clip 3.8.55.
Died 12.8.55.

Organising blood clot lay in relation to the left kidney. The left renal clip was in situ. Microscop-:ically, there was congestion of the left kidney but no infarction. The limbs, heart and lungs showed no significant abnormality.

Spleen: hyaline material in follicles.

Liver: contains a single focus of haemopoietic cells.

Suprarenals: normal.

GROUP 1 (A)

Rat 1. (died 9.3.56)

Kidney: Prominent glomerular capillary basement membranes. Occasional polymorphonuclear leukocytes scattered throughout glomeruli. Autolysis of tubular epithelium.

Lungs: multiple abscess formation.

Suprarenals, Heart, Knees, Ankles — no significant abnormality.

Rat 2. (died 26.3.56)

Heart, Lung, Suprarenal, Kidney, Liver — no significant abnormality

Rat 3. (died 6.4.56)

Lungs: peribronchial round cell cuffing.

Heart, Spleen, Suprarenals, Knee — no significant abnormality

Ankle: no significant abnormality.

Kidney: occasional dilated tubules contain eosinophilic hyaline material.

Liver: occasional clusters of round chronic inflammatory cells arranged in centre of lobules.

Rat 5.

Kidney, Heart, Spleen, Suprarenals, Liver, Lung, Knee, Ankle — no significant abnormality

Rat 6.

Kidney: some autolysis.

Lung, Liver, Heart, Spleen — no significant abnormality

Suprarenal: intense congestion, oedema and focal haemorrhages.

Knee, Ankle — no significant abnormality.

Rat 7.

Kidney: occasional perivascular round-cell aggregates.

Lung: peribronchial lymphocytic cuffing.

Heart, Spleen, Knee, Ankles, Suprarenal, Liver — no significant abnormality

Rat 8.

Kidney, Spleen, Heart, Liver — no significant abnormality

Lung: peribronchial lymphocytic cuffing

Knee, Ankle, Suprarenals — no significant abnormality

GROUP 1 (B)
(see table)

Rat 1.

Lung)
) no significant abnormality
Heart)

Kidney: There is slight dilatation of the collecting tubules and intratubular protrusion of both cytoplasm and nuclei in the proximal convolutions. Occasional collecting tubules contain small amounts of protein. There is very slight generalised enlargement of the glomerular tufts, due apparently to endothelial cell hyperplasia. No vascular changes.

Spleen: much autolysis.

Ankle)
) no significant abnormality
Knee)

Rat 2.

Kidney: Tubular dilatation is severe and generalised, but is most severe in the collecting tubules. In the proximal convolutions there is severe intratubular protrusion of both cytoplasm and nuclei while occasional collecting tubules contain small amounts of protein. In the glomeruli, endothelial cell proliferation, with slight ballooning, has caused slight enlargement of the tufts; there are no vascular

changes.

Spleen: concentric vascular thickening.

Lung, Suprarenal, Heart, Knee, Ankle — no significant abnormality

Rat 3.

Kidney: Tubular changes are very slight, being confined to minimal dilatation of the collecting tubules of the cortex. There are no significant glomerular or vascular changes.

Suprarenals, Heart, Lung, Spleen, Liver, Knee, Ankle — no significant abnormality

Rat 5.

Kidney: Slight, but generalised dilatation of all parts of the majority of the renal tubules. This is accompanied by slight cytoplasmic, and rather more severe nuclear, intratubular protrusion in the proximal

convolutions. Slight, generalised endothelial cell hyperplasia has resulted in minimal glomerular tuft enlargement. There are no vascular changes.

Spleen: Prominent follicles. Chronic inflammatory changes.

Knee, Ankle, Suprarenal, Lung, Heart, Liver — no significant abnormality

Rat 6.

Kidney: Tubular effects are confined to slight dilatation of the collecting tubules, together with minimal intratubular protrusion, of both cytoplasm and nuclei, in the proximal convolutions. Glomerular changes include only mild generalised enlargement, apparently due to endothelial cell hyperplasia. There are no vascular changes.

Liver: occasional small round cell foci.

Spleen: prominent germinal follicles.

Heart, Lungs, Knee, Ankle — no significant abnormality

Rat 8.

Kidney: Slight dilatation of the collecting tubules is accompanied by intratubular cytoplasmic and nuclear protrusion of the proximal convolutions. There is very slight generalised glomerular enlargement apparently due to endothelial cell hyperplasia. There are no vascular changes.

Lung, Spleen, Suprarenal, Liver, Knee, Ankle — no significant abnormality

Rat 10.

Kidney: Tubular changes are more severe, with severe dilatation of the collecting tubules, and slight to moderate dilatation of the proximal and distal convolutions. In the proximal convolutions, intra-:tubular cytoplasmic and nuclear protrusion is accompanied by a bizarre hypertrophy of many of the epithelial nuclei. The glomeruli, generally, are enlarged, apparently due to endothelial cell hyperplasia. There are no vascular lesions.

Rat 11.

Kidney: Tubular lesions are confined to moderately severe dilatation of the collecting tubules, and to moderate intratubular cytoplasmic and nuclear protrusion in the proximal convolutions. The glomeruli show minimal generalised enlargement, apparently due to endothelial cell hyperplasia. There are no vascular changes.

Lung, Suprarenal, Spleen, Heart — no significant abnormality

GROUP 2.

Rat 1. (died 24.1.56).

Kidney: Slight autolysis has occurred. There are no significant tubular lesions. The structure of the glomeruli is intact, and there are no vascular lesions.

Brain: in the choroid plexus are accumulations of mononuclear cells - the brain substance is unusually cellular.

Rat 2. (died 3.2.56).

Lung: perivascular foci of chronic inflammatory cells, many of them lymphocytes, but more plasma cells than are often seen. Many cells with "signet-ring" nuclei are also present. The reaction as a whole does not resemble that seen commonly in ageing rats in this colony; basophilic nuclear debris is present, located particularly at the vascular bifurcations, and between the pulmonary artery and bronchi.

Liver: occasional chronic inflammatory cells are present, and others with the signet-ring arrangement of nuclear material.

Rat 3.

Kidney: Minimal dilatation of the proximal

convoluted tubules is accompanied by slight intratubular cytoplasmic and nuclear protrusion of the proximal convolutions. There is minimal glomerular enlargement, of very doubtful significance, and no vascular lesions.

Lung, Heart, Liver, Brain, Suprarenals, Bones and Joints — no significant abnormality

Rat 6. (died 7.2.56).

Kidney: The tubular changes are identical with those seen in rat 3 and include slight dilatation of the proximal convolutions together with minimal intratubular cytoplasmic and nuclear protrusion of proximal convolu-:tions and minimal generalised glomerular enlargement. There are no vascular lesions.

Spleen: degenerate fragments of haematoxyphil material lying free in the germinal centres and in the pulp.

Lung, Liver, Brain, Suprarenal — no significant abnormality

Feet: one normal. The other contains an extensive area of muscle necrosis.

GROUP 3.

Rat 5. (died 24.1.56.)

Kidneys: There are no significant renal tubular lesions. Both the glomeruli and blood vessels are intact.

Suprarenals)
) no significant abnormality
Heart)

Brain: congestion, proliferation of bacilli (probably post mortem) in the tissue.

Spleen: many polymorphonuclear neutrophil leuko-:cytes.

Lung: oedema and congestion. Several small perivascular aggregates of polymorphs and small round cells.

Liver: some polymorphs and lymphocytes, in the portal tracts.

Joints: aggregates of histiocyte-like, deeply staining cells in synovia.

Rat 7. (died 7.2.56).

Kidneys: There is minimal proteinuria in the lower part of very occasional nephrons. There are no other significant tubular lesions. Both glomeruli and blood vessels are intact.

Muscle: occasional intramuscular foci of histio-:cytes and lymphocytes.

Heart)
)
Brain)
)
Spleen) no significant abnormality
)
Knee)
)
Foot)

Suprarenals: large, congested sinuses.

Liver: a chronic inflammatory focus is present, containing a single giant cell.

Rat 8. (died 4.1.56.)

Liver: a considerable number of small round, chronic inflammatory cells in perivascular spaces.

Spleen)
)
Heart)
) no significant abnormality
Brain)
)
Lung)

Rat 10. (died 17.12.55).

Kidney: Autolysis has occurred and it is difficult to define detailed structure.

Suprarenals: pale islands of eosinophilic material lie between the cortical cells.

Lung: extensive oedematous or fibrinous exudate lies in two alveoli. The usual peribronchial lymphocytic

cuffing is present.

Liver)
)
Heart) no significant abnormality
)
Spleen)

Rat 11. (died 7.2.56).

Kidney: There are no significant renal tubular, glomerular or vascular changes.

Liver: very occasional foci of round and polygonal, histiocytic cells, some in relation to the dilated portal veins.

Suprarenals: intensely haemorrhagic and oedematous. The haemorrhage is mainly cortical in distribution, while the zona glomerulosa is less severely involved than the remainder. A single focus of polymorphonuclear leuko-:cytes is present.

Muscle: occasional perivascular histiocytes but no other significant abnormality.

Knee)
)
Foot) no significant abnormality
)
Heart)

Brain: small aggregates of basophilic masses are present in the centre of the section - their significance is uncertain.

Lung: moderately severe peribronchial lymphocytic cuffing and aggregates of deeply basophilic cells closely

related to the pulmonary arteries and veins.

Spleen: A single old infarct. Occasional small fragments of basophilic nuclear debris.

Rat 11.

Kidney: There are no significant renal tubular, glomerular or vascular lesions.

Rat 12.(died 31.12.55).

Kidneys: The structure of both tubules and glomeruli is intact and the blood vessels normal.

Suprarenals)
) no significant abnormality
Heart)

Lungs: one contains a single focus of lipoid-laden macrophages.

Liver: considerable numbers of small round cells, among them many plasma cells, in the portal tracts.

Spleen: much haemosiderin pigment.

GROUP 4.

Rat 1. (died 2.4.56.)

Left kidney: Changes in the renal tubules are confined to the presence within a small number of collecting tubules of proteinaceous material. The majority of the glomeruli are intact, but at one margin of the kidney, partial infarction has occurred: in this area there is glomerular crowding and tubular atrophy. The blood vessels within the kidney are intact.

Spleen: congestion.

Suprarenals: severe congestion.

Lung: no significant abnormality.

Rat 2. (died 2.4.56.)

Kidney: Autolysis has occurred. At one margin of the kidney adjoining the inferior margin of the renal pelvis there is a segmental cortical infarct. Here both glomeruli and tubules have disintegrated. In several other smaller cortical zones segmental scars are present, representing the points of occlusion of the intralobular blood vessels.

Heart: left ventricular hypertrophy.

Liver: no significant abnormality.

Lung: pneumonia.

Spleen: prominent perifollicular envelopes.

Rat 4. (died 22.2.56.)

Kidney: The appearances are those of severe hypertension (malignant). They are superimposed on widespread ischaemic atrophy and partial infarction. There is thus a combination of fibrous glomerular obliteration, with fibrinoid arteriolar and intraglomerular change. Dilated tubules containing an excess of protein alternate with areas showing ischaemic tubular atrophy and interstitial replacement fibrosis.

Heart: basophilic disruption of the aortic media. Thickened coronary artery.

Rat 5. (died 28.1.56.)

Kidney: The kidney to which the renal arterial clip has been applied shows partly complete infarction, and partly incomplete infarction with glomerular crowding. There is significant proteinuria. The remaining kidney is intact, and shows no sign of hypertensive change.

Liver: intense congestion.

Lungs: bronchiectasis, with multiple abscess formation.

Heart: small foci of subpericardial lymphocytes.

Pancreas)
) no significant abnormality
Joints)

Rat 6. (died 13.1.56.)

Left kidney: The clipped kidney has undergone almost complete infarction. Within the degenerate tubules proteinaceous material can still be detected, but with this exception there is no detectable pre-existing abnormality.

Lung: purulent bronchitis and bronchiectasis

Liver, Spleen, Heart, Pancreas: no significant abnormality

GROUP 5.

Rat 1. (died 21.4.56.)

Kidney: the clip was found in situ on the right renal artery. Neither tubules, glomeruli, nor blood vessels show any significant abnormality in either kidney.

Heart)
Spleen)
Liver) no significant abnormality
Lung)
Knee)
Ankle)

Rat 2. (died 21.4.56.)

Kidneys: Macroscopically, the right renal clip was found in situ, and the right kidney appeared totally infarcted. The left was normal. Microscopically, the right renal infarction was less complete than had appeared probable. A thin rim of apparently normal glomeruli, crowded together by reduction in the amount of interstitial tissue surrounds a zone of complete infarction. In the left kidney, there is some intimal hyperplasia of the arcuate arterial walls, and occasional ischaemic glomeruli.

Lung)
)
Liver)
)
Spleen) no significant abnormality
)
Knee)
)
Ankle)

Rat 3. (died 20.4.56.)

Kidneys: Macroscopically, the right kidney appeared totally necrotic, while the left appeared normal. The right renal clip was in situ. Microscopically, no identifiable renal tissue was found at the site of the right kidney while the left appeared normal. In the liver were occasional portal foci of cells, some of which contained occasional clusters of fragmented basophilic material.

Spleen: prominent perifollicular envelopes.

Heart)
)
Knee) no significant abnormality.
)
Ankle)

Rat 5. (died 17.1.56.)

Death from anaesthesia.

Kidneys: The renal clip was found in situ on the right renal artery; this right kidney was congested and surrounded by granulation tissue representing the post-operative reaction. The structure of both kidneys does

not differ significantly from normal.

Rat 6. (died 21.4.56.)

Kidneys: The right kidney could not be identified - it appeared that total infarction had been followed by dissolution. The left kidney appeared normal. On the right, a calcified cystic focus was found closely applied to the liver margin: this represented the remains of the clipped right kidney. There were occasional chronic inflammatory cells in the loose connective tissue around the hepatic blood vessels.

Heart)
)
Lung)
) no significant abnormality
Knee)
)
Ankle)

Rat 8. (died 21.4.56.)

Kidneys: The clip was found in situ on the right renal artery. Both kidneys appeared normal. However, there was crowding of glomeruli in the right kidney and ischaemic changes of moderate severity, together with a reduction in the amount of cortical substance. The left kidney appeared normal.

The lung showed conspicuous peribronchial lympho-:cytic cuffing, and the splenic follicles were prominent.

Liver)
)
Knee) no significant abnormality
)
Ankle)

Rat 9. (died 21.4.56.)

Kidneys: The clip was found in situ on the right renal artery; both kidneys appeared externally normal. In the sections of the right kidney there was glomerular crowding due to ischaemic change and reduction in volume of cortex due to tubular atrophy. The left kidney appeared normal.

Spleen)
)
Liver)
)
Heart) no significant abnormality
)
Lung)
)
Knee)
)
Ankle)

Rat 10. (died 20.3.56.)

Kidneys: Both kidneys appeared normal. There was no evidence of right renal ischaemia.

Lung: prominent peribronchial and peribronchiolar muscular hypertrophy, with some peribronchial lymphocytic cuffing, but no evidence of recent infection.

Spleen)
)
Suprarenals) no significant abnormality
)
Liver)

GROUP 6.

Rat 1. (killed 30.7.56.)

Right Kidney: Tubular changes are confined to dilatation of the cortical collecting tubules. The glomeruli and blood vessels are intact.

Heart: near the apex of the left ventricle was found a group of pale staining rounded cells with oval nuclei.

Spleen: a single small haemorrhagic infarct.

Lung, Liver, Suprarenals, Muscle — no significant abnormality

Rat 2. (killed 2.8.56.)

Right Kidney: There are no tubular, glomerular or vascular changes.

In the thigh muscle, probably the site of previous injections of hydrallazine, occur bundles of necrotic muscle fibres among which replacement fibrosis has occurred.

Spleen, Liver, Lung, Suprarenals — no significant abnormality

Heart)
)
Knee) no significant abnormality
)
Ankle)

Rat 4. (died 14.8.56.)

Right Kidney: There are no tubular, glomerular or vascular changes.

Microscopically in the liver were found aggregates of periportal round cells.

Lung)
)
Spleen)
)
Muscle)
)
Suprarenals) no significant abnormality
)
Heart)
)
Knee)
)
Ankle)

Rat 9. (died 20.9.56.)

Kidney)
)
Suprarenals)
)
Lung) no significant abnormality
)
Heart)
)
Spleen)

Rat 12. (died 20.9.56.)

Kidneys: no significant abnormality.

Liver: one section shows no significant abnormality.

The other contains several areas of pale staining, small mononuclear cells, the significance of the presence of which is uncertain.

Lung)
) no significant abnormality
Heart)

Suprarenals: much cortical lipoid.

GROUP 7.

Rat 1.(died 27.7.56.)

Right Kidney: The tubular changes are confined to vacuolation of a small proportion of the cortical and outer medullary collecting tubules. No glomerular abnormalities are present, but thrombus of antemortem origin is present within the lumen of a principal renal arterial branch.

Suprarenals: some autolysis.

Lungs: peribronchial lymphocytic cuffing.

Muscle: extensive reaction at injection site, with necrosis, mononuclear cell infiltration and fibrosis.

Bone marrow: an unusually large number of megakaryocytes are present.

Heart: in the right ventricular muscle near the endocardial surface is a single focus of round cells.

Spleen)
)
Liver)
) no significant abnormality
Ankle)
)
Knee)

Rat 1 (a). (died 1.10.56.)

Right Kidney: Tubular changes are considerably more severe. There is generalised slight dilatation

of many of the nephrons, accompanied by intratubular protrusion of both cytoplasm and nuclei of epithelium of the proximal convoluted tubules. Many glomeruli have undergone slight enlargement and this is apparently the result of endothelial cell hyperplasia.

Suprarenals)
) no significant abnormality
Heart)

Spleen: minimal thickening of the small arteries.

Lung: branches of the pulmonary artery show extreme hypertrophy.

Liver)
)
Muscle)
) no significant abnormality
Forelimb)
)
Hindlimb)

Rat 2. (died 1.10.56.)

Right Kidney: Many collecting tubules are slightly dilated, and within some are aggregates of proteinaceous fluid together with hyaline casts. There is moderate intratubular cytoplasmic and nuclear protrusion of the epithelium of the majority of the proximal convoluted tubules, together with vacuolation of the epithelial cells of many collecting tubules. Slight generalised glomerular enlargement is the result of endothelial cell hyperplasia, and is occasionally accompanied by capsular adhesions.

In a significant proportion of glomeruli, segmental glomerular necrosis has occurred, and is seen in acute, subacute and late stages. In the acute stage, one half of the glomerulus only is usually involved, the other half being intact and apparently normal. The involved half is disrupted by the accumulation of fibrin and red blood cells, often arranged as a microaneurysmal bulge in the tuft capillaries. In others, polymorpho-:nuclear leukocytes are frequent, lying among the fibrin and debris. Occasionally the lesion may be seen in a stage of resolution, with histiocytic infiltration. Later, fibrosis occurs, and the lesion is represented as a segmental tuft scar. Crescent formation and epithelial proliferation are rare, although occasionally the entire tuft appears to be involved. In some (the minority) of instances the acute segmental lesion has allowed the escape of proteinaceous fluid which lies free in the subcapsular space and in the corresponding tubule. Where proteinuria is present it is associated, in a significant number of instances, with hyaline droplet change in the epithelial cells of the proximal convolution.

Lungs: prominent hyperplastic, pulmonary arteries. Peribronchial lymphocytic cuffing.

Spleen: active germinal follicular centres.

Heart)
)
Suprarenal)
) no significant abnormality
Forelimb)
)
Hindlimb)

Liver: congestion.

Muscle: the site of a previous intramuscular injection is included in the section. Many degenerate muscle fibres, haemosiderin-containing histiocytes and fibroblasts are present.

Rat 3. (died 1.10.56.)

Right Kidney: There is a moderate hydronephrosis accompanied by severe dilatation of the collecting tubules and by slight dilatation of the distal convolution. Severe cytoplasmic and nuclear protrusion of proximal convoluted epithelial cells is present and is accompanied by vacuolation of the cells of many medullary collecting tubules. There is proteinuria accompanied both by the presence of hyaline casts and by hyaline droplet change in many of the related cells.

The glomerular tufts are enlarged, as a result of endothelial cell hyperplasia and within the subcapsular space of some lies a proteinaceous transudate. Segmental glomerular lesions are less frequent than in the kidney of rat 2, but in other glomeruli there is diffuse fibrosis apparently of ischaemic origin.

Spleen)
)
Heart)
) no significant abnormality
Forelimb)
)
Hindlimb)

Muscle: The injection site has been included in the section; occasional muscle cells are vacuolated and degenerate, while small numbers of histiocytes are present.

Lung: peribronchial lymphocytic cuffing. The walls of many bronchial arteries are hyperplastic.

Rat 4. (died 1.10.56.)

Right Kidney: There is hydronephrosis of slight degree. Tubular changes include slight dilatation of the distal convoluted tubules accompanied by severe dilatation of the collecting tubules. Both cytoplasm and nuclei of the proximal convolutions protrude within the tubular lumina, and there is occasional vacuolation of the medullary collecting tubular epithelial cells. Proteinaceous fluid is present within the lumen of small numbers of the collecting tubules in their lower parts, while others contain hyaline casts.

The glomerular tufts as a whole are enlarged, as a result of endothelial cell hyperplasia. Some show the acute segmental hemiglomerular necrosis (described in detail under rat 2), and in this group protein

containing fluid is sometimes found to have escaped into the glomerular subcapsular space, and thence into the corresponding tubules. The destructive process appears to be of more recent origin in this animal since none of the segmental changes are quiescent or have undergone replacement fibrosis.

Muscle: the degenerate muscle from an injection site is included in the section.

Lung: some small pulmonary arteries have hyper-:plastic walls.

Pancreas)
)
Spleen)
)
Liver) no significant abnormality
)
Forelimb)
)
Hindlimb)

Rat 6. (died 1.10.56.)

Right Kidney: Tubular changes are widespread. They include slight dilatation of the proximal convoluted tubules accompanied by slight distal convolution and severe collecting tubule dilatation. In the lumina of the proximal convolutions, both cytoplasmic and nuclear protrusion is seen, of severe degree. Vacuolation of the epithelium of the collecting tubules has occurred but is minimal, and there is slight proteinuria.

Considerable generalised tuft enlargement,

apparently with endothelial cell hyperplasia, accompanies a high incidence of segmental tuft haemorrhagic necrosis. In some the changes are predominantly acute and of recent onset. In others, the changes are subacute or have resulted in the ultimate segmental proliferation of fibrous tissue in the form of a scar. Usually this scar is unaccompanied by crescent formation, epithelial proliferation, or adhesions, but capsular thickening with adhesion formation is present independently in a small number, while in others transuded protein-containing fluid has escaped into the subcapsular space. In a small number of tufts hyaline droplet change is present.

Lungs: the wall of many small pulmonary arteries and arterioles appears hyperplastic.

Muscle)
Heart)
Suprarenal) no significant abnormality
Forelimb)
Hindlimb)

Rat 8. (died 1.10.56.)

Kidney: Changes as a whole are considerably slighter than in rat 6. Tubular effects are represented by slight dilatation of the proximal and distal convolu-:tions and moderate dilatation of the cortical collecting

tubules. In the proximal convolutions severe intra-:tubular protrusion of both cells and cytoplasm has occurred, while there is vacuolation of occasional cortical collecting tubular cells, with proteinuria in some.

Many glomeruli have undergone enlargement due to endothelial cell hyperplasia, and in significant numbers segmental zones of acute haemorrhagic tuft necrosis have occurred, of a type described under rat 2.

Heart, Spleen, Muscle, Forelimb, Hindlimb — no significant abnormality

Lung: the pulmonary arterial branches in this animal do not show hyperplasia.

Liver: occasional lymphocytes form small collec-:tions of cells in the portal tracts.

Rat 9. (died 21.9.56.)

Right Kidney: Considerable autolysis has occurred. It is still possible to detect the presence of capsular adhesions, and of occasional subacute segmental glomerular lesions of a type described under rat 2. In some instances they are accompanied by the

transudation of protein into the subcapsular space.

Heart, Spleen, Lung, Forelimb, Hindlimb, Suprarenals — no significant abnormality

Rat 11. (died 1.10.56.)

Right Kidney: There is slight dilatation of the proximal and distal convolutions, accompanied by moderate dilatation of the collecting tubules. There is occasional vacuolation of the cells of the collecting tubular epi-:thelium with protrusion of both nuclei and of cytoplasm into the lumen of the proximal convolutions. Many glomeruli are slightly enlarged, with endothelial cell hyperplasia, and in a significant number there are acute and subacute segmental tuft necroses (see rat 2), acute changes being more common than subacute.

Liver: occasional small foci of necrosis are present.

Muscle, Suprarenals, Forelimb, Hindlimb — no significant abnormality

Rat 12. (died 21.9.56.)

Right Kidney: Tubular changes are confined to slight dilatation of many distal convoluted tubules and moderate dilatation of many collecting tubules, the lining cells of which are occasionally vacuolated.

Many glomeruli are enlarged, due to endothelial cell proliferation, while none shows segmental necroses.

Liver: a single cluster of small mononuclear cells is present in one section.

Suprarenals)
Heart)
Lung) no significant abnormality
Forelimb)
Hindlimb)

GROUP 8.

Rat 2.

Right Kidney: Autolysis has occurred. Nevertheless it is still possible to detect slight dilatation of the proximal convoluted tubules into the lumina of which both cytoplasm and nuclei protrude. There is also vacuolation (of doubtful significance) of the collecting tubule epithelial cells.

There are no significant glomerular or vascular lesions.

Rat 3.

Right Kidney: Autolysis has occurred. Recognisable changes are confined to moderately severe intratubular protrusion of both cells and nuclei in the proximal convolutions. There are no significant glomerular or vascular lesions.

Rat 4.

Right Kidney: Tubular changes are confined to severe intratubular protrusion of both cytoplasm and nuclei of the epithelium of the collecting tubules. Glomerular changes are confined to the presence, in very occasional glomeruli, of focal segmental acute tuft haemorrhagic necrosis, of a type described in more

detail in the kidney of rat 2 of Group 7. No vascular lesions.

Rat 5.

Right Kidney: There is slight to moderate intra-:tubular protrusion of both cytoplasm and nuclei of the epithelium of the proximal convolutions.

There are no significant glomerular lesions, and the blood vessels are normal.

Rat 7.

Right kidney: Hydronephrosis is present. This is accompanied by patchy, peritubular cortical fibrosis, and by slight to moderate intratubular protrusion of both cytoplasm and nuclei of the epithelial cells of the proximal convolution. There are no significant glomerular or vascular changes.

GROUP 9.

Rat 1. (28.11.56 - 5.2.57)

Right Kidney: H. & E. The kidney is the site of severe hydronephrosis. Scattered ischaemic foci are present in the outer part of the cortex, and in these there is both replacement fibrosis and tubular regenera-:tion. Beneath the pelvic epithelium there is a single focus of round cells.

Very occasional nephrons contain proteinaceous fluid, and there is patchy slight to moderate dilatation of collecting tubules and, to a less extent, of the proximal and distal convoluted tubules.

Elastic: no evidence of arterial hyperplasia or damage.

P.A.S.: no alteration in the amount of P.A.S.-positive material.

H.E.: no changes other than those described above.

Heart: patchy calcification of the aortic valve.

Liver, Lungs, Suprarenal, Spleen — no significant abnormality

Rat 2. (28.11.56 - 5.2.57).

Right Kidney: The changes are less severe than those seen in rat 1.

There is slight to moderate dilatation of the collecting tubules, mainly in the outer zone of the medulla but involving also the cortical segments. Dilatation of the convoluted tubules is less severe, and is least in the proximal parts. Nevertheless, the convoluted tubular cells show protrusion of the cytoplasm of many of the cells into the lumen of the tubules, which is occasionally accompanied by prominence of the nucleus which may lie in the protruded segment.

There are no glomerular lesions. The arteries and arterioles are intact. There is little proteinuria.

Elastic stain)
P.A.S.) provide no additional information.
H.E.)

Lung: there are occasional foci (probably normal) of proliferating lymphocytes.

Liver: the portal tracts contain an excess of small round cells.

Heart)
Spleen) no significant abnormality
Suprarenal)

Rat 3. (28.11.56 - 5.2.57.)

Right Kidney: The degree of dilatation of the tubular cells is no more severe than in rat 2. Never-:theless, the degree of intratubular protrusion of the cells of the proximal and, to a lesser extent, distal convolution is greater than in the previous animal. There is no proteinuria.

Only occasional glomeruli (three per section) show ischaemic obliteration and fibrosis. In one glomerulus this change is complete, and in one segment with crescent formation.

There are no acute necrotic or inflammatory glomerular lesions, and the arteries and arterioles are intact.

Elastic stain: confirms these observations.

P.A.S. ")
) no additional information
H.E. ")

Rat 4. (28.11.56 - 5.2.57.)

Right Kidney: The degree of renal tubular change is approximately the same as that in rat 3. Protein casts are present in many tubules, and in most instances these deposits lie in nephrons the glomerulus of which is apparently the site of a partial or segmental lesion.

The glomeruli contain a significant number of

lesions: these vary from massive necrosis, sometimes accompanied by tubular hyaline droplet change, to small segmental fibrous scar, occasionally with fibrosis and sometimes with degenerate local tissue.

Lung: peribronchial collections of nodes.

Heart, Lungs, Liver, Spleen, Suprarenal — no significant abnormality

Rat 5. (28.1.56 - 5.2.57.)

Right Kidney: The renal changes are on the whole less severe than in rat 4. A single whole exudative glomerular lesion is present in one section, the tuft being relatively slightly affected. The corresponding tubule contains however much protein. A single further flomerulus contains a fibrous hemiglomerular lesion.

There is moderately severe tubular dilatation, more advanced than in the previous 4 animals and accompanied by protrusion of the cytoplasm, and to a less extent the nuclei of the proximal convoluted tubules.

Heart, Lungs, Suprarenals — no significant abnormality

Liver)
) no significant abnormality
Spleen)

Rat 6. (28.11.56 - 5.2.57.)

Right Kidney: There is moderately severe hydronephrosis. The proximal convoluted tubules show slight dilatation with slight to moderately severe cytoplasmic cell intratubular protrusion, and less marked nuclear protrusion. Dilatation of the collecting tubules is rather more severe.

The glomeruli are intact but show slight generalised enlargement. There are no focal glomerular lesions, and no evidence of transcapillary exudation.

H.E.)
) stains confirm the absence of vascular
Elastic)
) lesions and of focal glomerular necrosis.
P.A.S.)

Heart)
)
Liver)
)
Lungs) no significant abnormality
)
Spleen)
)
Suprarenals)

Rat 8. (28.11.56 - 5.2.57.)

Right Kidney: Tubular changes are slightly less severe than those in rat 7, but both convoluted and collecting tubules show fundamentally the same type of

change. There is well marked tubular cytoplasmic protrusion, with prominent nuclear changes, but the glomeruli appear normal. In particular, there is evidence neither of focal glomerular necrosis nor of exudative capillary changes.

Elastic, P.A.S., H.E.) stains confirm the absence of vascular and of focal glomerular lesions.

Heart, Lungs, Liver, Spleen, Suprarenals) no significant abnormality

Rat 9. (28.11.56 - 5.2.57.)

Right kidney: There is slight to moderate tubular dilatation, affecting the collecting tubules of both outer cortex and outer medulla to a greater extent than the convoluted tubules. Cytplasmic and nuclear intra-:tubular cell protrusion is very much more severe in the convoluted tubules and in particular in the proximal parts.

Very occasional glomeruli show focal adhesions of tuft to capsule, but there are neither exudative nor segmental necrotic lesions.

Elastic, P.A.S., H.E. stains confirm the absence of vascular lesions and of focal glomerular damage.

Heart, Lungs, Liver, Spleen, Suprarenals: no significant abnormality

Rat 10. (28.11.56 - 5.2.57.)

Right Kidney: The appearances of the right kidney do not differ significantly from those of the right kidney of rat 9. There are generalised moderately severe tubular changes of the same character, while glomerular and vascular lesions are absent. There is slight generalised glomerular enlargement.

Elastic, P.A.S., H.E. stains confirm the absence of vascular lesions and of focal glomerular damage.

Heart, Lungs, Liver, Spleen, Suprarenals: no significant abnormality

GROUP 10.

(see Table)

Rat 2.

Right Kidney: The changes in the tubules are severe. They are most severe in the proximal convoluted tubules, where they take the form of nuclear and cytoplasmic intratubular protrusion, and of slight dilatation. There is more prominent dilatation of the collecting tubules, while here the cellular changes are less obvious.

There is slight generalised glomerular enlargement, but no evidence of segmental glomerular fibrosis, of focal necrosis or of exudative changes.

Elastic)
) stains confirm the absence of vascular
P.A.S.)
) and of focal glomerular lesions.
H.E.)

Rat 4.

Right Kidney: The tubular changes are similar to those of rat 2. There are moderately severe cellular changes in the epithelium of the proximal convoluted tubules, while the collecting tubules show principally dilatation.

There are occasional glomerular capsular adhesions,

with crescent formation in some, and a slight to moderate generalised glomerular enlargement. No focal or exudative glomerular lesions are seen.

Elastic)
) stains confirm the absence of vascular
P.A.S.)
) lesions and of focal glomerular damage.
H.E.)

Rat 5.

Right Kidney: The appearance of the tubules does not differ significantly from that of the tubules in rats 2 and 4; there is mainly cellular protrusion in the convoluted tubules, with slight dilatation, while the lower parts of the nephron show more conspicuous dilatation.

The glomeruli are generally enlarged. Many show capsular adhesions. An average of one glomerulus per section shows focal and segmental glomerular necrosis. None shows exudation. In others there is a widespread hyaline droplet change.

Elastic) stains confirm the absence of vascular
)
P.A.S.) damage and of a significant incidence
)
H.E.) of focal glomerular necrosis.

Rat 6.

Right Kidney: The tubular changes are slightly less severe than those in the previous 3 animals, but the convoluted tubules show detectable cytoplasmic

effects and there is significant collecting tube dilatation.

The glomeruli show minimal enlargement, and contain very occasional capsular adhesions. No focal or segmental necroses are seen.

Elastic) stains fail to show evidence of vascular
P.A.S.) damage and confirm the absence of
H.E.) glomerulonecrosis.

Rat 7.

Right Kidney: The renal changes are more severe than those in rat 6, but less severe than those of rat 5. There are widespread tubular changes, involving both the intratubular protrusion of cells of the proximal convoluted tubules and dilatation, in particular, of the collecting tubules.

There is considerable and widespread glomerular enlargement and 2 - 3 glomeruli in each section show fibrinoid foci, and some show capsular adhesions. No exudative lesions.

Elastic)
P.A.S.) stains confirm the absence of
H.E.) vascular changes.

Rat 9.

Right Kidney: The changes are less pronounced

than in the kidney of rat 7. Tubular effects are moderately severe, and some contain hyaline proteinaceous casts. There is mild proximal tubular dilatation, with intratubular cytoplasmic protrusion and more advanced collecting tubular dilatation.

There is generalised moderate glomerular enlarge-:ment and very occasional focal glomerular fibrosis affecting portions of the glomerular tuft only. No glomerular tuft only. No glomerulonecrosis, or exudative lesions.

Elastic)
) stains confirm the absence of vascular
P.A.S.)
) lesions and of glomerulonecrosis.
H.E.)

Rat 11.

Right Kidney: Tubular changes are of moderate severity only and include mainly intratubular cyto-:plasmic protrusion of the cells of the proximal, and, to a lesser extent, distal convoluted tubules, together with collecting tubule dilatation.

The glomeruli are enlarged, but show only occasional focal fibrous tuft lesions, and no evidence of necrosis.

Elastic)
) stains confirm the absence of vascular
P.A.S.)
) changes and of glomerulonecrosis.
H.E.)

Rat 12.

Right Kidney: There are moderately severe tubular changes, with intratubular cytoplasmic protrusion in the convoluted tubules, and moderate dilatation of the collecting tubules.

There is slight generalised glomerular enlargement, but no glomerulonecrosis and only very occasional capsular adhesions.

Elastic)
) stains confirm the absence of vascular
P.A.S.)
) lesions and of glomerulonecrosis.
H.E.)

GROUP 11.

Rat 2.

Right Kidney: The tubular changes are confined to slight dilatation of the proximal convoluted and of the cortical collecting tubules. In both there is protrusion of epithelial cytoplasm into the tubular lumina, but few nuclei are apparently displaced.

Glomerular changes are confined to minimal generalised enlargement of the tufts. No vascular abnormalities are seen.

Elastic)
) stained sections confirm these
P.A.S.)
) observations.
H.E.)

Rat 4.

Right Kidney: There is slight dilatation of the proximal convoluted and of the collecting tubules. In both there is minimal intratubular cytoplasmic protrusion unaccompanied by significant nuclear change. There is minimal generalised glomerular enlargement, although it is difficult to be certain that this can be attributed to endothelial cell proliferation. No vascular changes.

Elastic)
)
P.A.S.) stains confirm these observations.
)
H.E.)

Rat 6.

Right Kidney: There is minimal dilatation of the proximal convoluted and of the cortical collecting tubules together with intratubular protrusion of the epithelial cell cytoplasm. No enlargement of glomerular tufts is recognisable, and blood vessels are intact.

Elastic)
) stains provide no additional evidence
P.A.S.)
) of renal damage.
H.E.)

Rat 8.

Right Kidney: The right kidney is the site of a mild hydronephrosis, the cause of which is uncertain. No significant tubular dilatation can be recognised, while the tubular cell cytoplasm and nuclei are not protruded. Glomeruli and blood vessels are intact.

Elastic)
)
P.A.S.) stains provide no additional information.
)
H.E.)

Rat 9.

Right Kidney: The epithelial tubular cells are intact; there is dilatation neither of the proximal or distal convoluted tubules, nor of the collecting tubules. The glomeruli are not recognisably enlarged, and they and the blood vessels are intact.

Elastic)
) sections provide no additional information
P.A.S.)
) or evidence of renal damage.
H.E.)

GROUP 12.

Rat 1.(Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: There are moderate tubular changes, which include intratubular cytoplasmic protrusion in proximal, and to a less extent distal, convoluted tubules, and some dilatation of the collecting tubules. There are no focal glomerular lesions, and minimal evidence of glomerular enlargement.

Elastic)
) stains confirm these observations and
P.A.S.)
) the absence of vascular damage.
H.E.)

Rat 2.(Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: Tubular changes are severe and include pronounced cellular cytoplasmic protrusion of the proximal convoluted tubules and collecting tubular dilatation. Dilatation of the proximal convoluted tubules, by contrast, is slight. No tubular casts are present.

There is some glomerular enlargement, but no focal glomerular lesions are seen. Neither are there exudative glomerular changes.

Elastic)
) stains confirm the absence of vascular
P.A.S.)
) lesions and of glomerulonecrosis.
H.E.)

Rat 3.(Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: The changes are fundamentally the same as those in the kidney of rat 2.

The proximal convoluted tubules show relatively slight dilatation but pronounced intratubular cytoplasmic protrusion. The distal convoluted tubules show moderate to pronounced dilatation, and some cytoplasmic change. The collecting tubules show moderate to severe dilatation. Occasional areas of collecting tubules show cytoplasmic changes similar to those described in potassium depletion, but there are no casts. They resemble those described by Oliver in potassium deficiency. There are no casts.

Glomeruli show occasional but by no means invariable increase in size. There are no focal lesions, and no exudative changes.

Elastic)
)
P.A.S.) stains are not rewarding.
)
H.E.)

Rat 4.(Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: The generalised pattern of tubular

changes does not differ significantly from that seen in the kidney of rat 3.

The glomeruli show a generalised slight increase in size, but contain neither focal nor exudative lesions.

Elastic, P.A.S., H.E.) stains confirm the absence of vascular lesions and of glomerulonecrosis.

Rat 5. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: There is only moderate cellular change in the epithelium of the proximal convoluted tubules: the cells protrude into the lumen and where the tubules are cut tangentially, the epithelial cells stand out in islands lying within the lumina. Dilata-:tion of the proximal convolutions is slight, of the distal convolutions moderate and of the collecting tubules considerable. Generally, the evidence of dilatation is more conspicuous than that of epithelial cell change.

Small numbers of glomeruli show proliferation of the capsular epithelium, some with tubularisation or enlargement. Occasionally evidence of glomerular damage is confined to hyaline droplet change. Enlargement is not widespread.

Elastic) stains confirm the absence both of
P.A.S.) vascular damage and of glomerulonecrosis
H.E.) or exudative lesions.

Rat 6. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: The evidence of tubular damage is somewhat less than in rat 5, and is equivalent in intensity to that seen in the kidney of rats 3 and 4. The appearances include (1) slight dilatation of the proximal convolution, with clearly defined intratubular protrusion of the cytoplasm of many epithelial cells; (2) slightly more severe dilatation of the distal convolutions; and (3) moderately severe dilatation of the collecting tubules together with cytoplasmic changes of a kind described by Oliver in potassium deficiency. There are no casts.

The glomeruli show only a slight generalised increase in size. There are no focal lesions or evidence of necrosis or exudation.

Elastic)
) stains confirm both these observations
P.A.S.)
) and the absence of vascular lesions.
H.E.)

Rat 7. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: Tubular changes are only slight to moderate in intensity, and are generally very much milder than those seen in the kidneys of rats 4, 5 and 6. Cytoplasmic changes are present in the proximal convolutions, but here the degree of tubular dilatation is slight; dilatation, by contrast, is more pronounced in the collecting tubules.

Glomerular changes are limited to very slight generalised enlargement; there are no focal tuft or capsular lesions.

Elastic) stains confirm the absence of vascular
P.A.S.) lesions and of focal necrotic, or general-
H.E.) :ised exudative glomerular damage.

Rat 8. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: The changes in both tubules and glomeruli are indistinguishable from those seen in the kidneys of rats 5 and 6. They include moderately severe cytoplasmic intratubular protrusion of the proximal convolutions, slight dilatation of the distal convolutions with rather less severe cytoplasmic changes, and moderate dilatation of the collecting tubules.

Small numbers of glomeruli show epithelial capsular proliferation, and other changes similar to those seen in the kidneys of rats 5 and 6.

Elastic) stains confirm the absence of
P.A.S.) vascular changes and of glomerulo-
Picro-Mallory) :necrosis.

Rat 9. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: The renal tubules show changes which include proximal tubular cytoplasmic intratubular protrusion and collecting tubule dilatation, slightly less severe than those seen in rats 5, 6 and 8. There are no focal or segmental glomerular lesions, and little glomerular enlargement.

Elastic) stains confirm the absence of
P.A.S.) vascular lesions and of glomerulo-
Picro-Mallory) :necrosis.

Rat 10. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: The tubular changes do not differ significantly from those seen in rats 5, 6 and 8. There is moderately severe proximal convoluted tubular cyto-:plasmic intratubular protrusion, and slightly more

severe collecting tubule dilatation.

The glomeruli are occasionally enlarged, and some show capsular thickening, tubularisation, or hyaline droplet change. No focal necroses are seen, and there is no evidence of exudative lesions.

Elastic) stains confirm the absence of
P.A.S.) vascular damage and of glomerulo-
H.E.) :necrosis.

Rat 11. (Operation 8.4.57; injections 17.4.57; killed 24.6.57.)

Right Kidney: Severe tubular lesions are present. They include prominent intratubular protrusion of the cells of the proximal convolution, together with slight to moderate dilatation, and severe dilatation of the collecting tubules and to a less extent of the distal convolutions.

Moderate numbers of glomerular lesions are present. These are diffuse incharacter, invading the whole glomerulus; many glomeruli are enlarged, while some show hyaline droplet change. Occasionally there is fibrosis of a segment of the tuft, and in others partial crescent formation.

Elastic) stains confirm the absence of vascular
P.A.S.) lesions and of focal glomerular
H.E.) necrosis.

GROUP 13.

Males.

Rat 2.

Tubular changes are slight and confined to dilatation of the cortical collecting tubules. In the proximal convolutions there is minimal intratubular protrusion of the cytoplasm and occasionally of the nuclei. Glomeruli are very slightly enlarged and it seems probable that this enlargement is the result of endothelial cell hyperplasia.

Rat 3.

Tubular changes are confined to dilatation of the collecting tubules of the inner and outer cortical zones, and to intratubular protrusion of both cytoplasm and, less often, nuclei of the convoluted tubules. There is slight generalised glomerular enlargement and this appears to be due to endothelial cell hyperplasia.

Rat 4.

There is slight dilatation of the collecting tubules of both cortical zones, associated in some with the presence of proteinaceous casts. There is very slight intratubular protrusion of both cytoplasm and nuclei in the convoluted tubules. The glomeruli show a slight generalised increase in size and this is

apparently the result of endothelial cell proliferation.

Rat 5.

Associated with slight dilatation of the cortical collecting tubules is the presence in some of proteinaceous casts. Others (a very small number) show hyaline droplet change. In many places there is intratubular protrusion of both nuclei and cytoplasm of convoluted tubules, and less severely of collecting tubules. There is widespread slight increase in size of glomeruli apparently due to hyperplasia of endothelial cells.

Rat 6.

Changes in this kidney are less advanced than in the previous one. There is dilatation only of the cortical (outer zone) collecting tubules; the convoluted tubules are intact. There is generalised slight glomerular enlargement, and this is apparently due to endothelial cell hyperplasia.

Females.

Rat 1.

Changes in the kidneys of the female rats in this group appear as a whole to be significantly less severe than in the male. The tubules of the first animal are intact. The glomeruli show minimal enlargement, the

result of endothelial cell hyperplasia.

Rat 2.

Again, the renal tubules appear intact. There is very slight hyperplasia of the cells of the glomeruli, with consequent generalised glomerular enlargement.

Rat 3.

There is very slight dilatation of the collecting tubules of the cortex, together with slight glomerular enlargement due to endothelial cell hyperplasia.

Rat 4.

No significant changes are recognisable in the renal tubules of this animal. There is slight generalised glomerular enlargement due, apparently, to endothelial cell hyperplasia.

Rat 5.

The changes, as in rat 3 (female) are confined to slight dilatation of the collecting tubules of the cortex. There is minimal generalised glomerular enlargement, due apparently to endothelial cell hyperplasia.

Rat 6.

No recognisable tubular abnormalities are present and the changes in this kidney are confined to very slight generalised glomerular enlargement apparently associated with endothelial cell hyperplasia.

GROUP 14.

Males

Rat 1.

Right Kidney: moderately severe hydronephrosis is present. There are no other significant renal abnormal-:ities.

Rat 2.

Right Kidney: There is very slight dilatation of the distal convoluted tubules and of the collecting tubules. This is accompanied by slight intratubular protrusion of the cytoplasm and nuclei of many of the distal convolutions, and collecting tubules. There are no glomerular or vascular abnormalities.

Rat 3.

Right Kidney: There is slight dilatation of the ascending limbs of Henle, of the distal convoluted tubules and of the collecting tubules. There are no glomerular or vascular changes.

Rat 5.

Right Kidney: There is very slight intratubular protrusion of the cytoplasm and nuclei of cells of the proximal and distal convolution. There are no glomerular or vascular changes.

Rat 6.

Right Kidney: The presence of protein-containing fluid within a small number of collecting tubules accompanies the segmental acute necrosis of a very small number of glomeruli. There are no other significant changes.

Females

Rat 1.

Right kidney: There is very slight dilatation of both the ascending limbs of Henle and of the distal convoluted tubules. These changes accompany intratubular protrusion of both cytoplasm and nuclei of the distal convolutions. There are no glomerular or vascular lesions.

Rat 2.

Right Kidney: Cytoplasmic intratubular protrusion is slight and confined to the proximal convolutions. There are no other significant tubular changes. The glomeruli and blood vessels are normal.

Rat 3.

Right Kidney: There are no significant tubular, glomerular or vascular lesions.

Rat 4.

Right Kidney: Severe collecting tubule dilatation accompanies slight proteinuria and tubular hyaline droplet change and is associated with very small numbers of acute segmental glomerular necroses. There are no vascular changes.

Rat 5.

Right Kidney: Very slight dilatation of the distal convolutions, together with the intratubular protrusion of cytoplasm and nuclei. No glomerular or vascular lesions.

Rat 6.

Right Kidney: Very slight dilatation of the distal convoluted tubules and of the collecting tubules. No glomerular or vascular lesions.

GROUP 15.

Males.

Rat 1.

Right Kidney: The changes are mainly tubular: there is very slight dilatation of the proximal convoluted tubules, with slight intratubular protrusion of cytoplasm. The distal convoluted tubules are moderately dilated, often with vacuolation of the superficial part of the cytoplasm and preservation of the granularity of the basal subnuclear cytoplasm. Within their lumina is detritus of cytoplasmic origin. The collecting tubules are more severely dilated, with few cell changes.

The blood vessels are normal.

There is generalised slight increase in the glomerular size, but there are no glomerular necroses and no exudative lesions of the DCA type.

Elastic) stains confirm the absence of vascular
P.A.S.) lesions and of focal glomerulo-
H.E.) :necrosis.

Rat 2.

Right Kidney: There is autolysis: the recognisable changes are confined to the tubules and are of the general pattern and severity described in rat 1. Slight glomerular enlargement is seen but there are neither

vascular lesions nor glomerulonecrosis.

Elastic) stains confirm the absence of
P.A.S.) vascular lesions and of glomerular
H.E.) damage.

Rat 3.

Right kidney: Tubular dilatation is rather more severe than in animal 1. The dilatation is mainly of the collecting tubules, the epithelium of which is largely intact. The proximal convoluted tubules show slight dilatation only, and relatively slight cytoplasmic and nuclear protrusion. Glomeruli are slightly increased in size, but there is neither glomerulonecrosis nor exudative glomerular lesions. Occasional proteinuria is indicated by the presence of protein-containing mater-:ial in some of the collecting tubules.

Elastic) stains confirm both the absence of
P.A.S.) vascular damage and of glomerulo-
H.E.) :necrosis.

Rat 5.

Right Kidney: There is severe dilatation of the collecting tubules, associated with early vacuolation of the cytoplasm. In several nephrons, a succession of tubular segments can be followed in the cytoplasm of which there is hyaline droplet change and in whose lumen proteinaceous material has collected.

The glomeruli are large but their cells do not show cytoplasmic ballooning. There is no evidence of the exudative glomerular lesion of DCA, and none of glomerulonecrosis, segmental or complete.

Elastic) stains fail to reveal evidence of
P.A.S.) vascular damage and confirm the absence
H.E.) of glomerulonecrosis.

Rat 6.

Right Kidney: The tubular changes are similar to those seen in rat 5, but are slightly less severe: they are principally in the cortical collecting tubules, the convoluted tubules being slightly affected. There are no tubular hyaline droplet changes, and there is considerably less proteinuria than in animal 5. The glomeruli however are of almost similar size and none contains necrotic foci or exudative lesions.

Elastic) stains confirm the absence of
P.A.S.) vascular lesions and of glomerulo-
H.E.) :necrosis or transudation.

Females.

Rat 1.

Right Kidney: There is slight dilatation of the proximal convoluted tubules, and moderate dilatation of the distal convolutions. The cortical collecting

tubules are moderately severely affected but show few cell changes compared with those seen in the proximal convolutions.

No glomerular necrotic or exudative lesions are seen, although there is a slight generalised increase in the size of the glomeruli. The blood vessels are intact.

Elastic, P.A.S., H.E.) stains provide no additional information and confirm the normal structure of glomeruli and vessels.

Rat 4.

Right Kidney: The tubular changes generally are similar to those seen in rat 1, with slight dilatation and moderately severe cytoplasmic and nuclear intratubular protrusion in the convoluted tubules, and with severe dilatation and relatively slight cytoplasmic or nuclear change in the cortical and outer medullary collecting tubules. In some places papillary cellular proliferation of the epithelium of the collecting tubules is occurring.

Glomeruli are little enlarged, and there is neither transudation nor glomerulonecrosis.

Elastic, P.A.S., H.E.) stains confirm the absence of vascular and glomerular damage.

Rat 5.

Right Kidney: The tubular changes resemble those seen in rat 1, although the dilatation of collecting tubules is not as severe as that seen in rat 4. Further, there is no papillary intratubular epithelial proliferation. The cytoplasmic and nuclear changes are most severe in the proximal convolutions, while very occasional collecting tubules contain proteinaceous material.

Elastic)
P.A.S.) stains confirm the absence of vascular damage and of focal
H.E.) glomerulonecrosis.

Rat 6.

Right Kidney: The appearances of both convoluted and collecting tubules do not differ significantly from those in rat 5. The glomeruli generally are slightly enlarged. There are no vascular changes.

Elastic)
P.A.S.) stains provide no additional information.
H.E.)

GROUP 16.

Males.

Rat 1.

Right Kidney: There is slight but significant dilatation of the proximal and distal convoluted tubules and moderate dilatation of the collecting tubules of the cortex. In the convoluted and collecting tubules, cytoplasm of the epithelial cells protrudes into the tubular lumina; less often this is associated with intratubular protrusion of the cell nuclei. In a few nephrons there is proteimuria, with hyaline casts in the straight portions of the distal tubule and in the collecting tubules.

The majority of glomeruli are significantly enlarged, probably due to endothelial cell proliferation. In very occasional juxtamedullary glomeruli there is segmental tuft necrosis; rarely, glomeruli with fibrous segmental scars are seen. Blood vessels are intact.

Elastic)
)
P.A.S.) stains add no significant information.
)
H.E.)

Rat 2.

Right Kidney: There is slight dilatation of both

convoluted tubules, and of the cortical collecting tubules. In the proximal convolutions there is extremely severe protrusion of the cytoplasm into the tubular lumina, and less frequent protrusion of the epithelial cell nuclei. In the collecting tubules these changes are confined to slight cytoplasmic prominence of occas-:ional cells.

There is slight to moderate generalised glomerular enlargement, apparently due to endothelial cell prolifera-:tion. This is unaccompanied by segmental tuft necrosis, or by vascular changes.

Elastic)
) stains do not add significantly to
P.A.S.)
) these observations.
H.E.)

Rat 3.

Right Kidney: There is slight dilatation of the proximal and distal convolutions, and severe dilatation of the collecting tubules. These changes are associated with very frequent and obvious prominence of the epithelial cell cytoplasm in the convoluted tubules, the nuclei being less often involved. There is moderately frequent collecting tubule cell cytoplasmic protrusion.

The glomeruli, generally, are enlarged, apparently due to endothelial cell proliferation. There are no

segmental glomerular changes and no evidence of vascular damage.

Elastic)
) stains do not add significantly to
P.A.S.)
) these observations.
H.E.)

Rat 4.

Right Kidney: The kidney is the site of a mild hydronephrosis. The appearances of the cortex include patchy, but widespread peritubular fibrosis, slight dila-:tation of the convoluted and of the collecting tubules. In the former there is severe intratubular cytoplasmic protrusion, and moderate nuclear protrusion. In the latter both cytoplasmic and nuclear changes are only moderate in severity. Some collecting tubules contain proteinaceous fluid and occasional hyaline casts; careful examination of the collecting tubule cells reveals perinuclear vacuolation in some; in others the intra-:tubular cytoplasmic protrusion is accompanied by poly-:poidal epithelial cell proliferation.

There is slight generalised glomerular enlargement due apparently to endothelial cell proliferation and accompanied in some instances by glomerular hyaline droplet change. Blood vessels appear normal.

Elastic)
)
P.A.S.) stains are not informative.
)
H.E.)

Rat 5.

Right kidney: There is slight dilatation of the convoluted tubules and of the cortical collecting tubules. The changes in the former are accompanied by severe intratubular cytoplasmic protrusion and slightly less severe nuclear protrusion and in the latter by similar phenomena. Occasional nephrons show proteinuria.

There is generalised slight glomerular enlargement due apparently to endothelial cell hyperplasia. In very occasional tufts there are fibrous segmental scars. The blood vessels are normal.

Elastic)
)
P.A.S.) stains provide no additional information.
)
H.E.)

Rat 6.

Right Kidney: The kidney is the site of a mild hydronephrosis. Convoluted tubules are slightly dilated, while collecting tubules are moderately dilated. There is severe intratubular protrusion of the cytoplasm of the epithelial cells of both convolutions and of the collecting tubules, together with only moderately severe

protrusion of the nuclei of the convoluted tubules.

There is slight to moderate generalised glomerular tuft enlargement; this is probably the result of endothelial cell hyperplasia and is accompanied in very occasional instances by the presence in some glomeruli of segmental tuft fibrosis. Some of the smaller arteries show concentric medial hypertrophy.

Elastic)
)
P.A.S.) stains do not add materially to this analysis.
)
H.E.)

Females.

Rat 1.

Right Kidney: There is slight dilatation of the proximal convoluted tubules, and severe dilatation of the collecting tubules. These changes are associated with frequent protrusion of the epithelial cells of both tubules into the respective tubular lumina, but less often with intratubular protrusion of the nuclei of these cells. Occasionally, the tubules contain proteinaceous material.

The majority of the glomeruli have undergone slight increase in size, probably due to endothelial cell proliferation, and occasionally the enlarged glomeruli show a hyaline droplet change. No vascular lesions are seen. Occasional pyramidal shaped cortical scars

are present.

Elastic)
)
P.A.S.) stains provide no additional information.
)
H.E.)

Rat 2.

Right Kidney: The tubular changes are similar quantitatively and qualitatively to those seen in rat 1. They include slight dilatation of the proximal convoluted tubules, and moderate dilatation of the collecting tubules. Both regions of the nephron show the characteristic prominent intratubular cytoplasmic and nuclear protrusion, of moderate degree, observed in the previous animals of this group.

Scattered throughout the cortex are occasional small ischaemic fibrous scars. The blood vessels are intact, but the majority of the glomeruli have undergone slight enlargement, apparently due to endothelial cell hyperplasia.

Elastic)
) stained sections reveal no significant
P.A.S.)
) additional abnormality.
H.E.)

Rat 4.

Right Kidney: There are generalised renal tubular changes which include slight dilatation of the convoluted

and collecting tubules, together with apparent intratubular protrusion, of moderate degree, of both cytoplasm and nuclei in both regions of the nephron. Occasional tubules contain aggregates of proteinaceous material.

There is generalised slight glomerular enlargement, due mainly to endothelial cell hyperplasia, and in some of these enlarged glomeruli hyaline droplets are visible. The blood vessels appear intact.

Elastic)
) stains do not add materially to this
P.A.S.)
) assessment.
H.E.)

Rat 5.

Right Kidney: There is slight dilatation of the proximal convoluted tubules and moderate dilatation of the collecting tubules. The collecting tubules only show intratubular protrusion of their epithelial cyto-:plasm but this is not accompanied by apparent nuclear protrusion. These changes are accompanied by slight generalised glomerular enlargement due mainly to endo-:thelial cell hyperplasia. The blood vessels are intact.

Elastic)
)
P.A.S.) stains provide no additional information.
)
H.E.)

Rat 6.

Right Kidney: The renal tubular changes are similar to those seen in rat 5. They include slight dilatation of convoluted and of collecting tubules, with prominence and apparent intratubular protrusion of the cytoplasm in the latter only. Occasional tubules contain eosinophilic proteinaceous material. There is generalised slight glomerular enlargement, probably the result of endothelial cell hyperplasia, and some of these enlarged glomeruli show hyaline droplet change.

Elastic)
)
P.A.S.) stains do not give additional information.
)
H.E.)

GROUP 17.

Males.

Rat 3.

In occasional proximal convoluted tubules there is slight intratubular protrusion of both cytoplasm and nuclei. This is accompanied by slight proteinuria, by the presence of pale eosinophilic material within some proximal tubules, and by the presence of small amounts of proteinaceous fluid in the cortical interstitial intercellular spaces. The glomeruli and blood vessels are intact.

Rat 4.

In occasional proximal convolutions cytoplasm and nuclei protrude into the lumen of the tubules. The glomeruli and blood vessels are intact.

Females.

Rat 2.

There is slight dilatation of very occasional collecting tubules, while there is minimal intratubular protrusion of the cytoplasm of many proximal convoluted epithelial cells. No glomerular or vascular changes. Small amounts of interstitial protein-containing fluid lie between groups of cortical cells.

Rat 3.

Minimal dilatation of cortical collecting tubules, together with occasional interstitial aggregates of protein-containing fluid between groups of cortical cells. No glomerular or vascular changes.

Rat 4.

Slight collecting tubule dilatation associated with proteinuria; occasional hyaline casts and tubular hyaline droplet change. Minimal intratubular protrusion of the cytoplasm of proximal convoluted tubular epithelial cells. Occasional glomeruli show hyaline droplet change. No vascular anomalies.

GROUP 18.

Males.

Rat 1.

Right Kidney: Tubular changes are confined to slight intratubular protrusion of both epithelial cyto-:plasm and nuclei, principally of the proximal convoluted tubules. The glomeruli show no recognisable abnormality.

Rat 2.

Right Kidney: There is slight dilatation of the cortical collecting tubules, in their outer zone; some contain proteinaceous casts. There is slight intra-:tubular protrusion of the cytoplasm and occasionally of the nuclei of the proximal and distal convolutions. The glomeruli and blood vessels are intact.

Rat 3.

Right Kidney: Tubular changes in this kidney are slighter than in the previous one. Changes are confined to the presence in very occasional collecting tubules of proteinaceous casts. The glomeruli and blood vessels are intact.

Females.

Rat 1.

Right Kidney: (Generally, tubular changes in this

group of animals appear to be rather more prominent in females than in males.)

There is slight dilatation of both convoluted and of cortical collecting tubules associated, in the case of the former, with intratubular protrusion of both cytoplasm and of many nuclei. The glomeruli and blood vessels, however, are intact.

Rat 2.

Right Kidney: Again there is dilatation both of proximal convoluted and distal convoluted tubules, and of the cortical collecting tubules. These changes are commonly seen in association both with nuclear and with cytoplasmic intratubular protrusion in the convoluted tubules. The glomeruli and blood vessels are intact.

Rat 4.

Right Kidney: The changes are similar to those seen in the previous animal. They include slight dilatation of both collecting and of convoluted tubules, together in the latter with intratubular protrusion of cytoplasm and less commonly nuclei. The glomeruli and blood vessels are intact.

GROUP 19.

Males.

Rat 2.

Tubular changes are confined to moderate collecting tubule dilatation, with severe intratubular protrusion of both nuclei and cytoplasm of cells of proximal convolutions and of collecting tubules. There is occasional vacuolation of collecting tubular epithelial cells. Minimal enlargement of glomerular tufts is accompanied by endothelial cell swelling and sometimes ballooning. No vascular changes.

Rat 3.

Slight dilatation of cortical collecting tubules with occasional vacuolation of their convoluted portions. Intratubular cytoplasmic protrusion of cells of both proximal convolutions and of collecting tubules. Slight enlargement of glomerular tufts with apparent endothelial cell swelling. No vascular changes.

Rat 4.

Minimal dilatation of cortical collecting tubules, with slight vacuolation of cells of their medullary portions. Intratubular protrusion of cells of the collecting tubules of the cortex and of the proximal

convolutions. Slight generalised glomerular enlargement with endothelial cell swelling.

Females.

Rat 1.

Autolysis has occurred. No abnormalities can be detected.

Rat 2.

Occasional proteinuria. Intratubular cytoplasmic protrusion of the cells of the proximal convoluted tubules. Minimal generalised enlargement of the glomerular tufts. No vascular lesions.

Rat 3.

Moderate dilatation of cortical collecting tubules, with intratubular cytoplasmic protrusion of cells of both proximal convoluted and collecting tubules. Moderate glomerular tuft enlargement, associated with endothelial cell proliferation.

Rat 4.

Autolysis has occurred, but it is possible to discern mild intratubular cytoplasmic protrusion of both convoluted and cortical collecting tubular cells. No glomerular or vascular changes.

GROUP 20.

Males.

Rat 3.

Right Kidney: Tubular changes are confined to protrusion of cytoplasm and of nuclei into the lumina of the convoluted and collecting tubules, associated with slight proteinuria. The glomeruli and blood vessels are intact.

Rat 4.

Right Kidney: There is slight dilatation of the cortical collecting tubules. This is associated in some with proteinuria. The glomeruli appear generally to be slightly enlarged and "crowded". This crowding is not, however, due to ischaemia.

Females.

Rat 2.

Right Kidney: There is slight dilatation of the cortical collecting tubules. This is associated with intratubular protrusion of both nuclei and of cytoplasm in convoluted and collecting tubules, and with a characteristic vacuolation of many of the cells of the collecting tubules. Many of these vacuoles are peri-:nuclear. The glomeruli and blood vessels are intact.

Rat 3.

Right Kidney: Associated with dilatation of the collecting tubules is intratubular protrusion of both nuclei and cytoplasm. The glomeruli are intact.

Rat 4.

Right Kidney: There is slight dilatation of both proximal convolutions and of cortical collecting tubules. Cytoplasmic and sometimes nuclear intratubular protrusion is seen in both proximal convolutions and in the collecting tubules. The glomeruli and blood vessels are intact.

GROUP 21.

Males.

Rat 1.

Autolysis has occurred.

Rat 2.

Moderate intratubular cytoplasmic protrusion of cells of the proximal convoluted and cortical collecting tubules, together with nuclear protrusion of the cells of the proximal convolution. Slight enlargement of the glomerular tufts, a few of which show hyaline droplet change, and occasional small cortical interstitial protein aggregates. No vascular changes.

Rat 3.

Slight dilatation of cortical collecting tubules. Moderate cytoplasmic and nuclear protrusion of cells of the proximal convolutions and of the cortical collecting tubules. Occasional small cortical interstitial protein aggregates with enlargement of the glomerular tufts, some of which show hyaline droplet change.

Females.

Rat 1.

Slight dilatation of the cortical collecting

tubules. Intratubular protrusion of both nuclei and cytoplasm of proximal convoluted tubules and of collecting tubules, some of which are vacuolated. Minimal slight enlargement of glomerular tufts. Occasional small interstitial protein aggregates.

Rat 2.

Minimal dilatation of cortical collecting tubules, with intratubular protrusion of cytoplasm, and, less often, nuclei of cells of the proximal convolutions. Vacuolation of medullary collecting tubules. Generalised slight enlargement of glomerular tufts with endothelial cell hyperplasia. No vascular lesions.

Rat 3.

Slight cortical collecting tubular dilatation; intratubular protrusion of cytoplasm and nuclei of occasional proximal convoluted tubules. Vacuolation of occasional medullary collecting tubules. Slight generalised glomerular enlargement, with endothelial cell proliferation. No vascular lesions.

Rat 4.

Minimal dilatation of cortical collecting tubules, with moderate intratubular cytoplasmic protrusion and, less frequently, nuclear protrusion of both convoluted and

collecting tubules. Slight generalised glomerular enlargement and endothelial cell hyperplasia. No vascular lesions.

GROUP 22.

Males.

Rat 2.

Right Kidney: The kidney is the site of moderately severe hydronephrosis. There are no recognisable tubular changes, and the only significant abnormalities in the remaining renal cortical substance are the presence of occasional foci of polymorphonuclear leukocytes, forming small cortical abscesses, and a moderate proteinuria. The glomeruli and blood vessels are intact.

Rat 3.

Right Kidney: There is slight dilatation of the collecting tubules of the cortex. This is associated with slight intratubular protrusion of both cytoplasm and of nuclei in the proximal convoluted tubules. The glomeruli and blood vessels are intact.

Rat 4.

Right Kidney: The kidney is the site of a severe hydronephrosis. There is proteinuria, with proteinaceous casts in the collecting tubules which are significantly dilated. There is intratubular protrusion of both nuclei and cytoplasm in the proximal convoluted tubules.

Females.

Rat 1.

Right Kidney: There is slight dilatation of the proximal and distal convoluted tubules. This is associated with prominent vacuolation of the epithelium of the distal convoluted tubules. There is very slight generalised enlargement of the glomerular tufts, possibly due to endothelial cell hyperplasia. The glomeruli and blood vessels are in other respects normal.

Rat 2.

Right Kidney: Autolysis has advanced so far that it is not easy to be certain of the nature of tubular changes. The glomeruli show very slight generalised hyperplasia.

Rat 3.

Right Kidney: There is again severe autolytic change. Nevertheless it is possible to distinguish both dilatation of the cortical collecting tubules and moderate generalised glomerular enlargement. The blood vessels are intact.

Rat 4.

Right Kidney: There is slight autolysis. It is possible to distinguish both mild collecting tubular

dilatation, with intratubular cellular protrusion, and slight generalised glomerular enlargement. The blood vessels are intact.

GROUP 23.

Males.

Rat 1.

Right Kidney: The collecting tubules show slight to moderate widespread dilatation particularly in the outer part of the medulla, together with cellular changes which include protrusion of the vacuolated cytoplasm into the lumen of the tubules and papillary epithelial cell hyperplasia. The convoluted tubules are almost intact, but some distal convolutions contain proteinaceous material as do many of the collecting tubules.

The glomerular changes are relatively slight: some show the early endothelial cell vacuolation (ballooning) which is the hallmark of the electrolyte disturbances produced by severe DCA overdosage. Many, however, show only slight enlargement and minimal endothelial cell proliferation.

Elastic) stains confirm the absence of vascular
P.A.S.) damage, hyperplastic vascular sclerosis
H.E.) and of acute fibrinoid glomerular necrosis.

Rat 2.(8.11.57.)

Right Kidney: Tubular changes, particularly those

of the cortical and outer medullary collecting tubules, are very severe: they are grossly dilated. In many the epithelial cells protruding in polypoid fashion into the lumen have been cut across and lie as cytoplasmic islands within the lumen, forming a row of spheres lying parallel to the epithelial cells of the tubule. There is moderate dilatation of the proximal convolutions, and of the proximal part of the loop of Henle, and the convolutions particularly show prominent cell changes.

Occasional tubules contain hyaline casts, and the cytoplasm of some of these tubular cells shows hyaline droplet change. A few casts also lie in the loops of Henle. They are rare in the convoluted tubules.

Glomeruli are slightly but generally enlarged. In many there is both capillary dilatation, and ballooning of the endothelial cells of the tuft. None show either the severe transudative lesion of DCA overdosage, or focal tuft necrosis. There are no "burst" glomeruli or arteriolar changes: some however show epithelial capsular adhesions with partial obliteration of the subcapsular spaces.

Elastic)
P.A.S.) stains confirm the absence of vascular
H.E.) changes and of glomerulonecrosis.

Rat 3. (31.12.57.)

Right Kidney: The tubular changes are of the same general pattern as those seen in rat 2, but are much less severe. Proteinaceous casts are frequent.

By contrast, the glomeruli and small numbers of arterioles and small arteries (intratubular) are severely affected. Many glomeruli have passed beyond the stage of ballooning of the endothelial cells to a stage at which the centre of the glomerulus is sometimes represented only by a focus of fibrinoid necrosis and in which the remainder of the tuft, enlarged to an enormous size, occupies the entire glomerular subcapsular space. The enlargement is due to a proliferation and swelling of endothelial-like cells which however do not reach the proportions of those seen in the less severely affected animals, and were described as ballooned. Often the affected glomerulus lies close to a normal arteriole which sometimes shows no evidence of hyperplasia. Occasionally an arteriole showing fibrinoid necrosis leads directly into the "exploded" glomerulus and is involved equally in the disruptive process. The glomerular capsular basement membrane is distended but intact. Affected glomeruli of this type are distributed at random throughout the renal cortex, and often lie beside glomeruli which are either substantially normal or show ballooning

of the endothelial cells. Of the latter, some are associated with protein transudate which fills the remaining subcapsular space and lies within the related tubules.

Elastic) stains confirm the integrity of many of
P.A.S.) the interlobular and arcuate arteries and
H.E.) the fibrinoid nature of the change shown in many glomeruli, arteries and arterioles.

Females.

Rat 1. (31.12.57.)

Right Kidney: Tubular changes are only moderately severe and very much less striking than those seen in rat 2 (male). They include slight dilatation of the proximal convoluted tubules, with slight intratubular protrusion of the cytoplasm of many epithelial cells; dilatation of the distal convoluted tubules which is more severe than in the proximal tubules; and slight to moderate dilatation of the collecting tubules which show occasional polypoidal intratubular epithelial proliferation. Very many tubules contain eosinophilic proteinaceous material, especially those tubules the glomerulus of which is the site of transudation.

Many afferent arterioles and moderate numbers of intralobular arterial branches show fibrinoid medial change: sometimes this is accompanied by intravascular

thrombosis. The fibrinoid change is frequently segmental, and although the affected vascular segments vary considerably in length, they generally adjoin parts of the vessel which are entirely normal.

The majority of the glomeruli are abnormal. Many show only the enlargement and endothelial cell oedema described previously, and some of these are accompanied by evidence of transudation, hyaline droplet change and proteinuria. Others are more extensively damaged, and show both the fibrinoid centre and the concentric cellular hyperplasia described in rat 3 (male). The nature of this change is sometimes so diffuse that it is not easy to distinguish glomerulus from arteriole; the surrounding cellular proliferation is often similar. Nevertheless, an intact capsular basement membrane and the absence of elastic remnants help to make this distinction. In other instances, the damaged arteriolar segment merges into the distorted glomerular structure. Fundamentally, the disturbance differs only in site and not in nature.

In some instances a normal arteriole can be seen leading to a swollen glomerulus, with a necrotic centre while in others the arterial segment itself has undergone abrupt necrosis and can be seen lying alongside swollen glomeruli.

It is only occasionally possible to recognise with certainty transition between the enlarged ballooned transuding glomerulus and the "exploded" glomerulus with its necrotic centre. One explanation is that a sudden change may occur. The response is of a most unusual type. Finally, necrotic vessels are apparently not always associated with necrotic glomeruli, while fibrinoid foci in these "exploded" glomeruli may accompany normal arteries and arterioles. The last observation suggests that the explosive glomerular lesion is a local manifes-:tation of glomerular swelling and ischaemia caused perhaps by progressive swelling of the ballooned endothelial cells.

Elastic)
)
P.A.S.) stains confirm these observations.
)
H.E.)

Rat 2. (31.2.57.)

Right Kidney: There is slight proximal convoluted tubular dilatation, together with moderate intratubular cytoplasmic and nuclear protrusion. The collecting tubules are more severely dilated, but show less epithelial cell change. There is neither protrusion nor hyaline droplet change.

Glomerular effects are confined to widespread

glomerular enlargement with only moderate endothelial cell swelling. There is little or no ballooning, and no evidence of glomerulonecrosis, or "explosive" glomerular lesions.

Elastic) stains confirm both these findings
P.A.S.) and the absence of glomerulonecrosis
H.E.) and vascular damage.

Rat 3. (31.12.57.)

Right Kidney: The tubular changes are more severe than those in rat 2. Dilatation of the collecting tubules is considerable and cytoplasmic and nuclear protrusion of both proximal convolutions and of collecting tubules is severe. However, there is no proteinuria or hyaline droplet change.

The glomeruli are only slightly involved and the main changes are endothelial cell swelling and enlargement of the tufts. There is neither fibrinoid change nor necrosis of small blood vessels or of glomeruli, and there is no evidence of intimal arterial fibrosis.

Rat 4. (female). (31.12.57.)

Right Kidney: The changes do not differ significantly from those seen in rat 1 (female). There is slight proximal and distal convoluted tubule dilatation, with more severe dilatation of the collecting tubules,

accompanied by slight intratubular nuclear protrusion and more severe cytoplasmic protrusion in the convoluted tubules and slight cytoplasmic changes in the collecting tubules. Occasional hyaline droplet change is seen in the collecting tubules and this accompanies proteinuria.

Glomerular changes are widespread and severe. They are of the same general pattern as those seen in rat 1 (female) but less severe. Thrombus occludes some of the vessels whose walls show fibrinoid necrosis and some afferent arterioles are affected.

Elastic)
) stains confirm these changes and the
P.A.S.)
) absence of segmental glomerulonecrosis.
H.E.)

Rat 5.

Right Kidney: The tubular changes are slight and confined to dilatation of the collecting tubules with slight cytoplasmic changes.

The glomeruli show slight endothelial cell swelling and ballooning while the glomerular tufts are slightly enlarged.

Elastic) sections confirm the absence
)
P.A.S.) of vascular changes and
)
H.E.) glomerulonecrosis.

GROUP 24.

Males.

Rat 2. R. kidney. (Picro-Mallory).

Severe dilatation of the cortical collecting tubules, and to a lesser extent of the distal convoluted tubules, is the main cause for the extreme renal enlargement seen in this animal. There is slight dilatation of the proximal convoluted tubules, accompanied by protrusion of the cytoplasm and nuclei of many of the convoluted and collecting tubular cells into the respective tubular lumina. Many collecting tube cells are vacuolated and contain proteinaceous material, while in some the presence of hyaline casts is accompanied by epithelial hyaline droplet change.

There is generalised glomerular enlargement, due to endothelial cell proliferation and to "ballooning" of the tuft cells. In a small number of glomeruli transudation of protein into the subcapsular space has occurred and in others hyaline droplet change is present. No vascular lesions are seen.

Rat 3. R. kidney. (Picro-Mallory).

Tubular changes are rather more severe than in the previous animal. There is extreme collecting tubule dilatation - some contain proteinaceous material. Proximal and distal collecting tubules are moderately

dilated and occasionally their cells show both nuclear and cytoplasmic intratubular protrusion. The majority of glomeruli are enlarged, and show both moderate endothelial cell proliferation and severe endothelial cell "ballooning". In some the capsule is thickened, and occasionally crescent formation can be seen. Transudation of protein into the subcapsular space is rare, and hyaline droplet change is uncommon. There are no vascular changes.

Females.

Rat 1. Owing to autolysis histological detail is largely obscured.

Rat 3. R. kidney. (Picro-Mallory).

There is severe collecting tubule dilatation and slight dilatation of the distal convoluted tubules. These changes are accompanied by frequent and severe intratubular protrusion of the nuclei and cytoplasm of both proximal convoluted and collecting tubular epithelial cells. There is occasional proteinuria.

The majority of the glomeruli are enlarged, and endothelial cell hyperplasia is accompanied by frequent "ballooning" of cells. Some glomerular subcapsular spaces contain transuded proteinaceous fluid. There are no vascular changes.

Rat 4. R. kidney. (Picro-Mallory).

The distribution and severity of the changes are similar to those seen in Rat 3. Collecting and distal convoluted tubular dilatation is of moderate severity, while the intratubular nuclear and cytoplasmic cell protrusion is also of slighter degree than in Rat 3. A small number of tubules contain proteinaceous fluid.

There is generalised, moderately severe glomerular enlargement, due partly to endothelial cell hyperplasia and partly to cytoplasmic "ballooning". Small numbers of glomeruli contain transuded subcapsular proteinaceous fluid.

GROUP 25.

Males.

Rat 1.

Right Kidney (Picro-Mallory): Severe collecting tubule dilatation accompanies moderate dilatation of the distal convoluted tubules. There is slight dilata-:tion of the proximal convoluted tubules and of the ascending and descending loops of Henle. Protrusion of nuclei and cytoplasm into the tubular lumina is present only in the proximal convolutions. Occasional collecting tubules contain proteinaceous material.

Most glomeruli are enlarged, partly due to endothelial cell proliferation and partly to "ballooning". A few contain transuded proteinaceous fluid within their subcapsular spaces. Occasional small protein aggregates lie apparently free within interstitial tissue spaces.

There are no significant vascular lesions.

Rat 2.

Right Kidney (Picro-Mallory): Collecting, proximal and distal tubular dilatation are each slightly less severe than in the previous animal. Nuclei and cytoplasm occasionally protrude within the lumina of the

proximal convolutions, while some nephrons contain transuded fluid.

There is generalised slight glomerular enlargement with both endothelial cell hyperplasia and "ballooning". A single glomerulus shows a characteristic acute focal necrotic and aneurysmal lesion. There is widespread arteriolar fibrinoid change, and slight but significant arterial hyperplasia.

Rat 3.

Right Kidney (Picro-Mallory): Tubular changes are almost identical with those seen in rat 2; they include moderate collecting tubule dilatation, slight dilatation of both convolutions, and occasional proteinuria. Most glomeruli are enlarged, with endothelial cell hyperplasia and "ballooning", while arterial hyperplasia accompanies frequent acute fibrinoid arteriolar change.

Rat 4.

Right Kidney (Picro-Mallory): There is moderate dilatation of the collecting tubules, and slight dilatation of the distal convolutions. Occasional tubules contain proteinaceous fluid. Glomerular enlargement due both to endothelial cell hyperplasia and "ballooning" is accompanied by frequent arteriolar

fibrinoid necrosis, slight arterial hyperplasia, and the presence of proteinaceous fluid within occasional sub-:capsular spaces.

Females.

Rat 1.

Right Kidney (Picro-Mallory): There is severe collecting tubule dilatation. This accompanies slight intratubular cytoplasmic protrusion of proximal convoluted cells, and occasional proteinuria.

Generalised slight glomerular enlargement is due to endothelial cell proliferation and "ballooning". Many arterioles show fibrinoid change and there is slight arterial hyperplasia.

Rat 2.

Right Kidney (Picro-Mallory): The changes are relatively slight. There is only moderate collecting tubule dilatation, and slight enlargement of the glomerular tufts accompanies "ballooning" of a small proportion of endothelial cells. There are no significant vascular changes.

Rat 4.

Right Kidney (Picro-Mallory): There is moderate dilatation of the collecting tubules and slight dilatation

of the distal convolutions. Cytoplasm of occasional proximal convoluted epithelial cells protrudes into the tubular lumina, and there is proteinuria. Glomerular enlargement, which is widespread, is due to hyperplasia and "ballooning" of endothelial cells. There is frequent arteriolar fibrinoid necrosis and arterial hyperplasia.

GROUP 26.

Males.

Rat 1.

Right Kidney (Picro-Mallory): There is slight dilatation of the cortical collecting tubules, and slight generalised glomerular enlargement associated with terminal endothelial cell hyperplasia and endothelial cell "ballooning". No vascular changes.

Rat 2.

Right Kidney (Picro-Mallory): Slight dilatation of the distal convolution and of the cortical collecting tubules is associated with minimal intra-:tubular cytoplasmic and nuclear protrusion of the epithelial cells of the convoluted and collecting tubules. These changes accompany slight generalised glomerular enlargement due to endothelial cell prolifera-:tion. No vascular changes.

Rat 4.

Right Kidney (Picro-Mallory): Autolysis prevents reliable observations.

Females.

Rat 1.

Right Kidney (Picro-Mallory): Slight dilatation

of the cortical collecting tubules, a few of which contain proteinaceous fluid. Slight to moderate generalised glomerular enlargement with endothelial cell proliferation and occasionally "ballooning", accompanied in some cases by hyaline droplet change. No vascular lesions.

Rat 2.

Right Kidney (Picro-Mallory): Slight dilatation of collecting tubules with occasional proteinuria. Slight generalised glomerular enlargement, with minimal endothelial cell hyperplasia. No vascular lesions.

Rat 3.

Right Kidney (Picro-Mallory): Minimal dilatation of cortical collecting tubules, with occasional very slight protrusion of the cytoplasm and nuclei of both proximal convoluted and collecting tubules into the respective tubular lumina. Minimal generalised glomerular enlargement associated with endothelial cell hyperplasia. No vascular lesions.

Rat 4.

Right Kidney (Picro-Mallory): Minimal dilatation of the cortical collecting tubules, with occasional proteinuria, and moderate intratubular protrusion of both nuclei and cytoplasm of cells of the proximal,

distal and collecting tubules. Moderate generalised glomerular enlargement, with endothelial cell proliferation and "ballooning", occasional transuded protein-containing fluid in the subcapsular spaces, and very occasional fibrinoid arteriolar necrosis.

GROUP 27.

Males.

Rat 2.

R.) kidneys (Picro-Mallory).
L.)

Tubular changes are confined to minimal intra-:tubular cytoplasmic and nuclear protrusion in the proximal convolutions. These changes are so slight that they may be artefactual. Occasional slight glomerular enlargement. No vascular changes.

Rat 3.

R.) kidneys (Picro-Mallory).
L.)

Tubular and glomerular changes do not differ significantly from those seen in Rat 2. No vascular changes.

Rat 4.

R.) kidneys (Picro-Mallory).
L.)

The kidneys are the site of an extensive granulomatous infective process which makes valid comment on their detailed structure impossible.

Females.

Rat 1.

R.)
L.) kidneys (Picro-Mallory).

Brush borders of the proximal tubules are unusually prominent. There is minimal generalised glomerular enlargement. No vascular changes.

Rat 2.

R.)
L.) kidneys (Picro-Mallory).

The appearances do not differ significantly from those seen in rat 1.

Rat 3.

R.)
L.) kidneys (Picro-Mallory).

The appearances do not differ significantly from those seen in rat 1.

Rat 4.

R.)
L.) kidneys (Picro-Mallory).

The appearances do not differ significantly from those seen in rat 1.

GROUP 28.

Males.

Rat 3.

R.)
L.) kidneys (Picro-Mallory).

Occasional slight intratubular cytoplasmic protrusion in the proximal convolutions. Brush borders are unusually prominent. No glomerular or vascular changes.

Rat 4.

R.)
L.) kidneys (Picro-Mallory).

The appearances do not differ significantly from those in rat 3.

Females.

Rat 1.

R.)
L.) kidneys (Picro-Mallory).

The appearances do not differ significantly from those seen in rat 3 (male).

Rat 2.

R.)
L.) kidneys (Picro-Mallory).

The appearances do not differ significantly from

those in rat 3 (male).

Rat 3.

R.) kidneys (Picro-Mallory).
L.)

Severe autolysis prevents a reliable estimate of histological changes.

Rat 4.

R.) kidneys (Picro-Mallory).
L.)

The appearances do not differ significantly from those in rat 3 (male).

GROUP 32.

Males.

Rat 2.

R.)
L.) kidneys.

No glomerular or tubular abnormalities with the exception of occasional proteinuria. Normal blood vessels. No focal glomerular necroses.

Rat 3.

R.)
L.) kidneys.

Normal glomeruli and tubules. Occasional proteinuria, but no vascular lesions. No focal glomerular necroses.

Rat 4.

R.)
L.) kidneys.

No glomerular or tubular lesions. Normal blood vessels. Occasional proteinuria. In particular, no focal glomerular necroses.

Females.

Rat 1.

R.)
L.) kidneys.

The glomeruli are intact and there are no focal necroses. Normal tubules and blood vessels. Occasional proteimuria.

Rat 2.

R.) kidneys.
L.)

No focal glomerular lesions: glomeruli and tubules are intact. Normal blood vessels. Occasional proteimuria.

Rat 3.

R.) kidneys.
L.)

Glomeruli and tubules are intact. No focal glomerular necroses. Normal blood vessels. Occasional proteinuria.

Rat 4.

R.) kidneys.
L.)

No glomerular or tubular lesions. No focal glomerular necroses. Normal blood vessels. Occasional proteinuria.

GROUP 33.

R.)
L.) kidneys (Picro-Mallory).

The histological changes are minimal. Many convoluted tubules show intratubular protrusion of both cytoplasm and nuclei, but this is of minor degree and nowhere as severe as in the animals described in other groups. There are no significant glomerular or vascular changes.

GROUP 34.

R.)
L.) kidneys (Picro-Mallory).

Renal changes are minimal in degree and nowhere is there recognisable significant alteration in tubular, glomerular or vascular structure. Occasional aggregates of chronic inflammatory cells (lymphocytes and histiocytes) lie among the cortical tubules but their presence appears to be incidental to the present observations.

APPENDIX 6

Chronological account of experimental work

26.4.55. Right nephrectomy on 7 rats, using midline incision: gut displaced to one side, renal artery and vein clamped and ligated. Some difficulty in avoiding inferior vena cava. Right suprarenal apparently intact.

Rat 1 died at once (due anaesthetic)
" 2 " " " " "
" 3 died overnight (haemorrhage)
" 4 died 27.4.55)
" 5 " 27.4.55) delayed haemorrhage
" 6 " 27.4.55)
" 7 alive.

29.4.55. Rat 7 remains alive. Plethysmographic blood pressure recording device nearly ready.

2.5.55. Right nephrectomy, through lumbar incision, on 6 more rats. Gut displaced but not exteriorised. Adrenal avoided. Renal vessels ligated and divided. Massive bleeding followed the slipping of ligatures from the pedicles of two rats which subsequently died.

Rat 8 died (haemorrhage)
" 9 " "
" 10 alive
" 11 "
" 12 "

2.5.55. Introductory trial of modified Goldblatt clamp as cause of renal ischaemia.

Rat 14: L. renal artery easily identified, but animal died (anaesthesia).

Rat 15: L. renal artery not found: abdomen closed without further procedure.

Rat 16: L. renal artery identified. L. kidney mobilised through incision - 10 ml. syringe used as "sandbag" to support loin. Artery dissected out anteriorly and posteriorly. Clamp forceps slipped in around artery, but not vein, and silver wire clip closed (to leave gap of 0.3 mm.) around a guide wire which was then removed. Abdomen closed in layers with interrupted stitches.

Animal alive at close of operation.

Rat 17: Silver clip applied to this further rat today.

12.5.55. Rat 18)
" 19) Right nephrectomy, through right loin
" 20) incision, ether anaesthesia.
" 21) Alive on following morning.
" 22)
" 23)

16.5.55. Right nephrectomy performed on 6 further rats today (Nos. 24 - 29). All alive.

17.5.55. Right nephrectomy on seven further rats today,

one of which died from anaesthesia. Remainder alive. (Rats 30 - 36).

19.5.55. Right nephrectomy on six further rats today. Venous bleeding from one animal, but others well. (Rats 37 - 42).

24.5.55. Right nephrectomy on six further animals. All well. (Rats 43 - 48).

26.5.55. Right nephrectomy on six further animals today. (Rats 49 - 54). All well. Right nephrectomy also performed on the two animals to which the left renal arterial clamps had been applied successfully (nos. 16 and 17).

30.5.55. Plethysmograph now ready. Initial attempt at recording pressures, under ether anaesthesia. An easily detected end point was found, the depth of anaesthesia being important.

Blood pressures	Rat 16	Rat 17
	120)	100)
	) mm.Hg.	) mm.Hg.
	118)	94)
	) mean 118	) mean 97
	119)	96)
		)
		98)

2.6.55. Blood pressures recorded again.

Rat 16 (light anaesthesia)	Rat 17
156)	90)
) mm.Hg.	) mm.Hg.
150)	94)
) mean 153	) mean 92
154)	92)

(deep anaesthesia)

144)
) mm.Hg.
138)
) mean 141
140)

9.6.55. Blood pressures

Rat 16	Rat 17
140)	100)
) mm.Hg.	) mm.Hg.
140)	100)
) mean 139	) mean 99
138)	98)

13.6.55. Leaks occurring in manometer. Glass inserted in place of rubber manometer cuff.

Blood pressures

Rat 16	Rat 17
Animal died during anaesthesia.	114)
	) mm.Hg.
	110)
	) mean 111
	110)

Rat 17 then killed, to provide an index of efficacy of renal clamping.

14.6.55.- Silver wire clips applied to left renal arteries

of 6 animals upon whom right nephrectomy had been previously performed. Because of difficulty in applying clips, the renal veins were included. The kidneys of two animals at once became pale and ischaemic: one showed subcapsular haemorrhage. The kidneys of the remainder appeared unchanged.

16.6.55. One animal to whom renal clamp applied on 14.6.55 now dead.

20.6.55. Right nephrectomy performed on rats 55 - 61. All survived.

21.6.55. Right nephrectomy performed on rats 62 - 69. One died, the remainder survived.

3.8.55. Application of new wire in 6 rats, previously having had right nephrectomy, to left renal artery. Except in one animal in which the kidney was exposed anteriorly, the main renal arteries were exposed posteriorly through a lumbar incision, and separated from the veins. Bleeding from the veins was frequent but was always more or less controlled by packing. The artery was raised with an aneurysm needle, the clamp slipped round and rotated until the closed end lay distally. Artery forceps were then used to close the clamp, leaving a gap judged to be adequate. Only one kidney showed the immediate pallor of undue ischaemia; in one other, patchy ischaemia of the lower pole only occurred.

4.8.55. All rats survived.

5.8.55. Two rats found dead this morning. In the remain-:ing 4 animals the blood pressures were recorded.

	Rat 5	6	7	8
B.P.in mm.Hg.	128	144	114	110
	124	142	110	108
	130	141	110	107
	122	—	—	—
Mean	126	142	111	108

6.8.55. Further rat (5) died today.

Necropsy.

Rats	6	7	8
B.P. in mm.Hg.	124	110	114
	130	112	110
	128	114	110
Mean	127	112	111

8.8.55. Rat 6 dead this morning. Necropsy not possible.

Rats	7	8
B.P. in mm.Hg.	128	181
	130	175
	118	177
Mean	125	178

10.8.55. Rat 7 dead this morning. Necropsy.

11.8.55. Pressure readings in rat 8 today difficult and fluctuating.

12.8.55. Rat 8 dead this morning. Necropsy. At this point the method of recording blood pressures was reviewed, and it was decided that a new optical system (Byrom, 1947) should be introduced.

3.10.55. The new optical blood pressure recording device is now ready.

4.10.55. No success with new apparatus. Copper tubing substituted for rubber in many places.

11.10.55. After several days trial during which leaks were found and stopped, and apparatus made airtight, it was found possible to record human blood pressures. In the first two animals used no readings were available. Eventually in a third anaesthetised animal, fine, somewhat irregular pulsation became visible, with an amplitude of 5 - 10 mm. Regulation of the balance of pressures within and without the perspex chamber and the recording diaphragm enabled the point of optimal oscillation to be chosen. An initial pressure was measured, and the difference in pressure taken on the upward and on the downward movement of the manometric meniscus was found to be only 4 mm.Hg. Some interference from external vibration was experienced.

12.10.55. At this point it was judged suitable to make

definite plans for the experiment.

Group I constituted today. Each of 12 animals had

(Unilateral nephrectomy
(
(DOCA implant
(
(Repetitive injections of DCA in oil
(
(1% NaCl in place of drinking water.

Rats				Rats			
	1	Mean B.P.	113 mm.Hg.	7	Mean B.P.	125 mm.Hg.	
	2		113	8		129	
	3		121	9		117	
	4		104	10		102	
	5		112	11		101	
	6		103	12		95	

17.10.55. Injections of DCA in oil started to Group I. 50 mg. in 1.0 ml. under ether anaesthesia, in two divided amounts (0.5 ml. into each hindlimb).

Rat 10 died from anaesthetic.

18.10.55. All rats of Group I remain well with the exception of Rat 12 (dead).

20.10.55. Control group with unilateral nephrectomy established today (Group II).

Rat				Rat			
	1	Mean B.P.	122 mm.Hg.	7	Mean B.P.	119 mm.Hg.	
	2		109	8		118	
	3		128	9		118	
	4		118	10		114	
	5		117	11		105	

24.10.55. Group I injected with 5 mg. DCA in propylene glycol, owing to temporary shortage of commercial oily suspension.

29.10.55. Group I injected each with 5 mg. oily DCA.

5.11.55. Group I: 5 mg. DCA each I.M.I.

15.11.55. Blood pressures measured. 5 mg. DCA given each by I.M.I.

17.11.55. Renal clamp applied to the right renal artery in two animals, while in three more animals right renal clamping was unsuccessful.

22.11.55. Rats of Group I given 5 mg. DCA in oil by I.M.I.

25.11.55. 3 rats attempted application of right renal clamp. In each nephrectomy had to be performed on account of bleeding.

28.11.55. Blood pressures of Group I measured, and 10 mg. DCA in oil given to each.

30.11.55. Treatment of Control Group 2 begun today. Each having previously had a right nephrectomy, now started on I.M.I. Hydrallazine 4 mg. daily, equivalent in a 200 g. animal to a daily human dose of 1.4 g.

1.12.55. Group I. B.Ps. measured. 5 mg. DCA each. Group 2. 4 mg. Hydrallazine each.

2.12.55. Group 2. 4 mg. Hydrallazine each I.M.I.

3.12.55. Group 2. 4 mg. Hydrallazine each I.M.I.

5.12.55. Group I. B.Ps. measured. 5 mg. DCA each.
Group 2. Hydrallazine 4 mg. each I.M.I.

6.12.55. Group 2. Hydrallazine 4 mg. each I.M.I.

7.12.55. Group I. B.Ps. measured. 5 mg. DCA each.
Group 2. Hydrallazine 6.6 mg. each I.M.I.

9.12.55. Using merthiolate as antiseptic, subcutaneous implants of 25 mg. DCA inserted into each animal of
Group I. Group 2: Hydrallazine 6.6 mg. each I.M.I.

10.12.55. Yesterday's wounds intact.
Group 2: Hydrallazine 6.6 mg. I.M.I. each.

12.12.55. Group I. Implants intact.
Group 2. Hydrallazine 6.6 mg. I.M.I. each.

13.12.55. Group 2. Hydrallazine 6.6 mg. I.M.I. each.

A further control group (Group 3) started.

These were rats, whose blood pressures before treatment are known to be normal, and which are to receive Hydrallazine daily.

Blood pressures - Group 3 (Rats 97 - 108).

Rats	1 Mean B.P. 115 mm.Hg.	7 Mean B.P. 100 mm.Hg.
2	110	8 120
3	95	9 118
4	110	10 115
5	110	11 120
6	102	12 120

14.12.55. Group 1. Blood pressures measured.

Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

2 rats of group 3 dead today.

15.12.55. Group 1. Blood pressures measured.

Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I.

2 rats of Group 3 dead today.

16.12.55. Group 1. Blood pressures measured.

Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

17.12. 55. Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

19.12.55. Group 1. Blood pressures measured.

" 1. DCA 5 mg. each I.M.I.

" 2.)
" 3.) Hydrallazine 6.6 mg. each I.M.I.

1 rat of Group 3 killed selectively in an attempt

to determine why the mortality of this group is so high.

20.12.55. Group 2.) Hydrallazine 6.6. mg. I.M.I. each.
Group 3.)

21.12.55. Group 1. Blood pressures measured.
DCA mg. 5 I.M.I. each.
Group 2.) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

22.12.55. Group 2.) Hydrallazine 6 mg. I.M.I. each.
Group 3.)

23.12.55. Group 1. DCA 5 mg. I.M.I. each.
Group 2.) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

24.12.55. Group 2.) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)
1 rat of Group 3 dead today.

26.12.55. Group 1. DCA 5 mg. I.M.I. each.
Group 2.) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

27.12.55. Group 2.) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

28.12.55. Group 2.) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

29.12.55. Group 2.) Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

30.12.55. Group 1. Blood pressures measured. DCA 5 mg. I.M.I. each.

Group 2.) Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

Rat 4, Group 1 dead.

31.12.55. Group 2.) Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

2.1.56. Group 1. DCA 3.3 mg. I.M.I. each.

Group 2.) Group 3.) Hydrallazine 6.6 mg. each I.M.I.

3.1.56. Group 2.) Group 3.) Hydrallazine increased to 10 mg. I.M.I. each.

4.1.56. Group 1. Blood pressures measured. DCA 5 mg. I.M.I. each.

Group 2.) Group 3.) Hydrallazine 10 mg. I.M.I. each.

2 more rats of Group 2 dead today - the dose of Hydrallazine is now above the LD 50.

1 rat of Group 3 dead, and one other killed selectively. This afternoon, convulsions were observed in one animal. Two hours later recovery was complete. Decided to reduce Hydrallazine dosage to previous level.

5.1.56. All animals alive today.

Group 2.)
) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

6.1.56. Group 1. DCA 5 mg. I.M.I. each.

Group 2.)
) Hydrallazine 6.6 mg. each I.M.I.
Group 3.)

4 animals of the original Group operated on today. Clips applied to left renal artery, following previous right nephrectomy.

7.1.56. Group 2.)
) Hydrallazine 6.6 mg. I.M.I. each.
Group 3.)

9.1.56. One implant of animal in group 1 extruded today - question arises (a) as to whether the implants of 9.12.55 were adequate in amount, (b) as to whether they were still in situ.

10.1.56. Clips applied to left renal arteries of two further animals of original group, with previous right nephrectomy. Attempted application of right renal clamp to 4 other animals (Rats 75 - 78).

Rat 75 Right nephrectomy.
" 76 Left renal clamp.
" 77 Right renal clamp.
" 78 Died.

Group 2)
) Hydrallazine 6.6 mg. I.M.I. each.
Group 3)

11.1.56. Group 4 now established.

These animals had:

Previous right nephrectomy)
Left renal clamp) For B.P. see Table and Graph.

Group 1. Blood pressures recorded.

12.1.56. Right renal clamp applied to 6 more rats (Rats 79 - 84).

Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

13.1.56. Rat 6, Group 4 died today.

Group 1. DCA 5 mg. I.M.I. each.

Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

Group 5 now established.

These are young animals with right renal clamps applied on 10.1.56, on 12.1.56 and 5 more on 19.1.56.

14.1.56. Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

16.1.56. Group 1. DCA 5 mg. I.M.I. each.

Group 2.)
Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

17.1.56. Group 2.) Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

Group 5. Blood pressures recorded.

Rat 5 died from anaesthetic.

18.1.56. Group 2.) Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

Group 4.) Group 5.) Blood pressures recorded.

19.1.56. Group 1. Recording of blood pressures and injections of DCA temporarily discontinued, to await arrival of fresh implants, the addition of which may replace the I.M. injections.

20.1.56. Group 4.) Group 5.) Blood pressures recorded.

21.1.56. Group 2.) Group 3.) Hydrallazine 6.6 mg. I.M.I. each.

23.1.56. Group 2.) Group 3.) Hydrallazine 10 mg. I.M.I. each.

Group 5. Blood pressures recorded.

24.1.56. 1 rat of Group 2) 1 rat of Group 3) found dead this morning, probably the result of the increased dose of Hydrallazine yesterday.

25.1.56. Group 1. All alive.

Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

Group 4.)
Group 5.) Blood pressures recorded.

Leaks in pressure recording apparatus repaired.

26.1.56. Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

27.1.56. Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

28.1.56. Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

Rat 5, Group 4 dead today.

30.1.56. Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

31.1.56. Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

Convulsions observed today in 1 rat of Group 3, and in 2 rats of Group 2, beginning 1½ hours after injection and lasting 3 - 4 hours. No relief following the I.M. injection of 100 mg. glucose in 10% solution.

1.2.56. Group 2.)
Group 3.) Hydrallazine 10 mg. I.M.I. each.

Within 70 minutes of injection, tetanic convulsions developed in 1 rat of Group 2. No laryngeal stridor. Although spasms appeared to be spontaneous, they could often be initiated by sensory stimuli such as noise.

2.2.56. Establishment of further control group (Group I (B)).

New implants arrived. Each animal in this group received an implant of 100 mg. DCA, carefully stitched in position, following left nephrectomy, and was given 1% NaCl in place of tap water to drink.

Rats 85 - 96 used (as Group I (B)). Rat 96 died from anaesthesia.

Group 2.)
) Hydrallazine 10 mg. I.M.I. each.
Group 3.)

1 rat of Group 3 dead today: post-mortem examination not possible, number unknown.

3.2.56. Group 2.)
) Hydrallazine 10 mg. I.M.I. each.
Group 3.)

1 rat of Group 2 dead today (number unknown).

4.2.56. Group 2.)
) Hydrallazine 10 mg. I.M.I. each.
Group 3.)

6.2.56. Group 2.)
) Hydrallazine 10 mg. I.M.I. each.
Group 3.)

7.2.56. Rat 6, Group 2, dead today. At this point it was decided to kill the remaining 2 animals each, of Group 2 and Group 3, in order to obtain well fixed material.

Groups 2 and 3 thus concluded.

16.2.56. 1 rat of Group I (B), dead today - post mortem examination not possible.

17.2.56. 100 mg. DCA implanted into the 2 remaining animals of Group I (A), nos. 3 and 6.

19.2.56. 50 mg. more DCA implanted into each of rats 1, 2, 5, 7, 8, 9 and 11 of Group I (A); all of this group now have implants of 100 mg. DCA.

9.3.56. 1 rat of Group I (A) } dead today.
1 rat of Group I (B) }

20.3.56. Rat 10, Group 5 dead today.

23.3.56. 1 rat of Group I (A) dead today. Post mortem examination not possible.

26.3.56. It now appears that the rats of Group I (A) are dying off rapidly, possibly owing to age and to chronic lung infection.

Rat 2, Group I (A), dead today.

2.4.56. 1 rat of Group I (A))
1 rat of Group 4.) dead today.

Post mortem examination not possible. The remaining two rats of Group 4 killed today to provide well fixed material.

6.4.56. The remaining 5 rats of Group I (A) killed

20.4.56. Blood pressures of rats of Group I (B) and of Group 5 measured today. The blood pressure of the latter group was not raised, and owing to their obvious lack of response, it was decided to kill them.

21.4.56. Remaining 6 rats of Group 5 killed today.

15.5.56. Remaining 8 rats of Group I (B) killed today.

19.6.56. Group 6 established today.

These are young animals in whom left nephrectomy was combined with an implant of 100 mg. DCA, inserted through a separate left posterior cervical incision, and in whom 1% NaCl was substituted for tap water to drink.

Rat (series number)	Group No.	Markings
112	1	1 L.
113	2	2 L.
114	3	3 L.
115	4	1 R.
116	5	2 R.
117	6	3 R.
118	7	1 L. 1 R.
119	8	2 L. 1 R.
120	9	2 L. 2 R.
121	10	3 L. 1 R.
122	11	3 L. 2 R.
123	12	Unsatisfactory operation.

Rat 10 died following operation.

20.6.56. All remaining 10 rats of Group 6 well.

21.6.56. Group 7 started today. Left nephrectomy, 100 mg. DCA implant, 1% NaCl.

Rat (series number)	Group No.	Markings
124	1	1 L.
125	2	2 L.
126	3	3 L.
127	4	1 R.
128	5	2 R.
129	6	3 R.

23.6.56. (Group 7 continued.)

Rat (series number)	Group No.	Markings
130	7	1 L. 1 R.
131	8	2 L. 1 R.
132	9	2 L. 2 R.
133	10	3 L. 1 R.
134	11	3 L. 2 R.
135	12	3 L. 3 R.

24.6.56. All animals of Group 7 well today.

26.6.56. Injection of Hydrallazine started today. Each animal of Groups 6 and 7 injected I.M.I. under ether anaesthesia with 4 mg. of Hydrallazine.

27.6.56. Group 6)
Group 7) 4 mg. Hydrallazine each, I.M.I.

28.6.56. Dose of Hydrallazine increased to 6.6 mg. I.M.I. each today. The results were disastrous. Within 1½ hours of injection 95% of the animals of the two groups developed convulsions and 13 died; 9 remain alive.

At post mortem examination, the DCA implants were removed for further use.

29.6.56. To supplement the now depleted Groups 6 and 7, left nephrectomy and the implantation of 100 mg. DCA was now carried out in a further group of 8 rats (136 -143). The earlier group was amalgamated to form Group 6. The

latter, new group, was numbered Group 7.

2.7.56. All rats of Groups 6 and 7 remain alive and well.

16.7.56. The daily injection of both Groups 6 and 7 resumed. Hydrallazine 2 mg. I.M.I. each.

17.7.56. - 21.7.56. Groups 6 and 7. Hydrallazine 3 mg. I.M.I. each.

23.7.56. - 26.7.56. Groups 6 and 7. Hydrallazine 4 mg. I.M.I. each.

27.7.56. One rat of Group 7 found dead this morning (Rat 1).

28.7.56. Rats Groups 6 and 7 - Hydrallazine 4 mg. I.M.I. each.

30.7.56. 1 rat of Group 6 dead this morning (Rat 1).

Remainder of Groups 6 and 7 - Hydrallazine 5 mg. I.M.I. each.

31.7.56, 1.8.56 - 2.8.56. Groups 6 and 7 - Hydrallazine 5 mg. I.M.I. each.

1 rat of Group 6 (Rat 2) killed today.

3.8.56 - 4.8.56, 6.8.56 - 7.8.56. Groups 6 and 7 - Hydrallazine 5 mg. I.M.I. each.

1 rat of Group 6 dead this morning. Post mortem examination not possible.

Blood pressures of Groups 6 and 7 recorded (see Table).

10.8.56 - 11.8.56. Groups 6 and 7 - Hydrallazine 5 mg. I.M.I. each.

13.8.56 - 14.8.56. Groups 6 and 7 - Hydrallazine 6 mg. I.M.I. each.

1 rat (Rat 4, Group 6) died anaesthesia today.

15.8.56 - 16.8.56. Groups 6 and 7 - Hydrallazine 6 mg. I.M.I. each.

17.8.56 - 18.8.56, 20.8.56 - 23.8.56. Groups 6 and 7 - Hydrallazine 6.6 mg. I.M.I. each.

Blood pressure of Groups 6 and 7 measured today. Each given Hydrallazine 6.6 mg. I.M.I.

24.8.56. Groups 6 and 7 - Hydrallazine 6.6 mg. I.M.I. each.

24.8.56 - 20.9.56. Groups 6 and 7 injected daily (except Sundays), with 6.6 mg. each of Hydrallazine.

20.9.56. Rat 9, Group 7, dead today.

1.10.56. Remaining 7 rats of Group 7 killed today, to enable selective post mortem examinations to be performed.

14.10.56. Group 8 introduced today.

Each animal had left nephrectomy and the implantation of 200 mg. DCA, with 1% NaCl substituted for drinking water.

Rat (series number)	Group No.	Markings
144	1	1 L.
145	2	2 L.
146	3	3 L.
147	4	1 R.
148	5	2 R.
149	6	3 R.
150	7	1 L. 1 R.
151	8	2 L. 1 R.
152	9	3 L. 1 R.

For blood pressures of Group 8 see table.

15.10.56. 1 rat of Group 8 dead this morning. Post mortem examination not possible. Implants extracted.

20.10.56. Blood pressures Group 8 recorded.

22.10.56. Administration of Hydrallazine to rats of Group 8 begun. Today each received 3 mg. I.M.I. under ether anaesthesia.

23.10.56. - 27.10.56. Group 8 - Hydrallazine 3 mg. I.M.I. each.

Blood pressures recorded today (27.10.56).

29.10.56. Group 8 - Hydrallazine 4 mg. I.M.I. each.

30.10.56 - 31.10.56. Group 8 - Hydrallazine 5 mg. I.M.I. each.

31.10.56. 1 rat dead today. Post mortem examination not possible.

1.11.56. Group 8 - Hydrallazine 6 mg. each I.M.I.

1 rat dead today. Post mortem impossible.

2.11.56. Group 8 - Hydrallazine 6 mg. each I.M.I.

1 rat dead today. Post mortem again not possible owing to cannibalism.

2.11.56 - 20.11.56. Group 8 - Hydrallazine 6.6 mg. each daily (except Sundays) I.M.I.

20.11.56. Remaining 5 rats of Group 8 killed today.

28.11.56. Group 9 started today.

The rapid death of so many of the animals of Group 8, leaving only 5 upon whom post mortem examination could be performed, necessitated examining another similar group, kept in separate cages to prevent cannibalism.

Each animal, after left nephrectomy, had a subcutaneous implant of 200 mg. DCA.

Rat (series number)	Group No.	Markings
153	1	1 L.
154	2	2 L.
155	3	3 L.
156	4	1 R.
157	5	2 R.
158	6	3 R.
159	7	Both ears nicked
160	8	2 L. 1 R.
161	9	3 L. 1 R.
162	10	3 L. 2 R.

5.2.57. All rats of Group 9 alive.

Killed today (ether); post mortem.

6.2.57. Group 10 started. Left nephrectomy today. DCA 200 mg. implant with 1% NaCl + daily hydrallazine injections.

Series Number	Rat No.	Mark	Weights (in grams)
155	1	1 L.	220
156	2	2 L.	235
157	3	3 L.	250
158	4	1 R.	190
159	5	2 R.	270
160	6	3 R.	220
161	7	1 L. 1 R.	234

Series Number	Rat No.	Mark	Weights (in grams)
163	9	3 L. 1 R.	244
164	10	3 L. 2 R.	250
165	11	Both off	250
166	12	1 L. 2 R.	190

26.2.57. All rats of Group 10 survive.

Group 11 started today (Rats 167 - 178). 12 animals: 100 mg. DCA subcutaneous implant + daily hydrallazine injections.

4.3.57. Daily injections of hydrallazine begun in Groups 10 and 11.

Group 10)
) Hydrallazine mg. 2 I.M.I.
Group 11)

6.3.57)
) Group 10)
7.3.57)) Hydrallazine mg. 2 I.M.I. each day.
) Group 11)
8.3.57)

9.3.57 - 1.4.57. Gradually increasing doses of hydrallazine, rising from 2 to 5 mg. daily I.M.I. During this period 2 rats of Group 11 and one of Group 10 died.

1.4.57 - 5.4.57. Groups 10) Hydrallazine mg. 6
11) I.M.I.

6.4.57) Groups 10) Mg. 7)
)) Hydrallazine) daily
8.4.57) 11) mg. 6)

Group 12 started (Rats 179 - 190).

Left nephrectomy

1% NaCl.

Subcutaneous implant 100 mg. DCA.

Daily injections of sterile sodium chloride intramuscularly under ether anaesthesia.

9.4.57)
)
10.4.57)
)
11.4.57) Groups 10) Hydrallazine mg. 7)
)) daily
12.4.57) 11) mg. 6)
)
13.4.57)
)
15.4.57) Rat 5 Group 11 dead today

16.4.57 Groups 10) Hydrallazine mg. (7 I.M.I.
11) (6

Group 12 salt 0.2 ml. I.M.I.

At this point 9 rats of Group 10)
)
7 rats of Group 11) remain alive
)
11 rats of Group 12)

18.4.57. Group 10: rat 3 dead today.

Groups 10) (7
) Hydrallazine mg. (I.M.I.
11) (7

Group 12: Sodium chloride 0.3 ml. I.M.I.

19.4.57)
20.4.57) Groups 10) 7)
)) Hydrallazine mg.) I.M.I.
21.4.57) 11) 7)
22.4.57) 12 sodium chloride 0.3 ml. I.M.I.
23.4.57) 1 rat of Group 11 dead today, no post mortem possible.
24.4.57) Groups 10) 7
)) Hydrallazine mg.
25.4.57) 11) 7

12 sodium chloride 0.3 ml. I.M.I.

26.4.57 Groups 10) 8)
)) Hydrallazine mg.) I.M.I.
11) 8)

12 sodium chloride 0.3 ml. I.M.I.

27.4.57)
29.4.57) Groups 10)
))Hydrallazine mg.7
30.4.57) 1 rat Group 11 dead today, post mortem not possible 11) I.M.I.
1.5.57) 12 sodium chloride 0.3 ml. I.M.I.
2.5.57)
3.5.57)
4.5.57)
6.5.57)
Groups 10) 8)
7.5.57)) Hydrallazine mg.) I.M.I.
8.5.57) 11) 7)
) 12 sodium chloride 0.3 ml. I.M.I.
9.5.57)
10.5.57)
11.5.57)
13.5.57)

14.5.57. Remaining 5 rats of Group 11 killed today.

Heart) Kidneys) Liver) sectioned.
Lungs) Suprarenals) Spleen)

15.5.57)
16.5.57) Group 10 hydrallazine mg. 8 I.M.I.
17.5.57) Group 12 sodium chloride 0.3 ml. I.M.I.
18.5.57)
20.5.57)

21.5.57 Remaining 8 rats of Group 10 killed today.
Group 12 sodium chloride 0.3 ml. I.M.I.

22.5.57)
23.5.57) Group 12 sodium chloride 0.4 ml. I.M.I.
24.5.57)
25.5.57)

28.5.57)
29.5.57)
30.5.57)
31.5.57) Group 12 sodium chloride 0.5 ml. I.M.I.
1.6.57)
3.6.57)
4.6.57)

24.6.57. Remaining 10 rats of Group 12 killed today.

13.8.57. Group 13 started today (Rats 191 - 202).

6 Males) Each 100 mg. DCA by subcutaneous
6 Females) implantation, under ether anaesthesia.

Tap water to drink.

13.8.57.(Contd.) Group 14 started (Rats 203 - 214).

6 Males) Each 100 mg. DCA by subcutaneous
) implantation, under ether anaesthesia,
6 Females) with 1% sodium chloride substituted for drinking water.

17.8.57. All rats Group 13 and 14 alive. Blood pressures recorded.

21.8.57. Groups 13)) Blood pressure recorded.
14)

28.8.57. Groups 13)) Blood pressure recorded.
14)

19.9.57. Groups 13)) Blood pressure recorded.
14)

1 rat Group 14 died anaesthesia.

26.9.57. Groups 13)) Blood pressure recorded.
14)

4.10.57. Groups 13)) Blood pressure recorded.
14)

8.10.57. Group 15 started. (Rats 215 - 226).

Each Left nephrectomy.
Subcutaneous implant 100 mg. DCA.
1% sodium chloride to drink.
Daily injections of hydrallazine.

9.10.57. 1 rat Group 15 (female) found dead.

10.10.57. Group 13.) Blood pressures recorded.
Group 14.)

12.10.57. Group 15 blood pressures recorded.

14.10.57. Group 16 started (Rats 227 - 238)

Each Left nephrectomy

100 mg. DCA by subcutaneous implantation.

6 males)
)
6 females)

See tables of weights and blood pressure.

18.10.57. Group 16 blood pressures recorded.

22.10.57. Group 15 blood pressures) measured.
weights)

(On this occasion blood pressure measurements made only $2\frac{1}{2}$ - 3 hours after intramuscular hydrallazine mg. 2).

Group 19 started (Rats 239 - 246)

4 males)
)
4 females)

Each (left nephrectomy
(
(+ 1% sodium chloride to drink
(
(+ daily injections of increasing amounts
(
(of 9αfluorocortisol.

23.10.57. Blood pressures Group 13 measured.

Group 15. Hydrallazine mg. 2 I.M.I. (no anaesthesia)

19. 9 α-fluorocortisol mg.0.025 I.M.I.

24.10.57. Group 20 started (Rats 247 - 254)

4 males) Each left nephrectomy
4 females) 1% sodium chloride substituted for drinking water.

Daily injections of 9 α-fluorocortisol.

Group 15. Hydrallazine mg. 2 I.M.I.

19. 9 α-fluorocortisol mg. 0.025 I.M.I.

25.10.57. Group 15. Hydrallazine mg. 2 I.M.I.

19.)
) 9 α-fluorocortisol mg. 0.025 I.M.I.
20.)

26.10.57. Blood pressures Groups 19.)
) measured.
20.)

Groups 15. Hydrallazine mg. 4 I.M.I.

19.)
) 9 α-fluorocortisol mg. 0.05 I.M.I.
20.)

27,10.57.) Groups 15. Hydrallazine mg. 4 I.M.I.
28.10.57.)
29.10.57.)
30.10.57.) Blood pressures Groups 19.)
20.)

19.)
) 9 α-fluorocortisol mg. 0.05 I.M.I.
20.)

31.10.57. Groups 15. Hydrallazine mg. 4 I.M.I.

19.)
) 9 α-fluorocortisol mg. 0.1 I.M.I.
20.)

Group 14. 5 females killed (ether anaesthesia, bleeding), collection of blood for serum potassium estimations, and preservation of hearts, kidneys, adrenals, lungs, liver and spleens.

Weights (see table)

Electrolytes (see table)

Blood pressures group 15 measured.

1.11.57. Group 14 5 males killed. Procedure as with females.

Groups 15. Hydrallazine mg. 4 I.M.I.

19.)
) 9 α-fluorocortisol mg. 0.1 I.M.I.
20.)

16. blood pressures recorded.

Group 21 started. (Rats 255 - 258)

4 females. Each(Left nephrectomy
(
(Implantation of 70 mg. of

DCA, implants being made in such a way as to facilitate and increase absorption, by preserving looser texture and larger surface area of implant than previously.

2.11.57.) Groups 15.) Hydrallazine mg. 4)
3.11.57.) 20.) 2)
4.11.57.) 19.) 9 α-fluorocortisol mg. 0.1
20.) 0.1

4.11.57. Blood pressures groups 19 and 20 measured.

5.11.57. Blood pressures groups 21 and 22 measured.

Groups 15.) Hydrallazine mg. 4 I.M.I.
20.) mg. 2 I.M.I.

19.) 9 α-fluorocortisol mg. 0.15 I.M.I.
20.) 0.1 I.M.I.

Group 17 started (Rats 263 - 265)

3 males. Each(Bilateral adrenalectomy
(Left nephrectomy
(Daily injection 2.5 mg.cortisone
(1% sodium chloride to drink

Initial dose cortisone given at time of adrenalectomy.

Group 18 started (Rats 266 - 268)

3 males. Each(Bilateral adrenalectomy
(Left nephrectomy
(Daily injection 2.5 mg.cortisone
(1% sodium chloride to drink
(Daily injection of Hydrallazine.

6.11.57. Group 23 started (Rats 267 - 273)

2 females)
3 males)

6.11.57 (Contd.)

Each: (Left nephrectomy
(1% sodium chloride to drink
(Daily injections of DCA

Group 24 started (Rats 274 - 278)

2 females)
)
2 males)

Each: (Left nephrectomy
(1% sodium chloride to drink
(Daily injections of DCA
(Daily injections of Hydrallazine

Blood pressures group 15 measured.

Groups 15.)		4
)	Hydrallazine mg.	
20.)		2
19.)		0.15
)	9 α-fluorocortisol mg.	
20.)		0.1
17.)	2.5	
)	Cortisone mg.	
18.)	2.5	
23.)		2.5
)	agneous DCA suspension mg.	
24.)		2.5

7.11.57. Blood pressures Groups 19 and 20 measured.

Groups 15.)		4
)	Hydrallazine mg.	
20.)		2

7.11.57. (Contd)

Groups 19.) 0.15
) 9 α-fluorocortisol mg.
20.) 0.1

17.) 2.5
) Cortisone mg.
18.) 2.5

23.) 2.5
) DCA mg.
24.) 2.5

8.11.57. Blood pressures Groups 16 and 17 measured.

Group 17 4 additional females (Rats 279 - 282)

Each: (Bilateral adrenalectomy
(Left nephrectomy
(Daily cortisone injections
(1% sodium chloride to drink

Group 18 4 additional females (Rats 283 - 286)

Each: (Bilateral adrenalectomy
(Left nephrectomy
(Daily cortisone injections
(1% sodium chloride to drink

Group 23 2 additional females (Rats 287, 288)

Each: (Left nephrectomy
(1% sodium chloride to drink
(Daily injections DCA

Group 24 2 additional females (Rats 289,290)

Each: (Left nephrectomy
(1% sodium chloride to drink
(Daily injections DCA

8.11.57. (Contd)

Group 13 5 male rats killed.

Blood for serum potassium

Weights of whole animals, kidneys and heart.

Section of heart, kidneys, liver, lung, adrenals, spleen. (See tables).

Group 15.)
20.)
19.)
20.)
17.)
18.)
23.)
24.)
Injections as on 7.11.57

9.11.57. Groups injections as on 8.11.57.

10.11.57. Groups injections as on 8.11.57.

Group 22 Hydrallazine mg. 2.

11.11.57. Blood pressures groups 23 and 24 measured.

1 rat group 17.) found dead. No post mortem examination possible owing to autolysis.
1 rat group 20.)

Group 15.)		4
20.)	Hydrallazine	2
22.)		2

11.11.57 (Contd)

Group 19.)	9 α-fluorocortisol mg.	0.15
20.)		0.1
17.)	Cortisone mg.	2.5
18.)		2.5
23.)	DCA mg.	2.5
24.)		2.5

12.11.57. 1 rat (female) Group 15 dead today, following increased dose of Hydrallazine. Weights recorded.

Sections of heart, lungs, kidney, liver, spleen, adrenals. (See tables).

At necropsy the characteristic features of asphyxia were present: congestion, purple-red appearance of heart, haemorrhages on lung surfaces.

Groups 15.)	Hydrallazine	5
20.)		3
22.)		4
17.)	Cortisone mg.	2.5
18.)		2.5
19.)	9 α-fluorocortisol mg.	0.15
20.)		0.1
23.)	DCA mg.	2.5
24.)		2.5

13.11.57. Blood pressures Groups 15 and 18 measured.

Groups - doses of all drugs as on 12.11.57 except:

Group 15 - hydrallazine mg. 4

24 - hydrallazine mg. 4

14.11.57. Blood pressures groups 19 and 20 measured.

Groups: (as on 13.11.57 except Group 24 hydrallazine mg. 3)

15.11.57. Blood pressures Group 16 measured.

Groups: (as on 14.11.57 + Group 18 hydrallazine mg. 3)

1 Rat (female) Group 24 dead today.

Weight recorded.

Sections of heart, lungs, spleen, R.kidney, adrenals, liver.

16.11.57. Blood pressure Group 17 measured.

Groups 15.)		4
18.)		3
20.)	Hydrallazine mg.	3
22.)		4
24.)		4
17.) 18.)	Cortisone mg.	2.5
19.)	9 α.fluorocortisol mg.	0.15
20.)		0.15

16.11.57. (Contd)

Groups 23.)) DCA mg. 2.5
24.) 2.5

17.11.57. Blood pressures Groups 23 and 24 measured.

Groups - as for 16.11.57 except Group 20

hydrallazine mg. 4.

18.11.57. As for 17.11.57, except Groups 23 and 24

DCA mg. 3.75.

19.11.57. 1 rat (female) Group 16 found dead. Post mortem impossible owing to autolysis.

Groups: as for 18.11.57, except

Groups 17.)) Cortisone mg. 3.25
18.)

19.)) 9 α-fluorocortisol mg. 0.2
20.)

20.11.57. 1 rat (male) group 16 having convulsions (presumably hypertensive encephalopathy.) Killed (ether anaesthesia and bleeding).

Weights recorded.

R. kidney, heart, lungs, adrenals, spleen, liver sectioned.

Blood pressures Groups 16, 17 and 18 measured.

Groups, as on 17.11.57.

21.11.57. Blood pressures Groups 17 and 20 measured.

Groups as for 20.11.57, except:

Groups 17.) 18.) Cortisone mg. 3.75.

22.11.57. Groups as for 21.11.57

23.11.57. Groups as for 22.11.57, except:

Groups 17.) 18.) Cortisone mg. 2.5

19.) 20.) 9 α. fluorocortisol mg. 0.1 each.

24.11.57. 1 rat (male) Group 20 dead today: no necropsy possible.

Groups as for 23.11.57 except:

Groups 19.) 20.) 9 α. fluorocortisol mg. 0.15.

25.11.57. Group 13 6 female rats killed.

Blood for serum potassium.

Weights and organ weights (see tables)

Sections of heart, kidneys, adrenals.

Blood pressures Groups 23 and 24 measured.

Groups 15.)		4
16.)		3
20.)	Hydrallazine mg.	4
22.)		4
24.)		4

25.11.57. (Contd)

Groups 17.), 18.) Cortisone mg. 3.75

Groups 19.), 20.) 9 α-fluorocortisol mg. 3.75

Groups 23.), 24.) DCA mg. 3.75

Group 21. 4 new male rats (Rats 291 - 294)

Each: (Left nephrectomy
(70 mg. new DCA implants
(1% sodium chloride to drink

Group 22. 4 new rats (male) (Rats 295 - 298)

Each: (Left nephrectomy
(70 mg. new DCA implant
(1% sodium chloride to drink
(Daily injection hydrallazine.

26.11.57. Blood pressures groups 21 and 22 measured.

At this point it was noticed that several of the subcutaneous implants in animals of these two groups were being extruded through the skin wound.

Groups as for 25.11.57 except:

Groups 17.), 18.) Cortisone mg. 2.5

Groups 19.), 20.) 9 α-fluorocortisol mg. 0.15.

27.11.57. Group 19 killed today: no significant rise in blood pressure has occurred, and at the same time there has been extreme weight loss.

(see table of estimates of weights, organ weights, sodium and potassium made on these animals).

Rat 1 male Group 15 found dead this morning. Post mortem. Weight of whole animal, right kidney and heart recorded.

Rat 1 (female) Group 19 also found dead this morning. Post mortem. Weight of whole animal, right kidney and heart recorded.

Groups 15)		4
18.)		3
20.)	Hydrallazine mg.	4
22.)		4
24.)		4
17.)		
)	Cortisone mg. 2.5	
18.)		
19.)		
)	9 α-fluorocortisol 0.2	
20.)		
23.)		
)	DCA mg. 2.5	
24.)		

28.11.57. Blood pressures groups 17 and 18 recorded.

Groups: as 27.11.57, except:

28.11.57. (Contd)

Group 15. Hydrallazine mg. 5

23.) DCA mg. 3.75
24.)

29.11.57. Group 20 killed today. (1 rat (male) Group 20 found dead this morning. No necropsy possible. Decided to kill remainder.)

Groups 15.)		4.5
18.)	Hydrallazine mg.	4.5
22.)		4.5
24.)		4.5
17.) 18.)	Cortisone mg.	2.5
23.) 24.)	DCA mg.	3.75

30.11.57. Groups: as for 29.11.57, except Group 15:

Hydrallazine mg. 5.

31.11.57. Group 15.)		5
18.)	Hydrallazine mg.	5
22.)		5
24.)		5
17.) 18.)	Cortisone mg.	2.5
23.) 24.)	DCA mg.	3.75

2.12.57. Blood pressures groups 23 and 24 measured.

Groups: as on 31.11.57.

3.12.57. 1 rat (male) Group 22 dead this morning. No necropsy possible.

Group injections as on 2.12.57.

4.12.57. Group injections as on 2.12.57.

Blood pressures Groups 15 and 18 measured.

5.12.57. 1 rat (male) Group 18 dead this morning. Necropsy. Remainder becoming progressively emaciated, and decided to kill them.

Group 18. Remaining rats killed: weights recorded (whole animal, heart and R. kidney). Serum sodium and potassium estimated (see table).

All adrenals found to have been excised.

Groups 15.)		5.5
18.)	Hydrallazine mg.	5.5
22.)		5.5
24.)		5.5
17.)		2.5
18.)	Cortisone mg.	2.5
23.)		5
24.)	DCA mg.	5

6.12.57. Groups 15.) 6
22.) Hydrallazine mg. 6
24.) 6

23.)
) DCA mg. 5.
24.)

Groups 21 and 22. Started on 1.5% sodium chloride instead of 1% in order to expedite reaction.

Group 17. Remaining animals killed.

Weights, sections, serum sodium and potassium as for Group 18. (See tables).

Necropsy confirmed that all adrenals in animals of Group 17 had been satisfactorily removed. A single doubtful mass found at the site of adrenalectomy in one animal was sectioned and shown to be of inflammatory origin.

8.12.57. Groups 15.) 6.5
22.) Hydrallazine mg. 6.0
24.) 6.0

23.)
) DCA mg. 5.
24.)

9.12.57. Injections as for 8.12.57.

One rat group 22 (female) dead today. No necropsy possible, owing to autolysis.

10.12.57. Injections as for 8.12.57.

At this point it was observed that the animals of groups 15 and 15 were showing such lethargy and deterioration in condition that it was thought advisable to separate them into individual cages in order to be certain of material for histology.

11.12.57. Groups 15.) 7
22.) Hydrallazine mg. 6
24.) 6

23.)
24.) DCA mg. 5.

Rat 2 (female) of Group 15 dead to-day.
Weight 65 g.
R. kidney 1.52 g.
Heart 1.10 g.

Rat 2 (male) of Group 22 also found dead today.
Weight 85 g.
R. kidney 1.11 g.
Heart 0.65 g.

Heart, lungs, kidney, liver, spleen and adrenals of both these animals preserved for section.

12.12.57. Injections as for 11.12.57.

Rat 1 (male) Group 21 dead today. Weight 70 g.
R.kidney 1.11g.
Heart 0.65 g.

13.12.57. Groups 15.) 7
22.) Hydrallazine mg. 6
24.) 7

23.)
24.) DCA mg. 5.

Blood pressures Group 15 measured.

Rat 3 (male) group 22 dead today. Weight 60 g.

R.kidney 0.81 g.

Heart 0.76 g.

Group 25 started (Rats 299 - 306)

4 females) (Left nephrectomy
4 males) (Subcutaneous implant 100 mg. DCA
(1% sodium chloride in place of
(drinking water.
(Daily injections of DCA.

Mean weights: Males: mean 45 g.

Females: mean 37 g.

14.12.57. Injections as on 13.12.57

15.12.57. Injections as on 13.12.57.

Rat 1 (male) Group 23 found in convulsions, today and killed: blood for sodium and potassium estimations.

Weight 164 g.

Heart 1.06 g.

R. kidney 1.47 g.

15.12.57. (Contd).

The right kidney was found to be covered by many small pale foci, while petechial haemorrhages were scattered over the lungs. No mesenteric aneurysms found.

16.12.57. Injections as on 15.12.57.

17.12.57. DCA injections as on 16.12.57.

Hydrallazine: Groups 15 mg. 8
22 7
24 7

18.12.57. Injections as on 17.12.57.

Blood pressures group 16 measured.

Rat 2 (male) Group 23, which appeared ill yesterday killed today. Blood taken for serum potassium and sodium estimations. No blood pressure recordable.

At necropsy the right kidney was found to be greatly enlarged, pale and free from petechial haemorrhage. Bladder distended, and tense, and containing blood-stained urine. The heart was only slightly enlarged.

Weight

R. kidney 2.18 g.

Heart 0.91 g.

19.12.57. Injections as for 18.12.57.

20.12.57. Injections as for 19.12.57

Rat 1 (female) Group 22 dead this morning.

Weight 165 g.

R. kidney 1.23 g.

Heart 1.61 g.

Death apparently the result of hydrallazine convulsions.

21.12.57. Groups 15.) 8
22.) Hydrallazine mg. 8
24.) 8

23.)
24.) DCA mg. 5.

22.12.57.)
23.12.57.) Injections as on 21.12.57.

Rat 2 (male) Group 15 dead today.

Weight

R. kidney 1.19 g.

Heart 1.11 g.

24.12.57.)
26.12.57.) Injections as for 23.12.57.

27.12.57. Groups 15.) 7
22.) Hydrallazine mg. 8
24.) 8

23.)
24.) DCA mg. 5

Rat 2 (female) Group 22 dead today.

R. kidney 1.67 g.

Heart (? 1.61 g.)

28.12.57. Injections as on 27.12.57.

Group 26 started. (Rats 307 - 314).

4 males mean 90 g.

4 females mean 79 g.

Each: (Left nephrectomy
(
(100 mg. subcutaneous implant of DCA.
(
(1% sodium chloride in place of drinking water
(
(Daily injections of DCA
(
(Daily injections of pentolinium tartrate

29.12.57. Injections as on 28.12.57.

Group 27 started (Rats 315 - 322)

4 males

4 females

Each: (bilateral adrenalectomy
(
(tap water to drink
(
(daily cortisone injections

Group 28 started (Rats 323 - 330)

4 males

4 females

Each: (bilateral adrenalectomy
(
(tap water to drink
(
(daily injections cortisone
(
(daily injections hydrallazine

Dosage of hydrallazine now at convulsive levels.

2 rats of Group 22 observed in convulsions today; both recovered.

30.12.57. Injections as on 27.12.57.

3 rats dead this morning:

Rat 3 (male) group 15.

Weight 105 g.

R. kidney 1.39 g.

Heart 1.09 g.

Rat 3 (female) group 22.

Weight 200 g.

R. kidney 1.94 g.

Heart 1.47 g.

Rat 4 (female) group 22.

Weight 180 g.

R. kidney 1.72 g.

Heart 1.30 g.

Remaining 10 rats of Group 16 killed (ether and bleeding). Blood for serum potassium and sodium. Weights. Heart, lungs, R. kidney, adrenals, spleen and liver for section.

31.12.57. Remaining animals Groups 21, 22 and 23 killed today. Blood for serum sodium and potassium. Weights.

Heart, lungs, R. kidney, adrenals, spleen and liver for section.

Injections as for 30.12.57 (except Group 15 now

dead) and

Group 27.)
) cortisone mg. 2.5
Group 28.)

1.1.58. Groups 24.) 5
)
25.) DCA mg. 2.5
)
26.) 2.5

Groups 27.)
) cortisone mg. 2.5
28.)

24. hydrallazine mg. 8.

2.1.58. Injections as for 1.1.58 + Group 28 hydrallazine mg. 2.

1 Rat (male) Group 28 dead today following hydralla-:zine this morning. (This and subsequent experience confirmed that the animals with bilateral adrenalectomy are, as might be expected, unusually susceptible to the effects of slight hypotension, even when cortisone is given simultaneously.

3.1.58. Injections as for 2.1.58, but Group 28 hydralla-:zine mg. 1.

4.1.58.)
) Injections as for 3.1.58.
5.1.58.)

6.1.58. Injections as for 5.1.58 + Group 28 hydrallazine mg. 2 and Group 26 pentolinium mg. 0.025.

7.1.58) Rat 1 (female) Group 28 dead today. Weights:
8.1.58)
9.1.58) L. Kidney 0.42 g.
) R. Kidney 0.35 g.
) Injections as for 6.1.58.
10.1.58) Heart 0.25 g.
11.1.58)
12.1.58)
13.1.58) Blood pressures groups 27 and 28.

1 rat Group 24 (female) dead today. Weight 175 g.
R. Kidney 1.45 g.
Heart 2.02 g.

Groups 24.) 9
) Hydrallazine mg.
28.) 2

Groups 24.) 5
)
25.) DCA mg. 2.5
)
26.)

27.)
) cortisone mg. 2.5
28.)

26. pentolinium mg. 0.1.

14.1.58. Group 29 started today (Rats 331 - 335).

3 females of 5 remain alive.

Each: left renal arterial clamp.

Group 30 started (Rats 336 - 340)

4 males of 5 remain alive.

Each: left renal arterial clamp + daily injection.

hydrallazine.

Rat 1 Group 25 (female) dead today.

Weight 75 g.

Heart 0.95 g.

R. kidney 1.20 g.

Rat 2 Group 28 (female) dead today.

Weight 50 g.

R. kidney 0.35 g.

Heart 0.21 g.

15.1.58. Serial blood pressure readings on Group 24 made today. Starting at 1000 hours, the pressure was measured, 8 mg. hydrallazine injected and the pressure changes measured at intervals up to 24 hours.

Injections as on 14.1.58.

16.1.58. Groups 24.) 5
25.) DCA mg. 2.5
26.) 2.5
27.) Cortisone mg. 2.5
28.)
28.) 1
24.) Hydrallazine mg. 8

17.1.58.) Injections as on 16.1.58, but pentolinium
18.1.58.) (Group 26) now 0.3 ml. = 1.5 mg. daily.

19.1.58. 6 further rats Group 29) begun. (Rats 340-351)
5 further rats Group 30)

Rat 2 (female) Group 25 dead today.

Weight 70 g.

R. kidney 1.15 g.

Heart 0.75 g.

Kidney large and exceptionally pale.

1 rat (male) Group 29 found dead - no necropsy.

20.1.58. Injections as for 19.1.58.

Rat 1 (female) Group 26 dead today.

Weight 68.9 g.

R. kidney 1.05 g.

Heart 0.9 g.

21.1.58. Injections as for 20.1.58.

Blood pressures and weights Group 27.

22.1.58. Groups 24.) Hydrallazine mg. 9
28.) 1

24.) DCA. mg. 5
25.) 2.5
26.) 2.5

27.) Cortisone mg. 2.5
28.) 2.5

26. Pentolinium mg. 0.15

23.1.58. Injections as for 22.1.58.

24.1.58. Injections as for 23.1.58, except that Group 26 received mg. 0.2 Pentolinium each.

Rat 3 (female) Group 28 dead.

Weight 64 g.

Kidneys 0.45 g. 0.44 g.

Heart 0.43 g.

25.1.58. Injections as for 24.1.58.

Blood pressures Groups 29, 30.

26.1.58. Injections as for 25.1.58.

Rat 2 (male) Group 28 dead. No necropsy.

27.1.58. Remaining 3 animals Group 28 killed today. 2 animals of Group 25 also killed owing to progressive illness. Injections as for 26.1.58.

28.1.58. Injections as for 27.1.58.

Blood pressures recorded Groups 25, 26, 27.

29.1.58. Injections as for 28.1.58.

30.1.58. Injections as for 29.1.58.

31.1.58. Injections as for 30.1.58.

1.2.58. Groups 24.) 5
25.) DCA mg. 2.5
26.) 2.5

1.2.58 (Contd.)

Groups 26 Pentolinium mg. 2.5

27 Cortisone mg. 2.5

24 Hydrallazine mg. 11.

Rat 1 (male) Group 27 dead (see table).

2.2.58. Injections as for 1.2.58.

3.2.58. Groups 24.) 5
25.) DCA mg. 2.5
26.) 2.5

24 Hydrallazine mg. 12

26 Pentolinium 0.5 ml.

27 Cortisone mg. 2.5

Remaining 3 male rats of Group 25 killed today.

4.2.58. Group 26: Serial blood pressures following large dose Pentolinium.

2 male rats Group 26 dead (Rats 2 and 3) (one from anaesthesia).

Injections as for 3.2.58.

5.2.58. Injections as for 4.2.58, except Group 24 Hydrallazine mg. 13.

1 male rat (Rat 1) Group 27 dead.

6.2.58. Groups 24.) 5
) DCA mg.
26.) 2.5

27 Cortisone mg. 2.5

26 Pentolinium mg. 1.0

6.2.58. (Contd.)

Group 24 Hydrallazine mg. 15.

Remainder 5 rats Group 26 killed today.

Group 31 started (Rats 354 - 361 inclusive).

4 males) Left nephrectomy
4 females) DCA injection 2.5 mg. daily.
1% sodium chloride to drink.
Daily injections compound 14179.

Group 32 started (Rats 362 - 369 inclusive).

4 males) Daily injections of increasing
4 females) doses of picrotoxin beginning with 0.15 mg. I.M.I.

Group 33 started (Rats 370 - 379 inclusive).

4 males) Daily injections 2.5 mg. cortisone +
4 females) daily injections hydrallazine.

Group 34 started (Rats 378 - 385 inclusive).

4 males) Daily injections 9 α-fluorocortisol
4 females) + 1% sodium chloride to drink +
daily injections hydrallazine.

Two rats Group 24 died following convulsions due to increased dose of hydrallazine today. (Rat 1 male, rat 3 female).

7.2.58. Group 35 started (Rats 386 - 393 inclusive).

4 males) Left nephrectomy.
4 females) Daily injections 2.5 mg. DCA.
1% sodium chloride to drink.
Daily injections Reserpine.

7.2.58.(contd.)

Groups 24.) 5
31.) DCA mg. 2.5
35.) 2.5

32. picrotoxin mg. 0.3.

34. 9 α. fluorocortisol mg. 0.025.

27.) Cortisone mg. 2.5
33.)

24. Hydrallazine mg. 14.

8.2.58. Injections same as on 7.2.58, except that Group 24 received hydrallazine mg. 15.

9.2.58. Injections as for 8.2.58.

10.2.58. Injections as for 9.2.58.

11.2.58. 3 female animals Group 32 died following convulsions today (Females 1, 2 and 3).

Groups 24.) 5
31.) DCA. mg. 2.5
35.) 2.5

27.) Cortisone mg. 2.5
33.)

32. picrotoxin mg. 0.75.

33.) 1
24.) hydrallazine mg. 15
34.) 1

34. 9 α. fluorocortisol mg. 0.025 (ml.0.05)

35. reserpine ml. 0.1 (0.004 mg.)

31. 14179 mg. 1 (0.1 ml.)

12.2.58. Injections as for 11.2.58, except 3 animals in Group 24 now having respectively 17, 17 and 15 mg.

13.2.58. Rat 4 (female) Group 32 died today following convulsions.

Injections as for 12.2.58.

14.2.58. Injections as for 13.2.58.

15.2.58. Injections as for 14.2.58.

16.2.58. Injections as for 15.2.58.

17.2.58. Remaining 3 animals of Group 24 died this morn-:ing following convulsions.

Groups 29.) 3 females) (Rats 394 - 420) to which
30.) 4 males) renal arterial clamps applied on 13.2.58, given hydrallazine today.

Groups 30.) 2
33.) Hydrallazine mg. 3
34.) 3

31.)
) DCA mg. 2.5 each.
35.)

31. 14179 mg. 2.5.

32. picrotoxin mg. 1.35.

33. cortisone mg. 2.5.

34. 9 α-fluorocortisol mg. 0.05.

35. reserpine mg. 0.008.

18.2.58. Two animals of Group 33 dead following increased dose of hydrallazine.

Groups 30.) 4
33.) hydrallazine mg. 4
34.) 6

31.)
) DCA. mg. 2.5 each.
35.)

31. 14179 mg. 2.5.

32. picrotoxin mg. 1.35.

33. cortisone mg. 2.5.

34. 9 α fluorocortisol mg. 0.05.

35. reserpine mg. 0.008.

19.2.58. Group 32: picrotoxin mg. 1.5 following which 1 animal died (male).

Group 31: 14179 mg. 3.

Other injections as for 18.2.58.

20.2.58. Group 32: remaining 2 males of Group 32 died following 0.5 ml. (mg. 1.5) picrotoxin.

Remaining injections as for 19.2.58.

21.2.58. Injections as for 20.2.58.

22.2.58. Groups 30.)
33.) hydrallazine mg. 5
34.)

Other injections as for 21.2.58.

23.2.58. Injections as for 22.2.58.

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