Neurosecretory activity in the medulla terminalis X-organ

	Crab number	Staining index
PCB-treated	1	2
	2	3
	3	2
	4	3
	5	3
		Average 2.6*
Control	1	1
	2	1
	3	2
	4	2
	5	1
		Average 1.4

*Significantly greater than control ($p < 0.05$).

phloxin[8]. The secretory activity of the neurosecretory cell bodies in the medulla terminalis X-organ of the sectioned eyestalks was then evaluated by using the following index numbers to stage the cells: 0, neurosecretory granules absent; 1, very few granules; 2, intermediate between 1 and 3; 3, a large number of granules. This system is essentially that of Matsumoto, but he numbered his stages 0, 1, 3, and 5[9]. A cell whose cytoplasm is crowded with neurosecretory granules presumably contains more neurohormone than does a cell with few or no granules. Statistical evaluation of the data was performed using the Student's t-test.

Results and discussion. When the eyestalks were removed, the mean melanophore stage of the crabs that had been in the acetone-sea water for 24 h was 3.4 whereas that of the crabs in the PCB preparation was 1.7. This difference (3.4 versus 1.7) and the decrease from the initial melanophore stage (1.7 versus 3.0) were not only statistically significant ($p < 0.01$ for both) but were also consistent with the previous observation that Aroclor 1242 prevents the pigment in the melanophores from remaining as dispersed as in control crabs[5]. The slightly increased level of melanin dispersion, up from the original 3.0 to 3.4, in the control crabs was presumably due in large measure to the continuous exposure to the bright illumination (2100 lux) in contrast to the 422 lux the crabs had been exposed to in the stock tank prior to the start of the experiment. Bright illumination induces melanin dispersion in the fiddler crab[10].

Examination of the eyestalk sections revealed that the quantity of neurosecretory material in the neurosecretory cell bodies in the medulla terminalis X-organs of the crabs exposed to the PCB preparation was significantly greater than in the control crabs (table). This observation is consistent with the previously obtained data which showed that eyestalks of crabs exposed to Aroclor 1242 contained more MDH than did eyestalks of control crabs[5]. Of course, all of the neurosecretory granules stained were not MDH-containing alone. The eyestalk secretes several different neurohormones[11]. Additional experiments are being performed to determine what eyestalk neurohormones in addition to MDH are also affected by PCBs.

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Glomerular filtration rate and kidney blood flow in alloxan and streptozotocin diabetic rats

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Summary. Glomerular filtration rate (GFR), cardiac output, regional blood flow and kidney weight were measured in alloxan and streptozotocin diabetic rats at different times after the administration of diabetogen. A high GFR was found together with increased kidney weight and reduced blood flow.

The role of diabetic nephropathy as a cause of death in late diabetes mellitus underlines the importance of studying changes in renal structure and function early in the disease. A measurable thickening of the basement membrane and mesangial region has been clearly shown in recent studies using the electron microscope. It takes place long before the advent of any clinical evidence of nephropathy[1-3]. It is thought that this thickening is the forerunner of more advanced disease which leads to renal failure.

Recent clinical studies are agreed in showing an increase in the glomerular filtration rate (GFR) in early diabetes[4-7]. This elevation of GFR is accompanied by normal or slightly increased renal plasma flow (RPF) in some reports[5,6] and reduced RPF in others[4]. The exact mechanism behind this high GFR is not known. It has been variously suggested that it might be related to an increase in RPF, a decrease in RPF and hence a higher filtration pressure or to an increase in the permeability of the glomeruli. Increased kidney size was also reported to be behind this abnormality[8,9].

All the previously mentioned studies were carried out on human beings where ethical considerations limit the nature of the investigations capable of being executed. In particular, simultaneous estimation of cardiac output and renal blood flow, while extremely difficult in humans, is quite feasible in experimental animals, using the labelled microsphere technique previously described[10].

Previous work on experimental diabetes has been mainly directed to the toxic effects of the diabetogenic agents on the kidney itself. Alloxan and streptozotocin were both reported to cause renal lesions[11-14]. However, these lesions were also shown to be due to the diabetic condition or to

the use of excessively large doses rather than to the chemical nature of these agents[15,16]. The effects of these agents and experimental diabetes on kidney function are still to be investigated.

The present work reports measurements of GFR in experimental diabetes and in separate experiments correlates these with blood flow to the kidney. Differences in kidney weight in different stages of experimental diabetes were also recorded. The mechanisms which may be responsible for these abnormalities are discussed.

Materials and methods. Male CFE rats of approximately 200 g were rendered diabetic either by alloxan (50 mg/kg b.wt) or streptozotocin (60 mg/kg b.wt), both agents being given i.v. Control rats received i.v. saline instead. Only females, both diabetic and control, were used for creatinine clearance experiments as creatinine has been shown to be secreted by the renal tubules of male rats[17]. Experiments on alloxan diabetic rats were performed approximately 7 days after injection and the onset of diabetes, as measured by glycosuria (over 300 mg%). Streptozotocin diabetic rats were used after 3, 14 and 60 days respectively.

GFR was measured by creatinine and inulin clearances. The methods were essentially those of Harvey and Malvin[17] as modified by Barker and Browne[18]. Cardiac output and regional blood flow were measured by the method of Lucas and Foy[10].

Results. All animals used developed hyperglycaemia within 24 h of treatment with alloxan or streptozotocin. In both creatinine and inulin clearance experiments urine flow was markedly increased in diabetic rats compared with controls. Creatinine clearance (figure 1) was significantly enhanced in alloxan diabetic rats when compared with controls (0.48 ± 0.03 and 0.76 ± 0.02 respectively), whereas 14-day streptozotocin diabetic animals showed a mean clearance more than double that of the controls (1.21 ± 0.04).

In inulin clearance (figure 1) the same pattern was shown, alloxan diabetic rats giving a clearance double that of the controls (0.61 ± 0.02 and 0.3 ± 0.02). This ratio was exceeded in the 14-day streptozotocin diabetic rats (0.72 ± 0.04 ml/min/100 g) and when the rats were kept for 60 days the rise still persisted and a clearance nearly 3 times that of the controls resulted (0.85 ± 0.03 ml/min/100 g). All diabetic rats showed a big increase in kidney size (table), which reached nearly double the size of the control kidneys within 2 months.

Figure 2 shows the partial cardiac output received by the kidneys. In comparison with control rats the figures show no alteration in cardiac output during all stages of diabetes tested. Figure 2 also shows a sharp decrease in the amount of blood reaching the kidneys expressed as total blood flow per 100 g kidney weight. It is significant and progressive in all stages of the disease. This blood flow decreases with increasing time of the disease.

Discussion. This work on experimental diabetes confirms the reports of abnormally high GFR seen in human diabetes[4-6,8,9]. The results shown here may add some light to the confused picture of whether this high GFR is due to an increased RPF, a decreased RPF and so high filtration pressure in the glomerular capillaries or to an increase in the kidney size.

A large increase in the kidney size is seen in all groups of diabetic rats, reaching double the control size after 60 days of diabetes. This is in agreement with the increase in human kidney size in both juvenile and experimental diabetes[8,9,19]. The only report of a decreased kidney size[14] may be due to the effect of toxic doses of streptozotocin used, evident by the fact that more than half the animals died during the course of the experiment. We know of no former reports on the size of the kidney in alloxan diabetic animals.

Despite this large increase in kidney size in both alloxan and streptozotocin diabetic rats no increase in the partial cardiac output received by the kidney was noticed.

So the total blood flow to the kidney expressed as unit organ weight was very much reduced with the extent of the disease, a reduction which was significant in the early days of diabetes. This may be a sign of the atherosclerosis seen in renal arteries early in the disease[20] and of the thickening of capillary basement membrane which is also noticed in diabetic rats[1] and humans. The reduced blood flow to the kidney with no increase in cardiac output despite the hypertrophy, suggests a decrease in RPF. Decreased RPF was also seen by Ditzel and Junker[4] in human diabetics

The effect of experimental diabetes on the kidneys size expressed as weight of both kidneys (mean ± SE) in the rat. ADR are alloxan diabetic rats and SDR are streptozotocin diabetic rats

Rats tested	Kidneys size (g)
Controls	1.4 ± 0.04
7-day ADR	1.96 ± 0.04**
3-day SDR	1.61 ± 0.02*
14-day SDR	2.2 ± 0.04**
60-day SDR	2.52 ± 0.08**

* $p < 0.05$; ** $p < 0.01$.

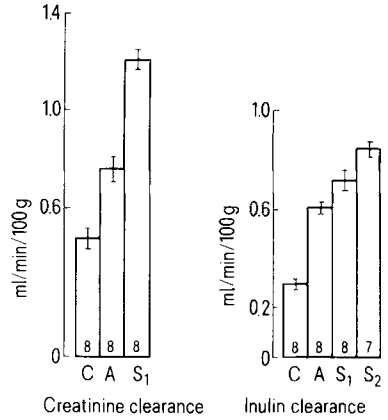

Fig. 1. Creatinine and inulin clearances (mean ± SE). C, control rats; A, 7-day alloxan diabetic rats; S_1 and S_2, 14- and 60-day streptozotocin diabetic rats. The number at the base of each column indicates the number of rats in each group. All test results are statistically different from controls ($p < 0.01$).

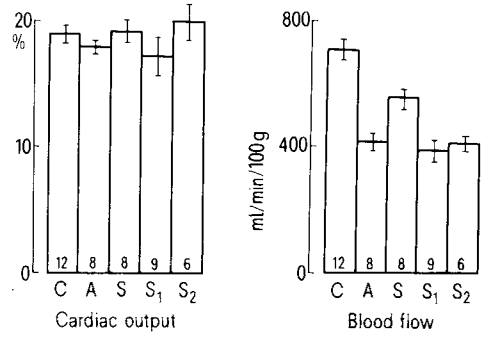

Fig. 2. The effect of experimental diabetes on the percentage cardiac output (mean + SE) and blood flow (ml/100 g/min, mean ± SE) to the kidney. C, control rats; A, 7-day alloxan diabetic rats; S, S_1 and S_2, respectively 3-day, 14-day and 60-day streptozotocin diabetic rats. The number at the base of each column indicates the number of rats in each group.

and although normal RPF was seen by Mogensen[5,6], no significant increase in the RPF was reported in diabetes. Most workers suggest either reduced RPF or an increased kidney size together with other metabolic and endocrine changes to be behind this high GFR. However, from these findings it can be said that at least in experimental diabetes it may be that both increased kidney size and reduced RPF and hence increased filtration pressure due to constriction of the vas efferens are responsible for the high GFR. Other metabolic and endocrine changes are not excluded.

These results also show that the kidney functions well during this period of diabetes, although the reduced blood flow to the kidney may indicate the growing tendency of atherosclerosis in renal vessels. Alloxan and streptozotocin in diabetogenic doses seem to have no direct effect on kidney function and the use of very high doses may be responsible for the lesions seen by some other workers. Work now in progress to investigate the response of the diabetic kidney to diuresis seems also to confirm these findings.

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Haemagglutinating activity of modeccin[1]

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Summary. Modeccin, the toxin of *Adenia digitata,* agglutinates erythrocytes from several mammalian species. The haemagglutinating activity is enhanced by neuraminidase and is inhibited by galactose and by galactose-containing sugars.

Modeccin is a highly toxic lectin purified recently from the roots of *Adenia digitata*[2-5], similar but not identical to ricin and abrin (for review on these see Olsnes and Pihl[6]).

Ricin and abrin are distinct from the agglutinins contained in the same plants, but still agglutinate red blood cells. It was observed in our laboratory that modeccin agglutinates rabbit erythrocytes[5], whereas Refsnes et al.[2], working with higher concentrations of the toxin, could observe only indirect agglutination of human erythrocytes. The present experiments were undertaken to elucidate further the haemagglutinating properties of modeccin and to compare them with the properties of ricin.

Materials and methods. Modeccin was prepared as described previously[5], and ricin as described by Nicolson et al.[7,8]. Blood was collected on 0.4% Na-citrate in 0.90% NaCl; erythrocytes were washed 4–5 times by successive resuspensions and centrifugations in 20 mM Na-phosphate buffer, pH 7.1, in 0.14 M NaCl containing bovine serum albumin (15 µg/ml) and were finally resuspended in the same solution at a concentration of 1.2%. Haemagglutinating activity was assayed in Greiner microtiter plates: each well contained 50 µl of erythrocyte suspension and 50 µl of resuspending buffer containing, when appropriate, 2.5 units of neuraminidase (from *Vibrio comma,* Boehringwerke)

Table 1. Haemagglutinating activity* of modeccin and ricin

Erythrocytes	Modeccin without neuraminidase	with neuraminidase**	Ricin without neuraminidase	with neuraminidase**
Human A	125	7.8	7.8	1.95
B	250	15.6	7.8	1.95
AB	250	15.6	3.9	1.95
0	250	15.6	15.6	1.95
Rabbit	31.2	31.2	1.95	1.95
Pig	62.5	31.2	3.9	3.9
Horse	125	7.8	7.8	0.98
Guinea-pig	125	15.6	15.6	0.98
Mouse	250	62.5	15.6	1.95
Rat	500	125	31.2	1.95
Sheep	No agglutination***	31.2	62.5	7.8
Ox	No agglutination***	31.2	250	31.2

*Expressed as the lowest concentration (in µg/ml) of the lectin giving visible agglutination. **2.5 units/ml. ***With modeccin up to 500 µg/ml.