GLYCEROL-INDUCED OLIGURIA AND REDUCED GLOMERULAR FILTRATION IN THE RAT

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A RECENT clinico-pathological study of acute oliguric renal failure in human beings (Finckh, Jeremy and Whyte, 1962) indicated that structural renal damage was unlikely to be responsible for the oliguria and that a functional cause was more probable. It was subsequently proposed that a fall in glomerular filtration is the principal cause of the oliguria and uraemia in these patients and that this results from a functional vasomotor disturbance within the kidneys (Finckh, 1962). The present work now provides experimental evidence that such a mechanism is at any rate possible, and describes the development of periods of oliguria with reduced glomerular filtration after the intraperitoneal injection of glycerol in the rat.

MATERIALS AND METHODS

Young adult female albino rats (170–210 g.) were used either as controls, or as test animals rendered oliguric by the intraperitoneal injection of single doses of 3·5 ml./kg. of glycerol given as a 12·5 per cent solution in distilled water. The urinary output of individual rats was measured at half-hourly intervals by observing the size of the " blots " formed by urine passed through the coarse wire floors of the cages on to absorbent paper of standard thickness, in the manner previously described (Finckh, 1960). The amount of endogenous creatinine passed in the urine by each rat was estimated by eluting it from the " blots " into distilled water and analysing the eluate. Pilot recovery experiments showed that the creatinine was easily and thoroughly eluted from the paper and a procedure was then adopted in which the blots were cut up into small pieces and thoroughly shaken up in jars with 50 times their estimated original volume of distilled water. Aliquots of these specimens were then estimated for their creatinine content by a modification of the method of Edwards and Whyte (1958).

Systolic and diastolic blood pressure recordings were made in the rats under light ether anaesthesia by means of a capacitance manometer connected to a 23-gauge hypodermic needle filled with isotonic sodium citrate solution and inserted by open operation upwards into the abdominal aorta. Most of these animals were also used for intrarenal blood flow study. This was done by withdrawing the needle until its tip was just below the level of the origin of the renal arteries and then injecting through it 0·3 ml. of fine colloidal indian ink (Pelikan ink, batch no. C11/1431a) diluted to 50 per cent in 0·85 per cent saline solution steadily but not forcibly against the stream of blood in the aorta. A ligature placed around the left renal pedicle was then tied at predetermined times after the injection and the right kidney was removed at selected intervals after the injection to observe the extent to which the pulse of ink had been cleared by the flushing through of unstained blood. The ligated left kidney was then removed and transverse slices were made across the centre of each kidney to include cortex and medulla with the papilla.

Haematoxylin and eosin stained sections of the kidneys of control and glycerol-injected rats were made after fixing transverse slices in Carnoy's fixative, paraffin embedding and cutting at 5–7 μ. Thick, cleared sections of the ink-injected kidneys were made by fixing transverse slices in formol saline, cutting by frozen section at 100 μ, clearing and mounting unstained.

Statistical procedures were performed to evaluate the changes in the output of urine and endogenous creatinine and the systemic blood pressures in the treated animals. Mean figures and standard deviations were obtained for the results of both control and test animals. The F-test was used to indicate whether the variations were homogeneous or not. When they were, " Student's " t-test for the differences of the means was used, but where they were not, a modified t-test was performed with the aid of Sukhatme's tables. For both methods differences were held to be significant when the significance level was 5 per cent or less.

RESULTS

Output of urine and endogenous creatinine in control rats

The mean volume of urine passed by 48 control rats over the test period of 6 hr. is shown in Fig. 1, together with the standard deviations from the mean. Brief ether anaesthesia before the test period caused no significant difference in

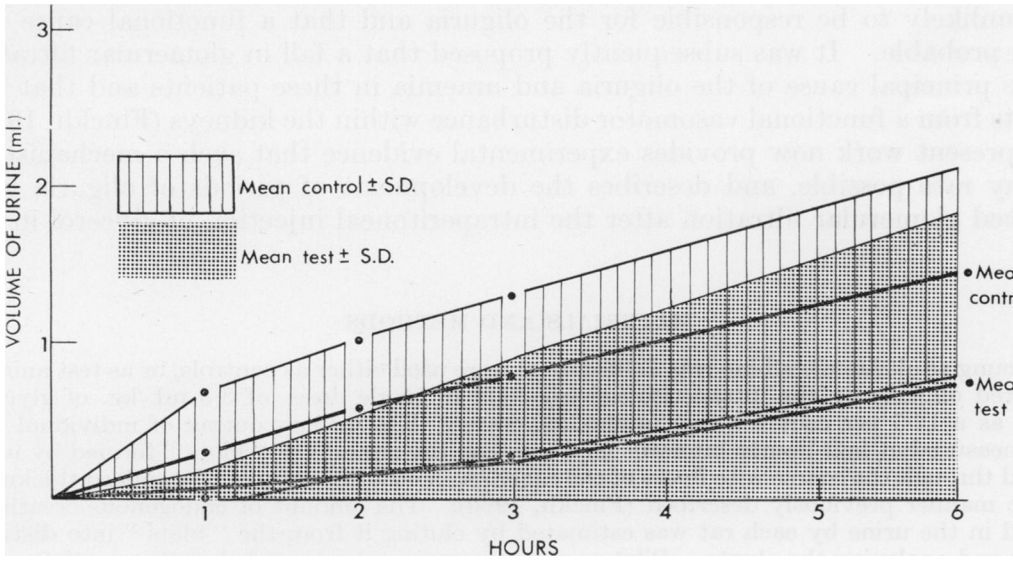


FIG. 1.—The cumulative urinary output of control and test rats, recorded over 6 hr. The mean output of the test rats is significantly lower than that of the controls for the whole period. (S.D., standard deviation.)

the output of urine. On the other hand it was found that unless the animals were allowed to become accustomed to their cages before the experiment was begun they became agitated and passed 2–3 times more urine than otherwise in the first 2 hr. The mean output of endogenous creatinine by the control rats was also found to be steady and continuous over the 6 hr. and to show little variation from the mean (Fig. 2).

Output of urine and endogenous creatinine in glycerol-injected rats

The effect of the single intraperitoneal injections of glycerol on the volume of urine and the output of endogenous creatinine in 51 rats is also shown in Figs. 1 and 2. Taken together this whole group had a considerably lowered output of urine and creatinine (significant at the 1 per cent level or less) for the entire 6 hr. Many rats were even more abnormal, as 37 rats were completely anuric for at least 3 hr. and 19 for 6 hr. or longer.

Histological appearances of the kidneys after glycerol-induced oliguria

The 51 rats which had been injected with glycerol were killed 24 hr. later and their kidneys were examined histologically. The principal abnormality noted was recent necrosis of proximal convoluted tubular cells. This had the appearance previously described for animals treated in this way (Finckh, 1959), but was present in small amounts and in some kidneys only, since the dosage of glycerol employed here was relatively small. Eight rats which had been anuric for 3 hr. or more and 2 rats which were anuric for over 6 hr. had only a few isolated necrotic cells in proximal tubules ; and 7 rats which were anuric for 3 hr. or more and 2 rats which were anuric for 5 hr. or more showed no histological abnormalities whatever in the kidneys.

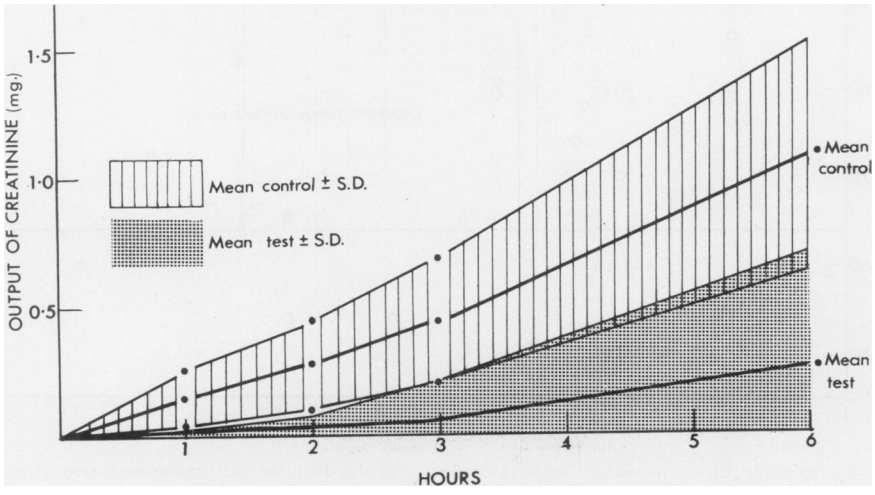


FIG. 2.—The cumulative endogenous creatinine output of control and test rats over 6 hr. The mean output of the test rats is much lower than that of the controls. (S.D., standard deviation.)

Systemic blood pressure and intrarenal blood flow in control rats

The blood pressure readings obtained by direct cannulation of the aorta in 82 control rats were found to show but moderate variation : the mean systolic pressure was 114 mm. Hg with a standard deviation of 8 mm. and the mean diastolic pressure was 82 mm. with a standard deviation of 9 mm. (Fig. 3).

When indian ink was injected into the aorta, the cortices of both kidneys were seen to darken immediately and then to become pale again within 2–5 sec. Thick, cleared sections of kidneys ligated just after the conclusion of the injection showed that the ink was principally in the glomeruli and adjoining cortical capillaries and that some was already in a few vasa recta in the medulla (Fig. 4). Similar sections of kidneys ligated 20–30 sec. later showed that most of the ink had by then passed out of the glomerular or other renal vessels and that the small amounts of ink remaining were mainly in the vessels of the deeper parts of the medulla, especially the papilla (Fig. 5).

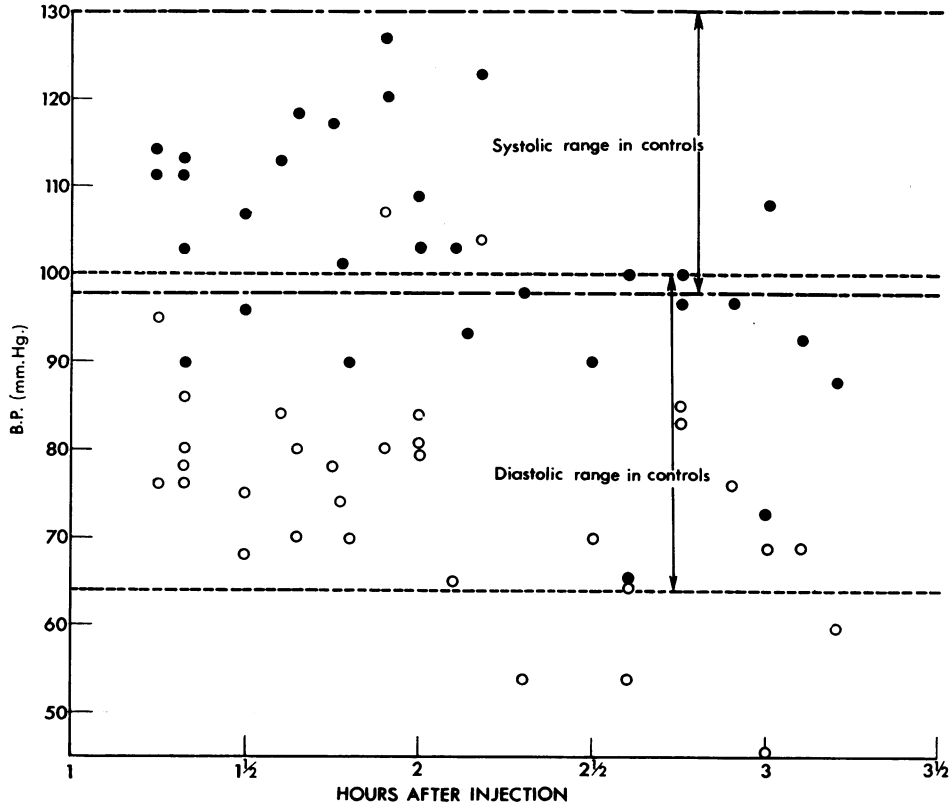


FIG. 3.—Systolic blood pressures (closed circles) and diastolic blood pressures (open circles) of glycerol-anuric rats compared with the ranges for normal controls (Normal range = mean ± twice standard deviation). The majority of readings during the 2nd hr. are within the normal range. During the 3rd hr. the means are significantly lower than normal, but quite a number of rats still have systolic and diastolic blood pressures within the normal range.

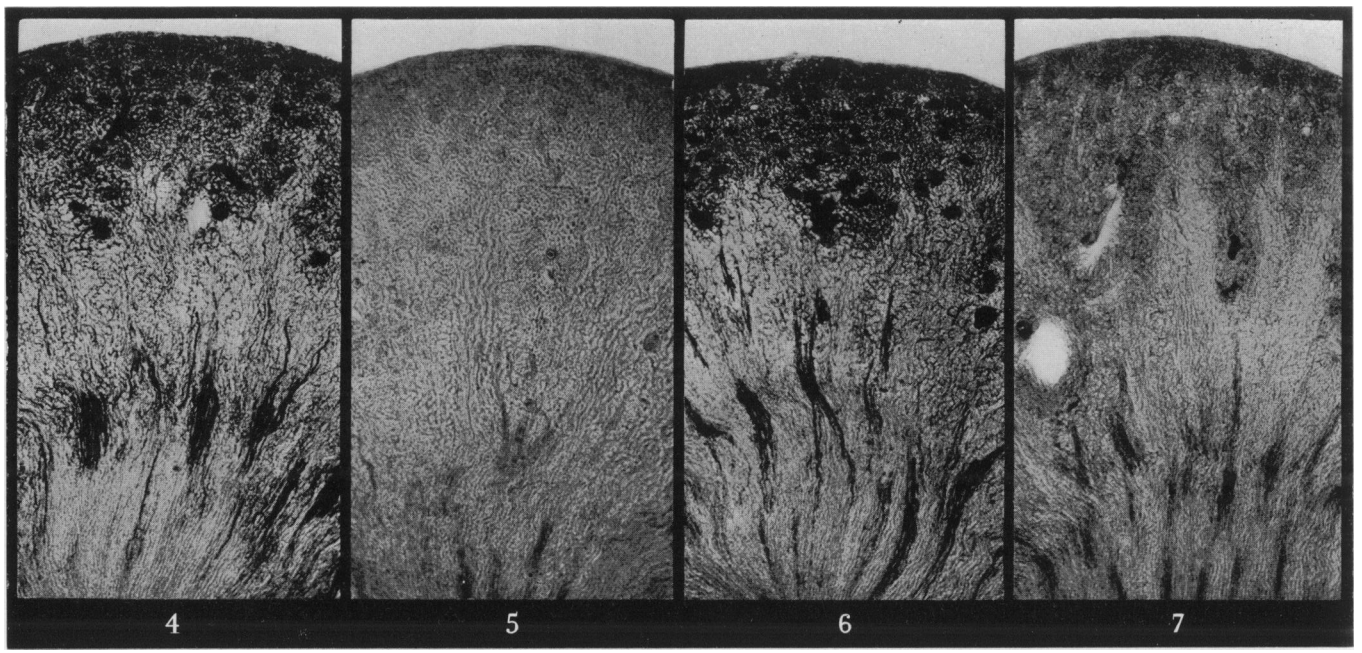
EXPLANATION OF PLATE

FIG. 4.—Portion of a thick (100 μ) cleared section of kidney from a control rat. The renal pedicle was tied at the conclusion of an injection of indian ink into the aorta. The glomerular and cortical capillaries are filled with ink and there is already some ink in the vasa recta of the medulla.

FIG. 5.—Portion of a thick cleared section of kidney from a control rat. This kidney was removed 25 sec. after the injection of indian ink into the aorta. The ink has by now almost entirely left the kidney, having been washed out by unstained blood, but a few vessels in the base of a papilla still contain some carbon.

FIG. 6.—Portion of kidney from a glycerol-injected rat which had been anuric for 2½ hr. The renal pedicle was ligated after the injection of indian ink into the aorta. The glomerular and cortical capillaries and vasa recta in the medulla are filled with ink to the same extent as in the controls (Fig. 4).

FIG. 7.—Portion of the opposite kidney from the same glycerol-anuric rat. This kidney was removed 25 sec. after the injection of indian ink. As in the controls (Fig. 5) the ink has been washed out of the cortical vessels; however, there is slightly more left in the medullary vasa recta, suggesting that the renal blood flow was slightly less than normal.



Finckh.

Systemic blood pressure and intrarenal blood flow in oliguric rats

The rats used for this part of the work were selected from a series given intraperitoneal injections of glycerol. Those which had passed urine into their cages or whose bladders contained urine at the time of operation were rejected and the remaining, anuric rats were then studied in the same way as the controls.

Both the systolic and the diastolic blood pressures showed more variation in this group than in the controls (Fig. 3). During the second hr. after injection of glycerol the mean systolic blood pressure had fallen slightly to 107 mm. Hg and the mean diastolic pressure to 80 mm. but statistical analysis showed that the difference was not significant at the five per cent level During the third hr. the blood pressures had fallen further in some animals, so that the mean blood pressures for the whole group were 102 mm. systolic and 76 mm. diastolic and there was now significant difference from normal.

The pattern of distribution of injected indian ink within the kidneys was generally similar to that in the control rats. The outer surface of the kidneys darkened immediately after the injection and then soon became paler again ; however, although accurate measurements were not made, it seemed that the process took a little longer in these animals than in the controls. Sections of kidneys ligated just after injection showed that the ink had filled all the glomeruli and nearby capillaries to the extent seen in the controls (Fig. 6). However, although in the kidneys ligated 20–30 sec. later the ink had left the cortical vessels in the same way as in the controls, slightly more remained in the medullary vessels, suggesting that the washing out of the ink was not as rapid or complete as in the controls (Fig. 7).

DISCUSSION

In these experiments the output of endogenous creatinine was used not only to show whether the urine was merely more concentrated than usual after the intraperitoneal injection of glycerol, but also to give an indication of changes in glomerular filtration after the manner of creatinine clearance studies. However plasma creatinine levels were not obtained as it was thought that the advantages of knowing these levels would be outweighed by the uncertain effects upon the general renal function of the excitement and changes in circulating blood volume which would be caused by the collection of sufficiently large blood samples. It was held that the plasma creatinine levels in young and healthy rats would deviate little from a constant figure during short term experiments if all the rats were of the same sex and roughly the same size and weight ; and that the plasma creatinine levels would then not need to be known for the urinary creatinine output to be used to indicate changes in glomerular filtration. This conclusion was given support by the relatively small deviation from the mean figure found in the series of control animals (Fig. 2).

These experiments showed that intraperitoneal injections of glycerol in the dosage employed led to periods of oliguria and even anuria for up to 6 hr., but without causing more than slight structural renal damage in a few rats. As judged by the endogenous creatinine output, glomerular filtration during the period of oliguria was seriously impaired, although the systemic blood pressure remained in most instances within the normal range and blood was shown still to be perfusing both cortex and medulla in near-normal manner.

The demonstration that oliguria can be produced in the absence of structural renal damage by a reduction of glomerular filtration provides support for the suggestion that acute oliguric renal failure in human beings may be principally brought about in this way (Allen, 1962; Finckh, 1962). However, the present experiments do no more than suggest the mechanism by which this may occur. It is hard to envisage how changes in glomerular permeability or intraglomerular back-pressure can be made to account for the results obtained, and a more likely explanation appears to be that the glomerular filtration pressure was lowered during the period of oliguria. Since the systemic blood pressure was not much reduced and since the renal cortices continued to be perfused, this in turn cannot be held to be the result of systemic hypotension or of a cortico-medullary shunt of the " Oxford " type (Trueta, Barclay, Daniel, Franklin and Pritchard, 1947; Sophian, 1953, 1962). There remains the possibility that an imbalance of afferent and efferent glomerular arteriolar tone might have permitted the continued perfusion of the kidneys without sufficient glomerular filtration pressure to produce much urine. This mechanism has already been proposed as a possible factor in acute renal failure in human beings (Finckh, 1962) and the present results accord with this explanation although they do not actually show that it does occur.

SUMMARY

Anuria or oliguria were produced in rats by the intraperitoneal injection of solutions of glycerol. The structural renal damage following this procedure was slight and during the period of oliguria there was evidence of grossly reduced glomerular filtration despite near-normal systemic blood pressures and continued blood flow through all parts of the kidneys.

These results provide experimental support for the contention that acute renal failure in human beings may be principally the result of reduced glomerular filtration of intrarenal vasomotor origin.

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