EFFECT OF CYCLOPHOSPHAMIDE ON TUBULAR SECRETION IN THE KIDNEYS

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A single intravenous injection of cyclophosphamide into rabbits (60 and 80 mg/kg) and dogs (40 mg/kg) caused marked inhibition of excretion of cardiotrast (diodone) by the kidneys, whereas diuresis and glomerular filtration were reduced by a lesser degree. The effect of cyclophosphamide on renal secretion was also confirmed by experiments with accumulation of cardiotrast by slices of renal cortex.

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Changes in renal activity under the influence of alkylating compounds are described in the literature [1, 3, 5]. They are probably not associated with structural damage to the kidneys [4]. However, in none of these investigations was a study made of tubular secretion, an important function in the kidneys along with filtration and reabsorption.

In the investigation described below the effect of cyclophosphamide on tubular secretion was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 6 dogs weighing 10-18 kg with ureters exteriorized by the Pavlov – Tsitovich method and on intact rabbits weighing 2.5-3.5 kg. During the experiments the dogs were kept in Pavlov frames, while the rabbits were fixed in special hammocks.

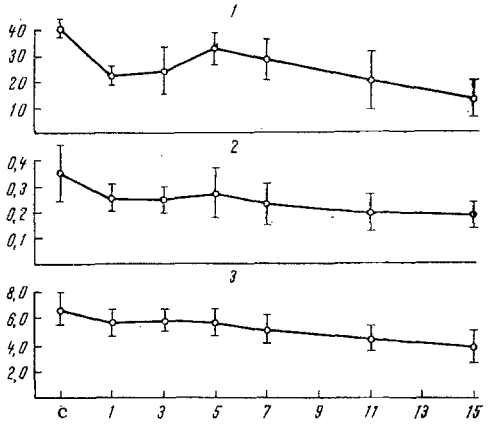

Fig. 1. Effect of cyclophosphamide (60 mg/kg) on kidney function in rabbits. Abscissa, day after injection of compound; ordinate: 1) excretion of cardiotrast in percent during first 30 min after injection; 2) diuresis (in ml/min); 3) glomerular filtration (in ml/min).

Cyclophosphamide was injected intravensously as single doses of 60 and 80 mg/kg into rabbits and 40 mg/kg into dogs. Altogether 109 experiments were performed on 33 animals.

The secretory power of the renal tubules was studied by determining the excretion of cardiotrast (diodone) in the urine after its intravenous injection in doses of 0.3-0.4 g/kg into dogs and 0.9-1.0 g/kg into rabbits. Previous investigations showed that these doses ensure maximal saturation of the tubular epithelium during the first 30 min after injection, and that within 2 h practically the whole of the cardiotrast had been excreted. Excretion was expressed as a percentage of the quantity injected. Cardiotrast was determined by the method of White and Rolfe as modified by Bak and co-workers [7]. Glomerular filtration was measured relative to endogenous creatinine.

In a separate series of experiments the effect of cyclophosphamide on the accumulation of cardiotrast by slices of renal cortex from rabbits was studied by the method of Cross and Taggart [8]. Two alternative forms of these experiments were used. In the first case cyclophosphamide was added directly to the incubation medium. The concentration of the com-

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TABLE 1. Effect of Cyclophosphamide (80 mg/kg) on Kidney Function in Rabbits

Index studied	Control	After injection of compound	P
Excretion of cardiotrast over 30 min	39,4±1,36	16,9±3,20	<0,001
2 h	80,8±3,01	60,4±5,27	<0,01
Filtration (in ml/min)	6,3±0,93	4,2±0,40	>0,05
Diuresis (in ml/min)	0,31±0,05	0,19±0,02	>0,05

TABLE 2. Effect of Cyclophosphamide (40 mg/kg) on Kidney Function in Dogs

Day after injection	Excretion of cardiotrast (in %)		Filtration (in ml/min/m^2)	Diuresis (in ml/min)
	over 30 min	over 2 h		
Control	34,8±1,36	83,9±2,29	36,8±4,44	0,58±0,16
1st	32,3±4,60	70,3±8,41	36,6±4,21	0,59±0,14
	P>0,5	P>0,05	P>0,5	P>0,5
3rd	34,4±5,13	73,7±10,65	32,4±5,52	0,65±0,19
	P>0,5	P>0,05	P>0,5	P>0,5
7th	14,7±5,63	40,7±11,67	29,5±7,71	0,34±0,09
	P<0,01	P<0,01	P>0,05	P>0,05

pound was chosen in accordance with recommendations for the selection of substances possessing cytotoxic action [6]. In these experiments the concentration was $1.5 \cdot 10^{-4}$ g/ml. In the second group of experiments the compound was injected intravenously into rabbits, which were sacrificed the next day and sections of the renal cortex cut. Kidneys taken from intact rabbits were used as the control. Absorption of oxygen was also determined in a Warburg apparatus in the experiments on slices.

EXPERIMENTAL RESULTS AND DISCUSSION

A single injection of cyclophosphamide into rabbits in a dose of 60 mg/kg sharply reduced the excretion of cardiotrast on the average by 46.2% compared with its initial level (Fig. 1) within 24 h after injection. During the next four days the excretion of cardiotrast increased, and on the 5th day it was approximately the same as in the control, although it was still significantly lower. Later still, the secretory function diminished progressively down to very low values. The animals died on the 20th-25th day. Filtration and diuresis in these experiments also fell slightly during the first day after administration of the compound, and thereafter they remained at about the same level until the 5th day of investigation, when a further considerable decrease began, accompanied by inhibition of secretory processes. Tubular secretion was disturbed more than filtration and diuresis, while reabsorption was virtually unaffected. The weight of the rabbits began to fall from the first day after administration of the compound, and by the 15th day it was 78.1±2.7% of its initial value.

With an increase in the dose of cyclophosphamide to 80 mg/kg (Table 1), the same changes in kidney function were observed as in the previous experiments, although they were more marked. The animals died sooner (during the first week). Excretion of cardiotrast in the first 30 min after injection was reduced by more than half, and this decrease was not compensated during observation for a period of 2 h.

The leukocyte count in the circulating blood (estimated in 1 mm^3) was reduced after injection of cyclophosphamide, and the decrease was particularly marked in the experiments when a large dose of the compound was given (from 13,000±1500 in the control to 5200±600 on the 3rd day after injection).

In the experiments with slices of rabbit kidney cortex, addition of cyclophosphamide to the incubation medium had virtually no effect on the ability of the slices to accumulate cardiotrast, or on the oxygen consumption of the kidney tissue. However, if the compound was injected intravenously into rabbits, the accumulation of cardiotrast by slices was substantially reduced (from 5.68±0.023 in the control to 3.09±0.23 in the experiment). Oxygen absorption also was significantly reduced (from 55.7±3.32 to 44.8±2.04 μliters/100 mg moist weight/h).

Following injection of cyclophosphamide into dogs in a dose of 40 mg/kg, slightly different results from those in rabbits were obtained (Table 2). The excretion of cardiotrast during the first few days after injection remained almost unchanged, and the decrease began only on the 7th day. The leukocyte count fell significantly on the 3rd day after administration of the compound. The life span of the dogs did not exceed two weeks. In the overall effect of cyclophosphamide, disturbances of secretory processes were more marked than changes in filtration and diuresis. This was perhaps because tubular secretion in an energy-dependent process [10, 11], and compounds with an alkylating action can disturb the transformation of energy in cells [2].

The pathogenesis of the inhibitory phase in the action of cyclophosphamide on kidney function is more complex. Possibly disturbance of synthesis of cell proteins and also extrarenal effects of cyclophosphamide may play a definite role in this case.

The experiments with slices of kidney cortex are interesting. The absence of changes in accumulation of cardiotrast by the slices after addition of cyclophosphamide directly to the incubation medium conforms to the view that cyclophosphamide must be activated in the body [6]. It is postulated that under the influence of phosphatases and phosphamidases, the compound is split up and an active derivative of chloroethylamine is liberated.

In fact, kidney slices from rabbits receiving the compound earlier accumulated cardiotrast with about half the intensity of kidney slices from intact animals.

The later disturbance of tubular secretion in dogs is probably a species characteristic. Pierce et al. [9] found that the dog's kidney differs in its sensitivity to certain antitumor compounds from the kidneys of other mammals.

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