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Research Article

THE PHARMACOLOGY OF CIRCULIN[1]

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In May, 1948, Murray and Tetrault reported a new antibiotic obtained from a culture of *B. circulans* which was more active against gram-negative than gram-positive bacteria (1). Further studies on identification of the organism, the assay method and the antibacterial properties were reported by Murray *et al.* (2) and the antibiotic was called circulin. Working in cooperation with the Purdue group, Colingsworth and Peterson (2) developed the fermentation and described a method for extracting and purifying the antibiotic. Although circulin resembled polymyxin D as a polypeptide type of antibiotic, it was differentiated from the latter on the basis of its antibacterial spectrum, chemical composition, and its reaction to crude trypsin. It was later found by Peterson and Reineke (3) that the inactivation of circulin was actually due to lipase. Preliminary acute toxicity tests in mice indicated that circulin was more toxic than polymyxin but less toxic than had been reported for aerosporin by Brownlee and Bushby (4).

In a current paper on the chemistry of circulin, Peterson and Reineke (3) have determined the amino acid components indicating a peptide structure containing D-leucine, L-threonine, L-α,γ-diaminobutyric acid and a fatty acid isomeric with pelargonic acid.

Since the earlier work (2) had indicated the effectiveness of circulin in protecting mice infected with *Salmonella typhosa* and *Klebsiella pneumoniae,* the pharmacologic and toxicologic studies here reported were undertaken as a requisite before proceeding to more extensive tests in infected animals and humans. The circulin used in this pharmacological study was prepared by the method previously described (2). Early work was done with preparation No. 8836 which assayed 2700 units per mg. or approximately 50 per cent pure. The balance of the work here reported was done with preparation 13-DHP-7 which assayed 6000 units per mg. and met the maximum standards of purity as described by Peterson and Reineke (3).

Acute Toxicity in Rats and Mice. Table I shows the approximate LD_{50} obtained in these species by the various routes of administration employed with comparable data reported in the literature for three other antibiotics. It is apparent from the LD_{50} obtained that the toxicity of these circulin preparations is almost directly proportional to their antibiotic content, and further, that the more purified material is several times as toxic as streptomycin, aureomycin or polymyxin. We were unable to obtain evidence that the toxicity of circulin could be reduced by increased purification.

TABLE I

Acute toxicity (LD_{50})

Species	Route	Circulin		Strep-tomycin	Poly-myxin	Aureo-mycin
		13-DHP-7 (6000 u./mg.)	8836 (2700 u./mg.)			
		mg./kg.	*mg./kg.*	*mg./kg.*	*mg./kg.*	*mg./kg.*
Mouse	I.V.	10	23	275*	—	134†
	S.C.	77	180	1200*	300†	>600‡
Rat	I.V.	—	20			
	I.M.	23	70			

* Molitor *et al.* (5)
† Stansly *et al.* (6)
‡ Harned *et al.* (7)

Chronic Toxicity in Guinea Pigs. Only the less pure circulin No. 8836 was available for this series. Eight guinea pigs, four of each sex, were injected subcutaneously with 10 mg. per kg. daily for 18 days. The only gross disturbances were a less rapid weight gain amounting to approximately 40 per cent of that observed for a comparable group of control animals and a moderately severe inflammatory reaction at the injection sites. Microscopic examination of heart, lung, liver, pancreas, spleen, lymph nodes, stomach, intestine, adrenal, kidney, spinal cord, and bone marrow revealed no morphological changes attributable to the antibiotic. The results of this preliminary

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series indicated that the toxicity of circulin was not cumulative and that it might be well tolerated under conditions of chronic administration which have been shown to bring out the nephrotoxicity of some of the polymyxins.

Chronic Toxicity in Mice. The pure material, circulin lot 13-DHP-7, was used for this series. Mice in groups of ten or more were injected daily for 14 days subcutaneously with graded doses ranging from the acute LD_{50} to approximately one-third of the LD_{50}. The maximum tolerated dose was found to be somewhat less than 33 mg. per kg., for only 80 per cent of the mice survived this dose. Survivals at the other dosage levels studied were 67 per cent at 41 mg. per kg., 60 per cent at 51 mg. per kg., 20 per cent at 64 mg. per kg., and 10 per cent at 80 mg. per kg. Local reactions to circulin were again apparent, for in this species sloughing of the skin occurred at the injection sites in almost all animals.

In view of the renal toxicity previously reported for polypeptides of the closely allied polymyxin series, special attention was given to the microscopical examination of kidney tissues. Morphological changes were limited to the presence of albumin in a few tubules at dosage levels greater than 51 mg. per kg., indicating an increase in glomerular permeability as a result of slight injury to the filtration mechanism. Only in kidneys obtained from mice surviving large single doses of circulin were any necrotic changes found. These studies confirm the guinea pig series in indicating a lack of specific toxicity to the kidney.

A Direct Comparison of Circulin and Polymyxin. This was done by chronic administration in rats of pure circulin lot 13-DHP-7 and a polymyxin D preparation No. 8899 prepared by D. R. Colingsworth and B. E. Leach in these laboratories from a culture of *Bacillus polymyxa,* Stamford strain supplied by Dr. P. G. Stansly. Polymyxin D lot No. 8899 assayed 1280 units per mg. and is estimated to be approximately two-thirds pure.

Five groups of ten or more 150-gram male rats were used. Each antibiotic was administered intramuscularly at 20 mg. per kg. and orally at 200 mg. per kg. once each day for seven consecutive days. These experiments were controlled by intramuscular injections of saline and administration of tap water by stomach tube to comparable numbers of untreated rats. A 20 per cent mortality occurred in the group treated with circulin parenterally and the surviving rats in this group lost an average of 1.5 grams per day. It should be noted, however, that this daily dose (20 mg. per kg.) was very close to the acute LD_{50} determined on a different group of rats. While no deaths occurred in the polymyxin group, these rats, too, lost an average of 1.5 grams per day.

No toxicity or histopathology was observed in either of the groups treated orally. The average weight gain per rat was the same as that for the controls; namely, 4 grams per day. Extensive histologic studies of skeletal muscle, kidney, adrenal, spleen, heart, liver, lung, stomach, intestine, and pancreas showed morphological changes limited to kidney, stomach and skeletal muscle of those animals receiving the antibiotics parenterally. The skeletal muscle injection sites of both groups showed marked degeneration, necrosis, and beginning regeneration. Stomach sections showed small foci of inflammatory cells associated with atrophy and necrosis of glands located at the base of the mucosa and also involving the submucosa. Kidney sections showed an occasional very slight degeneration of the convoluted tubules in the circulin-treated group. Mitotic figures were fairly prevalent in kidney sections from all groups indicating that the kidneys were practically not affected by the antibiotics.

Blood Levels and Urinary Excretion in Dogs. These studies were carried out using pure circulin lot 13-DHP-7 administered as a single dose by intravenous, intramuscular and oral routes. The method of assay employed was essentially that used by Stansly (8) for polymyxin in body fluids. Figure 1 shows the average blood levels obtained up to six hours after intravenous injections of 5 and 2.5 mg. of circulin per kg. into each of two dogs, and after intramuscular injections of 5 mg. per kg. into each of four dogs. At six hours blood levels were about the same following comparable doses by both routes of administration. Only 10 per cent of the circulin administered parenterally could be accounted for in the urine voided in the 48-hour period following injection.

Figure 2 shows the average blood levels obtained after oral administration of 10, 20 and 40 mg. of circulin per kg. to each of two dogs. These data indicate that circulin is absorbed from the gastro-

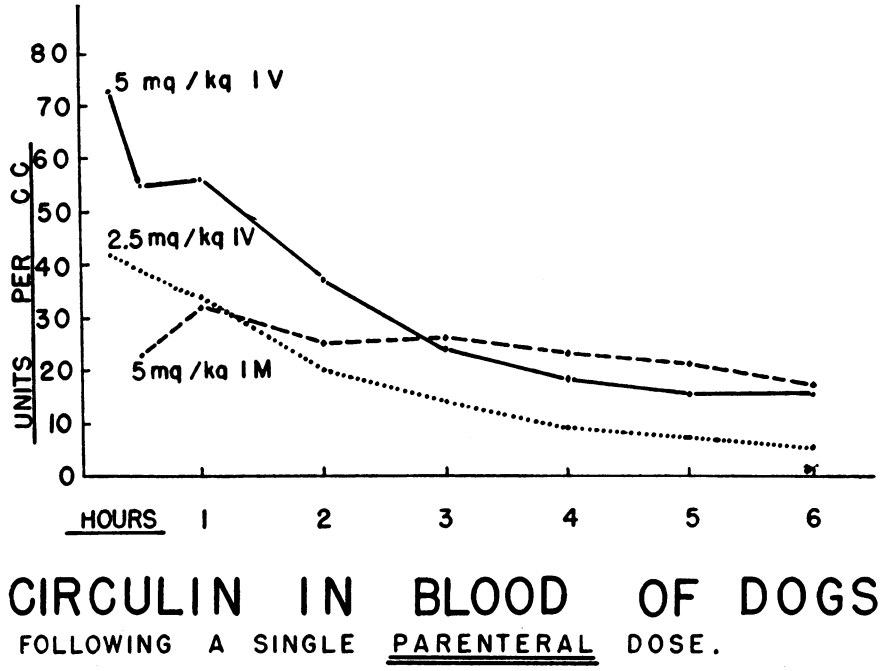

FIG. 1

intestinal tract but that from four to eight times as much is required by this route in order to obtain blood levels comparable to those obtained by parenteral administration. Only traces of circulin were present in the urine up to 80 hours at the lower dosage levels and at the 40 mg. per kg. dose not more than 6 per cent could be accounted for.

Blood Levels in Rats. Circulin lot 13-DHP-7

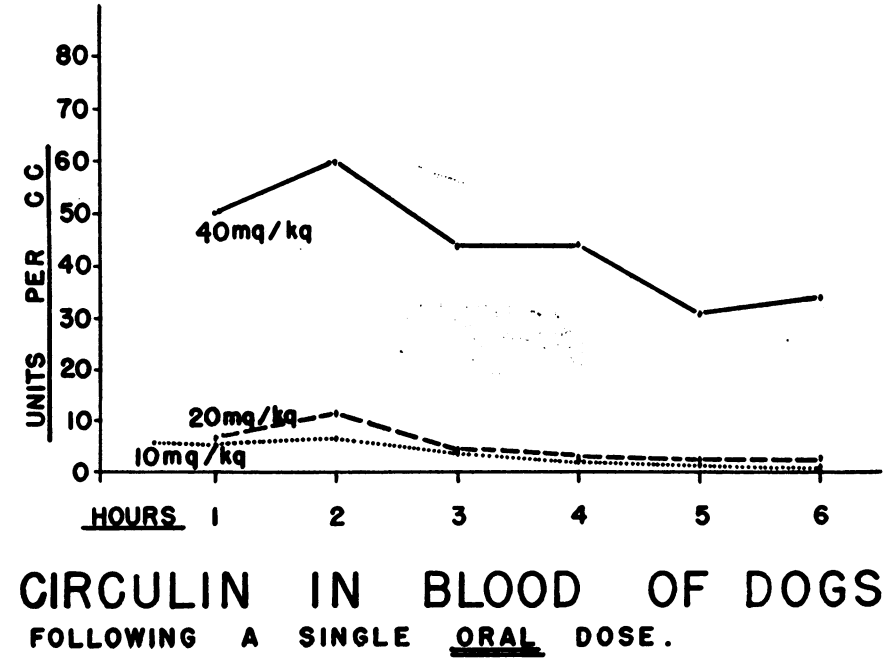

FIG. 2

was administered orally to 36 rats at dosages of 40, 100, and 200 mg. per kg. At intervals up to six hours after administration the rats in groups of three were bled by heart puncture and their pooled blood assayed for circulin. At all levels no activity was found. This indicates that intestinal absorption of circulin is poor in rats as contrasted to that in dogs and, no doubt, accounts for the very low chronic oral toxicity observed in rats.

Effects on Blood Pressure and Respiration. Blood pressure responses to intravenously injected circulin were determined using four dogs and three cats anesthetized with sodium phenobarbital. In general, doses of 1 to 3 mg. per kg. of lot 13-DHP-7 produced depressions amounting to 10–20 mm. Hg which lasted from 10 to 30 minutes. Occasionally a biphasic or pressor response alone was obtained. Doses between 5 and 10 mg. per kg. in unanesthetized dogs produced no ill effects except for a transient peripheral vasodilatation in one of two animals. Heart and respiratory rates were unaltered by doses of 1–3 mg. per kg. in the anesthetized animals.

SUMMARY

When compared with several other antibiotics active against gram-negative organisms, circulin was found to be more toxic. It did not, however, appear to exert its toxic action on the kidneys as judged by histopathological examination. A species difference was found to exist with respect to blood levels obtainable following oral administration of circulin to dogs and rats, indicating that in the former species it is well absorbed while in the latter it is either destroyed or poorly absorbed. The question of whether or not circulin will have an adequate margin of safety for therapeutic use in humans cannot be answered at this time but must await the results of clinical investigations now under way.

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