In the case of trypsin the anti-inflammatory action was found to be a function of proteolytic activity; it varied with the activity of partially and completely inactivated samples of this enzyme.

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Use of Artificial Kidney for Removal of Barbiturates in Dogs.* (21188)

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Barbiturates are responsible for one-third of all fatalities resulting from exposure to toxic substances and for 30 to 40% of all hospital admissions due to ingestion of poisons(1,2). Prompt recognition of barbiturate intoxication coupled with effective therapy can be life saving. With the advent of ultraviolet spectrophotometric methods(3-7), much of the difficulty previously involved in the laboratory determination of barbiturates in biological fluids has been eliminated. In 1 to 2 hours, one can not only determine the barbiturate level in biological material but also, in many cases, the specific barbiturate that was ingested. Thus, prompt chemical confirmation of barbiturate intoxication is now possible.

The removal of toxic substances, such as salicylates and bromides, by the use of hemodialysis has already been demonstrated(8, 9). There are relatively few available data to indicate the feasibility of this technic for the removal of barbiturates, although treatment of several patients has been reported (10). In a preliminary report from this laboratory hemodialysis was shown to be of limited value in pentobarbital intoxication in dogs(11).

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Methods. A series of dogs was given a known dose of a specific barbiturate intravenously or intraperitoneally. Using Goldbaum's technic(3) blood or plasma barbiturate levels were determined at 2-hour intervals, until 12 hours elapsed, then at 6-hour intervals until the animal recovered or died. Interspersed with the "control" dogs, another group of dogs, referred to hereafter as "dialyzed" dogs, were given the same dose of barbiturate and treated by hemodialysis 1 to 3 hours later. A Skeggs-Leonards(12) artificial kidney with a

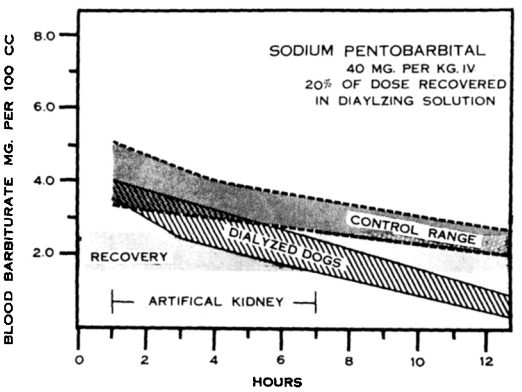

FIG. 1. Blood pentobarbital levels as a function of time. Control and dialyzed ranges represent all the values between maximum and minimum blood pentobarbital levels for all dogs in the respective groups. Recovery range represents spread of blood pentobarbital levels obtained when animals first began to move.

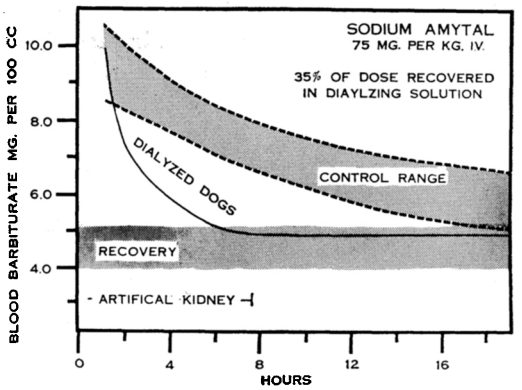

FIG. 2. Blood amobarbital levels as a function of time. Since all blood amobarbital levels for "dialyzed" dogs were so similar, the single line represents average value.

cellophane dialysis area of 20000 sq cm. operating with a blood flow of 200 ml/min. was used. The dialyzing solution had an electrolyte composition corresponding to the extracellular fluid of dogs(12). Generally, 200 to 300 litres of this dialyzing solution were used for each animal during the 4- to 8-hour dialysis period. The dog received no other therapy. In addition to the blood barbiturate levels, the amount of barbiturate in the total dialysate was also determined. The amount of barbiturate that can be removed by hemodialysis is a function of the "free" barbiturate, which is not bound to plasma proteins. Protein binding of various barbiturates was determined in some of the dogs by putting one litre of sterile dialyzing solution(12) in the dog's peritoneal cavity. The ratio of the barbiturate concentration in the peritoneal fluid and blood

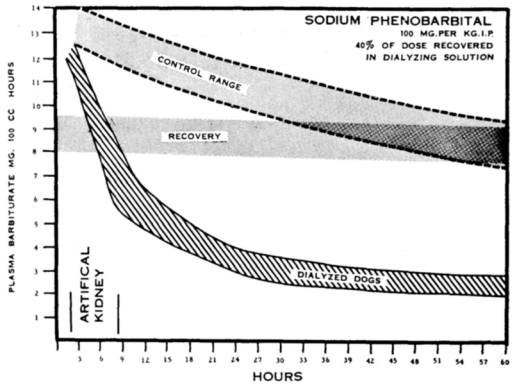

FIG. 3. Plasma phenobarbital levels as a function of time (100 mg/kg).

was determined at 4-hour intervals. After 12 hours, this ratio became constant and represented the percentage of "free" barbiturate.

Results. Sodium pentobarbital 40 mg/kg intravenously (Fig. 1). Three of the 8 "control" animals died as did 2 of 7 "dialyzed" dogs. Fig. 1 indicates that the "dialyzed" dogs began to move 5 hours sooner than the "controls". All the surviving animals completely recovered within approximately 22 hours after the original injection of the drug.

Amobarbital 75 mg/kg intravenously (Fig. 2). This amount of amobarbital administered intravenously over a 5- to 10-minute period is so close to the lethal dose that of the 15 dogs so injected, 7 died within 15 minutes. Of the 8 survivors, 5 were not treated. These "control" dogs were not asymptomatic until 36 to 48 hours had elapsed. One "control" dog died

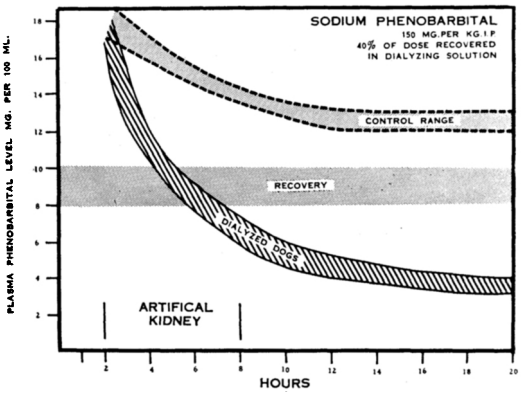

FIG. 4. Plasma phenobarbital levels as a function of time (150 mg/kg).

between 12 to 24 hours after the initial injection.

In the remaining 3 dogs, hemodialysis was started one hour after the initial injection of amobarbital and continued for 6 hours. By 19 hours, 2 dogs were completely recovered and the other recovered 28 hours after the initial injection.

Phenobarbital 100 mg/kg intraperitoneally (Fig. 3). Nine dogs were used in this series; 4 were controls and 5 were treated. All the dogs survived but the "control" dogs were not asymptomatic until 72 to 114 hours after the initial injection. In the 5 treated animals the plasma phenobarbital level dropped very sharply after hemodialysis was started. By

TABLE I. Renal and Artificial Kidney Barbiturate Clearances.

	Renal clearance (cc/min.)	Artificial kidney clearance (cc/min.)
Pentobarbital	0.7-1.7	15-26
Amobarbital	2.6-5.2	23-29
Phenobarbital	*	33-43

* Not analyzed. Barbital is not bound to plasma proteins. Since renal clearance of barbital in the dog is 6 cc/min.(14), one would expect the phenobarbital clearance to be somewhat less than this figure.

24 hours, 4 were standing and the last animal (heaviest) recovered completely by 72 hours.

Phenobarbital 150 mg/kg intraperitoneally (Fig. 4). Eleven dogs were used in this series, 5 were controls and 6 were treated. Four of the "control" dogs died at 6, 20, 70 and 72 hours respectively. Plasma phenobarbital levels at the time of death were all greater than 14 mg/100 ml. The surviving dog (13 kg) was completely anaesthesized for 3 days and could barely walk after 6 days. All the treated animals survived and began to move after 8 hours of hemodialysis. Four were asymptomatic after 24 to 30 hours, the other in 50 hours.

Protein binding. Experiments devised to determine the protein binding of the barbiturates in plasma indicate that 20 to 30% of the total phenobarbital is bound. In the case of pentobarbital, this value is 35% and for amobarbital it is 50 to 60%. Using another technic, Goldman(13), reported the protein binding of these barbiturates to be 20%, 37% and 37% respectively.

Clearance determinations. From the data obtained in these experiments, the renal barbiturate clearances, and the clearances of barbiturates by the artificial kidney, were calculated. No corrections were made for protein binding in the clearance data given in Table I.

Discussion. Kyle(10) used a Kolff-type artificial kidney to treat 3 human patients suffering from barbiturate intoxication. He concluded that "treatment of severe barbiturate poisoning by hemodialysis appears to be practical and effective."

There is no doubt that hemodialysis is a practical form of therapy. However, the effectiveness of this therapy is open to question. The data presented by Kyle indicate the artificial kidney will remove barbiturates from humans at the rate of 70 to 150 mg/hr. Our animal experiments corroborate this rate of removal. This is to be expected, since the area of the cellophane dialyzing surface is the same in the 2 types of artificial kidney. Thus, under optimum conditions, approximately 7 hours of hemodialysis would be required to remove one g of phenobarbital. The average amount of barbiturate ingested in poisoning incidents is approximately 5 g and in acute cases it may be as high as 10 g. Thus hemodialysis would only remove 10 to 20% of the original dose in a 7-hour period.

Another criterion for the efficacy of hemodialysis in barbiturate intoxication is the clinical response to this therapy. In the case of pentobarbital the "dialyzed" dogs began to move spontaneously approximately 4 hours sooner than the "controls." The rate of decline of blood pentobarbital levels was significantly greater in the "dialyzed" dogs. However, despite these benefits, all the animals took approximately the same time until they became asymptomatic and the relative death rate was the same for both series. It appears thus that hemodialysis is of questionable value in cases of pentobarbital intoxication.

In the case of amobarbital the blood amobarbital level declined rapidly while the animals were on the artificial kidney. Consequently the "dialyzed" dogs began to move spontaneously 10 hours before the "controls" and were completely recovered 17 to 20 hours sooner.

In the case of phenobarbital, there was no question about the beneficial effects of hemodialysis. At the lower dose level employed (100 mg/kg), "control" dogs did not begin to move spontaneously for at least 36 hours, while 4 of the 5 treated animals were able to walk, albeit unsteadily, in 24 hours. At a dose of 150 mg/kg, 4 of the 5 "control" dogs died, while all 6 of the treated animals recovered fully.

In view of these findings, hemodialysis therapy appears to be warranted in the treatment of severe intoxication due to a long-acting barbiturate, such as phenobarbital. The

expected beneficial result should be tempered by the knowledge that the rate of dialysis is slow and that the duration of treatment with the artificial kidney should be longer than the usual 6 hours used in treatment of renal insufficiency. This dialysis period could be shortened considerably if the existing artificial "kidneys" were enlarged to provide more dialyzing area. In the case of intoxication due to short-acting barbiturates, hemodialysis does not seem to be very helpful.

Hemodialysis may be life saving in those cases of acute barbiturate intoxication where the patient is comatose, areflexive with circulatory or respiratory difficulties and where the blood barbiturate level is elevated.

Summary. An artificial kidney of the Skegg-Leonards type was used successfully to lower blood barbiturate levels in dogs. Approximately 15 to 25% of the intravenously injected pentobarbital, 35% of intravenously injected amobarbital, and 40 to 70% of intraperitoneally injected phenobarbital were recovered in the respective dialysates. This therapy was life saving in the phenobarbital experiments, of some value in the amobarbital intoxication, but of questionable value in pentobarbital poisoning.

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Dehydrogenase Activities of Human Platelets.*† (21189)

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Although a good deal is known about the morphology and physical characteristics of platelets, little information is available concerning their biochemical properties. Platelets have been reported to contain certain enzymes such as esterases, acid phosphatase and β-glucuronidase(1), but, whereas according to some workers they have little or no intrinsic metabolism(2), others have found that they exhibit a very rapid metabolic activity(3). These activities, however, have usually been observed in freshly prepared platelets and always disappear completely following storage of the specimens for a period of 2 to 3 days (3). There is some indication that the methods used for the collection of blood and for the preparation and storage of platelets may exert an effect on the extent of their observable biochemical properties(4). It is

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