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The *in vitro* adsorption of drugs from horse serum onto carbon coated with an acrylic hydrogel

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In vitro studies have shown that uncoated carbon and carbon coated with an acrylic hydrogel are capable of adsorbing drugs from horse serum at 37°. Increase in the coating weight from 2 to 4% decreased the rate of adsorption but not the total capacity. *In vivo* data supports the concept of carbon haemoperfusion for use in the treatment of drug overdose.

The concept of carbon haemoperfusion for the treatment of severe drug overdose was first introduced by Yatzidis (1964). The wide adsorptive capability of activated charcoal would seem to make it an ideal choice as an adsorbent for the extracorporeal removal of blood borne toxins. It has been shown by Yatzidis (1964), Dunea & Kolff (1965), Hagstam, Larsson & Thysell (1966), and Chang (1969) that unless the carbon granules are covered by a biocompatible coating then excessive blood cell damage and carbon micro-embolization will occur.

In 1973 there were 2914 deaths in the United Kingdom from drug overdose (Editorial, *Pharm. J.*, 1975). A large proportion of these deaths were due to the barbiturates and the removal of these drugs from the blood by carbon haemoperfusion may lead to a reduction in mortality (Vale, Rees & others, 1975). Many drugs taken as an overdose are present at high plasma concentrations which might allow removal of clinically significant quantities by such a technique. However, some drugs are present at low concentrations and carbon haemoperfusion may be required for long periods to remove a clinically significant quantity.

I have screened a wide range of drugs for their adsorption onto uncoated and polymer coated carbon.

MATERIALS AND METHODS

Materials

Drug adsorption studies were from horse serum (Burroughs Welcome HS5), except that for ethchlorvynol which was adsorbed from fresh rabbit plasma.

The carbon granules, type 610 mesh size 6–10, were obtained from Sutcliffe Speakman, Leigh, Lancashire, England, and washed to remove 'fines' and chemical contamination. A coating of a biocompatible acrylic hydrogel was applied to give, by weight, a 2 or 4% coating.

Drugs used were: pentobarbitone, phenobarbitone, and paracetamol (Sigma); glutethimide (CIBA); salicylate and quinine (Hopkins and Williams); paraquat (ICI); imipramine (Geigy); chlorpromazine (May & Baker); chlordiazepoxide and diazepam (Roche); diphenhydramine and phenytoin (Parke-Davis); digoxin (Burroughs Wellcome); ethchlorvynol (Pfizer); orphenadrine and meprobamate (Poisons Unit,

Guy's Hospital, London). Tritium labelled digoxin was obtained from the Radio-Chemical Centre, Amersham.

Analytical reagents were prepared from A.R. grade chemicals obtained from standard laboratory suppliers.

Methods

The adsorption studies were made in 1 oz parallel-sided glass bottles, submerged in water thermostatically controlled at 37°. The bottles were shaken at a rate and stroke such that all the carbon granules moved freely within the test solution, but without damaging the polymer coating. The equivalent of 250 mg of carbon per test bottle was equilibrated with 5 ml horse serum, containing no drug, for 16 h at 4°. Horse serum (5 ml) containing twice the desired concentration of drug was then added to the test bottle, thus obtaining 10 ml of horse serum containing the required drug concentration.

The test bottles were sampled at ½, 1, 3, and 6 h from the time of adding the drug solutions. Separate bottles were used for each sampling if the assay required greater than 0·2 ml of serum.

Some drugs—methaqualone, glutethimide, meprobamate and diazepam—were first solubilized in a few drops of ethanol. On the addition of horse serum to these ethanolic solutions no precipitation of the drug or protein was apparent.

To obtain a suitable test solution containing ethchlorvynol (a light brown oil, not readily soluble in horse serum), a New Zealand rabbit (3 kg) was given by mouth about 2 g of drug in 5 ml of arachis oil. Two hours later the carotid artery was cannulated and after heparinization the animal was exsanguinated, the whole blood centrifuged and the plasma used for the adsorption study. Saline, which was used to 'wet' the test carbon before the adsorption, was replaced by 10 ml of the rabbit plasma.

Uncoated and 2% coated carbon was used with all the drugs examined and in addition 4% coated carbon was used with pentobarbitone, phenobarbitone, methaqualone, glutethimide, paracetamol, salicylate, imipramine and paraquat.

The analytical techniques used and the starting concentrations, which are associated with severe drug overdose, are listed in Table 1. An arbitrary value of 10 mg % was used for paraquat.

RESULTS

All 18 compounds were adsorbed by the carbon granules. The polymer coating, however, did present a barrier to the rate of adsorption but not the total capacity.

A typical result is shown in Fig. 1 for the adsorption of pentobarbitone. The percentage of drug remaining at ½, 1, 3 and 6 h for the 2% polymer coated granules is shown in Table 2.

At the end of the study the serum from bottles which contained uncoated carbon was contaminated by a large quantity of carbon fines produced by attrition of the carbon granules. The serum which had been in contact with the polymer coated carbon showed no visual evidence of fines.

DISCUSSION

All 18 drugs are adsorbed by uncoated, 2 and 4% coated carbon granules. The adsorption of pentobarbitone (Fig. 1) is typical, the adsorption rate being fastest

Table 1. *Drug assay techniques on starting concentrations.*

Drug	Assay	Reference	Concentration mg per 100 ml
Pentobarbitone	Gas liquid chromatography	Flanagan & Withers, 1972	10*
Phenobarbitone	,,	,,	20
Glutethimide	,,	,,	10
Methaqualone	,,	,,	10
Salicylate	Colorimetric	Trinder, 1954	50
Paracetamol	Ultraviolet absorption after ether extraction		100
Paraquat	Colorimetric	Calderbank & Yuen, 1965	10
Imipramine	Fluorescence spectroscopy after solvent extraction	Dingell, Sulser & Gillette, 1964	3
Diazepam	Ultraviolet absorption after ether extraction		1
Chlorpromazine	Ultraviolet absorption after chloroform extraction		1
Chlordiazepoxide	Ultraviolet absorption after ether extraction		0·5
Diphenhydramine	Fluorescence spectroscopy after solvent extraction	Martin, 1967	1
Digoxin	Radio-tracer		0·5 μg
Orphenadrine	Dye complexation after heptane extraction	Hespe, de Roos & Nauta, 1965	8·5
Quinine	Fluorescence after protein precipitation		2·5
Ethchlorvynol	Colorimetric	Algeri, Katsas & Luongo, 1962; Clarke, 1969	8·9
Meprobamate	Colorimetric	Hoffman & Ludwig, 1959; Felby, 1970	20
Phenytoin	Ultraviolet absorption after solvent extraction	Clarke, 1969	10

* Used as a model for buto-, seco- and quinalbarbitone.

for uncoated carbon and slowest for the 4% coated material. The adsorption varied between 9·1 and 55% after 1 h and between 0·2 and 26·4% after 3 h.

The first group of drugs in Table 2 represents those most frequently implicated in serious drug overdosage. For these drugs, a serious toxic dose may be several grams, giving plasma concentrations well in excess of 5 mg per 100 ml. In contrast, plasma concentrations in patients overdosed with drugs in the third group of Table 2 are not likely to exceed 1 mg per 100 ml, and are frequently much lower.

A high concentration of circulating drug might favour the use of haemodialysis or haemoperfusion in the treatment of drug overdose. Many poisons, although dialysable, have a low clearance across haemodialysis membranes (Bloomer, 1965; Hudson, Dennis & Hobbs, 1969; Rosenbaum, Winsten & others, 1970). However, drug clearances by haemoperfusion over various adsorbents, some of which were coated, have been shown to be superior to clearances obtained by conventional haemodialysis (De Myttenaere, Maher & Schreiner, 1967; Rosenbaum, 1971; Willson, Winch & others, 1973; Chang, Coffey & others, 1973; Widdop, 1975).

The drugs in the second group of Table 2 are not commonly encountered and represented only 1% of total deaths due to drug overdose in 1973 for the United Kingdom (Editorial, *Pharm. J.*, 1975).

The third group of compounds in Table 2 are infrequently the cause of death and are included to give a more complete picture of adsorptive capability of the coated

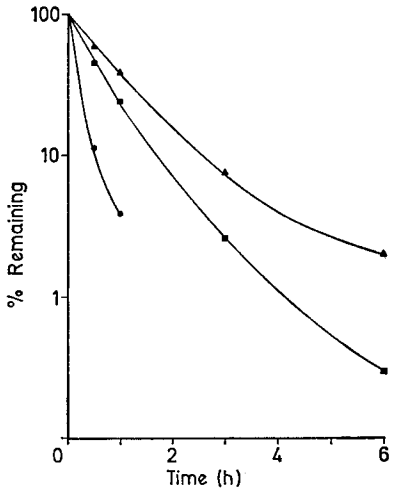

FIG. 1. The adsorption of pentobarbitone onto carbon granules. ●——● uncoated carbon; ■——■ 2% polymer coated carbon; ▲——▲ 4% polymer coated carbon.

carbon. It should be emphasized that the very low plasma concentration of drug found in patients overdosed with the compounds in the third group is likely to preclude the use of haemoperfusion, haemodialysis or peritoneal dialysis, in treatment programs aimed at lowering blood concentrations.

Although the adsorption of these drugs is most efficient with uncoated carbon, its use in clinical haemoperfusion is contra-indicated because of the release of micro-embolic fines and excessive blood cell damage (Dunea & Kolff, 1965; Hagstam & others, 1966). Coating the granules with an integral coating of a biocompatible acrylic hydrogel reduces the rate of absorption, but not capacity.

Table 2. *Percentage of drug remaining at* ½, 1, 3 *and* 6 *h for* 2% *polymer coated carbon granules.*

Drug	% Remaining ½ h	1 h	3 h	6 h
Group 1				
Pentobarbitone	45·8	23·9	3·3	0·3
Phenobarbitone	64·3	47·0	12·5	4·2
Glutethimide	54·1	27·6	2·7	0·1
Methaqualone	45·8	16·1	1·1	0·1
Salicylate	26·6	9·1	0·7	n.d.
Paracetamol	22·2	10·0	2·4	1·1
Group 2				
Orphenadrine	41·3	29·5	3·6	0·6
Quinine	44·8	18·9	3·4	0·4
Ethchlorvynol	64·0	55·0	26·4	9·0
Meprobamate	30·2	9·2	n.d.	n.d.
Phenytoin	43·5	19·6	0·2	n.d.
Group 3				
Paraquat	59·1	38·4	7·0	1·0
Imipramine	63·0	30·5	6·2	1·5
Chlorpromazine	40·9	28·0	13·4	11·2
Chlordiazepoxide	60·0	31·2	14·8	2·8
Diazepam	54·2	21·7	6·4	2·8
Diphenhydramine ..	26·6	16·4	8·0	5·0
Digoxin	69·2	53·2	19·7	3·9

n.d. None detected.

Although all 18 drugs are adsorbed from horse serum onto the polymer coated carbon, the difference in protein binding between horse serum and human blood and the tissue binding properties of these drugs may modify the rate of removal of drug by carbon haemoperfusion.

Optimization of efficacy and safety of carbon haemoperfusion has been established by extensive *in vitro* and *in vivo* studies on columns containing polymer coated carbons (Fennimore & Munro, 1975; Langley, Fennimore & others, 1975; Watson, Kolthammer & others, 1975).

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