A Study of the Inhalation of Pentachlorophenol by Rats Part V. A Protein Binding Study of Pentachlorophenol

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Previous studies of pentachlorophenol (PCP) exposures (CASARETT, et al., 1969a)(HOBEN, et al., 1975a) suggested to us that protein binding may be a contributory factor to the observed differences in PCP retention in the blood between man and the rat. Hence this study defines some of the properties of protein binding with PCP and compares PCP binding of human plasma and albumin with rat plasma and albumin.

MATERIALS AND METHODS

Equipment

MultiMagnet Stir, Labline Instruments Inc.
pH meter, Bendix
50 mm dialysis tubing, prepared according to the method of McMenamy (1968).
dialysis tubing clips, Spectrum Medical Industries
1" magnetic stir bars
250 ml beakers

Reagents and Solutions

Bovine albumin, 35% sterile solution, Sigma Chemical Co.
0.1% in buffer solution except in study where BSA was variable.
Human albumin cryst., Miles Laboratories, Inc.
0.1% in buffer solution.
Rat albumin cryst., Miles Laboratories, Inc.
0.1% in buffer solution.
Tromethamine buffer, Nutritional Biochemical Corporation
Adjusted to pH 7.5 with 1N HCl.
0.01 N solution used in all studies except where buffer was variable and except in pH study.
Barbiturate-Acetate buffer for pH study
0.712 g Na Acetate-$3H_2O$ + 14.714 g Na Barbiturate dissolved in 500 ml water.
50 ml of this stock solution was treated with X ml 1N HCl and 180-x ml of water to make solutions of various pH. (MICHAELIS, 1931)

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Pentachlorophenol (PCP), Eastman Kodak.
2% stock solution.
Water; distilled, deionized, hexane extracted, CO_2 free.
Rat plasma
Rat blood was drawn from the vena-cava of an etherized animal using 23 gauge needles on heparinized syringes. Blood was centrifuged at 4000 rpm for 15 minutes. 0.1% protein solution of plasma was prepared by diluting. 1.67 ml of plasma containing 6.0% protein to 100 ml with buffer.
Human plasma
Brachial artery blood was drawn into heparinized vacutainers. Centrifuged as above.
0.1% protein solution was prepared by diluting 1.47 ml of plasma containg 6.8% protein to 100 ml with buffer.

Method

Due the availability and cost, bovine serum albumin (BSA) was used in characterizing PCP binding to protein. Ten milliliters of a protein solution was pipetted into prepared dialysis bags, secured by a knot at the bottom and by a dialysis tube clip at the top. Care was taken to avoid contamination of the bags by touch. The bags were placed into 250 ml beakers containing 2000 µg PCP in 190 ml of 0.01N tris buffer at pH 7.35. (These were the standard values; however, depending on the particular test one parameter was varied. The varied values are given with each figure in the results.) A 1" magnetic stirring bar was added to each beaker which was placed on the multi-positional stir plate, sealed with parafilm and stirred for 22 hours. After equilibrium was attained, 1 ml samples from each bag and each buffer vessel were analyzed for PCP by GLC (HOBEN, et al. 1975b).

RESULTS AND DISCUSSION

The amount of PCP bound at varying time intervals indicated that binding equilibrium occurred at 12 hours (Fig. 1). Twenty-two hours was chosen simply as a working convenience.

Varying the PCP concentration within the reported range of occupationally exposed workers (CASARETT, et al. 1969b) resulted in a linear relationship to the amount of PCP bound per mg BSA (Fig. 2). Table I gives the experimentally derived values for the equation, $1/r = K/M\ (1/A + 1/M)$ which expresses the relationship free PCP (A), M, the number of bound species per protein molecule, the constant K, and r, the ratio of the moles of bound PCP to the total moles of protein (KLOTZ, 1946). From these data, M, the number of binding sites, was determined to be 13.

Increasing the protein concentration (Fig. 3) results in a decrease in the amount of PCP bound per mg protein. This suggests a rather non-specific binding being interfered with by protein-protein interaction.

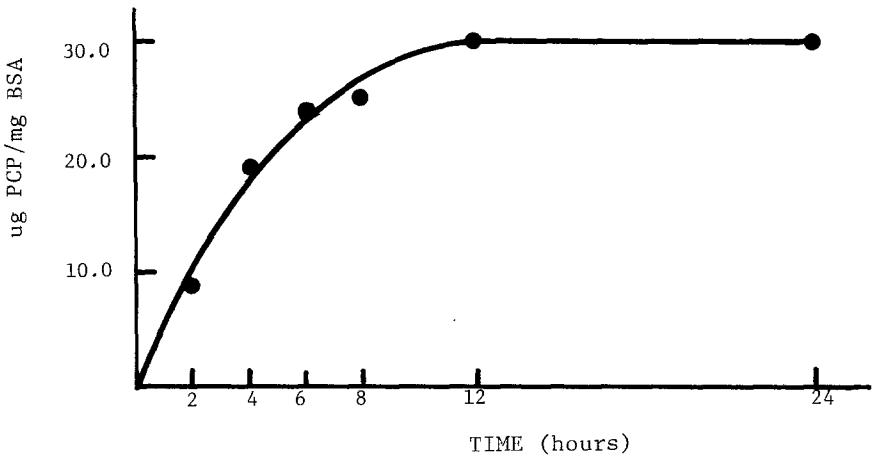

Fig. 1. Amount of PCP bound at different time intervals

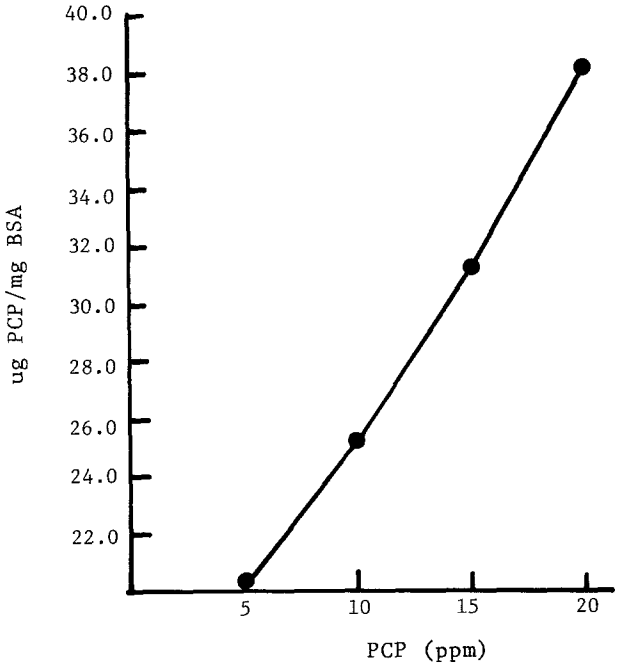

Fig. 2. Effect of PCP concentration on amount bound

TABLE I. Dialysis Equilibrium between BSA and Variable Concentrations of PCP

PCP Concentr.	µM PCP bound per per mg BSA	µM PCP bound µM BSA* = r	$\frac{1}{r}$	Free PCP µM	$\frac{1}{r}$
5 ppm	0.0759	5.24	0.1909	0.300	3.33
10 ppm	0.0944	6.512	0.1535	0.6575	1.5209
15 ppm	0.1248	8.614	0.1161	1.003	0.997
20 ppm	0.1432	9.885	0.1012	1.360	0.7353

* Mol. wt. BSA - 69,000 1 mg BSA - 1.449 µM

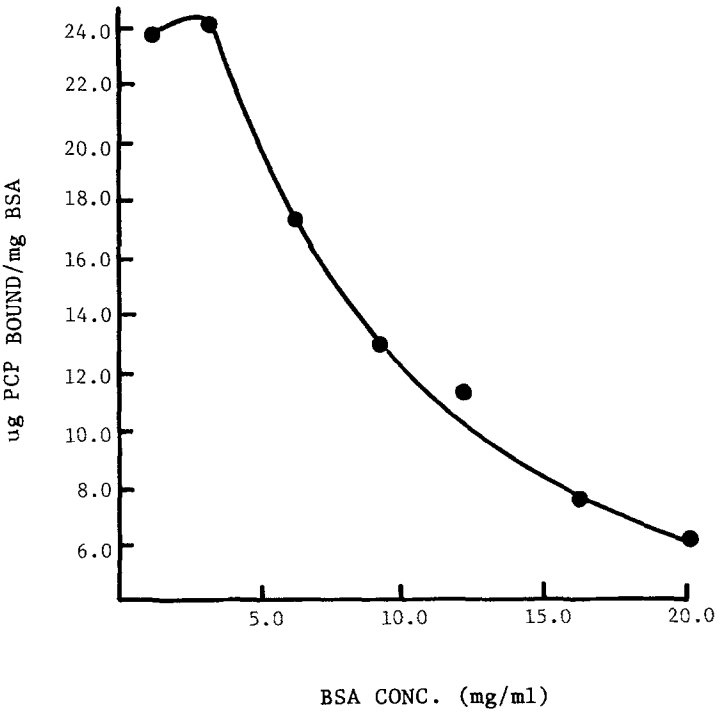

Fig. 3. Effect of protein concentration on PCP binding per mg BSA

There was an inverse relationship between PCP binding and both pH (Fig. 4) and ionic strength (Fig. 5). Thus at lower pH where PCP

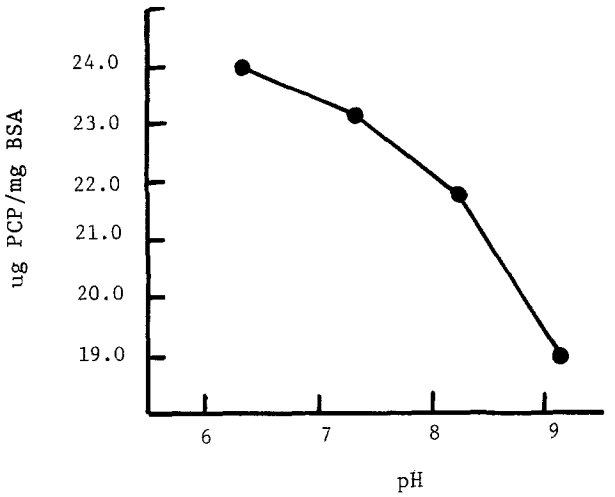

Fig. 4. Effect of pH on PCP binding

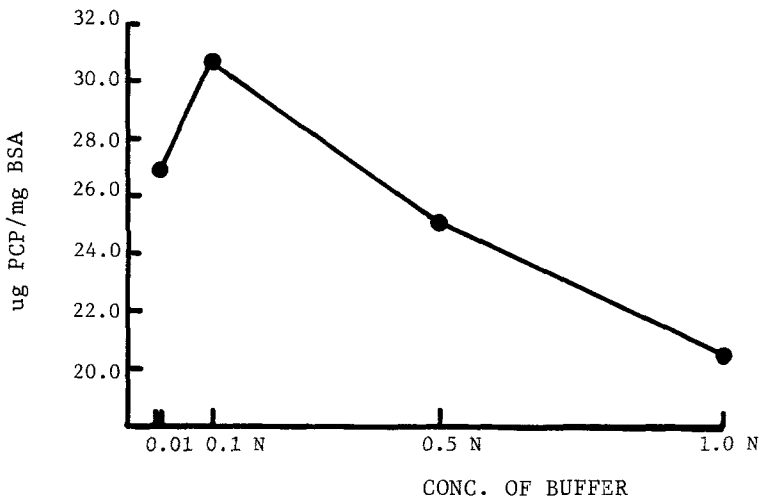

Fig. 5. Effect of buffer concentration on PCP binding

solubility is the least, PCP binding to protein is maximal. Binding studies below pH 6 (not shown) indicated a further increase in binding in most cases, but were not reproducible. One interpretation is that the unionized PCP may be responsible for binding since the binding increases as the pH approaches the pKa, 5.8 of PCP; however, the data can also be interpreted to support the PCP anion as the binding species since increasing the pH would increase the net negative charge of the protein resulting in a repulsion of the anion and consequent decrease in binding.

Figure 6 demonstrates the relationship between temperature and PCP binding. A decrease in temperature results in a decrease in available PCP. It is interesting to note that KEPLINGER, et al. (1959) demonstrated that there is also a relationship between the temperature of the environment and the toxicity of PCP. In this experiment the authors suggest that lowering the external temperature helps to decrease the PCP induced accelerated metabolic rates thereby decreasing this compound's toxicity. Whether these two temperature phenomena are directly related or not, they are both resulting in a decrease in toxicity.

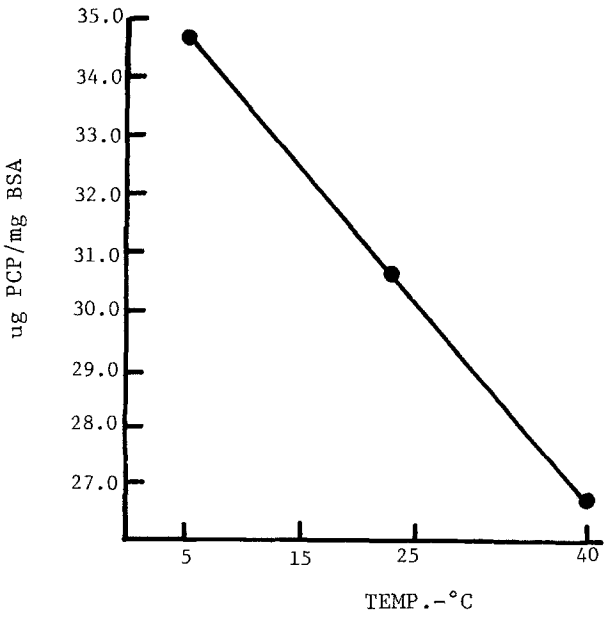

Fig. 6. Effect of temperature on PCP binding

Table II compares the binding properties of BSA, human albumin (HSA), rat albumin (RSA), human plasma and rat plasma.

TABLE II. The Binding of PCP to Some Plasmas and Albumin Fractions

Type of 0.1% Protein Solution	µg PCP bound	% PCP bound	µM PCP bound	Mol PCP Mol Albumin
Bovine Albumin[a]	29.3	14.6	1.10×10^{-1}	0.76
Human Albumin[a]	25.0	12.2	0.94×10^{-1}	0.65
Rat Albumin[a]	27.8	13.4	1.05×10^{-1}	0.72
Human Plasma[b]	25.5	17.8	0.96×10^{-1}	1.32
Rat Plasma[b]	16.5	8.5	0.62×10^{-1}	0.86

a - Mol Wt. 69,000 b - approx. 50% albumin

Examining just the albumin fractions there would be no reason to suspect that protein binding would give rise to the differences in blood retention observed between exposed humans and rats, as mentioned previously. The amount of PCP/albumin is very nearly the same for the albumin of these three species: cow, man, and rat. However, this ratio is significantly altered for human plasma suggesting that other factors in the plasma besides albumin play a role in the retention of this compound. These factors have yet to be determined. However, these data do support the idea that differences in binding between human plasma and rat plasma contribute to the longer retention and higher blood values observed in the human data (CASARETT, et al. 1969b) as compared to the rat data (HOBEN, et al. 1975a) (CASARETT, et al. 1969a).

SUMMARY

This study examined the effects on PCP binding to BSA by varying the temperature, pH ionic strength, PCP concentration and BSA concentration. It also compared the albumin binding of PCP to the plasma binding of PCP for the rat and human.

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