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Effect of hexane and carbon tetrachloride on microsomal cytochrome (P450)

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THIS communication reports that carbon tetrachloride, (CCl_4) chloroform, halothane, and hexane all produce a spectral change in microsomes, similar to that produced by hexobarbital. Remmer *et al.*[1] and Imai and Sato[2] have shown that when substrates are added to microsomal preparations, difference spectra can arise. Two types of difference spectrum are found, with hexobarbital there is a decrease of optical density around 420 mμ and an increase around 390 mμ, with aniline there is an increase around 430 mμ and decrease around 390 mμ. These changes are thought to be due to interaction between the haem of cytochrome P450 and the enzyme substrate complex.

MATERIALS AND METHODS

Male Wistar rats were fed a cube diet, and given a single injection of dicophane (DDT) 100 mg/kg s.c. 1–4 weeks before use, to stimulate synthesis of microsomal hydroxylating enzymes.[3, 4] The rats were starved overnight, killed by exsanguination, and the microsome fraction prepared by the method of Kato and Gillette.[5] The microsome fraction was diluted to 4 mg protein per ml in 0·1 M KCl with 50 mM Na–PO_4 buffer, pH 7·4.

Volatile solvents were added in Warburg flasks. Three ml of the dilute microsomal suspension was placed in the main compartment of the flask which was kept cold in a tray of ice. The solvent was added to the side arm, which was kept at room temperature, the flask was closed and the tray rocked gently for 20 min. Control preparations of microsomal suspensions differed only in the absence of solvent or substrate. If the control was not diluted, stored and handled in exactly the same way as the suspension containing solvent, a spurious difference spectrum was produced.

Difference spectra were recorded on a Unicam SP 800 double beam recording spectrophotometer at 20°, reading the microsomes plus solvent against the control microsomes. Solvents were added until a saturating concentration was reached, where further addition brought no additional spectral change. Spectral changes with aniline, hexobarbital and pyramidon were similarly investigated. These substrates were added in solution. Protein was estimated by the method of Lowry *et al.*[6]

RESULTS

(1) Fig. 1 shows that carbon tetrachloride (CCl_4) and hexane give typical difference spectra of the hexobarbital type. The same changes were produced whether CCl_4 was added in solution or through the gas phase.

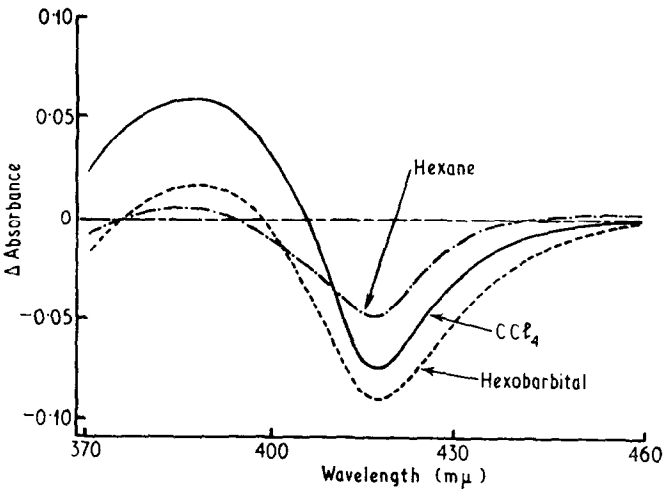

FIG. 1. Difference spectra of rat liver microsomes. Sample and control cuvettes contained 4 mg microsomal protein per ml. Hexobarbital, 3 mM, CCl_4 3 mM, and hexane, 20 mM were added to sample microsomes.

(2) Table 1 shows the magnitude and position of the maximal spectral changes produced by addition of some solvents and substrates to a microsomal preparation.

TABLE 1. MAXIMAL DIFFERENCE SPECTRA PRODUCED BY ADDITION OF SOLVENTS AND SUBSTRATES TO MICROSOMES

Substrate and concentration	Peak mμ	Trough mμ	Δ Absorbance peak to trough
Hexane 20 mM	388	417	0·21
Chloroform 4 mM	388	416	0·10
Halothane 3 mM	386	417	0·10
Acetone 20 mM	—	—	Nil
CCl_4 3 mM	388	417	0·20
Aniline 20 mM	429	396	0·24
CCl_4 3 mM + aniline 20 mM	429	396	0·24
Hexobarbital 3 mM	386	419	0·13
Pyramidon 20 mM	390	415	0·04
Hexobarbital 3 mM + pyramidon 20 mM	386	415	0·08

Microsomes were prepared and substrates and solvents added as described in the section on Methods. The concentrations of solvents are calculated on the assumption that all the solvent added has gone into the aqueous phase. Controls with microsomes plus substrate in both cuvettes or in neither, gave no apparent difference spectrum. The O.D. of control samples containing microsomes with no solvent or substrate rose from 0·6 at 700 mμ to 2·0 at 450 mμ.

None of the solvents or substrates had a detectable optical absorption in the 370–500 mμ range, in the concentrations used.

The maximal change produced by chloroform is only one half that produced by carbon tetrachloride although the wavelength and direction of the change is the same, and chloroform is more rapidly metabolized than carbon tetrachloride.[7]

(3) When aniline was added to a preparation already containing CCl_4, the CCl_4-difference spectrum disappeared, and the spectrum observed became indistinguishable from the aniline spectrum.

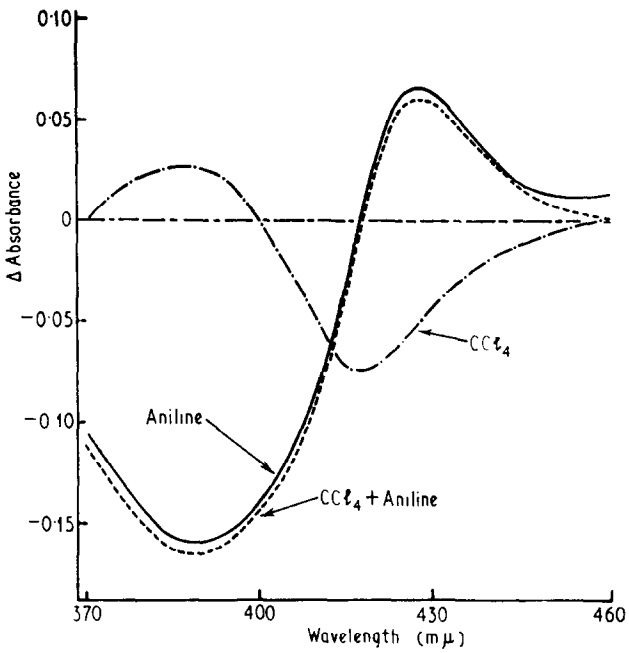

FIG. 2. Suppression of CCl_4 difference spectrum by aniline. Sample and control cuvettes contained 4 mg microsomal protein per ml. CCl_4 3 mM was added first, and followed by aniline 20 mM in the combined sample. Control cuvettes contained no substrate.

(4) Hexobarbital produced a spectral change of O.D. 0·13 but pyramidon at its saturating concentration produced an O.D. change of only 0·04.

When the suspension containing hexobarbital had pyramidon added, the total optical change decreased from 0·13 to 0·08.

DISCUSSION

The addition of these solvents to the list of substances that produce spectral change support the view that the microsomal hydroxylating system has low substrate specificity.

The reversal of the CCl_4 spectrum by aniline shows that the solvents do not act by denaturing the enzyme. CCl_4 is known to be a substrate for the microsomal hydroxylation system.[7]

The fact that different substrates produce different degrees of spectral change requires explanation. Either some substrates produce greater spectral change per occupied site than others, or there are different types of P450-enzyme complexes and each substrate can occupy only a limited range of these different types.

The abolition of the spectral change due to hexobarbital on adding excess pyramidon is compatible only with the first view. The same binding site must cause a greater optical effect when occupied by hexobarbital than when occupied by pyramidon. This finding, and the complete reversal of the CCl_4 spectrum by aniline argue in favour of all the substrates acting on the same population of P450 haemo

protein molecules. This could be either because the binding sites are non specific or else because the several specific binding sites converge onto the same population of P450 molecules.

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Fate of intravenously injected iron compounds: ferric–fructose complex, iron–EDTA, ferric hydroxide and iron–albumin labeled with ^{59}Fe

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Several authors have already studied the mechanism of iron intestinal absorption, especially concerning the effect of sugars and polyols in that absorption[1, 2] and the chelation by EDTA.[3] In order to obtain a more detailed view of the iron fate, a comparative study of the body distribution of four different radioiron labeled compounds has been performed.

The ^{59}Fe-labeled compounds were prepared as follows

(a) *^{59}Fe-ferric hydroxide.* To a 0·01 M $FeCl_3$ solution containing the ^{59}Fe activity as ferric chloride, 0·01 N NaOH was added to bring the pH to 10. Immediately 1 ml of 1% dextrose and 0·1 ml of 6% gelatin solution were added. The colloidal ferric hydroxide solution formed was heated for 10 min in a boiling water-bath and after cooling it down to room temperature it was dialyzed against distilled water until no basic reaction appears in the water.

(b) *^{59}Fe-ferric-fructose complex.* To 0·1 ml of 0·1 M $FeCl_3$ (with the ^{59}Fe activity incorporated) 0·1 ml of concentrated HCl and 2 ml of 10% fructose were added. The mixture was boiled for a few