THIN-LAYER CHROMATOGRAPHY OF RAT BILE AND URINE FOLLOWING INTRAVENOUS ADMINISTRATION OF PESTICIDAL SYNERGISTS

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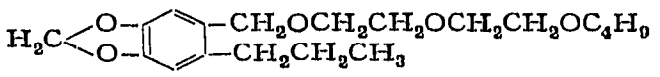
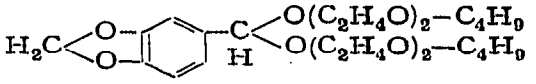
INTRODUCTION

A number of compounds possessing the methylenedioxyphenyl structure are of importance as insecticide synergists. For example, piperonyl butoxide (I) has been shown to be a potent synergist for pyrethrum[1,2], carbamate[3,4], chlorinated hydrocarbon insecticides[5,6], as well as an inhibitor of the epoxidation of aldrin to dieldrin[7] and dehydrohalogenation of DDT[8] and hexachlorocyclohexane. Tropital (II) has recently been reported as a new synergist for pyrethrins[9].



(I)



(II)

The purpose of this investigation was to follow by thin-layer chromatographic techniques the elimination of the synergists and their metabolites in rat bile and urine, resulting from single intravenous administrations of certain piperonyl compounds. Piperonyl butoxide* and tropital** as well as a probable degradation product, namely butyl carbitol, were used in these experiments.

EXPERIMENTAL

Preparation of the plates

Thin-layer plates (8 × 8 in.) 250 μ thick were prepared in the usual manner by mixing a slurry of Silica Gel DF-5*** and water in a ratio of 30 g of adsorbent to 72 ml water. They were air-dried for several hours, then oven-dried for one-half hour at 75°, washed by ascending chromatography with chloroform–methanol (1:1), then oven activated at 75° for one-half hour.

* 3,4-Methylenedioxy-6-propylbenzyl *n*-butyl-diethyleneglycol ether was obtained from Aldrich Chemical Co., Milwaukee, Wisc., U.S.A. and redistilled; b.p. 180°/1 mm.
** 3,4-Methylenedioxybenzaldehyde [2-(2′-*n*-butoxyethoxy)-ethyl]-acetal obtained from McLaughlin, Gormley King Co., Minneapolis, Minn., U.S.A.
*** Obtained from Camag, Muttenz, Switzerland.

Solvent systems

(A) Toluene–acetic acid–water (10:10:1)
(B) Ethyl acetate–acetic acid–methanol (70:10:20)
(C) Butanol–acetic acid–water (10:1:1)[10,11]
(D) Ethyl acetate–acetic acid (96:4)[12]

Detecting reagents

(1) Chromogenic agents:
(a) Conc. sulfuric acid–*n*-butanol (15:85)[12]
(b) Fuming iodine
(2) Radiation sources:
(a) U.V. 3660 Å — Mineralight, Blak-Ray Model UVL-22*
(b) U.V. 2537 Å — Mineralight, Model UVS-11*

Bile and urine sampling

Single intravenous injections of piperonyl butoxide (0.1 ml), tropital (0.1 ml) and butyl carbitol (0.02 ml) were given to adult male rats of the Sprague-Dawley strain averaging 350 g in weight. Bile samples were collected by fistula and urine samples by cannulation from each rat. Bile samples were collected for 4 to 5 h, 25 microdrops (6 λ each) per tube. In specific studies, samples were collected immediately after injection at one min intervals during a 10 min span, after which period samples were once again taken, 0.150 ml per tube. Details on the handling of the animals, anesthesia, surgery and sample collection and timing have previously been described[13].

During the shorter collection periods, only 0.03 ml samples were obtained as compared to 0.15 ml bile samples obtained during 6 to 7 min intervals.

At least three urine samples were collected, one before i.v. injection, a second at an appropriate interval after injection and a final sample at the termination of the bile collection. All samples were kept frozen until ready for analysis.

Photography of chromatoplates

Ektachrome-X 35 mm slides were obtained with a Honeywell Pentax, Model Hla camera fitted with a Kodak Wratten filter No. 2A. The visible light source was an Ultrablitz Pocket Electronic Flash.* The ultraviolet light sources were Mineralight 2537 Å and 3660 Å (two for each wavelength). All visible light photographs were exposed at 1/20 sec at f16. Both long and short wave photographs were exposed for 3 min at f8. Chromatograms A and B of Figs. 2, 4 and 6 (see legends for figures) were reproduced from 35 mm color slides from which black and white negatives were made.

PROCEDURE

Metabolite detection and characterization

Plates were first photographed in color under U.V. at 2537 Å, then treated with the specific chromogenic agent as follows:

* Obtained from Allied Impex Corp., New York, N.Y.

(1) *Sulfuric acid–butanol* (15:85). Detector was sprayed on the plate, then placed in a circulating air oven at 120° until color development was maximum (5–10 min). After cooling, the plates were photographed in color under both U.V. at 3660 Å and electronic flash visible light.

(2) *Fuming iodine.* Small crystals of iodine were sprinkled on the bottom of a suitable plastic tray and the plate to be fumed was turned silica gel side down, resting on glass stirring rods. An identical tray was used as a top. Fuming was continued until all the spots were colored brown. The plate was then removed from the tray and immediately photographed under U.V. at 2537 Å.

Time-study of metabolites formation

Twenty microliters (10 μl if less sample was available) of all bile samples (taken at prescribed intervals before and after i.v. injection of the synergist) were spotted on Silica Gel DF-5 plates. The plates were developed in the respective solvent, after the solvent was removed, photographed under 2537 Å, then sprayed with sulfuric acid–butanol and heated in a circulating air oven at 120° until color development was maximum (5–15 min). The plates were photographed in color under U.V. at 3660 Å and under visible light. Colors of each spot and R_F values were recorded directly from the photographs because many of the colors were unstable on the plate. One μl standard RBY-dye* was added to each plate as a reference.

RESULTS

Chromatographic differences in bile and urine samples resulting from i.v. administration of piperonyl butoxide, tropital and butyl carbitol are summarized in Tables I and II. Table I lists the R_F value of each component and data regarding its characterization (means of detection, color, etc.).

Table III depicts the color reactions and R_F values of free and conjugated bile acids on Silica Gel DF-5 utilizing solvents A–C

Fig. 1 illustrates diagrammatically the relative size, shape and R_F of each of the spots described in Table I. Piperonyl butoxide, "tropital"** and butyl carbitol are shown in this figure under columns 4, 5 and 6 respectively. Columns 2 and 3 of Fig. 1 show major bile components visible when examined under 2537 Å before acid spray and under 3660 Å after acid spray, respectively. Column 1 of this figure shows spots produced by cholesterol (A), lithocholic acid (B) and cholic acid (C) standards at 3660 Å after acid spray. Column 7 reveals a spot for a tropital standard visible under 2537 Å.

Piperonyl butoxide

As indicated in Table II, two spots (R_F 0.25 and 0.48) appear 2 and 3 min respectively after injection, utilizing toluene–acetic acid–water (10:10:1) as the developing solvent. (Note the appearance of both spots in chromatogram A in Fig. 2.) Both spots reach maximum intensity within 7 min but the latter spot disappears 36 min after injection while the former component remains constant for the remainder of the experiment (see Fig. 3). When the plates were sprayed with sulfuric acid–

* Obtained from Camag, Muttenz, Switzerland.

** Decomposition of tropital occurs at the pH of the solvent system.

TABLE I

SUMMARY OF RAT BILE AND URINE R_F VALUES RESULTING FROM SINGLE INTRAVENOUS ADMINISTRATION OF PIPERONYL SYNERGISTS AND DERIVATIVES

Solvent systems: (A) Toluene–acetic acid–water (10:10:1); (B) Ethyl acetate–acetic acid–methanol (70:10:20); (C) *n*-Butanol–acetic acid–water (10:1:1); (D) Ethyl acetate–acetic acid (96:4).
Detectors: (1) 2537 Å; (2) sulfuric acid–butanol, 3660 Å; (3) sulfuric acid–butanol, visible light; (4) iodine fuming.
Colors: Q = quench; B = blue; Bk = black; Bn = brown; Gr = grey; O = orange; V = violet; Y-G = yellow-green.

Compound	*Sample*	*Solvent system A*					*Solvent system B*				*Solvent system C*				*Solvent system D*			
		R_F	*Detector color*				R_F	*Detector color*			R_F	*Detector color*			R_F	*Detector color*		
			1	*2*	*3*	*4*		*1*	*2*	*3*		*1*	*2*	*3*		*1*	*2*	*3*
Piperonyl butoxide	Bile	0.25	Q	Bk	Bk	—	0.78	Q	Bk	Bk	0.60	Q	Bk	Bk				
		0.36	—	O	Bn	—												
Piperonyl butoxide	Std. (10 μg)	0.75	Q	—	Gr	—	0.89	Q	—	Gr	0.72	Q	—	Gr				
Tropital	Bile	0.11	Q	Bk	dk Bn	—	0.00	Q	Bk	—					0.00	Q	Y-G	—
		0.38	B	—	—	—	0.14	Q	Bk	—								
		0.72[a]	Q	Bn	Gr	—	0.92	B	—	—	0.68	B	—	—				
							0.95[a]	Q	Bn	Gr					0.84[a]	Q	Bn	Gr
Tropital	Urine	—	Not separable				0.82	B	—	—	0.68	B	—	—	0.48	B	—	—
Tropital	Std. (10 μg)	0.72	Q	Bn	Gr	—	0.95	Q	Bn	Gr					0.84	Q	Bn	Gr
Butyl carbitol	Bile	0.18	—	V	Gr	—	—	—	—	—		—	—	—				
		0.35	—	O	Bn	—												
		0.57	Q	Q	Tan	—												
Butyl carbitol	Std. (20 μg)	0.52	—	—	—	Bn	—	Not determined			—	Not determined						

[a] Believed to be tropital decomposition product.

butanol and viewed under 3660 Å (Chromatogram B, Fig. 2), the spot at R_F 0.28 appeared black while that of R_F 0.48 fluoresced yellow-green (second spot above 0.28 on Chromatogram B). In contrast to 2537 Å illumination, the R_F 0.48 spot showed little change in intensity for all bile samples including those taken before compound injection (see Fig. 3).

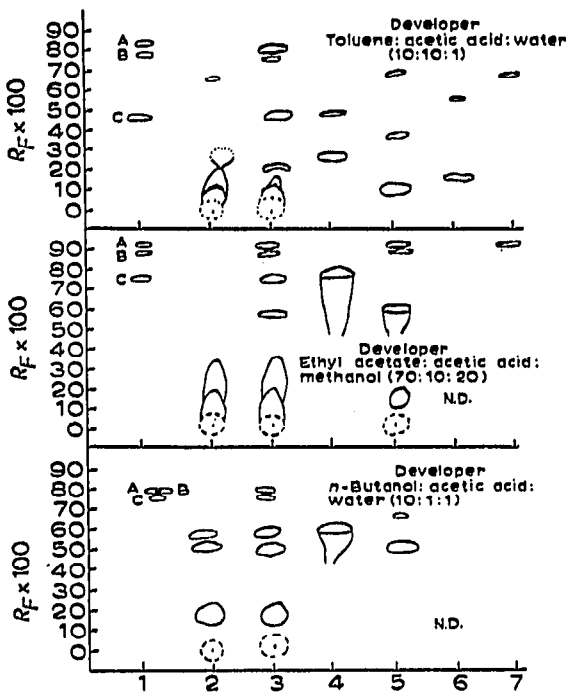


Fig. 1. Composite R_F values of rat bile components before and after intravenous administration of piperonyl butoxide, tropital and butyl carbitol. The data were obtained from Silica Gel DF-5 thin-layer plates developed with toluene–acetic acid–water (10:10:1), ethyl acetate–acetic acid–methanol (70:10:20) and *n*-butanol–acetic acid–water (10:1:1) as indicated. The numbered chromatograms are as follows: (1) 5 μg standards of cholesterol (A), lithocholic acid (B), and cholic acid (C) visible under 3660 Å after spraying with sulfuric acid–butanol. Columns (2) and (3) are major bile components before compound injection, visible at 2537 Å without acid spray and at 3660 Å after spray, respectively. Columns (4), (5), and (6) show only spots that appear when piperonyl butoxide, tropital or butyl carbitol is injected, under either 2537 Å before acid spray or at 3660 Å after acid spray, and column (7) is a 5 μg sample of tropital (probably decomposed) visible at 2537 Å.

From the data shown in Table III, column A, of R_F values of free and conjugated bile acids, it appears that the yellow-green fluorescent spot mentioned above at R_F 0.48 is cholic acid. Since none of the bile acids shown in Table III, however, were visible at 2537 Å it appears that a component other than cholic acid possessed the same R_F value.

A triangular orange fluorescent spot appeared initially before injection at R_F 0.36, whose concentration was inversely related to the appearance of the 0.28 and 0.45 (visible at 2537 Å) spots, since it disappeared 4 min after compound administration (see Chromatogram B, Fig. 2 and R_F 0.36 graph in Fig. 3).

A piperonyl butoxide standard possessed an R_F of 0.75 (see Table I, and Chromatogram B, Fig. 2, column 10) when developed with toluene–acetic acid–water (10:10:1). The compound exhibited strong U.V. absorption at 2537 Å to 2 μg and to

TABLE II

APPEARANCE TIME OF VARIOUS COMPONENTS IN RAT BILE RELATED TO INTRAVENOUS ADMINISTRATION OF PIPERONYL BUTOXIDE, TROPITAL AND BUTYL CARBITOL

Developer: toluene–acetic acid–water (10:10:1).

Sample	R_F	*Appearance time*	*Disappearance time*	*Comments*
Piperonyl butoxide	0.25	2 min after i.v.	None	Maximum intensity 7 min after i.v., then constant to end of experiment (145 min)
	0.36	Before i.v.	4 min after i.v.	Decreases rapidly after i.v.
	0.48[a]	3 min after i.v.	36 min after i.v.	Maximum intensity 7 min after i.v., then slowly decreases
Tropital	0.11	4.5 min after i.v.	None	Maximum intensity upon appearance. Constant to end of experiment (240 min)
	0.38	4.5 min after i.v.	None	Constant from 4.5–42 min after i.v. then slowly decreases to end of experiment (240 min)
	0.72[b]	4.5 min after i.v.	None	Increases from 4.5–24.5 min after i.v. then constant to end of experiment (240 min)
Butyl carbitol	0.18	7 min after i.v.	172 min after i.v.	Constant from 7–96 min then decreases to end of experiment (172 min)
	0.35	121 min after i.v.	None	Increases as R_F 0.18 spot decreases
	0.57	39 min after i.v.	None	Gradually increasing to end of experiment (172 min)

[a] Appears under 2537 Å before acid spray.
[b] Believed to be tropital decomposition product.

TABLE III

COLOR REACTIONS AND R_F VALUES OF FREE AND CONJUGATED BILE ACIDS ON SILICA GEL DF-5

Developers: (A) Toluene–acetic acid–water (10:10:1); (B) Ethyl acetate–acetic acid–methanol (70:10:20); (C) *n*-Butanol–acetic acid–water (10:1:1).
Detectors: (1) Sulfuric acid–butanol (15:85), visible light; (2) Sulfuric acid–butanol (15:85), U.V. 3660 Å.
Colors: B = blue; G = green; Gr = grey; Bn = brown; O = orange; P = purple; R = red; Y = yellow. All standards are fluorescent under 3660 Å.
Standards: 5 μg/μl methanol except cholesterol which is dissolved in chloroform. Applied 1 μl.

	Detected in rat bile	*Solvents*			*Detector*	
		A	*B*	*C*	*1*	*2*
Free bile acids						
Lithocholic acid	+	0.72	0.88	0.78	Gr	B
Deoxycholic acid	—	0.58	0.85	0.78	Y	Y-G
Chenodeoxycholic acid	—	0.57	0.84	0.77	Bn-Gr	B
Cholic acid	+	0.47	0.75	0.74	Y	Y-G
Hyocholic acid	—	0.48	0.77	0.74	Y	B
Conjugated bile acids						
Taurochenodeoxycholic acid	—	0.00	0.22	0.38	Y	B
Taurocholic acid	+	0.00	0.12	0.22	Y	B
Taurodeoxycholic acid	—	0.02	0.22	0.38	Y	B
Glycodeoxycholic acid	—	0.28	0.70	0.72	Y	B
Glycocholic acid	+	0.23	0.52	0.58	Y	B
Sterol						
Cholesterol	+	0.82	0.95	0.78	P-Gr	R-O
Reference RBY-Dye		0.85	0.95	0.79	R	—

less than 1 μg when sprayed with sulfuric acid–butanol. No evidence has been found in these studies for free piperonyl butoxide in the bile.

The equivalent metabolite of R_F 0.25 in toluene–acetic acid–water (10:10:1) appeared at R_F 0.78 and 0.60 when developed with ethyl acetate–acetic acid–methanol (70:10:20) and *n*-butanol–acetic acid–water (10:1:1) respectively. No other differences were noted utilizing these two solvent systems. No detectable differences between urine samples were noted when solvent systems A–C were employed.

Tropital

Selected bile and urine samples from a rat injected i.v. with tropital were developed in four solvent systems (see Table I). Ethyl acetate–acetic acid–methanol (70:10:20) (developer B) produced five spots, at R_F 0.00, 0.14, 0.45–60, 0.92 and 0.95 in bile, and one spot at R_F 0.82 in urine. Toluene–acetic acid–water (10:10:1) (developer A) resolved three spots in bile at R_F 0.11, 0.38 and 0.72 but effected no separation in urine. *n*-Butanol–acetic acid–water (10:1:1) (developer C) and ethyl acetate–acetic acid (96:4) (developer D) produced two spots each with the bile, samples at R_F 0.52, 0.68 and 0.00, 0.84 respectively; and one spot each at R_F 0.68 and 0.48 respectively with the urine sample.

Time studies were made with biles developed with toluene–acetic acid–water (10:10:1). As indicated in Table II and Fig. 5, all three spots appear in the bile 4.5 min after compound injection. Metabolite R_F 0.11 reaches maximum intensity immediately

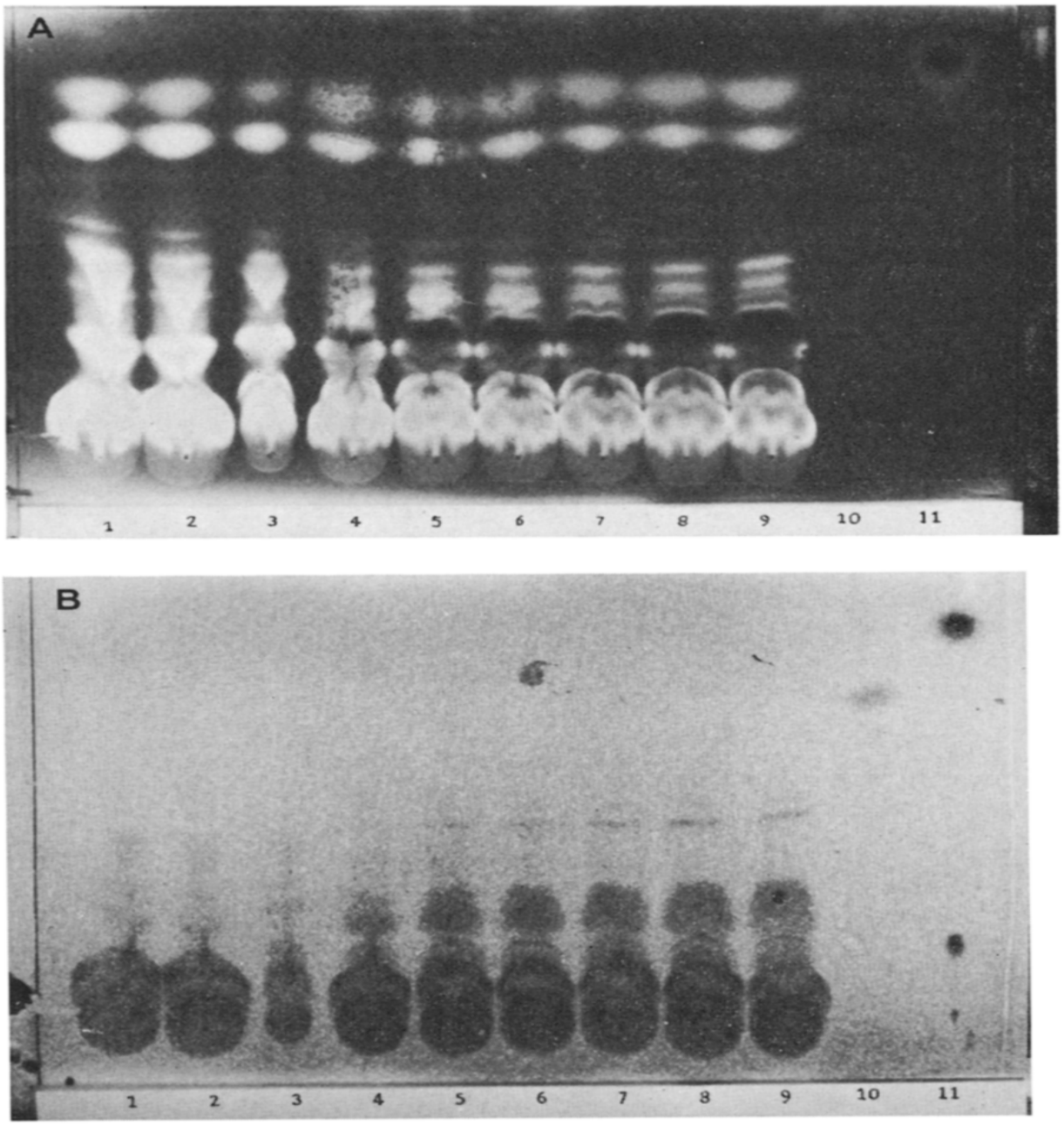


Fig. 2. Chromatograms of rat bile samples following intravenous administration of piperonyl butoxide. Chromatograms were developed with toluene–acetic acid–water (10:10:1) and photographed under 2537 Å (chromatogram A) and 3660 Å following sulfuric acid–butanol spray (15:85) (chromatogram B). Samples 1 and 2 were taken 2 min before injection and at injection time. Samples 3 to 9 were taken at one min intervals following injection. Sample 10 is a piperonyl butoxide standard (20 μg). Sample 11 is a RBY reference dye.

upon appearance and remains constant thereafter. The R_F 0.38 metabolite reaches maximum intensity immediately, remains constant for 42 min after injection, then slowly decreases to about one-half maximum in 6 h. The R_F 0.72 metabolite gradually increases to a peak 24 min after injection, then remains constant for the remainder of the experiment. The spot suspected to be cholic acid (R_F 0.48) in Fig. 5 shows an almost steady rise throughout the entire length of the experiment.

In Fig. 4 chromatograms A and B, tropital standards were compared with chromatograms of bile developed with toluene–acetic acid–water (10:10:1). The tropital "standard" at R_F 0.72 is identical to the R_F 0.72 found on the bile chromatogram when plates were viewed under 2537 Å illumination (Chromatogram A) and under 3660 Å after acid spray (Chromatogram B). However, because of the instability of tropital in the presence of acid the spot may represent a breakdown product and not the tropital itself. A similar correlation is presented in Table I with tropital "standards". The spots at R_F 0.95 and 0.84 in bile samples as developed with ethyl acetate–acetic acid–methanol (70:10:20) (developer B) and ethyl acetate–acetic acid (96:4) (developer D) respectively when examined under 2537 Å (detector 1), 3660 Å after acid spray (detector 2) and visible light after acid spray (detector 3) were found to be identical to the R_F and color characteristics of the tropital "standard".

No free tropital was found in any of the urine samples.

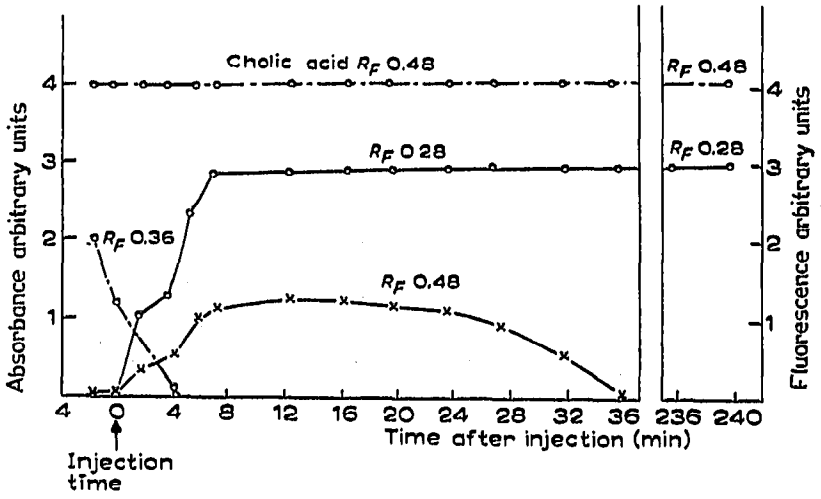


Fig. 3. Relationship of various bile components with time following intravenous administration of piperonyl butoxide. The data shown were taken from thin-layer plates developed with toluene–acetic acid–water (10:10:1). Concentrations were estimated visually as 1, 2, 3 or 4 degrees of intensity of absorbance or fluorescence. R_F 0.48 and 0.28 spots (solid lines) represent compounds that absorb at 2537 Å. R_F 0.36 and 0.48 (dashed lines) represent compounds that fluoresce at 3660 Å after detection with sulfuric acid–butanol.

Butyl carbitol

Initial studies utilizing sulfuric acid–butanol as a detector followed by heating of the chromatogram for 15–25 min at 105–110° failed to detect any metabolite or free butyl carbitol in the bile or urine of i.v. injected rats with butyl carbitol. Detection by fuming iodine was also tried unsuccessfully although this latter technique could detect faintly 20 μg of butyl carbitol standard. The butyl carbitol standard appeared as a brown spot at R_F 0.52 (as detected above) when developed with toluene–acetic acid–water (10:10:1) (see Table I). Metabolites, on the other hand, were detected when the sulfuric acid–butanol treated plate was heated for 10–15 min at 120° instead of 105°. Table I summarizes the results obtained. Bile samples developed with toluene–acetic acid–water (10:10:1) resulted in the separation of metabolites at R_F 0.18, 0.57 and 0.38. No metabolites were found in urine samples utilizing the above solvent system. In addition, no metabolites were detectable in bile and urine when

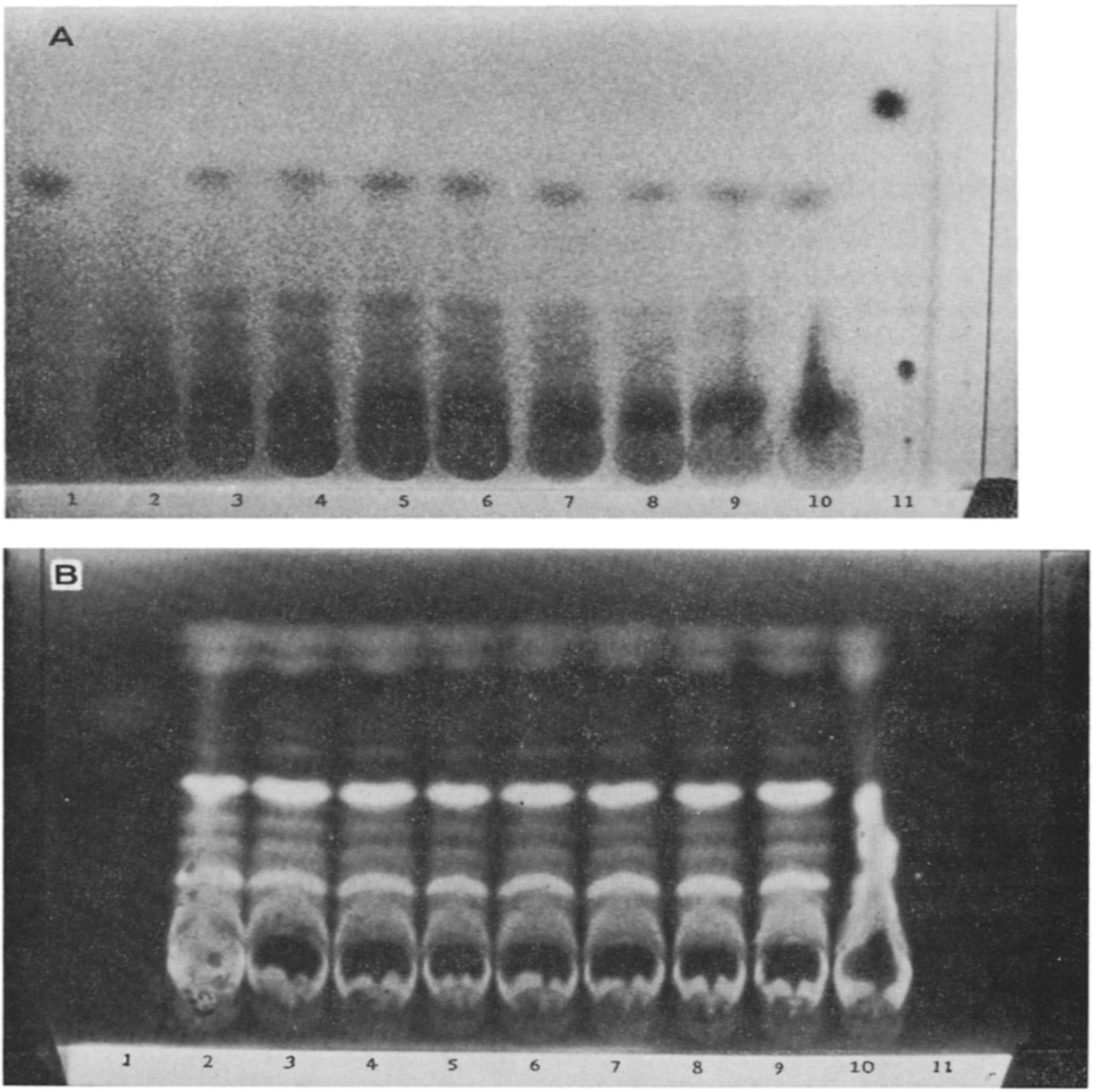


Fig. 4. Relationship of various bile components following intravenous administration of tropital. Chromatograms were developed with toluene–acetic acid–water (10:10:1) and photographed under 2537 Å (chromatogram A) and 3660 Å following sulfuric acid–butanol spray (chromatogram B). For chromatograms A and B, sample one (1) is a tropital sample (probably decomposed) 20 μg. Sample two (2) bile before injection, samples 3–10, biles after injection, and sample 11 RBY reference dye.

ethyl acetate–acetic acid–methanol (70:10:20) (developer B) and *n*-butanol–acetic acid–water (10:1:1) (developer C) were employed.

Fig. 6 depicts chromatograms of selected bile and urine samples developed with toluene–acetic acid–water (10:10:1) (developer A). Chromatogram A, viewed under 2537 Å revealed only the presence of a spot at R_F 0.57 in samples 3, 5 and 6 respec-

tively. Chromatogram B, viewed under 3660 Å after acid spray, showed a strong violet spot at R_F 0.18 in samples 2 and 3 and a weak spot at R_F 0.18 in samples 5 and 6. The triangular orange fluorescent bile component at R_F 0.35 was visible in samples 4, 5 and 6.

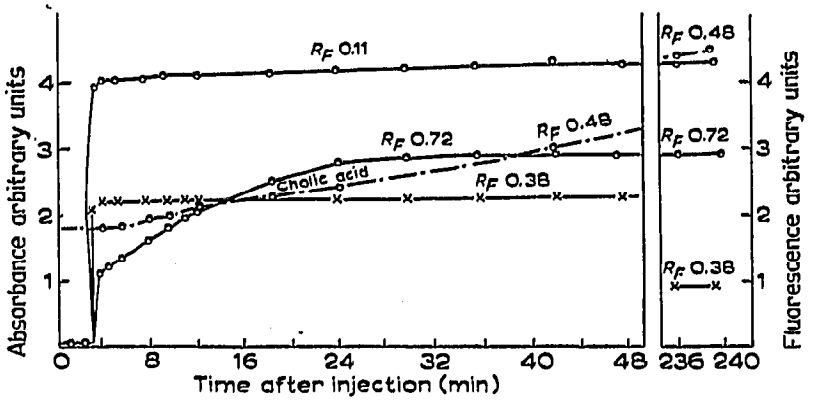


Fig. 5. Relationship of various bile components following intravenous administration of tropital. The data shown were taken from bile chromatograms developed with toluene–acetic acid–water (10:10:1). Concentrations were estimated visually as arbitrary degrees of absorbance or fluorescence. Solid lines represent changes in concentration, at the stated R_F of compounds that absorb at 2537 Å. The dashed line represents cholic acid as measured by fluorescence at 3660 Å after detection with sulfuric acid–butanol.

Fig. 7 depicts the relationship of bile components with time after i.v. administration of butyl carbitol. The changes in concentration of metabolites at R_F 0.18, 0.57 and the bile components at R_F 0.35 and cholic acid at R_F 0.48 are depicted utilizing toluene–acetic acid–water (10:10:1) (developer A).

It is of interest to note that the orange fluorescent bile component (R_F 0.35) appears in the bile at about the same interval that R_F 0.18 begins to decrease, and continues to increase as the component of R_F 0.18 decreases. This appears to be analogous to the orange fluorescent spot at R_F 0.36 that appears before injection in the piperonyl butoxide biles shown in Fig. 3, which decreases as R_F 0.28 metabolite increases. As also shown in Fig. 7, cholic acid (R_F 0.48) remains constant throughout the experiment, except when the component at R_F 0.18 begins to decrease, cholic acid increases. Its significance is so far not known.

DISCUSSION

In regard to the appearance of metabolites of various piperonyl derivatives and butyl carbitol in the bile, two factors should be considered, *viz.*, the effects produced by the injected compounds and the changes induced by cannulation *per se* on the composition of bile based on the blockage of the normal entero-hepatic circulation.

Normal rat bile is composed mainly of two bile acids, cholic and chenodeoxycholic acid, of which 90–95 % are present as the taurine conjugates. Lithocholic acid is present in smaller amounts. The precursor of each of these bile acids (which differ from one another only by hydroxyl groups in the 3α, 7α, and 12α position) is cholesterol. Of salient interest is the known intestinal bacterial conversion of cholic acid into deoxycholic acid. Deoxycholic acid has the unique characteristic of complexing with a

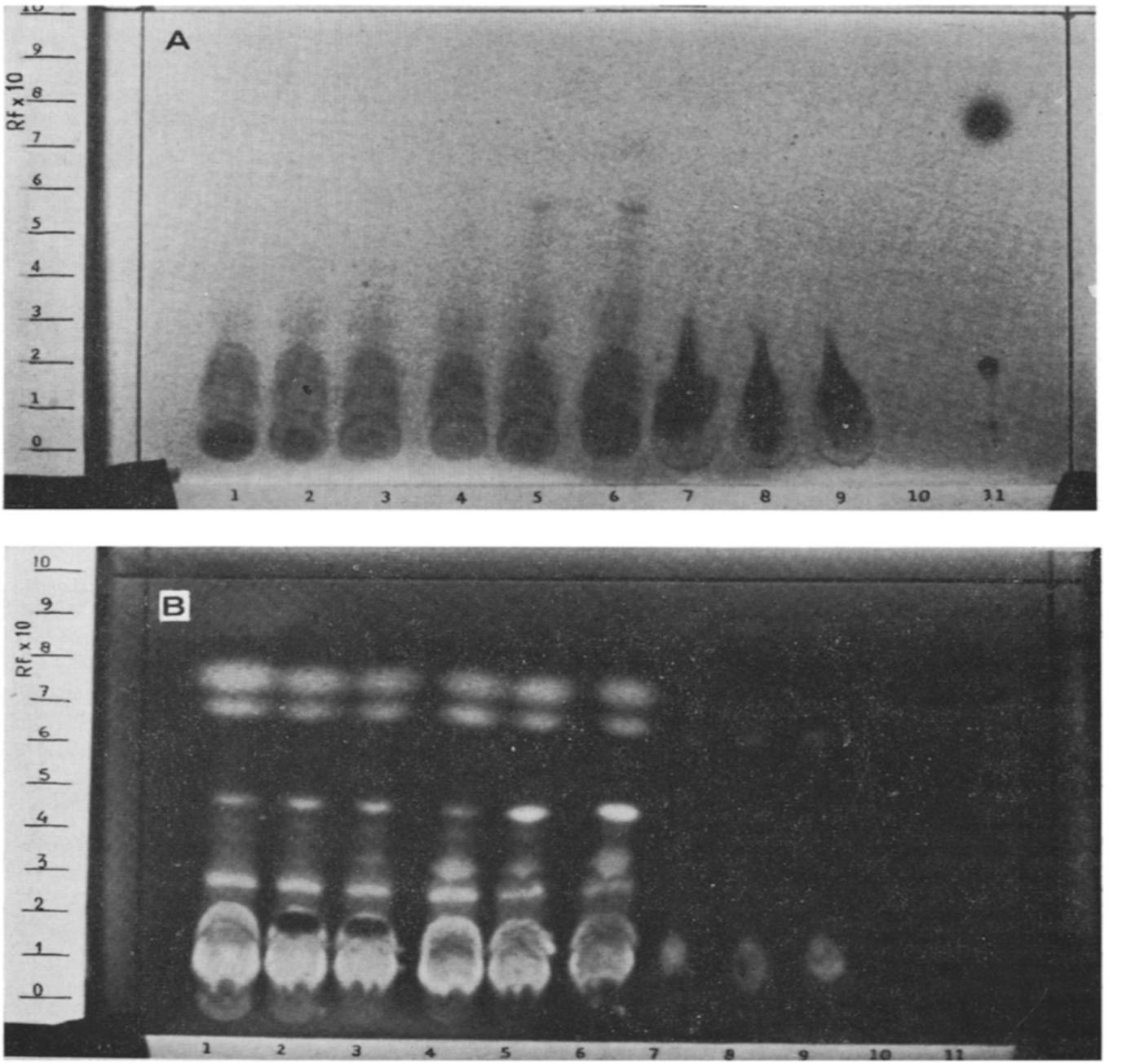


Fig. 6. Chromatograms of rat bile and urine samples following intravenous administration of butyl carbitol. Chromatograms were developed with toluene–acetic acid–water (10:10:1) and photographed under 2537 Å (chromatogram A) and under 3660 Å following sulfuric acid–butanol detection (chromatogram B). Bile samples 1 and 4 were taken before butyl carbitol injection, samples 2, 3, 5 and 6 were drawn 37, 70, 146 and 172 minutes after injection. Samples 7, 8 and 9 are from urine. Sample 7 was obtained before injection, and 8 and 9 after injection. Sample 10 is a 20 μg butyl carbitol standard (not detectable under the conditions employed). Sample 11 is a RBY reference dye.

variety of compounds, *e.g.*, fatty acids, vitamin K and drugs. No other bile component is known to possess these characteristics.

In the normal rat, the reabsorbed bile serves as an inhibitor for the syntheses of other bile salts by the liver. In the cannulated rat, however, this inhibition is consider-

ably reduced since all the excreted bile is drawn off. Except for that bile which is present in the intestine before cannulation no other bile acids are available. As was shown in this study, cannulated rats injected with tropital and butyl carbitol showed increasing amounts of cholic acid excretion with time. A similar study utilizing control biles also showed an increase of cholic acid with time. Since quantitation was only estimated visually it is uncertain whether or not the injected materials also influenced the cholic acid level. The orange fluorescent spot noted earlier with piperonyl butoxide and butyl carbitol bile samples was not observed in the control biles. Under the conditions of detection, no other bile components were observed to change in concentration with time. However, preliminary studies utilizing 1 % chromic acid in 50 % aqueous sulfuric acid as a detector have indicated changes in cholesterol concentration with tropital injected rats.

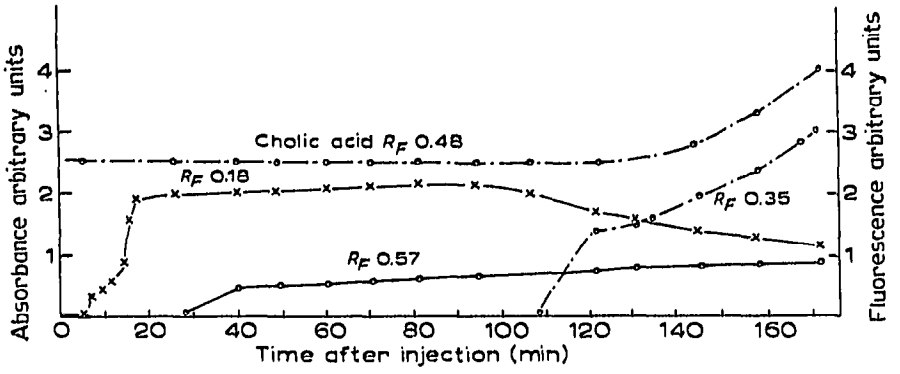


Fig. 7. Relationship of various bile components with time following intravenous administration of butyl carbitol. The data shown were taken from thin-layer plates developed with toluene–acetic acid–water (10:10:1). Concentrations were estimated visually as arbitrary degrees of intensity of absorbance or fluorescence. R_F 0.18 and 0.57 spots (solid lines) represent compounds that absorb at 2537 Å. R_F 0.35 and 0.48 (cholic acid) (dashed line) represent compounds that fluoresce at 3660 Å after detections with sulfuric acid–butanol.

CONCLUSION

The pesticide synergists studied in these experiments were found to be eliminated largely in the bile after i.v. injection. They were altered chemically and they appeared very rapidly in the bile reaching a steady rate for certain compounds or decreasing in concentration slowly with time. As expected, bile acids and cholesterol were also detected in bile and were found to increase with time in certain cases. These studies serve to illuminate certain hazards which may be encountered on repeated and prolonged inhalation or contact with piperonyl synergists, which need further clarification.

The rate of elimination of the compounds from the organism, although it is high in these experiments does not reach a rapid peak with rapid decline thereafter but suggests prolonged elimination of the metabolites from the body in the bile. Inhibition of certain detoxification mechanisms and delayed elimination from the body of these detoxified chemicals is, therefore, a hazard as suggested in an earlier publication[14].

It remains to be shown, however, whether the synergists still possess this enzyme inhibitory activity after absorption from the lung, skin or gastrointestinal tract. These experiments are now under way.

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SUMMARY

Chromatographic differences in rat bile and urine samples resulting from single intravenous administration of piperonyl butoxide, tropital and butyl carbitol were elaborated utilizing four solvent systems, *viz.* toluene–acetic acid–water (10:10:1), ethyl acetate–acetic acid–methanol (70:10:20), ethyl acetate–acetic acid (96:4), and butanol–acetic acid–water (10:1:1). Detection was accomplished with concentrated sulfuric acid–*n*-butanol (15:85) and fuming iodine as well as 2537 Å and 3660 Å ultraviolet sources.

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