Table VI. Analysis of Fluvalinate Stereoisomers as Menthyl Ester Derivatives

fluvalinate sample	% yield of menthylation	% of isomers			
		αR,2R	αS,2S	αR,2S	αS,2R
standard[a]	40	21	17	34	28
renal fat	39	31	14	42	13
feces (24–36 h)	35	37	13	41	10
milk (12–24 h)	25	25	3	50	12

[a] That is, [*trifluoromethyl*-^{14}C]fluvalinate.

milk, and feces was considerably reduced whereas the αR,2R and αR,2S isomers were more abundant (Table VI). Hence, it appears that the αS,2R isomer of fluvalinate is degraded more rapidly than the other stereoisomers; coincidentally, this is by far the most potent insecticidal isomer (Anderson et al., 1980). Since the cholate conjugate of **2** consisted of a 91:9 ratio for (*R*)-**2**:(*S*)-**2**, bile acids appear to selectively remove (*R*)-**2**, which may offer a partial explanation for enhanced disappearance of (αS,2R)-fluvalinate.

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Metabolism of Fluvalinate by Laying Hens

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[*trifluoromethyl*-^{14}C]Fluvalinate (**1**) is rapidly metabolized when given as a single oral dose to laying hens. Within 2 days, 88–100% of the applied dose is excreted with the major excretion products being fluvalinate, the anilino acid (**2**), methylhydroxylated **2**, the taurine conjugate of **2**, and the taurochenodeoxycholic acid conjugate of **2**. Eggs collected for 2 weeks after dosing accounted for ~0.6% of the applied dose with the majority of the radiolabel found in the yolk. Major yolk products included fluvalinate, the anilino acid, and the taurochenodeoxycholic acid conjugate of **2**. Radioactivity did not accumulate significantly in any tissue examined.

Fluvalinate [α-cyano-3-phenoxybenzyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate] is an insecticide with pyrethroid-like activity that is currently being developed by Zoecon Corp. for pest control on numerous field crops. Due to the potential exposure of chickens to fluvalinate residues in processed agricultural products used as feed, we now report the metabolic fate of fluvalinate in laying hens [for the preceding report in this series, see Quistad et al. (1982a)].

EXPERIMENTAL SECTION

Radioassay and Chromatography. Radioactivity was quantitated by liquid scintillation counting (LSC) alone or in conjunction with sample combustion to $^{14}CO_2$ (Quistad et al., 1982a). The extraction of excreta, yolks, and tissues utilized a Polytron homogenizer (Brinkmann). The quantitation of radiolabeled residues in extracts was achieved by using gradient-elution reversed-phase liquid chromatography (LC) by coinjection of a known amount of radiolabeled extract together with authentic metabolite standards and collection of timed fractions for subsequent assay by LSC. The conditions for reversed-phase and normal-phase LC have been described (Quistad et al., 1982a). The following mixtures of methanol–0.1% acetic acid were used for reversed-phase LC: SS 1 (gradient 60–70% methanol over 15 min, 70–90% over 10 min, isocratic at 90% for 10 min); SS 2 (gradient 65–90% methanol over 25 min); SS 3 (isocratic at 80% methanol); SS 4 (gradient 70–90% methanol over 20 min). For normal-phase LC, a Zorbax SIL column (25 × 0.46 cm, 5 μm) was used with elution at ca. 1.7 mL/min with half-water-saturated ether–pentane: 75:25 (SS 5), 80:20 (SS 6), and 2:98 (SS 7). The following solvents were used for thin-layer chromatography (TLC) on silica gel GF (Analtech, Newark, DE): SS 8 (hexane–ethyl acetate, 1:2), SS 9 (ethyl acetate–isobutyl alcohol–acetic acid, 10:3:1), SS 10 (hexane–ethyl acetate–acetic acid, 10:30:0.3), SS 11 (hexane–ethyl acetate, 8:1), SS 12 (hexane–ethyl acetate, 2:1), SS 13 (ethyl acetate), SS 14 (hexane–ethyl acetate, 5:1), SS 15 (hexane–ethyl acetate, 4:1), SS 16 (hexane–ethyl

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Table I. Dosages and Recoveries of Radiolabel from Laying Hens Given a Single Oral Dose of [*trifluoromethyl*-^{14}C]Fluvalinate

	chicken									
	4	7	14	16	1	5	6	8	11	12
fluvalinate dosage: mg/kg	1.08	0.92	1.03	1.0	1.16	0.91	97	102	10.8	0.1
μCi	5.18	5.14	29.0	28.3	5.63	53.2	6.17	311	49.5	15.7
wt of chicken, kg	1.53	1.78	1.72	1.78	1.55	1.97	1.68	1.99	1.60	1.67
duration, days	1	1	1	1	14	14	14	14	14	14
recoveries, % of applied dose										
excreta										
0–24-h extract	85.3	86.0					77.4	45.8		
0–24-h residue	6.4	7.5			100.4[a]	98.6[a]	5.4	3.5		
24 h to sacrifice					3.7	6.5	18.3[b]	46.5[b]		
total in excreta	91.7	93.5			104.1	105.1	101.1	95.8		
eggs (total for 14 days)					0.7	0.5	0.6	0.4	0.5	0.4
carcass: extract	2.8	1.5								
residue	1.5	1.0			0.1[a]	0.1[a]				
tissues	0.4	0.2								
total	96.4	96.2			104.9	105.7	101.7	96.2		

[a] Not extracted. [b] Majority of ^{14}C excreted at day 2 and little thereafter.

acetate, 10:1), and SS 17 (hexane–ethyl acetate–acetic acid, 12:9:0.1).

Mass spectra were obtained by using a Hewlett-Packard Model 5985 GC/MS instrument in the electron impact (EI) mode. Certain field desorption mass spectra were determined by Drs. Y. Naya and H. Naoki (Suntory Institute for Bioorganic Research). The ^{1}H nuclear magnetic resonance (NMR) spectra were determined by using a Bruker WM-300 instrument at 300 MHz (Dr. M. Maddox, Syntex Corp.), a Nicolet 200-MHz spectrometer (Dr. C. Schramm, Occidental Research Corp.), or a Varian T-60.

Synthetic Standards. The preparation of [*trifluoromethyl*-^{14}C]fluvalinate (1) has been reported previously (Quistad et al., 1982a) and was a mixture of αR,2R, αS,2S, αR,2S, and αS,2R stereoisomers. Analysis by normal-phase LC (SS 7) revealed a ratio of 49:51 for [(αR,2R) + (αS,2S)]:[(αR,2S) + (αS,2R)]. The radiochemical purity of combined isomers was 99% as judged by reversed-phase LC with a specific activity of 48.3 mCi/mmol prior to dilution with carrier.

The preparations of the anilino acid (2), the hydroxy acid (3), and the glycine conjugate of 2 (i.e. 4) will be reported elsewhere (Quistad et al., 1980) as will that for the methylated, peracetylated glucuronide of 2 (Quistad et al., 1982a).

The taurochenodeoxycholic acid conjugate of 2 (as its methyl ester, i.e., methylated 5) was synthesized from 2 (16 mg, 0.055 mmol) and the methyl ester of taurochenodeoxycholic acid (28 mg, 0.055 mmol) in the presence of dicyclohexylcarbodiimide (11 mg, 0.055 mmol) and 4-(dimethylamino)pyridine (2 mg, 0.02 mmol) in CH_2Cl_2 (2 mL). The product was purified by TLC (SS 8, R_f = 0.28, 88% yield). This product was found to be somewhat unstable and would decompose slowly to the free sulfonic acid (i.e., 5) whether neat or in acetone solution.

The synthesis of the taurine conjugate of 2 (i.e., 6) was as follows: the acid chloride of 2 (112 mg, 0.34 mmol) was dissolved in ether (1 mL). To this was added taurine (98 mg, 0.78 mmol) dissolved in 1 M NaOH (0.78 mL, 0.78 mmol). The mixture was stirred at room temperature for 2 h and then evaporated to dryness. The taurine conjugate of 2 was isolated in quantitative yield by TLC (R_f = 0.27, SS 9). A portion of this conjugate was methylated in 8% yield; the methyl ester was identified by its ^{1}H NMR and mass spectra.

For the synthesis of the ornithine bis adduct of 2 (i.e., 7), ornithine hydrochloride (29 mg, 0.17 mmol) was dissolved in 1 M NaOH (340 μL, 0.34 mmol). After cooling in ice, this mixture was added to the acid chloride of 2 (112 mg, 0.34 mmol). Additional 1 M NaOH (340 μL, 0.34 mmol) was added after 5 min and the reaction mixture was stirred for 1 h at room temperature. Water was added and the product was extracted into ethyl acetate. Preparative TLC (SS 10) gave the ornithine bis adduct of 2 (R_f = 0.23, 43 mg, 36% yield). Methylation (CH_2N_2) gave the methyl ester of the bis adduct [R_f = 0.44, SS 11; EI mass spectrum m/z (rel intensity) 702 (1.1, M^+), 700 (1.5, M^+ for both Cl = 35), 451 (5), 424 (2), 408 (8), 392 (4), 252 (67), 250 (100); diastereomers separable by GLC with a 1-m OV-17 column].

Dosage. White leghorn hens (Jensen Farms, Tracy, CA) weighing 1.53–1.99 kg were used in these studies. Each was housed in a laying cage with food (Albers Trip-L-Duty Mash, Carnation Co.; ground oyster shell supplement) and water provided ad libitum. The chickens were maintained in a greenhouse (24–35 °C) and when necessary, fluorescent lighting extended daylight from 5:00 a.m. to 9:00 p.m. The dose was prepared by adsorbing [^{14}C]fluvalinate of the appropriate specific activity onto chicken feed (1.7 g) and transferring the feed to a gelatin capsule ($^1/_8$ oz, Central City Medical, San Carlos, CA). Each chicken was weighed prior to dosing and after administration of the capsule was returned to the cage equipped with a metal pan for total collection of excreta. The exact dose and weight of each chicken are given in Table I.

Excreta Analysis. The excrement was collected at 24-h intervals and quantitated by LSC following combustion of aliquots (70–700 mg). Selected fecal samples were extracted with methanol (3 × 300 mL) for quantitation followed by metabolite analysis using reversed-phase LC (SS 1).

The presence of fluvalinate and several metabolites in excreta was verified by TLC and reversed-phase LC using the 1-day extract from chicken 4 (1 mg/kg). Fluvalinate (1) was eluted from a silica gel TLC plate (R_f = 0.76, SS 17) and shown to be coincident with an authentic standard upon LC (SS 1, k' = 21.7). Similar analysis confirmed the presence of the anilino acid (2, R_f = 0.44, k' = 12.7 in SS 1) and the glycine conjugate of 2 (i.e., 4, R_f = 0.17, k' = 7.3 in SS 1). The methyl ester of hydroxy acid (3) was isolated by TLC (R_f = 0.53) and further purified by additional TLC (SS 12, R_f = 0.36) and LC (SS 1, k' = 9.7). The structural assignment was verified by EI mass spectrometry of the methyl ester: m/z (rel intensity) 327 (0.5, M^+ for Cl = 37), 325 (2.0, M^+ for Cl = 35), 269 (40), 267 (82), 254 (38), 252 (78), 208 (87), 206 (100), 179 (61), 59 (88).

The taurine and taurochenodeoxycholate conjugates of anilino acid 2 were isolated from the 1-day excreta of

chicken 8 (102 mg/kg dose). These metabolites were isolated from the methanol extract of 44 g of excreta that was purified by column chromatography (silica gel 60, E. Merck; 2.5 × 24 cm column). After elution of the column with ethyl acetate to remove nonpolar impurities, methanol eluted both of these sulfonic acids. The taurine conjugate of **2** (i.e., **6**, $k' = 2.2$ in SS 1) was methylated and purified by TLC (SS 13, $R_f = 0.80$) and LC (SS 1, $k' = 9.9$). The structure was verified by EI mass spectrometry of the methyl ester: m/z (rel intensity) 418 (1.2, M^+), 416 (2.8, M^+), 252 (34), 251 (13), 250 (100), 206 (10), 55 (15).

The taurochenodeoxycholate conjugate of **2** (i.e., **5**) was also isolated from the methanol eluate of the silica gel column. Methylation of this fraction with CH_2N_2 gave the methyl ester of **5** which was purified by TLC (SS 8, $R_f = 0.38$) and reversed-phase LC (SS 3, $k' = 15.5$). Since methylation of the sulfonic acid moiety of **5** was inefficient, it was necessary to recycle unmethylated polar products (i.e., the TLC origin zone) for several treatments with CH_2N_2 in order to acquire ca. 300 μg of the methyl ester of **5**. The structure of this metabolite was verified by ^1H NMR and field desorption (FD) mass spectrometry (Quistad et al., 1982b). A synthetic standard of the methyl taurochenodeoxycholate conjugate of **2** had the same ^1H NMR and mass spectral characteristics as the methylated metabolite. Normal-phase LC (SS 5) resolved the metabolite (as its methyl ester) into diastereomers ($k' = 17.8$ and 19.2).

Analysis of Eggs. After dosage certain chickens were maintained for 14 days with a daily collection of eggs. For preliminary analysis the egg whites and yolks were separated and weighed, and duplicate aliquots (0.5–1 g) were quantitiated for ^{14}C by LSC. The 1-day egg white from chicken 8 was extracted with ethyl acetate, and metabolites were analyzed by reversed-phase LC (SS 1).

Extraction of yolks with acetonitrile effectively gave a fraction containing fluvalinate and its nonpolar metabolites (principally **2**) with a low mass of lipid. Further extraction of the yolk with methanol was necessary in order to recover polar ^{14}C-labeled residues (mostly **5**). The methanol filtrate was delipidized with a short silica gel column and then combined with the acetonitrile extract to give a single fraction that was examined by TLC. In general, this procedure gave a >88% recovery of yolk ^{14}C with substantial reduction of biomass. [^{14}C]Fluvalinate is stable in this extraction procedure. Analysis by TLC (SS 17) gave only three zones of radioactivity that were coincident with fluvalinate, **2**, and **5**. Subsequent analysis of fluvalinate and **2** by reversed-phase LC (SS 1) revealed no other significant radiolabeled metabolites in these TLC zones. Hence, subsequent quantitations of **1** and **2** were based on TLC analysis alone. Although **5** was the major component at the origin after TLC of yolk extracts, LC analysis was necessary for accurate quantitation of **5** in each yolk. The retention time of **5** (free sulfonic acid) was somewhat variable upon reversed-phase LC ($k' = 13$–18 for SS 1). Although **5** is rather refractory to methylation, two successive treatments with CH_2N_2 gave a 65% conversion of the metabolite **5** to its methyl ester ($k' = 19.7$ in SS 1). Normal-phase LC of this methylated ^{14}C-labeled metabolite showed radioactivity coincident with a synthetic standard of the methyl ester of **5** ($k' = 9.0$ and 9.7 in SS 6 for the diastereomers).

Tissue Analysis. At 1 or 14 days after treatment chickens were sacrificed with ether. Selected tissues were removed and frozen for subsequent quantitation of aliquots (68–281 mg) by combustion and then LSC. Certain carcasses were minced manually and with a meat grinder (Univex). The ground carcass was thoroughly mixed, and aliquots (200–500 mg) were quantitated by LSC after combustion. The carcasses of chickens 4 and 7 were extracted with methanol and CH_2Cl_2 (total volume ~2800 mL). Aliquots of the extract (1–2 mL) were quantitated by LSC as were the dried residual solids (~200 mg) following combustion.

Aliquots of muscle (two sites, 34–53 g) and fat (two sites, 3–13 g) were removed from chickens 14 and 16 and quantitated by LSC following combustion (0.08–0.5-g aliquots). The identity of ^{14}C-labeled residues in leg muscle from both chickens and breast fat from chicken 14 was investigated. The muscle (18–20-g aliquots) was mixed with Celite and extracted (2 × 100 mL of methanol, 1 × 100 mL of chloroform), and an aliquot of the filtrate was quantitated by LSC. After concentration of the solvent, an aliquot of the extract was fractionated by TLC (SS 17), and zones were eluted from the silica gel for quantitation. Radioactivity coeluting with fluvalinate (**1**) and the anilino acid (**2**) was further examined on LC (SS 4 and 2, respectively, chicken 16 only).

Chicken 14 breast fat (2.5 g plus 2 g of Celite) was extracted with acetonitrile (2 × 100 mL) and methanol (2 × 100 mL), and aliquots of the separate filtrates were quantitated by LSC. After being stored in the freezer overnight, the methanol extract was filtered to remove precipitated solids and combined with the acetonitrile extract. An aliquot of the combined extracts was fractionated by TLC (SS 17), and zones were eluted for quantitation. The fluvalinate zone was repurified by TLC (SS 14, $R_f = 0.43$) and eluted from the silica gel for subsequent LC analysis (SS 4, $k' = 13.6$). Aliquots of all dried residues (after extractions) were quantitated by LSC following combustion.

Fluvalinate Stereoisomer Analysis. For examination of the possibility of preferential metabolism of any of the four fluvalinate isomers, an egg yolk sample (chicken 5, 6-day yolk) and an aliquot of excreta (chicken 1 1-day excreta, 10 g) were extracted with acetonitrile (2 × 30 mL), and the fluvalinate was purified by TLC (SS 15, $R_f = 0.48$). The fluvalinate stereoisomers were derivatized to menthyl esters for determination of the isomeric composition (Quistad et al., 1982a). The menthyl esters were purified by silica gel TLC (SS 16, $R_f = 0.40$) and then analyzed by normal-phase LC [SS 7, $k' = 4.9$, 6.4, 7.9, and 9.4 for (αR,2R)-, (αS,2S)-, (αR,2S)-, and (αS,2R)-fluvalinate derivatives respectively]. At the same time, a sample of the [*trifluoromethyl*-^{14}C] fluvalinate used for the chicken doses was derivatized in order to compare recoveries.

RESULTS AND DISCUSSION

Excreta. As shown in Table I, at a dose of 1 mg/kg the majority of the radiolabel is excreted within 1 day, and a small amount (<7% applied dose) in next 13 days. Table II summarizes the products found in excreta. At a dose of 1 mg/kg, fluvalinate is rapidly metabolized since only 9% of the excreted ^{14}C was parent compound. As expected at a higher dose (100 mg/kg) significantly more fluvalinate was found (44% of 1-day excreta ^{14}C). Anilino acid **2** and its oxidation product (hydroxy acid **3**) represented 8 and 10% of the ^{14}C in the 1-day excreta. Although taurine is a relatively minor amino acid in chicken urine (Sykes, 1971), the taurine conjugate of **2** contributed 15% of the fecal ^{14}C-labeled residue. A similar taurine conjugate of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid has been reported from permethrin metabolism in laying hens (Gaughan et al., 1978). Glycine and ornithine are two of the more abundant amino acids in chicken urine (Skyes, 1971), but despite efforts to identify conjugates of

Table II. Distribution of Metabolites in Excreta from Laying Hens Dosed with [*trifluoromethyl*-^{14}C]Fluvalinate

	% of total ^{14}C in sample		
	chicken 4 (1 mg/kg), 1-day excreta[b]	chicken 8 (102 mg/kg) 1-day excreta[c]	chicken 8 (102 mg/kg) 2-day excreta[d]
methanol extract	93.0	92.9	88.2
fluvalinate (1)	8.7	44.2	12.0
anilino acid (2)	8.0	12.1	18.5
hydroxyanilino acid (3)	9.8	2.6	*a*
glycine conjugate of 2 (i.e., 4)	1.0	⩽0.6	⩽2.6
taurine conjugate of 2 (i.e., 6)	15.3	5.7	11.3
taurochenodeoxycholic acid conjugate of 2 (i.e., 5)	11.9	9.6	11.4
glucuronide conjugate of 2	⩽2.9	⩽1.2	*a*
4′-hydroxyfluvalinate	⩽0.1	⩽0.3	⩽0.3
2-chloro-4-(trifluoromethyl)aniline	⩽0.5	⩽0.5	*a*
nonpolar unknown	⩽0.6	0.4	⩽0.4
polar unknowns	~18	~5	~5
residual solids	7.0	7.1	11.8

[a] Products not resolved. [b] 91.7% of applied dose. [c] 49.3% of applied dose. [d] 38.9% of applied dose.

these amino acids only the glycine conjugate of **2** (i.e., **4**) could be identified (representing only 1% of the fecal ^{14}C). The apparent absence of the bis adduct of **2** with ornithine (i.e., **7**) is interesting since ornithuric acids are major urinary metabolites of aromatic acids in chickens (Bridges et al., 1970; James et al., 1972). The ornithine bis adduct of **2** represented a maximum of 0.3% of the 1-day excreta from chicken 8. There is the possibility that **2** could form a mono adduct with ornithine; however, synthesis of a standard to facilitate the search for such a metabolite proved unsuccessful. This latter product may be present in small amounts, but its TLC and LC retention behavior are unknown.

A major, polar unknown was present in the excreta of a chicken dosed at 1 mg/kg (12% of excreta ^{14}C), and giving a chicken a high dose of [^{14}C]fluvalinate (102 mg/kg, 311 μCi) allowed isolation of 300 μg of this product (as its methyl ester). As the free acid, this metabolite failed to give pertinent information by ^{1}H NMR (300 MHz), field desorption, or direct-inlet, EI mass spectrometry. Methylation was inefficient, yet sufficient methylated sample was obtained to allow identification of the metabolite as the taurochenodeoxycholic acid conjugate of **2** (i.e., **5**). The acylation of the bile acid by **2** occurred at the 3α-hydroxyl group (not the 7α-hydroxyl) based on ^{1}H NMR spectra of the metabolite and selected synthetic standards (Quistad et al., 1982b).

Chenodeoxycholic acid is regarded as the predominant bile acid in fowl (Haslewood, 1971), and it is generally conjugated with taurine (Anderson et al., 1957). The taurochenodeoxycholate adduct was by far the most abundant bile acid conjugate of **2** found; no other bile acid conjugate represented more than 0.5% of the radioactivity in the excreta.

The glucuronide conjugate of **2** was a major urinary product in a lactating dairy cow (Quistad et al. 1982a), and such glucuronide conjugates of xenobiotics are known in fowl (Skyes, 1971; Gaughan et al., 1978). However, no conclusive evidence (either by derivatization or β-glucuronidase treatment, followed by chromatographic analysis) was found for the glucuronide of **2** in chicken excreta (maximum of 1.2% of chicken 8, 1-day excreta).

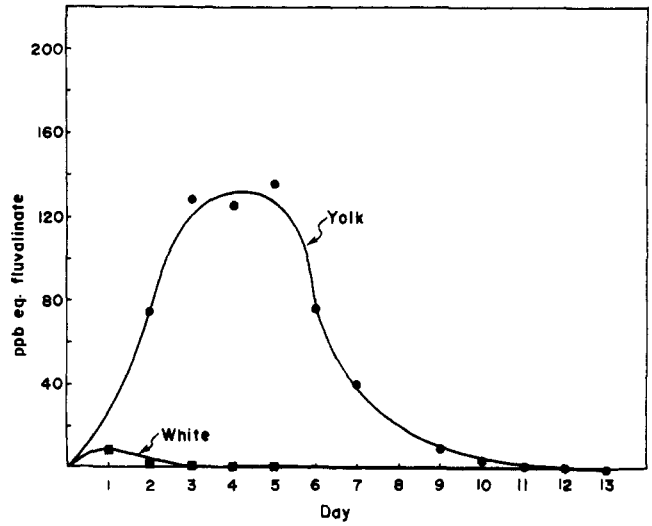

Figure 1. ^{14}C-Labeled residues in eggs after dosage at 1 mg/kg with [^{14}C]fluvalinate.

Other metabolites that have been identified from previous fluvalinate metabolism studies include 2-chloro-4-(trifluoromethyl)aniline and 4′-hydroxyfluvalinate. These metabolites are either absent or present in small amounts (<0.5% of excreta ^{14}C). Additional unkown *polar* radiolabeled products represented as much as 18% of the ^{14}C in 1-day excreta, no single product being more than 5% of the 1-day ^{14}C-labeled residue.

Eggs. The levels of radiolabel found in eggs are shown in Table I. Residues in the eggs were low, peaking 1–2 days after dosing for whites and 4–5 days for yolks (Figure 1). The appearance of peak ^{14}C-labeled residue levels in eggs after ca. 5 days have also been reported for dermal and oral administration of permethrin to laying hens (Hunt et al., 1979; Gaughan et al., 1978). As indicated in Figure 2 the residues in egg yolks are roughly proportional to the applied dose. Although much less ^{14}C-labeled residue was found in egg white relative to the yolks, the anilino acid (**2**) was the major ^{14}C-labeled component of egg white residue. Since egg white (albumin) consists primarily of protein formed in the oviducal tissues (Gilbert, 1971), the very low levels of ^{14}C-labeled residues in that fraction are understandable. The yolk substances, on the other hand, are formed in the liver and transported via the blood to the ovary, allowing potentially more contact with absorbed metabolites.

Identification of radiolabel in yolks was difficult due to the paucity of ^{14}C and high lipid content. A combination of extractions allowed quantitation of major metabolites, and as shown in Table III, both fluvalinate (**1**) and the anilino acid (**2**) were identified. Considerable radiolabel was found as a polar product, which by coelution with an authentic standard was shown to be the taurochenodeoxycholic acid conjugate of **2** (i.e., **5**). Also found was a minor, unknown, nonpolar product (2.2% of yolk ^{14}C), possibly the methyl ester of **5**, formed as a result of extraction with methanol.

Examination of the yolk residues from a chicken dosed at 1 mg/kg indicated that the ratio of the three major products was similar from 2–7 days posttreatment (Table III). Fluvalinate decreased slightly with increased time (from 32 to 25% of yolk ^{14}C), while the anilino acid and **5** increased slightly. A similar product ratio was found in the 5-day yolk from a chicken dosed at 0.1 mg/kg, but significantly less fluvalinate (as compared to its metabolites) was found in the yolks when chickens were dosed at 10 or 100 mg/kg. There was little evidence of any glyceride

Table III. Distribution of Metabolites in Egg Yolks from Laying Hens Dosed with [*trifluoromethyl*-^{14}C]Fluvalinate

	% of total ^{14}C in egg yolk									
chicken	12	5	5	5	1	5	5	11	11	8
dose, mg/kg	0.1	0.9	0.9	0.9	1.2	0.9	0.9	10.8	10.8	102
days posttreatment	5	2	3	4	4	5	7	4	5	6
% of applied dose in yolk	0.15	0.06	0.10	0.07	0.19	0.11	0.05	0.14	0.10	0.08
extract	93.4	91.2	93.0	91.5	89.1	92.0	89.9	91.0	91.5	80.8
fluvalinate (1)	33.1	31.6	25.8	30.1	33.9	28.5	24.5	15.0	16.1	10.5
anilino acid (2)	5.1	4.6	3.5	5.1	9.9	8.2	11.7	4.6	4.7	3.3
taurochenodeoxycholate conjugate of 2 (i.e., 5)	39.4	39.0	39.4	32.6		42.0	47.6	58.0	48.0	38.9
residual solids	6.6	8.8	7.0	8.5	10.1	8.0	10.1	9.0	8.5	19.2

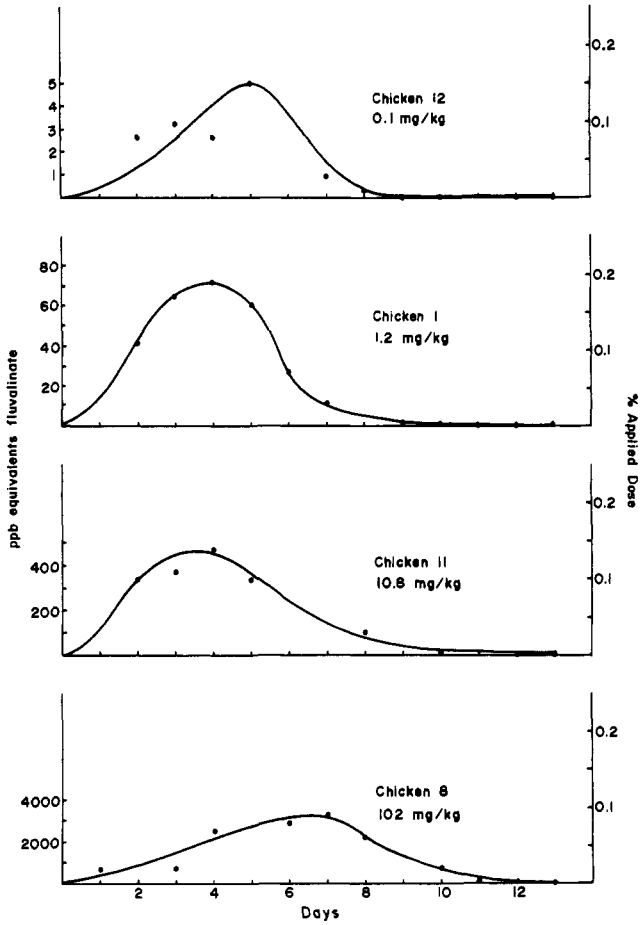

Figure 2. ^{14}C-Labeled residues in egg yolks as a function of dose rate with [^{14}C]fluvalinate.

conjugates of 2, even though these have been identified in rats dosed with fluvalinate in corn oil (Quistad et al., 1980).

Tissues. Examination of tissues from chickens sacrificed only 1 day after dosing indicated no apparent accumulation of radiolabel (Table IV). While the liver contained the highest residue (0.14–0.28% of applied dose), the remaining carcass after removal of selected tissues contained 3–4% of the applied dose that was probably associated with the alimentary canal awaiting elimination. After 14 days essentially 100% of the applied dose had been excreted, with the carcass containing small ^{14}C-labeled residues (0.1% of applied dose).

Residues in fat and muscle only one day after dosing at 1 mg/kg were extremely low (10–48 and 2–13 pbb equiv as fluvalinate, respectively), and consequently, identification of the ^{14}C-labeled residues in these tissues was difficult. Two chickens were given a dose of fluvalinate at 1 mg/kg (28 μCi) and sacrificed 1 day thereafter in order to maximize ^{14}C-labeled residues in tissues. This allowed purification of sufficient radiolabel to facilitate analysis

Table IV. Distribution of ^{14}C-Labeled Residues after 1 Day in Tissues of Laying Hens Dosed with [*trifluoromethyl*-^{14}C]Fluvalinate at 1 mg/kg

	ppb equiv as fluvalinate (% of applied dose)			
	chicken 4	chicken 7	chicken 14	chicken 16
liver	100 (0.28)	45 (0.14)		
kidneys	83 (0.06)	27 (0.02)		
heart	30 (0.01)	10 (<0.01)		
lungs	71 (0.03)	26 (0.01)		
gizzard	26 (0.05)	7.5 (0.02)		
skin	34	24		
muscle: leg	9.8	6.6	3.4	13
breast	6.5	2.6	1.7	5.7
fat: leg	24	9.4	40	29
breast	9.5	22	48	24
carcass: extract	34 (2.8)	14 (1.5)		
residue	19 (1.5)	10 (1.0)		

Table V. Isomer Ratios of Fluvalinate Isolated from Chicken Excreta and Egg Yolk

sample	% yield of menthyl-ation	% isomer			
		αR,2R	αS,2S	αR,2S	αS,2R
standard (prior to dosage)	~40	20.9	16.7	34.1	28.3
excreta (1 day, chicken 1)	40.6	23.1	17.6	37.1	22.2
egg yolk (6 day, chicken 5)	42.5	26.7	17.9	25.0	30.3

of major residues. Fluvalinate (1) was the major residue in breast fat (72% of fat ^{14}C) and was a major component of leg muscle radiolabel (18–20% of muscle ^{14}C). Also important in the muscle was the anilino acid (2, 15–44% of muscle ^{14}C) and unidentified polar radiolabel (17–27% of muscle ^{14}C).

Stereoisomer Ratios. Conversion of (αRS,2RS)-fluvalinate into the four menthyl ester derivatives allowed analysis of possible selective metabolism of stereoisomers of fully racemic 1 by chickens. The results from the analysis of the four fluvalinate isomers in excreta and an egg yolk sample, as well as from the standard derivatization of a standard, are shown in Table V. The ratios indicate little or no preferential degradation of any of the isomers of fluvalinate.

Normal-phase LC allowed separation of the diastereomers of the methyl taurochenodeoxycholate conjugate of the anilino acid since the bile acid group functions as a resolving agent for the (*R*)- and (*S*)-anilino acid. The ratio of the diastereomers of this conjugate isolated from excreta was 43:57 (elution order unknown). When isolated from egg yolk, the ratio of isomers was 11:89; however, the LC separation of the isomers in this instance was incomplete. Since the fluvalinate isomers show no preferential metabolism, and the bile acid conjugate from excreta shows

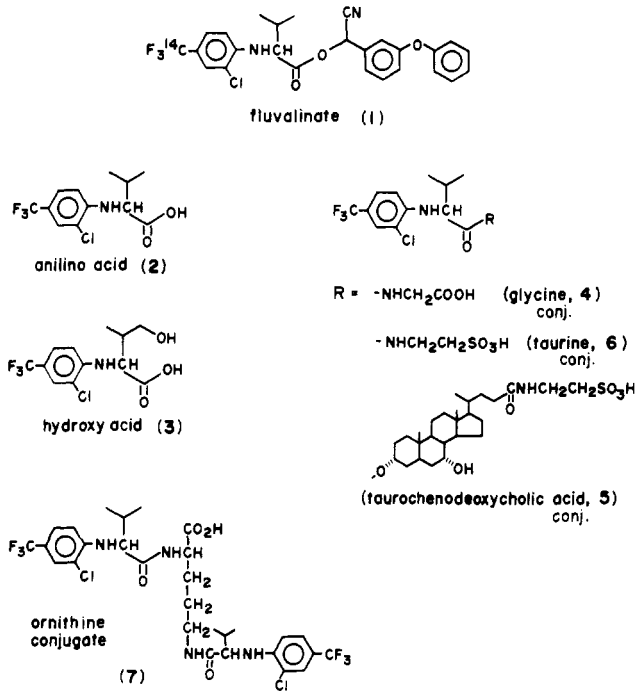

Figure 3. Structures of potential metabolites of fluvalinate from a laying hen.

little difference in formation of either diastereomer, it is likely that the observed unequal isomer ratio of the bile acid conjugate from egg yolk is a result of poor resolution and not differential occurrence of one isomer.

CONCLUSIONS

The rapid metabolism and excretion of radiolabel from chickens dosed with [*trifluoromethyl*-^{14}C]fluvalinate are probably indicative of the high metabolic rate and the high rate of food passage in chickens. Laying hens are known to excrete a meal of mash within 8 h (Hill, 1971). Fluvalinate itself is rapidly metabolized as evidenced by the low levels of parent compound in the excreta. The major products in excreta are the anilino acid (2), hydroxylated 2 (i.e., 3), and polar conjugates of 2 (adducts of taurine and taurochenodeoxycholic acid). The low levels of radiolabel in the eggs may be evidence for the lack of absorption of fluvalinate or its metabolites. The major products in egg yolks are fluvalinate, the anilino acid, and the taurochenodeoxycholic acid conjugate of the anilino acid. Low ^{14}C-labeled residues were found in all tissues examined, and analysis of fat and muscle indicated fluvalinate to be the major residue, but accompanied by some anilino acid and unknown polar radiolabel. The major metabolites from fluvalinate metabolism are summarized in Figure 3. There is no evidence for selective metabolism of any stereoisomers of fluvalinate.

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Reversibility of Thermal Degradation of Betacyanines under the Influence of Isoascorbic Acid

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Isoascorbic acid was effective in reversing the thermal degradation of betacyanines, the red pigments of beet. Heating an aqueous solution of red pigments cleaved the betacyanine molecule, causing the loss of red color. When isoascorbic acid was added either before or after heating, the red color of thermally degraded betacyanine in solution was restored almost completely after 24 h of storage in the dark at 25 °C. Isoascorbic acid did not produce a similar result with thermally degraded yellow betaxanthine pigments.

The red beet (*Beta vulgaris*) is a potential source of valuable water-soluble pigments, the so-called betalaines, for use as food colorants (von Elbe and Maing, 1973; von Elbe et al., 1974a,b; Pasch et al., 1975; Sapers and Hornstein, 1979). However, the instability of these natural colorants is a major obstacle to practical use (von Elbe et al., 1974b). Heat is one of the principal factors that causes loss of color through the degradation of betalaines. A

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