with lipoproteins *in vitro*(9,10,11). Our work shows that cholesterol is also bound with both alpha and beta lipoproteins *in vitro*. The mechanism of this combination is not clear at the present time, and this could possibly represent an exchange reaction. However, such observations suggest an interesting possibility that blood levels of various lipid substances may be partially determined by binding capacity of serum lipoproteins. A substance similar to that produced after removal of lipids with ether might serve as a basic lipid binding material, and quantity of this substance present might influence amount of lipid bound in the serum. Since little is known concerning mechanisms regulating serum lipid levels, the possible role of this mechanism might deserve further investigation.

Summary. 1. Extraction of serum lipoproteins of density less than 1.063 g ml with ether produces 2 distinct ultracentrifugal components. One is associated with higher density lipoproteins and the other with lower density lipoproteins in the density group less than 1.063. In a case of essential hyperlipemia and with hyperlipemic rabbit serum only the moiety associated with lowest density lipoproteins was present. It would appear that both components are necessary in transformation of lower density lipoproteins to higher ones. Absence of the higher density moiety might result in an accumulation of lipid in lower density lipoproteins as occurred in the case of essential hyperlipemia and lipemic rabbit serum. 2. Also, it was demonstrated that both alpha and beta lipoproteins bind cholesterol *in vitro*. This finding suggests the possibility that serum cholesterol levels may partially depend upon this mechanism of combination.

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Plasma Levels, Excretion and Metabolic Disposition of 4-(p-Dimethylaminostyryl)-quinoline Dihydrochloride in Dogs.*† (24751)

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The efficacy of a series of 4-substituted p-aminostyryl quinoline dyes against experimental carcinomas and lymphomas in rats has been demonstrated by Haddow *et al.*(3) and by Hughes, *et al.*,(4). Bahner(1) has shown that 4-(p-dimethylaminostyryl)quinoline dihydrochloride (4M20) is among the most effective members of the series. The present report presents details of a specific, sensitive, fluorimetric method for estimation of 4M20 in biological materials and results of studies on plasma levels, excretion and metabolic fate of the drug in dogs.

Methods. Estimation of 4M20 in plasma, urine and feces. To 2 ml of plasma, urine, or

* Portions of these data were presented in abstract form in *J. Pharmacol. and Exp. Therap.*, 1958, 122.

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TABLE 1. Buffer-Ethylene Dichloride Distribution Studies of Authentic and Apparent 4M20 from Dog Plasma.†

Buffer pH	% extraction of apparent 4M20 from dog plasma*	% extraction of authentic 4M20*
1.09	19.4	18.1
1.24	23.8	23.9
1.48	33.1	32.9
1.78	49.0	45.8
1.99	61.7	61.7

* Averaged results of triplicate determinations.

† Aqueous solutions of authentic 4M20 as well as dog plasma from animals receiving 30 mg/kg intrav. were submitted to $EtCl_2$ extraction procedure. The $EtCl_2$ extracts were washed with buffers of known pH (determined on line-operated Beckman pH meter) and their 4M20 content estimated as described under Methods.

diluted, homogenized feces in 35 ml glass stoppered centrifuge tubes was added 1 ml of 2 N NaOH and 10 ml of ethylene dichloride. The mixture was shaken mechanically 30 minutes according to the method of Woods *et al.*(7), centrifuged and the aqueous layer removed by aspiration. The drug in the ethylene dichloride layer was estimated in the Aminco-Bowman Spectrophotofluorometer at activation wave length of 415 mμ and fluorescence wave length of 519 mμ. Parallel standards were carried through each determination and specificity of the method was demonstrated by means of buffer-ethylene dichloride distribution studies. The method is sufficiently sensitive to permit accurate estimation of 0.05 μg/ml of solution. Plasma levels, urinary and fecal excretion in dogs. A dose of 30 mg/kg of 4M20 was administered orally or intravenously to female mongrel dogs weighing approximately 15 kg. Samples of plasma and urine were collected prior to and after administration of the drug. Urine was collected by means of indwelling catheter for the first 8 hours and cage collected thereafter. Chromatography and characterization of 4M20 metabolites. Samples of urine, feces, and bile were collected from animals which received 30 mg/kg of 4M20 intravenously. Urine and bile in amounts of 0.05 ml were spotted directly on paper (Whatman No. 1). An aqueous alcoholic extract of feces was similarly spotted on the paper. A solvent mixture of n-butanol, acetic acid and water (40, 10, 20) was used for development. Chromatograms of known 4M20 added to urine, feces, and bile were run simultaneously as controls. For separation of larger amounts of the metabolites, bile from dogs which received 30 mg/kg of 4M20 intravenously was spread in uniform line on Whatman 3MM paper 18¼″ x 24½″. The solvent mixture used for development was the same as that described above. The major metabolite isolated in this manner was extracted from the paper with three 200 ml portions of 1:4 mixture of 0.5 N HCl and methanol. The extract was evaporated to dryness in hood and dissolved in 50 ml of the HCl-methanol mixture. Sufficient acetone was added to precipitate any salts present. The mixture was centrifuged and the pink supernatant fluid containing the metabolite was decanted and the solvent removed under vacuum. The metabolite was compared to 4M20 with respect to its solubilities, ultraviolet absorption spectrum in methanol, infra red absorption spectrum, and the melting point of its picrate derivative.

Results. The results of buffer distribution studies (Table I), indicate that the method of analysis is specific for unchanged 4M20. The results of experiments on plasma levels after administration of 30 mg/kg of 4M20, intravenously are shown in Fig. 1. Following administration there was rapid disappearance of the drug from the plasma. The levels ranged from 1.1 to 2.1 μg/ml of plasma initially and were less than 0.1 μg/ml in 5 hours. After oral administration of the same dose, the drug was barely detectable in plasma.

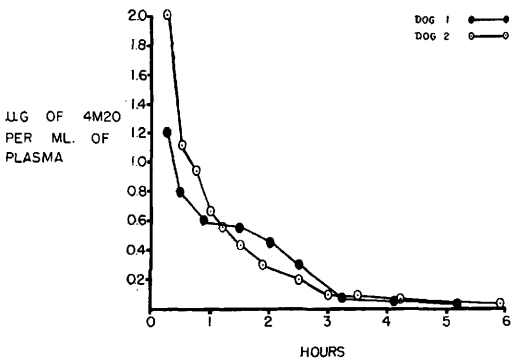


FIG. 1. Plasma levels of 4M20 after intrav. administration of 30 mg/kg of dihydrochloride salt. Figures in graph represent averaged results of duplicate determinations.

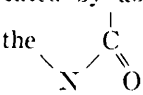
TABLE II. Percent of Total Dose of 4M20 Excreted in Urine and Feces of Dogs after 30 mg/kg Intravenously and Orally.

	I.V. admin.		Oral admin.	
	Urine	Feces	Urine	Feces
Dog 1	.5	Trace*	Trace	Trace
2	"	"	"	"

* Trace = Detectable on paper chromatograms.

Urinary and fecal elimination of 4M20 after 30 mg kg administered intravenously and orally is summarized in Table II. Following administration of the drug by either route, less than 0.5% of the dose of the unchanged drug was found in the urine. Acid hydrolysis of urine did not alter these results significantly. Transient proteinuria was demonstrated after intravenous but not after oral administration. Only traces of unchanged drug were found in feces after intravenous and oral administration.

Paper chromatograms of urine, feces, and bile indicated the presence of at least 6 metabolites (Rf = .06, .10, .13, .21, .37, .48) and traces of unchanged 4M20. These materials range from red to yellow in color and can be readily detected in very small quantities, since they fluoresce intensely under ultraviolet light. There is present in urine, feces and bile a common major metabolite which constitutes approximately 50% of the colored materials detectable on paper chromatograms.

The major metabolite was isolated as the hydrochloride salt as described above. Its properties are summarized and compared with the properties of the hydrochloride salt 4M20 in Table III. Infra red analysis of the metabolite as compared with the spectrum of 4M20 indicated the following changes. (a) one or more oxidations as indicated by presence of the hydroxyl group, (b) introduction of a hydroxyl group at position 2 which is tautomeric with quinolone at position 2 (indicated by absorption band characteristic for the  configuration).

The possibility of a second oxidation cannot be ruled out. Synthesis of the 2-hydroxy-4-(p-dimethylaminostyryl) quinoline resulted in a product whose properties were quite different from those of the metabolite, indicating that some further change has occurred in the molecular structure beyond a simple oxidation at position 2. Furthermore, the high solubility of the metabolite in NaOH as compared to the insolubility of 4M20 in the base would indicate that the metabolite forms a water soluble salt of a phenol. Further attempts to complete the characterization of the metabolite were not successful due primarily to the difficulty in obtaining a crystalline product in a completely pure form.

Discussion. Rapid and almost complete destruction of 4M20 by the dog is quite apparent. The material is metabolized in a variety of ways which is all the more remarkable since in our hands the material appears to be quite stable chemically. The obvious question arises whether one or more of the metabolites might possess anti-tumor activity. This question remains unanswered.

Bahner(2) has studied metabolism of 4-(p-dimethylaminostyryl)-quinoline methiodide and related compounds in the mouse. He

TABLE III. Comparison of Properties of 4M20 and Its Major Metabolite from Dog Bile.

Property		4M20	Metabolite
Solubility of HCl salt	H_2O	Yes	Yes
	dil. HCl	"	"
	dil. NaOH	No	"
	Methanol	Yes	"
Melting point of dihydrochloride salt		178° with decomposition	210-215°C with decomposition
" " " pierate derivative		260-263° with decomposition	276-278°C
" " " free base		133-134°C	Not obtained in crystalline form
Ultraviolet and visible absorption maxima		240 mμ, 290 mμ, 450 mμ, 490 mμ	500 mμ
Rf in butanol, acetic acid, water mixture (40, 10, 20)		.81	.48

reported a 36-47% recovery of this material in feces after oral administration and only trace amounts in urine. It is possible that the methiodide is metabolized in a different manner than is the dihydrochloride and/or that the mouse metabolizes these materials differently than does the dog. However, it should be pointed out that the method of extraction in mouse studies (hot ethanol) is not specific for the unchanged methiodide and that when a similar extraction was performed on dog feces after 4M20 administration the presence of at least 4 similarly colored metabolites was demonstrated.

Kelsey *et al.*(5) and Mead and Koepfli(6) have followed detoxication of quinine by liver slices *in vitro* and found a product whose properties suggest an oxidation occurring at 2 position in the quinoline moiety. This metabolic change would appear to be analogous to that which occurs with 4M20.

Summary. 1. A specific sensitive method for detection in biological materials of 4M20 has been presented. 2. Plasma levels, urinary and fecal excretion of 4M20 in the dog after intravenous and oral administration were studied. These studies indicate almost complete destruction of the material after administration by both routes. Less than 0.5% of unchanged material was recovered in urine after intravenous administration and only traces were found in feces. After oral administration only traces of unchanged material were found in urine and feces. 3. The major metabolite of the drug was isolated by means of chromatography. It has been partially characterized as oxidation product with hydroxylation occurring at position 2. Other undetermined changes also occur in the molecule.

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Effects of Croton Oil on Sugar Absorption by Isolated Surviving Guinea Pig Intestine.* (24752)

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Croton oil has been known to act as a tumor induction promoter or cocarcinogen since 1944(1,2,3). It also has mild carcinogenic properties(4,5,6). In an attempt to ascertain what metabolic mechanisms may be influenced by the presence of croton oil, the effects of this substance on intestinal absorption of sugars, in the presence and absence of some amino acids, have been studied. The experiments were carried out using the perfusion technic of Darlington and Quastel(7). This technic has been fully described and will not be described further except to point out that it consists of the circulation at 37°C of an oxygenated Ringer-bicarbonate medium (the mucosal solution) through the lumen of a segment of surviving guinea pig intestine. The intestinal segment is also bathed by an oxy-

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