cephalexin. The introduction of an amino group in the lateral chain evidently reduced considerably the hydrophobicity of the molecule and, therefore, its retention by the octadecylsilyl column. In penicillins, capacity factors decreased in the order XIV > XIII > XII > XI.

Influence of Methanol Content in Mobile Phase—As expected, the retention of cephalosporins and penicillins by the nonpolar octadecylsilyl stationary phase decreased as the methanol concentration of the mobile phase increased. For the various penicillins and cephalosporins studied, the representation of $\log k'_0$, $\log k'_{-1}$, or $\log k'_{+1}$ *versus* the methanol concentration in the mobile phase, expressed in volume percent, shows a reasonable linear relationship with similar slopes.

Yamana *et al.* (8) used $\log k'$ as a lipophilic index for certain cephalosporins and penicillins, considering it to be analogous to the R_m index determined for cephalosporins and penicillins by means of reversed-phase TLC and comparable to $\log P$, the logarithm of the distribution ratio, determined in *n*-octanol–water systems, which is generally accepted as a lipophilic index. Similarly, $\log k'_0$, $\log k'_{-1}$, and $\log k'_{+1}$ could represent the lipophilic character of each form of penicillins and cephalosporins.

Influence of Salt Concentration—The effect of ionic strength on the capacity factor for I, V, and X was studied (Fig. 5). The mobile phases used were 0.05 *M* phosphate buffers at pH 2.5, 3.5, and 7.0; the ionic strength was varied from 0.51 to 2.0 for each pH value. The influence of the ionic strength on capacity factors at pH 2.5 and 3.5, where I, V, and X are in cationic or zwitterion forms, was very small. At pH 7.0, where these substances are in anionic form, this influence was important, especially for V; it decreased in the order V > X > I.

According to Horvath *et al.* (6), the increase in k' with ionic strength can be attributed to an increase in water surface tension when the salt concentration increases. On the contrary, the ionic form of cephalosporin C does not affect the influence of ionic strength on capacity factors. The k'/μ plots are straight lines of similar slope for each pH value studied, except at pH 2.5 and a very low ionic strength (Fig. 6).

The influence of ionic strength on the retention of II and XII–XIV also was studied at pH 2.6 and 6.0 (Fig. 7). The mobile phase was a mixture of 0.05 *M* phosphate buffer and methanol (2:1 v/v). Plotting k'/μ resulted in lines with negative slopes at the lowest acid pH, where the penicillins are in cationic or undissociated form. At pH 6, where the penicillins and the cephalosporins are in anionic form, the slopes of the k'/μ lines are positive.

It must be emphasized that the ionic strength of the mobile phase has a greater influence on the capacity factors of charged forms than on those of uncharged ones, and it is in the opposite direction. In fact, an increase of the ionic strength increases the retention of the charged forms while decreasing the retention of the uncharged acid forms.

CONCLUSION

The effect of cephalosporin and penicillin ionization on the chromatographic retention by nonpolar stationary phases can be interpreted in terms of hydrophobic interactions between the solute and the hydrocarbon function bound to the support surface. This quantitative study permitted determination of the capacity factors of each molecular form (k'_0, k'_{-1}, and k'_{+1}) of the investigated drugs.

The results given in this work will aid in the separation and analysis of some of the investigated substances.

The logarithms of the capacity factors of each molecular form, being independent of pH, probably will represent more adequately the lipophilic or hydrophobic characteristic of a compound having ionizable groups.

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Hypocholesterolemic Agents VII: Inhibition of β-Hydroxy-β-methylglutaryl-CoA Reductase by Monoesters of Substituted Glutaric Acids

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Abstract □ A series of 1-(4-biphenylyl)pentyl hydrogen 3-alkylglutarates and 3-hydroxy-3-alkylglutarates was synthesized and assayed for inhibition of rat liver β-hydroxy-β-methylglutaryl-CoA reductase. Limited solubility of the monoesters in the enzyme assay system prevented the determination of the I_{50} values. However, the limited data indicated no significant changes in the activity of the analogs when they were assayed at identical concentrations.

Keyphrases □ Hypocholesterolemic agents—inhibition of β-hydroxy-β-methylglutaryl-CoA reductase, monoesters of substituted glutaric acids, rat liver microsomes □ Microsomes, rat liver—inhibition of cholesterol biosynthesis, monoesters of substituted glutaric acids □ Enzyme inhibition—effect of monoesters of glutaric acids on cholesterol biosynthesis, rat liver microsomes

An approach to the design of cholesterol biosynthesis inhibitors as potential hypocholesterolemic agents was discussed previously (1, 2). The rationale for the inhibition of the enzyme β-hydroxy-β-methylglutaryl-CoA reductase was presented (1). Structure–activity relationships indicated that maximum activity was observed when R_1 = biphenylyl, R_2 = *n*-butyl, and n = 1–4 in a series of arylalkyl hydrogen alkanedioates (I). In addition, the incorporation of a β-hydroxy-β-methyl moiety into the acid portion of the glutarate analog provided II, which was seven times more active than the glutarate analog of I.

This paper describes the synthesis and assay of a series of 1-(4-biphenylyl)-*n*-pentyl hydrogen 3-alkylglutarates (III) and 3-hydroxy-3-alkylglutarates (IV). The 3-alkyl series (III) was designed specifically to determine if there was a hydrophobic binding site on the enzyme where the 3-alkyl group of the glutaric acid moiety could bind. The

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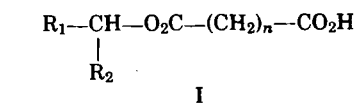
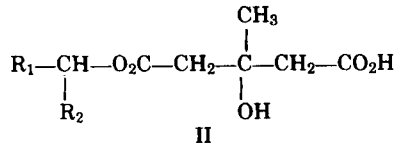
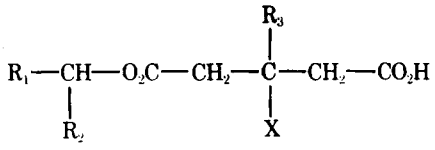


I



II

3-hydroxy-3-alkyl series (IV) was synthesized and evaluated to determine if binding to the hydrogen-bonding site *via* the hydroxyl group and to the hydrophobic site *via* the 3-alkyl group on the enzyme could occur simultaneously.



III: R_3 = alkyl, X = H
IV: R_3 = alkyl, X = OH

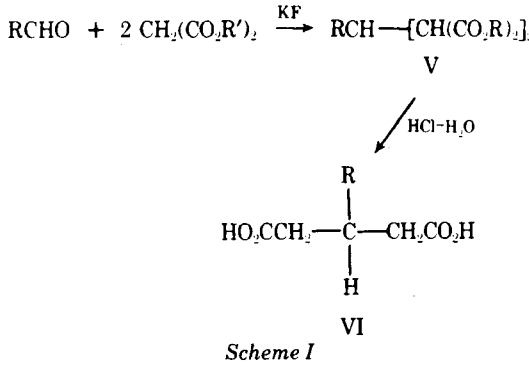
EXPERIMENTAL[1]

Chemistry—The desired monoesters (III and IV) were prepared from 1-(4-biphenylyl)-*n*-pentanol (1) and the appropriate substituted glutaric acid using the method of Büchi *et al.* (3). Synthesis of the required 3-alkylglutaric acids (VI) was performed *via* the pathway illustrated in Scheme I. Condensation of diethyl malonate with aliphatic aldehydes in the presence of potassium fluoride gave the alkylidenedimalonates (V) (4) in 25–85% yields. Acid hydrolysis and decarboxylation of V afforded the desired acids (VI) in 60–80% yields.

The 3-hydroxy-3-alkylglutaric acids (VIII) were synthesized using the pathway illustrated in Scheme II. Treatment of the appropriate ester with the allyl Grignard reagent, as described by Tschesche and Machleidt (5), gave the 4-hydroxy-4-alkyl-1,6-heptadienes (VII) in good yields (80–87%). Ozonolysis of VII followed by treatment with hydrogen peroxide gave the required hydroxy acids (VIII) in 70% yields.

1-(4-Biphenylyl)-n-pentyl Hydrogen 3-Alkyl- and 3-Hydroxy-3-alkylglutarates—The monoesters (IIIa–IIId and IVa–IVd) of the 3-alkylglutaric acids (VI) and 3-hydroxy-3-alkylglutaric acids (VIII) were prepared using the procedure of Büchi *et al.* (3) as described previously (2). The crude oily monoesters were chromatographed on silicic acid. Elution with 20% ether–petroleum ether (bp 60–75°) afforded colorless oily monoesters. The monoesters were characterized as their solid *S*-benzylthiuronium salts using the method of Donleavy (6) as described previously (1). Table I lists the analytical data.

3-Alkylglutaric Acids (VI)—The 3-*n*-propyl- (7), 3-isopropyl- (8), and 3-isobutyl- (9) glutaric acids were prepared by acid hydrolysis and decarboxylation of the appropriate tetraethyl alkylidenedimalonate (4). The preparation of 3-*n*-butylglutaric acid serves as an example.



Scheme I

A mixture of 26.0 g (0.067 mole) of tetraethyl *n*-butylidenedimalonate (4), 250 ml of water, and 250 ml of concentrated hydrochloric acid was refluxed for 24 hr. The resulting solution was extracted with ethyl acetate. The organic phase was washed three times with water, washed with a saturated sodium chloride solution, and then dried over anhydrous sodium sulfate. The solvent was removed to give 9.9 g (78%) yield of a pale-yellow oil; NMR ($CDCl_3$): δ 0.35–0.82 (m, 9H, C_4H_9), 1.2 (s, 4H, CH_2CCH_2), 1.93–2.17 (m, 1H, CH), and 5.80 [s, 2H, $(CO_2H)_2$] ppm. The oil was characterized analytically as the white *S*-benzylthiuronium salt (1), mp 165–167°.

Anal.—Calc. for $C_{25}H_{36}N_4O_4S_2$: C, 57.7; H, 6.9; N, 10.8. Found: C, 57.8; H, 7.1; N, 10.8.

3-Hydroxy-3-alkylglutaric Acids (VIII)—The acids were prepared by ozonolysis followed by hydrogen peroxide oxidation of the appropriate 4-hydroxy-4-alkyl-1,6-heptadiene to afford the desired 3-hydroxy-3-alkylglutaric acid (VIII). The 4-hydroxy-4-alkyl-1,6-heptadienes were prepared using the method of Tschesche and Machleidt (5). The preparation of 4-hydroxy-4-isobutyl-1,6-heptadiene serves as an example.

To a stirred suspension of 4.62 g (0.19 g atom) of magnesium turnings in 8 ml of dry ether, 8 ml of dry tetrahydrofuran, and a crystal of iodine was added dropwise a solution of 6.5 g (0.05 mole) of ethyl isovalerate and 14 ml (0.16 mole) of allyl bromide in 19 ml of dry ether and 56 ml of dry tetrahydrofuran. The reaction mixture was maintained at a gentle reflux during the addition and was refluxed for 3 hr after the addition was complete. The resulting mixture was poured onto ice–hydrochloric acid and then extracted with ether.

The organic phase was washed with water, 5% NaOH, water, and finally saturated sodium chloride solution. It then was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 9.4 g of an orange liquid. Vacuum distillation afforded 7.8 g (81% yield) of a colorless liquid, bp 50–51° (0.65 mm Hg); NMR ($CDCl_3$): δ 0.95 [d, 6H, $C(CH_3)_2$], 1.39 (d, 2H, CH_2), 1.56 (s, 1H, OH), 1.69 (m, 1H, R_3CH), 2.25 [d, 4H, $(C{=}CCH_2)_2$], 4.89–5.43 [m, 4H, $(C{=}CH_2)_2$], and 5.54–6.31 [m, 2H, $(C{=}CH)_2$] ppm.

Anal.—Calc. for $C_{11}H_{20}O$: C, 78.5; H, 12.0. Found: C, 78.4; H, 12.0.

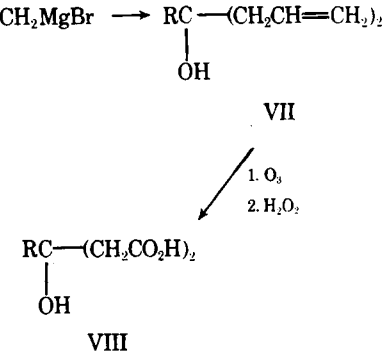
The following 4-hydroxy-4-alkyl-1,6-heptadienes were prepared using the described procedure: 4-hydroxy-4-*n*-propyl-1,6-heptadiene, bp 39–41° (2.0 mm Hg) [lit. (10) bp 70° (17 mm Hg)]; 4-hydroxy-4-isopropyl-1,6-heptadiene, bp 48–51° (1.5 mm Hg) [lit. (10) bp 43–45° (1.3 mm Hg)]; and 4-hydroxy-4-*n*-butyl-1,6-heptadiene, bp 93–95° (6 mm Hg) [lit. (10) bp 90–92° (6 mm Hg)].

3-Hydroxy-3-n-propylglutaric Acid—The method of Tschesche and Machleidt (5) was used. A solution containing 4.16 g (0.027 mole) of 4-hydroxy-4-*n*-propyl-1,6-heptadiene, 3.5 ml of acetic acid, and 43 ml of methylene chloride was cooled in an isopropanol–dry ice bath. Ozone was passed through the solution for 2 hr, and the resulting blue solution was allowed to warm to 25°. To the clear, colorless solution was added 25 ml of acetic acid, and the methylene chloride was removed under reduced pressure.

To the residue were added 25 ml of acetic acid and 25 ml of 30% H_2O_2. The solution was heated at reflux for 8 hr, and removal of the solvent under reduced pressure afforded 3.69 g of a colorless oil. Trituration with petroleum ether (bp 60–75°) afforded 3.6 g (70% yield) of a white solid, mp 90–94°. Two recrystallizations from ether–petroleum ether (bp 60–75°) afforded a white solid, mp 96–98°; NMR (dimethyl sulfoxide-d_6): δ 0.54–1.77 [m, 7H, $CH_3(CH_2)_2$], 2.51 [s, 4H, $(CH_2COO)_2$], and 6.11 [broad s, 3H, OH, $(COOH)_2$] ppm.

Anal.—Calc. for $C_8H_{14}O_5$: C, 50.5; H, 7.4. Found: C, 50.5; H, 7.4.

3-Hydroxy-3-isopropylglutaric Acid—The procedure described for



VII

VIII

Scheme II

[1] Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were carried out by Atlantic Microlaboratories, Atlanta, Ga. IR spectra were determined using a Perkin-Elmer model 257 spectrophotometer. NMR spectra were obtained using a Perkin-Elmer model R-24 spectrometer in the solvent specified with tetramethylsilane as the internal reference.

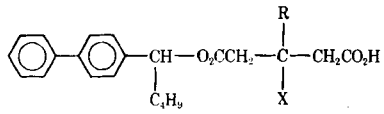


Table I—Properties of Monoesters of Substituted Glutaric Acids

Compound	R	X	Yield[a], %	Melting Point (Salt)	Formula	Analysis, %	Calc.	Found
III*a*	$CH_3(CH_2)_2$	H	35	116–117°	$C_{33}H_{42}N_2O_4S$	C	70.4	70.4
						H	7.5	7.5
						N	5.0	5.0
III*b*	$(CH_3)_2CH$	H	34	122–123°	$C_{33}H_{42}N_2O_4S$	C	70.4	70.5
						H	7.5	7.5
						N	5.0	5.0
III*c*	$CH_3(CH_2)_3$	H	38	106–108°	$C_{34}H_{44}N_2O_4S$	C	70.8	70.7
						H	7.7	7.7
						N	4.9	4.9
III*d*	$(CH_3)_2CHCH_2$	H	32	136–138°	$C_{34}H_{44}N_2O_4S$	C	70.8	70.6
						H	7.7	7.7
						N	4.9	4.8
IV*a*	$CH_3(CH_2)_2$	OH	20	110–112°	$C_{33}H_{42}N_2O_5S$	C	68.4	68.4
						H	7.3	7.3
						N	4.8	4.8
IV*b*	$(CH_3)_2CH$	OH	22	118–120°	$C_{33}H_{42}N_2O_5S$	C	68.4	68.2
						H	7.3	7.4
						N	4.8	4.9
IV*c*	$CH_3(CH_2)_3$	OH	17	101–103°	$C_{34}H_{44}N_2O_5S$	C	68.9	68.8
						H	7.5	7.5
						N	4.7	4.7
IV*d*	$(CH_3)_2CHCH_2$	OH	19	121–122°	$C_{34}H_{44}N_2O_5S$	C	68.9	68.7
						H	7.5	7.5
						N	4.7	4.7

[a] The monoesters were colorless, viscous oils. The IR (chloroform) and NMR (deuterochloroform) spectra were consistent with the assigned structures.

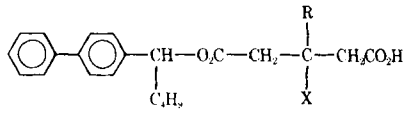


Table II—Inhibition of β-Hydroxy-β-methylglutaryl-CoA Reductase

Compound	R	X	Activity (I/S)[a] 0.5	1.0	2.0	2.5
IX	CH_3	H	—	—	50[b]	—
III*a*	$CH_3(CH_2)_2$	H	19	28	Insoluble	—
III*b*	$(CH_3)_2CH$	H	13	24	Insoluble	—
III*c*	$CH_3(CH_2)_3$	H	16	27	Insoluble	—
III*d*	$(CH_3)_2CHCH_2$	H	17	28	Insoluble	—
X	CH_3	OH	—	—	50[c]	—
IV*a*	$CH_3(CH_2)_2$	OH	—	17	52	Insoluble
IV*b*	$(CH_3)_2CH$	OH	—	15	43	Insoluble
IV*c*	$CH_3(CH_2)_3$	OH	17	22	28	Insoluble
IV*d*	$(CH_3)_2CHCH_2$	OH	—	22	36	Insoluble
XI	H	H	—	—	58[d]	—

[a] The inhibition index, I/S, equals the ratio of the micromolar concentration of the inhibitor to the micromolar concentration of the substrate required to give the percentage inhibition shown. At least two sets of duplicate determinations were used. [b] I/S = 9 as reported previously (2). [c] I/S = 1.5 as reported previously (2). [d] A standard inhibitor used routinely to provide comparability of each assay set (2); I/S = 7.

3-hydroxy-3-*n*-propylglutaric acid was used to give 3-hydroxy-3-isopropylglutaric acid (73% yield) as a white solid, mp 87–92°. Two recrystallizations from ethyl acetate–petroleum ether (bp 60–75°) afforded a white solid, mp 99–101°; NMR (dimethyl sulfoxide-d_6): δ 0.93 [d, 6H, $C(CH_3)_2$], 1.30–2.12 (m, 2H, OH, R_3CH), 2.63 [s, 4H, $(CH_2COO)_2$], and 8.00 [broad s, 3H, OH, $(COOH)_2$] ppm.

Anal.—Calc. for $C_8H_{14}O_5$: C, 50.5; H, 7.4. Found: C, 50.4; H, 7.4.

*3-Hydroxy-3-*n*-butylglutaric Acid*—The procedure described for 3-hydroxy-3-*n*-propylglutaric acid was used to give 3-hydroxy-3-*n*-butylglutaric acid (68% yield) as a white solid, mp 66–76°. Two recrystallizations from ethyl acetate–petroleum ether (bp 60–75°) afforded a white solid, mp 76–78°; NMR ($CDCl_3$): δ 0.67–2.23 [m, 9H, $CH_3(CH_2)_3$], 2.78 (s, 4H, $(CH_2COO)_2$], and 8.70 [broad s, 3H, OH, $(COOH)_2$] ppm.

Anal.—Calc. for $C_9H_{16}O_5$: C, 52.9; H, 7.9. Found: C, 53.0; H, 7.9.

3-Hydroxy-3-isobutylglutaric Acid—The procedure described for 3-hydroxy-3-*n*-propylglutaric acid was used to give 3-hydroxy-3-isobutylglutaric acid (70% yield) as a white solid, mp 90–98°. Recrystallization from ethyl acetate–petroleum ether (bp 60–75°) afforded a white solid, mp 106–108°; NMR (dimethyl sulfoxide-d_6): δ 0.93 [d, 6H, $C(CH_3)_2$], 1.49 (d, 2H, CH_2), 2.06 (m, 1H, R_3CH), 2.61 [s, 4H, $(CH_2COO)_2$], and 5.66 [broad s, 3H, OH, $(COOH)_2$] ppm.

Anal.—Calc. for $C_9H_{16}O_5$: C, 52.9; H, 7.9. Found: C, 52.8; H, 7.9.

Preparation of Rat Liver Microsomes—Rat liver microsomes containing β-hydroxy-β-methylglutaryl-CoA reductase activity were prepared according to the method of Shapiro and Rodwell (11) as described previously (2).

Enzyme Activity and Inhibition—The assay (2) was modified with regard to the isolation of mevalonolactone. The enzyme incubation mixture consisted of $NADP^+$ (9.7 μmoles)[2], glucose-6-phosphate[2] (59.7 μmoles), torula yeast glucose-6-phosphate dehydrogenase[2] (5 units), microsomal protein (2 mg) as determined by a modification of the method of Lowry *et al.* (2, 12), 0.1 ml of ethylene glycol monoethyl ether containing the inhibitor, and sufficient assay buffer [30 m*M* ethylenediaminetetraacetic acid, 70 m*M* NaCl, and 10 m*M* β-mercaptoethanol (pH 6.8)] to give a total volume of 2.9 ml.

Enzymatic activity was initiated by the addition of 0.1 ml of a solution containing 94 nmoles (31 μ*M*) of *dl*-β-hydroxy-β-methyl[3-^{14}C]glutaryl-CoA[3] (specific activity of 920 dpm/nmole). After 25 min of shaking incubation at 37°, the reaction was stopped by the addition of 0.2 ml of concentrated hydrochloric acid. Fifty microliters of an acetone solution containing 7×10^5 dpm (24 μmoles) of *dl*-4-[^{3}H]mevalonolactone[4] was added to the incubation mixture, and the mixture was incubated at 37°

[2] Sigma Chemical Co.
[3] P-L Biochemicals.
[4] Amersham Corp.

for 30 min. To the mixture was added 3.2 g of anhydrous sodium sulfite, and this mixture was extracted twice with 10-ml portions of benzene (13).

The benzene extract was evaporated to dryness in a liquid scintillation vial at 50° under a nitrogen stream. Twenty milliliters of Bray's scintillation solution (14) was added, and tritium and carbon 14 were determined simultaneously in a liquid scintillation counter[5]. Counting efficiency was determined by the external standard method. Some variability in the inhibition data was noted between tubes of microsomes (even from the same liver preparation). For this reason, a standard inhibitor, 1-(4-biphenylyl)pentyl hydrogen succinate (IX), was utilized routinely.

RESULTS AND DISCUSSION

The activity of IIIa–IIId and IVa–IVd as inhibitors of rat liver β-hydroxy-β-methylglutaryl-CoA reductase is shown in Table II. Unfortunately, the low solubility of these compounds in the enzyme assay prevented the determination of I/S values required for 50% inhibition. Thus, no definite conclusions can be made concerning the effect of increasing the size of the alkyl group at the C-3 position of the glutaric acid moiety with or without a C-3 hydroxyl group. However, the limited data do indicate that no substantial changes in inhibitory activity resulted from these modifications [compare 3-hydroxy-3-methyl (X) to 3-hydroxy-3-*n*-propyl (IVa) and the similar degree of inhibition shown by the analogs when assayed at identical I/S values].

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Comparison of Dye Dilution Method to Radionuclide Techniques for Cardiac Output Determination in Dogs

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Abstract □ A study was undertaken to identify the most accurate ^{99m}Tc-labeled radiopharmaceutical and to determine the accuracy of a noninvasive radionuclide technique (antecubital injection and precordial detection) for cardiac output determinations. Phase I employed sodium pertechnetate, stannous pyrophosphate with sodium pertechnetate, technetium-99*m* red blood cells, and technetium-99*m* human serum albumin as radionuclide tracers. Cardiac output was determined by the dye dilution method and then by the invasive radionuclide technique. The radiopharmaceutical was injected into the same intracardiac catheter used in the dye dilution method. Seven to 10 mongrel dogs were used to test the accuracy of each radiopharmaceutical. A paired *t* test and regression analysis indicated that technetium-99*m* human serum albumin was the most accurate radiopharmaceutical for cardiac output determinations, and the results compared favorably to those obtained by the dye dilution method. In Phase II, technetium-99*m* human serum albumin was used as the radionuclide tracer for cardiac output determinations with the noninvasive technique. The results compared favorably to those obtained by the dye dilution method. Regression analysis indicated a correlation coefficient of 0.91. A paired *t* test demonstrated that the difference between the two methods was not statistically significant ($p > 0.05$). The data suggest that a noninvasive radionuclide technique using an intravascular radiopharmaceutical may be safe and nontraumatic for cardiac output determinations in humans.

Keyphrases □ Cardiac output—determination, comparison of dye dilution method and radionuclide techniques, dogs □ Dye dilution method—determination of cardiac output, comparison to radionuclide techniques, dogs □ Radionuclides—use in determination of cardiac output, comparison to dye dilution method, dogs

Measurement of cardiac output using readily detectable tracers such as indocyanine green[1] or suitable radionuclides is based on the principle of conservation of mass (1–3). The direct Fick method involves measuring the amount of oxygen extracted from the inspired air by the lungs and the arteriovenous oxygen difference (4–6). The Stewart–Hamilton dye dilution method is analogous to the direct Fick method (7–9). The validity and reproducibility of this method were verified by several investigators (10–14). The relative ease of detection and quantitation of radioactive tracers have resulted in the use of radiopharmaceuticals for cardiac output determinations (15–29). However, the necessity for cardiac catheterization and cannulation usually limits their usefulness.

[1] Cardio-green, Hynson, Westcott and Dunning, Baltimore, Md.

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