ticular note was the failure of many attempts to obtain crystalline products of I or analogs of I when they were attached to Co(III) or Cr(III), although the iminodiacetic acid and methyl iminodiacetic acid complexes are prepared and isolated easily. It is hoped that the approach outlined here for gaining information on the structure of ^{99m}Tc-labeled radiopharmaceuticals will be useful for understanding the mechanisms of localization of currently used radiopharmaceuticals and in the design of new radiopharmaceuticals.

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ACKNOWLEDGMENTS

Supported by U.S. Public Health Service Grants GM10548 and HL17655.

Radiolabeled Benzoylcholine Derivatives as Possible Myocardial-Imaging Agents

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Received February 22, 1980, from the *Division of Nuclear Medicine and the Department of Chemistry, Johns Hopkins University, Baltimore, MD 21205.* Accepted for publication September 25, 1980.

Abstract □ Two radioiodinated analogs of benzoylcholine were investigated as possible myocardial-imaging agents. *O*-([2-^{125}I]Iodobenzoyl)-choline and *N*-([2-^{125}I]iodobenzoyl)cholamine were prepared by nucleophilic substitution of sodium [^{125}I]iodide for stable iodine in *O*-(*N,N*-dimethylaminoethyl)-2-iodobenzoate and *N*-(*N',N'*-dimethylaminoethyl)-2-iodobenzamide, respectively, and by methylation with methyl iodide. The *in vivo* distribution of each compound in mice was determined as a function of time. Favorable heart-to-blood and heart-to-lung ratios were obtained with *N*-([2-^{125}I]iodobenzoyl)cholamine.

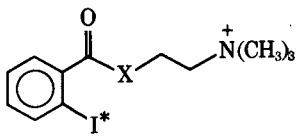
Keyphrases □ Benzoylcholine, radioiodinated analogs—synthesized and evaluated as possible myocardial-imaging agents, biodistribution studies in mice □ Radionuclide imaging, myocardial—radioiodinated benzoylcholine analogs synthesized and evaluated for use as myocardial-imaging agents, mice □ Radiopharmaceuticals, iodinated—benzoylcholine analogs, synthesis and evaluation for use as myocardial-imaging agents, mice □ Biodistribution—radioiodinated benzoylcholine analogs synthesized and evaluated for use as myocardial-imaging agents, mice

The use of radiolabeled enzyme inhibitors as potential organ-imaging agents has been suggested by several investigators. Wieland *et al.* (1) studied the distribution of several radiolabeled inhibitors of the adrenal cortex enzymes, 20α-hydroxylase, 11β-hydroxylase, and 17α-hydroxylase. The finding that some of these inhibitors localized in the adrenal cortex suggested that radiolabeled enzyme inhibitors might be useful in the design of new diagnostic radiopharmaceuticals.

BACKGROUND

Recently, Burns *et al.* (2) studied the distribution of a simple, positively charged inhibitor of acetylcholinesterase. Their results suggested that labeled inhibitors of acetylcholinesterase might be useful as myocardial-imaging agents due to relatively high levels of acetylcholinesterase activity in the heart of some species.

Another study demonstrated that the cholinesterases of erythrocytes and plasma were different (3). The work of several investigators (4–8) clearly established the distinguishing characteristics between acetylcholinesterase (true cholinesterase or acetylcholine hydrolase) and pseudocholinesterase (butyrylcholinesterase or acylcholine acylhydrolase). Acetylcholinesterase predominates in erythrocytes, the central nervous system, and the motor endplates of skeletal muscle. Pseudocholinesterase predominates in the liver, plasma, and many types of smooth muscle. Both enzymes occur in high concentrations in autonomic ganglia (9). In some species, the distribution of pseudocholinesterase activity (10) indicates that an appropriate radiolabeled substrate or inhibitor might be a useful imaging agent for the myocardium.



V: X = O
VI: X = NH

Many compounds are hydrolyzed by pseudocholinesterase including butyrylcholine, acetylcholine, and benzoylcholine. The fact that benzoylcholine is a substrate for butyrylcholinesterase, coupled with the observation that iodoaryl compounds are generally resistant to *in vivo* deiodination (11), prompted the investigation of two radioiodinated analogs of benzoylcholine as potential myocardial-imaging agents. This report details the syntheses and preliminary biological studies of *O*-([2-^{125}I]iodobenzoyl)choline (V) and *N*-([2-^{125}I]iodobenzoyl)cholamine (VI).

EXPERIMENTAL[1]

***O*-(*N,N*-Dimethylaminoethyl)-2-iodobenzoate (I)**—2-Iodobenzoic acid (19.7 g, 0.077 mole), thionyl chloride (19.2 g, 0.162 mole), and a catalytic amount of dimethylformamide (50 μl) were warmed gently in a water bath. The evolution of hydrogen chloride and sulfur dioxide ceased after 60 min, and the solution was evaporated under reduced pressure to remove excess thionyl chloride. Benzene (100 ml) was added to the

[1] PMR spectra were obtained on a Varian T-60 spectrometer, with chemical shifts reported relative to tetramethylsilane or 3-(trimethylsilyl)propionic acid sodium salt as noted. IR spectra were recorded on a Pye-Unicam SP-1000 spectrometer. Melting points were determined in a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Robertson Microanalytical Laboratory, Florham Park, N.J. Radioactive iodine was obtained from New England Nuclear Corp. as a carrier-free solution of sodium [^{125}I]iodide in a pH 8–10 aqueous solution (reductant free) at a specific activity of ~350 mCi/ml. The solution was diluted with normal saline to a specific activity of 10 mCi/ml.

0022-3549/81/0400-0439$01.00/0

light-yellow oil that remained, and 2-dimethylaminoethanol (6.59 g, 0.074 mole) was added dropwise with stirring. After refluxing for 120 min, the solution was allowed to cool to room temperature.

The hydrochloride salt of the product was extracted into water (75 ml). Sodium hydroxide (5 ml, 10%) was added to the aqueous phase, which was extracted with ether (25 ml). This process was repeated five times. The ether extracts were dried (magnesium sulfate) and then concentrated under reduced pressure to yield 14.6 g (62%) of pale-yellow oil. The product was isolated as the hydrochloride salt for analysis, mp 162–163.5°; IR (mineral oil): 1725 (C=O) cm^{-1}; PMR [deuterium oxide, 3-(trimethylsilyl)propionic acid sodium salt]: δ 3.00 [s, 6H, $N(CH_3)_2$], 4.50–4.73 (m, 4H, CH_2CH_2), 6.80–7.43 (m, 2H, aromatic H-4,H-5), and 7.60–7.82 (m, 2H, aromatic H-3,H-6) ppm.

Anal.—Calc. for $C_{11}H_{14}INO_2 \cdot HCl$: C, 37.15; H, 4.25; N, 3.94. Found: C, 36.95; H, 4.40; N, 3.81.

***O*-(2-Iodobenzoyl)choline Iodide (II)**—Methyl iodide (7.1 g, 50 mmoles) was added to a solution of I (4.0 g, 12.5 mmoles) in chloroform (50 ml). The solution was refluxed for 60 min and then allowed to cool to room temperature. The precipitate that formed was collected by filtration, washed twice with ether, and dried *in vacuo*, yielding 5.48 g (95%) of white needles, mp 148–149°; IR (mineral oil): 1720 (C=O) cm^{-1}; PMR (dimethyl sulfoxide-d_6, tetramethylsilane): δ 3.40 [s, 9H, $^+N(CH_3)_3$], 4.05 (m, 2H, CH_2N), 4.80 (broad s, 2H, $COOCH_2$), 7.12–7.70 (m, 2H, aromatic H-4,H-5), and 7.80–8.13 (m, 2H, aromatic H-3,H-6) ppm.

Anal.—Calc. for $C_{12}H_{17}I_2NO_2$: C, 31.26; H, 3.72; N, 3.04. Found: C, 31.11; H, 3.72; N, 2.99.

***N*-(*N'*,*N'*-Dimethylaminoethyl)-2-iodobenzamide (III)**—2-Iodobenzoic acid (5.0 g, 0.02 mole), thionyl chloride (5.05 g, 0.042 mole), and a catalytic amount of dimethylformamide (50 μl) were warmed gently in a water bath. The solution was treated as was I to yield a light-yellow oil, which was added to a solution of *N*,*N*-dimethylethylenediamine (1.70 g, 0.019 mole) in 10% NaOH (50 ml). After stirring for 30 min at room temperature, the precipitate that formed was collected by filtration and recrystallized from hot ethanol–water to yield 4.27 g (70%) of white crystals, mp 113.5–115.5°; IR (mineral oil): 1640 (C=O) and 3160 (N–H) cm^{-1}; PMR (chloroform-*d*, tetramethylsilane): δ 2.10 [s, 6H, $N(CH_3)_2$], 2.45 (t, 2H, CH_2N), 3.44 (q, 2H, $CONHCH_2$), 6.73 (broad s, 1H, CONH), 6.90–7.42 (m, 3H, aromatic H), and 7.78 (d, 1H, aromatic H *ortho* to amide) ppm.

Anal.—Calc. for $C_{11}H_{15}IN_2O$: C, 41.53; H, 4.75; N, 8.80. Found: C, 41.54; H, 4.80; N, 8.69.

***N*-(2-Iodobenzoyl)cholamine (IV) Iodide**—Methyl iodide (1.12 g, 7.8 mmoles) was added to a solution of III (2.5 g, 7.8 mmoles) in chloroform (50 ml), and then the solution was stirred for 60 min at room temperature. The precipitate that formed was collected by filtration and recrystallized from 1-propanol to yield 2.56 g (71%) of white crystals, mp 156–157.5°; IR (mineral oil): 1650 (C=O) and 3300 (N–H) cm^{-1}; PMR (dimethyl sulfoxide-d_6, tetramethylsilane): δ 3.35 [s, 9H, $^+N(CH_3)_3$], 3.75 (broad s, 4H, CH_2CH_2), 7.11–7.63 (m, 3H, aromatic H), 7.93 (d, 1H, aromatic H *ortho* to amide), and 8.97 (broad s, 1H, CONH) ppm.

Anal.—Calc. for $C_{12}H_{18}I_2N_2O$: C, 31.33; H, 3.94; N, 6.09. Found: C, 31.46; H, 4.04; N, 6.02.

***O*-([2-^{125}I]Iodobenzoyl)choline (V) Chloride**—A small aliquot of an aqueous stock solution of sodium [^{125}I]iodide (10 mCi/ml) containing 0.1 mCi was mixed with methanol (100 μl) and evaporated to dryness in a 1-ml serum vial. Compound I (10 mg, 0.03 mmole) was added to the vial, which was sealed with a vented polytef-lined stopper. The vial was heated in an oil bath at 103° for 20 min and then was allowed to cool to room temperature. A saturated sodium metabisulfite solution (250 μl) was added to the vial, and the pH of the resulting solution was adjusted to 11 with 10% NaOH. The solution was extracted three times with chloroform (500 μl). The chloroform extracts, which contained 60% of the initial activity, were combined and dried over magnesium sulfate.

Methyl iodide (100 μl, 1.6 mmoles) was added to the solution, which was refluxed for 30 min and then cooled to room temperature. The chloroform was extracted twice with normal saline (1 ml). More than 95% of the remaining activity was found in the saline extracts, resulting in an overall radiochemical yield of 57%. The saline extracts were passed through a 1 × 3-cm column of anion-exchange resin[2] eluted with normal saline. The solution was passed through a 0.22-μm filter[3] and diluted with saline to an activity of 10 μCi/ml. The specific activity of the product was ~1.9 mCi/mmole. The radiochemical purity was verified by TLC prior to the biodistribution studies.

***N*-([2-^{125}I]Iodobenzoyl)cholamine (VI) Chloride**—A small aliquot (0.1 mCi) of an aqueous stock solution of sodium [^{125}I]iodide was prepared as described for V. Compound III (10 mg, 0.03 mmole) was added to the vial, which was sealed with a vented polytef stopper and immersed in an oil bath at 120° for 15 min. After the vial was allowed to cool to room temperature, the reaction mixture was treated as described for V. The chloroform extracts contained 48% of the initial activity, and the saline extracted >95% of the remaining activity, resulting in an overall radiochemical yield of 46%. The specific activity of the product was ~1.6 mCi/mmole. The radiochemical purity was verified by chromatographic methods prior to use in the biodistribution studies.

TLC[4]—The radiochemical purity of the products was verified using TLC with the following solvent systems: acetone–water (4:1), chloroform–methanol (4:1), and chloroform–methanol–30% ammonium hydroxide (10:1.5:0.1). Table I shows the R_f values determined for I–IV, 2-iodobenzoic acid, sodium [^{125}I]iodide, and 2-iodohippuric acid.

Biodistribution Studies—The tissue distributions of V and VI were determined as a function of time in normal ICR mice. The mice, 30–40 g, were divided in groups of six and sacrificed under ether anesthesia at 5, 30, 60, and 120 min following the intravenous administration of 1 μCi of either compound in 0.1 ml of saline. Samples of blood, heart, lungs, kidneys, liver, spleen, and muscle were taken and counted in a scintillation well counter. Care was taken to prevent urine from contaminating the carcass when the bladder was removed. The results were expressed as the percentage of injected dose per organ and per gram of wet tissue.

Samples of urine were collected at various intervals and developed on TLC using the described solvent systems. The radiochromatograms were compared with the original samples.

RESULTS AND DISCUSSION

Standard methods of electrophilic carrier-free iodinations (*e.g.*, lactoperoxidase or *N*-chloro-*p*-toluenesulfonamide sodium salt) are not useful for preparation of iodoaryl compounds where the aromatic ring contains deactivating groups. However, halogens bound to an aromatic ring in a position *ortho* to an electron-withdrawing group are activated with respect to nucleophilic substitution. While this method does not produce the high specific activity product produced by electrophilic iodination, it does allow for the rapid incorporation of radioactive iodine into a molecule of known structure.

Labeling of both I and III by nucleophilic isotopic exchange under high temperature conditions was achieved without significant decomposition, as confirmed by TLC. The reaction products were methylated easily with methyl iodide and extracted into saline for use in the biodistribution studies. Compound V was stable in aqueous solution for several weeks when refrigerated at 5°, and VI was stable for several months under the same conditions.

The biodistributions of V and VI were determined at 5, 30, 60, and 120 min postinjection (Tables II–V). Compound VI cleared rapidly from the blood, with only 1.7% of the injected dose remaining in circulation 5 min after injection. More than 30% of the injected dose was excreted *via* the kidneys after 5 min postinjection. Chromatography of urine samples at various intervals indicated that VI was excreted unchanged, with no evidence of amide hydrolysis or deiodination. Its accumulation in the heart was significant, 6.4% of the dose/g at 5 min postinjection. Although the activity in the heart did not remain constant, the compound cleared slowly enough that heart-to-blood ratios of >6 and heart-to-lung ratios of >2 were maintained at least 2 hr after administration (Table VI).

Unlike VI, V exhibited no significant accumulation in the heart, with heart-to-blood and heart-to-lung ratios of <1 at all times studied (Table VI). Compound V cleared slowly from the circulation, with 17.4% of the injected dose remaining after 5 min and ~3% remaining after 30 min. The clearance from the body was rapid, with only 25% of the injected dose remaining after 30 min and <10% remaining after 60 min.

Excretion of V was primarily *via* the kidneys. Chromatography of urine samples at various intervals indicated that the compound was hydrolyzed and excreted in the form of [2-^{125}I]iodobenzoic acid. The fate of the hydrolyzed choline could not be determined by these experiments. The observation that 2-iodobenzoic acid, which is generated by the hydrolysis of V, was excreted intact was unexpected. Substituted benzoic acids are conjugated *in vivo* with glycine to form the corresponding substituted hippuric acids prior to excretion (12). However, TLC of an authentic sample of 2-iodohippuric acid[5] in acetone–water and chloroform–methanol showed that it was excreted as 2-iodobenzoic acid.

[2] Bio-Rad AG-1X8, chloride form.
[3] Type GS, 0.22 μm, Millipore Corp.
[4] Chromatography was performed on Baker-flex silica gel IB-F (7.5 × 2.5 cm).
[5] Hippuran, Mallinckrodt Chemical Works, St. Louis, Mo.

Table I—TLC R_f Values

System[a]	I	II	III	IV	Sodium [^{125}I]Iodide	2-Iodobenzoic Acid	2-Iodohippuric Acid
A	0.60	0.24	0.25	0.20	0.71	0.75	0.49
B	0.60	0.11	0.55	0.12	0.31	0.50	0.11
C	0.66	0.04	0.70	0.06	0.60	0.10	0.00

[a] A = acetone–water (4:1), B = chloroform–methanol (4:1), and C = chloroform–methanol–ammonium hydroxide (10:1.5:0.1).

Table II—Biodistribution of *O*-([2-^{125}I]Iodobenzyl)choline in Mice

Organ	Mean Percent of Dose per Organ ± *SD*[a]			
	5 min	30 min	60 min	120 min
Blood[b]	17.44 ± 1.97	3.05 ± 1.02	0.45 ± 0.25	0.18 ± 0.07
Heart	0.52 ± 0.07	0.08 ± 0.02	0.01 ± 0.01	—[c]
Lungs	1.07 ± 0.15	0.23 ± 0.06	0.03 ± 0.01	0.02 ± 0.01
Kidneys	14.38 ± 1.94	4.60 ± 2.80	0.34 ± 0.17	0.18 ± 0.24
Liver	5.64 ± 0.85	1.40 ± 0.36	0.22 ± 0.08	0.09 ± 0.03
Spleen	0.19 ± 0.03	0.07 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
Carcass	41.37 ± 4.19	15.70 ± 4.72	5.52 ± 3.92	1.86 ± 0.72
Total	80.6	25.1	6.5	2.3

[a] Six mice studied at each time. [b] Based on 7% of the body weight. [c] Not significantly above background.

Table III—Biodistribution of *O*-([2-^{125}I]Iodobenzoyl)choline in Mice

Organ	Mean Percent of Dose per Gram ± *SD*[a]			
	5 min	30 min	60 min	120 min
Blood	6.08 ± 1.28	1.43 ± 0.44	0.19 ± 0.12	0.09 ± 0.03
Heart	3.36 ± 0.31	0.81 ± 0.29	0.09 ± 0.05	—[b]
Lungs	4.56 ± 0.48	0.99 ± 0.25	0.16 ± 0.06	0.07 ± 0.04
Kidneys	27.88 ± 3.59	12.36 ± 6.99	0.80 ± 0.51	0.36 ± 0.47
Liver	2.91 ± 0.47	0.77 ± 0.19	0.13 ± 0.06	0.05 ± 0.01
Spleen	1.69 ± 0.43	0.54 ± 0.20	0.10 ± 0.04	0.05 ± 0.02
Muscle	1.15 ± 0.12	0.40 ± 0.17	0.07 ± 0.06	0.05 ± 0.08

[a] Six mice studied at each time. [b] Not significantly above background.

Table IV—Biodistribution of *N*-([2-^{125}I]Iodobenzoyl)cholamine in Mice

Organ	Mean Percent of Dose per Organ ± *SD*[a]			
	5 min	30 min	60 min	120 min
Blood[b]	1.68 ± 0.23	0.59 ± 0.18	0.57 ± 0.13	0.23 ± 0.08
Heart	1.10 ± 0.20	0.71 ± 0.12	0.45 ± 0.09	0.10 ± 0.03
Lungs	0.85 ± 0.17	0.35 ± 0.27	0.17 ± 0.02	0.06 ± 0.02
Kidneys	7.90 ± 4.37	1.75 ± 0.88	1.12 ± 0.44	0.48 ± 0.49
Liver	24.43 ± 3.02	9.95 ± 2.79	6.53 ± 1.70	0.99 ± 0.28
Spleen	0.10 ± 0.03	0.08 ± 0.02	0.06 ± 0.01	0.03 ± 0.01
Carcass	30.68 ± 6.63	20.31 ± 4.14	19.16 ± 3.69	14.19 ± 4.36
Total	66.7	33.7	28.1	16.0

[a] Six mice studied at each time. [b] Based on 7% of the body weight.

Table V—Biodistribution of *N*-([2-^{125}I]Iodobenzoyl)cholamine in Mice

Organ	Mean Percent of Dose per Gram ± *SD*[a]			
	5 min	30 min	60 min	120 min
Blood	0.61 ± 0.12	0.21 ± 0.06	0.21 ± 0.05	0.08 ± 0.03
Heart	6.40 ± 1.59	4.64 ± 0.63	3.20 ± 0.75	0.51 ± 0.21
Lungs	2.80 ± 0.66	1.29 ± 0.59	0.69 ± 0.16	0.21 ± 0.09
Kidneys	14.54 ± 10.03	3.43 ± 1.84	2.26 ± 0.72	0.74 ± 0.75
Liver	12.95 ± 1.71	4.96 ± 1.23	3.67 ± 0.95	0.48 ± 0.19
Spleen	0.78 ± 0.19	0.66 ± 0.09	0.56 ± 0.14	0.20 ± 0.06
Muscle	0.34 ± 0.08	0.26 ± 0.14	0.21 ± 0.04	0.19 ± 0.07

[a] Six mice studied at each time.

Table VI—Heart-to-Blood and Heart-to-Lung Ratios as a Function of Time in Mice

	5 min	30 min	60 min	120 min
V				
Heart/blood	0.6	0.6	0.5	NA[a]
Heart/lung	0.7	0.8	0.6	NA
VI				
Heart/blood	10.5	22.1	15.2	6.4
Heart/lung	2.3	3.6	4.6	2.4

[a] Not available.

Positively charged quaternary ammonium compounds can bind to the negatively charged anionic pocket of the cholinesterase active site by a combination of coulombic and van der Waals' dispersion forces (13). Compounds V and VI were designed to interact with the enzyme as a result of these interactions. It is possible that V is a substrate for pseudocholinesterase (6) in light of the active hydrolysis of benzoylcholine by that enzyme. Enzymatic hydrolysis may account for the high blood activity found in the biodistribution studies and the urinary excretion of [2-^{125}I]iodobenzoic acid. On the other hand, by virtue of its amide functionality, which is more stable to hydrolysis by esterase enzymes, VI is not expected to be a substrate for either pseudocholinesterase or

acetylcholinesterase. Although VI was prepared as an inhibitor of pseudocholinesterase and may be an inhibitor of acetylcholinesterase, there is no evidence that the uptake of VI by the heart is related to binding to either enzyme. *In vitro* studies are in progress to investigate the specific mechanism responsible for the accumulation of VI in heart muscle.

The simple, rapid synthetic technique employed in the preparation of VI would easily allow the 13-hr half-life of iodine 123, which has excellent imaging characteristics, to be substituted for the stable iodine in the molecule. The apparent *in vivo* stability and the high heart-to-blood and heart-to-lung ratios obtained in mice with VI suggest that iodine 123-labeled VI may be useful for imaging the heart in humans and warrants further study as a myocardial-imaging agent.

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ACKNOWLEDGMENTS

Supported in part by U.S. Public Health Service Grants GM-10548 and HL-17655.

Effect of Macromolecules on Aqueous Solubility of Cholesterol and Hormone Drugs

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Received August 6, 1980, from the *Department of Pharmaceutics, School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication October 1, 1980.

Abstract □ The solubility of cholesterol and some hormone drugs in aqueous macromolecule solutions was investigated. Polyvinylpyrrolidone, dextrans, and heparin increased the solubilities of progesterone, testosterone, and diethylstilbestrol, while acacia, pectin, and carrageenans decreased their solubilities. Dextrans increased the solubilities of cholesterol and the three hormone drugs. Acacia and pectin greatly increased cholesterol solubility; however, these macromolecules significantly decreased progesterone and diethylstilbestrol solubilities and slightly decreased testosterone solubility. Heparin decreased cholesterol solubility and increased progesterone, testosterone, and diethylstilbestrol solubilities. Carrageenans significantly decreased cholesterol, progesterone, and testosterone solubilities and had little effect on diethylstilbestrol solubility. A strong bathochromic shift in the absorption spectra of progesterone and testosterone in polyvinylpyrrolidone solutions indicated an attachment between the polymer and the C=O group of the steroids.

Keyphrases □ Cholesterol—effect of macromolecules on aqueous solubility □ Hormone drugs—effect of macromolecules on aqueous solubility □ Solubility—cholesterol and hormone drugs, effect of macromolecules □ Macromolecules—effect on aqueous solubility of cholesterol and hormone drugs

Several macromolecular compounds have been reported to have hypercholesterolemic effects. Acacia and pectin (1–6) and dextran (6–11) have been reported to lower serum cholesterol in humans and animals. Heparin reportedly retards atherosclerosis development and minimizes the degree of hypercholesterolemia (12–14). Carrageenans have been found effective in suppressing hyperlipidemia and atherosclerosis in cholesterol-fed rabbits (15, 16). Polyvinylpyrrolidone was reported to have hypocholesterolemia activity (17).

These reports contain conflicting statements concerning the *in vivo* effect and mechanism of action of these macromolecules for lowering serum cholesterol. A previous article from this laboratory (18) reported on the interesting *in vitro* effects of carbohydrate macromolecules on the aqueous solubility of cholesterol. The large increases in the apparent solubility of cholesterol in 0.5% pectin and acacia solutions provided a possible explanation for the serum cholesterol-lowering ability of these hydrocolloids and prompted further solubility studies involving other macromolecules. This report concerns the effect of various macromolecules on the solubility behavior of cholesterol and the related hormone drugs, testosterone, progesterone, and diethylstilbestrol.

EXPERIMENTAL

Materials—Diethylstilbestrol[1], progesterone[2], and testosterone[3] were recrystallized from ethanol. Cholesterol[4] was purified by recrystallization from acetic acid, and the crystals were dried at 90°. The sample was then recrystallized from 70% alcohol–water and dried in a vacuum desiccator. The melting points and IR spectra of the compounds were in good agreement with literature values. Dextrans[5], pectin (citrus)[6], acacia[1], polyvinylpyrrolidone[7], heparin sodium[2], and carrageenans[8] were used as received.

Solubility Experiments—A stock solution of cholesterol in benzene was prepared containing 1.0 mg of cholesterol and 10 μCi of [26-^{14}C]-cholesterol[9]. Stock solutions of testosterone and progesterone were

[1] Matheson, Coleman and Bell.
[2] Sigma Chemical Co.
[3] Supplied by Schering Laboratories.
[4] Fisher Scientific.
[5] Dextran T_{40} and T_{70}, Pharmacia Laboratories.
[6] Eastman Kodak Co.
[7] NP-K30, GAF Corp.
[8] Seakem 11 and 21, Marine Colloids.
[9] Nuclear Chicago.

0022-3549/81/0400-0442$01.00/0