Biological Activity of the Hypolipidemic Agent, N^2-*n*-Butylindazolone

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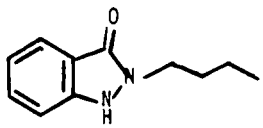
Abstract □ Previously, a series of *N*-substituted indazolone derivatives proved to be effective hypolipidemic agents in rodents. The most effective agent, N^2-*n*-butylindazolone, at 20 mg/kg/d was shown to suppress the levels of cytoplasm acetyl coenzyme A required for cholesterol and fatty acid synthesis as well as *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase activities. Lipid content of the liver, small intestine, and serum lipoprotein fractions was lowered, whereas lipid content was increased in fecal excretions by drug treatment for 14 d. The absorption of orally administered cholesterol from the intestine was severely reduced after drug administration. The mode of action of N^2-*n*-butylindazolone appears to be similar to cyclic imides possessing hypolipidemic activity.

Keyphrases □ N^2-*n*-Butylindazolone—hypolipidemic activity in rodents, cholesterol and triglycerides reduction □ Antihyperlipidemic agents—N^2-*n*-butylindazolone, activity in rodents, cholesterol and triglycerides reduction

A series of *N*-substituted indazolone derivatives were previously screened for hypolipidemic activity in mice and were observed to be potent hypolipidemic agents at the low dose of 20 mg/kg/d (1). *N*-Substitutions of four carbon atom chain lengths in the indazolone (1) as well as in the phthalimide (2) and saccharin (2) series afforded the best hypolipidemic activity. Thus, N^2-*n*-butylindazolone (I) was selected for a more in-depth investigation of its effects on lipid synthesis and distribution in rodents.



I

EXPERIMENTAL SECTION

The N^2-*n*-butylindazolone was synthesized by the method of Schwartz *et al.* (3). The details of the synthesis and physical characteristics have been reported previously (1).

Antihyperlipidemic Screens in Normal Rodents—N^2-*n*-Butylindazolone was suspended in 1% carboxymethylcellulose–water, homogenized, and administered to CF_1 male mice (~25 g) intraperitoneally for 16 d or to Sprague–Dawley male rats (~350 g) orally by an intubation needle for 14 d. On days 9 and 14 or 16, blood was obtained by tail vein bleeding and the serum was separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann–Burchard reaction (4). Serum was also collected on day 14 or 16 and the triglyceride content was determined by a commercial kit[1].

Testing in Hyperlipidemic Mice—CF_1 male mice (~25 g) were placed on a commercial diet[2] which produced a hyperlipidemic state. After the serum cholesterol and triglyceride levels were observed to be elevated, the mice were administered test drugs at 20 mg/kg/d intraperitoneally for an additional 14-d period. Serum cholesterol and triglyceride levels were measured.

Animal weights were obtained periodically during the experiments and were expressed as a percentage of the animal's weight on day zero. After dosing for 14 d with test drugs, selected organs were excised, trimmed of fat, and weighed.

The acute toxicity (LD_{50} values) (5) was determined in CF_1 male mice by administering test drugs at 100 mg–2 g/kg ip as a single dose. The number of deaths were recorded over a 7-d period for each group.

In vitro enzymatic studies were determined using 10% homogenates of CF_1 male mouse liver with 50–200 μM of test drug. *In vivo* enzymatic studies were determined using 10% liver homogenates (prepared in 0.25 M sucrose + 0.001 M ethylenediaminetetraacetic acid, pH 7.2) from CF_1 male mice obtained after administering the agents at a 10–60 mg/kg/d i.p. dose for 16 d. The enzyme activities were determined by following literature procedures (2) for: acetyl coenzyme A (CoA) synthetase (6); ATP-dependent citrate lyase (7); mitochondrial citrate exchange (8, 9); cholesterol side-chain oxidation (10); 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) (11, 12); acetyl CoA carboxylase activity (13); fatty acid synthetase activity (14); *sn*-glycerol-3-phosphate acyl transferase activity (15); phosphatidate phosphohydrolase activity (16).

Rat Studies—In Sprague–Dawley rats that had been administered test drugs in 14 d, the liver, small intestine, and fecal materials (24-h collection) were removed, extracted (17, 18), and analyzed for cholesterol levels (4), triglyceride levels[3], neutral lipid content (19), and phospholipid content (20).

Sprague–Dawley rats (~400 g) were administered test agents for 14 d orally. On day 13, 10 μCi of [^{3}H]cholesterol was administered, orally in rats, and according to procedures described previously (2), tissue samples were combusted[4] or plated on filter paper, dried and digested for 24 h in base[5] at 40°C, and counted[6]. Results were expressed as disintegration/min (dpm) per total organ.

Sprague–Dawley rats (~400 g) were administered the test drug intraperitoneally for 14 d at 20 mg/kg/d. On day 13, 10 μCi of [1,2-^{3}H]cholesterol (40.7 Ci/mmol) was administered to the rat, orally. Twenty-four hours later, the blood was collected and the serum was separated by centrifugation (21).

Sprague–Dawley male rats (~350 g) were treated with test drugs at 20 mg/kg/d orally for 14 d. After anesthetizing the animal, the bile duct was cannulated as previously described (2). [1,2-^{3}H]Cholesterol (40.7 mCi/mmol) (10 μCi) was administered orally 18 h prior to commencing the surgery. The bile was collected over the next 6 h and the volume (mL) measured. Aliquots were counted[6] and analyzed for [^{3}H]cholesterol content (4).

Sprague–Dawley male rats (~350 g) were administered test drugs at 20 mg/kg/d orally. Blood was collected from the abdominal vein and lipoprotein fractions were obtained by the method of Hatch and Lees (22) and Havel *et al.* (23). Each fraction was analyzed for cholesterol (4), triglyceride[3], neutral lipids (19), phospholipids (20), and protein levels (24).

RESULTS

N^2-*n*-Butylindazolone proved to be a potent hypolipidemic agent in mice and rats after intraperitoneal and oral administration, respectively. In mice, the dose affording the maximal lowering effect was 20 mg/kg/d, reducing serum cholesterol 53% and serum triglyceride levels 56%. This dose was slightly less effective in rats, lowering the levels of cholesterol 44% and triglycerides 53% (Table I).

Examination of *in vitro* enzymatic activities in CF_1 mice (Table II) demonstrated that mitochondrial citrate exchange was effectively reduced 34% at 100 μM and 43% at 200 μM. *In vitro* ATP-dependent citrate lyase activity was suppressed 35% at 200 μM. Acetyl CoA synthetase activity was lowered 46% at 100 μM and 49% at 200 μM. HMG CoA reductase activity was reduced 28% at the lower concentration (50 μM) but returned to more normal levels of activity at 100–200 μM. Cholesterol side-chain oxidation was reduced

[1] Hycel Triglyceride Test Kit; Fisher.
[2] Basal Atherogenic Test Diet; U.S. Biochemical Corp.
[3] Triglyceride Kit (bmc); Bio-Dynamics.
[4] Tissue-Oxidizer; Packard.
[5] Hyamine Hydroxide; New England Nuclear.
[6] Fisher Scintiverse in a Packard Scintillation Counter.

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Table I—Hypolipidemic Activity of N^2-*n*-Butylindazolone in Male CF_1 Mice and Sprague–Dawley Rats [a]

Compound	Mice (Intraperitoneal Dose)			Rats (Oral Dose)		
	Serum Cholesterol		Serum Triglyceride	Serum Cholesterol		Serum Triglyceride
	Day 9	Day 16	Day 16	Day 9	Day 14	Day 14
N^2-*n*-Butylindazolone						
10 mg/kg/d	86 ± 7[b]	58 ± 6[c]	64 ± 6[c]	—	—	—
20 mg/kg/d	86 ± 6[b]	47 ± 4[c]	44 ± 4[c]	92 ± 8	56 ± 6[c]	47 ± 5[c]
40 mg/kg/d	72 ± 6[c]	56 ± 5[c]	50 ± 4[c]	—	—	—
60 mg/kg/d	67 ± 6[c]	55 ± 4[c]	50 ± 4[c]	—	—	—
Control (1% Carboxymethylcellulose)	100 ± 7[d]	100 ± 6[e]	100 ± 7[f]	100 ± 8[g]	100 ± 7[h]	100 ± 7[i]

[a] Expressed as percent of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.010$. [c] $p \leq 0.001$. [d] 118 mg %. [e] 122 mg %. [f] 137 mg %. [g] 73 mg %. [h] 78 mg %. [i] 110 mg %.

Table II—*In Vitro* Effects of N^2-*n*-Butylindazolone on CF_1 Mouse Liver-Enzyme Activities [a]

Enzyme Assay	Control	50 μM	100 μM	200 μM
Mitochondrial citrate exchange	100 ± 7[d]	71 ± 6[c]	66 ± 5[c]	57 ± 6[c]
ATP-dependent citrate lyase	100 ± 6[e]	101 ± 7	78 ± 6[c]	65 ± 6[c]
Acetyl CoA synthetase	100 ± 6[f]	73 ± 6[c]	54 ± 7[c]	51 ± 5[c]
HMG CoA reductase	100 ± 9[g]	72 ± 5[c]	81 ± 7[b]	92 ± 8
Cholesterol side-chain oxidation	100 ± 5[h]	—	71 ± 6	—
Acetyl CoA carboxylase	100 ± 6[i]	91 ± 7	90 ± 6	90 ± 7
Fatty acid synthetase	100 ± 8[j]	91 ± 7	85 ± 7	73 ± 6[c]
sn-Glycerol-3-phosphate acyl transferase	100 ± 7[k]	73 ± 6[c]	45 ± 5[c]	53 ± 5[c]
Phosphatidate phosphohydrolase	100 ± 7[l]	100 ± 8	74 ± 6[c]	57 ± 7[c]

[a] Expressed as percent of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.01$. [c] $p \leq 0.001$. [d] 30.8% exchange of mitochondrial citrate. [e] 30.5 mg of citrate hydrolyzed/g wet tissue/20 min. [f] 28.5 mg of acetyl CoA formed/g wet tissue/20 min. [g] 384,900 dpm of cholesterol formed/g wet tissue/60 min. [h] 6080 dpm of CO_2 formed/g wet tissue/18 h. [i] 32,010 dpm/g wet tissue/30 min. [j] 37,656 dpm/g wet tissue/20 min. [k] 537,800 dpm/g wet tissue/20 min. [l] 16.7 μP_i/g wet tissue/15 min.

29% at 100 μM. The activities of regulatory enzymes of triglyceride synthesis, *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase were significantly inhibited by N^2-*n*-butylindazolone. The former was inhibited 55% at 100 μM and 47% at 200 μM. The latter was suppressed 26% at 100 μM and 43% at 200 μM. The effects of N^2-*n*-butylindazolone on *in vivo* enzyme activities of mouse liver (Table III) were a 38% suppression of acetyl CoA synthetase activity at 60 mg/kg/d and a 20% inhibition of HMG CoA reductase activity at 10 mg/kg/d. Acetyl CoA carboxylase activity was suppressed 20–30% for all of the doses tested. Fatty acid synthetase activity was reduced 25% at 20 mg/kg, but the agent had no effect at other doses. Phosphatidate phosphohydrolase activity was reduced in a dose-dependent manner with 60 mg/kg/d resulting in a 59% reduction. *sn*-Glycerol-3-phosphate acyl transferase activities were inhibited >20% at 10 and 20 mg/kg/d.

Lipid content was lowered 20% in the liver and 65% in the small intestine after dosing rats 20 mg/kg/d for 14 d. Cholesterol content in the liver was lowered to 29%, triglyceride level and neutral lipids were reduced 21 and 34%, respectively; phospholipid content was elevated 24%. In the small intestine, cholesterol, triglycerides, and neutral lipid levels were lowered 27, 84, and 80%, respectively. In the feces, lipid content and cholesterol levels were elevated 5% and 8% which is not significant, but there were elevations in the levels of neutral lipids and phospholipids which were significantly different from the control (Table IV).

The serum lipoprotein fractions obtained from rats treated for 2 weeks showed decreased cholesterol, triglyceride, and neutral lipid levels in all four lipoprotein fractions. Phospholipid levels were generally not affected with the exception of the high density lipoprotein which had a 14% reduction (Table IV).

[^{3}H]Cholesterol distribution after 2-weeks dosing in rats showed that cholesterol was not accumulated in the brain, lung, heart, liver, or spleen. On the other hand, higher concentrations of cholesterol were found in the small intestine (52%), large intestine (129%), chyme (80%), and feces (60%) when compared with control values. Bile excretion studies showed a 28% increase in [^{3}H]cholesterol excretion in the bile on day 14, and studies also demonstrated a 95% suppression of absorption of [^{3}H]cholesterol from the intestine after oral administration over a 24-h period (Table V).

Organ weights of the control and treated animals did not differ significantly although those of the treated animals were slightly lower. The weight of the control and treated animals increased 9.76% and 5.86%, respectively, over two weeks. Daily food intake (g/d) was 30.39 for the control and 28.75 for the treated group. Adrenal weights were not altered by drug treatment indicating that there was no compensating hypertrophy of the adrenal cortex to produce increased steroidogenesis.

Data in Tables I–V are expressed as percent of control ± *SD*. The probable significance level (p) between each test group and the control group was determined by the t test.

DISCUSSION

In comparing the mode of action of N^2-substituted indazolone to *N*-substituted cyclic imides and derivatives [e.g., phthalimide (2), saccharin (2), and 1′,8′-naphthalimide (25)], some similarities exist. All of these agents inhibit mitochondrial citrate exchange decreasing the availability of the key intermediate, acetyl CoA, which is required for fatty acid and cholesterol synthesis. All of the *N*-substituted imides block the two regulatory enzymes of triglyceride synthesis (*sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase) and showed marginal inhibition of HMG CoA reductase, the regulatory enzyme of cholesterol synthesis. Differences which exist between *N*-substituted indazolone and *N*-substituted saccharin, phthalimide, and 1′,8′-naphthalimide cyclic imides include marginal inhibition

Table III—*In Vivo* Effects of N^2-*n*-Butylindazolone on CF_1 Mouse Liver-Enzyme Activities [a]

Compound	ATP-Dependent Citrate Lyase	Acetyl CoA Synthetase	HMG CoA Reductase	
N^2-*n*-Butylindazolone				
10 mg/kg/d	97 ± 8	99 ± 7	80 ± 7[c]	
20 mg/kg/d	100 ± 6	98 ± 6	91 ± 7	
40 mg/kg/d	101 ± 7	91 ± 7	91 ± 6	
60 mg/kg/d	92 ± 7	62 ± 5[c]	98 ± 8	
Control (1% carboxymethylcellulose)	100 ± 8[e]	100 ± 7[f]	100 ± 6[g]	
	Acetyl CoA Carboxylase	Fatty Acid Synthetase	Phosphatidate Phosphohydrolase	*sn*-Glycerol-3-Phosphate Acyl Transferase
N^2-*n*-Butylindazolone				
10 mg/kg/d	77 ± 6[c]	93 ± 7	75 ± 7[c]	79 ± 6[c]
20 mg/kg/d	69 ± 5[c]	75 ± 6[c]	68 ± 6[c]	72 ± 7[c]
40 mg/kg/d	78 ± 5[c]	94 ± 7	58 ± 5[c]	84 ± 8[b]
60 mg/kg/d	80 ± 6[c]	102 ± 8	41 ± 5[c]	88 ± 7
Control (1% carboxymethylcellulose)	100 ± 5[i]	100 ± 6[j]	100 ± 7[l]	100 ± 7[k]

[a] See legend for Table II.

Table IV—*In Vivo* Effects of N^2-*n*-Butylindazolone on Lipid Levels in Liver, Small Intestine, Feces, Bile, and Serum Lipoprotein Fractions of Rats[a]

	Lipid, mg	Cholesterol	Triglycerides	Neutral Lipids	Phospholipids	Proteins
Liver						
Control	100 ± 8	100 ± 7[d]	100 ± 6[e]	100 ± 7[f]	100 ± 9[g]	100 ± 8[h]
Treated	80 ± 7[b]	71 ± 6[c]	81 ± 5[c]	66 ± 6[c]	124 ± 8	102 ± 8
Small intestine						
Control	100 ± 7	100 ± 6[i]	100 ± 7[j]	100 ± 8[k]	100 ± 8[l]	100 ± 7[m]
Treated	35 ± 4[c]	73 ± 6[c]	16 ± 3[c]	20 ± 4[c]	75 ± 6[c]	104 ± 6
Feces						
Control	100 ± 8	100 ± 7[n]	100 ± 6[o]	100 ± 8[p]	100 ± 8[q]	100 ± 8[r]
Treated	105 ± 7	107 ± 8	114 ± 5[c]	125 ± 7[c]	131 ± 7[c]	76 ± 7
Serum lipoprotein						
Chylomicron						
Control	—	100 ± 7[s]	100 ± 5[t]	100 ± 7[u]	100 ± 7[v]	100 ± 6[w]
Treated	—	66 ± 6[c]	65 ± 4[c]	70 ± 7	102 ± 8	108 ± 7
Very low-density lipoprotein						
Control	—	100 ± 7[x]	100 ± 6[y]	100 ± 7[z]	100 ± 8[aa]	100 ± 5[bb]
Treated	—	33 ± 3[c]	42 ± 5[c]	60 ± 6[c]	107 ± 9	88 ± 6
Low-density lipoprotein						
Control	—	100 ± 6[cc]	100 ± 7[dd]	100 ± 8[ee]	100 ± 9[ff]	100 ± 7[gg]
Treated	—	33 ± 2[c]	78 ± 5[c]	67 ± 6[c]	115 ± 6	92 ± 6
High-density lipoprotein						
Control	—	100 ± 6[hh]	100 ± 7[ii]	100 ± 6[jj]	100 ± 7[kk]	100 ± 7[ll]
Treated	—	23 ± 2[c]	34 ± 4[c]	60 ± 5[c]	86 ± 6[b]	106 ± 7

[a] After 14 d of dosing, expressed as percent of control (mean ± *SD*), $n = 6$. [b] $p \leq 0.010$. [c] $p \leq 0.001$. [d] 24.03 mg cholesterol/g tissue. [e] 44.11 mg neutral lipid/g tissue. [f] 6.37 mg triglyceride/g tissue. [g] 7.19 mg. [h] 4.5 mg protein/g wet tissue. [i] 7.82 mg/g. [j] 6.98 mg/g. [k] 1.12 mg/g. [l] 2.06 mg/g. [m] 42 mg/g. [n] 28.47 mg/g. [o] 33.94 mg/g. [p] 1.86 mg/g. [q] 1.39 kg/g. [r] 6.99 mg/g. [s] 337 μg/mL. [t] 67 μg/mL. [u] 420 μg/mL. [v] 149 μg/mL. [w] 184 μg/mL. [x] 190 μg/mL. [y] 98 μg/mL. [z] 22 μg/mL. [aa] 26 μg/mL. [bb] 50 μg/mL. [cc] 210 μg/mL. [dd] 10 μg/mL. [ee] 45 μg/mL. [ff] 41 μg/mL. [gg] 122 μg/mL. [hh] 544 μg/mL. [ii] 620 μg/mL. [jj] 27 μg/mL. [kk] 153 μg/mL. [ll] 657 μg/mL.

Table V—Effects of N^2-*n*-Butylindazolone on [^{3}H]Cholesterol Distribution in Rats[a]

Organ	Organ Weight, g		Total Organ dpm	
	Control	Treated	Control	Treated
Brain	1.866	1.766	29,115	12,956
Lung	1.766	1.667	15,350	12,838
Heart	1.333	1.001	24,582	21,868
Liver	12.000	11.682	47,429	25,756
Kidney	2.900	2.466	5,875	5.191
Spleen	0.566	0.666	19,388	15,988
Adrenal	0.036	0.034	—	—
Stomach	2.066	2.100	11,375	40,005
Small intestine	8.333	8.400	15,291	37,298
Large intestine	4.100	5.000	47,109	108,044
Chyme	5.866	3.233	102,772	185,024
Feces	5.702	6.566	320,454	512,301

[a] 24 h after administration for 14 d; $n = 6$.

of fatty acid synthesis at the lower doses by the *N*-substituted indazolone; the latter agent also suppresses appetite. The *N*-substituted indazolone did lower lipid levels in the liver and small intestine of the rat with increased lipid levels in the fecal material after 14 d of dosing. Lower levels of cholesterol, triglycerides, and neutral lipids of the four blood lipoprotein fractions were evident after drug therapy. The *N*-substituted indazolone derivatives more consistently lowered lipid content of the four lipoprotein fractions when compared with cyclic imides. Whereas the ratio of cholesterol level of high density lipoprotein to low density lipoprotein probably did not change significantly, lower levels were noted in both lipoprotein fractions. Supposedly, lower levels of cholesterol in the low density fraction and higher levels of cholesterol in high density lipoprotein fraction protect against lipid deposition in the atherogenic plaque of the blood vessel and leads to clearance of lipoproteins from the blood.

Like *N*-substituted cyclic imides, [^{3}H]cholesterol distribution and excretion was altered by N^2-*n*-butylindazolone. First, after oral administration of cholesterol, GI tract absorption over a 24-h period was reduced markedly. Second, there was less cholesterol in the major organs, *e.g.*, brain, liver, heart, lung, and kidney. Third, drug therapy resulted in larger amounts of labeled cholesterol being excreted by the fecal route compared with the control. Fourth, the increase in fecal [^{3}H]cholesterol may reflect the decreased absorption of cholesterol caused by the drug and the increase in biliary excretion. The *N*-substituted indazolone derivatives appeared to be more effective than cyclic imides in reducing cholesterol absorption and appeared to be slightly less effective in reducing liver regulatory enzyme activity than cyclic imides. For example, phthalimide and saccharin reduced cholesterol absorption 56% and 33%, respectively, whereas N^2-*n*-butylindazolone resulted in a 95% reduction. However, phosphatidate phosphohydrolase activity was reduced 97% by phthalimide at 60 mg/kg/d and 70% by saccharin at 100 mg/kg/d, whereas N^2-*n*-butylindazolone at 60 mg/kg/d only lowered the activity 59%, its maximum effect. Thus, the modes of action of cyclic imides in lowering body lipids vary from compound to compound, usually affecting the same biochemical parameter but having different magnitudes of response. All of the tested cyclic imides to date appear to have multiple sites in metabolism on which they act to lower serum lipid levels in the body.

In conclusion, N^2-*n*-butylindazolone appears to be a potent hypolipidemic agent and, like cyclic imides tested, more potent than clofibrate which is commercially available. The therapeutic index was well within safe utilization of the compound and the compound[7] effectively suppressed both serum cholesterol and triglyceride levels.

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Estimation of Tris(2-butoxyethyl) Phosphate in Biological Fluids: Novel Intersubject Variability in Recovery from Human Serum

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Abstract □ Tris(2-butoxyethyl) phosphate (I), a plasticizer commonly found in evacuated blood collection tubes, displaces many basic drugs from their binding sites on serum proteins and causes them to redistribute from serum into red blood cells (*i.e.*, artificially lowering serum or plasma drug concentration). Thus, the ability to quantitate I in serum or plasma may be helpful in establishing the suitability of various lots of evacuated blood collection tubes for use in drug level monitoring and pharmacokinetic studies. In the process of establishing a minor modification of an assay which has been reported, remarkable and reproducible interindividual variability ($n = 10$) in the slope of standard curves was observed (range, 0.0143–0.0486). This variability appeared to be caused by differences in the recovery of I from the serum of these individuals. The source of this difference seemed to be related to serum lipoprotein concentration since the slope of standard curves was highly correlated with serum triglyceride concentration ($r = -0.800$) as well as with the sum of serum triglyceride and cholesterol concentrations ($r = -0.881$). These observations suggest that the examination of interindividual differences in the recovery of drugs and related compounds from serum should be a routine part of assay development.

Keyphrases □ Tris(2-butoxyethyl) phosphate—recovery variability, human serum, correlation to lipoprotein, triglyceride and cholesterol levels

Tris(2-butoxyethyl) phosphate (I), a plasticizer found in the rubber stoppers of many evacuated blood collection tubes and in some catheter materials, has been shown to significantly displace basic drugs such as propranolol (1), quinidine (2), lidocaine (3), imipramine (4), and alprenolol (4) from their binding sites on α_1-acid glycoprotein (the principal base binding protein in human serum). Spuriously low (typically 25%) plasma or serum concentrations accompany this displacement due to increased partitioning of the drugs into red blood cells (1, 3). Artifacts related to this phenomenon may pervade much of the published data describing the pharmacokinetics of this type of compound. Furthermore, this artifact may adversely affect therapeutic drug monitoring. Specifically, it is likely that different lots of evacuated blood collection tubes cause varying amounts of "displacing activity" (3) and that different lots of tubes may be used to collect blood during a hospital admission. Thus, the capacity to approximate the concentration of I in plasma or serum samples is important.

A second reason to examine the analysis of I in biological fluids was that preliminary studies in this laboratory suggested remarkable intersubject variability in the slopes of standard curves for I in human serum. It is well known that the recovery of some compounds is dependent upon the biological fluid (urine, blood, serum, *etc.*) being extracted. For example the lidocaine metabolites, monoethyl-glycinexylidide and glycinexylidide, have a significantly lower recovery from serum than from urine (5). The present text describes the evidence for large (approximately threefold) interindividual differences in the recovery of I from serum, provides preliminary observations regarding the plasma constituents which exhibit covariance with these interindividual differences, and presents a description of analytical methods which allow the estimation of I in serum samples.

EXPERIMENTAL SECTION

Tris(2-butoxyethyl) phosphate[1] and dioctyl phthalate[1] were used as received. All solvents were spectral or HPLC grade.

Standard Assay of I in Serum—The concentration of I in serum samples was determined using a minor modification of a previously published procedure (4). The original extraction method was modified by increasing the organic to aqueous phase volume ratio from 2:1 to 10:1. To a 0.50-mL serum sample was added 5 mL of dichloromethane containing 1.0 μg/mL of dioctyl phthalate as an internal standard. The mixture was agitated on a fixed-angle rotary mixer (20 rpm) for 10 min and centrifuged (1000×*g*). The organic phase was transferred to a clean tube and evaporated to dryness. The residue was redissolved in 25 μL of ethyl acetate, and 1 μL was injected into a gas chromatograph equipped with flame-ionization detectors[2]. This instrument contained glass columns (122 × 0.2 cm) packed with 3% OV-101 on 80/100-mesh chromosorb W(HP)[3]. Helium was used as a carrier gas at a flow rate of 30 mL/min. The analysis was run isothermally at a column temperature of 220°C with injector and detector temperatures of 250°C. The flow rates of hydrogen and air were 30 and 300 mL/min, respectively.

[1] Aldrich Chemical Company, Inc., Milwaukee, Wis.
[2] Model 5730A; Hewlett Packard.
[3] Alltech Associates, Arlington Heights, Ill.

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