Sodium Polyethylene Sulfonate: Tissue Distribution and Effect on C^{14}-1-Palmitic Acid Oxidation. (27500)

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There is considerable evidence that heparin plays an important role in lipemic clearing and fat transport(1). Whether or not it also affects the rate of oxidation of triglycerides and fatty acids directly has not been determined. A marked increase in respiratory $C^{14}O_2$ occurred in rats administered a C^{14}-labeled soybean oil emulsion preceded by heparin but it was not clear whether heparin might also exert a direct effect on the oxidation of fats in addition to making the fat more readily available to oxidative processes(2). We became interested in investigating this aspect of the action of heparin while working on a synthetic heparin-like substance, sodium polyethylene sulfonate (PES)*, which is active orally and has been shown to possess a higher ratio of plasma lipemic clearing activity to anticoagulant activity than heparin in several animal species(3). Chronic administration of PES is toxic to laboratory animals (3) and therefore the present study was designed to include experiments using tritiated PES to obtain data on excretion and tissue localization of the drug in animals.

Materials and methods. C^{14}-1-palmitic acid and C^{14}-1-tripalmitin were incorporated into an emulsion consisting of 5% olive oil, 1% Demal,† 0.5% Pluronic F-68,‡ 4% dextrose and water to contain approximately 4.4 μc/ml. Groups of 6 male rats of Sprague-Dawley ancestry weighing 150-175 g were fasted overnight, injected I.P. with 3 mg/kg sodium heparin or PES (mol wt 12,000-14,500) and ½ hour later given 1 ml of the appropriate emulsion per 150 g body weight by femoral vein. Rats were placed in glass metabolic units where the respired CO_2 was collected. The exhaled $C^{14}O_2$ was counted as $BaC^{14}O_3$ by a procedure previously outlined (4).

For excretion and distribution studies in dogs and rats, PES was tritiated with an exposure period of 10 days(5). The resulting mixture was purified to constant specific activity (9.6 μc/mg) by dialysis, treated with NaCl and absolute ethanol, and dehydrated with 1:1 ethanol-acetone. Labeled PES had good biological activity measured by a lipemic clearing response in dogs. Tritium samples were counted in a liquid scintillation spectrometer§ at tap 9. One-tenth ml samples of urine were added directly to 10 ml of counting mixture made of 700 ml distilled toluene, 300 ml absolute ethanol, 3 g diphenyloxazole and 100 mg 1,4-di(2-[5-phenyloxazolyl])benzene. One-tenth ml samples of either citrated plasma or 20% aqueous pooled tissue homogenates were dissolved in 1 ml of 1 M Hyamine solution by warming before 10 ml of counting mixture were added(6); appropriate internal standards were used throughout the counting procedure.

Results and discussion. Oxidation rates by fasted rats. In general, all rats oxidized C^{14}-1-palmitic acid much faster than those given the labeled acid in the triglyceride form (Table I). Neither PES nor heparin altered the specific activity of expired $C^{14}O_2$ from rats given C^{14}-1-palmitic acid. Both agents, however, increased the specific activity of $C^{14}O_2$ from rats given C^{14}-1-tripalmitin. Heparin acted faster, showing the calculated peak increase of 44% at ½ hour while PES gave a peak increase of 59% at 1 hour; both treated groups gave similar specific activities at 2 hours and thereafter. Lack of effect of PES and heparin on oxidation of C^{14}-1-palmitic acid but stimulation of oxidation of C^{14}-1-tripalmitin is interpreted to mean that PES and heparin increased the rate of hydrolysis of the triglyceride by clearing factor thus yielding fatty acids which in turn were rap-

* Obtained from Farbwerke Hoechst, Frankfurt-am-Main, Hoechst, Germany.

† Polyglycerol ester of oleic acid, Emulsol Corp., Chicago, Ill.

‡ Wyandotte Chemicals Corporation, Wyandotte, Mich.

§ Packard Tri-Carb model 314-DC.

TABLE I. Oxidation of Labeled Palmitic Acid Injected as the Acid or as the Triglyceride.*

Group	Cumulative specific activity of exhaled CO_2† ½ hr	1 hr	2 hr	3 hr	5 hr
C^{14}-1-tripalmitin					
Control	41 ± 3.4‡	61 ± 2.4	80 ± 2.9	80 ± 3.0	63 ± 2.9
PES	60 ± 3.2	97 ± 6.2	101 ± 5.1	94 ± 4.2	71 ± 4.0
Heparin	59 ± 2.0	83 ± 4.8	101 ± 3.5	93 ± 2.9	75 ± 3.8
C^{14}-1-palmitic acid					
Control	261 ±14	224 ±13	145 ±10	113 ± 5	76 ± 4
PES	291 ±33	232 ±22	153 ±11	115 ± 6	84 ± 4
Heparin	253 ±15	226 ± 4	151 ± 4	117 ± 8	84 ± 2

* Six rats/group (36 in all), fasted overnight.

† Given as counts/min./mg $BaCO_3$ corrected; 1 μc = 4.4 × 10^5 cpm for gas flow counter used. Cumulative indicates total $C^{14}O_2$ exhaled from inj. time 0.

‡ Stand. error of mean.

idly oxidized. Another interpretation, although less plausible, is that PES and heparin increase oxidation of intact triglycerides.

Excretion and distribution of PES. Total urinary excretion of radioactivity following administration of labeled PES to dogs accounted for only a fraction of the dose (Table II) and no excretion *via* the bile was observed after I.V. administration (Table II, footnote). Urinary excretions were essentially complete after 3, 8 and 12 hours for I.V., I.M. and oral administrations respectively; total excretion was complete after 24 hours for all routes of administration (Table II, footnote). Total amounts excreted, although at different rates, were similar and increased with increased doses for both I.V. and I.M. administrations which suggested good absorption from the I.M. site. However, after oral administration excretion was extremely small, indicating rather poor absorption from the G.I. tract, on a percentage basis. The major share of injected radioactivity could not be accounted for in urine and bile, hence it appeared that much of the PES was stored; capacity for the suggested storage was considerable since daily I.M. injections of unlabeled PES for one month did not alter the excretion following an additional dose of radioactive PES (Table II, footnote).

TABLE II. Excretion of Tritium after a Single Dose of Labeled PES to Dogs.*

Dose, mg/kg	No. of dogs	Route	24 hr urine tritium,† % of dose
.5	2	I.V.	7.2 ± 5.5
10.0	3	"	34.8 ± .6
2.5‡	2	I.M.	8.5 ± 1.4
5.0	2	"	29.0 ± 1.5
250.0	3	Oral	.4 ± .3

* No radioactivity was found in bile collected for 7 hr from 2 anesthetized dogs given 10 mg/kg I.V. of labeled PES.

† Tritium excretion complete within 24 hr for all routes of administration. After I.V. 95% of total excretion complete by 3 hr; after I.M. 88% complete by 8 hr and after oral administration 90% complete by 12 hr.

‡ After 1 mo of daily injections (2.5 mg/kg I.M.) of unlabeled PES to these same dogs, tritiated PES was inj. again; total excreted in urine was 11.0 ± 2.4% of the dose.

Rats also excreted only part of an I.V. dose of radioactive PES and since the supply of tritiated PES was limited, tissue concentrations of apparent PES were determined in rats. A large dose of radioactive PES (60 mg/kg I.V.) was used in order to insure adequate tissue concentration of the isotope for counting. After an acute dose, about 50% was found in the urine and the rest in various organs, of which the liver, kidney and skeletal muscle contained the most (Table III). After 2 weeks, no further excretion took place but radioactivity in the kidney decreased while skeletal muscle content increased. With repeated doses a greater per cent of the dose seemed to concentrate in the liver (Table III, column 3). Although heparin given I.V. is concentrated in the liver of dogs up to 24 hours after administration, the entire dose is excreted in different forms within 48 hours (7). Prolonged tissue concentrations of PES are, therefore, different from those following heparin injection and could explain in part the toxicity observed after chronic administration to laboratory animals(3).

Summary. Neither injected sodium poly-

TABLE III. Tissue Distribution of Tritium after Injection of Labeled PES to Rats.*

Pooled tissue	Tissue radioactivity (% of dose) 24 hr	14 days	48 hr after 4 injections
Urine	52.8	0	40.0
Plasma	0	0	0
Liver	19.6	20.0	40.6
Kidney	8.3	2.0	3.7
Skeletal muscle†	7.0	17.8	10.4
Fat (perirenal & genital)‡	4.1	4.4	3.4
Intestine (small & large)	3.8	3.7	3.8
Spleen	.3	.3	.4
Heart	.2	.2	.2
Lungs	.2	.5	.7
Total retained by tissues	43.5	48.9	63.2

* Tritiated PES given I.V. (60 mg/kg) to 6 rats; 3 sacrificed after 24 hr; 3 sacrificed after 14 days. Four successive daily I.V. (60 mg/kg) injections of labeled PES given 4 additional rats; sacrificed 48 hr after last inj.

† Taken from foreleg; total skeletal muscle calculated as 50% of total rat wt (wet).

‡ Calculated as representative of total fat considered 12.6% of total rat wt (wet)(8).

ethylene sulfonate (PES) nor heparin affected the rate of oxidation of intravenously administered C^{14}-1-palmitic acid in rats whereas both agents increased the oxidation of C^{14}-1-tripalmitin. These findings are interpreted as indicating that PES and heparin increase the rate of oxidation of triglyceride fatty acids indirectly by increasing the rate of hydrolysis to fatty acids, which in turn are rapidly oxidized. When tritiated PES was given to dogs and rats, only a fraction of the dose was found in the urine. In rats about 50% was excreted in the urine and the remainder could be accounted for in various tissues; liver and skeletal muscle contained the most.

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Comparative Serum Binding, Distribution and Excretion of Tetracycline and a New Analogue, Methacycline. (27501)

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Of the many tetracycline analogues, only 4, chlortetracycline, oxytetracycline, tetracycline (TC), and demethylchlortetracycline (DMCT), are currently available for clinical use. These analogues share virtually the same antimicrobial spectrum, differing only in relative activity and in properties such as stability, binding to serum proteins, relative distribution in body and renal excretion(1,2). Recently, a new analogue, 6-methylene oxytetracycline, generic name methacycline (MC), has been reported to have antimicrobial and pharmacologic properties similar to DMCT(3,4).

In a previous report dealing with various penicillin analogues(5), it was pointed out that oral absorption studies provide only limited information concerning the effect of serum protein binding on blood levels. Some workers have attributed higher blood concen-