ppm (br s, 1, C-1 ArH); ^{13}C-NMR: 24.5 (C-4), 47.4 (C-3), 56.0 (C-7 OCH_3), 61.8 (C-8 OCH_3) 115.0 (C-6), 121.7 (C-8a), 122.4 (C-5), 129.2 (C-4a), 147.2 (C-7), 151.3 (C-8), and 155.8 ppm (C-1) (10-12); MS M^+: *m/z* 191 (83%) (calc. for $C_{11}H_{13}NO_2$, 191.0946; obs., 191.0947), 176 (65), 161 (66), 146 (70), 133 (35), 116 (79), 105 (26), 89 (49), 77 (86), 62 (47) and 51 (100).

7,8-Dimethoxy-3,4-dihydroisoquinoline Methiodide (IV)—To 7,8-dimethoxy-3,4-dihydroisoquinoline (**III**) (100 mg) in acetone (5 mL) was added methyl iodide (0.25 mL), and the resulting solution was allowed to stand overnight. The resulting yellow crystals were removed by filtration, washed with cold acetone, and recrystallized twice from acetone–methanol to afford IV as yellow needles (112 mg), mp 165–166°C; UV λ_{max} (MeOH) (log ϵ): 375 (3.03), 297 (3.77), and 218 (4.06); IR ν_{max} (KBr): 1668, 1582, 1498, 1270, 1254, 1080, 1033, 954, and 820 cm^{-1}; ^{1}H-NMR (CD_3OD): δ 3.39 (2 closely overlapping poorly defined triplets, 4, J = 8 Hz), 3.86 (s, 3, N-2 N^+CH_3), 3.95 (s, 3, C-7 $ArOCH_3$), 4.10 (s, 3, C-8 $ArOCH_3$), 7.15 (d, 1, J = 8 Hz, C-5 ArH), 7.52 (d, J = 8 Hz, C-6 ArH), and 9.27 ppm (br s, 1, C-1 ArH); MS M^+: *m/z* 191 (100%) ($M^+ - CH_3I$), 142 (52), 127 (57), and 77 (23).

7,8-Dimethoxy-1,2,3,4-tetrahydroisoquinoline (**II**)—To 7,8-dimethoxy-3,4-dihydroisoquinoline (III) (60 mg) in methanol (30 mL) was added, with stirring, sodium borohydride (200 mg) over a period of 30 min. The mixture was stirred for an additional 8 h and was then filtered. The filtrate was evaporated to give a residue which was dissolved in HCl (5%) (20 mL), basified with NH_4OH to pH 8–9, and extracted with chloroform (50 mL) (4×). The chloroform extracts were combined, dried (anhydrous sodium sulfate), filtered, and the filtrate was evaporated to afford a residue (50 mg) which was dissolved in methanolic HCl (5%) (4 mL). The solvent was subsequently removed by evaporation and the resulting residue treated with a mixture of acetone–methanol (12:1) (3 mL) to afford yellowish-white crystals of II HCl, mp 189–191°C, identical with authentic 7,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride by direct comparison [mp, UV, IR (1)].

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Determination of Heparin Activity by a New Laser Nephelometric Method and Comparison to the USP Method

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Abstract □ A new method for the determination of heparin activity in solutions has been developed, based on the increase in light scattering observed during the clotting of plasma. The recalcification clotting time (RCT) of sheep plasma is measured in the presence of heparin, using a laser nephelometer. The activity of heparin in the sample is determined from a standard curve of the logarithm of RCT *versus* heparin activity. The results indicate that this method is simpler, faster, and more reproducible than the current USP grading method.

Keyphrases □ Heparin—activity, laser nephelometry method, comparison to USP method, recalcification clotting time □ Laser nephelometry—heparin activity, comparison to USP method, recalcification clotting time □ Recalcification clotting time—heparin activity, laser-nephelometry, comparison to USP method

Heparin is an anticoagulant widely used in the treatment of myocardial infarction and thromboembolic disease (1). The anticoagulant effect of the drug cannot be predicted by chemical analysis because it has not been related to any specific functional group or to molecular weight (2). Therefore, the activity of heparin, rather than its concentration, must be measured.

Evaluation of the anticoagulant activity of heparin is based on the ability of the drug to prolong the clotting of recalcified sheep plasma (*i.e.*, sheep plasma to which calcium ions are added to promote coagulation). This ability can be measured instrumentally and visually. Reed *et al.* (3) developed an instrumental method based on measurement of the time needed for a sample of recalcified sheep plasma to reach a pre-established viscosity, *i.e.*, a predetermined degree of clotting. This time is then related to the times needed for a set of standards to reach the same viscosity. The USP method, which does not use instruments, is based on the visual evaluation of the degree of clotting of samples as compared with that of a set of standards.

This work describes the development of a nephelometric method to determine heparin activity and compares this method with the USP procedure. Woziwodzki (4) used nephelometric techniques to study the clotting of plasma. More recently Tanaka (5) used laser nephelometric techniques to study the structure of polyacrylamide gels. Because a clot is essentially a gel composed of a fibrin polymer (1), it was postulated that a laser nephelometer could be used to measure the clotting time.

EXPERIMENTAL SECTION

Apparatus and Reagents—The cuvette compartment of a laser nephelometer[1] was modified to allow external circulation of water, thus maintaining

[1] Hyland PDQ Laser Nephelometer, Northbrook, Ill.

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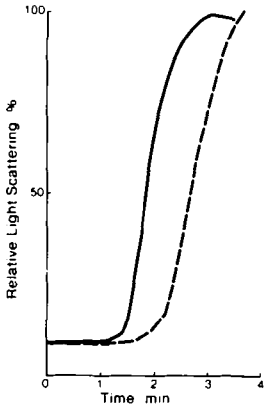

Figure 1—*Strip-chart recording of light scattering during clot formation. Key: (——) 0.88 U of heparin/mL; (- - -) 1.06 U of heparin/mL; 0% is 0.9% NaCl.*

a temperature of 37 ± 0.5°C. The following were used as received from the manufacturers: heparin sodium USP reference standard, sodium chloride[2], calcium chloride[2], and frozen sheep plasma[3].

Standard Preparation—A 3-U/mL (USP units) heparin stock solution in 0.9% NaCl was prepared from a sodium heparin reference standard. The working standards were prepared from this stock solution using 0.9% NaCl as a diluent. Because the activity range of the working standards depends on the lot of sheep plasma used, the range was determined by measuring the clotting times of several working standards having activities between 1 and 3 U/mL. For the plasma used in these studies, the activities ranged between 1.10 and 1.40 U/mL with clotting times between 100 and 200 s. Clotting times higher or lower than this range showed deviations from linearity for the logarithm of clotting time *versus* activity.

Procedure—Samples were diluted with 0.9% NaCl to an activity within the range of the standard curve. The sheep plasma was thawed at room temperature, filtered, and kept in an ice bath during the analysis.

The instrument was standardized by setting zero light scattering with a solution of 1 mL of 0.9% NaCl mixed with 0.5 mL of sheep plasma. Maximum light scattering was set at 190 using a fully clotted sample prepared by incubating a mixture of 0.5 mL of 0.9% NaCl, 0.5 mL of sheep plasma, and 0.5 mL of 0.02 M $CaCl_2$ for 5 min at 37°C.

A 0.5-mL aliquot of the diluted sample was mixed with 0.5 mL of sheep plasma in a 10 × 75-mm test tube and incubated at 37°C for at least 3 min. Immediately after addition of 0.5 mL of 0.02 M $CaCl_2$ (prewarmed to 37°C), a timer was started and the contents of the tube were remixed. The tube was placed in the nephelometer, and the time was recorded when the display reached 100. This is defined as the recalcification clotting time (RCT). Three determinations were made on each solution. The activity of the test solution was determined from a standard curve of the logarithm of mean RCT *versus* heparin activity.

Comparison of the Nephelometric and USP Methods—Three aqueous solutions of sodium heparin[4] buffered with citric acid[5] and sodium monohydrogen phosphate[3], with activities of 45.07, 49.97, and 54.91 U/mL, were prepared. These solutions were analyzed for heparin activity using both the USP XX (6) and nephelometric methods. On each of 3 d, two technicians analyzed each solution using a randomized order, making three determinations on duplicate dilutions of each sample.

RESULTS AND DISCUSSION

Figure 1 illustrates the increase in light scattering during clot formation for two levels of heparin activity. The two curves differ only in the amount of time elapsed before clot formation begins. The midpoint of the clotting curve was chosen as the end point for clotting time because it lies in the linear section of greatest slope, allowing timing instruments to be stopped with the greatest precision.

Reed *et al.* showed that the logarithm of clotting time varies linearly with heparin activity (3). The nephelometric method produced similar results. Good correlation with a straight line at the 95% confidence level was obtained with a standard curve consisting of at least four points. For the comparison study, the average correlation coefficient for the nephelometric standard curves was 0.9901.

The results indicate that the nephelometric assay is more reproducible than the USP assay. The coefficients of variation, calculated at an activity of 50 U/mL, were 2.79% for the nephelometric assay and 5.94% for the USP method. For each method, the total observed variation in assay results was statistically analyzed in terms of each of the underlying sources of assay imprecision. The results of this variance component analysis and the relative contribution of each source of variation are shown in Table I. For the nephelometric assay, determination of clotting time is the largest contributor to variance. This variability is due to normal changes in the clotting response of sheep plasma. The greatest sources of assay imprecision for the USP assay are daily shifts in assay results and technician differences. The technician variance seems to be due to the subjectivity of the grading system, indicating not only differences in the way two technicians grade, but also differences in the way a single technician grades from day to day. This factor seems to be eliminated with the instrumental method.

Table I—Comparison of the Precision of the Nephelometric and USP Methods

Variance Components	Nephelometric Method: Variance Estimate, U/mL²	Nephelometric Method: Relative Contribution to Total Variance, %	USP Method: Variance Estimate, U/mL²	USP Method: Relative Contribution to Total Variance, %
Date	0.000	0.0	4.414	48.6
Concentration * date	0.000	0.0	0.045	0.5
Tech	0.000	0.0	2.292	25.2
Concentration * tech	0.000	0.0	0.000	0.0
Date * tech	0.612	23.5	1.563	17.2
Concentration * date * tech	0.898	34.5	0.259	2.9
Dilution (concentration * date * tech)	0.097	3.7	0.000	0.0
Determination (dilution)	0.998	38.3	0.513	5.6
Total variance	2.60	100.0	9.09	100.0
SD, U/mL	1.39[a]	—	2.97[b]	—
CV[c] at heparin activity of 50 U/mL	2.78	—	5.94	—

[a] Mean of three determinations per dilution. [b] Mean of two determinations per dilution. [c] Calculated using the formula: $CV = (SD/\text{heparin activity}) \times 100$.

Table II—Comparison of the Accuracy of the Nephelometric and USP Methods

Technician	Theoretical Heparin Activity, U/mL	Mean Bias[a]: Nephelometric	Mean Bias[a]: USP	Recovery, %: Nephelometric	Recovery, %: USP
1	45.07	−0.64	−1.25	98.6	97.2
	49.97	−0.83	−2.87	98.3	94.3
	54.92	−0.75	−2.83	98.6	94.9
2	45.07	−0.25	1.43	99.5	103.2
	49.97	−1.91	−0.41	96.4	99.2
	54.92	−1.14	−0.82	97.9	98.5

[a] Difference between theoretical and experimentally determined activity.

The accuracy of each method was evaluated by comparing observed assay values with theoretical heparin activity (Table II). Although both methods demonstrate a negative mean bias at nearly all concentration levels for both technicians, the observed biases were within the precision limits found for each method. This negative bias is most likely a systematic error in the experimental procedure (*e.g.*, preparation of the test solutions) because it was observed in all results except one. The two methods were tested for equivalency by analyzing the ranks of the mean biases. This analysis showed no significant difference between methods ($p > 0.05$).

The nephelometric method has definite advantages over the USP method. It is faster (a single assay can be completed in less than half the time the USP method takes) and simpler (it does not involve the subjective grading, lengthy procedure, and calculations of the USP method). The nephelometric method is also more sensitive. Activities of 1 U/mL of heparin can be detected with the nephelometer *versus* 3 U/mL with the USP assay.

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Comparative Pharmacokinetics of [^{65}Zn]Zinc Sulfate and [^{65}Zn]Zinc Pantothenate Injected Intravenously in Rabbits

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Received March 14, 1980, from the **Laboratory of Biochemistry and* [‡]*Pharmacology Jean Bernard Hospital, BP 577, 86021 Poitiers Cedex, France.* Accepted for publication December 12, 1983.

Abstract □ The pharmacokinetics of zinc sulfate were compared with those of a new zinc salt, pantothenate, in rabbits. Each salt was administered at a dosage of 3.3 μCi of zinc-65/kg of body weight. The measured pharmacokinetics of the two compounds responded to a two-compartment open model. The urinary elimination of the two salts was similar, as was their localization in the skin and fur, but zinc pantothenate was fixed by the liver to a lesser extent than was zinc sulfate.

Keyphrases □ [^{65}Zn]Zinc sulfate—intravenous administration to rabbits, pharmacokinetics □ [^{65}Zn]Zinc pantothenate—intravenous administration to rabbits, pharmacokinetics □ Pharmacokinetics—comparison of [^{65}Zn]zinc sulfate and [^{65}Zn]zinc pantothenate injected intravenously, rabbits □ Zinc salts—zinc sulfate and zinc pantothenate, comparative pharmacokinetics, intravenous administration to rabbits

Zinc sulfate is used to treat acrodermatitis enteropathica (1, 2), a condition associated with abnormal zinc metabolism. Zinc pantothenate, which we synthesized in our laboratory, had a much lower toxicity when administered orally to animals than did zinc sulfate and exhibited better gastric tolerance (3). We report here a comparative pharmacokinetic study of the intravenous injection of the two radioactive zinc salts.

EXPERIMENTAL SECTION

Reagents—[^{65}Zn]Zinc pantothenate was prepared as previously described (4). The principle of the reaction is an exchange between calcium pantothenate and [^{65}Zn]zinc sulfate with a specific radioactivity of 3.5 mCi/mg.

Animal Treatment—Six male rabbits (mean weight, 2.5 kg) were isolated in metabolism cages and placed on a water diet 12 h prior to the experiment. The necks of the animals were shaved, and a 0.5% solution of lidocaine was applied as a light topical anesthesia. The jugular vein was freed, and a catheter was inserted. Bleeding was prevented by placing a small sponge imbibed with anesthetic under the suture. The protruding catheter was fixed to the skin with a bandage, thus permitting both injection of the products tested and blood sampling.

Protocol and Analytical Procedures—Each zinc salt was injected into the jugular vein at a dose of 3.3 μCi of zinc-65/kg, and blood samples were taken at 5, 10, 15, 30, and 60 min and 2, 4, and 6 h postinjection. Blood samples (4 mL) were taken at the indicated times in a dry polypropylene tube (5), and serum was separated by centrifugation for 10 min at 3000 rpm. Animals were sacrificed 6.5 h after injection, and various organs or tissue fragments were removed and weighed: liver, kidneys, whole skin with fur, and, after washing with physiological saline, the small intestine.

Radioactivity was determined with a liquid scintillation counter[1]. Serum activity is expressed as disintegration per minute after quench correction (Fig. 1). For urine elimination and tissue and organ samples, the results are expressed as the percentage of the total quantity administered (Table I).

[1] Model 9000 γ; Beckman.

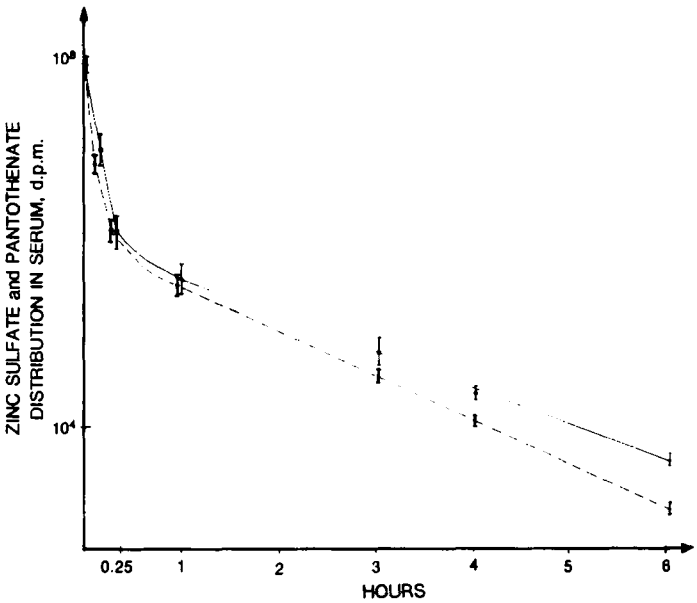

Figure 1—*Kinetics of the blood distribution of [^{65}Zn]zinc sulfate (—) and [^{65}Zn]zinc pantothenate (- -) after intravenous injection to six rabbits. The radioactivity is expressed as disintegrations per minute (d.p.m.). Results are expressed as the mean* ± SE.

RESULTS AND DISCUSSION

The pharmacokinetic data indicate that the distribution half-life (α) of zinc pantothenate is shorter (0.11 h^{-1}) than that of zinc sulfate (0.134 h^{-1}) (Fig. 1). Urinary elimination is very low and does not exceed 1% of the administered dose, which confirms results of previous studies with rats (6) and dogs (7) with zinc sulfate alone.

Both salts exhibit a similar distribution in the kidneys, the small intestine, the skin, and the fur. In the present study, there is a clear difference in hepatic

Table I—Tissue Distribution and Urine Elimination of [^{65}Zn]Zinc Pantothenate and [^{65}Zn]Zinc Sulfate after Intravenous Administration [a]

Tissue or Fluid	Radioactivity, Percentage of Total	
	[^{65}Zn]Zinc Pantothenate	[^{65}Zn]Zinc Sulfate
Liver	8	17
Kidney	2	2
Whole skin and fur	4	2
Small Intestine	1	1
Urine elimination	1	1

[a] Results were obtained 6.5 h after administration to six rabbits; results are expressed as percentage of the total activity.

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