The serum decay curves for coralyne in dogs and monkeys exhibited a rapid distribution phase followed by a much slower second phase. Pharmacokinetic values derived by computer analysis are summarized in Table I. Values for the first phase were similar to those derived for rats given an intravenous dose of coralyne (5). As observed for rats, however, a distribution phase may be missing, because distribution of the dose in the total body water of dogs and monkeys would produce a larger serum concentration (15 μg/ml) at t_0 (y intercept).

In dog serum, but not monkey serum, a faintly fluorescent metabolite of coralyne appeared after 45 min at R_f 0.80. This nonpolar metabolite may be the same as that observed in the bile of rats (5). Based on TLC using 10–50-fold more extract than was used to detect coralyne, the contribution of fluorescence of this metabolite to total fluorescence is small.

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Rapid Determination of Gold in Whole Blood of Arthritis Patients Using Flameless Atomic Absorption Spectrophotometry

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Abstract □ An assay for gold in whole blood of arthritis patients was developed using the graphite furnace atomic absorption spectrophotometer. This method involves no pretreatment of the whole blood except for simple dilution, thereby eliminating some variables and saving laboratory time and expense.

Keyphrases □ Gold—atomic absorption spectrophotometric analysis in whole blood □ Atomic absorption spectrophotometry—analysis, gold in whole blood □ Metals—gold, atomic absorption spectrophotometric analysis in whole blood

Gold salts have been used in rheumatoid arthritis for over 40 years, but few concise and accurate assays for the determination of gold in biological fluids have been developed. Since varying amounts of gold are found in the red blood cells of some patients receiving chrysotherapy (1, 2), an analysis of whole blood rather than blood fractions should be used to determine optimally the total gold content in the circulation.

Most methods of gold analysis (*i.e.*, chemical, UV emission, spectrography, polarography, and neutron activation) require extensive laboratory work to remove interfering fractions and repeated extractions (3, 4). Neutron activation analysis for the estimation of gold is the most sensitive method available (5); but since samples must be dried, sealed, irradiated for several hours, and cooled for several days, there is a possibly prohibitive time delay in reporting results. Furthermore, the assay requires expensive neutron sources with licensed operators and multichannel analyzers, both of which are relatively unavailable (5). This assay is beyond the capabilities of most clinical laboratories, thus rendering it rather impractical (4).

The lack of simple and accurate assay techniques for gold in biological fluids made it difficult to determine absorption, distribution, deposition, and excretion throughout dosing intervals and inhibited evaluation of the pharmacology and the pharmacodynamics of gold (4, 6–8). Atomic absorption spectrophotometric results compare well with those of neutron activation analysis (5), and its simplicity, speed, and range make it ideal for clinical work.

EXPERIMENTAL

Apparatus—The analysis was done using an atomic absorption spectrophotometer[1] with a graphite furnace accessory[2]. Power for the furnace was supplied by a controller[2] with a temperature ramp accessory[2] (Table I) to minimize elemental loss due to rapid gross temperature changes. All samples were injected *via* an automatic sampling system[3]. Since the instrument digital readout did not respond rapidly enough to the atomization signal, a strip-chart recorder[4] was implemented. The chosen emission source was a hollow cathode lamp[5].

Reagents and Standards—The water used in all dilutions was previously deionized by passage through a mixed-bed, ion-exchange column[6] with subsequent filtration through a 5-μm membrane filter[7] to remove any dislodged resin and other particulates.

[1] Perkin-Elmer model 403.
[2] Perkin-Elmer model HGA-2100.
[3] Perkin-Elmer model AS-1.
[4] Perkin-Elmer model 36.
[5] Perkin-Elmer Intensitron (No. 303-6031).
[6] Barnstead Ultrapure (No. D0809).
[7] Metricel.

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Table I—Preset Conditions on the Temperature Controller and Ramp Accessory

Parameter	Temperature	Cycle Time[a], sec	Ramp Time, sec
Dry	125°	30	10
Char	800°	90	10
Atomize	2200°	5	0

[a] Includes ramp time for that step.

Table II—Precision of the Gold Analysis at Different Whole Blood Concentrations [a]

Actual, μg/dl	Observed[b], μg/dl	*CV*, %
100	112.6	10.4
300	289.2	5.1
500	493.4	1.8

[a] Using an average calibration curve selected from actual data. [b] Mean of 18 determinations from 3 separate days.

All standards were prepared by the addition of atomic absorption standards[8] to quantities of blank human whole blood. To separate 20-ml aliquots of whole blood were added 20, 60, and 100 μl of 1000-ppm standards, making the final concentrations of the standard gold–whole blood solutions 1, 3, and 5 ppm, respectively.

The atomic absorption spectrophotometer settings were: emission wavelength, 242.8 nm; slit width, 7 nm; lamp current, 10 mamp; argon flow rate, 40 ml/min; and water flow rate, 2–2.5 liters/min.

Procedure—All patient blood samples were received in glass tubes[9] with edetic acid as the anticoagulant. Each sample was both vortexed and inverted gently to distribute the formed constituents evenly throughout, and then a 500-μl portion was withdrawn using a micropipet[10]. This portion was placed in a polypropylene centrifuge tube[11] containing 9.5 ml of the deionized, distilled water. The resulting mixture was then vortexed for 10 sec to ensure complete dispersion and hemolysis of the blood.

The procedure was repeated for all patient and standard samples. The final step involved placing diluted samples, done in duplicate, into sampling cups from which the automatic sampling system withdrew 20 μl for each determination.

RESULTS AND DISCUSSION

The study was conceived to develop a simple, rapid method of determining blood gold levels in arthritis patients and to improve clinician monitoring of chrysotherapy.

Since chrysotherapy is becoming more widely used in the treatment of active rheumatoid arthritis, the clinician needs to be able to adjust the dosing based on blood level data rather than subjective data such as patient opinion and range of motion evaluation. If the patient shows no clinical improvement when therapeutic blood levels (300–600 μg/dl) are reached, therapy can be stopped rather than continued in the hope of subjective improvement.

Study data also indicate that a considerable portion of the so-called therapeutic failures may be due to undetected subtherapeutic blood gold levels. Reproducible quantitative detection of gold at picogram levels was attained (Table II) without complicated analytical procedures involving extraction, chelate formation, and sample transfers. Gold concentrations (300–600 μg/dl) in the normal therapeutic range do not require procedures sensitive to picogram levels. Clinically useful analytical procedures must be simple, specific, and capable of being performed rapidly.

Operation of the atomic absorption spectrometer in the therapeutic gold concentration range results in a good signal-to-noise ratio with suitable amplitude of the recorder response for data reduction.

The preset conditions on the controller[2] (Table I) for the programmed temperature time cycles were critical to the assay precision and accuracy. The cycle timing primarily affected the assay sensitivity, while the assay reproducibility was primarily affected by the temperature settings.

[8] Fisher Scientific (1000 ppm).
[9] Vacutainers, Becton-Dickinson Co.
[10] Eppendorf, Brinkmann.
[11] Nalgene.

Table III—Example of Individualized Patient Blood Level Analysis

Standard Curve Data	
Concentration, ppm	Absorbance Units
1	11
1	11
3	32
3	33
5	52
5	53
Slope	10.375
Y-Intercept	0.875
SE	0.165
Correlation coefficient	0.999
Confidence limits	9.915–10.835
Patient Data	
Patient name	Doe, John
Time	Hour 4
Sample date	3/29/76
Gold concentration (whole blood), μg/dl	411
Error, %	1.659
Average absorbance	43.5

Table IV—Example of Cumulative Blood Level Data for Physician from One Dosing Interval (1 Week)

Time	Date Drawn	Whole Blood Level, μg/dl
Hour 0	3/29/76	213
Hour 1	3/29/76	469
Hour 2	3/29/76	430
Hour 3	3/29/76	425
Hour 4	3/29/76	411
Day 1	3/30/76	293
Day 2	3/31/76	258
Day 3	4/1/76	230
Day 4	4/2/76	194

Volunteer blood and a suitable commercial source of whole blood were used to run whole blood standard curve samples and were compared. There was no difference in instrumental response to freshly drawn or commercial standard curve whole blood samples at any time. The use of different anticoagulants such as edetate disodium, ammonium pyrrolidinedithiocarbamate, heparin, citrates, and oxalates did not alter the test results. Therefore, the whole human blood used for blank determinations could be used with the usual anticoagulants employed by commercial blood suppliers. Anticoagulant citrate phosphate dextrose solution was used in the human whole blood units.

The standard curve was linear from 0 to 600 μg/dl. If the standard curve correlation coefficient (Table III) was less than 0.950, the calibration procedure was repeated. If a patient's whole blood gold level was outside the limits of the chosen standard (roughly the normal therapeutic blood level range), the whole blood sample was diluted more or less than the standard procedure (0.5 ml of whole blood diluted with 9.5 ml of water) to bring the absorbance values within the specified range.

The analytical procedure developed utilizes an ordinary whole blood sample with routinely used anticoagulants, one dilution with water, mixing, and injection into the instrument. The method meets the clinically desirable parameters of simplicity, speed, appropriate sensitivity, and presentation of the data in a format[12] suitable for research (Table III) or clinical (Table IV) utilization.

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Synthesis and β-Adrenergic Blocking Action of a New Thiazolylthiopropanolamine Derivative

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Received September 14, 1977, from the *Research and Development Center, Pharmaceuticals Division, Sumitomo Chemical Co., Ltd., Takatsukasa 4-2-1, Takarazuka, Hyogo, Japan.* Accepted for publication January 11, 1978.

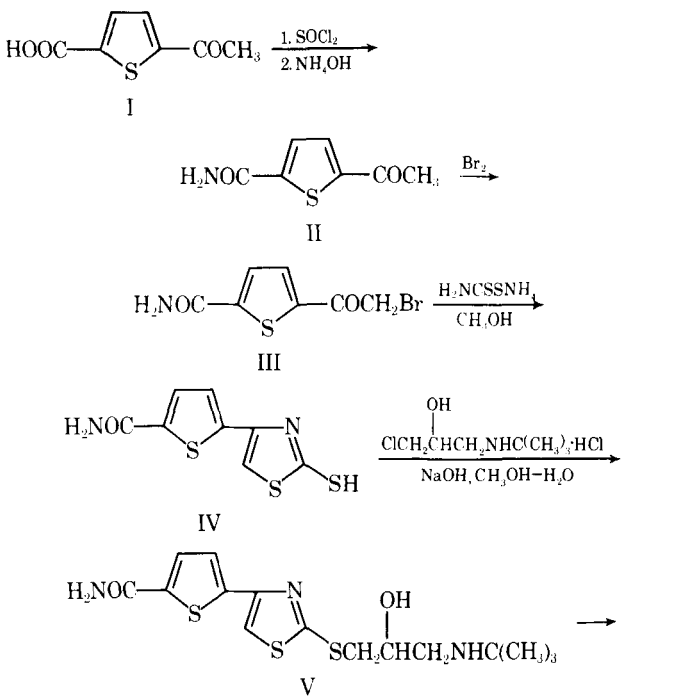
Abstract □ The synthesis of (±)-2-(3′-*tert*-butylamino-2′-hydroxypropylthio)-4-(5′-carbamoyl-2′-thienyl)thiazole hydrochloride is described. The new compound antagonized the cardiovascular effects, such as positive chronotropic, positive inotropic, or depressor arterial blood pressure responses, elicited by intravenous isoproterenol; it was 9–14 times as potent as propranolol in anesthetized open chest dogs. The oral administration of the compound reduced isoproterenol tachycardia in conscious dogs. It was about five times as potent as propranolol in this test, with maximal action after 1 hr, and its duration was significantly longer than that of propranolol.

Keyphrases □ Thiazolylthiopropanolamine derivative—synthesized, β-adrenergic blocking activity evaluated in dogs □ β-Adrenergic blocking activity—thiazolylthiopropanolamine derivative evaluated in dogs

Many amino alcohol derivatives have been described to have a β-blocking action, and some have been of interest for the treatment of angina pectoris and cardiac arrhythmias (1). Most compounds available for clinical use belong to the aryloxypropanolamine series, which is considered the second generation of β-blocking agents, in contrast with the arylethanolamine series, which includes original β-blocking agents such as dichloroisoproterenol and pronethalol (2).

During a search for drugs affecting the peripheral autonomic nervous system, various compounds with an *N*-substituted thiopropanolamine moiety attached to a heterocyclic nucleus were synthesized and tested for β-blocking action as well as the other pharmacological actions. A new compound, (±)-2-(3′-*tert*-butylamino-2′-hydroxypropylthio)-4- (5′-carbamoyl-2′-thienyl)thiazole hydrochloride[1] (VI), showed remarkable β-blocking action with low toxicity and was virtually devoid of other pharmacological actions.

The present report describes the chemical synthesis and β-blocking activity of this derivative compared with that of propranolol (3) in *in vivo* experiments.



Scheme I

EXPERIMENTAL[2]

Chemistry—Reaction of 5-carboxy-2-acetylthiophene (I, Scheme I) (4) with thionyl chloride in toluene and successive treatment with aqueous ammonia furnished the carbamoyl derivative (II), which was converted to its monobromide (III). Reaction of the bromide with ammonium dithiocarbamate resulted in isolation of 2-mercapto-4-(5′-carbamoyl-2′-thienyl)thiazole (IV). Condensation of IV with 1-chloro-3-*tert*-butylaminopropanol hydrochloride (5) by means of base in an equivalent mixture of methanol and water provided 2-(3′-*tert*-butylamino-2′-hydroxypropylthio)-4-(5′-carbamoyl-2′-thienyl)thiazole (V), from which its crystalline hydrochloride (VI) was derived.

5-Carbamoyl-2-acetylthiophene (II)—To a suspension of I, 22 g (0.129 mole) of thionyl chloride in 200 ml of toluene was added dropwise with vigorous stirring. The mixture was heated under a gentle reflux for 2 hr and evaporated *in vacuo* to dryness. The residue was dissolved in 150 ml of toluene again and treated with aqueous ammonia below 10°.

The white precipitate was collected by suction filtration, washed with water, and dried, yielding 20 g (92.8%), mp 226–228° (methanol); NMR:

[1] S-596.

[2] Melting points were obtained on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. IR spectra were determined in mineral oil mulls on a Hitachi EPI-G3 IR spectrometer. NMR spectra were taken in deuterated dimethyl sulfoxide on a Varian Associates T-60 instrument with tetramethylsilane as the internal standard.

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