# Distribution and Urinary Excretion of Furosemide in the Rat

SETSUYA SENO*, STANLEY M. SHAW, and JOHN E. CHRISTIAN

**Abstract** □ Using $^{35}$S-labeled furosemide, the distribution and excretion of furosemide was investigated. Emphasis was placed upon the possibility of accumulation of the compound resulting from daily administration. The excretion and distribution of furosemide and/or metabolites (expressed as furosemide) were determined at various time intervals after termination of dosing. Of the tissues studied, the level of furosemide was the highest in the liver and kidney. At the termination of drug administration, furosemide levels in liver and kidney of single-dose animals were not significantly different from levels observed in repeatedly treated rats; however, 10 days later the retention of furosemide was significantly greater in the repeatedly treated rats. The results of the excretion studies indicated, as did the distribution data, that furosemide may have accumulated in repeatedly treated animals.

**Keyphrases** □ Furosemide, $^{35}$S-labeled—distribution, excretion, rats □ Tissue, plasma levels—furosemide □ Excretion, urine, feces—furosemide □ Accumulation, furosemide—repeated administration □ Scintillometry, liquid—radioactivity determination □ Autoradiography—furosemide and metabolites

Furosemide is a sulfonamide derivative of anthranilic acid. It is a relatively new diuretic which is chemically distinct from organomercurials, thiazides, and other heterocyclic compounds. Furosemide has been used for the acute and chronic treatment of edema associated with various diseases. Considerable literature related to the properties and therapeutic effects of furosemide is available (1–11).

The object of this investigation was to study the distribution and excretion of furosemide in the rat. Emphasis was placed upon the possibility of accumulation of the compound from daily administration. For an investigation of this nature, radiotracer techniques offer unsurpassed advantages and consequently were utilized in the study.

## EXPERIMENTAL

Female strain albino rats[1] (175-200 g.) were divided into groups of six animals each. Animals were housed individually in stainless steel screen wire bottom metabolism cages. Distilled water was given *ad libitum* throughout the experiment. Food was allowed *ad libitum* except for 3 hr. before administration of labeled furosemide. Sulfur-35-labeled furosemide[2] (1 μc./mg.) was administered as an aqueous suspension in acacia and carboxymethylcellulose at a dosage level of 20 mg./rat/day. Radiation dose to the experimental animals from the labeled furosemide was calculated and shown to be insignificant.

The investigation involved groups of animals administered either a single dose of labeled furosemide or a total of 10 doses of labeled compound given over a period of 10 days. Single-dose animals received the labeled compound at the same time as the final dose was administered to the repeatedly treated rats. From certain groups, urine and feces were collected during treatment and for varied time intervals following cessation of treatment. For tissue distribution studies, groups of animals were sacrificed at varied time intervals following the termination of drug administration. Tissues were prepared for liquid scintillation counting by adding 2 ml. of a tissue-solubilizing base mixture[3] to approximately 50 mg. of tissue contained in counting vials. The vials were heated at 65° for 30 min. with occasional shaking. After cooling the sample to room temperature, 0.2–0.5 ml. (depending upon coloration) of 30% hydrogen peroxide solution was added to decolorize the sample. Upon completion of the reaction, 1 ml. of glacial acetic acid and 15 ml. of the XDC scintillator solution[4] were added to the vial.

Urine samples collected from individual animals were measured by volume and diluted to a definite volume with distilled water. Accurately measured aliquots of the diluted urine were placed in counting vials and mixed with 1 ml. of a solubilizing base mixture. The samples were then treated in the same manner as the tissue samples. Feces samples were dried at room temperature, weighed, and pulverized. Accurately measured aliquots of approximately 50 mg. of pulverized feces were transferred into counting vials and 2 ml. of a solubilizing base mixture added. The samples were treated in the same manner as the tissue samples except for the amount of hydrogen peroxide solution, which was approximately 1 ml.

All the samples in this study were analyzed for total $^{35}$S activity using an automatic liquid scintillation spectrometer[5] with a counting error of 5% or less. An internal standard technique, in which the samples were counted before and after the addition of a nonquenching $^{35}$S furosemide reference solution, was employed to obtain absolute disintegration rates. The counting data were mathematically treated so as to express the $^{35}$S activity as the concentration or total quantity of furosemide in each sample. The average and *SD* values of each set of six animals data were computed. The significance of differences was tested by the Student *t* test. Computations were accomplished with a computer (IBM 7090) with Fortran programming.

## RESULTS AND DISCUSSION

With the exception of a brief urinary metabolite investigation, no attempt was made to separate furosemide and metabolites in the various samples. The data are expressed as furosemide although furosemide and/or metabolites may have been present.

**Distribution**—Throughout all experiments, the furosemide level in kidney tissue was greater than that observed in other tissues studied (Table I). Six hours following final drug administration, the level of furosemide in kidney tissue of animals administered a single dose of the drug, was 33 mcg./g. of kidney, while the concentration of the diuretic agent for repeatedly treated rats was 54 mcg./g. of kidney tissue. The relative difference in furosemide concentration in kidney tissue between single-dose and repeatedly treated animals was approximately 40%, 6 hr. following the final dose; however, the difference was not statistically significant. The difference was larger and statistically significant ($p < 0.01$) after 10 days, with the furosemide level in the kidney tissue of repeatedly treated rats being three times larger than that of single-dose animals. The second highest concentration of furosemide was found in liver tissue. The concentration of the diuretic agent for single-dose and repeatedly treated animals was approximately 9 mcg./g. of liver 6 hr. following final drug administration. Four days later, approximately twice as great a concentration of furosemide was

[1] Sprague-Dawley, Inc., Madison, Wis.
[2] Synthesized by Farbwerke Hoechst AG, Frankfurt, Germany, and supplied by Hoechst, Cincinnati, Ohio.
[3] A mixture of three parts of 1 *M* *p*-(diisobutylcresoxyethoxyethyl)-dimethylbenzyl ammonium hydroxide solution in methanol and one part of 30% aqueous potassium hydroxide solution.
[4] The XDC scintillator solution consisted of 80 g. of naphthalene, 10 g. of PPO, and 0.5 g. of POPOP dissolved in 1 l. of solvent prepared with one part of xylene, three parts of dioxane, and three parts of ethylene glycol monoethyl ether.
[5] Tri-Carb, Packard Instrument Co., Inc., LaGrange, Ill.

**Table I**—Furosemide[a] in Various Tissues of Single-Dose and Repeatedly Treated Rats

| Sacrifice,[b] days | Liver | Kidney | Spleen | Brain | Muscle | Plasma |
|---|---|---|---|---|---|---|
| | | | **Single-Dose**[c] | | | |
| 0.25 | 8.62[d] ±1.31 | 32.82 ± 5.64 | 2.24 ±0.91 | 0.31 ±0.18 | 0.63 ±0.18 | 12.25 ± 4.39 |
| 4 | 0.85 ±0.82 | 8.91 ± 9.95 | 0.29 ±0.05 | 0.10 ±0.06 | 0.12 ±0.08 | 0.27 ± 0.43 |
| 10 | 0.19 ±0.05 | 1.89 ± 0.06 | 0.10[e] ±0.06 | 0.01 ±0.05 | 0.01 ±0.04 | 0.04 ± 0.01 |
| 15 | 0.07 ±0.03 | 0.78 ± 0.21 | 0.05 ±0.06 | — — | — — | — — |
| 20 | 0.04 ±0.04 | 0.43 ± 0.08 | 0.04 ±0.04 | — — | — — | — — |
| | | | **Repeatedly Treated**[f] | | | |
| 0.25 | 8.72 ±5.19 | 53.97 ±32.75 | 3.96 ±4.53 | 0.23 ±0.28 | 0.82 ±0.45 | 6.23 ± 7.44 |
| 4 | 1.60 ±0.45 | 13.49 ± 1.44 | 0.80 ±0.33 | 0.25 ±0.21 | 0.14 ±0.27 | 0.21 ± 0.09 |
| 10 | 0.87 ±0.29 | 5.98 ± 1.75 | 1.32 ±0.12 | 0.19 ±0.23 | 0.00 ±0.00 | 0.05 ± 0.06 |
| 15 | 0.17 ±0.03 | 3.08 ± 0.57 | 0.07 ±0.03 | — — | — — | — — |
| 20 | 0.09 ±0.07 | 1.49 ± 0.25 | 0.05 ±0.05 | — — | — — | — — |

[a] Expressed as mcg./g. of tissue. Data expressed as furosemide may include a certain fraction of metabolic products, as no attempt was made to separate furosemide and metabolites. [b] Time of sacrifice following cessation of treatment. [c] Animals received a single dose (20 mg./rat) of labeled furosemide. [d] Average of six animals per group ± *SD*. [e] A furosemide level of 0.20 mcg./g. of tissue or less may be regarded as negligible (0.03 mcg./g. or less for blood plasma). [f] A total of 10 doses of labeled furosemide (20 mg./rat/day) administered over a period of 10 days.

found in the liver of repeatedly treated animals as that found in single-dose rats. The difference in the liver drug level was statistically significant ($p < 0.01$) in animals sacrificed 10 days after cessation of furosemide treatment.

Furosemide levels in the blood plasma of single-dose and repeatedly treated rats were statistically equivalent throughout the investigation. The furosemide concentration diminished quite rapidly to an insignificant level within 10 days after final dosing. No significant differences in furosemide levels between single-dose and repeatedly treated animals were observed in the spleen, brain, or muscle tissue. Observations were discontinued 10 days after final dosing.

In Table II, the data are expressed as furosemide in the entire organ. In general, differences in the furosemide content in single-dose and repeatedly treated rats are in agreement with the results obtained when the data are expressed as furosemide per gram of tissue. Although, furosemide found in the liver of repeatedly treated rats was significantly higher ($p < 0.01$) than that detected in single-dose animals 4 days and thereon after termination of treatment, statistical significance was not obtained until 10 days after termination of dosing when the data are expressed as furosemide per gram of tissue. Similar results may be observed for the kidney.

It is interesting to note that no significant difference in furosemide content in the kidney or liver was observed between repeatedly treated and single-dose animals 6 hr. following the final dose, while significant differences occurred in animals sacrificed 4 days or later after cessation of treatment. The data suggest a delay in the elimination of the drug which was given previous to the final dose and which was retained somewhere in the animal, thus suggesting an accumulation of minor fractions of furosemide and/or metabolites in the animal.

**Excretion**—Exemplitory data describing the excretion of furosemide in the urine and feces of repeatedly treated rats is shown in Table III. Four days after the final dose, 95% of the total furosemide administered (200 mg.) during the study had been accumulated in the excreta. Of the total furosemide excreted, 47.5% was accounted for in the urine while 52.5% was found in the feces.

Table IV summarizes the results of the study of furosemide excreted in the urine following the termination of single or multiple

**Table II**—Furosemide[a] in Various Organs of Single-Dose and Repeatedly Treated Rats

| Organ | Time of Sacrifice, days[b] 0.25 | 4 | 10 | 15 | 20 |
|---|---|---|---|---|---|
| | | | **Single-Dose**[c] | | |
| Liver | 53.70[d] ± 9.52 | 4.43 ± 2.81 | 1.34 ± 0.30 | 0.42 ±0.15 | 0.26 ±0.24 |
| Kidney | 49.76 ±11.19 | 12.10 ±12.11 | 2.78 ± 0.93 | 1.09 ±0.30 | 0.62 ±0.15 |
| | | | **Repeatedly Treated**[e] | | |
| Liver | 48.17 ±11.15 | 13.96 ± 3.48 | 7.62 ± 2.64 | 1.17 ±0.25 | 0.55 ±0.39 |
| Kidney | 87.44 ±42.40 | 24.65 ± 3.15 | 10.66 ± 2.99 | 4.58 ±0.48 | 2.20 ±0.28 |

[a] Expressed as mcg./organ. Data expressed as furosemide may include a certain fraction of metabolic products as no attempt was made to separate furosemide and metabolites. [b] Time of sacrifice following cessation of treatment. [c] Animals received a single dose (20 mg./rat) of labeled furosemide. [d] Average of six animals per group ± *SD*. [e] A total of 10 doses of labeled furosemide (20 mg./rat/day) administered over a period of 10 days.

**Table III**—Excretion of Furosemide[a] in the Urine and Feces of Repeatedly Treated[b] Rats

| Time,[d] days | Furosemide Excreted,[c] mg. Urine | Feces | Total |
|---|---|---|---|
| 1 | 6.17 | 2.45 | 8.62 |
| 2 | 14.27 | 3.36 | 17.63 |
| 3 | 15.59 | 3.01 | 18.60 |
| 4 | 15.10 | 10.95 | 26.05 |
| 5 | 12.65 | 12.34 | 24.99 |
| 6 | 6.69 | 14.51 | 21.20 |
| 7 | 6.81 | 15.92 | 22.73 |
| 8 | 4.21 | 12.74 | 16.95 |
| 9 | 4.92 | 8.28 | 13.20 |
| 10 | 2.89 | 12.13 | 15.03 |
| 11 | 0.77 | 3.51 | 4.28 |
| 12 | 0.46 | 0.64 | 1.11 |
| 13 | 0.27 | 0.35 | 0.62 |

[a] Data expressed as furosemide may include a certain fraction of metabolic products as no attempt was made to separate furosemide and metabolites. [b] A total of 10 doses of labeled furosemide (20 mg./rat/day) administered over a period of 10 days. [c] Excreta collected at 24-hr. intervals and data expressed as the average of six rats. [d] Time after the intial dose was administered.

**Table IV**—Excretion of Furosemide[a] in the Urine of Single-Dose and Repeatedly Treated Rats

| Time[d] days | Single-Dose[b] Urine Volume, ml. | Single-Dose[b] Furosemide, mg. | Repeatedly Treated[c] Urine Volume, ml. | Repeatedly Treated[c] Furosemide, mg. |
|---|---|---|---|---|
| 1 | 5.2 | — | 20.2 | 3.472 ± 0.553 |
| 2 | 5.2 | — | 27.7 | 6.263 ± 1.886 |
| 3 | 5.3 | — | 28.3 | 6.572 ± 0.946 |
| 4 | 6.9 | — | 30.3 | 6.990 ± 2.064 |
| 5 | 6.6 | — | 23.5 | 6.926 ± 2.354 |
| 6 | 9.2 | — | 24.5 | 6.942 ± 2.429 |
| 7 | 10.7 | — | 23.5 | 6.958 ± 2.668 |
| 8 | 10.3 | — | 20.0 | 7.119 ± 3.170 |
| 9 | 11.0 | — | 22.2 | 6.282 ± 2.004 |
| 10 | 24.2 | 4.728 ± 1.432 | 30.5 | 7.979 ± 4.001 |
| 11 | 14.0 | 0.940 ± 0.320 | 15.2 | 1.845 ± 0.678 |
| 12 | 12.7 | 0.099 ± 0.036 | 10.8 | 0.531 ± 0.195 |
| 13 | 13.3 | 0.024 ± 0.006 | 13.5 | 0.174 ± 0.072 |
| 14 | 14.0 | 0.017 ± 0.007 | 13.5 | 0.128 ± 0.047 |
| 15 | 12.2 | 0.011 ± 0.005 | 13.8 | 0.072 ± 0.038 |
| 16 | 11.5 | 0.009 ± 0.004 | 13.5 | 0.054 ± 0.023 |
| 17 | 15.2 | 0.011 ± 0.003 | 19.2 | 0.082 ± 0.040 |
| 18 | 10.7 | 0.013 ± 0.003 | 17.3 | 0.088 ± 0.038 |
| 19 | 12.5 | 0.011 ± 0.004 | 13.5 | 0.074 ± 0.030 |
| 20 | 13.0 | 0.010 ± 0.003 | 12.2 | 0.077 ± 0.038 |
| 21 | 12.7 | 0.010 ± 0.003 | 13.0 | 0.078 ± 0.019 |
| 22 | 10.7 | 0.008 ± 0.005 | 11.8 | 0.078 ± 0.031 |
| 23 | 16.2 | 0.007 ± 0.003 | 17.0 | 0.068 ± 0.025 |
| 24 | 12.5 | 0.006 ± 0.002 | 11.7 | 0.070 ± 0.037 |
| 25 | 13.3 | 0.007 ± 0.004 | 16.8 | 0.085 ± 0.059 |
| 26 | 12.0 | 0.003 ± 0.002 | 18.0 | 0.045 ± 0.024 |
| 27 | 12.0 | 0.002 ± 0.001 | 12.7 | 0.025 ± 0.015 |
| 28 | 14.8 | 0.004 ± 0.002 | 9.8 | 0.023 ± 0.009 |
| 29 | 11.8 | 0.003 ± 0.002 | 13.0 | 0.021 ± 0.007 |

[a] Data expressed as furosemide may include a certain fraction of metabolic products as no attempt was made to separate furosemide and metabolites. [b] Single dose of labeled furosemide (20 mg./rat) administered to a group of six animals on Day 9. Data are expressed as the average of six rats per group ± *SD*. [c] A total of 10 doses of labeled furosemide (20 mg./rat/day) administered over a period of 10 days to a group of six animals. [d] Time after intial dose was given to the repeatedly treated rats. Urine collected at 24-hr. intervals

dosing. After the final dose, the amount of furosemide excreted in the urine decreased rapidly for 4 days, was maintained at a plateau for the next 10 days, and was decreasing at the termination of the study. During the entire collection period, higher levels of furosemide were present in the urine of repeatedly treated animals than observed in the urine of single-dose rats. As may be seen in Table IV, a definite diuretic effect was elicited by furosemide during the interval of drug administration. After termination of drug treatment the daily urinary excretion of furosemide was 5 to 10 times higher in the repeatedly treated animals than in single-dose animals, although the urinary volume was essentially the same for both groups. The larger excretion of furosemide from repeatedly treated animals may have resulted from an overlapping effect of the daily excretion of the drug given previous to the final dose, or suggests that furosemide and/or metabolites may have accumulated in the animal.

After cessation of drug administration for single-dose and repeatedly treated animals, urine volume continued to be elevated for both groups in relation to that observed in animals previous to dosing. The increased urine volume did not correspond to the furosemide and/or metabolite level detected in the urine or kidney of the single-dose and repeatedly treated rats. In fact, the urine volume was essentially equal within 24 hr. after the final dose while the amount of furosemide and/or metabolites in the urine of repeatedly treated rats was 5 to 10 times higher than that detected in the urine of single-dose rats. The repeated administration of furosemide may have resulted in an induction of enzymes responsible for the metabolism of furosemide, with the enhanced activity of the enzymes producing a decrease in active compound and a subsequent increase in inactive metabolites. As will be cited later in this article, chromatographic analysis of urine excreted from furosemide-treated rats showed numerous metabolites of the parent compound. Also, repeatedly treated rats had significantly ($p < 0.01$) larger livers than single-dose rats 4 days (8.88 ± 1.19 *versus* 5.98 ± 1.13 g.) and 10 days (8.75 ± 0.38 *versus* 6.96 ± 0.58 g.) after termination of dosing. Increased mean liver weights, for rats under stress or pretreatment with drugs, are often used as an indication of increased enzyme synthesis or a self-stimulating type of enzyme induction. Since no attempt was made to differentiate between furosemide and metabolites in tissue and urine samples, a greater percentage of the radioactivity detected in the urine and kidney from repeatedly treated rats may have been present as inactive metabolites than in the single-dose rats. Essentially equal amounts of active compound may have been present in both groups after cessation of drug administration. However, the above data and comparisons may indicate that the equal diuresis, yet unequal furosemide and/or metabolite levels, resulted from the storage of large amounts of inactive metabolite in the rat during the period of repeated dosing.

In a limited study two female rats (212 and 215 g.) were individually housed in metabolism cages. A single dose of labeled furosemide (1 μc./mg.) was administered by intubation as an aqueous suspension at a dose level of 20 mg./rat. Urine excreted was collected under toluene at 2, 6, 12, and 24 hr. after dosing. Aliquots of urine were applied to thin-layer chromatographic plates prepared from precipitated silicic acid[6] and distilled water. Methanol solutions of $^{35}S$ furosemide and 4-chloro-5-sulfamyl anthranilic acid were applied as references. The compound, 4-chloro-5-sulfamyl anthranilic acid is a known metabolite of furosemide (12). Chromatographic development was conducted in a chromatographic chamber containing a solvent mixture of *n*-butanol–acetone–phosphate buffer of pH 6.8 (50:20:30). The separated compounds were located by applying a 0.2% methanol solution of *N*-dimethyl-α-naphthylamine. Autoradiograms were obtained by exposing X-ray film[7] to the developed plates. The adsorbed labeled compounds on the thin-layer chromatogram located by autoradiography were scraped quantitatively into separate counting vials. Fifteen milliliters of scintillator gel[8] was added to each vial, the silica gel powder containing the radioactive sample suspended in the scintillation gel by shaking, and cooled previous to counting with a (liquid) scintillation spectrometer. The radioactivity in each spot was expressed in terms of the percent of the total radioactivity. Total radioactivity

[6] Silica Gel G.
[7] Kodak No-Screen, Eastman Kodak Co., Rochester, N. Y.
[8] Four percent of Cab-O-Sil (Cabot Corp., Boston, Mass.) in the XDC scintillator solution.

**Table V**—Relative Amount of Furosemide and Metabolites in Urine[a]

| Collection[b] Interval, hr. | Labeled Compound ($R_f$) (0.50)[c] | (0.41)[d] | (0.36) | (0.27) | (0.24) | (0.14) | (0.00) |
|---|---|---|---|---|---|---|---|
| 0–2 | 74.05[e] | 10.83 | 1.78 | 0.44 | 10.29 | 1.87 | 0.75 |
| 2–6 | 69.55 | 11.14 | 1.58 | 0.81 | 16.10 | 0.44 | 0.38 |
| 6–12 | 53.24 | 23.30 | 2.15 | 2.41 | 17.24 | 1.05 | 0.61 |
| 12–24 | 46.95 | 27.06 | 2.89 | 3.12 | 17.68 | 1.46 | 0.84 |
| 0–2 | 55.27[e] | 19.35 | 2.68 | 0.81 | 18.87 | 2.24 | 0.77 |
| 2–6 | 57.16 | 19.96 | 1.76 | 1.63 | 18.47 | 0.46 | 0.56 |
| 6–12 | 49.32 | 23.87 | 2.40 | 4.88 | 18.33 | 0.67 | 0.52 |
| 12–24 | 39.18 | 29.29 | 2.84 | 5.56 | 20.76 | 1.82 | 0.55 |

[a] Data expressed as percent of total radioactivity from a sample applied to the thin-layer chromatographic plate. [b] Time elapsed from furosemide administration (20 mg./rat). [c] Furosemide. [d] The metabolite 4-chloro-5-sulfamyl anthranilic acid. [e] Data from a single rat.

was obtained by summation of radioactivity in each spot from a sample applied to the thin-layer plate.

As may be observed in Table V, from five to seven labeled compounds were separated from urine samples collected at various intervals after dosing. The compound at $R_f$ 0.50 corresponded to intact furosemide while the compound at $R_f$ 0.41 was 4-chloro-5-sulfamyl anthranilic acid. The remaining labeled compounds were not identified. The relative amount of furosemide decreased with the passage of time, while metabolites of furosemide increased. The 24-hr. urine sample contained 40 to 47% of total radioactivity as furosemide and approximately 30% as 4-chloro-5-sulfamyl anthranilic acid. The labeled compound at $R_f$ 0.24 contained up to 20% of the total radioactivity in the 24-hr. urine sample. The methanol solution of $^{35}$S-labeled furosemide applied as a reference to the chromatographic plate contained 2.5% 4-chloro-5-sulfamyl anthranilic acid as a radiochemical impurity.

## SUMMARY

The data observed during the investigation of the distribution of labeled furosemide would seem to suggest that a portion of the diuretic agent and/or metabolites was distributed in various tissues, amassed in the body, and released gradually over a period of time. The daily administration of labeled furosemide resulted in increased concentrations of the drug and/or metabolites in the liver, kidney, and urine of experimental animals in relation to rats given a single dose of the compound, thus suggesting accumulation in the rat from chronic administration.

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* Sankyo Co., Tokyo, Japan.

# Isolation of the Anti-inflammatory Principles from *Achillea millefolium* (Compositae)

**ARTHUR S. GOLDBERG*, EVANGELYNNE C. MUELLER, EDWARD EIGEN, and SALVATORE J. DESALVA**

**Abstract** □ An aqueous extract of the dry flower heads of *Achillea millefolium* (yarrow) has been found to possess anti-inflammatory activity as measured by the mouse paw edema test. Fractionation has resulted in the isolation of a material which reduces inflammation by 35%. This concentrate is water-soluble, nonsteroidal, and has a very low order of toxicity. Physical and chemical studies show this active fraction to be a mixture of protein-carbohydrate complexes.

**Keyphrases** □ Anti-inflammatory constituents—*Achillea millefolium* flower heads □ Column chromatography—separation, identity □ Electrophoresis, disc—identification □ Colorimetry—analysis □ Pharmacological screening—*A. millefolium* extracts

The isolation, partial characterization, and pharmacology of the water-soluble, nonsteroidal, anti-inflammatory constituents of *Achillea millefolium* are reported.

*Achillea millefolium* L. (colloquially referred to as yarrow) is a wild-growing flowering plant belonging to the family Compositae and is found abundantly throughout America and Europe (1). The many uses of yarrow in folk medicine date back to Achilles who used the plant to heal the wounds of his warriors (2). The chemical investigation of *A. millefolium* for the past 200 years has led to the isolation of a large number of compounds, but the principles responsible for the anti-inflammatory properties of the plant are still unknown. The volatile oil of *A. millefolium* has been tested for anti-inflammatory activity and found to be inactive (3).

Preliminary screening in these laboratories of an aqueous extract of the flower heads of *A. millefolium* showed that it possessed both topical and systemic anti-inflammatory activity.

## EXPERIMENTAL

**Materials**—The flower heads of *A. millefolium*, collected in New Jersey during the summer of 1966, were used in this investigation.

**Preparation of the Initial Extract for Biological Testing**—The initial evaluation of *A. millefolium* for anti-inflammatory activity was carried out on a cold aqueous extract of the dry flower heads. The extract was dried by lyophilization and tested.

**Isolation and Characterization of the Active Principles**—The dry flower heads of *A. millefolium* (1.3 kg.) were extracted with $CHCl_3$ for 24 hr. in a continuous extraction[1] apparatus. The marc was air-

[1] Soxhlet.