and by the fact that compounds of different polarographic stabilities exhibited correspondingly different inhibitory activities on trypsin proteolysis (work in progress).

This view of mercury activity proposes a mechanism by which organic groups may produce selectivity by controlling the electron density on the mercury moiety.

REFERENCES

(1) O'Donnell, M. L., Sebulak, A. M., and Kreke, C. W., THIS JOURNAL, 50, 764(1961).
(2) Benesch, R. E., and Benesch, R., in P. D. Boyer "The Enzymes," Vol. 1, 2nd ed., Academic Press, Inc., New York, N. Y., 1959, p. 538.
(3) Benesch, R. E., and Benesch, R., *J. Phys. Chem.*, **56**, 648(1952).
(4) Benesch, R., and Benesch, R. E., *J. Am. Chem. Soc.*, **73**, 3391(1951).
(5) Wuggatzer, W. L., and Cross, J. M., THIS JOURNAL, **41**, 80(1952).
(6) O'Donnell, M. L., and Kreke, C. W., *ibid.*, **48**, 268(1959).
(7) Gilman, H., "Organic Chemistry," John Wiley and Sons, New York, N. Y., 1947, p. 1071.
(8) Benesch, R., and Benesch, R. E., *Arch. Biochem. Biophys.*, **38**, 425(1952).
(9) Friedman, H. L., *Ann. N. Y. Acad. Sci.*, **65**, 461(1957).
(10) Weiner, I. M., and Müller, O. H., *J. Pharmacol. Exptl. Therap.*, **113**, 241(1955).

A New Oral Gelatinized Sustained-Release Dosage Form

By NOBUO TANAKA, SETSUKO TAKINO, and ISAMU UTSUMI

A new sustained-release micropellet was prepared which takes advantage of the relationship of gelatin solubility to the hardness of the gelatin caused by formalin treatment. This new gelatin micropellet could be produced more easily than the coated micropellet employed in the usual sustained-release dosage forms.

THE SUSTAINED-RELEASE principle has universal application in the field of practical pharmacy. It is generally understood that gelatin is digested in the human gastrointestinal tract and that the rate of hydrolysis of the gelatin can be varied by hardening. This suggested that if medication was dissolved or suspended in a gelating sol and the gelatin treated with formalin-isopropanol, the rate of hydrolysis in the gastrointestinal tract would decrease. In this study, the relationship of the hydrolysis to the grade of gelatin hardening was applied to produce a sustained-release gelatin micropellet.

As described in the *Experimental* section, the technique utilized to produce the gelatin sustained-release micropellet is easier than the more complicated and painstaking process required to produce the conventional coated micropellet. The gelatin micropellet (containing medication) had a diameter of 0.3 to 0.5 mm. To obtain the desired sustained-release effect, the gelatin micropellet was stored in 10% formalin-isopropanol at 2–5° for varying periods of time. From the results of the *in vitro* dissolution test, the treated gelatin micropellet was found to prolong protease hydrolysis.

Urinary excretion, blood concentration, and biological kinetic theory data have been employed to evaluate oral sustained-release dosage forms and such studies have been reported recently in the literature (1–5). We used chemical determinations of the amount of medication in the blood or urine to examine the delay in absorption and urinary excretion of the medication imbedded in the treated gelatin micropellets. As gelatin can be digested in the gastrointestinal tracts of carnivorous and omnivorous animals, five dogs and three humans were used to evaluate the *in vivo* sustained-release of sulfanilamide (SA) and riboflavin (RF) through blood concentration determinations.

EXPERIMENTAL

Preparation of Dosage.—Table I shows the physical properties of gelatin and mineral oil used in the production of the micropellets.

An apparatus to produce the micropellets also is shown in Fig. 1. The apparatus consists of a stainless steel vessel, stirring wing, and motor. Two-hundred grams of water was added to 60 Gm. of gelatin. After the gelatin swelled 40 Gm. of SA powder (less than 50 μ in diameter) was added.

TABLE I.—PHYSICAL PROPERTIES OF GELATIN AND MINERAL OIL

	Viscosity, cps.	pH	M. p., ° C.	Sol-gel Transformation ° C.
Gelatin	23	6.70	30.5	25.7
		Sp. Gr.		Viscosity, cps.
Mineral Oil		0.853 (15.5° C.)		13.0 (35° C.) 22.0 (20° C.)

Received August 1, 1962, from the Osaka Research Laboratory, Tanabe Seiyaku Co., Ltd., 962 Kashima-cho, Higashiyodogawa-ku, Osaka-shi, Japan.
Accepted for publication November 13, 1962.
The authors thank Dr. M. Fujisawa and Dr. N. Sugimoto for permission to publish this paper and Mrs. Y. Kondo for assistance in this work.

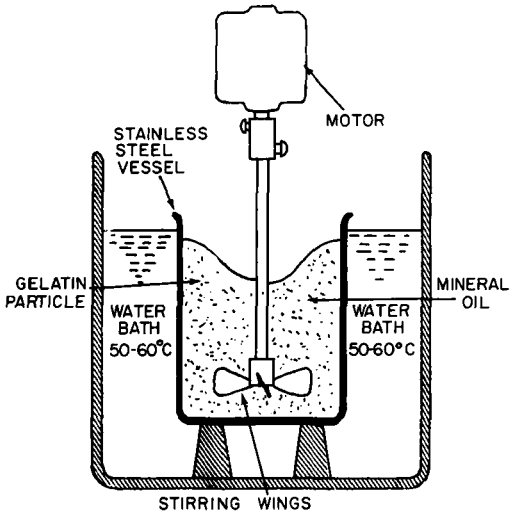

Fig. 1.—Apparatus used to produce gelatin micropellets.

TABLE II.—CHARACTERISTICS OF SA AND RF MICROPELLETS

Size	Yield, %	Content, %
SA		
Less than 24 mesh	7	38.08
24–35 Mesh	72	31.12
35–50 Mesh	17	20.32
More than 50 mesh	4	10.54
RF		
Less than 24 mesh	4	5.15
24–35 Mesh	81	5.10
35–50 Mesh	13	5.25
More than 50 mesh	2	5.30

The mixture was warmed on a steam bath to form a sol. Gelatin sol was poured into 600 Gm. of mineral oil heated previously to about 55–60° and stirred at 18–22 r.p.m. After 5 or 6 minutes, the vessel was steeped in ice water, cooled quickly to less than 5°, and kept at the same temperature until the gelatin microdrops became a perfect gel. After 300 Gm. of about 5° isopropanol was added to the vessel for dehydration, the solution was stirred about 5 minutes; then the gelatin micropellets were separated. The micropellets were then washed two times with 100 Gm. of 5° isopropanol and dried until the alcohol disappeared. The sustained-release effect of micropellets were obtained by immersing 1 Gm. of the micropellets into 10 ml. of 10% formalin-isopropanol in a covered vessel and hardened in a refrigerator at 2–5° for 24 hours and dried.

The method of RF was almost the same as with SA. One-hundred and seventy grams of water was added to 76 Gm. of gelatin. After the gelatin swelled, 4 Gm. of RF powder (less than 50 μ in diameter) was added, and the same procedure with SA was used. The hardening time of the micropellets was 24, 48, or 72 hours. These micropellets are shown in Tables II and III.

In Vitro Dissolution Experiment.—Micropellets equivalent to 100 mg. of SA and 200 ml. of simulated gastric fluid (dissolution medium) were put into a flask and immersed in thermostat adjusted to 37 ± 1°.

At appropriate intervals of time, 20 ml. was removed from the flask for analysis; a similar volume of dissolution medium was added each time to maintain constant volume. Five milliliters of 20% trichloroacetic acid was added to a 20-ml. quantity used for analysis.

Animal Experiment.—Five mongrel dogs weighing approximately 7 Kg. were fasted overnight. The mixture of micropellets and a piece of wet bread were given orally to the dogs. Blood samples of 2 ml. were then withdrawn from the vein after 1, 2, 4, 6, 7, and 24 hours, respectively. Dogs were used repeatedly with a rest period of more than 6 days for their complete recovery.

Method of Analysis for SA.—Tsuda's (6) modification of the Bratton and Marshall (7) method was used. The blood was hemolyzed by spraying into distilled water. Color was developed using 0.1% Tsuda reagent (β-diethylamino-ethylaminophthalene oxalate). Equivalent quantities (2 ml.) of blood were used in the preparation of the standard curve.

Method of Analysis for RF.—The method of analysis for RF was Yagi's method (8). Hemolyzed blood was decomposed with exposure to light. After acidifying by acetic acid, it was extracted with chloroform. Standard lumiflavin solution was prepared using 1 mcg./ml. standard RF solution instead of blood.

RESULTS AND DISCUSSION

As shown in Table IV, it is obvious from the results on various samples *in vitro* that hardened

TABLE III.—DOSAGES AND CONTENTS OF SA AND RF MICROPELLETS

Sample	Dosage and Contents
I	Gelatin micropellet containing 33.2% SA
II	Micropellet, treated for 24 hr., and containing 19.0% SA
III	RF-phosphate solution
IV	Gelatin micropellet containing 5% RF
V	Micropellet, treated for 24 hr., and containing 5% RF
VI	Micropellet, treated for 48 hr., and containing 5% RF
VII	Micropellet, treated for 72 hr., and containing 5% RF

TABLE IV.—PERCENTAGE OF ACCUMULATIVE SA RECOVERED IN THE *In Vitro* DISSOLUTION TEST

Sample I		Sample II	
Time	%	Time	%
5 min.	32.9	5 min.	5.9
10 min.	59.5	10 min.	9.9
15 min.	76.5	20 min.	30.7
35 min.	80.0	30 min.	39.6
1 hr.	89.6	45 min.	53.4
2 hr.	99.5	1 hr.	61.5
3 hr.	99.7	2 hr.	82.0
		3 hr.	86.0
		5 hr.	91.6
		7 hr.	94.1
		23 hr.	98.2
		30 hr.	99.6

TABLE V.—TOTAL SA CONCENTRATION IN BLOOD (MG.%). DATA FOR FIVE DOGS AFTER ORAL ADMINISTRATION OF SAMPLE I (250 MG. OF SA PER 1 KG. OF BODY WEIGHT)

Time, Hr.	Dog 1	2	3	4	5	Av.
2	6.56	6.44	6.17	6.08	6.00	6.27
4	13.88	10.20	8.77	8.78	9.62	10.25
6	9.21	9.41	9.35	9.16	9.62	9.35
7.5	7.63	8.38	7.94	7.47	8.53	7.99
16	2.00	2.93	2.24	1.83	5.25	2.95
20	2.25	1.77	1.22	1.05	3.11	1.88
24	1.15	1.16	1.03	0.08	1.76	1.18
30	0.84	0.62	0.46	0.34	0.64	0.58

TABLE VI.— TOTAL SA CONCENTRATION IN BLOOD (MG.%). DATA FOR FIVE DOGS AFTER ORAL ADMINISTRATION OF SAMPLE II (250 MG. OF SA PER 1 KG. OF BODY WEIGHT)

Time, hr.	Dog 1	2	3	4	5	Av.
2	0.47	0.49	0.45	0.43	0.51	0.47
4	1.56	1.85	1.43	1.13	1.68	1.59
6	3.73	3.69	4.01	3.88	4.14	3.84
8	6.35	5.07	5.86	6.65	4.77	5.77
10	7.00	7.42	7.13	8.29	7.46	7.43
16	7.09	9.88	10.00	9.35	11.03	9.46
18	8.00	9.10	10.78	9.35	8.22	9.09
20	9.14	8.53	9.14	6.44	8.15	8.08
22	8.60	8.34	8.99	8.00	7.87	8.36
24	8.57	7.99	8.81	7.46	6.54	7.87
25	7.90	7.26	7.87	7.01	5.01	7.01
28	6.25	6.89	6.75	6.67	4.14	6.68
30	6.42	5.73	6.73	5.99	3.03	5.58

micropellets have the prolongation of hydrolysis against protease in the simulated gastric fluid. In the RF micropellets, RF could not be measured by the same method as the *in vitro* test of SA micropellets because of the slight solubility of RF in simulated gastric fluid.

Dogs were given Sample I and Sample II orally at a dose of 250 mg. of SA per Kg. of body weight, and SA concentrations in blood were measured at the times shown. Blood concentration of SA reached a peak 3–4 hours after administration of Sample I. On the other hand, in Sample II maximum peak of SA concentration in blood appeared after 16

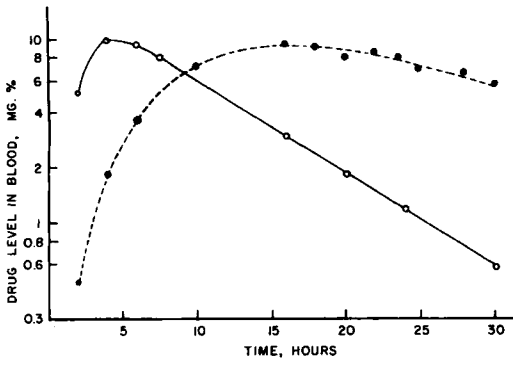

Fig. 2.—Logarithm of SA levels in blood against time after administration of SA gelatin micropellets to dogs. —O—O—, untreated micropellets; —●—●—●—, micropellets treated with formalin-isopropanol for 24 hours.

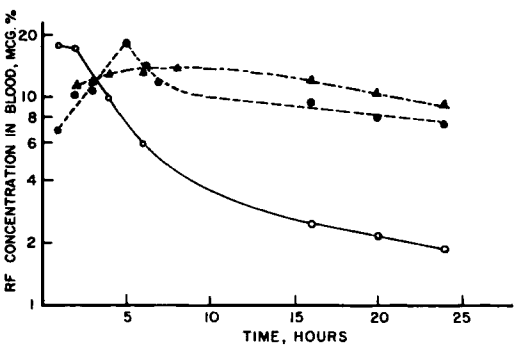

Fig. 3.—Logarithm of RF concentration in blood after administration of RF solution and its gelatin micropellets to dogs. —O—O—, RF solution; —●—●—●—, untreated micropellets; —▲—▲—▲— micropellets treated with formalin-isopropanol for 24 hours.

hours. Since the blood concentration of Sample II decreased more gradually than that of Sample I, it is certain that Sample II fulfills prolongation. The results of these experiments appear in Tables V and VI.

The decline of blood concentration of SA and its homologs after they had reached a maximum concentration, followed a pseudo first-order rate (5,9,10). To examine whether samples stayed in the digestive tracts, the logarithm of SA concentration in blood was plotted as a function of time (Fig. 2). A straight-line relationship of decrease resulted in Sample I after the maximum blood concentration. The possible interpretation is that Sample I almost dissolved in the gastrointestinal tracts in 3–4 hours. Sample II is considered to stay in the digestive tract for a long time since blood concentration increased until the 16th hour and decreased slowly after this. Judging from the above results, it is obvious that

TABLE VII.—TOTAL RF CONCENTRATION IN BLOOD (MCG.%). DATA FOR FIVE DOGS AFTER ORAL ADMINISTRATION OF SAMPLE III (1 MG. OF RF PER KG. OF BODY WEIGHT)

Time, Hr.	Sample III, mcg. % 1	2	3	4	5	Av.
1	17.5	16.0	19.0	18.7	18.8	18.0
2	17.0	15.5	18.1	18.1	18.8	17.5
4	9.8	9.2	11.1	10.7	9.2	10.0
6	5.8	5.3	7.2	7.2	4.5	6.0
16	2.5	2.2	2.6	2.5	2.7	2.5
20	2.3	2.0	2.4	2.0	2.3	2.2
24	1.8	1.9	1.5	1.9	2.4	1.9

TABLE VIII.—TOTAL RF CONCENTRATION IN BLOOD (MCG.%). DATA FOR FIVE DOGS AFTER ORAL ADMINISTRATION OF SAMPLE IV (1 MG. OF RF PER KG. OF BODY WEIGHT)

Time, hr.	Sample IV, mcg. % 1	2	3	4	5	Av.
1	5.0	4.0	8.4	9.2	8.4	7.0
2	10.5	9.3	11.2	12.0	9.5	10.5
3	10.9	9.7	11.2	12.4	10.8	11.0
5	17.0	15.3	18.5	19.7	22.0	18.5
6	9.8	16.0	15.1	13.7	15.3	14.0
7	8.7	15.2	13.3	12.0	10.8	12.0
16	8.8	9.6	11.3	8.8	9.0	9.5
20	6.2	7.6	10.0	8.3	7.9	8.0
24	6.8	7.5	7.4	7.0	8.8	7.

TABLE IX.—TOTAL RF BLOOD CONCENTRATION OF FIVE DOGS AFTER ORAL ADMINISTRATION OF SAMPLE V (1 MG. OF RF PER KG. OF BODY WEIGHT)

Time, hr.	Sample V, mcg. % 1	2	3	4	5	Av.
2	12.0	9.0	11.0	10.3	15.2	11.5
3	12.7	10.5	13.1	12.9	10.8	12.0
4	13.2	12.9	13.6	13.0	13.3	13.2
6	13.3	13.9	14.3	11.0	13.5	13.2
8	13.3	12.1	15.9	14.6	14.1	14.0
16	11.9	10.8	13.4	12.2	12.7	12.2
20	10.5	9.3	12.0	8.7	13.5	10.8
24	9.0	8.7	10.2	9.5	10.6	9.6

TABLE X.—THE RELATION OF RF QUANTITY (MCG.) EXCRETED IN URINE TO HOURS OF FORMALIN TREATMENT IN THREE ADULT HUMANS

Subjects→		A	B	C
Sex→		Man	Man	Woman
Age→		35	20	25
Sample	Collection Time, hr.	Subjects A	B	C
III	0–2	1092	1853	4208
	2–4	511	689	1867
	4–6	87	112	335
	6 to 7.5	57	91	221
IV	0–2	595	836	1689
	2–4	825	1071	2426
	4–6	161	199	509
	6 to 7.5	32	51	76
V	0–2	243	408	661
	2–4	884	1749	3142
	4–6	250	555	1098
	6 to 7.5	142	216	538
VI	0–2	113	216	274
	2–4	754	1168	2400
	4–6	368	595	1062
	6 to 7.5	183	255	644
VII	0–2	43	94	150
	2–4	463	760	1129
	4–6	339	520	1003
	6 to 7.5	205	362	950

gelatin micropellets hardened with formalin produce a sustained-release effect.

Both medication transport into blood and excretion in urine were determined on RF gelatin micropellets; the urinary excretion of RF is quicker than that of SA. Using the same dogs that were used in the experiment of SA micropellets, 1 mg. of RF per Kg. of body weight was administered to each dog and RF concentration in blood was determined. The results are shown in Fig. 3 and Tables VII–IX.

When Sample III was given to dogs (Fig. 3), the blood concentration gave a maximum level within 1 hour and then dropped quickly.

With Sample IV, RF concentration in blood reached a peak at about 4 hours; even 24 hours after dosing, RF concentration was higher than that of Sample III. In Sample V, a gradual rise occurred through 4 hours, reached a plateau and remained there for 3 hours, and 24 hours after

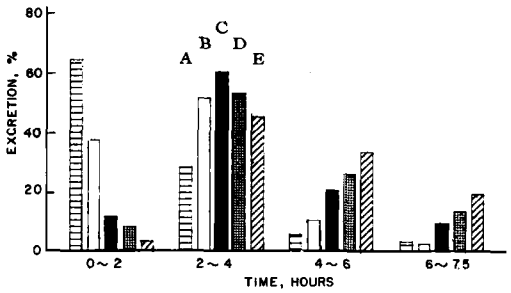

Fig. 4.—Ratio of RF in urine against time (after oral administration to three humans) if amount of accumulative RF excreted into urine for 7.5 hours is presumed 100%. A, RF solution, B, no treatment micropellets, C, micropellets treated for 24 hours, D, micropellets treated for 48 hours, E, micropellets treated for 72 hours.

dosing dropped to a blood concentration similar to Sample IV. Then the behavior of urinary excretion of RF in the load test on humans was examined. Results similar to those of the dogs were expected. The results are shown in Table X and Fig. 4.

When Sample III was administered, RF concentration in urine had a maximum value after 2 hours, but in other cases maximum excretion was shown about 4 hours after dosing. Table X shows that the recovered quantities of Samples VI and VII are lower than those of the other samples. This phenomena is probably due to the delay of excretion since the excreted quantities from 6 to 7.5 hours after administration rose proportionally to the time of treatment by formalin.

In these experiments there was a distinct difference in blood concentration response among Sample III, Sample IV, and Sample V. In the urinary excretion test, peak level of Sample II was observed 2 hours after dosing, while those of the other three samples appeared after 4 hours. Therefore, the sustained release of a certain drug cannot always be determined only by the peak of the urinary excretion, since the quantity excreted in urine is not directly related to blood concentration.

REFERENCES

(1) Nelson, E., THIS JOURNAL, 46, 572(1957).
(2) Robinson, M. J., and Swintosky, J. V., *ibid.*, 48, 473(1959).
(3) Nelson, E., *ibid.*, 50, 181(1961).
(4) Heimlich, K. R., Macdonnell, D. R., Flanagan, L. T. and O'Brien, P. D., *ibid.*, 50, 232 (1961).
(5) Kakemi, K., Arita, T., and Koisumi, T., *Yakugaku Zasshi*, 81, 172(1961).
(6) Tsuda, K., and Matsunaga, S., *ibid.*, 62, 362(1942).
(7) Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, 128, 537(1939).
(8) Yagi, K., "New Analytical Method of Vitamin Series," Ishiyaku Shuppan Co., Ltd., 32 Komagome Katamachi, Bunkyo-ku, Tokyo, Japan, 1954, p. 28.
(9) Analyzing the data of Northey, E. H., "The Sulfonamides and Allied Compounds," Reinhold Publishing Corp., New York, N. Y., 1948, p. 442.
(10) Swintosky, J. V., THIS JOURNAL, 45, 395(1956); *Ibid.*, 46, 399(1957).