Absorption, Metabolism, and Excretion of the Semisynthetic Penicillin 6-(2-Ethoxy-1-naphthamido)penicillanic Acid (Nafcillin)

By SIDNEY S. WALKENSTEIN, ROBERT WISER, EDITH LEBOUTILLIER, CATHRYN GUDMUNDSEN, and HAZEL KIMMEL

Nafcillin, 6-(2-ethoxy-1-naphthamido)penicillanic acid, a semisynthetic penicillin effective against penicillin G-resistant staphylococci, was labeled with carbon-14, and its absorption, metabolism, and excretion studied in dogs and rats after oral and intramuscular administration. Maximum plasma levels were attained within 15 minutes of an intramuscular dose and within 2 hours of an oral dose, although detectable levels appeared in plasma within 30 minutes in the latter case. Low levels of activity persisted in plasma up to 8 days, although about one-third was excreted in feces and about one-sixth in urine within 48 hours. Thirty minutes after injection, the drug was found in all major organs and tissues examined. Within the first 6 hours, about half the carbon-14 excreted in urine was due to nafcillin, about 30 per cent was due to a metabolite of similar R_f to unchanged drug, and the remaining 20 per cent was divided equally between two other metabolites.

AMONG THE NEWER penicillins synthesized from 6-aminopenicillanic acid, 6-(2-ethoxy-1-naphthamido)penicillanic acid, nafcillin (Wy-3277), has been found effective *in vitro* (1) and *in vivo* (2) against penicillin G -resistant staphylococci.

To enable us to investigate the metabolism, absorption, and excretion of the drug more completely than was possible in a non-isotopic study (3), C^{14}-labeled nafcillin was employed.

METHODS

2-Ethoxy-1-naphthoic acid-carboxyl-C^{14} (I) was synthesized by C^{14}-carbonation of the Grignard reagent prepared from 7.5 Gm. (0.03 mole) of 2-ethoxy-1-bromonaphthalene in tetrahydrofuran. The carbonation was performed in a manner previously described (4). Radioactive carbon dioxide was generated from 5.9 Gm. (0.03 mole) $BaC^{14}O_3$ (10 mc.). The product was isolated by acidification of the reaction mixture to pH 2 with 10% sulfuric acid, separation of the organic layer, and evaporation of the tetrahydrofuran. The residue was treated with 10% sodium hydroxide and cyclohexane, warmed to 50°, and cooled, the aqueous layer removed and adjusted to pH 7 with 5 *N* sulfuric acid. This solution was heated to 70° and filtered. The warm filtrate was acidified to pH 2, cooled, the solid filtered off and dried at 60°, m.p. 141–145°. The yield was 4 Gm. (61%).

The preparation of labeled 6-(2-ethoxy-1-naphthamido)penicillanic acid and its sodium salt involve only slight modifications of procedures described in the patent literature (5–7). Four grams of I were diluted with 1.2 Gm. of non-labeled acid (0.024 mole total) for conversion to the acid chloride. Thionyl chloride (1.9 ml.) was added dropwise to a stirred mixture of acid, 0.1 ml. dimethylformamide, and 9 ml. of dichloromethane. After addition was complete, the mixture was refluxed for 1 hour. The excess thionyl chloride was removed and the dichloromethane solution of the acid chloride used for the reaction with 6-APA.

6-APA (5.1 Gm., 0.024 mole) and triethylamine (5.1 Gm., 0.05 mole) were combined in 24 ml. of dichloromethane, cooled, and treated with the solution of the acid chloride. The mixture was stirred for 1.5 hours and then adjusted to pH 2 with 1 *N* sulfuric acid. The dichloromethane layer was separated, washed with water, and carefully extracted with 24 ml. of 1 *N* sodium bicarbonate. The phases were separated and the dichloromethane extracted again with 10 ml. of 1 *N* sodium bicarbonate. The sodium bicarbonate extracts were combined, treated with 3 ml. of methyl isobutyl ketone and acidified to pH 2 with 1 *N* sulfuric acid. After standing a short time, the water was decanted from the gummy precipitate and 5 ml. of methyl isobutyl ketone was added to the residue. Stirring caused the product to crystallize. The solid was filtered off, washed with 1.7 ml. of cold methyl isobutyl ketone, 6.8 ml. of cold butanol, and finally with 17 ml. of cold ether. The yield of light tan product, air dried overnight (m.p. 138–141°) was 4.1 Gm. (41%). This material was suspended in 3.5 ml. of acetone to which 5.5 ml. of 2 *N* sodium 2-ethylhexanoate in butanol was added, and the mixture stirred until solution was complete. The solution was treated with Darco and filtered. After dilution with *n*-butanol (17.5 ml.), cooling and stirring promoted crystallization of the salt. The product was washed several times with ether and recrystallized by dissolving in a minimum volume of acetone and diluting with four volumes of absolute ethanol. The yield was 2.2 Gm. of sodium salt with a specific activity of 1210 disintegrations per mcg. A second batch of labeled material was obtained by concentrating the filtrate to incipient dryness, adding 2 Gm. of non-labeled nafcillin and recovering the salt (407 disintegrations per mcg.)

Received November 6, 1962, from Wyeth Laboratories, Inc., Philadelphia 1, Pa.
Accepted for publication December 17, 1962.
The authors are indebted to James King and Charles A. Lung for technical assistance, to Sanford Rosenman, Larry Gordon, and Paul Horwitz for the bioassays, and to Felix Rauzzino, Alfonse Dervinis, and Robert Stewart for surgery in the bile collection experiments.

as described above. Both samples of radioactive product were chromatographically pure and when assayed microbiologically found to correspond with nonradioactive nafcillin (200 penicillin G units per mg.). The salt is hygroscopic.

Samples of tissue, plasma, and urine, ranging in size from 50 to 200 mcg. were placed in counting vials containing 2 ml. of Hyamine-10X, 1 *M* solution in methanol (Packard Instrument Co.) and kept at 55° for 48 hours. Each sample was then treated with 100 μl. of 30% hydrogen peroxide, and after 10 minutes at room temperature, the samples were acidified with concentrated hydrochloric acid. To each sample, 2 ml. of absolute ethanol and 10 ml. of scintillator solution (5 Gm. of 2,5-diphenyloxazole plus 100 mg. of 1,4-bis-2-(5-phenyloxazolyl)benzene per liter of toluene) was then added. The vials were read in a liquid scintillation spectrometer (Packard Instrument Co.) at 2°.

Feces were converted to carbon dioxide by wet Van Slyke combustion and assayed as such in a Nuclear-Chicago Dynacon electrometer. Urine, plasma, and bile were submitted for microbiological assay.

C^{14}-Nafcillin was given orally in gelatin capsules and intramuscularly in 10% water solution to dogs at a dose of 10 mg. of sodium salt per Kg. body weight. Samples of urine, feces, and plasma (heparinized) were taken at various time intervals for 8 days from the six dogs in each group. The bile ducts of four other dogs were cannulated and bile and urine collected continuously for 6 hours while the animals were kept under sedation with sodium pentothal. At the end of the sixth hour, collection balloons were affixed to the cannulae and the sedation was ended. At 24 hours the dogs were sacrificed and the bile was removed from the collection balloons. Two of the animals had been given 10 mg. per Kg. orally in gelatin capsules prior to the operation and two were injected with a similar dose intramuscularly following cannulation of the bile ducts, a procedure which took 15 minutes.

For the study of the distribution in the tissues of rats the drug was given in aqueous solution orally (stomach tube) and intramuscularly at a dose of 23.6 mg. per Kg. In each group, three rats were sacrificed per time interval up to 48 hours and dissected for radioassay of tissues.

Ascending paper chromatograms of urine samples were made on Whatman No. 1 paper developed in butanol, acetic acid, water (4:1:5, upper phase) and in butanol, pyridine, water (45:25:20). After development, the chromatograms were radioautographed on NO Screen X-ray film and subsequently cut into 1/2-in. sections for assay in a thin-window flow counter, or assayed microbiologically by application to the surface of agar seeded with *Staphylococcus aureus* followed by incubation at 37° for 16 hours.

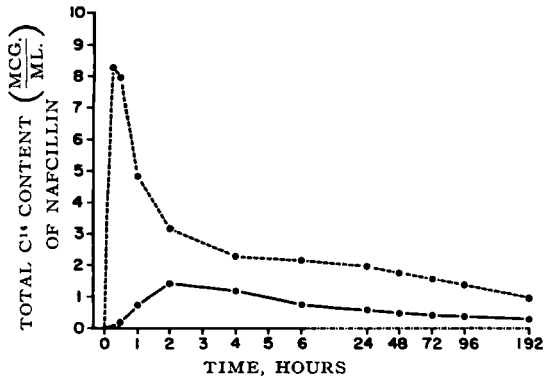

Fig. 1.—Plasma levels of C^{14} following a single dose of 10 mg. per Kg. C^{14}-nafcillin in dogs.

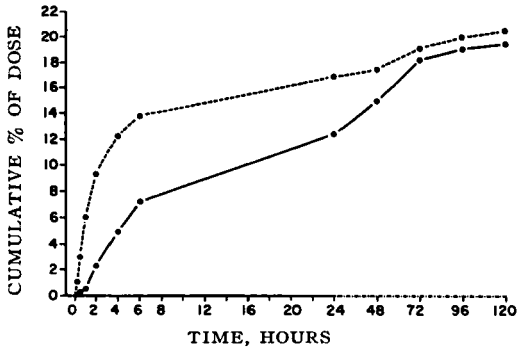

Fig. 2.—Urinary excretion of C^{14} following a single dose of 10 mg. per Kg. of C^{14}-nafcillin in dogs.

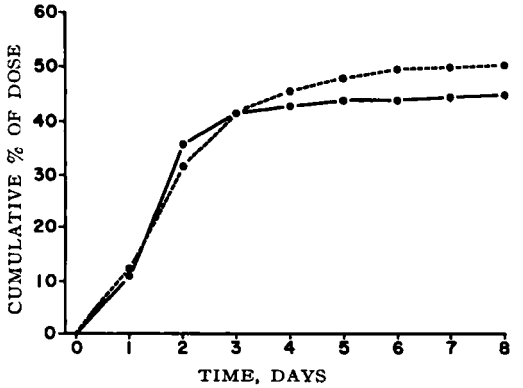

Fig. 3. Fecal excretion of C^{14} following a single dose of 10 mg. per Kg. of C^{14}-nafcillin in dogs.

RESULTS

Plasma Levels in Dogs.—Virtually all the radioactivity present in whole blood resided in the plasma fraction. Rapid absorption from the intramuscular injection site was indicated by a peak plasma level of C^{14} equivalent to 8.2 mcg. per ml. of nafcillin at 15 minutes, followed by a decline at a rate of about 2.5 mcg. per ml. per hour to the second hour (Fig. 1). After 8 days, the amount of radioactivity present in plasma amounted to the equivalent of approximately 1 mcg. per ml. Given by the oral route, the plasma level of C^{14} rose to about 1.4 mcg. per ml. by the second hour and then fell linearly to about 0.25 mcg. per ml. at the eighth day.

Urinary Excretion in Dogs.—Appreciable radioactivity amounting to an average of 1% of an intramuscular dose appeared in the urine within 15 minutes, 6% at 1 hour (Fig. 2). Following an oral dose, about 0.3% was excreted in the urine within the first hour. By the sixth hour 14% of the intra-

TABLE I.—C^{14}-TISSUE LEVELS[a] IN RATS AFTER A SINGLE INTRAMUSCULAR DOSE (23.6 MG./KG.) OF C^{14}-NAFCILLIN

Tissue	1/2 hr.	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.	48 hr.
Plasma	17.5	10.4	1.3	0.6	0.45	0.32	0.27
Lung	6.7	5.8	0.4	0	0	0	0
Liver	145.9	243.6	37.8	11.6	5.9	0.99	0.7
Brain	0.3	0.3	0	0	0	0	0
Heart	5.0	2.2	0.2	0	0	0	0
Spleen	2.6	1.9	0.1	0	0	0	0
Kidney	97.5	58.4	4.9	0.5	0.62	0	0
Stomach	4.7	1.8	1.4	1.1	1.7	0	1.76
Muscle	1.5	1.7	0	1.1	0	0	0
Skin	4.0	2.5	0.9	0.7	0.1	0	0
Bladder	11.4	82.3	13.0	0.5	0.5	0	0.13
Cecal wall	3.8	2.1	3.7	166.5	752.2	112.5	153.0
Small intestine	289	795	587.3	375	101.3	5.9	16.9
Fat	2.0	0	1.4	0	0	0	0
Urine[b]	...	...	...	...	2.2%	11.5%	23.2%
Cecal contents[b]	0	0	0.17%	16.6%	33.3%	30 %	8.3%
Feces[b]	0	0	0	...	...	21.5%	36.2%

[a] Average values, three animals per group, expressed as equivalent amount of nafcillin in mcg./Gm. of tissue. [b] Total collection, expressed as per cent of dose.

muscular dose and 7% of the oral dose was excreted. However, by the third day, regardless of route of administration, roughly the same fraction of the dose of radioactivity, 18–19%, appeared in urine. After the third day, little additional radioactivity appeared and the total amounts excreted in the urine by the eighth day after intramuscular and oral dosing were 21 and 19.4%, respectively.

Fecal Excretion in Dogs.—Approximately the same amount of radioactivity was excreted in feces after both intramuscular and oral administration (Fig. 3), about 10% in the first 24 hours, 20–25% in the next 24 hours. The total by the eighth day amounted to almost 50% of the dose.

Distribution in Tissue of Rats.—Within 30 minutes of an intramuscular dose, radioactivity was found in all major organs and tissues (Table I). The highest concentrations appeared in small intestine, liver, and kidney. Since cecal wall was rinsed free of its contents prior to assay, the high values for this tissue indicate a fair degree of binding. The plasma level at 30 minutes, 17.5 mcg. per ml., was what one might anticipate from the results in dogs if one considers that the latter received half the dose given the rats. However, at 24 and 48 hours, the plasma levels were only one-eighth those of intramuscularly injected dogs. The relative amounts of radioactivity appearing in feces and urine correspond roughly with those of dog excreta, with a 2:1 ratio of fecal to urinary content.

Tissue levels following an oral dose of C^{14}-nafcillin in rats were lower than after an intramuscular dose (Table II). That these lowered values are partly the result of the slow rate of gastric emptying in the rat can be seen by the values for stomach which, at 4 hours, still contained an appreciable amount of the drug. The drug seems to be more poorly absorbed from the gut of the rat than from that of the dog (since plasma levels were lower than in dogs, though the dose was higher) and at 48 hours the rats had excreted only half as much radioactivity in the urine as had the dogs. Another indication of the poor absorption from the gut of the rat is the ratio of fecal to urinary excretion, about 10:1 (including cecal contents) in the oral-dosed rats compared with about 2:1 with the intramuscular-dosed rats. It will be recalled that the ratio was 2:1 in dogs for both routes of administration.

Metabolism.—It was shown in Fig. 2 that 14% of injected radioactivity was excreted in urine

TABLE II.—C^{14}-TISSUE LEVELS[a] IN RATS AFTER A SINGLE ORAL DOSE (23.6 MG./KG.) OF C^{14}-NAFCILLIN

Tissue	1/2 hr.	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.	48 hr.
Plasma	0.57	0.71	0.75	0.30	0.15	0	0.08
Lung	0.76	0.10	1.82	0	0	0	0
Liver	19.5	21.5	19.8	8.77	5.55	2.57	0.11
Brain	0.08	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0
Spleen	0.27	0	0	0	0	0	0
Kidney	1.06	1.31	1.53	0.42	0.10	0	0
Stomach	693	759	426	140	12	0.57	1.0
Muscle	0.10	0.26	1.32	0	0	0	0
Skin	0	0	0	0	0	0	0
Bladder	0.98	1.13	0.28	1.30	0.15	0.06	0
Cecal wall	0.16	0	18.22	514	926	222	111
Small intestine	499	588	655	183	72.3	3.88	1.86
Fat	0.06	0.12	0.25	0	0	0	0
Urine[b]	0.03%	0.16%	0.60%	1.47%	1.3%	2.8%	6.7%
Cecal contents[b]	0	0	2.7 %	32.9 %	40.5%	32.0%	9.0%
Feces[b]	...	...	...	...	...	12.7%	58.2%

[a] Average values, three animals per group, expressed as equivalent amount of nafcillin in mg./Gm. of tissue. [b] Total collection expressed as per cent of dose.

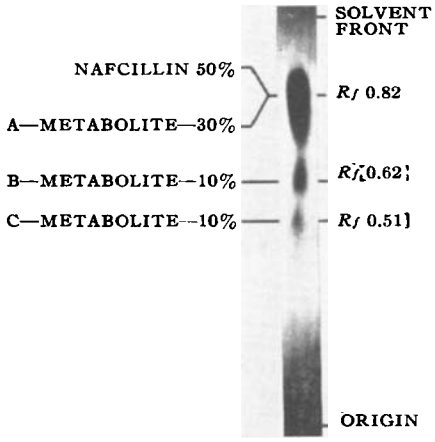

Fig. 4.—Ascending radioautograph of paper chromatogram of metabolites of nafcillin in urine. Solvent: butanol, glacial acetic acid, water (4:1:5) (upper phase).

TABLE III.—$\left[\frac{\text{MICROBIOLOGICAL}}{\text{RADIO-}}\right]$ ACTIVITY OF DOG URINE AND PLASMA SAMPLES[a] AFTER AN INTRAMUSCULAR DOSE

Time, hr.	Urine, %	Plasma, %
1/4	64	30
1/2	50	40
1	62	20
2	57	5
4	67	0
6	53	0
24	14	0

[a] Values are per cent of total radioactivity contributed by unchanged nafcillin as determined by bioassay. Each value is the average from six dogs (see Figs. 1 and 2).

within 6 hours of an intramuscular dose of C^{14}-nafcillin. Bioassay of this urine indicated that only half of the C^{14} present was contributed by microbiologically active drug. From this, it was inferred that the major area of radioactivity at the R_f of nafcillin (0.82), representing 80% of the radioactivity on the paper chromatogram (Fig. 4), contained, in addition to nafcillin, one or more metabolites accounting for 30% of the C^{14} in urine. Two other metabolites, at R_f 0.62 and R_f 0.51, each accounting for 10% of the C^{14} in urine, were found by bioassay of the paper chromatogram to be microbiologically inactive, while the area at R_f 0.82 showed an amount of microbiological activity consistent with the idea that the C^{14} present was divided between nafcillin and metabolite in the ratio 5:3.

One substance considered as a possible metabolite, 2-ethoxynaphthoic acid, was excluded because of its high R_f value, 0.92, as compared with the highest zone of radioactivity at R_f 0.82. The possibility that 2-ethoxynaphthoic acid was formed, but escaped detection by being further metabolized was considered, but ruled out on two counts: when 2-ethoxynaphthoic acid was administered to dogs both unchanged acid and a metabolite (presumably the naphthuric acid) appeared in the urine in about equal quantities, and therefore, one would expect to find some free ethoxynaphthoic acid present if hydrolysis did occur; secondly, nafcillin is resistant to hydrolysis by cultures of *Alcaligenes faecalis* under conditions in which benzylpenicillin is cleaved.

Attempts to identify metabolite *A* as either the penicilloic or penilloic acid of nafcillin have thus far been inconclusive.

Bioassay/Radioassay Ratios.—Values obtained by bio- and radio-assay in urine and plasma showed a consistently lower ratio (bioassay/radioassay) in

TABLE IV.—EXCRETION OF NAFCILLIN IN BILE AND URINE OF DOGS

Time hr.	Animal No.	Bile: Cumulative % of Dose	Bile: Bioassay, % / Radio-	Urine: Cumulative % of Dose	Urine: Bioassay, % / Radio-	Plasma: Total Activity, mcg./ml.	Plasma: Bioassay, % / Radio-
			Intramuscular[a]				
1/2	1	17.4	72	2.0	84	2.7	8
	2	11.6	109	0.05	...	2.4	20
1	1	30.0	71	3.3	80	2.3	20
	2	32.9	70	1.40	82	3.0	12
2	1	48.1	60	6.3	58	1.6	13
	2	61.6	77	4.9	57	2.2	6
4	1	63.1	32	9.8	50	1.3	10
	2	75.3	100	10.6	42	2.0	0
6	1	70.2	37	11.6	33	1.2	0
	2	79.8	50	12.3	55	1.6	0
24	1	79.8	...	13.6	0	1.1	0
	2	82.6	5	...	...	1.4	0
			Oral[a]				
1/2	3	0.11	80	0	...	0.2	0
	4	1.43	81	0.03	...	0.4	40
1	3	3.02	48	0.20	0	0.4	0
	4	5.24	83	0.12	...	1.1	10
2	3	5.80	34	1.14	4	0.4	0
	4	11.91	52	1.48	25	1.3	5
4	3	6.83	16	2.63	0	0.3	0
	4	19.18	46	3.68	10	1.5	0
6	3	7.52	3.6	3.72	0	0.2	...
	4	22.74	41	5.19	6	1.1	0
24	3	10.80	0	8.20	0	0.7	0
	4	42.24	14	...	...	1.1	0

[a] Dose: 10 mg./Kg.

plasma (Table III) than in urine. A ratio of one-half to two-thirds was maintained in the urine for the first 6 hours after intramuscular injection while the drop in plasma bioassay accompanied a change in the ratio from one-third to zero.

When bile and urine were collected after intramuscular administration of nafcillin in a separate experiment, bile and urine maintained fairly high bioassay/radioassay ratios for the first 6 hours (the bioassay ranging from 32 to 100% of the radioassay) but plasma samples bioassayed 20% or less for the first 4 hours and *nil* at the sixth hour. By comparing the amounts of the drug excreted in bile (Table IV) with those finally appearing in feces (Fig. 3), a fair degree of reabsorption of nafcillin from gut is apparent.

REFERENCES

(1) Rosenman, S. B., and Warren, G. H., "Antimicrobial Agents and Chemotherapy—1961," American Society for Microbiology, Detroit, Mich., 1962, p. 611.
(2) Yurchenko, J. A., Hopper, M. W., and Warren, G. H., *op. cit.*, p. 620.
(3) Yurchenko, J. A., Hopper, M. W., and Warren, G. H., *Antibiot. Chemotherapy*, in press.
(4) Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., and Yankwich, P. E., "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 178–179.
(5) Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., U. S. pat. 2,951,839(1960).
(6) Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., South African pat. 60/1738(1960).
(7) Childress, S., and Mascitti, A. A., U. S. pat. 3,053,831 (1962).

Physics of Tablet Compression XIII

Development of Die-Wall Pressure During Compression of Various Materials

By JOHN J. WINDHEUSER†, JAGDISH MISRA, STUART P. ERIKSEN, and TAKERU HIGUCHI

A method for determining the pressure transmitted to the die wall during tablet compression is described. Basically, the method involves strain-gauge measurement of die expansion and the relation of this to the transmitted pressure. The pressure transmitting behavior of a number of compounds, both organic and inorganic, have been studied; some correlations between observed response and physical properties are suggested. Limited studies on the effect of the addition of a lubricant to the crystals are also reported. It was noted that change in the magnitude of transmission to the die wall was not a simple linear function of lubricant concentration.

EARLIER reports (1–6) of measurements of several variables involved during compression of pharmaceutical tablets did not attempt to determine the relative magnitude of the lateral forces developed during formation of compressed tablets on the die wall by various types of materials. Nelson (7), reporting earlier from these laboratories, proposed and tested a method for possible measurement of these forces but did not study the comparative behavior of different materials. Results of direct measurements carried out on a number of organic and inorganic substances by a relatively simple technique are presented in this communication.

The perpendicular force developed in a die cavity during tablet formation obviously is related to the flow characteristics of the compressed material. If the confined substance acted simply as a hydraulic fluid, the lateral pressure should be essentially equal to the compressional pressure. Compaction of any common granular solid, on the other hand, would lead to development of much lower sideward pressure—the extent of lowering being (in a sense) a measure of ease of lateral distortion or flow of the compressed material. This property would be reflected in the relative ease of ejection of the formed tablet and the moldability of the compacted mass. All this may depend on such

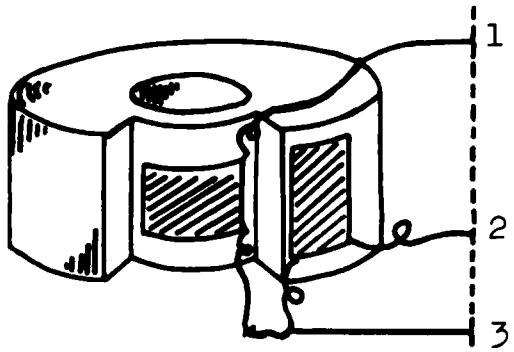

Fig. 1.—Standard steel die modified to accept strain gauges. Numbers refer to connections in circuit diagram in Fig. 2.

Received November 27, 1962, from the School of Pharmacy, University of Wisconsin, Madison.
Accepted for publication December 28, 1962.
This study was supported in part by a grant from the American Chicle Co., Long Island City, N. Y.
† Present address: Sandoz Pharmaceuticals, Hanover, N. J.