survival prolonged 70% by previous intraperitoneal injection of 3.33 mg of β-mercaptoethylamine per 20 g body weight.

1. Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P., and Fenn, W. O., *Science,* in press.

2. Sixth International Congress of Radiology, *Brit. J. Radiol.,* 1951, v24, 416, 422, and 428.

3. Barron, E. S. G., Seki, L., and Johnson, P., *Arch. Biochem. Biophys.,* 1952, v41, 188.

4. Chapman, W. H., Sipe, C. R., Eltzholtz, D. C., Cronkite, E. P., and Chambers, F. W., *Radiology,* 1950, v55, 865.

5. Chapman, W. H., and Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 318.

6. Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science,* 1949, v110, 213.

7. Patt, H. M., Smith, D. E., Tyree, E. B., and Straube, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 18.

8. Cole, L. J., and Ellis, M. E., *Am. J. Physiol.,* 1952, v170, 3.

9. Bacq, Z. M., Dechamps, G., Fischer, P., Hervé, A., LeBihan, H., Lecomte, J., Pirotte, M., and Rayet, P., *Science,* 1953, v117, 633.

10. Rugh, R., and Wang, S. C., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 411.

11. Snell, E. E., Brown, G. M., Peters, V. J., Craig, J. A., Wittle, E. L., Moore, J. A., McGlohon, V. M., and Bird, O. D., *J. Am. Chem. Soc.,* 1950, v72, 5349.

12. Baddiley, J., and Thain, E. M., *J. Chem. Soc.,* 1951, 2253.

13. Gregory, J. D., and Lipman, F., *J. Am. Chem. Soc.,* 1952, v74, 4017.

14. Bacq, Z. M., and Hervé, A., *Arch. Internat. de Physiol.,* 1953, v61, 434.

15. Stadie, W. C., *et al., J. Biol. Chem.,* 1945, v161, 153, 175, 181, 189.

16. Mann, P. J. G., and Quastel, J. H., *Biochem. J.,* 1946, v40, 139.

17. Dickens, F., *Biochem. J.,* 1946, v40, 145 and 171.

18. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol.,* 1949, v32, 537.

19. Stadie, W. C., and Haugaard, N., *J. Biol. Chem.,* 1946, v164, 257.

20. Patt, H. M., *Physiol. Rev.,* 1953, v33, 35.

21. Dawson, R. M. C., *Biochem. J.,* 1953, v55, 507.

22. Penrod, K. E., *Fed. Proc.,* 1953, v12, 108.

23. Mead, J. F., *Science,* 1952, v115, 470.

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Excretion of Carbon-14 by Man after Administration of Morphine-N-Methyl-C^{14}.*† (20791)

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In contrast to the considerable information available concerning the excretion of morphine in the human addict(1-3), little attention has been directed to morphine excretion in non-addicted man following administration of a single therapeutic dose. Oberst has presented data on urinary concentration of free and bound morphine in normal man but figures for complete excretion are lacking(3). Thus most of our present knowledge regarding morphine excretion in the normal animal has been derived from data on dogs(4-6) and rats(7). In both these species, it has been shown that with the development of tolerance there is a concomitant alteration in the excretion of morphine metabolites as evidenced by a marked reduction in the recovery of bound morphine from urine. Although it is possible that a parallel alteration occurs in man following the development of addiction, nevertheless, precise data on normal man are desirable. In view of the relatively small amount of morphine contained in a therepeutic dose, it was felt that the application of tracer technics to this problem would be helpful.

Experiments on rats using N-C^{14}H$_3$ labeled morphine indicated that excretion of radio-

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activity is virtually complete in 48 hours(8). This suggested that excretion might be rapid enough in man to warrant the injection of radioactive morphine. Since only limited amounts of carbon-14 can be administered to normal humans, the present study was initiated to determine the feasibility of doing tracer studies in normal humans using one microcurie or less of radioactive carbon and as a preliminary step toward isolation and identification of metabolic products of morphine. This report deals with the quantitative aspects and time relations involved in the urinary, fecal and pulmonary excretion of carbon-14 by cancer patients and normal human subjects following the administration of N-$C^{14}H_3$ labeled morphine contained in a single therapeutic dose.

Methods. The morphine-N-methyl-C^{14} used in these studies was synthesized by Rapoport, Lovell and Tolbert(9). It was converted to the sulfate and diluted with commercial morphine sulfate for intramuscular injection. The cancer patients received a dose of 15 mg containing 17.2 microcuries of carbon-14. The normal subjects were given 10 mg containing a total of 0.9 microcuries of carbon-14. The subjects included 3 terminal cancer patients and 2 normal adult volunteers as follows: A. C., ♀, age 49, carcinoma of the colon with metastases: H. B., ♂, age 68, multiple myeloma; H. E., ♂, age 32, normal; P. R., ♀ age 33, normal. The cancer patients were not markedly debilitated and had no gross disturbance of liver, kidney, or bowel function with the exception of A. C. who had a persistent colitis partially controlled by terramycin. A. C. and H. B. received no morphine type analgetics for 48 hours prior to radio-morphine injection. In addition to these non-addicted subjects, one cancer patient designated as a morphine addict was included in this study. This patient, V. M., ♀, age 31 with carcinoma of the colon with metastasis, had been taking morphine or similar analgetics for 2 years and at the time of this experiment had been taking daily oral doses of up to 90 mg of morphine.

One of two methods was used for determination of respiratory $C^{14}O_2$ depending on the amount of radioactivity administered. For the cancer patients it was sufficient to have the subject rebreathe for 1 minute into a 9 liter rubber bag containing about 6 liters of oxygen. This CO_2 thus collected was absorbed in a NaOH tower and subsequently precipitated as $BaCO_3$ which was weighed to determine the per minute output of CO_2. Samples with a specific activity of greater than 10 dis./min./mg $BaCO_3$ were counted with a proportional counter (Nucleometer, Radiation Counter Laboratory, Chicago. Ill.), others were converted to CO_2 and measured in an ionization chamber. The instruments were calibrated with samples prepared from standard carbon-14 obtained from the National Bureau of Standards, Washington, D. C. Breath samples from the normal adults used in this study were obtained by having the subjects breathe directly into a vacuum line designed for the collection and purification of CO_2(10). Three- to 6-minute breath samples were collected and stored in one liter bulbs. Radioactivity was then measured in a 500 cc ionization chamber using the rate of drift method with a vibrating reed electrometer (Applied Physics Corp., Pasadena, Calif.) and a continuous recorder (Brown Potentiometer Recorder, Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.) The sensitivity of this method was about 0.004 dis./min./mg $BaCO_3$. *Urine samples* were collected at varying intervals for 3 days following drug injection. Aliquots were dried and combusted to CO_2 using a chromic-sulfuric acid oxidizing mixture(11). Feces were collected for 3 days and aliquots of each 24-hour specimen combusted as above. The CO_2 obtained from urine and feces combustions was precipitated as $BaCO_3$ and radioactivity measured using 100 cc ionization chambers.

Results. The output of $C^{14}O_2$ in the breath was estimated by plotting the total radioactivity obtained from each breath sample versus time and mechanically integrating the rate plot thus obtained. The area under the curve was cut into strips corresponding to one or two hours on the time axis and the weight of each compared with the weight of a section of the graph paper which was equivalent to a convenient number of disintegrations per minute. The peak rate of $C^{14}O_2$ excretion

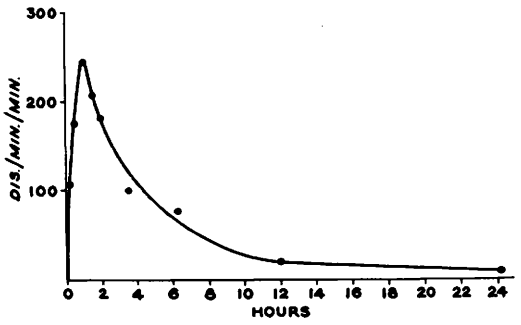

FIG. 1. Typical rate plot of pulmonary $C^{14}O_2$ excretion (subject H.E.).

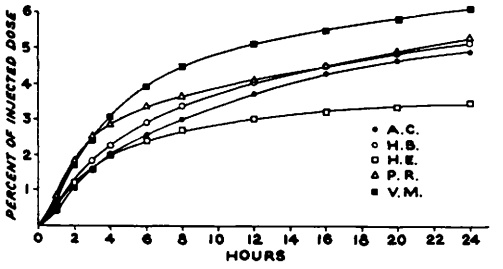

FIG. 2. Pulmonary excretion of $C^{14}O_2$ for the 24 hr period following the administration of labeled morphine.

in the breath occurs between 30 and 90 minutes after drug administration, corresponding to the time when the pharmacologic actions of morphine are most profound (see Fig. 1), and then falls off rapidly. The cumulative 24-hour pulmonary $C^{14}O_2$ excretion is shown for all subjects in Fig. 2. The 24-hour pulmonary excretion of carbon-14 ranged between 3.4 and 5.3% for the nontolerant subjects. Although more pulmonary $C^{14}O_2$, 6.1%, was recovered from the addict (V.M.) further studies are required to determine whether this represents a significant difference.

Measurable amounts of radioactivity were present in breath samples obtained from H.B. 5 days after injection and from V.M. 4 days but not 8 days after injection. This suggests that to a limited extent a transfer of $C^{14}H_3$ groups from the morphine molecule to the metabolic pool may occur from which $C^{14}O_2$ is slowly liberated by normal catabolic processes. In the metabolism of glycine in man (12,13) approximately 40% of the methylene carbon is converted to CO_2 in 24 hours. If one assumes that the rate of metabolism of the N-methyl group of morphine and the N-methylene group of glycine are comparable then one can estimate that the 5% pulmonary excretion of $C^{14}O_2$ in these subjects represents about 12.5% breakdown of the morphine to produce methyl groups for general body metabolic processes. There is also the possibility that a small amount of the intact morphine molecule is held in some firm combination which is slowly metabolized, since Gross and Thompson(4) have shown that the nontolerant dog continues to excrete small amounts of morphine for at least 6 days. It should be further noted that an interesting species difference between rat and man exists in the pulmonary excretion of radioactivity. The sex difference which occurs in the rat(8) was not found in our studies on humans.

Considerable differences are observed in total urinary excretion of radioactivity (Fig. 3) which may be related in part to urine output. The addict (V.M.) maintained a high urinary output during the entire course of the experiment and exhibited a marked diuresis for the first 6 hours during which her rate of Carbon-14 excretion was well above that of the other subjects. Two subjects, A.C. and P.R., had low urine outputs for the first 6 hours with correspondingly low carbon-14 outputs. A similar coupled trend was observed by Plant and Pierce(14) in their studies on dogs and by Oberst(1) in his studies on human addicts. Appreciable amounts of activity were eliminated in the feces of non tolerant subjects and, in contrast to urinary excretion, the amounts are fairly

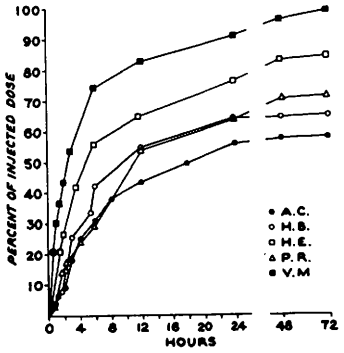

FIG. 3. Urinary excretion of carbon-14 following administration of labeled morphine.

TABLE I. Fecal Excretion of Carbon-14 as Related to Feces Output.

Hr		Non-addict A.C.	Non-addict H.B.	Non-addict H.E.	Non-addict P.R.	Addict V.M.
0-24	g feces	412	109	159	0	0
	% dose	6.91	2.24	7.34	0	0
24-48	g feces	375	151	170	443	123
	% dose	.27	4.22	1.60	9.46	.04
48-72	g feces	353	22	350	0	0
	% dose	.06	.27	1.44	0	0
72-96	g feces	—	—	—	74	49
	% dose	—	—	—	.88	.03
Total	g feces	1140	282	679	517	172
	% dose	7.24	6.73	10.38	10.34	.07

TABLE II. Summary of Carbon-14 Recovery from All Sources.

Subject	Non-addict A.C.	Non-addict H.B.	Non-addict H.E.	Non-addict P.R.	Addict V.M.
Urine	57.5	64.7	83.7	71.1	98.3
Feces	7.2	6.7	10.4	10.3	.1
Breath	4.9	5.2	3.4	5.3	6.1
Blood	.2	.1	—	—	—
Vomiting & duodenal drainage	.7	—	—	—	—
Total	70.5	76.7	97.5	86.7	104.5

TABLE III. Concentration of Carbon-14 in Duodenal Secretions of Subject A.C.

Hr	.5	1.5	3.0	6.0	12.0	24.0
Dis./min./ml	391	508	5469	10360	3670	4760

constant (Table I). Fecal excretion of carbon-14 by the addict V.M. was negligible and since this represents a significant difference from the nontolerant subjects it should be further studied.

Total carbon-14 recoveries from all sources ranged from 71-105% (Table II). The highest values were obtained on those subjects who had high urinary carbon-14 outputs, V.M. and H.E., and in these the pulmonary excretion of $C^{14}O_2$ was already quite slow at the end of 24 hours. Since the slope of the pulmonary excretion curve (Fig. 2) for the other three subjects indicates that there is continued production of $C^{14}O_2$ at the end of 24 hours, one can postulate that there is here a more extensive incorporation of the N-methyl group of the morphine molecule into the general body carbon pool. This could account in part for the low radioactivity recovery in these cases. To determine the role of the intestinal tract in the disposition of morphine, a duodenal tube was passed in subject A.C. and samples obtained at intervals for 24 hours. The concentration of carbon-14 in duodenal secretions at various times is shown in Table III. Assuming that duodenal carbon-14 originates in the biliary excretion and that the 24-hour bile output is 500 ml (15), integration of the curve obtained by plotting concentration versus time (Table III) gives a total of 7.4% of the injected dose potentially available for excretion via the intestinal route. Since this figure closely approximates the experimentally determined figure of 6.9% for the 24-hour fecal excretion the inference may be drawn that the total fecal output arises from bilary excretion. The reabsorption of this material from the intestines is apparently negligible, since 24-hour retention of feces by subject P.R. did not result in a decreased fecal carbon-14 output as compared with other nontolerant subjects.

Previous work by Oberst(3) has shown that morphine appears in the bile in conjugated form but is recovered from the feces in free form only. It would appear from our results, therefore, that hydrolysis of conjugated morphine must take place in the intestine. This is supported by the finding that the intestinal flora of the rat are able to hydrolyze glucuronides(16). It is of interest to note that a striking species difference exists between rat and man in the disposition of morphine as evidenced by a 5-fold greater fecal excretion and at least a 9-fold greater

biliary excretion in the rat(8). It would appear that in the rat much intestinal morphine of biliary origin is subsequently reabsorbed.

Summary. 1. Therapeutic doses of morphine-N-methyl-C^{14} have been administered to 5 human subjects. The major portion of the radioactivity is excreted in 24 hours. Urinary excretion accounted for most of the recovered activity, and there was some correlation between urine volume and carbon-14 excretion when these volumes were abnormally high or low. 2. Fecal excretion accounted for 7 to 10% of the dose and the possibility that this material originates in the bile as "bound" morphine with subsequent hydrolysis in the intestine is discussed. Intestinal excretion is not as important in man as in rats. 3. Pulmonary excretion of $C^{14}O_2$ ranged from 3½ to 6% of the injected dose in 24 hours. There was no sex difference in excretion by this route, as has been observed in the rat. 4. The carbon-14 excretion pattern for the one addict was similar to the nontolerant subjects, except in the case of the feces. 5. It has been shown that it is feasible to do tracer studies in normal subjects using less than a microcurie of carbon-14.

1. Oberst, F. W., *J. Pharmacol. and Exp. Therap.*, 1940, v69, 240.
2. *Idem,* 1941, v73, 401.
3. *Idem,* 1942, v74, 37.
4. Gross, E. G., and Thompson, V., *J. Pharmacol. and Exp. Therap.,* 1940, v68, 413.
5. Thompson, V., and Gross, E. G., *ibid.,* 1941, v72, 138.
6. Gross, E. G., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 61.
7. Zauder, H. L., *J. Pharmacol. and Exp. Therap.,* 1952, v104, 11.
8. March, C. H., and Elliott, H. W., *Fed. Proc.,* 1952, v11, 373.
9. Rapoport, H., Lovell, C. H., and Tolbert, B. M., *J. Am. Chem. Soc.,* 1951, v73, 5900.
10. Baker, E. M., Marcus, M., and Tolbert, B. M., to be published.
11. Skipper, H. E., Bryan, C. E., White, L. Jr., and Hutchinson, O. S., *J. Biol. Chem.,* 1948, v173, 371.
12. Berlin, N. I., Tolbert, B. M., and Lee, H. C., *J. Clin. Invest.,* 1953, v32, 1.
13. Berlin, N. I., Tolbert, B. M., and Lawrence, J. H., *ibid.,* 1951, v30, 73.
14. Pierce, I. H., and Plant, O. H., *J. Pharmacol. and Exp. Therap.,* 1932, v46, 201.
15. Gamble, J. L., *Extracellular Fluid,* Cambridge, Mass., 1950 Chart 37.
16. Glazko, A. L., Dill, W. A., and Wolf, L. M., *J. Pharmacol. and Exp. Therap.* 1952, v104, 452.

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Effect of Restraint on Temperature Regulation in the Cat.* (20792)

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Hypothermia produced by restraint in mice has been previously reported(1), and a brief review of the literature dealing with this subject presented. To the authors' knowledge only the smaller thermolabile laboratory animals have been studied for this response. This group includes the rabbit with thermal instability commonly noted in routine work (2). In the present series, cats, generally thermostable animals, have been stressed by restraint and the effect on body temperature noted. It is known that stresses, which are partially emotional in nature, result in a loss of temperature regulation of smaller mammals, with a resultant body temperature drop when the animals are exposed to cold(1). If such a response to stress, either emotional or physical, existed in man, the lowering of body temperature in the cold would impose a serious handicap on such individuals as mili-

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