used in this laboratory less frequently. The NTI and proteose soy bean trypsin inhibitor both inhibit chymotrypsin much more actively than does the GSTI.

Summary. Soy and navy bean trypsin inhibitors considerably reduce the incidence of fatal trypsin embolism in rabbits with the retention of marked thromboplastic activity by the inhibitor-enzyme complex. The thromboplastic effect is observed with little or no proteolytic activity and the relative protection against embolism is apparent even with extensive proteolytic activity. Similar observations were made with the bean trypsin inhibitors and hypochlorite-treated trypsin, the latter having been shown earlier to have considerably less hypotensive effect than untreated trypsin. The differentiation of the soy and navy bean trypsin inhibitors is briefly considered and an improved method of preparing the navy bean inhibitor is given.

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Distribution and Excretion of Radioactivity after Administration of Morphine-N-methyl C^{14} to Rats.* (21145)

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Our knowledge of the tissue distribution and excretion of morphine is limited because available analytical methods are not sensitive enough to determine tissue levels after the administration of a single therapeutic dose. The synthesis of morphine-N-methyl-C^{14}(1) has made it possible to follow the drug through the body using tracer technics. This report deals with tissue distribution and excretion of carbon-14 in rats after administration of minimal analgetic doses, as well as an investigation of N-demethylation of morphine *in vitro*.

Methods. In vivo studies: Adult Wistar-type rats of both sexes weighing approximately 200 g were given 5 mg/kg of suitably diluted morphine-N-methyl-C^{14}HCl subcutaneously. This constituted a minimal analgetic dose in the animals studied. The carbon-14 in the tissues, excreta and expired air was determined as previously described(2). For biliary tract excretion studies a rat was anesthetized with ether and a polyethylene catheter was inserted into the common bile duct through an uppermidline abdominal incision. The abdomen was closed, the animal was placed in a restraining cage and labeled morphine was administered after the animal had fully recovered from the anesthetic and bile was flowing freely.

Six female rats were given a total of 45 mg cyclopentyl testosterone propionate in three 15 mg subcutaneous doses over a 38-day period prior to studies on the effect of androgens. Two rats were made tolerant to morphine by daily injections for 7 weeks beginning

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TABLE I. Percent Radioactivity Recovered from Tissues, Excreta and Expired Air after Injection of 5 mg/kg Morphine-N-methyl-C^{14} Subcutaneously.

Time of sacrifice Sex	1 hr ♂	6 hr ♀	24 hr ♀	48 hr ♂
				(1st 7 hr)
Expired air	1.1	.2	.6	8.2
Liver	2.3	.4	.3	
Stomach	6.0	.1	.04	
Small intestine	15.7	3.4	6.5	.3
Large "	.5	19.6	16.5	.2
Feces			24.0	26.7
Kidneys and bladder	1.3	.8	.2	
Urine	17.8	63.6	52.1	Lost
Left leg	9.8	1.1	.2	
Other	39.0	5.4	1.2	<.5
Total	93.5	94.6	101.6	

with a dose of 10 mg/kg and working up to 40 mg/kg after 5 weeks. *In vitro* studies: Liver slices approximately 0.5 mm thick were cut freehand and 800-1000 mg were suspended in 10 ml of extracellular Krebs-Ringer solution in modified Warburg flasks at 37.2°C. Oxygen or other gas as specified was passed through the flasks into NaOH towers for absorption of carbon dioxide. At the end of the experiment carbon dioxide in solution was liberated by acidification of the Ringer's solution. Labeled morphine was added to make a final concentration of 10 μg per gram of liver, a value derived from the *in vivo* studies.

Results. 1) Distribution of radioactivity after subcutaneous injection: These results have been reported in part(3) and are summarized in Table I. Up to 20 individual tissues were analyzed from some animals but for simplicity of presentation only those containing significant amounts of radioactivity are tabulated. Absorption was not quite complete at one hour; the left hind leg (site of injection) contained 9.8% of the dose compared to 2.5% in the opposite leg. Brain and spinal cord at one hour contained only traces of activity by the method used; other tissues not directly concerned with excretion showed low levels of activity. Concentration occurred chiefly in organs of excretion as evidenced by the large amounts of activity present in the urine and gastrointestinal tract one hour after drug administration. By 6 hours almost two-thirds of the activity was excreted in the urine and by 24 hours 99% was in the urine, feces or gastrointestinal tract. The expired air of the males in this series contained appreciable amounts of activity indicating an N-demethylating or transmethylating process while females excreted minimal amounts by this route.

2) Biliary excretion: 62.6% of the injected carbon-14 was recovered from bile collected from a female rat during the 6-hour period following injection of 5 mg/kg of labeled morphine. Urine collected during the same period contained 18.1% of the dose. These figures are practically the reverse of those obtained in the intact animal and suggest an entero-hepatic circulation of morphine or its metabolites following the initial excretion by the liver in the bile. No appreciable amount of activity was present in the gastrointestinal tract of this animal. The material excreted in the bile consisted of both free and bound morphine. Colorimetric determinations(4) before and after acid hydrolysis under 15 lb pressure showed that the major portion of the morphine recovered in the bile is present in the bound form. Similar results have been reported for dogs(5) but in man all the morphine in the bile was conjugated(6).

3) Pulmonary excretion of $C^{14}O_2$ by intact, androgen-treated and morphine-tolerant rats is shown in Table II and Fig. 1. The expired air from untreated males and females, from a male and females treated with cyclopentyltestosterone propionate and from a tolerant male rat was collected for 6 hours after subcutaneous administration of 5 mg/kg labeled morphine and its radioactivity measured. A 1-hour collection was made on another tolerant male rat. The amount of $C^{14}O_2$ liberated by the treated females was similar to that excreted by the males while the amount from untreated females was minimal, and that from the treated male quite high, confirming the data obtained previously(3). Pulmonary excretion by morphine-tolerant rats 1 and 6 hours after injection was similar to that of normal rats.

4) *In vitro* studies: The liberation of $C^{14}O_2$ from rat liver slices treated with morphine-N-methyl-C^{14} under various conditions is shown in Table III. It is apparent that the sex difference observed *in vivo* is reproduced *in*

TABLE II. Percent Radioactivity Recovered from Expired Air during 6 Hr after Injection of 5 mg/kg Radioactive Morphine Subcutaneously.

Untreated rats		Cyclopentyl testosterone treated rats		Morphine-tolerant rats
Males (5)	Females (8)	Males (1)	Females (4)	Males (2)
4.77	.50	11.4	4.1	7.4-6 hr .7-1 hr

vitro. Furthermore, the amount of $C^{14}O_2$ liberated by liver slices approximates that recovered from expired air *in vivo*. Added testosterone failed to increase $C^{14}O_2$ liberation by liver slices from female rats and depressed it in the liver slices from male rats. The latter effect is probably related to the depression of tissue respiration produced by testosterone(7). Methionine in the amount used did not alter the rate of "demethylation", nor was it appreciably depressed by an amount of ethionine sufficient to make a 20:1 ethionine-methionine ratio based on the amount of methionine in liver(8). Dibucaine in the concentration used produced only inhibition of "demethylation". This amine oxidase(9) and cytochrome(10) inhibitor was included in this series because independent experiments showed that it produced a diphasic effect on the QO_2 of liver slices from fed rats, first stimulating and then inhibiting oxygen uptake. No parallel effect was observed on $C^{14}O_2$ liberation. Finally, the rate of "demethylation" was not affected by N-allylnormorphine but was completely inhibited by anaerobic conditions or homogenization in a Potter homogenizer.

Discussion. The general pattern of morphine excretion in the rat was followed by tracer methods. The presence of $C^{14}O_2$ in the expired air shows that morphine is in part "demethylated" in the male rat. This was confirmed in man(11) although no sex differences were found. Thus, some morphine is probably converted to normorphine which may be excreted either free or conjugated. This process resembles that found with meperidine (12) and probably with codeine(13), but is probably relatively unimportant insofar as the detoxification of morphine is concerned. This belief is strengthened by the finding that tolerant animals do not show increased "demethylation" and that morphine has been reported to be more toxic to mature male rats than to mature females(14). It is of interest that N-allylnormorphine which is conjugated by the liver at the same rate as morphine, and acts as a competitive inhibitor(15), does not seem to be effective in blocking the "demethylation" of morphine.

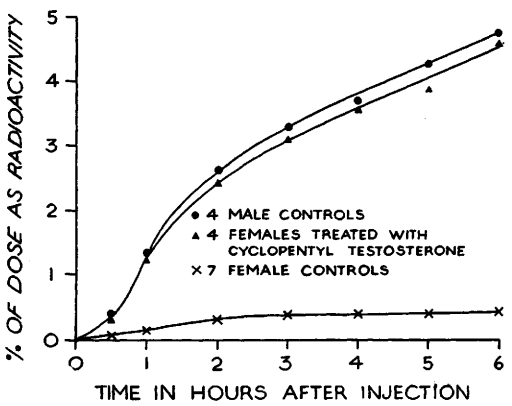

FIG. 1. Radioactive CO_2 excreted by pulmonary route after subcutaneous administration of 5 mg/kg labeled morphine.

It is most interesting to find a process in the liver which is stimulated by androgens. Very little is known regarding the influence of castration or androgen administration on liver enzyme content(16). Data of this type might help in determining the systems involved in "demethylating" morphine. Other compounds such as the xanthines, cocaine, mesantoin, mephobarbital, aminopyrine, and ephedrine have been shown to be demethylated in the body but no sex differences were reported.

Pulmonary excretion of carbon-14 by tolerant animals is not markedly different from that by normal animals as was also found in man(11). In the rat and dog, previous work has shown that an important fraction of the administered morphine is not recoverable from the excreta of tolerant animals(5,17). By using labeled morphine and comparing radioactive and colorimetric values it should be possible to locate this "missing" fraction so as better to understand the changes induced by

TABLE III. Percent Radioactivity Liberated in One Hour as $C^{14}O_2$ by Rat Liver Slices *In Vitro*.

Animals	Substances in flask	%
Males, untreated (7)	10 γ M*	Avg 14.8 (range 12.0 -22.0)
Females, " (3)	10 γ M	" .29(" .17- .52)
" , treated with testosterone, cyclopentyl propionate (2)	10 γ M	21.0, 23.7
Male, untreated	10 γ M 10 mg testosterone propionate suspension	3.6
Female, "	10 γ M 1 mg testosterone propionate suspension	.33
Male, "	100 γ M	13.6
	10 γ M 10 mg methionine	12.0, 12.7
	10 γ M 10 mg ethionine	10.5
	10 γ M—control	12.1
	Atmosphere of nitrogen 10 γ M	0
	10 γ M .002 M dibucaine	4.2, 3.1
	10 γ M 20 γ N-allyl normorphine	12.6
	10 γ M Liver ground to a brei	0

* M = Morphine-N-methyl-C^{14}.

the development of tolerance.

Summary. After the administration of morphine-N-methyl-C^{14} to rats, radioactivity concentrated chiefly in the urinary and intestinal tracts with only minimal amounts in the central nervous system. Urinary excretion predominated but there is evidence for an entero-hepatic circulation of morphine or its metabolites following initial excretion by the liver in the bile. Male rats excreted significantly more radioactivity via the pulmonary route than did females. Treatment of females with cyclopentyl testosterone increased their pulmonary excretion to the male level. Liberation of $C^{14}O_2$ by liver slices from male and female rats to which morphine-N-methyl-C^{14} was added *in vitro* followed the same pattern.

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