For a quantitative study of the transfer of PAEB from plasma into bile, the drug was administered intravenously to two groups of 8 rats in which the renal pedicles had been ligated to help maintain a fairly constant plasma level of drug. In one group, bile was collected for 30 min, and plasma obtained at the end of the collection period. In the other group, bile was collected for two consecutive half-hour periods, and the plasma then obtained. The results (Table 1) show that during both half-hour periods, the concentration of unchanged PAEB in bile was about 80 times that in plasma; and the conjugates of the drug, presumably formed in the liver, were also more concentrated in bile than in plasma.

The secretion into bile of PAEB at high concentrations suggests that the liver may have a specialized transport process for organic cations as well as for organic anions. Preliminary studies with the tertiary amine derivative of PAEB and with three other organic cations, decamethonium, tetraethylammonium, and Darstine® (mepiperphenidol), suggest that only the Darstine® is concentratively transported from blood to bile.

Further work is in progress to characterize the hepatic transport process for organic cations.

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Differential binding and release of norepinephrine and tachyphylaxis

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THE phenomenon of tachyphylaxis to sympathomimetic amines has been a classical problem in pharmacology for many years. Burn and Rand have provided indirect evidence showing that certain sympathomimetic amines exert their effects by the liberation of norepinephrine from stores.[1] Recently, several investigators have shown that after the development of tachyphylaxis the actions of sympathomimetic amines can be restored by the administration of norepinephrine.[2, 3] We have previously demonstrated that the spontaneous release of H^3-norepinephrine from the heart is rapid at first and then becomes progressively slower.[4] This suggests that H^3-norepinephrine is taken up by a store with rapid turnover rate and then gradually enters stores with slower turnover. Blaschko and Welch observed that only one-fifth of the pressor amines in the undenatured granule of the adrenal medulla had an immediate physiological effect after its intravenous administration.[5] This communication will provide direct evidence showing two kinds of stores of norepinephrine, one in which it is easily released and another in which it is tightly bound. Tyramine produces tachyphylaxis by depleting the more easily releasable store of norepinephrine.

Previous work in this laboratory has shown that tyramine releases H^3-norepinephrine from the rat heart.[6] A significant reduction of endogenous catecholamines in the rat heart was also found after the administration of 10 mg of tyramine per kg (Fig. 1). Tyramine was given repeatedly at 15-min intervals beginning 15 min after the injection of H^3-norepinephrine. The hearts were assayed for H^3-norepinephrine[7] and endogenous catecholamines[8] 60 min after the injection of H^3-norepinephrine.

Rats were given 10 μc of H^3-norepinephrine per 100 g, intravenously. In Experiment 1, rats were given per kg, 10 mg of tyramine HCl intramuscularly 30 min after the H^3-catecholamine; and in Experiment 2, the same dose of tyramine was given 48 hr after the H^3-catecholamine. Animals were killed 30 min after the tyramine injection, and the hearts assayed for H^3-norepinephrine and endogenous catecholamine. Control animals did not receive tyramine but were killed at the same time

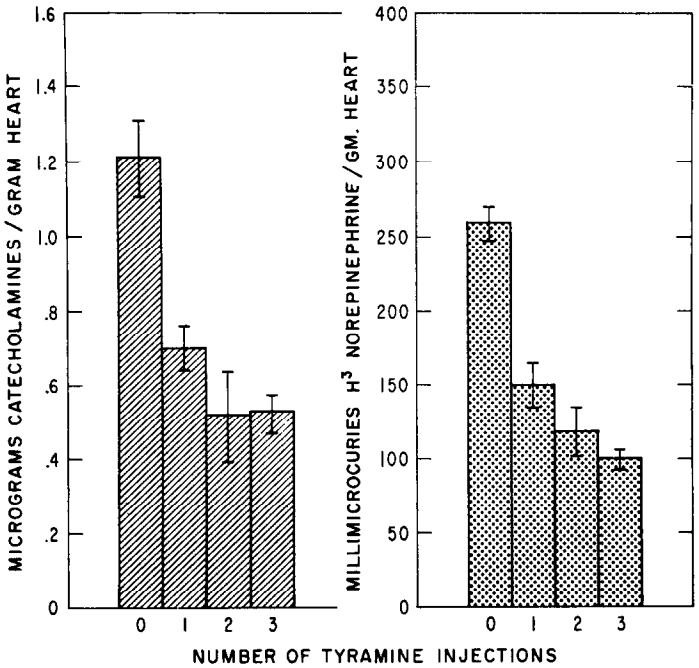

FIG. 1. Male rats (Sprague–Dawley) weighing 130–140 g were given 10 μc of H^3-norepinephrine (20 mc/mg) per 100 g in the tail vein. Fifteen minutes later, groups of six rats received 1, 2, or 3 successive injections of tyramine (10 mg/kg) intramuscularly at 15-min intervals. A control group received no tyramine. Animals were killed 60 min after the administration of H^3-norepinephrine and the hearts were assayed for endogenous catecholamines and H^3-norepinephrine.

TABLE 1. DIFFERENTIAL RELEASE OF CATECHOLAMINES BY TYRAMINE

	Experiment 1			Experiment 2		
	Control	Tyramine	% Released	Control	Tyramine	% Released
mμc H^3-norepinephrine g heart	419 ± 17	243 ± 10	42	19·5 ± 0·9	16·2 ± 1·6	17
μg catecholamine g heart	0·87 ± 0·03	0·52 ± 0·02	40	0·89 ± 0·04	0·53 ± 0·03	40
$\frac{m\mu c}{\mu g}$ (specific activity)	438 ± 8	468 ± 8		22·1 ± 1·0	29·9 ± 2·9*	

* Change in specific activity $P < 0{\cdot}05$.

intervals. From eight to ten rats were used in each group. Results are expressed as mμc of H^3-norepinephrine and μg of endogenous catecholamine per g heart ± s.e.m.

With each successive injection of tyramine, the decrease of endogenous and H^3-norepinephrine in the heart was smaller and smaller (Fig. 1). After the third injection, there was a negligible decrease in the

endogenous catecholamines and H^3-norepinephrine concentration. Blood pressure was also measured in rats during three repeated injections of tyramine as above and tachyphylaxis was demonstrated. After the third injection of tyramine, there was little or no blood pressure elevation, yet considerable amounts of the catecholamine were present in the heart. These observations indicate that the reduced response to tyramine after its repeated administration is a result of the depletion of bound catecholamines easily available for release.

To examine further the nature of the different types of binding of catecholamines, the effect of tyramine on H^3-norepinephrine in the heart for short and long periods of time was compared. Rats received H^3-norepinephrine as above and 30 minutes or 48 hours later 10 mg of tyramine per kg was given, and the animals were killed 30 min after the tyramine administration. After 48 hr, the specific activity of the norepinephrine was significantly elevated in the animals treated with tyramine (Table 1). Thus, tyramine preferentially released unlabelled norepinephrine. On the other hand, tyramine given 30 min after H^3-norepinephrine released labelled and unlabelled norepinephrine to about the same extent (Table 1). These results provide additional evidence for the presence of more than one pool of stored norepinephrine. After 48 hr the H^3-norepinephrine remaining in the heart is predominantly confined to stores having a slow turnover rate. Since tyramine results in an increase in specific activity at this later time, the catecholamines released must be derived from the stores that are turning over more rapidly and have a smaller percentage of the remaining H^3-norepinephrine.

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Consideration of chemical reaction mechanisms in relationship to the biological action of "dual antagonists"*

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THE term "dual antagonists" was introduced by the author and his co-workers several years ago[1, 2] to describe certain chemical compounds incorporating the biologically essential structural features of two different, but synergistic, inhibitors into a single molecule. Use of such compounds as chemotherapeutic agents may be considered as a new form of "combination chemotherapy", a potentially more effective one because it provides for synchronized action and, possibly, more selective localization of the synergistic drug-components.[2] Somewhat similar suggestions were made by Wooley[3], who showed that "aggregate analogues" of *p*-aminobenzoic acid and 1,2-dimethyl-4,5-diaminobenzene inhibited the growth of *Staphylococcus aureus* in an "irreversible" manner. This relatively simple concept has been proving fruitful as a working hypothesis in the design of effective chemotherapeutic agents; additional results obtained through its application, and the conclusions derived from these results, are discussed in the present paper.

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