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# STUDIES OF UPTAKE OF l-NOREPINEPHRINE-$^{14}$C

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**Abstract**—During a constant slow infusion, L-norepinephrine-$^{14}$C accumulates in the tissues. The rate of accumulation from the circulation depends upon the relative blood flow to each tissue and the ability of the tissue to extract the delivered compound. The efficiency of extraction from the circulation seems to be related to the norepinephrine content of the tissue which, in turn, reflects sympathetic nerve density. The heart, which has a very high relative blood flow and a high norepinephrine concentration, accumulates the labeled catecholamine most rapidly. The seminal vesicle, which has a high norepinephrine concentration but a low blood flow, extracts norepinephrine efficiently so that its rate of norepinephrine-$^{14}$C accumulation is about the same as that of the intestine, which has a high blood flow but a low norepinephrine concentration.

After a long infusion period, there is a steady state in which the ratio of the specific activities of norepinephrine in the tissue and urine can be used to estimate the proportion of the catecholamine in the tissue derived from the circulation. Only 20 per cent of the norepinephrine in the heart appears to be derived from the circulation. In other tissues less than 10 per cent comes from the circulation. Thus synthesis appears to be the main source of tissue catecholamine, with uptake from the circulation playing only a minor role in maintaining stores of this catecholamine.

INTRAVENOUSLY administered norepinephrine is rapidly inactivated. Some of the catecholamine is destroyed, predominantly by o-methylation, and some is taken up and retained in the tissues.[1] Norepinephrine that remains in the tissues is held in the sympathetic nerves[2] and appears to mix with endogenous norepinephrine.[3] During sympathetic nerve stimulation endogenous norepinephrine is released into the circulation.[4] The behaviour of administered labeled catecholamine should be the same as that of endogenous norepinephrine released into the circulation. Administered norepinephrine is not uniformly distributed. The heart retains a relatively large portion and has been thought to have a specific affinity for the sympathetic transmitter.[5]

In the studies to be described, some of the variables that may determine the uptake of administered norepinephrine from the circulation have been examined. The proportion of endogenous norepinephrine derived from the circulation has been estimated in several tissues.

## MATERIALS AND METHODS

L-Norepinephrine-7-$^{14}$C was prepared enzymatically from dopamine-7-$^{14}$C (25 mc/mmole), prepared from dopamine-$\beta$-oxidase purified from bovine adrenal medulla.[6]

The labeled norepinephrine was infused slowly (0·9 $\mu$g/hr) into tail veins of Sprague-Dawley rats, weighing 180–220 g, placed in small restraining cages, which facilitated collection of urine samples. Animals were killed at various times after beginning the infusion. Tissues were removed rapidly, homogenized in cold 0·4 N perchloric acid,

and the endogenous norepinephrine and norepinephrine-$^{14}$C assayed[7] and specific activities calculated. The specific activity of urinary norepinephrine was similarly determined.

The proportion of the cardiac output delivered to a given organ was estimated 1 hr after starting infusion of L-norepinephrine, by determining the fraction of an i.v. dose of $^{42}$K present in each of the tissues about 20 sec after injection.[8] Similar data were obtained from animals perfused with norepinephrine for up to 24 hr, but were not significantly different from the blood flows obtained at 1 hr.

## RESULTS

*Rate of uptake of* l-*norepinephrine-*$^{14}$*C by various tissues.* During the first 4 hr of norepinephrine-$^{14}$C infusion the tissue content of the labeled catecholamine increased linearly (Fig. 1). The uptake rate was greatest in heart and least in skeletal muscle.

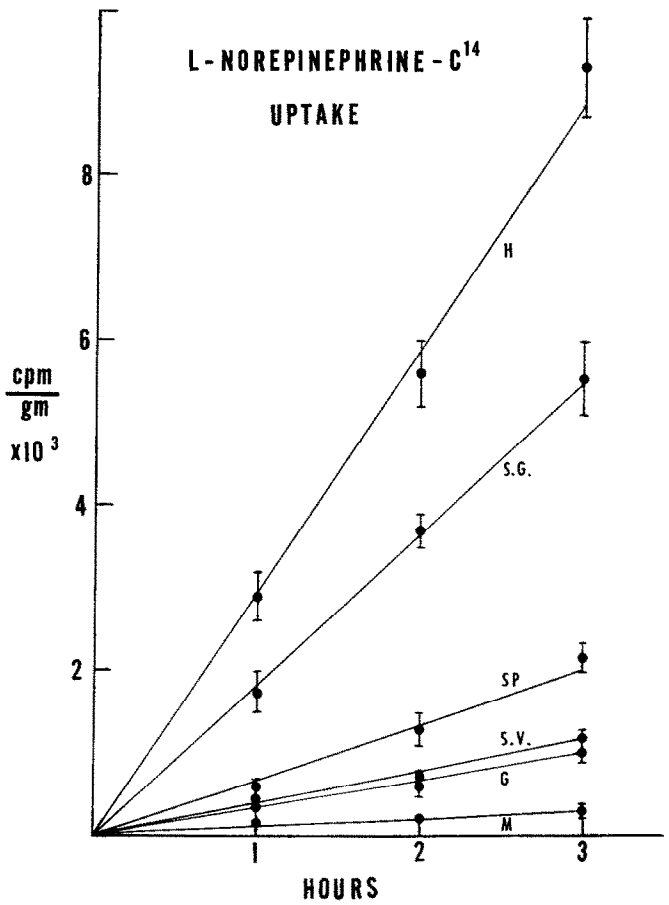


FIG. 1. Uptake of *l*-norepinephrine-$^{14}$C (25 mc/mmole) during a constant slow (0·9 mg/hr) i.v. infusion. The tissues examined were heart (H), salivary gland (S.G.), spleen (SP), seminal vesicle (S.V.), intestine (G), and skeletal muscle (M). Each point represents the mean (± S.E.M.) for six animals.

*Relative blood flow to the various tissues.* Tissue content of $^{42}K$, 20 sec after its i.v. administration, has been used to estimate the portion of the cardiac output to that tissue.[8] The percent of injected $^{42}K$ found in an organ approximates the percent cardiac output delivered to it. Of the organs examined, heart receives the greatest portion of cardiac output relative to its weight (Table 2).

*Extraction of norepinephrine.* Since norepinephrine is removed rapidly from the circulation, the proportion delivered to an organ is about the same as the proportion of the cardiac output reaching the organ. The ratio of the rate of norepinephrine-$^{14}C$ uptake (slope of the lines in Fig. 1) to the proportion of cardiac output expresses the extraction efficiency of an organ. This ratio (the relative extraction rate) was calculated for each tissue examined (Table 1). The heart, seminal vesicle, and salivary gland

TABLE 1. RELATION OF BLOOD FLOW TO L-NOREPINEPHRINE-$^{14}C$ UPTAKE

The percent of intravenously administered $^{42}K$ found in a tissue 20 sec after injection equals the percent of cardiac output (% c.o.) to that tissue.[8] The rate of norepinephrine uptake is the slope of the functions illustrated in Fig. 1. The relative extraction rate is the ratio of the norepinephrine-$^{14}C$ accumulation rate to the relative blood flow.

| | Accumulation rate (cpm/g/hr) | Relative blood flow (% c.o./g) | Relative extraction rate (cpm/hr/% c.o.) |
|---|---|---|---|
| Heart | 2,957 | 1·98 | 1,490 |
| Salivary gland | 1,838 | 1·20 | 1,530 |
| Spleen | 603 | 1·61 | 1,010 |
| Seminal vesicle | 400 | 0·30 | 1,330 |
| Intestine | 360 | 1·04 | 346 |
| Skeletal muscle | 110 | 0·40 | 275 |

extract circulating norepinephrine best; spleen less well; and intestine and skeletal muscle least efficiently.

*Relation of norepinephrine-$^{14}C$ extraction and norepinephrine content.* There is an apparent relationship between endogenous norepinephrine content and the relative rate of norepinephrine-$^{14}C$ absorption by a tissue (Fig. 2). Heart (H), salivary gland (SG) and seminal vesicle (SV) have the greatest norepinephrine concentrations, and they extract norepinephrine-$^{14}C$ more efficiently than skeletal muscle (M) or intestine (G), which have low norepinephrine concentrations. Spleen (SP) is intermediate in norepinephrine content and extraction.

*Specific radioactivity of norepinephrine in the urine and tissues after infusion of labelea catecholamine for 36 hours.* Shortly after beginning a continuous slow infusion of L-norepinephrine-$^{14}C$, the specific activity in urine (and presumably plasma) becomes constant.[9] A steady state develops in which the rate of norepinephrine-$^{14}C$ entry into the tissues from the circulation equals its rate of release and destruction, yielding a constant tissue specific activity. In the heart this equilibration takes about 24 hr.[9] The specific activity of norepinephrine in urine, heart, spleen, muscle, adrenal gland, and salivary gland after 36 hr of slow i.v. infusion of the labeled catecholamine is shown in Table 2. During this time a steady state probably occurs or is approximated. In this steady state the ratio of specific activity of the norepinephrine in tissue to that in urine is equal to the proportion of norepinephrine in the tissue derived from the

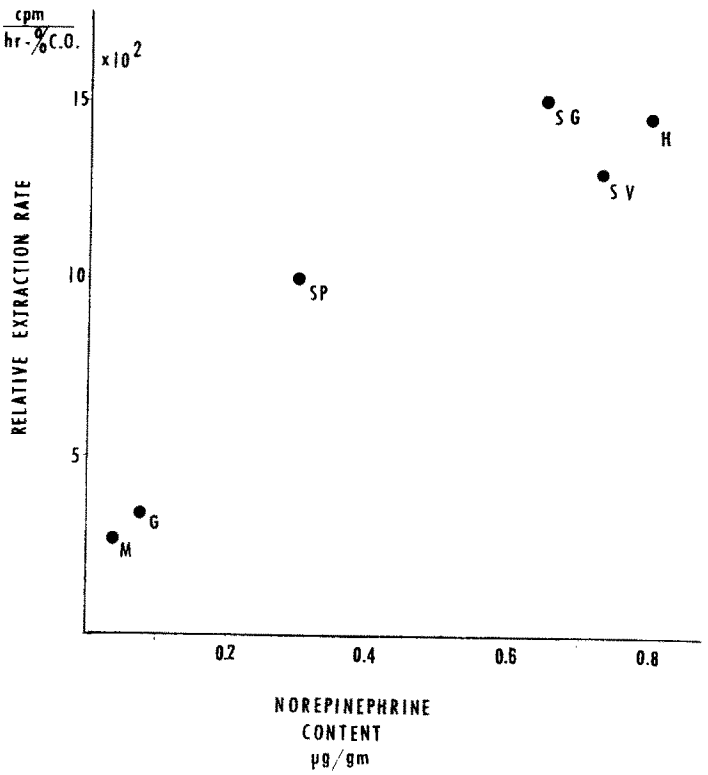


FIG. 2. The relationship between relative extraction rate shown in Table 1 (ordinate) to tissue norepinephrine concentration (abscissa). The letter next to each point represents the tissues shown in FIG. 1. Tissue norepinephrine content is the mean for ten animals.

## TABLE 2. ORIGIN OF NOREPINEPHRINE IN TISSUES

*l*-Norepinephrine-$^{14}$C (130 cpm/m$\mu$g) was infused i.v. slowly (0·90 $\mu$g/hr) for 36 hr. Specific activities (S.A.) in urine and tissues were determined at the end of the infusion period. From these, the proportion of norepinephrine derived from the circulation and from synthesis was calculated (see Appendix).

| | S.A. (cpm/m$\mu$g) | Per cent of NE from | |
|---|---|---|---|
| | | Circulation | Synthesis |
| Urine | 60 | 100·0 | 0·0 |
| Heart | 12 | 20·0 | 80·0 |
| Spleen | 4·8 | 3·0 | 92·0 |
| Muscle | 2·3 | 4·0 | 96·0 |
| Salivary gland | 1·8 | 3·0 | 97·0 |
| Adrenal gland | 0·05 | 0·1 | 99·9 |

circulation (see Appendix). These ratios have been calculated (Table 2). The portion of catecholamine not derived from the circulation must have been synthesized in the tissue.

## DISCUSSION

There is a difference in the rate at which administered norepinephrine-$^{14}$C enters various tissues (Fig. 1). The administered compound must reach a tissue via the circulation before it is extracted. Organs receiving a greater proportion of the cardiac output receive a greater fraction of the administered compound. The relative ability to remove catecholamine from the circulation can be estimated from the rate of accumulation of norepinephrine-$^{14}$C in the tissue, if this is corrected for relative blood flow. This correction has been made by dividing the rate of norepinephrine-$^{14}$C accumulation by the fraction of the cardiac output perfusing a tissue (Table 1). The relative extraction rate thus obtained is similarly high for heart, salivary gland, and seminal vesicle, but skeletal muscle and intestine do not extract norepinephrine from the circulation as efficiently. Seminal vesicle accumulates norepinephrine-$^{14}$C more rapidly than intestine, but it receives only one third as much catecholamine via the circulation and must, therefore, extract it more efficiently than intestine.

Administered norepinephrine is taken up into the sympathetic nerves.[2] If the concentration of norepinephrine throughout the sympathetic nervous system does not vary greatly, then the norepinephrine content of a tissue must parallel the density of sympathetic nerves there. The correlation between norepinephrine content and efficiency of norepinephrine-$^{14}$C extraction is consistent with the view that sympathetic nerve density is a factor in accumulation of norepinephrine in the tissues. The heart, salivary gland, and seminal vesicles have relatively high norepinephrine concentrations, and they remove labeled norepinephrine from the circulation relatively efficiently. Differences in the rate of norepinephrine-$^{14}$C accumulation correlate with differences in distribution of the cardiac output as well as with norepinephrine concentration. The heart accumulates norepinephrine most rapidly, presumably because it has a high density of sympathetic nerves and receives a high proportion of the cardiac output. The seminal vesicle is almost as efficient in removing norepinephrine from the circulation but receives a smaller fraction of the cardiac output. The intestine accumulates no more than seminal vesicles even though it receives a greater share of the cardiac output.

Sympathetic nerves discharge norepinephrine into the circulation and thus maintain a circulating pool of catecholamine which has the same fate as intravenously administered labeled norepinephrine. On the other hand, since a portion of administered norepinephrine is taken up by tissues, circulating norepinephrine contributes to the catecholamine stores in tissues. Synthesis of norepinephrine can occur in the isolated heart at a rate sufficiently rapid to maintain catecholamine stores.[10] Thus, both synthesis and uptake may be sources of tissue norepinephrine.

The extent to which the circulating pool of norepinephrine contributes to tissue stores of catecholamine can be estimated by examining the specific activities of norepinephrine-$^{14}$C in the tissues during the steady state which develops after slow i.v. infusion of L-norepinephrine-$^{14}$C for a long time. At the end of 36 hr a steady state is reached in the heart[9] and, presumably, in other tissues in which as much norepinephrine-$^{14}$C enters an organ as exits by release or metabolism.

Urinary norepinephrine is derived largely, if not entirely, from the circulating catecholamine. If all the urinary norepinephrine were derived from the plasma, then its specific activity would be equal to the specific activity of norepinephrine in the arterial blood. If some norepinephrine were synthesized and excreted by the kidney tubules without entering the plasma, then the specific activity of urinary norepinephrine would be lower than that of the plasma. It is unlikely, however, that norepinephrine synthesized in the kidney is a major source of urinary norepinephrine. If there is no isotope effect favoring excretion of labeled norepinephrine-$^{14}$C, it is not possible for the specific activity of urinary norepinephrine to exceed that of plasma norepinephrine. It is therefore assumed that the specific activity of norepinephrine in the urine approximates that of the plasma. Because of the difficulty in accurately assaying rat plasma norepinephrine this assumption could not be tested experimentally. If all the norepinephrine in a tissue were derived from the circulation, its specific activity there should be the same as in the plasma. If, during a steady state, tissue specific activity be lower than that of plasma, the ratio of specific activities is equal to the proportion of endogenous norepinephrine derived from the circulating pool of catecholamines (see Appendix).

From the ratios of tissue to urinary norepinephrine specific activities, the proportion of endogenous norepinephrine derived from the circulating pool of catecholamines is calculated. Under the conditions of this experiment approximately 20 per cent of

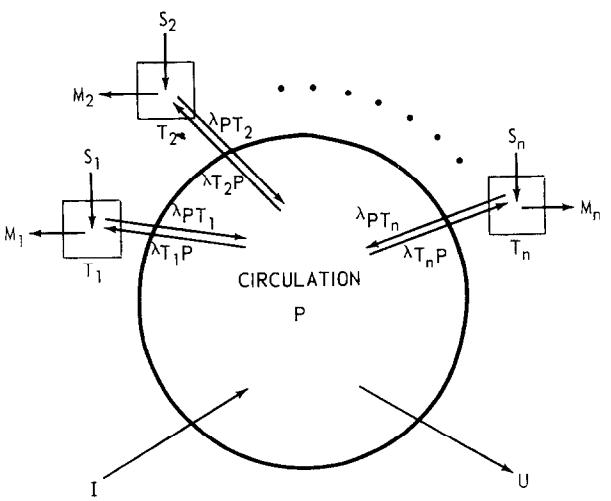


FIG. 3. Diagrammatic representation of the exchange of norepinephrine between circulation and tissues, illustrating relationships discussed in Appendix. *I* represents infusion; *U* urinary excretion; the symbol $\lambda$ is a transfer constant such that $\lambda ij$ represents the rate of transfer of norepinephrine from any compartment, *j*, to another compartment, *i*; $S_n$ refers to the rate of synthesis, $M_n$, to the rate of metabolism in the tissue, $T_n$.

cardiac catecholamine is derived from the circulation, and less than 10 per cent of norepinephrine in other tissues is so derived. In the tissues examined, most of the norepinephrine appears to be derived from synthesis.

Thus the uptake by tissues of norepinephrine from the circulating pool depends on the distribution of the cardiac output and the density of sympathetic nerves. Uptake

from the circulation, however, does not appear to be an important mechanism for maintaining norepinephrine stores.

## APPENDIX

After a long period of intravenous infusion, I, there is a steady state. In the equilibrium (Fig. 3) the amount of norepinephrine-$^{14}$C entering a tissue, $T_n$, is equal to the total amount of norepinephrine-$^{14}$C taken from the plasma, $\lambda_{T_nP}$, multiplied by its specific activity, [S.A.]$_P$, and must equal norepinephrine-$^{14}$C loss. The rate of tissue norepinephrine-$^{14}$C loss depends on the rate of norepinephrine release, $\lambda_{PT_n}$, plus norepinephrine metabolism, $M_{T_n}$, and upon the specific activity of norepinephrine in the tissue, [S.A.]$_{T_n}$. Thus:

$$\lambda_{T_nP}\,[\text{S.A.}]_P = [\lambda_{PT_n} + M_{T_n}]\,[\text{S.A.}]_{T_n}$$

The sum of the rates of uptake, $\lambda_{T_nP}$, and synthesis, $S_{T_n}$, equals the sum of the release, $\lambda_{TP_n}$, and metabolism, $M_{T_n}$.
Thus,

$$\lambda_{T_nP}\,[\text{S.A.}]_P = [\lambda_{T_nP} + S_{T_n}]\,[\text{S.A.}]_{T_n}$$

and

$$\frac{[\text{S.A.}]_{T_n}}{[\text{S.A.}]_P} = \frac{\lambda_{T_nP}}{\lambda_{T_nP} + S_{T_n}} = \text{proportion of norepinephrine derived from the circulating pool}$$

$$1 - \frac{\lambda_{T_nP}}{\lambda_{T_nP} + S_{T_n}} = \frac{S_{T_n}}{\lambda_{T_nP} + S_{T_n}} = \text{proportion of norepinephrine derived from synthesis}$$

In the steady state these equations hold even if there is a segregation of tissue norepinephrine stores into several compartments without uniform mixing. It is assumed that the specific activities of norepinephrine-$^{14}$C in plasma and urine are approximately equal.

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