AN ELECTRON MICROSCOPE AUTORADIOGRAPHIC STUDY OF THE NEURONAL AND EXTRANEURONAL LOCALIZATION OF LABELED AMINE IN THE HEART OF THE BAT AFTER ADMINISTRATION OF TRITIATED NOREPINEPHRINE

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ABSTRACT
The localization of labeled amine in the heart of the bat after administration of tritiated norepinephrine (NE) was studied by means of electron microscope autoradiography. Monoamine oxidase was inhibited so that the distribution of amine in both neuronal (Uptake1) and extraneuronal (Uptake2) sites could be analyzed. Labeling was nonrandom in both the atrial and ventricular myocardium. The highest relative specific activity was found in neural processes which showed morphological criteria of terminal adrenergic axons. Analysis of the distribution of label around the labeled axonal varicosities indicated that the radioactive amine was more concentrated peripherally than centrally in these structures. Label was also found over cardiocytes in both atrium and ventricle. The pattern of this labeling indicated that the radioactive amine was associated with myofilaments. In the ventricle, I bands were most heavily labeled, indicating a probable association of radioactive amine with thin filaments. Labeling was prevented by administration of phenoxybenzamine and decreased only in cardiocytes by normetanephrine. The nonrandom distribution of labeled amine within cardiocytes supports the view that Uptake2 represents not only a second mechanism of inactivation of the sympathetic neurotransmitter, but may also be involved in the mediation of some of the action of NE on cardiac muscle.

A variety of mechanisms have evolved in different synaptic systems subserving the function of terminating the action of neurotransmitter substances. These include diffusion of the substance away from the synapse, metabolic conversion of the released transmitter into pharmacologically inert substances, or removal of the transmitter by transport across a membrane in the vicinity of the synapse. For this latter purpose, transport back into the presynaptic element, transport into the postsynaptic cell, or transport into the surrounding glial or Schwann elements all could do. It is well known...
that sympathetic adrenergic axons take up exogenous norepinephrine (NE) and it is also believed that uptake of NE by presynaptic axons is important in terminating the action of transmitter when it is released endogenously. The physiological significance of extraneuronal uptake of NE is not clear. This subject has recently been reviewed by Iversen (1971).

The uptake of NE by the heart has been particularly well studied (Iversen, 1963; see also the review by Iversen, 1971). At low ambient concentrations of NE, the bulk of the NE is taken up into adrenergic terminal axons and is virtually abolished by treatments such as immunosympathectomy of animals at birth (Iversen et al., 1966) or by chemical sympathectomy with 6-hydroxydopamine (Thoenen and Tranzer, 1968). However, in addition to this neuronal uptake of NE a second mediated uptake by non-neuronal tissues in the heart has also been defined, and termed Uptake$_2$ (Iversen, 1965). Uptake$_2$ was initially found when the heart was exposed to very high perfusion concentrations of NE. However, Uptake$_2$ has recently been shown to be operative at all ranges of concentration of NE. At low concentrations the amine is metabolized by monoamine oxidase and catechol-O-methyl transferase (Lightman and Iversen, 1969). Histochemical evidence based on perfusion of the heart with high concentrations of NE indicates that cardiac muscle cells (Farnebo and Malmfors, 1969) as well as arterial smooth muscle, connective tissue fibers, and fibroblasts may take up NE by Uptake$_2$ (Jacobowitz and Brus, 1971). In order to further investigate the sites of uptake of NE in the heart, the technique of electron microscope autoradiography has been used in the present study. This technique has the advantage of high resolution and permits a more precise identification of structures to which NE binds than does histochemical localization of NE. A relatively small dose of NE was used and monoamine oxidase was inhibited in order to study both neuronal and extraneuronal uptakes of NE at ambient concentrations of the amine that might be of physiological significance. An inhibitor of catechol-O-methyl transferase was not given because the combination of inhibition of this enzyme with simultaneous inhibition of monoamine oxidase and administration of NE produces toxic changes in cardiac muscle cells (Gershon et al., 1974). Experiments were done on aroused bats and on hibernating animals. In order to test the relationship of the observations to Uptake$_2$ the effect of phenoxycetamine and normetanephrine on the pattern of labeling was determined. It was hoped that more knowledge of the intracellular distribution of the NE taken up by cardiocytes may help resolve whether Uptake$_2$ represents simply another mechanism (in addition to Uptake$_1$) of inactivation of NE or whether it is involved in mediation of some of the cardiac effects of NE.

**MATERIALS AND METHODS**

14 adult male bats *Myotis lucifugus* were used in this study. Four were in the homoiothermic or active phase of their annual life cycle and were caught in June. The animals were kept in the laboratory for 1 wk before use. During this time they were maintained on a diet of meal worms and hamburger meat. Ten animals were caught while they were hibernating. They were transported over ice to the laboratory. These bats were allowed to equilibrate in continued hibernation for 1 wk at 4°C. Two animals were used while still hibernating and eight were aroused before use.

Monoamine oxidase was inhibited with pheniprazine (10 mg/kg) which was injected intraperitoneally. 1 h later the animals were lightly anesthetized. Two active, two hibernating, and two aroused bats were each injected with 2.5 mCi (0.064 mg) of tritiated D$_1$-norepinephrine into a wing vein for autoradiography. Two active bats were given 0.1 mCi of tritiated NE for measurement of the radioactive compounds present in the heart. The animals were decapitated 2.5 h after administration of the label. Two aroused animals were given phenoxycetamine (20 mg/kg; intraperitoneally), two received normetanephrine (30 mg/kg, i.p.), and two control animals were injected with saline 30 min before injection with tritiated NE (0.5 mCi). Pheniprazine was given as before and hearts were processed as above except that they were used both for autoradiography and for biochemical determinations.

For autoradiography, tissues were fixed by perfusion of the anesthetized animals through the heart with hypertonic 6.5% glutaraldehyde (containing 3% sucrose and 0.1 M phosphate buffer, pH 7.4) before decapitation. This procedure is similar to that used for the autoradiographic localization of tritiated 5-hydroxytryptamine (5-HT) and preserves the labeled amine while permitting radioactive metabolites to wash out of the tissues (Gershon and Nunez, 1973). Aghajanian and Bloom (1967) have shown that not all of the labeled NE is preserved by the methods like the ones used here. Autoradiography thus preferentially demonstrates bound NE and cannot be relied upon to reflect all of the physiological sites of residence of NE. After fixation tissues were postfixed in osmium tetroxide, dehydrated, and embedded in Epon 812 as described previously (Gershon and Nunez, 1973). Portions of the atria and the ventricle were embedded.
separately. Thin sections were obtained and picked up on copper grids coated with Formvar. A monolayer of Ilford L4 emulsion, diluted 1:4, was applied to grids with a platinum wire loop. The grids were exposed for 4-5 wk in a dry atmosphere of 100% CO₂. Parallel control grids containing nonradioactive sections of the bat's cardiac ventricle were similarly coated and served to assist in the determination of background. After exposure, Kodak Microdol-X for 5 min, was used for development. Finally, after staining with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) the grids were examined in a Philips EM 200 electron microscope.

Autoradiographs were analyzed by a combination of the “circle analysis” method of Williams (1969) and the analysis of grain distribution described by Salpeter et al. (1969). The methods used were identical to those described in detail in a prior publication (Gershon and Nunez, 1973) except that the radius of the circles superimposed over silver grains as well as that of the random circles which were used to determine the effective area of various cellular components was equal to 1.5 times the half distance (HD; the distance from a point count planimetry. The “effective area” (Williams, 1969) occupied by each cellular component was obtained by noting the frequency with which the components occurred in the circles of a regular grid of circles, identical in size to those used in scoring grains. A total of 3,242 circles was scored. Relative specific activity of each component was determined by dividing the final count of grains over the component by its effective area. The frequency of occurrence of components in the random grid circles was used as the distribution which would have been expected if the isotope and thus the silver grains were spread randomly. Grid..
Figure 1 A portion of the atrial myocardium of a bat. Silver grains are associated with a small bundle of unmyelinated nerves (N) coursing through the intercellular connective tissue. A labeled varicose axonal expansion (V) contains granulated vesicles. A portion of this varicosity is not enclosed by Schwann cell cytoplasm and fronts on the extracellular space (arrow). A region of the cytoplasm of a cardiocyte (C) is also shown. Note that the muscle is partially contracted and that due to the extensive overlap of thin and thick filaments the I bands are not distinctive. The marker = 1 μm. × 26,000.
Figure 2 A portion of the ventricular myocardium. Label is associated with three of the axons (arrows) in a small bundle of unmyelinated nerves (N) coursing through the intercellular connective tissue. Note that the musculature is more stretched than that of the atrium and that the I bands of the longitudinally sectioned cardiocyte (C) are distinct. The marker = 1 μm. × 25,480.
FIGURE 3. Additional examples of axonal labeling in the ventricular myocardium. Note in Fig. 3 A that label is found in close proximity to a varicosity (arrow) which contains granulated vesicles, exhibits a region not covered by the process of a Schwann cell, and is probably therefore part of a terminal adrenergic axon. × 52,000. In Fig. 3 B a heavily labeled varicosity is found adjacent to an unlabeled varicosity in the atrial myocardium. This latter axonal expansion (CV) contains small clear vesicles and is probably cholinergic. The marker = 0.5 μm. × 52,000.
circle and grain frequency distributions were compared by a $\chi^2$ test and were highly nonrandom ($P < 0.001$).

The corrected distribution of silver grains over various components of the atrial tissue is shown in Table I. Note that many more grains were associated with extramuscular components (84.8%) than cardiocytes (15.2%), despite the greater effective area of the cardiocytes (62.9% vs. 37.1%). The highly labeled neural components (69.0% of grains; relative specific activity 17.25) accounted for this. However, since background was negligible, the grains which were found over the cardiocytes, though not concentrated, could not be ignored.

Therefore, the grain distribution over the cardiocytes was separately analyzed. This latter distribution is shown in Table II. The grain distribution as judged by comparison with the grid circles by a $\chi^2$ test again does not seem random ($P < 0.01$).

However, the number of grains observed was small. In order to improve the statistical comparison, components were combined and grains associated with myofilaments were pooled and compared with the grain frequency over all other cytoplasmic regions of the cardiocytes. This comparison is shown in Table II (B). Note the relatively high specific activity of the myofilaments. More grains than could be accounted for by a random distribution ($P < 0.01$) were associated with the myofilaments, and fewer with the other cytoplasmic constituents. When only those grains which were associated with the myofilaments were analyzed (Table II [C]), however, the distribution appeared random with respect to A and I bands.

For the ventricular myocardium, 761 grains and 3,060 circles were scored and analyzed (Table III). The corrected distribution of silver grains appeared nonrandom by $\chi^2$ comparison with the

![Figure 4](image-url)
FIGURE 5 Portions of the ventricular myocardium. The relatively rare occurrence of grains over cardiocytes near their sarcolemma is illustrated. In Fig. 5 C one grain appears over an axon in the intercellular connective tissue (arrow). × 26,000.

expected distribution derived from analysis of grid circles ($P < 0.001$). Again, many more grains were associated with the extramuscular components than with the cardiocytes (69% vs. 31%) although a higher percentage of grains appeared over ventricular than atrial cardiocytes (31% vs. 15.2%). Again, the high relative specific activity (26.0) of the neural elements largely accounted for the
### Table I

**Grain Distribution Over the Atrial Myocardium**

| Cardiocyte components | Silver grains | Grid circles (effective area) | Relative specific activity |
|-----------------------|---------------|-------------------------------|---------------------------|
| Mitochondria          | 4.8           | 20.5                          | 0.23                      |
| A band                | 2.8           | 6.5                           | 0.43                      |
| Glycogen-rich areas   | 0.2           | 3.3                           | 0.06                      |
| Atrial granules       | 0             | 0.2                           | 0                         |
| Lipid droplets        | 0             | 0.2                           | 0                         |
| Sarcoplasm (cytoplasmic structures not listed above) | 0 | 9.9 | 0 |
|                       | **15.2**      | **62.9**                      |                           |

| Extramuscular components | Silver grains | Grid circles (effective area) | Relative specific activity |
|--------------------------|---------------|-------------------------------|---------------------------|
| Extracellular space      | 12.0          | 27.3                          | 0.44                      |
| Endothelium              | 0.8           | 2.4                           | 0.33                      |
| Vascular luminal space   | 3.0           | 3.4                           | 0.88                      |
| Axon and Schwann cells   | 69.0          | 4.0                           | 17.25                     |
|                         | **84.8**      | **37.1**                      |                           |

* Nuclei were not included in the tabulation of silver grains, circle analysis, or point count planimetry.

### Table II

**Grain Distribution Over Atrial Cardiocytes**

|                   | Silver grains | Grid circles (effective area) | Relative specific activity |
|-------------------|---------------|-------------------------------|---------------------------|
|                   | %             | %                             |                           |

**A. Individual components**

1) Mitochondria    32.0 32.7 1.0
2) I bands         18.0 10.3 1.7
3) A bands         49.0 35.5 1.4
4) Glycogen-rich areas 1.3 5.2 0.3
5) Atrial granules 0 0.3 0.8
6) Lipid droplets 0 0.1 0
7) Sarcoplasm      0 15.8 0

* $X^2 = 22.3, P < 0.01$

|                   | Observed | Expected | $X^2$ | Relative specific activity |
|-------------------|----------|----------|-------|---------------------------|
|                   | grains   | grains   |       |                           |

**B. Combined components**

Items numbered 1, 4, 5, 6, 7 (nonmyofilaments) 26 42 6.1 0.62
Items numbered 2, 3 (myofilaments) 52 36 7.1 1.44

* $X^2 = 13.2, P < 0.1$

**C. Myofilaments (52 grains; 933 circles)**

|                | Observed | Expected | $X^2$ | Relative specific activity |
|----------------|----------|----------|-------|---------------------------|
| A band         | 38       | 40       |       |                           |
| I band         | 14       | 12       |       |                           |

* $X^2 = 0.4, P - NS$
gic axons (Hökfelt, 1968). Sections through the ventricular myocardium appear in Figs. 2-5. Examples of grains found over extramuscular structures are shown in Figs. 2 and 3. Grains over cardiocytes are shown in Fig. 4 where they were most often found in close proximity to myofilaments. In Fig. 5 the rare appearance of grains close to the sarcolemma is shown.

Since labeling in extramuscular areas was so concentrated in neural elements the distribution around these structures was studied by the method of Salpeter et al. (1969). A histogram showing the distribution of grains around the plasma membrane of axons is shown in Fig. 6 A and B. The curve superimposed on the histograms is the predicted distributions of grains (Salpeter et al., 1969) that would result if the labeled source acted as a hollow circle with a radius of 4 HD (Fig. 6 A) or a hollow band with half band width of 4 HD (Fig. 6 B). The typical radius of axon terminals was determined as described by Budd and Salpeter (1969) and ranged between 230 and 340 nm. Some terminals used in this analysis appeared circular in profile while others were cut longitudinally and appeared more elongate, like bands. Thus it is difficult to say whether the appropriate expected distribution should be approximated by a circular or a band source. However, as can be seen in Fig. 6 A and B the distribution fits either of these hollow sources moderately well. The histogram does not fit the distribution that would have been expected.

### Table III

**Grain Distribution Over the Ventricular Myocardium**

| Component                      | Silver grains | Grid circles (effective area) | Relative specific activity |
|--------------------------------|---------------|-------------------------------|---------------------------|
| Cardiocyte components          |               |                               |                           |
| Mitochondria                   | 9.3           | 27.5                          | 0.34                      |
| I band                         | 6.0           | 7.4                           | 0.81                      |
| A band                         | 15.5          | 30.9                          | 0.50                      |
| Glycogen-rich area             | 0.1           | 4.7                           | 0.02                      |
| Lipid droplets                 | 0             | 1.5                           | 0                         |
| Sarcoplasm                     | 0.1           | 10.4                          | 0.01                      |
| Extracellular space            | 11.4          | 10.1                          | 1.13                      |
| Endothelium                   | 0.4           | 1.6                           | 0.25                      |
| Vascular luminal space         | 2.6           | 3.8                           | 0.69                      |
| Axon and Schwann cells         | 54.6          | 2.1                           | 26.00                     |
|                               | 60.0          | 17.6                          |                           |

### Table IV

**Grain Distribution Over Ventricular Cardiocytes**

| Component                      | Silver grains | Circles (effective area) | Relative specific activity |
|--------------------------------|---------------|--------------------------|---------------------------|
|                                | %             | %                        |                           |
| A. Individual components       |               |                          |                           |
| Mitochondria                   | 28.3          | 35.5                     | 0.80                      |
| I band                         | 18.3          | 9.6                      | 1.91                      |
| A band                         | 47.0          | 40.0                     | 1.17                      |
| Sarcoplasm                     | 6.4           | 13.0                     | 0.49                      |
|                                | X² = 35.8, P <0.01 |

|                                | Observed grains | Expected grains | X² |
| B. Combined components         |                |                |    |
| Mitochondria                   | 71             | 89             | 3.6 | 0.80 |
| Myofilaments                   | 164            | 124            | 12.9 | 1.32 |
| Sarcoplasm                     | 16             | 33             | 8.7  | 0.49 |
|                                | X² = 25.2, P <0.01 |

| C. Myofilaments (164 grains)   |                |                |    |
| A band                         | 118            | 132            | 1.48 |
| I band                         | 46             | 32             | 6.13 |
|                                | X² = 7.6, P <0.01 |
If the actual source had been either a solid band or a solid disk. Thus it seems likely that axon terminals are labeled and, as they appear to behave as hollow sources, the label appears to be concentrated around the periphery of the terminals.

The radioactive compounds present in the hearts were identified by thin-layer chromatography. Greater than 85% of the radioactivity existed as NE and less than 15% as normetanephrine. Deaminated metabolites of NE could not be detected.

**Figure 6** An analysis of the distribution of radioactivity around axon terminals. The shortest distance was measured from the center of the smallest circle necessary to encompass a silver grain to the plasma membrane of the axon. A histogram of the experimental distribution of developed grain density relative to a random grid of placebo points has been plotted on the ordinate which has been normalized so that density is 1 at the plasma membrane. The distance from the plasma membrane is plotted on the abscissa in units of HD (negative inside; positive outside). The superimposed curves were taken from the study of Salpeter et al. (1969) and show the expected distributions for a hollow circular source (A) and a hollow band source (B) of radius, or half band width, respectively, of 4 HD.

**Figure 7** (A) An autoradiograph of a portion of the ventricular myocardium of an active aroused bat injected with tritiated NE. When viewed by dark-field light microscopy, silver grains stand out and appear white. The arrows point to heavily labeled axons. × 912. (B) An autoradiograph of a portion of the ventricular myocardium of a hibernating bat treated as in Fig. 7 A. When viewed by dark-field light microscopy the pattern and intensity of labeling of cardiocytes and axons (arrows) are similar to that of the aroused animal. × 885.
FIGURE 8 (A) An autoradiograph of a portion of the ventricular myocardium of an aroused bat treated with phenoxybenzamine before injection of tritiated NE. Even when viewed by dark-field light microscopy, no labeling of any structure more intense than background is apparent. × 912. (B) A portion of the ventricular myocardium of an aroused bat treated as in Fig. 8 A except that saline was substituted for phenoxybenzamine. Labeling of cardiac muscle cells can be seen. No axons are present in this field. × 885.

indicating that monoamine oxidase had been effectively inhibited by the dose of pheniprazine used in these experiments. Increasing the amount of administered tritiated NE fivefold did not change the pattern of radioactive metabolites in the hearts. Administration of phenoxybenzamine decreased the total radioactivity found in the heart to less than half that of control, but injection neither of phenoxybenzamine nor normetanephrine altered the relative ratio of radioactive NE to radioactive O-methylated compounds in the heart.

The effect of hibernation, phenoxybenzamine, and normetanephrine on the uptake and distribution of label was assessed by dark-field light microscopy. Both cardiac muscle cells and axons were labeled in the hearts of hibernating animals. No differences in amount or pattern could be found between these and the hearts of aroused animals (Fig. 7 A and B). Phenoxymazine pretreatment, however, completely prevented labeling of both cardiocytes and axons (Fig. 8).

Normetanephrine was not as effective as phenoxybenzamine but it did appear to decrease labeling of cardiocytes while having little or no discernible effect on the labeling of axons.

DISCUSSION

The observations made in this study are in accord with the findings of previous workers that both nerve and cardiac muscle are sites of uptake of amine when the heart is exposed to exogenous NE (Farnebo and Malmfors, 1969; Jacobowitz and Brus, 1971; Iversen, 1971; Sachs, 1970; Wolfe and Potter, 1963). The distribution of label in both atrial and ventricular myocardium was found to be nonrandom. Axonal varicosities containing small dense-cored vesicles accounted for the predominant labeling of extramuscular components. Significant label was also found within cardiocytes. The distribution of label in this latter site is of particular significance with respect to Uptake2.

It is likely that $Uptake_2$ accounted for the
penetration of label into cardiac muscle cells. Other investigators have found by histochemical means that this process mediates uptake of NE by cardiac muscle cells and other extraneuronal tissues (Farnebo and Malmfors, 1969; Jacobowitz and Brus, 1971). Moreover, despite the initial view of Uptake 2 as a threshold process, the transport system has now been shown to be active even at low concentrations of NE and can be detected when metabolism of NE is blocked (Lightman and Iversen, 1969; Iversen, 1971). Thus, it would be expected to be operative in these experiments. In confirmation of this view, phenoxybenzamine, a drug which powerfully inhibits Uptake 2 (Burgen and Iversen, 1965; Iversen, 1971), abolished labeling of cardiac muscle cells. Normetanephrine, another inhibitor of Uptake 2 (Burgen and Iversen, 1965; Iversen, 1971), decreased labeling of cardiac muscle cells although it was not so effective in this action as phenoxybenzamine. Since 2.5 h elapsed between injection of tritiated NE and sacrifice of the animals, it is possible that the labeling of cardiac muscle cells results not from the initial pulse of injected label, but from continued normal activity of the labeled adrenergic axons. Material taken up into non-neuronal tissues by Uptake 2 is generally not tightly bound and washes relatively quickly out of cells (Iversen, 1971).

It also seems unlikely that the labeling of cardiac muscle cells results from an artificial linking of tritiated NE to the cells. Glutaraldehyde can bind free amino acids to tissues (Peters and Ashley, 1967). After injection of tritiated NE, when catechol-O-methyl transferase is intact, there is a rapid fall in the plasma concentration of labeled amine, reflecting its metabolism and uptake by tissues (Axelrod and Tomchick, 1960; Whitby et al., 1961). After 2.5 h, the time interval between injection of tritiated NE and sacrifice of the animals in these experiments, there is little free NE left for uptake. Labeled O-methylated amines are less tightly bound in tissues, and their concentration falls more quickly than that of the labeled unmetabolized amine (Whitby et al., 1961). Therefore the content of free amine not already bound to structure at the time of fixation is very small. Thus, the chance of glutaraldehyde fixation artificially accounting for the localization is minimized. The blocking of labeling by phenoxybenzamine supports this view and indicates that labeling reflects a physiological phenomenon. It is interesting that bat tissues are able to take up radioactive amine even when hypothermic during hibernation. Both neural and muscle uptake mechanisms must therefore be more resistant to cooling (at least during hibernation) in the bat than in other species. It is possible that some linking of free amine still occurred and contributed to the diffuse background (Descarries and Droz, 1970). Labeling of connective tissue structures was detected, but this, too, probably represents a physiological phenomenon as NE is known to bind to connective tissue, especially to collagen and elastin (Powis, 1973).

If Uptake 2 represents simply an additional mechanism of inactivation of the sympathetic transmitter, and the amine taken up in Uptake 2 by cardiocytes has no action within these cells, then one might expect to find a random intracellular distribution of the amine. In fact, if the amine did not bind in some way to intracellular components its autoradiographic localization by the techniques used in this study might not have been possible (Aghajanian and Bloom, 1967). On the other hand, a preferential localization of label to a given intracellular structure would indicate binding of amine to or within that structure. Such a finding would suggest that it might be profitable to search for an action of NE or perhaps normetanephrine on that structure.

In this study, we did find a nonrandom distribution of label within cardiocytes. Autoradiographic silver grains were associated with the myofilaments in both atrium and ventricle. In the atrium this distribution appeared random with respect to the A and I bands but in the ventricle the grain density appeared higher over the I than the A band. This distribution is what one would expect if the label were associated with the thin filaments. In the sections of atrium the overlap between thin and thick filaments was extensive, whereas the sarcomeres were more extended in the ventricle and the overlap of filaments consequently less. Thus, an association of label with thin filaments would not show up as a greater grain density in the I band in sections of atrium because of the extensive overlap of thin and thick filaments but would do so in sections of ventricle.

It is impossible to say from these observations which, if any, of the myofibrillar proteins of the thin filaments has an affinity for the amine taken up by the cardiocytes. Actin, tropomyosin, and troponin are all associated with the thin filaments of muscle (Katz, 1970) and are thus potentially the
responsible substances. However, there is some indirect evidence for a beta adrenoceptor site on troponin although beta-adrenergic drugs have not been found to have a direct effect on the contractile proteins (Katz, 1970). Nevertheless, the findings of the present study are consistent with the view that NE might bind to troponin.

It seems probable that the autoradiographic silver grains observed in this study indicated the presence of labeled NE. NE and normetanephrine were the only radioactive compounds present in tissue at the time of fixation. NE predominated, greater than 85% of the radioactive material being unchanged tritiated NE. Moreover, normetanephrine washes out of tissues more readily than does NE, and NE is thus more likely to have been bound and preserved during histological processing (Gershon and Thompson, 1973). However, the possibility that some silver grains resulted from the presence in the tissue sections of labeled normetanephrine has not been entirely excluded.

The distribution of silver grains around labeled axonal varicosities was also interesting. As expected, labeled terminals contained small dense-cored vesicles and were thus adrenergic. Varicosities which contained predominantly small clear vesicles were not labeled and thus the radioactive NE was not found nonspecifically in axons which were probably cholinergic. However, label did not appear to be distributed uniformly throughout the adrenergic varicosity but instead appeared around the periphery of these structures. A similar peripheral concentration of labeling by [3H]noradrenaline around adrenergic axon terminals was found by Budd and Salpeter (1969) in the adrenal capsule and pineal glands of the mouse. However, in these locations, in mice, the typical terminal appeared larger (the typical radius was 600 nm) than those observed in this study of the heart of the bat (230–340 nm). Due to the dense packing of vesicles within labeled varicosities it was impossible to ascertain whether label was associated with the dense-cored vesicles themselves. The vesicles showed no marginal distribution similar to that of the label. Thus, if labeled NE is within the vesicles, then those vesicles nearest the plasma membrane rather than all vesicles must preferentially contain newly taken up NE. This observation is in accord with the conclusions of other studies that there is more than one pool of stored NE in adrenergic axon terminals (Kopin et al., 1968; Gewirtz and Kopin, 1970; Stjarne and Wennmalm, 1970; Thoa et al., 1971) and that newly taken up NE enters the readily releasable pool of the transmitter (see also Drakontides and Gershon, 1972). It may be that the peripheral concentration of label reflects the entry of newly taken up NE into the releasable pool of transmitter. If so, then it would be quite possible that the vesicles nearest the plasma membrane of axonal varicosities represent the location of the releasable transmitter pool.

These studies have thus provided some additional insight into adrenergic mechanisms in the heart. Exogenous NE can be taken up by cardiocytes and adrenergic axons. The distribution within cardiocytes suggests binding of the amine to thin filaments, a localization consistent with the hypothesis that NE binds to troponin. This uptake of NE, probably by the uptake, may thus be related to the inotropic action of the amine. In axons the labeled amine is preferentially distributed around the periphery of adrenergic varicosities. This distribution may reflect the entrance of labeled exogenous NE into the releasable pool of transmitter and suggests that peripheral vesicles may represent the location of this transmitter pool.

The authors are grateful to Mrs. Maria Demeri for technical assistance and to Mr. Alfred E. Revzin for electronic management.

This study was supported in part by United States Public Health Service grants NS 07436, HL 14713, and AM 12957. Dr. Michael D. Gershon is a Markle Foundation Scholar in Academic Medicine.

Received for publication 16 October 1973, and in revised form 27 March 1974.

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