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# *In vitro* studies on uptake, storage and disappearance of norepinephrine in spleen of white leghorn chicken

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**Abstract.** *In vitro* studies on uptake, storage and disappearance of norepinephrine were conducted on spleen slices of chicken. Uptake process was found to be time and concentration dependent. Maximum uptake of norepinephrine occurred at $10^{-4}$ M concentration after 45 min of incubation. Cocaine, metanephrine, phenoxybenzamine and a combination of cocaine and metanephrine, all inhibited the accumulation of norepinephrine, the last being most effective, indicating that both $uptake_1$ and $uptake_2$ processes are operative simultaneously and independently in disposition of amine. Disappearance studies following maximum accumulation indicated that the release was monophasic from a single pool. Reserpine pretreatment significantly reduced the accumulation and enhanced the release of norepinephrine.

**Keywords.** Norepinephrine; uptake; disappearance; chicken; spleen; cocaine; metanephrine; phenoxybenzamine; reserpine.

## 1. Introduction

The neurotransmitter of the sympathetic nerves in mammals is norepinephrine (NE) whereas it is epinephrine (E) in amphibians. In poultry, the fact is yet to be established. Several investigators have reported higher levels of NE than E in avian blood and other tissues of chicks (Von Euler 1963; Ignarro and Shideman 1968; Sturkie *et al* 1970). However, others have reported higher levels of E than NE in various tissues of chicken (Lin and Sturkie 1968; Kumar 1980; Uppal *et al* 1981; Rana 1984). It has also been proposed that in this species a mixture of both NE and E may be serving as neurotransmitter (De Santis *et al* 1975; Komori *et al* 1979). The mechanisms involved in the synthesis, storage, uptake and release of adrenergic neurotransmitter in various mammalian tissues have been studied extensively. But, in poultry such information is not adequately available. The present study was, therefore, undertaken for gaining more insight in the uptake, storage and disappearance of NE in spleen slices of chicken.

## 2. Materials and methods

Female white leghorn chicken (20–25 weeks old) weighing between 1·5–2 kg were used. The birds were acclimatized in the animal house of the department for 3–4 days before the start of experiment. Feed and water were provided *ad lib.*

The birds were sacrificed by decapitation, spleen was removed and kept in the ice-cold Kreb's Hauseleit physiological salt saline (PSS). Slices of uniform thickness and diameter, weighing between 100–150 mg, were prepared with perspex tissue hand microtome.

2.1 *Uptake of norepinephrine*

The spleen slices were preincubated for 15 min at 37°C in 10 ml of PSS containing iproniazid ($10^{-4}$ M) and tropolone ($10^{-4}$ M) as monoamineoxidase (MAO) and catechol-O-methyl transferase (COMT) inhibitors respectively. Thereafter, NE was added in 3 different concentrations ($10^{-6}$ M, $10^{-5}$ M or $10^{-4}$ M) in different tubes and incubated for varying intervals of 5, 10, 15, 30, 45 and 60 min. The slices treated with iproniazid and tropolone but not with NE were taken as controls. The NE content in all the tissue slices was determined fluorometrically by the method of Ansell and Beeson (1968) after extracting catecholamines in acid butanol as described by Sadavongvivad (1970) using internal standard and the net uptake of accumulated amine at various time intervals was calculated. Concentration curves were constructed by plotting the net uptake at different concentrations of amine against time. In the preliminary studies, the maximum accumulation of NE was observed at a concentration of $10^{-4}$ after 45 min of incubation. For all subsequent studies, the spleen slices were incubated with $10^{-4}$ M concentration of NE for 45 min.

2.2 *Disappearance of accumulated norepinephrine*

Following maximum accumulation, the slices were incubated in amine free medium for different time intervals of 5, 10, 15, 30, 45 and 60 min. The NE content in these slices was measured as described earlier. Disappearance curves were constructed by plotting the tissue amine concentration against time. Rate constant of disappearance ($K$) and half-life ($t_{\frac{1}{2}}$) of NE was calculated by regression analysis.

2.3 *Disappearance of accumulated NE from spleen slices in reserpine treated birds*

Birds were given reserpine at 5 mg/kg intramuscularly for two consecutive days. Twenty four h after the second injection, the birds were sacrificed and *in vitro* disappearance of accumulated NE in spleen slices was studied as described earlier.

2.4 *Effect of drugs on uptake of NE*

After preincubation with iproniazid ($10^{-4}$ M) and tropolone ($10^{-4}$ M), the tissue was exposed to cocaine, metanephrine, phenoxybenzamine or a combination of cocaine and metanephrine at a concentration of $10^{-4}$ M each. Thereafter, the uptake of NE was studied as described earlier.

## 3. Results and discussion

The accumulation of NE in spleen slices was found to be concentration and time dependent, maximum being 8·62 $\mu$g/g $\pm$ 0·52 (SE) at $10^{-4}$ M concentration of NE after 45 min of incubation (figure 1). The effect of various uptake blockers viz. cocaine, metanephrine, phenoxybenzamine and a combination of cocaine and metanephrine is shown in figure 2. Cocaine, a selective and competitive uptake$_1$ blocker (Furchgott *et al* 1963; Farmer and Petch 1963), caused significant inhibition

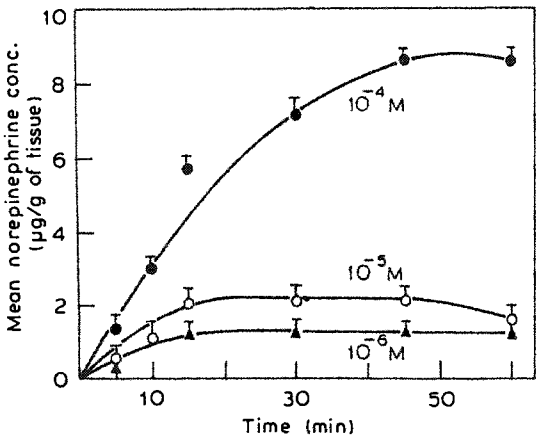


**Figure 1.** Accumulation of norepinephrine in spleen slices incubated with different concentrations of norepinephrine in the presence of tropolone and iproniazid ($10^{-4}$ M each). Vertical bars denote ±SE ($n=6$).

(59·83%) of NE uptake. Inhibition of uptake$_1$ by cocaine has been shown in chicken heart (Ignarro and Shideman 1968; Kumar 1980), liver (Agarwal *et al* 1987) and cat brain (Denglar *et al* 1961). Cocaine has also been reported to have no effect on uptake$_1$ process in rat uterus (Wurtman *et al* 1963) and chicken heart *in vivo* (Rana 1984).

Metanephrine, a potent uptake$_2$ blocker (Iversen 1965), exhibited 63·4% inhibition of accumulation of NE. Inhibition of uptake$_2$ process by metanephrine in other poultry tissues like heart (Kumar 1980; Rana 1984) and liver (Agarwal *et al* 1987) has also been reported.

The above results indicated that both uptake$_1$ and uptake$_2$ processes are almost equally involved in terminating the effects of exogeneous NE. The maximum inhibition of 79·19% was observed with the combination of cocaine and metanephrine further indicating that both uptake$_1$ and uptake$_2$ processes are operating simultaneously. Further, phenoxybenzamine which is known to inhibit both uptake$_1$ and uptake$_2$ processes nonspecifically (Axelrod *et al* 1962; Lightman and Iversen 1969) exhibited 66·67% inhibition of NE accumulation supporting the above results. Similar observations on chicken heart (Kumar 1980; Rana 1984) and liver (Agarwal *et al* 1987) are on record. The fact that the combination of cocaine and metanephrine or phenoxybenzamine could not cause complete inhibition of accumulation of NE indicates that some other process may also be involved in dissipating NE in chicken spleen.

In the spleen slices obtained from reserpine treated birds, a significantly lower accumulation (5·20 µg/g ± 0·37 SE) of NE was found as shown in figure 3. However, the disappearance of accumulated NE was linear with respect to time in normal as well as in reserpine treated birds (figure 3). The elimination was faster in spleen slices from reserpinised birds as compared to the normal birds. The disappearance rate constants were found to be $2{\cdot}80 \times 10^{-2}$ min$^{-1}$ and $3{\cdot}50 \times 10^{-2}$ min$^{-1}$ and the elimination half lives were 24·57 and 19·80 min in normal and reserpine treated birds, respectively. The significant reduction of catecholamines accumulation in reserpine pretreated chicken heart (Kumar 1980; Rana 1984) and liver (Agarwal *et al*

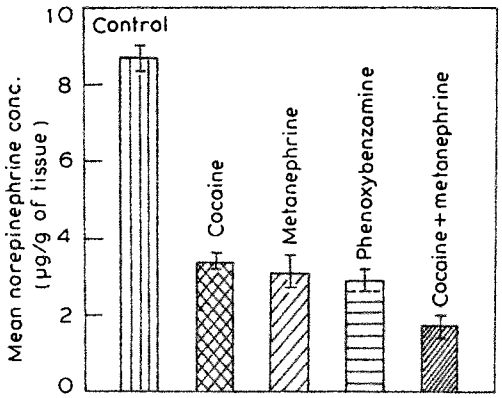


**Figure 2.** Accumulation of norepinephrine in spleen slices of 45 min incubation in the absence and presence of various uptake blockers ($10^{-4}$ M). Vertical bars denote ±SE ($n=6$).

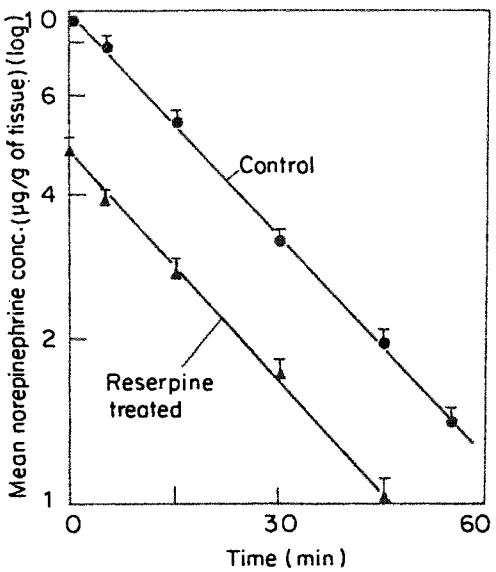


**Figure 3.** Disappearance of norepinephrine in control and reserpine treated chicken slices after maximum accumulation of norepinephrine. Vertical bars denote ±SE ($n=6$).

1987) has also been reported from our laboratory. Rana (1984) in the *in vivo* studies in chicken heart, reported a biphasic release of recemic NE. In a number of mammalian tissues, Montanari *et al* (1963) also reported similar type of biphasic release. Contrary to these, a monophasic disappearance linear with respect to time was observed in the present investigation. Similar monophasic linear release of NE has been reported in chicken heart (Kumar 1980) and liver (Agarwal *et al* 1987).

The present study is an attempt to fill the gap in our knowledge of how NE is handled by the sympathetic nerves in the chicken spleen. The results show some interesting variations in this species as compared to mammals. The differences

between spleen and other poultry tissues like heart and liver, are not very significant.

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