Br. J. Pharmac. (1969), **35**, 418–427.

An uptake of radioactivity from (±)-^{3}H-isoprenaline and its inhibition by drugs which potentiate the responses to (−)-isoprenaline in the guinea-pig isolated trachea

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1. The uptake of radioactivity derived from (±)-^{3}H-isoprenaline by the guinea-pig isolated trachea has been measured, and the assumption is made that all the radioactivity is due to isoprenaline.

2. 40% of the total (±)-^{3}H-isoprenaline taken up was loosely bound to the tissue while 25% was firmly bound.

3. The firmly bound component of the uptake was more susceptible to inhibition by drugs than the loosely bound component.

4. Desipramine and cocaine did not reduce the accumulation of firmly bound isoprenaline.

5. Cooling to 23° C, guanethidine and phentolamine caused a moderate reduction in the accumulation of firmly bound isoprenaline.

6. Phenoxybenzamine and (±)-metanephrine caused a highly significant reduction in the accumulation of firmly bound isoprenaline.

7. These findings are discussed in relation to previous studies of the uptake of isoprenaline and of other processes which may be related.

8. The inhibition of uptake by the agents examined correlated with their potentiation of the action of (−)-isoprenaline found previously.

9. It is suggested that a tissue uptake can significantly modify the pharmacological response to isoprenaline *in vitro*.

A correlation between the effects of six drugs and of cooling on the uptake of (±)-^{3}H-noradrenaline and on the responses to (−)-noradrenaline in the guinea-pig isolated trachea has been demonstrated (Foster, 1968). This is consistent with the general hypothesis that there is a causal relationship between inhibition of uptake and potentiation of noradrenaline *in vitro*.

In both the cat isolated atria (Schneider & Gillis, 1966) and the guinea-pig isolated tracheal chain (Foster, 1967) the response to isoprenaline was potentiated as much as that to noradrenaline by cooling. This raises the questions whether there is an uptake of isoprenaline in such isolated tissues and, if so, whether it is inhibited by the drugs which potentiate the effects of isoprenaline.

The uptake of radioactivity from (±)-^{3}H-isoprenaline has now been measured in the guinea-pig isolated trachea. This uptake consists of two components and the effects of six drugs and of cooling on the firmly bound component have been assessed. The results of this assessment have been correlated with the previously reported potentiation of the action of (−)-isoprenaline caused by these procedures.

Methods

The Krebs solution, drugs and guinea-pigs used were as described by Foster (1967).

The preparation of the trachea, its incubation, exposure to (±)-^{3}H-isoprenaline and drugs, washing and extraction were as described by Foster (1968) for (±)-^{3}H-noradrenaline.

Radioactive solutions

(±)-Isoprenaline-7-^{3}H (5.03 c/mmole in 0.1 N acetic acid) was obtained from New England Nuclear Corporation. It was reported to have radiochemical purity greater than 99%. It was diluted with distilled water to yield a stock solution of 25 μc/ml. in 0.0025 N acetic acid. This was further diluted with ascorbic acid solution, 2.3×10^{-2}M in distilled water, to produce a working stock such that 0.1 ml. added to 4 ml. of Krebs solution gave a final concentration of 189 nc/ml. and 37.56 pmole/ml. (±)-isoprenaline and 5.7×10^{-4} M ascorbic acid.

These stock solutions were stored at −10° C.

Radioassay

The phosphor used was a slight modification of that described by Bray (1960). It had the following composition: naphthalene, 100 g; 2,5-diphenyloxazole, 7 g; 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene, 0.3 g; methanol (absolute), 100 ml.; ethane-diol, 20 ml.; 1,4-dioxan to 1 l. The effect of the modification was to increase from 10 to 13% the mean tritium-counting efficiency of the system using 1 ml. aqueous samples.

To 10 ml. of this phosphor was added either 1 ml. of Krebs incubation medium or 1 ml. of a 0.4 N perchloric acid extract of trachea to which had been added sufficient 5 N NaOH to neutralize the acid. Samples were counted for 20 min or until 100,000 counts had accumulated (whichever was the shorter) using a liquid scintillation coincidence counter. Automatic external standardization was used to correct for quenching.

Expression of results

Although only radioactivity was actually measured, all results of uptake experiments are quoted in terms of (±)-^{3}H-isoprenaline. The apparent volume of distribution (ml./g) was derived by dividing radioactivity in tissue (pmole/g of blotted wet tissue) by radioactivity in medium (pmole/ml.).

Statistical methods

All measures of variation of the means quoted are standard errors. Student's *t* test was used to assess the significance of a difference between means while the

significance of a correlation was assessed non-parametrically by the Kendall rank correlation coefficient (Siegel, 1956).

Results

Separation of total tissue uptake of (±)-^{3}H-isoprenaline into components

Table 1 shows the steps which were taken to subdivide the total uptake of (±)-^{3}H-isoprenaline into fractions and the amounts of (±)-^{3}H-isoprenaline found in each fraction.

Dependence on time of total tissue content of (±)-^{3}H-isoprenaline

Figure 1 shows the time course of the total tissue accumulation of (±)-^{3}H-isoprenaline after exposure to 37.56 pmole/ml. Also shown is the (±)-^{3}H-isoprenaline associated with the cellular and structural elements of the trachea. In both cases the apparent isoprenaline space after 90 min significantly exceeds 1 ml./g ($P<0.02$).

The firmly bound (±)-^{3}H-isoprenaline is included for comparison. Of the total tissue content at 15 min, 35% lies extracellularly, 25% is firmly and 40% is loosely bound.

On which fraction should the effects of drugs be assessed?

It was possible that drugs might affect either the firmly or loosely bound components, or both. The results of a pilot experiment seeking to decide which fraction should be examined more closely are shown in Table 2.

This suggests that the fraction of the total uptake which is most affected by these drugs is the firmly bound and not the loosely bound fraction.

Firmly bound (±)-^{3}H-isoprenaline in tracheae exposed to drugs and to cooling

The conditions for inhibition of uptake were derived from the data on potentiation of the actions of (−)-isoprenaline (Foster, 1967). Each drug was used at the highest concentration used previously and after a contact time which would be expected to allow equilibrium conditions to develop. Several of the normal values were repeated during the course of the present study to confirm the validity of using previously obtained data.

TABLE 1. *Total tissue content of (±)-^{3}H-isoprenaline and its components*

	Content (pmole/g) Mean±S.E.	*N*	Comment
Total	43·56±1·15	5	Measured without washing
Extracellular	15·40±0·94	—	Extracellular fluid space of 0·41 ±0·025 ml./g (Foster, 1968) assumed to contain 37·56 pmole/ml.
Cellular and structural	28·16±1·49	—	By subtraction
Firmly bound	11·09±1·03	5	Measured after washing
Loosely bound	17·07±1·81	—	By subtraction

The total content was assayed after exposure of tracheae (*N*) to (±)-^{3}H-isoprenaline, 37·56 pmole/ml., for 15 min. The firmly bound component was measured after a similar exposure, followed by five washes of 10 ml. for 5 min each. This washing regime has been shown to remove 98·7±6·1% of ^{14}C-sorbitol (Foster, 1968).

Neither desipramine nor cocaine potentiated the action of isoprenaline, metanephrine and phenoxybenzamine (3.3×10^{-6}M) potentiated it strongly, while phentolamine, guanethidine and cooling to 23° C caused a moderate potentiation. Phenoxybenzamine, at a concentration of 6.6×10^{-7}M, caused about half-maximal potentiation of the action of isoprenaline.

Table 3 presents the results of measurements of the firmly bound (±)-^{3}H-isoprenaline made in these conditions. Neither cocaine nor desipramine inhibited the accumulation of firmly bound (±)-^{3}H-isoprenaline, phenoxybenzamine (higher concentration) and metanephrine inhibited it strongly ($P=0.005$), while cooling to 23° C, phenoxybenzamine (lower concentration), guanethidine and phentolamine caused a moderate inhibition. Only with the last two drugs, however, did this inhibition achieve statistical significance ($P<0.05$).

One possible criticism of this experimental design is that the use of a variable time of exposure may introduce a bias if the activity of the uptake process alters

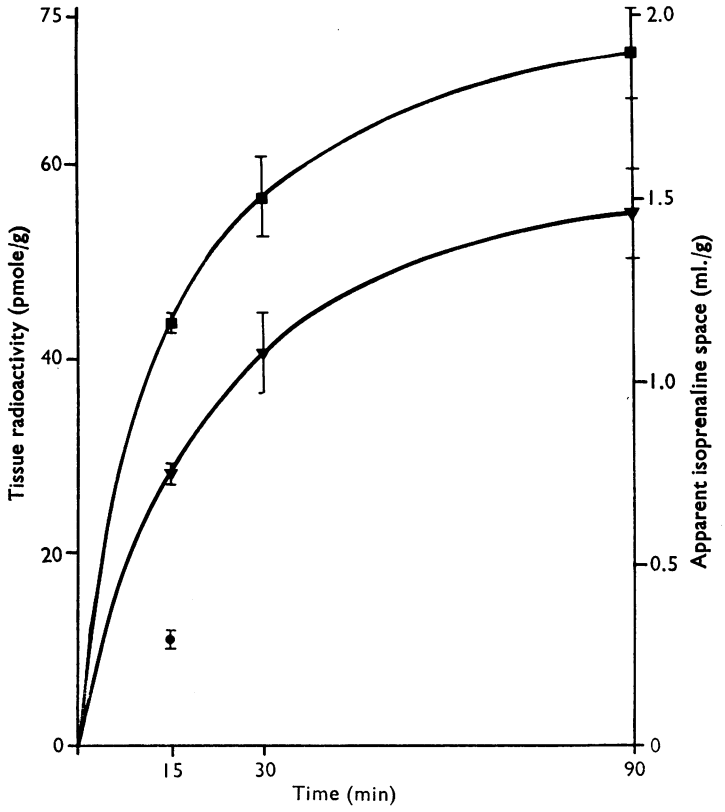

FIG. 1. Time course of tracheal accumulation of (±)-^{3}H-isoprenaline after exposure to 37.56 pmole/ml. ■——■, Total tissue content. ▼——▼, Radioactivity associated with the cellular and structural elements of the tissue, derived from total tissue content by subtracting the radioactivity in the extracellular fluid space. ●, Firmly bound radioactivity, determined by 15 min exposure to 37.56 pmole/ml. followed by five 10 ml. washes at 5 min intervals. Means and standard errors are plotted. Groups of five tracheae were used.

TABLE 2. *Comparison between effects of drugs on total (±)-^{3}H-isoprenaline uptake, and on the firmly and loosely bound fractions*

	Conc. (M)	Total content Mean±S.E. (pmole/ml.)	N	2P	Firmly bound Mean±S.E. (pmole/ml.)	N	2P	Loosely bound Mean±S.E. (pmole/ml.)	N	2P
Control	—	34·57±1·61	3	—	7·36±0·64	6	—	11·81±1·97	3	—
Desipramine	$1·2\times10^{-5}$	35·34±0·47	3	0·7	7·50±0·31	5	0·85	12·43±1·10	3	0·8
Guanethidine	$1·0\times10^{-5}$	33·15±0·75	3	0·5	6·39±0·37	6	0·2	11·35±1·26	3	0·9
Phenoxybenzamine	$3·3\times10^{-6}$	32·89±1·40	3	0·5	5·84±0·27	5	0·05	11·65±1·71	3	0·95
Metanephrine	$8·1\times10^{-5}$	33·20±2·19	3	0·6	5·33±0·20	6	0·01	12·47±2·39	3	0·8

Tracheae were exposed to the drugs stated for 240 min and for the last 15 min of this to (±)-^{3}H-isoprenaline 37·56 pmole/ml. They were removed for assay of total content or washed for 25 min and removed for assay of firmly bound radioactivity. The loosely bound radioactivity was derived as in Table 1. *N*, number of tracheae in each group; 2*P*, probability, on Student's *t* test (two-tailed), that a mean is different from the control mean.

with time. The whole assessment of drug activity was therefore repeated using the same conditions as in Series 1, except that a standard time of exposure of 240 min was used. The results of this second series of experiments are presented in Table 4. Again, neither desipramine nor cocaine inhibited the accumulation of firmly bound (±)-^{3}H-isoprenaline, metanephrine inhibited it strongly ($P=0.01$), while cooling to 23° C, guanethidine, phentolamine and both concentrations of phenoxybenzamine caused a moderate inhibition. In the last three cases this achieved statistical significance.

The two series of mean uptake results were compared using the Kendall rank correlation coefficient (Siegel, 1956): the probability was $P=0.0029$ that the correlation between them arose by chance.

Correlation between inhibition of accumulation of firmly bound (±)-^{3}H-isoprenaline and potentiation of the effect of (−)-isoprenaline

Figure 2 shows the mean firmly bound (±)-^{3}H-isoprenaline plotted against the mean potentiation of the effect of (−)-isoprenaline for all the different treatments used and for both series of experiments. The best fitting straight lines are included. The significance of the correlation was examined using the Kendall rank correlation coefficient (Siegel, 1956). In both series the correlation was significant ($P=0.038$ for Series 1; $P=0.0063$ for Series 2). One may therefore conclude that the two variables—inhibition of accumulation of firmly bound (±)-^{3}H-isoprenaline and

TABLE 3. *Effects of drugs and cooling an accumulation of firmly bound (±)-^{3}H-isoprenaline (measured as pmole/g)*

Drug	Conc. (M)	Time (min)	*N*	Mass mg±S.E.		Firmly bound radioactivity pmole/g±S.E.		2*P*
Control	—	80	5	117	8	11·09	1·03	—
Desipramine	$1·2\times10^{-5}$	160	8	145	11	9·66	0·66	0·25
Cocaine	$1·3\times10^{-4}$	80	6	123	11	11·26	1·26	0·99
23° C	—	80	6	150	6	8·77	0·52	0·08
Guanethidine	$1·0\times10^{-5}$	200	7	137	13	7·49	1·05	0·04
Phentolamine	$7·1\times10^{-5}$	160	6	162	25	7·45	0·36	0·01
Phenoxybenzamine	$6·6\times10^{-7}$	240	5	206	25	8·62	0·92	0·1
Phenoxybenzamine	$3·3\times10^{-6}$	240	7	163	11	7·35	0·36	0·005
Metanephrine	$8·1\times10^{-5}$	120	6	130	7	6·96	0·42	0·005

Trachea were exposed to the drugs or conditions stated and for the last 15 min of this exposure to (±)-^{3}H-isoprenaline 37·56 pmole/ml. They were then washed for 25 min and removed for assay. *N*, Number of tracheae in each group; Mass, blotted wet weight of trachea, 2*P*, probability, on Student's *t* test (two-tailed), that a mean is different from the control mean.

TABLE 4. *Effects of drugs and cooling using standard time of exposure*

Drug	Conc. (M)	*N*	Mass mg±S.E.		Firmly bound radioactivity pmole/g ±S.E.		2*P*
Control	—	6	184	23	7·36	0·64	—
Desipramine	$1·2\times10^{-5}$	5	189	29	7·50	0·31	0·85
Cocaine	$1·3\times10^{-4}$	5	167	8	6·84	0·49	0·55
23° C	—	6	223	9	6·44	0·66	0·3
Guanethidine	$1·0\times10^{-5}$	6	193	21	6·39	0·37	0·2
Phentolamine	$7·1\times10^{-5}$	8	207	21	5·94	0·29	0·05
Phenoxybenzamine	$6·6\times10^{-7}$	5	211	22	5·88	0·26	0·05
Phenoxybenzamine	$3·3\times10^{-6}$	5	167	8	5·84	0·27	0·05
Metanephrine	$8·1\times10^{-5}$	6	184	18	5·33	0·20	0·01

As Table 3, but all tracheae exposed to the drugs or conditions stated for 240 min. Some of these data have already appeared in Table 1.

potentiation of the effect of (−)-isoprenaline—are associated in the population from which these sample means were drawn.

Discussion

Accumulation of isoprenaline by the guinea-pig isolated trachea has been demonstrated, if it is assumed that all the radioactivity found is due to isoprenaline. A 90 min exposure to (±)-^{3}H-isoprenaline resulted in an apparent volume of distribution of 1.9 ml./g. Even after subtraction of the isoprenaline calculated to lie in the extracellular fluid space the apparent volume of distribution still significantly exceeded 1 ml./g.

The quantitative features of the tissue distribution of radioactivity found in the present study are compared with the results found after exposure to (±)-^{3}H-noradrenaline (Foster, 1968) in Table 5. The general features of the isoprenaline

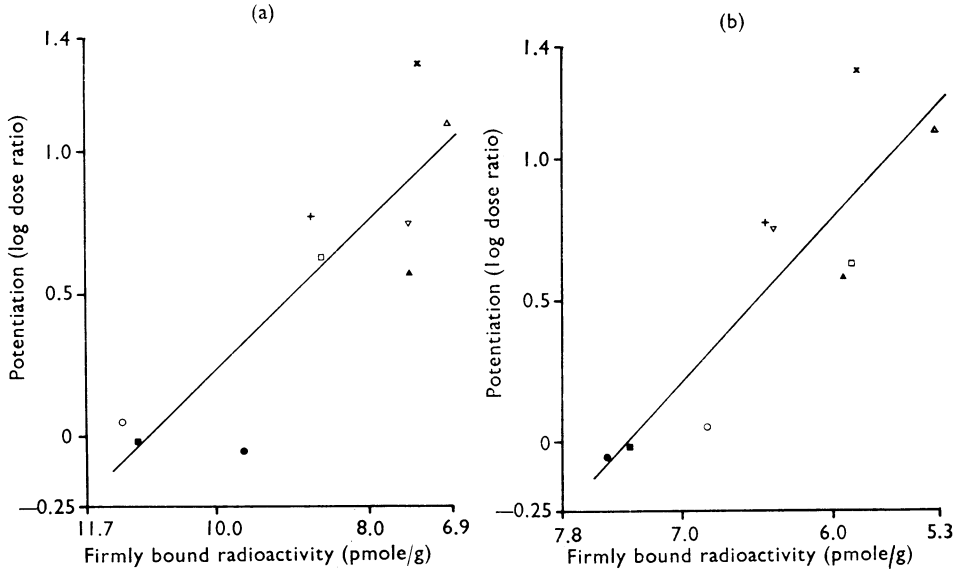

FIG. 2. Correlation between mean potentiation of the action of (−)-isoprenaline (measured as log. dose ratio, Foster, 1967) and mean accumulation of firmly bound (±)-^{3}H-isoprenaline (measured as pmole/g) for (a) Series 1 (Table 3) and (b) Series 2 (Table 4) of uptake experiments. Each point is the mean of at least five experiments. The best-fitting straight lines are included. ■, Control; ○, cocaine, 1.3×10^{-4}M; ●, desipramine, 1.2×10^{-5}M; +, cooling to 23° C; □, phenoxybenzamine, 6.6×10^{-7}M; ×, phenoxybenzamine, 3.3×10^{-6}M; ▽ guanethidine, 1.0×10^{-5}M; ▲, phentolamine, 7.1×10^{-5}M; △, metanephrine, 8.1×10^{-5}M.

TABLE 5.

	% of total radioactivity		Amount of isoprenaline as % of amount of noradrenaline
	Noradrenaline	Isoprenaline	
Extracellular fluid space	16	35	80
Total uptake	100	100	36
Loosely bound	25	40	56
Firmly bound	59	25	15

uptake described here—rather low total uptake, with relatively less in the firmly and relatively more in the loosely bound fractions, compared with noradrenaline—may be compared with previous studies of isoprenaline uptake.

Anden, Corrodi, Ettles, Gustafsson & Persson (1964) found that phenoxybenzamine did not potentiate the action of isoprenaline on the rabbit isolated heart and that no significant accumulation of isoprenaline (assayed by differential spectrophotofluorimetry) could be detected in the heart after perfusion for 60 min with the catecholamine followed by 5 min of washout. Phenoxybenzamine also failed to potentiate the action of isoprenaline on the rabbit isolated left ventricular papillary muscle preparation. Hertting (1964) administered (±)-^{3}H-isoprenaline or an equal amount of (±)-^{3}H-noradrenaline to rats intravenously and either 10 min or 2 hr later assayed the blood, heart, spleen and lungs. For the three latter tissues the ratios of unchanged isoprenaline in 1 g of tissue to that in blood at 10 min were 6.8, 4.9 and 7.0, respectively. The similar ratios for unchanged noradrenaline were 29.4, 1.9 and 2.0. By 2 hr the noradrenaline content of most tissues had halved, whereas their isoprenaline content was 90% lower than at 10 min. He postulated that isoprenaline entered the noradrenaline-binding sites but was incapable of being bound there.

Ross & Renyi (1966) found that the apparent volume of distribution of (±)-^{3}H-isoprenaline in mouse brain slices was 1.0 ml./g; it was not reduced by ouabain (20 μg/ml.) but was significantly increased to 1.2 ml./g by pretreating the animals with pheniprazine. Callingham & Burgen (1966) showed that the rat isolated perfused heart accumulated (±)-^{3}H-isoprenaline to an apparent volume of distribution of 1–3 ml./g, over the range 1.2×10^{-7} to 9.5×10^{-5}M. Washing caused a rapid loss of much of the accumulated isoprenaline, but even after 60 min washing 7% remained.

These results on the uptake of isoprenaline are not inconsistent with the present findings that isoprenaline is accumulated but to a smaller extent than is noradrenaline and that much of that which is accumulated is rather readily washed out of tissues.

The uptake found in the trachea could be regarded as comprising two fractions, loosely and firmly bound, so it was necessary to discover which fraction might be susceptible to drug-induced inhibition. Some pilot studies using desipramine, guanethidine, phenoxybenzamine and (±)-metanephrine compared their effects on the total (±)^{3}H-isoprenaline uptake and on the firmly and loosely bound fractions. It was clear that the percentage inhibition of uptake with phenoxybenzamine and metanephrine was much larger (and significant) when measured on the firmly bound component than on the total uptake or loosely bound components (when it was not significant). Consequently it was decided to use the firmly bound fraction to assess the effects of drugs which potentiate the actions of catecholamines on isoprenaline uptake.

Cocaine and desipramine did not potentiate the action of (−)-isoprenaline on the guinea-pig isolated tracheal chain (Foster, 1967) and caused no inhibition of the accumulation of firmly bound (±)-^{3}H-isoprenaline. Callingham & Burgen (1966) found no inhibition by cocaine of the uptake of isoprenaline which they examined; they related this process to the Uptake$_2$ described by Iversen (1965) for noradrenaline and adrenaline and shown by him to be insensitive to both cocaine and desipramine.

In sharp contrast to cocaine and desipramine is the effect of metanephrine; this drug caused marked potentiation of the action of (−)-isoprenaline on the tracheal chain and caused a highly significant inhibition of the uptake of (±)-^{3}H-isoprenaline. Callingham & Burgen (1966) found that normetanephrine (10^{-5}M) caused a 49% inhibition of the uptake of isoprenaline from a medium containing 1.2×10^{-7}M and Iversen (1965) and Burgen & Iversen (1965) found (±)-metanephrine to be the most potent inhibitor of Uptake$_2$ which they studied.

Phenoxybenzamine is another agent which has been shown to potentiate the action of isoprenaline and strongly inhibit its uptake in the trachea and also inhibit the Uptake$_2$ mechanism in the rat isolated heart (Iversen, 1965). In spite of the marked differences in conditions between the present study and that of Callingham & Burgen (1966) there may be an underlying similarity in the processes of isoprenaline accumulation under investigation which gives rise to this similarity in sensitivity to inhibitors. There may also be relationships with two other recent studies. Eisenfeld, Axelrod & Krakoff (1967) found that the rat isolated perfused heart exposed to cocaine (10^{-4}M) took up about 5% as much (±)-^{3}H-noradrenaline as did the control heart. Inclusion of metaraminol in the perfusate reduced the amount of unchanged noradrenaline found in the heart but not the amounts of metabolites. Phenoxybenzamine and phentolamine, on the other hand, reduced the amounts of both noradrenaline and metabolites. Simmonds & Gillis (1968) examined this process further and found that the heart accumulated (±)-^{3}H-normetanephrine as well as noradrenaline and that most of this (90%) was removed by washing for 10 min. They found this uptake to be insensitive to cocaine and suggested its equivalence to Uptake$_2$.

The other procedures examined in the present study, cooling to 23° C, guanethidine and phentolamine each caused a moderate potentiation of the action of (−)-isoprenaline (Foster, 1967) and a moderate inhibition of the accumulation of firmly bound isoprenaline.

For the nine different treatments examined in Series 1 there was a significant ($P=0.038$) correlation between the amounts of potentiation and inhibition of uptake of isoprenaline observed. This significance loses some of its force, however, when one realizes that (a) the time of exposure was variable and may therefore introduce a distortion, and (b) means have been ranked (treated as different) even though the difference between them was not statistically significant. In order to answer these criticisms the experiments were repeated using a contact time for all drugs equal to the longest used in Series 1. In this second series there was still a significant ($P=0.0063$) correlation between the amounts of potentiation and inhibition of uptake observed, and the two series of uptake results correlated with a probability $P=0.0029$. Thus it is unlikely that the ranks assigned to the means are seriously in error.

Callingham & Burgen (1966) had to "leave unanswered the question whether the tissue uptake by Uptake$_2$ significantly modifies pharmacological responses to isoprenaline." The concentration of (±)-^{3}H-isoprenaline used in the present study lies on the lower part of the log. concentration : effect curve of isoprenaline in the guinea-pig isolated tracheal chain (it represents approximately an ED20; Foster, 1966). It may be that the demonstration of a correlation between the inhibition of uptake from such a concentration of isoprenaline and the potentiation of the response to isoprenaline answers this question.

I am grateful to the Chest and Heart Association for an award in support of this work, to Geigy (U.K.) Ltd. for desipramine and to CIBA Laboratories Ltd. for guanethidine.

REFERENCES

ANDEN, N.-E., CORRODI, H., ETTLES, M., GUSTAFSSON, E. & PERSSON, H. (1964). Selective uptake of some catecholamines by the isolated heart and its inhibition by cocaine and phenoxybenzamine. *Acta pharmac. tox.*, **21**, 247–259.

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.*, **1**, 279–285.

BURGEN, A. S. V. & IVERSEN, L. L. (1965). The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br. J. Pharmac. Chemother.*, **25**. 34–39.

CALLINGHAM, B. A. & BURGEN, A. S. V. (1966). The uptake of isoprenaline and noradrenaline by the perfused rat heart. *Mol. Pharmac.*, **2**, 37–42.

EISENFELD, A. J., AXELROD, J. & KRAKOFF, L. (1967). Inhibition of the extraneuronal accumulation and metabolism of norepinephrine by adrenergic blocking agents. *J. Pharmac. exp. Ther.*, **156**, 107–113.

FOSTER, R. W. (1966). The nature of the adrenergic receptors of the trachea of the guinea-pig. *J. Pharm. Pharmac.*, **18**, 1–12.

FOSTER, R. W. (1967). The potentiation of the responses to noradrenaline and isoprenaline of the guinea-pig isolated tracheal chain preparation by desipramine, cocaine, phentolamine, phenoxybenzamine, guanethidine, metanephrine and cooling. *Br. J. Pharmac. Chemother.*, **31**, 466–482.

FOSTER, R. W. (1968). A correlation between inhibition of the uptake of ^{3}H from (±)-^{3}H-noradrenaline and potentiation of the responses to (−)-noradrenaline in the guinea-pig isolated trachea. *Br. J. Pharmac. Chemother.*, **33**, 357–367.

HERTTING, G. (1964). The fate of ^{3}H-iso-proterenol in the rat. *Biochem. Pharmac.*, **13**, 1119–1128.

IVERSEN, L. L. (1965). The uptake of catecholamines at high perfusion concentrations in the rat isolated heart; a novel catecholamine uptake process. *Br. J. Pharmac. Chemother.*, **25**, 18–33.

ROSS, S. B. & RENYI, A. L. (1966). Uptake of some tritiated sympathomimetic amines by mouse brain cortex slices *in vitro*. *Acta pharmac. tox.*, **24**, 297–309.

SCHNEIDER, F. H. & GILLIS, C. N. (1966). Hypothermic potentiation of chronotropic responses of isolated atria to sympathetic nerve stimulation. *Am. J. Physiol.*, **211**, 890–896.

SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioural Sciences.* New York: McGraw-Hill.

SIMMONDS, M. A. & GILLIS, C. N. (1968). Uptake of normetanephrine and norepinephrine by cocaine-treated rat heart. *J. Pharmac. exp. Ther.*, **159**, 283–289.

(*Received July* 19, 1968)