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THE TRANSMITTER RELEASED BY STIMULATION OF THE BRONCHIAL SYMPATHETIC NERVES OF CATS

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A study has been made of the transmitter released in a cat heart-lung preparation when the sympathetic chains were stimulated. The nervi accelerantes were always sectioned before stimulation. The transmitter appeared first in the pulmonary venous blood. In its actions on the heart-lung preparation, it resembled isoprenaline and not adrenaline. Chromatographic studies using three different solvents showed that 80 to 100% of this transmitter consisted of a catechol amine which had R_F values which were identical with those of isoprenaline. Pharmacological studies failed to distinguish between the actions of this amine and those of isoprenaline, but clearly differentiated between those of the pulmonary amine, adrenaline, and noradrenaline.

The discovery of trace amounts of a third sympathomimetic amine in saline extracts of the adrenal glands of cats, monkeys, and man has been reported by Lockett (1954). This amine could not be differentiated from isoprenaline (*N*-isopropylnoradrenaline) by its colour reactions, by its chromatographic behaviour, or by its pharmacological activity; it could, however, readily be distinguished from adrenaline and from noradrenaline both in R_F values and by pharmacological means.

The possible physiological significance of this trace amine required study. The chemical transmitter of the postganglionic sympathetic nerve fibres supplying bronchi was examined because isoprenaline (Konzett, 1940a) and the third amine of the adrenal gland (Lockett, 1954) had proved more active than adrenaline as dilators of previously constricted bronchioles. Cats were selected for this investigation because the third amine of the adrenals was known to occur in this species (Lockett, 1954).

METHODS

The experiments recorded involved 123 cats. Anaesthesia was induced with chloroform and ether (1:4 parts v/v) and was maintained with chloralose, 8 ml./kg. of a 1% w/v solution in 0.9% w/v NaCl in water, given by femoral venous cannula. The anticoagulant used was heparin (Liquemin, Roche), 500 units/kg. body weight. Donor blood, containing very little adrenaline and noradrenaline, was obtained as follows: anaesthetized cats were made spinal, the adrenals were excluded from the circulation, and the sympathetic chains were removed from just above the stellate ganglia to the brim of the pelvis. After an interval of 20 min. the cats were bled from a carotid arterial cannula.

Heart-lung preparations were made in adrenalectomized spinal cats by a technique essentially similar to that used by Knowlton and Starling (1912). Systemic outflow was recorded from a simple syphon placed between the peripheral resistance and the reservoirs. The circuit was arranged to allow the return of blood from the syphon recorded to either of two reservoirs. These reservoirs were small double surface condensers, each of 60 ml. maximum capacity. Blood from either reservoir was returned to the heart through a 4 in. Liebig condenser which adjoined the cannula in the superior vena cava. The reservoirs and the Liebig condenser were warmed with water which was circulated by a Stewart Turner aquarium pump from an electrically heated, thermostatically controlled water bath. Air pressure in the Starling type of peripheral resistance and brachiocephalic mean arterial pressure were recorded from mercury manometers in the usual way. Right atrial pressures and venous filling pressures were recorded from mercury manometers, the floats of which moved frontal writing levers; the latter magnified the movements of the mercury surfaces 8 to 13 times. Change in heart size was recorded by a cardiometer connected with a bellows recorder. Oxygen consumption was measured by means of a float recorder which formed part of an oxygen-filled closed circuit. A Starling pump supplied the preparation with oxygen drawn from this float, and returned the expired gas to it through a cannister filled with soda lime. Changes in bronchiolar resistance were inferred from records of tidal air made by Konzett's method (1940a).

Electrical Stimulation of the Sympathetic Chains.—Loose ligatures were first placed round the great

vessels ready for the establishment of the heart-lung preparation. Next, the nervi accelerantes were divided and the azygos vein and subclavian arteries were tied. Rapid incisions were then made through the sternum and intercostal spaces such that shielded Palmer electrodes could lie horizontally below the level of the great vessels. These electrodes were placed between the ganglia and round the sympathetic chains from just below the stellate ganglia to the neck of the 8th rib on each side. The wiring was so arranged that current should pass through each ganglion from one electrode to the next. Rectangular stimuli of 0.5 to 1 msec. duration were delivered at a rate of 12 to 16/sec. from a 5 to 10 v. source through all the electrodes on both sides simultaneously.

Electrical Stimulation of the Heart.—Hearts were driven at constant high rate by means of rectangular stimuli of 2 msec. duration delivered through small light shielded electrodes made of platinum wire mounted in perspex. These were screwed gently on to the edge of the right auricular appendage.

Concentrated Protein-free Extracts of Plasma.—These were prepared for chromatography by a method essentially similar to that described by Vogt (1952). Two modifications of this method were found advisable in adapting the method for use with plasma volumes which varied from 35 to 110 ml. First, the residues obtained after evaporation to dryness *in vacuo* were dissolved in known volumes of solvent, and the process was continued in each case with as large a measured volume of the solution as was practicable. Secondly, it was found necessary to repeat the extraction of the residues with ethanol saturated with sodium chloride.

Chromatography and Elution.—Concentrated acetone-alcohol extracts of plasma were quantitatively transferred to acid-washed Whatman No. 1 filter paper. The area of the paper to be traversed by plasma samples destined to be tested biologically was sprayed with aqueous ascorbic acid 50 mg./100 ml. In some experiments phenol containing 15% w/v 0.1N-HCl was used as solvent. In others the solvent was butanol saturated either with 0.5N-HCl (Crawford, 1951) or with 16.7% v/v aqueous acetic acid (Shepherd and West, 1953). Ascending chromatograms were made in an atmosphere of CO_2. Solvents were removed by washing with benzene, and the whole process, including drying, was carried out at room temperature.

Adrenaline, noradrenaline, and isoprenaline were used as reference compounds. 10 μg. of each amine was added to 20 ml. of control plasma, from which a protein-free concentrated extract of plasma was prepared. The alcohol-acetone extract of this plasma was added to a part of the paper washed with acid, but unsprayed with ascorbic. This practice was adopted because the R_F values given by these reference compounds when applied to the paper in plasma extract were usually slightly greater than the R_F values determined simultaneously for these amines applied to the paper in aqueous or alcohol-acetone solution (see Table I).

Chromatograms of one plasma sample for elution and of mixed reference compounds in plasma were made in parallel on one sheet of paper. The solvent front was allowed to advance beyond the line of application by not less than 16 cm. (phenol) or 22 cm. (butanol) to ensure good separation of the amines. The reference chromatogram was developed by spraying with 0.44 g. potassium ferricyanide dissolved in 100 ml. 0.2 M-phosphate buffer at *p*H 8.0 after drying. The methods used for elution and for the preparation of solutions for biological assay were described by Vogt (1952).

Drugs.—(−)-Adrenaline tartrate (Burroughs Wellcome), (−)-noradrenaline and (±)-isoprenaline (Sterling Winthrop) were obtained commercially.

RESULTS

The Effect of Bilateral Stimulation of the Sympathetic Chains after Division of the Nervi Accelerantes in the Cat Heart-Lung Preparation.—Electrical stimulation of those ganglia of the sympathetic chains which lie below the stellate ganglia and above the upper border of the body of the ninth dorsal vertebra was followed by an increase in the tidal air of the cat heart-lung preparation. This evidence of a decrease in bronchiolar resistance appeared 15 to 20 sec. after the onset of stimulation, and was only rarely accompanied by any significant change in the performance of the heart before blood, which had passed through the preparation during stimulation of the chains, had begun to re-enter the heart from the venous reservoir (Fig. 1). In occasional

TABLE I

R_F VALUES OF REFERENCE AMINES COMPARED AFTER EXTRACTION FROM PLASMA AND EXTRACTION FROM WATER IN THREE DIFFERENT SOLVENTS

Amine	R_F Values*					
	Phenol—0·1N-HCl		n-Butanol—0·5N-HCl		n-Butanol—16·7% Acetic v/v	
	Water	Plasma	Water	Plasma	Water	Plasma
Noradrenaline	0·20±0·02 (5)	0·21±0·03 (5)	0·11±0·08 (4)	0·12±0·07 (4)	0·25±0·08 (4)	0·26±0·07 (4)
Adrenaline	0·49±0·04 (5)	0·52±0·05 (5)	0·16±0·05 (4)	0·18±0·03 (4)	0·32±0·09 (4)	0·36±0·10 (4)
Isoprenaline	0·67±0·05 (5)	0·71±0·08 (5)	0·34±0·06 (4)	0·39±0·09 (4)	0·57±0·12 (4)	0·63±0·08 (4)

* Mean R_F values are given with standard error. The number of experiments shown in brackets.

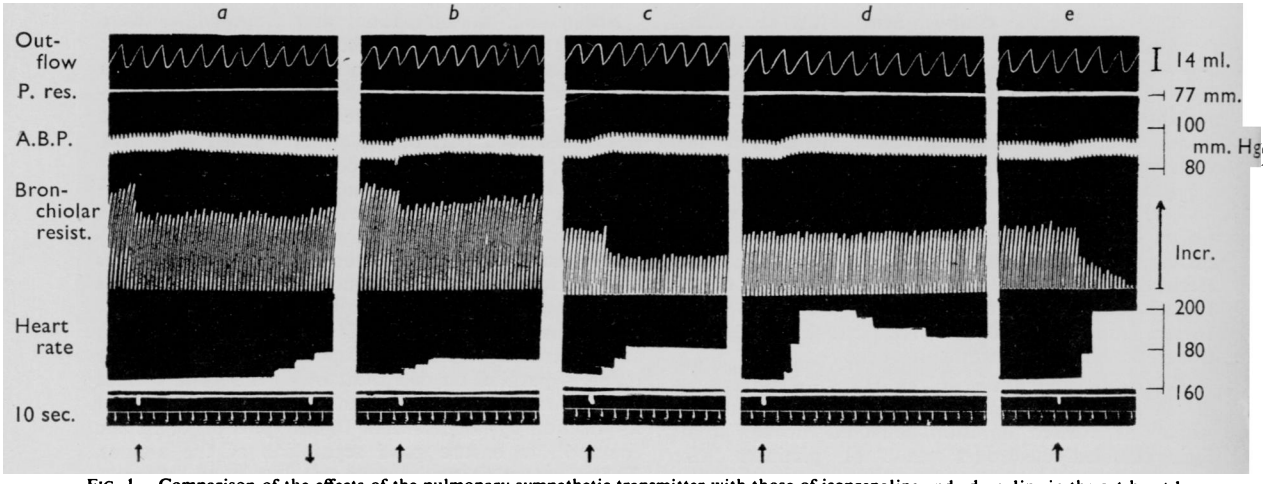

FIG. 1.—Comparison of the effects of the pulmonary sympathetic transmitter with those of isoprenaline and adrenaline in the cat heart-lung preparation. Records show, from above downwards, systemic outflow, peripheral resistance, mean brachiocephalic pressure (A.B.P.), bronchiolar resistance, heart rate, signal. Temp. 38° C. *a*, Effect of bilateral stimulation (between the arrows) of the sympathetic chains from which the nervi accelerantes have been divided. Blood was allowed to recirculate. Between *a* and *b*, this stimulation was repeated; blood passing through the preparation during this stimulation was collected in a separate reservoir. *b* shows the change (at arrow) from normal blood to blood collected during stimulation. *c*, 2 μg. (±)-isoprenaline. *d*, 4 μg. (−)-adrenaline. *e*, 3 μg. (±)-isoprenaline, each added to the venous reservoir at arrows.

preparations, however, mild tachycardia, associated with some decrease in heart size and a slight fall in right atrial pressure, was seen 30 sec. after the beginning of stimulation. Such preparations were discarded for the study of the predominant transmitter released by stimulation of the chains, because they were not typical. Their incidence may perhaps be related to the observation of Cannon, Lewis, and Button (1926) that bilateral removal of the stellate ganglia is insufficient, at least in some cats, to cause complete sympathetic denervation of the heart.

The Site at which Transmitter was Released in the Cat's Heart-Lung Preparation in Response to Stimulation of the Chains.—A series of experiments was carried out in which simultaneous serial blood samples were withdrawn from the brachiocephalic arterial cannula, from a pulmonary vein, and from the coronary sinus during stimulation of the chains. The brachiocephalic sample was collected in a syringe which was allowed slowly and continuously to fill with blood through a needle inserted through the rubber just beyond the end of this cannula. The other two samples collected were outflows from polythene cannulae. One of these had been inserted into a left upper lobe vein pointing toward the lung. The other cannula had been passed through the right auricular appendage, and its tip extended for about 1 cm. within the coronary sinus. Plasma was separated from each blood sample without delay and was tested for adrenaline-like activity on the rat uterus by the method of Gaddum, Peart, and Vogt (1949).

The results of these experiments are summarized in Table II, Expts. 1, 2, and 3. Cols. 5, 6, and 7 in Table II show the concentrations of (±)-isoprenaline in μg./ml. which were equally effective with the plasma samples in inhibiting the responses of the rat uterus to acetylcholine. The periods during which these plasma samples were collected are found in col. 2 measured in sec. from the start of stimulation. It can be seen that transmitter appeared in the pulmonary venous blood some 15 to 20 sec. after the beginning of chain stimulation (Table II, Expts. 1, 2, 3, and 6). Only after a lag period of 6 or more sec. did a measurable concentration of transmitter appear in the brachiocephalic sample. The cause of this delay is indicated by reference to cols. 3 and 4 (Table II). Col. 3 shows that the systemic output of these hearts was low; it had been deliberately reduced in order that the concentrations of transmitter in the plasma samples should be great enough for measurement. Col. 4 gives minimum estimated values for the dead space between the pulmonary veins and the point of collection beyond the brachiocephalic cannula. Mixing of pulmonary venous blood with the contents of this dead space may have delayed the arrival of measurable amounts of amine in the brachiocephalic sample. This hypothesis was tested by making one altera-

TABLE II

CONCENTRATIONS OF SYMPATHETIC TRANSMITTER IN PLASMA, DURING STIMULATION OF THE THORACIC SYMPATHETIC CHAINS, ASSAYED ON THE RAT UTERUS IN TERMS OF μG. (±)-ISOPRENALINE/ML. PLASMA

Expt. No.	Sampling Period. Sec. from Onset of Stimulation	Systemic Output ml./min.	Minimum Estimate of Innominate Dead-space (ml.)	Concentration of Transmitter Expressed as μg. (±)-Isoprenaline/ml. Plasma		
				Pulm. Vein	Innom. Art	Coronary Sinus
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	0–13	33	4·5	Trace	Nil	Nil
	13–24	33		0·08	Trace	,,
2	0–15	40	4·0	Trace	,,	Trace
	15–25			0·07	0·05	0·03
3	0–12	38	5·5	Nil	Nil	Nil
	12–20			0·065	,,	,,
4	120–160	46	—	0·125	0·110	0·085
5	20–40	54	3·0	0·08	0·05	0·03
	120–150			0·13	0·12	0·08
6	10–15	36	2·0	0·04	0·03	Nil
	15–20			0·07	0·06	Trace

tion in technique. The brachiocephalic sample was collected through a fine polythene cannula which passed through the tip of the brachiocephalic cannula (Table II, Expt. 5) and on into the aorta to open a little above the aortic valves (Table II, Expt. 6). The results confirm the hypothesis. Reduction in the dead space between the pulmonary veins and the point of brachiocephalic sampling greatly reduced the delay in the appearance of transmitter in the latter sample.

Whereas, after 2 min. of chain stimulation, the concentration of transmitter in the brachiocephalic sample did not differ significantly from that in the pulmonary venous sample, that in the coronary sinus sample was lower. This fact, coupled with the slow onset of tachycardia during stimulation of the chains (Fig. 1a), indicated partial destruction of the transmitter during circulation through the heart.

The results from four other experiments have been excluded from Table II because unidentified stimulant activity in the plasma, especially in that from coronary sinus blood, made the estimation of sympathomimetic activity in untreated plasma impossible.

The experiments described above proved that, in cats, stimulation of the sympathetic chains caused the liberation of an adrenaline-like substance into the pulmonary circulation. That this substance originated from postganglionic nerve fibres was established by the use of choline 2:6-xylyl ether bromide (TM10) provided by Professor W. A. Bain for this purpose. TM10 has been shown selectively to prevent the liberation of transmitter from postganglionic adrenergic nerve fibres (Exley, 1956; Bain and Fielden, 1956). Since the action of TM10 takes 30 to 35 min. to develop, and as the effects of stimulation of the sympathetic chains on tidal air cannot be shown with certainty for more than 45 min. after ligature of the aorta, TM10 was added to the blood in the external reservoir just before the external heart-lung circuit was established. Fig. 2 shows that TM10, so used, blocked concurrently the effects both of stimulation of the peripheral ends of the nervi accelerantes on the heart, and the effects of chain stimulation on the bronchiolar resistance, without affecting the actions of adrenaline or isoprenaline, and without blocking the effects of vagal stimulation.

Anatomical evidence indicates that the postganglionic sympathetic fibres to the smooth muscle of the bronchi must be considered the most probable source of the amine liberated into the pulmonary veins of the cat on stimulation of the sympathetic chains. The normal drainage of most of the bronchial venous blood is known to be by way of the pulmonary veins in this species. There is, however, commonly a very small additional bronchial vein on the right, opening into the azygos vein near the heart, and, less commonly, another still smaller bronchial vein on the left, opening into the superior intercostal. The combined flow was therefore measured in an animal in which both these veins were present; with the chest open, during positive-pressure ventilation, the total flow was less than 4 ml./hr. The ligature of these two accessory bronchial veins, and of all other minute twigs entering or leaving the great vessels, was a routine practice during the construction of the heart-lung preparations, and was not attended by change in tidal air. In the final preparations, the reservoir volumes decreased by less than 6 ml./hr., and the chest cavities remained

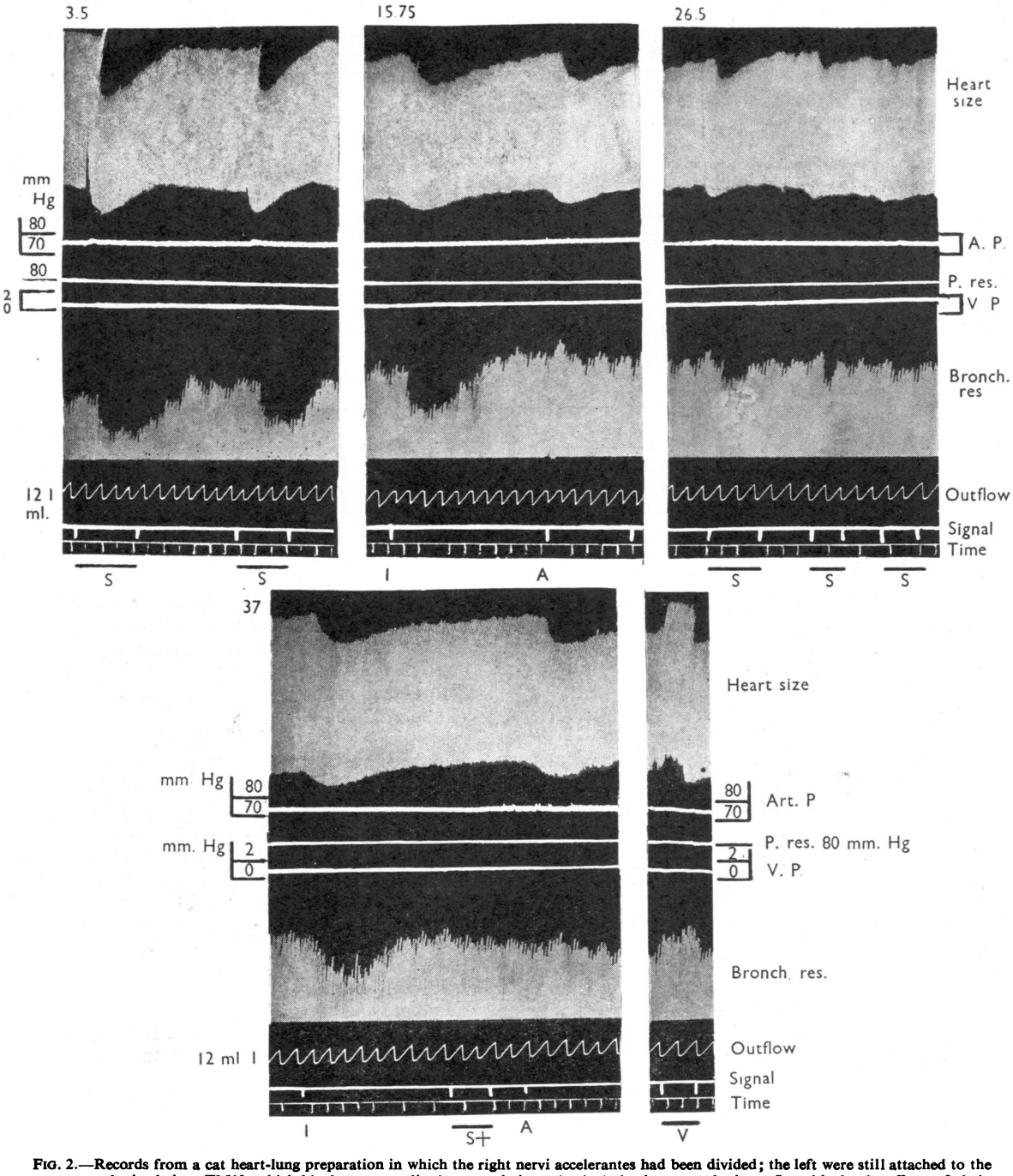

FIG. 2.—Records from a cat heart-lung preparation in which the right nervi accelerantes had been divided; the left were still attached to the sympathetic chain. TM10, which blocks postganglionic transmission selectively in the sympathetic outflow, blocks the effects of chain stimulation and of stimulation of the nervi accelerantes concurrently. TM10, 1 mg./100 ml., and pilocarpine nitrate 75 mg./100 ml. in circuit blood. Records from above downward show heart size, mean arterial pressure, peripheral resistance, venous filling pressure, bronchiolar resistance, systemic outflow, signal, and time trace recording irregularly 30 or 60 sec. The numerals above the records denote time in min. from ligature of the aorta. S=bilateral stimulation of the sympathetic chains from just above the stellate ganglia to T8, 7 v., 250 μsec., 15/sec. I=5 μg. (±)-isoprenaline. A=5 μg. (−)-adrenaline. S+=stimulation of the chains as before but with stimulation of peripheral right nervi accelerantes (2 v., 250 μsec., 15/sec.). V=stimulation of peripheral right vagus (2 v., 250 μsec., 15/sec.). Note that the effects of adrenaline and isoprenaline added to the circulating blood are unaffected by TM10.

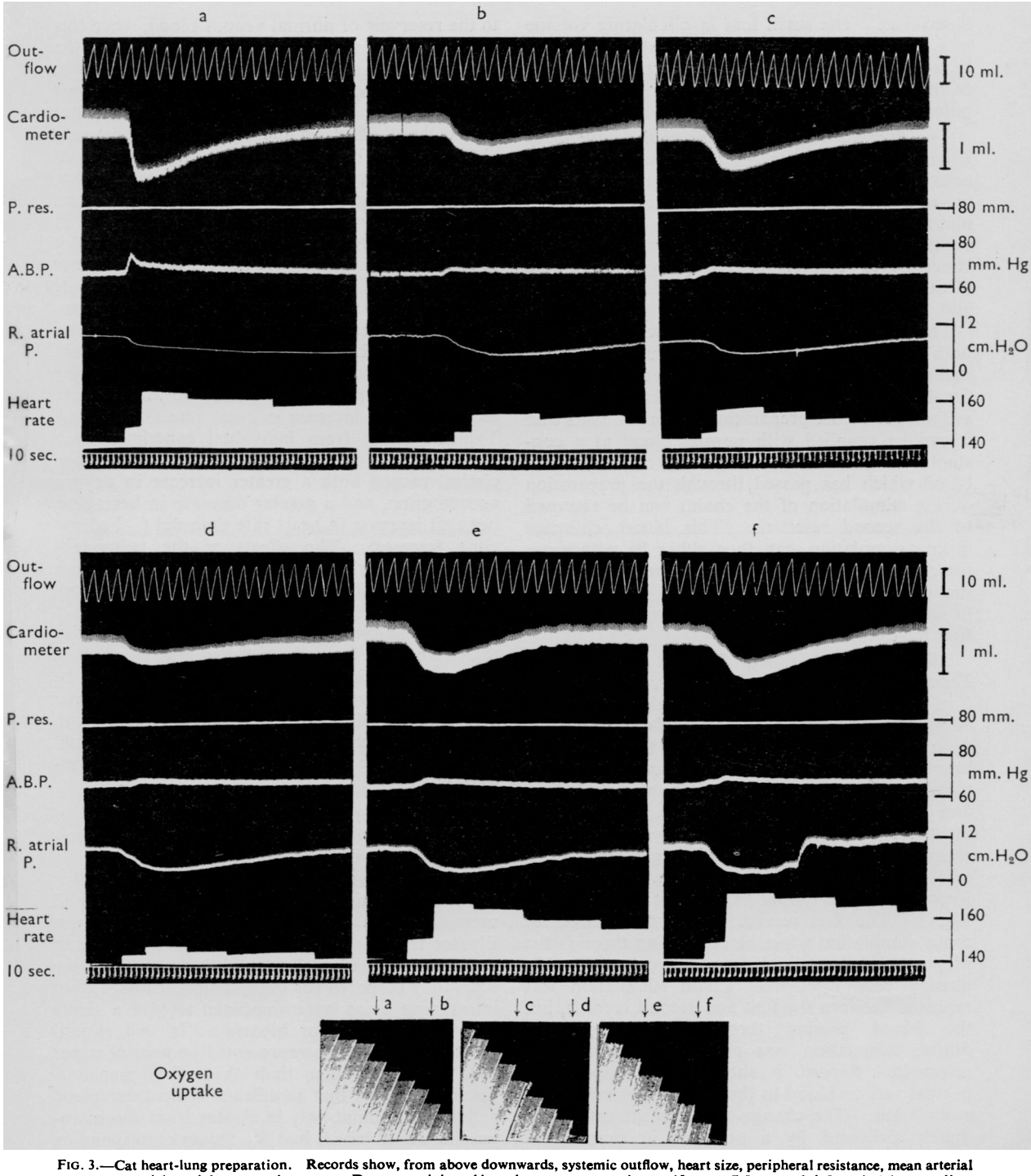

FIG. 3.—Cat heart-lung preparation. Records show, from above downwards, systemic outflow, heart size, peripheral resistance, mean arterial pressure, right atrial pressure, heart rate. Drugs were injected into the venous reservoir at *a*, 10 μg., *c*, 7.5 μg., and *d*, 5 μg. (±)-isoprenaline. *e*, 10 μg., and *f*, 20 μg. (−)-adrenaline. *b*, a change from normal blood to blood collected during chain stimulation. Below, a record of oxygen consumption: each drop in the record is an arrest of the trace for 5 min.

blood-free. The small loss in circulating volume was therefore attributed to evaporation from the lung surfaces, and it was concluded that the whole of the bronchial venous drainage had entered the pulmonary circulation.

The Pharmacological Actions of the Pulmonary Transmitter Substance. — The pharmacological activity of this transmitter was first examined in the untreated whole blood into which it had been liberated. Then the constituent amines were separated chromatographically from protein-free concentrated extracts of the plasma, and were identified by their R_F values, colour reactions, and biological activity.

The cat heart-lung preparation was chosen for both series of experiments although the sympathetic chains in this preparation only transmitted for 45 min. after ligature of the aorta. The great advantages of the preparation lay in the facts that it can be supplied with normal blood at a constant rate from the first of the two reservoirs, and blood which has passed through the preparation during stimulation of the chains can be returned to the second reservoir. This blood, collected during stimulation, can then either be withdrawn for chromatographic work, or can be fed back into the preparation for the study of its pharmacological actions after the direct effects of stimulation have worn off.

The Action of Blood Containing Pulmonary Transmitter on the Heart-Lung Preparation of the Cat.—The effects of (−)-adrenaline, (−)-noradrenaline, and (±)-isoprenaline added to normal venous blood were compared in the cat heart-lung preparation with those of blood containing transmitter. The temperature, the venous inflow, and the peripheral resistance were kept constant in these experiments. In four experiments, changes in heart rate, tidal air, right atrial pressure, and mean arterial pressure were measured. Records from a typical experiment are shown in Fig. 1. The first record, *a*, shows the effect of chain stimulation when blood passing through the preparation was allowed to recirculate; it has already been discussed. Chain stimulation was repeated between the first and second records, but the blood passing through the preparation during stimulation was collected in a separate reservoir. Record *b* shows the change from normal venous blood to the blood collected during stimulation. The change-over was almost immediately followed by a decrease in bronchiolar resistance and a moderate increase in heart rate. Record *c* shows that very similar changes were induced by the addition of 2 μg. (±)-isoprenaline to the reservoir of normal venous blood, when this was supplying the preparation. In contrast, the addition of 4 μg. (−)-adrenaline to this reservoir, record *d*, caused a much greater increase in heart rate than did the blood containing transmitter, but was without effect on the bronchiolar resistance.

Changes in heart size, heart rate, right atrial pressure and oxygen uptake were measured in five other experiments which all gave similar results. One such experiment is illustrated in Fig. 3 in which the tracings show that the changes recorded in right atrial pressure were too variable, and those in mean arterial pressure too small, to differentiate with any certainty between the actions of adrenaline and isoprenaline. The effects of these amines did, however, become clearly distinguished when graphs were plotted for each experiment relating changes in oxygen consumption and in heart size as ordinates to increase in heart rate as abscissae. Typical graphs from individual experiments are shown in Fig. 4*a* and *b*. (±)-Isoprenaline (open circles) caused both a greater increase in oxygen consumption, and a greater decrease in heart size, per unit increase in heart rate than did (−)-adrenaline (crosses). The effects of the pulmonary transmitter (dark squares) resembled much more closely those of (±)-isoprenaline (o) than those of adrenaline (x).

Noradrenaline is well known to be less effective than adrenaline in producing change in bronchiolar resistance and in oxygen consumption, but to be equally effective as adrenaline on heart rate. These facts, which were confirmed by experiment, made it very unlikely that noradrenaline would prove a major component of the pulmonary transmitter.

Chromatographic Separation of the Amines of Pulmonary Sympathetic Transmission.—Successive portions of chromatograms, prepared as described above, were eluted and tested for sympathomimetic action both on the blood pressure of the spinal cat and on the rat uterus. Eluates from areas corresponding in R_F value to noradrenaline, adrenaline, and isoprenaline were separately prepared for examination. Eluates from intervening areas were combined to give a single "blank" solution for bioassay. In two experiments this blank also represented an area of paper of R_F value greater than that of isoprenaline. Table III shows that significant sympathomimetic activity was found only in eluates from chromatographic zones which had R_F values corresponding to those of noradrenaline, adrenaline, and isoprenaline. This activity has been expressed in μg. of the reference compound of corresponding R_F value

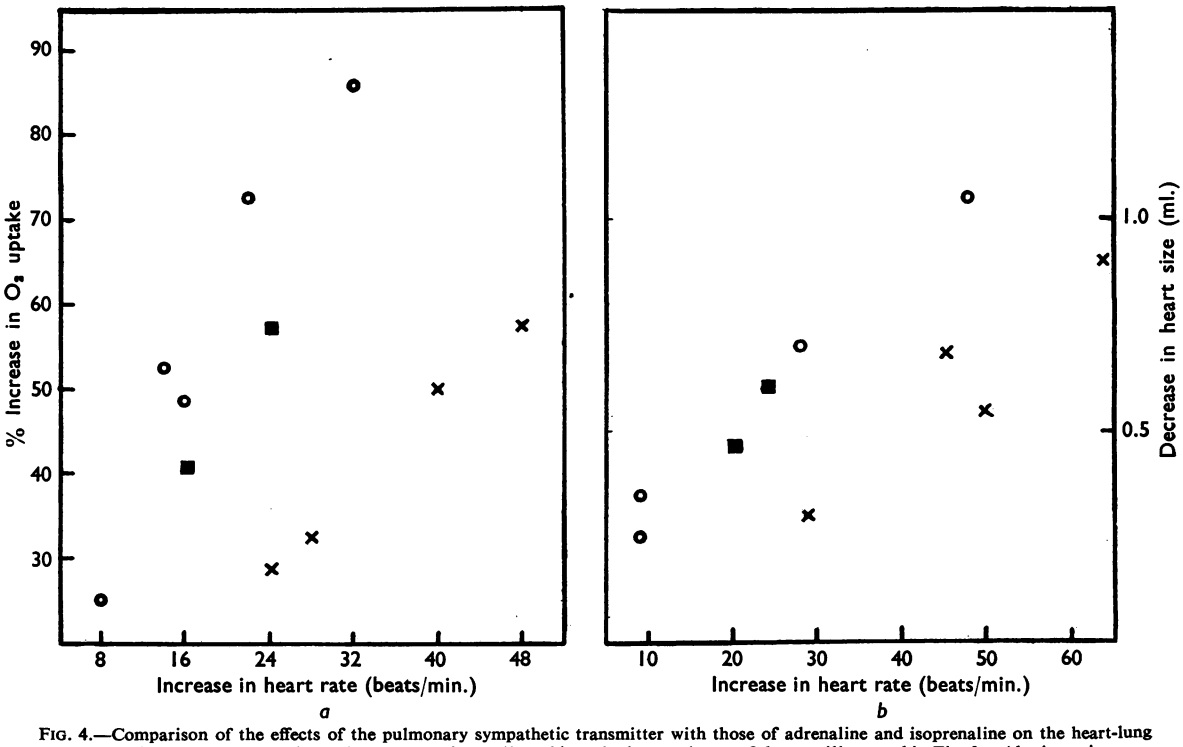

FIG. 4.—Comparison of the effects of the pulmonary sympathetic transmitter with those of adrenaline and isoprenaline on the heart-lung preparation of the cat. Each graph represents data collected in a single experiment of the type illustrated in Fig. 3. Abscissae, increase in heart rates. Ordinates, % increase in O_2 consumption (*a*), and decrease in heart size (*b*). Open circles, (±)-isoprenaline; crosses, (−)-adrenaline; and black squares, blood collected during stimulation of the sympathetic chains.

per 100 ml. plasma. (±)-Isoprenaline was used as standard for the assay of "blank" activity. Where none was demonstrated, a figure has been entered which represents the concentration of amine which might have remained undetected.

The important fact shown in Table III is the great increase of activity in the fraction with R_F value equivalent to isoprenaline that resulted from bilateral stimulation of sympathetic chains from which the nervi accelerantes had been severed. This

TABLE III

CONCENTRATIONS OF SYMPATHOMIMETIC AMINES IN THE PLASMA OF BLOOD WHICH HAD PASSED THROUGH A CAT HEART-LUNG PREPARATION IMMEDIATELY BEFORE ("C" SAMPLE) AND DURING STIMULATION OF THE THORACIC SYMPATHETIC CHAINS FROM WHICH THE NERVI ACCELERANTES HAD BEEN DIVIDED ("S" SAMPLE)

These amines were assayed after chromatographic separation in terms of the reference amine of corresponding R_F value.

Expt. No.	Chromatographic Solvent	Systemic Blood Flow (ml./min.)	Plasma Sample		Activity of Serial Chromatographic Eluates against Different Reference Compounds, by Different Assay Methods (see Text) (μg./100 ml. Plasma)				Preparation of Blood Donor
			Nature	Vol. Used (ml.)	(−)Noradrenaline	(−)Adrenaline	(±)-Isoprenaline	Blank (±)-isoprenaline	
					Cat B.P.	Rat Uterus	Rat Uterus	Rat Uterus	
1	Phenol-HCl	45	C	20	7·6	2·8	<0·3	<0·3	Spinal adrenalectomized
			S	35	7·5	3·5	30·0	<0·4	
2	,,	61	C	30	6·4	12·2	1·5	<0·5	Spinal
			S	35	6·5	12·0	9·0	<0·5	
3	,,	38	C	25	<3·5	1·0	3·0	1·0	Spinal sympathectomized, adrenalectomized
			S	40	<2·0	1·0	22·0	1·0	
4	Butanol-acetic	34	C	25	<3·0	<0·3	<0·3	<0·3	,, ,,
			S	35	<2·5	2·4	23·0	<0·8	
5	,,	43	C	27	<3·0	0·5	4·0	<0·2	,, ,,
			S	52	<1·5	3·8	18·0	<0·1	

fraction undoubtedly contained the predominant amine of the pulmonary transmitter. A much smaller, but probably significant, increase in adrenaline was observed under the same circumstances (Table III, Expts. 4 and 5), but in no case was there an increase in the noradrenaline.

The varying concentrations of adrenaline and of noradrenaline in the control plasma samples should probably be related to differences in the methods used to collect blood for these experiments (Table III, last column). Variation in the amine content of donor blood is highly likely to have been reflected in these experiments, because the time elapsing between ligature of the aorta and the completion of blood sampling was short, and varied only between 18 and 21 min. The small amounts of activity in the isoprenaline R_F fractions from control plasma (Table III, Expts. 2, 3, 5) may have been due to slight traction of the electrodes on the chains.

Since isoprenaline was used successfully as a chromatographic reference compound for the predominant amine released during stimulation of the sympathetic nerves to the lungs throughout these and all other experiments, it must be concluded that the R_F values for these two compounds did not differ significantly either with phenol-hydrochloric or with butanol-acetic as solvents.

Colour Reactions.—The predominant amine released by stimulation of the sympathetic nerves to the lungs, like isoprenaline, gave an adrenochrome reaction when treated with oxidizing reagents in weakly alkaline solution. Adrenochrome formation is typical of catechol amines with primary or secondary amino nitrogen in the side chain. The reaction product with ferricyanide was fluorescent under ultraviolet light; this would not occur were the hydroxyl group in the side chain substituted (Lund, 1949).

The Pharmacological Actions of the Predominant Amine of Pulmonary Adrenergic Transmission.—The predominant amine released by stimulation of the adrenergic nerves to the lungs was separated chromatographically, and was then compared with isoprenaline in a series of parallel assays. In the four experiments shown in Table IV use was made of their vasodilator action in cats under chloralose anaesthesia and of their antagonism to the action of acetylcholine on the rat uterus and colon. This type of parallel assay was used for the characterization of the third amine of the adrenal gland and has already been illustrated (Lockett, 1954, Figs. 3 and 4). Throughout the present work either the uterus or the colon was taken from a strain of rats which were normally sensitive to isoprenaline, but which were abnormally insensitive to adrenaline (Lockett, 1954). Table IV shows that the amine released by stimulation of adrenergic nerves to the lungs could not be distinguished from isoprenaline, but was clearly differentiated from adrenaline by these methods.

TABLE IV

THE AMINE OF PULMONARY SYMPATHIC TRANSMISSION OF R_F VALUE EQUIVALENT TO ISOPRENALINE IS DIFFERENTIATED FROM (−)-ADRENALINE, BUT NOT FROM (±)-ISOPRENALINE, BY PARALLEL ASSAYS ON THE UTERUS AND COLON OF THE RAT, AND ON THE MEAN ARTERIAL PRESSURE OF CATS ANAESTHETIZED WITH CHLORALOSE

Expt. No.	Activity of Eluates Expressed as: μg. (−)-Adrenaline/ml.			μg. (±)-Isoprenaline/ml.		
	Rat Uterus	Rat Colon	Cat B.P.	Rat Uterus	Rat Colon	Cat B.P.
1	40*	8·0	1 ml. depressor; 2 μg.(−) adren. pressor	4·0*	4·0	4·0–4·5
2	3·0	12·0*	1 ml. depressor; 1 μg.(−) adren. pressor	3·5	3·0*	3·0–3·5
3	5·5	30·0*	1 ml. depressor; 3 μg.(−) adren. pressor	7·0	8·0*	7·0–8·0
4	26·0*	5·0	1 ml. depressor; 4 μg.(−) adren. pressor	4·5*	4·0	4·0–4·5

* Tissue taken from a strain of rats normally sensitive to isoprenaline but abnormally insensitive to adrenaline.

In all subsequent experiments, eluates of the pulmonary amine were first standardized in terms of half the equally active dose of (±)-isoprenaline on rat uterus and colon. Thereafter, the assumed identity of the two amines was tested on a variety of preparations. The errors which must result from attributing the activity of (±)-isoprenaline solely to the laevorotatory component are not large (Lands, Luduena, and Tullar, 1954) and lie within the error of the rat tissue assays. The results obtained are summarized in Table V.

TABLE V

RATIOS OF THE WEIGHTS OF AMINES WHICH PROVED EQUALLY EFFECTIVE IN MODIFYING THE PERFORMANCE OF CAT HEART-LUNG PREPARATIONS

The preparations were maintained at constant peripheral resistance, systemic flow, and temperature. Weights quoted for the pulmonary amine of R_F value equivalent to isoprenaline are in terms of (±)-isoprenaline ÷ 2, and were obtained by standardization of eluates on the rat uterus and colon.

Response Measured	Ratios of Equally Effective Doses			No. of Expts.
	(−)-Adrenaline	(±)-Isoprenaline ÷2	Pulmonary Amine	
Increase in heart rate..	1·0	0·27–0·45	0·27–0·40	5
Decrease in heart size	1·0	0·50–0·67	0·50–0·67	5
Bronchiolar dilation ..	1·0	0·12–0·20	0·12–0·18	5
Increase in O_2 uptake (driven hearts 210 beats/min.)	1·0	0·33–0·50	0·33–0·50	4

The heart-lung preparations (Table V) were all maintained at constant temperature, systemic output, and peripheral resistance. In the first five experiments, pilocarpine nitrate, 50 μg./100 ml., was added to the circuit blood. This caused the moderate bronchiolar constriction required, but also slowed the hearts by 8 to 14 beats/min. In these preparations, isoprenaline and the pulmonary amine equated in their actions on heart rate and size and in the relief of bronchiolar constriction. In the last four experiments in Table V, pilocarpine was omitted and the hearts were driven at constant rates which varied from 208 to 212 beats/min. The changes in oxygen consumption produced by these amines were measured; again isoprenaline and the pulmonary amine proved equally active (Table V). The latter two amines also showed great similarity in duration of action. Their effects on heart rate and on oxygen consumption lasted more than twice as long as those of adrenaline. In the normal heart-lung preparation isoprenaline and the pulmonary amine had actions qualitatively similar but quantitatively different from those of adrenaline (Table V). By contrast, after pentobarbitone, when the heart could no longer adapt to changes in venous inflow without marked change in right atrial pressure, both pulmonary amine and isoprenaline caused dilatation of the heart and a steep rise in right atrial pressure, whereas adrenaline still caused a fall in atrial pressure and a diminution of heart size.

Finally, the pulmonary amine, like isoprenaline, was shown to dilate the vessels of the rabbit ear, and to cause relaxation of the rabbit uterus, whereas adrenaline constricted the vessels of the rabbit ear and caused contraction of the rabbit uterus (Lands, 1947).

Discussion

The evidence presented shows that a catechol amine was released into the pulmonary circulation of the cat when the adrenergic fibres to the lungs were stimulated. This amine proved, after chromatographic separation, indistinguishable from isoprenaline in R_F values, colour reactions, and pharmacological activity. In addition, the actions of this separated amine very closely resembled those of total pulmonary adrenergic transmitter in blood when examined on the heart-lung preparation of the cat. This fact indicates that the chemical procedures used in the preparation of plasma extracts, in chromatography, and in elution, produced no artifact.

The pulmonary amine is not only differentiated from noradrenaline by its R_F values. Its actions on the rat uterus, the blood pressure of the cat, the rabbit ear, and the rabbit uterus are in sharp contradistinction to those of noradrenaline.

Clear differentiation of this amine from adrenaline is also evident in R_F values, in parallel assays on the cat's blood pressure, rat uterus, and rat colon (Table IV) and in actions on the cat heart-lung preparation (Table V). The values obtained for the relative potency of adrenaline and isoprenaline on heart rate, heart size, and oxygen consumption in the cat heart-lung preparation differ quantitatively from, but are similar qualitatively to, values obtained for the cat heart perfused through the coronary vessels with oxygenated salt solution (Marsh, Pelletier and Ross, 1948). This discrepancy is almost certainly to be related to the wide divergence of the experimental conditions.

The type of action shown by the pulmonary amine resembles, in general, that of noradrenaline with a single *N* substituent of chain length equal to or exceeding three carbon atoms (Konzett, 1940b). The great potency of the pulmonary amine as an antagonist of pilocarpine-induced bronchospasm restricts the possible identification of this amine to isoprenaline (*N*-isopropylnoradrenaline) or to *N*-tertiary butyl noradrenaline amongst those amines of this series which have been fully investigated—namely, those with two, three, and four carbon atoms in single *N* substitution (Marsh *et al.*, 1948). *N*-propyl and *N*-isobutyl noradrenaline are less active in this respect (Konzett, 1940b). *N*-tertiary butyl noradrenaline has been shown twice as active as isoprenaline in accelerating the heart and in increasing the force of its contraction in Langendorf preparations of cat heart (Marsh *et al.*, 1948), but it is possible that this difference between the two amines would be decreased in the cat heart-lung preparation. R_F values for *N*-tertiary butyl noradrenaline are not available. Failure to differentiate between the pulmonary amine and isoprenaline by pharmacological means cannot therefore be regarded as proof of the identity of these amines. Such an hypothesis receives support from the identical R_F values obtained for these two substances in three different solvents.

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