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# Pharmacological significance of biogenic amines in the lungs: 5-hydroxytryptamine

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## Summary

1. A technique for the spectrofluorometric analysis of 5-hydroxytryptamine, histamine, noradrenaline and dopamine in a single lung sample has been developed. The method is a combination of the extraction procedure of Shore & Olin (1958), plus the modification introduced by Hogans (1967) for the analysis of three of the four amines. The fourth amine, histamine, was analysed by the method of Shore, Burkhalter & Cohn (1959), which was combined with the analysis of the other three amines. The combined procedure consisted principally of disintegrating the lung tissue in butanol and subsequent separation of amines for measurement of fluorescence, either directly, as for 5-hydroxytryptamine, or after formation of fluorophores, as for histamine, noradrenaline and dopamine. It has been possible to analyse all four amines from a sample of the lung in which the bronchopulmonary responses have been investigated.

2. The concentration of 5-hydroxytryptamine in the lung of seven species ranges from about 0·5 $\mu$g/g in the guinea-pig, dog and man to about 7 $\mu$g/g in the rabbit. The pulmonary resistance is increased after an injection of 5-hydroxytryptamine in the rat and guinea-pig.

3. The guinea-pig is more sensitive to 5-hydroxytryptamine than the rat. In both animal species, the content of 5-hydroxytryptamine in the lung is elevated by administration of either 5-hydroxytryptamine or 5-hydroxytryptophan. In the rat, the administration of *p*-chlorophenylalanine reduces the content of 5-hydroxytryptamine in the brain but not in the lung.

4. It is suggested that the 5-hydroxytryptamine contained in the lung tissue is stored mainly in the platelets and in the mast cells.

## Introduction

The importance of biogenic amines in the effects of drugs has been demonstrated in recent years for the central and autonomic nervous systems. The blockade of the sympathetic innervation of blood vessels by reserpine is explained by depletion of noradrenaline in the nerve endings. The antidepressant effect of monoamine oxidase inhibitors such as iproniazid is attributed to the inhibition of the breakdown of catecholamines and increase in the content of noradrenaline and 5-hydroxy-

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tryptamine (5-HT) in the brain. More recently, dopamine has become important in the management of a nervous disease that hitherto has been difficult to control. Hornykiewicz (1965) found that there is a gross depletion of dopamine, noradrenaline and 5-HT in the basal ganglia and substantia nigra of patients suffering from Parkinson's disease. Administration of 3,4-dihydroxyphenylalanine (DOPA) has been found to increase dopamine in the brain (Carlsson, 1959). A slight improvement has been noted in patients with Parkinson's disease when DOPA was given (McGeer & Zeldowicz, 1964).

The interest in the content of biogenic amines in the lung has preceded the interest in their content in the nervous system, yet the significance of their presence in the lung is poorly understood. Histamine was first demonstrated by Best, Dale, Dudley & Thorpe (1927) to be present in the lung tissue. There is adequate demonstration of the release of histamine from the lung tissue under special conditions (see references cited by Rocha e Silva, 1966). 5-HT is found in relatively high concentrations in many mammalian lungs and is released in pulmonary embolism (Smith & Smith, 1955) and anaphylaxis (Waalkes, Weissbach, Bozicevich & Udenfriend, 1957). The enzyme that synthesizes 5-HT, 5-hydroxytryptophan decarboxylase, and the enzyme that destroys 5-HT, monoamine oxidase, are also known to be present in the lung (Weissbach, Waalkes & Udenfriend, 1957). There is, however, no acceptable hypothesis for the role of either 5-HT or histamine in the regulation of lung function.

As for the catecholamines, noradrenaline is believed to be associated with sympathetic innervation in the lung tissue. In some animal species, dopamine is the major form of catecholamine present (Euler & Lishajko, 1957). The influence of receptor blocking drugs on the noradrenaline content of the lung and the significance of dopamine have not been elucidated.

The information at present available for the autonomic and central nervous systems is being used as a model for outlining the procedures applied to the investigation. This report is concerned with the development of a technique to analyse all four biogenic amines in a single sample of lung. The technique has been applied to the investigation of 5-HT in this report, of histamine (Aviado & Sadavongvivad, 1970a) and of noradrenaline and dopamine (Aviado & Sadavongvivad, 1970b).

## Methods

### *Extraction and analysis of biogenic amines*

The procedure adopted in this investigation was a direct offshoot of the method developed by Hogans (1967). The method consists basically of homogenizing the tissues in butanol saturated with dilute acid. One portion of the butanol extract was used for noradrenaline and dopamine assay and the other portion for 5-HT assay. To Hogans' method, the extraction and analysis of histamine described by Shore, Burkhalter & Cohn (1959) was added. The addition proved successful and the details of the procedure are as follows.

*Extraction of amines.* Tissue samples, approximately 0·5 g, were weighed in a glass tissue grinder. Immediately after weighing the tissue, 10 ml of butanol was added, followed by a volume of 0·01 N hydrochloric acid, which resulted in a volume of 1·5 ml water. The tissue was ground with a motor-driven Teflon pestle while being cooled in an ice bath. The homogenate was transferred to a centrifuge tube and centrifuged for 15 min.

*Analysis of histamine and 5-HT.* Four ml of butanol extract was transferred to a test tube containing 5 ml of 0·1 M borate buffer, pH 10, saturated with sodium chloride and butanol. The contents were shaken vigorously for 30 s and centrifuged for 5 min. Two ml of the washed butanol layer was transferred to another test tube containing 4 ml of *n*-heptane and 2 ml of 0·1 N hydrochloric acid. After shaking and centrifuging, the upper butanol-heptane layer was removed by aspiration. One ml of the hydrochloric acid was transferred to a quartz cuvette and the fluorescence of 5-HT was measured in the Aminco-Bowman spectrofluorometer with the activation wavelength set at 295 mμ and the emission wavelength at 330 mμ (Udenfriend, Weissbach & Clark, 1955). After reading the 5-HT fluorescence, 0·2 ml 1 N sodium hydroxide and 0·1 ml 1% *o*-phthalaldehyde in absolute methanol were added. Four min later, 0·1 ml of 3 N hydrochloric acid was added and then after another minute the fluorescence derived from histamine was measured with activation wavelength set at 360 mμ and emission wavelength at 450 mμ.

*Analysis of noradrenaline and dopamine.* Four ml of the butanol extract was transferred to a centrifuge tube containing 3 ml of 0·1 M phosphate buffer, pH 6·5. The contents were shaken for 20 s and centrifuged for 10 min. The butanol layer was aspirated leaving about 2·5 ml buffer solution containing the catecholamines. To a 1 ml portion, 0·25 ml of 4% w/v disodium edetate (pH 6·5) was added, followed by 0·2 ml iodine solution (4·8% w/v potassium iodide and 0·25% w/v iodine in water). After 2 min, 0·25 ml alkaline sulphite solution was added (0·125 g sodium sulphite in 1 ml water and 4 ml 5 N sodium hydroxide). After another period of 2 min, 0·3 ml 5 N acetic acid was added. The tissues blank was prepared in the same way except for reversing the addition of iodine and alkaline sulphite. The tubes were boiled in a water bath for 5 min and cooled immediately in ice water for 1 min to develop the fluorophores of noradrenaline and dopamine. The fluorescence of noradrenaline is read immediately after cooling, with the activation wavelength at 385 mμ and emission wavelength at 485 mμ (Shore & Olin, 1958). According to Hogans (1967) the fluorescence of noradrenaline develops quickly, reaching maximum at about the end of boiling. The fluorescence of dopamine develops slowly, reaching a maximum about 20 min later and remaining stable for hours. Hence the fluorescence of dopamine was measured at least 20 min after the measurement of noradrenaline fluorescence. The activation wavelength was set at 320 mμ and the emission wavelength at 370 mμ.

## *Procedures in the rat*

Seven series of experiments were conducted on the rat. (1) Fifteen rats were divided into three groups to settle the question of the most suitable method for removal of the lung. (2) Six rats were used to determine the recovery of amines added to the lung. (3) Eighteen rats were divided into three groups; six rats for control saline injection; six rats for injection of 5-HT (25 mg/kg); and six for injection of 5-hydroxytryptophan (50 mg/kg). The injections were given 30 to 45 min before removal of the lung while the rats were anaesthetized with diallylbarbituric acid (60 mg/kg) and urethane (240 mg/kg). (4) Twelve rats were divided into two groups, one group was used as a control and in the other group each animal received an injection of reserpine (5 mg/kg) 18 hr before being killed. (5) Ten rats were used to characterize the bronchopulmonary effects of intravenous injection

of 5-HT (10 mg/kg). The details for measurement of their responses are described below.

(6) Forty rats were divided into six control groups and six experimental groups. In the first two groups, one received a control injection of 0·5% w/v methyl cellulose and the other received *para*-chlorophenylalanine (PCPA) 500 mg/kg in 0·5% w/v methyl cellulose, 60 to 90 hr before killing the rats. In the next two groups, one received the same compound suspended in 0·9% w/v sodium chloride solution adjusted to pH 2·6, 60 to 90 hr before the rats were killed ; the other was used as a control. In the last eight groups, the treated animals received injections of PCPA in methyl cellulose suspension from one to five times. (7) Twenty-four rats were divided into six groups. In the first two, the treated rats received PCPA (300 mg/kg) 3 days before the experiment. In the second two, the treated animals received PCPA and 5-HT (25 mg/kg), and in the third two they received PCPA and 5-hydroxytryptophan (50 mg/kg). In each pair of groups, one group remained as control and did not receive PCPA.

### *Procedures in the guinea-pig*

Four series of experiments on the guinea-pig were completed. In all experiments, the lungs were removed while the animals were anaesthetized with diallylbarbituric acid (60 mg/kg) and urethane (240 mg/kg). (1) Sixteen guinea-pigs were divided into four groups: a control group, a treated group that received 5-HT (30 mg/kg) injected intraperitoneally daily for 7 days ; a group that received one injection of 5-HT (30 mg/kg) intraperitoneally 40 min before killing ; and the last group received one injection of 5-hydroxytryptophan (50 mg/kg). The lungs were analysed for their content of the four amines. (2) Thirty-six guinea-pigs, of which half remained as controls and the other half received 5-HT (30 mg/kg) intraperitoneally daily for 6 days, were used. A control and an experimental group were investigated on each of the following days: on day 1 after the last injection of 5-HT, on day 2, on day 3, on day 4, on day 5 and on day 8. The lungs were analysed only for histamine and 5-HT.

(3) Five guinea-pigs received an intravenous injection of 5-HT (1 to 5 $\mu$g/kg) to examine its effect on the pulmonary resistance. (4) Of thirteen guinea-pigs six were used as controls and the remaining seven received 5-HT (30 mg/kg) intraperitoneally daily for 6 days. During the following week, one animal from each group was anaesthetized before eliciting the bronchopulmonary response to intravenous injections of 5-HT (1, 2, 5 and 10 $\mu$g/kg).

### *Bronchopulmonary responses in the rat and guinea-pig*

The animal was anaesthetized with diallylbarbituric acid (60 mg/kg) and urethane (240 mg/kg) intraperitoneally. A catheter of polyethylene tubing, 1·8 mm inner diameter and 42 cm long, was inserted to measure intrapleural pressure. The outside end of the catheter was attached to a differential pressure transducer with the other part of the transducer connected to the trachea. Air flow was not measured by the usual attachment of a pneumotachometer to the tracheal cannula. Instead, the animal was placed in a cylinder-shaped body plethysmograph made of clear acrylic resin and the pneumotachometer was installed on one end of the chamber. The tracheal cannula was attached via another hole to an outside tube, which was

continuously flushed with either room air or a gas mixture at a rate of 1 litre/min. The respiratory movement of the animal caused a flow of air in and out of the plethysmograph via the pneumotachometer. The air flow arising from the chest motion in the body plethysmograph, which was usually similar to tracheal flow, was integrated to derive tidal volume. The signals of transpulmonary pressure and air flow were displayed on an oscilloscope screen to measure pulmonary compliance by a method described in an earlier paper (Ito & Aviado, 1968).

## Results

After preliminary testing of the method of Hogans, it was decided to proceed with the analysis of the lungs removed from rats killed in one of three ways. In one group, five rats were decapitated without anaesthesia, in a second group, five rats were decapitated under ether anaesthesia, and in a third group, five rats were decapitated under anaesthesia with diallylbarbituric acid and urethane. Table 1 summarizes the mean values for 5-HT, noradrenaline and dopamine in the lungs. Although the mean values for the three groups of animals were about equal, the standard error of the mean and coefficient of variation were different. The group decapitated under diallylbarbituric acid and urethane anaesthesia had lower values for the standard error of the mean and the coefficient of variation than the other groups. The high variability in the group without anaesthesia and in the group under ether anaesthesia indicates that the stimulation of the sympathetic nervous system during decapitation was intense and variable enough to influence the results. Subsequently, all rats were decapitated under diallylbarbituric acid and urethane anaesthesia.

In the next experiment, the lungs were analysed for four amines and the recoveries of the amines were investigated. Six rats were used, the lung was excised and two sets of samples were prepared from each lung: one set of samples had no amine added and the other set had histamine, 5-HT, noradrenaline and dopamine added. A comparison of values from both sets allowed an estimation of the recovery to be made (Table 1). The addition of histamine did not interfere with the analysis of

TABLE 1. *Extraction of biogenic amines from rat lung*

| Procedure | No. of animals | Concentration of amine (mean±S.E.M.) μg/g lung; coefficient of variation (%) | | | |
|---|---|---|---|---|---|
| | | Histamine | 5-HT | Noradrenaline | Dopamine |
| Decapitation no anaesthetic | 5 | — | 5·31<br>±1·72<br>64% | 0·18<br>±0·03<br>75% | 0·12<br>±0·01<br>17% |
| Decapitation under ether anaesthesia | 10 | — | 5·68<br>±1·88<br>64% | 0·11<br>±0·04<br>73% | 0·09<br>±0·03<br>67% |
| Decapitation under diallyl-barbituric acid and urethane | 15 | — | 6·78<br>±0·84<br>25% | 0·10<br>±0·00<br>0 | 0·19<br>±0·00<br>0 |
| Recovery of added amines | 6 | 3·99 | 2·42 | 0·22 | 0·11 |
| Endogenous concentration | | ±0·70 | ±0·52 | ±0·01 | ±0·02 |
| % Recovery* | | 83%<br>±3·3 | 90%<br>±0·4 | 61%<br>±1·2 | 59%<br>±0·6 |

* 0·25 μg of the amine was added per 0·25 g of lung sample ; the value for endogenous amine was corrected for percent recovery.

5-HT, noradrenaline and dopamine. The recovery for these three amines was about the same as the recovery without addition of histamine reported in applying the original method for three amines.

*Injection of 5-HT or 5-hydroxytryptophan in the rat.* The results of the analysis of histamine, 5-HT, noradrenaline and dopamine in the lungs are summarized in Table 2. The six rats that received 5-HT showed an increase in the quantity of 5-HT in the lung tissue, but the amounts of the other three amines were essentially unchanged. The rats that received 5-hydroxytryptophan also showed an increase in 5-HT content.

*Injection of reserpine in rats.* The effects of reserpine on the concentrations of biogenic amines in the lung were studied (Table 2). Only the content of 5-HT and that of noradrenaline was reduced. There were essentially no changes in the concentrations of histamine and dopamine.

*Responses to 5-HT in the rat.* The acute effects of 5-HT (10 $\mu$g/kg, injected intravenously) were elicited in a group of ten rats (Table 3). A consistent pattern was observed throughout, namely, a mean increase in pulmonary resistance of 23%, a mean fall in pulmonary compliance of 18% and a mean rise in aortic blood pressure of 28%. Doses of less than 10 $\mu$g/kg gave inconsistent results.

*Injection of 5-HT and* para*-chlorophenylalanine in the rat.* In these experiments only the concentration of 5-HT was estimated. *Para*-chlorophenylalanine (PCPA) was selected because it is known to block an enzyme involved in the synthesis of 5-HT (Koe & Weissman, 1966). PCPA is insoluble in water and had to be injected as a suspension. Two vehicles were tested: methyl cellulose and an acid solution of sodium chloride (0·9% w/v). The two control groups of rats did not differ as regards the 5-HT content of the lung and the brain (Table 4). Among the experimental rats that received a single intraperitoneal injection of PCPA (500 mg/kg),

TABLE 2. *Content of biogenic amines in the lung of rats receiving 5-HT, 5-hydroxytryptophan or reserpine*

| Procedure | No. of animals | Concentration of amine (mean ± S.E.M.) $\mu$g/g lung | | | |
|---|---|---|---|---|---|
| | | Histamine | 5-HT | Noradrenaline | Dopamine |
| Control | 6 | 5·00 ±0·17 | 1·91 ±0·16 | 0·21 ±0·02 | 0·19 ±0·02 |
| 5-HT 25 mg/kg | 6 | 4·80 ±0·16 | 6·03* ±0·76 | 0·18 ±0·02 | 0·18 ±0·02 |
| 5-hydroxytryptophan 50 mg/kg | 6 | 4·96 ±0·17 | 4·88* ±0·38 | 0·18 ±0·02 | 0·18 ±0·01 |
| Control | 6 | 5·04 ±0·12 | 3·76 ±0·40 | 0·14 ±0·01 | 0·19 ±0·01 |
| Reserpine 5 mg/kg | 6 | 4·83 ±0·16 | 0·98* ±0·12 | 0·08 ±0·00 | 0·19 ±0·01 |

* $P < 0·001$ difference from mean of control animals.

TABLE 3. *Maximum responses to intravenous injection of 5-HT in rats*

| Procedure | No. of animals | Body weight (g) | Mean ± S.E.M. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Resistance | | Compliance | | Resp. min. vol. | |
| | | | Control cm $H_2O$/ml/s | % Change | Control ml/cm $H_2O$ | % Change | Control ml/min | % Change |
| 5-HT 10 $\mu$g/kg | 10 | 335 ±23 | 0·30 ±0·04 | +23 ±20 | 0·38 ±0·03 | −18 ±2 | 99 ±2 | +28 ±6 |

the brain 5-HT concentration was decreased to as low as 10% of the control when methyl cellulose was the vehicle. In the animals that received acid sodium chloride solution as the vehicle, the fall of the 5-HT in the brain was less pronounced; the concentration was 30% of the control level 90 hr after injection. In both groups of rats receiving PCPA, 5-HT concentration in the lung was not affected.

The results from additional groups of rats that received repeated injections of PCPA for several days are also summarized in Table 4. Two days after a single injection of PCPA (300 mg/kg), the brain 5-HT concentration had already reached the minimum value, as can be seen by the comparably low concentration in the groups receiving injections for longer periods. The concentration of 5-HT in the lung remained unchanged even after five injections of PCPA, which amounted to a dose of 1·5 g/kg over a period of 15 days.

The interaction between the enzyme inhibitor, PCPA, exogenously administered 5-HT and 5-HT formed from 5-hydroxytryptophan was studied. The results summarized in Table 4 indicate that the uptake of 5-HT and decarboxylation of 5-hydroxytryptophan by the lung were not impaired by the injection of PCPA.

*Injections of 5-HT or 5-hydroxytryptophan in the guinea-pig.* The results are summarized in Table 5. The injection of 5-HT or its precursor did not alter the contents of histamine, noradrenaline and dopamine in the lung of the guinea-pig. The 5-HT content of the lung in the groups receiving either 5-HT or its precursor was increased significantly. The group receiving repeated injections of 5-HT had a higher concentration of 5-HT in the lung than did the groups receiving a single injection of 5-HT or the precursor shortly before removal of the lung. This observation suggests that 5-HT is taken up by the lung and is not destroyed rapidly.

The rate of reduction of the artificially increased level of 5-HT in the lung was studied in another group of guinea-pigs. In this series, thirty-six guinea-pigs were

TABLE 4. *Content of 5-HT in the lung and brain of rats receiving* para-*chlorophenylalanine (PCPA)*

| Procedure | No. of animals | Concentration of 5-HT (mean±S.E.M.) μg/g tissue | | | |
|---|---|---|---|---|---|
| | | Control animals | | PCPA-treated animals | |
| | | Lung | Brain | Lung | Brain |
| PCPA 300 mg/kg in methyl cellulose | 8 | 1·86 ±0·19 | 0·57 ±0·02 | 1·79 ±0·31 | 0·06* ±0·00 |
| PCPA 300 mg/kg in acid saline | 8 | 1·68 ±0·17 | 0·58 ±0·01 | 1·33 ±0·11 | 0·19* ±0·08 |
| PCPA 300 mg/kg (one injection) | 6 | 3·65 ±0·66 | 0·50 ±0·02 | 3·33 ±1·08 | 0·08* ±0·01 |
| PCPA 300 mg/kg (two injections) | 6 | 2·40 ±0·21 | 0·55 ±0·04 | 2·30 ±0·13 | 0·07* ±0·01 |
| PCPA 300 mg/kg (three injections) | 6 | 2·61 ±0·38 | 0·50 ±0·01 | 2·39 ±0·24 | 0·05* ±0·01 |
| PCPA 300 mg/kg (five injections) | 6 | 2·82 ±0·10 | 0·62 ±0·02 | 2·67 ±0·12 | 0·06* ±0·01 |
| PCPA 300 mg/kg (one injection) | 8 | 2·02 ±0·09 | — — | 2·24 ±0·18 | — — |
| PCPA 300 mg/kg plus 5HT 25 mg/kg (one injection) | 8 | 8·44 ±0·33 | — — | 8·32 ±0·28 | — — |
| PCPA 300 mg/kg plus 5-hydroxytryptophan 50 mg/kg (one injection) | 8 | 8·38 ±0·26 | — — | 7·46 ±0·69 | — — |

* $P < 0·001$ difference from mean of controls.

used. Half of the animals served as controls; the other half received 5-HT (30 mg/kg) intraperitoneally each day for 6 consecutive days. After the sixth day of injection, three control animals and three treated animals were killed on the first, second, third, fourth, fifth and eighth days. The results of the analysis of the lung for 5-HT are summarized in Fig. 1. The 5-HT content of the treated group remained significantly higher than the control even on the eighth day after the last injection. It can be noted also that the return of the 5-HT content towards normal concentration occurred in three phases. There was a rapid fall between day 1 and day 2, followed by a plateau phase, and a slow return to normal concentrations thereafter. These results indicate the existence of different pools of 5-HT in the lung. The meaning and importance of these pools were not further investigated.

After intraperitoneal injection of 5-HT all animals developed dyspnoea, which started within 30 to 60 s after the injection. This latent period varied between animals and also varied from day to day in each animal. There was no progressive prolongation of the latent period. The gross signs were also highly variable;

TABLE 5. *Content of biogenic amines in the lung of guinea-pigs receiving injections of 5-HT or 5-hydroxytryptophan*

| Procedure | No. of animals | Concentration of amine (mean±S.E.M.) μg/g lung | | | |
|---|---|---|---|---|---|
| | | Histamine | 5-HT | Noradrenaline | Dopamine |
| Control | 4 | 6·74 ±0·70 | 0·23 ±0·01 | 0·16 ±0·02 | 0·22 ±0·03 |
| 5-HT 30 mg/kg daily for 7 days | 4 | 6·58 ±0·26 | 0·93* ±0·08 | 0·15 ±0·02 | 0·18 ±0·01 |
| 5-HT 30 mg/kg (one injection) | 4 | 6·08 ±0·31 | 0·53* ±0·31 | 0·18 ±0·01 | 0·21 ±0·01 |
| 5-Hydroxytryptophan 50 mg/kg (one injection) | 4 | 5·91 ±0·09 | 0·60* ±0·01 | 0·20 ±0·01 | 0·21 ±0·01 |

* $P < 0{\cdot}001$ difference from mean of controls.

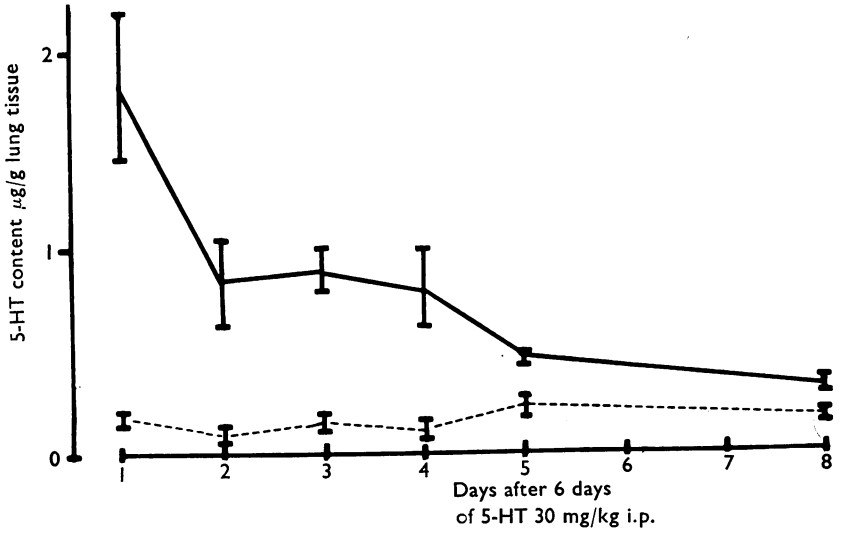


FIG. 1. Changes in content of 5-HT in the lungs of guinea-pigs from 1 to 8 days after a 6 day period of daily intraperitoneal injections of 5-HT (30 mg/kg). The mean concentration and S.E.M. are given for lung content of 5-HT-treated guinea-pigs (solid line) and controls (dotted line).

dyspnoea occurred either associated with or without cyanosis and convulsions. There was no predictable pattern of response from day to day, apart from the appearance of dyspnoea. The dyspnoea lasted between 30 min and 1 hr; there was no progressive shortening of the duration of dyspnoea in any of the animals. There were therefore no signs of tolerance developing after intraperitoneal administration of 5-HT.

*Responses to 5-HT in the guinea-pig.* The responses of five guinea-pigs to the intravenous injection of various doses of 5-HT are summarized in Table 6. With a dose of 1 $\mu$g/kg, the pulmonary resistance was doubled. One animal did not respond to this dose. With the higher doses of 2 and 5 $\mu$g/kg, all animals responded with an increase in pulmonary resistance, which in some cases was as high as a five-fold increase. The maximum response appeared within 15 s of the injection. The shape of the time response curves for pulmonary resistance was similar in all animals. Figure 2 shows the shape of such curves and the tachyphylaxis which developed after five intravenous injections of 5 $\mu$g/kg, spaced at 10 min intervals, and two additional injections at 5 min intervals. Ten min after the last dose, an injection of 5-HT in the same dosage as before failed to elicit any increase in pulmonary resistance. Twenty min later, 30 mg/kg 5-HT was injected intraperitoneally. Pulmonary resistance started to rise during the first min, reached a peak of about 80% higher than the control in 5 min, then declined slowly. At 20 min after the intraperitoneal injection, a dose of 5 $\mu$g/kg 5-HT, injected intravenously, elicited a reduced response. These observations were confirmed in two additional animals.

The effects of repeated injections of 5-HT on the response to this amine were tested in a final group of guinea-pigs. Half of the animals received injections of 5-HT (30 mg/kg) daily for 6 days, as before. On days 1, 2, 3, 4, and 8 after the last injection, control and pretreated animals were anaesthetized and a study made of the response of pulmonary resistance to intravenous injection of 5-HT. The responses are compared in Table 7. On day 1, the treated animal was more sensitive to 5-HT than the control. On day 2, two of the treated animals were less sensitive than the one control tested. On day 3, two controls and two treated animals were tested: the two controls were not equally sensitive; one of the treated animals was more sensitive than both the controls and the other less sensitive than both the controls. On day 4, one of each was tested: the treated was less sensitive than the control. Finally, on day 8, one treated animal was more sensitive than the control. In all these animals the maximum responses were reached within 15 s of the intravenous injection. It can be concluded that tolerance to 5-HT injection could not be demonstrated in this experiment.

TABLE 6. *Changes in pulmonary resistance following intravenous injection of 5-HT in guinea-pigs*

| | | | Pulmonary resistance (means ± S.E.M.) percentage change from control | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Time after injection | | | | | |
| Procedure | No. of animals | Control cm $H_2O$/ml/s | 15 s | 30 s | 45 s | 1 min | 1·5 min | 2 min |
| 5-HT 1 $\mu$g/kg | 3 | 6·0 ±1·0 | + 65 ± 37 | + 11 ± 5 | +20 ±13 | +20 ±13 | +20 ±13 | +20 ±13 |
| 5-HT 2 $\mu$g/kg | 4 | 10·0 ±1·0 | +275 ± 43 | +622 ± 14 | +11 ±10 | + 8 ±10 | +15 ±12 | +17 ±11 |
| 5-HT 5 $\mu$g/kg | 5 | 7·0 ±1·0 | +242 ± 73 | + 20 ± 11 | +13 ±13 | +13 ±13 | + 8 ±14 | + 8 ±14 |

## Discussion

The experiments reported above represent the first report of the successful analysis of four biogenic amines contained in a single sample of lung. The dependability of the analysis has been demonstrated by the recovery of the amines added to the sample (Table 1). The spectrofluorometric techniques for 5-HT, noradrenaline and dopamine have been adequately investigated by others. Michaelson & Coffman (1967) reported the occurrence in the brain of spermidine as a contaminant for

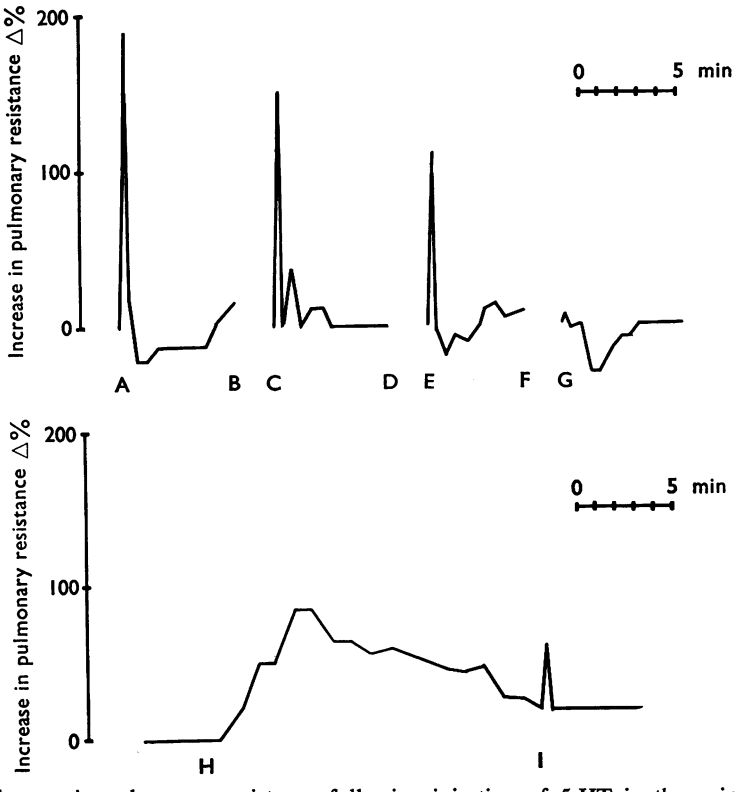


FIG. 2. Changes in pulmonary resistance following injection of 5-HT in the guinea-pig. At A, C, E, G, and I, 5-HT 5 μg/kg was injected intravenously. Between F and G, two injections of 5-HT (5 μg/kg) were given at 10 min intervals, followed by two more injections of the same dose at 5 min intervals. Ten min after the last injection, 5-HT (5 μg/kg) was given at G. At H, 5-HT (30 mg/kg) was injected intraperitoneally.

TABLE 7. *Responses to intravenous injection of 5-HT in guinea-pigs pretreated with 5-HT (20 mg/kg per day) for 6 days*

| Procedure (days after last 5-HT injection) | No. of animals | Mean % change in pulmonary resistance following intravenous 5-HT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | 5-HT (1 μg/kg) | Control | 5-HT (2 μg/kg) | Control | 5-HT (5 μg/kg) | Control | 5-HT (10 μg/kg) |
| 1 | 2 | 0 | 0 | +136 | +141 | +909 | +1,100 | | |
| 2 | 2 | 0 | 0 | +166 | + 75 | +909 | + 161 | | |
| | 1 | — | 0 | — | 0 | — | + 52 | | |
| 3 | 2 | 0 | +44 | + 22 | + 75 | +150 | + 243 | | |
| | 2 | 0 | 0 | 0 | 0 | +118 | 0 | +5,163 | +150 |
| 4 | 2 | +67 | 0 | +233 | 0 | +878 | 0 | + 709 | +650 |
| 8 | 2 | +50 | +11 | +108 | +112 | +360 | + 519 | | |

histamine. The occurrence of spermidine in the lung tissue and its influence on the spectrofluorometric analysis of histamine is under investigation in this laboratory.

The concentration of 5-HT in the rat and guinea-pig lungs, together with the concentration in six additional animal species reported elsewhere (Aviado & Sadavongvivad, 1970a, b) are summarized in Table 8. The values reported by other investigators are also included in this table. The guinea-pig lung contains the lowest amount of 5-HT. The human and the dog lungs contain slightly higher concentrations than the guinea-pig lung. In these three species, a 5-HT concentration higher than 1 $\mu$g/g has not been encountered. The rat lung contains slightly more 5-HT. The cat and goat lungs contain more than the rat lung. The mouse lung contains more than the goat lung, and the rabbit lung has the highest concentration of 5-HT, which approaches 7 $\mu$g/g.

The procedures that influence the 5-HT content in the lung are as follows: The content in the lungs of the rat and the guinea-pig was increased by injection of either 5-HT or 5-hydroxytryptophan. In the rat, PCPA did not influence the 5-HT content of the lung even after repeated administration for up to 2 weeks. Reserpine consistently caused a fall in the 5-HT content of the lung not only in the rat, reported above, but also in the rabbit, cat, dog and goat (Aviado & Sadavongvivad, 1970b). It appears, therefore, that the content of 5-HT in the lung is readily influenced by procedures that increase its uptake and release but is rather resistant to procedures that influence the pathways of synthesis and degradation.

That the injection of 5-HT can cause an increase in content of 5-HT in the lung indicates that this tissue has an intrinsic ability to take up and store the amine. There has been no suggestion as to how storage is brought about. In the mouse and rat, it is known that 5-HT appears in the mast cells. In all other species, the mast cells do not contain 5-HT (Parratt & West, 1957). It may be assumed that in the mouse and rat, part of the 5-HT in the lung is located in the mast cells.

The observations that reserpine consistently reduces the 5-HT content of the lung

TABLE 8. *Content of 5-HT in mammalian lungs*

| Animals | 5-HT ($\mu$g/g) | Method of assay | Authors |
|---|---|---|---|
| Mouse | 2·95 | Bioassay | Parratt & West (1957) |
| | 1·95 | Fluorometric | Weissbach, Waalkes & Udenfriend (1957) |
| | 5·30±0·55 | Fluorometric | Aviado & Sadavongvivad (1970a) |
| Rat | 2·3 | Fluorometric | Weissbach *et al.* (1957) |
| | 2·7 | Fluorometric | Weissbach, Waalkes & Udenfriend (1958) |
| | 3·5 | Fluorometric | Weissbach *et al.* (1958) |
| | 1·91±0·16 | Fluorometric | This article |
| Guinea-pig | 0·2 | Fluorometric | Weissbach *et al.* (1957). |
| | <0·2 | Fluorometric | Weissbach *et al.* (1958) |
| | <0·3 | Fluorometric | Weissbach *et al.* (1958) |
| | 0·23±0·01 | Fluorometric | This article |
| Rabbit | 2·1 | Fluorometric | Weissbach *et al.* (1957) |
| | 1·61 | Bioassay | Parratt & West (1957) |
| | 6·78±1·04 | Fluorometric | Aviado & Sadavongvivad (1970a) |
| Cat | 0·62 | Bioassay | Parratt & West (1957) |
| | 3·22±0·48 | Fluorometric | Aviado & Sadavongvivad (1970a) |
| Dog | 0·26 | Bioassay | Parratt & West (1957) |
| | 0·53±0·47 | Fluorometric | Aviado & Sadavongvivad (1970b) |
| Goat | 1·56 | Fluorometric | Pepeu & Giarman (1962) |
| | 3·69±0·47 | Fluorometric | Aviado & Sadavongvivad (1970b) |
| Human | 0·1 | Fluorometric | Sanders, Waalkes, Gilbert & Terry (1959) |
| | 0·30±0·03 | Fluorometric | Aviado & Sadavongvivad (1970b) |

of the rat indicate that the 5-HT stored in the lung in various species is uniformly sensitive. A similar depletion has been demonstrated for platelets (see references cited by Erspamer, 1966). The rapidity of the action of reserpine supports the suggestion that 5-HT in the lung could be stored in the platelets.

In the experiments reported above, 5-HT produced bronchoconstriction in the rat and guinea-pig. Bronchospasm due to 5-HT has been reported previously in the isolated guinea-pig trachea (Sinha & West, 1953 ; Balzer, Greeff & Westermann, 1956) as well as in the intact cat (Reid & Rand, 1952 ; Comroe, Lingen, Stroud & Roncoroni, 1953 ; Konzett, 1956) and dog (Rahamimoff & Bruderman, 1965). The predominant effect of 5-HT in most animal species is therefore bronchoconstriction.

Herxheimer (1955, 1957) observed the development of tolerance to 5-HT in the unanaesthetized guinea-pig by measuring the preconvulsion time, which is the period between exposure of the animal to 5-HT and the appearance of convulsion. The preconvulsion time was prolonged after several daily exposures to 5-HT. In the present investigation, however, the repeated intraperitoneal injection of a large dose of 5-HT did not reduce the intensity of the airway response to intravenous injection of 5-HT. Tachyphylaxis developed rapidly after several intravenous injections of 5-HT and also after a single large dose injected intraperitoneally. This indicates that, although acute tachyphylaxis can appear rapidly, the repeated daily injection of large doses of 5-HT does not produce tolerance. The mechanism that underlies the prolongation of the preconvulsive time in Herxheimer's experiments cannot be the same as the mechanism underlying the tachyphylaxis. It is therefore likely that the mechanism for the prolongation of preconvulsion time is conditioned by a slower absorption of 5-HT aerosol, or by physiological adaptation.

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