richest. These two eugenol-containing barks both contained acicular calcium oxalate crystals, but their fibres were greater than 40 μ in diameter. Hoppe (1958) gives eugenol as the main constituent of the oil of *C. culilawan*, but the specimen I examined yielded neither eugenol nor cinnamaldehyde.

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Effects of the amphetamine group on intraneuronal brain amines *in vivo* and *in vitro*

SIR,—Previous studies have shown that (+)-amphetamine in large doses causes a decrease in the brain content of noradrenaline (Smith, 1965). The present investigation was made to study the action of (+)-amphetamine at the cellular level with the help of the histochemical fluorescence method of Hillarp & others. The existence of central dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) neurones has recently been demonstrated by this technique. These neurones have been shown to contain specific mechanisms for uptake and storage of the amines. They have an uptake mechanism, probably localized at the level of the cell membrane and sensitive to, for example, desipramine or cocaine (see review by Hillarp, Fuxe & Dahlström, 1965). Furthermore, they possess a reserpine-sensitive storage mechanism localised in specific granules.

Single injections (i.p.) of (+)-amphetamine (5–60 mg/kg), (±)-amphetamine (15–60 mg/kg), methamphetamine (30 mg/kg) and benzylamphetamine (30 mg/kg) have been given to male, albino rats (Sprague-Dawley, 200–300 g). The animals were killed at 1, 2 or 3 hr after the injection. Pieces from all parts of the brain were dissected, freeze-dried and treated with formaldehyde gas (Dahlström & Fuxe, 1964). Fluorescence microscopic examination showed that (+)-amphetamine, 15–60 mg/kg, caused a fairly marked to marked decrease in number and intensity of the very fine catecholamine (mainly noradrenaline) terminals in, for example, the neocortex, the gyrus cinguli and the formatio reticularis of the lower brain stem. The fine to fairly thick catecholamine terminals of the hypothalamus, on the other hand, remained unaffected even with the higher doses. The dopamine and 5-HT terminals exhibited a normal appearance after all doses except with 60 mg/kg. After this dose, the dopamine terminals showed a distinct decrease in their amine contents. Somewhat less marked changes occurred after (±)-amphetamine and methamphetamine, while benzylamphetamine did not cause any definite decrease in the intraneuronal amine levels of the noradrenaline terminals.

These results are supported by *in vitro* studies on the central monoamine neurone with brain slices (for technical details, see Hamberger & Masuoka, 1965). Noradrenaline terminals in brain slices from neocortex after preincubation with noradrenaline, 10 μg/ml, and rinsing were markedly decreased in

number and intensity with a concentration of 1 μg (+)-amphetamine/ml in the incubation medium, while the noradrenaline terminals in hypothalamic brain slices were not significantly affected even with a concentration of 100 μg/ml. The dopamine terminals in the nucleus putamen were found to be intermediate in sensitivity, showing a distinct decrease of endogenous amine with a concentration of 10μg/ml.

Biochemical determination of noradrenaline and dopamine (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962) in whole brain after treatment with large doses of amphetamine (15–30 mg/kg, 1 and 2 hr before killing) demonstrated a distinct decrease in the brain content of noradrenaline (down to 50% of the normal value) but no certain changes of the dopamine levels.

TABLE 1. EFFECT OF (+)-AMPHETAMINE ON L-DOPA INDUCED MONOAMINE ACCUMULATION IN RAT BRAIN AND HEART

Drugs mg/kg			Heart		Brain		
Nialamide i.p.	(+)-Amphetamine i.p.	Dopa s.c.	Noradrenaline	Dopamine	Noradrenaline	Dopamine	3-Methoxytyramine
150	15	50	0·05	6·21	0·06	2·31	3·02
150	0	50	0·13	7·44	0·21	4·70	2·22
100	5	25	0·03	1·46	0·04	0·65	1·49
100	0	25	0·11	1·74	0·14	1·10	0·94
100	5	25	0·04	1·48	0·05	0·87	1·38
100	0	25	0·10	2·14	0·15	1·08	0·79

(+)-Amphetamine was given 45 min, dopa 30 min before killing. The rats were pretreated with reserpine (10 mg/kg i.p.) and nialamide (in doses indicated) 22 and 4 hr before death, respectively. Control rats were treated in the same way as the experimental animals except that no (+)-amphetamine was given. The values are single values of 3 pooled organs expressed in μg/g tissue.

To study the effect of the (+)amphetamine on the so called "membrane pump" another kind of experiment was made. Rats of the same stock and the same techniques were used. (+)-Amphetamine, 5–20 mg/kg i.p., was administered to animals pretreated with reserpine (10 mg/kg i.p., 24 hr before killing) and nialamide (100 mg/kg, i.p., 4–6 hr before killing), 15 min before a dose of 3,4-dihydroxyphenylalanine (L-dopa) (50 mg/kg s.c., 30 min before killing). Brains of animals treated only with reserpine - nialamide - L-dopa showed in the fluorescence microscope large amounts of strongly green fluorescent dopamine and noradrenaline terminals in most parts, due in all probability to an uptake and decarboxylation of L-dopa (*cf.* Fuxe, 1965). The amines formed are, however, not bound to the granules, since these have been blocked by reserpine. If (+)-amphetamine is administered in doses down to 5 mg/kg, and possibly lower, before the L-dopa injection, the fluorescence microscopic picture is found to be quite different. The noradrenaline and dopamine terminals become only weakly fluorescent after the L-dopa injection or remain non-fluorescent. Thus, considerably less amounts of catecholamines are detected in the terminals. Biochemical determinations showed (Table 1) that the noradrenaline and dopamine levels in the brain were considerably reduced and that the levels of 3-methoxytyramine (Carlsson & Lindqvist, 1963; Carlson & Waldeck, 1964) were increased after reserpine-nialamide-(+)-amphetamine-L-dopa treatment compared to the levels obtained after reserpine-nialamide-L-dopa treatment. The noradrenaline levels in the heart were also reduced, while no consistent effect on the dopamine levels was observed.

These results support the view that amphetamine at least partly acts by blocking the membrane pump of both the noradrenaline and dopamine neurones. This conclusion is also supported by the results from *in vitro* studies on brain

slices from neocortex and brain stem of reserpinized animals, which showed a marked to complete inhibition of uptake of α-methyl noradrenaline (1 μg/ml) in the catecholamine terminals if amphetamine was present in the bath. The concentration of amphetamine ranged from 100 μg/ml down to 1 μg/ml.

Studies on peripheral adrenergic terminals in slices of vas deferens from reserpinized animals show that the uptake of α-methylnoradrenaline (1 μg/ml) is markedly inhibited also after incubation with (+)-amphetamine (1 μg/ml). These results agree with previous studies on the effect of (+)-amphetamine on the peripheral adrenergic nerve terminals (Carlsson & Waldeck, 1965; Malmfors, 1965), demonstrating a block of the membrane pump after treatment with (+)amphetamine. Thus, no fundamental differences seem to exist between the peripheral and central catecholamine neurones in their reaction to amphetamine.

The present investigation indicates that at least one of the sites of action of amphetamine is on the membrane pump. The experiments also suggest an effect of large doses of the drug directly on the granules. This assumption is based on the fact in contrast to (+)-amphetamine the potent membrane blocker desipramine has never been observed to decrease the noradrenaline levels of the brain, not even with very high doses. The present experiments also demonstrate differences in sensitivity towards amphetamine between the various noradrenaline terminal systems of the brain, which cannot be accounted for by differences in impulse flow, since these differences were present also *in vitro*. There remains to be explained, however, why the pharmacological effects of amphetamine occur after reserpine treatment (Rech, 1964; Smith, 1965). This need not be due to a direct stimulatory effect on the receptor site since the synthesis of amines continues in spite of reserpine treatment and, thus, (+)-amphetamine by means of its action on the membrane pump or some other unknown actions may release sufficient quantities of amines to act on the postsynaptic membrane.

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An improvement on Vane's stomach strip preparation for the assay of 5-hydroxytryptamine

SIR,—Vane (1957) described a practical and sensitive preparation for the assay of 5-hydroxytryptamine (5-HT) using a strip of fundus from the rat stomach. Since then, this preparation has been widely used by others. Two difficulties were encountered in practical application: the slowness of the muscle to relax after responding to 5-HT and the fluctuation in the resting length of the muscle. These were overcome by stretching the muscle for 15 sec after each contraction. A working cycle required at least 4 min with this procedure.

Because of a need to assay over 30 samples of intestinal perfusate for 5-HT at one time, we have modified Vane's preparation to obviate the need for stretching the muscle after each contraction and also to shorten the time cycle.

The modifications we have made are:

(1) The manner of cutting the stomach strip was slightly different, five instead of six incisions being made, three from the fundus end of the opened-out plate of tissue alternating with two incisions made from the pyloric end of the plate.

(2) An auxotonic frontal writing pendulum lever was used. The small counterweight can be varied to give the required tension to the strip. The baseline position of the lever was set at about 10° below the horizontal and had a load of 2·5 g at this position.

(3) On setting up the preparation, it was left to stretch in the organ bath for 2 hr with the physiological solution flowing through at a rate of about 30 drops/min.

(4) A magnesium-free Krebs solution was used: (g/litre) NaCl, 6·92; KCl, 0·353; $CaCl_2$, 0·282; KH_2PO_4, 0·161; $NaHCO_3$, 2·1; glucose, 2·0. The solution was oxygenated with a 3% carbon dioxide and 97% oxygen mixture.

(5) A temperature of 39·5° for the organ bath was used. At this temperature the muscle responded and relaxed more rapidly than at 37°.

Under these conditions the sensitivity of response of the muscle to 5-HT was adequate, a 1 ng dose (in 6·5 ml bath volume) normally causing a recorded

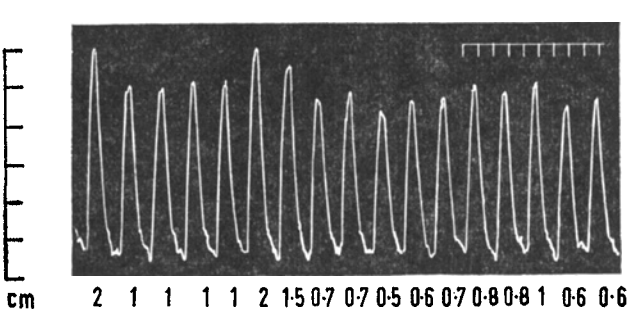

FIG. 1. Record of response of the stomach strip to various doses of 5-HT in ng added to the organ bath (volume 6·5 ml). Time in min. Scale in cm.