The high activity of tryptophan mustard suggested the possibility that this alkylating agent was competing with the transport of tryptophan through the blood brain barrier. However, the administration of L-tryptophan (800 mg/kg i.p. or 100 μg/rat s.c.) did not protect against the neurotoxicity of 1 or 5 μg/rat of tryptophan mustard given intracerebrally. These observations may influence the choice of drugs to be injected locally in the chemotherapeutic treatment of brain tumours.

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Two different mechanisms for incorporation of ^{3}H-metaraminol into the amine-storing granules

SIR,—In a previous paper it was reported that metaraminol is incorporated into the adrenal medullary granules *in vitro* by a mechanism which does not utilise Mg^{++} and ATP (Lundborg, 1966). This mechanism is not influenced by reserpine. But it has also been shown that the ability of the heart to retain metaraminol is considerably blocked by reserpine (Shore, Busfield & Alpers, 1964; Carlsson & Waldeck, 1965). To elucidate this apparent discrepancy between *in vitro* and *in vivo* evidence the following experiments were made.

Mice, in groups of six, were given ^{3}H-metaraminol 0·04 mg/kg i.v. alone or preceeded 6 hr before by reserpine 10 mg/kg i.p. At various intervals after ^{3}H-metaraminol had been given the animals were killed. The hearts were removed and homogenised with a plastic pestle in 0·25 M sucrose containing

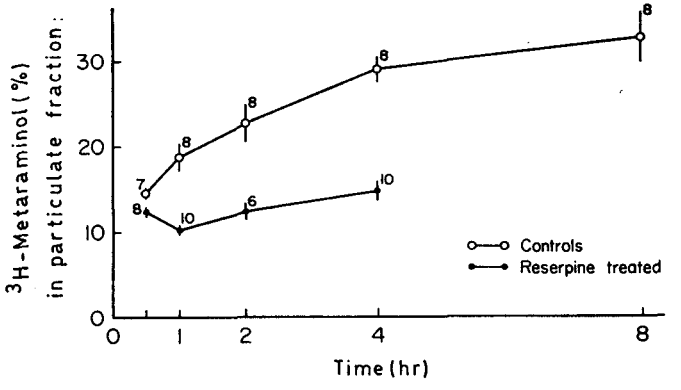

FIG. 1. Subcellular distribution of ^{3}H-metaraminol in the mouse heart. The results are expressed as ^{3}H-metaraminol in the particulate fraction as percentage of ^{3}H-metaraminol in the particulate + supernatant fractions. The bars indicate s.e.m. and the figures the number of experiments. For experimental details see text.

0·005 M phosphate buffer at pH 7·4 and 0·001 M $MgCl_2$. The homogenate was centrifuged at 2,000 × *g* for 10 min at 4° and the supernatant fluid was re-centrifuged at 100,000 × *g* in a Spinco 40 rotor for 60 min. The amount of ^{3}H-metaraminol in the "coarse" fraction (sediment 1), "particulate" fraction (sediment 2) and supernatant fractions was determined by liquid scintillation counting.

In the coarse fraction the amount of ^{3}H-metaraminol was almost constantly 30% of the total. Our results, therefore, will be expressed as the amount of ^{3}H-metaraminol in the particulate fraction as percentage of ^{3}H-metaraminol in the particulate fraction + supernatant fraction.

If ^{3}H-metaraminol is given alone there is a gradual increase of ^{3}H-metaraminol in the particulate fraction, from 14·5% after ½ hr to 32·7% after 8 hr (Fig. 1). During the same time the total amount of ^{3}H-metaraminol in the pooled 6 hearts of every group changed from 33·9 ng ± 3·5 (s.e.m.) to 27·6 ng ± 1·3. When reserpine was administered before metaraminol the percentage of metaraminol in the particulate fraction was constantly at a level of 10 to 15% while the total amount of metaraminol in the hearts changed from 26·0 ng ±1·3 after ½ hr to 7·6 ng ±0·7 after 4 hr.

Apparently there is an uptake of metaraminol in the particulate fraction even after reserpine pretreatment. This is in agreement with the *in vitro* data mentioned above, that there is a reserpine-resistant uptake of metaraminol in the amine granules. If not reserpine-treated, however, the granules seem to gradually incorporate more metaraminol. This reserpine-sensitive process is apparently so slow that it is hardly observed in an *in vitro* experiment.

These observations suggest the possible existence of at least two different mechanisms, both of importance for the incorporation of amines into the storage granules. The first, a reserpine-resistant uptake, the equilibrium being achieved rather soon. The second, a reserpine-sensitive mechanism, probably identical with the ATP-Mg^{++}-dependent uptake mechanism observed *in vitro* by Carlsson, Hillarp & Waldeck (1962, 1963) and by Kirshner (1962a, b). In the instance of ^{3}H-metaraminol (Lundborg, 1966) the incorporation is too slow to be observed *in vitro*. *In vivo*, however, ^{3}H-metaraminol may be gradually incorporated by a reserpine-sensitive mechanism resulting in an efficient retention of the amine.

A slow incorporation of metaraminol in the particulate fraction has also been observed by Giachetti & Shore (1965).

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The relation between vanilyl mandelic acid and 5-hydroxyindoleacetic acid excretion in a patient with a carcinoid tumour

SIR,—There is convincing evidence from experiments with several species (Werle & Aures, 1959; Rosengren, 1960; Lovenberg, Weissbach & Udenfriend, 1962; Reid, Riley & Shepherd, 1963; Reid & Shepherd, 1964) that the non-specific histidine decarboxylase, dopa- and 5-hydroxytryptophan decarboxylases are, in fact, one and the same enzyme. An opportunity to study this relationship in man was afforded recently by a carcinoid tumour showing an exceptionally high output of 5-hydroxyindoleacetic acid (5-HIAA).

5-HIAA was estimated by the method of Macfarlane and others (1956) and vanilylmandelic acid (VMA) by Connelian & Godfrey's (1964) modification of the method of Pisano, Crout & Abraham (1962), in 24-hr urine specimens collected from the patient (F.N., male, age approx. 50 years) over a period of six months During most of this time the patient was under treatment with methysergide to reduce the symptomatic effects of released 5-HT. The results are recorded, in chronological order, in the Table 1.

TABLE 1. VMA AND 5-HIAA CONTENT OF URINE SPECIMENS FROM A MALE WITH CARCINOID SYNDROME DURING SIX MONTHS

	Urinary excretion, mg/24 hr	
Date	VMA	5-HIAA
25/3/65	7·12	336
26/3/65	8·00	417
27/3/65	9·92	510
28/3/65	9·18	538
9/4/65	10·20	680
21/4/65	9·18	500
4/5/65	8·88	410
7/5/65	10·08	590
31/5/65	9·02	548
12/7/65	11·00	575
5/9/65	13·90	692
1/10/65	7·16	275
Upper limit of normal	7·00	10·0
	Correlation between VMA and 5-HIAA: $r = 0{\cdot}859$; $P = <0{\cdot}01$	

The highly significant correlation of the excretion levels of the metabolites of 5-HT and catechol amines supports the theory of a common decarboxylase responsible for the formation of these amines. On the other hand, in the carcinoid tumour, 5-HIAA formation is very much more rapid than VMA formation, since, in this particular patient, the output of 5-HIAA is increased to as much as 70 times the normal level, whereas the highest value for VMA excretion is only twice that of the upper limit of normal. In fact, there is no noticeable increase in VMA excretion above the normal range until the 5-HIAA excretion rises to 300–400 mg/24 hr, so that it would be unlikely that this relation would be observed in the average case of carcinoid, in which the 5-HIAA output rarely exceeds 100 mg/24 hr. This would suggest that there is a big difference in the