produced typical pressor responses. In three of the animals given pargyline, this response was decreased in magnitude by doses of 40 mg/kg. In the two other cats, iproniazid (50 mg/kg) caused similar effects.

These experiments indicate that the presence of the medullary vasomotor centre is necessary to prevent the peripheral hypertensive action of pargyline. Further, reflexly-induced hypertensive responses are reduced by both pargyline and iproniazid.

Pargyline (as well as some other monoamine oxidase inhibitors) may have two antagonistic actions, a peripheral hypertensive effect resulting from an increase in the concentration of circulating catecholamines and a stronger depressant action on vasomotor centres in the central nervous system. The result is a reduction in blood pressure and the postural hypotension which is observed clinically.

Department of Pharmacology, T. E. LYNES
Georgetown University School of Medicine,
Washington, D.C., U.S.A.
August 5, 1966

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On the neurotoxic effects induced by alkylating agents

SIR,—During some experiments made to evaluate the chemotherapeutic effect of DL-sarcolysine applied topically to a cerebral tumour of the rat, signs of neurotoxicity were observed. We therefore investigated the central effect of several alkylating agents injected intracerebrally in normal rats. The symptoms observed, the relative potency, and some attempts to protect the animals are reported.

Male Sprague-Dawley rats, 160 $\pm$10 g, were kept in Makrolon cages (47 $\times$ 26 $\times$ 15 cm) 6 per cage at a constant temperature of 22° and relative humidity of 60%. They had free access to food (Diet Alal 56 Alal, Milan) and water until the beginning of the experiment.

The intracerebral injection was made under light ether anaesthesia through the squamopetrosal fissure of the temporal area of the skull (Valzelli, 1964).

The drugs were dissolved in distilled water and injected in 0·02 ml volumes. The intracerebral injection of the solvent never induced any appreciable symptoms and the animals completely recovered from anaesthesia in about 10 min. The mortality was calculated after a period of 24 hr.

The drugs used were: DL-sarcolysine, L-sarcolysine, D-sarcolysine, glycine mustard, alanine mustard, cyclophosphamide, chlorambucil, 6-diazo-5-oxo-L-norleucine (DON), azaserine (all from CCNSC, N.I.H. Bethesda), tryptophan mustard (Dr. L. Otis, Stanford Research Institute, Palo Alto, California), degranol (Medimpex, Budapest), mustine, and tretamine (Simes, Milan), phenobarbitone sodium (Bayer), thiourea (Erba, Milan), phenytoin sodium (Recordati, Milan).

Several alkylating agents, when injected intracerebrally in rats, produce signs of neurotoxicity. The symptomatology is not entirely comparable for all drugs. However, a typical pattern, as with DL-sarcolysine, includes a latency time of about 30–60 min, signs of central stimulation, inco-ordinate movements, sterotyped behaviour ending with clonic convulsions and occasional tonic extensions. By increasing the dose, the latency time decreased and eventually the animals died. With DL-sarcolysine, aggregation of the animals did not change the latency time. Thus the time before the appearance of the first convulsion was 52 $\pm$6 min when the rats were isolated and 70 $\pm$9 min when the rats were kept 5 in a cage.

Table 1 summarises the neurotoxic dose (ED50) and the lethal dose (LD50) for each drug injected intracerebrally. Mustine, with a latency time of about 10 hr, induced only signs of spasm which never reached the stage of convulsions.

TABLE 1. NEUROTOXICITY OF VARIOUS ANTITUMOUR DRUGS GIVEN INTRACEREBRALLY

No. of rats	Drug	Neurotoxic activity ED50 (μg/rat i.c.)	LD50 (μg/rat i.c.)
150	DL-Sarcolysine	23 (39 – 13)**	40 (72 – 22)**
36	L-Sarcolysine	20 (27 – 14)	32 (48 – 21)
60	D-Sarcolysine	13 (22 – 7)	18 (30 – 11)
36	*m*-DL-Sarcolysine	47 (77 – 28)	~100
53	Glycine mustard	18 (23 – 14)	18 (21 – 15)
78	Alanine mustard	25 (29 – 21)	36 (46 – 28)
48	DL-Tryptophan mustard	1·1 (1·4 – 0·8)	4·3 (7·3 – 2·5)
18	Mannitol mustard	>100	>100
48	Mustine	*	20 (48 – 8)
18	Tretamine	>100	>100
18	Cyclophosphamide	>100	>100
18	Chlorambucil	>100	>100
18	DON	> 50	> 50
18	Azaserine	>100	>100

* = A non typical symptomatology was observed with doses from 10 to 100 μg/rat.
** 95% fiducial limits.

All the mustard derivatives of amino-acids showed a typical pattern of neurotoxicity. The most active drug was the tryptophan mustard followed by D-sarcolysine and then DL-sarcolysine, L-sarcolysine and the nitrogen mustards of alanine and glycine. *m*-DL-Sarcolysine was less convulsant than the corresponding isomer *p*-DL-sarcolysine. The mannitol mustard and other alkylating agents including tretamine, cyclophosphamide and chlorambucil were not neurotoxic up to the dose of 100 μg/rat. DON and azaserine were tested for comparative purposes because of their chemical relation with amino-acids, but they did not show any marked effect. When DL-sarcolysine was inactivated by boiling, it lost its neurotoxic effect.

Attempts were made to protect against this neurotoxic action with anticonvulsant drugs. Phenytoin sodium (100 mg/kg i.p.) was ineffective, while sodium phenobarbitone showed a clear protective effect. In one experiment the ED50 of DL-sarcolysine changed from 15 to 50 μg/rat when phenobarbitone was given at the dose of 100 mg/kg i.p. 30 min before the intracerebral injection. The LD50 of DL-sarcolysine injected intracerebrally changed from 29 to 100 μg/rat. Also, radioprotectors were given in an attempt to decrease the neurotoxicity of DL-sarcolysine, but cysteine (1 g/kg i.p.) or thiourea (1 g/kg i.p.) were found to be inactive. It is interesting to note that previous experiments demonstrated an antagonism between these two radioprotectors and DL-sarcolysine when the latter was given intraperitoneally (Garattini, Palma & Reyers, 1965; Garattini, Palma, Reyers-Degli Innocenti & Guaitani, 1966).

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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Istituto di Ricerche Farmacologiche, "Mario Negri,"
Via Eritrea, 62, Milan,
Italy.

M. G. DONELLI
R. ROSSO
S. GARATTINI

August 4, 1966

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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

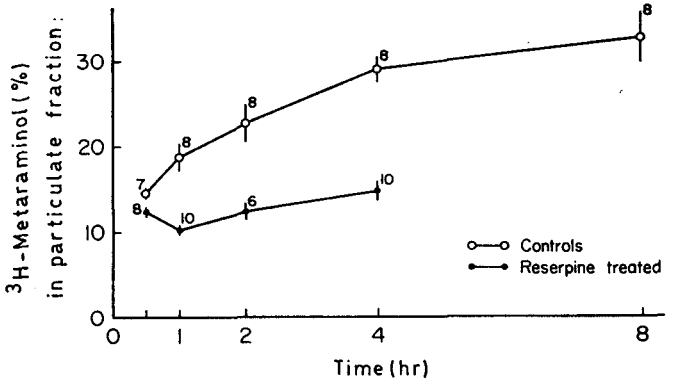

FIG. 1. Subcellular distribution of ^{3}H-metaraminol in the mouse heart. The results are expressed as ^{3}H-metaraminol in the particulate fraction as percentage of ^{3}H-metaraminol in the particulate + supernatant fractions. The bars indicate s.e.m. and the figures the number of experiments. For experimental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