& Conti, 1964). The results obtained with some selected compounds—reported in Table 1—show the following:

(1) The presence of the NH_2-group on the C-6 of the nucleus increases the antiphlogistic activity independently from the length and the shape of the side-chain. The acute toxicity is unchanged or diminished.

(2) The replacement of the methyl group on the side-chain with an ethyl-group led to an increase of the antiphlogistic activity.

(3) The branching of the side-chain led to a reduction of the antiphlogistic activity.

(4) The presence of the chloroethyl-group on the side-chain confers a more pronounced anti-inflammatory activity, particularly when its presence is accompanied by the introduction of NH_2-group on the C-6 of the nucleus.

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Role of the polymethylene chain in derivatives of demecarium bromide on the inhibition of monoamine oxidase

SIR,—Inhibition of monoamine oxidase has been shown by several structurally unrelated compounds. We have recently reported (Pant, Parmar & Bhargava, 1964) that demecarium bromide [decamethylene-bis(3-dimethylaminophenyl *N*-methylcarbamate)dimethobromide], a potent inhibitor of brain acetylcholinesterase inhibits monoamine oxidase present in isolated rat liver mitochondria. In the present study the effect of the tetramethylene, hexamethylene, octamethylene and dodecamethylene derivatives as well as the decamethylene derivative has been investigated on monoamine oxidase in rat liver mitochondria to show the effect of the number of methylene groups connecting the two neostigmine molecules present in these compounds.

Monoamine oxidase activity was determined by the conventional Warburg manometric method (Creasey, 1956). The oxygen uptake has been shown by Parmar & Nickerson (1961) to reflect the true enzyme activity during oxidative deamination of tyramine. The effect of these compounds was also investigated on the oxidation of tryptamine. The inhibition of monoamine oxidase produced by these compounds at the final concentration of 8×10^{-5}M using tyramine and tryptamine as substrates is shown in Table 1. Inhibition of the enzyme increased with increase in the number of methylene groups in the compounds during oxidative deamination of tyramine or tryptamine. The compound with 4 methylene groups had no inhibitory effect on the enzyme activity at this concentration. Further increase in its concentration to $3{\cdot}2 \times 10^{-3}$M also produced no

inhibition of enzyme. In the absence of substrate no oxygen uptake could be observed with these agents even at 3×10^{-3}M. Furthermore, the degree of inhibition was unaltered by pre-incubation of the enzyme preparation with these compounds for varying length of time before the addition of either tyramine or tryptamine. The parent substance, neostigmine, has been shown to have no inhibitory effect on monoamine oxidase under similar experimental conditions (Pant, Parmar & Bhargava, 1964).

TABLE 1. INHIBITION OF RAT LIVER MITOCHONDRIAL MONOAMINE OXIDASE

Substrate	Inhibition %				
	$[CH_2]_4$	$[CH_2]_6$	$[CH_2]_8$	$[CH_2]_{10}$	$[CH_2]_{12}$
Tyramine	0·0	15·6	17·9	37·0	55·4
	0·0	11·0 (13·5)	16·1 (17·0)	41·0 (39·0)	52·2 (52·9)
	0·0	14·0	17·1	39·2	51·2
Tryptamine	0·0	9·8	18·0	41·0	88·4
	0·0	6·0 (8·1)	11·3 (15·3)	42·2 (41·3)	71·0 (78·7)
	0·0	8·6	16·5	40·8	76·8

Per cent inhibition was calculated from the decrease in the oxygen uptake. Each reaction vessel contained mitochondria equivalent to 250 mg of fresh rat liver tissue; 0·01M tyramine or tryptamine in a total volume of 3·0 ml with 0·066M phosphate buffer pH 7·4. The inhibitors present in the side arm at the final concentration of 8×10^{-5}M were incubated with the enzyme preparation for 10 min before adding either tyramine or tryptamine. The enzyme system was then incubated at 37° for 1 hr in an atmosphere of oxygen. Figures in parentheses are averages.

On the basis of these observations it can be assumed that the polymethylene chain seems to be involved in the binding of these compounds to the active site(s) to produce enzyme inhibition. A similar explanation on the role of an alkyl chain in monoamine oxidase inhibition was put forward by Barsky, Pacha, Sarkar & Zeller (1959) who found monosubstitution of the second nitrogen atom of isonicotinic acid hydrazide produced optimal inhibition with the butyl derivative. At present it is difficult to provide an explanation for increase in the inhibition of the enzyme along with increase in the number of methylene groups present in the chain and also why the compounds should inhibit two different enzymes, monoamine oxidase and brain acetylcholinesterase, the former being an oxidative enzyme which chances to be a flavoprotein (Belleau & Moran, 1963) and the latter a hydrolase without any cofactor requirements. Further studies with these compounds may prove useful in the elucidation of the nature of the active site(s) of the enzyme monoamine oxidase and its role in physiological functions.

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