

Table 1. Frequency distribution of attack scores for *D. tigrina* as a function of length of fast and time of day tested.

Score interval	Length of fast			
	45 to 90 hr		98 hr	
	Day	Night	Day	Night
$s = 0$	6	6	5	2
$0 < s \leq 0.1$	0	2	3	1
$0.1 < s \leq 0.4$	0	0	6	2
$0.4 < s \leq 0.6$	0	0	2	4
$0.6 < s \leq 0.8$	0	0	1	2
$0.8 < s \leq 1.0$	0	0	1	8
$1.0 < s \leq 1.5$	0	0	0	3
$1.5 < s \leq 3.0$	0	0	0	6

or illumination. Available facilities did not permit conclusion in regard to the "driven oscillator" model (4) as the means of synchronization to the solar day. Other species (5) exhibit similar diurnal rhythms in behavior (6).

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#### References and Notes

- Species supplied as *D. dorotocephala* by Carolina Biological Supply Co., Elon College, N.C., but although black pigmented do not otherwise resemble these and have been identified as *C. foremani* by C. A. Miller and W. H. Johnson, *Ann. N.Y. Acad. Sci.* **77**, 87 (1959).
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- A. M. Mood, *Introduction to the Theory of Statistics* (McGraw-Hill, New York, 1950), p. 395.
- C. S. Pittendrigh, et al., *Proc. Natl. Acad. Sci. U.S.A.* **44**, 965 (1958); V. G. Bruce and C. S. Pittendrigh, *Am. Naturalist* **92**, 295 (1958).
- G. P. Wells, *Symp. Soc. Exptl. Biol.* No. 4, 127 (1950); F. M. Baldwin, *J. Animal Behavior* **7**, 187 (1917); J. Arbit, *Science* **126**, 654 (1957); F. A. Brown, Jr., et al., *J. Exptl. Zool.* **123**, 29 (1953); *Sci. American* **190**, 34 (1954).
- I wish to acknowledge the assistance of Mr. David Schucker in conducting these observations.

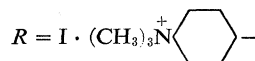
15 March 1960

### New Quaternary Ammonium Compounds with Adrenomimetic Action

**Abstract.** Quaternary salts of two triesters of *p*-dimethylaminothiophenol and phosphoric acid or thiophosphoric acid have effects similar to epinephrine on blood pressure, heart rate, and the nictitating membrane of the anesthetized cat. Two analogous compounds derived from phenol are depressor. None is a potent anticholinesterase. Further investigation is desirable because of the possibility of a relatively specific action on sympathetic ganglia.

In the course of a study of anticholinesterases, certain triaryl phosphate esters bearing quaternary ammonium groups were synthesized (1) which failed to show the expected activity. For four of these, the code number,

structure, and relative activity in vitro as cholinesterase inhibitors compared to neostigmine are: CP46,  $(RO)_3P=S$ , 1.8; CP99,  $(RS)_3P=O$ , 0.53; CP98,  $(RS)_3P=S$ , 3.8; CP27,  $(R'O)_3P=S$ , 0.25; neostigmine 100, where



and  $R'$  is the corresponding *meta* isomer of  $R$ . This is in marked contrast to related quaternary ammonium phosphate esters, such as the dialkoxyposphoryl esters of *m*-dimethylaminophenol methiodide (phosphostigmines) (2), which are potent inhibitors of cholinesterase. The low activity of compound 27 indicates that this is not a question of *meta* as against *para* orientation. All of these compounds were tested on the anesthetized cat for effects on blood pressure, respiration, heart rate, and the nictitating membrane.

Animals were anesthetized with Dial. Blood pressure was recorded with the Sanborn electromanometer; respiration, with a Krogh spirometer recycled four times per minute, from which rate and minute volume could be read; heart rate was recorded with a Thorp counter integrated over a 10-second interval; and contractions of the nictitating membrane, with an isotonic lever. All injections were given into the femoral vein. Test doses of acetylcholine (1  $\mu$ g/kg) and of epinephrine (1 to 8  $\mu$ g/kg) were given before and after injection of the experimental compound.

None of these substances induced muscle fasciculation or the typical profound muscarinic effects characteristic of systemically administered anticholinesterases. Compounds 27 and 46 were found to be moderately long-acting depressors. Compound 27 has been studied clinically in peripheral vascular disease as a vasodilator (3). Its mechanism of action is in doubt and does not fall into any obvious classification. Compounds 98 and 99, on the other hand, were pressor and their actions were as follows: compound 99 showed a minimal effect on the blood pressure at 0.1 mg per kg which consisted in a slight and transient rise or fall or biphasic response. Similar effects were seen with epinephrine at 1  $\mu$ g/kg. At higher doses of compound 99 the blood pressure was consistently elevated, sometimes showing a bimodal curve as illustrated in Fig. 1. The onset and duration of the pressor action appeared to be the same for compound 99 and epinephrine at equally pressor doses. The heart rate did not change markedly and moved in the same direction as after test doses of epinephrine. In small to moderate doses, compound 99 caused a transient stimulation of respiratory minute volume without alteration in rate. At high doses, 6 to 12 mg/kg,

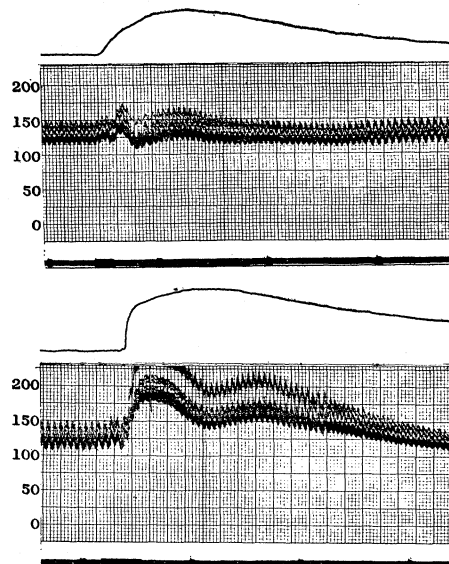


Fig. 1. Female cat, 2.7 kg, Dial anesthesia. From top to bottom: isotonic contraction of the nictitating membrane, blood pressure in millimeters of mercury, time in minutes. At the signal in the upper tracing epinephrine (0.004 mg/kg) was injected. At the signal in the lower tracing compound 99 (2 mg/kg) was injected. Drugs were given intravenously.

the animals died as a result of immediate respiratory arrest. Contraction of the nictitating membrane occurred at dosage levels showing effects on blood pressure, and the two effects had the same duration. A fall in blood pressure was observed with both compound 99 and epinephrine in animals previously given 15 mg of dibenamine per kilogram intravenously in three divided doses. Duration was comparable to that of the pressor effect before dibenamine.

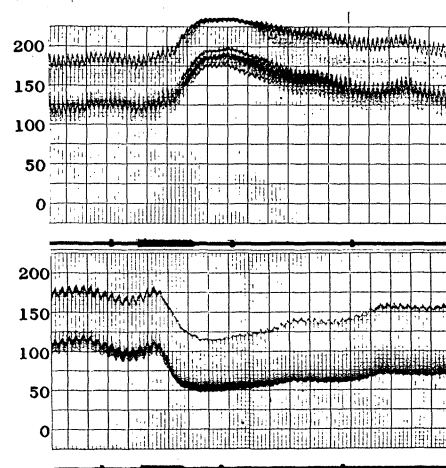


Fig. 2. Female cat, 3.2 kg, Dial anesthesia. From top to bottom: blood pressure in millimeters of mercury, time in minutes. At each signal compound 98 (5.3 mg/kg) was injected. Between the upper and lower tracings three doses of dibenamine (5 mg/kg) were given. Drugs were given intravenously.

The actions of compound 98 on heart rate, blood pressure, respiration, and nictitating membrane responses were, in general, the same as for compound 99, except that minimal effects were first seen at about 0.5 to 1 mg/kg, and death from respiratory arrest occurred at from 10 to 20 mg/kg. The effect of dibenamine on the blood pressure response to compound 98 is shown in Fig. 2.

Neither compounds 98 nor 99 exhibited tachyphylaxis or potentiation as a result of repeated doses given over a period of a few hours, and neither modified the control response to test doses of epinephrine or acetylcholine.

The mechanism of action of compounds 98 and 99 remains to be determined, but certain presumptions may be made on the evidence available about the effects observed in the cat. Since they are quaternary ammonium compounds, they are in all probability excluded from the central nervous system. For the same reason, action on sympathetic nerve endings is unlikely. Hence their pressor effect would appear to be due to a nicotinic action on autonomic ganglia. A relative predilection for sympathetic ganglia is suggested by the absence of marked parasympathetic phenomena. It is of interest that there was no evidence of depression of ganglia after repeated doses. Rapid destruction of these phosphate esters seems likely because of their short duration of action and lack of cumulative effects. The pharmacologic actions observed do not appear to have been reported previously among phosphate esters and specificity of any degree for sympathetic ganglia is rare. Further study of this class of compounds would therefore seem to be indicated.

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#### References and Notes

1. The compounds described were prepared in these laboratories by Dr. H. M. Fitch from the appropriate phenols and chlorophosphates, using conventional methods.
2. A. S. V. Burgen and F. Hobbiger, *Brit. J. Pharmacol.* **6**, 593 (1951).
3. O. A. Rose and A. Ebel, *J. Am. Geriatrics Soc.* **4**, 142 (1956).
- 4 March 1960

#### Self-Absorption Correction for Carbon-14 Assay

R. W. Hendler has proposed a method to derive self-absorption corrections for carbon-14 assay [*Science* **130**, 772 (1959)]. His method consists in the determination of the apparent specific activities for various weights of

Table 1. Slopes and intercepts of tangents to the initial and final part of the self-absorption correction curves  $F$ . The tangents were constructed by drawing a line connecting the two lowest and two highest integer values on the curve. (Thus for No. 1, 5 and 6 mg, and 21 and 22 mg.)

No.	Source	Preparation	Area (cm <sup>2</sup> )	Range (mg)	Tangents of $F$			
					Initial		Final	
					Slope	Intercept	Slope	Intercept
1	J. Katz	BaC <sup>14</sup> O <sub>3</sub> on filter paper	3.14	5-22	0.018	0.68	0.049	0.17
2	J. Katz	BaC <sup>14</sup> O <sub>3</sub> on filter paper	2.55	6-18	0.023	0.65	0.071	0.04
3	Veterans Administration Hospital, Los Angeles, California	BaC <sup>14</sup> O <sub>3</sub> on filter paper	4.50	8-38	0.016	0.58	0.035	0.30
4	Department of Physiology, Univ. of California, Berkeley	BaC <sup>14</sup> O <sub>3</sub> on filter paper	19.5	10-85	0.011	0.60	0.013	0.37
5	Department of Biology, California Institute of Technology, Pasadena	C <sup>14</sup> protein on steel	2.85	5-21	0.027	0.91	0.046	0.61
6	Katz and Golden [J. Lab. Clin. Med. <b>53</b> , 658 (1959)]	BaS <sup>35</sup> O <sub>4</sub> on glass filter paper	3.14	4-25	0.032	0.59	0.048	0.35

the radioactive compound. An apparent specific activity at a convenient arbitrary weight is taken as the reference activity, and the ratio of the apparent specific activities at any weight to the reference activity is designated as the conversion (or correction) factor  $F$ . By multiplying by  $F$ , the apparent specific activity at any weight can be converted into the standard specific activity. The novel aspect of Hendler's paper is the finding that when  $F$  is plotted against weight of sample, a straight line results.

The empirical procedure described by Hendler has been in common use in many laboratories to determine self-absorption corrections for BaC<sup>14</sup>O<sub>3</sub> and other C<sup>14</sup> compounds, and it has also been described in detail for BaS<sup>35</sup>O<sub>4</sub> [J. Katz and S. Golden, *J. Lab. Clin. Med.* **53**, 658 (1959)]. However, as far as I am aware,  $F$  has never been found to be a linear function of sample weight.

I have at hand six conversion tables (summarized in Table 1); four tables are for BaC<sup>14</sup>O<sub>3</sub>, one for BaS<sup>35</sup>O<sub>4</sub>, and one for C<sup>14</sup>-protein. Two of the tables for BaC<sup>14</sup>O<sub>3</sub> and the one for BaS<sup>35</sup>O<sub>4</sub> were prepared by myself; the rest have been in use for many years in three other laboratories. The BaCO<sub>3</sub> and BaSO<sub>4</sub> were prepared by collecting the precipitate with suction on filter paper; the C<sup>14</sup>-protein, by evaporating aliquots in planchets. The diameter of the samples ranges from 18 to 48 mm, and the weight ranges from 5 to 85 mg. For radioassay mica end-window and gas-flow Geiger tubes of several types were used, one of the tubes (the Micromil gas-flow counter) being identical with one used by Hendler.

When the values of the conversion factor for all six tables were plotted against sample weight, the resulting curves were not linear, but increased continuously in slope. The tangent to the upper portion of these curves tended to pass through the origin. While it was difficult to extrapolate to zero mass, it seemed that, at least with two curves, the curve tends to become parallel to the abscissa.

An elementary consideration of the character of self-absorption explains the nature of the curves described above. When the region of "infinite thickness" is approached the count becomes maximal and constant, and the apparent specific activity, when the weight increases, becomes simply inversely proportional to the sample weight.  $F$  then becomes directly proportional to weight. (For instance, take the count rate at infinite thickness, at 50 mg, as 500 count/min and the reference specific activity as 20 count/min per milligram. Then  $F$  at 50, 100, 200, and 400 mg would be 2, 4, 8, and 16). Obviously, then, in this region the plot of  $F$  is a linear function of weight and when extrapolated will pass through the origin.

Consider now the region of very low mass, where the apparent specific activity is almost independent of mass. In that region  $F$  will tend to become constant and the slope will be very small.  $F$  will intersect the ordinate above the origin (see Hendler). The curve between the two regions obviously will increase in slope, reaching a constant maximal value at the region of infinite thickness, and only then become linear, and when extrapolated will intersect the origin. Our experi-