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Action of Prostigmine, Carbaminoyl-choline (Doryl) and Acetyl-B-methyl-choline (Mecholyl) on Intestine of a Cladoceran.

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In a previous work it was demonstrated by Obreshkove[1,2] that it is possible to study the physiological action of acetylcholine and physostigmine by observing their effects on the intestine and the heart of *Daphnia magna*. The results obtained were of such a nature as to suggest strongly some known facts pertaining to the role which these drugs have been said to play in the transmission of nervous impulses. Although there has not been demonstrated in Cladocera anything which corresponds morphologically to the autonomic nervous systems in vertebrates, if the intestine of *Daphnia magna* is touched with a fine glass needle at the bend of the digestive tube where the intestine enters the stomach, the heart immediately stops beating and the posterior portion of the digestive tube commences to exhibit powerful intestinal contractions. The heart in this respect behaves like the inhibition produced after electrical excitation of the vagus nerve in vertebrates. These and other observations lead us to extend this type of work to other drugs which have been said to play a similar role in the body as acetylcholine and physostigmine. In this work 3 more recently introduced drugs were utilized for a study, namely, carbaminoyl-choline (Doryl), acetyl-B-methyl-choline (Mecholyl) and prostigmine. The nature of their action was compared with that of acetylcholine and physostigmine. Our interest is primarily centered on the comparative physiological evaluation of these chemicals with regard to their stability, potency of action, and their pharmacological effects as observed on the intestine of the *Simocephalus vetulus,* a cladoceran. In spite of the comparatively low structural organization of this organism, these drugs induce such unmistakably dramatic effects on the musculature of the intestine that their action is remarkably similar to the therapeutic value ascribed to them in the treatment of atony and intestinal distention.[3]

Methods. In this work *Simocephalus vetulus* young in their second instar were employed. The method of rearing this animal and other cladocera, and the method employed in the selection of animals which are in the same stage of development have been described elsewhere.[4] The animals were subjected to experimentation separately; in each case a single individual was transferred to a micro culture slide for treatment with the specific drug employed. Animals which were found to be in an excited state (probably due to unfavorable culture media) were not accepted for the experimental work. Fresh solutions of the drugs were prepared daily, usually just before their utilization. The dilutions recorded in this paper are expressed in per cent concentration.

Results. When a *Simocephalus vetulus* young is treated with prostigmine, the muscular peristaltic and antiperistaltic contractions of the intestine become extremely violent. When the drug becomes effective, it exhibits an abrupt, powerful contractile wave of considerable amplitude and the effects persist for some hours thereafter. Prostigmine showed graded action for a wide range of concentrations, namely, a definite relation was established between the time which elapses from the application of the drug to the onset of the initial vigorous peristaltic wave and the concentrations employed.

The time required to induce the initial

1 Obreshkove, V., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 427.

2 Obreshkove, V., *Biol. Bull.*, 1941, **81**, 105.

3 Fraser, F. R., *Brit. Med. J.*, 1938, pp. 1293-1299.

4 Obreshkove, V., *Physiol. Zool.*, 1930, **3**, 271.

TABLE I.
Onset of Vigorous Intestinal Contractions in *Simocephalus vetulus* After Treatment with Prostigmine of Various Concentrations.

Prostigmine 1×10^{-1} Sec.	Prostigmine 1×10^{-2} Min.	Prostigmine 1×10^{-3} Min.	Prostigmine 1×10^{-4} Min.	Prostigmine 1×10^{-7} Min.
35	2.5	6.1	21	86
55	1.9	5.6	25	78
60	2.7	9.1	11	63
40	1.2	6.4	24	88
63	1.6	5.8	35	72

effect of the drug varied from less than one minute for an abnormally high prostigmine concentration (1×10^{-1}) to more than one hour for more dilute solutions (1×10^{-7}). This graded action is well illustrated in Table I. The analogue of prostigmine, namely, physostigmine, was observed to produce a similar action on the intestine of *Daphnia magna*.[5] It stimulated the intestinal peristalsis in *Daphnia magna* as well as in *Simocephalus vetulus* as powerfully as prostigmine. However, the period which elapsed from the application of the drug to the onset of the vigorous peristaltic waves was found to be much longer for prostigmine than for physostigmine (Table V) for the same concentration of the drugs. With every concentration of these two drugs, it was observed that the amplitude of the peristaltic waves increased with the lapse of time.

TABLE II.
Onset of vigorous intestinal contractions in *Simocephalus vetulus* after treatment with acetylcholine (1×10^{-5}) and also the time of action of the same concentration of the drug following the administration of prostigmine (1×10^{-5}) for 2 minutes.

Acetylcholine 1×10^{-5} min.	Acetylcholine 1×10^{-5} after treatment with prostigmine (1×10^{-5}) sec.
12.3	35
15.0	40
13.0	38
19.0	35
12.8	45
14.0	60
17.0	44

Prostigmine and Acetylcholine. Prostigmine causes in *Simocephalus vetulus* intensification and prolongation of the effects of acetylcholine. Individuals which were treated with prostigmine prior to the application of acetylcholine gave a reaction time for the onset of the characteristic effects which was considerably less than the time required for the action of acetylcholine and prostigmine alone. This was found to be true for a wide range of concentrations of these two drugs. The reduction in the time of action of acetylcholine due to prior application of prostigmine was found to be considerable. When acetylcholine (1×10^{-5}) was administered to the animal alone the time of action was found to be on the average about 15 minutes. Prostigmine reduced this period to less than one minute (Table II). This action of prostigmine as well as physostigmine has been explained on the basis that, in mechanisms in which the transmission of nervous impulses have been associated with acetylcholine, they delay the destruction of acetylcholine by cholinesterase. In view of the demonstration by Artemov and Mitropolitanskaja[6] of the presence in whole daphnia of an acetylcholine-like substance and cholinesterase in the hemolymph of crustacea, as well as the demonstration of the occurrence of acetylcholine in the nervous tissue in crustacea, the observations recorded in Table II acquire added significance. In man prostigmine stimulates intestinal peristalsis as powerfully as physostigmine.[7] Prostigmine has been recommended by the manufacturers for therapeutic use as an intestinal stimulant in the case of post-operative intestinal atony,

[5] Obreshkove, V., *Biol. Bull.*, 1941, **81**, 105.

[6] Artemov, N. M., and Mitropolitanskaja, R. L., *Bull. de Biol. ed. de Méd. Expér. U. S. S. R.*, **5**, 378.

[7] Ammon, A., *Arch. Ges. Physiol.*, 1933, **233**, 486.

TABLE III.
Onset of Vigorous Intestinal Contractions in *Simocephalus vetulus* After Treatment with Doryl* of Various Concentrations.

Doryl 1×10^{-3} Sec.	Doryl 1×10^{-4} Sec.	Doryl 1×10^{-6} Sec.	Doryl 1×10^{-9} Sec.	Doryl 1×10^{-12} Sec.
26	32	20	20	21
24	27	27	25	30
55	36	31	28	25
30	39	28	32	40
28	45	24	35	30
35	28	35	24	33

* Doryl—trade name for carbaminoyl-choline.

and it appears that its effect may be due to the potentiation of the acetylcholine effects normally occurring in the body.[8]

Carbaminoyl-choline (*Doryl*). Since its introduction by Kreitmair,[9] carbaminoyl-choline has been observed to produce the same qualitative effects as acetylcholine.[9] In the mammal it has been shown to act typically as a mimic of parasympathetic stimulation, and its effects, as in the case of acetylcholine, are inhibited by atropine. Carbaminoyl-choline produces the same effects on the intestine of the *Simocephalus vetulus* as was demonstrated for acetylcholine, prostigmine and physostigmine. It differs from acetylcholine primarily in its greater stability, in its more powerful action and in its greater effectiveness as judged by the time which elapses between the application of the drug and the onset of the characteristic effect. Various concentrations of this drug were employed in the experimental work, ranging from concentrations of 1×10^{-3} through 1×10^{-12} (Table III). The drug was so effective and the characteristic effect was produced with such rapidity that the graded action so characteristic of acetylcholine and prostigmine could not be demonstrated within the range of concentrations employed in this work. Very dilute solutions (1×10^{-12}) produced the characteristic effect in approximately the same length of time as that of considerably stronger solutions (Table III). The action of a comparatively weak solution of carbaminoyl-choline required a high concentration of atropine (1×10^{-1}) to abolish its effects. Even after the application of very high concentrations of atropine the effects of carbaminoyl-choline persisted in some cases for over 2 hours or more before they were completely abolished. The effects of stronger solutions of acetylcholine (1×10^{-3}), on the other hand, were abolished in the majority of cases in less than 2 minutes by a much weaker solution of atropine (1×10^{-5}). The antagonistic effect of atropine on acetylcholine and carbaminoyl-choline respectively is shown in Table IV. It is evident from an inspection of this table that atropine acts more slowly and less effectively in antagonizing the action of carbaminoyl-choline. It has been said concerning this drug that it is not susceptible to destruction by cholinesterase and that its activity is not affected by antiesterase drugs such as physostigmine and prostigmine.

Acetyl-B-methyl-choline (*Mecholyl*). The intestine of the *Simocephalus vetulus* after treatment with acetyl - B - methyl - choline straightens out as a result probably of the much increased muscle tonus of the organ. The lumen of the entire digestive tube decreases and there appears a sphincter-like constriction just posterior to the rectal region. These effects were observed with all the dilutions employed, and the time of their appearance was found to vary, as in the case of the graded action demonstrated for acetylcholine and prostigmine, with the strength of the drug employed. Prior to the formation of the rectal constriction, much of the contents of the digestive tube is emptied in a few seconds after the introduction of the drug due to the

[8] Fraser, F. R., *Brit. Med. J.*, 1938, pp. 1349-1354.

[9] Kreitmair, H., *Arch. ex. Path. Pharmak.*, 1932, **164**, 346.

TABLE IV.
Onset of Vigorous Intestinal Contractions in *Simocephalus vetulus* After Treatment with Acetylcholine, Doryl,* and Mecholyl† and the Time of Abolishing the Characteristic Effects by Atropine.

Acetylcholine 1×10^{-3} Sec.	Atropine 1×10^{-5} Sec.	Doryl 1×10^{-4} Sec.	Atropine 1×10^{-1} Min.	Mecholyl 1×10^{-1} Sec.	Atropine 1×10^{-8} Sec.
21	95	50	78	25	134
25	60	45	48	40	75
23	69	60	110	33	82
26	133	46	117	35	240
27	90	42	134	28	317
29	144	35	94	34	340

* Doryl—trade name for carbaminoyl-choline.
† Mecholyl—trade name for acetyl-B-methyl-choline.

TABLE V.
A Comparative Study of the Time of Action in Inducing Vigorous Intestinal Contractions in *Simocephalus vetulus* After Treatment with the Same Concentration (1×10^{-4}) of Doryl, Acetylcholine, Physostigmine, Prostigmine, and Mecholyl.

Doryl 1×10^{-4} Sec.	Acetylcholine 1×10^{-4} Min.	Physostigmine 1×10^{-4} Min.	Prostigmine 1×10^{-4} Min.	Mecholyl 1×10^{-4} Min.
27	8.2	9.5	21	26
42	7.8	8.8	26	14
52	8.6	9.0	11	24
46	7.8	9.2	24	27
32	10.0	9.0	35	40
35	7.6	8.5	25	36

very powerful initial peristaltic waves. There was also observed a very rapid palpitation of the anal portion of the digestive tube posterior to the constriction, but no relaxation of the sphincter effect appeared as long as the intestine was under the influence of the drug. Starr[10] reports similar effects induced by Doryl and Ritvo[11] by Mecholyl on the human esophagus and stomach as those described above for Mecholyl on the intestine of the *Simocephalus vetulus*.

In contrast with carbaminoyl-chloride a very clear antagonism was found to exist between acetyl-B-methyl-choline and atropine. A very weak atropine solution (1×10^{-8}) very quickly overcame the effects of abnormally high concentration of acetyl-B-methyl-choline (1×10^{-1}). This is in marked contrast with the period required to abolish the effects produced by relatively weak carbaminoyl-choline by strong concentrations of atropine (Table IV). The time of action of a wider range of concentrations of atropine, as well as the time of the re-establishment of strong intestinal contractions by acetylcholine following atropine, has been recorded for *Daphnia magna*.[12]

The existence of this marked antagonism between acetyl-B-methyl-choline and atropine was further demonstrated. In a series of 5 experiments a preliminary application of atropine 1×10^{-5} for 5 minutes prevented the appearance of the characteristic effect ascribed to this drug. Myerson, Loman and Dameshek[13] have demonstrated that acetyl-B-methyl-choline in the human induces secretory and vaso-motor effects. They, too, have observed that small doses of atropine when given prior to the administration of acetyl-B-methyl-choline prevented the appearance of the characteristic Mecholyl effects.

The comparative physiological and pharmacological effects of acetylcholine, prostigmine, carbinoyl-choline, and acetyl-B-methyl-choline are shown in Table V. From an inspection of

10 Starr, J., *Am. J. Med. Sc.*, 1937, **193**, 393.
11 Ritvo, M., *Am. J. Roentgen.*, 1936, **36**, 868.
12 Obreshkove, V., *Biol. Bull.*, 1941, **81**, 105.
13 Myerson, A., Loman, J., and Dameschek, W., *Am. J. Med. Sci.*, 1937, **193**, 198.

the table, it is seen that, when individuals are subjected to drugs of the same concentration (1×10^{-4}), Doryl produces vigorous intestinal contractions in the shortest period of time. Acetylcholine and physostigmine come next in order of effectiveness and appear to be equally potent. Prostigmine and acetyl-B-methyl-choline are the least effective of all the drugs utilized in this experimental work.

Summary. 1. Prostigmine, acetylcholine, carbaminoyl-choline and acetyl-B-methyl-choline produce in *Simocephalus vetulus* vigorous intestinal contractions.

2. The period which elapses between the addition of the drugs and the onset of the characteristic effect is definitely dependent, with the exception of carbaminoyl-choline, on the concentration of the drug.

3. Carbaminoyl-choline did not exhibit a graded action within the range of concentrations employed in this work.

4. Atropine blocks the action of the drugs employed in this work. However, it acts more slowly and less effectively in antagonizing the action of carbaminoyl-choline.

5. Prostigmine causes intensification and prolongation of the effects of acetylcholine and it reduces considerably the time which elapses between the administration of acetyl-choline and the appearance of the vigorous intestinal contractions.

6. In contrast with carbaminoyl-choline, a marked antagonism was found to exist between acetyl-B-methyl choline and atropine.

7. Carbaminoyl-choline differs from acetyl-choline primarily in its greater stability and more powerful action.

8. The action of prostigmine, acetyl-choline, carbaminoyl-choline and acetyl-B-methyl-choline is remarkably similar in certain respects to the action ascribed to them on the digestive tube for a number of mammals.

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A Latent Pneumotropic Pasteurella of Laboratory Animals.

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Laboratory mice are known to harbor a variety of latent pneumotropic agents including bacteria,[1] pleuropneumonia-like organisms[2,3,4] and viruses.[5-12] Increasing interest in human respiratory infections has led to many attempts to adapt agents causing such diseases to small laboratory animals. Blind passage of lung material by the respiratory route frequently leads to the production of lung lesions in the experimental animals due to the activation of latent agents. Eliminating such a possibility is an important step in ascertaining the relationship of the agent studied to the lesions produced.

In the course of attempts to adapt to Swiss

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3 Edward, D. G. F., *J. Path. and Bact.*, 1940, **50**, 409.

4 Edward, D. G. F., *J. Path. and Bact.*, 1947, **59**, 209.

5 Andrewes, C. H. and Glover, R. E., *Brit. J. Exp. Path.*, 1945, **26**, 379.

6 Dochez, A. R., Mills, K. C., and Mulliken, B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 683.

7 Gonnert, R., *Klin. Wochenschr.* 1941, **20**, 76.

8 Gordon, F. B., Freeman, G., and Clampit, J. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 450.

9 Heinzmann, K., *Klin. Wochenschr.*, 1941, **20**, 910.

10 Horsfall, F. L. and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

11 Nelson, J. B., *J. Exp. Med.* 1937, **65**, 833.

12 Nigg, C., *Science*, 1942, **95**, 49.