effect became apparent even after seven days' incubation. However, by contrast, almost pure cultures of epithelial cells were obtained when the kidney tissue of puppies six months of age or older was used. As best as could be determined, satisfactory cultures were regularly produced with kidney tissue from pups at least 3 months old.

The effect of the tissue culture virus on susceptible puppies is being studied at the present time, and will be reported later.

Summary. The serial propagation of ICH virus by the roller tube method in dog kidney tissue culture through 15 consecutive passages is reported. The growth of the virus under these conditions causes a specific cytopathogenic degeneration of the tissue growth, similar to that reported for poliomyelitis virus in the presence of human or monkey tissues. Identification of the tissue culture virus was made by both the complement-fixation and serum neutralization methods using a known infectious canine hepatitis fox immune serum.

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Effect of 1,1-Dimethyl-4-Phenylpiperazinium Iodide on Peristaltic Reflexes of Isolated Guinea Pig Ileum. (20844)

GRAHAM CHEN AND RUTH PORTMAN.

From the Research Laboratories, Parke, Davis & Co., Detroit, Mich.

1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP) was shown to be a potent stimulant of the autonomic ganglia(1). The question has been raised as to whether or not this compound produces paralysis of the ganglia at high concentrations as does nicotine. The inquiry is of theoretical interest in studying the chemical transmission of nerve impulses, since the ganglionic stimulating and paralyzing actions of nicotine may be explained by depolarization and overdepolarization at the synapses induced by low and high concentrations respectively of the liberated acetylcholine(2). In our experiments on pentobarbitalized dogs, no difference in hypertensive response was noticed upon repeated intravenous injections of submaximal doses of DMPP (20-30 μg/kg). However, by previous administration of 0.75 mg/kg of N-(2-bromoethyl)-N-ethylnaphthalene methylamine.HBr (SY 28, an adrenergic blocking agent) in order to suppress partially the hypertensive effect of DMPP, there was produced some diminution in hypertensive response to the second injection of 0.1 mg/kg of DMPP 5 minutes later(3). These results seem to indi-

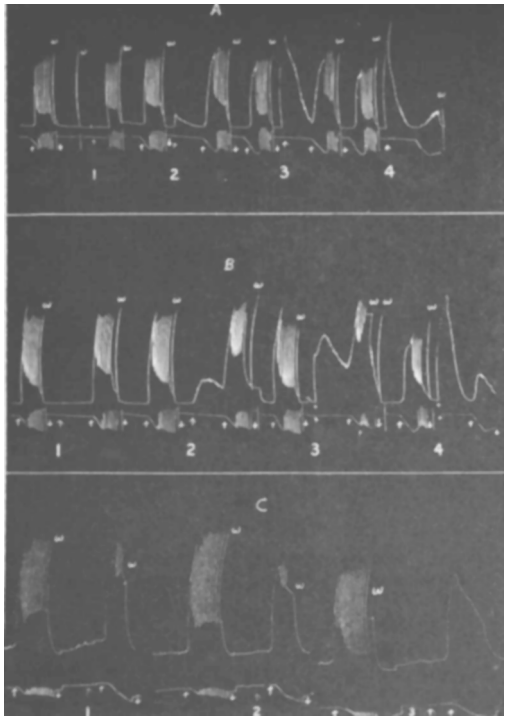

FIG. 1. Comparison of effects of DMPP, nicotine, and hexamethonium on peristaltic reflexes of an isolated guinea pig's ileum. Upper tracing—contractions of longitudinal musculature. Lower tracing—contractions of circular musculature; ω—change to fresh Tyrode solution. Peristalsis was induced by the increase of hydrostatic pressure in the lumen (in cm) for 1 or 2 min. as indicated by the arrow. The test compound in milligram quantities was added and left in the bath for 3 min. prior to the induction of peristaltic reflexes. In graphs A and B, DMPP and nicotine are shown to produce a spasmodic effect on the quiescent intestine; they increase the peristaltic reflex activity at low concentrations (0.1-0.2 mg) but suppress it at higher concentrations (0.4 mg). No stimulating effect is evident with "C_6" at any concentration (Graph C).

The concentrations of the added compounds are indicated by numbers as follows:

(A) 1, 2, 3, 4 = 0.05, 0.10, 0.20, 0.40 mg DMPP respectively.

(B) 1, 2, 3, 4 = 0.05, 0.10, 0.20, 0.40 mg nicotine respectively.

(C) 1, 2, 3 = 0.4, 0.5, 0.6 mg "C_6" respectively.

cate that DMPP is either rapidly eliminated from the site of action or a high concentration of DMPP is required to paralyze the ganglion. In view of such possibilities, any paralyzing action of DMPP appears to be best studied in an isolated organ whereby a constant and high concentration of it may be maintained.

The effect of DMPP on the peristaltic reflexes of an isolated guinea pig's ileum was then investigated. The results obtained for DMPP together with those for nicotine, hexamethonium ("C_6"), d-tubocurarine, and physostigmine are given in this report.

Experimental. For recording the peristaltic reflexes of the intestine, the method of Trendelenburg was employed(4). A clean strip of guinea pig ileum about 6 cm long was placed in a 100 ml muscle chamber with Tyrode solution at 37°C. The upper end of the intestine was closed and tied to a muscle lever to record contractions of the longitudinal muscle. The lower end was connected with

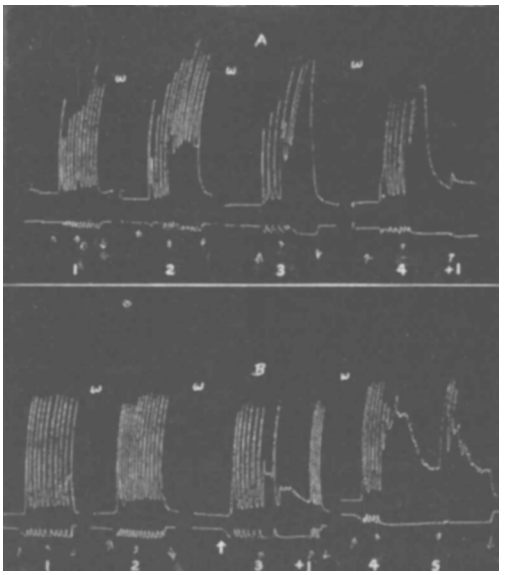

FIG. 2. The effects of DMPP and "C_6" added to the bath during peristalsis. These recordings are similar to those in Fig. 1. Peristaltic activity was increased by DMPP at low concentrations (0.1-0.2 mg), but suppressed at higher concentrations (0.4 mg). Once suppressed, there was no resumption of peristalsis by further increase of pressure (3 cm to 4 cm). Peristaltic activity was suppressed by "C_6" at all concentrations (0.4-0.6 mm); normal peristalsis was reinduced by the increase of pressure (3 cm to 4 cm) and by DMPP (0.4 mg).

The concentrations of the added compounds are shown by numbers as follows:

(A) 1, 2, 3, 4 = 0.10, 0.20, 0.30, 0.40 mg DMPP respectively.

(B) 1, 2, 3, 4 = 0.20, 0.30, 0.40, 0.60 mg "C_6" respectively.

5 = 0.4 mg DMPP.

A further increase of pressure in the lumen is indicated by the "+1" sign.

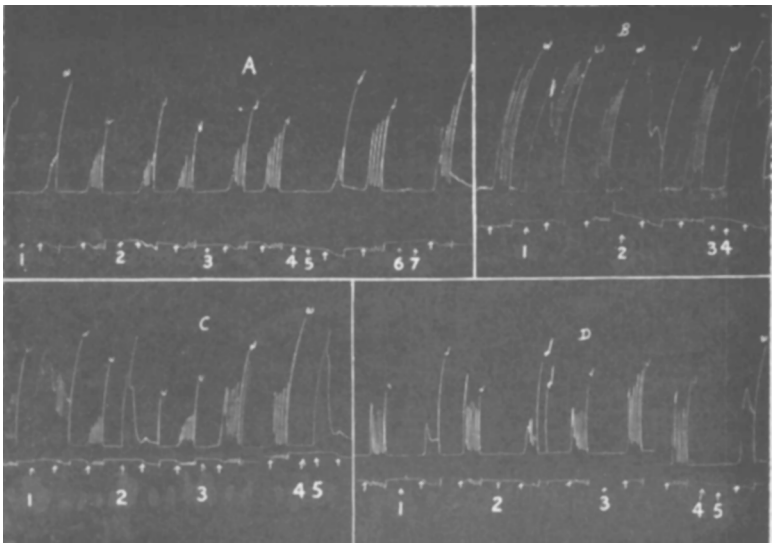

FIG. 3. Effect of physostigmine on peristaltic reflex of an isolated guinea pig ileum in response to d-tubocurarine, nicotine, DMPP, or "C_6." The recordings were made as were those in Fig. 1. The time intervals in min. between the addition of each substance and the induction of peristaltic reflexes, and the duration of peristalsis (1 min.), are given below. As shown by the last part of each graph, physostigmine (5 and 10 μg) antagonized only the paralyzing effect of d-tubocurarine (1 mg, graph A), but did not affect that of nicotine (0.25 mg, graph B), DMPP (0.4 mg, graph C) or "C_6" (0.15 mg, graph D).

The concentrations of the added compounds are indicated by numbers as follows:

(A) 1, 2 = 1.0, 0.5 mg d-tubocurarine respectively; 2 and 4 = 2.5 μg physostigmine; 5 = 1 mg d-tubocurarine; 6 = 5.0 μg physostigmine; 7 = 1 mg d-tubocurarine.
(B) 1, 2 = 0.10, 0.25 mg nicotine respectively; 3 = 5 μg physostigmine; 4 = 0.25 mg nicotine.
(C) 1, 2 = 0.30, 0.40 mg DMPP respectively; 3 and 4 = 10 μg physostigmine; 5 = 0.4 mg DMPP.
(D) 1, 2 = 0.20, 0.10 mg "C_6" respectively, 3 and 4 = 5 μg physostigmine; 5 = 0.15 mg "C_6."

tubing to the base outlet of a pressure bottle containing Tyrode solution. Through the top of the bottle, the changes in volume of the intestine were recorded with a tambour. The bottle was set on a stand with adjustable knobs so that the pressure in the ileum could be raised gradually at a constant rate. The intestine was allowed to stand in the muscle bath for ½-1 hour before experimentation. The minimal pressure required to induce a uniform peristalsis was then determined by raising the pressure bottle at 0.5 mm per second. The peristaltic contractions were recorded graphically at this pressure for one or 2 minutes. A consistent peristaltic reflex to the same increase of pressure at 5-minute intervals was taken as a control response. A test compound was added to the bath one or 3 minutes before the pressure in the ileum was raised to the control level. The strip was washed twice after each dose, and allowed to recover at least 5 minutes between each determination.

Results. In Fig. 1 are presented the kymographic recordings of peristalsis of isolated ilea under the influence of DMPP, nicotine tartrate, and "C_6". The record shows also the spasmodic effect of DMPP and nicotine on the gut at the quiescent stage as they were added to the bath 3 minutes before the pressure in the lumen was increased. The spasmodic effect of DMPP, as shown previously, may be blocked by "C_6" but not by the antimuscarinic action of atropine(1). It is seen on these graphs that DMPP like nicotine augmented the peristaltic reflex of the ileum at low concentrations (0.1-0.2 mg/100 cc) by an increase in the force of contraction with less relaxation of both the longitudinal and the circular musculature. At higher concentrations, however, DMPP prevented, as nicotine did, the induction of reflex peristalsis by pres-

sure. On the other hand, a pure blocking effect on peristalsis is indicated by "C_6" at all concentrations.

Results of a similar nature are shown in Fig. 2, obtained in experiments by adding DMPP or "C_6" during peristalsis. These graphs are given to show particularly the return of peristalsis, which had been suppressed with "C_6", by a further increase of pressure in the gut or by DMPP. When once suppressed by DMPP, on the other hand, no resumption of peristalsis occurred by the further increase of pressure.

In view of the antagonistic action of physostigmine toward d-tubocurarine at the neuromuscular junction, the influence of physostigmine upon the suppressive effect of DMPP, nicotine, "C_6" and d-tubocurarine on peristaltic reflexes was investigated. As shown by the recordings in Fig. 3, physostigmine antagonized the blocking effect of d-tubocurarine on peristaltic reflexes but did not affect that of DMPP, nicotine, or "C_6". It suggests the possibility that the latter agents and d-tubocurarine inhibit the ganglionic response of the ileum to increasing pressure in the lumen by different mechanisms. This awaits further investigation.

Summary. DMPP has been shown, like nicotine, to augment the peristaltic reflex of an isolated ileum to increasing pressure at low concentrations but inhibits it at high concentrations. This is apparently due to its stimulating and paralyzing action on the parasympathetic ganglion at low and high concentrations, respectively. The fact that the paralyzing effect of DMPP on the ganglia was not evident on repeated administrations in animals of submaximal doses may be explained by the rapid elimination of it from the site of action. Whereas physostigmine antagonized the blocking effect of d-tubocurarine, it does not influence that of DMPP on peristaltic reflexes of the ileum.

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Influence of Incubation Time and pH Changes on Uptake of P-aminohippurate by Renal Slices. (20845)

E. CARHART,* F. KODA, AND A. FARAH.

From the Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, N. Y.

Cross and Taggart(1) have determined the various factors which influence the uptake of p-aminohippurate (PAH) in rabbit kidney slices. These authors have stated that the maximal uptake of PAH by renal slices occurs following a 120-minute experimental period. Furthermore, they state that pH variations between 7.0 and 7.8 have no effect on PAH slice:medium ratio (S:M Ratio) of renal slices of the rabbit. Under certain conditions the time factor as well as the pH of the buffer solution have marked effects on the S:M ratio and this report represents the data on the influence of these factors on the PAH S:M ratio determined with dog renal slices.

Methods. In some of the experiments the phosphate buffer devised by Cross and Taggart(1) was employed. In another series of experiments a bicarbonate buffer (NaCl = 0.92%, KCl = 0.042%, $CaCl_2$ = 0.024%, $NaHCO_3$ = 0.05%(2) and a 95% oxygen, 5% CO_2 gas phase were used. About 100-150 mg of kidney slices obtained from 9-18 kg dogs were placed in 20 cc beakers containing 2.8 cc of buffer and 0.0013 M PAH. Various substrates were neutralized and added in a

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