extracted muscle could cause only negligible tension developments in the presence of ATP and ACh (10^{-4} to 10^{-3} g/ml). In 16 preparations out of 22 preparations, choline (10^{-4} g/ml) or histamine (10^{-4} g/ml) induced little tension development with ATP (Fig. 1-f and g). Both drugs did not contract the isolated muscle strips of the dog small intestines even at so high concentrations as 10^{-5} g/ml. So the activities of drugs tested in this subcellular experiment were very similar to those shown in intact muscle strips of the dog small intestines. The concentrations of drugs were, however, very high compared with the physiological concentration and so simple extrusion of GEDTA-chelatable cations (i.e. Ca ions) from the fraction by competition with high doses of cationic drugs might be supposed. The facts, however, that choline, histamine and atropine could not show any tension development even at high doses may deny the above mechanism. The reproducibility was not sufficient even in these experiments using glycerol extracted skeletal muscle, but investigation with glycerol extracted dog small intestinal smooth muscle showed smaller and slower tension development and it is lacking considerably in reproducibility even when ATP and Ca ions were used. Therefore as a model of contracting protein glycerol extracted skeletal muscle was utilized.

Of course, there are no evidences that the fraction keeps unique organizations of ACh receptors in the cells, but we could reproduce some receptor mechanisms in this contraction system consisting of the subcellular fraction (P_s), ATP and glycerol treated rabbit psoas similar to those found in muscle strips of the small intestine. In addition, the other two fractions which were supposed to contain little ACh receptors from the labelling experiment (1, 2) could not produce any tension development with ATP and ACh.

It may be probably concluded from the above results that Ca ions can be released from P_s fraction by ACh because the tension development was abolished by adding GEDTA (Fig. 1-b). It is not easy to interpret true pharmacomechanical coupling from our experiments. There are considerable evidences that, in striated and in smooth muscles, a rise in free intracellular Ca activates contraction (5, 6). This activator Ca may arise from an increased influx of the extracellular ion or from translocation of Ca bound at the surface membrane or concentrated in an intracellular storage site. Our results are supposed to emphasize the ability of ACh to translocate Ca ions into the cytoplasm from Ca binding sites in membranes.

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EFFECTS OF METOCLOPRAMIDE ON SERUM AND ERYTHROCYTE CHOLINESTERASES

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Metoclopramide (MT) produces anti-emetic and anti-arrhythmic activities and, with reference to the dose, a stimulating or depressing activity on the gastro-intestinal muscle and that of urinary bladder, uterus and gall bladder.

A large number of investigations was carried out with MT on the parasympathetic efferential periphery. According to Boissier et al. (1) MT (20 mg/kg i.v.) slightly increases the hypotensive effect by acetylcholine (ACh). According to Kosaka et al. (2) the piloric hypermotility by MT (0.2 mg/kg i.v.) in man is not antagonized by atropine (10 μ g/kg s.c.) or N-butyl-hyoscine bromide (0.2 mg/kg i.v.). According to Katsuki et al. (3) the gastric hypermotility by MT (10 mg i.v.) in man is blocked by atropine (0.5 mg i.m.). According to Shimamoto et al. (4) MT does not produce in vitro anti-ACh effects on several organs. According to Coullaud et al. (5) the stimulating effects by MT on the rabbit colon and ileum are not modified, neither in vivo nor in vitro by atropine or hexamethonium. According to Jacoby et al. (6) the stimulating effects by MT on the gastric and duodenal muscle would be antagonized by benztropine in the non-anesthetized dog and by atropine in the anesthetized dog; MT (2 mg/kg i.v.) would not potentiate the effects by ACh on the gastric muscle of the dog. According to Eisner (7) MT in conc. of 10^{-6} – 10^{-5} potentiates the stimulating effects by ACh on bands of human stomach, pylorus, duodenum, ileum and colon and partly antagonizes the inhibitory effect by atropine to ACh; always according to Eisner (8) the stimulating effects by MT on the gastro-intestinal muscle in man would be antagonized by atropine, therefore the hypothesis is advanced that MT has a common receptor with ACh. According to Hukuhara et al. (8, 9) the stimulating effect by MT on the rat duodenum and jejunum is not modified in vitro by a previous treatment with hexamethonium or atropine or nicotine; always according to Hukuhara et al. (8, 9) MT in high conc. (5×10^{-5} – 10^{-4}) would have ganglioplegic effects. According to Mantegazza et al. (10), on the gastro-intestinal muscle MT produces some peripheral effects on the intramural nervous elements with a non well definable mechanism. According to Beani et al. (11) and Bianchi et al. (12), on the rabbit colon, MT in conc. of 10^{-6} – 10^{-5} inhibits some nervous structures which may be stimulated with 5-hydroxytryptamine, appointed to depress the cholinergic exciting influences, and sensibilizes the muscular effector to ACh without modifying the release of ACh. According to Marmo (13) and Marmo et al. (14–17), MT does not produce in vitro anti-muscarinic effects on several smooth muscles of different animals; and the stimulating effects by MT on the gastro-intestinal and uterine muscle and that of urinary bladder would prevalingly be due to a direct effect of the smooth muscle. In previous researches (14) we observed that MT, already in a dose as high as 100 μ g/kg i.v. potentiates in the anesthetized cat (ethylurethane, 1 g/kg i.m.), even if bivatogomized, the hypotensive effect by ACh, leaving unchanged the one by oxotremorine; therefore, we investigated the effects by MT on the serum- and erythrocyte-cholinesterases of the rabbit.

TABLE 1. In vitro effects by metoclopramide (MT) and procainamide (PC) on serum- and erythrocyte-cholinesterases of the rabbit.

Treatment		% inhibition of serum cholinesterases	% inhibition of ery- throcyte cholinesterases	IC ₅₀ of serum cholinesterases	IC ₅₀ of erythro- cyte cholinesterases
MT	10^{-7}	3.8 $\pm$ 0.1 (4)	2.8 $\pm$ 0.1 (4)		
"	10^{-6}	20.5 $\pm$ 1.2 (4)	19.4 $\pm$ 1.5 (4)		
"	10^{-5}	36.2 $\pm$ 1.8 (4)	34.7 $\pm$ 2.9 (4)	4.22×10^{-5}	5.31×10^{-5}
"	10^{-4}	68.4 $\pm$ 3.5 (4)	67.1 $\pm$ 4.3 (4)		
"	10^{-3}	69.3 $\pm$ 3.8 (4)	72.4 $\pm$ 4.6 (4)		
P C	10^{-7}	2.3 $\pm$ 0.1 (4)	1.9 $\pm$ 0.01 (4)		
"	10^{-6}	13.9 $\pm$ 1.4 (4)	14.2 $\pm$ 1.3 (4)		
"	10^{-5}	15.8 $\pm$ 1.3 (4)	16.1 $\pm$ 1.7 (4)	2.23×10^{-4}	1.78×10^{-4}
"	10^{-4}	31.1 $\pm$ 1.9 (4)	29.5 $\pm$ 1.8 (4)		
"	10^{-3}	66.2 $\pm$ 3.7 (4)	68.2 $\pm$ 5.3 (4)		

Each value is given as the mean $\pm$ standard error, the number of experiments being given in parentheses.

The cholinesterases activity was evaluated according to the method of Ellman et al. (18) in the rabbit serum and erythrocytes in the absence or presence of metoclopramide hydrochloride (MT) or procainamide hydrochloride (PC); the incubation lasted 45 minutes; readings were made at 25°C. The determination of IC_{50} (inhibitory conc. 50) was carried out with the probits method according to Burn et al. (19). In a second group of researches, the hypotensive effects by ACh (10–100 μ g), incubated for 30 minutes at 37°C with 0.2 ml of rabbit serum added to 50 μ g of MT, PC, procaine hydrochloride (P) or prostigmine sulfate (PR), was investigated in cats (δ , 2.500–2.600 g) anesthetized with ethylurethane (1 g/kg i.m.).

Fig. 1. Cat δ , 2.5 kg. Carotid artery pressure. 1–2 = ACh (0.25 μ g i.v.) ; 3 = rabbit serum (0.2 ml i.v.) ; 4 = rabbit serum (0.2 ml i.v.) + ACh (10 μ g i.v.) ; 5 = rabbit serum (0.2 ml i.v.) + ACh (100 μ g i.v.) ; 6 = rabbit serum (0.2 ml i.v.) + ACh (100 μ g i.v.) + PR (50 μ g i.v.) ; 7 = rabbit serum (0.2 ml i.v.) + ACh (10 μ g i.v.) + MT (50 μ g i.v.) ; 8 = rabbit serum (0.2 ml i.v.) + ACh (100 μ g i.v.) + MT (50 μ g i.v.) ; 9 = rabbit serum (0.2 ml i.v.) + ACh (100 μ g i.v.) + PC (50 μ g i.v.) ; 10 = rabbit serum (0.2 ml i.v.) + ACh (100 μ g i.v.) + P (50 μ g i.v.). 50 μ g i.v. of PR or MT or PC or P + 0.2 ml of rabbit serum don't modify the slight hypotensive effect by 0.2 ml i.v. of rabbit serum.

Metoclopramide produces an anti-cholinesterasic effect as documented by the investigations carried out on the serum- and erythrocyte-cholinesterases of the rabbit (Table 1) and by the ones carried out while studying the hypotensive effect by ACh incubate in rabbit serum in the presence or not of MT (Fig. 1); the anti-cholinesterasic effect by MT resulted to be more intensive of that by procainamide or procaine, but less intensive by that of prostigmine (Table 1 and Fig. 1). We appropriate to emphasize that the potentiating effects of the responses by ACh, observed by some authors, must be interpreted not as an activation of the muscarinic receptor by MT, but in the sense found by us in the present work (namely, inhibition of the cholinesterases). However, this does not exclude that MT may also inhibit the nervous structures appointed to depress the cholinergic exciting influences [Beani et al. (11), Bianchi et al. (12)].

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EFFECT OF LITHIUM SUBSTITUTION FOR SODIUM ON OXYGEN CONSUMPTION OF GUINEA PIG TAENIA COLI IN HIGH K MEDIUM

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It was reported that high potassium solution (isotonic, 40 mM) induced a tension development in guinea pig taenia coli accompanied by an increase in oxygen consumption (1). Intracellular Ca space increased in high K medium as well as cellular Ca fraction which did not exchange within 4 minutes. The latter is referred to "tightly bound fraction (TBF)" (2). By addition of ouabain (2.5×10^{-8} M) to high K medium the increased oxygen consumption was maintained, although the developed tension was almost abolished, and both changes were dependent on external calcium (3). Moreover, the elevated intracellular Ca space was maintained by addition of ouabain, while the increased TBF returned to the control level in the high K medium (2).

When lithium was substituted for sodium at a level above 50 mM in high K (hypertonic, 40 mM) medium, the developed tension of the muscle gradually decreased to the original level (4, 5). In the present