pressed area contracts, the scybalum now moves antiperistaltically and may be expelled at the oral cut or enter the colon oral to the cut.

The antiperistaltic wave shows the following characteristics:

1. A contraction wave of the circularis 0.5 to 1 cm long, produces a grayish-white bloodless cord, while its surface longitudinal layer appears pink. The length of the progressing contraction remains more or less constant. The contraction wave passes without noticeable pause through the cut to the oral colon.

2. A wave of inhibition 1 to 2 cm long precedes the scybalum; this portion of the colon is pink-red, relaxed and shows longitudinal folds; no contraction occurs in this area if it is stroked with a blunt dissecting needle or if digitally compressed for a short time. This inhibitory wave passes the cut and relaxes the colon oral to the cut so that frequently the scybalum enters without difficulty. When alternate peristalsis and antiperistalsis occurs in the segment, then the grayish-white, bloodless, contracted circularis on one side of the scybalum, is seen becoming pink and then relaxing permitting progress of the scybalum, while the circularis on the other side progressively contracts.

Changes seen over the scybalum during its transit: on the face of the scybalum adjacent to the traveling, contracted circularis, a series of 3 to 4 transverse ridges may be seen; they are produced by contraction of the longitudinal fibers and lift the relaxed circularis bands over the scybalum. This is clearly seen when the scybalum enters the cut: in antiperistalsis the aboral lip of the oral cut is curled back 3 mm plus by the contraction of the longitudinal fibers and the relaxed, inhibited, circularis is lifted over the scybalum, and then the circularis contracts and the scybalum enters the inhibited colon oral to the cut. After its entry the circularis of this section contracts powerfully; the oral lip of the cut, however, shows but little curling, usually 1 plus mm. Inspection of the lips of a cut shows thus whether a peristaltic or antiperistaltic wave has recently passed.

The speed of the antiperistaltic wave is much slower than that of a peristaltic wave.

No clear-cut antiperistalsis was ever seen after pithing the spinal cord, combined with subdiaphragmatic section of both splanchnics and vagi.

Antiperistalsis in our experiments, therefore, obeys the same basic conditions developed by Bayliss and Starling[1] for peristalsis, if allowance is made for the change in direction.

[1] Bayliss, W. W., and Starling, E. H., *J. Physiol.*, 1899, **24**, 99; *ibid.*, 1900, **26**, 107 and 125.

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Inhibition of Choline Acetylase by α-Keto Acids.*

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Evidence has been provided for the assumption that the energy released by the breakdown of phosphocreatine is adequate to account for the electric energy released by the nerve action potential.[1] Hence if the primary event responsible for the alterations of the nerve membrane during the passage of the impulse is the release of acetylcholine, as recently suggested,[2-4] energy rich phosphate

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[1] Nachmansohn, D., Cox, R. T., Coates, C. W., and Machado, A. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 97; *J. Neurophysiol.*, 1943, **6**, 383.

[2] Nachmansohn, D., Cox, R. T., Coates, C. W., and Machado, A. L., *J. Neurophysiol.*, 1942, **5**, 499.

[3] Nachmansohn, D., *Collecting Net*, 1942, **17**, 61; *Biol. Bull.*, 1944, **87**, 158.

TABLE I.
Inhibitory Effect of α-ketoacids on Choline Acetylase Prepared from Fresh Brain or from Acetone Dried Powder (1 g of powder = 5-6 g of fresh brain).

Compound	Extracts from fresh brain		Extracts from dried brain	
	Conc., M × 10^{-4}	ACh formed μg/g/hr	Conc., M	ACh formed μg/g/hr
Pyruvic acid	0.0	108	0.0	600
	3.15	63	2×10^{-3}	440
	6.3	49		
α-Keto glutaric acid	0.0	128	0.0	390
	3.15	101	1.25×10^{-4}	200
	6.3	85	2.5×10^{-4}	160
Oxyphenyl pyruvic acid	0.0	155	control	820
	2.5	102	no eserine	780
	5.0	93	no fluoride	810

bonds should be used for reversing the process and rebuilding the ester. In accordance with this hypothesis an enzyme, choline acetylase, has been extracted from brain which in presence of adenosine-triphosphate under strictly anaerobic conditions synthesizes acetylcholine in cell free solution. From 1 g fresh rat brain an enzyme solution may be prepared which forms 100-150 μg of acetylcholine (ACh) per hour. Presence of eserine and fluoride is necessary to inhibit the action of choline esterase and adenosinetriphosphatase. The enzyme contains active sulfhydryl groups which may be easily oxidized and are readily inactivated by monoiodoacetic acid or Cu in low concentrations.[5] On dialysis choline acetylase rapidly loses its activity. Addition of the natural *l* (+) glutamic acid reactivates it partly.[6] With potassium + glutamic acid 50-80% of the original activity may be restored; further addition of cyanide or replacement of glutamic acid by cysteine may reactivate the enzyme nearly completely.[7,8] The experiments suggest that the enzyme may require besides active –SH groups and potassium the presence of an amino acid group.

It has now been found that oxidized amino acids, *i.e.*, α-keto acids, are strong inhibitors of choline acetylase. Pyruvic acid, α-ketoglutaric acid, phenyl- and oxyphenyl pyruvic acid have been tested so far. They inhibit choline acetylase in concentrations of 10^{-3} to 10^{-4} M. Table I gives a few significant data. The effect has also been tested on extracts prepared from powder of acetone dried brain. In these extracts the enzyme is about twice as pure as in those obtained from fresh tissue, 1 g of protein forming 3-4 mg of ACh in 60 min; the choline acetylase is separated from adenosinetriphosphatase so that no addition of fluoride is required;[7] choline esterase is largely or sometimes almost completely removed so that addition of eserine has either a small effect or practically no effect. Two observations are reproduced in the table; the last experiment recorded shows the small effect of eserine and fluoride.

α-keto acids occur in the living cell in concentrations close to those found to have an inhibitory effect. Moreover, pyruvic acid is known to be a "physiological anticonvulsant."[9] The strong inhibitory effect of these compounds on choline acetylase appears therefore to be of general physiological as well as of clinical interest.

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