Action of Reserpine on Isolated Intestinal Muscle

Certain intermediates of the tricarboxylic acid cycle antagonize the reserpine inhibition of contractions induced by drugs of isolated guinea pig ileum[1] (Table 1). We have observed that the most effective reserpine antagonists were those added as the free acids. These reduced the reaction of the bath-fluid to about *p*H 5. When the reaction was reduced to similar levels by the addition of small volumes of 0·2 *N* hydrochloric acid, antagonism to reserpine was also seen. This was less marked, however, than that following addition of the most effective metabolites, which included α-ketoglutaric acid (1 mgm. per ml.) and *cis*-aconitic acid anhydride (1 mgm. per ml.). At a bath *p*H of 7·4, no antagonism to reserpine was seen.

Hydrochloric acid produced a similar degree of antagonism to reserpine as did 1 mgm. per ml. of DL-leucine, which was tested along with other amino-acids and intermediates of fat metabolism (Table 1). It was concluded that these compounds are effective by virtue only of their ability to increase the hydrogen ion concentration of the bath fluid. Sodium pyruvate (1 mgm. per ml.) and sodium fumarate (1 mgm. per ml.), previously found to be inactive, were rendered active by reduction of the bath *p*H to 5 with hydrochloric acid. Furchgott and Wales[2] have suggested, on the basis of experiments on isolated rabbit intestinal muscle, that succinate, α-ketoglutarate and fumarate are more effective at lower bath *p*H values as sources of energy for the activity of this tissue.

Using Locke's solution, isolated rabbit duodenum showed[3] a reduction of tone and depression of spontaneous activity following addition of 10 μgm. per ml. of reserpine, which reduced the bath *p*H from 7·4 to 6·9. When the *p*H change of the bath-fluid which follows addition of reserpine (dissolved in ascorbic acid/sodium ascorbate mixture) is prevented by the use of Krebs–Henseleit solution[4], a stimulant action precedes the reduction in tone and rhythmic activity (Fig. 1). This effect is prevented by the prior addition to the bath of 1 μgm. per ml. of atropine sulphate or 1 μgm. per ml. of hexamethonium bromide but not by 2 μgm. per ml. of 2-brom D-lysergic acid diethylamide (*BOL* 148). These observations on rabbit gut support the findings of Plummer and his colleagues on dog gut *in situ*[5].

Table 1. Activity of Compounds tested for Antagonism to the Action of Reserpine on Guinea Pig Ileum

Intermediates of	Effect: Alone	Effect: With hydrochloric acid
(*a*) *Fat metabolism*		
3-Hydroxybutyric acid	0	+
Sodium propionate	0	+
(*b*) *Protein metabolism*		
L-Glutamic acid	0	+
DL-α-Alanine	0	+
DL-Leucine	+	
(*c*) *Carbohydrate metabolism*		
Glucose-1-phosphate	0	+
Fructose-1,6-diphosphate	0	+
Glucose-6-phosphate	0	+
3-Phosphoglyceric acid	++	
Sodium pyruvate	0	++
Sodium succinate	0	++
Sodium fumarate	0	+
Citric acid	++	
cis-Aconitic acid anhydride	++	
*iso*Citric acid	0	++
α-Ketoglutaric acid	++	
Malic acid	++	
Maleic acid	++	
Oxaloacetic acid	++	

0 represents no activity
+ represents activity equal to that of hydrochloric acid
++ represents activity greater than that of hydrochloric acid

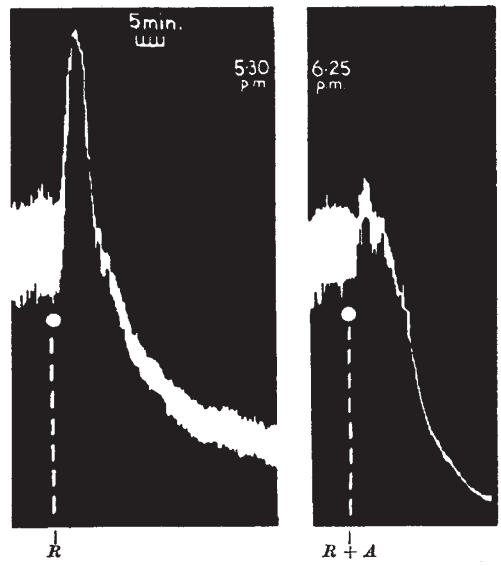

Fig. 1. Isolated rabbit duodenum suspended in 5 ml. Krebs–Henseleit solution. At *R*, 10 μgm. per ml. reserpine added; at *R* + *A*, 1 μgm. per ml. atropine sulphate added 5 sec. before 10 μgm. per ml. reserpine

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Slow Freezing of Carp Muscle and Inosinic Acid Formation

It has been noticed that when fresh muscles are frozen at very low temperature, for example, by sudden immersion in liquid propane or in liquid nitrogen, there is no significant change found in the amounts of adenosine triphosphate or creatine phosphate[1]. We have observed[2] the changes which take place in fresh muscle of carp during storage in an ice chest at − 8° C. Under these conditions, the material was frozen slowly and it was found that the amounts of adenosine triphosphate and creatine phosphate decrease very rapidly, and those of compounds such as inosine monophosphate increase greatly. In the present work, an attempt has been made to observe the formation of inosine monophosphate during the course of slow freezing of carp muscle.

Experiments were carried out on dorsal muscle of carp. 1 gm. of fresh muscle was solidified by freezing by storage at − 8° C. for 5 hr. The frozen muscle was homogenized with 20 ml. of 4 per cent perchloric acid solution at low temperature and the perchloric acid extract was filtered and separated by ion-exchange chromatography.

The degree of separation and optical densities of separated nucleotides are shown in Fig. 1. For each