THE EFFECTS OF VARIABLE INTERVALS OF COOLING UPON THE RESPONSES OF THE LAPIN INTESTINE AND UTERUS AND THE FELINE INTESTINE TO HISTAMINE, ACETYLCHOLINE, PITUITRIN AND BARIUM CHLORIDE

BRADFORD N. CRAVER, M. D. AND WALTER E. BARRETT, Summit, N. J.

THE EXPERIMENTS herein reported represented an attempt to repeat in part results described by Ambache in 1946 (1). He determined the responses of small intestinal strips of the rabbit, mouse and guinea pig to various drugs and cations and then studied the effects upon those responses of various periods of cooling in Tyrode's solution (0-2° C) up to eight days. The longest interval applied only to the more durable rabbit gut. The gut of the mouse yielded no responses after more than a few hours of cooling; the gut of the guinea pig was intermediate in its viability. From these results he concluded that the neurogenic components of the gut degenerated more rapidly than the myogenic and that on that basis the actions of drugs might be distinguished that acted directly on the muscle, such as acetylcholine in higher doses, from those acting through a release of endogenous acetylcholine, such as barium ions and histamine. He noted that the responses to the last two stimulants disappeared first, as the period of cooling lengthened, since they depended for their effects upon the integrity of Auerbach's plexus in the gut. Higher doses of acetylcholine, on the other hand, acted directly upon the musculature and so continued to cause responses until the muscular elements themselves had degenerated.

METHODS:

The arrangements in our laboratory for studying the action of isolated tissues have already been described (2). The tissues employed in this study included the small intestine and uterus of the rabbit and the small intestine of the cat.

Research Department, Division of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

The gut of the guinea pig was not studied, since that from our strain of animals has proven almost entirely unresponsive to all stimulants after 12 hours of cooling. In the work with the intestines, but not the uterus, parallel experiments were performed as follows:

1. One of each of the two species was kept in a minimal amount of Tyrode's solution with air overlying the solution;

2. A second pair was kept in Tyrode's solution which had been well gassed with a 95 per cent oxygen — 5 per cent carbon dioxide mixture. In this second case the overlying air was replaced by the same gaseous mixture after which the bottle was tightly stoppered. The pH's of the solutions were taken at the end of the experiment and compared with that of freshly made Tyrode's solution.

For each day's work successive 2 cm. segments of each gut were removed, beginning with the ileal end. That was designed, insofar as possible, to eliminate as a variable in the study the gradient of diminishing intestinal activity from duodenum to ileum (3). The drugs studied included acetylcholine hydrobromide, histamine diphosphate, surgical Pituitrin and barium chloride.

RESULTS:

The results have been summarized in Tables 1-3; at the end of the experiment the pH of the Tyrode's solution with the feline gut exposed to air was 7.63, that exposed to oxygen with CO_2, 6.78, and that of Tyrode's without tissue, 8.28. The corresponding figures for the lapin gut were 8.01, 6.87 and 8.28. The pH of fresh Tyrode's solution is 8.0. It would appear the tissue was per se a better buffer than the Tyrode's solution, a not unexpected result in view of the very weak buffering capacity of that solution. The solution exposed to oxygen and carbon dioxide maintained a lower pH as expected. The pH changes themselves can probably be viewed as inconsequential for isolated tissues, a conclusion confirmed by the remarkable parallelism in the rates at which the re-

TABLE I
CHANGES IN THE RESPONSES OF ISOLATED TISSUES TO DRUGS AFTER VARIABLE INTERVALS OF COOLING

LAPIN GUT

Gaseous Phase	*Hours 0-2° C*	*Histamine*		*Acetylcholine*		*Pituitrin*		*Barium Chloride*		*Remarks*
		Dose	*Response*	*Dose*	*Response*	*Dose*	*Response*	*Dose*	*Response*	
AIR	0	40	***	0.2	***	0.4	---	100	**	
O_2-CO_2	0	40	***	0.2	***	0.4	---	100	**	
AIR	24	40	***	0.2	***	0.4	---	100	**	
O_2-CO_2	24	40	***	0.2	***	0.4	---	100	**	
AIR	50	40	*	0.2	*	0.4	–	100	*	Secondary relaxation after Ach.
O_2-CO_2	50	40	**	0.2	**	0.4	– –	100	*	Segmental movements almost gone — both strips
AIR	74	40	*	0.2	*	0.4	–	100	–	Secondary relaxation after Histamine and Ach.
O_2-CO_2	74	40	*	0.2	*	0.4	–	100	*	Secondary relaxation after Histamine and Ach.
AIR	98	1000	0	0.2	*	0.4	0	1000	*	
O_2-CO_2	98	1000	0	4	*	Not Tested		1000	*	
AIR	120									No Responses
O_2-CO_2	120									

All doses are in microgm./ml. except for Surgical Pituitrin, which is in Units/ml.
* = contraction.
– = relaxation.

TABLE II

CHANGES IN THE RESPONSES OF ISOLATED TISSUES TO DRUGS AFTER VARIABLE INTERVALS OF COOLING

LAPIN UTERUS (NON-VIRGIN)

Hours	*Histamine*		*Acetylcholine*		*Pituitrin*		*Barium Chloride*		*Remarks*
0-2° C	*Dose*	*Response*	*Dose*	*Response*	*Dose*	*Response*	*Dose*	*Response*	
1	50	***	0.2	***	0.005	***	100	***	Pituitrin & Barium Chloride elicited chain of responses
					Delayed			Delayed	
22	50	***	5	***	0.005	***	100	***	See Above
					Delayed			Delayed	
46	50	***	0.2	***	0.005	***	100	***	See Above
					Delayed			Delayed	
70	50	***	0.2	***	0.005	**	100	**	Chain of less marked, more widely spaced reactions after Pituitrin & Barium Chloride
					Delayed			Delayed	
99	50	**	2	(*)	0.005	*	200	*	Same as Above.
143	50	*	2	*	0.005	*	200	**	Same as Above.
166	50	*	500	0	0.01	*	200	*	Same as Above.

All doses are in microgm./ml. except for Surgical Pituitrin, which is in Units/ml.
* = contraction.
– = relaxation.

TABLE III

CHANGES IN THE RESPONSES OF ISOLATED TISSUES TO DRUGS AFTER VARIABLE INTERVALS OF COOLING

FELINE GUT

Gaseous Phase	*Hours*	*Histamine*		*Acetylcholine*		*Pituitrin*		*Barium Chloride*		*Remarks*
	0-2° C	*Dose*	*Response*	*Dose*	*Response*	*Dose*	*Response*	*Dose*	*Response*	
AIR	22	1	***	0.04	***	0.2	–––	60	***	Secondary Stimulation after washing out Pituitrin
O_2-CO_2	22	1	***	0.04	***	0.2	–––	60	***	
AIR	50	1	***	0.04	***	0.2	–––	60	**	See above.
O_2-CO_2	50	1	***	0.04	***	0.2	–––	60	**	
AIR	73	1	***	0.04	***	0.2	–––	1000	*	See above.
O_2-CO_2	73	1	***	0.04	***	0.2	–––	1000	*	
AIR	92	1	*	0.04	*	0.2	–	1000	*	See above.
O_2-CO_2	92	1	**	0.04	**	0.2	––	1000	*	
AIR	117	1	*	0.04	*	0.2	–	60	*	Secondary Stimulation after Pituitrin gone
O_2-CO_2	117	1	*	0.04	*	0.2	–	60	*	
AIR	141	200	0	0.4	**	0.2	–	60	(*)	
				0.2	0					
O_2-CO_2	141	10	***	0.04	**	0.2	–	60	(*)	
		200	***	0.4	**					
AIR	163	200	0	0.4	**	0.2	–	60	(*)	
				0.2	0					
O_2-CO_2		10	***	0.04	**					
	163	200	***	0.4	**	0.2	–	60	(*)	
AIR	197	200	0	0.4	*	0.2	–	60	*–0	
O_2-CO_2		10	**	0.04	*	0.2	0	60	0	
	197	200	*	0.4	*	0.2	–	60	*–0	
AIR	221	200	0	0.4	(*)	0.2	0	60	0	
O_2-CO_2	221	200	(*)	0.4	(*)	0.2	0	60	(*)	
AIR	245									No responses.
O_2-CO_2	245	200	(*)	0.4	(*)	0.2	0	60	(*)	
AIR	270									No responses.
O_2-CO_2	270									No responses.

All doses are in microgm./ml. except for Surgical Pituitrin, which is in Units/ml.
* = contraction.
– = relaxation.

sponses of the two differently treated intestines declined.

DISCUSSION:

The decline in the responsiveness of all three tissues to histamine and acetylcholine as the interval of storage at 0-2°C lengthened cannot be viewed as significantly different. Two of the tissues (feline gut and lapin uterus), and to a less extent, the lapin gut, appeared to have lost their sensitivity to barium chloride at a significantly faster rate than they did to the other two stimulants. This would appear to confirm, as Ambache contended, that barium chloride does not act directly upon the muscular contractile mechanism. Our results yield no evidence concerning the mechanism of barium chloride's action. Feldberg and Emmelin (4) in experiments designed to elucidate this mechanism, could find no support for Ambache's hypothesis. In an extensive study of the toxicity of various metallic ions upon fungi, Seifriz (5) concluded their toxicity was best correlated with the physical properties of the respective ions: valence, hydration, solubility, dimensions, etc. Such physical properties probably account for the non-specific effect of the barium ion, since no physiological role for this ion has ever been demonstrated. By altering the potential of the cell's membrane, it could temporarily distort the cell's equilibrium. Some indirect evidence suggests that a disturbance in the local concentration of the potassium ion may be the focus of its action. Capraro and Barilli (6) found the response of the guinea pig's intestine to potassium in the presence of a fixed concentration of barium ion increased within a limited range as the concentration of the potassium ion was augmented. Local shifts in the concentrations of potassium ions have been considered at least partly responsible for the reaction to other drugs. D'Silva (7) and Schwartz (8) first demonstrated on animals that epinephrine causes an increase in the serum's concentration of potassium ion. This was confirmed by Marenzi and Gerschman (9) for animals. Later it was independently shown for man also by D'Silva (10) and by Brewer, Larson and Schroeder (11). The earlier failure to detect an increase in the serum's concentration of the potassium ion in humans (12, 13, 14) had been due to the use of venous blood for analysis. The elevation in the concentration of the potassium ion was so fleeting it could be detected only by an analysis of the arterial blood. It had subsided by the time the blood had arrived at the venous end of the circulation. These facts, with others, have led Brown, Bülbring and Burns (15) to suggest epinephrine may act by altering the distribution of the potassium ion in muscle. It is thus possible that the barium ion may act by causing a shift in the concentration of some other cation. It does seem most unlikely that it acts directly on the cell's contractile mechanism, since the responses to histamine and acetylcholine persisted somewhat longer than did those to the barium ion. That a many fold increase in the dose failed to augment the response of still active tissue indicates that it acts on a labile component or element of the structure, which, once exhausted or destroyed, leaves the barium ion in reasonable doses without a focus of attack. This suggests the focus of the barium ion's action is considerably removed from the final link in the enzymatic chain of reactions terminating in the contractile response.

SUMMARY:

A study was made of the effects of cooling upon the responses of the isolated intestines of cats and rabbits and the isolated uterus of the rabbit to acetylcholine, histamine, Pituitrin, and barium chloride. Since the responses of the tissues to the last spasmogenic agent were characterized by marked tachyphylaxis and an earlier loss than were the responses to the first two, it has been suggested that the focus of the barium ion's action is relatively far removed from the final contractile response. It may lead to an alteration in the local concentration of some other cation such as potassium.

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