Technical Papers

Thermal Stimulation of Isolated Organs and its Inhibition by Pharmacological Agents

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The effect of temperature variations on biological systems has been examined from various standpoints: sensitivity of isolated organs to drugs (*1–3*), thermodynamics of muscle contraction (*4*), biochemical changes (*5*), and reaction of the organism *in toto* (*6–10*). We investigated the effect of controlled temperature variations on the isolated intestine with the anticipation that this might give some clue to the mechanism of the thermal effect on the organism, especially so far as the possible intervention of a mediator is concerned. In exploring this possibility, we searched to obtain an inhibition of the thermal effect by pharmacological agents. About 150 experiments carried out so far permit a certain number of conclusions which we consider it worth while to report, while our *in vivo* studies are progressing.

Isolated intestinal strips were suspended, and recorded kymographically, in a Ringer solution containing, per liter: 9 g NaCl, 0.25 g KCl, 0.25 g $CaCl_2$, 0.5 g glucose, and 0.5 g $NaHCO_3$.

Care was taken to avoid exposure of the organs to uncontrolled cooling by collecting and suspending the strip as rapidly as possible from the guinea pig or rabbit just sacrificed, or by making the autopsy in a thermostatic chamber at 38°. The required temperature variation on the suspended strip was obtained by adding to the external water bath ice water or water at a temperature above 38°. The internal temperature of the glass tube containing the organ was continuously recorded.

Our findings regarding cold contractions were:

1) A sudden decrease of the temperature—for instance, from 37° to 24° C—induces a contraction of the isolated guinea pig intestine. This contraction, which resembles those induced by histamine and acetylcholine, has a broader (more prolonged) shape than the latter, however. The fall of temperature which in all cases gave a typical cold contraction occurred at the rate of 10°/min. The deeper and more rapid the temperature fall, the more constant and typical is the outcome of the experiment.

2) It is possible to lower the temperature of the isolated guinea pig intestine to + 4° without obtaining a cold contraction, if the temperature is decreased gradually (e.g., at a rate of 1° C/min).

3) The cold contraction is inhibited by synthetic antihistaminics (Benadryl, Pyribenzamine, Thenylpyramine) and by spasmolytic drugs such as Trasentine and Pavatrine, but it is not influenced by atropine. For example, Benadryl 1: 200,000–1: 400,000 inhibited completely the cold contraction (Fig. 1); atropine concentrations as high as 1: 20,000 were ineffective.

4) The isolated guinea pig intestine conserves its sensitivity to histamine and acetylcholine until the temperature reaches + 8°–10°. Its sensitivity to histamine and $BaCl_2$ becomes nil, or almost, at about + 4°, whereas that to acetylcholine is still maintained at this low temperature.

5) When the temperature of the cooled guinea pig intestine is raised again to 37°, it reacquires its usual behavior and sensitivity not only to biochemical (ACH, histamine) stimulators, but also to another thermal (cold or heat) stimulation. In fact, in our experiments with intermittent temperature changes, the thermal contraction has been obtained repeatedly over periods of 4–8 hr.

6) At low temperatures (+ 4°) the contraction curve caused by acetylcholine and histamine, whenever it occurs, is greatly prolonged; the muscular contraction has a tendency to become spastic.

In contrast to the guinea pig intestine, that of the rabbit gives only a very short contraction, if any,

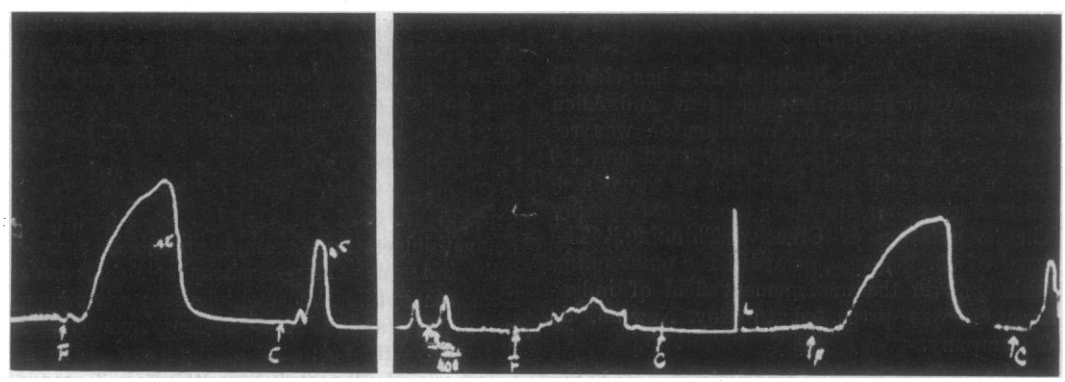

FIG. 1. Guinea pig intestine: *F*, cold contraction caused by sudden decrease from 38° to 4°, is followed by heat contraction (*C*) after bringing the temperature back to 38°. *F* and *C* are inhibited by 1: 400,000 Benadryl. After washing with Ringer's (*L*) the initial thermal effect reappears.

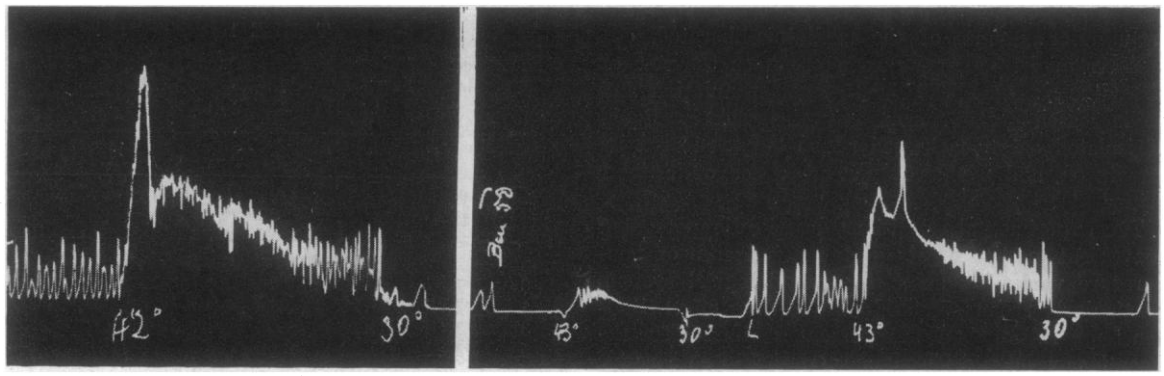

FIG. 2. "42°," heat contraction obtained by raising the temperature from 30° to 42°; "30°," intestine has been brought back to 30° C. Benadryl 1 : 50,000 inhibits the heat effect. After washing (*L*) the heat effect reappears.

upon lowering the temperature. Furthermore, at low temperatures (+4°) it loses its spontaneous contraction and its sensitivity to acetylcholine, epinephrine (1:100,000,000), histamine. When the temperature is brought back to the physiological zone (37°), the rabbit intestine reacquires its spontaneous contractions and its original sensitivity to the above reagents.

Regarding heat contractions, our findings were:

1) A sudden increase of temperature gives a contraction of the isolated guinea pig intestine, which characteristically can be obtained without surpassing the body temperature (e.g., by an increase from 25° to 37°). However, the contraction is more intense if the final temperature is above 38° (e.g., from 30° to 43°). The heat contraction occurs less regularly than that induced by cold; in our experience it has been obtained in about 60% of the trials.

2) The form of the heat-contraction curve is different from that obtained by temperature decrease; it is generally represented by a higher initial peak, followed in most cases by a number of rapid contractions, as illustrated in Fig. 2.

3) The heat contraction is greatly diminished or completely abolished by Benadryl, Trasentine, and Pavatrine and is not influenced by atropine. The initial peak contraction is more sensitive to the effect of these drugs than the more prolonged and lower part of the contraction curve. Thus, in favorable experimental conditions, it is possible to inhibit the first, higher contraction and to maintain the successive ones.

4) A certain differentiation between cold and heat stimulation is given by the behavior of the rabbit intestine, which reacts only to the latter. The heat contraction of rabbit intestine is inhibited by Benadryl.

Sudden temperature variations induce contractions of the isolated intestine which resemble those determined by chemical stimulators (acetylcholine, histamine, $BaCl_2$).

The duration of the cold-contraction curve is more prolonged than that obtained with chemical stimulators at physiological temperatures. The prolonged shape of the curve depends probably on the final temperature condition of the experiment rather than on the nature of the stimulus, since at low temperatures histamine and acetylcholine also induce a more prolonged contraction of the guinea pig intestine. A decreased activity of the enzymes responsible for the destruction of the stimulators (histaminase, cholinesterase), determined by the drop of temperature, could account for the prolonged effect of the stimulation.

The responsiveness of the rabbit intestine to heat but not to cold stimulation can be related to the insensitivity of this organ to chemical stimulators at low temperatures.

Thermal stimulation depends on the *rate* of the temperature variation (i.e., on the *variation* of the energy content) and is independent of the absolute temperature reached. Thus, low (4°) as well as high (42°–44°) temperatures can be reached without contraction, by changing the temperature gradually.

The contraction induced by *thermal stimulation* is inhibited by the antihistaminics Benadryl, Pyribenzamine, and Thenylpyramine, and by the antispastic agents Pavatrine and Trasentine.

On the isolated intestine, Benadryl (benzhydryl-ether of dimethylaminoethane) possesses general antispastic properties, since it is effective against acetylcholine, histamine, and $BaCl_2$, whereas the ethylendiamine derivatives (Pyribenzamine, Thenylpyramine) have activity mainly against histamine (*11, 12*).[1] The inhibiting effect of the latter drugs on the thermal contraction leads to the conclusion that *antihistaminic activity* is a sufficient condition to bring about inhibition. As a corollary, it may be assumed that histamine is the mediator responsible for the effect of thermal stimulation. In confirmation of this assumption atropine, the most effective antagonist of the acetylcholine effect on the guinea pig intestine, does not inhibit thermal contraction. The antithermal activity of Pavatrine and Trasentine might be attributed to their depressant action on the muscular fiber.

Direct transfer experiments, using the tissue-bath fluid after contraction, have not so far revealed (biologically) detectable amounts of any mediator. This, however, could depend on a rapid enzymatic destruction, on an intracellular liberation and fixation, or

[1] A correspondingly wider range of activity has been observed also *in vivo* for Benadryl (*13*). This drug, in contrast to the ethylendiamine antihistaminics, has a high protective action against the lethal cholinergic shock of guinea pig.

on minute amounts of the mediator liberated, etc.

There might be some relation between the role of histamine as a mediator of thermal stimulation and the increased histamine content in the perfusion liquid of isolated cat liver heated above 38°, reported by Rawlinson and Kellaway as an effect of cellular injury (*5*).

The experimental conditions described represent a convenient method of studying the influence of temperature variations on a reactive biological system, particularly from the standpoint of drug effect. Sudden temperature changes represent a stimulus for the smooth muscle, which contracts, probably as a result of the liberation of a histaminelike mediator. The contraction is inhibited by antihistaminic and antispastic drugs, but not by atropine.

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Effects of an Exogenous Growth Regulator on Proteolytic Enzymes of the Soybean Plant[1, 2]

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In recent years it has been shown that 2,4-dichlorophenoxyacetic acid (2,4-D) decreases the amount of protein nitrogen in the leaves and increases the level of this fraction in the stems and roots of treated plants (*1–6*). Accordingly, it was considered possible that this growth regulator affects proteolytic enzymes differently in the leaves and in the stems and roots. To test this hypothesis soybean plants were treated with 2,4-D and analyzed for proteinase and polypeptidase activity in leaves, stems, and roots.

Plants were grown in solution culture until they were 38–40 cm tall and had 6 fully expanded trifoliate leaves. At that stage the soybeans were treated by placing them in nutrient solutions containing 5 ppm of 2,4-D for an exposure period of 24 hr, after which the treated plants were returned to fresh nutrient solutions containing no 2,4-D.

TABLE 1

Effect of 2,4-D on Proteinase and Polypeptidase Activity in Leaves, Stems, and Roots of Soybean Plants

Days after treatment	Leaves		Stems		Roots	
	Controls	Treated	Controls	Treated	Controls	Treated
	*Proteinase Activity**					
0	1.97	—	0.14	—	0.43	—
1	1.88	1.48	.10	0.19	.59	1.01
3	1.68	1.47	.04	.52	0.42	1.28
5	1.82	1.22	0.09	0.34	—	—
	*Polypeptidase Activity**					
0	2.55	—	0.77	—	0.86	—
1	—	—	.84	0.90	1.16	1.25
3	2.49	2.35	.66	0.79	0.81	1.65
5	2.35	1.55	0.75	1.10	—	—

* Measured as ml 0.05 *N* NaOH required to neutralize the increase in carboxyl groups after 24 hr incubation of plant enzyme extract with substrate of gelatin for proteinase activity or peptone for polypeptidase activity.

Within 6 hr of the start of exposure to 2,4-D, twisting of stem tips and epinasty of petioles of treated plants were observed. By the third day after treatment, these symptoms were more pronounced, although all leaves were still completely turgid. By the fifth day, some of the leaves of the treated plants had wilted permanently and were just starting to turn dry. Most of the leaf tissue was still not dehydrated or dead, as indicated by the fact that the average percentage dry matter of all leaves of treated plants at this stage had reached only 19.5% as compared with 17.3% for the controls. Stems and bases of petioles of treated plants were definitely enlarged by this time.

Plants harvested before and after treatment were separated into the tissue fractions indicated in Table 1 and dried at 45° C. At each harvest reported in Table 1 all the leaves from 9 plants were composited into one sample for analysis. Each stem and root sample also represented all the respective tissue fraction from 9 plants. Glycerol extracts of the dried tissue were used for measurement of proteinase and polypeptidase activity according to methods described by Blagowestschenski and Melamed (*7*), Lauffer *et al.* (*8*), and Moundfield (*9*). Duplicate aliquots were analyzed, and determinations were repeated when good agreement between replicates was not obtained.

The results shown in Table 1 indicate that, with 2,4-D treatment, proteinase and polypeptidase activity decreased in the leaves by the end of the experiment, whereas in the stems and roots the activity increased considerably. The magnitude of the changes in proteolytic enzyme activity following treatment with 2,4-D indicates a significant influence of the growth regulator on this phase of nitrogen metabo-

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Plant Physiology.

[2] This paper is based upon work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Md., under Contract No. DA-18-064-CML-450 with Rutgers University.