60% of animals, maximum transport occurred in the pH range 5.90–6.12. Over 90% of animals showed maximum transport between pH 5.50–6.50. Mean transport rates at pH 4.80 and pH 7.26–7.82 were significantly lower than at pH 5.50–6.12.

Comment. Although several studies, in both man and animals, have been concerned with the effect of pH on glucose absorption, the pH optimum has remained controversial. A major difficulty in such *in vivo* studies is the maintenance of constant intraluminal pH during the study period. It is now well recognized that intraluminal pH tends toward neutrality during perfusion, owing to the buffer capacity of enteric secretions. The present study was therefore performed in everted gut sacs in which pH could be precisely maintained.

In the present studies, pH was maintained by means of phosphate buffers. Although it is possible that phosphate per se may have specific effects on glucose transport, this seems unlikely since Laszt (1), Ponz and Larralde (4) and Darlington *et al.* (10) have shown that phosphate does not have measurable effects.

The data indicate that pH has a pronounced effect on glucose transport, with a pH optimum of about 5.9. It is concluded that intraluminal pH may be of considerable importance in the intestinal absorption of glucose.

Summary. The effect of pH on glucose transport was studied in 131 everted gut sacs of upper small bowel from 25 hamsters, using phosphate buffered solutions over the pH range 4.80–7.82. The relationship between pH and glucose transport rate revealed a bell-shaped curve with a pH optimum of 5.9. Under these conditions, in which intraluminal pH adjustment by the small bowel was eliminated, a pronounced effect of pH on glucose absorption was demonstrated.

1. Laszt, L., Biochem. Z. **76**, 40 (1935).
2. Groen, J., J. Clin. Invest. **16**, 245 (1937).
3. Goldenberg, J. and Cummins, A. T., Gastroenterology **45**, 189 (1963).
4. Ponz, F. and Larralde, J., Nature **164**, 912 (1951).
5. Broitman, S. A. and Zamcheck, N., Federation Proc. (Abstract) **23**, 259 (1964).
6. Magee, L. D. and Hastings, A. B., J. Biol. Chem. **142**, 893 (1942).
7. Swallow, J. H. and Code, C. F., Am. J. Physiol. **212**, 717 (1967).
8. Wilson, T. H. and Weisman, G., J. Physiol. (London) **123**, 116 (1954).
9. Dahlquist, A., Anal. Biochem. **7**, 18 (1964).
10. Darlington, W. A. and Quastel, J. H., Arch. Biochem. Biophys. **43**, 194 (1953).

Received Sept. 21, 1967. P.S.E.B.M., 1968, Vol. 127.

The Protective Effect of Guanylic Acid against Isoproterenol Toxicity* (32787)

JOHN KABAL AND ESTELLE R. RAMEY (Introduced by J. W. Johnston, Jr.)
Department of Physiology and Biophysics, Georgetown Medical Center, Washington, D.C. 20007

Kokas and co-workers (1) found that pretreatment of intact rats with a guanylic acid derivative decreased the cardiac hypertrophy produced during a chronic forced swimming experiment. More recently, work from this laboratory (2) showed that adrenalectomized rats given injections of guanylic acid (GA) had a significantly extended survival time after tourniquet shock. Our data also indicated (3) that this guanine nucleotide could suppress the cardiac hypertrophy, the elevation in plasma FFA and the decrease in liver glycogen which results from adrenalin injection. Similarly, guanylic acid has been shown by Sydow (4) to have significant cardiovascular effects. The mechanism of these effects of guanylic acid remains unknown.

To further elucidate the cardiovascular effects of these nucleotides, we investigated and compared the effect of chronic and acute administration of guanylic and adenylic acid

* Supported in part by Washington Heart Association Grant and USPHS Grant No. AM-04714.

(AA) on the survival time during tourniquet shock when the functional capacity of the heart had been greatly reduced by use of isoproterenol.

Methods. Male, albino rats of the Sprague-Dawley strain, weighing 220 ± 20 (SD) gm were used throughout the experiments. The animals were maintained on Purina Rat Chow and drinking water ad libitum. The room temperature was maintained at 72°F ± 2° (SD). The rats were kept in individual cages, large enough to prevent restraint-hypothermia.

Limb ischemia was produced by ligation of the kind limbs at the inguinal level with two tightly bound rubber bands (Fisher Scientific Company; rubber tubing gooch, Size B, 1.25 inches under light ether anesthesia.

The drugs were administered as follows: (i) Guanylic or adenylic acids (2′ (5) mixed isomers of Na Salt; Pabst Laboratories, Milwaukee, Wisconsin) were injected in the dose of 15 mg/kg sc either daily for a period of nine days or as a single injection before the application of tourniquets. The control groups were treated with 0.9% NaCl solution (0.2 ml/rat sc) during the same period of time. (ii) Isoproterenol HCl (Winthrop Laboratories, New York) was given in two single injections (50 mg/kg sc per injection) on 2 consecutive days on the last 2 days of the nucleotide-pretreatment or 2 days prior to the single nucleotide injections.

Tourniquets were applied 1 day after the last drug injections except in the case of the single injections of nucleotides, which were injected just before application of the tourniquets.

Total body oxygen consumption was measured during anesthesia (30 mg pentobarbital/kg ip) in a M.O.U.S.E. spirometer (model 160, Custom Eng. & Dev. Co., St. Louis, Mo.) and expressed in ml of O_2/hour per 100 gm of body weight.

The heart rates of the anesthetized rats were calculated using the ECG (Sanborn Twinviso recorder). The animals were then decapitated and the hearts were quickly removed, blotted and weighed. The cardiac index was expressed as the heart weight in grams per 100 gm of body weight.

For statistical evaluation we calculated the standard deviation and the significance of the

TABLE I. Cardiovascular Effects of Isoproterenol and Nucleotides.

	No. of rats	Body wt.	Cardiac index: Heart wt. (mg) /100 gm of body wt. (± SD)	*p* value	Total body O_2 uptake (ml of O_2/hour per 100 gm of body wt. (± SD)	Heart rate/min
1. Normal, control	11	225 ± 14	362 ± 18	—	196 ± 16	435 ± 26
2. Isoproterenol treated	14	218 ± 11	526 ± 29	to 1 = <.01	234 ± 22	416 ± 32
3. Isoproterenol and guanylic acid pretreatment	12	229 ± 8	465 ± 21	to 2 = <.025	221 ± 19	428 ± 29
4. Isoproterenol and adenylic acid pretreatment	10	221 ± 15	531 ± 35	to 2 = NS	227 ± 15	422 ± 38

TABLE II. Effect of Nucleotides on Survival After Bilateral Limb Ischemia.

Groups	No. of rats	Survival time[a] (hours)	p value
1. Untreated controls	21	11.2 ± 1.6	—
2. Isoproterenol	16	3.4 ± 0.8	to 1 = <.01
3. Isoproterenol and GA pretreatment	15	5.3 ± 0.7	to 1 = <.01 to 2 = <.01
4. Isoproterenol and AA pretreatment	14	2.8 ± 1.1	to 1 = <.01 to 2 = NS to 3 = <.01
5. GA single injection	15	10.8 ± 1.1	to 1 = NS
6. GA pretreatment	10	10.9 ± 1.4	to 1 = NS
7. AA single injection	12	11.6 ± 0.8	to 1 = NS
8. AA pretreatment	8	12.1 ± 1.6	to 1 = NS
9. Isoproterenol and GA single injection	15	3.7 ± 0.5	to 1 = <.01 to 2 = NS
10. Isoproterenol and AA single injection	15	2.5 ± 0.7	to 1 = <.01 to 2 = NS

[a] Mortality was 100% in all groups.

difference between two means by the Student t test (6).

Results. Two consecutive days of subcutaneous injection of 50 mg/kg of isoproterenol caused extensive damage to the myocardium. Histological sections show marked edema. This is also manifested in the increased cardiac index as compared to normal controls (Table I). Seven-days pretreatment with guanylic acid before injecting the isoproterenol significantly lowered the edema and the cardiac index accordingly was also lower. Similar pretreatment with adenylic acid had no significant effect (Table I). Total body O_2 consumption and heart rate was not affected by isoproterenol treatment.

In the second part of the experiment we investigated the effect of isoproterenol on the survival time after bilateral or unilateral ischemia with or without nucleotide pretreatment. We have found consistently that 5-hours bilateral limb ischemia produces irreversible shock and subsequent lethality within about 11 hours after releasing the tourniquets (Table II). The survival time of the isoproterenol treated animals is lowered to approximately 3 hours following the 5-hour-bilateral limb ischemia. Single injections of the nucleotides at the time of applying the tourniquets with or without the previous isoproterenol treatment did not alter the survival time significantly. However, when the isoproterenol treated animals were pretreated with GA 7 days prior to the isoproterenol injection, the survival time was significantly extended. Adenylic acid pretreatment, on the other hand, did not have a significantly protective effect.

When unilateral limb ischemia was maintained for 5 hours, all normal animals survived the procedure following removal of the tourniquet. Isoproterenol injection concomitant with the unilateral tourniquet application led to 100% mortality within 7 hours after removal of the tourniquet (Table III). Guanylic acid pretreatment for 7 days before the exposure to tournique stress and isoproterenol produced some increase in survival time following tourniquet release. Adenylic acid had no effect.

Discussion. Our purpose in this experiment was to use the combined isoproterenol and tourniquet stress as a test of the effectiveness of certain nucleotides in the maintenance of homeostasis. We had earlier data which indicated that guanine nucleotides could alter the metabolic responses to stress and confer some degree of protection on the animal subjected to tourniquet application (2, 3).

In our earlier work we had found that

TABLE III. Effects of Nucleotides on Tourniquet and Isoproterenol Stressed Animals.

Groups	No. of rats	Survival times (hours)	Mortality (%)	p value
1. Untreated control	16	∞	0	—
2. Isoproterenol	19	7.1 ± 1.3	100	to 3 = <.01
3. Isoproterenol and GA pretreatment	12	11.7 ± 1.4	100	to 2 = <.01 to 4 = <.01
4. Isoproterenol and AA	9	6.2 ± 1.5	100	to 2 = NS

intact rats were not significantly protected by nucleotide treatment when subjected to tourniquet application alone. Adrenalectomized rats, however, showed an increased viability when guanine nucleotides were administered (2). In addition, the intact animals did maintain certain metabolic parameters closer to unstressed levels with guanine nucleotide treatment (3). This suggested to us that the availability of endogenous nucleotides to the stressed intact animal might be optimum at one degree of stress but inadequate if the intensity of the stress required more nucleotides than could be made available by either liver production or lymphatic involution (5).

In acute experiments using isoproterenol injections in resting animals, Beznak and coworkers could find no significant change in systolic or diastolic pressure, heart rate, peripheral resistance, systolic output, and systolic work (7). In our experiments, we had already challenged the capacity of the cardiovascular system to compensate for the extravasation of fluid from the damaged hind limbs. We then added the insult of isoproterenol injection and under these circumstances the cardiovascular adjustments were so limited that even unilateral limb ischemia led to shock and death of the animals. With these conditions we were able to demonstrate that guanylic acid but not adenylic acid could significantly extend the survival time of the stressed rats. The mechanism whereby this was accomplished is obscure. It is possible that the availability of additional nucleotides may contribute to maintenance of more adequate protein synthesis via increased RNA synthesis. There must be a degree of specificity in this action because adenine nucleotides did not have this effect. Thus, we are studying the effect of the guanine nucleotides on RNA synthesis in cardiac muscle under the conditions of the experiments described above.

Summary. Subcutaneous injections of isoproterenol in rats produces myocardial edema and other histologic evidence of toxicity. If guanylic acid is injected daily for 7 days preceding the administration of isoproterenol there is significantly less cardiac muscle edema and the cardiac index (heart weight/body weight) is lowered. Similar pretreatment with adenylic acid had no effect. When bilateral or unilateral hind limb tourniquet is added to the stress of isoproterenol injection the survival time of the animals following tourniquet removal is markedly decreased. Seven days of pretreatment with guanylic acid significantly extended the survival time of these doubly stressed animals. Again, adenylic acid was without effect. It is suggested that guanine nucleotides may be utilized to a greater degree during severe stress and administration of exogenous guanine nucleotides may produce more effective protein anabolism under these circumstances.

1. Kokas, E., Miczban, I., and Toro, I., Acta Physiol. Hung. 7, 409 (1955).

2. Kabal, J. and Ramey, E. R., Proc. Soc. Exptl. Biol. Med. 104, 95 (1960).

3. Kabal, J. and Ramey, E. R., Endocrinology 77, 188 (1965).

4. Sydow, V. L. and Ahlquist, R. P., J. Am. Pharm. Assoc. 43, 166 (1954).

5. Hollis, V. W., Jr. and Ramey, E. R., Am. J. Physiol. 211, 1339 (1966).

6. Bailey, N. T. J., "Statistical methods in biology." Wiley, New York, 1959.

7. Beznak, M., Can. J. Biochem. Physiol. 40, 25 (1962).

Received Sept. 22, 1967. P.S.E.B.M., 1968, Vol. 127.