IGF's have a simultaneous inhibitory effect on GRF. Furthermore, in view of recent reports demonstrating that centrally administered peptides rapidly enter peripheral blood (*19*), it is possible that intracerebroventricularly administered ILA's also feed back at the pituitary level by transport from cerebrospinal fluid to anterior pituitary.

In our experiments, central injection of ILA's caused a diminution in body weight associated with decreased food intake, but large doses of insulin did not have a similar effect, suggesting that the response is mediated by somatomedin receptors rather than insulin receptors in the CNS. This finding is not at variance with that of an earlier study (*20*) suggesting a role for centrally administered insulin in the control of appetite since, in that study, animals received insulin infused centrally over a 14-day period and showed no significant effects after 24 hours. Since many studies indicate that somatomedins are depressed in undernutrition (*8, 17*), our findings raise the possibility that IGF's participate in the regulation of feeding and appetite and suggest that IGF's should be further investigated in human obese states. Our observations should be confirmed with more highly purified somatomedins before the IGF's are included in the ever-growing list of putative satiety factors (*21*).

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Trypsin Inhibition by Tapeworms: Antienzyme Secretion or *p*H Adjustment?

Abstract. *The tapeworm* Hymenolepis diminuta *releases proteins that inhibit trypsin activity. These proteins may be either antienzymes or nonspecific macromolecules that interfere with trypsin. Saline solutions with initial* p*H values ranging from 5.5 to 10.0 were all acidified to* p*H 5.0 by tapeworms. If the initial* p*H was lower than 5.0, it was raised. Because trypsin activity is inhibited at* p*H 5.0, this intestinal parasite can protect itself from digestion by regulating its environmental* p*H or releasing trypsin inhibitors, or both.*

It is not clear how intestinal parasites such as tapeworms live and thrive in an environment of hydrolytic enzymes without being digested. One hypothesis is that they produce and release hydrolase inhibitors—the so-called antienzymes (*1*). We report that *Hymenolepis diminuta* releases proteins that appear to inhibit trypsin activity. These proteins could be antienzymes or nonspecific macromolecules that interfere with trypsin activity or assays. This tapeworm can also regulate the *p*H of its environment to about 5.0 by excreting organic acids. Because trypsin activity is minimal at *p*H 5.0, this endoparasite can protect itself from trypsin digestion by acidifying its environment. Indeed, the rat intestine is more acidic when *Hymenolepis* is present (*2*).

Tapeworms were obtained from young rats infected in the laboratory and washed in buffered Krebs-Ringer saline (*3*). Groups of six to eight worms were incubated at 37°C in 10 ml of saline for 15 minutes, transferred to 5 ml of fresh saline for further incubation at 37°C for various periods, and removed. Replicate samples of the conditioned saline were pooled (total volume, 15 ml), and the *p*H was readjusted to 7.4 (All *p*H adjustments were made by appropriate additions of HCl or NaOH.) Samples of the pooled saline were used for measuring protein (by the Lowry method) and trypsin inhibitory activity, with azoalbumin used as the substrate for trypsin (*4*). The buffer, tris(hydroxymethyl)aminomethane maleate, was replaced isosmotically with NaCl when unbuffered saline was used.

Buffered saline (initial *p*H 7.4) conditioned with tapeworms for 2 hours contained both Lowry-positive material (95 μg/ml) and trypsin inhibitory activity. Trypsin digestion of azoalbumin was 16 percent less in conditioned saline than in control saline. Both Lowry-positive material and trypsin inhibitory activity appeared during the second 30 minutes of incubation and increased progressively for up to 2 hours. Dialysis (cutoff, 3500 daltons) of conditioned saline adjusted to *p*H 7.4 against 3 liters of buffered saline at *p*H 7.4 and 4°C rapidly removed Lowry-positive material. After 5 hours of dialysis 65 percent of the Lowry-positive material was removed; a maximum of 75 percent was removed after 16 hours. However, even after 48 hours of dialysis there was no significant decrease in trypsin inhibitory activity in the nondialyzable fraction. After a 5-minute exposure of this fraction to 95°C, 50 percent of the

trypsin inhibitory activity was removed, and after 15 minutes at 95°C all inhibitory activity disappeared. This suggests that about 75 percent of the Lowry-positive material released by the worms does not affect trypsin activity.

Throughout the experiments we observed that the *p*H of saline containing tapeworms changed during the incubation period. To determine whether tapeworms can regulate ambient *p*H, we incubated worms for 2 hours in buffered saline having an initial *p*H of 2.0 to 8.0. When the initial *p*H was below 5.0 it was increased by the tapeworms, but if the initial *p*H was above 5.0 it decreased (Fig. 1). An initial *p*H of 4.0 was increased in 30 minutes to 4.7, where it was maintained for the remaining 90 minutes of incubation. The *p*H of saline with initial values of 3.0 and 5.0 increased and decreased, respectively, to 4.7. In control saline, trypsin digestion of azoalbumin was inhibited 95 percent when the *p*H was lowered from 7.4 to 5.0 and was negligible at *p*H 4.5.

We then determined the buffering capacity of the substances excreted by the tapeworms. Solutions of unbuffered saline (initial *p*H 5.5, 7.4, and 10.0) containing 5.0 m*M* glucose were incubated with worms for 2 hours and then titrated (Fig. 2). During the incubation period the worms decreased the *p*H of each solution to approximately 5.0. As expected, these *p*H changes occurred more rapidly than those shown in Fig. 1. In fact, all the solutions were at or below *p*H 6.0 after 30 minutes. Color and motility of the worms appeared normal. Before titration the final glucose concentrations, as determined by the glucose oxidase method, were 3.47 m*M* (initial *p*H 5.5), 3.06 m*M* (initial *p*H 7.4), and 2.50 m*M* (initial *p*H 10.0). Thus, in lowering the ambient *p*H to 5.0 from 5.5, 7.4, and 10.0, the worms consumed progressively more glucose.

The titration curves for the conditioned saline (Fig. 2) show effective buffer regions between *p*H 3.5 and 5.5. For example, with the addition of 20 μmole of OH^-, there was an increase of over 5 *p*H units in control saline but an increase of only 1 *p*H unit in conditioned saline. In addition, the relative buffer concentrations in conditioned saline increased with increasing initial *p*H. For example, the *p*H of conditioned saline with initial values of 5.5 and 10.0 increased to 9.0 and 6.0, respectively, after the addition of 45 μmole of OH^-. Thus, the buffering capacity of the saline increased as glucose consumption by the worms increased. An approximate *p*K value (*p*H 4.5) of conditioned saline is shown in Fig. 2. Once conditioned saline was outside the *p*H range of 3.5 to 5.5, its buffering capacity was rapidly lost.

Our experiments demonstrate that *Hymenolepis* releases heat-labile, nondialyzable protein having "antitrypsin" activity. Although it is tempting to interpret this as evidence for the antienzyme hypothesis, we have not completely characterized this protein. It may not be a true enzyme inhibitor like the proteolytic inhibitor secreted by the larval tapeworm *Taenia pisiformis* (*5*). *Hymenolepis* does release proteins in vitro that interfere in nonspecific ways with certain enzyme assays (*6*). Moreover, previous attempts to identify or isolate an inhibitor from trypsin inactivated by *Hymenolepis* have been unsuccessful (*7*). Thus, the apparent trypsin inhibitor demonstrated in this study might be a nonspecific macromolecule that simply interferes with the trypsin assay. If we presume that this substance is nonspecific, we are led to conclude that protection from trypsin involves some mechanism other than the production and release of antitrypsin.

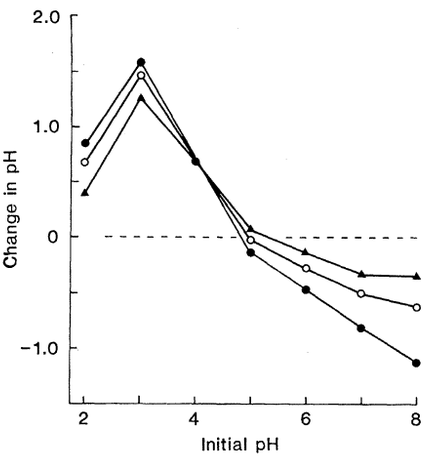

Fig. 1. Changes in the *p*H of buffered saline during 2 hours of incubation with *Hymenolepis diminuta*. Ten worms were incubated per each 12 ml of saline. Measurements of *p*H were made after 30 minutes (▲), 90 minutes (○), and 120 minutes (●) and are plotted relative to the initial *p*H (2.0 to 8.0). At *p*H 3.0 to 8.0, worm color and motility remained normal throughout the incubation period. After 10 minutes at *p*H 2.0 the worms were moribund, as indicated by their lack of movement and change in color.

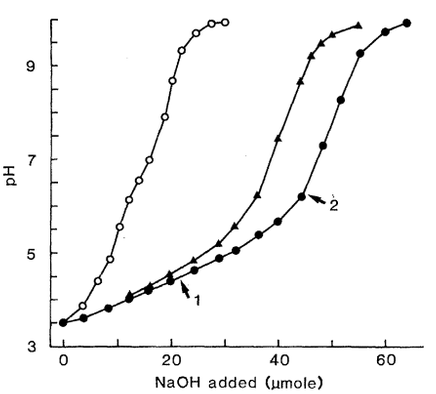

Fig. 2. Titration curves for unbuffered saline (○) and unbuffered saline conditioned for 2 hours with *Hymenolepis diminuta* (▲, initial *p*H 5.5; ●, initial *p*H 10.0). After incubation, the worms were removed and all solutions were adjusted to *p*H 3.5 and titrated with NaOH. Increases in *p*H from 3.5 to 10.0 following the addition of OH^- are plotted. Values for a solution with an initial *p*H of 7.4 fell between those for the other two test solutions and were omitted for clarity. Arrow 1 indicates the approximate center of the buffer regions (*p*K). At arrow 2, buffering capacities were lost.

Our studies show that the tapeworm can regulate the acidity of its environment to about *p*H 5.0. The intestinal lumen of a rat infected with *Hymenolepis* is strongly acidic (*p*H 5.5) in the region occupied by the worms, being some 1 to 2 *p*H units more acidic than the lumen of an uninfected rat intestine (*2*). Because trypsin activity in this *p*H range is minimal, the primary mechanism of trypsin inhibition (and self-protection) may well be acidification of the ambient medium by the worms. Thus, the production of antitrypsin, if it occurs at all, would be redundant.

The remarkable ability of the tapeworm to regulate the *p*H of its environment is linked directly to its anaerobic form of energy metabolism. Glucose is metabolized to lactic, succinic, and acetic acids, which are excreted through the body surface or the excretory canals (*8*). Because of the *p*K values of these acids, they would form an effective polybasic buffering system in the worm's vicinity, with a buffering range centered between *p*H 4.0 and 5.0. Indeed, the *p*H of the fluid in the excretory canals is 4.5 (*9*). The regulatory nature of this system is supported by our observation that glucose consumption and net acid excretion (*10*) by the worms are not constant, but increase markedly as ambient *p*H is raised from 5.0.

The absence of aerobic pathways in many endoparasites has long been thought to stem from the limited O_2 in their environment. The partial pressure of oxygen (PO_2) in regions of the host favored by parasites is often low (*11*), and anaerobiosis may well be the most appropriate means for energy transformation. However, many endoparasites live in fully aerobic situations and still depend on anaerobic fermentation. The selection pressure for anaerobiosis in these organisms must therefore involve factors other than the environmental PO_2. If endoparasites such as tapeworms

are protected from enzyme activity in an acidic environment, then their mode of life would place a premium on their capacity to produce and excrete organic acids. Selection would favor anaerobiosis independent of environmental PO_2.

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Normalization of Depressed Heart Function in Rats by Ribose

Abstract. *Severe constriction of the abdominal aorta and simultaneous injection of isoproterenol in rats induced depression in heart function and reductions in cardiac adenosine triphosphate and total adenine nucleotides. When ribose was continuously infused for 24 hours, biosynthesis of cardiac adenine nucleotides was stimulated to such an extent that the reductions in adenosine triphosphate and total adenine nucleotides were prevented and left ventricular hemodynamic parameters were normal. These results support the hypothesis that adenosine triphosphate is primarily responsible for depression in myocardial contractility and that ribose is cardioprotective through its pronounced effects on adenine nucleotide metabolism in heart muscle.*

Various interventions have been proposed to improve myocardial adenine nucleotide metabolism in situations in which there is a discrepancy between energy supply and demand. In such a pathophysiological situation, the degradation of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) further proceeds to adenosine, inosine, and hypoxanthine (*1*), which are released (*2*) and thus lost from the myocardial cell for restitution of adenine nucleotides through the "salvage pathways." These pathways include the phosphorylation of adenosine to AMP through adenosine kinase and the conversion of hypoxanthine to inosine monophosphate, a reaction catalyzed by hypoxanthine-guanine phosphoribosyltransferase. In this reaction, 5-phosphoribosyl-1-pyrophosphate, which is also an important precursor substrate for the biosynthesis of adenine nucleotides (*3*), is consumed.

One procedure for studying myocardial adenine nucleotide metabolism is designed to stop the total breakdown of adenine nucleotides and bases. This may be accomplished by applying allopurinol, an inhibitor of xanthine oxidase (*4*). Such an inhibition may have been responsible for the improvement of function and metabolism in dogs with experimental myocardial infarction (*5*) and hemorrhagic shock (*6*). Another approach is to inhibit adenosine transport within or efflux from the myocardial cell by administering dipyridamole (*7*), which also has pronounced dilatory effects on the coronary vascular system (*8*).

Apart from affecting degradation and transport of adenine nucleotides and their breakdown products, there are basically two ways to stimulate the synthesis of myocardial adenine nucleotides. The first involves the administration of adenosine (*9*), inosine (*10*), and adenine (*11*), all of which can be used to restore cardiac adenine nucleotides through the salvage pathways, which depend on the availability of 5-phosphoribosyl-1-pyrophosphate as regards inosine and adenine. The second approach is aimed at enhancing the biosynthesis of adenine nucleotides with ribose (*12*). It is based on the fact that the rate of biosynthesis is very low (6 nmole/g per hour) compared with the total content of adenine nucleotides, and that stimulation with ribose is possible not only under control conditions (*12*) but also in such situations as recovery from lack of oxygen (*13*), cardiac hypertrophy (*14*), and stimulation with catecholamines (*15*). The pronounced effect of ribose can be attributed primarily to the increased availability of 5-phosphoribosyl-1-pyrophosphate, a substrate that appears to be the major limiting factor for biosynthesis of myocardial adenine nucleotides (*3*, *12*). In the experimental conditions mentioned, the stimulation of adenine nucleotide biosynthesis is so considerable that the decline in adenine nucleotides is attenuated or even prevented. Although the effects of ribose on adenine and uridine nucleotide metabolism (*16*) and on the morphology of the myocardium have been documented (*15*), it has not been known whether the impairment of heart function associated with ATP depletion can be prevented by ribose.

To examine this, a reduction of myocardial adenine nucleotides was induced in rats (240 to 260 g) experiencing impaired heart function. This combined effect was elicited by constricting the abdominal aorta to a final diameter of 0.65 mm (*17*) and by simultaneously adminis-

Table 1. Effect of aortic constriction (AC) and simultaneous injection of isoproterenol (ISO; 25 mg/kg, subcutaneously) on the myocardial content of ATP and total adenine nucleotides, left ventricular systolic pressure (LVSP), maximum rate of increase in left ventricular pressure, and the product of LVSP and heart rate (HR) in rats that had received a continuous intravenous infusion of 0.9 percent NaCl or ribose (200 mg/kg per hour) for 24 hours. Values are means ± standard errors for the number of experiments given in parentheses.

Treatment	ATP (μmole/g)	ATP, ADP, and AMP (μmole/g)	LVSP (mmHg)	Maximum rate of increase in LVSP (mmHg/sec)	LVSP × HR (mmHg/min)
Control	4.4 ± 0.07 (30)	5.8 ± 0.10 (30)	142 ± 4 (19)	6,073 ± 187 (19)	58,342 ± 1,897 (19)
AC + ISO + NaCl	3.4 ± 0.10 (14)*	4.7 ± 0.16 (14)*	111 ± 5 (11)*	4,699 ± 338 (11)*	34,764 ± 4,600 (11)*
AC + ISO + ribose	4.2 ± 0.10 (18)	5.7 ± 0.12 (18)	133 ± 6 (12)	6,231 ± 308 (12)	52,569 ± 2,637 (12)†

*Significantly different from corresponding control value ($P < .0005$, unpaired t-test). †$P < .05$.