1,2-Propanediol-2-Phosphate in Ascaris lumbricoides

Abstract. *No phosphagen is present in the body-wall muscle of* Ascaris lumbricoides. *A stable phosphorus compound, which on the basis of its chemical reactions seems to be 1,2-propanediol-2-phosphate, constitutes more than half of the acid-soluble phosphorus compounds.*

During a study of the acid-soluble phosphorus compounds in the body-wall musculature of *Ascaris* we found little, if any, phosphagen; the amount of phosphorus set free by 1-minute hydrolysis in 0.06*N* sulfuric acid (treatment which leads to complete hydrolysis of phosphoarginine) scarcely exceeded that expected to be liberated from adenosine triphosphate. Colorimetric determinations of free and bound arginine confirm these findings. Stable phosphorus compounds were present in unusually high amount; in 20 experiments, the content of acid-soluble phosphorus compounds was 32.0 ± 7.1 μmole/g and only 11.8 ± 3.2 μmole/g were hydrolyzed by boiling in 1.0*N* sulfuric acid for 180 minutes.

The bulk of stable phosphate was not precipitated either by barium alone or after addition of alcohol (*1*). The amount of nonhydrolyzable phosphorus remaining in the supernatant depended on final concentration of the solution: in two extracts, 7.7 and 6.7 μmole/g, respectively, remained in solution; the corresponding values for the same extracts after a 1:10 dilution were 14.5 and 13.4 μmole/g. Apparently the stable compound in *Ascaris* muscle forms a more soluble salt than most other phosphates, but, even so, its solubility is slight.

Alcohol was evaporated from the supernatant, barium ions were removed by a cation-exchanger (Dowex 50 W), the acetic acid remaining after addition of barium acetate was extracted with ether, and the sample was concentrated by lyophilization and subjected to electrophoresis on Whatman No. 3 paper. Salicylate buffer (0.1*M*, *p*H 3.9) was used because it allows the paper to be stained with sulfosalicylic acid–ferric chloride without decomposing compounds on the paper. The strip was cooled with carbon tetrachloride saturated with salicylic acid to prevent extraction of the salicylic acid from the buffer, which was also saturated with this acid. The compound appeared as a single spot with a mobility of 0.83 with respect to inorganic phosphate. It was very stable, judged by its resistance to hydrolysis in 5*N* sulfuric acid at 100°C; after 2, 4, 8, and 16 hours respectively, the corresponding degree of hydrolysis was 3.85, 5.65, 11.2, and 23.9 percent.

Properties of the unknown compound indicated that it might be propanediol phosphate, which is also very stable to acid hydrolysis (*2, 3*); its barium salt is a little more soluble in alcohol than the barium salts of other phosphorus compounds of biological origin (*1, 4, 5*). Both the extracted compound and synthetic propanediol phosphate prepared from propylene oxide and phosphate (*6*) had identical mobility values.

Upon paper chromatography with a mixture of propanol, ammonia, and water (*7*), the synthetic compound forms one main spot (R_F with respect to inorganic phosphate, 1.57) and sometimes another spot with an R_F of 1.28 with respect to inorganic phosphate. Formation of two spots is believed to be caused by the presence of two isomers of propanediol phosphate (*6*). The extract from *Ascaris* formed a spot with an R_F of 1.28, which suggests the presence of 1,2-propanediol-2-phosphate.

The synthetic compound was treated with bromine (*8*), and the reaction product was subjected to ionophoresis. Two spots appeared: a large one (mobility 0.90), corresponding presumably to acetol phosphate formed from the 1-isomer (that is, propanediol-1-phosphate) and a smaller spot (mobility 1.10), corresponding to lactic acid-2-phosphate derived from propanediol-2-phosphate (*8*). The compound extracted from *Ascaris lumbricoides* and purified by ion-exchange chromatography gave only the spot with a mobility of 1.10 when treated with bromine. This is further evidence that the extracted compound is 1,2-propanediol-2-phosphate.

Propanediol phosphate found in small amounts in brain (*2, 5*), liver and kidney (*1, 9*), Flexner-Jobling carcinoma (*4, 10*), and in the lens (*11*) has been reported to be the 1-phosphate, but little is known of its physiological function (*8, 12, 13*). The high accumulation of the compound in *Ascaris lumbricoides* suggests other than metabolic functions. It may, for example, contribute as nonpermeating anion to the osmotic balance of the cells.

J. KUBIŠTOVÀ
D. SETH*

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

References and Notes

1. J. Sacks, *J. Biol. Chem.* **181**, 655 (1949).
2. O. Lindberg, *Arkiv Kem. Mineral. Geol.* **21B**, No. 3, 1 (1945).
3. O. N. Miller, C. G. Huggins, K. Arai, *J. Biol. Chem.* **202**, 263 (1953).
4. G. A. Le Page, in *Manometric Techniques and Tissue Metabolism*, W. W. Umbreit, R. H. Burris, J. F. Stauffer, Eds. (Burgess, Minneapolis, Minn., 1949), p. 185.
5. H. Rudney, *J. Biol. Chem.* **210**, 353 (1954).
6. G. P. Lampson and H. A. Lardy, *ibid.* **181**, 697 (1949).
7. C. S. Hanes and F. A. Isherwood, *Nature* **164**, 1167 (1949).
8. E. Huff and H. Rudney, *J. Biol. Chem.* **234**, 1060 (1959).
9. O. Lindberg, *Arkiv Kem. Mineral. Geol.* **A23**, No. 2, 45 (1946).
10. D. P. Groth and G. A. Le Page, *Cancer Res.* **14**, 837 (1954).
11. J. Nordman and P. Mandel, *Ann. Oculist. (Paris)* **185**, 929 (1952).
12. W. Sakami and J. M. Lafaye, *J. Biol. Chem.* **193**, 199 (1951); H. Rudney, *ibid.* **210**, 361 (1954).
13. O. N. Miller and G. Bazzano, *Ann. N.Y. Acad. Sci.* **119**, 957 (1965).

* Present address: Parasitological Laboratory, Ciba Research Center, Bombay, India.

26 September 1966

Glucagon, Starvation, and the Induction of Liver Enzymes by Hydrocortisone

Abstract. *Glucagon selectively potentiates an effect of hydrocortisone: when injected into adrenalectomized rats it increases fourfold the induction by hydrocortisone of tyrosine transaminase, but not of tryptophan pyrrolase. Glucagon alone doubles the basal level of tyrosine transaminase and decreases that of tryptophan pyrrolase. The effects of glucagon on both enzymes resemble those of starvation.*

Since the first observation on the induction of rat liver tyrosine transaminase by a glucocorticoid (*1*) in 1955, additional inducers have been found (*2*), but none more effective than hydrocortisone. The enhancement, by starvation, of the hydrocortisone induction of tyrosine transaminase that was recently observed (*3*) led us to identify glucagon as a new regulatory factor of this enzyme.

Figure 1 shows the different effects

of high doses of glucagon on two enzymes, tryptophan pyrrolase and tyrosine transaminase, in two kinds of adrenalectomized rats: untreated ones and those injected with hydrocortisone. Maximal induction, a 15-fold rise in tyrosine transaminase in 7 hours, occurs in the latter group; hydrocortisone or glucagon alone induces only fourfold and twofold, respectively. Tryptophan pyrrolase is also induced fourfold by hydrocortisone, but glucagon does not modify this. Glucagon by itself significantly decreases the level of tryptophan pyrrolase (but not at the lower doses used in Fig. 2). All these effects of glucagon, as illustrated in Fig. 1, are qualitatively similar to those of starvation.

In the experiments of Fig. 2, the amount of glucagon, injected once or twice, was varied. It may be seen that doses of glucagon that do not induce tyrosine transaminase become effective in conjunction with hydrocortisone: only the highest dose (1 mg twice) increases the basal level of tyrosine transaminase, but a fifth of this dose is sufficient to enhance the induction of this enzyme by hydrocortisone. The enhancement by two injections of 1 mg of glucagon is greater than that by one, particularly if the second dose is given early (2 hours after the first). The effect of approximately 0.5 mg of glucagon is quantitatively similar to that of starvation (indicated by the broken line in Fig. 2): it doubles the induction by hydrocortisone.

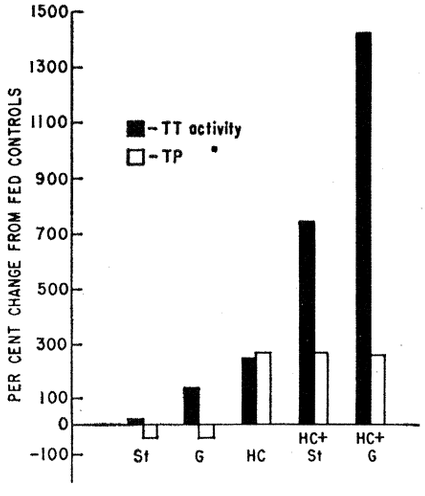

Fig. 1. The induction of tyrosine transaminase (TT, dark bars) and tryptophan pyrrolase (TP, light bars) after starvation or treatment with glucagon. Three-month-old inbred (NEDH strain) female rats were used 5 to 10 days after bilateral adrenalectomy. The heights of the bars show the percent changes in enzyme from those in fed controls (TT = 66, TP = 3.9 μmoles/hr per gram of body weight, at 25°C), caused by 24 to 48 hours of starvation (St), by glucagon (G), by hydrocortisone (HC), by hydrocortisone in starved rats (HC + St), and by the combination of hydrocortisone and glucagon (HC + G). Glucagon (1 mg per 100 g of body weight) was injected intraperitoneally twice, 7 and 5 hours before assay, and hydrocortisone (2.5 mg of the hemisuccinate per 100 g of body weight) once, 5 hours before assay. Tryptophan pyrrolase and tyrosine transaminase were assayed in the freshly prepared soluble fraction of individual livers by methods previously described (*6*). Each value is a mean of results with 5 to 15 rats. The magnitudes of the standard errors of the means were similar to those in Fig. 2.

It is well recognized that in intact rats various experimental treatments may stimulate the pituitary-adrenal system, with the result that the secondary secretion of glucocorticoids induces the tyrosine transaminase. The present results suggest that other secondary effects can occur even in adrenalectomized rats: a variety of factors (for example, dietary changes) may well induce or enhance the induction of tyrosine transaminase by stimulating the secretion of glucagon.

Since the secretion of glucagon is inversely related to the level of blood glucose (*4*), starvation might increase it. Although glucagon has not been measured in the starved rats used here, the results are consistent with the effect of starvation being mediated through release of glucagon. This possibility is reinforced by the fact that starvation and glucagon have more than one effect in common: (i) both increase the basal level of tyrosine transaminase, (ii) both decrease the basal level of tryptophan pyrrolase, (iii) neither affects the induction of tryptophan pyrrolase by hydrocortisone, and (iv) both enhance the induction of tyrosine transaminase by hydrocortisone. The last one of these is the largest and physiologically the most interesting effect. In fed adrenalectomized rats, hydrocortisone induces tryptophan pyrrolase and tyrosine transaminase to a similar extent. In the presence of glucagon, with its striking effect on tyrosine transaminase, the similarity between the two enzymes disappears. Such selective action on enzymes regulated by hydrocortisone suggests that glucagon can qualitatively modify the metabolic effect of glucocorticoids.

It has been reported that in isolated, perfused livers hydrocortisone itself does not stimulate urea production but enhances the stimulatory effect of glucagon on urea production (*5*). The large increase in tyrosine transaminase, seen in the present studies, with hydrocortisone plus glucagon may contribute to this catabolic effect of the combined hormones.

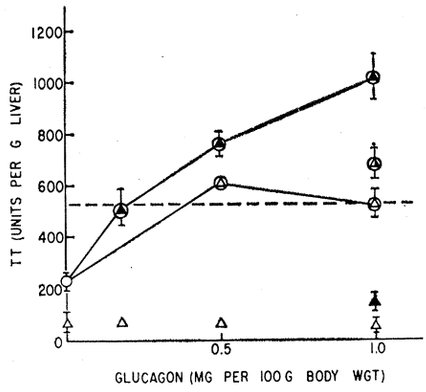

Fig. 2. The effect of different doses of glucagon on tyrosine transaminase and on its induction by hydrocortisone. Glucagon was injected into adrenalectomized rats (in amounts indicated on the abscissa) once, 7 hours before assay (△), or twice, 7 and 5 hours (▲) or 7 and 3 hours before assay (◮). Hydrocortisone (2.5 mg of hemisuccinate per 100 g of body weight) was administered 5 hours before assay alone (○) or with the indicated regimen of glucagon (circles around triangles). The points are means; ± standard errors of the means are shown by vertical lines, except for the groups comprising less than four rats. For comparison, the level of tyrosine transaminase in rats starved for 24 to 48 hours and given hydrocortisone 5 hours before asssay is shown by the broken horizontal line.

OLGA GREENGARD
GEORGE T. BAKER

Cancer Research Institute,
New England Deaconess Hospital,
Boston, Massachusetts 02215

References and Notes

1. E. C. C. Lin and W. E. Knox, *Biochim. Biophys. Acta* **26**, 85 (1957).
2. W. E. Knox and O. Greengard, *Advance Enzyme Regulat.* **3**, 247 (1965).
3. O. Greengard, G. T. Baker, M. L. Horowitz, W. E. Knox, *Proc. Nat. Acad. Sci. U.S.*, **56**, 1303 (1966).
4. R. H. Unger, A. M. Eisentraut, M. S. McCall, L. L. Madison, *J. Clin. Invest.* **41**, 682 (1962).
5. L. L. Miller, in *Recent Progr. Hormone Res.* **17**, 539 (1961).
6. W. E. Knox, M. M. Piras, K. Tokuyama, *J. Biol. Chem.* **241**, 297 (1966); E. C. C. Lin, B. M. Pitt, M. Civen, W. E. Knox, *ibid.* **233**, 668 (1958).

7. Supported in part by USPHS grant CA-08676, by a grant from the American Cancer Society (Massachusetts Division), Inc., and by U.S. Atomic Energy Commission contract AT(30-1)-901 with the New England Deaconess Hospital (NYO-901-64). We are grateful to W. N. Shaw of the Lilly Research Laboratories for the gift of insulin-free glucagon.

6 October 1966