

or failure of the infection. The results for histones and total protein support the view that parasitically induced changes in host nuclei occur in the early stages of rust development and may lie at the core of this and other similar host-parasite relationships. This interpretation assumes that there is no significant change, as the infection progresses, of any masking effect of other groups on the dye-binding capacity of amino groups on the histones—that is, that the measured decreases in histones are real (13).

P. K. BHATTACHARYA  
J. M. NAYLOR  
MICHAEL SHAW

Department of Biology,  
University of Saskatchewan,  
Saskatoon, Saskatchewan

## Increased Activities of Glycogenolytic Enzymes in Liver after Splanchnic-Nerve Stimulation

**Abstract.** *Electrical stimulation of the splanchnic nerve of rabbits caused a marked increase, within 30 seconds after the onset of stimulation, in liver-glycogen phosphorylase and glucose-6-phosphatase activities. The increased activity of liver phosphorylase after splanchnic-nerve stimulation was likewise observed in adrenalectomized and pancreatectomized rabbits. Glycogen content of the liver decreased only slightly after 5-minute stimulation.*

Evidence has accumulated that the enzymatic composition of mammalian cells is not constant but can be altered by factors such as the type of nutrients, hormonal changes, and the administration of certain foreign chemical agents. Shimazu has reported that certain liver enzymes are influenced by the highest autonomic center of the hypothalamus (1), and has shown that the hypothalamic influence on the liver enzymes may be communicated through peripheral autonomic nerves (2).

We have demonstrated that the amounts of blood glucose and liver glycogen in rabbits are changed by electrical stimulation of the hypothalamus (3). Thus, electrical stimulation of the ventromedial hypothalamic nucleus (one of the nuclei in the sympathetic area of the hypothalamus) caused an increase in blood glucose followed by a pronounced decrease in liver glycogen, and electrical stimulation of the lateral hypothalamic nucleus (the parasympathetic area of the hypothalamus) caused a slight decrease in blood glucose with an insignificant change of liver glycogen.

We now report the effect of electrical

- References and Notes**
- H. H. Flor, *Advan. Genet.* **8**, 29 (1956).
  - R. J. Allen, *J. Agr. Res.* **21**, 571 (1923).
  - C. Person, *Can. J. Genet. Cytol.* **2**, 102 (1960).
  - H. S. Whitney, M. Shaw, J. M. Naylor, *Can. J. Botany* **40**, 1533 (1962).
  - K. Patau, *Chromosoma* **5**, 341 (1952); ———, H. Swift, *ibid.* **6**, 149 (1953).
  - M. H. Flax and M. Himes, *Physiol. Zool.* **25**, 298 (1952).
  - H. Swift, in *Nucleic Acids: Chemistry and Biology*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), vol. 2.
  - M. Alfert and I. I. Geschwind, *Proc. Nat. Acad. Sci. U.S.* **39**, 991 (1953).
  - F. Schrader and C. Leuchtenberger, *Exp. Cell. Res.* **1**, 421 (1950).
  - H. Swift, in *The Nucleohistones*, J. Bonner and P. Ts'o, Eds. (Holden-Day, San Francisco, 1964).
  - M. Shaw, *Annu. Rev. Phytopathol.* **1**, 259 (1963); ———, *Phytopathol. Z.* **50**, 159 (1964).
  - R. C. Huang, and J. Bonner, *Proc. Nat. Acad. Sci. U.S.* **48**, 1216 (1962); V. G. Alfrey, V. C. Littau, A. E. Mirsky, *ibid.* **49**, 414 (1963); J. Bonner, R. C. Huang, R. V. Gilden, *ibid.* **50**, 893 (1963); B. C. Goodwin and I. W. Sizer, *Science* **148**, 242 (1965).
  - Supported by a grant from the National Research Council of Canada to M.S.
- 4 October 1965

Male rabbits weighing about 2300 g were lightly anesthetized with pentobarbital sodium (10 mg/kg, intravenously). Laparotomy was made under additional anesthesia with ether. The left splanchnic nerve was exposed and freed from surrounding adipose tissue just under the diaphragm, and a bipolar platinum electrode, fitted in a tiny plastic plate, was placed at the nerve. Fifteen to twenty minutes after placement of the electrode, one lobe of the liver was quickly removed by ligation and immediately immersed in liquid nitrogen. Stimuli delivered from an electronic stimulator (square pulses of 0.3 msec duration; frequency, 100 per second; amplitude, 50 volts) were applied to the splanchnic nerve through an isolation unit. After the indicated time of stimulation, the other lobe of the same liver was quickly removed and similarly frozen in liquid nitrogen while the stimulus was still being delivered.

For assay of phosphorylase, a portion (2 g) of each frozen liver was promptly pulverized and ground at  $-20^{\circ}\text{C}$  with 2 volumes of 60-percent glycerol solution containing 0.05M NaF and 0.005M EDTA (ethylene diamine tetraacetic acid, adjusted with NaOH to pH 6.1) which had been chilled to near freezing state. The material was ground for about 10 minutes and then was diluted to 20 ml with cooled aqueous solution of the same salts. The suspension was centrifuged at 15,000g for 5 minutes at  $-5^{\circ}\text{C}$ , and the supernatant was immediately analyzed for phosphorylase activity. The reaction mixture for assay contained 0.025M glucose-1-phosphate, 0.5 percent glycogen, 0.05M sodium-citrate buffer (pH 6.1), 0.025M NaF, 0.0025M EDTA (pH 6.1) and a suitable volume of the centrifuged liver extract in a total volume of 1 ml. Inorganic phosphate liberated during 5-

stimulation of the splanchnic nerve, the peripheral sympathetic nerve innervating the liver, on glycogen phosphorylase and glucose-6-phosphatase activities of rabbit liver. The activities of these two enzymes were greatly increased within 30 seconds after the onset of stimulation of the splanchnic nerve. This result would properly explain the previous observations that blood glucose increases rapidly, whereas liver glycogen decreases markedly by hypothalamic stimulation of the sympathetic area (3).

Table 1. Effect of electrical stimulations of the splanchnic nerve on phosphorylase and glucose-6-phosphatase activities, and glycogen content of rabbit liver. S, stimulation; P<sub>1</sub>, inorganic phosphate. Each row represents one rabbit.

Enzyme activity [ $m\mu\text{mole (P}_1\text{) mg}^{-1}$ (protein) $\text{min}^{-1}$ ]										
Phosphorylase				Glucose-6-phosphatase				Glycogen (mg/g of liver)		
Be-fore	After S			Be-fore	After S			Be-fore	After	
S	30 sec	1 min	5 min	S	30 sec	1 min	5 min	S	S	
	7.2		20.5	23.0	12.2		14.3	19.4	37.9	27.4
	9.1		21.9	6.7	12.2		16.3	16.9		
	4.3	16.1	9.5	23.9	15.1	21.5	21.9	18.0	26.3	21.4
	5.5	20.0	11.7	24.5	19.4	24.0	28.3			
	10.4	24.5	30.8	33.4	20.2	29.5	24.8	26.7	44.3	43.6
*P values						.02>P>.01	.02>P>.01	.02>P>.01		.2>P>.1

\* The *t*-test was made by paired observations (after stimulation compared to before stimulation).

Table 2. Response to splanchnic-nerve stimulation of liver phosphorylase in adrenalectomized and pancreatectomized rabbits. Bilateral adrenalectomy and pancreatectomy of the rabbits were carried out by laparotomy. Then 15 to 20 minutes later, the splanchnic nerve was electrically stimulated. Results are given as  $m\mu\text{mole } (P_i) \text{ mg}^{-1} (\text{protein}) \text{ min}^{-1}$ .  $P_i$ , inorganic phosphate.

Before	After		
	30 sec	1 min	5 min
<i>Adrenalectomized rabbits</i>			
4.9	10.0	16.3	26.4
6.9	24.0	20.9	29.2
5.7	8.1	12.8	13.7
4.3	12.0	9.6	16.4
<i>Pancreatectomized rabbits</i>			
9.6	19.9	23.8	32.3
7.9	19.0	13.3	21.1
5.9	13.1	16.5	20.1

or 10-minute incubation at 37°C was determined (4).

For assay of glucose-6-phosphatase, each frozen liver was homogenized in a glass homogenizer with nine volumes of cold isotonic KCl solution. The homogenates were centrifuged at 12,000g for 10 minutes at 0°C. The reaction mixture contained 0.02M glucose-6-phosphate, 0.04M sodium maleate buffer (pH 6.5), 0.01M  $\text{MgCl}_2$  and a suitable volume of the supernatant fraction of the centrifuged liver homogenate in a total volume of 1 ml. Inorganic phosphate released during 10-minute incubation at 37°C was measured as described above. Liver glycogen was determined as described (3).

The activity of the phosphorylase in liver was markedly increased by electrical stimulation of the splanchnic nerve (Table 1), attained nearly the maximum within 30 seconds after the onset of stimulation of the splanchnic nerve, and remained fairly constant at least up to the end of a 5-minute period of stimulation. In each case the stimulation caused an approximately threefold increase in phosphorylase activity ( $P < .01$ ) as compared with that before stimulation (except in case No. 2, 5-minute stimulation), though minor variations of the activity existed between animals before stimulation. Similar observations on muscle phosphorylase have been reported by Danforth *et al.* (5) and Posner *et al.* (6). They showed that the activity of phosphorylase *a* of frog sartorius muscle and rat gastrocnemius muscle was rapidly and markedly increased by direct stimulation in vitro of the muscle preparation or by electrical stimulation of the posterior tibial nerve. Independently of their observations, essentially the same results

on phosphorylase of rabbit gastrocnemius muscle were obtained in this laboratory by electrical stimulation of the sciatic nerve of rabbits: the phosphorylase *a* of gastrocnemius muscle was increased approximately threefold over that of the control while the total activity of phosphorylase (phosphorylase *a* and *b* combined) was not affected by stimulation of the sciatic nerve (7). In the studies reported here, although only active phosphorylase of the liver was assayed, the increased activity of phosphorylase after splanchnic-nerve stimulation might be due to a conversion of inactive phosphorylase (dephosphophosphorylase) into active form during the stimulation.

The activity of liver glucose-6-phosphatase was also increased about 40 percent by stimulation of the splanchnic nerve as compared with that before stimulation in each case ( $.02 > P > .01$ ). The response of this enzyme to splanchnic-nerve stimulation was observed within 30 seconds after the onset of stimulation and the increase in activity continued at least for the 5 minutes following stimulation.

Glycogen content of the liver after a 5-minute period of stimulation of the splanchnic nerve was only slightly decreased as compared with that before stimulation ( $.2 > P > .1$ ). A much greater decrease in liver glycogen has already been observed when the sympathetic area of the hypothalamus was stimulated electrically for a longer period (3).

Electrical stimulation of the vagus nerve had, if any, a suppressing effect on liver phosphorylase and glucose-6-phosphatase activities.

Table 2 shows the effect of adrenalectomy and pancreatectomy upon the response of liver phosphorylase to the splanchnic-nerve stimulation. The activity of liver phosphorylase was likewise increased markedly. It is unlikely that the effect of splanchnic-nerve stimulation on liver glycogenolytic enzymes is due solely to hormonal factors such as epinephrine released from the adrenal or glucagon released from the pancreas.

Our results show that sympathetic stimulation results in an acceleration of glycogenolysis in liver; they give a further support, in addition to the findings previously reported (1, 2), to the concept that certain liver enzymes are under the control of the autonomic nervous system. In relation to this concept, Suzuki has recently demon-

strated with his silver-impregnation method that nerve fibers ending to the liver enclose the surface of the hepatic cells in a reticular form through an intermediation of the "transmittal cells" which lie outside the sinusoid and function as relay stations of the nerve fibers (8).

TAKASHI SHIMAZU

AOI FUKUDA

Department of Anatomy, Osaka University Medical School, Osaka, Japan

#### References and Notes

1. T. Shimazu, *Biochim. Biophys. Acta* **65**, 373 (1962); *J. Biochem.* **55**, 163 (1964); *Gann* **56**, 143 (1965); *Biochim. Biophys. Acta* **105**, 377 (1965).
2. T. Shimazu, *Proc. Symp. Chem. Physiol. Pathol.*, in press.
3. T. Shimazu, A. Fukuda, T. Ban, *Nature*, in press.
4. T. Takahashi, *Protein Nucleic Acid Enzyme Tokyo* **2**, 464 (1957).
5. W. H. Danforth and E. Helmreich, *J. Biol. Chem.* **239**, 3133 (1964).
6. J. B. Posner, R. Stern, E. G. Krebs, *ibid.* **240**, 982 (1965).
7. T. Shimazu and A. Fukuda, unpublished.
8. K. Suzuki, *Proc. Gen. Assembly Japan Med. Congr. 16th* **4**, 13 (1963).

8 October 1965

#### Caterpillar Feeding on a Sundew Plant

Abstract. *The caterpillar of a plume moth (Trichoptilus parvulus) was found feeding on a sundew plant (Drosera capillaris) in Florida. The larva eats the stalked glands with their sticky secretion, the leaf blades, and even dead insects trapped by the plant.*

Appropriately known as sundews because of the glistening droplets of secretion borne by the stalked glands on their leaves, the species of the family Droseraceae are among the more fascinating of carnivorous plants. Their mechanism of capture of prey is well known: insects or other small animals, when trapped in the viscid secretion, eventually succumb and are digested by the plant. We now report the discovery of a remarkable moth larva that actually lives and feeds on a sundew plant.

The caterpillars were found on specimens of their host plant, *Drosera capillaris*, collected on open sandy terrain, among tall grasses and sedges, near Lake Placid, Florida. What follows is based on observation of six larvae. Two of them, which initially measured only 1.5 mm in length and might have been in their late first or early second instar, were observed al-