existence of a similar alteration in the hypothalamus of the female rat with diabetes. These results and the abundant literature existing on reproductive and gonadal alteration in the diabetic male[4,5] and female rats[2-4] may suggest that an impairment in the synthesis and/or the release of pituitary gonadotropins may occur. A relationship between the gonadotropin levels and the oxidative metabolism of the hypothalamus[20-22] has already been suggested. Evidence is accumulating which indicates that the oxidative activity of the hypothalamus is also associated with the 'short' feed-back mechanism[23] controlling the secretion of FSH[24] and of LH[25]. VELASCO and TALEISNIK[26] have also shown that the limbic structures are functionally associated and participate in the ovulatory processes. However, more data are needed in order to establish whether the metabolic alterations which have been observed in the diabetic rats are produced by an impairment at the central (hypothalamic) level or at the peripheral (gonadal) level of the hypothalamic-hypophyseal-gonadal axis.

Oxidative metabolism of hypothalamus, amygdala and hippocampus in diabetic rat

Groups	QO_2 (µl O_2/mg wet tissue/h) Hypothalamus	Amygdala	Hippocampus
D-diestrus	1.30 ± 0.07 (14)	1.09 ± 0.09 (19)	1.49 ± 0.08 (29)
E-estrus	1.59 ± 0.08 (17)	1.59 ± 0.11 (28)	1.21 ± 0.06 (35)
P-pancreatectomized	0.98 ± 0.08 (15)	1.45 ± 0.06 (12)	1.54 ± 0.06 (15)
Analysis of variance			
F ratio	16.62	6.38	5.98
P value	0.01	0.01	0.01
Multiple comparisons test			
$P < 0.05$ between	D vs. E D vs. P E vs. P	D vs. E D vs. P –	D vs. E – E vs. P

Mean ± S.E. Figures in parentheses are number of determinations.

Resumen. Se determinò el consumo de oxìgeno de hipotàlamo, amigdala e hipocampo en ratas hembras a las que se les habia producido una diabetes experimental por pancreatectomìa sub-total (95%). Se usaron las ratas diabeticas durante la fase de diestro y como controles se utilizaron animales normales en distintas etapas del ciclo sexual. Los animales diabeticos en hipotàlamo presentaron valores de consumo de oxìgeno inferiores a los màs bajos registrados en los animales controles (diestro). La amigdala, de las ratas diabeticas, mostrò un metabolismo oxidativo similar al presentado en estro por los controles normales, a pesar de encontrarse en la fase diestro en el momento del sacrificio. Por el contrario el consumo de oxìgeno del hipocampo de los animales diabeticos no mostrò modificaciones en comparaciòn con los controles normales.

O. SCHIAFFINI[27], B. MARIN and V. G. FOGLIA

Catedra 1ª de Fisiología, Facultad de Medicina de Madrid, Madrid-3 (Spain), and Instituto de Fisiología, Facultad de Medicina de Buenos Aires (Argentina), 29 December 1970.

[20] J. A. MOGUILEVSKY, N. GHITTONI and O. SCHIAFFINI, Acta physiol. latinoam. *16a*, 86 (1966).
[21] J. A. MOGUILEVSKY, Revta argent. Endocr. Metab. *13*, 25 (1967).
[22] C. LIBERTUN, J. A. MOGUILEVSKY, O. SCHIAFFINI and V. G. FOGLIA, Experientia *25*, 196 (1969).
[23] L. MARTINI, F. FRASCHINI and M. MOTTA, *Endocrinology and Human Behaviour* (Ed. R. P. MICHAEL; Oxford University Press, London 1968), p. 175.
[24] F. FRASCHINI, M. MOTTA and L. MARTINI, Experientia *24*, 270 (1968).
[25] M. A. DÁVID, F. FRASCHINI and L. MARTINI, Endocrinology *78*, 55 (1966).
[26] M. E. VELASCO and S. TALEISNIK, Endocrinology *84*, 132 (1969).
[27] Investigator of Instituto de Fisiología, Facultad de Medicina de Buenos Aires (Argentina). Research Fellow of Fundación Marqués de Urquijo (Spain). Present address: Istituto di Farmacologia e di Terapia dell'Università, Via Vanvitelli 32, I-20129 Milano (Italy).

Transport of Whole Protein Molecules from Blood to Saliva of a Plant-Bug

An unresolved problem in the transmission of plant viruses in the saliva of Homoptera is how the virus penetrates cellular barriers in the insect: whether as whole particles or as components that are eventually reassembled. A number of metabolites are transferred rapidly to the saliva when injected into the haemolymph of plant-bugs[1], and the haem group from haemoglobin finds its way into the salivary glands of the blood-sucking bug *Rhodnius prolixus* (Heteroptera: Reduviidae)[2]. But the protein of the salivary 'haemalbumin' of *Rhodnius* is of unknown origin and history; and, to the knowledge of the present writers, no evidence has yet been presented of the direct transfer of particles as large as protein molecules from the haemolymph to the saliva of Hemiptera.

Horseradish peroxidase is a highly stable enzyme with a mol wt. of about 40,000; and it is easily detectable with a benzidine reagent[3]. Peroxidases occur naturally in insects[4], and in the present study were found in the salivary 'sheath material' (the solidifying fraction of the saliva[5]) of the rose aphid, *Macrosiphum rosae* (Homoptera: Aphididae) and in the haemolymph, watery saliva[1], and sheath material of the peanut trash bug, *Elasmolomus sordidus* (Heteroptera: Lygaeidae). On the other hand, the fifth instar larva of *Eumecopus punctiventris* (Heteroptera: Pentatomidae) had barely detectable amounts of peroxidase in its haemolymph, and no detectable enzyme in its sheath material. This insect did not produce watery

[1] P. W. MILES, J. Insect Physiol. *13*, 1787 (1967).
[2] V. B. WIGGLESWORTH, Proc. R. Soc. B. *131*, 313 (1943).
[3] A. G. E. PEARSE, *Histochemistry Theoretical and Applied* (Churchill, London 1960).
[4] M. LOCKE, Proc. Electron Microscopy Soc. of America, 26th Annual Meeting, 1968, p. 154.
[5] P. W. MILES, J. Insect Physiol. *11*, 1261 (1965).

saliva readily in these experiments, but could be made to do so by injection of 100 µg pilocarpine nitrate in 1 µl saline. The secretion induced in this way also lacked detectable peroxidase.

When *Eumecopus* was injected with pilocarpine and 100 µg horseradish peroxidase (Koch-Light, U.K.), watery saliva collected 1–2 min after the injection gave a strong reaction for the enzyme; and, on dissection, a short length of the long, tubular, accessory salivary gland[1] stained deeply with the benzidine reagent. Sheath material secreted within 12 h of injection of horseradish peroxidase, either with or without pilocarpine, also reacted with the reagent. These reactions were inhibited by 0.005 *M* KCN or phenylthiourea. The injections were made in such a way[1] that accidental injury of the salivary apparatus would not have occurred, and experiments with 50 individuals gave consistent results. Insects injected with bovine blood albumin instead of horseradish peroxidase showed no reaction for the enzyme in haemolymph, salivary secretions, or salivary glands.

Plant-bugs, including *Eumecopus*, secrete a polyphenol oxidase in their saliva, and tests were made using DOPA as a substrate[5] to determine whether this enzyme had interfered in tests for peroxidase. A positive reaction for polyphenol oxidase was found in the haemolymph, sheath material, and parts of the accessory salivary gland of *Eumecopus* irrespective of the reaction for peroxidase, which occurred as indicated above only after injection of horseradish peroxidase.

These experiments did not indicate how the horseradish peroxidase passed into the salivary glands. It is conceivable that the apoenzyme and prosthetic group[6] could enter independently and recombine in the saliva; but, in the absence of evidence that the apoenzyme can be reduced in size and still retain its activity, it may be assumed that protein molecules of about 40,000 mol. wt. are transferable via the accessory gland directly from the haemolymph to saliva of *Eumecopus*. The insect belongs to a family that is not known to transmit virus diseases to plants; but there are sufficient similarities in the physiology of feeding and salivation throughout the Heteroptera: Pentatomorpha and Homoptera to permit analogies to be drawn[5]. The results presented here indicate that relatively large colloidal particles may pass unchanged directly through cellular barriers in these insects.

Zusammenfassung. Es wird nachgewiesen, dass grosse Proteinmoleküle mit einem Molekulargewicht von 40000 unverändert aus der Hämolymphe von *Eumecopus* in deren Speichel gelangen und damit ausgeschieden werden.

P. W. MILES and DANUTA SLOVIAK

Waite Agricultural Research Institute, Department of Entomology, University of Adelaide, Glen Osmond (South Australia), 23 December 1969.

[6] K. G. PAUL, in *The Enzymes* (Eds. P. D. BOYER, H. LARDY and K. MYRBÄCK; Academic Press, New York 1963), vol. 8, p. 227.

Transmembrane Secretory Potentials in the Cat Submandibular Gland During Perfusion with Potassium-Free and Low Sodium Locke Solutions

It was recently shown that the type I secretory potential, i.e. the hyperpolarization of the basal acinar cell membrane occurring during stimulation of the cat submandibular gland, was unaffected when all extracellular chloride was substituted by sulphate[1,2]. It is therefore likely that the ion transport producing the secretory potential is a cation transport from the acinar cells to the extracellular fluid. The potassium equilibrium potential across the basal acinar cell membrane is greater than the membrane potential during stimulation of the gland. The sodium equilibrium potential is, however, much smaller than the resting membrane potential[3]. An outward potassium current being partly short-circuited by an inward sodium current could thus explain the secretory potential on the basis of passive movements of ions down their electrochemical gradients. If this were true, the size of the secretory potential should increase during perfusion with potassium-free solutions and solutions in which most of the sodium was substituted by a bulky non-permeating cation. The experimental results were in accordance with this expectation.

Methods. Cats (2.4–3.6 kg) anaesthetized with chloralose (80 mg/kg i.p.) were used. The experiments were carried out as described previously[1,4], with the exception that the impalement of the acinar cells with the microelectrode was done by using a stepping motor micromanipulator remotely controlled (Transvertex, Sweden) and that the potentials were recorded on an UV-recorder (S.E. 3006). The glands were stimulated by close intra-arterial injections of 1 µg acetylcholine. Each time a secretory potential had been recorded, the number of drops of saliva secreted was counted. The control Locke solution contained (m*M*): 140 NaCl, 4.0 KCl, 2.4 Na_2HPO_4, 0.6 NaH_2PO_4, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 5.5. glucose. In the potassium-free solution all KCl was substituted by NaCl. In the tetraethylammonium (TEA) solution all NaCl was substituted by TEACl. The TEA Locke solution was prepared freshly for each experiment. The perfusion fluids were equilibrated with pure oxygen. After each shift of perfusion fluid at least 5 min passed before stimulating the gland.

Results. Perfusion with TEA Locke solution. In the Figure the results from 1 experiment are shown. It is seen that the salivary secretion was abolished during perfusion with TEA solution, while the sizes of the secretory potentials were enhanced. Table I shows the data from all experiments.

Perfusion with potassium-free Locke solution. Table II shows the data from all the experiments. The sizes of the secretory potentials recorded during perfusion with

[1] O. H. PETERSEN and J. H. POULSEN, Experientia *24*, 919 (1968).
[2] O. H. PETERSEN and J. H. POULSEN, in *Exocrine Glands* (Eds. S. Y. BOTELHO, F. P. BROOKS and W. B. SHELLEY; University of Pennsylvania Press, Philadelphia 1969)., p. 34.
[3] A. S. V. BURGEN, in *Handbook of Physiology*, Section 6 (American Physiological Society, Washington 1967), vol. II, p. 574.
[4] O. H. PETERSEN and J. H. POULSEN, Acta physiol. scand. *70*, 293 (1967).