steroids is the interesting feature of the ganglia. The cholesterol content during development of the sensory ganglia shows 2 important steps (Figure 2): one after 8 days and a less pronounced increase after 11 days. This change in the synthesis is most probably due to the myelinization process, as has also been shown by MEDDA and BOSE[5], who reported that in the spinal cord of chick embryos there was an increase of cholesterol formation up to 8 days of incubation. Comparing the increase of cholesterol per ganglion with the increase of protein/ganglion, one observes that cholesterol increases 30 times and protein only 5 times. This observation agrees with the statement of FOLCH-PI[6] that during the myelinization process the protein content decreases relatively compared with lipid synthesis. Further it is interesting to note that the sum of cholesterol and sterol-like compound per protein remains almost constant at approximately 85×10^{-3} during the whole period from 6 until 14 days. It may be possible that the sterol-like compound is a biological precursor of cholesterol, which ultimately takes the place of the unknown compound after a certain stage of cellular development has been reached. Already FUMAGALLI and PAOLETTI[7] have observed that during embryonic growth there is a change in the steroid pattern. However, chemically the sterol-like compound does not seem to be a precursor of cholesterol because this compound has an absorption maximum in the UV-region at 286 nm in ethanol indicating a relative high state of insaturation, and it is known in steroid biosynthesis that all the dehydrogenation steps occur after the formation of cholesterol. Furthermore, orientative mass spectrometric measurements have indicated a molecular weight of the sterol-like compound which coincides with a C_{21}-steroid.

In the case that the sterol-like compound is not a biochemical precursor of cholesterol, it is difficult to envisage its role in the myelinization process. To this observation one can add the results obtained during previous work[1] showing that a specific nerve growth factor (NGF)[8] has an inhibitory effect in vitro on the synthesis of this sterol-like compound in 8-day-old chick embryo sensory ganglia, reducing its quantity of about 25% in a 4 h incubation period.

The coincidence between the period, in which the nerve cells are responsive to NGF, which elicits nerve fibre outgrowth between the sixth and tenth day of development and the maximum concentration of the sterol-like compound in the ganglia between the eighth and ninth day may suggest a possible relationship between the action of NGF and the disappearance of the sterol-like compound in the ganglia[9].

Riassunto. Sono stati misurati per via gascromatografica i livelli di colesterolo e di un composto steroidico a struttura finora sconosciuta nei gangli sensitivi di embrione di pollo di 6 fino a 14 giorni di età. I risultati ottenuti mostrano che durante lo sviluppo lo steroide a struttura sconosciuta ha una massima concentrazione a 8–9 giorni per poi decrescere fino a 14 giorni, mentre il colesterolo presenta una tipica curva di accrescimento ad *S* con due punti di flesso, uno a 8 ed uno a 11 giorni.

F. H. FOPPEN and ANTONIA LIUZZI

Laboratori di Chimica Biologica, Centro di Neurobiologia, Istituto Superiore di Sanità, Roma (Italy), 15 May 1968.

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β-Fructofuranosidase Activity in Pea Seeds

Dormant seeds of Leguminosae contain a large amount of reserve oligosaccharides of the raffinose family which are hydrolyzed in the first phases of germination. Enzymes responsible for their splitting, β-fructofuranosidase (β-D-fructofuranoside – fructohydrolase 3.2.1.26)[1-3] and α-galactosidase, were found in dormant seeds and their activities increased during germination. In *Phaseolus vulgaris*, 2 types of β-fructofuranosidase were found[1,2]: in dormant seeds, alkaline β-fructofuranosidase with a pH optimum at 7.7 was present only, while during germination the activity of acid β-fructofuranosidase with pH optimum at 5.0 increased. In dormant *Vicia faba* seeds the alkaline β-fructofuranosidase was found too, but the substrate specificity was different[3]. β-Fructofuranosidase from *P. vulgaris* seeds was specific to sucrose. Substrate specificity of *V. faba* enzyme was similar to the specificity of β-fructofuranosidase of other sources. Transferase activity of β-fructofuranosidase was observed in the case of acid β-fructofuranosidase from seedlings of *P. vulgaris*[2] and in the case of alkaline β-fructofuranosidase of *V. faba* seeds[3]. Alkaline β-fructofuranosidase from dormant *P. vulgaris* seeds did not exhibit transferase activity[2].

In the course of the study on the metabolism of saccharides in pea seedlings, the nature of enzyme hydrolyzing sucrose present in dormant seeds and seedlings was investigated. In dormant pea seeds, only the alkaline β-fructofuranosidase activity was determined; in 10-day pea seedlings both types of β-fructofuranosidase activity – alkaline and acid – were found. Properties of alkaline β-fructofuranosidase were studied in detail.

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Materials and methods. Pea seeds (*Pisum sativum* L., var. Edelperle) were ground and extracted with 0.1 *M* phosphate buffer pH 7.0. After centrifugation proteins were fractionated with ammonium sulphate. Active fraction was chromatographed on a hydroxylapatite column. The lyophilized enzyme preparation thus obtained was found to be purified 4-fold and was used for subsequent experiments. No further purification could be achieved by chromatography on Sephadex G-150 and DEAE-Sephadex A-50 course. The enzyme activity was estimated by determination of reducing sugars, produced in the course of β-fructofuranosidase action, by means of the FISHER-KOHTÈS method[4].

Results and discussion. Alkaline β-fructofuranosidase had a pH optimum at 7.6–7.7 (Figure 1) and hydrolyzed sucrose, raffinose and stachyose. The rate of raffinose hydrolysis was 4 times lower and that of stachyose 11 times lower, than with sucrose.

It was found that alkaline β-fructofuranosidase exhibited transferase activity. The formation of methyl-β-D-fructofuranoside was determined by paper chromatography in a mixture containing sucrose or raffinose (4 *M*), methanol (9%) and enzyme preparation (20 mg/ml of mixture).

Alkaline β-fructofuranosidase activity was activated by compounds containing an –SH group (L-cysteine, 2-mercaptoethanol, glutathion) (Figure 2) and was inhibited by –SH inhibitors (*p*-chloromercuribenzoate, iodoacetamide, *N*-ethylmaleimide) (Figure 3) and by heavy metal ions (Ag[I], Cu[II]). Activation was also achieved by NaN_3, and disodium salt of EDTA. Acid β-fructofuranosidase activity was also activated by –SH compounds.

The results of our experiments showed that the substrate specificity and transferase activity of alkaline β-fructofuranosidase from dormant pea seeds are similar to those of *V. faba* seeds described by PRIDHAM[3] and differ from those of *P. vulgaris* described by COOPER and GREENSHIELDS[1,2]. The enzyme preparation catalyzed hydrolysis of sucrose, as well as of raffinose and stachyose. Melezitose and maltose were not split under the same conditions, which proved that β-fructofuranosidase activity was responsible for sucrose hydrolysis. Hydrolysis of raffinose and stachyose could not be caused by the action of α-galactosidase, because at pH 7.7 the α-galactosidase activity of the preparation used was negligible. Melibiose under the same conditions was not hydrolyzed.

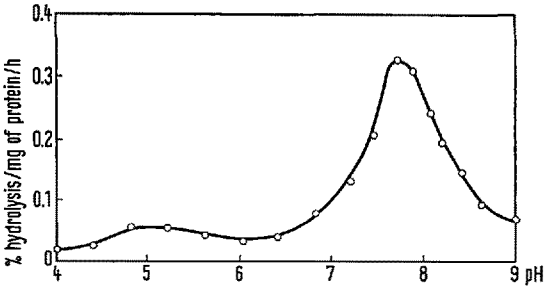

Fig. 1. Dependence of alkaline β-fructofuranosidase activity on pH. The enzyme preparation (5 mg) was incubated with 0.1 *M* sucrose in 0.05 *M* phosphate and/or veronal buffer solutions in a total vol. 1 ml for 1 h at 20 °C. Hydrolysis of sucrose at pH 5.0 was caused by the presence of α-glucosidase activity in enzyme preparation. At pH 7.7 maltose and methyl-α-D-glucopyranoside were not hydrolyzed.

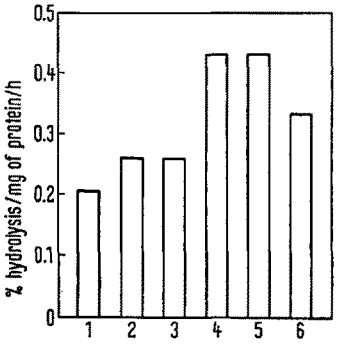

Fig. 2. Activation of alkaline β-fructofuranosidase. The enzyme preparation (5 mg) was incubated with 0.1 *M* sucrose in 0.05 *M* phosphate buffer pH 7.7 in a total vol. 1 ml at 20 °C for 1 h in the presence of 10 μmoles of the activator: (1) without activator; (2) EDTA; (3) NaN_3; (4) glutathion; (5) L-cysteine; (6) 2-mercaptoethanol.

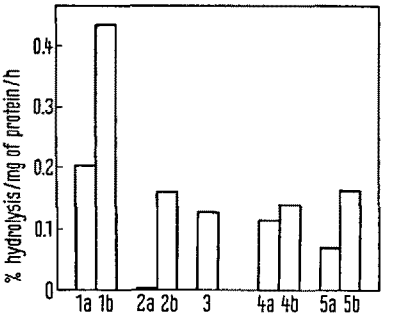

Fig. 3. Inhibition of alkaline β-fructofuranosidase. The enzyme preparation (5 mg) was incubated with 0.1 *M* sucrose in 0.05 *M* phosphate buffer pH 7.7 in a total vol. 1 ml at 20 °C for 1 h in the presence of: (1a) without inhibitor and activator; (1b) 10 μmoles of L-cysteine; (2a) 10 μmoles of *p*-chloromercuribenzoate; (2b) 10 μmoles of *p*-chloromercuribenzoate + 10 μmoles of L-cysteine; (3) 1000 μmoles of iodoacetamide; (4a) 10 μmoles of *N*-ethylmaleimide; (4b) 10 μmoles of *N*-ethylmaleimide + 10 μmoles of L-cysteine; (5a) 1000 μmoles of L-cystine; (5b) 1000 μmoles of L-cystine + 10 μmoles of L-cysteine.

Zusammenfassung. In trockenen Erbsensamen wurde alkalische β-Fruktofuranosidase mit Transferaseaktivität gefunden. Ein angereichertes Enzympräparat, durch L-Cystein, 2-Merkaptoethanol und Glutathion aktiviert und durch *p*-Chlormerkuribenzoat, Iodoacetamid, *N*-Ethylmaleimide und L-Cystin inhibiert, wurde hergestellt.

M. PROŠKOVÁ, M. TICHÁ, G. ENTLICHER and J. KOŠTÍŘ

Department of Biochemistry, Charles University, Praha 2 (Czechoslovakia),
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The β-Receptors in the Rat Pancreas

The stimulatory effect of isoproterenol (ISO) perfusion, a predominant β-stimulatory agent, upon insulin secretion in human beings has been reported by PORTE[1,2]. This stimulation was not accompanied by any hyperglycemia. MALAISSE et al.[3,4] have reported the effect of this drug in vitro, describing a blocking or stimulating action. The latter was obtained only when an α-blocking agent and glucose in a concentration of at least 100 mg/100 ml ($0.6 \times 10^{-2} M$ glucose) were present in the incubation medium together with ISO. The present investigation is intended to determine whether the pancreas response to ISO could be obtained under different experimental conditions as the previously reported.

Slices of rat pancreas (norvegicus male rats of about 100–150 g body weight) were incubated following a technique described in some previous reports[5]. After an equilibration period of 30 min in Krebs Ringer bicarbonate buffer (pH 7.4) with $0.3 \times 10^{-2} M$ glucose at 37 °C, the pancreas slices were placed in incubation flasks with 3 ml of the same buffer and incubated with continuous gassing, using a mixture of 95% oxygen and 5% carbon dioxide during 15 min (baseline). After this period, the slices were transferred to a second incubation medium (stimulation) with (a) $1.7 \times 10^{-2} M$ glucose or (b) $0.3 \times 10^{-2} M$ glucose plus 8.07×10^{-4} mM ISO. Release of insulin into the incubation medium (baseline and stimulation) was determined by the immunoassay method of HERBERT[6].

The results are summarized in the Table. In all cases the amounts of insulin are expressed in μU/mg of tissue/15 min.

These results show that when glucose in the medium increases from $0.3 \times 10^{-2} M$ to $1.7 \times 10^{-2} M$ a significant stimulation of the release of insulin from the pancreas was obtained. ISO, in the above mentioned concentration, was able to elicit an insulin response similar to the one observed with high glucose concentration.

On the basis of these results, several conclusions may arise: (a) the existence of β-receptors in the rat pancreas; (b) these receptors can be stimulated by ISO in the presence of low concentrations of glucose and in abscence of α-blockers; (c) release of insulin is obtained as a result of the stimulation of β-receptors[7].

Pancreas response. Insulin expressed in μU/mg tissue/15 min

Baseline	Response (test substance added)		Δ	P
$0.3 \times 10^{-2} M$ glucose	$1.7 \times 10^{-2} M$ glucose	$0.3 \times 10^{-2} M$ glucose + 8.07×10^{-4} mM ISO		
22.0 ± 2.1 (9)	41.0 ± 3.2 (9)		+ 19.0	< 0.01
21.2 ± 2.6 (7)		35.6 ± 1.9 (7)	+ 14.4	< 0.01

Figures represent mean value ± S.E.M. No. of cases in brackets.

Resumen. Se estudió el efecto del isoproterenol (ISO) sobre la secreción de insulina in vitro. Los resultados indican que el ISO es capaz de estimular la secreción de insulina en forma similar a la glucosa en altas concentraciones.

J. J. GAGLIARDINO[8], R. E. HERNANDEZ and R. R. RODRIGUEZ

Comisión de Investigación Científica de la Provincia de Buenos Aires and Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata (Argentina),
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Distribution of Noradrenaline in the Genital Organs of the Female Rat with a Remark on Dopamine in the Cervix and Vagina

Recent investigations have revealed a distinct adrenergic innervation to the different female reproductive organs of several mammals, e.g. rabbit[1], cat[2], guinea-pig[3] and human female[4]. Furthermore, a considerable variation of the amount of adrenergic innervation to various parts of the female genital tract has been demonstrated[5]. In contrast to the species mentioned, the female rat has been reported to receive adrenergic innervation to the genital organs almost exclusively as blood vessel innervation[6,7]. However, histochemical findings have indicated that at least the isthmic part of the rat oviduct may receive adrenergic nerves to the smooth muscular wall as well[6,8]. Because of this finding it seemed to be of interest to make a quantitative estimation of the adrenergic innervation to various parts of the female rat genital tract by determination of their noradrenaline (NA) content.

Material and methods. 60 adult female Sprague-Dawley rats weighing 180–250 g were used. No determination of the stage of estrus cycle was performed since it has been

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