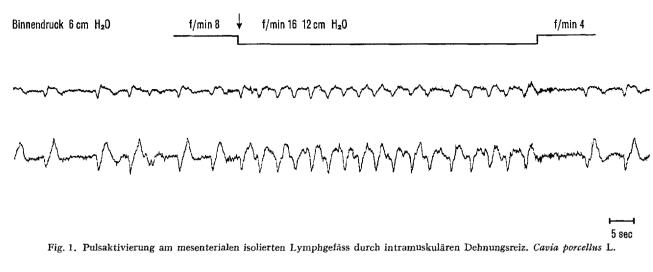
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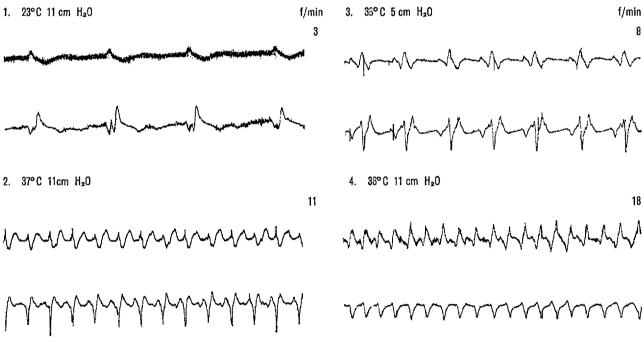


Fig. 2. Kurvenbeispiele für Temperatur- und Druckabhängigkeit isolierter Lymphgefässe. Cavia porcellus L.

H. Mislin<sup>5</sup>

Zoologisches Institut der Universität Mainz, 31. Oktober 1960.

Benachbarte Klappensegmente im Alternansrhythmus.

## Fructose-1,6-Dephosphatase in the Liver of Chickens' Embryos

The researches performed by me¹ have demonstrated the presence of glucose-6-phosphatase in the liver of chickens' embryos starting from the first day of the egg's hatching. On the next day one could be noticed a gradual increase of the enzyme activity which reached its maximum in the embryo's liver after 16 days. The activity then slowly decreased, but without ever reaching the rate of the adult animal. This observation seemed to support the concept of a glucose synthesis, at least for the hepatic tissue of chicken embryo, through a cycle contrary to that of anaerobic glycolysis starting from the pyruvic acid. In fact, glycides are almost completely lacking in the chicken's egg<sup>2</sup>. Another interesting enzyme in this way is the fructose-1,6-dephosphatase. I have therefore thought it proper to investigate the behaviour of the liver of chickens' embryos at different stages of development; at the same time, I have also determined the quantity of glucose and glycogen in the embryo hepatic tissue.

<sup>&</sup>lt;sup>1</sup> M. T. Rinaudo, Estratto dal vol. XXXV, fasc. 24 bis (1959) del Boll. Soc. ital. Biol. sperim.

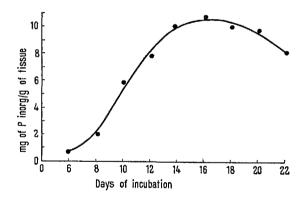
<sup>&</sup>lt;sup>2</sup> M. TOMITA, in B. FLASCHENTRÄGER, and E. LEHNARTZ, *Physiologische Chemie*, II. Band, Zweiter Teil, Bandteil c (Springer, Berlin-Göttingen-Heidelberg 1959), p. 460.

These researches have been carried out on embryo liver obtained from 'Plymouth' hens, developed in an electric incubator at  $37^{\circ}$ C. The fructose-1,6-dephosphatase was determined according to the method of Poggel and McGilvery³ on homogenate of liver in a  $0.05\,M$  boric acid-NaOH buffer, in the presence of fructose-1,6-dephosphate  $(0.05\,M)$ , MgSO<sub>4</sub>  $(0.05\,M)$ , MnCl<sub>2</sub>  $(0.005\,M)$ , and cysteine  $(0.05\,M)$ , all at pH 9.5.

I have also performed on the liver a quantitative determination of glycogen according to Montgomery's modification of the method of Good, Kramer, and Somogy15, after digestion with KOH 30% and glycogen precipitation with ethanol 95%. Glucose was determined according to the methods of Nelson and Somogy17.

The results showed that, from the sixth day of the life of the embryo, a fructose-1,6-dephosphatase activity is present in the liver with rates inferior to those of the adult animal. The rate increases rapidly in the following days, however, until between the 14th and 16th day of the chick embryo's life it reaches twice the rate found in the adult chicken. At that time the maximum rate of activity is observed. In the succeeding days there is a gradual decrease in activity. Only a few hours after hatching, however, the fructose-1,6-dephosphatase activity is superior to that of the adult chicken.

The Figure shows the development of the phenomenon in the embryo liver from the 6th to the 20th day. Each point of the curve represents the average of three determinations. The enzyme activity is expressed in mg of inorganic phosphorus delivered per g of fresh tissue.



Fructose-1,6-dephosphatase activity in the liver of chick embryos Test composition: homogenate (1:30) ml 0.2; fructose-1,6-dephosphate (0.05 M) ml 0.1; MgSO<sub>4</sub> (0.05 M) ml 0.1; MnCl<sub>2</sub> (0.005 M) ml 0.1; cysteine (0.05 M) at pH 9.5 ml 0.1; 0.05 M boric acid-NaOH buffer at pH 9.5 ml 0.4.

In the new born chicken I have noticed rates almost similar to those of 20-days old embryos. The average rate for new born chickens (4 cases) is 8.15 mg of inorganic phosphorus freed/g of tissue as compared with 9.75 mg in the 20-days old embryos (4 cases). In the adult chickens, weighing 1 kg, we have found an average rate (4 cases) of 5.36 mg of inorganic phosphorus freed/g of tissue. The enzyme activity of the chicken at birth is therefore 32% greater than that of the adult chicken.

It is quite significant to notice that, parallel to the increase of the enzyme activity, there is also an increase of the contents of the hepatic glycogen in different development stages of the embryo starting from the 12th day. In the Table below, the embryo liver glycogen rates are recorded expressed in mg/g of tissue. Each figure is the average of three determinations.

Glycogen contained in the liver of embryo chickens, g of tissue 12th day embryo 5.72 mg 18th day embryo 33.00 mg 14th day embryo 5.50 mg 20th day embryo 42.75 mg 16th day embryo 29.60 mg

After a few hours after the opening of the egg, the glycogen undergoes a remarkable decrease in the chicken. The glycogen content, 1 h after hatching is 4 mg; after 12 h (4 cases each) 1.70 mg after 36 h (4 cases) 0.78 mg. In the adult chicken, after 12 h fasting (7 cases), 2.7 mg of glycogen/g of tissue were found.

The glucose in the liver of embryos of different ages, on the contrary, is found in constant amounts (about 3 mg/g of tissue). However, glucose is observed only in embryos that are 14-days old. In the adult animal (10 cases), a medium rate of 8 mg/g of tissue has been found.

The experimental data reported support the possibility that, in the liver of embryo chickens, the glucose and glycogen synthesis proceeds from compounds with 3 atoms of carbon, in a manner that is similar to a reversal of anaerobic glycolysis.

Riassunto. Una fruttoso-1,6-difosfatasi compare nel tessuto epatico di embrioni di pollo già al 6° giorno di incubazione dell'uovo; in seguito aumenta rapidamente e raggiunge il massimo fra il 14° ed il 16° giorno; quindi tende a diminuire, pur mantenendosi sempre superiore a quella del fegato dell'animale adulto. Di pari passo all'aumentare dell'attività dell'enzima si osserva un incremento della concentrazione del glicogeno epatico, che cade però bruscamente all'atto della schiusa.

M. T. RINAUDO

Istituto di Chimica Biologica, Università di Torino (Italy), September 12, 1960.

- <sup>3</sup> B. M. Pogell and R. W. McGilvery, J. biol, Chem. 208, 149 (1954).
- <sup>4</sup> R. Montgomery, Arch. Biochem. Biophys. 67, 378 (1957).
- <sup>5</sup> C. Good, H. Kramer, and M. Somogyi, J. biol. Chem. 100, 485 (1933).
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## Partial Purification of a Plasma-Kinin-Forming Enzyme from Horse Urine<sup>1</sup>

The generic name plasma-kinins has been proposed <sup>2</sup> for a group of polypeptides with similar pharmacological and chemical properties. The first kinin to be described was kallidin, so named by Werle <sup>3</sup> in 1947; it is a basic <sup>4</sup> polypeptide <sup>5</sup>, with unknown structure, characterized in 1937 to 1939 by its hypotensive and smooth-muscle-stimulating properties <sup>6,7</sup>. Peptides related to bradykinin, a plasma-kinin discovered in 1949 by Rocha E Silva and Beraldo <sup>8</sup>

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- <sup>7</sup> E. WERLE and M. GRUNZ, Biochem. Z. 301, 429 (1939).
- <sup>8</sup> Cf. M. Rocha E Silva, Polypeptides which Affect Smooth Muscles and Blood Vessels (Pergamon Press, London 1960), p. 210.