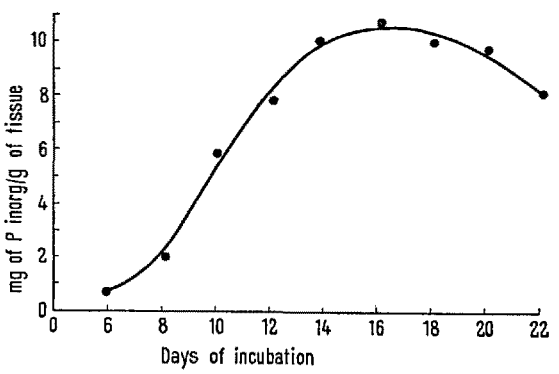
These researches have been carried out on embryo liver obtained from 'Plymouth' hens, developed in an electric incubator at 37°C. The fructose-1,6-dephosphatase was determined according to the method of POGGEL and MCGILVERY[3] on homogenate of liver in a 0.05 *M* boric acid-NaOH buffer, in the presence of fructose-1,6-dephosphate (0.05 *M*), $MgSO_4$ (0.05 *M*), $MnCl_2$ (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[4] of the method of GOOD, KRAMER, and SOMOGYI[5], after digestion with KOH 30% and glycogen precipitation with ethanol 95%. Glucose was determined according to the methods of NELSON[6] and SOMOGYI[7].

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; $MgSO_4$ (0.05 *M*) ml 0.1; $MnCl_2$ (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.

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Partial Purification of a Plasma-Kinin-Forming Enzyme from Horse Urine[1]

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

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were recently synthesized[9]. In 1952, substance U, a depressor and smooth-muscle-stimulating principle present in urine, was reported by BERALDO[10].

Normal urine contains kallikrein[11], a kallidin-forming-enzyme. Although kallikrein was considered as a possible liberator of substance U, which would then be identical with kallidin, some doubts were raised about this possibility because dog's urine has no effect on ox serum or globulins[12], and because the concentration of the liberator of substance U in urine does not diminish following pancreatectomy[13].

In the experiments reported here, the kinin-liberating activity of horse urine, or of enzyme preparations obtained from it, was assayed in the isolated guinea pig ileum[14] suspended in an 8 ml bath of atropinized (1 µg/ml) Tyrode solution at 37°C. The assay consisted of incubating the unknown preparation with an excess of dialyzed horse plasma in the smooth muscle chamber for 2 or 3 min, and comparing the spasmogenic effect with that of a crude enzyme powder taken as standard.

The urinary kinin-liberating-enzyme was purified as follows: the proteins separating from horse urine at 3–4°C, pH 7.0, between 0.40 and 0.70 of saturation with ammonium sulfate, were refractionated at the same pH to isolate the material precipitated between 0.45 and 0.55 ammonium sulfate saturation. The proteins were then dialyzed in the cold against distilled water followed by equilibration of the solution against a 0.075 *M* phosphate buffer, pH 6.0. The resulting solution was run through a column of diethylaminoethyl (DEAE)-cellulose; the active material remained in the column and was eluted with the same buffer, 0.15 *M*, pH 6.0. This material was further purified by rechromatography on DEAE-cellulose under slightly different conditions. Considering as unity the activity per mg of N of the first ammonium sulfate precipitate, an eighty fold (80×) purification was finally reached.

This purified material liberates a kinin either from fresh horse plasma or from plasma heated for 3 h at 56–58°C; it also acts on bovine, human, dog, and rat plasmas, although not with the same intensity. Fresh plasma globulins (precipitated by ammonium sulfate between 0.25–0.40 of saturation), or globulins heated for 3 h at 56–58°C, and bovine fraction IV-4[15] also serve as substrates for kinin formation; by contrast, fraction IV-4 of swine plasma produced no kinin. Kinin formation is not inhibited by soy-bean trypsin inhibitor or by ovomucoid which, under the same conditions, completely inhibited trypsin. Using preparations with different degrees of purity, a parallelism was observed in quantitative experiments between their ability to liberate kinin and to hydrolize *p*-toluenesulfonyl-L-arginine methyl ester (TAME). All these properties, including the last one[16], are shared by the kallikreins. The stability of the enzyme activity was studied at different temperatures (30, 50, 70, and 98°C) and pHs (1, 3, 5, 7, 9), using a commercial preparation of kallikrein (Padutin, Bayer) for comparison. A maximum stability around neutrality was found for both enzymes.

We thus believe that the liberator of substance U is urinary kallikrein and that substance U itself is probably identical with kallidin; the inability to liberate a kinin by incubating ox globulin with dog's urine[12] is possibly explainable by the species specificity of the kallikreins which is already known[6,7] and which was here confirmed (horse urinary kallikrein did not liberate kallidin from swine plasma globulins). The failure to observe a decrease in the urinary releaser of substance U following pancreatectomy[13] was probably due to elimination in urine of kallikreins from extra-pancreatic sources. The presence of a substance U-liberating-factor in the urine produced by heart-lung-kidney-preparations[13] may be explained by the fact that lung tissue contains kallikrein[17], and kidney tissue probably also does, because renin solutions seem to be contaminated with kallikrein[18]. We have also confirmed HORTON and LEWIS'[19] observation that urokinase is not the rapid kinin-liberator studied here. Further purification of the kinin-forming-enzyme from horse urine appears necessary; the purest preparation obtained is still impure as revealed both by paper electrophoresis studies and by the fact that it contains a kinin-destroying factor which differs from the kinin liberator.

Zusammenfassung. Durch Chromatographie mit Diäthylamino-äthyl-cellulose nach Ammoniumsulfat-Fraktionierung wurde ein Plasma-Kinin-freisetzendes Ferment aus Pferdeharn teilweise (80×) gereinigt. Das so gereinigte Präparat war gegen Pferde-, Menschen-, Rinder (Fraktion IV-4)- und Ratten-Blutplasma, nicht aber gegen Schweine-(Fraktion IV-4)-Globuline wirksam. Durch Sojabohnen-Trypsininhibitor oder Ovomucoid erfolgt keine Hemmung. Präparate von verschiedenem Reinheitsgrad zeigten entsprechende *p*-Toluenesulfonyl-L-arginin-methylester-spaltende und Kinin-freisetzende Wirksamkeit.

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A Novel Reaction at Low Temperature between Nucleotides and Protein Enhancing Biological Activity[1]

The present chemically defined basal medium used in the axenic culture of the nematode *Caenorhabditis briggsae* is not adequate by itself to support continuous growth. Serial subcultures can, however, be maintained with small additions of organic supplements, 5–10% by volume. A supplement used extensively in our work is material precipitated from homogenate of liver by ammonium

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