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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sulfate (NICHOLAS et al.[2]). This material, precipitated three times between 40 and 60% saturated ammonium sulfate, is used after dialysis against $M/15$ potassium phosphate buffer at pH 7 and sterilization by filtration through asbestos pads (Hercules ST grade).

The effectiveness of this supplement has now been found to be increased about 50 fold upon freezing it at 2% by volume with the basal medium. Activation does not occur at 4°C, 20°C, or by preincubation at 37°C, but only upon freezing. Freezing conditions of −17°C for periods of one day to two weeks have been used with no apparent differences in effectiveness. The activation is lost by holding at 4°C overnight, but is restored by refreezing; the process, then, is a reversible one. Preliminary experiments indicate that a shift in a peak of the absorption spectrum accompanies activation, as well as a change in optical rotation.

As shown in a previous paper (DOUGHERTY and HANSEN[3]), the concentration of a growth requirement can be related by the usual log dose-response curve to the number of days a newly hatched larva takes to reach maturity. Young larvae hatched into phosphate buffer were inoculated into medium immediately after it had thawed and warmed to 20°C, and the rate of growth to maturity determined (DOUGHERTY et al.[4]). The activated supplement permitted successful growth and reproduction when added to our chemically defined basal medium in an amount corresponding to 5 μg of nitrogen per culture.

Six preparations of this protein fraction, two from horse liver and four from lamb liver, have shown this 'freeze-activation'. These ranged in dry weight 34–73 mg/ml, nitrogen content 3.6–6.8 mg/ml, optical density ratio at 280/260 mμ– 1.31–1.57.

Preliminary attempts to activate supplements from chick embryo extract, serum, and autolyzed bacteria have been unsuccessful so far.

The 'freeze-activation' of the liver protein fraction did not occur in water, but initially, only with the chemically defined medium. This is a multi-component medium, designed for maximum growth response of the nematode (in publication). Since it seemed likely that only a part of the medium was involved in the activation, the components were investigated first in groups and then singly. Freezing with amino acids, salts, vitamins, or glutathione was ineffective. Only the nucleotide-group produced activation, and it was subsequently obtained with each of the nucleotides (adenylic, guanylic, cytidylic, and uridylic acids) and the base (thymine), tested individually at 1 *mM* concentration, their level in the basal medium (Table). The final tests were made by freezing these solutions with 2% liver protein fraction and then adding the activated mixture to an equal volume of chemically defined medium at 2 fold concentration.

The nature of this 'freeze-activation' process remains obscure; there is clearly an interaction between some component of the supplement and one of the nucleotides or the nucleic acid base during freezing and thawing. This interaction could be: 1. an alteration of some spatial configuration by rearrangement of hydrogen bonding to make the material more accessible to the enzyme systems of the nematode, 2. the displacement of some small molecule from a more complex material, or 3. an unfolding of the protein and subsequent interaction with the heterocyclic activator to produce a new and transient material of high biological activity.

An interesting speculation is that this 'freeze-activation' could, perhaps, play a significant role in the survival of micrometazoa in the polar regions.

Activation of liver protein fraction as a nutritional supplement by freezing and thawing

Liver protein[a] %	Activator	Days to mature: Freeze-activated	Days to mature: Fresh Mix
10	EM-62[b]	21	21
5	EM-62	7	non-maturing
2	EM-62	5	non-maturing
1	EM-62	5	
0.5	EM-62	11	
0.25	EM-62	non-maturing	
0.1	EM-62	non-maturing	
1	4 nucleotides[c] + thymine	7	
1	individual nucleotides[c] or thymine	8–9	
1	EM (less nucleotides and thymine)	non-maturing	

Concentrations of supplement less than 2% were equally effective whether obtained by diluting the 'freeze-activated' 2% mixture or by freezing at lower dilutions.

[a] Fraction-C; dry weight 38.3 mg/ml; N 4.6 mg/ml; O.D. 280/260 mμ = 1.53.

[b] Chemically defined medium containing amino acids, vitamins, salts, nucleotides, glucose, and glutathione (in publication).

[c] Adenylic, guanylic, cytidylic, and uridylic acids.

Résumé. Une protéine du foie, en partie purifiée, congelée en présence de nucléotides ou de thymine a montré un accroissement d'activité. Ceci résulte en une augmentation de 50 fois la valeur nutritive, lorsque ajoutée au milieu chimique défini pour la culture du nématode *Caenorhabiditis briggsae*. Un changement physique de la protéine est postulé, à cause du changement de la rotation optique de l'absorption aux UV qui accompagne l'accroissement d'activité.

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Changes in the Liver Protein Pattern due to Lysergic Acid Diethylamide (LSD)

While studying the effects of lysergic acid diethylamide (LSD), we carried out some investigations on the electrophoretic pattern of organ-extracted proteins. Although such studies are still in course, we should like to anticipate a few data concerning the behaviour of proteins extractable from liver by low ionic strength buffers. In fact, 1 mg/kg of the drug injected intraperitoneally causes a sudden and remarkable change of the free electrophoretic protein pattern so that any attempt to calculate the mobility values is often unreliable (Fig.).