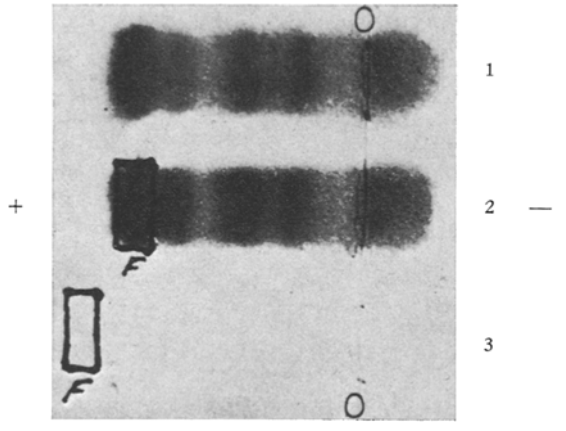
After the run, the paper was air dried and moistened with 5% acetic acid. The wet strip was then viewed under UV light for green-yellow fluorescence (characteristic of citrinin). The fluorescing portions were marked F. The strip was then stained for proteins overnight using bromophenol blue. 5% acetic acid was employed for destaining.



1, Human serum (dialyzed); *2*, Human serum–citrinin mixture (dialyzed); *3*, Citrinin; *F*, Fluorescence portions (marked before staining).

Results and discussion. From the figure it can be seen that a fluorescing portion was present in the electrophoretic path of human serum-citrinin mixture, and this corresponded with the albumin band, when stained. The only other fluorescence marked was that of pure citrinin (run alongside) and this had moved towards the anode faster than the adjacent albumin fraction of serum. These observations suggest that a) citrinin is capable of binding to at least one of the human serum proteins-albumin, and b) the binding of citrinin to albumin is fairly stable because the complex was not affected by dialysis. In a separate experiment it was shown that undialyzed human serum-citrinin mixture exhibited 2 fluorescent spots, one corresponding to free citrinin and the other to that of albumin portion. All the above observations could be noted again when agar gel was employed for electrophoresis. Moreover, studies with ^{14}C-citrinin confirmed the binding of citrinin to serum albumin.

It is felt that the observed in vitro binding of citrinin to serum protein fraction may help in understanding the possible transport mechanism of toxic citrinin. Similar binding studies with cellular components will throw light on the possible mode of action of citrinin with reference to its toxicity in animals and plants[7–9].

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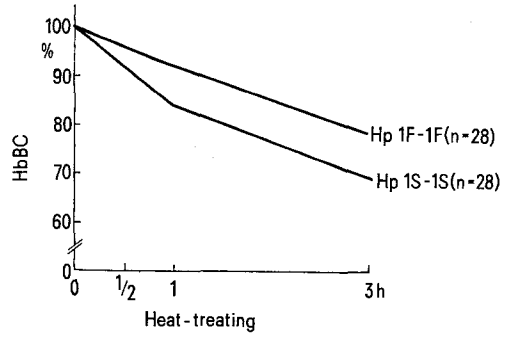
Hemoglobin binding capacity of heat incubated sera of different haptoglobin subtypes

Ö. Hevér

Laboratory Service of the State Institute for Medical Rehabilitation, P. O. Box 123, H–1528 Budapest (Hungary), 6 October 1976

Summary. Sera of haptoglobin subtypes Hp 1F-1F and Hp 1S-1S incubated at 56 °C show a different degree of reduction in the hemoglobin binding capacity. The difference is small but significant.

Earlier it was demonstrated that in serum incubated at 56 °C the hemoglobin binding capacity (HbBC) is reduced and the extent of reduction varies with the Hp phenotype in the order of decreasing Hp 1-1 < Hp 2-1 < Hp 2-2[1]. The duration of the heat incubation also influences the extent of reduction in the HbBC[2]. Agar-gel electrophoretic investigations proved that a smaller quantity of Hp-Hb complexes are formed in heat-treated serum and this quantity depends on the phenotype[3]. On the basis of examinations of complexes formed with sera of the phenotype Hp 1-1, one may conclude that even within these phenotypes there is an additional variation among the individual sera[4]. These findings suggested different heat-stability for sera of different Hp subtypes.



Change of the HbBC mean value of sera heat-treated at 56 °C as compared in percent to their not-treated controls.

Materials and methods. 28 sera of Hp 1F-1F and Hp 1S-1S subtypes were analyzed. Subtypifying was carried out according Pastewka et al.[5]. Photometric measurement of the peroxidase activity of the complex formed: the sera were divided into 4 parts. One remained untreated, this represented the native control sample. The other 3 were kept for half, 1 and 3 h respectively in water bath at 56 °C. The placing of samples into the water bath was carried out in such a manner that heat-incubation of all samples was finished at the same time. Following this,

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the value of HbBC was determined with the guaiacole peroxid method according to Owen et al.[6] both in the native and heat-incubated samples. The original manual method was mechanized with pipettors, and flowthrough cuvette. The samples were prepared in 6-sec-intervals and measured after 10 min standing in waterbath at 25 °C in the same order with the same intervals. In this way the sera of Hp 1F-1F and Hp 1S-1S phenotypes were analyzed together in the same serial procedure to avoid possible inaccuracy and divergence of the results originating from different reagents and reactions executed in different serials.

Results. The figure summarizes the results of examination. It may be noted that, as compared to the native, the HbBC value was reduced less in sera of the subtype Hp 1F-1F than in those of subtype Hp 1S-1S. Between the mean values of the 2 groups, there is a significant difference after the heat-incubation for 1 h as evidenced by the double-t-test of Student: $t = 4.09$; $p < 0.001$. The alpha polypeptide chains of Hp molecules determined by the alleles Hp^{1F} and Hp^{1S} differ in only one amino acid. In position 54, there is lysine in the alpha-1F chain and glutamine in the alpha-1S chain[7]. This causes a difference in the electrophoretic mobility of the 2 polypeptide chains on the basis of which subtyfying is carried out. According to present knowledge, the beta chains do not differ in individual phenotypes[8]. It may be supposed that this chemical difference in the alpha-chains of Hp causes also a difference in the behaviour of the Hp molecule to heat.

The phenomenon was investigated in whole human serum and not with purified Hp. However, the decrease of the HbBC in the serum under effect of heat may support to the altered behaviour of the Hp. This is justified by the dependence of subtypes and by the earlier demonstrated dependence of usual phenotypes[1,3].

At present there is no explanation for the HbBC reduction of Hp occurring upon the effect of heat. Most probably 56 °C of heat does not cause major changes in the Hp molecule. In the course of experiments made with Laurell's rocket method, there was no degradation product to be seen in heat incubated sera[9]. It is more likely that the change is configurational. The Hp molecules have a structural similarity to Bence Jones proteins[7]. There is a similarity between the 2 proteins in presenting a special behaviour against heat, too. The heat sensibility of the Hp molecules concerns one of its functions, the most characteristic and the most important – as we regard it nowadays –, the hemoglobin binding capacity. The further investigation of the decreasing hemoglobin binding capacity of the haptoglobins exposed to heat may lead to new data concerning haptoglobin-hemoglobin interaction.

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Zinc biochemistry in normal and neoplastic growth processes[1]

B. L. Vallee

Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, and the Division of Medical Biology, Peter Bent Brigham Hospital, Boston (Massachusetts 02115, USA), 12 October 1976

Summary. Zinc is essential for the growth of all species. Growth arrest results from its deficiency and presumably reflects important roles for this metal at critical points of metabolism. Studies of zinc metalloenzymes show that zinc serves as a coenzyme to more than 80 enzymes, among which are the reverse transcriptases which cause leukemia in many species. Its role in nucleic acid metabolism is emphasized.

Zinc is essential for the growth of all species. Growth arrest results from its deficiency and presumably reflects important roles for this metal at critical points of metabolism. Thus, it was found 25 years ago that normal leukocytes contain substantial quantities of zinc while leukemic cells contain less than 10% of the zinc content of normal leukocytes[2]. The consequences to cellular growth and proliferation of such intracellular decreases in zinc content have only begun to be investigated recently. We have utilized various cell lines including E. gracilis[3], and human leukemic lymphoblasts to examine the involvement of zinc in the biochemical events of cell division. The biological essentiality of zinc can be discerned at various steps of cell growth and development in both normal and neoplastic cells.

The unicellular eukaryote, E. gracilis, has proved most suitable in this regard and has served to define the approaches required to study zinc metabolism in cells cultured in vitro. Importantly, the zinc content of this organism decreases to less than 10% that of normal, zinc sufficient cells when the zinc concentration of the culture medium is less than 10^{-7} M[3]. The resultant deficiency severely limits its growth. A number of striking morphological and chemical derangements are the consequence. Both RNA and protein synthesis are markedly depressed. The DNA content and cellular volume doubles while β-1,3 glucan (paramylon) aggregates. Further, proteins and peptides of unusual composition, amino acids, nucleotides and pyrophosphate accumulate. In addition, the intracellular content of Mg, Mn and Fe increases markedly. Addition of zinc to the culture media completely restores the chemical. morphological and growth patterns to those characteristic of normal E. gracilis cells[4]. These dramatic results show that in zinc-deficient cells both biochemical and morphological processes of the cell cycle, i.e. DNA and/or RNA synthesis as well as function and cell division are markedly disturbed, perhaps as the consequence of the essentiality of zinc to the primary molecular events, responsible for those events. Consequently, we have em-

1 This work was supported by Grant-in-Aid GM-15003 from the National Institutes of Health of the Department of Health, Education and Welfare.
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