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A NEW BLEEDING TENDENCY DUE TO HERITABLE HYPER α_2-MACROGLOBULIN-
EMIA (α_2-MACROGLOBULIN-AKITA). Y. Endo, K. Iwamoto, S. Mamiya, H.
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A heritable elevation in α_2-macroglobulin (α_2M) was identified
in a 9-year-old girl with bleeding tendency and prolonged APTT to
49.1 sec (normal 27-38) and recalcification time to 438 sec
(<180). Plasma α_2M level in her family tree i.e. sister, mother,
maternal grand mother, father and paternal grand mother was 406,
380, 352, 339, 166 and 236 mg/dl (140-285), respectively. Thus it
is thought to be an autosomal dominant disease, but other rela-
tives show no apparent clinical syndrome. The significance of a
possible causal association between elevated α_2M and prolonged
APTT was showed.

Relationship between plasma concentration
of α_2-macroglobulin and APTT

α_2-macroglobulin (mg/dl)	50	100	150	200	250	300	350
APTT (sec)		38.6	40.3	41.9	43.3	44.3	48.0	54.3

The activity of α_2M of the patient determined as trypsin-protein
esterase was 351 mg/dl (197%), also. The α_2M of the patient showed
normal mobility to anti-α_2M plasma with acceralated anodal mobility
by crossed immunoelectrophoresis and normal mobility by immuno-
fixation electrophoresis. In addition, by the analysis with SDS-
polyacrylamidegelelectrophoresis we demonstrated no qualitative
abnormality in α_2M of the patient. The new familial abnormality is
tentatively designated "α_2-Macroglobulin .-Akita".

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ACTIVATION OF HEPARIN COFACTOR II BY PHOSVITIN, A PHOSPHOGLYCO-
PROTEIN, AND OTHER PHOSPHATE-CONTAINING POLYANIONS. F.C.
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We are characterizing the specificity of the polyanion-
binding domain of the heparin/dermatan sulfate-dependent plasma
protease inhibitor, heparin cofactor II (HCII). Various phos-
phate-containing polyanions accelerate the HCII-catalyzed inhi-
bition of thrombin (T). Phosvitin, a phosphoprotein, enhances
the HCII/T reaction at 25°C and pH 8.0 with the apparent sec-
ond-order rate constant value (k_2) increasing from 5×10^4 M^{-1}
min^{-1} (in the absence of phosvitin) to 8×10^7 M^{-1} min^{-1} as
phosvitin increased from 0.05 to 30 ug/ml and then decreases as
phosvitin is increased above 30 ug/ml. Apparent dissociation
constant values for phosvitin-HCII and phosvitin-T are 450 nM
and 10 nM, respectively. Polynucleotides accelerate the HCII/T
reaction and have the following specificity (concentrations
examined from 1-200 ug/ml): poly(guanylic acid) >> poly(adeny-
lic acid, guanylic acid) > poly(inosinic acid) > poly(guanylic
acid, uridylic acid) > poly(uridylic acid) = poly(adenylic
acid) > poly(cytidylic acid). Polyphosphate anions (phosphate
chain length, n, ranging from 5-100) enhance the HCII/T reac-
tion. When compared at an equimolar phosphate concentration
(1 mM), the rate was saturated at n = 50 with a maximum k_2 of
about 5×10^7 M^{-1} min^{-1}. Ca^{2+} (or Mg^{2+})-phosvitin/polyphosphate
anion complexes and salmon protamine-polynucleotide complexes
have lost the ability to enhance the HCII/T reaction. Phospho-
pyridoxylated-HCII (lysine modified), with greatly reduced
heparin cofactor activity, has lost its accelerating effect
with phosvitin, polynucleotides and the polyphosphate anions.
None of the above mentioned polyphosphate-containing compounds
are effective at accelerating either the HCII-catalyzed inhi-
bition of chymotrypsin or the antithrombin III-catalyzed T
reaction. Our results suggest that (i) HCII is activated by the
multiple negative charges of phosphate polyanions but they
alone are not sufficient; (ii) the effective phosphate poly-
anions must also possess a specific conformation for maximum
activity; and (iii) the phosphoserine-containing protein, phos-
vitin, can serve as a "template" for HCII/T.

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INACTIVATION OF HEPARIN COFACTOR II (HC II) BY POLY-
MORPHONUCLEAR LEUKOCYTES (PMNL). P. Sié, D. Dupouy, F.
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Several authors have shown that antithrombin III
(AT III) was catalytically inactivated by neutrophil
elastase, an observation relevant to pathophysiologi-
cal processes in the vicinity of inflammatory sites.
The aim of this study was to investigate whether HC
II, another natural thrombin inhibitor, is also inac-
tivated by PMNL.
A rapid loss of HC II activity occured upon in-
cubation with fresh human PMNL stimulated by phorbol
myristate acetate (T1/2 1 uM HC II, 0.35 10^6 cells/mm^3
: ~2 min) or with PMNL extracts prepared by nitrogen
cavitation. Antithrombin (dermatan sulfate cofactor)
and antichymotrypsin activities of HC II were lost at
the same rate. Resting PMNL were ineffective. Inacti-
vation was prevented by several serine-protease inhi-
bitors but was Ca^{++}/Mg^{++} independent. Inactivation
coincided with the formation of a 54 KD peptide after
a first non-inactivating degradation into a 62 KD
peptide (native HC II : 76 KD). These reaction
products are reminiscent of those described upon in-
cubation with proteinase I from Echis carinatus venom.
HC II was inactivated more rapidly than AT III (T 1/2
of AT III in the same conditions ~15 min). However,
heparin (1-10 ug/ml) strongly accelerated the rate of
AT III inactivation and slightly protected HC II, thus
reversing the order of inactivation. Dermatan sulfate
had no effect on this process.
In conclusion, this study shows : 1) both AT III
and HC II are rapidly inactivated by PMNL enzymes,
thus favoring locally thrombin-mediated processes ; 2)
heparin increases AT III degradation by PMNL, a possi-
ble route of catabolism operating in patients with low
AT III levels during heparin treatment.

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MOLECULAR HETEROGENEITY IN FAMILIAL HEPARIN COFACTOR II
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In our normal material of 379 blood donors,3 indivi-
uals had values below mean -2.5 SD (below 56%).Further
studies revealed hereditary deficiency in two of these
individuals. In the family study,5 out of 7 individuals
had heparin cofactor II (HC II) values below 56%. In
only one of these 5 a history of DVT was obtained. In
deceased members of her family,however,frequent epi-
sodes of thromboembolic disease had occurred.
Crossed immunoelectrophoresis (CIE) was performed in
plasma from deficiency individuals from both families.
Heparin in the first dimension gave a pattern similar
to that observed with normal pooled plasma. The inacti-
vation of thrombin by HC II is preferentially accele-
rated by dermatan sulfate (DS).HC II consumption in
in vitro coagulation is increased by DS rather than by
heparin. This prompted the addition of DS to the first
dimension,which makes the antigen move faster, but pro-
duced no alteration in the antigenic pattern or size,
neither in pooled plasma nor in a family member with
normal activity. In family members with low HC II acti-
vity,DS in the first dimension,resulted in an abnormal
CIE pattern with two distinct precipitation arcs with
identity pattern. Molecular heterogeneity of HC II has
previously not been reported.
Screening 70 individuals who had sustained throm-
bosis before the age of 50,no values suggesting con-
genital deficiency were encountered.
Conclusion. The CIE findings suggest that plasma of
affected family members contain two types of HC II
molecules of immunological identity. One type shows the
normal accelerated mobility in the presence of DS and
the other lacks this effect. The abnormal electrophore-
tic pattern becomes,however,first apparent when DS is
added to the first dimension. When looking for molecu-
lar defects in congenital HC II deficiency,it is impor-
tant that the first dimension is run with DS.