unlikely since PMN from animals judged free of IBR exposure (and antibody negative as measured by virus neutralization and ADCC), still expressed CDNC and, in fact, comparable degrees of cytotoxicity were observed with PMN from antibody positive and antibody negative animals (table 2). Furthermore, CDNC was apparent with 4 separate batches of FCS all of which lacked detectable antiviral antibody activity (table 2). In addition the complement source lacked detectable antibody to IBR and did not support CDNC following heat inactivation (table 1). Finally, the CDNC effect could be demonstrated against cells infected with HSV or VSV infected cells (table 3), viruses against which cattle are not normally exposed and do not have antibodies to. Finally, pretreatment of PMN to elute any putative cytophilic antibody, failed to ablate the cooperative PMN and complement cytotoxicity (table 2). Taken together, these results indicate that cells infected with certain viruses become susceptible to destruction by the combined presence of PMN and complement. If a similar effect occurs in vivo, it could provide a mechanism of recovery from infection before the time when specific components of immunity become active.

Since only virus infected cells acted as targets, it seems likely that some component on the surface of such cells, was activating complement. Candidate substances to cause this activation could be a viral antigen or perhaps a protease[20]. Thus some viruses, including the herpesvirus Epstein-Barr virus can activate complement by the alternative pathway[21]. In addition, it was recently shown that the G protein present on the viral envelope of VSV can activate the classical complement pathway without the participation of antibody[22]. Whether or not IBR and HSV viruses can activate complement has not been determined. The idea of a membrane bound protease activating complement was recently elaborated by Dierich and Landen[20] and it has been reported that certain virus infected cells may express increased protease activity[20,23]. Although complement activation alone may be sufficient to destroy virus infected cells[24] and did occur to a degree at the highest level of complement used in our study, our results showed a marked increase of cytotoxicity in the presence of PMN. It would be that these cells, which bear complement receptors[25], were being anchored to the target cells and as a result delivered a lytic signal to the targets. This lytic signal could in turn involve the elaboration of certain activated complement components as suggested by Mayer[26]. Alternatively, the PMN could be activating complement, perhaps by proteases present in their membrane. Those activated components generated could bind by different receptors to both PMN and virus infected cells with the binding to the latter cells being followed by the elaboration of the lytic unit. Clearly more work is needed to resolve the mechanism of CDNC and to clarify the possible in vivo significance of a defense mechanism which could operate before antibodies and sensitized T cells build up to protective levels.

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The concentration of high molecular weight kininogen antigen in homogenates of various human tissues[1]

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Summary. The concentration of high molecular weight kininogen, measured in human tissue homogenates, was 2–3 times higher in kidneys, adrenals and thyroid than in homogenates of lung, heart, liver and spleen. No measurable quantities of this protein were found in homogenates of brain and skeletal muscles.

Of the 2 plasma kinin precursors only high molecular weight kininogen (HMW-kininogen), but not low molecular weight kininogen (LMW-kininogen) plays a key role in Hageman factor-dependent activation of clotting, fibrinolysis and prekallikrein activation[3–6]. The site of synthesis of this protein, and its distribution in the organism, are unknown. The present report deals with the results of the measurement of HMW-kininogen antigen in human tissue homogenates.

Material and methods. Fragments of selected organs, obtained during routine postmortem examination of 40 bodies, maintained at 4 °C, were homogenized in a Potter

Fig. 1. Double immunodiffusion. The numbered wells were filled with tissue homogenates as follows: *1* a mixture of equal parts of gray and white brain matter; *2* skeletal muscle; *3* heart; *4* lung; *6* spleen; *7* liver; *8* kidney cortex; *10* kidney medulla; *11* adrenal; *12* thyroid. *5* and *9* were filled with 1:4 diluted normal human plasma. Central wells were filled with rabbit antiserum to kininogen.

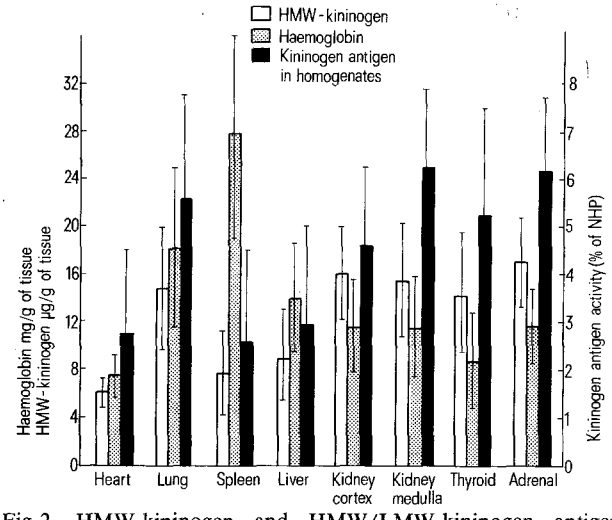

Fig. 2. HMW-kininogen and HMW/LMW-kininogen antigen concentration in tissue homogenates. Pooled plasma of 6 healthy subjects was used as a standard for the calculation of HMW/LMW-kininogen concentration in tissue homogenates.

type polyethylene homogenizer in 0.3 M NaCl containing 0.5 mg/ml of soya bean trypsin inhibitor. After centrifugation the clear supernatants were concentrated in dialysis sacs in the presence of polyethyleneglycol 20,000 to a volume equal to that of the homogenized tissue, followed by dialysis in phosphate-citrate buffer[7]. HMW-kininogen was isolated from human plasma, and rabbit antiserum to this protein was prepared as described[7,8]. The concentration of HMW-kininogen was estimated with the test of hemagglutination inhibition[7,9] and the concentration of the antigen common to HMW and LMW-kininogens was measured by radial immunodiffusion[10]. Protein concentration was estimated according to Lowry et al[11] and hemoglobin concentration by the method of Drabkin[12].

Results. Figure 1 shows that homogenates of all the tissues tested, with the exception of brain and skeletal muscles, formed precipitation lines with antiserum to kininogen.

The concentrations of HMW-kininogen (figure 2) in the homogenates of the lung, renal medulla and cortex, adrenal and thyroid were similar, and higher than the concentration in the homogenates of heart, liver and spleen. A similar pattern was found when the concentrations of HMW/LMW-kininogen antigen in various tissue homogenates were compared.

Figure 3 demonstrates that the value of HMW-kininogen/Hb concentration was nearly twice as high for the homogenates of both renal tissues, adrenals and thyroid as for the homogenates of the heart, lung and liver.

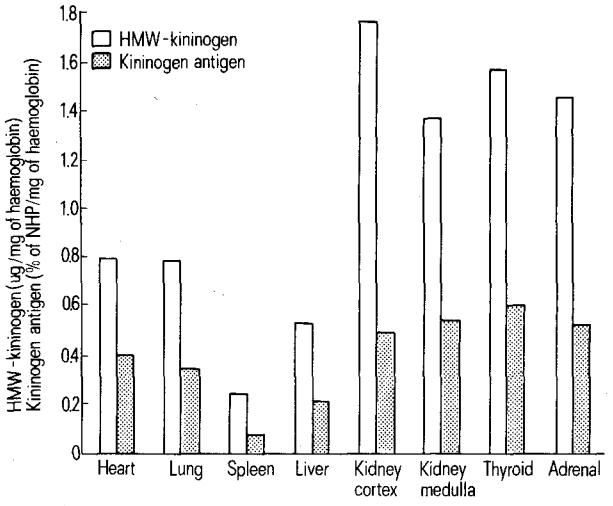

Fig. 3. The coefficients of HMW-kininogen and HMW/LMW-kininogen antigen concentration per mg of hemoglobin.

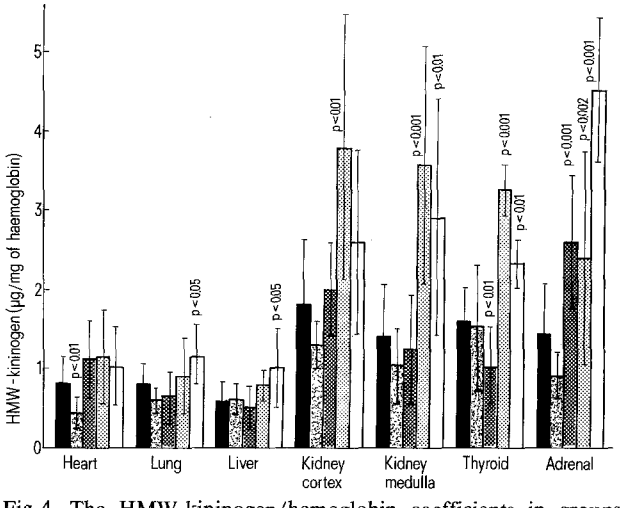

Fig. 4. The HMW-kininogen/hemoglobin coefficients in groups formed according to the cause of death. ▨ chronic circulatory insufficiency; ▩, myocardial infarction; ▨, neoplasma; □, pneumonia; ■, various.

Figure 4 presents the values of HMW-kininogen/Hb concentration in tissue homogenates of patients dying from chronic circulatory insufficiency (n = 7), myocardial infarction (n = 8), malignant neoplasms (n = 7), pneumonia (n = 5) compared with the values for 'control group' of patients (n = 13) dying from other diseases. Of particular interest in this figure are the high values of the coefficient calculated in both parts of the kidneys and in adrenals and thyroid, in patients dying from malignant neoplasms, pneumonia and myocardial infarction.

Discussion. The lack of correlation between hemoglobin concentration, representing the blood remaining in the tissues, and the concentration of HMW-kininogen (correlation coefficient 0.21) suggests that a part of the detected HMW-kininogen antigen may be derived from another source. This is supported by the fact that after calculation of HMW-kininogen concentration per weight unit of hemoglobin different values were obtained and not similar values as might be expected assuming that the blood remaining in the organs was the only source of the antigen detected. In a separate study only insignificant changes in HMW-kininogen concentration were observed when plas-

ma mixed with tissue homogenates was incubated at 4 °C for 24 h. This observation, and the results presented, indicate that the differences in organ distribution of HMW-kininogen does not depend directly on the amount of blood remaining, or on different rates of postmortem digestion of this protein. On the basis of this study it could be discussed whether HMW-kininogen may be synthesized in the kidneys, adrenals and thyroid or whether the results reported were due to an effect of accumulation of HMW-kininogen in these organs caused by the disease or processes preceding death, although the protein is produced elsewhere.

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H-Y Evolution

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Summary. The female: male sex ratio in litters born to C57 black mice immunized with male spleen preparations is 2:1 and males were stillborn. In addition *Drosophila busckii* males are H-Y antigen positive. It is argued that H-Y is the primary testicular determinant and that this role is evolutionarily ancient.

H-Y antibodies are produced by inbred female mice after immunization with male cells from the same strain[1]. H-Y antigen is considered to be the primary sexual determinant and the antigen is thought to be the product of the mammalian testis determining gene[2]. H-Y appears to be Y linked in mammals[3], but H-Y cross reaction has been observed in males lacking a Y-chromosome[4]. These findings suggest that progenies of immunized females should be primarily or entirely female. Attempts to alter the sex ratio of mice at birth by immunizing the mother against H-Y antigen were, however, unsuccessful when skin transplantation was the method of immunization[5]. The sex ratio in litters born to C57 black mice immunized with male spleen preparations is 2:1; moreover, all males were stillborn and showed signs of runting and developmental abnormalities (unpublished data). This result supports Wachtel's prediction of a primary sex determining role for H-Y in vertebrates[2].

Most evolutionary biologists see gene exchange as the essence of sex[6], and it has recently been suggested that cellular recognition signals evolved from sexual recognition signals similar to those seen in *E. coli*[7]. Assuming that sex evolved but once and that H-Y is the male determinant, leads to the prediction that cross reaction should be observed in an array of taxonomically diverse organisms. Murine H-Y antiserum gives positive cross reactions with male rat, guinea-pig, rabbit, human, leopard frog[8] and fish[9]. The antigen has also been located in *Xenopus laevis*, White Leghorn chicken[8] and trout females[10]. More recently Pechan (unpublished data) has obtained positive cross reaction with male cells from *Drosophila busckii*. This, in conjunction with the other observations, suggests that H-Y is evolutionarily very old. While Wachtel's[2] suggestion that H-Y stimulates gonadal differentiation of the heterogametic (XY males or ZW females) sex appears to hold in a wide variety of organisms, the suggestion that H-Y directs different developmental pathways in XY and ZW sex determining systems can be questioned. The male determining to female determining switch of H-Y would have to have occurred at least twice, once in the lineage leading to birds and once in that leading to Lepidopterans. Given the apparent evolutionary conservatism of H-Y, this seems unlikely. Agreeing that H-Y is the primary sex determinant, we would like to suggest that the functional H-Y gene exists only in the male of all species. In this scheme heterogametic female positive cross reaction with H-Y murine antiserum is due to spurious cross reaction with cell surface communication components which are closely related to H-Y in that they have a common evolutionary origin. Indeed steric interference between H-2 and H-Y has been reported[11].

In unicellular organisms where mating type is determined by a single gene, the gene product is, most likely, a membrane component which facilitates fertilization or gene exchange. 2 evolutionary schemes relating H-Y antigens to other cell surface communication components may be considered. First, H-Y is derived directly from one of these primitive sex determining alleles, and the spurious cross reacting components developed as cellular recognition signals related to H-Y through gene duplications. Second, the primitive sex determining alleles, *m* and *f*, produced the H-Y gene by duplication. Isogamety requires only single gene sex determination; thus, in arguing for cell surface communication components and H-Y molecular relatedness we start at the evolution of 2 sexes from the point at which isogametes were produced[12]. The advantages accruing to those individuals producing larger than average gametes (female) and to those producing smaller than average gametes (male) were maximized when a small gamete united with a big gamete. In this union the advantages of motility and nutritional resources were combined. Larger × larger and smaller × smaller fertilizations would be selected against by improbability of fertilization and inadequate nutrition for initial development. Heterogamety