Synthesis of Plasma Antihæmophilic Factor

In *Nature* of May 12, 1962, L. I. Woolf offered an explanation of 'severe' and 'mild' *X*-linked hæmophilia *A* while discussing gene expression in heterozygotes. He proposed that the plasma-level of antihæmophilic globulin (*AHG*, *AHF*, Factor VIII) regulated its rate of synthesis, that is, that 'end-product (feedback) inhibition' controlled the synthetic rate. 'Feedback inhibition' is known to be a very important regulatory mechanism in biosynthetic pathways, producing small anabolites such as amino-acids, vitamins, etc.[1]. We can find no documented evidence, however, that 'feedback inhibition' is a controlling mechanism in protein synthesis. 'Feedback inhibition' should be sharply distinguished in this respect from 'feedback repression', which is recognized to be a highly important genetic mechanism concerned with regulating the synthesis of enzymes (proteins) in bacteria[1-4].

Woolf apparently has not considered the implications of the existence of another disorder in which there is *AHF* deficiency. This condition, von Willebrand's disease (*v.W.D.*), clearly results from a dominant mutation occurring on an autosomal chromosome, and its main features have been confirmed by many investigators[5-10]. The bleeding time is prolonged in *v.W.D.* but is normal in hæmophilia *A*. Thus, another plasma component, tentatively designated 'vascular factor' (*V.F.*), apparently controls the bleeding time and is reduced in *v.W.D.* along with *AHF*.

One of us pointed out several years ago that the occurrence of these two disorders of *AHF* synthesis clearly distinguishable on genetic grounds, one of which is dominantly inherited, makes it difficult to understand the regulation of the *AHF*-level[11]. It would appear that an explanation of *AHF* regulation must take into account that: (*a*) genes on at least 2 chromosomes are concerned; (*b*) at least two plasma factors are involved; (*c*) 'feedback inhibition' is almost certainly not the controlling mechanism.

Recently, French workers[8] have clearly documented an important earlier observation by a Swedish group[10]. When fresh plasma from persons with hæmophilia *A* (low *AHF*, normal *V.F.*) is transfused into persons with *v.W.D.* (low *AHF*, low *V.F.*), a remarkable result is obtained. Within a few minutes the bleeding time becomes normal as might have been expected; but there is also a progressive and completely unexpected increase in circulating *AHF*. The rising *AHF*-level may reach a peak as much as 24 h later and only slowly returns to the low, pre-transfusion level. This 'paradoxical' effect is observed neither when the plasmas are mixed *in vitro* nor when *v.W.D.* plasma is transfused into persons with hæmophilia *A*. The observations suggest that the low *AHF*-levels of hæmophilia *A* and *v.W.D.* are achieved by quite different biochemical mechanisms.

One explanatory model which comes to mind is one which might be called the 'activator' model. In this model an activator or co-factor of *AHF* is produced from information carried on the autosomal chromosome, while the structural information for a pro-*AHF* is coded on the *X*-chromosome. In *v.W.D.* the amount of activator is reduced and only a small quantity of the structurally and qualitatively normal *AHF* precursor becomes activated. When hæmophilia *A* plasma, rich in activator, is infused into a patient with *v.W.D.* the activator reaches the site where pro-*AHF* is activated. This is followed by a rising plasma *AHF*-level, until the activator becomes exhausted. When *v.W.D.* plasma, presumably containing normal pro-*AHF* molecules, is transfused into persons with hæmophilia *A*, only the expected rise in plasma *AHF* is observed, probably because the pro-*AHF* cannot reach the site where activation occurs.

To explain the dominance of the mutation this model assumes either that the genes normally producing activator are working at maximum speed and that one normal allele does not produce enough of the competent activator, or that the mutant activator substance acts as an inhibitor. It also assumes that the activator is a molecule which can enter and leave cells with ease, while pro-*AHF* is a molecule which is expelled from cells but does not enter them readily. The simplest situation would be one in which the *V.F.*, which is responsible for the normal bleeding time, is also the activator of the *AHF* system, but this is not essential. *V.F.* might be a product of an associated reaction.

The model described here is not without precedent. It has recently been observed that the activity of xanthine dehydrogenase in *Drosophila melanogaster* is controlled by two loci on different chromosomes (I and III). Mutation at either locus results in decreased xanthine dehydrogenase activity; however, *in vitro* mixture of extracts from the mutant flies results in return of enzymatic activity[12].

Another model can be imagined which operates in accordance with the system proposed by Monod and colleagues for regulating protein synthesis in bacteria[13]. In this 'inducer' model a dominant regulator gene mutation of the i^s type would explain *v.W.D.* The altered repressor substance produced by this autosomal gene would not be inactivated by its inducer, and its operon would always be repressed. Since this operon, which would ordinarily synthesize the inducer for the *X*-chromosomal *AHF* operon, is repressed, the plasma-level of *AHF* is reduced. This model also would be most elegant if the *V.F.* were also the inducer; but this is not essential. Here again the inducer would be required to be a small molecule because known inducers are small, and it is required to pass from blood of hæmophilia *A* into nuclei of persons with *v.W.D.* if *de novo* *AHF* synthesis is to occur.

The chemical nature of *V.F.* is unknown. It is interesting to note, however, that both models require a highly mobile molecule which probably implies small size. Coincidentally, several small molecular species are known to have striking physiological effects on blood vessel walls (serotonin, pitressin, adrenaline, acetylcholine). It is also of interest that *AHF*-levels in normal persons increase temporarily following adrenalin injections[14] or heavy exercise[15]. The presence of the inducer in Fraction I-0[10] may be explained by the occurrence of a protein carrier or by its particular solubility characteristics.

Sex-linked hæmophilia could be due to a structural mutation under either model. Severe sex-linked hæmophilia might result either from an operator mutation (0^c type) or a major change in the structural gene in the 'inducer' model. 'Mild' hæmophilia, on the other hand, might be a 'mis-sense' structural alteration with mild but not complete loss of the activity of the molecule.

It is difficult to imagine tests which could be carried out and lead to a decisive choice between these models. It is also possible that neither model is entirely correct; but we believe their heuristic value justifies their description.

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