

There are several possible explanations for this discrepancy, such as the fact that energy distribution may not be uniform, and that many breaks may rejoin or yield viable aberrations. However, if chromosome breakage or resultant deletions are the major cause of growth inhibition or death, a very small fraction of total energy absorbed per nucleus would actually be involved in the production of the lethal lesion(s). The correlation described indicates that death depends upon a critical amount of energy being absorbed on the average by each chromosome. However, the fact that the structural organization and thus the actual nuclear volume occupied by the elements of an interphase chromosome are not well known raises important theoretical questions about just where and how critical amounts of energy are absorbed by the nuclear material. Since the amount of DNA per chromosome is not constant in different species but the energy absorbed per chromosome is almost constant, the ultimate biological damage is not due to the production of a constant proportion of damaged DNA molecules but, presumably, to the production of a relatively constant number of some kind or kinds of deleterious event(s). We cannot, at this time, conclude whether these events are gross chromosomal damage (breakage and aberration), more subtle molecular disturbances, or a combination of both.

It has been pointed out that an adequate knowledge of the role of nuclear variables in determining radiosensitivity should allow one to make predictions of expected radiation responses of species or biological material for which no radiobiological data were available (2). Such predictions have been made (8) and have been in part quite successful. The graphs presented in Figs. 1 and 2 can be used to estimate the expected lethal dose for any plant species for which interphase chromosome volume (interphase nuclear volume divided by somatic chromosome number) is known. The ability to make such predictions should be of considerable theoretical and practical value in radiobiology, radioecology and, if the method can be successfully extended to animal cells, possibly also in radiotherapy (9).

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Amino Acid Composition of Hemerythrin in Relation to Subunit Structure

Abstract. *Determination of the amino acid composition of coelomic hemerythrin from Golfinia gouldii shows 3 arginine residues and 10 to 11 lysine residues per protein subunit of 13,500 molecular weight. On this basis, the 28 to 30 major peptide spots revealed by electrophoresis and chromatography of tryptic hydrolysates would indicate two kinds of subunit. However, similar evidence from chymotryptic hydrolysates is not unequivocal, since the number of peptide spots is also compatible with an assumption of only one kind of chain. In addition to indicating the possible existence of two types of subunit, the peptide maps of enzymic digests of hemerythrin from individual animals shows at least one and perhaps more differences in peptide composition.*

Hemerythrin, the oxygen-carrying pigment of sipunculid worms, is constituted of eight subunits held together by noncovalent bonds (1, 2). The native protein of 107,000 molecular weight can be dissociated into its subunits, merohemerythrins (3), by a number of chemical or environmental modifications including succinylation, treatment with sulfhydryl-blocking reagents, addition of urea and increase or decrease

in pH. Thus, because it is made up of separable polypeptide chains, hemerythrin shows macromolecular features analogous to those of hemoglobin.

In comparisons of hemerythrin to hemoglobin, the question immediately arises whether all eight merohemerythrins are basically identical with some occasional changes in constitution resulting from genetic modification (4) or whether there are more far-reaching differences between the chains.

A tentative answer to this question of the basic identity or nonidentity of the chains is available from assays of amino acid content of the protein combined with information on the number of peptides occurring in tryptic and chymotryptic digests.

Hemerythrin was isolated from *Golfinia gouldii* (also known as *Phascolosoma gouldii*) and crystallized (5). The protein was obtained from the pooled blood of 100 worms. Total amino acids in native protein as well as in hemerythrin from which iron had been removed by treatment with acidified acetone were determined by standard hydrolytic and analytical methods and with the use of a Beckman-Spinco amino acid analyzer (model 120 B). The results are summarized in Table 1.

Samples of pooled hemerythrin and of crystallized protein from individual worms were hydrolyzed by trypsin. Hemerythrin, in 0.02M ammonium carbonate of pH 8.3, was denatured by heating. Acid-treated trypsin (6) was added in small increments over a period of about 4 hours until its total amount was 5 percent with respect to the weight of hemerythrin. The precipitated proteins generally went into solution at the end of 1 hour. Larger proportions of enzyme were also used without significant changes in the results. Controls with trypsin alone showed no peptides. All hydrolysates were lyophilized and then dissolved in a small amount of water. An aliquot of this solution was subjected to electrophoresis on Whatman 3MM paper in pyridine-acetate buffer of pH 6.4. This was followed by chromatography with a mixture of butanol, acetic acid, and water (200:35:75 by volume) (7). The separated peptides were revealed with a ninhydrin reagent containing collidine (7) and with specific reagents for histidine, tyrosine, and tryptophan.

The amino acid analyses show three arginyl residues and 10.5 lysyl residues in each merohemerythrin unit of 13,500 molecular weight. If these units are all

Table 1. Amino acid composition of hemerythrin.

Amino acid	Residues per 13,500 g of protein
Lysine	10.5
Histidine	6.5
Arginine	3.03
Aspartic acid	16.2
Threonine	4.35
Serine	3.38
Glutamic acid	9.5
Proline	4.03
Glycine	6.2
Alamine	5.3
½ Cystine	0.96
Valine	3.72
Methionine	0.85
Isoleucine	8.34
Leucine	7.36
Tyrosine	4.8
Phenylalanine	8.5
Ammonia	12.2

alike one would expect 14 to 15 peptides in a peptide map of a tryptic hydrolysate, whereas the number actually found is 28 to 30. The same number of tryptic peptides has been reported by Manwell (4).

The most obvious interpretation of these results is that the subunits of hemerythrin are of two distinct types with major differences in the respective sequences of amino acids. On the other hand, peptide maps of chymotryptic hydrolysates of either pooled hemerythrin or protein from individual worms do not show substantially more peptide spots than can be accounted for by the sum of the number of amino acid resi-

dues providing peptide bonds known to be susceptible to cleavage by this enzyme. All attempts to separate the presumed different subunits have failed. These attempts included (i) chromatography on DEAE-Sephadex, CM-Sephadex, and Amberlite IRC-50 under a variety of conditions, with and without urea, and (ii) electrophoresis in starch gel under different conditions and with a variety of dissociating reagents. Some of these efforts have produced resolvable peaks but these peaks have shown peptide maps identical with those from the original protein. An alternative interpretation must therefore be borne in mind, namely, that the subunits are basically identical (4) but that the linkages susceptible to attack by trypsin are so grouped that more peptides are produced than would be expected from the number of susceptible bonds, because of occasional partial hydrolysis.

A further complication in the structure of hemerythrin arises from observations of differences in peptide maps of enzymic digests of the protein of individual animals. One such difference has been reported by Manwell (4). We have found in the chymotryptic digests, differences indicative of substitutions of amino acids in at least one, and possibly more, positions in the chain. Figure 1 shows the peptide map of the chymotryptic digest of pooled hemerythrin. When similar chymotryptic digests of the hemerythrin of individual worms are examined, a class of worms may be distinguished, in which the peptides marked 13B (basic, tryptophan-containing) and 3A (acidic, tyrosine-containing) are present, and another in which they are absent. These two peptides are invariably either present or absent together. This suggests the substitution of a residue by either tyrosine or tryptophan, thus affording a new point of chymotryptic attack, or the deletion of a portion of the peptide chain.

The variation in peptides 13B and 3A is the most obvious because they reveal amino acids which are easily detected by specific color reagents. It is probably not the only one that exists in hemerythrins from the species *Golfingia gouldii*. Manwell (4) has found variations in electrophoretic behavior in the protein from individual worms of the same species which he suggests may be due to a single substitution of an acidic residue for an uncharged one.

Thus while the results of tryptic hydrolyses indicate the presence of two kinds of subunits, this conclusion is not unequivocal since the observations from chymotryptic hydrolyses could also be rationalized in terms of a single chain. In either event it is clear that variations in peptide constitution of the subunits do occur from one individual to another (8).

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Adaptation to Chromatic Aberration by the Human Visual System

Abstract. Prolonged exposure to the chromatic dispersion produced by prism spectacles leads to a perceptual adaptation. The adaptation develops rapidly in the first two days of the wearing of the spectacles, and seems to be a special reaction to the relative change in illuminance at intensity gradients on the retina.

Perceptual adaptation to changes of the eyes' normal optical linkage to the environment may be experienced by anyone who, wearing a new pair of glasses, is at first disturbed by the distortional side-effects. Such adaptation suggests the existence of regulating mechanisms underlying normal vision, and provides a novel perspective on the dependence of perception on past experience. Among the most interesting of these adaptation phenomena are those reported by Gibson (1) and Kohler (2) for the image aberrations

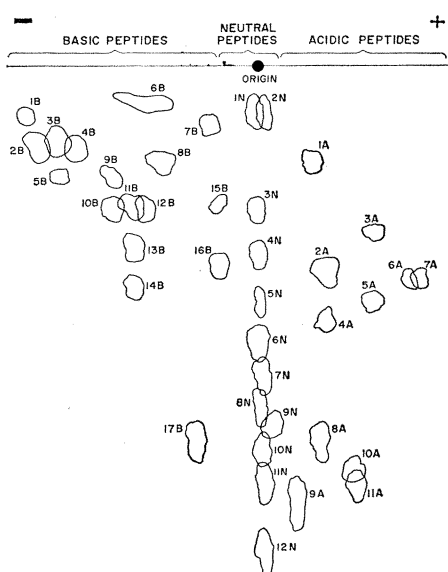


Fig. 1. Peptide map of a chymotryptic hydrolysate of hemerythrin.