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U.S. ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE

BIOCHEMICAL ASPECTS OF ERYTHROPOIESIS IN
CHRONIC IRRADIATION

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For the contract period beginning November 15, 1969 the study of the Biochemical Aspects of Erythropoiesis in Chronic Irradiation involved: (a) isolation of globins from 6 groups of animals; (b) characterization of globins by (1) polyacrylamide gel electrophoresis, and (2) peptide mapping; (c) separation and isolation of globin chains from the 6 groups of animals; (d) characterization of isolated globin chains: - (1) test of purity by absorption spectrophotometry; (2) polyacrylamide gel electrophoresis; (3) N-terminal amino acids; (4) amino acid composition; and, (5) molecular weight determinations.

1. ISOLATION OF GLOBINS: 6 groups of animals

Globin was prepared from adult female rats:

- (1.) non-irradiated, non-splenectomized animals (control rats)
- (2.) non-irradiated, stabilized splenectomized rats (100 days post splenectomy)
- (3.) continuously irradiated rats (70 rads/day) for 90 days
 - a. non-splenectomized animals
 - (1.) ambient (normal) oxygen environment
 - (2.) hypoxic (10% oxygen plus 90% nitrogen)
 - b. splenectomized animals
 - (1.) ambient (normal) oxygen environment
 - (2.) hypoxic (10% oxygen plus 90% nitrogen)

Heme cleavage was accomplished by the acid-acetone procedure [Fanelli et al., Biochem. Biophys. Acta 30:608 (1958); Schroeder et al., Biochem. 2:992 (1968)]. Some evidence of incomplete heme-globin cleavage was apparent when blood was obtained from all continuously irradiated animals. This observation was in contrast to the experience with non-irradiated rats, i.e., clean preparations were produced. Differences in strength of chromophore binding is suggested since the technical procedures were meticulously and reproducibly carried out. The dry material was stored at -20°C for further studies.

2. CHARACTERIZATION OF GLOBINS

a. Polyacrylamide gel electrophoresis (cationic migration).

All of the globins were insoluble above pH 7.0. They were soluble on the acid side, i.e., pH 4.0. Thus, it was possible to observe cationic migration. Six distinct components were obtained from globins of non-irradiated, non-splenectomized; non-irradiated splenectomized, and irradiated splenectomized rats (normal oxygen and hypoxic). Five components were seen in the globins of irradiated non-splenectomized (normal oxygen and hypoxic) animals. This confirms the differences seen in the anionic migration of hemoglobin from the same groups of animals previously reported (see COO-2005-5).

b. Finger printing (peptide mapping). Preliminary exploration of peptide mapping of globins using tryptic digests with descending paper chromatography first in one direction followed by high voltage electrophoresis in the second direction was carried out. The finger prints from the various groups of globins manifested differences. These, however, require further study. It was felt that finger printing of isolated globin chains would be more conclusive. This aspect is currently in progress.

3. SEPARATION AND ISOLATION OF GLOBIN CHAINS

Separation of rat globin into its component chains has been accomplished by column chromatography using a cation exchange resin (IRC-50) at conditions of low pH and ionic strength. An interrupted urea gradient (3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 M) was used. Satisfactory nadirs were obtained and appropriate fractions could be selected and pooled. The pooled fractions were dialyzed overnight against running distilled water in the cold room at 3°C. The dialyzed samples were lyophilized. The residues were dissolved in a small volume of distilled water and redialyzed to remove, traces of urea. The redialyzed samples were lyophilized. This procedure proved uniformly satisfactory since no precipitation occurred during dialysis steps and all of the dried products

were water soluble. The dried material was stored at -20°C until further use.

Five well defined elution peaks were repeatedly obtained. They were designated by greek letters according to the literature. This, however, does not imply that they are identical with those obtained from human sources. Further studies will establish their identity.

The peaks were eluted from the column at the following urea concentrations - alpha - 4.0 M; beta - 4.8 M; gamma - 6.0 M; delta - 7.6 M and epsilon - 9.2 M.

4. CHARACTERIZATION OF GLOBIN CHAINS

a. Absorption spectra. Absorption spectra using a Carey recording spectrophotometer were run on each of the separated chains to ascertain the presence or absence of chromophore groups. The alpha, beta and gamma chains from non-irradiated animals showed no absorption peaks suggestive of a chromophore group. The delta and epsilon chains showed traces of a chromophore group at 395 millimicrons. Similarly, the alpha, beta chains of the hypoxic irradiated animals showed no significant absorption at 395 millimicrons. The gamma chain exhibited a trace and the delta and epsilon presented a definite peak at 395 millimicrons. By contrast, the gamma, delta and epsilon chains revealed significant amounts of

chromophore. These findings indicate that in certain instances the chromophore is more tightly bound and is not dissociated during the experimental procedure used. The nature of the binding is being investigated.

b. Polyacrylamide gel electrophoresis. Cationic migration of the five globin chains revealed heterogeneity and differences in the patterns between hypoxic continuously irradiated and the non-irradiated control were noted. Multiple components were observed. These data are being critically examined.

Urea gels were prepared according to Sakol et al., (Arch. Biochem. Biophys. 130:337 (1969)). The patterns of the globin chains were examined and multiplicity of banding was observed. A comparison of three series [non-irradiated (N); continuously irradiated hypoxic (RIH) and continuously irradiated splenectomized (RSP)] is shown in the photographs in Figure 1.

c. N-terminal amino acids. The Edman degradation has presented some technical problems when applied to the globin chains of the rat. These are now being satisfactorily resolved.

All of the chains from the various groups of animals show valine to be the N-terminus. It is anticipated that in the near future data will be available for the sequence of the first four amino acids.

Dansylation is to be used for confirmation of the end group.

Figure 1. Disc gel (15% acrylamide, 2.5 M urea, 0.9 N acetic acid, pH 2.7, stained with amido schwarz 10 B) electrophoresis patterns of five globin chains from three groups of animals :- non-splenectomized, non-irradiated (N); non-splenectomized, irradiated, hypoxic (RIH); and splenectomized, irradiated (RSP). The chains are designated as :- α , β , γ , Δ , and ϵ (at top of the photographs). Each chain within a group reveals a characteristic heterogeneous pattern. Note the difference in the electrophoretic patterns in the different groups.

d. Amino acid composition. The amino acid composition of the chains from the groups is being ascertained using an amino acid analyzer. The data to date are somewhat incomplete. The evidence indicates that there is a significant decrease in the number of aspartic acid residues in the alpha chain of the continuously irradiated hypoxic animals compared to controls.

Two sets of globin chains, i.e.; continuously radiated hypoxic and non-irradiated controls. The analysis for the globin chains from irradiated animals is complete, whereas, the delta and epsilon chains of the controls is in progress.

e. Molecular weights. The molecular weights of the globin chains are in the process of being run with a spinco analytical centrifuge.

The investigation of the biosynthetic pathway for the modified hemoglobin to ascertain the derivatives from the sequences involved in the normal entity was not undertaken during the past period. The effort involved in the accumulation of basic data from control and experimental animals militated against such an exploration.

The principal investigator devoted 50% of his efforts during the academic year (November to June) and 100% for July and August to the project. One junior undergraduate student devoted 12 hours per week to the project during both semesters

of the academic year. The junior student has spent 10 weeks full time during the summer months. This student will write an undergraduate thesis during the coming academic year.

The principal investigator will devote 50% of his effort for the remainder of the current term (September, October and November). It is anticipated that cogent progress will be made during the remainder of the current term. Effort will be directed toward the completion of manuscripts in preparation for publication.

Effort is to be directed to: (1) peptide mapping of isolated globin chains from the different groups of animals; (2) continued work on separation and isolation of globin chains (a) improve resolution of the ion exchange column chromatography by modifying the urea gradient; (b) accumulate material for further studies, i.e. amino acid analysis and peptide isolation; (c) complete the molecular weight determinations of isolated globin chains.