A DEAE- Cellulose Chromatographic Separation of Co^{57} Vitamin B_{12} Binders in Normal Human Serum.* (32511)

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At least two vitamin B_{12} binders are present in normal human serum, one with electrophoretic mobility of an alpha-globulin and the other of a beta-globulin(1,2,3). In this communication we describe a DEAE- cellulose chromatographic method for the separation of Co^{57} B_{12} radioactive peaks from normal serum to which Co^{57} B_{12} had been added *in vitro*. Protein fractions enriched in beta-globulins and alpha-globulins without contamination from each other were obtained by this method.

Materials and methods. The method described by Adams, Yoder and Free(4), modified to give maximum separation of beta-globulins and alpha-globulins, was used. The DEAE- cellulose (Schleicher and Schull, Keene, N. H., No. 70, standard) was prepared as described by Peterson and Sober(5) and packed into a 15 × 4 cm column. Either 300 or 2,000 $\mu\mu$g of Co^{57} B_{12} per ml were added to thawed frozen serum and the latter dialyzed against 0.005 M sodium phosphate buffer, pH 8.0, for 24 hours at 4°C. After completion of dialysis, the serum was chromatographed on a DEAE- cellulose column which had been equilibrated with 0.005 M buffer and eluted stepwise at 4°C with 300 ml of 0.005 M pH 8.0, 0.0175 M pH 6.3, 0.04 M pH 5.9, 0.1 M pH 5.8 and 0.4 M pH 5.2 sodium phosphate buffers in the presence of 0.09% methylparaben and 0.01% propylparaben (Tenneco Chemical, N.Y.). Fractions of 20 to 30 ml were collected at a flow rate of 60 ml/hr and 4 ml samples were counted in a Nuclear Chicago well-type scintillation counter. Concentrated dialyzed samples from radioactive peaks were subjected to electrophoresis on cellulose aceptate membranes in 0.075 M veronal buffer, pH 8.6.

Results. Two radioactive peaks appeared (Fig. 1) when 300 $\mu\mu$g/ml Co^{57} B_{12} had been added to normal serum corresponding to the findings of Hall and Finkler(1). With the addition of 2,000 $\mu\mu$g/ml of Co^{57} B_{12} to the serum, five radioactive peaks appeared (Fig. 1). Peak 1 was composed of gamma-globulins, beta-globulins and albumin (Fig. 2); peak 2 of beta-globulins; peaks 3 and 4 of albumin and trace of other proteins; peak 5 of alpha-globulins and albumin (Fig. 2). Thus peaks

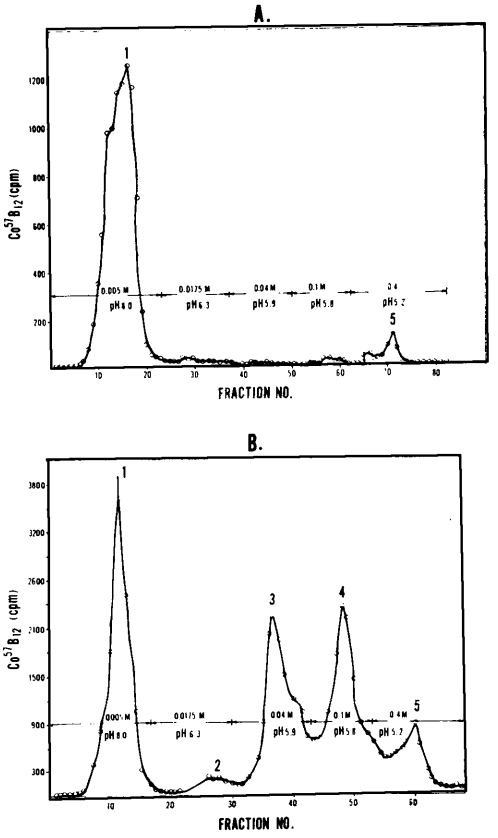

FIG. 1. Stepwise separation of radioactive peaks in human serum. Twenty ml of serum applied to a DEAE- cellulose column (15×4 cm). Adsorbent and serum equilibrated with 0.005 M sodium phosphate buffer, pH 8.0. Fractions of 20 ml collected in (A) and 22 ml in (B). Four ml samples of fractions were counted in a well-type scintillation counter. Temperature, 4°C. (A) Three hundred $\mu\mu$g of Co^{57} B_{12} per ml added to serum. (B) Two thousand $\mu\mu$g/ml Co^{57} B_{12} added to serum.

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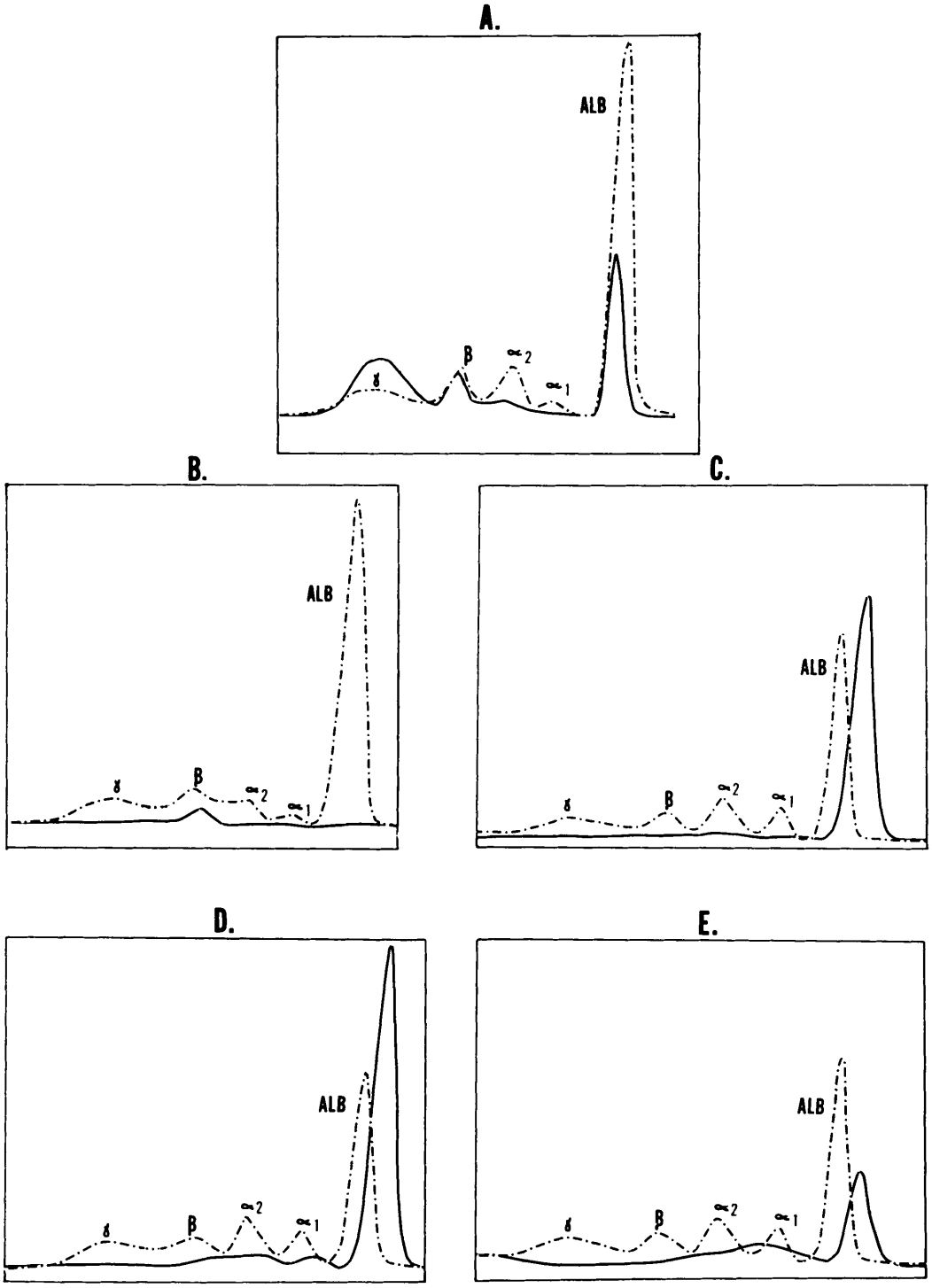

FIG. 2. Electrophoretic patterns of the radioactive peaks on cellulose acetate membrane, in 0.075 M veronal buffer, pH 8.6. The dotted line represents the pattern from normal serum. (A) peak 1; (B) peak 2; (C) peak 3; (D) peak 4; (E) peak 5.

2, 3, 4 were observed only when large amounts of radioactive Co^{57} B_{12} were added. Since the peaks consist of mixtures of proteins, identification of the specific proteins binding Co^{57} B_{12} cannot be delineated.

Summary. A method was described for the separation of vit B_{12} binders in normal serum on a 15 × 4 cm DEAE- cellulose column by stepwise elution with 0.005 M pH 8.0, 0.0175 M pH 6.3, 0.04 M pH 5.9, 0.1 M pH 5.8 and 0.4 M pH 5.2 sodium phosphate buffers. Two radioactive peaks appeared when 300 $\mu\mu$g Co^{57} B_{12} per ml of serum had been added *in vitro*. With the addition of 2,000 $\mu\mu$g/ml of Co^{57} B_{12} to the serum, 5 radioactive peaks appeared.

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Serotonin Antagonist Increases Longevity in Mice With Hereditary Muscular Dystrophy.* (32512)

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The occurrence of hereditary muscular dystrophy in an inbred strain of mice was first reported by Michelson *et al*(1), and intensive research on these animals since that time has indicated that, in addition to histologic changes, a number of biochemical, histochemical, and physiologic differences exist in their skeletal muscle. Several recent investigations have implicated a high content of the biogenic amine, serotonin (5-hydroxytryptamine), with this myopathy and with other skeletal muscle defects. Gordon and Dowben(2), while studying the distribution of adrenaline and noradrenaline in mice with hereditary muscular dystrophy, found approximately twice the concentration of serotonin in the spleen of afflicted mice as in normal mice, and its presence was thought possibly to represent a physiologic response to enhanced sympathetic activity or to a general disorder in amine metabolism. When normal mice were given daily injections of serotonin for over 3 weeks, focal lesions, characterized by hyaline sarcoplasmic degeneration and myofibrillar destruction, developed throughout the skeletal musculature(3). Studies of other endocrine or humoral factors in dystrophic mice are rare, but Alger and Boccabella(4) found that sexual maturity was delayed in both sexes.

The present study was undertaken to establish if treatment with a specific serotonin antagonist would modify in a short period of time such characteristics as body weight and weights of several endocrine organs and the kidneys, and if treatment with such an agent over extended periods of time would influence survival of afflicted mice and the progressive development of the myopathy.

Materials and methods. Strain 129/Re male and female mice were obtained from Jackson Memorial Laboratories, and animals with normal *(DyDy* or *Dydy)* and dystrophic *(dydy)* genotypes were divided into control and treated groups. All animals, which were 4 to 6 weeks old on arrival from the supplier, were maintained until the 7th week, when treatment was begun, to standardize the beginning of the experimental period. Groups of male and female mice of normal and dystrophic phenotypes were injected intraperitoneally daily with either 2.0 or 5.0 mg/kg body weight of the serotonin antagonist, 1-methyl-

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