and in subcutaneously implanted polyvinyl sponge has been shown to vary with age and diet. A tryptophan-deficient diet significantly reduced the rate of skin collagen synthesis in young animals. However, the greater loss of more labile components of the skin resulted in a relative greater percentage of skin collagen in this group. Rate of synthesis in the sponge was not significantly affected. The deficient animals showed an increased specific activity in liver and serum proteins, suggesting that protein synthesis probably occurs at a faster rate in the liver simultaneously with increased catabolism or utilization.

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Identification of 24-Dehydrocholesterol in Human Blood Vessels Following MER-29 Therapy.* (26839)

DAVID H. BLANKENHORN
Department of Medicine, University of Southern California School of Medicine, Los Angeles

Avigan *et al.*(1) have demonstrated that inhibition of sterol biosynthesis by MER-29 (Triparanol) results in accumulation of 24 dehydrocholesterol in rat livers. 24-dehydrocholesterol has been identified by Steinberg *et al.*(2) in the blood of humans given MER-29. Little is known of the biologic activity of 24-dehydrocholesterol in man except that it does not appear spontaneously in human blood(3). The use of MER-29 for treatment of hypercholesterolemia in man makes it important to learn if 24 dehydrocholesterol accumulates in blood vessel walls as well as in blood.

Materials and methods. Vessels were obtained at autopsy from a 16-year-old white male whose diagnoses were: familial hypercholesterolemia, congenital aortic stenosis and recent myocardial infarction. The patient had received 250 mg per day of MER-29 for 79 days. Extensive atheromatosis covered 90% of the area of the aortic wall. Specimens of normal vessel wall were taken from between atheromas and stripped of adventitia. Atheromas were graded(4), freed from surrounding tissue, and stripped of adventitia.

Serum was saponified and extracted with petroleum ether as described by Abell *et al.* (5). Tissue specimens were saponified in 20% ethanolic KOH and extracted with one volume of water and 2 volumes of petroleum ether. Petroleum ether residues were taken up in chloroform for gas liquid chromatography (GLC).

GLC was performed in ⅛ inch glass columns, packed with Gas Chrom P† coated with neopentyl adipate terminated (NAT), neopentyl glycol sebacate (NGS) as described by Lipsky(6), or QF-1 (10,000 C.S.) as described by VandenHeuvel *et al.*(7). An argon ionization detector containing radium was used and was calibrated with a known mixture of cholesterol and 24-dehydrocholes-

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† Applied Science Laboratories, Inc., State College, Pa.

TABLE I. Appearance Time of Peak Concentration, Liver, and Normal Aortic Wall Sterols.

Charge		Appearance time (min.) NAT*	QF-1†	NGS‡
Liver sterols (2 peaks)	1.	13	70	16.3
	2.	17	88	20.0
Aortic sterols (2 peaks)	1.	13	70	16.3
	2.	17	88	20.0
Cholesterol		13	70	16.3
24-dehydrocholesterol		17	88	20.0

* 4 ft ⅛ inch column, 3% NAT, 231°C, 300 ml/min. flow.
† 6 ft ⅛ inch column, 1% QF-1, 217°C, 300 ml/min.
‡ 3 ft ⅛ inch column, 1.5% NGS, 231°C, 400 ml/min.

terol.‡ Relative peak areas of cholesterol and 24-dehydrocholesterol in unknown mixtures were measured, corrected in proportion to peak areas of the standard mixture, and 24-dehydrocholesterol was expressed as a percent of cholesterol plus 24-dehydrocholesterol.

Serum sterols were also assayed by the method of Abell *et al.*(5), modified as follows: Color developed was read after 30 min at 620 mμ; and 90 min at 420 mμ. Apparent desmosterol and cholesterol were calculated from the data of Avigan *et al.*(8) and Frantz *et al.*(9).

Results. During a 3-month period prior to MER-29 therapy total serum sterols estimated colorimetrically ranged from 390 to 525 mg% (6 determinations) and there was no evidence of 24-dehydrocholesterol. During 79 days of MER-29 therapy total serum sterols estimated colorimetrically ranged from 407 to 512 mg % (6 determinations). During the last 40 days of MER-29 therapy, 24-dehydrocholesterol could be demonstrated in serum. By colorimetric determination, 24 dehydrocholesterol was estimated to be 19-24% of total serum sterols; separation by GLC indicated that 24-dehydrocholesterol comprised 25 to 32% of total serum sterols.

The day preceding death, total serum sterols were 407 mg% and 24 dehydrocholesterol was 32% of total (determined by GLC). Sterols from liver and normal blood vessel wall removed at autopsy were separated by GLC and 2 peaks were found. These peaks had appearance times identical with that of cholesterol and 24-dehydrocholesterol when chromatographed on columns of NAT, NGS, and QF-1 (Table I).

In 3 liver specimens, 24-dehydrocholesterol was 23-29% of total sterol. In normal aorta, from 3 locations, 24-dehydrocholesterol was 13-16% of total sterol. In normal iliac arterial wall, 24-dehydrocholesterol was 8.8% of total sterol. Ten atheromas (4 grade II, 6 grade III) demonstrated no evidence of 24 dehydrocholesterol.

Summary and conclusions. This report indicates that 24-dehydrocholesterol accumulated in human blood vessel walls and it seems probable that treatment with MER-29 was responsible. Although 24-dehydrocholesterol was not demonstrable in the extensive atheromatosis which covered 90% of the aorta, it accounted for 13-16% of the sterols in normal aortic wall. The magnitude of accumulation of 24-dehydrocholesterol in normal aortic wall would not have been apparent if assays had been performed on sterols extracted from the aorta as a whole because the sterols of normal wall would have been greatly diluted by atheromatosis lipid containing no 24-dehydrocholesterol. Because these vessels were examined only 79 days after onset of therapy with MER-29, the presence of 24-dehydrocholesterol in normal wall but not in atheromas seems best explained by a more rapid accumulation in normal wall. However, it is possible that 24-dehydrocholesterol may only accumulate in normal vessel walls.

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Assay of Serum Vitamin B_{12} Concentration Using Co^{57}-B_{12} and Intrinsic Factor. (26840)

SHELDON P. ROTHENBERG* (Introduced by S. A. Berson)

Hematology Service, Departments of Medicine and Laboratories, Long Island Jewish Hospital, New Hyde Park, N. Y., and Radioisotope Service, Bronx Veterans Hospital, N. Y.

Athough many proteins are capable of binding Vit. B_{12}, the greatest avidity for the vitamin is exhibited by intrinsic factor (I.F.). Barlow and Frederick(1) reported that purified intrinsic factor concentrates reveal several distinct protein components on paper electrophoresis but the most intense binding of Co^{60}-labeled Vit. B_{12} was observed in the region with the least amount of stainable protein material.

Bunge and coworkers(2) have clearly shown that Co^{60}-B_{12} (B_{12}*) and unlabeled Vit. B_{12} are biologically identical and compete for the binding sites of normal human gastric juice.

In agreement with observations of other investigators(1) initial studies in this laboratory using paper electrophoresis demonstrated that in mixtures of I.F. and B_{12}*, where there was insufficient I.F. to bind all the B_{12}*, 2 peaks of radioactivity could be demonstrated; the free B_{12}* remaining close to the origin and the B_{12}*-I.F. complex moving toward the anode. If concentrations of I.F. and B_{12}* were kept constant while concentration of unlabeled Vit. B_{12} was varied, the ratio of bound B_{12}* to free B_{12}* (B/F) decreased progressively with increase in concentration of unlabeled B_{12} in consequence of the competitive inhibition of binding of the labeled vitamin.

These results are analogous to the observations of Berson, Yalow *et al.*(3) on binding of insulin-I^{131} to insulin binding antibodies, in studies employing paper electrophoresis and chromatography. The observed inverse relationship between the ratio of bound to free I^{131}-insulin (B/F) and total concentration of insulin present was exploited in development of an immunoassay for insulin in plasma(4).

In the present communication the application of these principles to assay of Vit. B_{12} in plasma is described.

Methods. Since the specific activity of commercially available labeled B_{12} is at present too low to yield adequate counting rates in small amounts of serum extracts containing tracer quantities of B_{12}*, electrophoretic separation of free and bound B_{12}* proved inexpedient. Therefore, attempts were made to separate bound and free Vit. B_{12}* by precipitation of I.F.-B_{12}* complexes from larger quantities of serum extract than could be conveniently analyzed by paper electrophoresis. Several protein precipitants (TCA, phosphotungstic acid, etc.) produced co-precipitation of free Vit. B_{12} and could not be used for the present purpose. The Somogyi, barium hydroxide-zinc sulfate method(5) proved satisfactory.

Because of its higher available specific activity, Co^{57}-B_{12} (5 $\mu c/\mu g$)† was used instead of the Co^{60}-labeled vitamin.

Preparation of plasma extracts. Most or all of the Vit. B_{12} in serum is present as a heat-labile Vit. B_{12}-protein complex. When serum is acidified and heated the proteins are precipitated and Vit. B_{12} is liberated. Ac-

* Dazian Foundation Fellow in Hematology, Long Island Jewish Hospital, New Hyde Park, N. Y. Present address: Department of Medicine, New York Medical College, Fifth Ave. at 106th St., New York, N. Y.

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