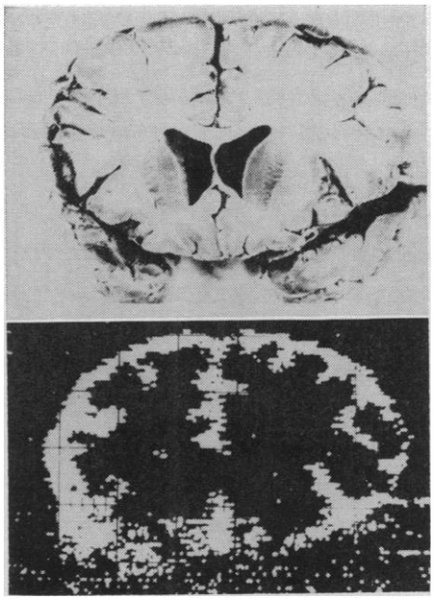

Fig. 2. Coronal section of brain (top) and an ultrasonic scan thereof by the new scanning system.

tate the use of a reflecting system to focus the ultrasonic energy.

A multireflector transducer-array system has been developed, of which Fig. 1 is a diagrammatic cross-section. The basic reflecting surface is a section of a prolate spheroid yielding an elliptic section in two dimensions. Energy from the first focal point of the spheroid is reflected to the second focal point. The spheroidal reflector is illuminated by a paraboloidal reflector whose focal point coincides with the focal point of the spheroid. An unfocused transducer that generates a plane wave front is directed along the major axis of the spheroid. The paraboloid converts the plane wave front into a spherical wave front diverging from the focal point of the spheroid. The spheroidal section reflects this energy to the target focal point which can be at a depth in tissue approximately equal to the separation between the foci of the spheroid. By reciprocity, echoes are returned along similar propagation paths. This focusing system accomplishes the objects of focusing a plane wave to a point, maintaining a constant length of propagation path to the target and return, and converging the energy to the target point through a large solid angle.

Initial results with such a system in prototype indicate a considerable improvement in image resolution. A spheroidal reflector 25 cm in diameter and with a spacing of 20 cm between foci has been excited with pulsed ultrasound at a frequency of 2.25 Mcy/sec. The ultrasonic receiver has been electronically time-gated to pass only echoes returning within 0.5 μsec of those returning from the target focal point; this provides a target volume 0.75 mm in diameter. The transducer is transported in two dimensions, and position information is taken from linear-motion potentiometers to the oscilloscope used for image display. The presence of an echo from the target volume is used to intensify the oscilloscope beam, and the image is generated by the storage-type oscilloscope. With this system resolution within 1 mm has been obtained in each of three dimensions; a relatively thin sectional image can thus be developed with improved image resolution.

A two-dimensional image obtained with an early prototype of the new scanning system appears in Fig. 2; resolution is clearly improved in this scan of a coronal section of brain, and gyri and sulci are plainly shown. Resolution in this image was limited to some degree by the resolution capability of the storage oscilloscope used to display it. Time spent in producing this image was 8 to 10 minutes, which was determined mainly by the mechanical transport system used rather than by limitations in the actual scanning.

Figure 2 also illustrates a problem presented by this scanning technique. In this scan, echoes returning from considerable depth in tissue were much lower in amplitude than those returning from near the surface; thus the lateral ventricles are not clearly outlined. Yet the wide angle of incidence from the transducer array permitted presentation of images from both sides of the brain fairly readily. Since the echoes used to generate the image display return to the transducer all at the same time following a transmitted pulse, the usual technique for controlling the gain of the ultrasonic receiver as a function of time, in order to provide increased gain at increased depth in tissue, cannot be used with this system.

Underway is an attempt to develop a method of controlling the echo amplitude so that the images developed will have more uniform illumination. Techniques under study include automatic control of receiver gain and control of the amplitude of the transmitted pulse; the control information is being taken from the echo amplitudes that return from the region of the target focal point.

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21 April 1965

Double Beta-Lipoprotein: A New Genetic Variant in Man

Abstract. *A β-lipoprotein variant, in which two bands appear after electrophoresis, has been found in three generations of a family. The variant, immunologically also a β-lipoprotein, differs in molecular size, density, and charge from normal β-lipoprotein. Individuals showing the variant appear to be heterozygous for an uncommon mutant gene.*

Genetically determined variations have been detected in many proteins of normal human serum. Some variations, such as those in haptoglobins, transferrins, and γ-globulins, represent common polymorphisms (*1*); others, such as double albumin, in which two electrophoretically separable albumins occur in the same serum (*2*), are rare. Inherited variations in serum lipoproteins, for which differences in the protein moieties may be responsible, include antigenic polymorphism (see *3*), a-β-lipoproteinemia (*4*), and absence of high-density lipoprotein (*5*). Variations in mobility and staining characteristics of lipoprotein bands detected by pa-

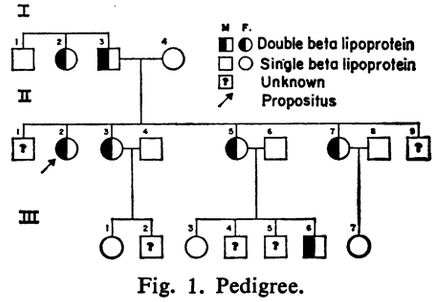

Fig. 1. Pedigree.

per electrophoresis have been associated with disorders of lipid metabolism, either primary or secondary to other diseases (*6*). In normal serum a single β-lipoprotein band is demonstrated by electrophoretic separation at an alkaline *p*H and by subsequent staining for lipids. When separated on paper under usual conditions (*7*) this component appears in the β-globulin region; in starch gel the band lies between the origin and the α-2 macroglobulin (*8*). Pre-β-lipoproteins, which may occur in persons with hyperlipemia, appear under routine conditions of paper electrophoresis as a lipid-staining area just ahead of (of greater mobility than) the normal β-lipoprotein band; modified techniques may improve their resolution on paper (*9*), but they are not distinguishable components in starch gel.

We have found a lipoprotein variant in which two distinct bands staining for lipid are present in the β-lipoprotein region, both on paper and in starch gel. This abnormality was first detected on routine paper electrophoresis (*7*) of serum from a 42-year-old woman with anemia following gastrectomy. Serums from available relatives revealed the variant in her father, paternal aunt, three sisters, and nephew (Fig. 1). Hyperlipemia and hypercholesterolemia occurred in some members of the family but were not associated with the β-lipoprotein abnormality. The characteristic seemed to occur in individuals heterozygous for an uncommon gene, but information regarding this woman's family was insufficient to establish whether the trait was autosomal or sex-linked.

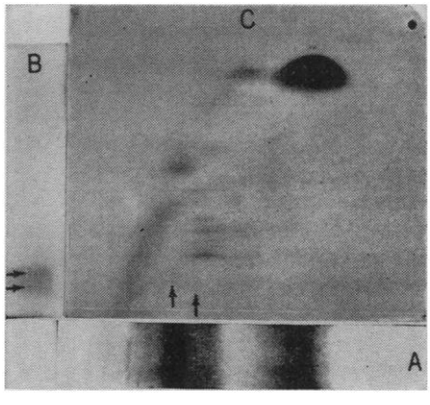

Fig. 2. Electrophoretic separation of whole serum from propositus. *A*, Paper strip stained for lipoproteins (Sudan Black); anode at right. Wide dark band closest to anode is α-lipoprotein. Two bands further from anode are β-lipoprotein; the wider and darker of the two corresponds to normal β-lipoprotein, the lighter is the abnormal component of greater mobility than normal β-lipoprotein. *B*, Starch-gel strip stained for lipoproteins (Lipid Crimson); anode at top, origin at bottom. The band corresponding to normal β-lipoprotein has moved slightly further toward the anode than the lighter-staining abnormal component. *C*, Two-dimensional separation; gel stained for all protein (Nigrosin). Anode was at right for the first separation, on paper; on top for the second separation, in starch gel. Arrow indicates two β-lipoprotein spots: the one toward the right locates the abnormal component, which moved further toward the anode than did normal β-lipoprotein in the first dimension (on paper) and not as far as the normal in the second dimension (in starch gel).

Methods of detection and characterization of the variant were as follows. After paper electrophoresis and staining of strips with Sudan Black B (Fig. 2*A*), two bands were seen in the region in which normally only a single β-lipoprotein band appears. The more densely stained band corresponded to normal β-lipoprotein. The other, less densely stained, had greater mobility toward the positive electrode than the normal band. In starch-gel electrophoresis (*10*) the β-lipoprotein band corresponding to the normal had greater mobility toward the anode than the weaker additional component, a reverse of their relative positions on paper (Fig. 2*B*). This suggested that in addition to differing in charge, as manifested by increased mobility on paper, the abnormal component was of greater molecular size, as indicated by its relatively greater retardation in starch gel. This was confirmed by two-dimensional electrophoresis, whereby a strip of paper on which serum has been fractionated electrophoretically is inserted into a starch gel and subjected to further separation in the second medium (*11*); the component having the greater mobility on paper thus has the lesser mobility in starch gel (Fig. 2*C*). In starch-block electrophoresis in borate buffer at *p*H 8.6, the unusual band was found between normal β-lipoprotein and the α-globulins, as on paper; this was verified by starch-gel electrophoresis of fractions eluted from the block.

Serum from the propositus was also fractionated by preparative ultracentrifugation in potassium bromide solutions of successively increasing densities (*12*). The fraction of density 1.019 to 1.063, which normally contains all β-lipoprotein appearing as a distinct band in starch-gel electrophoresis, contained only one band, corresponding to the normal (stronger) band seen in electrophoresis of whole serum. The weaker component, which moved more slowly in starch gel and faster on paper, appeared in the fraction of density 1.063 to 1.21, which normally contains only alpha (high-density) lipoproteins. Immunodiffusion techniques showed that this high-density fraction contained both the expected α-lipoprotein and a β-lipoprotein; the latter appeared to be immunologically identical with that found in the fraction of lower density. When serum from normal individuals or from patients with hypercholesterolemia or hyperlipemia was centrifuged and tested simultaneously or under identical conditions, it showed no β-lipoprotein in the high-density fractions.

It thus appeared that this abnormal component was immunologically a β-lipoprotein, differing from normal β-lipoprotein in charge, molecular size, and density. These properties suggest that the protein moiety of the variant might be a polymer of the normal molecule that binds less lipid. This apparently different class of molecules, detectable in other members of the woman's family, appears to be genetically determined. The variant provides further evidence that the protein moiety of β-lipoprotein is subject to simple genetic control.

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13. Supported by PHS (grants HE 08556 and HD 00542) and the American Heart Association. Carried out during the tenure of an Advanced Research Fellowship (W.S.) and an Established Investigatorship (K.H.) of the American Heart Association.

8 April 1965