tests and the focal islet lesions among many ALS-injected and irradiated euglycemic littermates are interpreted as evidence that ALS did not completely protect against the hypothesized cell-mediated autoimmune process, even when therapy preceded the detection of glycosuria. It is not known whether insulitis and β cell injury may have preceded ALS or irradiation therapy, since these animals were not tested for glucose tolerance prior to the experiments (*14*). It is conceivable that complete or more lasting protection of the pancreatic β cell mass might result from different modes or schedules of immunosuppression. The data presented, however, do establish that empiric immunosuppression may ameliorate or prevent spontaneous diabetes in the BB/W rat, in a manner analogous to the immunological prevention of virus-induced (*15*) or chemically induced (*16*) diabetes in mice.

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Clonal Characteristics of Experimentally Induced "Atherosclerotic" Lesions in the Hybrid Hare

Abstract. *The female hybrid hare (*Lepus timidus × Lepus europaeus*) is heterozygous for eletrophoretically separable, X-linked isoenzymes of glucose-6-phosphate dehydrogenase. The isoenzymes of this animal have been used as cellular markers in the study of the clonal origins of experimentally induced atherosclerotic lesions. Aortic lesions produced in the hybrid hare by feeding cholesterol and injuring the aortic wall with a catheter have been shown to have polyclonal characteristics and in this way are fundamentally different from atherosclerotic fibrous plaques in man.*

The X-linked enzyme, glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49), has been used as a cellular marker to investigate the clonal origins of a variety of human lesions. Studies carried out on tissue from black American females heterozygous for G6PD isoenzymes showed that a number of types of tumors, both benign and malignant, originate from a single clone of cells (*1*). Other studies have been performed on atherosclerotic lesions, leading to the observation that the majority of atherosclerotic plaques have monoclonal characteristics (*2*).

Progress in our understanding of the biology of human atherosclerosis has been hindered by the lack of appropriate animal models. The rabbit has been used extensively, and "atherosclerotic" lesions have been produced by feeding the animal cholesterol (*3*) or by injury to the intimal surface of an artery from a catheter (*4*). However, these lesions differ

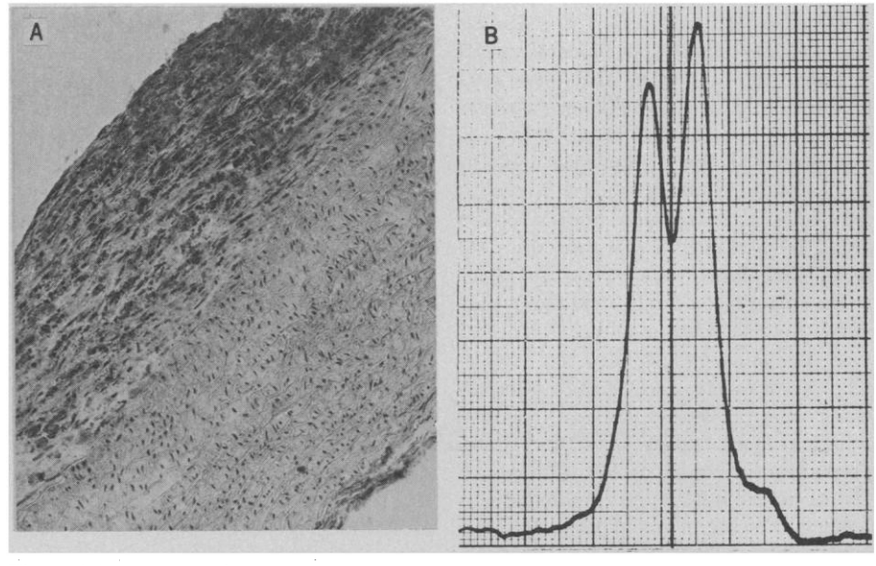

Fig. 1. (A) Frozen section of "atherosclerotic" lesion showing abundant lipid and elongated cells. Oil Red O stain; ×110. (B) Densitometric scan of G6PD isoenzyme bands from an "atherosclerotic" lesion of animal 1. Dissection and cellulose acetate electrophoresis were carried out with the use of standard techniques (*2*). A tris-EDTA-glycine buffer (*p*H 9.2; Gelman) was used. Eight samples were applied to each electrophoresis plate, and electrophoresis was carried out at 360 V for 23 minutes in a Helena electrophoresis chamber. Following specific enzyme staining, the amount of enzyme activity in each isoenzyme band was quantitated with the use of a Helena Quick-Scan densitometer. This densitometric scan of G6PD bands shows a very adequate separation of isoenzyme bands. The results are expressed as the percentage contribution of the slower (*L. timidus*) isoenzyme band (right side) to the total G6PD activity.

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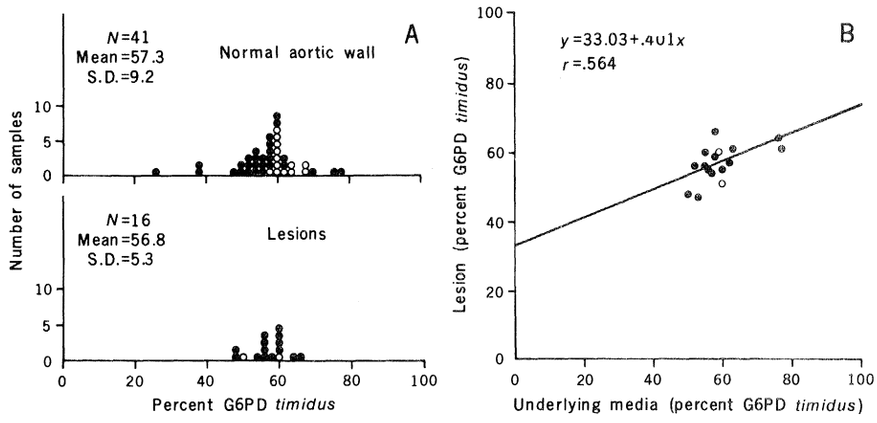

Fig. 2. (A) Isoenzyme values for samples of underlying media and "atherosclerotic" lesions from two hybrid hares. Each circle represents the percentage contribution of the *L. timidus* isoenzyme band to the total G6PD activity for one portion of media or lesion. Closed circles represent portions from animal 1; open circles, from animal 2. Portions of lesion and media from both animals cluster between 40 and 60 percent *L. timidus*, consistent with a polyclonal origin of their cellular constituents. (B) The correlation of the isoenzyme values (percent *timidus*) between samples of "atherosclerotic" lesions and samples of underlying media. Each circle represents the isoenzyme values for a portion of lesion paired with the value for the media sample lying immediately beneath it. The significant correlation ($r = .564$, $P < .025$) shows that the isoenzyme values of the lesions resemble normal arterial wall.

from atherosclerosis in man in several important respects. This is particularly true of those cholesterol-feeding experiments in which animals are given very large amounts of cholesterol that accumulates not only in the aortic intima but in the liver, spleen, eye, skin, and other sites as well. The aortic lesions that develop lack certain features of the human disease, such as ulceration and hemorrhage into the plaques (*5*). In an earlier study we suggested that the monoclonal character of the fibrous plaque should be regarded as the hallmark of the disease and that any valid experimental model should produce lesions that show this characteristic. This led us to search for a laboratory animal in which X-linked, electrophoretically separable isoenzymes of G6PD could be used as a cellular marker and in which atherosclerosis could be produced experimentally. We found that Ohno *et al.* (*6*) had described such an animal—the hybrid hare produced by the mating of the European hare (*Lepus europaeus*) and the Scandinavian snowshoe hare (*Lepus timidus*) (*7*).

The present report describes (i) the production of "atherosclerotic" lesions in the hybrid hare by a combination of feeding cholesterol and balloon catheterization and (ii) the enzyme studies performed to determine whether the lesions produced were monoclonal and therefore acceptable as true atherosclerotic lesions. The combination of cholesterol feeding and balloon-catheter injury was used to produce lesions of maximal thickness, which facilitated the clean dissection of the intimal lesion off the underlying media; contamination of the intimal lesion by small fragments of media would lead to the demonstration of both isoenzymes in the sample whether the lesion itself contained one or both isoenzymes. Lesions were produced in the aorta by feeding rabbit chow containing 2 percent cholesterol by weight for 2 weeks and then 1 percent cholesterol by weight for 6 weeks to each of two hybrid female hares (weight, 2.5 kg). Two weeks after initiation of cholesterol feeding, the animals were anesthetized and a No. 5 French balloon catheter was inserted via the femoral artery to the level of the aortic arch, inflated, and drawn down the aorta to injure the intimal surface. This procedure was repeated 3 weeks later, but the opposite femoral artery was used. Three weeks after the final catheterization the animals were killed. At the time of death, serum cholesterol concentrations, by the enzymatic method (Bio-Dynamics/BMC) (*8*), were 1392 mg/dl for animal 1 and 514 mg/dl for animal 2.

The aortas of both animals showed well-developed changes. Animal 1 had severe "atherosclerosis" involving the iliac arteries and aorta, including the aortic arch. The lesions were diffuse, raised, and whitish yellow. Animal 2 had less extensive "atherosclerosis" limited to the distal aorta and iliac arteries. These lesions were raised and pearly white. By light microscopy, the lesions contained numerous elongated, lipid-containing cells (Fig. 1A). Examination with electron microscopy showed large numbers of smooth muscle cells, many of which contained lipid vacuoles in their cytoplasm. Smooth muscle cells were defined as those showing cytoplasmic myofibrils, limiting basement membrane, and numerous micropinocytotic vesicles along the plasma membrane.

The "atherosclerotic" areas could be clearly separated from the underlying media by microdissection. One-half of each lesion was fixed for histologic examination to ensure that contamination by media had not occurred. Small portions (surface area, 1 to 4 mm^2) of the lesion and of the media underlying it, as well as red blood cells from each hare, were assayed for G6PD isoenzymes by using standardized methods (*2*). Although only a 7 percent difference in relative electrophoretic mobility between the two hare isoenzyme bands (human beings have a 10 percent difference) is obtained with this method, an adequate separation of the bands was achieved (Fig. 1B). The results of electrophoresis were expressed in terms of the percentage contribution of the slower (*L. timidus*) isoenzyme band to the total amount of enzyme activity.

Results were obtained on a total of 16 "atherosclerotic" lesions, 14 from animal 1 and two from animal 2 (Fig. 2A). None of these lesions was contaminated by underlying media. The isoenzyme values for samples of underlying media showed approximately equal amounts of each isoenzyme and in this way were similar to samples of human media. Values for samples of "atherosclerotic" lesions were also indicative of polyclonal origin, unlike the fibrous atherosclerotic plaques in man whose values show the predominance of only one of the isoenzyme types. None of the samples of "atherosclerotic" lesions in the hare met the criteria for monoclonality used in human studies (*2*).

A comparison was made between the isoenzyme distribution of each "atherosclerotic" lesion and that of the particular hare's blood. The mean isoenzyme values for blood (animal 1, 67.9 ± 3.6 percent *timidus*; animal 2, 63.7 ± 1.0 percent *timidus*) were significantly higher than the values for the aortic lesions. However, the isoenzyme distributions of the lesions were similar to, and correlated significantly with, the values of the immediately underlying media (Fig. 2B). The clonal characteristics of the aortic lesions, therefore, resemble more closely those of the underlying media than those of the blood.

The clonal characteristics of these experimentally induced atherosclerotic lesions clearly differ from those observed in human fibrous atherosclerotic plaques that are predominantly monoclonal (*2*).

Our results, therefore, suggest that the cellular populations in the experimentally induced lesions are fundamentally different in origin from those in the human lesions. The lesions produced in the hares more closely resemble the human fatty streak, whose values cluster in the center of the range. However, isoenzyme values for human fatty streaks have a wider spread, and a small but significant minority (3.0 percent) of these lesions have been shown to be monoclonal (*2*).

It is concluded that the lesions produced in the hares' arteries by a combination of cholesterol feeding and balloon-catheter injury lack monoclonal characteristics and therefore are not analogous to naturally occurring atherosclerotic plaques in man. However, the hybrid hare provides an animal model in which both the production of "atherosclerotic" lesions and the quantification of the G6PD enzyme markers are feasible, but further studies, including longer term experiments, are needed to determine which experimental procedure, if any, provides a true atherosclerotic lesion.

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Perception of Size of One Object Among Many

Abstract. *Adaptation to a grating of properly chosen frequency may lead to two apparently conflicting observations: Another grating may then appear to be of increased frequency (compared with its "unadapted" frequency) while the individual bars of the grating appear to have widened. This perceived widening parallels previous results with single bars. By attending to only one grating bar, the subject effectively seems to change the grating frequency spectrum to that of a single bar.*

The concept of size is basic to all science. One does not "define" length, width, height, or depth, in other, more primitive terms. One simply provides standard units and prescribes the operations whereby size may be measured in their terms.

Size might be a psychological primitive as well. We report experimental results that appear paradoxical if size is indeed primitive. The paradox appears resolvable if a model currently popular in vision research is applied, with one additional, ad hoc hypothesis.

The paradoxical observation is that when, by a process of adaptation, a set of bars (a "grating") is made to appear to be of a higher spatial frequency (more bars per centimeter), the bars also appear to be wider (more centimeters per bar). Geometrically, this is impossible in a grating with light and dark bars of equal width. If either bar, light or dark, has width w cm, the spatial frequency f is given by $f = 1/(2w)$ cycles per centimeter. If either frequency or width increases, the other must decrease. Our results indicate that the psychological correlates of f and w do not fit the equation (more correctly, the definition of f).

Figure 1 [based on figure 1 of (*1*)] offers a demonstration of an adaptation procedure that leads to a shift in perceived spatial frequency. An observer adapts to the large high- and low-frequency gratings by holding the page about a foot away and scanning back and forth across the horizontal fixation bar for at least 1 minute. A glance at the fixation square between the short gratings should then demonstrate the apparent difference of spatial frequency between them, with the upper one (which falls within the retinal region that has been adapted to the upper, low-frequency grating) appearing higher in spatial frequency. (The effect is fleeting when the adaptation time is only 1 minute.)

To measure perceived width, we allowed the viewer to adjust the height of the grating bars to equal their widths, both before and after adaptation. This "make-a-square" method has previously (*2*) enabled viewers to report the perceived widths of single bars (rectangles). The methods of this experiment were identical to those in an earlier report (*2*).

Figure 2 shows data derived from individual subjects' settings to make the bars in the short test grating look square. After adaptation to gratings whose bars are narrower than, equal to, or wider than the test grating bars (that is, higher, equal, or lower in spatial frequency), the

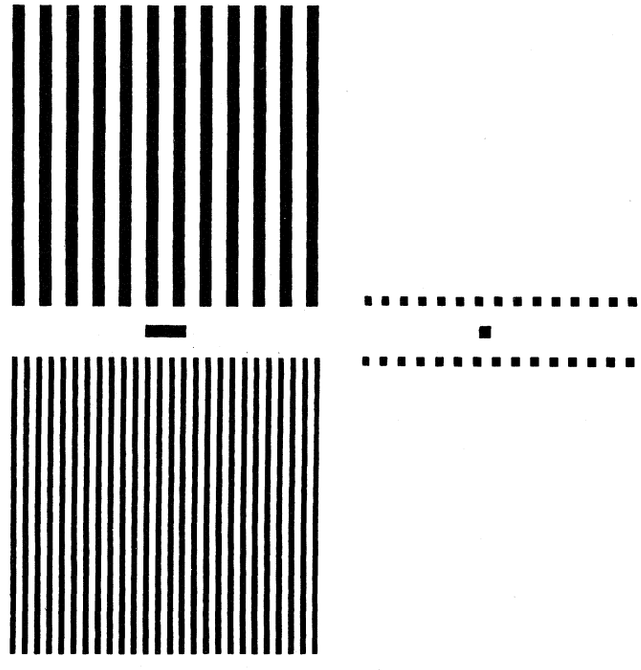

Fig. 1. The Blakemore and Sutton (*1*) demonstration with short gratings.

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