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19. In an unpublished study by M. Essex and M. Hirsch (Massachusetts General Hospital), 2 of 93 kidney transplant patients had antibodies to HTLV-MA when their sera were tested with previously described methods (*8, 16*). In another unpublished study by M. Essex, J. Bailey, and T. Guthrie (Medical College of Georgia), none of 17 patients with systemic lupus erythematosus, a disease of immunoregulatory dysfunction, had positive results for the antibodies.
20. We thank the many physicians, blood banks, and health departments that assisted in the investigations described in this report.
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27 February 1984; accepted 6 March 1984

Interferon-β–Related DNA Is Dispersed in the Human Genome

Abstract. *Interferon-β_1 (IFN-β_1) complementary DNA was used as a hybridization probe to isolate human genomic DNA clones λB3 and λB4 from a human genomic DNA library. Blot-hybridization procedures and partial nucleotide sequencing revealed that λB3 is related to IFN-β_1 (and more distantly to IFN-α_1). Analyses of DNA obtained from a panel of human-rodent somatic cell hybrids that were probed with DNA derived from λB3 showed that λB3 is on human chromosome 2. Similar experiments indicated that λB4 is not on human chromosomes 2, 5, or 9. The finding that DNA related to the IFN-β_1 gene (and IFN-α_1 gene) is dispersed in the human genome raises new questions about the origins of the interferon genes.*

A single human interferon-β (IFN-β) gene located on human chromosome 9 (designated IFN-β_1), a cluster of IFN-α_1–hybridizing genes on chromosome 9, and a single IFN-γ gene on chromosome 12 have been identified and characterized (*1*). Other studies of human IFN-β production and studies of lengths of translationally (and biologically) active human IFN-β messenger RNA (mRNA) that can be expressed in appropriately induced human-rodent somatic cell hybrids have indicated that functional IFN-β genes are present on human chromosomes 2, 5, and 9 (*2, 3*). Using full-length, sequence-confirmed IFN-β_1 complementary DNA (cDNA) as a hybridization probe, we isolated two human genomic DNA clones, λB3 and λB4, from a human genomic DNA library in λ phage Charon 4A (*4*). The relatedness of λB3 and λB4 to IFN-β_1 was confirmed by additional hybridization tests and, in the case of λB3, by partial nucleotide sequencing. We have now used blot-hybridization procedures to assign λB3 to human chromosome 2. In similar experiments, we found that λB4 is not on human chromosomes 2, 5, 9, or 12.

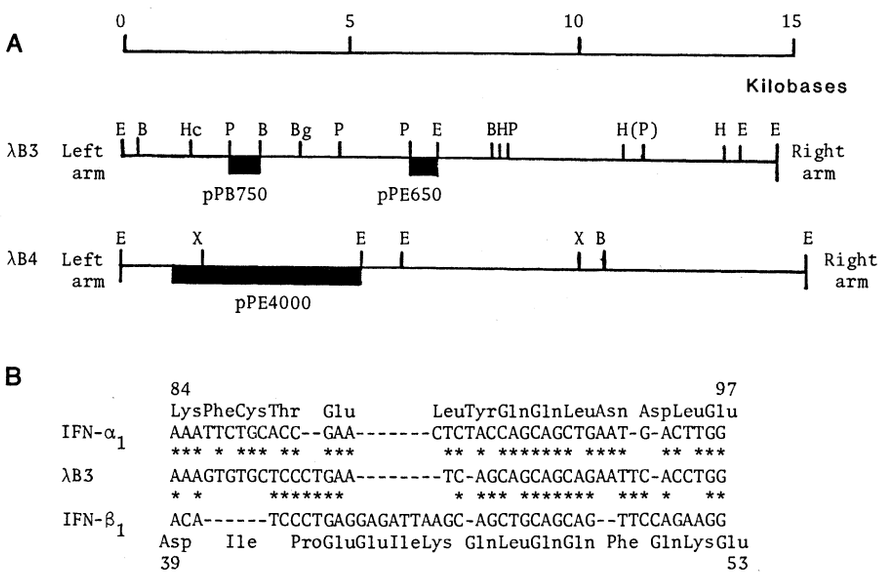

Fig. 1. An example of nucleotide sequence relatedness between λB3, IFN-α_1 and IFN-β_1. (A) Schematic restriction maps of λB3 and λB4. The restriction sites are: *B*, Bam HI; *Bg*, Bgl II; *E*, Eco RI; *H*, Hind III; *Hc*, Hinc II; *P*, Pst I; and *X*, Xba I. Only those sites that can be clearly identified are indicated. The origins of the various relevant subclones are also shown. The origin of the 105-nucleotide pPP-105 subclone of λB3 has not yet been unambiguously determined. (B) The nucleotide sequence of a portion of the pPB-750 area in the map of λB3 compared with the nucleotide and amino acid sequences of IFN-α_1 and IFN-β_1 (*1, 9, 10*). Asterisks indicate nucleotide matches. Amino acid residue numbers correspond to those for the mature proteins.

Interferons have customarily been classifed as α, β, or γ on the basis of their antigenicity and the relatedness of their nucleotide sequences (*1*). For example, IFN-α and IFN-β proteins are not neutralized by antiserums to each other, and IFN-α_1 cDNA probes do not cross-hybridize with IFN-β_1 DNA [the relatedness of their nucleotide sequences is only 43 percent (*1*)]. We discovered that λB3 DNA straddles the IFN-α and -β systems. Although λB3 appears (by serology) to represent an IFN-β gene located on chromosome 2, it cross-hybridizes with IFN-β_1 cDNA (strongly) and IFN-α_1 DNA (weakly). The discovery of DNA that straddles the IFN-α and IFN-β gene families dispersed in the human genome adds a new dimension to the description of the human interferon system.

The inference that there are a number of distinct human IFN-β genes was supported by the detection of translationally active (in *Xenopus laevis* oocytes) human IFN-β mRNA species of different lengths; the IFN-β mRNA species were detected by subjecting polyadenylated RNA obtained from induced human and human-rodent somatic cell hybrids to electrophoresis through agarose–methyl mercury gels (*3, 5*). These studies produced data consistent with the earlier assignment by others (*1*) of the 0.9-kilobase (kb) IFN-β_1 mRNA to chromosome 9 and suggested the assignment of the 1.3-kb IFN-β_2 mRNA to chromosome 5 and 1.8-kb IFN-β_3 mRNA to chromosome 2. Additional translationally active IFN-β mRNA species of lengths 0.35, 0.65, 3, 5, and 8 kb have since then been detected in both induced human lymphoblastoid and fibroblast cells (*5*).

We isolated three distinct human genomic DNA clones from a human genomic DNA library in λ phage Charon 4A that cross-hybridized with IFN-β_1 cDNA (*4*). Two of these (λB3 and λB4) are distinct from each other and from the IFN-β_1 gene, although they both strongly cross-hybridized with IFN-β_1 cDNA. Blot-hybridization tests indicated that poly(I) · poly(C)–induced (I, inosine; C, cytosine) human diploid fibroblasts can contain polyadenylated RNA species 1.8, 3, 5, and 8 kb long that hybridize with λB3 DNA and a 12-kb species that hybridizes with λB4 (*4*). These data suggested that λB3 DNA may reside on chromosome 2 and correspond to the 1.8-kb IFN-β_3 mRNA. We tested this possibility with blot-hybridization analyses of DNA derived from a panel of human-mouse and human-hamster so-

matic cell hybrids; for these analyses, a 650-nucleotide DNA fragment derived from λB3 and subcloned into pBR322 (pPE-650) was used as a hybridization probe.

That λB3 is related to IFN-β_1 has been confirmed by (i) nucleic acid hybridization (see Fig. 2 and lane 1 of Fig. 3 for examples) and (ii) partial nucleotide sequencing, which reveals sequence homology between several sections of λB3 and sequences coding for the IFN-β_1 and IFN-α_1 proteins. Figure 1A shows a schematic restriction map of λB3, and Fig. 1B illustrates the nucleotide sequence of a portion of the area designated pPB-750 in the map. This nucleotide sequence is homologous to both IFN-α_1 and IFN-β_1. Protein domains containing amino acids 45 to 50 and 90 to 96 are highly conserved between the IFN-β_1– and the IFN-α_1–related genes. The nucleotide sequence of λB3 in the region illustrated in Fig. 1B is approximately 90 percent homologous with the nucleotide sequences of the well-known α and β interferons (when both IFN-α_1 and IFN-β_1 are simultaneously included in the comparison); the homology is approximately 75 percent with the sequence of IFN-α_1 alone and approximately 60 percent with the sequence of IFN-β_1 alone. In blot-hybridization experiments, several restriction enzyme fragments of λB3 strongly hybridized with an IFN-β_1 cDNA probe but showed relatively weak (but clear) hybridization with an IFN-α_1 cDNA probe (Fig. 2). This observation is consistent with nucleotide sequence data (adding up to approximately 6 kb of sequence), indicating that λB3 may be more closely related to IFN-β_1 than to IFN-α_1 and that these regions of homology are scattered over large distances in λB3. Similar blot-hybridization results have been obtained with λB4 (not shown). Under the conditions of these phage blot-hybridization experiments there is no detectable cross-hybridization between a coding-region IFN-α_1 cDNA probe and a coding-region IFN-β_1 DNA piece (not shown), even though the two coding-region DNA segments used are distinctly related (approximately 43 percent relatedness between the nucleotide sequences) (*1*).

Table 1. Assignment of λB3. Hybrid lines with inconclusive karyotype and isozyme data for a particular chromosome are not included. Symbols indicate the presence (+/) or absence (−/) of hybridization signal as related to the presence (/+) or absence (/−) of a particular human chromosome. The number of concordant responses is the sum of the +/+ and −/− responses; the number of discordant responses is the sum of the +/− and −/+ responses.

Human chromosome	Number of hybrids showing response				Ratio of discordant to total responses
	+/+	−/−	+/−	−/+	
2	11	12	1	0	0.042
20	10	9	2	3	0.208
3	8	9	4	1	0.227
X	11	7	1	5	0.250
17	7	10	5	1	0.260
6	7	10	5	2	0.291
12	10	7	2	5	0.291
16	5	10	7	0	0.318
4	7	9	5	3	0.333
10	6	10	6	2	0.333
18	8	8	4	4	0.333
9	4	11	8	0	0.347
5	5	9	6	2	0.363
11	9	6	3	6	0.375
15	4	11	8	1	0.375
1	4	10	8	2	0.416
7	3	11	9	1	0.416
8	4	10	8	2	0.416
14	5	9	7	3	0.416
13	5	7	7	5	0.500
21	7	5	5	7	0.500
19	5	6	7	6	0.541
22	2	8	10	3	0.565
Y	0	2	8	0	0.800

Appropriate λB3-derived DNA probes also hybridize polyadenylated RNA of lengths 1.8 (IFN-β_3), 3, 5, and 8 kb obtained from poly(I) · poly(C)–induced human diploid fibroblasts (FS-4 strain). Transcripts of these lengths show antiviral interferon-β activity (by serologic typing) after microinjection into *Xenopus* oocytes (*3, 5*). Furthermore, λB3 and λB4 are related to each other because DNA probes derived from λB3 cross-hybridized with λB4, and vice versa, in blot-hybridization analyses of phage DNA digests under low-stringency conditions (not shown).

The λB3 DNA probe pPE-650 hybridized with a 13-kb Eco RI fragment (Fig. 3, lane 2) and a 5.6-kb Bam HI fragment (not shown) in digests of total human DNA analyzed in blot-hybridization experiments. [In experiments in which total human DNA digests are probed for unique DNA sequences under high-stringency conditions, λB3 and λB4 DNA probes do not exhibit detectable cross-hybridization with each other or with other related DNA sections because these hybridizations do not reach the high values of C_0t (C_0, DNA concentration; t, time) that can be reached when blots of pure phage DNA digests of one kind are hybridized with pure heterologous insert DNA probes, as in Fig. 2.] However, under exceptional conditions we have observed that the 13-kb Eco RI human DNA fragment that hybridized with pPE-650 also hybridized with the IFN-β_1 cDNA probe in experiments in which the same blot was hybridized sequentially with the two probes (experiments similar to that shown in Fig. 3, lane 1). Under the stringent hybridization and washing conditions we used, the human pPE-650 DNA probe hybridized only faintly with DNA fragments in digests of murine and hamster DNA (Fig. 3, lanes 3 and 4). The strong human DNA signal can be readily distinguished from this faint rodent signal.

DNA preparations from 25 human-mouse and human-hamster somatic cell hybrids were digested either with Eco RI (Fig. 3) or Bam HI (not shown) and were analyzed for the presence of the 13-kb Eco RI (Fig. 3, lanes 5 to 14) or the 5.6-kb Bam HI (not shown) DNA fragments that hybridized with pPE-650. The human chromosome composition of these

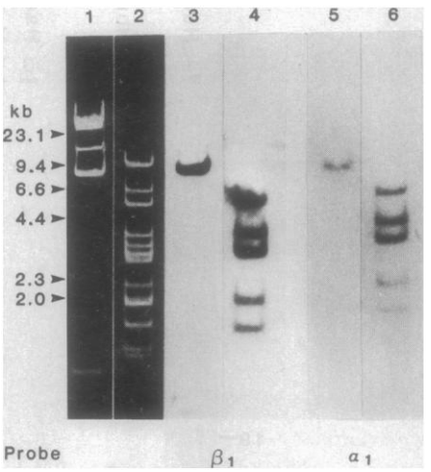

Fig. 2. Hybridization of λB3 DNA with both IFN-β_1 and IFN-α_1 cDNA probes. λB3 DNA (0.7 μg) was digested with either Eco RI or with Pst I. Half of each sample was subjected to electrophoresis through 1 percent agarose gel and blotted on Gene Screen Plus (New England Nuclear). The remaining half of each digest was used to prepare a companion blot in a like manner (*4*). Lanes 1 and 2 represent the ethidium bromide stain of the Eco RI– and Pst I–digested DNA shown in lanes 3 and 4. The DNA in lanes 3 and 4 was hybridized with a ^{32}P-labeled, nick-translated Bgl II–Pst I DNA fragment, 150 nucleotides long, that was derived from an IFN-β_1 cDNA clone [pD19 in (*4*)]. Lanes 5 and 6 represent a companion blot that was hybridized with ^{32}P-labeled Pvu II–Eco RI DNA fragment, 560 nucleotides long, that was derived from an IFN-α_1 cDNA clone [pAS1-1 (*5*)]. Hybridization was essentially in fivefold-strength standard saline citrate (SSC) (SSC is 0.15*M* NaCl and 0.015*M* sodium citrate, *p*H 7.5) at 65°C for 16 to 18 hours and washing was for approximately 1.5 hours in double strength SSC, also at 65°C (*4*). Approximately one-tenth as much probe (2×10^6 Cerenkov count/min) was used in the experiment of lanes 3 and 4 as was used for that of lanes 5 and 6 (15×10^6 Cerenkov count/min). The autoradiograms were exposed for 1.5 hours (Kodak XAR-5 film). Results similar to those in lanes 3 and 4 have also been obtained with a coding region Hinc II–Bgl II DNA fragment from IFN-β_1 cDNA used as a probe.

hybrids was compared with the presence of pPE-650–specific DNA in them (Table 1). From this analysis it is apparent that pPE-650–specific human DNA, and thus λB3 DNA, resides on human chromosome 2. Hybridization of the somatic cell hybrid DNA blots with another λB3-derived DNA subclone (pPP-105) that hybridizes with a 7.5-kb Eco RI fragment in human DNA leads to the same conclusion.

To determine whether λB4 DNA was present on the same chromosome as λB3, we digested DNA from the same set of somatic cell hybrids (Fig. 3) with Kpn I and performed blot-hybridization analyses with a 4-kb section of λB4 subcloned in pBR322 (pPE-4000). Under the experimental conditions used, the pPE-4000 probe detected one strongly hybridizing DNA fragment of approximate length 20 kb and three weaker fragments of lengths 11, 7.8, and 6.4 kb in human DNA (Fig. 4, lane 2). Specific fragments of lengths 5.8 and 7.2 kb were also detected in the hamster and mouse parental cell lines (Fig. 4, lanes 3 and 4), but these are clearly distinguishable from the human DNA fragments. Comparison of Fig. 4 (lanes 5 to 14) with Fig. 3 demonstrates that λB3 and λB4 cannot be on the same chromosome. Thus λB4 cannot be located on human chromosome 2. Furthermore, a comparison of these data with the human chromosome compositions of the hybrids tested indicates that λB4 is not on chromosomes 5 or 9. The absence of a human DNA hybridization signal in lanes 7 and 8 of Fig. 4 also indicates that pPE-4000–related DNA is not on chromosome 12, to which the IFN-γ gene was assigned (*1*). Since four Kpn I human DNA fragments of lengths 6.4 to 20 kb hybridized with the 4-kb λB4 probe pPE-4000, which

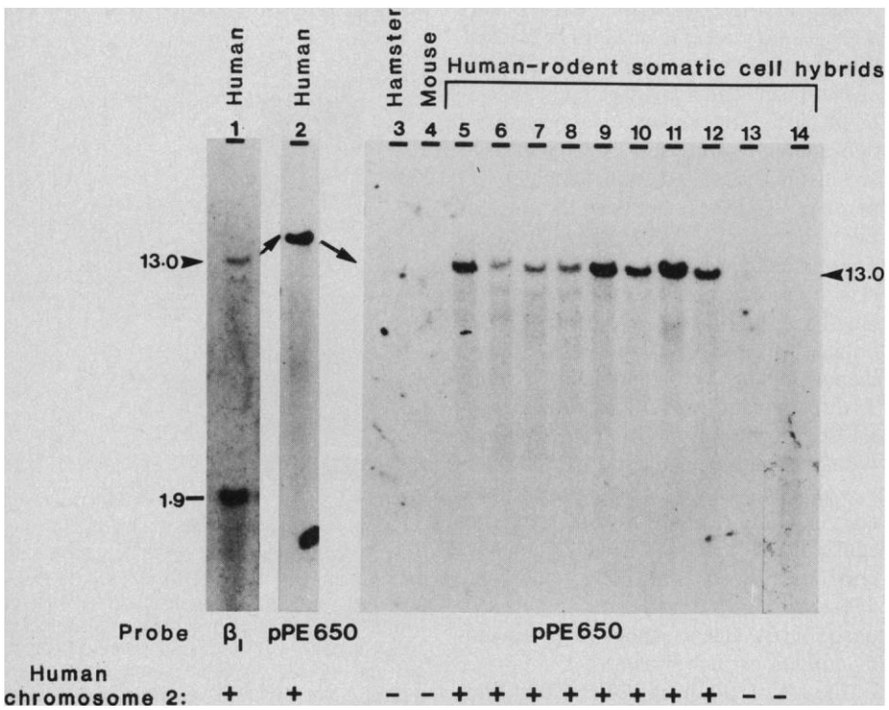

Fig. 3. Blot-hybridization analyses of DNA from human, mouse, and hamster cells and from human-rodent somatic cell hybrids were performed with human IFN-β–related DNA probes (IFN-β_1 cDNA and the pPE-650 subclone of λB3). DNA samples (20 μg in lanes 1 and 2 and 30 μg in lanes 3 to 14) were restricted with Eco RI (New England Biolabs, 4 units per microgram of DNA) for 24 hours as specified by the manufacturer. The DNA was then subjected to electrophoresis through 0.8 or 1 percent agarose gels, blotted onto nitrocellulose paper (BA85, Schleicher and Schuell), and probed with nick-translated (Bethesda Research Laboratories kit; α^{-32}P-labeled deoxycytosine triphosphate from New England Nuclear), ^{32}P-labeled IFN-β_1 cDNA pD19 (lane 1) (*4*) or the pPE-650 subclone derived from λB3 (lanes 2 to 14) (*4*). Briefly, hybridization was in sixfold-strength SSC (see legend to Fig. 2) and 10 percent (weight to volume) dextran sulfate (Pharmacia) at 65°C for 20 to 24 hours. The hybridized blots were washed in double-strength SSC at room temperature for 30 minutes and then in 0.3-strength SSC at 65°C for 1.25 hours. Autoradiography usually required an overnight exposure on Kodak XAR5 film with intensifying screens. (Lanes 1 and 2) Human lymphoblastoid (Namalwa) cell DNA (*11*); (lane 3) hamster E36; (lane 4) mouse A9; (lane 5) somatic cell hybrid BDA 17b17; (lane 6) BDA 17b17/Dpt-2; (lane 7) AIM 15aB1/38; (lane 8) AIM 15aB1/36/DpT; (lane 9) BDXE 36-10; (lane 10) BDXE 36-4a; (lane 11) BDA 10a3; (lane 12) BDA 10a3/DpT; (lane 13) 21-30b SR; and (lane 14) BDA 14b25. Lanes 9 and 10 represent human-hamster hybrids. The human chromosome composition of these hybrids has been described earlier (*3*, *12*) and was deduced with standard procedures (*13*). The presence (+) or absence (−) of human chromosome 2 in each of the cell lines is indicated.

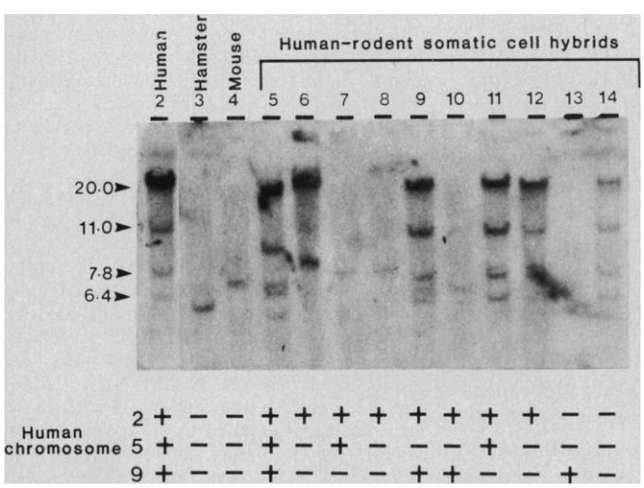

Fig. 4. Blot-hybridization analyses of DNA from human, mouse, and hamster cells and from human-rodent somatic cell hybrids with the pPE-4000 subclone of human λB4 genomic DNA. DNA samples (30 μg) were digested with Kpn I and analyzed as described in the legend to Fig. 3 except that ^{32}P-labeled pPE-4000 was used as the hybridization probe. Lane numbers correspond to those in Fig. 3. The presence (+) or absence (−) of human chromosomes 2, 5, and 9 in each of the cell lines tested is also indicated and is based on data summarized in (*3*) and (*12*), except that BDA 17b17/Dpt-2 (lane 6) is taken to contain human chromosomes 2, 4, 13, and 21. The somatic cell hybrid AHA 3d2-1 represents another example of a hybrid that appears to contain chromosome 5 but lacks the λB4 hybridization signal (not shown). Only hybrids indicated in lanes 5, 7, 8, 9, 11, 12, and 14 contain chromosome 12. Thus lanes 6, 7, and 8 exclude the assignment of pPE-4000 to chromosome 12.

does not contain a Kpn I site (Fig. 4), three or more section of human DNA very closely related to pPE-4000 must be present on a chromosome other than 2, 5, 9, or 12. An unambiguous assignment of the various λB4-specific human DNA fragments has not yet been achieved.

Our data demonstrate that IFN-β–related DNA is dispersed in the human genome. The murine IFN-β system also appears to be complex. Skup *et al.* (*6*) reported the isolation of two distinct partial cDNA clones (20/11 and 3/10) that appear to correspond to murine IFN-β mRNA's. They based this conclusion on experiments in which the cDNA clones were immobilized on nitrocellulose filters and hybridized with IFN mRNA preparations, after which the hybridized mRNA was eluted and analyzed for IFN-β activity with a translation assay. Clones 20/11 and 3/10 are different from each other and are different from well-known murine IFN-β_1 cDNA (*7*). Finally, because the human 0.8-kb IFN-β_1 gene hybridized with DNA sections of length greater than 5 kb in each of the λB3 and λB4 clones, and because the IFN-β_1 gene lacks introns (*1*), the possibility arises that the IFN-β_1 gene may have arisen from the IFN-β–related DNA located on the other chromosomes through a process of gene conversion, as has been found in several other gene families (*8*). The observation that λB3 DNA not only hybridizes with an IFN-β_1 cDNA probe but also hybridizes with an IFN-α_1 cDNA probe reveals an unexpected facet of the human interferon gene family—the origins of the α and β interferon systems must be more closely intertwined than has been recognized thus far.

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8 November 1983; accepted 4 January 1984

Magnesium Deficiency and Hypertension: Correlation Between Magnesium-Deficient Diets and Microcirculatory Changes in situ

Abstract. *Rats maintained for 12 weeks on diets moderately or more severely deficient in magnesium showed significant elevations in arterial blood pressure compared to control animals. Examination of the mesenteric microcirculation in situ revealed that dietary magnesium deficiency resulted in reduced capillary, postcapillary, and venular blood flow concomitant with reduced terminal arteriolar, precapillary sphincter, and venular lumen sizes. The greater the degree of dietary magnesium deficiency the greater the reductions in microvascular lumen sizes. These findings may provide a rationale for the etiology, as well as treatment, of some forms of hypertensive vascular disease.*

Numerous hypotheses have been suggested to account for the development of primary hypertension in man (*1*), including salt (NaCl) intake, overall nutrition, and genetic make-up (*1, 2*). It has also been proposed that increased blood pressure is due to a supersensitivity of blood vessels to the constrictor actions of endogenous neurohumoral substances (for example, adrenergic amines, angiotensin, or vasopressin) or to a decreased sensitivity of blood vessels to endogenous vasodilator substances (for example, prostaglandins) (*1*). All of these hypotheses have generated some controversy, and exactly how a sustained increase in arteriolar and venular vascular tone is brought about in a variety of clinical and experimental forms of hypertensive disease remains a mystery (*1*).

Several recent studies point to a causal relation between decreased concentrations of magnesium ion (Mg^{2+}) in blood or tissues and hypertension; the incidence of hypertension is high in geographic areas with soft drinking water or magnesium-poor soil (*3–6*). Since 1925, it has been known that pharmacologic doses of magnesium salts can somehow produce hypotension and attenuate high blood pressure in hypertensive patients; more recently, long-term administration of lower doses of magnesium salts has decreased requirements for antihypertensive drugs (*7*). Hypomagnesemia has been reported in a number of hyperten-