pears to be capable of discriminating between DF5HT-containing cytoplasmic areas and intracellular vacuoles. Qualitatively, the mapping mode is extremely useful in selecting fluorine-rich areas for subsequent study by specific probing. Although the spatial resolution of 10 to 30 nm and the signal-to-noise levels obtained here by mapping cannot be compared directly to those obtainable by either x-ray microanalysis or electron microscope radioautography, they appear to be superior to those obtained from biological specimens with the latter two techniques. A combination of mapping and probing with energy-loss spectroscopy should thus permit the determination in biological material of the intracellular disposition and translocations of a wide range of fluorinated organic molecules.

JONATHAN L. COSTA
Clinical Neuropharmacology Branch, National Institute of Mental Health, Bethesda, Maryland 20014
DAVID C. JOY
DENNIS M. MAHER
Bell Laboratories, Murray Hill, New Jersey 07974
KENNETH L. KIRK
Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014
S. W. HUI
Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York 14263

References and Notes

1. P. Goldman, *Science* **164**, 1123 (1969); D. F. Loncrini and R. Filler, *Adv. Fluorine Chem.* **6**, 43 (1970); R. Filler, Ed., *Biochemistry Involving Carbon-Fluorine Bonds* (American Chemical Society, Washington, D.C., 1976).
2. G. A. Boswell, Jr., W. C. Ripka, R. M. Scribner, *Org. React.* **21**, 1 (1974); C. M. Sharts and W. A. Sheppard, *ibid.*, p. 125; M. J. Shaw, J. A. Weil, H. H. Hyman, R. Filler, *J. Am. Chem. Soc.* **92**, 5096 (1970); K. L. Kirk and L. A. Cohen, *ibid.* **95**, 4619 (1973).
3. R. Woldseth, *X-Ray Energy Spectrometry* (Kevex Corp., Burlingame, Calif., 1973).
4. M. Isaacson and D. Johnson, *Ultramicroscopy* **1**, 33 (1975); C. Colliex, V. E. Cosslett, R. D. Leapman, P. Trebbia, *ibid.* **1**, 301 (1976); M. S. Isaacson and A. V. Crewe, *Annu. Rev. Biophys. Bioeng.* **4**, 165 (1975).
5. K. L. Kirk, *J. Heterocyclic Chem.* **13**, 1253 (1976).
6. J. L. Costa and D. L. Murphy, *Nature (London)* **255**, 407 (1975).
7. ———, *Life Sci.* **18**, 1413 (1976).
8. ———, M. Kafka, *Biochem. Pharmacol.* **26**, 517 (1977).
9. J. L. Costa, K. L. Kirk, H. Stark, D. L. Murphy, in preparation.
10. J. L. Costa, T. S. Reese, D. L. Murphy, *Science* **183**, 537 (1974); J. L. Costa, Y. Tanaka, K. D. Pettigrew, R. J. Cushing, *J. Histochem. Cytochem.* **25**, 1079 (1977).
11. J. L. Costa, T. C. Detwiler, R. D. Feinman, D. L. Murphy, C. S. Patlak, K. D. Pettigrew, *J. Physiol. (London)* **264**, 297 (1977).
12. To maximize the signal-to-noise ratio, the electron spectrometer was operated with an acceptance angle of 5 mrad and a slit width chosen to give a resolution of 20 eV. These values were chosen to permit good separation of the *K* and *L* edges of all elements [D. C. Joy and D. M. Maher, *Proc. Electron Microsc. Soc. Am. 35th* (1977), p. 244].
13. During the recording of a single spectrum, cells were exposed to the 10-nm² spot for approximately 10 seconds (specimen-level current density, 10 A/cm²). Recording a second and third spectrum with the beam positioned identically gave fluorine absorption edges with the same integral area as that seen in the first spectrum. Thus, under the conditions employed here, loss of fluorine due to radiation damage appears not to present a problem.
14. S. W. Hui and J. L. Costa, in preparation.
15. We also examined platelets incubated for 60 minutes with an initial concentration of $10^{-5}M$ of the sulfur analog of serotonin [the benzo[*b*]thiophene derivative of 5HT; see E. Campaigne, D. R. Knapp, E. S. Neiss, T. R. Bosin, *Adv. Drug Res.* **5**, 1 (1970); T. R. Bosin and E. Campaigne, *ibid.*, in press]. Spectra recorded from dense bodies in these cells contained large sulfur ionization edges at 240 eV, but no fluorine edges. In addition, dense bodies were lucent when cells were mapped in the 240 eV-loss mode, but not in the 680 eV-loss mode.
16. J. G. White, *Ser. Haematol.* **3**, 17 (1970); J. L. Costa and S. W. Hui, *Biophys. J.* **19**, 307 (1977).
17. S.W.H. is the recipient of a career development award (CA 00084) from the National Cancer Institute. We thank M. A. Smith for technical assistance.

13 October 1977; revised 9 January 1978

Ah Locus: Genetic Differences in Susceptibility to Cataracts Induced by Acetaminophen

Abstract. *The Ah^b/Ah^b homozygous and the Ah^b/Ah^d heterozygous inbred mouse strains from the (C57BL/6)(DBA/2)F_1 × DBA/2 backcross are genetically responsive to 3-methylcholanthrene. They both also develop, within 6 hours after a large intraperitoneal dose of acetaminophen, an irreversible opacity in the anterior portion of the lens. Such cataract formation does not occur in similarly treated nonresponsive inbred strains or nonresponsive Ah^d/Ah^d individuals from the same backcross. Differences in acetaminophen metabolism and toxicity are associated with the Ah locus in the mouse, and differences in heritability at the Ah locus exist in the human. Our ophthalmologic findings may be important clinically to certain patients receiving either a single large overdose of this drug or high doses over a long period.*

Lenticular opacification or cataract results from senility, congenital defects, viral infections, metabolic disorders, and various types of physical and chemical insult to the lens (*1*). Cataracts can also be induced in lenses in organ culture (*2*). Because of the diversity in cataractogenic agents, no single mechanism can account for the different forms of cataract. Osmotic imbalance produced by polyol accumulation within the lens was suggested to be responsible for sugar-induced cataract (*3*), and light-scattering by aggregated protein was suggested as a cause of senile cataracts (*4*). Cataracts induced by chemicals and drugs, especially naphthalene, have been extensively investigated because of their similarity to senile cataracts (*5*).

The Ah locus in the mouse controls the ability of polycyclic and halogenated aromatic compounds to induce the monooxygenase activities associated with cytochrome P_1-450 (*6*, *7*). The Ah

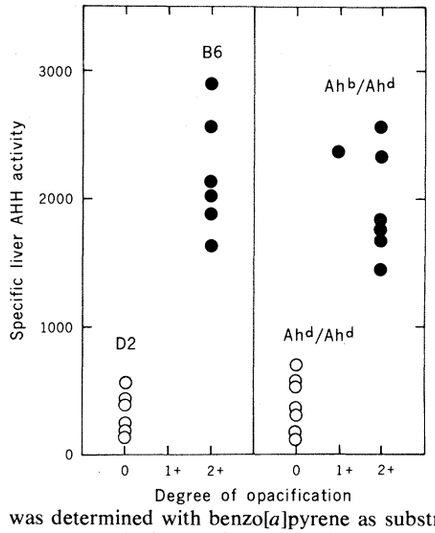

Fig. 1. Correlation between hepatic AHH inducibility and cataractogenesis in the nonresponsive D2 inbred strain, the responsive B6 inbred strain, and the nonresponsive Ah^d/Ah^d and responsive Ah^b/Ah^d progeny from the B6D2F_1 × D2 backcross. All inbred mice were obtained from The Jackson Laboratory (Bar Harbor), and all breeding was done within our own mouse colony. Sexually immature (5- to 6-week-old) mice of either sex were used. The mice were treated intraperitoneally with MC (200 mg per kilogram of body weight) in corn oil (25 ml/kg) 48 hours prior to acetaminophen (1000 mg/kg) in warm water (25 ml/kg). The acetaminophen was completely dissolved at the time of injection. No mice died before 6 hours at this dose. Eyes were evaluated with an ophthalmology slit lamp 5 hours later: 0, no signs of opacification; 1+, about 50 percent opacification; 2+, complete opacification. Mice were then immediately killed, and liver microsomal AHH activity was determined with benzo[*a*]pyrene as substrate (*18*). One unit is defined as that amount of enzyme catalyzing the formation of the hydroxylated product per minute at 37°C causing fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo[*a*]pyrene (*18*). Specific AHH activity denotes the number of units per milligram of microsomal protein. Seven nonresponsive Ah^d/Ah^d and seven responsive Ah^b/Ah^d weanlings from the B6D2F_1 × D2 backcross were genotyped with respect to the Ah locus by zoxazolamine paralysis time (*19*); these mice were then given MC and acetaminophen 2 weeks later.

0036-8075/78/0505-0539$00.50/0

locus can be correlated with the difference in responsiveness to such compounds between C57BL/6 (B6, responsive; Ah^b) and DBA/2 (D2, nonresponsive; Ah^d) inbred strains, although regulation of this response probably involves several alleles at more than one locus (*8*). Heterozygotes (Ah^b/Ah^d) are responders (*6, 8*). Responsiveness occurs not only in the liver but also in numerous nonhepatic tissues such as lung, kidney, bowel, skin, bone marrow, lymph nodes, retinal pigmented epithelium of the eye, ovary, testis, and mammary gland (*6*). Other effects of the Ah locus have recently been reviewed (*6*).

The presence of the Ah^b allele in mice is most easily determined by the induction by 3-methylcholanthrene (MC) of one of the P_1-450–mediated monooxygenase activities, aryl hydrocarbon hydroxylase (AHH) (*8, 9*). Figure 1 shows the correlation between the Ah^b allele and acetaminophen-caused cataracts. Hence, individuals in the same family develop or fail to develop cataracts after the same dose of the same drug, depending on this single allelic difference. In some studies (data not shown), we found that acetaminophen causes cataracts in other MC-treated responsive inbred strains such as A/J, CBA/J, and C3H/HeJ but not in other MC-treated nonresponsive inbred strains such as RF/J, AKR/J, SJL/J, and SWR/J.

Figure 2 illustrates the physical appearance of these mice and the histological appearance of their ocular tissues. A thin opalescent layer just anterior to the equatorial cortex of the lens is present, extending anteriorly to the visual axis. Subepithelial vacuolation was seen within the fibers of the opaque lens. The cornea remained clear, and the retina and retinal pigmented epithelium were normal by light microscopic examination. No vacuoles in the subepithelial layer of the lens of Ah^d/Ah^d mice were found.

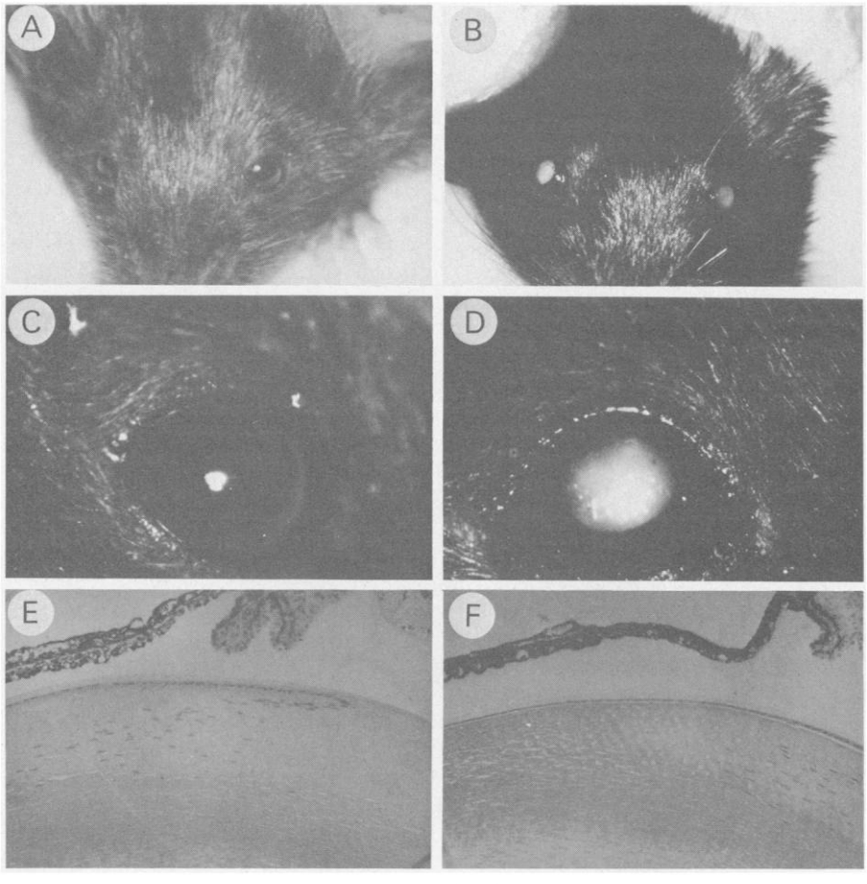

Fig. 2. (A and C) Genetically nonresponsive Ah^d/Ah^d homozygote; (B and D) genetically responsive Ah^b/Ah^d heterozygous sibling from the $B6D2F_1 \times D2$ backcross 5 hours after receiving acetaminophen and 53 hours after receiving MC, as described in Fig. 1. (E and F) Hematoxylin- and eosin-stained sections of ocular tissue from mice in (A) and (B), respectively (×500). The tissues from top to bottom are iris, cornea, and lens. Vacuoles seen in the subepithelial layer of the lens in (F) are the result of hydration of the lens cells and are always associated with cataract formation (*1*). Doses of more than 1000 mg of acetaminophen per kilogram of body weight were almost always fatal to Ah^b/Ah^d mice within the first 8 hours after acetaminophen administration, but these doses were not lethal, nor did they cause lens opacification to Ah^d/Ah^d mice. At lower doses of acetaminophen (400 to 800 mg/kg), the ocular opacity developed more slowly in Ah^b/Ah^d mice. If a cataract did not appear within 10 hours after acetaminophen administration, however, no cataract developed subsequently. The degree of opacification that had developed within these 10 hours was never reversible.

The mechanism of acetaminophen-induced cataract formation is uncertain. However, reactive intermediates of labeled acetaminophen, formed predominantly in the liver, are bound covalently at much higher levels in the lens and in numerous other tissues of the genetically responsive individual than in nonresponder mice (*10*). Prior treatment with phenobarbital, which enhances glucuronide conjugation of acetaminophen principally in the liver (*11*) and is independent of the Ah locus (*6, 12*), prevents cataracts in these responsive mice (*10*). There is much less acetaminophen-induced hepatotoxicity in phenobarbital-treated than in MC-treated animals (*11*). A reactive metabolite of acetaminophen may therefore pass into the aqueous humor from the bloodstream; the steady-state level of this reactive intermediate may be decreased by phenobarbital, in spite of more acetaminophen hepatotoxicity in phenobarbital-treated animals than in controls (*11*). The fact that the anterior portion of the lens shows opacification is consistent with the circulation pathway of aqueous humor from the posterior chamber across the front of the lens to the anterior chamber (*13*).

There is evidence (*6, 14*) for heritability of aromatic hydrocarbon responsiveness in the human. Moreover, cigarette smoking (*15*) and ingestion of charcoal-cooked foods (*16*) are known to induce these drug-metabolizing enzyme activities and thus may enhance acetaminophen metabolism to reactive intermediates. Chlorinated dibenzo-*p*-dioxins and dibenzofurans, and polychlorinated and polybrominated biphenyls are also known (*17*) to be potent inducers of these drug-metabolizing enzymes. A clinical history of cigarette smoking, ingestion of charcoal-cooked foods, and exposure to halogenated hydrocarbons that are environmental pollutants might be important in assessing differences in genetic susceptibility to drug-induced cataract formation.

Acetaminophen is a clinically proven analgesic and antipyretic and is indicated in diseases accompanied by discomfort and fever, such as the common cold and viral infections. It should be considered that long-term administration of high doses of acetaminophen may contribute to cataract formation. This possibility might be considered even more seriously in the elderly patient, in whom lenticular opacities may be diag-

nosed as senile cataracts. Drug-induced cataracts are similar in morphology to senile cataracts (*5*). Moreover, a straightforward causal relationship might be very difficult to prove clinically, if risk for cataract development is dependent on individual differences at the Ah locus in the elderly population.

HITOSHI SHICHI
Laboratory of Vision Research, National Eye Institute, Bethesda, Maryland 20014
DOUGLAS E. GAASTERLAND
Clinical Branch, National Eye Institute
NANCY M. JENSEN
DANIEL W. NEBERT
Developmental Pharmacology Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20014

References and Notes

1. R. van Heyningen, *The Eye* (Academic Press, New York, 1969), vol. 1, p. 381; *Cataract and Abnormalities of the Lens* (Grune & Stratton, New York, 1975).
2. H. Obazawa, L. O. Merola, J. H. Kinoshita, *Invest. Ophthalmol.* **13**, 204 (1974); J. Piatigorsky and T. Shinohara, *Science* **196**, 1345 (1977).
3. J. H. Kinoshita, *Invest. Ophthalmol.* **13**, 713 (1974).
4. A. Spector, L.-K. Li, R. C. Augusteyn, A. Schneider, T. Freund, *Biochem. J.* **124**, 337 (1971); J. A. Jedziniak, J. H. Kinoshita, E. M. Yates, L. O. Hocker, G. B. Benedek, *Invest. Ophthalmol.* **11**, 905 (1972); D. Roy and A. Spector, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3484 (1976).
5. D. R. Adams, *Br. J. Ophthalmol.* **14**, 49 (1930); R. van Heyningen and A. Pirie, *Biochem. J.* **102**, 842 (1967); J. R. Rees and A. Pirie, *ibid.*, p. 853; R. van Heyningen, *Invest. Ophtalmol.* **15**, 685 (1976).
6. S. S. Thorgeirsson and D. W. Nebert, *Adv. Cancer Res.* **25**, 149 (1977); R. E. Kouri and D. W. Nebert, in *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winston, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), vol. 4, p. 811.
7. Cytochrome P_1-450 is defined as those forms of microsomal CO-binding hemoprotein that increase when mice are treated with polycyclic aromatic inducers such as MC, β-naphthoflavone, benzo[*a*]pyrene, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*6*).
8. D. W. Nebert, J. R. Robinson, A. Niwa, K. Kumaki, A. P. Poland, *J. Cell. Physiol.* **85**, 393 (1975).
9. With benzo[*a*]pyrene as the in vitro substrate, AHH activity is measured by the rate of formation of 3-hydroxybenzo[*a*]pyrene and other phenols such as 9-hydroxybenzo[*a*]pyrene having similar wavelengths of fluorescent activation and emission (*6, 8*).
10. H. Shichi, N. M. Jensen, D. W. Nebert, in preparation.
11. D. J. Jollow, J. R. Mitchell, W. Z. Potter, D. C. Davis, J. R. Gillette, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **187**, 195 (1973); J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette, B. B. Brodie, *ibid.*, p. 211; W. Z. Potter, S. S. Thorgeirsson, D. J. Jollow, J. R. Mitchell, *Pharmacology* **12**, 129 (1974); J. R. Mitchell, S. S. Thorgeirsson, W. Z. Potter, D. J. Jollow, H. Keiser, *Clin. Pharmacol. Ther.* **16**, 676 (1974).
12. S. S. Thorgeirsson, P. J. Wirth, W. L. Nelson, G. H. Lambert, in *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winston, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1977), vol. 4, p. 869.
13. We have demonstrated an association between the Ah^b allele and AHH induction by polycyclic hydrocarbons in the pigmented epithelium of the eye [H. Shichi, S. A. Atlas, D. W. Nebert, *Exp. Eye Res.* **21**, 557 (1975)]. Other ocular tissues such as the ciliary body may possess a similar drug-metabolizing system and contribute directly to formation of a reactive acetaminophen metabolite within the eye.
14. G. Kellermann, M. Luyten-Kellermann, C. R. Shaw, *Am. J. Human Genet.* **25**, 327 (1973); S. A. Atlas, E. S. Vesell, D. W. Nebert, *Cancer Res.* **36**, 4619 (1976); S. A. Atlas and D. W. Nebert, in *Drug Metabolism—from Microbe to Man*, D. V. Parke and R. L. Smith, Eds. (Taylor & Francis, London, 1976), p. 393.
15. R. M. Welch, Y. E. Harrison, A. H. Conney, P. J. Poppers, M. Finster, *Science* **160**, 541 (1968); D. W. Nebert, J. Winker, H. V. Gelboin, *Cancer Res.* **29**, 1763 (1969); O. Pelkonen, P. Jouppila, N. T. Kärki, *Toxicol. Appl. Pharmacol.* **23**, 399 (1972); E. J. Pantuck, R. Kuntzman, A. H. Conney, *Science* **175**, 1248 (1972); J. Kapitulnik, W. Levin, P. J. Poppers, J. E. Tomaszewski, D. M. Jerina, A. H. Conney, *Clin. Pharmacol. Ther.* **20**, 557 (1976).
16. E. J. Pantuck, K.-C. Hsiao, A. H. Conney, W. A. Garland, A. Kappas, K. E. Anderson, A. P. Alvares, *Science* **194**, 1055 (1976); A. H. Conney, E. J. Pantuck, K.-C. Hsiao, W. A. Garland, K. E. Anderson, A. P. Alvares, A. Kappas, *Clin. Pharmacol. Ther.* **20**, 633 (1976).
17. D. R. Bickers, A. Kappas, A. P. Alvares, *J. Pharmacol. Exp. Ther.* **188**, 300 (1974); A. Poland and E. Glover, *Mol. Pharmacol.* **10**, 349 (1974); G. E. R. Hook, J. K. Haseman, G. W. Lucier, *Chem. Biol. Interact.* **10**, 199 (1975); A. P. Poland and E. Glover, *Mol. Pharmacol.* **13**, 924 (1977).
18. D. W. Nebert and J. E. Gielen, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1315 (1972).
19. J. R. Robinson and D. W. Nebert, *Mol. Pharmacol.* **10**, 484 (1974).
20. We thank co-workers G. H. Lambert and S. S. Thorgeirsson for discussions concerning early aspects of this work and Dr. J. H. Kinoshita for his critical reading of this manuscript.

11 November 1977; revised 6 January 1978

Delayed Hypersensitivity in Man: Effects of Systemic Anticoagulation

Abstract. *Skin test reactivity, lymphocyte transformation, and mononuclear cell tissue factor generation were evaluated both before and during systemic anticoagulation in 24 volunteers. Anticoagulation with warfarin decreased skin test induration and tissue factor generation, but lymphocyte transformation remained unchanged. An intact coagulation mechanism, including tissue factor generation, appears to be important for the development of skin test induration in humans.*

Various inflammatory lesions induced in experimental animals are characterized by fibrin deposition. These lesions or the fibrin deposition (or both) can be prevented by preliminary treatment of the animal with anticoagulants or fibrinolytic agents (*1–3*). Studies in animals and in man have suggested a correlation between the induration of delayed hypersensitivity skin lesions and intralesional fibrin deposition. No correlation has been found, however, between induration and the accumulation of mononuclear cells in the lesions (*4*). Immune-mediated deposition of fibrin polymers may participate in the formation of skin test induration by trapping fluid within the developing lesions (*1, 5*). Although the blood coagulation activation mechanism has not been elucidated, anticoagulation has been effective in decreasing both fibrin deposition and induration in experimental animals (*1–3, 6*). The evidence for interaction between the immune system and the intrinsic coagulation pathway via Hageman factor has been reviewed (*7*); however, it remains equally plausible that the coagulation cascade in these lesions is activated via the extrinsic pathway (that is, via the tissue factor pathway). Tissue factor is generated by human mononuclear cells in response to stimulation in vitro by mitogens or antigens, and has been found in association with mononuclear cells obtained from areas of intense in vivo immune reaction (*8, 9*).

We have studied skin test reactivity, in vitro lymphocyte transformation, and mononuclear cell tissue factor generation before and during the course of systemic anticoagulation in 24 normal volunteers. Our study supports the hypothesis that an intact coagulation mechanism, possibly involving mononuclear cell tissue factor, is requisite for optimal delayed hypersensitivity skin reactivity in man.

Twenty-four normal volunteers (mean age 36) were skin tested with four common antigens (*10*) and their blood was drawn for in vitro evaluation of lymphocyte transformation and mononuclear cell tissue factor generation after stimulation with the same antigens or two mitogens, phytohemagglutinin and pokeweed. Subsequently, each volunteer began a 9-day course of anticoagulation with oral sodium warfarin (Coumadin, Endo Laboratories). On day 7, after we determined that therapeutic levels of anticoagulation had been achieved as defined by prolongation of the prothrombin time to two to two and-a-half times the control value, skin testing and in vitro delayed hypersensitivity studies were repeated. Skin tests were evaluated on day 9, and warfarin was discontinued.

Regardless of whether 5- or 10-mm induration was the criterion of a positive skin test, there was a significant decrease in the total number of positive tests elicited during the period of anticoagulation (Table 1) (*11*). Table 1 also demonstrates a significant increase in the number of subjects who manifested anergy.

The mean diameter of skin test induration was decreased during anticoagulation (Fig. 1A) and was statistically significant for SK-SD (*10*) and mumps

0036-8075/78/0505-0541$00.50/0