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

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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

antigens (*12*). Skin test erythema, however, was not significantly altered. The PPD (*10*) antigen produced too few positive skin tests to allow evaluation of the data.

Although in vitro lymphocyte transformation was unaffected by anticoagulation (Fig. 1B), mononuclear cell tissue factor generation decreased significantly for PPD and SK-SD, and was of borderline significance for the mitogens (Fig. 1C). No correlation could be detected among units of tissue factor generated, prothrombin time, and degree of skin test induration in individuals. Warfarin affected neither skin test induration nor in vitro tissue factor generation after stimulation by the monilia antigen. This suggests the possibility that the immune response to monilia may be different from the response to either bacterial antigens or mitogens and may be independent of fibrin deposition. In vitro mononuclear cell cultures could not be evaluated for tissue factor generation after stimulation with mumps antigen because this antigen has an inhibitory effect on the assay system, and the inhibitor could not be separated by dialysis or filtration.

Table 1. The effect of anticoagulation on skin test reactions. Anergy was defined as a failure to develop skin test induration of 10 mm in response to one or more of four antigens. Numbers in parentheses represent the range.

Time of test	Prothrombin time* (seconds)	Factor II* (%)	Skin tests ≥ 10 mm† (*N*)	Skin tests ≥ 5 mm† (*N*)	Anergic patients‡ (*N*)
Before anticoagulation	11.3 ± 0.5 (10.2 to 12.4)		29/96	33/96	2/24
During anticoagulation	23.4 ± 2.2 (19.2 to 28.6)	25.8 ± 7.0 (18 to 35)	13/96	20/96	14/24
		P values§	< .01	< .05	< .01

*Mean ± 1 S.D. †Denominators refer to the total number of skin tests performed. ‡Denominator refers to the total number of patients studied. §*P* values were determined by chi-square tests.

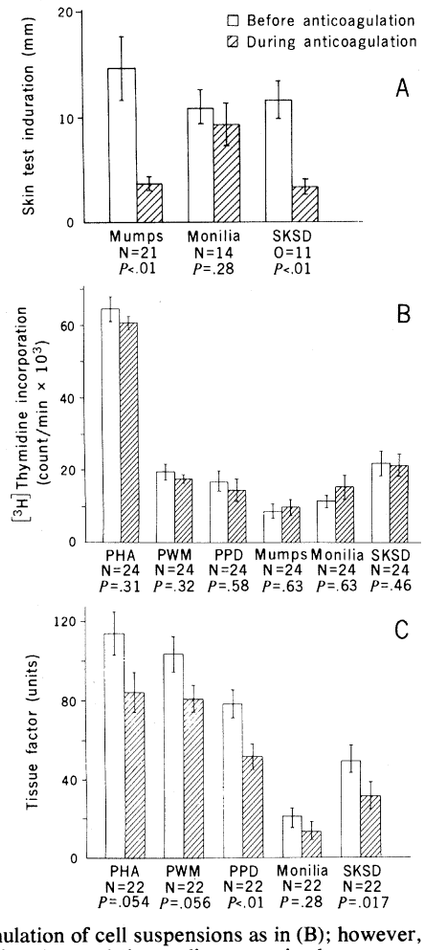

Fig. 1. Effect of anticoagulation on tests of delayed hypersensitivity. Statistical analysis was performed using a Student's *t*-test for paired data. (A) Skin test induration was evaluated 48 hours after intradermal injection of 0.1 ml of antigen (*10*) as the maximum diameter of palpable induration. Skin test antigens were the same strength and lot number both before and during anticoagulation; *N* refers to the number of skin tests evaluable (only tests which demonstrated measurable induration before anticoagulation were included). Each bar represents the mean diameter of induration (± 1 S.E.M.) in response to the indicated antigen. (B) Lymphocyte transformation measured [^{3}H]thymidine incorporation following in vitro stimulation of mononuclear cell suspensions before and during anticoagulation. Mononuclear cells separated from whole blood by isopycnic centrifugation (*8, 19*) were 97 to 99 percent viable as determined by trypan blue dye exclusion, and contained less than 3 percent polymorphonuclear leukocytes. Quadruplicate cultures at a concentration of 1×10^6 cell/ml were grown in RPMI 1640 medium plus 10 percent autologous serum in 0.2-ml round-bottomed microtiter wells, and were stimulated with optimal concentrations of mitogens or antigens (frozen in portions at −70°C). After the cultures were incubated in a 5 percent CO_2 atmosphere for 3 days (mitogens) or 5 days (antigens), 0.5 μCi [^{3}H]thymidine was added to each and incubation was continued for 4 hours before harvesting on glass fiber filters (*20*). Each bar represents the mean incorporation of radioactivity (count/min ± 1 S.E.M.) in cultures stimulated by the indicated mitogen or antigen. Unstimulated cultures contained < 500 count/min. (C) Mononuclear cell tissue factor generation was evaluated in vitro following stimulation of cell suspensions as in (B); however, the cells were grown in 0.5-ml cultures in plastic tubes and the medium contained no serum. Tissue factor activity after 24 hours of incubation was measured by the ability of a sonicated aliquot of the cell suspension to shorten the clotting time of Celite-adsorbed human plasma (*19*). Results were recorded in arbitrary units compared to a standard curve prepared from dilutions of a brain thromboplastin suspension. Each bar represents the mean tissue factor production (± 1 S.E.M.) in cultures stimulated by the indicated mitogen or antigen.

The delayed hypersensitivity reaction involves activation of a variety of mediators of inflammation (for example, migration inhibition factor, chemotactic factor, and so forth) (*13*). Blood coagulation and fibrin deposition have also been noted to be prominent in inflammatory reactions. The hypothesis that an intact clotting mechanism is important for the development of a maximum skin test response to foreign antigens is further supported by the observed inhibition of skin test induration following anticoagulation. We suggest that the sequence of events leading to positive delayed hypersensitivity skin tests may involve the following: (i) recognition of the injected material as foreign and attraction of cells to the skin test site, (ii) production of increased amounts of mononuclear cell tissue factor, (iii) local initiation of fibrinogen conversion to fibrin via the extrinsic pathway, and (iv) trapping of water in the fibrin meshwork resulting in increased induration. Since warfarin impairs the synthesis of vitamin K–dependent clotting factors (II, VII, IX, and X), it may inhibit fibrin deposition, trapping of water, and subsequent skin test induration. Warfarin may also decrease fibrin deposition by inhibiting tissue factor generation.

We have reported here that systemic warfarin, given in a dose that produces therapeutic hypoprothrombinemia, inhibits tissue factor generation in stimulated mononuclear cells and maximal development of delayed hypersensitivity skin test induration in humans. These results suggest a role for tissue factor in the immune-related fibrin deposition of skin test reactions although further experiments are needed to demonstrate its absolute requirement in the delayed hypersensitivity response. The mechanism responsible for the decrease of in vitro tissue factor generation is not clear but could be due to a direct effect of warfarin on cellular function (for example, uncoupling of oxidative phosphorylation, inhibition of mitosis, cell killing) (*14*), or interference with vitamin K–dependent γ-carboxylation of glutamic acid residues in an intermediary protein or in tissue factor itself (*15*).

The discovery that therapeutic doses of warfarin inhibit both tissue factor generation and skin test induration suggests a potential rationale for its use in the treatment of immune diseases characterized by fibrin deposition. Renal allograft rejection, lupus nephritis, glomerulonephritis, and experimental encephalomyelitis have been treated with anticoagulant drugs (*16, 17*).

Skin test reactivity is widely used as a screen for specific diseases; it is also used to assess the integrity of the cell-mediated immune system (*18*). Our results suggest that skin test induration must be interpreted with care in anticoagulated patients.

RICHARD L. EDWARDS
FREDERICK R. RICKLES
Department of Medicine, University of Connecticut School of Medicine and *Veteran's Administration Hospital, Newington, Connecticut 21077*

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11. Five subjects had initial SK-SD skin tests more than 2 weeks prior to initiation of anticoagulation, leading to a prolonged interval between initial and repeat SK-SD skin tests. This length of time may allow deterioration of the protein antigen which is subject to autodigestion. The other subjects had all skin tests within a 10-day period, and all antigens utilized were known to be stable for at least that length of time. The statistical significance of the results was found to be unchanged if all skin test results were included in the analysis.
12. When the results for all four skin test antigens were pooled, the decrease in induration was also significant ($P < .01$); however, the paired t-test may not be applicable to the analysis of data pooled in this fashion (Dr. Joseph Sheehan, Biostatistician, Department of Research in Medical Education, University of Connecticut School of Medicine, personal communication).
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* Address reprint requests to Building 5, Research, Newington VA Hospital, Newington, Conn. 06111.

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Saccharin-Induced Sister Chromatid Exchanges in Chinese Hamster and Human Cells

Abstract. *Since the induction of sister chromatid exchanges in cultured cells has been shown to be the most sensitive mammalian system to detect the effects of mutagenic carcinogens, Chinese hamster ovary cells and human lymphocytes were exposed to the sodium saccharin found to induce bladder cancer in rats. Both that saccharin and a highly purified extract of it increased the yield of sister chromatid exchanges in both types of cells. The results, which were repeatable and statistically highly significant, indicated that the weak carcinogen, saccharin, is also mutagenic in the sense that it induces cytogenetic changes.*

The noncaloric artificial sweetener saccharin has recently been shown to induce bladder cancer in rats (*1*). This was particularly true for saccharin-fed male offspring of females that also had been fed saccharin. The carcinogenicity of the substance has led to the proposal that its use be banned in the United States, where in 1967 it was estimated that 75 percent of the population used an average of 20 mg per person per day [see (*2*)].

An excellent correlation has been found between the carcinogenicity of organic compounds and their mutagenicity

Table 1. Induction of SCE's in CHO cells by saccharin.* Abbreviation: NG, no growth.

Treatment	Number of SCE's/ number of chromosomes	SCE's per chromosome	SCE's per cell
	Experiment 1		
Control	875/2027	0.432 ± 0.015	8.75 ± 0.30
Saccharin (S1022) (%)			
0.1	953/2003	0.476 ± 0.015†	9.53 ± 0.30
0.5	995/2027	0.491 ± 0.016‡	9.95 ± 0.32‡
1.0	1246/2028	0.614 ± 0.017§	12.46 ± 0.35§
5.0	NG	NG	NG
	Experiment 2		
Control	845/1967	0.430 ± 0.015	8.45 ± 0.29
Saccharin (S1022) (%)			
1.0	1294/1982	0.653 ± 0.018§	12.94 ± 0.36§
1.5, 2.0, 2.5, 3.0	NG	NG	NG
	Experiment 3		
Control	855/1947	0.439 ± 0.015	8.55 ± 0.29
Saccharin (pure) (%)			
0.5	1021/1969	0.519 ± 0.016‡	10.21 ± 0.32§
1.0	1121/2006	0.559 ± 0.017§	11.21 ± 0.33§
1.5, 2.0, 5.0	NG	NG	NG
	Experiment 4		
Control	872/1979	0.441 ± 0.015	8.72 ± 0.30
Saccharin (pure) (%)			
0.8	1105/1987	0.556 ± 0.017§	11.05 ± 0.33§
1.0	1196/1996	0.599 ± 0.017§	11.96 ± 0.35§
1.2, 1.4	NG	NG	NG

*100 cells per point. †$P < .05$. ‡$P < .01$ (P values from t-test). §$P < .001$.

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