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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10. Purified protein derivative of the tubercle bacillus (PPD, 5 tuberculin units per 0.1 ml; Connaught Laboratories, Toronto); mumps antigen (Eli Lilly, Indianapolis, Ind.); Monilia (Dermatophyton "O", 1/100 dilution; Hollister-Steir, Yeardon, Pa.); and streptokinase-streptodornase (SK-SD), 0.5 units per 0.1 ml (Varidase, Lederle Laborotories, Pearl River, N.Y.).
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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in short-term microbial tests that can detect many of the various types of mutations (*3*). Because mutations that are the result of large chromosomal changes cannot be induced in bacteria, however, and because of differences in the spectrum of mutations that can be induced in prokaryotic and eukaryotic cells, short-term cytogenetic tests have been used to detect the ability of compounds to induce aberrant chromosomes. Saccharin has been found to be weakly mutagenic in several of these cytogenetic tests, which are not very sensitive [for review see (*2*)].

Recently, a far more sensitive cytogenetic test than the standard chromosome aberration test has been used extensively to detect the attack by mutagenic carcinogens on the DNA in mammalian chromosomes. This is the induction of sister chromatid exchanges (SCE's) in cells that have been cultured so that the sister chromatids in a chromosome will stain differently from one another (*4–9*). We have performed short-term SCE tests both of the sodium salt of saccharin (lot S1022, Maumee process, Sherwin-Williams) used in the carcinogenicity study cited above and of a highly purified extract of the same material (impurities, 1 to 5 ppm). The test was carried out in cultured Chinese hamster ovary (CHO) cells and in human lymphocytes. In both types of cells the original and the purified saccharin increased the numbers of SCE's induced.

To see sister chromatid exchanges, cells were grown for two rounds of DNA replication in the presence of 10 μM 5-bromodeoxyuridine (BrdUrd). At the end of this time each chromosome contained one unifilarly and one bifilarly substituted chromatid. These sister chromatids stained differently from one another with the fluorochrome plus Giemsa, or harlequin chromosome technique of Perry and Wolff (*10*), which consists of staining the cells with the fluorochrome Hoechst 33258, exposing them to light, and then restaining with Giemsa. In these preparations (Fig. 1) SCE's can be clearly seen and scoring can be done with great ease and precision (*11*).

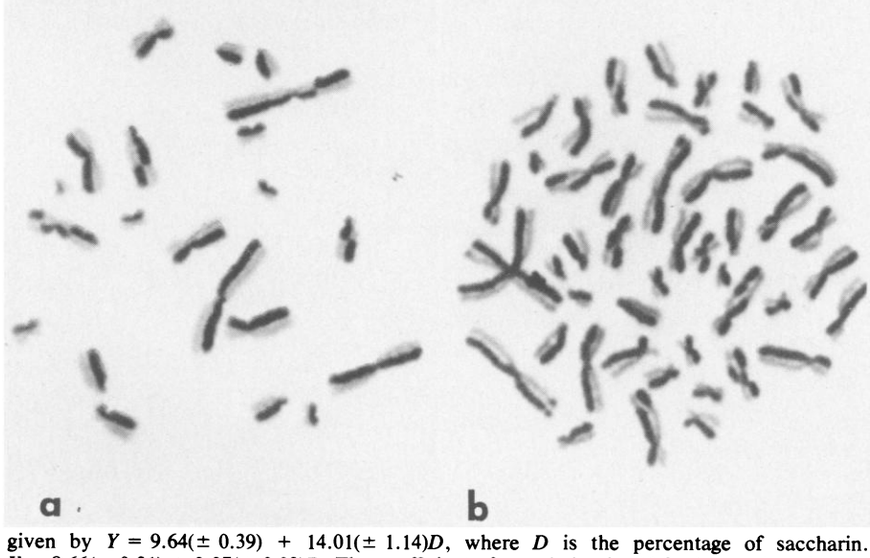
Corning plastic tissue flasks (75 cm^2) were seeded with 5×10^5 CHO cells in 10 ml of McCoy's 5A medium containing 15 percent fetal calf serum, 100 units of penicillin per milliliter, and 100 μg of streptomycin per milliliter. The cells were grown at 37°C in 5 percent CO_2. After 20 to 24 hours the medium in the exponentially growing cultures was replaced with the same medium supplemented with 10 μM BrdUrd and various concentrations of the saccharin. Twenty-two hours later, when the cells had replicated twice in the presence of BrdUrd, $2 \times 10^{-7} M$ Colcemid was added and 2 hours after this the mitotic cells were collected.

Cultures containing 0.2 ml of freshly drawn human blood in 5 ml of McCoy's 5A medium with the same supplements as those used for the CHO studies were grown at 37°C in 5 percent CO_2 for 72 hours. The medium contained 20 μM BrdUrd and various concentrations of saccharin. Colcemid was added for the last 4 hours and the cells were collected by centrifugation. In such cultures a large proportion of the dividing lymphocytes have replicated twice and contain harlequinized chromosomes.

Table 2. Induction of SCE's in human lymphocytes by saccharin. Cells cultured 72 hours in 20 μM BrdUrd. Abbreviation: NG, no growth.

Treatment	Number of SCE's/ number of chromosomes	SCE's per chromosome	SCE's per cell
	Experiment 1		
Control	981/4588	0.214 ± 0.007	9.81 ± 0.31
Saccharin (S1022) (%)			
0.1	1169/4592	0.255 ± 0.007*	11.69 ± 0.34*
0.5	1711/4594	0.372 ± 0.009*	17.11 ± 0.41*
1.0, 1.5	NG	NG	NG
	Experiment 2		
Control	950/4592	0.207 ± 0.007	9.50 ± 0.31
Saccharin (S1022) (%)			
0.3	1311/4595	0.285 ± 0.008*	13.11 ± 0.36*
0.5	1607/4598	0.349 ± 0.009*	16.07 ± 0.40*
0.6 to 0.9†	NG	NG	NG
Saccharin (pure) (%)			
0.3	1322/4590	0.288 ± 0.008*	13.22 ± 0.36*
0.5	1745/4596	0.380 ± 0.009*	17.45 ± 0.42*
0.6 to 0.9†	NG	NG	NG

*$P < .001$ by t-test. †0.6, 0.7, 0.8, and 0.9 percent.



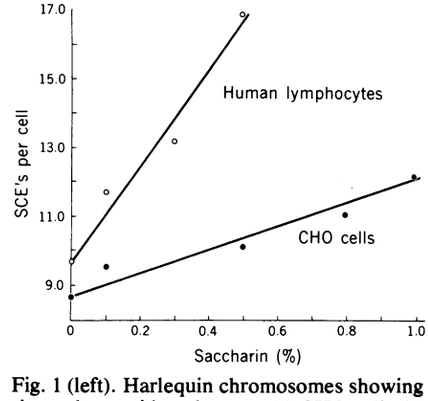


Fig. 1 (left). Harlequin chromosomes showing sister chromatid exchanges. (a) CHO cell. (b) Human lymphocyte. Fig. 2 (right). Linear regressions of SCE's per cell versus concentration of saccharin. All data combined. For human lymphocytes the linear regression is given by $Y = 9.64(\pm 0.39) + 14.01(\pm 1.14)D$, where D is the percentage of saccharin. For CHO cells the regression is given by $Y = 8.66(\pm 0.21) + 3.37(\pm 0.32)D$. The coefficient of correlation is .98 for human lymphocytes and .96 for CHO cells.

Both CHO cells and human lymphocytes were treated with 0.075*M* KCl to spread the chromosomes and were fixed in a mixture of methanol and acetic acid (3:1). The cells were concentrated by centrifugation and drops of the concentrated fixed cells were placed on clean dry microslides, which were then stained for 15 minutes in 0.5 μg of Hoechst 33258 per milliliter in 0.067*M* Sorensen's buffer (*p*H 6.8), washed, mounted with the buffer and a cover glass, and exposed to approximately 30,000 J/m² of light from a Hanovia 100-watt mercury bulb (exposure, 30 seconds to 1 minute). The slides were then washed in distilled water and stained for 10 to 20 minutes in 3 percent Giemsa (Gurr's R66 in Sorensen's buffer, *p*H 6.8). One hundred differentially stained cells were scored at each point.

Because 5 percent saccharin was fed to the rats in the carcinogenicity test, this was the highest concentration used. In fact, no growth was observed either in CHO cells treated with more than 1 percent saccharin or in human lymphocytes treated with more than 0.5 percent saccharin (Tables 1 and 2). In no case did the saccharin change the *p*H of the medium as evidenced by its color. The experiments were highly repeatable. Four control experiments with CHO cells contained 8.45 to 8.75 SCE's per cell. This increased to 11.21 to 12.94 SCE's per cell after treatment with 1 percent saccharin.

In human lymphocytes the number of SCE's per cell increased from a control value of 9.5 to 9.8 to a value of 16.01 to 17.45 at 0.5 percent saccharin. In both cases the *P* values, as determined by the *t*-test of statistical significance, were far lower than .001. Lower concentrations of saccharin induced fewer SCE's.

The linear regressions of SCE's versus dose of saccharin are presented in Fig. 2; a greater increase was found in human lymphocytes than in CHO cells. It is not certain, however, whether this represents a difference in sensitivity between the two cell types, for, although the slopes of the curves differ by a factor of 4, the human lymphocytes were exposed to saccharin three times longer than were CHO cells. The increase with dose seen for both cell types stands in contrast to a recently published study with Chinese hamster cells from the DON line (*12*) in which the saccharin results were variable. Only a slight increase was found at all doses, leading the authors to conclude that saccharin "did not cause a pronounced increase in the frequency of SCE."

The present results show that saccharin can induce SCE's and that, in human and other mammalian cells, the highly sensitive short-term SCE test is capable of detecting cytogenetic effects of a weak carcinogen that does not induce reversions at the histidine locus in the *Salmonella* test (*13*).

SHELDON WOLFF
BRITA RODIN
Laboratory of Radiobiology and *Department of Anatomy, University of California, San Francisco 94143*

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Myoadenylate Deaminase Deficiency: A New Disease of Muscle

Abstract. *Five cases of a new disease presented with muscular weakness or cramping after exercise; three of the cases also had an elevated serum creatine phosphokinase. Muscle biopsies were histologically normal but lacked adenylate deaminase by stain and solution assay, while the erythrocyte isozyme was normal. A clinical diagnostic test has been developed, and the human enzyme was separated by acrylamide-gel electrophoresis.*

Application of a new histoenzymatic stain (*1*) to frozen muscle biopsies has uncovered a new disease: myoadenylate deaminase deficiency. The occurrence of five cases in a series of 250 biopsies suggests that the disease must be fairly common, although it has not been recognized in the past. The main clinical and laboratory features are listed in Table 1. All were young males with a chief complaint, often since childhood, of muscle weakness or cramping after exercise (or both). Physical and neurological examinations were normal except for decreased muscle mass, hypotonia, and weakness in some cases. Skeletal growth and dentition were normal. Blood cell counts and blood chemistry, urinalysis, x-rays, and other laboratory data have regularly been normal, except for mildly elevated creatine phosphokinase values and nonspecifically abnormal electromyograms, which have generally been the reasons for the subsequent muscle biopsy.

Biopsies were evaluated at the Armed Forces Institute of Pathology with a series of histochemical stains: hematoxylin and eosin, periodic acid Schiff, crystal violet, modified Gomori trichrome, alkaline adenosinetriphosphatase (with and without prior incubation with acid), alkaline phosphatase, NADH (reduced nicotinamide adenine dinucleotide)-tetrazolium reductase, succinate dehydrogenase, phosphorylase, and nonspecific esterase on the fresh-frozen specimen; and electron microscopy on the clamped, glutaraldehyde-fixed specimen (*2*). Four of the cases had entirely normal histology and one had evidence of mild type 1 atrophy. Histograms were prepared (*2*) for cases 1, 2, and 3 where fiber orientation was suitable but showed no impressive abnormalities. Case 1 had a normal distribution and mean size of fiber types for his age and biopsy site but an increased coefficient of variation for type 2B fibers (32 percent). Case 3 had only 3 percent type 2B fibers, and 2 percent of the type 1 fibers were smaller than 40 μm in diameter. Case 2 had only 9 percent type 2B fibers, and 5 percent of the type 1 fibers were less than 40 μm in diameter. A normal complement of type 2B fibers appeared to be present in cases 4 and 5.

All five cases showed no reaction in the adenylate deaminase stain, and this was confirmed quantitatively by solution assay of homogenized microtome sections (*3*) (Table 2) compared to nine controls. To verify tissue integrity, two other highly soluble enzymes were also assayed: adenosine deaminase (*3*), which is very low in muscle, and creatine phos-

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