sensitivity to T_3, particularly with replacement doses. The capacity to respond, however, seemed not to be altered because high doses of T_3 elicited the same response in starved and fed animals. The decrease of $\dot{V}O_2$ in starvation, in the presence of adequate replacement doses of T_3, favors the hypothesis that the sensitivity of $\dot{V}O_2$ to T_3 provides a finer and more efficient control on thyroid hormone activity than that brought about by alterations in thyroid hormone metabolism.

Three groups of investigators (*6*) have recently found a decrease in hepatic nuclear T_3 receptors in starved rats. Tata *et al.* (*7*) described in this situation a lack of stimulation of protein synthesis, even when supraphysiological doses of T_3 (50 μg/100 g) were given. More specifically, Tarentino *et al.* (*8*) and Dillmann *et al.* (*9*) reported that the induction of the cytosolic enzyme, malic dehydrogenase, known to be inducible by thyroid hormones, is blocked by starvation. The similarity between these subcellular changes and thermogenesis is striking. Even though thermogenesis is considered to be one of the major actions of thyroid hormones, it represents a late event and many unrelated factors could modify the interaction of these hormones and thermogenesis. Although this relationship may be complex, its physiological relevance can hardly be doubted and might serve as the basis for more analytical studies of the mechanisms involved in thyroid hormone action.

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5. The values for $\dot{V}O_2$ before and 6, 12, 48, and 72 hours after the injection were, respectively, as follows. Hypothyroid rats (when fed): 1.32 ± 0.01; 1.34 ± 0.03; 1.58 ± 0.03 ($P < .001$); 1.38 ± 0.02 ($P < .05$); and 1.28 ± 0.02 ml of O_2 per minute per 100 g of body weight. Hypothyroid rats (when starved): 1.16 ± 0.02; 1.15 ± 0.02; 1.17 ± 0.03; 1.07 ± 0.03, and 1.04 ± 0.03. Euthyroid rats (when fed): 1.66 ± 0.03; 1.69 ± 0.03; 1.74 ± 0.03; 1.68 ± 0.04, and 1.60 ± 0.04 (analysis of variance and multiple comparisons of means by Scheffé). Unless otherwise specified, the values were not significant in comparison to the baseline.
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Sister Chromatid Exchanges in Human Lymphocytes After Exposure to Diagnostic Ultrasound

Abstract. *The frequency of sister chromatid exchanges increased in freshly isolated human lymphocytes as well as in a continuously growing lymphoblast line by exposure to diagnostic levels of ultrasound for 30 minutes. The results confirm previous findings indicating that ultrasound of diagnostic intensities can affect the DNA of animal cells.*

The use of ultrasonic studies in medical diagnosis has greatly increased, particularly in obstetrics. It is estimated that by the mid-1980's virtually all infants in the United States will have been exposed to ultrasound in utero (*1*). Diagnostic levels of ultrasound are now regarded as innocuous to the developing fetus. Investigators have been unable to demonstrate deleterious effects of ultrasound on human chromosomes (*2–7*). However, as recently reported (*8, 9*), exposure to pulsed ultrasound generated by a commercial diagnostic instrument appeared to have deleterious effects on rapidly growing mammalian and insect tissues. We have detected disturbances in HeLa cell DNA and in the growth characteristics of C3H mouse cell cultures exposed to ultrasound in the diagnostic range (*10*). Here we report the effects of ultrasound on the incidence of sister chromatid exchange (SCE), a sensitive direct indicator of chromosome damage (*11, 12*). Exposure to diagnostic levels of ultrasound increased the frequency of SCE's in freshly isolated human lymphocytes as well as in a continuously growing human lymphoblast line (SKL-7).

Human blood containing heparin (10 U/ml) was allowed to stand for 60 minutes so that the leukocytes were separated by gravity from the erythrocytes. The supernatant serum containing lymphocytes was dispersed into growth medium at a concentration of approximately 2×10^5 white cells per milliliter of medium. The lymphocytes were grown in the dark in McCoy's 5A medium containing 15 percent fetal bovine serum, phytohemagglutinin-16 (PHA-16, 1 μg/ml; Burroughs Wellcome), and heparin (5 to 10 U/ml). Bromodeoxyuridine (BrdU), at a final concentration of 5 to 10 μg/ml (Sigma), was added approximately 22 hours after the culture was initiated. The lymphocytes were harvested 72 hours later, after completion of two rounds of DNA replication. Colcemid (0.2 μg/ml) was added for 2 hours to arrest cells in metaphase, and the cells were then resuspended in 0.075*M* KCl for 10 minutes and fixed in three changes of a 3:1 mixture of absolute alcohol and glacial acetic acid. The human SKL-7 lymphoblast line was grown under dark conditions in McCoy's 5A medium containing 20 percent fetal bovine serum in the presence of 10 μg of BrdU per milliliter for two division cycles (approximately 36 hours) before the cells were harvested, as in the experiments with peripheral blood lymphocytes.

Differential staining was obtained by a modification of the fluorescence plus Giemsa technique (*13*). Metaphase spreads were stained with Hoechst 33258 (1 μg/ml) for 10 minutes, exposed to an ultraviolet light source (General Electric, G8T5) for 15 minutes at a 5-cm distance, and then stained with 10 percent Giemsa solution in phosphate buffer at *p*H 7 for 5 minutes.

The cells were exposed to ultrasound between their first and second divisions (48 hours after addition of the mitogen in the fresh lymphocyte culture, and at 18 hours in the lymphoblast line). This was done because it has been shown that cells must pass through S-phase in order to express ultraviolet damage by SCE (*14, 15*). In a number of experiments, 0.1 μg of mitomycin per milliliter was added to portions of the lymphocytes in separate cultures to provide control preparations with high frequencies of SCE's.

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The arrangements for the ultrasonic exposure of the cell suspensions and for the determination of the exposure dosimetry have been described (*10*). In brief, a sterile polypropylene test tube filled to the brim with the cell suspension was covered with a freshly prepared parafilm strip and placed in a 37°C water bath. In these experiments, ultrasonic exposure was provided by a Reflectoscope UM727 (Sperry Rand Co.) clinical diagnostic ultrasonoscope operating at 700 pulses per second, with an unfocused 2.0 MHz transducer having a 13-mm piezoceramic disk. The transducer was coaxially aligned on the parafilm for the 30-minute exposure period; a commercial transmission gel was used as a couplant.

When measured on a radiation force balance, the total time-averaged acoustic power of the ultrasonic beam was estimated to be 4.5 mW. Schlieren study of the sound field within the test tube has shown that little of the sound field leaks out of the side walls of the tube and approximately 35 percent of the incident beam is reflected back into the tube from the bottom. The reflected wave does not superpose on the incident wave. Thus, if one assumes that all of the sound field is confined to the tube, the average intensity of the cross section of the 17 mm diameter tube is 2.7 mW/cm^2. At the focal point of the spherical bottom, however, the intensity of the reflected wave could be approximately 50 percent greater than that of the incident wave, so that in this region the temporal average intensity may be as high as 5.0 mW/cm^2.

Fresh human lymphocytes exposed to diagnostic ultrasound showed a small but significant increase in the number of SCE's (Table 1); similar results were obtained with the lymphoblast line (Table 2). In the last two experiments with lymphocytes (Table 1), $5 \times 10^{-4}M$ caffeine was added to the culture at 22 hours to enhance the sensitivity of human lymphocytes to a possible mutagenic action (*16*). However, no such enhancement occurred.

The number of background SCE's varied in lymphocytes from different donors and in different experiments. Cells with the lowest spontaneous incidence of SCE's showed the greatest response to ultrasound. The addition of mitomycin caused a four- to fivefold increase in SCE incidence.

Metaphase spreads were examined in each experiment (Tables 1 and 2) and karyotyped under the microscope at the time the SCE's were scored. Since the SCE's were not specific for particular chromosomes, it is unlikely that the small increase in SCE frequency can be accounted for by an increased susceptibility to damage by ultrasound in a particular chromosome or group of chromosomes.

The mechanism of SCE is unknown. According to Kato (*12*), single-strand breaks in both chromatids occur in the genesis of SCE as a direct result of damage or as the initial step in the repair process. However, certain powerful mutagens, such as ionizing radiation, evoke only a slight increase in SCE frequency (*11*). *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), a known carcinogen, causes only small increases in SCE frequency that are neither dose-related nor enhanced by the addition of caffeine to human lymphocytes (*16*). Ethyl methanesulfonate is mutagenically more potent than methyl methanesulfonate, yet it is a less powerful inducer of SCE's (*12*). Paradoxically, the effect of some weak carcinogens can be detected readily by the induction of SCE (*17*). By contrast, a study of azaguanine resistance as a genetic marker for mutation in Chinese hamster ovary (CHO) cells indicated a linear relationship exists between induced SCE's and mutations in the CHO cell system for four chemicals that were tested (*18*). Although linear relationships between SCE frequency and mutagenic potential have not been demonstrated in human lymphocytes, most investigators believe that an increased SCE frequency indicates chromosome damage.

In our earlier studies, ultrasonic exposure at medical diagnostic levels induced immunoreactivity to rabbit antibodies to nucleosides in the G_1 phase of HeLa cells (*10*). Since the antibodies reacted only with single-stranded DNA, the results suggested that ultrasound

Table 1. Induction of sister chromatid exchanges (SCE's) in peripheral blood human lymphocytes by ultrasound.

Treatment	Number of SCE's/number of chromosomes	SCE's per chromosome	SCE's per cell	*P*
		Experiment 1		
Control	253/1380	0.183 ± 0.014	8.43 ± 0.64	< .0005
Ultrasound	426/1150	0.370 ± 0.021	17.04 ± 0.96	
		Experiment 2		
Control	456/3220	0.142 ± 0.006	6.51 ± 0.26	< .001
Ultrasound	633/3220	0.197 ± 0.007	9.04 ± 0.33	
		Experiment 3		
Control	130/920	0.141 ± 0.010	6.50 ± 0.50	< .002
Ultrasound	180/920	0.196 ± 0.013	9.00 ± 0.60	
		Experiment 4		
Control	574/2300	0.249 ± 0.013	11.48 ± 0.61	< .01
Ultrasound	521/1748	0.298 ± 0.015	13.71 ± 0.68	
		Experiment 5		
Control	203/920	0.221 ± 0.014	10.15 ± 0.65	< .05
Ultrasound	260/920	0.283 ± 0.015	13.00 ± 0.67	
		Experiment 6		
Control	277/1840	0.151 ± 0.009	6.93 ± 0.42	< .001
Ultrasound	408/1840	0.222 ± 0.011	10.20 ± 0.49	
		Experiment 7		
Control plus caffeine	317/1840	0.172 ± 0.011	7.93 ± 0.51	< .002
Ultrasound plus caffeine	412/1840	0.224 ± 0.012	10.30 ± 0.55	
		Experiment 8		
Control plus caffeine	315/1932	0.163 ± 0.008	7.50 ± 0.39	< .0005
Ultrasound plus caffeine	424/1932	0.219 ± 0.011	10.10 ± 0.49	

*P values from Student's t-test.

Table 2. Induction of sister chromatid exchanges (SCE's) in SKL-7 human lymphoblast line by ultrasound.

Treatment	Number of SCE's/number of chromosomes	SCE's per chromosome	SCE's per cell	*P*
		Experiment 1		
Control	470/2313	0.203 ± 0.008	9.35 ± 0.38	< .0005
Ultrasound	574/2296	0.255 ± 0.009	11.72 ± 0.44	
		Experiment 2		
Control	754/2384	0.32 ± 0.008	14.72 ± 0.39	< .0005
Ultrasound	892/2350	0.38 ± 0.013	17.48 ± 0.62	

*P values from Student's t-test.

damaged DNA. Such damage was confirmed by autoradiography of cells exposed to [^{3}H]thymidine, which indicated that DNA repair had been initiated by ultrasound (*10*). Both single-strand scission and repair mechanisms have been implicated in the basic mechanism of SCE production, and it is not surprising that ultrasound increased the frequency of SCE in the present studies. The magnitude of the effect was small, but the increase was detectable in every experiment.

Our results with cells exposed to ultrasound in vitro cannot be directly extrapolated to the clinical situation where the ultrasound probe is in constant motion and no one region is continuously exposed to the beam. Most clinical examinations of the fetus are of short duration and the surrounding maternal tissues attenuate the ultrasonic beam. However, our findings suggest that ultrasound may not be entirely innocuous.

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Thyrotropin-Releasing Hormone Selectively Depresses Glutamate Excitation of Cerebral Cortical Neurons

Abstract. *The microiontophoretic application of thyrotropin-releasing hormone causes a selective reduction in neuronal excitation evoked by* L-*glutamate but not by acetylcholine in rat cerebral cortex. Thyrotropin-releasing hormone has no influence on the activity of acetylcholinesterase or on choline uptake and release from cerebral synaptosomes. This evidence for a selective interaction between a centrally acting peptide and an excitatory amino acid neurotransmitter may indicate a specific locus of thyrotropin-releasing hormone action at glutamate-activated receptor sites.*

Thyrotropin-releasing hormone (TRH) can directly influence central nervous system (CNS) function, independent of its role as a releasing hormone (*1*). The proposal that this direct CNS effect results from the action of TRH as a neurotransmitter or neuromodulator has been supported by several observations: (i) TRH is present in the brain of several vertebrate and nonvertebrate species, including those that lack a thyroid gland (*2*); (ii) TRH is localized within neurons (*3*); (iii) TRH is concentrated within, and can be released from, preparations of synaptosomes (*4, 5*); (iv) high-affinity stereoselective TRH binding sites are present within the brain (*6*); and (v) exogenous applications of TRH can alter central neuronal excitability (*7, 8*). In previous studies (*8*), we indicated that microiontophoretic application of TRH is associated with a decrease in spontaneous or glutamate-evoked activity in several brain regions—an observation that has prompted further examination of the specificity of the action of TRH. We now report that in the cerebral cortex of the rat, TRH selectively antagonizes neuronal excitation evoked by L-glutamate. TRH has less of an effect on L-aspartate-evoked excitation and no significant effect on acetylcholine-evoked excitation.

Extracellular recordings were obtained from neurons in the sensorimotor cortex of male Sprague-Dawley rats anesthetized with pentobarbital (35 mg/kg, intraperitoneally) or urethane (1.25 g/kg, intraperitoneally). Conventional amplification methods were used to record extracellular activity from micropipettes filled with 2*M* NaCl that were rigidly attached to multibarreled micropipettes filled with solutions for testing by microiontophoresis (*9*). TRH from different sources and in different concentrations and solvents (*10*) was applied as a cation; currents ranged up to a maximum of 100 nA. Additional channels contained sodium chloride (0.2*M*) for use as a current control and sodium chloride (2.0*M*) for use in an automatic current balancing circuit (*11*).

Experiments on 42 neurons examined the influence of TRH on the excitatory actions of two putative amino acid neurotransmitters, L-glutamate and L-aspartate. In 35 of these neurons, the application of TRH was associated with a rapid, readily reversible reduction in amino acid-induced excitation, without any significant alteration in spike amplitude or shape. When applied with stronger ejection currents, TRH appeared to diminish excitation evoked by both amino acids; however, when applied with weaker ejection currents, the action of TRH was more specific and most pronounced in the glutamate-evoked responses (Fig. 1). This action of TRH on amino acid-evoked excitability was considerably more prominent than its relatively weak depressant action on the spontaneous activity of 3 of 26 neurons tested.

To examine the specificity of this depressant action, TRH was applied during excitations induced by acetylcholine on cholinoceptive cortical neurons (*12*).

Table 1. Thyrotropin-releasing hormone and acetylcholinesterase activity, uptake of [^{14}C]choline in pellet, and K^+-evoked release of ^{3}H from [^{3}H]choline. The number of experiments is given in parentheses.

TRH (μg/ml)	AChE activity* (μmole/hour-mg protein)	Uptake of [^{14}C]choline† (nmole/5 min-mg protein)	K^+-evoked release of ^{3}H from [^{3}H]choline
Control	3.09 ± 0.68 (6)	1.05 ± 0.05 (9)	2505 ± 475 (3)
1	3.20 ± 0.62 (6)		
5		1.03 ± 0.09 (3)	
10	2.90 ± 0.70 (6)		
20		1.02 ± 0.02 (3)	
50	3.00 ± 0.50 (6)		2510 ± 610 (3)

*Values represent average activity (mean ± standard error) of three assays from the same P_2 preparation. †Protein concentration, 1 to 3 mg/ml.

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