case), in 8- by 8- by 8-inch wire-mesh cages (*8*). The cage groups were maintained in the animal colony until they had reached assigned test ages, when they were exposed to 100 r of x-radiation per day, 5 days per week, for the duration of life. The cages were transported to the x-ray cubicle and were irradiated; the mice that they contained were in a vertical x-ray beam that delivered about 15 r/min 0.5 inch above the cage floor. Dose rates were measured with Victoreen condenser chambers. Radiation factors were: 200 kvp (peak) at 15 ma from a half-wave generator; 0.5 mm Cu and 1.0 mm Al added filtration; half-valve layer, 0.9 mm Cu.

The distributions of survival times after the beginning of treatment are given in Table 1. Mean after survivals (MAS), their standard error (SE), the standard deviations of the survival curves (SD), and the mean accumulated doses (MAD) are also given.

In the period from 100 to 600 days of age, the mean accumulated dose shows comparatively little dependence on age, but in the period from 600 to 800 days of age, it decreases rapidly.

The results found here are in fairly good accord with those of two LD_{50} studies that covered the same age ranges. Zirkle *et al.* (*1*) and Kohn and Kallman (*3*) found little change in LD_{50} in the first year, and Hursh and Casarett (*2*) found LD_{50}'s of 715 r for 6-month-old, and 600 r for 16-month-old, rats. Studies currently under way indicate that in mice there is a small but significant increase in LD_{50}/30 days and MAD/30 days during the period from 100 to 400 days of age (*9*). All of these data are consistent in indicating that the change of radiosensitivity with age is small during the first half of the adult life span.

The dependence of radiosensitivity on age in the later part of the life span has not been reported previously. The data presented here indicate that sensitivity

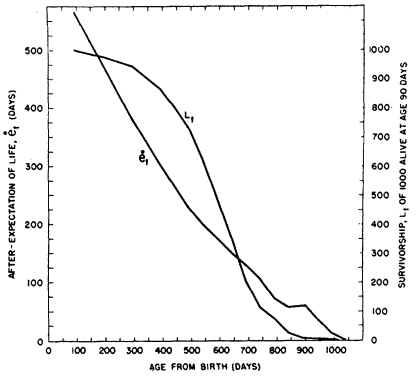

Fig. 1. Survivorship and after-expectation curves for untreated CF No. 1 female mice that were maintained in the same laboratory environment as the mice that were tested for radiosensitivity.

increases rapidly with age in this period. Although the samples available for test at advanced ages were small, the downward trend observed at ages greater than 600 days is highly significant. This is confirmed by current studies (*9*).

The survivorship curve and the after-expectation of life curve for untreated CF No. 1 female mice is given in Fig. 1. These curves are based on a large sample of mice that were maintained in the same laboratory environment contemporaneously with the mice that were tested for radiosensitivity.

One of the reasons for making this study on the age-dependence of radiosensitivity was to test the adequacy of a set of postulates about the lethal action of ionizing radiations. In brief, the postulates specified that injury is proportional to dose, that recovery is proportional to amount of injury, that all kinds of radiation injury sum with the effects of age, and that injury due to aging accumulates as a linear function of age. These postulates had been enunciated by me (*8*) and, in a basically similar form, by Blair (*10*). An important deduction was that the curve of LD_{50} versus age should have approximately the form of the curve of after-expectation of life; that is, it should be approximately linear during the first half of life and concave upward throughout adult life. The curve of mean accumulated dose versus age (Table 1) departs markedly from this form. In view of the available data on LD_{50} versus age, and of the form of the radiosensitivity curve found in this study, it is concluded that one or more of the postulates referred to in the first part of this paragraph must be revised (*11*).

GEORGE A. SACHER

Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois

Table 1. Survival times of CF No. 1 female mice exposed to 100 r 5 days per week, mean after-survival times (MAS), standard errors of means (SE), standard deviations of survival times (SD), and mean accumulated dose (MAD). Day 0 was the day of first exposure.

Age at exposure (day)	Survival time (day)											*n*	MAS	SE	SD	MAD
95	16	18	21	21	23	25	26	28	29	30	36	11	24.8	1.8	5.8	1827
151	24	31	32	35	36	36	37	38	39	41		10	34.9	1.6	4.9	2560
263	13	21	27	28	36	37	38	38	38	41		10	31.7	2.9	9.1	2320
375	14	25	32	36	38	44	44	49				8	35.3	4.1	11.5	2562
375	18	31	33	36	36	36	38	39	42	48		10	35.7	2.5	7.8	2620
487	18	32	32	34								4	29.0	3.7	7.4	2175
543	24	28	29	30	32	35	46					7	32.0	2.5	6.6	2329
599	15	16	23	32	35	41						6	27.0	4.3	10.6	1983
599	27	32	33									3	30.7	1.9	3.2	2300
711	4	18	28									3	16.7	6.9	12.0	1267
711	16											1	16.0			1200
823	3	6										2	4.5			400

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5. This work was performed under the auspices of the U.S. Atomic Energy Commission. I am indebted to John Sackis, Betty Halstead, and Mildred Ogden for valuable assistance in the performance of the experiment.

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18 February 1957

Oxidation of Chlorpromazine by Peroxidase and Catalase

The *in vivo* and *in vitro* conversions of chlorpromazine to its sulfoxide have been observed (*1, 2*). The mechanism of oxidation has not been described. This report is concerned with the oxidation of the drug by hydrogen peroxide in the presence of the enzymes peroxidase and catalase. Peroxide oxidation of phenothiazines generally has long been known (*3*); however, under the conditions prevailing in biological systems, the uncatalyzed peroxidation is sluggish.

It was found that in the range *p*H 3 to 6.3 chlorpromazine was readily converted to a deep red substance by hydrogen peroxide and peroxidase. A similar result was obtained with catalase over a more restricted *p*H range, no color appearing above *p*H 4.5. In both cases, the solutions bleached at rates dependent on the relative concentrations of the reagents. Both the extent of color production and the rate of bleaching

were appreciably lower in the presence of catalase than they were when peroxidase was used. At very low peroxide concentrations and fairly high chlorpromazine concentration, the bleaching was retarded, and the absorption spectrum of the red material could be examined (Fig. 1*A*). When bleaching was complete, the ultraviolet absorption spectra of the enzymic reaction mixture, chlorpromazine, and chlorpromazine sulfoxide were compared. The spectral comparison (Fig. 1*B*) indicates that the sulfoxide was probably present in the product mixture.

Descending paper chromatograms were developed with the solvent mixture recommended by Salzman and Brodie (*2*). Aliquots of the enzymic reaction mixture were taken at several intervals after the reaction was started. Within 10 minutes, a spot identified as that of the sulfoxide was detectable, and within 30 minutes the sulfoxide spot was strongly colored, while the chlorpromazine spot showed a greatly reduced color intensity. Sampling of the completely bleached reaction mixture several hours later resulted in the appearance of a very strong sulfoxide spot. In no case did more than two spots appear. Either the red product was not detectable with the sulfuric acid spray, or its migration was identical with that of chlorpromazine. Catalase oxidation produced the same result, giving spots having R_f values of 0.82 to 0.84 and 0.93 to 0.96. Control R_f values for the sulfoxide and chlorpromazine run under the same conditions were, respectively, 0.77 to 0.86 (0.82 average) and 0.89 to 0.97 (0.93 average).

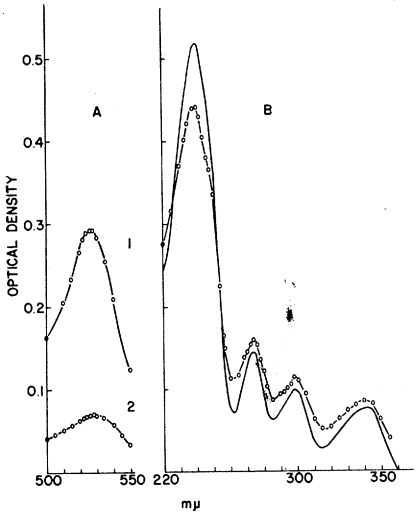

Fig. 1. Absorption spectra of enzymic oxidation products. (*A*) Curve 1: H_2O_2 ($1.7 \times 10^{-5}M$), chlorpromazine hydrochloride ($9.2 \times 10^{-4}M$), and horse radish peroxidase (Worthington; 1.3 μg/ml) in 0.1*M* acetate buffer at *p*H 4.5. Curve 2: H_2O_2 and chlorpromazine as for curve 1 and crystalline beef liver catalase (Worthington; 13 μg/ml) in 0.06*M* acetate buffer at *p*H 4.5. The blanks contained all components except H_2O_2. (*B*) Solid line: chlorpromazine sulfoxide ($10^{-5}M$). Curve showing experimental points: the initial reaction mixture contained chlorpromazine · HCl ($1.6 \times 10^{-4}M$), H_2O_2 ($3.8 \times 10^{-3}M$), and peroxidase (3 μg/ml) in 0.06*M* acetate buffer at *p*H 5.5. After 30 minutes, a sample was diluted 1/15 with buffer for spectrophotometry. Both curves represent measurements made in the same buffer. The blanks contained all components except chlorpromazine.

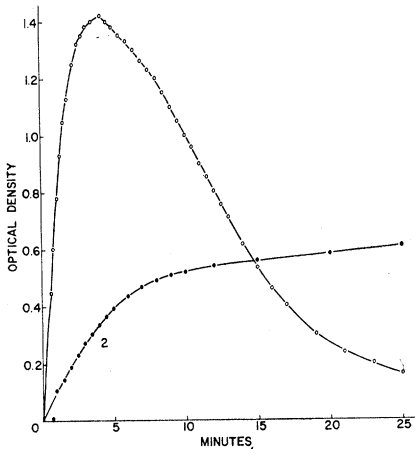

Fig. 2. Time courses of formation and decay of enzymic oxidation products. Curve 1: red intermediate; chlorpromazine · HCl ($9.7 \times 10^{-4}M$), H_2O_2 ($1.76 \times 10^{-4}M$), and peroxidase (2.3 μg/ml) in 0.1*M* acetate at *p*H 4.5. Measurement was made with the photomultiplier attachment at 527 mμ, slit 0.09 mm, and approximately 25°C. The blank contained all components except the enzyme. Curve 2: same conditions as in curve 1 except that chlorpromazine and enzyme concentrations were reduced to $9.7 \times 10^{-5}M$ and 0.23 μg/ml, respectively. Measurements were made at 274 mμ, slit 2.1 mm, no photomultiplication. The blank contained all components except the enzyme.

A qualitative indication of the time-courses of formation and decay of the red product and the formation of the sulfoxide was obtained by following absorbancy changes at 527 and 274 mμ (Fig. 2). The rapidity of the former reaction and the relative slowness of the latter suggested that the red product was a precursor of the sulfoxide. Since the blank cuvettes in these experiments contained all components except the enzyme, compensation for any nonenzymic peroxidation was made automatically. Indeed, no slit adjustment was necessary during either experiment, and it was concluded that no measurable uncatalyzed oxidation had occurred. It has been shown *in vivo* (*2*) that the sulfoxide itself undergoes further metabolism. Since the systems described here yielded the sulfoxide as the terminal product, it was clear that, at best, these oxidations could not account for any steps beyond sulfoxide formation in the over-all metabolism.

The metabolic significance of catalase and peroxidase is not entirely clear, nor is it certain that mammalian peroxidases would carry out the reactions described here for the horse radish enzyme. It is doubtful that these reactions represent more than interesting models of the actual mammalian oxidations. It is of interest that Tauber (*4*) has also shown that crystalline catalase is capable of oxidizing various large molecules.

It may be noted that, in tissues that are capable of generating respective amounts of hydrogen peroxide metabolically, the possible effects of catalase or peroxidases (even free hemoglobin) would be difficult to eliminate in studies on phenothiazine oxidation (*5*).

D. J. Cavanaugh
Biochemical Laboratory, Southeast Louisiana Hospital, Mandeville

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5. Chlorpromazine hydrochloride (Thorazine) and chlorpromazine sulfoxide were generously supplied by the Smith, Kline and French Laboratories, Philadelphia, Pa.

25 February 1957

Sphingosine as an Inhibitor of Blood Clotting

One of us has isolated from brain tissue a powerful clot-delaying substance which has been identified as sphingosine (*1, 2*). This substance is a component of cerebrosides and sphingomyelins, but it is also found free as a contaminant in preparations of the latter, thus acounting for their inhibitory effect in blood clotting (*2*). Pure sphingomyelin is indifferent in blood-clotting reactions.

Sphingosine has also been isolated from the lipid activator of pig brain (*3*), and it seems to be the active principle of antithromboplastin (also called anticephalin) (*4*). According to Tocantins and Carroll (*5*), antithromboplastin appears in the blood of hemophiliacs in abnormally high quantities. With sphingosine and chicken plasma, interesting reactions are observed. With small quantities, clotting times are prolonged to 40 hours or more. However, in the presence of the lipid activator from pig brain, the inhibitory effect of sphingosine is abolished, and the clotting times may even be shorter than those obtained with optimal concentrations of the lipid activator alone. This may be called "sphin-