$$(\pi'\pi'') = [f(1)g(2) + f(2)g(1)] \cos(\varphi_1 - \varphi_2),$$

where $\pi' \equiv fe^{i\Phi}$; $\pi'' \equiv ge^{i\varphi}$.

[5] Usually the stability in the AO method of the symmetric form $(1s_a\ 1s_b)$, as compared with $1s_a(1)\ 1s_b(2)$ alone, is said to be due to the occurrence of a *negative* exchange integral instead of the usual positive integral found for atoms. A detailed analysis reveals, however, that the apparent negative exchange energy in $(1s_a\ 1s_b)$ is the sum of a positive exchange energy as for atoms, plus other numerically larger negative terms of the same nature as the *resonance* terms which make H_2^+ stable as compared with H plus H^+ (cf. R. S. Mulliken, *Chem. Rev.*, **9**, 354 (1931)).

[6] An interesting short-cut toward BPAO's is that proposed by Gurnee and Magee (*J. Chem. Phys.*, **18**, 142 (1950)), who took σ as $1s^{(Z)}$ centered about a point somewhat displaced from nucleus a toward nucleus b.

[7] Hirschfelder, J. O., and Linnett, J. W., *J. Chem. Phys.*, **18**, 130 (1950).

[8] Coulson, C. A., and Fischer, I., *Phil. Mag.* [7], **40**, 386 (1949); also Slater, J. C., *Phys. Rev.*, **82**, 538 (1951).

[9] The amount of configuration interaction, i.e., the value of γ, is of course considerably greater here than if BMO's had been used for $1\sigma_g$ and $1\sigma_u$; the same sort of thing is always expected when wave functions are not based on BAO's or BMO's.

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THE RADIATION SENSITIVITY OF CATALASE AS A FUNCTION OF TEMPERATURE

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Introduction.—A complete solution to the problem of the direct action of radiation on large molecules necessitates the knowledge of three things: (1) the amount and form of energy lost in passing through the material being investigated, (2) the manner in which this energy is dissipated in the material and (3) the response of the system to the energy put into it. If one uses a type of radiation whose physical effects are well known, then the sensitivity of large molecules to this radiation can give information about the structure of the molecule. If the radiation employed is a beam of fast deuterons, we can be reasonably certain of the first item.[1] The manner in which the energy released in a molecule is dissipated is not well known, but an extension of the present work should shed additional light on this problem. We will be primarily concerned here with the response of an aggregate of catalase molecules to the energy released by the passage of deuterons through them. Charged particle irradiation of large molecules with specific functions usually gives the result that one primary ionization inactivates the entire molecule.[2, 3] However, previous work on the irradiation of catalase by deuterons[4] indicated that an ionization within

the catalase molecule did not destroy all its catalytic activity. Some viruses show results qualitatively similar to those obtained for catalase, and the usual reason given for the failure of an ionization to inactivate them is that the released energy only destroys a part of the structure, and the rest is able to function by itself.[1] In the case of T-1 bacteriophage it has been shown that the radiation sensitivity increases as the bombardment temperature increases.[5] The present work reports somewhat similar results for the irradiation of catalase by deuterons. The results will be analyzed in terms of structure of the catalase molecule.

Experimental Procedure.—In order to eliminate any indirect effects due to a suspending medium, all the irradiations were carried out upon dry catalase. The catalase used was derived from beef red cells.† Samples for bombardment were prepared by pipetting 0.05 ml., containing amounts up to 100 μg. solids, onto small glass coverslips. The coverslips were dried in a vacuum desiccator and then placed around the periphery of a circular copper disk which was put in an insulated bombardment chamber of the cyclotron in which the pressure was $<10^{-4}$ mm. Hg. The disk could be rotated from outside the chamber so as to expose different samples to the deuteron beam. The current carried by the deuteron beam was measured by a galvanometer. The area of the beam was measured from the blackening of photographic film placed in the beam, and the time of exposure was controlled by an electrically operated shutter. Further details may be found elsewhere.[3]

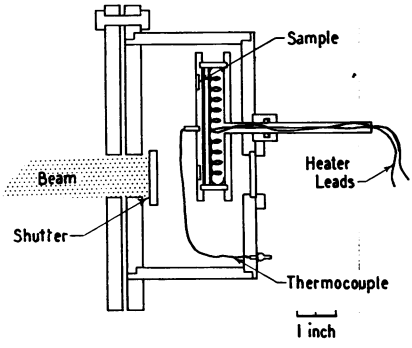

FIGURE 1

The bombardment chamber with provision for raising the temperature during irradiation.

For irradiations above room temperature, the bombardment disk was heated electrically, as shown in figure 1. The temperature during the bombardment was measured by a calibrated chromel-alumel thermocouple. The temperature was kept within ± 2°C. of the average temperature. For bombardments below room temperature a device similar to that shown in figure 1 was used, except that the electrical heater was absent. The inside of the bombardment disk could then be filled with liquids at different temperatures, such as liquid air or a water-ice mixture.

After bombardment the samples were removed from the cyclotron, the catalase dissolved off the coverslips in water and assayed for enzymatic activity by comparing the amount of hydrogen peroxide decomposed in a

known time with a previously constructed calibration curve. The activity of control samples, which underwent the same treatment except for irradiation, was also determined. The hydrogen peroxide was determined either by the amount of I_2 it formed from KI[4] or from its absorption at 247mμ as found in a Beckman spectrophotometer.[6]

If the samples are deposited on quartz disks, it is possible to determine how much material is left on the coverslip after immersion in water by measuring the absorption of the disk in the ultra-violet region of the spectrum. Data on irradiated and control samples indicated that at least 90 per cent of the material was dissolved off.

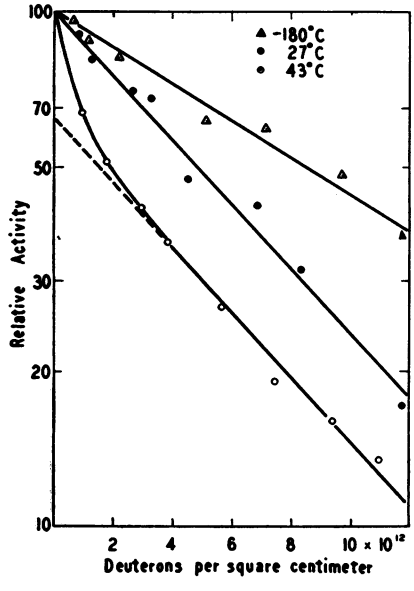

FIGURE 2

A semilog plot of the relative activity of catalase vs. the number of incident deuterons/cm.^{2} for several irradiation temperatures.

The energy of the deuterons used was 3.8 m. e. v. It is possible to calculate from published data[7] that these deuterons, in passing through protein, will lose on the average 235 e. v./100A. It is known that fast charged particles can lose their energy in three ways: by ionization, excitation and nuclear recoils. For 3.8-m. e. v. deuterons only the first two are of significance, and because of the low quantum yield for excitation,[8] we shall neglect the second and consider that the important energy loss item is that due to ionization. It is known from analysis of energy loss by charged particles in gases that the average energy lost per ionization is about 110 e. v. We shall assume the same value holds for solids.[1] This primary ionization can of course give rise to energetic electrons which can produce ionizations of their own.

Results.—Some of the experimental results are shown in figure 2. The relative activity of catalase is plotted on a logarithmic scale against the number of deuterons per cm.^{2}. It is seen that the room temperature curve is a straight line. This indicates that the relative activity may be given by an expression of the form $\frac{A}{A_0} = e^{-SD}$, where A_0 is the activity of the controls, A the activity of the samples irradiated by a dose of D deuterons per cm.^{2} and S is a proportionality constant which we shall call the inac-

tivation cross-section. This expression of course simply indicates that the loss of activity under irradiation is a random affair, just as is radioactive decay. If we want to be literal about it, we can give the proportionality constant S physical significance. S has the units of an area, and thus it can represent the cross-section of a volume which is inactivated by the passage of a deuteron through this volume. For example, from the data taken at room temperature, one can compute that the cross-section corresponds to 1420 A^2. This is seen by finding the dose for which $\frac{A}{A_0} = e^{-1} = 0.368$. The cross-section is then simply the reciprocal of this dose. Since catalase has a molecular weight of 225,000,[9] and thus a cross-sectional area of about 6000 A^2, the inactivation cross-section is obviously too small. But a deuteron can pass through a catalase molecule without losing any energy at all, since the distribution of primary ionizations along a deuteron path is a random one. The chance of such a miss may be found by using bombarding particles of different velocities.[2] Previous work with different energy deuterons and with 2-m. e. v. electrons,[10] which because of their very sparse ionization give an inactivation volume instead of cross-section, indicates that a cross-section of 1420 A^2 corresponds to a volume whose molecular weight (assuming a density of 1.33 g./cm.^{3}) is about 110,000. Thus if we assume catalase has a molecular weight of 225,000, then a primary ionization at room temperature knocks out one-half of its activity. It is seen in figure 2 that irradiation at liquid-air temperature yields a significantly smaller cross-section than at room temperature.

Data taken at 43°C. give a more complex curve than at room temperature. This curve, however, can be resolved into at least two components, one of which has the same cross-section as the room temperature curve. The rapid decline in activity at low doses has been associated with a partial heat inactivation of catalase.[4] It should be pointed out, however, that dry catalase is not appreciably inactivated for several days at a temperature of 43°C. Since the reasons for this initial rapid decline in some of the curves obtained are not well understood at present, we shall neglect these data and concentrate on the part with the small cross-section. Other typical data are shown in figure 3. While cross-sections from these data may be obtained with high precision, it is only fair to recognize that the absolute accuracy is nowhere nearly as good. The major source of this error lies in the possible lack of uniformity in the cross-section of the deuteron beam and in the calibration of the beam galvanometer.

Figure 4 is a compilation of data obtained from runs similar to those already presented. Each point represents one run except for 300° and 90°K. which represent averages of five runs each. The general trend is for the cross-section to increase as the temperature goes up. This increase, however, is not by any means uniform. There definitely seem to

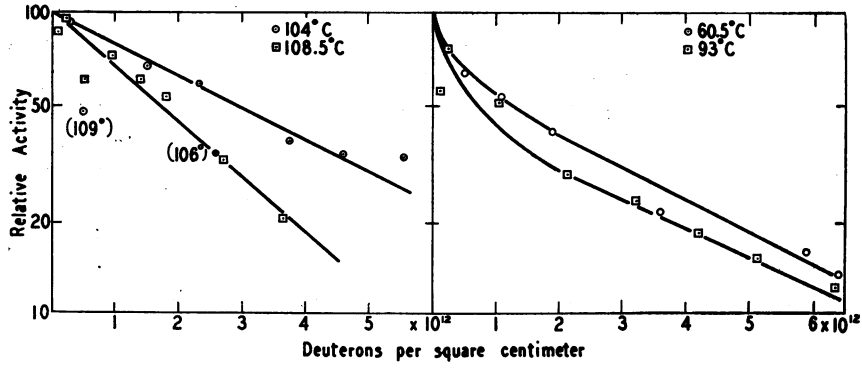

FIGURE 3

A semilog plot of the relative activity of catalase vs. the number of incident deuterons/cm.^{2} for several irradiation temperatures. Note the similar slopes for 60.5° and 93°C. and the big difference between 104° and 108.5°C. The points of the 104°C. run which were at a higher temperature are indicated.

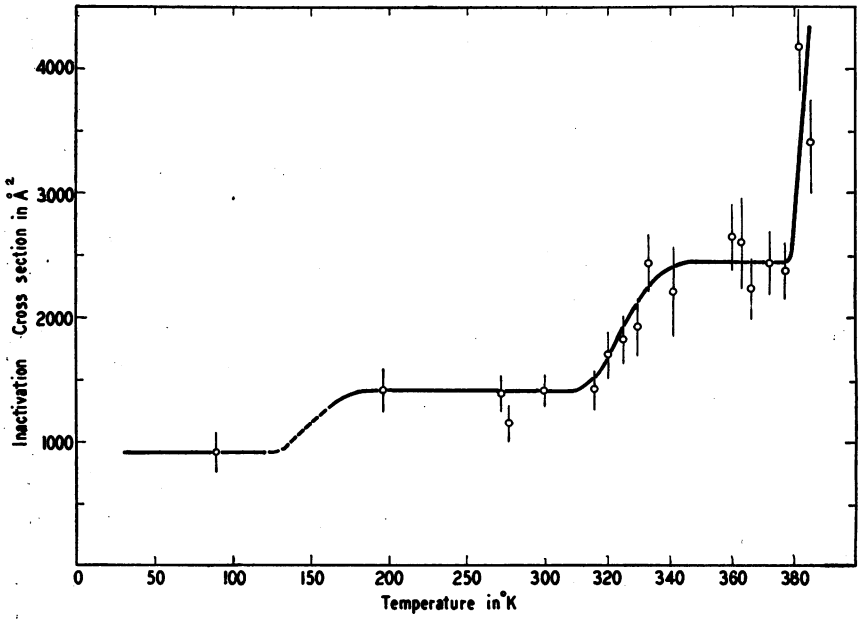

FIGURE 4

The inactivation cross-section for 3.8-m. e. v. deuterons vs. the temperature during irradiation. The temperature scale is not uniform.

be temperature intervals over which the cross-section remains constant. Somewhat similar results have been found for invertase.[11] At the highest temperatures indicated the rate of heat inactivation is appreciable. At 385°K. the specific rate constant for inactivation is about 2×10^{-3} sec.$^{-1}$.[4]

Discussion.—In the analysis that follows we shall assume that the molecular weight of catalase is 225,000. As mentioned previously, we can take other data to indicate that a cross-section of 1420 A^2 corresponds to the inactivation of a molecular weight of 110,000, or just about one-half the molecule, by a primary ionization.

The only thing that we are varying in this experiment is the manner of degradation of the energy released within the catalase molecule by an ionization. Since the average energy released for all the temperatures involved is the same, the relative cross-sections at different temperatures are measures of the sensitivity of the molecule at these different temperatures. Because a molecule is a three-dimensional structure, it is more significant to calculate the volumes associated with these cross-sections. Since the volume depends upon the 3/2 power of the area, it is a simple matter to find the volume inactivated by a primary ionization at various temperatures, from the experimental observation that 1420 A^2 corresponds to a molecular weight of 110,000 inactivated per primary ionization. The results have been put in terms of molecular weights since these are more recognizable than volumes. We thus obtain the values presented in table 1.

TABLE 1

THE MOLECULAR WEIGHT INACTIVATED PER PRIMARY IONIZATION FOR DIFFERENT TEMPERATURES

TEMPERATURE, °K.	CROSS-SECTION, A^2	MOLECULAR WEIGHT PER PRIMARY IONIZATION	MOLECULAR FRACTION PER PRIMARY IONIZATION
300	1420	110,000	½
90	925	58,000	¼
350	2450	250,000	1
385	4200	500,000	2

Qualitatively there is a simple explanation for these results. At liquid-air temperatures all the bonds in the molecules are in their ground vibrational states. Any large amount of localized energy introduced into the molecule will be degraded to thermal energy within a relatively small volume. As the temperature is increased the energy will still fall off rapidly with distance from the site of introduction, but because some of the higher vibrational levels are now excited, it is easier for a new molecular configuration to arise. The data indicate that the catalase molecule can function in parts. If this were not so, the cross-section versus temperature curve would show a steady increase with temperature. Since catalase has

four heme groups, the correlation between the molecular fraction inactivated and parts of the molecule is excellent. It is of interest that there is no evidence for a cross-section plateau corresponding to three-quarters of the molecule. This may mean that a quarter catalase molecule is inactive, as has been suggested,[12] or that inactivation of three-quarters of the molecule alters it so drastically that its entire structure is changed, and it no longer is a closely knit entity. At temperatures above 380°K. the cross-section rises above that corresponding to the whole molecule. At these temperatures the heat inactivation rate is large, and any slight additional energy could cause inactivation of the entire structure. If this energy comes from a deuteron passing outside the molecule, owing, for example, to a secondary electron or an energetic excitation, then obviously one would measure a volume greater than the size of the molecule.

Summary.—The sensitivity of catalase to irradiation by 3.8-m. e. v. deuterons depends on the temperature at which the bombardment is performed. From 90* to 385°K. three different regions of constant sensitivity have been found. They correspond to inactivation of one-quarter, one-half and the entire enzymatic activity of the molecule. There is no evidence for the inactivation of three-quarters of the molecule.

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† Armour Powdered Catalase 30 from Armour and Company, Chicago, Ill.

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