ENZYME SUBSTRATE REACTIONS IN HIGH MAGNETIC FIELDS

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ABSTRACT The reaction rates of two enzyme substrate systems, ribonuclease-RNA and succinate-cytochrome c reductase, were followed as a function of magnetic field from zero to 48,000 gauss. The reaction rates remained constant to within 10 per cent.

INTRODUCTION

The literature of biomagnetic phenomena has recently been surveyed by Jacobius (1) who concluded that it "combines the oldest with the newest, the highly speculative with the factual, and the pseudoscientific with the scientifically sound. To make it worse, the validity of most of the findings in this field is still a matter of controversy, and there appears to be no clear concept as to what biological and physiological effects are to be ascribed to magnetism proper or to thermal and other incident factors." In spite of this bleak summation, two events have caused renewed interest in the subject. The first is the commercial availability of superconducting magnets which are capable of generating fields to 50,000 gauss and higher. Such fields were hitherto not generally available to experimenters. The second, and potentially the more important, is the theoretical work of Little (2) on the possible existence of superconductivity in certain kinds of macromolecules[1] and its relationship to enzyme reaction mechanisms and specificities.

Little chose for theoretical analysis a hypothetical macromolecule consisting of a long, one dimensional, conjugated chain of carbon atoms to which were attached, in a periodic fashion, side groups of easily polarizable dye molecules. His results show that there may exist an attractive potential among the delocalized electrons of the conjugated spine analogous to that which gives rise to superconductivity in metals according to the theory of Bardeen, Cooper, and Schrieffer (3). Whereas in a metal the attractive potential results from interactions between electrons and lattice ions, the governing interactions in the polymer are those of the mobile electrons of the spine with the polarizable side groups. Perhaps the most remarkable property of such a polymer is that the transition temperature, between the normal

[1] This possibility was first raised by F. London, *in* Superfluids, volume I, New York, John Wiley & Sons, Inc., 1950.

and superconducting state, is much higher than room temperature and is of the order of 2000 K. A property common to all superconductors is that the transition temperature depends on the ambient magnetic field so that a superconductor may revert to the normal state when a magnetic field of sufficient strength is applied. An empirical relationship between H_c, the critical magnetic field required to quench superconductivity at $T = 0$, and T_c, the transition temperature for the onset of superconductivity at zero magnetic field, has been found[2] to be $H_c = A\ T_c^{\frac{1}{2}}$. For metallic superconductors the constant, A, lies in the range 60 to 110 (for H_c in gauss and T_c in degrees Kelvin). If we assume that this formula may be extrapolated from low temperatures ($T \sim 1$ to 10 K) to high temperatures ($T \sim 2000$ K), a value of $H_c = 2 \times 10^5$ gauss should then characterize the superconducting state of the macromolecule.

The pertinent feature of superconductors that is sensitive to, and ultimately destroyed by, magnetic fields is the high correlation among electrons. According to Little's calculation, the correlation extends over distances comparable to macromolecular dimensions (~30 A). Reactions, taking place in one part of a macromolecule could then conceivably influence the reactivity of other, even remote, parts of the molecule. Such a mechanism, if indeed operative, might well play a critical role in the highly specific catalytic reactions of enzymes. Accordingly, it can be reasoned that in the presence of a magnetic field greater than the critical value, any enzyme that utilizes superconducting properties for achieving high catalytic rates will be catastrophically denatured and a marked reduction in reaction rate should then be manifest.

On the basis of this reasoning we have searched for an effect of magnetic fields on the rates of two enzymatically catalyzed reactions; the hydrolysis of RNA catalyzed by the single enzyme, ribonuclease, and the reduction of cytochrome c by succinate, catalyzed by the enzyme complex, succinate-cytochrome c reductase. The highest field we could employ was 50 kgauss, which admittedly, was a factor of about 5 less than the required value as estimated above for transition temperatures of 2000 K. Nevertheless, the search was undertaken in the hope that some expression of superconductivity might be detected.

In earlier work, Akoyunoglou (4) reported an effect of a 20 kgauss magnetic field on the activity of carboxydismutase. In this experiment, the loss of activity due to denaturation proceeded at a rate 14 to 20 per cent slower in the field than outside it. Activation of trypsin by a field of 5 kgauss was reported by Smith and Cook (5). Both of these effects, however, await confirmation and further characterization.

MATERIALS AND METHODS

The experiments were performed in a Varian 50 kgauss superconducting magnet (Varian Associates, Palo Alto, California). The magnet system, shown in Fig. 1, consisted of a 6

[2] See C. Kittel, Introduction to Solid State Physics, New York, John Wiley & Sons, Inc., 1956.

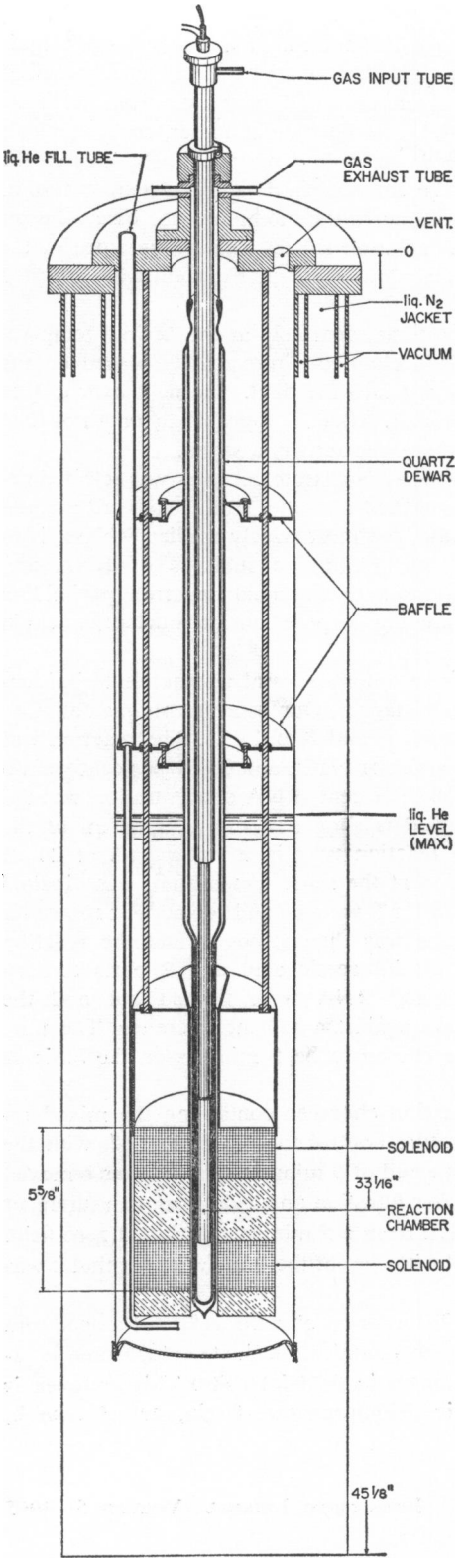

FIGURE 1 Liquid helium Dewar, superconducting magnet, and reaction chamber assembly.

inch compensated solenoid suspended just above the bottom of a stainless steel liquid helium chamber surrounded by a liquid nitrogen jacket. A slender quartz Dewar provided access to the solenoid from the top of the Dewar and insulated the sample from the liquid helium bath in which the solenoid was immersed. The reaction chamber, covered with a thin-walled stainless steel radiation shield, was lowered into place at the center of the solenoid on the end of the tube. Fig. 2 shows the chamber in detail. The temperature of the reaction mixture was regulated by flowing gas down the mounting tube over a heater coil and past the reaction chamber inside the radiation shield. The temperature of the reaction was monitored with a thermocouple. Gas flow and heater current were adjusted manually.

After equilibrating enzyme and substrate solutions separately at the desired temperature they were mixed together at zero time in a chamber, immediately placed on the end of the temperature control tube, and lowered into the field. The field over a 3 cc sample at the center was homogeneous to 1 per cent. After an appropriate length of time the tube was removed, the reaction stopped, and enzyme activity assayed.

RNA-Ribonuclease. The ribonuclease-RNA substrate complex was selected because the system had been extensively studied (6) and offered a straightforward method of assay. The action of bovine pancreatic RNase, the most widely studied RNase, is to attack the phosphodiester bond associated with pyrimidine subunits of RNA and produce, through a nucleoside-2′, 3′-cyclic phosphate intermediate, free pyrimidine nucleotides or a pyrimidine nucleotide terminating a pure purine nucleotide chain (oligonucleotide) through a 5′ linkage.

The spectrophotometric assay was chosen from among several alternative techniques because of the geometry of the superconducting magnet. Our assay technique followed with slight modifications, that of Dickman, Aroskar, and Kropf (7). The reaction was carried out in a solution containing 0.05 M Tris, 0.2 M NaCl, and 0.001 per cent gelatin at pH 7.6. Enzyme concentration was 4.0×10^{-5} per cent; RNA concentration was 0.6 per cent. A time course experiment at zero field is shown in Fig. 3. One ml of the enzyme solution was first equilibrated in the reaction tube in a water bath at 24 ± 0.25°C. At time zero, 1 ml of substrate solution at the same temperature was pipetted into the reaction tube. The solutions were mixed for 5 seconds and replaced in the water bath for the requisite length of time. The tube was then removed and the reaction stopped with 3 ml of a solution of 1 part glacial acetic acid and 2 parts tertiary butanol. Acid-precipitated protein and undigested RNA were filtered out and the absorbancy of the solution measured at 260 mμ against a solvent reference. The time interval for the magnetic field experiment was chosen to be 5 minutes on the basis of the data in Fig. 3.

For the magnetic field experiments, the reaction chamber containing the mixed reactants, was placed on the end of the temperature control tube and lowered, with the radiation shield in place, into the solenoid. At the end of 5 minutes the tube was removed and the acid was added to stop the reaction. After filtration an absorbancy measurement was taken at 260 mμ against a reference solution from a 5 minute reaction at zero field. The reference was treated in a manner identical with that of the sample except that it was not immersed in a magnetic field.

Succinate-Cytochrome c Reductase. In order to observe as broad a spectrum of effects as possible, the activity of the enzyme complex succinate-cytochrome c reductase, was measured as a function of the magnetic field intensity. This complex is known to consist of the components succinic dehydrogenase, flavin, cytochrome b,

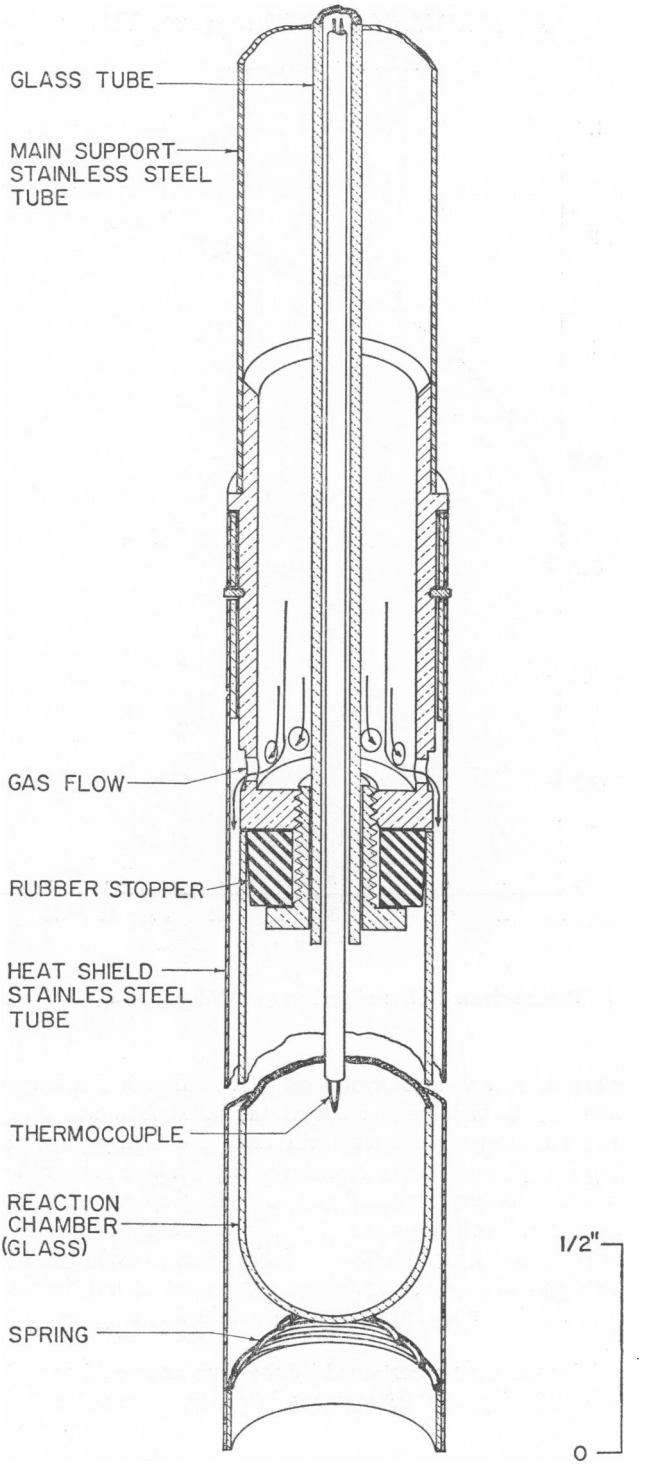

FIGURE 2 Reaction chamber and temperature regulating system.

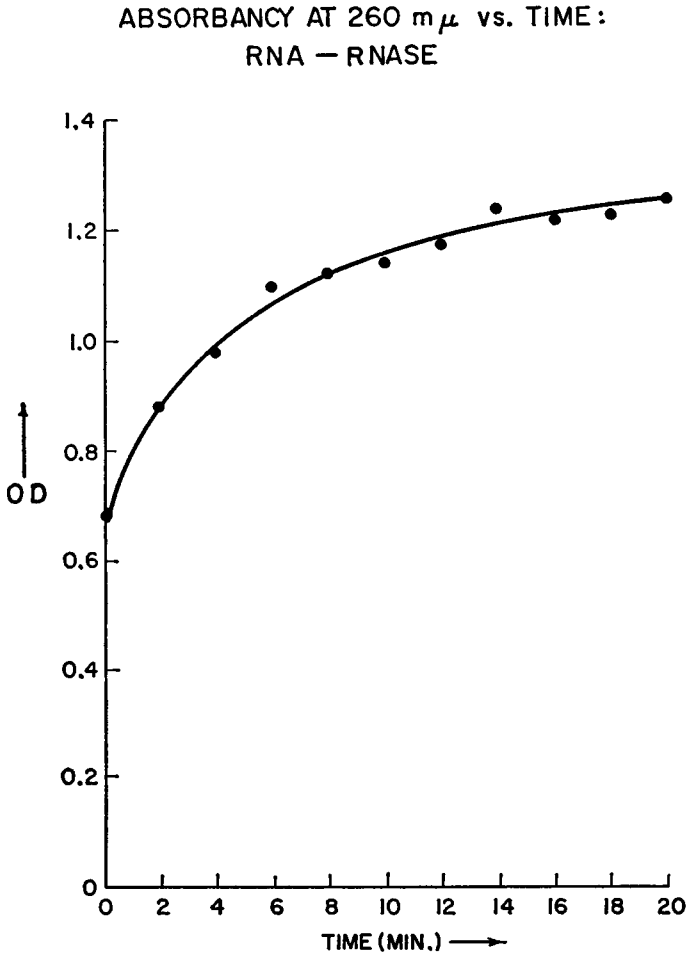

FIGURE 3 Ribonuclease-RNA: absorbancy at 260 mμ as a function of time.

ubiquinone, cytochrome c_1, and non-heme iron (8). Although this enzyme complex can be purified considerably from its native mitochondrial state it was thought to examine its activity while still close to the latter form since the highly purified enzyme is not capable of coupling phosphorylation to electron transport. The preparation of choice was a suspension of rat liver mitochondria that had been prepared in 0.25 M sucrose and then washed with water. Such a preparation allows the full expression of succinate-cytochrome c reductase activity and still sustains coupled phosphorylation in that region of the electron transport chain (9, 10). Respiratory control has been sacrificed by the water treatment but the latter was necessary to obtain full expression of cytochrome c reductase activity.

The enzyme activity was first ascertained by the time course of optical density change at 550 mμ following addition of 0.03 mg protein of the preparation described above to

a cuvette containing 2 ml of 0.001 M KCN[3] (to block cytochrome oxidase activity), 0.1 per cent cytochrome c, 0.01 M sodium succinate, and 0.01 M potassium phosphate at pH 7.0. The reduction of cytochrome c was shown to be linear with time during the first 6 minutes of the reaction (Fig. 4) and this time was used in all subsequent experiments. The reaction was stopped at 6 minutes by the addition of 10 γ-antimycin. It was shown that this amount of antimycin completely blocked the reduction of cytochrome c for at least 5 minutes. The actual experiments in the presence of the magnetic field were then carried out according to the following procedure. The cuvette was loaded with 2 ml of the solution described above, with the cyanide and enzyme added last. The cuvette was placed in the magnetic field for 6 minutes, removed, antimycin added, and the

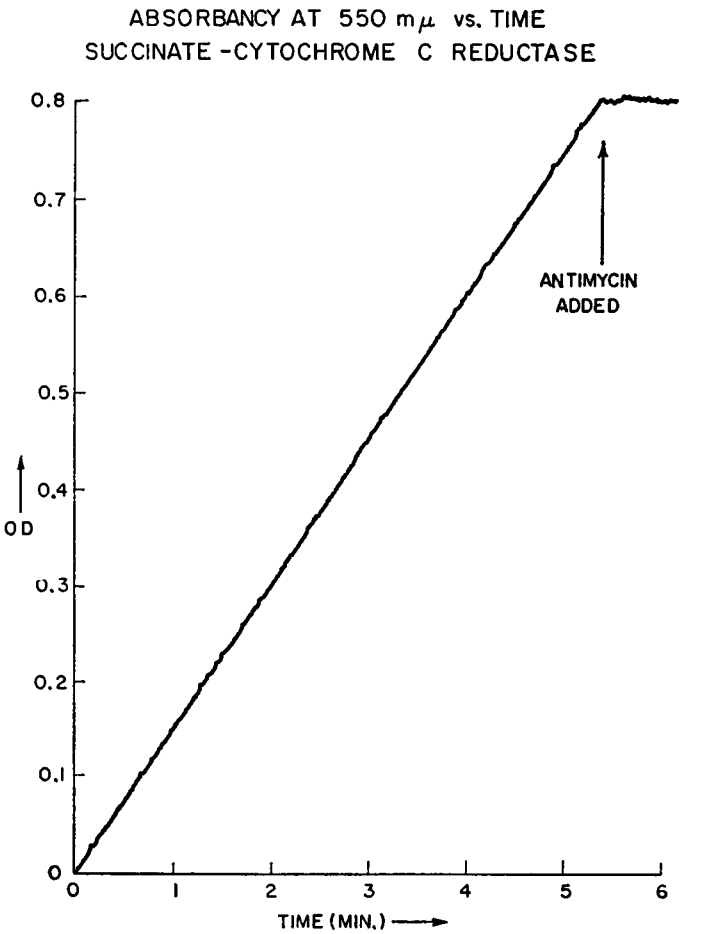

FIGURE 4 Succinate-cytochrome c reductase: absorbancy at 550 mμ as a function of time.

[3] The KCN was added to the cocktail containing the cytochrome c immediately before addition of the enzyme and initiation of the reaction, in order to avoid decomposition of the cytochrome c by the KCN.

optical density change at 550 mμ that occurred during this time immediately measured in a Cary spectrophotometer against a reference containing all ingredients except enzyme. This number was taken as the measure of the enzymatic activity of the complex.

RESULTS

Fig. 5*a* and *b* show optical density data characterizing ribonuclease activity for trials at 17 field values between 0 and 48 kgauss, with temperature variation and the average temperature for each trial. With two exceptions the temperature variation

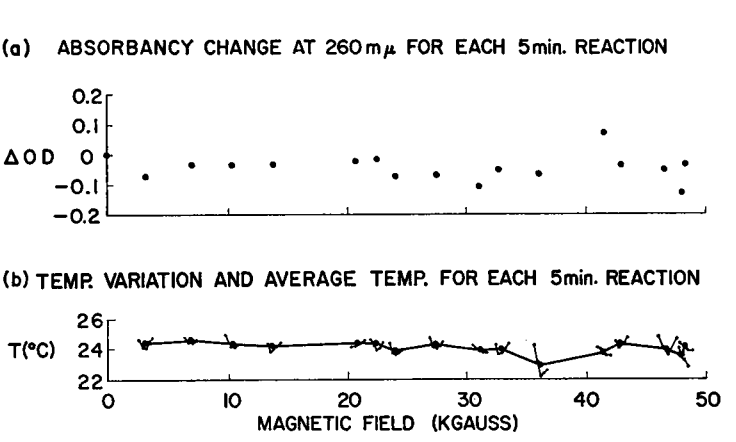

FIGURE 5 Ribonuclease-RNA: (*a*) absorbancy change at 260 mμ for each 5 minute reaction as a function of magnetic field, (*b*) temperature variation for each 5 minute reaction.

over each 5 minute trial was within ±0.5°C of the mean value shown. In two cases the temperature varied ±1.0°C from the mean. A temperature correction to the optical density data was not made.

The data on succinate-cytochrome c reductase are shown in Fig. 6 which represents four groups of experiments. In two of the groups the activity of the preparation was found to decay in time at a rate of about 0.1 OD units per hour. This correction was applied. In the remaining two groups fresh enzyme solution was used for each field trial thereby eliminating the need for a correction. Another correction was applied to take account of temperature variations. This correction was made using the relation

$$A_{T_1} = A_{T_2} \exp\left\{\mu\left(\frac{1}{kT_2} - \frac{1}{kT_1}\right)\right\}$$

where A_{T_1} is the activity at temperature T_1, A_{T_2} is the activity at T_2, and μ is an activation energy. T_1 was taken to be 23°C; μ was chosen according to Sizer (11) to be

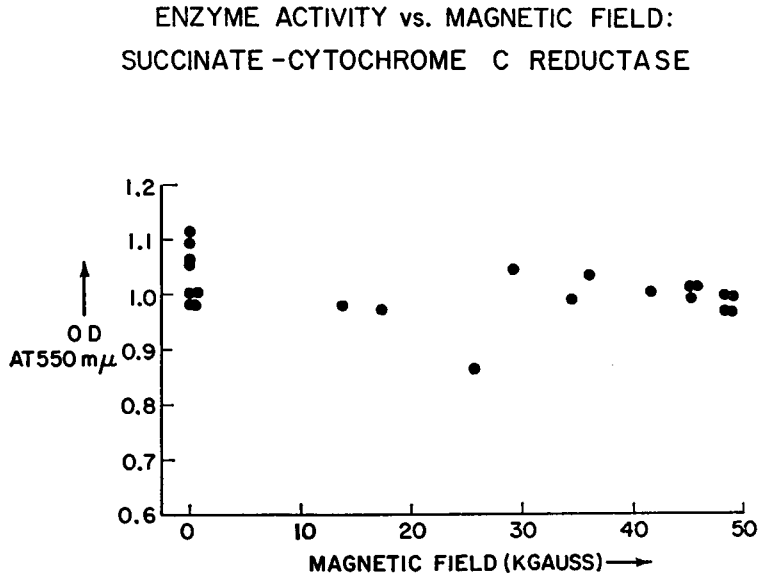

FIGURE 6 Succinate-cytochrome c reductase: absorbancy change at 550 mμ for each 5 minute reaction as a function of magnetic field.

2.0×10^4 cal/mole. The range of temperature variation was similar to that of the ribonuclease-RNA system.

It is apparent from Figs. 5*a* and *b* and 6 that in both enzyme-substrate systems there is no significant deviation from the zero field reaction rate up to a 48 kgauss field. An estimate of the inherent error in these experiments is provided by the spread of zero field rates in the assay of succinate-cytochrome c reductase activity. The variation is confined to about 10 per cent of the measured value. The main source of error is probably in the temperature variation rather than in the technique of carrying out the reaction. Fig. 4 shows the reaction rate to be strictly linear with time and the starting and stopping of the reaction was easily done on schedule. It was not deemed particularly useful to carry out a statistical analysis of the data in view of the fact that, except at zero field, only a few measurements per field value were possible. However, it is clear from Fig. 6 that all of the points lie within 10 per cent (the zero field spread) of a straight line parallel to the x axis. A least-squares calculation of the best straight line to draw through the points does not significantly modify this conclusion.

DISCUSSION

The experiments of Akoyunoglou and those of Smith and Cook are sufficiently different from ours so that a direct comparison is not possible. The basis of their positive effects remains unexplained in the light of our negative findings.

The suggestion of a possible connection between superconductivity, as applied to

macromolecules, and enzyme reactivity still awaits experimental support. It would be wrong, however, to interpret our experiments as a general reputation of the hypothesis that superconductivity may be a property of certain types of biological molecules. Certainly enzymes do not resemble the model macromolecule envisaged by Little. Their spine is only intermittently conjugated and their side groups are not nearly polarizable to the extent required by the model. Furthermore, even if these limitations were not unduly restrictive to the operation of a superconducting state, it is possible that we may not yet have reached the field strengths necessary for its detection by this type of experimental approach.

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