

# ON THE ELECTRON PARAMAGNETIC RESONANCE OF ADENOSINE TRIPHOSPHATE\*

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Recent experiments from this laboratory<sup>1</sup> have suggested that triphosphate compounds might be electron acceptors. Since adenine is a good electron donor<sup>2</sup>, the possibility exists that in an adenosine triphosphate molecule the adenine group might donate an electron to the triphosphate. The molecule would then exist in a peculiar internal charge transfer condition, the triphosphate having one electron in excess and the adenine being deficient in one. If such a state is a possible one, then it is likely that the actual state would involve a resonance between two electronic configurations. In one there would be no electron in excess on the triphosphate, while in the other state there would be.

For such a possibility to exist there must be some overlap between the ground state orbital of adenine and an excited state orbital of the triphosphate group. In the extended state of an ATP molecule there can be no overlap. It is therefore of interest to note that, as has been shown by the Courtauld atomic model, folded configurations of ATP may exist in which the triphosphate group is in close proximity to the adenine.<sup>3</sup> In one of these configurations it may be shown that the triphosphate group fits very neatly on the face of the adenine ring structure. In this folded configuration there may be appreciable overlap between the orbitals of the adenine and the triphosphate moieties.

Thanks to the kindness of Professor Hartmut Kallmann and Dr. Neil Wotherpoon of the Institute of Mathematical Sciences at New York University, we were able to make some preliminary studies of ATP using the techniques of electron paramagnetic resonance. The electron paramagnetic resonance assembly was a Varian model, V-4500, equipped with a Varian six-inch magnet. Approximately 50 mg of crystalline ATP (Disodium Salt, Pabst) was placed in a Pyrex test tube of 1.5 mm inside diameter. The tube was evacuated and sealed. The tube was then placed in the microwave cavity and a spectrum run.

A low signal of complex shape was obtained. This signal is shown in Figure 1 along with the spectrum obtained from ADP prepared in a similar fashion. It may be seen that the spectrum of ADP is quite low and is a simple one showing no structure. This is important because atomic models demonstrate that in ADP the phosphates cannot be fitted nearly as well onto the adenine face as in ATP. Furthermore, diphosphate does not have the acceptor properties of triphosphate.<sup>1</sup>

The ATP signal was integrated and compared with an integrated signal from DPPH (diphosphopicyryl hydrazyl) which contains one unpaired electron per molecule. On an equivalent molecular basis the ATP signal was lower than that of DPPH by a factor of  $10^{-4}$ .

Several possibilities present themselves for understanding the weakness of the ATP signal.

(1) In a charge transfer complex, the fact that there is resonance between a charge transfer state and a no-charge transfer state means that the spins will

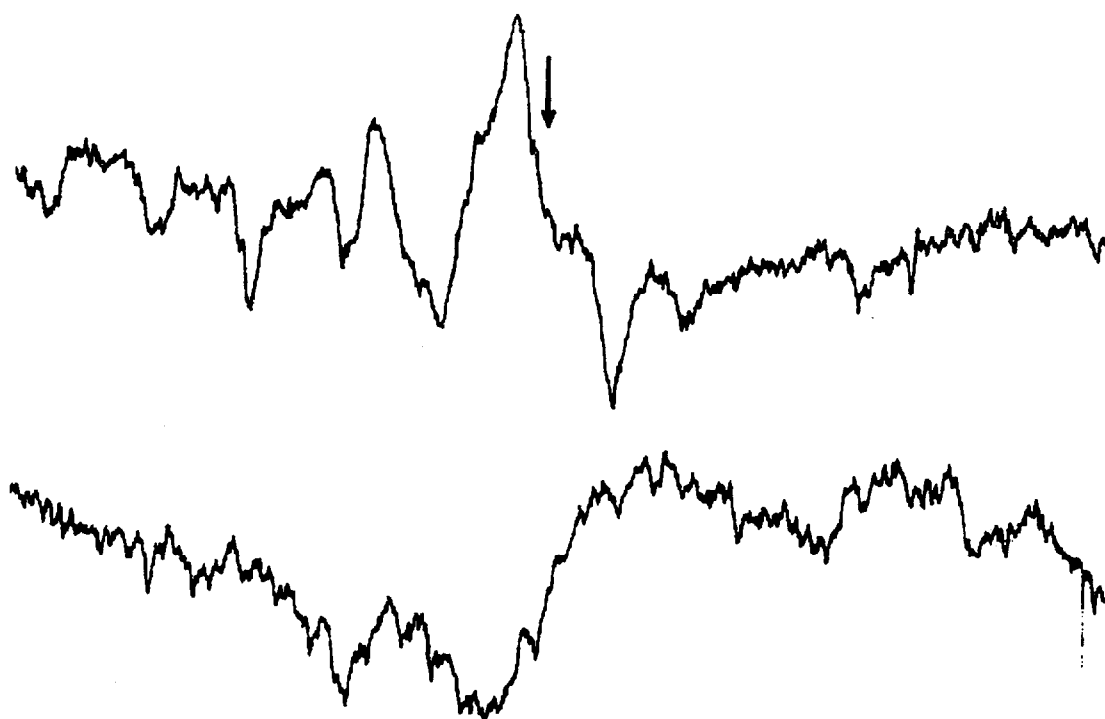


FIG. 1.—First derivative of electron paramagnetic resonance absorption. Upper curve is ATP. Lower curve is ADP. The arrow indicates the free electron resonance. The figure covers 800 gauss.

tend to remain parallel even in the charge transfer state. Only if one of the spins flips due to spin-orbit coupling or some other reason will the complex exist in a state in which e.p.r. absorption is possible. Thus, even if there were quite appreciable charge transfer there might still be relatively low e.p.r. absorption.

(2) In a dry ATP powder only a small fraction of the ATP molecules may be in the folded configuration and only molecules in the folded configuration may show an internal charge transfer.

(3) Even if a relatively large fraction of the ATP molecules were folded in a dry powder the resonance might be such that the contribution of the internal charge transfer state might be small compared to the no-charge transfer state.

(4) A combination of the above might be operative.

(5) The signal may be due to a transition metal impurity in the form of a metal-ATP complex. Judging from the procedure for the manufacture of ATP,<sup>4</sup> such an impurity would be quite a large one. This possibility, though not likely, cannot be ruled out at present.

It is clear that further work must be done on this problem. However, in view of the importance of ATP in cellular metabolism the authors felt that this preliminary note should be published at this time.

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<sup>1</sup> These PROCEEDINGS, (to be published soon).

<sup>2</sup> Pullman, Bernard, and Alberte Pullman, these PROCEEDINGS, **44**, 1197 (1958).

<sup>3</sup> Szent-Györgyi, Albert, *Bioenergetics* (Academic Press, 1957).

<sup>4</sup> United States Patent No. 2,700,038. Pabst Brewing Company.