received up-link signal. When no up-link is received, however, the down-link frequency is derived from a free-running crystal oscillator in the spacecraft. The radio frequency signal is amplified and transmitted from a high-gain spacecraft antenna.

The ground transmitter and receiver system employs an 85-foot (26-m) parabolic antenna with a Cassegrainian simultaneous-lobing feed. A traveling-wave maser cooled by a closed-cycle helium refrigerator operating at 4.2°K is used for the receiver front end. After amplification by the maser, the signal is split into two separate receiver channels. The first channel consists of a triple-conversion phase-locked receiver. It is operated in the standard DSIF receiver configuration. This receiver's VCO is kept in phase synchronism with the received signal. By a series of frequency multiplications, divisions, and additions, the transmitter's exciter frequency is coherently compared to the receiver's VCO to obtain the two-way Doppler frequency. The receiver's automatic gain control (AGC), which is a received-signal power-level tracking servo, is used to determine received-power level. Appropriate AGC voltages were recorded on magnetic tape, and the Doppler count was digitized. This system yielded frequency information in real time. This channel is also used as the sum channel of the pointing system for the simultaneous-lobing antenna.

The second receiver channel—a manually tuned, constant-gain, triple-conversion superheterodyne—is operated in a nonstandard configuration. It amplifies and translates the down-link signal to the audiofrequency region of the spectrum and then records it on magnetic tape. The local-oscillator (LO) signals for this receiver were derived from the rubidium frequency standard, which drives a pair of synthesizers. The first LO frequency was periodically stepped to keep the signal in the receiver's passband. The second and third LO's were derived from the second synthesizer operating at 19.996 Mcy/sec. The output of the third mixer had a passband of 1 to 3 kcy/sec, which was recorded on magnetic tape. Since the LO frequencies are derived from the rubidium standard, the frequency integrity of the Doppler is maintained. The analog information on the magnetic tape was digitized after the mission for use in a digital computer.

Figure 8 is a power spectrum of the audio open-loop signal made from the digitized tape on an IBM 7094 computer. The time interval from 03:25:16 to 03:25:17 was chosen. During this second, the one-way frequency, which was first observed as the vehicle reappeared at 03:25:08, was switched off. (This signal component can be seen at 2900 cy/sec.) After the one-way signal disappeared, the two-way signal was recorded at an audio frequency of 1900 cy/sec. The phase modulation sidebands at ± 150 cy/sec can be seen on both signals.

It should again be pointed out that these numbers are the results of less than 1 month's analysis with relatively crude techniques. As the analysis proceeds, the results will be refined, taking into account additional data as well as more sophisticated theoretical investigations of the physical characteristics of the atmosphere and ionosphere.

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7. We thank the Mariner Project and Deep Space Net personnel of JPL for their excellent performance in the preparation for the experiment and the acquisition of data. Also, special gratitude is due T. W. Hamilton of JPL and B. B. Lusignan of Stanford University for their many contributions to the success of the experiment. This paper presents the results of one phase of research carried out at the JPL, California Institute of Technology, under NASA contract NAS 7-100. The Stanford contribution was supported under NASA contracts NGR 05-020-065 and NsG-377.

23 August 1965

Heat Stabilities of Acid Phosphatases from Pinto Bean Leaves

Abstract. *Two acid phosphatases were demonstrable by polyacrylamide gel electrophoresis. They had different mobilities and different heat stabilities in 1.0M acetate buffer, pH 5.2. Both phosphatases had the same electrophoretic mobility in tris buffer at pH 7.5, gave one boundary in the analytical ultracentrifuge in tris or acetate buffer, and had the same sedimentation coefficient. The difference in these properties suggests an alteration in conformation of the proteins by the buffer systems.*

Newer techniques of protein chemistry, especially starch gel and polyacrylamide gel electrophoresis, have spurred the study of protein changes that occurr in plant and animal organs during disease or ontogeny. We have been studying acid phosphatase changes in bean leaves after removing the terminal buds. The heat stability of this enzyme was found useful in its characterization. These studies raised the possibility that there were two acid phosphatases which differed in their stability to heat. Heat stability studies also suggest the occurrence of multiple acid phosphatases in other tissues (*1*).

Acid phosphatase was extracted from bean leaves (*Phaseolus vulgaris* L. var. Pinto) and highly purified (*2*). A 1.0*M* acetate buffer, *p*H 5.2, was employed as the extraction medium and a freeze-thaw cycle, carried out at *p*H 5.2, was introduced before column chromatography on diethylaminoethyl cellulose at *p*H 7.5 in 0.01*M* tris (hydroxymethyl) aminomethane-HCl (tris) buffer. Enzyme activity was assayed by the method of Torriani (*3*) and protein was determined by the micro-Kjeldahl technique (*4*). The enzyme had a broad substrate range, *p*H optimum at 5.2, and hydrolysis of *p*-nitrophenyl-phosphate was noncompetitively inhibited by fluoride (*2*). Thus the enzyme is a typical type II phosphomonoesterase.

Loss in enzyme activity on heating in 1.0*M* acetate buffer, *p*H 5.2, is shown in Fig. 1. The initial decay in acetate buffer is approximately first order, but the drop in activity stops after 40 minutes, which suggests that two enzymes were present in the enzyme preparation, one of which was less heat

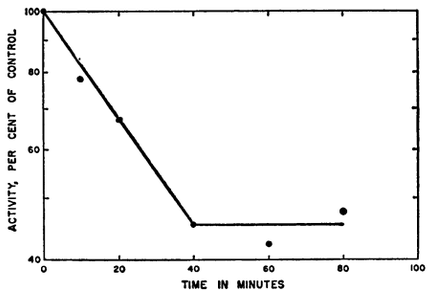

Fig. 1. Heat stability of acid phosphatase in 1.0*M* acetate, *p*H 5.2, at 50°C.

stable than the other at *p*H 5.2. After heating for 100 minutes at 50°C and centrifuging to remove the precipitate, 53 percent of the activity with *p*-nitrophenylphosphate and 55 percent of the protein remained. On the basis of these analyses and an examination of the heat stability curves for the point of interception of the slopes of the two phases of the curves, it is concluded that the heat-labile portion comprised about half of the acid phosphatase preparation.

An examination of the enzymes by polyacrylamide gel electrophoresis was carried out; there was only one enzymatically active zone when the enzymes were prepared in tris buffer (Fig. 2). After the purified preparation was dialyzed overnight against 2 liters of 1.0*M* acetate buffer at *p*H 5.2, two zones were detected on the gels. After dialysis to remove tris buffer, the formation of two isozymes in acetate is readily reversible. Only one band could be detected when the same preparation was again dialyzed against tris buffer at *p*H 7.5, but two bands were always found when tris buffer was replaced by acetate (*p*H 5.2). When the enzyme was heated in acetate buffer for 10 minutes at 50°C the new acid phosphatase could not be detected.

When acid phosphatase was centrifuged in an analytical ultracentrifuge, only one boundary was apparent, regardless of which buffer was used. Thus the new acid phosphatase does not appear to be produced by aggregation. The sedimentation coefficient ($s_{20,w}$) was 4.7 in tris buffer (*p*H 7.5), but 2.5 in acetate buffer (*p*H 5.2).

The results may suggest an expansion of the molecule at the lower *p*H similar to that which was reported for serum albumin (*5*). Both phosphatases seem to have the same mass, so that their separation on polyacrylamide gels reflects either the acquisition of an increased positive charge by the heat-labile protein when in acetate buffer or a change in conformation, possibly brought about by the lower *p*H or altered buffer composition. Both inorganic and organic salts are important in controlling protein conformation (see *6*).

Apparently the two phosphatases are isozymes and are not simply interconvertible forms of the same enzyme. Once heated, additional amounts of the labile acid phosphatase are not produced.

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30 June 1965

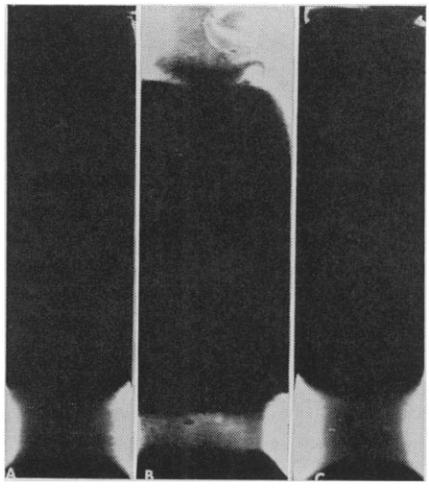

Fig. 2. Photographs of polyacrylamide gels stained with Gomori stain (*3*) using β-glycerophosphate. Phosphatase was heated in appropriate buffer on a water bath at 50°C for 100 minutes, centrifuged to remove precipitate, and placed on the gels for electrophoresis. Anode is at the bottom. (*A*) Enzyme in 0.1*M* tris, *p*H 7.5. (*B*) Enzyme in 1.0*M* acetate, *p*H 5.2, not heated. Note second band at top. (*C*) Enzyme in 1.0*M* acetate, *p*H 5.2, heated.

Inhibitory Oxidation Products of Indole-3-Acetic Acid: Enzymic Formation and Detoxification by Pea Seedlings

Abstract. *Extracts of etiolated pea seedlings oxidize indole-3-acetic acid, a plant auxin, to 3-hydroxymethyloxindole. At physiological* p*H this compound is dehydrated to 3-methyleneoxindole, a highly reactive sulfhydryl reagent and inhibitor of cell growth. 3-Methlyeneoxindole is in turn detoxified by an enzymatic, triphosphopyridine nucleotide–linked reduction to 3-methyloxindole, a nontoxic compound. These enzymatic conversions may be responsible for some of the responses to auxin, particularly sensitivity to its inhibitory effects on growth.*

A search for the biochemical basis for the action of indole-3-acetic acid, a plant auxin, with *Escherichia coli* as a model organism, revealed that both photooxidation of indole-3-acetic acid by riboflavin and its enzymatic oxidation by horseradish peroxidase yield a potent inhibitor of the growth of microorganisms as well as of higher plants (*1*). Although the earliest detectable product of the oxidations, 3-hydroxymethyloxindole, has no toxic effect of its own, it is rapidly dehydrated to 3-methyleneoxindole, a potent sulfhydryl reagent and inhibitor of sulfhydryl enzymes. The bacteriostatic effect of 3-methyleneoxindole is transitory as it is reduced to 3-methyloxindole, a nontoxicant, by a bacterial enzyme utilizing the reduced form of diphosphopyridine nucleotide as the reductant (*2*). Reactions similar to those of the model system (Fig. 1), if found in plants, could maintain precise adjustment of the intracellular level of the highly active 3-methyleneoxindole, and might therefore be involved in the regulatory action of indole-3-acetic acid. We now describe such reactions in extracts of pea seedlings.