[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF TORONTO, AND THE NATIONAL RESEARCH COUNCIL, ATOMIC ENERGY PROJECT, CHALK RIVER, ONTARIO]

# On the Mechanism of the Action of Ionizing Radiations on Sodium Thymonucleate[1,2]

BY D. B. SMITH AND G. C. BUTLER

The action of ionizing radiations on aqueous solutions of sodium thymonucleate has been investigated several times.[3–6] Reduction in the high viscosity increment due to the nucleate has been the principal finding and also the principal criterion of alteration in the nucleate. Scholes, *et al.*,[6] have investigated the nature of chemical changes following massive X-ray doses. Butler[5] investigated the effect of X- and $\gamma$-rays on the viscosity of sodium thymonucleate solutions and presented evidence that it was an indirect one mediated by the water of the solutions.

It is generally held[7–9] that the chemical effects of ionizing radiations on aqueous solutions are caused by hydrogen atoms and hydroxyl radicals resulting from the primary ionization of water molecules. These free atoms and radicals may react with each other to produce hydrogen molecules, oxygen atoms and oxygen molecules and, especially if dissolved oxygen were initially present, hydroperoxyl radicals and hydrogen peroxide.

The present report describes some attempts to identify which of these components of "activated water" is responsible for the effects of X- and $\gamma$-rays on the thymonucleate solutions.

While this report was being prepared for publication three communications bearing on our work appeared. Collinson, Dainton and Holmes[10] showed that chemically produced hydroxyl radicals could simulate the action of X-rays in inactivating ribonuclease. Limperos and Mosher[11] and J. A. V. Butler and K. A. Smith[12] demonstrated a reduction in the viscosity of solutions of thymonucleate under the action of chemically produced free radicals, including hydroxyl radicals.

## Experimental

**Preparation of Sodium Thymonucleate.**—Sodium thymonucleate was prepared from calf thymus nucleohistone as described by Smith and Sheffer.[13]

**Methods of Viscosity.**—Viscosity measurements were made by the two methods described by Butler.[5]

**Attempts to Detect Oxygen Produced during Irradiation.**—The evolution of oxygen from irradiated nucleate solutions would indicate a preferential reaction of hydrogen atoms, the oxygen arising from the dismutation of excess hydroxyl radicals.[14] An aqueous solution (0.5%) of sodium thymonucleate was irradiated with 200 kv. X-rays in a closed vessel previously evacuated to remove dissolved oxygen. After doses of 25,000 r. in one experiment and 40,000 r. in another, the gas phase above the solution was brought to atmospheric pressure and tested for oxygen by introducing a solution of reduced indigo monosulfonate. There was no change in the color of the dye, indicating that the nucleate was not acted upon by H atoms so as to leave an excess of hydroxyl radicals.

**Effect of Dissolved Oxygen.**—The presence in a solution of a second substance capable of reacting with radiochemically produced radicals may exert a "protective" effect by reducing the number of radicals available for reaction with the original solute.[15] Oxygen is reduced by hydrogen atoms first to hydroperoxyl radicals ($HO_2$) and then to hydrogen peroxide.[8] The presence of dissolved oxygen might therefore reduce the effect of X-rays on thymonucleate if this effect was due wholly or partly to hydrogen atoms and if hydroperoxyl radicals were ineffective or less effective than hydrogen atoms. If, on the other hand, the radiochemical reaction with thymonucleate were due to hydroperoxyl radicals or hydrogen peroxide the presence of dissolved oxygen would be expected to increase the effect. No differences in viscosity reduction could be detected, however, when 0.5% sodium thymonucleate solutions were irradiated with 17,000 r. of 200 kv. X-rays in the presence of air and in the presence of oxygen-free nitrogen after careful removal of oxygen.

**Effect of Hydrogen and Palladium**—In an attempt to study directly the effect of H atoms, 0.5% solutions of sodium thymonucleate were treated with hydrogen and an active palladium black catalyst but no change in viscosity resulted. Although the hydrogen molecule is considered to be dissociated at the palladium surface this system does not reproduce accurately the distribution of hydrogen atoms in an irradiated solution. Failure of hydrogen and palladium to affect the viscosity of thymonucleate solutions does not, therefore, provide conclusive evidence concerning the reactivity of hydrogen atoms with thymonucleate.

**Effect of Hydroxyl Radicals.**—Hydroxyl radicals may be produced conveniently in neutral aqueous solutions through the photolysis of hydrogen peroxide by ultraviolet light of wave length less than about 3700 Å. according to the reaction $H_2O_2 + h\nu \rightarrow 2OH$.[16] Evidence for the effectiveness of hydroxyl radicals produced in this way in simulating the action of X-rays in initiating the polymerization of some vinyl compounds has been offered by Dainton.[17] We have found that the viscosity of thymonucleate solutions containing hydrogen peroxide decreased markedly on exposure to ultraviolet light (Fig. 1).

The effect of hydrogen peroxide, without irradiation, at 25° on thymonucleate (0.06%) dissolved in 0.12 *M* sodium chloride solution was investigated at two different peroxide concentrations. In both cases the relative viscosity decreased linearly with time; with 0.1 *M* peroxide the relative viscosity diminished by 2.5% in 80 minutes while with 0.15 *M* peroxide it decreased 10% in the same period.

Thymonucleate solutions in rectangular quartz cells were irradiated with a beam of light from a General Electric CH-4 100 watt mercury vapor lamp. Between the light source and the cell was placed a filter system consisting of a potassium dichromate solution (0.15 g./l.) in a "Plexiglass"

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[1] This communication forms part of a thesis submitted to the School of Graduate Studies of the University of Toronto by D. B. Smith in partial fulfillment of the requirements for the degree of Doctor of Philosophy, January, 1950. It was presented in part before the Canadian Physiological Society at Montreal in October, 1949.

[2] The financial support of the National Research Council of Canada is acknowledged.

(3) A. H. Sparrow and F. M. Rosenfeld, *Science*, **104**, 245 (1946).

(4) B. Taylor, J. P. Greenstein and A. Hollaender, *ibid.*, **105**, 263 (1947); *Arch. Biochem.*, **16**, 19 (1948).

(5) G. C. Butler, *Can. J. Research*, **B27**, 972 (1949).

(6) G. Scholes, G. Stein and J. Weiss, *Nature*, **164**, 709 (1949).

(7) J. Weiss, *Nature*, **153**, 748 (1944); *Trans. Faraday Soc.*, **43**, 314 (1947).

(8) A. O. Allen, *J. Phys. Colloid Chem.*, **52**, 479 (1948).

(9) C. B. Allsop, *Brit. J. Radiol.*, **21**, 72 (1948).

(10) E. Collinson, F. S. Dainton and B. Holmes, *Nature*, **165**, 266 (1950).

(11) G. Limperos and W. A. Mosher, *Am. J. Roentg. and Rad. Ther.*, **63**, 681 (1950).

(12) J. A. V. Butler and K. A. Smith, *Nature*, **165**, 847 (1950).

(13) D. B. Smith and H. Sheffer, *Can. J. Research*, **B28**, 96 (1950).

(14) J. Weiss, *Trans. Faraday Soc.*, **36**, 856 (1940).

(15) W. M. Dale, W. J. Meredith and M. C. K. Tweedie, *Nature*, **151**, 280 (1943).

(16) H. C. Urey, L. H. Dawsey and F. O. Rice, THIS JOURNAL, **51**, 1371 (1929).

(17) F. S. Dainton, *J. Phys. Colloid Chem.*, **52**, 490 (1948).

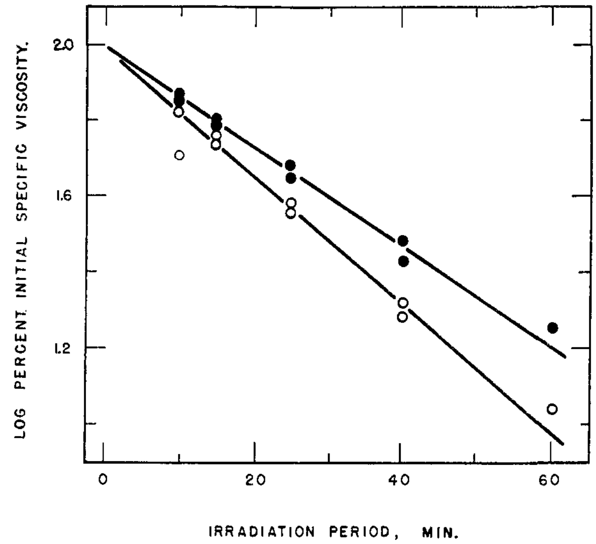

Fig. 1.—Survival curves obtained on ultraviolet irradiation of solutions containing thymonucleate, batch XIII, (0.06%), sodium chloride (0.02 *M*) and hydrogen peroxide (0.1 *M*): ●, irradiation cell no. II; ○, irradiation cell no. IV.

cell (20-mm. light path), a Pyrex glass filter (Corning no. 7740) and a red-purple Corex A filter (Corning no. 9863). The light passed by this filter system ranges in wave length from 2900 to 3500 Å. and is principally of wave length 3135 Å. It was necessary to use light of this wave length so that it would be absorbed by the hydrogen peroxide and not by the nucleate and so that it would be completely absorbed by the uranyl oxalate-oxalic system[18] that we used for chemical actinometry.

Rates of change were calculated as

proportional rate = % decrease in viscosity/irradiation period

absolute rate = % decrease in viscosity × nucleate concn./irradiation period

In all of our work the rate of irradiation was constant and in all the experiments except the one dealing with the effect of peroxide concentration on rate, the hydrogen peroxide concentration was the same (0.01 *M*). Under such conditions the rates mentioned above are related to the yield by a constant factor which is evaluated in the discussion.

In any one experiment all viscosity measurements were made in one viscometer on solutions of the same concentration; irradiated solutions were diluted, where necessary, to the concentration chosen for viscometry. For a series of irradiations on one day a stock solution containing nucleate, sodium chloride and hydrogen peroxide[19] was prepared and stored at 5°; samples for irradiation were taken from this stock solution as required. Cold storage was necessary to avoid the loss of viscosity due to the action of peroxide at 25° described previously. The viscosity of a 0.06% thymonucleate solution in 0.02 *M* sodium chloride and 0.1 *M* hydrogen peroxide fell by only 6% on storage for 16 hours at 5°. In all control experiments care was taken to keep the control solution at room temperature for the same length of time as the irradiated solution. When duplicate irradiations were carried out simultaneously the results were assessed using two different viscometers.

From the representative survival curve shown in Fig. 1 it can be seen that there is an exponential relation between residual specific viscosity and dose of radiation. Such a survival curve has been predicted by Lea,[20] and Dale, *et al.*,[15] for radiochemical reactions in which the original solute and its decomposition products have the same affinity for the active radicals.

The effect of altering the peroxide concentration on absolute rate is illustrated in Fig. 2. The constant maximum in the rate is thought to be due to competition for hydroxyl radicals between thymonucleate on the one hand and hydrogen peroxide or some transient product of its photolysis on the other.[8]

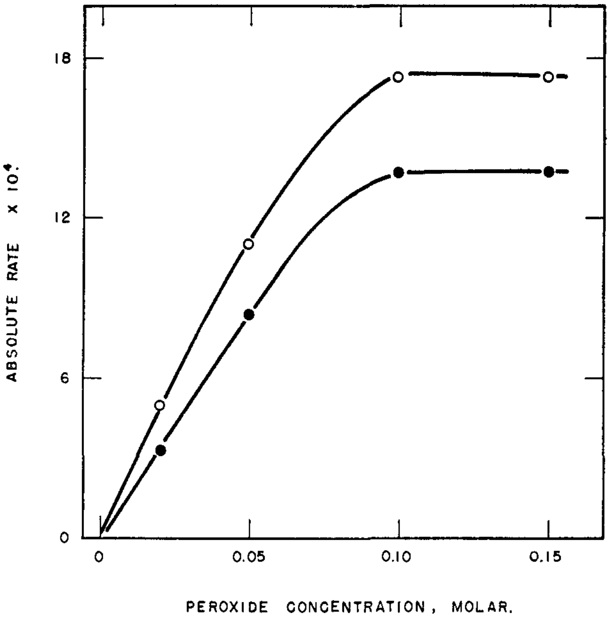

Fig. 2.—Effect of peroxide concentration on rate: solutions contained thymonucleate, batch XIII (0.06%), sodium chloride (0.02 *M*) and hydrogen peroxide: ●, irradiation cell no. II; ○, irradiation cell no. IV.

In studying the effect of varying the nucleate concentration on absolute rate it was found that above a nucleate concentration of 0.03% the rate was constant but that below this concentration the rate fell off sharply.

Low concentrations of glucose and methanol exerted a protective effect (*cf.* refs. 5, 21) but it differed from that with γ-rays[5] in that with peroxide and ultraviolet light no more than 50% protection could be obtained and this limit was reached at a concentration of 0.05 *M* for both glucose and methanol. Thymonucleate previously treated with peroxide and ultraviolet light so as to reduce its specific viscosity almost to zero, also had a protective action but there was no limit to this action as in the cases of glucose and methanol; furthermore the results indicated that degraded and un-

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[18] W. G. Leighton and G. S. Forbes, THIS JOURNAL, **52**, 3139 (1930).

[19] "Superoxol," Merck and Co., Inc., 30% $H_2O_2$ containing no stabilizer.

[20] D. E. Lea, "Action of Radiations on Living Cells," Cambridge, 1946, p. 61.

[21] W. M. Dale, *Biochem. J.*, **36**, 80 (1942).

degraded nucleate had equal affinities for hydroxyl radicals.

More concentrated solutions of thymonucleate (0.5%) were treated with hydrogen peroxide and ultraviolet light and viscosity measurements were expressed in terms of $m$ (*cf.* ref. 5). (a) Solutions with four different concentrations of hydrogen peroxide were irradiated for 30 minutes, with water replacing dichromate in the filter cell, and (b) stock solutions of nucleate and hydrogen peroxide were irradiated for different periods employing two different concentrations of peroxide (0.1 and 0.05 $M$) and either water or chromate solution in the filter system. In all cases, the survival curves, in which log per cent. control $m - 1$ was plotted against the variable, appeared to be approximately linear.

**Inhibition of Oxygen Production by Thymonucleate.**—The final products of the ultraviolet irradiation of hydrogen peroxide solutions are water and oxygen, produced in a chain reaction involving hydroxyl radicals.[8] If thymonucleate reacts with and removes hydroxyl radicals, this chain would be interrupted and a lower yield of oxygen would result. The extent to which thymonucleate interfered with these reactions was tested by comparing oxygen production in the presence and absence of nucleate. In Table I are given the volumes of gas (oxygen) produced when the solutions listed were irradiated in an evacuated vessel with ultraviolet light; each volume of oxygen recorded is corrected for the amount produced by a similar solution kept in the dark as a control.

TABLE I

THE EFFECT OF DISSOLVED THYMONUCLEATE ON OXYGEN PRODUCTION BY 10 ML. OF A 0.1 $M$ SOLUTION OF HYDROGEN PEROXIDE IRRADIATED WITH ULTRAVIOLET LIGHT

| Nucleate concn., % | Oxygen produced, ml. |
|---|---|
| 0 | 0.355 |
| .001 | .071 |
| .0022 | .025 |
| .005 | .037 |

## Discussion

Some estimates can be made of the number of hydroxyl radicals required for a given viscosity reduction. Butler[5] found, in two experiments, that to reduce the specific viscosity of thymonucleate solutions to half their initial value, productions within the solution of 57 and 80 $\mu M$ respectively, of active radicals of one kind per gram of sodium thymonucleate were required for two different batches of thymonucleate.

With a 0.05% nucleate solution containing sodium chloride (0.02 $M$) and hydrogen peroxide (0.1 $M$) the half-survival time was 22.5 minutes. The light intensity on the front surface of the solution was found by chemical actinometry[18] to be $1.2 \times 10^{-9}$ einstein/sec. and the peroxide absorbed 26% of the incident light of wave length 3135 Å. If the quantum yield for the initial photolysis of hydrogen peroxide is assumed to be unity and if all hydroxyl radicals react with the nucleate, it may be calculated that 250 $\mu M$ of hydroxyl radicals react per gram of nucleate during 22.5 minutes. The assumptions involved in this estimate lead to a maximum value and the hydroxyl radical requirements for the same viscosity change are thus of the same order of magnitude for the two methods of degradation. This strengthens the conclusion that the mechanism in both cases is similar. The lower requirements in the radio-chemical degradations may indicate that hydrogen atoms are also reacting.

### Physical Investigations of the Degraded Nucleate

The nature of the product resulting from the action of photochemically-produced hydroxyl radicals on thymonucleate was investigated in three ways.

A report has already been made[13] on measurements of particle weight and shape by the light scattering method. These showed that the average particle size of the nucleate was greatly reduced following ultraviolet irradiation in the presence of hydrogen peroxide.

Electron micrographs were made[22] of the residue left after freeze-drying drops of dilute thymonucleate solutions and shadowing with chromium or palladium. The appearance of the undegraded nucleate closely resembled some electron micrographs of sodium thymonucleate already published.[23] The material had the form of long fibers and ribbons. The widths of the narrowest strands were estimated to be about 100 A., well above the limits of resolution of the instrument. Since the width of a single polynucleotide chain is probably not greater than 15 Å., considerable lateral aggregation may be indicated. In electron micrographs of nucleate that had been degraded with hydroxyl radicals, much shorter fibers were seen. There was also considerable background debris not observed in photographs of undegraded nucleate.[24]

A few sedimentation runs[25] were made using undegraded nucleate and one run using nucleate that had been treated with hydroxyl radicals.[26] The boundary in the undegraded nucleate solution was initially very sharp and was broadened very little as centrifugation proceeded. The boundary in the degraded nucleate solution was much less sharp at the beginning and very quickly became indistinct.[27]

The results of these three physical methods of investigating the type of change occurring in the thymonucleate concur therefore with viscometry in indicating that the material is changed from a state of very high particle weight with a very great particle asymmetry to one wherein both these characteristics are greatly reduced.

## Summary

It has been found that the chemical reactions by which X- and $\gamma$-rays degrade sodium thymo-

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(22) An R. C. A. type EMU electron microscope was used.

(23) J. F. Scott, *Biochim. Biophys. Acta*, **2**, 1 (1948).

(24) The authors wish to thank Dr. D. Scott of the Department of Physics, University of Toronto, for taking these electron micrographs and for his interest and advice.

(25) A "Spinco" ultracentrifuge was used.

(26) The authors thank Dr. A. F. Graham of the Connaught Laboratories, University of Toronto, for making these runs and for advice in their interpretation.

(27) Taylor, Greenstein and Hollaender[4] reported similar effects following X-irradiation of thymonucleate solutions.

nucleate in solution do not result in the production of oxygen nor are these reactions affected by oxygen.

The failure of hydrogen, in the presence of palladium black, to affect the viscosity of thymonucleate solutions has been taken to indicate, but not to prove, that the degradation is not due to reaction with hydrogen atoms. On the other hand, when thymonucleate solutions containing hydrogen peroxide are irradiated with ultraviolet light in the range of wave lengths where most of the light absorption is due to the peroxide, there has been found a sharp reduction in solution viscosity that can be attributed to the action of free hydroxyl radicals formed in the photolysis. With constant nucleate and hydrogen peroxide concentrations the logarithm of viscosity reduction is proportional to time of irradiation. The viscosity reduction is not a constant function of the amount of light absorbed under all conditions since the rate of reduction increases with hydrogen peroxide concentration only over a limited range. The constant maximum in the rate attained at higher peroxide concentrations may be ascribed to competition for hydroxyl radicals between nucleate on the one hand and hydrogen peroxide and its photolytic decomposition products on the other. The finding that a very small amount of nucleate causes a marked reduction in the amount of oxygen produced from hydrogen peroxide solutions by ultraviolet irradiation is an additional indication that the nucleate reacts with hydroxyl radicals.

Further evidence for the free radical nature of the decomposition is provided by the observations that the rate of degradation decreases at very low nucleate concentrations and also when other solutes, such as glucose, methanol and irradiated nucleate, are present.

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[CONTRIBUTION NO. 20 FROM THE THERMODYNAMICS LABORATORY OF THE PETROLEUM EXPERIMENT STATION, BUREAU OF MINES]

# Thermodynamic Properties and Rotational Isomerism of 2-Thiabutane[1]

BY D. W. SCOTT, H. L. FINKE, J. P. MCCULLOUGH, M. E. GROSS, K. D. WILLIAMSON, GUY WADDINGTON AND H. M. HUFFMAN[2]

The experimental work reported in this paper consisted of calorimetric and vapor-pressure studies of 2-thiabutane (methyl ethyl sulfide) which yielded values of the entropy and the heat capacity of this substance in the ideal gas state. These data were correlated and extended to other thermodynamic functions and to higher temperatures by calculations based on spectroscopic and molecular structure information. The interpretation of the spectroscopic and thermal data led to the conclusion that the two rotational isomers of 2-thiabutane have nearly the same energy.

## Experimental

**Material.**—The 2-thiabutane used for the experimental measurements consisted of a small sample of highly purified material and a larger sample of material of somewhat lower purity. These were prepared and purified at the Laramie Station of the Bureau of Mines as part of A.P.I. Research Project 48A. The high-purity sample was used for the low-temperature studies and vapor-pressure measurements. From observations of the melting point as a function of per cent. melted, the purity was determined to be 99.9984 ± 0.0005 mole per cent. This determination involved the usual assumptions of liquid soluble-solid insoluble impurities and ideal solutions. The melting-point data are summarized in Table I. In the ebulliometric studies, the difference between the boiling and condensation temperatures of this sample was found to be 0.008°. The second sample, of lower purity, was used for determining the heats of vaporization and the vapor heat capacities, because these measurements required a larger volume of material. The purity of this second sample, determined at the Laramie Station by the time-temperature freezing-point method, was reported to be 99.49 ± 0.18 mole per cent.

**Low-Temperature Studies.**—Measurements of the heat capacity of 2-thiabutane in the solid and liquid states and its melting point and heat of fusion were made in an apparatus similar to that described by Ruehrwein and Huffman.[3] The calorimeter used to contain the sample was constructed of platinum and had internal vanes of gold to promote more rapid attainment of thermal equilibrium. The results of the heat-capacity measurements are listed in Table II. The precision of the measurements was, in general, better than 0.1%, and above 30°K. it is believed that the accuracy uncertainty should not be greater than 0.2%. Values of the heat capacity at integral temperatures, as selected from a smooth curve through all the data, are given in Table III.

TABLE I

MELTING POINT OF 2-THIABUTANE; 0°C. = 273.16°K.

Triple point: 167.23 ± 0.05°K. $N_2/F = 0.0420\ \Delta T$ where $N_2$ is the mole fraction of impurity, $F$ the fraction of sample in liquid form, and $\Delta T$ the melting point lowering. Impurity: 0.0016 ± 0.0005 mole per cent.

| Melted, % | $T$, °K. Obsd. | $T$, °K. Calcd. |
|---|---|---|
| 6.5 | 167.2268 | 167.2247 |
| 26.9 | 167.2291 | 167.2291 |
| 50.7 | 167.2297 | 167.2298 |
| 71.0 | 167.2301 | 167.2300 |
| 91.3 | 167.2301 | 167.2301 |
| 100.0 | | 167.2301 |
| Pure | | 167.2305 |

Duplicate determinations each gave a value of 2,333 cal./mole for the heat of fusion.

**Vapor Pressure.**—The vapor pressure of 2-thiabutane was measured from 23 to 101° by an ebulliometric method, using the apparatus that has been described in a previous publication from this Laboratory.[4] The results of the measurements are given in Table IV. An Antoine equation was fitted to these data by a least-squares adjustment, and the equation which was obtained is

$$\log_{10} p = 6.93849 - 1182.562/(t + 224.784) \quad (1)$$

The normal boiling point given by this equation is 66.65°. Values of the vapor pressure calculated by means of the

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[1] This investigation was performed as part of the work of American Petroleum Institute Research Project 48A on "The Production, Isolation, and Purification of Sulfur Compounds and Measurement of their Properties," which the Bureau of Mines conducts at Bartlesville, Okla., and Laramie, Wyo. Article not copyrighted.

[2] Deceased.

[3] Ruehrwein and Huffman, THIS JOURNAL, 65, 1620 (1943).

[4] Waddington, Knowlton, Scott, Oliver, Todd, Hubbard, Smith, and Huffman, THIS JOURNAL, 71, 797 (1949).