I

AN ATTEMPT TO PREPARE ANTIBODIES TO HISTAMINE *IN VITRO*

II

AN INVESTIGATION OF A POSSIBLE METHOD FOR PREPARING LARGE SINGLE CRYSTALS OF ALUMINUM ORTHOPHOSPHATE

III

INVESTIGATIONS OF THE CRYSTAL STRUCTURES OF AMINO ACIDS BY X-RAY DIFFRACTION

A. AN INVESTIGATION OF THE SPACE-GROUP AND LATTICE CONSTANTS OF *dl*-SERINE

B. AN INVESTIGATION OF THE CRYSTAL STRUCTURE OF *l*-THREONINE

Thesis by

David Powell Shoemaker

In Partial Fulfilment
of the Requirements for the Degree of
Doctor of Philosophy

California Institute of Technology
Pasadena, California

1947

TABLE OF CONTENTS

ACKNOWLEDGEMENT

Elsewhere in this thesis I have acknowledged the cooperation and assistance given me by those who have supervised my research and by those who have made contributions to its progress. I wish here to emphasize my gratitude to Professor Linus Pauling for his interest in my work and for his valuable advice and suggestions, and to Dr. Robert B. Corey for his continued advice and encouragement and especially for the very friendly association that existed between us in the amino acid work. I also wish to thank Dr. Corey and Professor Verner Schomaker for many helpful criticisms during the preparation of this thesis.

I

AN ATTEMPT TO PREPARE ANTIBODIES TO HISTAMINE *IN VITRO*

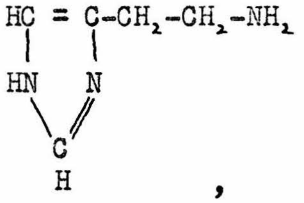
1. Introduction

The object of the investigation here described is to prepare antibodies to histamine *in vitro*, by a method suggested by Professor Linus Pauling. Although the experiments failed to give evidence of antibody formation, a description of the work will be given here.

In 1940 Professor Pauling[1] published a theory of the structure of antibodies and of the mode of their formation. He assumed that antibody molecules differ from molecules of serum globulin only in the way the ends of the polypeptide chain are coiled, and suggested that in the formation of an antibody in a living organism the ends of a polypeptide chain similar to that of globulin coil in contact with the antigen molecule in such a way that the completed antibody, which is not significantly different in stability from the related globulin molecules, has over part of its surface a shape complementary to that of some region or regions of the antigen molecule. The close mechanical fit that is possible between an antigen and the related antibody makes possible bonding of adequate strength, though the bonding forces consist only of weak van der Waals forces and hydrogen bonding forces, and accounts for the great specificity of antibody-antigen interactions. As each polypeptide chain is double-ended, the antibodies may be bivalent, and precipitates consisting of three dimensional networks may form when the antibodies interact with multivalent antigens.

Professor Pauling also suggested[1] that the necessary conditions for antibody formation might be obtained *in vitro* as well as *in vivo*. As a partial uncoiling of the polypeptide chain may be expected to occur when a protein is subjected to conditions that tend to denature it, antibodies might be formed by partially denaturing globulin in the presence of the antigen, subsequently removing the denaturing agent or condition under circumstances favoring a re-coiling of the chains in contact with the antigen molecules, and finally dissociating the antibody-antigen complexes in order to enable the removal of the antigen from the preparation. Experiments which were carried out along these lines by Professor Pauling and Professor Dan H. Campbell[2] indicated that this method may be a feasible one for the artificial production of antibodies.

In 1942 Professor Pauling suggested that the *in vitro* method be used in an attempt to prepare antibodies to histamine,



,

which is the decarboxylation product of the amino acid histidine. Histamine is a metabolic product of living organisms which ordinarily is destroyed rapidly by the organisms. When injected into an animal it produces a shock-like response, and many of the characteristics of anaphylactic and traumatic shock are ascribed to the produce of histamine by the organism[3]. Antibodies capable of binding and inactivating histamine and histamine-like substances may be of therapeutic value in the treatment of shock and allergy, and investigations involving their use may aid in the elucidation of the relation of histamine to shock.

The in vitro method was chosen for this work in part because of the difficulty of preparing a suitable antigen for injection into animals by coupling histamine, as a hapten, to a protein molecule. After the investigation described below was completed Fell, Rodney, and Marshall[4] reported that antisera to histamine azoprotein (histamine azo-despicated-horse-serum-globulin) and histamine carbamido protein had been prepared and that these preparations inhibited anaphylaxis in vivo and in vitro, precipitated the corresponding antigens, and gave positive hapten inhibition tests. These workers, however, coupled the histamine molecule to the protein molecule through the aliphatic amino group; a much better procedure would be to couple at the ring in order to leave virtually unchanged the molecular fragment that is believed to give the molecule its physiological activity[5]. Additional work is being carried out along these lines by Professor Carl Niemann and coworkers.

Further discussion of the in vivo method is beyond the scope of this report.

I devoted three months in the summer of 1942 to an attempt to prepare an antibody to histamine by the in vitro method. This was carried out under the supervision of Professor Carl Niemann. It was recognized at the outset that probability of success was not high, as the general method was very novel and the best conditions had not been established. The investigation was nevertheless carried out because of the high importance of obtaining information regarding the relation of histamine to shock, and because of the therapeutic value that antibodies to histamine might possess.

2. The general method

In these experiments, preparations to which we will refer as histamine-treated globulin (HTG) were prepared by heating bovine serum globulin with a molar excess of histamine at 56°C, which is about ten degrees below the temperature of irreversible denaturation, in order to allow the polypeptide chains to uncoil partially. The mixtures were slowly cooled in order to favor the re-coiling of the chains at the surfaces of the histamine molecules and dialyzed to dissociate the complexes and remove the histamine.

As the histamine molecule is very small in comparison to antibody molecules its immunological valence is at most two. Accordingly we would not expect an antibody-antigen precipitate to form on addition of histamine to the HTG preparation. As a precipitation test with histamine would not be expected to be useful, it is necessary to use some other method of evaluating the anti-histamine activity of the HTG preparations.

For this purpose we used smooth muscle protection tests with guinea pig ileic strips[6] in a Schultz-Dale apparatus. The applicability of this test for this purpose derives from the fact that in the presence of histamine, even in very low concentrations, strips or lengths cut from the ileum of the guinea pig undergo a contraction sufficient to activate a recording device, and that under the optimum conditions the magnitude of the response is an approximately linear function of the histamine content at low concentractions. If an HTG preparation has antibody-like activity against histamine, the addition of the preparation to a histamine solution should reduce the effect of the solution on the smooth muscle, at least relative to the control experiments.

3. Experimental

(a) Materials

Histamine was obtained in the form of histamine phosphate, in which two molecules of phosphoric acid are combined with each molecule of histamine. For use in assay work aqueous solutions containing one gamma of histamine, calculated as the free base, per ml. were prepared. More concentrated solutions were used in the preparative work.

Bovine γ-globulin * was obtained from the laboratories of Armour and Company. This material (G) was used for most of our experiments in a two-percent aqueous solution. In an attempt to remove substances having histamine-like activity against smooth muscle, a part of our supply was dialyzed in a two-percent solution against 0.9-percent sodium chloride solution for several days and dried by the lyophil procedure to obtain a product which we shall designate dialyzed and lyophiled globulin (DG). In later experiments the dialysis was carried out against a phosphate buffer at pH 7.5 in order to obtain a product (BG) with higher pH stability. The molecular weights of these materials were assumed[7] to be about 160,000.

Smooth muscle for the assay work was obtained from guinea pigs which weighed about 200 to 400 grams. Each animal was killed by a blow on the head and the ileum was removed and flushed with nutrient solution until clean. The ileum was then stored in a refrigerator between cotton pads saturated with nutrient solution, and was cut into one-inch lengths as needed.

* Run 21, Fraction II % III.

Tyrode's solution[8] was used as the nutrient solution in all of this work.

(b) Partial denaturation in the presence of histamine.

Solutions containing two percent of the untreated globulin (G) or of the dialyzed and lyophiled globulin (DG or BG)and 0.0015 to 0.003 percent of histamine (calculated as the free base) were heated at 56°C. in vaporstats over boiling acetone for periods ranging from two days to a week. The solutions, together with any precipitates that formed, were then placed in Dewar flasks and set aside to cool. They were then dialyzed in cellophane bags against 0.9-percent sodium chloride solutions for four to eight days at 4°C. with daily changes of the dialysates. Any precipitate that was present was then centrifuged off. The solutions are designated HTG, HTDG, or HTBG, depending on the protein preparation used. Corresponding control preparations, designated CTG, CTDG, and CTBG, were prepared by a similar procedure but without the addition of histamine. To obtain additional controls other variations were made in this procedure; the most important one is the omission of the dialysis operation.

(c) Schultz-Dale tests

The apparatus used in this work is similar to that designed by Dr. Gordon Alles of this Institute. In a single all-glass unit are provided a muscle bath with a capacity of 20 ml., a reservoir for storage of nutrient solution, a stopcock for draining the contents of the muscle bath and refilling the bath from the reservoir, and a jacket through which water is pumped from a thermostat to maintain the contents of the bath and reservoir at 37°C. The contents of the bath and the reservoir are continually aerated by a stream of air bubbles from a hypodermic needle or glass capillary tube.

In each run a one-inch length of the smooth muscle was tied at both ends with a silk thread and mounted in the muscle bath. One end was tied to a kymograph lever by means of which the contractions of the muscle could be amplified and recorded on a kymograph drum. After an hour in the bath the muscle was ready for use. Its response was calibrated with known amounts of histamine, 0.05 to 0.25 gamma, at the beginning of the run and frequently during the run. In each test about 1 ml. of the solution to be assayed was introduced into the bath with a hypodermic syringe, and when the response became steady the bath was drained, rinsed three times with nutrient solution, and refilled. Tests were made in cycles in which solutions to be tested for antibody activity were alternated with the controls. In each cycle four or more different solutions were tested.

Very erratic behavior was often observed; the calibration usually changed considerably with time, and consecutive runs were often quite unreproducible.. For this reason it was necessary to make several tests with each solution and to compare the results for different solutions only within a single cycle of tests.

(d) Preliminary experiments

Measurements of pH were made on the protein solutions at various stages of their treatment. The untreated solutions of raw protein were found to have pH values of about 7.2. The addition of histamine and two days of heating at 56°C usually did not significantly affect the pH value, although in a few cases pH values as low as 6.1 were observed. After dialysis the pH value dropped often as low as 4.8. Similar results were obtained during the treatment of <u>DG</u>, but in the experiments with <u>BG</u>

the pH value remained at 6.9 to 7.2 during the entire procedure. The amounts of precipitate that formed during heating and especially during dialysis were smallest in the experiments carried out with *BG* and largest in those with *DG*.

Some experiments were carried out to determine the histamine-like activities of the unheated and heated protein preparations. We found that one milliliter of the two-percent globulin solution (*G*) is equivalent to about 0.15 or 0.16 gamma of histamine. The activity was found to decrease to about 0.12 on heating at 56°C. for several hours. The *DG* had an activity equivalent to about 0.11 gamma of histamine. As these activities are of the order of the activity of histamine in the amounts used in the assay work, the need for controls is clear. To estimate the degree of non-specific binding of histamine by protein, mixtures of histamine with heated and unheated globulin were compared with undialyzed *HTG* preparations and with histamine itself. No positive indication of non-specific binding was obtained.

(e) Tests for antibody-like activity

A total of 229 solutions was prepared for tests of antibody-like activity. Most of these were prepared by mixing the histamine stock solution (one gamma per milliliter) with the various protein preparations (*HTG*, *HTDG*, *HTBG*, and the corresponding control preparations). On the assumption that all of the protein molecules in each preparation had been converted into bivalent antibodies, one milliliter of the antibody solution is calculated to be equivalent to 28 ml. of the histamine solution. The proportion of antibodies in the preparations is not expected to be large, however; therefore many of the mixtures tested were prepared with a much larger ratio of protein solution to histamine solution. The mixing ratios that we employed ranged from 9 : 1 to 1 : 99.

Some of the solutions tested were: undialyzed HTG preparations, solutions of histamine that had been heated at 56°C. under conditions similar to those of the treatment of the protein preparations, mixtures of untreated protein with histamine solution, and water controls similar in composition to the other test solutions but with water substituted for protein solution.

These solutions were tested in groups of four to six by the Schultz-Dale technique as described above. Each group was selected to represent a particular set of conditions determined by the choice of starting material, the conditions of treatment of the protein, and the ratio of the protein preparation to the histamine solution in the solutions to be tested. Each group usually contained one mixture prepared from HTG, one prepared from undialyzed HTG, one prepared from CTG, a solution of the untreated protein, a water control, often a heated histamine control, and sometimes others that we will not mention here[9].

4. Results

The amplitudes of the contractions were measured with a millimeter scale. The results for each testing cycle were judged as a group, and on a relative basis.

When, through the group, the solutions to be tested for antibody activity assayed less than the corresponding controls (except for the water control which was virtually always low), the result of the group was designated a positive antibody response (+). When they were consistently above the controls, the result was designated an anomalous antibody *response* (-). Intermediate designations (±, 0, ∓) were assigned to groups that gave inconsistent or inconclusive results. The criterion for *positive antibody response* may be, for example, the satisfaction of all of the inequalities

$$R\ (\underline{HTG} + \underline{H}) < R\ (\underline{CTG} + \underline{H}),$$

$$R\ (\underline{HTG} + \underline{H}) < R\ (\underline{HTG}) + R(\underline{H}),$$

$$R\ (\underline{HTG} + \underline{H}) < R\ (\underline{G}) + R(\underline{H}), \text{ and}$$

$$R\ (\underline{HTG}\ \text{undialyzed}) < R\ (\underline{CTG}) + R\ (\underline{HH}),$$

where $\underline{R}$ designates smooth muscle response and where corresponding total quantities of protein and of histamine (assuming no decomposition of the latter) are the same on both sides of each inequality.

The results of these tests are presented in Table 1. The symbols used in this table and elsewhere in the text may be summarized as follows:

G = globulin (usually in a 2% aqueous solution),

DG = dialyzed and lyophiled globulin,

BG = globulin dialyzed against a buffer and then lyophiled,

H = histamine (usually in a solution containing one gamma per ml.),

prefix *HT* = histamine-treated (Sec. 3b),

prefix *CT* = control-treated (Sec. 3b), and

HH = heated histamine (H heated at 56°C.).

Table 1

Results of Tests for Antibody Activity

Ratio of protein preparations* to histamine solution** in mixtures tested	Major groups of test solutions			
	HTG HTDG G	HTG HTDG CTG HH G	HTG CTG G	HTBG CTBG HH BG
9 : 1		∓		
7 : 1	---0			
1 : 1	00--+0-	-		
1 : 3	--	--	∓-	-∓±±+00
1 : 7	+000-			
1 : 9		00--∓ ---0	∓∓	-0--+0
1 : 19	--			
3 : 97		00++0 -0--00	∓∓-0	++
1 : 49	-0			
1 : 99		∓00+00+		
Experiments with undialyzed preparations		-00-+00 000-000-		0000

* 2% aqueous solution.
** Aqueous solution containing one gamma of histamine per ml.

5. Discussion and summary

It is apparent from the results given in the table that the preparations possessed no ability to protect smooth muscle against histamine. The preponderance of anomalous (-) results ("probody" response) is very difficult to explain, for it is hard to see any mechanism whereby any of these preparations could <u>enhance</u> the activity of histamine when it is introduced into the muscle bath in a mixture with histamine. A simple explanation of these results might be that the histamine molecules are so bound by the modified protein molecules that their activity toward smooth muscle is not decreased, and that the histamine-protein complexes are absorbed by the smooth muscle. This hypothesis is an extremely tenuous one for it is almost inconceivable that the histamine molecule could be bound by an antibody without losing its activity through blocking of an essential part of the active molecular fragment.

We must conclude that the results of this experiment indicate that this method of preparing antibodies to histamine was unsuccessful, and that otherwise the results are inconclusive.

It is possible that because of the comparatively small size of the histamine molecule as an antigen, the histamine molecules were completely surrounded or enclosed by the re-coiling polypeptide chains in such a way that the complexes could not be dissociated by dialysis. <u>In vitro</u> experiments in which the antigen molecules comprise histamine molecules as haptenic groups coupled by azophenyl linkages to protein molecules or to resorcinol molecules might be more successful; <u>in vivo</u> work along these lines has already been done[4]. The use of antigens of this kind would permit the use of precipitation tests and possibly hapten inhibition tests.

I am very grateful to Professor Linus Pauling for suggesting this problem, and to Professor Carl Niemann for valuable guidance and suggestions.

References

1. Linus Pauling, *J. Am. Chem. Soc.* 62, 2343 (1940).
2. Linus Pauling and D. H. Campbell, *Science* 95, 440 (1942); *J. Exper. Med.* 76, 221 (1942).
3. C. H. Best and E. W. McHenry, *Physiol. Rev.* 11, 371 (1931).
4. N. Fell, G. Rodney, and D. E. Marshall, *J. Immunol.* 47, 237, 251 (1943).
5. Carl Niemann and J. T. Hayes, *J. Am. Chem. Soc.* 64, 2288 (1942).
6. M. Guggenheim, and W. Löffler, *Biochem. Z.* 72, 303 (1916).
7. E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides", A.C.S. Monograph. New York: Reinhold Publishing Corp., 1943. p 428.
8. T. Sollman, "A Manual of Pharmacology". Philadelphia: W. B. Saunders Co., 1942. p. 854.
9. For details see a report by D. P. Shoemaker, "An Attempt to Prepare an Antibody to Histamine", submitted to Professor Carl Niemann, September 23, 1942.

II

AN INVESTIGATION OF A POSSIBLE METHOD FOR PREPARING LARGE SINGLE CRYSTALS OF ALUMINUM ORTHOPHOSPHATE

1. Introduction

Large native crystals of α-quartz constitute a very important raw material in the electronics and communications industry where these crystals are used in the manufacture of piezoelectric elements for frequency controls and filters. They are also important in the chemical industry for the manufacture of clear fused silica, and they have uses in the optical industry as well. The usefulness of quartz for piezoelectric elements arises from the lack of a center of symmetry, a crystal structure that results in a piezoelectric effect of appreciable magnitude, a degree of hardness that enables the crystals to withstand severe mechanical vibrations of very high frequencies, and the high degree of homogeneity, purity, and perfection that can be found in native crystals of this substance.

A large proportion of the crystals that are used in the electronics industry are imported from Brazil. Although the supply was adequate for many years, the additional demands imposed by the recent war made native quartz crystals of satisfactory size and quality a critical material. As no means existed for preparing suitable crystals of quartz synthetically, it became very desirable to search for a suitable substitute.

In the spring of 1943 Professor Linus Pauling pointed out that crystals of aluminum orthophosphate ($AlPO_4$) might serve as a satisfactory substitute for quartz. This suggestion was based on the close structural similarity of crystals of this compound to those of quartz and on the apparent feasibility of growing them from a solution. At Professor Pauling's suggestion, and with the approval of Dr. J. B. Conant, then Chairman of the National Defense Research Committee, I undertook to study the conditions by which such crystals could be prepared, with the hope that if the results showed promise the work would become the subject of an NDRC contract. I carried out this work under the supervision of Dr. Reuben E. Wood.

My preliminary work on this problem is described below. Although crystals up to 8 mm. in length and 4 mm. in diameter were prepared, and although the method appeared to be promising for the preparation of larger crystals, the work was abandoned after two and one-half months on receipt of word from Dr. Conant that the major problem had been solved by other methods.

2. Previous work

Aluminum orthophosphate ($AlPO_4$) appears to have been first prepared in the anhydrous crystalline state by de Schulten[1], who in 1884 reported that he obtained crystals by adding concentrated phosphoric acid in excess to a saturated aqueous solution of sodium aluminate and heating the mixture in a sealed tube for several hours at 250° C. The crystals appeared as hexagonal prisms at most 2 mm. in length and 0.1 mm. in diameter. Examination with the polarizing microscope showed them to be optically uniaxial and positively birefringent.

Strada[2] obtained powder photographs of both the orthophosphate and the orthoarsenate ($AlAsO_4$) of aluminum, and indexed them on the basis of a tetragonal cell. He concluded that the space-group of both compounds is $S_4^2 - I\bar{4}$ and that the crystals are similar in structure to crystobalite.

The orthoarsenate was reinvestigated by Machatschki[3], who found the conclusions of Strada with regard to this compound incorrect. From comparisons of powder photographs of this substance with photographs of α-quartz Machatschki concluded that aluminum orthoarsenate, and probably aluminum orthophosphate which was claimed to be isomorphous with the orthoarsenate, are rhombohedral in symmetry and similar to α-quartz in structure. A similarity to the quartz structure should not be surprising, for the crystallographic properties of Al^{+3}, P^{+5}, and As^{+5} are in some ways analogous to those of Si^{+4}, particularly with regard to the tendency for oxygen atoms to coordinate tetrahedrally around these kernels.

Huttenlocher[4] undertook to verify this conclusion for the orthophosphate. By a method similar to that of de Schulten he obtained small crystals with prismatic and rhombohedral faces similar to those of quartz. The angle between adjacent prismatic and rhombohedral faces in the zone of a two-fold axis was found by goniometric measurements to be 21°05'; this is very close to the angle (2021)/(1010) for quartz, which is 21°29'. The lattice constants were determined from powder photographs. These and other properties of this substance as determined by Huttenlocher are compared in the following table with the cell constants and properties of α-quartz as determined by Wyckoff[5] and others, and with the cell constants and density of aluminum orthoarsenate as determined by Machatschki[3].

Table 2

Lattice Constants and Physical Properties of Aluminum Orthophosphate, Aluminum Orthoarsenate, and α-Quartz

	AlPO$_4$[4]	AlAsO$_4$[3]	α-Quartz
Lattice constants, Å			
a	4.93	5.03	4.90[5]
c	2 x 5.47	2 x 5.61	5.39
c/a (x-ray)	2 x 1.11	2 x 1.11	1.10
c/a (optical)	2 x 1.123		
Density, g./cm.^{3}	2.56	3.34	2.65
Hardness	Considerable		7*
Indices of refraction			
Ordinary ray	1.524		1.544[6]
Extraordinary ray	1.530		1.553
Double refraction	.006		.009

* On Mohs' scale

It may be inferred from the data given in this table that the two aluminum compounds are very similar in structure to α-quartz. The similarity is further borne out by comparisons, made by Huttenlocher and Machatschki, of intensities of x-ray reflections with those of quartz. In accordance with the doubled c-axis found for the aluminum compounds, it is probable that positions corresponding to those of silicon atoms in quartz are occupied alternately in the c-direction by atoms of aluminum and phosphorus or arsenic.

Huttenlocher also observed that aluminum orthophosphate undergoes a transition at high temperatures to a form which is very nearly optically isotropic. In order to determine whether this transition is analogous to the transition between α- and β-quartz, Professor J. H. Sturdivant in an unpublished investigation carried out an x-ray investigation of aluminum orthophosphate at room temperature and at high temperatures. His work substantiated the conclusions of Huttenlocher regarding the structure of the low temperature form and indicated that the high temperature form is similar in structure to β-quartz.

The method employed by Professor Sturdivant for preparing crystals of aluminum orthophosphate is similar to that of de Schulten and is the one which was adopted as the basis of the present investigation. His best crystals, which ranged in size up to one or two millimeters, were obtained by dissolving 1/3 mole of aluminum turnings in a solution of 0.5 mole of sodium hydroxide in 100 ml. of water, mixing the filtered solution with 3 to 5 moles of phosphoric acid (used as the 85% solution), and heating the mixture at 250° C. in sealed tubes.

In the experiments described below solutions of the same kind were used, and the compositions of the solutions and the conditions of crystallization were varied in order to determine the compositions and conditions which favor the growth of the largest and best formed crystals of aluminum orthophosphate.

3. Apparatus and materials

In this work it was necessary to heat aqueous solutions at temperatures as high as 250° C. in sealed glass tubes. Accordingly some attention was given to the design of tubes which are able to withstand internal pressures as high as 50 atmospheres at this temperature without bursting. Tubes with inside diameters as large as 20 mm. were prepared from pyrex combustion tubing of 2-mm. wall thickness. These had body lengths of about 2.5 inches, were closed at one end, and were tapered at the other end to a junction with a length of 7-mm. pyrex tubing of 1-mm. wall thickness. In addition, many tubes of smaller diameter were made from stock pyrex tubing with a wall thickness of about 1 mm. All tubes were carefully annealed.

Since the surface of the glass becomes etched during contact with the hot solution, each tube could be used only once. In use, each tube was filled about two-thirds full, and the open end sealed off. It was then enclosed in a length of steel pipe to minimize hazards due to bursts, and placed horizontally in an electric furnace.

The electric furnace used in these experiments was lined on the inside with a copper pipe 2.5 inches in diameter and 24 inches long. The ends of the furnace were stopped with copper and asbestos plugs which were provided with holes or notches to accomodate a thermometer and a specially constructed mercury thermoregulator. By means of this thermoregulator the furnace temperature could be maintained at any desired temperature up to 300° C. within one or two degrees.

The solutions that were heated in the furnace were prepared in the following way. A solution of sodium aluminate was prepared by adding 27 g. of aluminum turnings to a solution of 60 g. of sodium hydroxide in 300 ml. of water. The reaction vessel was cooled under the cold water tap until the reaction was complete. The solution was then filtered and made up to 300 ml. by the addition of water to replace that lost by evaporation. This stock solution was designated solution _A_. A stock solution designated as solution _B_ was prepared by adding 75 ml. of 85% phosphoric acid to 30 ml. of solution _A_ and stirring until the precipitate of aluminum hydroxide, which first formed on the addition of acid, redissolved to give a solution which contained aluminum ions and primary phosphate ions ($H_2PO_4^-$) as well as sodium ions, water, and excess phosphoric acid.

Another solution, C, which contained aluminum and phosphate in the same total concentrations but no appreciable amount of sodium ion, was prepared by dissolving a well washed precipitate of aluminum hydroxide in an excess of 85% phosphoric acid and diluting the resulting solution with water.

Best results were obtained when these solutions were freshly prepared before each experiment.

4. Preparation of aluminum orthophosphate crystals

In order to obtain a stock of crystalline material to be used in solubility studies and as seeds for the growth of larger crystals, glass tubes were charged with solution B or solution C, sealed off, and placed in the furnace where they were maintained at 200°- 250° C. for a day or more. The tubes were then removed from the furnace, cooled, and opened. The crystals were washed into a Buchner funnel where they were further washed with water and dried with alcohol and ether. Crystals prepared in this way ranged in size from microscopic particles to rhombohedra 2 mm. or 3 mm. long and large multicrystalline clumps. Many of the crystals had white surfaces which apparently resulted from etching by the solution.

5. Variations in the conditions of crystallization

It was desired to determine the effect of composition, water content, and temperature on the rate of production of crystals and on the size and quality of the crystals obtained.

In parallel experiments in which stock solutions B and C were heated at 220° C. for 15 hours, solution B gave a 15% yield of well-formed crystals which ranged in size up to nearly 3 mm., while solution C gave a 60% yield of somewhat smaller crystals. The reason for the difference in behavior may be that in solution B, which contains an excess of primary phosphate ions, the aluminum ions are more completely complexed with primary phosphate ions than in solution C. This hypothesis must be taken as tentative, however, for the results of this experiment would not be much different if solution C contained a much larger number of nuclei on which crystals can grow, either by accident or as a fundamental result of some step in the preparation of the solution. In the absence of other evidence the results of this experiment may be taken to indicate that the use of sodium aluminate rather than aluminum hydroxide in the preparation of the crystallizing solution is favorable for the growth of large, well-formed crystals.

Other experiments showed that the addition of water to solution B increases the yield of crystals and the rate of their growth, but decreases their size. Reduction of the water content by the addition of phosphorus pentoxide considerably reduced the rate of crystal growth.

In the short time available, no radical changes in the composition of the crystallizing medium were tested. In the remaining experiments solution B was used without alteration, except in some cases in which the concentration was increased by dissolving previously prepared crystals in the solution. Reduction in the rate of crystallization by addition of phosphorus pentoxide appeared to be inadvisable because over the long period of time that would be required to grow a large crystal many additional centers of crystallization are likely to appear.

Experiments with solution B at different temperatures showed that no crystals form below about 190° C. Above 190° crystals were found to grow at a rate which increased with the temperature. At 250° C. the crystals that appeared were usually small in size. Moreover, at this temperature the glass tubes were strongly etched by the solution. From the standpoint of size and perfection of the crystals the optimum temperature appeared to be in the range 195° to 200° C.

6. Solubility of crystals

At room temperature the crystals are apparently insoluble in water or phosphoric acid, but at temperatures between 100° and 180° C. they dissolve in phosphoric acid or in solution B at an appreciable rate. Hence the successful use of a seed crystal requires that the crystallizing solution be heated rapidly to temperatures above 190° C. and that the seed not be too small. Moreover, tubes containing finished crystals should be cooled rapidly to prevent etching of the crystal faces.

Experiments showed that at 110° C. solution B is capable of dissolving powdered crystals of aluminum orthophosphate to the extent of about 0.2 g. per ml. It may therefore be possible to grow crystals of this compound at temperatures below 180° C. by the simple expedient of first saturating the crystallizing solution with it at still lower temperatures.

7. Growth of large crystals from seeds

Several experiments were carried out in which seed crystals were placed in tubes charged with solution B and the tubes heated at 196° C. for periods ranging from four days to several months.

In one experiment three tubes were charged with solution B and with respectively no seed crystal, one seed crystal, and several seed crystals. These were heated at 196° C. for two weeks. At the end of this time each of the tubes contained a large number of small crystals, which were for the most part well formed. No crystals larger than 3 mm. in size were found in the unseeded tube, one large crystal 8 mm. long and about 4 mm. in diameter was found in the tube to which a single seed had been added, and three or four crystals having maximum dimensions of 5 mm. to 8 mm. were found in the tube to which several seeds had been added. The crystals were rhombohedra of varying degrees of perfection; their lengths were approximately twice their diameters.

The results indicate that the growth of large crystals from seeds is feasible, but that care must be taken to eliminate other possible centers of crystallization. It is apparent from the relative sizes of the crystals that the small crystals in each tube must have begun to grow only after several days of heating; during this time the glass walls of the tubes may have been etched by the solution to such an extent that they provided unwanted centers of crystallization.

In another experiment a tube was charged with 35 ml. of solution

B and with one seed crystal, and was heated at 196° C. for two months. At the end of that time there were many crystals present in the tube and it was impossible to determine which of them had grown on the seed.

8. Properties of the crystals

The crystals are similar in shape and features to those prepared by Professor Sturdivant, though many of them were much larger in size. The largest single crystal was 8 mm. in length and 4 mm. in diameter.

The crystals are rhombohedral in form, though few of them approached perfection. Prismatic faces were not observed. The faces are etched in varying degrees, and the etch patterns on the rhombohedral faces of well formed crystals indicated that the crystals have the point-group symmetry D_3 - 32, as does quartz.

A crystal about 4 mm. long and in shape a very nearly perfect rhombohedron was mounted on an optical goniometer with its three-fold axis vertical, and the latitude and azimuthal angles for five of the faces were measured. The results are given in the following table.

Table 3

Goniometric Examination of a Crystal of Aluminum Orthophosphate

Indices		Character of reflection	Azimuth	Latitude
Hex. axes	Rhomb. axes			
1011	100	Good	0° 00'	21° 24'
1101	010	Rather fuzzy	120° 00'	21° 25'
0111	001	*		
1011	100	Very fuzzy**	179° 48'	- 21° 50'

Table 3 (continued)

Indices		Character of reflection	Azimuth	Latitude
Hex. axes	Rhomb. axes			
1101	010	Excellent	300° 04'	- 21° 25'
0111	001	Rather fuzzy	60° 08'	- 21° 22'

* Only a blur was observed; this face apparently was in contact with the wall of the tube.

** This poor reflection was not used in orienting the crystal.

The average absolute value of the latitude angle, which corresponds to the angle between rhombohedral and prismatic faces in the zone of a two-fold axis, is 21° 24' if the data for (0111) and (1011) are excluded. This is to be compared with the value 21° 05' reported by Huttenlocher[4]. With the use of hexagonal axes the azial ratio c/a is found to be 2.210, in good agreement with the values previously found by x-ray measurements and listed in Table 2.

The density of the crystals at 20° C. was measured by displacement of water in a pycnometer, and was found to be 2.61, in rough agreement with the value 2.56 given by Huttenlocher.

The hardness of the crystals was found to be about 4 on Mohs' scale. They are accordingly considerably softer than quartz, which has a hardness of 7. This result appears to contradict that of Huttenlocher, who did not, however, refer the hardness of his crystals to Mohs' scale.

On heating strongly in a gas-oxygen flame the crystals usually turned white and crumbled. When the crystals were fused, small bubbles were observed in the melt, and on resolidifying the product did not have a glass-clear appearance.

Lack of time prevented the study of the piezoelectric properties of the crystals. Moreover, quantitative piezoelectric studies would require crystals larger in size than those that were available. Qualitative or semi-quantitative measurements might be carried out with the present crystals by one or more of the methods outlined by Wooster[7].

9. Discussion of the technique

The results of this study indicate that it is feasible to grow crystals of aluminum orthophosphate that are large enough to use for piezoelectric purposes. The following conclusions may be drawn with regard to method:

(a) The crystals may be grown from a concentrated mixture of sodium aluminate and phosphoric acid in excess. The use of sodium aluminate rather than aluminum hydroxide appears to give better results, possibly due to better complexing of the aluminum ions. The solutions should be at least as concentrated as those used in the present investigation, but the results of these studies do not appear to suggest radical changes in their composition.

(b) Large crystals may be best grown from seeds. Great care should be exercised to exclude additional centers of crystallization. If glass tubes are to be used, the experimental conditions may require modification to permit work at temperatures low enough so that etching of the glass does not take place. It may be desirable to use tubes of fused silica, or to carry out the crystallization in a fused silica beaker within an autoclave.

(c) The temperature at which the crystals are grown should not be much higher than that of incipient crystallization.

If further experiments are attempted, variations in the experimental arrangement should be tested. For example, the crystals might be grown in a tube that is heated non-uniformly in order to obtain stirring by convection. If a considerable amount of the crystalline material is placed in the cool end of the tube, this material may continuously replenish the solution as it is depleted by the growth of a larger crystal at the warmer end of the tube. If an autoclave is to be used in the way mentioned above, the bottom of the beaker might be covered with small crystals, and an electrically heated finger, dipping into the solution and holding a seed crystal, could serve as the site for the growth of a large crystal.

It should be pointed out that the relative softness of the aluminum orthophosphate crystals (4 on Mohs' scale) may be a barrier to their use as a general substitute for quartz in piezoelectric applications. If the crystals are found to have piezoelectric properties comparable to those of quartz, they may nevertheless find application in electronic circuits that do not subject them to mechanical vibrations of high amplitude.

10. Acknowledgements

I am indebted to Dr. Reuben E. Wood, who supervised this work, for many valuable suggestions. I am very grateful to Professor James H. Sturdivant for information concerning his previous work, and to Professor Verner Schomaker for several valuable discussions.

I wish to thank Professor Linus Pauling for suggesting this study.

11. Summary

A method of preparing crystals of aluminum orthophosphate has been tested with a view to the development of a procedure for growing large single crystals of this substance for possible use as a substitute for quartz in the manufacture of piezoelectric elements. In this method, a mixture of sodium aluminate and phosphoric acid is heated in a sealed tube at about 200° C.

This work was dropped before completion on receipt of information that other methods were being used to solve the problems caused by the wartime scarcity of large quartz crystals, and the effort which might otherwise have been spent in continuing this investigation was directed to war work of higher priority.

Enough work was done to indicate that the preparation of large single crystals of aluminum orthophosphate is feasible.

References

1. A. de Schulten, Compt. Rend. **98**, 1583 (1884).

2. M. Strada, Gazz. Chimica ital. **64**, 653 (1934).

3. F. Machatschki, Z. Krist. **90**, 314 (1935).

4. H. F. Huttenlocher, Z. Krist. **90**, 508 (1935).

5. R. W. G. Wyckoff, "The Structure of Crystals" (Second Edition), ACS Monograph No. 19. New York: Chemical Catalog Co., 1931. p 239.

6. Handbook of Chemistry and Physics (Twenty-ninth Edition). Cleveland, Chemical Rubber Publishing Co., 1945. p 452.

7. W. A. Wooster, Crystal Physics. Cambridge: At the University Press, 1938. Chapter VI.

III

INVESTIGATIONS OF THE CRYSTAL STRUCTURES OF AMINO ACIDS BY X-RAY DIFFRACTION

1. Introduction

The work presented in this section will be discussed under two headings: (A) An Investigation of the Space-Group and Lattice Constants of dl-Serine, and (B) An Investigation of the Crystal Structure of ℓ-Threonine.

Information regarding the structure of amino acids and simple peptides is of considerable interest in connection with the study of the structure of protein molecules, a study which is the subject of a research program of the Gates and Crellin Laboratories of Chemistry of this Institute[1,2].

X-Ray diffraction investigations of amino acids and simple peptides are undertaken primarily to obtain knowledge of the structures of the amino acid residues as they probably exist in the protein molecule, of the manner of combination of these residues in peptide chains, and of possible modes of packing of the peptide chains in the protein molecule. The structural information given by an x-ray diffraction study of an amino acid concerns bond distances and bond angles among the atoms of the amino acid molecule, and the manner of packing and hydrogen bonding among the molecules that make up the crystal. Information of this kind has been given by the few complete structure determinations that have been carried out in the amino acid field; this information should be supplemented by thorough studies of many other amino acids and simple peptides which represent a wide variety of modes of packing and of intermolecular bonding, substituent groups, spacial relations among residues in peptides, and so forth. Needless to say these investigations are only a part of the work which must be done on the problem of protein structure; this work must also include x-ray studies of protein molecules in crystals and fibers, physical measurements of the shapes, molecular weights, and properties of these molecules, chemical analysis for amino acid residues, and perhaps electron microscopy.

The structures of only two amino acids have been investigated in detail by x-ray diffraction methods. The crystal structure of dl-glycine has been determined by Albrecht and Corey[3], and the structure of nickel glycine dihydrate has been determined by Stosick[4]. The crystal structure of dl-alanine has been determined by Levy and Corey[5]. Quantitative work on peptides is limited to the determination by Corey[6] of the crystal structure of diketopiperazine, which is a cyclic dipeptide of glycine, and the work (still in progress) by Hughes and Moore[7] on β-glycylglycine. Preliminary work has been done by Bernal[8] and by Albrecht and coworkers[9] on a number of other amino acids. This work includes determinations of space-groups, lattice constants, densities, and in many cases cleavage and optical properties. The amino acids studied by Bernal are α-glycine, β-glycine, d-alanine, dl-alanine, d-phenylalanine, ℓ-cystine, asparagine monohydrate, ℓ-aspartic acid, ℓ-glutamic acid, and diketopiperazine; those studied by Albrecht and coworkers are dl-valine, dl-threonine, dl-serine, dl-norleucine, and dl-methionine[2]. The space-group reported for dl-thneorine by Albrecht and coworkers is apparently incorrect, and will be discussed further in a later section. The occurrence of this error indicates the need for a careful review of the preliminary work on some of the other amino acids.

I had originally planned to undertake a reinvestigation of the space-groups and lattice constants of some of the amino acids mentioned above, as well as to carry out similar studies of some amino acids for which data on space-groups and lattice constants are inadequate or lacking. The latter group includes tyrosine, iodogorgoic acid, thyroxine, leucine, isoleucine, hydroxyglutamic acid, arginine, lysine, histidine, proline, hydroxyproline, and tryptophane. It soon became apparent that it would be impossible for me to carry out a complete investigation of a single amino acid if I were to make studies of space-groups and lattice constants of any significant number of compounds. The work described in this section is therefore limited to a determination of the space-groups and lattice constants of *dl*-serine and *ℓ*-threonine, and to a more detailed investigation of the structure of *ℓ*-threonine.

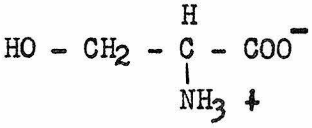
References

1. R. B. Corey, *Chem. Rev.* 26, 227 (1940).
2. R. B. Corey, *Advances in Protein Chemistry* (to be published).
3. Gustav Albrecht and R. B. Corey, *J. Am. Chem. Soc.* 61, 1087 (1939).
4. A. J. Stosick, *J. Am. Chem. Soc.* 67, 365 (1945).
5. H. A. Levy and R. B. Corey, *J. Am. Chem. Soc.* 63, 2095 (1941).
6. R. B. Corey, *J. Am. Chem. Soc.* 60, 1598 (1938).
7. E. W. Hughes and W. J. Moore, *J. Am. Chem. Soc.* 64, 2236 (1942).
8. J. D. Bernal, *Z. Krist.* 78, 363 (1931).
9. G. Albrecht, G. W. Schnakenberg, M. S. Dunn, and J. D. McCullough, *J. Phys. Chem.* 47, 24 (1943).

A. AN INVESTIGATION OF THE SPACE-GROUP AND LATTICE CONSTANTS OF dl-SERINE

1, Introduction

This section is a description of a redetermination of the space-group and lattice constants of dl-serine:



$$HO - CH_2 - \overset{H}{\underset{NH_3^{+}}{C}} - COO^{-}$$

This compound is of frequent occurrence in proteins, notably silk-fibroin. It may be regarded as a hydroxyl derivative of alanine. Its study by x-ray diffraction is an attractive problem because of the relatively small number of atoms in the molecule.

The space-group and lattice constants of dl-serine crystals were first determined by Albrecht and coworkers[1]. These investigators found the crystals on goniometric examination to be monoclinic holohedral, with axial ratios a:b:c = 1.194 : 1 : 0.522 and with $\beta = 73^\circ 47'$. The authors found that their results may be reconciled with those reported by Groth[2] who made a different choice of a and c axes*. From measurements of layer-lines of rotation photographs and from measurements of high-order pinacoid reflections Albrecht and coworkers obtained the following values for the lattice constants:

$$\begin{aligned} a_o &= 10.74\ \text{Å} \\ b_o &= 9.15\ \text{Å} \\ c_o &= 4.78\ \text{Å} \\ (\beta &= 73^\circ 47') \end{aligned}$$

By the usual convention the supplement of the reported value of β, $106^\circ 13'$,

---

* The authors' assertion that Groth made different choices of both the a axis and the c axis is apparently incorrect; only the a axis was chosen differently.

would be given. It is not clear whether this angle was calculated from the x-ray data; the mode of presentation of the results suggests that this angle was merely taken from the optical data. The density value of 1.537 g. per cm^3. indicates that there are four (4.00 calculated) molecules in the unit cell. The systematic absences of odd order *0k0* reflections and of *h0l* reflections with *h* odd led the authors to assign the space-group $C_{2h}^5 - P2_1/a$ to *dl*-serine.

A quantitative investigation of the structure of *dl*-serine was begun in these laboratories by Dr. Chia-Si Lu. Dr. Lu subsequently moved to Amoy, China, where he plans to complete this work. His data are not immediately available to us, but he has recently informed me (in a private communication) that his results for the space-group and lattice constants agree with those of Albrecht.

In view of the importance of this amino acid I undertook to make a redetermination of its space-group and lattice constants, with a view to the possibility of carrying out a complete structure determination at a later date. The work which I have completed is described below. Additional work on this amino acid in these laboratories has been postponed, and will probably be undertaken if Dr. Lu is unable to complete the work in China.

2. Preparation of crystals

The *dl*-serine used in this work was prepared by Roland N. Icke, and was kindly made available to us by Professor Carl Niemann.

Crystals of *dl*-serine were prepared in the following way. A solution saturated with the amino acid at about 50° C. was prepared by adding finely ground crystals in small portions to about 30 ml. of water. The solution was then divided into two or three portions in 50-ml. beakers. Each beaker was covered with a watch glass and immersed in a water bath consisting simply of a one-liter beaker filled with water heated at 50° C. The water baths were allowed to cool for one to three days; as a result of the cooling, and also of the accompanying loss of water from the solutions by evaporation, amino acid crystals of a wide variety of sizes and degrees of perfection appeared. Promising crystals were removed from the beakers with an eye-dropper, dried on filter paper, and transferred to a circular paper pillbox for storage. Each beaker was then warmed to 50° C. in its water bath, enough water was added to obtain complete solution, and the bath was again allowed to cool; this process was repeated until a sufficient number of satisfactory crystals was obtained.

Selected crystals of dl-serine were mounted on stiff glass fibers with the aid of clear lacquer. The crystals that were mounted for x-ray work were usually less than 0.5 mm. in their longest dimension; frequently it was necessary to resort to cleavage to obtain crystals of suitable shape and size because of the plate-like character of the virgin crystals. Larger crystals were mounted with goniometer wax for optical work.

3. Optical examination

dl-Serine crystallized in monoclinic tablets; a drawing of a typical crystal is presented in Figure 1. Our choice of axes is the same as that of Albrecht[1]. The faces in the zone [001] possessed many striae in the zone direction; this was especially true of (100) and ($\bar{1}$00).

Two crystals about 1 mm. in size and several 0.5 mm. or less in

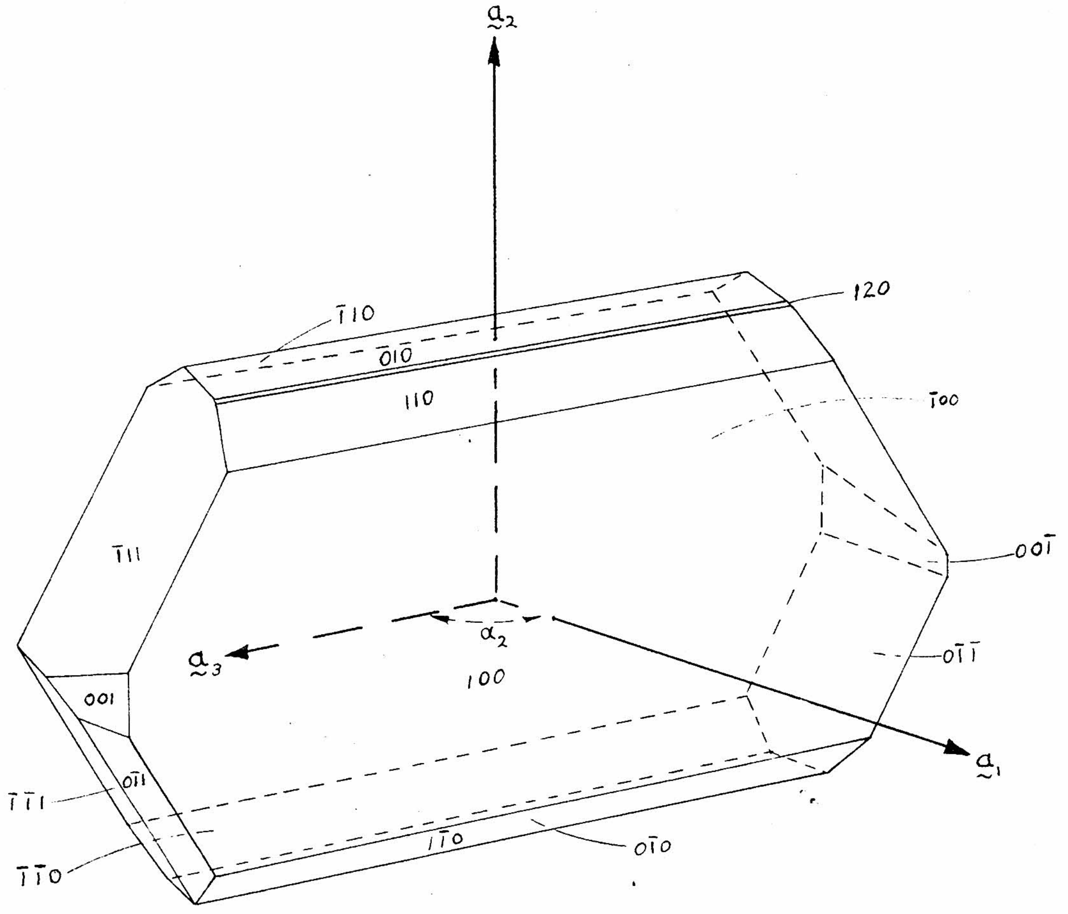


Figure 1. A typical crystal of dl-serine.

size were examined with the optical goniometer. Good reflections were obtained from all faces not in the zone [001]. Most of the faces in the zone [001] gave streaky reflections. Faces (110), ($\bar{1}10$), ($1\bar{1}0$), and ($\bar{1}\bar{1}0$), however, gave fair reflections.

The face development and the characteristics of the individual faces indicate that the crystals have the point-group symmetry C_{2h} (monoclinic prismatic).

In Table 4 are presented the averaged results of the most important goniometric measurements for three crystals and the values of the axial ratios calculated from them.

Table 4

Optical Goniometry of dl-Serine Crystals

Crystal faces	Number of measurements*	Angle between face normals		Data of Groth[2,3,4]	
		Average value	Mean deviation		
100/001	14	73°29'	5'		
011/001	6	26°51'	9'		
110/100	5	48°40'	28'	100/110	48°16'
$11\bar{1}/10\bar{1}$	8	27°40'	12'	011/001	27°27'
$10\bar{1}/100$	9	80°10'	10'	001/100	79°16'

Computed values of axial ratios and monoclinic angle:

$$a_1 : a_2 : a_3 = 1.176 : 1 : 0.528 \quad (= a_o:b_o:c_o)$$

$$a_3 : a_1 = 0.449 : 1$$

$$\alpha_2 = 106^\circ 31' \quad (= \beta)$$

* Number of equivalent pairs of crystal faces measured among the three crystals examined.

Groth[2], in summarizing studies of serine by Erlenmeyer and Stoop[3] and by Haushofer[4], reports that crystals of dl-serine are monoclinic prismatic, with axial ratios of 1.141 : 1 : 0.5235 and a monoclinic angle $\beta = 100^\circ 44'$. These results correspond to a choice of $\underset{\sim}{a}_2$ and $\underset{\sim}{a}_3$ similar to ours, but $\underset{\sim}{a}_1$ is along $[101]$ with respect to our axial system. By our choice of axes, axial ratios of 1.163 : 1 : 0.5235 and a monoclinic angle of 105°30' may be derived from Groth's data.

4. Density

The density of serine was determined approximately by displacement of 60°-70° ligroin in a small pycnometer, and was found to be 1.52 g./ml.

5. Cleavage

The crystals were found to give good cleavage parallel to the *c* face. Attempts to cleave the crystal in other orientations gave unsatisfactory results. Groth[2] reports cleavage parallel to the *a* face.

6. Laue symmetry

A Laue photograph taken with $\underline{a_2}$ parallel to the incident beam indicated the presence of a two-fold axis; several Laue photographs taken with a_2 perpendicular to the incident beam indicated the presence of a mirror plane. The Laue symmetry is therefore C_{2h}, and the crystal must belong to this point group or to one of the subgroups C_2 or C_s.

7. Layer-line measurements

Rotation photographs were taken with each of the three principal crystal axes in turn oriented parallel to the rotation axis. CuK radiation was used. Approximate values of the lattice constants were obtained from layer-line measurements. The results are presented in Table 5.

Table 5

Results of Layer-Line Measurements on dl-Serine

$a_1 = 10.0$ Å

$a_2 = 9.1$ Å

$a_2 = 4.4$ Å

8. Precise determination of lattice constants

Precise values of the unit cell dimensions were obtained from oscillation and rotation photographs; the results are presented in Table 6. CuK radiation filtered through nickel foil was used. The effective film-radius of the camera was calibrated by means of powder photographs of sodium chloride.

Table 6

Precise Determination of Lattice Constants for dl-Serine

A. Calculation of b_1

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	h_{100}	Weighting factor
200	0.1954 kX^{-1}	0.09770 kX^{-1}	1
400	.3895	.09738	2
10.0.0	.9738	.09738	5 x 2*
12.0.0	1.1682	.09735	6 x 2*
801	0.8654	.09733**	4
801	.7474	.09744**	4
10.0.1	1.0554	.09733**	5 x 2*
10.0.1	0.9397	.09780**	5
12.0.1	1.1254	.09730**	6

Average value of $h_{100} = b_1 = 0.09740$
Mean deviation .00009

* The α_1 α_2 doublet was resolved in the indicated reflections; hence these reflections were weighted doubly.

** These values were calculated with the aid of the value given below for b_3 and with a preliminary value of $_2$ (73°30').

B. Calculation of b_2

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	h_{010}	Weighting factor
020	0.2195 kX^{-1}	0.1098 kX^{-1}	1
040	.4381	.1095	2
060	.6571	.1095	3
061	.6925	.1096*	3
051	.5893	.1096*	2
062	.7874	.1096*	3

Average value of $h_{010} = b_2 = 0.1096$
Mean deviation .00005

* These quantities were calculated with the aid of the value given below for b_3 .

Table 6 (continued)

C. Calculation of b_3

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	h_{001}	Weighting factor
002	0.4432 kX^{-1}	0.2166 kX^{-1}	2
003	.6505	.2168	3
004	.8658	.2164	4
005	1.0824	.2165	5

Average value of $h_{001} = b_3 = 0.2165$

Mean deviation .0001

D. Calculation of β_2 from b_1, b_3, and $h0l$ reflections

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	$\cos \beta_2$	Weighting factor
605	1.0748 kX^{-1}	0.28327	1
201	0.2466	.28357	3
402	.4933		
603	.7405		
804	.9880		
403	.8454	.27846	(not included in average)
604	.8978	.28216	1
404	.8424	.28496	1
803	.8616	.28327	1
802	.9922	.28119	1
404	1.0454	.28305	1

Average value of $\cos \beta_2 = 0.28318$

Mean deviation .00059

Calculated values of β_2 73°33'

Mean deviation 2'

Table 6 (continued)

E. Summary

$b_1 = 0.0974 \pm .0001 \text{ kX}^{-1}$

$b_2 = 0.1096 \pm .00005 \text{ kX}^{-1}$

$b_3 = 0.2165 \pm .0001 \text{ kX}^{-1}$

$\beta_2 = 73^\circ 33' \pm 2'$

Adding an uncertainty of 0.1% for error in camera calibration and reading of fiducial mark, we obtain for the unit cell dimensions:

$a_1 = 10.70 \pm .02 \text{ kX}$

$a_2 = 9.12 \pm .02 \text{ kX}$

$a_3 = 4.815 \pm .006 \text{ kX}$

$\alpha_2 = 106^\circ 27' \pm 4'$

$a_1 : a_2 : a_3 = 1.173 : 1 : 0.528$ $\qquad$ $a_3 : a_1 = 0.450 : 1$

9. Weissenberg photographs

Well exposed equatorial Weissenberg photographs were taken with each of the three crystal axes in turn oriented parallel to the axis of rotation. Unfiltered CuKα radiation was used. In addition, equi-inclination Weissenberg photographs were taken of the first and second layer-lines with $\underset{\sim}{a}_3$ parallel to the rotation axis and of the first layer line with $\underset{\sim}{a}_2$ parallel to the rotation axis. The photographs were examined by means of an indexing chart and the reflections and extinctions were noted; these are tabulated below.

Table 7

Systematic Extinctions from Weissenberg Photographs

Orders of 0k0 observed

020, 040, 060, 0.10.0

Orders of 0k0 absent

030, 050, 070, 090; 080

Orders of h0ℓ observed

200, 201, 202, 203, 205, $20\bar{1}$, $20\bar{2}$, $20\bar{3}$, $20\bar{4}$, $20\bar{5}$, 400, 402, 403, 404, $40\bar{1}$, $40\bar{2}$, $40\bar{3}$, $40\bar{4}$, $40\bar{5}$, 600 (?), 601, 602 (?), 604, $60\bar{1}$, $60\bar{2}$, $60\bar{3}$, $60\bar{5}$, 801, 802, $80\bar{2}$, $80\bar{3}$, $80\bar{4}$, 10.0.0, 10.0.1, 10.0.2, $10.0.\bar{1}$, $10.0.\bar{2}$, $10.0.\bar{3}$, $10.0.\bar{4}$, 12.0.0, $12.0.\bar{1}$, $12.0.\bar{2}$, $80\bar{1}$

Orders of h0ℓ absent

101, 102, 103, 104, 105, $10\bar{2}$, $10\bar{3}$, $10\bar{4}$, $10\bar{5}$, 300, 301, 302, 303, 304, $30\bar{1}$, $30\bar{2}$, $30\bar{3}$, $30\bar{4}$, $30\bar{5}$, 500, 501, 502, 503, 504, $50\bar{1}$, $50\bar{2}$, $50\bar{3}$, $50\bar{4}$, $50\bar{5}$, 700, 701, 702, 703, 704, $70\bar{1}$, $70\bar{2}$, $70\bar{3}$, $70\bar{4}$, $70\bar{5}$, 900, 901, 902, $90\bar{1}$, $90\bar{2}$, $90\bar{3}$, $90\bar{4}$, 11.0.0, 11.0.1, $11.0.\bar{1}$, $11.0.\bar{2}$; 204, 401, 603, $60\bar{4}$, 800, 803, $80\bar{5}$

Observed 0k0 reflections with k odd: none

Observed h0ℓ reflections with h odd: none

These data indicate that the symmetry elements of dl-serine include a twofold screw axis parallel to $\underset{\sim}{a}_2$ and an *a*-glide plane perpendicular to it; the first demands that no 0k0 reflections with k odd should appear, and the second demands the absence of h0ℓ reflections with h odd. Although the number of reflections permitted by the use of CuKα radiation is not large, it is felt that the generality of the extinctions is reasonably certain.

It is apparent that the lattice is primitive as the extinctions required by centered lattices are violated.

It may be concluded that the space group is $C_{2h}^{5} - P2_1/a$, as none of the other primitive space groups isomorphous with C_{2h} or its subgroups C_2 or C_s show the above systematic extinctions. This space group is in agreement with the previous finding of Albrecht and coworkers[1].

10. Laue photographs

Four Laue photographs of <u>dl</u>-serine have been indexed by means of gnomonic projections on the basis of the lattice constants given above, and the value of $n\lambda$ was calculated for each reflection from the measured value of $\sin\vartheta$ and the (relatively prime) indices of the reflection. The results of this work are summarized in Table 8.

Table 8

Summary of Laue Photographs of <u>dl</u>-Serine

Orientation of crystal	Number of first order reflections	Total number of observed reflections
$\underset{\sim}{a}_1 \parallel \underset{\sim}{s}_o$	85	252
$\underset{\sim}{a}_2 \parallel \underset{\sim}{s}_o$	62	233
$\underset{\sim}{a}_3 \parallel \underset{\sim}{s}_o$	207	449
$\measuredangle\ \underset{\sim}{a}_3, \underset{\sim}{s}_o = 6^\circ 55'$, $\underset{\sim}{b}_1 \perp \underset{\sim}{s}_o$	103	297

Only three first order reflections were observed for which the apparent value of $n\lambda$ was less than 0.24 Å, the short wavelength limit of the x-ray tube, and of these the lowest value found for $n\lambda$ was 0.214 Å.

We may conclude that within experimental error all of the values of nλ are greater than the short wavelength limit of the x-ray tube, and that the lattice constants given above are correct within the experimental error of their determination.

11. Summary of work on dl-serine

The results of the redetermination of the space-group and lattice constants of dl-serine are summarized in Table 9, where they are compared with the results of Albrecht's original determination[1].

Table 9

Summary of Work on dl-Serine

	Present investigation	Results of Albrecht
Space group	C_{2h}^5 - $P2_1/a$	C_{2h}^5 - $P2_1/a$
Lattice constants		
a_1	10.70 ± .02 kX	10.74 Å
a_2	9.12 ± .02 kX	9.15 Å
a_3	4.815 ± .006 kX	4.78 Å
α_2	106°27' ± 4'	(106°13')
Density in g. per cm.^{3} at 25°C.	1.52	1.537
Number of molecules per unit cell	4 (4.14 calc.)	4 (4.00 calc.)

Table 9 (continued)

	Present investigation	Results of Albrecht
Axial ratios (x-ray)		
$a_1 : a_2 : a_3$	1.173 : 1 : 0.528	1.174 : 1 : 0.5224
$a_3 : a_1$	0.450 : 1	0.445 : 1
Axial ratios and monoclinic angle (optional)		
$a_1 : a_2 : a_3$	1.176 : 1 : 0.528	1.194 : 1 : 0.522
$a_3 : a_1$	0.449 : 1	0.437 : 1
α_2	106°31'	106°13'

Because of the precision of the x-ray data from which the unit cell was determined, and because of the surprisingly good agreement with the data obtained optically, it may be concluded that the values found in the present investigation for the axial lengths and the monoclinic angle are at least as accurate as those of Albrecht.

12. Acknowledgements

I am indebted to Professor Carl Niemann for the sample of dl-serine used in this work. I am happy to acknowledge the assistance given by Miss Lillian Casler in the preparation of the gnomonic projections, and to acknowledge the generous cooperation and valuable suggestions given by Dr. Robert B. Corey, under whose guidance this work was done.

References

1. G. Albrecht, G. W. Schnakenberg, M. S. Dunn, and J. D. McCullough, *J. Phys. Chem.* **47**, 24 (1943).

2. Groth, "Chemische Krystallographie," Vol. 3. Liepzig, Wilhelm Engelmann, 1910. p. 221.

3. Erlenmeyer and Stoop, *Ann. d. Chemic.* **337**, 257 (1904).

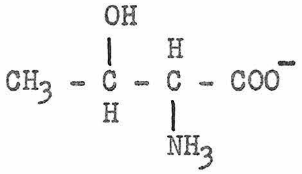
4. Haushofer, *Z. Krist.* **3**, 74 (1879).

B. AN INVESTIGATION OF THE CRYSTAL STRUCTURE OF ℓ-THREONINE

1. Introduction

In this section, work that has led to the determination of the space group, lattice constants, and approximate structure of crystals of ℓ-threonine is described.

Threonine, or α-amino-β-hydroxy-n-butyric acid, has the molecular structure



$$CH_3 - \overset{OH}{\underset{H}{C}} - \overset{H}{\underset{NH_3}{C}} - COO^-$$

with a trans configuration of the amino and hydroxyl groups with respect to the extended aliphatic chain (similar to threose). The diastereoisomer, allothreonine, was not studied in the present investigation.

Threonine was probably first isolated from a protein hydrolysate by Schryner and Buston[1], who isolated from a hydrolysate of glutenin (from oats) an amino acid $C_4H_9O_3N$ which gave a dibenyoyl derivative and a phenylisocyanate derivative. Later McCoy, Meyer, and Rose[2] isolated from commercial fibrin an amino acid of the same empirical formula, which was found to stimulate growth of rats and to be an essential amino acid. They showed that this amino acid is one of the four isomeric α-amino-β-hydroxy-n-butyric acids. In a later investigation[3], Meyer and Rose found that treatment of the natural product with nitrous acid yields the dihydroxy-n-butyric acid that corresponds to d(-)-threose. Accordingly they named the naturally occurring isomer d(-)-threonine. The diastereoisomer allothreonine was so named by West and Carter[5].

In a series of investigations West and Carter prepared mixtures of the four isomers (*d*- and *l*-threonine, and *d*- and *l*-allothreonine)[4], separated the isomers[5], and found that *d*(-)-threonine is the only one of the four isomers that stimulates the growth of rats[6]. Their method of synthesis was as follows. Crotonic acid was mercurated with mercuric acetate in hot methanol to form anhydro-α-hydroxymercuri-β-methoxy-*n*-butyric acid. The potassium salt of this compound was treated with bromine and potassium bromide in the presence of light, and the α-bromo-β-methoxy acid thereby obtained was aminated by treatment with ammonia. The methoxy group was removed by refluxing with hydrobromic acid to give a mixture containing all four α-amino-β-hydroxy-*n*-butyric acids. In order to obtain *dl*-threonine free from *dl*-allothreonine, the methoxy precursors were formylated or benzoylated and the acylated O-methylthreonine was crystallized or precipitated from the solution. The acyl group and the methyl group were removed by refluxing with hydrobromic acid. Threonine was resolved into its optical isomers by treatment of N-formyl-O-methylthreonine with brucine, separating the salts by crystallization, and removing the brucine and the formyl and methyl groups in the usual manner.

H. B. Milne[7] of the laboratories of this Institute has prepared *l*-threonine by the following method. From an aqueous solution of N-benyoyl-O-methyl-*dl*-threonine, the phenylhydrazide of the *l*-isomer was enzymatically precipitated. The precipitate was hydrolyzed with hydrobromic acid and *l*-threonine was precipitated from the neutralized solution by the addition of ethanol. The material that he prepared was used in our x-ray investigation.

According to Cohn and Edsall[8], threonine has been found in the following proteins: insulin (2.66%), casein (3.5%), gelatin (1.4%), and silk fibroin (1.36%). Brand[9] reports that it is also present in β-lactoglobulin (5.85%), egg albumin (4.0%), lactalbumin (5.1%), bovine serum albumin (6.5%), human serum albumin (5.0%), and human γ-globulin (8.4%).

Hence threonine is a protein constituent of considerable importance. It is, moreover, comparatively simple in molecular structure, and is therefore an attractive subject for an x-ray crystallographic study.

The ℓ-isomer was chosen for x-ray work instead of the d-isomer because the former is more easily prepared. There is no doubt that the crystal structures of the two isomers are identical except for the enantiomorphic difference; indeed, much of the work described below was done on crystals grown from the racemic mixture, with results that were identical to those obtained with ℓ-threonine.

The work described below was done under the supervision of Dr. Robert B. Corey.

2. Discussion of previous crystallographic work

Preliminary crystal structure work on threonine was reported by Albrecht and coworkers[10] in 1943. From an aqueous solution of dℓ-threonine they obtained flat, six-sided plates which they found on goniometric examination to be orthorhombic hemimorphic hemihedral (C_{2v}-mm) with axial ratios 1.675 : 1 : 0.6644. From layer-line measurements on rotation photographs these authors obtained the lattice constants

$$\begin{aligned} a_o &= 13.64\ \text{Å}, \\ b_o &= 7.75\ \text{Å}, \\ \text{and } c_o &= 5.16\ \text{Å}. \end{aligned}$$

The density was found to be 1.437 g./cm.^{3}; this value indicates that there are four molecules per unit cell (3.99 calculated). Laue photographs that were taken along the three axes showed that the cell constants are correct and revealed no systematic (hkl), (0kl), (h0l), or (hk0) absences. With the assumption of two molecules of each optical isomer in the unit cell, these results lead uniquely to the space-group C_{2v}^1 - Pmm2.

On the basis of our present-day knowledge of the principles governing crystal structures it is immediately apparent that the space-group assigned by these workers is incorrect. As the individual molecules can have no planes of symmetry, no molecule can extend through a mirror plane. Hence with the proposed space-group each molecule must be confined within a region bounded by mirror planes and of rectangular cross-section only 6.82 Å by 3.87 Å in size. It is hard to see how a non-planar molecule like threonine can be confined between mirror-planes only 3.87 Å apart. It is even more difficult to see how a structure can exist in which groups of the same electrical charge are as close together as they would have to be with this space-group, or how two adjacent molecules touching each other only at a mirror plane are prevented from sliding or rotating against each other to produce a better fit and to fill the holes in the structure.

The authors' assertion that crystals of threonine are orthorhombic hemimorphic hemihedral is not borne out by our morphological studies of crystals of this substance, nor in any obvious way by the microphotograph that they published. In all other respects we have verified the data on which their space-group assignment was based: the Laue symmetry (D_{2h}), the size of the unit cell and the number of molecules in it, the primitive character of the lattice, and the absence of glide planes. If we reject the space group C_{2v}^1 - Pmm2 and accept the other results, we are led uniquely to the four primitive space-groups of the orthorhombic enantiomorphic hemihedry (D_2 - 222), and to the corollary that the crystals which these authors studied were not crystals of *dl*-threonine but were instead crystals of *d*-threonine and of *l*-threonine which either formed separately or grew together as twins. Their examination of Laue photographs alone for evidence of systematic extinctions due to glide planes gave these authors no opportunity to observe possible systematic extinctions of the type (h00), (0k0), and (00*l*).

The only apparent alternative to the acceptance of an enantiomorphic space group is the assumption that the crystals are twins of pseudo-orthorhombic individuals having a true symmetry lower than orthorhombic. This alternative cannot easily be rejected with certainty, but the work described later makes it appear very improbable. The enantiomorphic character of the space group can be tested, however, by an x-ray investigation of one of the isomers alone. We have carried out such an investigation and have obtained x-ray photographs identical with those obtained with crystals grown from the racemic mixture. This evidence, together with additional evidence from systematic extinctions and from the interpretation of our intensity data, has led us to the assignment of the space-group $D_2^4 - P2_12_12_1$ to crystals of *d*- or *l*-threonine.

3. Materials

The preparations of *dl*-threonine and *l*-threonine that were used in this investigation were kindly supplied by Professor Carl Niemann. The *dl*-threonine was prepared by Dr. P. L. Nichols, Jr. The method of preparation was essentially that of West and Carter[4,5].

The *l*-threonine was prepared by H. B. Milne as described above. Determinations of the optical activities of these two preparations were made with small samples; *dl*-threonine showed no significant rotation, and *l*-threonine showed a rotation of $\alpha_D = 27^\circ$ in rough agreement with the previously reported value of 28.4° at 26° C.[11]

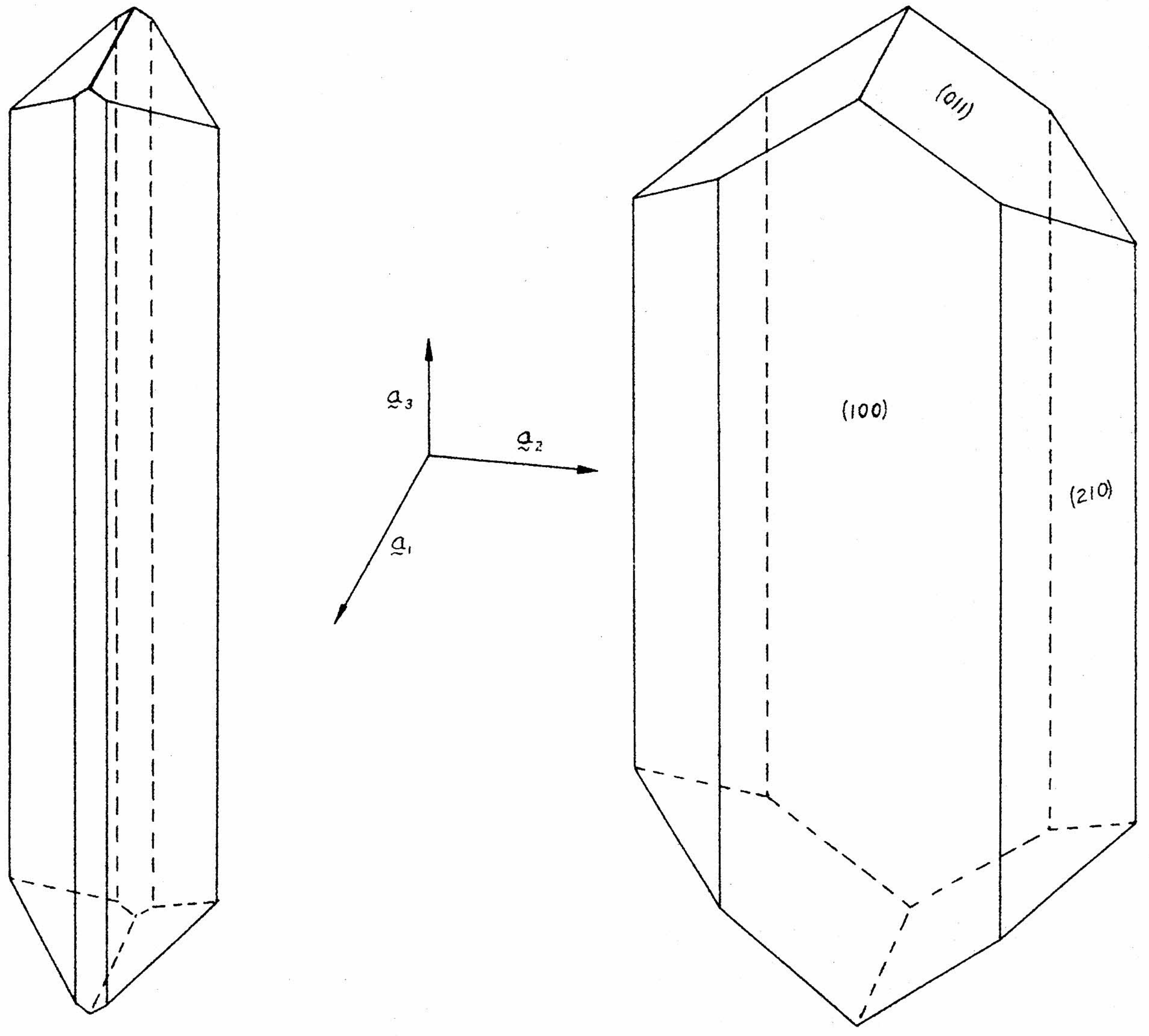
4. Growth of crystals

Crystals of both preparations were obtained by crystallization from water by the method described above in connection with the investigation of serine.

5. Morphological study

Examination of the crystals with the microscope and the optical goniometer indicated that the crystals are orthorhombic. No morphological characteristics that would indicate symmetry less than that of the holohedry were observed. Crystals grown from the two preparations were very similar; they differed only in that the crystals prepared from *dl*-threonine were usually long needles whereas those prepared from *l*-threonine had much more pronounced (100) faces and were lath-like in shape. Drawings of typical crystals of the two preparations are given in Figure 2 .

It is very difficult to evaluate the axial ratios precisely from our goniometric data because of the very poor agreement among the data. Discrepancies of one degree or more were observed among measurements of a given interfacial angle, but no systematic tendencies indicative of lower symmetry were observed. The crystals of *l*-threonine gave results of somewhat greater consistency than did crystals grown from the racemic mixture. Goniometric data obtained from the best crystals are given in the following table. The value enclosed by parentheses was not included in the average.



a. dl-Threonine

b. l-Threonine

Figure 2. Crystals prepared from dl-threonine and l-threonine. Actual crystals are usually much longer in proportion to their other dimensions than those shown.

Table 10

Goniometric Data for Threonine

A. Crystals prepared from dl-threonine

Crystal 7	100/210	(39°41')	Crystal 5	011/010	56°12'
	100/2$\bar{1}$0	41°25'		01$\bar{1}$/010	56°06'
	$\bar{1}$00/$\bar{2}\bar{1}$0	41°05'			
	$\bar{1}$00/$\bar{2}$10	41°18'	Crystal 1	011/010	56°30'
				0$\bar{1}$1/0$\bar{1}$0	56°10'
Crystal 5	100/210	41°16'			
	$\bar{1}$00/$\bar{2}$10	41°17'	Crystal 15	011/010	56°06'
				0$\bar{1}$1/0$\bar{1}$0	56°43'

B. Crystals prepared from l-threonine

Crystal 2	100/210	41°00'	Crystal 1	011/010	56°2'
	100/2$\bar{1}$0	40°51'		0$\bar{1}$1/0$\bar{1}$0	56°12'
	$\bar{1}$00/$\bar{2}\bar{1}$0	40°53'			
	$\bar{1}$00/$\bar{2}$10	41°13'	Crystal 2	011/010	56°1'

C. Summary

Average value of 100/210 etc.	41°09'
Average value of 011/010 etc.	56°14'

Axial ratios:

$$a_1 : a_2 : a_3 = 1.748 : 1 : 0.669$$

We may note that Albrecht's values of the axial ratios correspond to the following values of the two most important angles:

100/210	39°57'
011/010	56°24'

While the last is in agreement with our results, the first is not. Angles as small as 39°57' were, however, not infrequently observed for 100/210 in our crystals.

6. Cleavage

Good cleavage parallel to (100) was observed, and good cleavage faces were obtained. Attempts to cleave crystals on (010) were unsuccessful. When attempts were made to cleave crystals parallel to (001) conchoidal fractures usually resulted, with slight indications of (001) cleavage in some instances.

7. Density

The density of the crystals was determined by flotation. Two crystals grown from the two respective threonine preparations were suspended together in a vial containing a mixture of methylene iodide and benzene, and the composition of the mixture was adjusted until the crystals showed no significant tendency to rise or fall. A very slight indication of a difference in density between the two crystals was observed; the ℓ-threonine crystal tended to rise very slowly while the other remained stationary, but the difference was smaller than the probable error due to changes in the temperature during the experiment. The density of the final mixture was measured with a pycnometer, and was found to be 1.464 g. per cm.^{3}.

8. Laue symmetry

Laue photographs of crystals of the first preparation, taken with the three principal axes in turn oriented parallel to the x-ray beam, showed in each case the presence of two mirror planes. The Laue symmetry is therefore D_{2h}. Because of the possibility that the crystals may be twins of monoclinic or triclinic individuals, many crystals and fragments of crystals were examined in this way; all showed the D_{2h} Laue symmetry. No evidence of twinning was obtained from Laue photographs of this preparation.

Laue photographs of crystals of the ℓ-threonine preparation were substantially identical to those of the first preparation in the positions and relative intensities of the spots, and differed from them only in that some of the photographs of ℓ-threonine showed streaky or elongated spots indicative of bending or twinning. The character of these spots was not such as to suggest that their peculiarities resulted from interpenetrating of individuals of symmetry lower than orthorhombic.

In view of the very good evidence of the identity of the two preparations from the point of view of x-ray diffraction, no distinction has been made between them in the selection of crystals for the remaining work, and no differences were observed between them in this work.

9. Determination of lattice constants

(a) Layer-line measurements

Well exposed rotation photographs taken around the three principal axes with copper Kα radiation gave well-defined layer lines. From measurements of the positions of the layer lines the following values of the lattice constants were calculated:

$$a_1 = 13.63 \text{ Å}$$
$$a_2 = 7.71 \text{ Å}$$
$$a_3 = 5.13 \text{ Å}$$

These values are in agreement with those of Albrecht[1] within the limits of experimental error.

(b) Precise determination of lattice constants

Equatorial reflections on several oscillation and rotation photographs taken around the three principal axes with copper Kα radiation were indexed and their positions measured. Preliminary values of the reciprocal lattice constants b_1 and b_2 were obtained from orders of (100) and (010). These values were used in the calculation of b_3 principally from (10ℓ) and (20ℓ) reflections, as only one order of (001), namely (002), has been found to have appreciable intensity. With the aid of this value of b_3 and the preliminary values of b_1 and b_2 additional reflections were used in the calculation of final values of b_1 and b_2. In averaging the results for a given reciprocal lattice vector b_i, the value obtained from each reflection was weighted with a factor equal to the product of the index h_i by the number of individual measurements made on that reflection (counting α_1 and α_2 measurements as separate). The lattice constants are given in both kX units and Ångström units, and the reciprocal lattice constants are given in reciprocal kX units.

Table 11

Precise Determination of Lattice Constants for ℓ-Threonine

(a) Determination of b_1

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	b_1	Weighting factor
600	0.4411	0.07352	6
800	.5879	.07349	8
10·0·0	.7359	.07359	10
12·0·0	.8842	.07368	24
14·0·0	1.0314	.07367	28
16·0·0	1.1774	.07359	64

Preliminary value of b_1 (weighted mean of above determinations) 0.07362

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	b_1	Weighting factor
810	0.6042	0.07377	8
10·1·0	.7503	.07390	(omitted from mean)
10·0·1	.7617	.07364	20
11·1·0	.8226	.07384	(omitted from mean)
11·0·1	.8335	.07367	11
12·0·1	.9042	.07358	24
13·1·0	.9672	.07373	13
14·0·1	1.0491	.07363	42
16·0·1	1.1938	.07361	32

Final value of b_1 (weighted mean of all determinations) 0.07363
Mean weighted deviation .00004

(b) Determination of b_2

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	b_2	Weighting factor
020	0.2599	0.1300	(omitted from mean)
040	.5180	.1295	8
060	.7769	.1295	6
080	1.0356	.1294	8

Preliminary value of b_2 (weighted mean of above determinations) 0.1295

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	b_2	Weighting factor
061	0.8018	0.1296	12
071	.9268	.1294	7
081	1.0538	.1295	8
190	1.1678	.1295	18
091	1.1810	.1294	18

Final value of b_2 (weighted mean of all determinations) 0.1295
Mean weighted deviation .00005

Table 11 (continued)

(c) Determination of b_3

Reflection	$\frac{2 \sin \vartheta}{\lambda}$	b_3	Weighting factor
002	0.3900	0.1950	2
105	.9758	.1946	5
205	.9852	.1948	5
106	1.1713	.1948	30
206	1.1786	.1949	12

Final value of b_3 (weighted mean of all determinations)	0.1948
Mean weighted deviation	.00005

(d) Summary; calculation of a_1, a_2, a_3($a_i = 1/b_i$)*

$b_1 = 0.07363$ kX^{-1} $a_1 = 13.58 \pm .02$ kX $= 13.61 \pm .02$ Å (cgs)
$b_2 = 0.1295$ kX^{-1} $a_2 = 7.72 \pm .01$ kX $= 7.74 \pm .01$ Å
$b_3 = 0.1948$ kX^{-1} $a_3 = 5.13 \pm .01$ kX $= 5.14 \pm .01$ Å

$a_1 : a_2 : a_3 = 1.759 : 1 : 0.665$

* 1 kX = 1.0020 Å (cgs)

From the volume of the unit cell (541.8 Å^3), the density of the crystal (1.464 g. per cm.^{3}), and the molecular weight of threonine (119.12) it was found that there are four molecules per unit cell (4.01 calculated).

(c) Verification by means of gnomonic projections

Four Laue photographs of ℓ-threonine have been successfully indexed, by means of gnomonic projections, on the basis of the lattice constants given above. This work is summarized in the following table.

Table 12

Gnomonic Projections of Laue Photographs of ℓ-Threonine

No.	Orientation	Number of first-order reflections	Total number of reflections
1	$\underset{\sim}{a}_1 \parallel \underset{\sim}{s}_0$*	125	391
2	$\underset{\sim}{a}_2 \parallel \underset{\sim}{s}_0$	133	308
3	$\underset{\sim}{a}_3 \parallel \underset{\sim}{s}_0$	170	338
4	$\underset{\sim}{a}_1 \perp \underset{\sim}{s}_0$; $\measuredangle\, \underset{\sim}{a}_3, \underset{\sim}{s}_0 = 7^\circ 55'$	415	779
		843	1816

* $\underset{\sim}{s}_0$ denotes a unit vector in the direction of the incident beam

The value of $n\lambda$ was calculated for each reflection. Only two values of $n\lambda$ appeared to be lower than 0.24 Å, the short-wavelength limit of the x-ray tube; (561) and (561), on the fourth film, gave $n\lambda$ values of 0.227 Å and 0.229 Å. As these are lower than 0.24 Å by an amount that is within the experimental error of the measurement of small angle reflections, we may conclude that the indexing is correct and that the unit cell has been correctly chosen.

10. Systematic extinctions

Well-exposed Weissenberg photographs have been taken around the three principal axes with copper Kα radiation. No systematic extinctions of the types (hkℓ), (0kℓ), (h0ℓ), and (hk0) were observed. Reflections of the type (h00), (0k0), and (00ℓ) were examined with the following results.

Table 13

Observed and Unobserved Reflections for ℓ-Threonine

(h00) observed: 200, 400, 600, 800, 10·0·0, 12·0·0, 14·0·0, 16·0·0

(h00) not observed: 100*, 300, 500, 700, 900**, 11·0·0, 13·0·0, 15·0·0

(0k0) observed: 020, 040, 060, 080

(0k0) not observed: 010, 030, 050, 070, 090

(00ℓ) observed: 002, (004)

(00ℓ) not observed: 001, 003, 005, 006

* The reflection angle of (100) is too small for observation of this reflection on Weissenberg photographs, as the beam stop interferes; however, this reflection was not observed on rotation and oscillation photographs.

** A $\mathrm{CuK}\beta$ reflection from (10·0·0) lies at the expected position for (900) on Weissenberg photographs; no (900) reflection was observed, however, on a well exposed oscillation photograph.

Five-degree oscillation photographs were taken with copper $K\alpha$ radiation in an attempt to obtain some of the odd order reflections. No such reflections have yet been found, with the single exception of a spot obtained at the expected position for (700) on oscillation photographs taken around the $\mathbf{a}_2$ axis. The spot is of a very remarkable character and is unquestionably not a (700) reflection; it may be a Laue spot.

Photographs taken with molybdenum $K\alpha$ radiation, in an attempt to obtain higher orders of (001), were not sufficiently well exposed to be useful.

11. Summary of data on the space-group and lattice constants

The crystal morphology and the Laue symmetry indicate that the crystals are orthorhombic. As there can be no symmetry plane in a crystal containing only ℓ-threonine molecules, the space-group must be of the orthorhombic enantiomorphic hemihedry (D_2 - 222). All of the atoms must lie in general positions because the molecule does not have a two-fold symmetry axis. As there are no systematic absences of the type (hkl), the lattice must be primitive and the number of molecules must be equal to the order of the point group, which is four, or to some integral multiple of this number. As a value of four is given by experiment, there can be only one set of crystallographically equivalent molecules in the crystal.

The systematic absence of odd order (h00) and (0k0) reflections strongly indicates that there are two-fold screw axes parallel to $\underset{\sim}{a}_1$ and $\underset{\sim}{a}_2$. The existence of a third screw axis parallel to $\underset{\sim}{a}_3$ is also indicated by the extinctions but with less certainty; with copper Kα radiation the only odd orders of (00ℓ) that can be examined are (001), (003), and (005). From the apparent absences it was concluded that the space-group is probably D_2^4 - $P2_12_12_1$, though there still remained the possibility that the absences of odd order (00ℓ) reflections is accidental and that the space-group is accordingly D_2^3 - $P2_12_12$. It is of course also possible that the (h00) and (0k0) extinctions are accidental but the likelihood is small. Accordingly, pending verification by the analysis of intensity data, the space-group was assumed to be D_2^4 - $P2_12_12_1$. As will be shown later, the analysis of the intensity data has confirmed this space-group beyond reasonable doubt.

The data on the space-group and lattice constants are summarized in Table 14, where these results are compared with those of Albrecht[10].

Table 14

Data on the Space-Group and Lattice Constants for ℓ-Threonine

	ℓ-Threonine, this investigation	dl-Threonine, Albrecht[10]
Crystal Form	Orthorhombic	Orthorhombic Hemimorphic Hemihedry
Laue Symmetry	D_{2h}	D_{2h}
Axial ratios, optical		
$a_1 : a_2 : a_3$	1.748 : 1 : 0.669	1.675 : 1 : 0.6644
Cleavage	(100)	
Density, g.per cm.^{3}	1.464	1.437
Lattice constants		
a_1	13.61 ± .02 Å	13.64 Å
a_2	7.74 ± .01 Å	7.75 Å
a_3	5.14 ± .01 Å	5.16 Å
Axial ratios, x-ray		
$a_1 : a_2 : a_3$	1.759 : 1 : 0.665	1.760 : 1 : 0.666
Number of molecules per unit cell	4 (4.01 Calc.)	4 (3.99 Calc.)
Systematic extinctions	(h00), h odd (0k0), k odd (00), ℓ odd (?)	None observed.
Space-group	D_2^4 - $P2_12_12_1$	C_{2v}^1 - Pmm2

12. Collection of intensity data

(a) Weissenberg photography

Three crystals were used in the collection of intensity data. They were mounted, respectively, with the three principal axes parallel to the axis of rotation of the Weissenberg x-ray goniometer. They may be described as follows:

$\underset{\sim}{a}_1$. The crystal to be mounted on this axis was prepared from *l*-threonine. It was shaped into a cylinder (with its axis parallel to $\underset{\sim}{a}_1$) about 0.3 mm. high and 0.3 mm. in diameter by dissolving away excess material with a camel's-hair bruch moistened with water.

$\underset{\sim}{a}_2$. This crystal was also prepared from *l*-threonine, and was shaped roughly into a cylinder 0.4 mm. high and 0.24 mm. in diameter.

$\underset{\sim}{a}_3$. This crystal is a needle about 1 mm. in length, 0.25 mm. in its largest cross-sectional dimension, and 0.18 mm. in its smallest such dimension. The crystal was grown from a solution of *dl*-threonine and was used without any further treatment. It is similar in shape to the crystal shown in Figure 3a.

The crystals were mounted on stiff pyrex fibers with the use of flake shellac, which was melted with an electrically heated wire. They were oriented by means of the optical goniometer or with Laue photographs.

The reasons for the use of crystals as small as this are that we desired to obviate the necessity for applying absorption corrections and that we wished also to reduce the effect of extinction. As the calculated linear absorption coefficient to threonine for copper $K\alpha$ radiation is 1.04 mm.$^{-1}$, the absorption is approximately 22% for a cylindrical crystal 0.3 mm. in diameter and is very nearly the same for all scattering angles in the equatorial plane[12]. The deviations from average absorption that are encountered in crystal $\underset{\sim}{a}_3$, which is not cylindrical, are of the order of $\pm 3\%$ of the intensity of the scattered beam. These approximations may be expected to hold for the layer lines to a precision adequate for our work.

Equatorial and equi-inclination Weissenberg photographs were taken of the following layer lines with copper $K\alpha$ radiation:

a_1: $h_1 = 0, 1, 2, 3, 4, 5, 6, 7, 8$
a_2: $h_2 = 0, 1, 2, 3, 4, 5$
a_3: $h_3 = 0, 1, 2, 3$

These are all of the layer lines that can be photographed with an equi-inclination angle of less than 30°, which is the limit of the capacity of the Weissenberg goniometer that we used. On each layer line reflections may be obtained out to 1.20 Å^{-1} in reciprocal space ($\sin \vartheta_{max} = 0.925$); the limiting circumstance is in this case geometric interference by the pinhole system. With the use of the three crystals all of the 618 reflections in an octant of a sphere of radius 1.20 Å^{-1} in the reciprocal latice were made available for estimation of intensity. All but 70 of these reflections occur on two or more layer lines (around different axes), and 191 reflections occur on three layer lines.

To facilitate the estimation of intensities, a multiple film technique similar to that of deLange, Robertson, and Woodward[13] was employed in obtaining these photographs. The camera of the Weissenberg goniometer was modified to permit the simultaneous use of four or five films. Eastman No-Screen Duplitized Safety X-Ray Film (Code No. 5133) was used throughout.

(b) Estimation of intensities

The intensities of all of the 1357 reflections on 19 sets of photographs were estimated visually. A novel scheme, which turned out to be very successful, was employed. Instead of being estimated directly, the intensities were estimated first on a logarithmic scale which had as its base of logarithms the so-called film factor. The film factor is the reciprocal of the factor by which the intensity of the reflected x-ray beam is decreased by absorption in passing through a single film. The most convenient procedure, with this method, is as follows. First a single strong reflection spot is chosen as a primary standard and arbitrarily assigned a number, or <u>intensity index</u>, say 6.00. A number of secondary standards with intensity indices of 5.00, 4.00, 3.00, etc., are then found by intercomparison of the films in the set; use is here made of the fact that the intensity index of a given reflection decreases by 1.00 on going from one film to the next. As the film factor is approximately equal to 4, it is possible to find tertiary standards with intensity indices of 5.50, 4.50, etc., by superposition of primary standards, for a doubling of intensity corresponds approximately to an increase of 0.5 in the intensity index. The tertiary standards may be checked by superposing them and comparing with secondary standards. The intensity indices of the remaining reflections may be obtained by interpolation among these standards, and by making use of the decrement of 1.00 from film to film. It is convenient in this work to cut a piece of tracing paper to the shape and size of the first film of the multiple-film set, draw on it an indexing net, and write the intensity indices directly on the paper.

A minor difficulty which was experienced when the number of spots on a film was comparatively small was the scarcity of spots suitable for use as standards. This difficulty was often removed by making use of spots on other sets of films. More serious difficulties, which are independent of the method of estimation, were the variations in the shape and character of the spots due to the resolution of the $\alpha_1 \alpha_2$ doublet, compression or extension of spots close to the base-line of layer line photographs, and the splitting which often resulted from backlash of the camera drive screw on the Weissenberg goniometer.

The relative intensity, i, of each reflection on the film was determined from the intensity index, x, by use of the relation

$$i = b^x \tag{1}$$

in which b is the film factor. This was at first assumed to be equal to 4; this value was determined several years ago by Dr. Chia Si Lu of these laboratories and has been in general use in this laboratory.

(c) Correction and correlation of intensities

For a set of films that represents a given layer line or reciprocal lattice net, the values of i determined visually are assumed to be proportional to the integrated intensities of the respective reflections. The constant of proportionality is not known, and is in general different for each set of films. It is possible, however, to put the intensities obtained from all of the films on the same relative basis, for reflections from a given plane were usually obtained with more than one orientation of the crystal. From the intensities for each set of films, values of F_{hkl}^2 (the square of the structure factor) corresponding to a given net in the reciprocal lattice may be calculated on a relative basis peculiar to that net. As each point of the reciprocal lattice may lie on two or three such nets, scale factors may be obtained which may be applied to the different nets in order to tie all of the data on these nets to a common relative basis.

Because the absolute intensities are not known, all constant factors in the expression[14] for scattering of unpolarized monochromatic x-rays by a small, rotating, ideally imperfect (mosaic) crystal may be absorbed into an unknown constant of proportionality $\underline{k}$, and we may write

$$i = kF_{hkl}^2 \; \frac{1 + \cos^2 2\vartheta}{\sin 2\vartheta} \cdot \frac{1}{\sqrt{1 - \frac{\sin^2 \nu}{\sin^2 \vartheta}}} \, , \tag{2}$$

where we make use of the form developed by Tunell[15] for the Lorentz factor to be used in equi-inclination Weissenberg photography. In this equation ϑ is the Bragg angle, ν is the equi-inclination angle, and $\underline{F}_{hkl}^2$ is the square of the absolute value of the structure factor for the (hkl) plane; we will henceforth refer to $\underline{kF}_{hkl}^2$ as the <u>corrected</u> <u>intensity</u>.

With this equation all of the data were converted into corrected intensities. To facilitate the computations, a plot of

$$\frac{1 + \cos^2 2\vartheta}{\sin 2\vartheta}$$

against $\sin \vartheta$ and a plot of

$$\frac{1}{\sqrt{1 - \frac{\sin^2 \nu}{\sin^2 \vartheta}}}$$

against the ratio $\sin \nu / \sin \vartheta$ were prepared.

By making all possible comparison among the reciprocal lattice nets and averaging the scale factors obtained, a set of 19 scale factors for the 19 respective nets was obtained. By applying these scale factors all of the corrected intensities were placed on the same relative basis. The corrected intensities were then averaged for each plane with weights based upon the quality of the reflections.

We now have an opportunity to observe the agreement that was obtained among the two or three determinations of the corrected intensity for each reflection. As the empirical procedure of establishing the relative bases determined only one parameter for each 50 to 100 spots, the individual intensity determinations are virtually independent. In Table 18 on page 110 the individual determinations are compared for each of several planes that are now being used in structure factor work. In quality of agreement these planes are representative of the remaining reflections, except that the agreement for reflections of very low intensities or high indices is not quite as good, in general, as that shown in the table, but is certainly adequate for our work.

The general agreement is surprisingly good and justifies considerable confidence in the intensity data.

(d) Revision of the film factor

The precision that can be obtained in visual interpretation of x-ray photographs appears to be high enough to suggest that other factors that influence the estimation of intensity should be brought under closer control. One of the most important of these is the film factor. A small error in the film factor has a negligible effect on reflections of comparable strengths but it may have a large effect when reflections of considerable different strengths are compared. Dr. Herman E. Seemann of the Eastman Kodak Co. has informed me[16] that the sensitivity of different lots of No-Screen film may depart as much as 10% from the mean; a similar departure may be expected for the film factor. For intensities that differ by a factor of only four, this deviation would produce a 10% error in the intensity ratio. It is therefore desirable to determine the film factor for each lot of film, or at least to determine the <u>average</u> film factor to an accuracy much better than 10%. The latter course is the only practical one available to us in our study of ℓ-threonine.

During the collection of intensity data for ℓ-threonine it became apparent that the accepted value of 4 is too high. Measurements made with a densitometer on multiple film oscillation photographs indicated a film factor of about 3.5, and microphotometric measurements of a set of powder photographs indicated a factor of only 3.2. These methods are, of course, of limited precision.

Dr. Seemann[16] made direct measurements of the transmission of No-Screen film with K fluorescence radiation from copper and molybdenum[15]. The measurements were made with an air ionization chamber, and included estimates of the magnitude of scattering by the film. His results for percentage transmission are as follows:

	With scattered radiation	Without scattered radiation
Copper	27.3	26.7
Molybdenum	73.8	72.7

These results are claimed to be good to within ± 3% for the sample tested. The film factor indicated for copper Kα radiation by these results is 3.7. This value is probably the best so far obtained, and was used subsequently in our work. All of our values of kF^2_{hkl} are now being corrected in accordance with the new film factor.

(e) Powder photography

In order to determine the effect of extinction on the intense reflections, a multiple-film powder photograph was prepared. A few crystals were ground to an extremely fine powder in an agate mortar. A thin-wall pyrex capillary tubing about 0.25 mm. in inside diameter was loaded with the powder and mounted vertically on a goniometer head by means of a close-fitting glass fiber which was inserted into one end of the tube. By pulling the capillary tube downward the fiber was made to extrude from the top of the tube a compressed cylindrical rod of powder. This rod was placed in the path of an x-ray beam of low divergence and was rotated during the exposure.

The lines on the films were indexed and equatorial photometric tracings of the films were made with a Fred Henson microphotometer. As the resolved lines were not widely different in half-width, the optical density of each line in comparison to the background was taken as a measure of the integrated intensity. Comparisons among the data obtained from the first three films of the set showed that the density is a very nearly linear function of the intensity over the density range encountered (the maximum density was 0.3). The integrated intensity, per unit length of the line at the equator, is given[17] by the equation

$$i' = n K F^2_{hkl} \frac{1+\cos^2 2\vartheta}{\sin 2\vartheta} \cdot \frac{1}{\sin \vartheta}, \tag{3}$$

where K is an unknown constant, and n is the number of planes of identical type that belong to the form (hkl) and thereby contribute to the powder line. The calculated values of $K\ F^2_{hkl}$ for the well resolved lines were multiplied by a scale factor in order to bring the values for all except the very strongest reflections into correspondence with the values of $k\ F^2$ obtained from single-crystal photographs. The results are shown in Table 15 .

Table 15

Comparisons of Strong Reflections on
Single Crystal and Powder Photographs

hkl	Corrected Intensities	
	Single crystal	Powder
210	480	430
101	1350	1600
011	2500	3270
120	2090	2730
211	990	1030
121	3270	3580
320	1850	1920
221	1800	2000
620	1550	1390

It is evident that the effect of primary and secondary extinction is unusually small; indeed it does not greatly exceed the probable error of the intensity determinations. This is largely due to our use of small crystals, but it does nevertheless seem to indicate a high degree of imperfection in the crystals.

Small corrections were made in the values of $\underline{k}\ \underline{F}^2_{hkl}$ for the very strongest planes. As the magnitude of such small extinctions is practically independent of the scattering angle it is only necessary to multiply each value of $\underline{k}\ \underline{F}^2_{hkl}$ by a factor

$$(1 + g\ Q)$$

where $\underline{g}$ is an empirically determined constant and $\underline{Q}$ is proportional to the integrated intensity of the reflection.

(f) Reduction to an absolute basis

The final reduction of structure factors to an absolute basis is best done at the later stages of the structure determination. An approximate method, which does not depend on a knowledge of the structure, was developed in order to bring the structure factors close enough to an absolute basis to facilitate the testing of trial structures.

As will be shown later, the amplitude of the peak at the origin of a Patterson distribution is given by

$$\sum_{h=-\infty}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l=-\infty}^{\infty} F_{hkl}^{2} = v_a \sum_{j} P_{jj}(0), \tag{4}$$

in which the summation on the right is taken over all atoms in the unit cell, and

$$P_{jj}(0) = 4\pi \int_{0}^{\infty} f_j'^{2}(H)\, H^{2}\, dH. \tag{5}$$

The term $P_{jj}(0)$ represents the contribution of atom j to the amplitude of the peak at the origin. In equation (5)

$$H = \frac{2\sin\theta}{\lambda} \tag{6}$$

and $f'(H)$ is the atomic form factor combined with the temperature factor. For equation (5) to be strictly valid it is necessary that the temperature factor be isotropic. It is also required that there be no significant amount of interference from neighboring Patterson peaks.

The heretofore unknown constant of proportionality is determined by requiring that equation (4) be satisfied. It is necessary to assume a value for the temperature factor; the value chosen for all atoms was

$$e^{-A\left(\frac{\sin\theta}{\lambda}\right)^{2}}$$

where

$$A = 1.7 \times 10^{-16}$$

at room temperature. This is the value that was found in the determination of the structure of glycine by Albrecht and Corey[18]. The form factors that we used were those compiled by James and Brindley[12b]. The term F_{000}^2 is, for convenience, subtracted from both sides of equation (5); it is simply the square of the number of electrons in the unit cell.

This method was applied to the structure factors that were used later for comparison with calculated structure factors. The application of the method to the new set containing the new value of the film factor is now in progress.

13. Application of a three-dimensional Patterson synthesis

(a) Introduction

Since the publication of Patterson's 1935 paper[19] on the direct Fourier inversion of F_{hkl}^2 data, the method which he proposed has been widely used by workers in the field of crystal structure. It is possible, in principle, to carry out the Fourier inversion in three dimensions, but with the calculation aids available the computations have been heretofore regarded as much too involved. The method has been used almost entirely in one- or two-dimensional syntheses of all of the orders of a given plane or of all of the planes in a given zone; the Patterson projections obtained in this way are projections of all of the Patterson density in the unit cell onto a line or plane. These projections are very useful if the structure contains heavy atoms in special positions or if there are only a few atoms in the unit cell, but their interpretation may be extremely difficult if there are a large number of atoms of comparable scattering power in the unit cell.

A closer approach to a three-dimensional synthesis is that of Harker[20], who showed that lines or planar sections of Patterson space which have, as their constant coordinates, the corresponding translation components of symmetry elements have properties similar in some ways to Fourier projections. When the number of atoms in the unit cell is large the interpretation of these Harker sections may be difficult because many peaks may accidentally be present that are not due to the symmetry relations on which these sections are based. A Harker section which we prepared from the data for ℓ-threonine failed to give much useful information concerning the structure until it was examined in connection with other features of the three-dimensional Patterson distribution.

The great reduction in calculation effort that was introduced by the use of punched-card methods with the International Business Machines[21] suggested that it might be feasible to carry out Patterson computations in three dimensions without undue effort. The Hollerith punched-card method[22] has already been used in England for three-dimensional Fourier calculations. The development of the L cards, which correspond to the Beevers-Lipson strips, by Professor Verner Schomaker[23] has provided the most convenient means yet found for carrying out two-dimensional summations. Professor Schomaker suggested that these cards be used in the calculation of the three-dimensional Patterson distribution for ℓ-threonine. These calculations have been carried out by Professor Schomaker and myself, and the Patterson distribution that we obtained proved to be of great value in the determination of the approximate structure of ℓ-threonine.

Although use has been made of Patterson sections by other workers, to our knowledge the work described below represents the first time that a complete three-dimensional representation of the Patterson function has been calculated and used in the determination of a crystal structure.

As a background for this work we shall discuss the theory of the

Patterson distribution with special regard for its use in three dimensions.

(b) Theoretical discussion

It was shown by Patterson[19] that a direct Fourier inversion of the experimentally determined set of $\underline{F}_{hkl}^2$ data*,

$$P(u,v,w) = \frac{1}{v_a} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} F_{hkl}^2 \cos 2\pi(hu+kv+lw), \tag{7}$$

yields a density distribution in *uvw* space which may be interpreted in terms of interatomic vectors in the crystal. A peak which has coordinates $(\underline{u},\underline{v},\underline{w})$ represents a vector between two atoms in the crystal; this vector is

$$\underset{\sim}{P} = u\underset{\sim}{a}_1 + v\underset{\sim}{a}_2 + w\underset{\sim}{a}_3 \tag{8}$$

where $\underset{\sim}{a}_1$, $\underset{\sim}{a}_2$, and $\underset{\sim}{a}_3$ are the lattice vectors corresponding to the particular choice of the unit cell. As

$$P(u,v,w) = P(\bar{u},\bar{v},\bar{w}), \tag{9}$$

each interatomic vector is represented in Patterson space ($\underline{u},\underline{v},\underline{w}$ space) by two peaks related by a center of symmetry at the origin. All interatomic vectors that are related to a given vector by the operations of the space group are represented by related peaks in Patterson space. The symmetry of the distribution of Patterson peaks is related to that of the crystal. The Patterson distribution always has a center of symmetry in consequence of the double-ended character of the vectors, but the Patterson symmetry is not simply that of the crystal space group plus a center of symmetry except in special cases. The addition of a center of symmetry to D_2^4 - $P2_12_12_1$, for example, gives D_{2h}^{15} - Pbca or D_{2h}^{16} - Pnma, while the space group of the Patterson distribution is D_{2h}^{1} - Pmmm. There is also in the Patterson distri-

---

* $\underline{F}_{hkl}^2$ is written for convenience instead of $|\underline{F}_{hkl}|^2$; v_a = volume of unit cell.

bution an inner symmetry comprising the necessary relations that must exist among the coordinates of peaks representing various types of interatomic vector interactions, for the non-equivalent peaks are not independent in general.

It can be shown[19] that the Patterson function $P\ (u,v,w)$ may be written

$$P\ (u,v,w\ ) = v_a \int_0^1 \int_0^1 \int_0^1 \rho\,(x,y,z)\,\rho(x+u,y+v,z+w)\ dxdydz, \tag{10}$$

where $\rho\,(x,y,z)$ is the electron density distribution in the crystal, in terms of electrons per unit volume. We may expand the electron density as a Fourier series in two ways:

$$\rho(x,y,z) = \frac{1}{v_a} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} F_{hkl}\, e^{-2\pi i (hx+ky+lz)} \tag{11}$$

and

$$\rho\,(x+u,y+v,z+w) = \frac{1}{v_a} \sum_{h'=-\infty}^{\infty} \sum_{k'=-\infty}^{\infty} \sum_{l'=-\infty}^{\infty} F^{*}_{h'k'l'}\, e^{2\pi i (h'[x+u]+k'[y+v]+l'[z+w])} \tag{12}$$

When these expressions are multiplied together and integrated term by term in equation (10), all terms for which h, k, and l are not all equal respectively to h', k' and l' vanish by orthogonality, and we obtain

$$P(u,v,w) = \frac{1}{v_a} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} F_{hkl}\, F^{*}_{hkl}\, e^{2\pi i (hu+kv+lw)}, \tag{13}$$

which gives equation (7) because

$$F^{2}_{hkl} = F^{2}_{\bar{h}\bar{k}\bar{l}}\ .$$

The definition of $P\ (\,u,\ v,\ w)$ given by equation (10) gives this quantity the dimensions of reciprocal volume, and $P\ (u,\ v,\ w)$ may be interpreted as the number of electron-electron interactions per unit volume of Patterson space where the interactions to be considered are those between all of the electrons in a single unit cell of the crystal and all of the electrons in the entire crystal.

The electron density may be written

$$\rho(x,y,z) = \sum_{j=1}^{\infty} \rho_j(x,y,z)$$

where ρ_j represents the electron density to be ascribed to atom j, and where the summation is taken over all of the atoms in the crystal. On replacing the electron density functions in the integrand of equation (10) by the summations we obtain

$$P(u,v,w) = \sum_{i=1}^{\infty}\sum_{j=1}^{\infty} \tilde{P}_{ij}(u,v,w)$$

where both sums are taken over all of the atoms in the crystal, and

$$\tilde{P}_{ij}(u,v,w) = v_a \int_0^1\int_0^1\int_0^1 \rho_i(x,y,z)\,\rho_j(x+u,y+v,z+w)\,dx\,dy\,dz.$$

The only terms $\tilde{P}_{ij}$ that are significantly large are those representing interactions among the atoms that contribute significantly to the electron density in the unit cell covered by the integration.

Let us attempt to obtain this result in a more useful form. In virtue of the translation group of the crystal lattice,

$$\sum_{j}^{\infty} \tilde{P}_{ij}(u,v,w) = \sum_{m=-\infty}^{\infty}\sum_{n=-\infty}^{\infty}\sum_{p=-\infty}^{\infty}\sum_{j}^{N} \tilde{P}_{ij}(u+m, v+n, w+p)$$

$$P(u,v,w) = \sum_{i}^{\infty}\sum_{j}^{\infty} \tilde{P}_{ij}(u,v,w) = \sum_{m=-\infty}^{\infty}\sum_{n=-\infty}^{\infty}\sum_{p=-\infty}^{\infty}\sum_{i}^{N}\sum_{j}^{N} P_{ij}(u+m, v+n, w+p)$$

where we define P_{ij} as

$$P_{ij}(u,v,w) = v_a \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \rho_i(x,y,z)\,\rho_j(x+u,y+v,z+w)\,dx\,dy\,dz. \tag{14}$$

Further

$$P(u,v,w) = \sum_{i=1}^{N}\sum_{j=1}^{\infty} P_{ij}(u,v,w) = \sum_{m=-\infty}^{\infty}\sum_{n=-\infty}^{\infty}\sum_{p=-\infty}^{\infty} P_N(u+m, v+n, w+p), \tag{15a}$$

where

$$P_N(u,v,w) = \sum_{i=1}^{N}\sum_{j=1}^{N} P_{ij}(u,v,w), \tag{15b}$$

and where we have again made use of the translation group. The Patterson function is represented in equation (15a) as the folding of the crystal lattice with the distribution P_N due only to the N atoms in a single unit cell. Now P_{ij} is the contribution to the Patterson distribution made by the vector that extends from a single atom i and a single atom j in the same unit cell. The N terms of kind P_{ii} in the summation of equation (15b) represent the interactions of each of the N atoms in the unit cell with itself, and the $N^2 - N$ terms of kind P_{ij} represent the $N(N-1)$ interactions of these atoms with each other.

We shall now investigate the nature of P_{ij}. To do this we shall first transform the coordinates of ρ_i and ρ_j to coordinates which have their origins at the centers of the respective atoms. Let

$$\rho_i(x,y,z) = \bar{\rho}_i(\xi,\eta,\zeta) \tag{16}$$

where

$$\xi_i = x - x_i, \quad \eta_i = y - y_i, \quad \zeta_i = z - z_i \tag{17}$$

and x_i, y_i, z_i are the coordinates of the center of atom i. Now

$$\rho_j(x+u, y+v, z+w) = \bar{\rho}_j(\xi_j + u, \eta_j + v, \zeta_j + w) = \bar{\rho}_j(\xi_i + x_i - x_j + u, \ldots)$$

because

$$x = x_i + \xi_i = x_j + \xi_j$$

etc. Now if we let

$$\left.\begin{aligned} u &= u_{ij} + \lambda_{ij}, & u_{ij} &= x_j - x_i, \\ v &= v_{ij} + \mu_{ij}, & v_{ij} &= y_j - y_i, \\ w &= w_{ij} + \nu_{ij}, & w_{ij} &= z_j - z_i, \end{aligned}\right\} \tag{18}$$

then

$$\rho_j(x+u, y+v, z+w) = \bar{\rho}_j(\xi_i + \lambda_{ij}, \eta_i + \mu_{ij}, \zeta_i + \nu_{ij})$$

and we can write

$$P_{ij}(u,v,w) = \bar{P}_{ij}(\lambda,\mu,\nu) = v_a \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \bar{\rho}_i(\xi,\eta,\zeta)\, \bar{\rho}_j(\xi+\lambda, \eta+\mu, \zeta+\nu)\, d\xi\, d\eta\, d\zeta \tag{19}$$

where for convenience we have dropped the subscripts on the coordinates. If

$\bar{\rho}_i$ and $\bar{\rho}_j$ are monotonically decreasing functions of the distance

$$|\xi \underset{\sim}{a}_1 + \eta \underset{\sim}{a}_2 + \zeta \underset{\sim}{a}_3|$$

from the nucleus of the atom, it is evident that $\bar{P}_{ij}$ monotonically decreases with the distance

$$|\lambda \underset{\sim}{a}_1 + \mu \underset{\sim}{a}_2 + \nu \underset{\sim}{a}_3|$$

from the point in Patterson space which has the coordinates (u_{ij}, v_{ij}, w_{ij}), and that this point is the center, or at any rate the position of maximum density, of a peak. The N^2 interactions of the atoms with themselves have in common that

$$u_{ii} = v_{ii} = w_{ii} = 0\ ;$$

the peaks due to these interactions are all superposed at the origin, and in fact at each lattice point. The $N\ (N-1)$ interactions of atoms with one another give rise to peaks that are, in general, separated from one another. When peaks overlap or superpose, their amplitudes are additive.

It is possible to calculate the *shape* of the peak, i.e. the variation of Patterson amplitude as a function of the vector distance from the center of the peak. To do this we will make use of a theorem on Fourier transforms.[24] If

$$f(x) = \int_{-\infty}^{\infty} F(t) e^{-2\pi i t x} dt, \qquad F(t) = \int_{-\infty}^{\infty} f(x) e^{2\pi i t x} dx$$

and

$$g(x) = \int_{-\infty}^{\infty} G(t) e^{2\pi i t x} dt, \qquad G(t) = \int_{-\infty}^{\infty} g(x) e^{-2\pi i t x} dx$$

are two sets of Fourier transforms, then

$$\int_{-\infty}^{\infty} F(u) G(u+t) du = \int_{-\infty}^{\infty} f(x) g(x) e^{-2\pi i t x} dx$$

The integral on the left represents a type of *folding* of the functions F and G, and the integral on the right provides the means of carrying out this folding by use of the Fourier transforms of F and G. This may be easily generalized to three dimensions. In our case the Fourier transform of the electron density, $\bar{\rho}_i(\xi, \eta, \zeta)$, is the *form factor*, $f_i\ (h, k, l)$, of atom *i*. We will here regard the Miller indices as continuous variables. We may assume that

$$\bar{\rho}_i(\xi,\eta,\zeta) = \bar{\rho}_i(-\xi,-\eta,-\zeta)$$

and accordingly

$$f_i(h,k,l) = f_i(-h,-k,-l)$$

so that we use the cosine transforms:

$$\bar{\rho}_i(\xi,\eta,\zeta) = \frac{1}{v_a}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f_i(h,k,l)\cos 2\pi(h\xi+k\eta+l\zeta)\,dh\,dk\,dl \tag{20}$$

and

$$f_i(h,k,l) = v_a\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \bar{\rho}_i(\xi,\eta,\zeta)\cos 2\pi(h\xi+k\eta+l\zeta)\,d\xi\,d\eta\,d\zeta. \tag{21}$$

We now have

$$\bar{P}_{ij}(\lambda,\mu,\nu) = \frac{1}{v_a}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f_i(h,k,l)\,f_j(h,k,l)\cos 2\pi(h\lambda+k\mu+l\nu)\,dh\,dk\,dl. \tag{22}$$

We may further assume that the atoms are spherically symmetrical in electron density and that the form factors accordingly have spherical symmetry. Let

$$\left.\begin{aligned} \underset{\sim}{R} &= \lambda\underset{\sim}{a}_1+\mu\underset{\sim}{a}_2+\nu\underset{\sim}{a}_3 \\ \underset{\sim}{H} &= h\underset{\sim}{b}_1+k\underset{\sim}{b}_2+l\underset{\sim}{b}_3 \end{aligned}\right\} \tag{23}$$

where $\underset{\sim}{b}_1$, $\underset{\sim}{b}_2$, and $\underset{\sim}{b}_3$ are the basic vectors of the reciprocal lattice, and let

$$\left.\begin{aligned} \bar{P}_{ij}(\lambda,\mu,\nu) &= P_{ij}(R) \\ f_i(h,k,l) &= f_i(H) \end{aligned}\right\} \tag{24}$$

where R and H are the scalar quantities corresponding to equations (23). The quantity H is related to the Bragg angle of x-ray reflection by equation (6). The quantity R represents distance from the center of the Patterson peak. Equation (22) may now be transformed into our final result

$$P_{ij}(R) = 4\pi\int_0^{\infty} f_i(H)\,f_j(H)\,\frac{\sin 2\pi HR}{2\pi HR}\,H^2\,dH \tag{25}$$

by standard methods.

If we assume that each atom has a spherically symmetrical temperature factor of known magnitude, we may multiply it into the form factor,

$$f_i'(H) = f_i(H)\, e^{-\frac{1}{2} B_i(H)\cdot T} \tag{26}$$

and we may calculate for the shape of the Patterson peak

$$P_{ij}'(R) = 4\pi \int_0^\infty f_i'(H)\, f_j'(H)\, \frac{\sin 2\pi HR}{2\pi HR}\, H^2 dH. \tag{27}$$

If the temperature factor is anisotropic, it is necessary to make use of a more general expression analogous to equation (22).

For the special case of $R = 0$ and $i = j$, equation (27) gives the expression that was used in putting the experimentally determined values of F^2_{hkl} on an absolute basis (equations (4) and (5)).

Patterson[19] has shown that the peaks may be sharpened by dividing an average form factor out of the F^2_{hkl} data before carrying out the Fourier summation. The coefficients used in the summation were

$$F^2/\bar{f}^2.$$

When this is done, a Patterson distribution is obtained which corresponds to a crystal structure in which the atoms have an electron density distribution such that their form factors are equal to

$$f_i/\bar{f}.$$

The peaks will therefore have a shape given by

$$P_{ij}''(R) = 4\pi \int_0^\infty \frac{f_i f_j}{\bar{f}^2}\, \frac{\sin 2\pi HR}{2\pi HR}\, H^2 dH. \tag{28}$$

In the limit where $f_i f_j/\bar{f}^2$ is a constant the peak assumes the characteristics of a three-dimensional *delta function*: infinite height and infinitesimal width. Practical limits are imposed on the attainable degree of sharpening by the limited accuracy of the data and, more importantly, by the degree of convergence that can be obtained with the amount of data at hand. In order to prevent the occurrence of large periodic variations in amplitude at appreciable distances from the center of the Patterson peak it is necessary that $f_i f_j/\bar{f}^2$ be rapidly converging toward zero at the maximum value of $\underline{H}$ for which data have been obtained. The problem of convergence is analogous to that which arises in electron diffraction work.

Dr. Jurg Waser and Professor Schomaker have investigated the problems of convergence and of resolving power for the field of electron diffraction in a way that is more general than the simple method of Patterson, and van Reijen[26] has investigated these problems in a somewhat similar way for the field of crystal structure. Dr. Waser and Professor Schomaker introduced the *modification function*, which for our purposes we may write as $\underline{M}(\underline{H})$. This is a function that is multiplied into the experimental data before the Fourier synthesis is carried out. Its form is so chosen as to obtain a satisfactory compromise between convergence and resolving power. Both of these properties may be determined for a given modification function by calculating its Fourier transform, which is related to the shapes of the peaks that will be obtained in the electron diffraction radial distribution function or in the Patterson distribution obtained for a crystal.

For use in the Patterson calculation we will assume that before the modification function is applied the coefficients $\underline{F}_{hkl}^2$ have been divided by the square of an average value of the combined form factor and temperature factor to obtain new coefficients that show no systematic tendency to rise or fall in magnitude with increasing $\underline{H}$. This is a sharpening procedure similar to that of Patterson. After the application of the modification function the coefficients are

$$C_{hkl} = \frac{M(H)}{\bar{f}^2} F_{hkl}^2 .$$

After the Fourier synthesis

$$P''(u,v,w) = \frac{1}{v_a}\sum_{h=-\infty}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l=-\infty}^{\infty} C_{hkl} \cos 2\pi(hu+kv+lw) \tag{29}$$

is carried out, the peaks are expected to have shapes given by the equation

$$P_{ij}''(R) = 4\pi\int_0^\infty M(H)\,\frac{f_i'(H)\,f_j'(H)}{\overline{f'}^2(H)}\,\frac{\sin 2\pi HR}{2\pi HR}\,H^2\,dH \tag{30}$$

$$\cong \text{const} \times \int_0^\infty M(H)\,\frac{\sin 2\pi HR}{2\pi HR}\,H^2\,dH\,. \tag{30a}$$

It is assumed that the ratio

$$f_i f_j/\bar{f}^2$$

is spherically symmetrical in H. If there is a marked anisotropy in the temperature factor, the anisotropy should be removed in the sharpening procedure.

As an example of the type of modification function that could be used, the artificial temperature factor, which is a Gaussian function,

$$M(H) = e^{-\alpha^2 H^2} \tag{31}$$

might be cited. The Fourier transform of this function is likewise a Gaussian function

$$\text{const.} \times P_{ij}(R) \cong N(R) = \frac{\pi^{3/2}}{\alpha^3}\, e^{-\pi^2 R^2/\alpha^2} \tag{32}$$

In the use of any modification function, allowance must be made for the fact that the integration in equation (28) can be carried out only to the limit of the data. The shape of the peak will contain the effect of the limit or cutoff, so the cutoff must be regarded as an essential part of the modification function unless the modification function itself goes to zero before the limit of the data is reached. In the use of the artificial temperature factor the parameter α must be taken large enough to obtain satisfactory convergence. On the other hand, as α is made larger in an effort to obtain good convergence, the Patterson peaks become broader and the resolving power accordingly becomes smaller.

Although the artificial temperature factor may be a convenient modification function to use, it does not necessarily enable the best compromise between convergence and resolving power to be made. Professor Schomaker has shown that functions of the type*

$$M_n(H) = H^n e^{-\alpha_n^2 H^2}, \quad n > 0 \tag{33}$$

give, at comparable convergence, peaks that are sharper than those given by the artificial temperature factor. They have the somewhat objectionable feature, however, that each peak is surrounded by a shallow negative area of large extent. This may be less objectionable than the more slowly damped periodic variations due to non-convergence that arise when an artificial temperature function is used at the same resolving power.

Another consideration that affects the choice of a modification function is the relative reliability of the data at different values of H;

---

* The Fourier transforms of $M_n(H)$ for n - 0, 2, and 4 are

$$n = 0 : \quad N_0(R) = \frac{\pi^{3/2}}{\alpha^3} e^{-\rho^2} \tag{34}$$

$$n = 2 : \quad N_2(R) = \frac{\pi^{3/2}}{\alpha^5} e^{-\rho^2}\left[\frac{3}{2} - \rho^2\right] \tag{35}$$

$$n = 4 : \quad N_4(R) = \frac{\pi^{3/2}}{\alpha^7} e^{-\rho^2}\left[\frac{15}{4} - 5\rho^2 + \rho^4\right] \tag{36}$$

where

$$\rho = \frac{\pi}{\alpha} R \tag{37}$$

We may write

$$P_{ij}(R) = \text{const} \times N(R)$$

if $f_i' f_j' / \bar{f}'^2 = \text{const.}$ If (after Professor Schomaker) we we take as the criterion of convergence the smallness of the it is advantageous for the modification function to have its highest values where the data are most reliable. If the intensities of strong reflections at low $\underline{H}$ are uncertain because of extinction, it may be desirable to choose a modification function that is small at low $\underline{H}$. Small values at high $\underline{H}$ are desired both to obtain good convergence and to take account of the greater uncertainty in the estimation of intensities for the weaker reflections. Finally, the use of a modification function which has small values at low $\underline{H}$ may have the advantage of considerably reducing the contributions due to hydrogen atoms, because the form factor for hydrogen decreases more rapidly with increasing $\underline{H}$ than do the form factors of carbon, nitrogen, and oxygen atoms.

Before proceeding with an account of the calculation of the three-dimensional distribution for threonine, we shall mention another contribution of Patterson that was of use in our work. Patterson showed that it is possible to carry out the calculation in such a way that the peak at the origin does not appear. In our case the elimination of the peak at the origin was a matter of convenience in the calculations, for its presence would require

---

quantity

$$S = 1 - \frac{\int_0^{H_{max}} H\,M(H)\,dH}{\int_0^{\infty} H\,M(H)\,dH} \tag{38}$$

and as the criterion for resolving power the magnitude of the quantity

$$T = -\frac{1}{4\pi^2}\left[\frac{1}{RN(0)}\frac{\partial^2}{\partial R^2}RN(R)\right]_{R=0} \tag{39}$$

which is related to the curvature of the peak, we obtain, for modifications functions $H^n e^{-\alpha_n^2 H^2}$,

$$\begin{aligned} n = 0 :&\quad S_0 = e^{-\alpha^2 H_{max}^2} &\quad T_0 &= \frac{3}{2}\cdot\frac{1}{\alpha^2} \\ n = 2 :&\quad S_2 = (1+\alpha^2 H_{max}^2)\,e^{-\alpha^2 H_{max}^2} &\quad T_2 &= \frac{5}{2}\cdot\frac{1}{\alpha^2} \\ n = 4 :&\quad S_4 = (1+\alpha^2 H_{max}^2+\tfrac{1}{2}\alpha^4 H_{max}^4)\,e^{-\alpha^2 H_{max}^2} &\quad T_4 &= \frac{7}{2}\cdot\frac{1}{\alpha^2} \end{aligned} \tag{40}$$

For two given values of the convergence we obtain for the relative resolving the handling of rather large numbers in the punched card calculations.

To eliminate the peak at the origin it is only necessary to subtract the Fourier transform of the peak from the data. If the average form-temperature factor used for the sharpening operation is chosen in an appropriate way,

$$\overline{f'}^{2} = \frac{1}{N}\sum_{j=1}^{N} f'^{2}_{j}, \qquad (42)$$

we need only subtract from the data a constant times the modification function. This may be done by subtracting a constant from the *sharpened* data before applying the modification function, so that the coefficients for the Fourier summation are

$$C'_{hkl} = \left[\frac{F^2_{hkl}}{\overline{f'}^{2}(H)} - A\right] M(H) \qquad (43)$$

The constant A is chosen to make

$$P''(0,0,0) = \frac{1}{v_c}\sum_{h=-\infty}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l=-\infty}^{\infty} C'_{hkl} \cong 0$$

If it is not necessary that the peak vanish quantitatively, satisfactory results may be obtained by equating A to the arithmetic mean of all of the sharpened coefficients:

$$A = \overline{F^2_{hkl}/\overline{f'}^{2}}$$

It is important in this procedure to make sure that the data have been correctly sharpened; otherwise the shape of the peak subtracted from the Patterson distribution may be different from the natural shape of the peak, so that although the amplitude may be zero at the origin there may

---

powers of the three modification functions:

$$S_o = S_2 = S_4 = 0.03\,, \qquad T_o : T_2 : T_4 = 1 : 1.09 : 1.18 \qquad (41)$$
$$S_o = S_2 = S_4 = 0.01\,, \qquad T_o : T_2 : T_4 = 1 : 1.15 : 1.25$$

be enough over- or under-compensation in the neighborhood of the central peak to affect the heights and positions of nearby peaks.

The characteristics of the Patterson distribution that are useful in the determination of the structure of a crystal are described later for the space-group $P2_12_12_1$ in connection with their application to ℓ-threonine.

(c) The mechanics of the synthesis for ℓ-threonine

Because of the crystal symmetry $P2_12_12_1$ it was necessary to calculate the Patterson distribution only for one octant of the unit cell: $u = 0$ to $\frac{1}{2}$, $v = 0$ to $\frac{1}{2}$, $w = 0$ to $\frac{1}{2}$.

The data available for the Patterson synthesis for ℓ-threonine were the 618 non-equivalent corrected intensities kF^2_{hkl}, that had been calculated from the intensity data with a film factor of 4.0 and placed on the same relative basis. The new value (3.7) of the film factor was not known at that time, and the extent of extinction in strong reflections had not been determined. The limit of the data was

$$\sin \vartheta_{max} = 0.925, \quad H_{max} = 1.20\ \text{Å}^{-1}.$$

It was now necessary to decide what form of modification function to use. As the extent of the data is rather limited* we need to use the data in such a way that the maximum resolving power is obtained consistent with reasonable freedom from complications due to non-convergence. As the extent of extinction was not then known, we desired to suppress the strong reflections at low H. On the basis of these considerations, Professor Schomaker suggested that we use the modification function

$$M(H) = H^4 e^{-\alpha^2 H^2} \tag{44}$$

---

* $1/H_{max} = 0.83$ Å, which is an appreciable fraction of the interatomic distances in the molecule.

with

$$\alpha^2 H^2_{max} = 6.97,$$

corresponding to

$$\delta = 0.03$$

in equation (38). This modification function has a linear resolving power about 18% greater than that of the artificial temperature factor at comparable convergence. In order to improve the convergence the modification function was reduced in magnitude near the cutoff at $H = 1.2\text{Å}^{-1}$. A plot of the modification function finally adopted is given in Figure 3, and the radial distributions of amplitude for oxygen-oxygen, nitrogen-nitrogen, and carbon-carbon peaks, as calculated from equation (30) are given in Figure 4. The amplitude scale for the plots of peak-shapes is the same as the amplitude scale of the finished Patterson.

The corrected intensities $\underline{kF}^2_{hkl}$ were first sharpened by dividing out the square of the combined form factor and temperature factor for nitrogen. In order to eliminate the peak at the origin, the sharpened intensities were averaged and the average value was subtracted from each sharpened intensity. The modification function was applied to obtain the coefficients given by equation (43).

The Patterson summation may be written as follows:

$$P''(u,v,w) = \frac{1}{v_a} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} C'_{hkl} \cos 2\pi(hu+kv+lw)$$

$$= \frac{1}{v_a} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} C'_{hkl} \cos 2\pi hu \cdot \cos 2\pi kv \cdot \cos 2\pi lw.$$

The second form of writing follows from the fact that because of the orthorhombic symmetry the coefficients are even functions of h, k, and l. Further, in virtue of this property,*

---

* The term C'_{000} does not appear in the following equation because $M(0) = 0$.

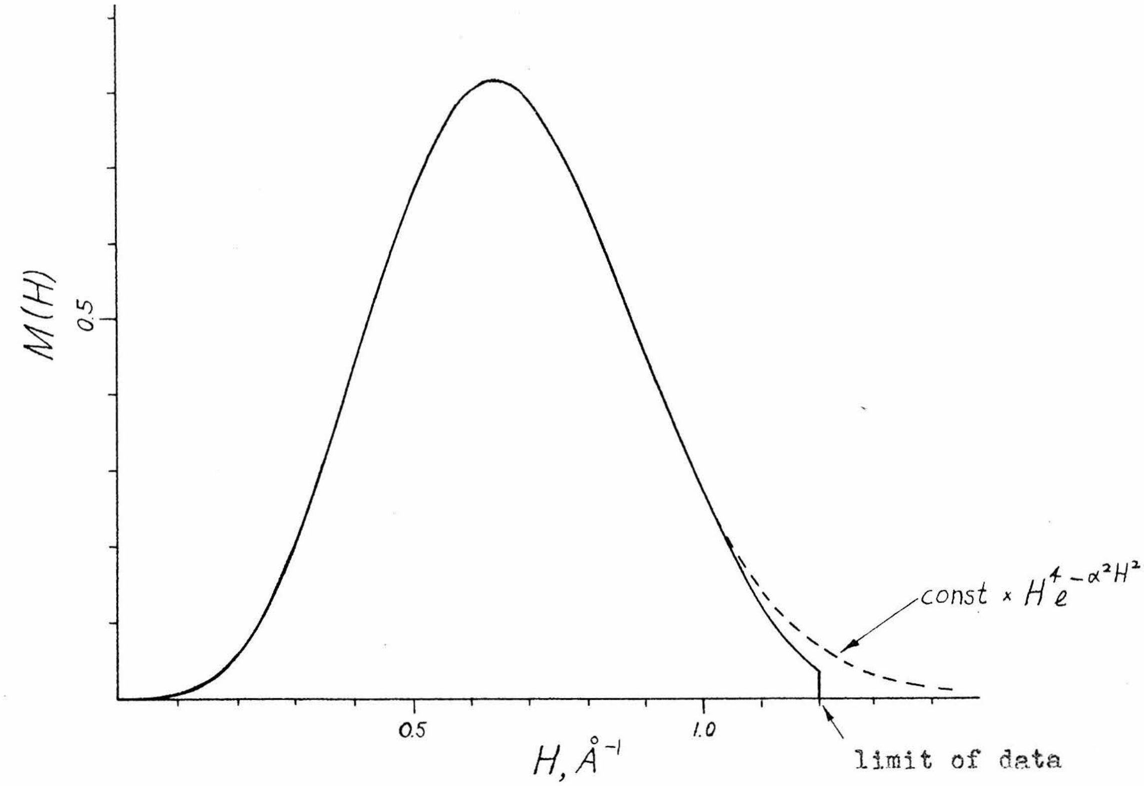


Figure 3. Modification function used in the calculation of the Patterson function.

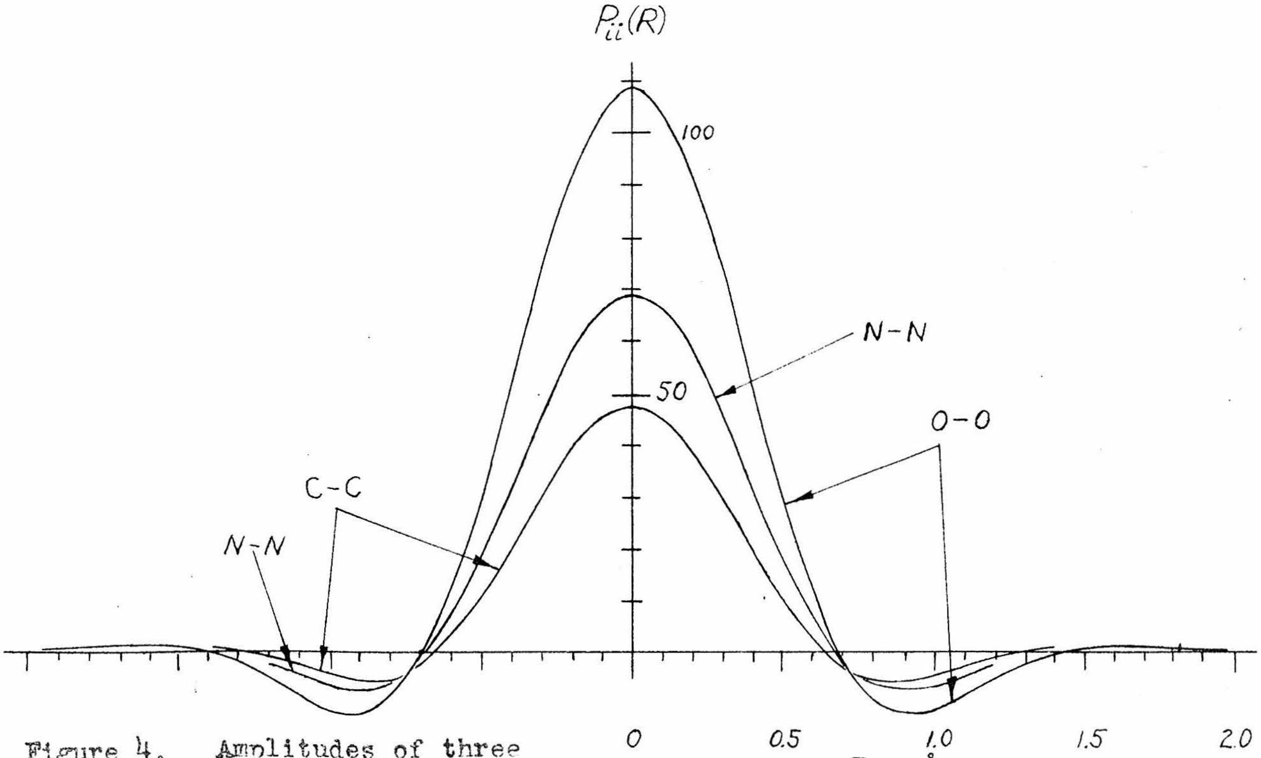


Figure 4. Amplitudes of three kinds of Patterson peaks, as functions of the radial distances from the centers of the peaks. Calculated by means of equation (30) from the modification function given in Figure 3.

$$v_a P''(u,v,w) = 2\sum_{h=1}^{\infty} C'_{h00}\cos 2\pi hu + 2\sum_{k=1}^{\infty} C'_{0k0}\cos 2\pi kv$$

$$+ 2\sum_{l=1}^{\infty} C'_{00l}\cos 2\pi lw$$

$$+ 4\sum_{k=1}^{\infty}\sum_{l=1}^{\infty} C'_{0kl}\cos 2\pi kv\cdot\cos 2\pi lw + 4\sum_{h=1}^{\infty}\sum_{l=1}^{\infty} C'_{h0l}\cos 2\pi hu\cdot\cos 2\pi lw$$

$$+ 4\sum_{h=1}^{\infty}\sum_{k=1}^{\infty} C'_{hk0}\cos 2\pi hu\cdot\cos 2\pi kv$$

$$+ 8\sum_{h=1}^{\infty}\sum_{k=1}^{\infty}\sum_{l=1}^{\infty} C'_{hkl}\cos 2\pi hu\,\cos 2\pi kv\,\cos 2\pi lw$$

$$= \sum_{h=0}^{\infty}\sum_{k=0}^{\infty}\sum_{l=0}^{\infty} C''_{hkl}\cos 2\pi hu\,\cos 2\pi kv\,\cos 2\pi lw \qquad (45)$$

where

$$C''_{h00} = 2\,C'_{h00}\text{, etc.}$$
$$C''_{0kl} = 4\,C'_{0kl}\text{, etc.}$$
$$C''_{hkl} = 8\,C'_{hkl}.$$

After taking account of this multiplicity, we were ready to carry out the summation with the **L** cards on the International Business Machines. Each card contains values of $A\cos 2\pi hx$ or $A\sin 2\pi hx$ for

$$x = 0,\ 1/60,\ 2/60,\ \ldots,\ 15/60$$

and when it is run through the tabulator with each of four different lead cards it provides values of the function from $x = 0$ to $x = 1$ in intervals of 1/60. Cards are available for all combinations of the amplitudes

$$A = \pm 1, \pm 2, \ldots, \pm 5, \pm 10, \ldots, \pm 50, \pm 100, \pm 200, \pm 500$$

with the frequencies

$$h = 0, 1, 2, \ldots 20.$$

Each value of the function is rounded off to the nearest integer. The amplitudes of our coefficients ranged from -57 to $+367$ but must of them were less than 50 in absolute magnitudes. The frequencies ranged as follows:

$$h = 0, 1, 2, \ldots, 16$$
$$k = 0, 1, 2, \ldots 9$$
$$l = 0, 1, 2, \ldots 6 .$$

We calculated the Patterson function over the following ranges in u, v, w and with the following intervals between points:

$u = 0$ to $\frac{1}{2}$,	$\Delta u = 1/60$,	$a_1 \Delta u = 0.227$ Å,	31 values
$v = 0$ to $\frac{1}{2}$	$\Delta v = 2/60$,	$a_2 \Delta v = 0.258$ Å,	16 values
$w = 0$ to $\frac{1}{2}$	$\Delta w = 3/60$,	$a_3 \Delta w = 0.257$ Å,	11 values

Number of points calculated = 5456.

The calculations were done in the following way. A summation over the index l was carried out for each hk combination to obtain the function

$$B_{hk}(w) = \sum_{l=0}^{6} C''_{hkl} \cos 2\pi l w. \qquad (46)$$

For each of eleven values of w from 0 to $\frac{1}{2}$ and for each value of h the B's were summed over the index k:

$$D_h(v,w) = \sum_{k=0}^{9} B_{hk}(w) \cos 2\pi k v. \qquad (47)$$

Finally the D's were summed over h for each of the sixteen values of v and the eleven values of w:

$$v_a P''(u,v,w) = \sum_{h=0}^{16} D_h\,(v,w) \cos 2\pi h u \qquad (48)$$

There were 134 summations, of one to seven terms each, in the first step. In the second step there were 187 summations of one to ten terms each, and in the final step there were 176 summations of fifteen to seventeen terms each. The total number of summations was 497 and the number of terms was estimated to be about 4500. This necessitated the handling of more than ten thousand cards. The cards were pulled from files containing three or more cards of each amplitude-frequency combination, and were checked once before putting them on the tabulator. The terms were then listed by the tabulator and checked again for errors. The summations were then carried out twice with the tabulator to guard against machine errors. Finally, the cards were sorted by machine back into the files to be used again.

Professor Schomaker and I, working together, completed the operations with the punched cards in three and a half days of work; a total of 62 man-hours were spent in these operations. This is certainly less than the total time spent in other operations related to the Patterson synthesis: the preparatory operations of sharpening the data, eliminating the peak at the origin, and applying the modification function, and the final operations of plotting the results and drawing in contours.

The completed Patterson distribution is presented in an appendix to this thesis.

(d) Interpretation of the Patterson distribution

(1) General considerations

In the interpretation of the Patterson distribution two distinct types of vector interactions are recognized.

First, there are interactions among crystallographically equivalent atoms; these have characteristics which depend upon the symmetry elements in the crystal. The interactions of atoms interrelated by the translation group, for example, give peaks at the lattice points in Patterson space; these are similar to the peak at the origin due to interactions of atoms with themselves. Interactions among atoms interrelated by symmetry planes and symmetry axes give rise to peaks having special values for one or two of their coordinates; these peaks are restricted, therefore, to special lines or planes the constant parameters of which correspond to the constant translational components of the respective symmetry elements. As these interactions provided the basis of Harker's method[20] of determining atomic parameters, we shall refer to the corresponding peaks as *Harker peaks*. The lines or planes on which these peaks are constrained to lie are known as *Harker lines* and *Harker sections*.

Second, if there are more than one set of equivalent atoms in the crystal, interactions between pairs of non-equivalent atoms will exist and will give rise to peaks that have no special values for their coordinates; these peaks will be referred to as *non-Harker peaks*.

Unless it accidentally lies on a Harker section, a non-Harker peak may be immediately recognized as such. If the symmetry axis is a two-fold screw axis or ~~any~~ rotation axis, the Harker plane coincides with a mirror plane of the Patterson distribution, and any non-Harker peak that accidentally lies on the Harker section will superpose with the peak that is related to it by the mirror plane to give a peak twice as high as a generally situated peak due to a similar interaction. Since a Harker peak has a height equal to that of a generally situated peak due to non-equivalent atoms of the same kind, it may be possible to recognize Harker peaks by their height as well as by the apparent rigor of their constraint to the Harker plane. The heights of oxygen-oxygen, nitrogen-nitrogen, and carbon-carbon peaks that occur on our Patterson distribution (see Appendix) are given for both Harker and non-Harker peaks under various multiplicity conditions in Table 16 .

Table 16

Relative Heights of Patterson Peaks*

	Positions of Non-Harker Peaks				Positions of Harker Peaks		
	General Position	Mirror Plane	Two-fold axis	Center of Symmetry	Harker Plane	Two-fold axis	Center of Symmetry
O-O	109	218	436	872	109	218	436
N-N	69	139	278	555	69	139	278
C-C	47	95	190	380	47	95	190

* On the same relative scale as the contour heights for the Patterson distribution as given in the appendix

From the symmetry of the crystal and the number N of atoms in the unit cell it is possible to calculate the number of peaks of each kind to be expected in the Patterson cell. For the space-group D_2^4-$P2_12_12_1$, to which we will hereafter restrict our discussion, there are three Harker-sections, one of which corresponds to each of the three two-fold screw axes. Each of the atoms interacts* with the three other atoms crystallographically equivalent to it so as to give three Harker peaks, one on each section. There are

---

* We use this term in the following sense: between atoms X_i and X_j there are two interactions P_{ij} and P_{ji} representing respectively the vectors $X_i \longrightarrow X_j$ and $X_j \longrightarrow X_i$.

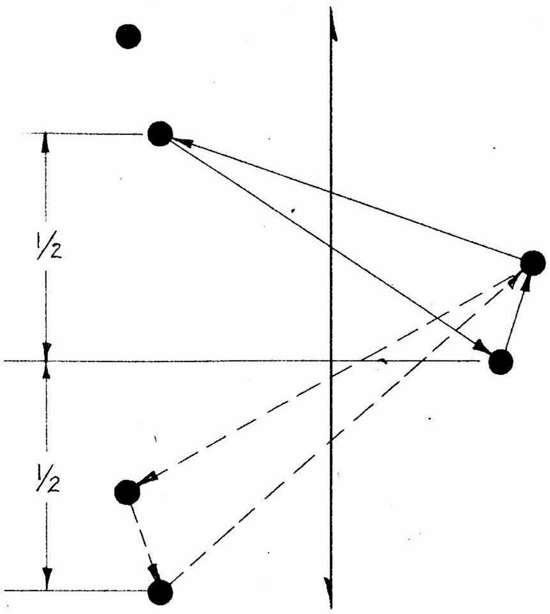
therefore on each section four equivalent peaks (of which, in the Harker method, one may be assigned to a given atom) for each of the $\underline{N}/4$ kinds of non-equivalent atoms. There are $\underline{N}$ Harker peaks on each section, or $3\underline{N}$ in the unit cell. Each of the $\underline{N}$ atoms in the unit cell interacts with the $4(\underline{N}/4-1)=\underline{N}-4$ atoms that are not equivalent to it to give altogether $\underline{N}(\underline{N}-4)$ non-Harker peaks. The total number of peaks, aside from the peak at the origin, is seen to be $\underline{N}(\underline{N}-1)$, in agreement with the result previously obtained. If these numbers agree with actual counts of the peaks obtained from the Patterson synthesis, the analysis of the Patterson distribution may be carried out with the assurance that each peak has a unique interpretation. For threonine, with 32 atoms in the unit cell, we expect a total of 992 peaks of which 896 are non-Harker peaks and 96 are Harker peaks. In each quadrant of each of the three Harker sections we expect to find eight Harker peaks and within the octant that we have calculated we expect to find 112 non-Harker peaks; the total number of peaks for which we must account is 136. Approximately half that number of resolved peaks has been found; a considerable amount of overlapping and superposition of peaks has evidently occurred, and we must take this into account.

The necessary relations, other than those given only by the operations of the space group on individual interatomic vectors, that exist among the positions of Patterson peaks are expected to follow a pattern, characteristic of the space group, that we may call the inner symmetry of the Patterson distribution. The essential principle of the inner symmetry is: for any closed circuit of interatomic vectors in the crystal, the vectors represented by the corresponding Patterson peaks must add vectorially to zero; i.e. the respective coordinates of the corresponding peaks must add to zero.

The features of the inner symmetry that are characteristic of the crystal space group arise when this principle is applied to vector circuits which contain Harker vectors, i.e. vectors between atoms that are equivalent by virtue of the operations of the space group. For example, the apparent symmetry of the Patterson distribution obtained for a crystal with any primitive orthorhombic space group is D_{2h}^{1}-Pmmm. The characteristic differences among the Patterson distributions for these space groups involve the existence and locations of Harker lines and sections, the relative positions of Harker peaks representing interactions between equivalent pairs of equivalent atoms, and the relations between Harker peaks and non-Harker peaks; differences in these features constitute differences in inner symmetry. We shall discuss some of these features briefly in connection with the space-group D_2^4-$P2_12_12_1$.

We may first examine the relations between Harker and non-Harker peaks in this space group. If two non-equivalent Harker peaks are to be ascribed to interactions between equivalent atoms in two respective non-equivalent pairs, then two non-equivalent non-Harker peaks must be found which correspond to the two kinds of interaction between non-equivalent atoms. The way in which the inner symmetry principle applies in the case of a two-fold screw axis is shown in Figure 5.

A special case, pointed out by Albrecht and Corey[18] is illustrated in Figure 6. If an interatomic vector between non-equivalent atoms is parallel to a Harker section, a non-Harker peak is found on the Harker section half way between the two Harker peaks. It will, moreover, be the superposition of two peaks (the limiting case of the overlapping of two peaks, one on either side of the Harker plane). Every peak on the section $\underline{w}=0$, for example, must give rise to this phenomenon on the section $w=\frac{1}{2}$.



Two non-equivalent sets of atoms grouped around a two-fold screw axis.

For convenience in presentation, all atoms are assumed to lie in a plane which includes the screw axis.

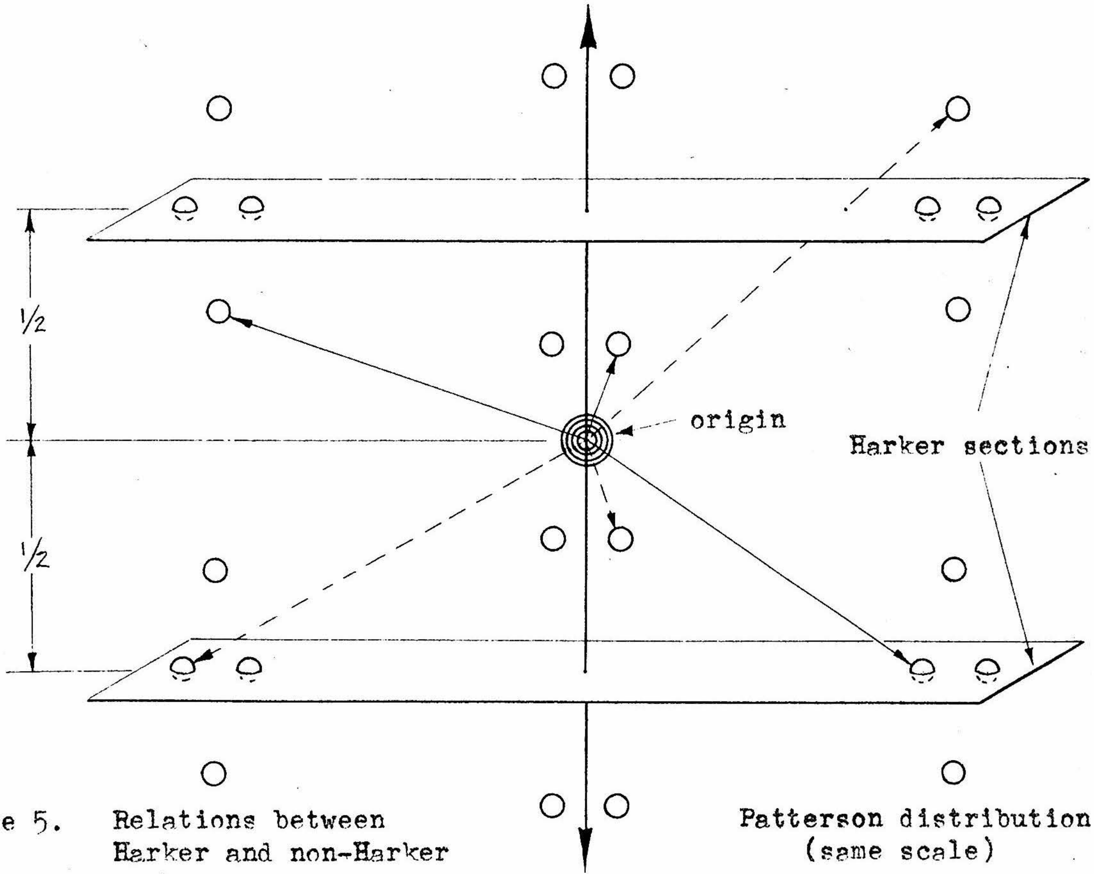
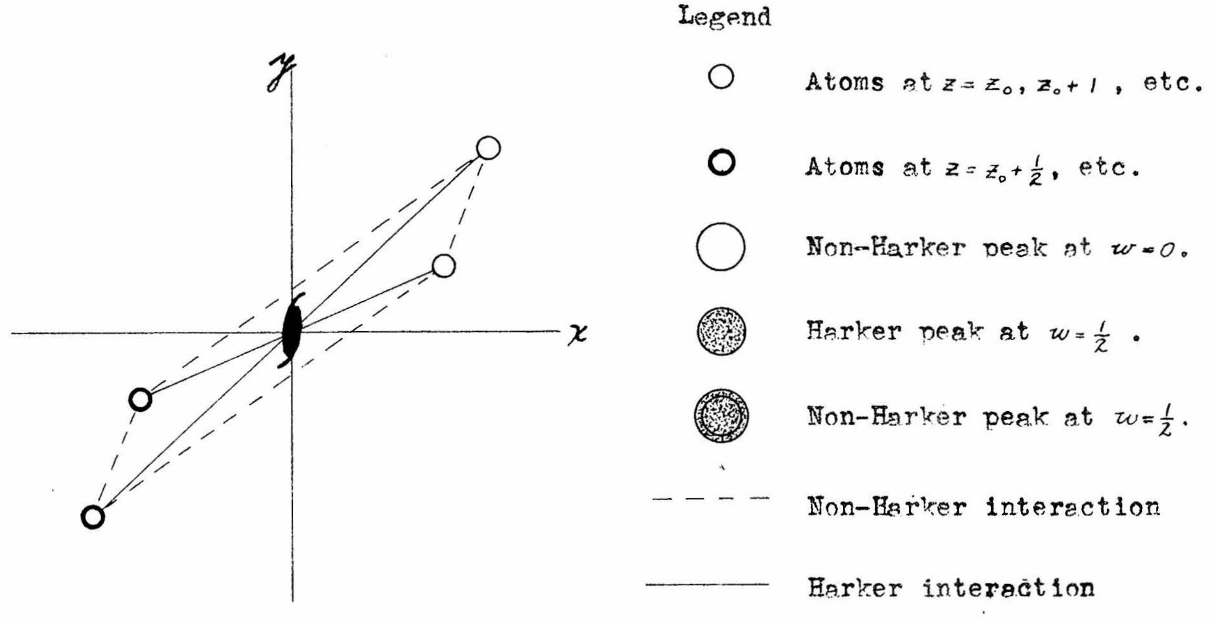
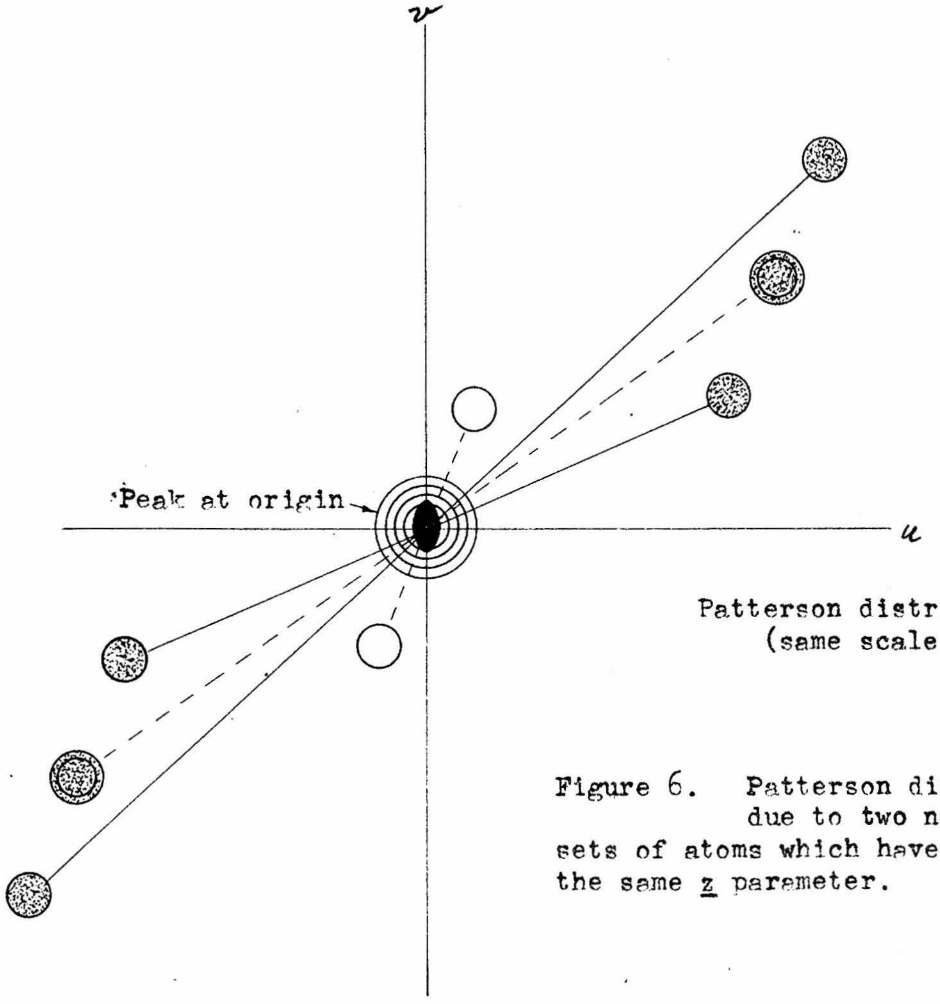


Figure 5. Relations between Harker and non-Harker peaks.

Patterson distribution (same scale)



Two non-equivalent sets of atoms grouped around a screw axis



Patterson distribution (same scale)

Figure 6. Patterson distribution due to two non-equivalent sets of atoms which have, by accident, the same z parameter.

Let us determine the inner symmetry relations among Harker peaks. With the space-group D_2^4-$P2_12_12_1$, the position of a Harker peak due to a given pair of equivalent atoms depends on the vector distance between each atom and the screw axis; twice this distance gives the vector distance of the Harker peak from the origin of coordinates for the section. The atom is not, in general, equidistant from each of the screw axes; corresponding components of the vectors between an atom and three differently oriented screw axes have a sum or difference of $(\underline{n}/2 + \frac{1}{4})$ where n is an integer. This is obvious from Figure 7 in which a diagram of the symmetry elements of the space group is given.[12b] If Harker peaks corresponding to a given atom on the three different Harker sections are given the coordinates $(\frac{1}{2}, v', w')$, $(u'', \frac{1}{2}, w'')$, and $(u''', v''', \frac{1}{2})$, the necessary relations may be written

$$\begin{aligned} u'' \pm u''' &= n' \pm \tfrac{1}{2} \qquad n', n'', n''' = \text{integers} \\ v' \pm v''' &= n'' \pm \tfrac{1}{2} \\ w' \pm w'' &= n''' \pm \tfrac{1}{2} \end{aligned} \tag{49}$$

An important application of the inner symmetry was the confirmation of the space group of ℓ-threonine. The small number (three) of odd orders of (001) that could be examined with copper $K\alpha$ radiation did not allow much certainty in the conclusion that the symmetry axis parallel to $\underset{\sim}{a}_3$ is a two-fold screw axis. A search for peaks with the expected character of Harker peaks on the sections $w = 0$ and $w = \frac{1}{2}$, an examination of the relations among the peaks on the two sections, a test of the equations (49), and finally the discovery of several other vectorial relations consistent with the inner symmetry of the space group D_2^4-$P2_12_12_1$, all verified the conclusion

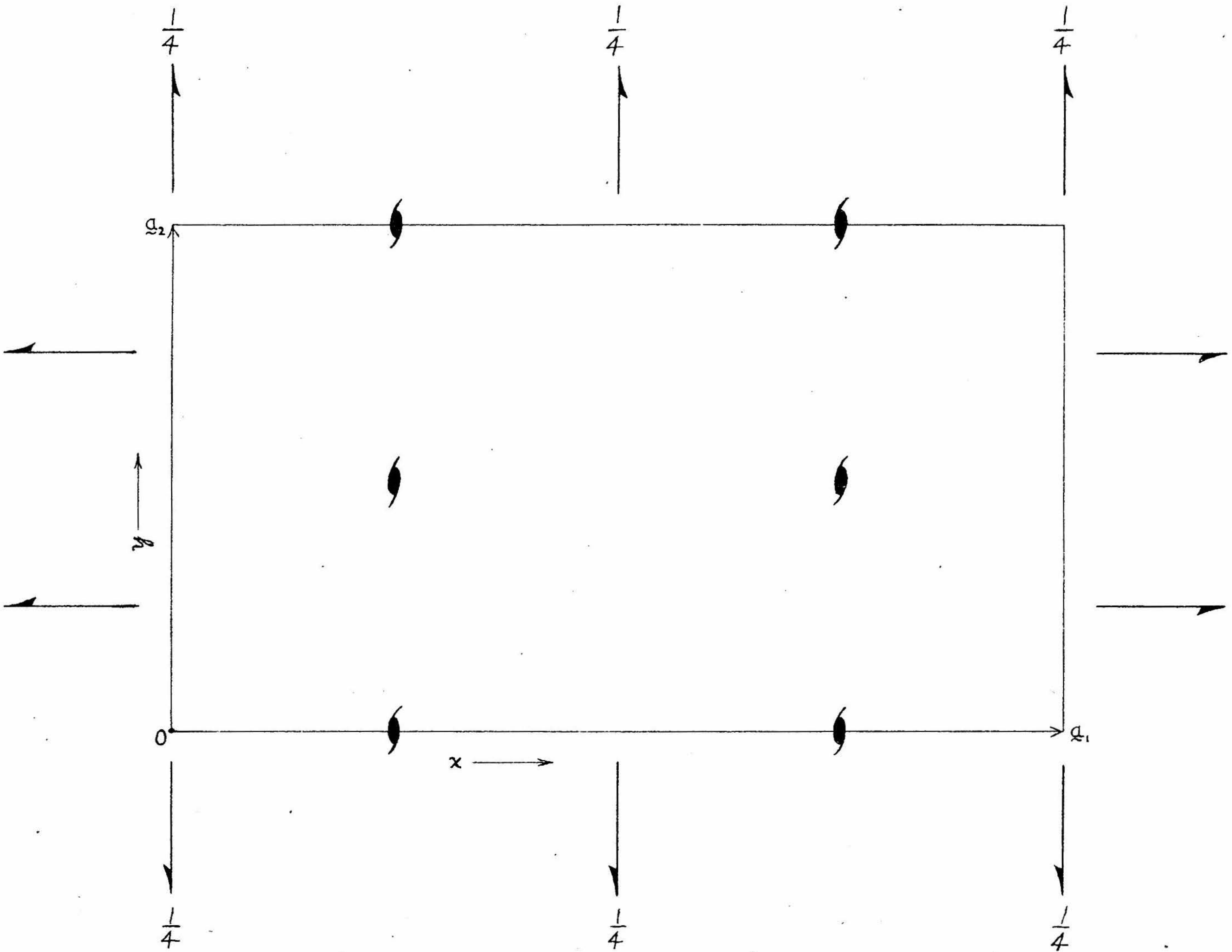


Figure 7. Symmetry elements of the space group D_2^4 - $P2_12_12_1$.

Adapted from Internationale Tabellen but with a change in the orientation of the axes. One unit cell is shown. The $\underset{\sim}{a}_3$ axis is perpendicular to the plane of the paper and is directed toward the reader.

that this space group is correct.

We have by no means exhausted the study of the inner symmetry. With the background given above, however, we may proceed to the analysis of the Patterson distribution for information regarding the structure of ℓ-threonine.

(2) Examination of peaks close to the origin

In the octant we should expect to find seven peaks, due to pairs of atoms bonded with covalent bonds, within 1.6 Å of the origin; nine, each due to a pair of atoms bonded indirectly through a third atom, in the range 2.2 Å to 2.7 Å; and in the range 2.7 Å to 3.0 Å we expect to find some peaks corresponding to hydrogen bonds. If the peaks for these short vectors can be identified, the orientation and configuration of the threonine molecule can be determined immediately.

On inspection of the region within 1.6 Å of the origin it is difficult to account for seven interactions due to bonded pairs of atoms. A peak at ($\underline{u}\underline{v}\underline{w}$) = (0.08, 0.12, 0.00) is about 1.5 Å from the origin; it has an extension in the $\underline{w}$ direction and is high enough to account for two carbon-carbon interactions. An elongated peak centered at (0.00, 0.06, 0.25) is about 1.4 Å from the origin and could accomodate two or three carbon-carbon, carbon-nitrogen, or carbon-oxygen interactions.

We find in the range 2.2 A to 2.7 A a high peak at ($\underline{u}$, $\underline{v}$, $\underline{w}$,) = (0.055, 0.00, 0.40) that is elongated in the $\underline{w}$ direction, probably by overlapping with the equivalent peak at (0.055, 0.00, 0.60), but is otherwise very symmetrical. Its center is 2.25 Å from the origin, and its height is about that expected for an oxygen-oxygen peak. The only other peak centered at 2.2 Å to 2.3 Å is a peak at (0.00, 0.27, 0.00) which is much too low, in view of the multiplicity, to represent any interactions except those involving hydrogen atoms unless its small amplitude can be accounted for by negative contributions of other peaks. Other peaks in this range are a high peak at (0.17, 0.13, 0.07), 2.7 Å from the origin; a broad, moderately strong peak at (0.10, 0.25, 0.20), 2.6 Å from the origin; and an elongated peak of moderate strength at (0.09, 0.16, 0.35), 2.5 Å from the origin. Each of these is high enough to account for at least two interactions; together with the one good peak 2.2 Å from the origin they can probably account for the nine indirectly bonded interactions expected in this region.

When the calculation of a three-dimensional Patterson distribution was first contemplated it was thought that the peaks near the origin would be the most useful feature of the distribution. However, with a single exception which is discussed below, we were unable to make a clear-cut interpretation of these peaks without additional evidence.

Perhaps the most useful single clue to the structure was provided by one of these peaks, however. Among the intramolecular interatomic interactions the ones which should give the strongest peaks are the three oxygen-oxygen interactions. One of these, moreover, has a known distance that is unique among all of the possible interatomic distances in the structure; it is the oxygen-oxygen interaction of the carboxyl group; glycine[18] and alanine[27] have oxygen-oxygen distances very close to 2.20 Å. No other pairs of atoms not directly bonded together can easily have interatomic distances less than 2.4 Å. The peak at (0.055, 0, 0.40) is the only peak, high enough to account for an oxygen-oxygen interaction that occurs at a distance close to 2.2 Å from the origin. This peak may therefore be identified with the carboxyl oxygen-oxygen interaction with fair certainty.

(3) Determination of some atomic parameters

With the assumption that our assignment of the peak (0.055, 0.00, 0.40) to the interaction of the carboxyl oxygen atoms is correct, we proceeded to look for the Harker peaks corresponding to these two atoms. In the analysis that follows, we shall take the non-Harker vector corresponding to these two atoms as

$$O_{II} \rightarrow O_{I} = (-0.055,\ 0.00,\ 0.40)$$

in which the negative sign for the first coordinate is arbitrarily chosen.

The first requirements for the Harker peaks are that they have the respective vector separations (Δu, Δv, Δw):

$$\underline{w} \quad \text{Harker} \quad (O_{II} \rightarrow O_{I}) = (\pm 1.10,\ 0.00,\ 0.00)$$

$$\underline{v} \quad \text{Harker} \quad (O_{II} \rightarrow O_{I}) = (\pm 1.10,\ 0.00,\ \pm 0.80)$$

$$\underline{u} \quad \text{Harker} \quad (O_{II} \rightarrow O_{I}) = (0.00,\ 0.00,\ \pm 0.80)$$

and that the peaks have coordinates that satisfy equations (49). After exhaustive examination of the sections the only assignment of peaks which we found to be compatible with these requirements is as follows. The coordinates given are ($\underline{u}$, $\underline{v}$, $\underline{w}$); the values given do not correspond exactly to the centers of the peaks but have been shifted slightly in the light of further developments. The reader may locate these peaks on the sections given in the appendix. The prime marks represent atoms equivalent to the unprimed atoms by the respective screw axes.

$$\begin{array}{lll} \underline{w} & \text{Harker} & O'''_{I} \to O_{I} = (0.37,\ 0.47,\ \tfrac{1}{2}) \\ & & O'''_{II} \to O_{II} = (0.48,\ 0.47,\ \tfrac{1}{2}) \\[1em] \underline{v} & \text{Harker} & O''_{I} \to O_{I} = (-0.13,\ \tfrac{1}{2},\ 0.40) \\ & & O''_{II} \to O_{II} = (-0.02,\ \tfrac{1}{2},\ -0.40) \\[1em] \underline{u} & \text{Harker} & O'_{I} \to O_{I} = (\ \tfrac{1}{2},\ -0.03,\ 0.90) \\ & & O'_{II} \to O_{II} = (\ \tfrac{1}{2},\ -0.03,\ 0.10) \end{array}$$

This assignment may now be tested by calculating the expected coordinates of non-Harker vectors from the requirements of closure:

$$\begin{aligned} O'''_{I} \longrightarrow O_{II} &= (0.425,\ 0.47,\ 0.10) \\ O'''_{II} \longrightarrow O_{I} &= (0.425,\ 0.47,\ 0.90) \\[1em] O''_{I} \longrightarrow O_{II} &= (-0.075,\ 0.50,\ 0.00) \\ O''_{II} \longrightarrow O_{I} &= (-0.075,\ 0.50,\ 0.00) \\[1em] O'_{I} \longrightarrow O_{II} &= (0.555,\ -0.03,\ 0.50) \\ O'_{II} \longrightarrow O_{I} &= (0.445,\ -0.03,\ 0.50) \end{aligned}$$

To illustrate the closure of vectors we may add the vectors in a typical circuit:

$$\begin{array}{l} O'''_{I} \longrightarrow O_{II} = (0.425,\ 0.47,\ 0.10) \\ O_{II} \longrightarrow O_{I} = (-0.055,\ 0.00,\ 0.40) \\ O_{I} \longrightarrow O'''_{I} = (-0.37,\ -0.47,\ -\tfrac{1}{2}) \\ \hline O'''_{I} \longrightarrow O'''_{I} = (0.00,\ 0.00,\ 0.00) \end{array}$$

All of these points lie on peaks in the Patterson distribution; hence the assignment conforms with the requirements of inner symmetry. The coordinates of the atoms are, from this assignment,

	x	y	z
O_{I}	0.435	0.235	0.45
O_{II}	0.49	0.235	0.05

These are obtained by substituting the coordinates of the Harker peaks for the unprimed atoms into the relations

$$
\begin{aligned}
x &= \frac{u'''}{2} + \frac{1}{4} = \frac{u''}{2} + \frac{1}{2} \\
y &= \frac{v'''}{2} \qquad\quad = \frac{v'}{2} + \frac{1}{4} \\
z &= \frac{w''}{2} + \frac{1}{4} = \frac{w'}{2} \qquad ,
\end{aligned}
\tag{50}
$$

which are consistent with equations (49).

On examination of the section $v=0$ we find peaks at (0.25, 0.00, 0.00) and at (0.195, 0.00, 0.40). As the vector connecting these peaks is just the $O_{II} \rightarrow O_I$ vector (-0.055, 0.00, 0.40) it appears likely that these peaks represent interactions of some other atom in the structure with O_I and O_{II}. This atom which we will designate X_I, must be represented on the v Harker section by a Harker peak, and on this same section there must be two non-Harker peaks involving atom X_I because the atom apparently has the same y coordinate as the two oxygen atoms. We observe on the v Harker section the phenomenon which we expect to find when three atoms have equal y parameters: the three Harker peaks which represent these atoms lie at the vertices of a triangle, the edges of which are bisected by non-Harker peaks. This is illustrated in Figure 8.

Let us start with the assignment

$$O_I \rightarrow X_I = (0.25,\ 0.00,\ 0.00)$$

$$O_{II} \rightarrow X_I = (0.195,\ 0.00,\ 0.40).$$

We may expect to find Harker peaks as follows:

$$X_I''' \rightarrow X_I = (0.87,\ 0.47,\ \tfrac{1}{2})$$

$$X_I'' \rightarrow X_I = (0.37,\ \tfrac{1}{2},\ 0.40)$$

$$X_I' \rightarrow X_I = (\tfrac{1}{2},\ -0.03,\ 0.90).$$

We indeed find that these points lie on peaks. The positions of non-Harker peaks are expected to be:

Figure 8. Harker section $v = \frac{1}{2}$, showing Harker peaks due to atoms O_I, O_{II}, and X_I and non-Harker peaks that arise from interactions among these atoms.

$X_I''' \rightarrow O_I = (0.62,\ 0.47,\ 0.50)$, $X_I''' \hookrightarrow O_{II} = (0.675,\ 0.47,\ 0.10)$

$X_I'' \rightarrow O_I = (0.12,\ 0.50,\ 0.40)$, $X_I'' \rightarrow O_{II} = (0.175,\ 0.50,\ 0.00)$

$X_I' \rightarrow O_I = (0.25,\ -0.03,\ 0.90)$, $X_I' \hookrightarrow O_{II} = (0.305,\ -0.03,\ 0.50)$

All of these points lie on peaks, as the reader may verify. From equations (50) we obtain the parameters

	x	y	z
X_I	0.685	0.235	0.90

In a similar way we find an atom X_{II} which has coordinates

	x	y	z
X_{II}	0.560	0.115	0.90

by making use of Harker peak $(0.715,\ 0.23,\ \frac{1}{2})$, and the non-Harker peaks $(0.065,\ 0.13,\ 0)$ and $(0.80,\ 0.37,\ 0.50)$ which relate it approximately to the *w* Harker peak for atom X_I $(0.87,\ 0.47,\ \frac{1}{2})$. In this case the correspondence of the expected interactions with Patterson peaks is not as good as that for X_I.

We have established tentative coordinates for other atoms but have hesitated to rely on them as in some cases the agreement with observed peaks is poor. At this point we turned to examination of evidence of other kinds, which we shall discuss in the next section.

Our analysis of the Patterson distribution is by no means complete. We expect that during the refinement of parameters, frequent checking of parameter changes against the Patterson distribution will be useful. We have already checked our promising trial structure, which is described in the next section, against the Patterson distribution by comparing the calculated and observed positions of peaks. The calculated points are plotted in red on the sections given in the appendix. The result, which we will not discuss in detail here, was a promising fit in which nearly all of the expected peaks fell on or close to actual peaks although many of the peaks were not utilized in the determination of parameters. A few bad disagreements exist, but there is good promise of obtaining a satisfactory fit eventually.

14. The determination of the approximate structure*

From the analysis of the three-dimensional Patterson distribution we have obtained parameters for four atoms. We must now attempt to interpret this information in the light of other evidence. This includes steric considerations, possible modes of hydrogen bonding, some requirements imposed by structure factors, and cleavage properties.

We started with the assumption that the atoms for which we have parameters are all in the same molecule. This assumption is based partly on evidence obtained from structure factors which indicated that the molecule is strung out approximately in the $\underset{\sim}{a}$ direction, and partly on the difficulty of obtaining a plausible mode of hydrogen bonding between the amino groups and the carboxyl groups if the contrary assumption is made.

The mutual electrostatic attraction of the carboxyl oxygen atoms and the amino group may tend to bring the nitrogen atom close to the plane of the carboxyl group. In the structures of alanine[27] and glycine[18] the nitrogen atom is not far out of the plane of the carboxyl group. Placement of the nitrogen atom in approximately the position shown by Figure 9 in line with this argument is not incompatible with the Patterson distribution and provides an excellent opportunity for hydrogen bonding to O_{II} of the

---

* Frequent reference to Figure 9 may be of help in following this discussion.

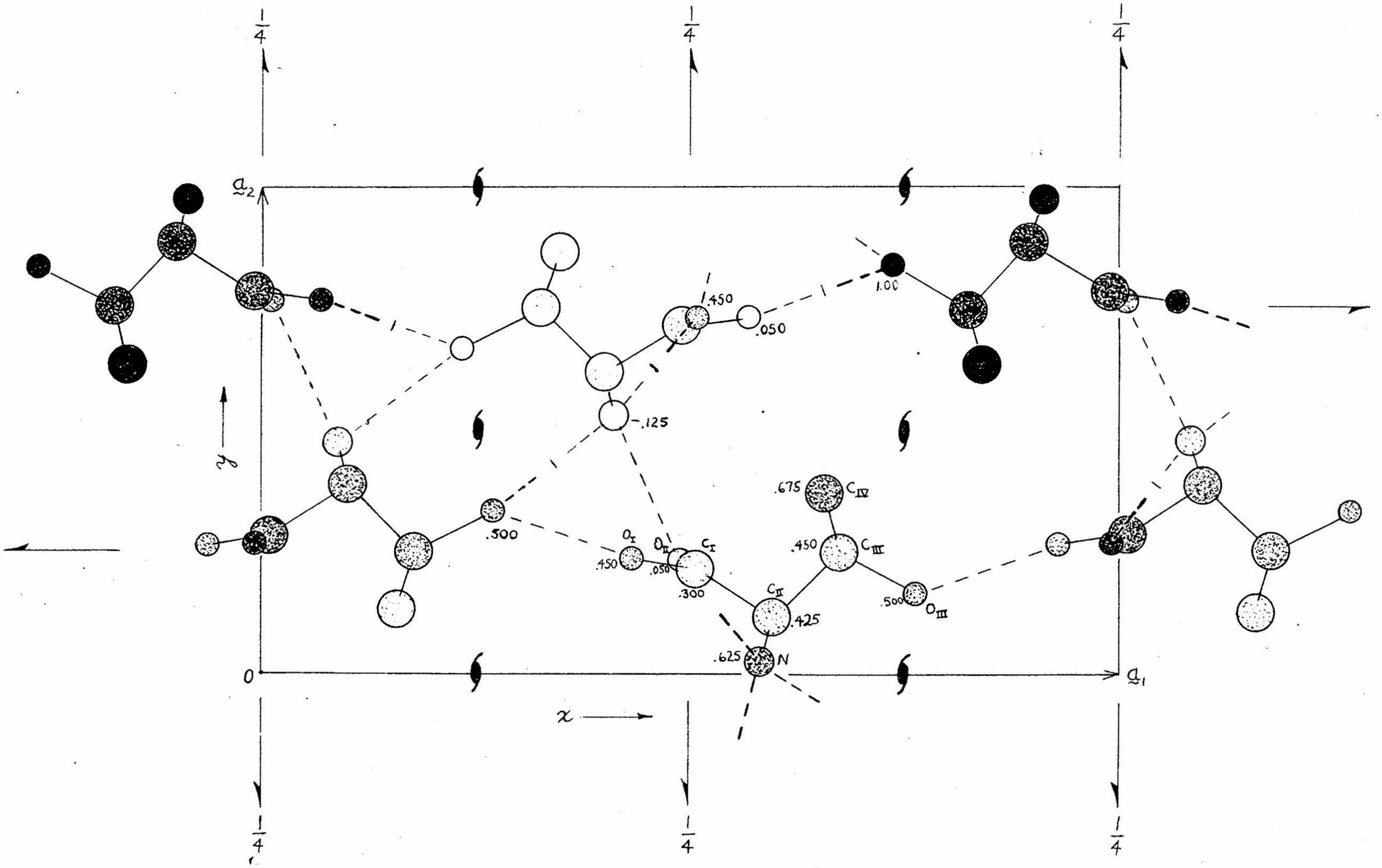


Figure 9a. Probable approximate structure of d- or l-threonine. One unit cell is shown; the a_3 axis is perpendicular to the plane of the paper (toward the reader). In decreasing order of their sizes, the circles represent carbon, nitrogen, and oxygen atoms. Hydrogen atoms are not shown. Bonds between atoms in a molecule are shown as solid lines; hydrogen bonds are shown as dashed lines. See also Figure 9b.

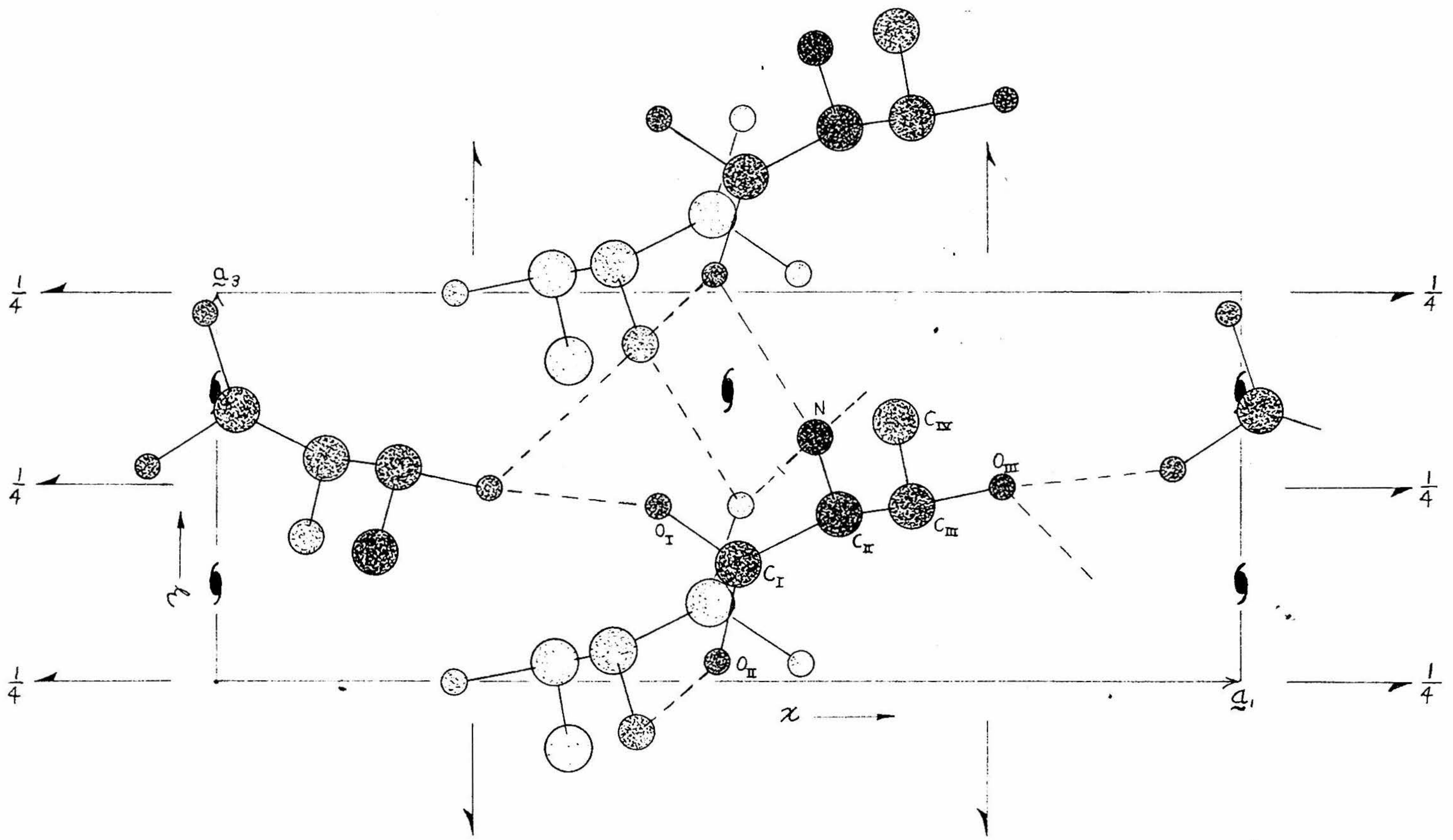


Figure 9b. In this view of the approximate structure, the a_2 axis is normal to the plane of the paper (away from the reader).

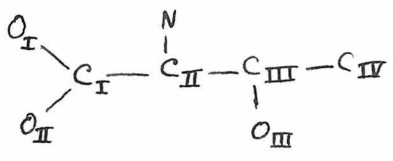
molecule above (in the *z*-direction) and to O_{II} of another molecule. Moreover, with this placement of the nitrogen atom, C_{II} and C_{III} can be made to occupy the atomic positions X_{II} and X_{I} respectively.* The position of C_{I} is now fixed, and we are left with the task of placing C_{IV} and O_{III} .

At this point we made an arbitrary assignment of parameters to these two atoms and calculated structure factors for some of the low-index planes. The agreement between observed and calculated structure factors was extremely poor, but the results yielded a very useful piece of information: the calculated value of (200) was much too high. A very large contribution to this plane is made by the carboxyl group. In order to reduce the structure factor to the experimental value, it is necessary that the atoms O_{III} and C_{IV} have *x* parameters as near as possible to 3/4 in order to cancel partially the contribution of the atoms near $x = \frac{1}{2}$.
This consideration, further, made it very unlikely that the molecule has a diagonal orientation with an appreciable component in the *y* direction.

The approximate structure shown in Figure 9 was then proposed.

---

* The numbering of the atoms is as follows:



In this structure the ammonium group forms two good hydrogen bonds to O_{II} atoms in two different adjacent molecules and a third weaker bond to the oxygen atom, O_{III}, of the hydroxyl group in a third molecule. The hydroxyl group has an opportunity to form a hydrogen bond to O_{I} of another molecule. Thus all of the capacity of the amino group and the hydroxyl group for hydrogen bonding is utilized, and both carboxyl oxygen atoms are involved in the hydrogen bonding. The packing of the molecules appears to be good; a rather short intramolecular $N-C_{IV}$ distance (3.0 Å) is the only objectionable feature, and there is considerable liklihood that this distance will be lengthened in more refined models.

The strong $N-O_{II}$ hydrogen bonds form a two-dimensional network of molecules that would resist cleavage on (001) and (010) planes; in addition, nearly all of the ionic bonding is confined to the virtually two-dimensional array of positively charged amino groups and negatively charged carboxyl groups. Cleavage on (100), however, would be resisted only by hydrogen bonds involving the hydroxyl group and by weak van der Waals forces. The cleavage properties expected for this structure are in agreement with experiment; good cleavage was obtained only on (100).

After making small changes in the parameters predicted for the atoms by the Patterson distribution, we calculated the structure factors of low index $(0k\ell)$, $(h0\ell)$, and $(hk0)$ reflections for this trial structure.

The structure factor may be written

$$F_{hk\ell} = \sum_{i=1}^{N} f_i'(h,k,\ell)e^{+2\pi i(hx+ky+\ell z)} \tag{51}$$

where the sum is taken over all of the atoms in the unit cell. In the space group $P2_12_12_1$ the atoms occur in sets of four, which are crystallographically equivalent and have the coordinates[12b],

$$xyz; \quad \tfrac{1}{2}-x,\ \bar{y},\ \tfrac{1}{2}+z; \quad \tfrac{1}{2}+x,\ \tfrac{1}{2}-y,\ \bar{z}; \quad \bar{x},\ \tfrac{1}{2}+y,\ \tfrac{1}{2}-z. \tag{52}$$

The structure factor reduces to the form

$$F_{hkl} = \sum_{i=1}^{N/4} f_i(hkl) \left[A_{hkl} + i\, B_{hkl}\right]$$

where[12b]

$$\begin{aligned} A_{hkl} &= 4 \cos 2\pi \left[h(x-\tfrac{1}{4})+\tfrac{k}{4}\right]\cdot \cos 2\pi \left[k(y-\tfrac{1}{4})+\tfrac{l}{4}\right]\cdot \cos 2\pi \left[l(z-\tfrac{1}{4})+\tfrac{h}{4}\right] \\ B_{hkl} &= -4 \sin 2\pi \left[h(x-\tfrac{1}{4})+\tfrac{k}{4}\right]\cdot \sin 2\pi \left[k(y-\tfrac{1}{4})+\tfrac{l}{4}\right]\cdot \sin 2\pi \left[l(z-\tfrac{1}{4})+\tfrac{h}{4}\right] \end{aligned} \tag{53}$$

It is now necessary to sum only over N/4 non-equivalent atoms, as for example those in a single molecule of threonine. For calculation of (0kl), (h0l), and (hk0) reflections it is convenient to take the origin of each respective set of coordinates on the respective screw axis in order to make B vanish. We finally obtain

$$A_{0k\ell} = \begin{cases} 4 \cos 2\pi k y_1 \cos 2\pi \ell z_1, & k+\ell \text{ even} \\ -4 \sin 2\pi k y_1 \sin 2\pi \ell z_1, & k+\ell \text{ odd} \end{cases}$$

$$y_1 = y-\tfrac{1}{4}; \quad z_1 = z$$

$$A_{h0\ell} = \begin{cases} 4 \cos 2\pi h x_2 \cos 2\pi \ell z_2, & h+\ell \text{ even} \\ -4 \sin 2\pi h x_2 \sin 2\pi \ell z_2, & h+\ell \text{ odd} \end{cases} \tag{54}$$

$$z_2 = z-\tfrac{1}{4}, \quad x_2 = x$$

$$A_{hk0} = \begin{cases} 4 \cos 2\pi h x_3 \cos 2\pi k y_3, & h+k \text{ even} \\ -4 \sin 2\pi h x_3 \sin 2\pi k y_3, & h+k \text{ odd} \end{cases}$$

$$x_3 = x-\tfrac{1}{4}, \quad y_3 = y$$

$$B_{0k\ell} = B_{h0\ell} = B_{hk0} = 0$$

The parameters that were assigned to the atoms of the trial structure are given in Table 17, and the structure factors that were calculated with these parameters are compared with the experimentally determined structure factors in Table 18.

Table 17

Approximate Parameters of Atoms

Atom	x	y	z
O_I	0.433	0.233	0.450
O_{II}	0.492	0.233	0.050
O_{III}	0.767	0.167	0.500
N	0.588	0.025	0.625
C_I	0.508	0.217	0.300
C_{II}	0.608	0.117	0.425
C_{III}	0.675	0.250	0.450
C_{IV}	0.658	0.367	0.675

Table 18

Corrected Intensities, and Observed and Calculated Structure Factors

hk0	Relative F^2_{hkl}			F_{obs}	F_{calc}
EVEN	1	2	3		
110	1500		1680	29	-24
310		1680	1750	30	14
220	310	335	290	13	23
510	400	385	430	15	-18
420	4940	4950	5070	52	-42
130	50		48	5	6
330	2190	1980	2430	34	31
620	2140	2320	2720	36	25
710		1660	1660	30	-24
530	6	< 4	< 9	2	10
240	1390	1130	1510	27	-37
440	87	107	80	7	2
820	< 4	< 7	< 11	< 2	-6
730	256	243	252	12	12
150	21		14	3	-6
200		1090	1080	24	-36
400		4030	4060	47	18
600		363	341	14	3
800		78	74	6	10
10.0.0		615	578	18	-10
12·0·0		631	680	19	28

Table 18 (continued)

hk0	Relative F^2_{hkl}			F_{obs}	F_{calc}
	1	2	3		
<u>ODD</u>					
210	690	630	750	19	15
120	3150		3420	42	-23
410	2990	3020	2970	40	48
320		2840	2970	39	24
230	570	530	590	17	26
520	740	680	660	19	18
610	320	370	340	14	-27
430	2400	2220	2740	36	-30
140	1100		1060	24	9
340	2260	1890	2350	35	-21
720	580	680	690	19	3
630	82	75	59	6	0
810		38	36	5	-14
540	418	426	434	15	8
250	27	30	29	4	-4

Table 18 (continued)

h0l	Relative F^2_{hkl}			F_{obs}	F_{calc}
	1	2	3		
EVEN					
101	2060	2090		33	-20
301		24	22	1	-30
202	7	<5	3	2	3
501	2760	2680	3010	39	-18
402	650	680	630	19	-16
701		210	194	10	-8
103	920	1000		23	20
602	162	180	169	10	16
303	<4	<8	<5	<2	0
002	3260	3320		42	-53
004	3			1	8
006	<2	<7		<1	-2

Table 18 (continued)

h0l	Relative F^2_{hkl}			F_{obs}	F_{calc}
	1	2	3		
ODD					
201	470	480	420	16	-26
401	8	9	6	2	8
102	570	680		18	-8
302	3	5	4	1	-17
601		1850	1940	32	21
502	1040	950	930	23	-7
203	680	790	700	20	-17
801			<11	<1.5	-14
702	73	77	67	6	4
403	1190	1200	1100	25	-5

Table 18 (continued)

0kl	Relative F^2_{hkl}			F_{obs}	F_{calc}
	1	2	3		
EVEN					
011	3910	3740	4430	46	-62
031	11	14	$\geq$ 8	2	2
022	3160	2610	3330	40	36
013	304	322	311	13	-4
042	80	98	91	7	14
033	18	$\leq$ 26	23	3	-5
020	5020		5360	53	74
040	371		324	14	-46
060	189		174	10	9
080	52		44	5	-17
ODD					
021	63	69	63	6	23
012	11	7	11	2	1
032	1070	1110	1000	24	-22
041	198	180	171	10	15
023	237	234	243	11	6

Notes to Table 18 :

(1) The values of relative F^2_{hkl} were obtained with the film factor 4.0. Under the headings 1, 2, and 3 are given the values obtained with crystals rotated around the axes $\underset{\sim}{a}_1$, $\underset{\sim}{a}_2$, and $\underset{\sim}{a}_3$ respectively.

(2) The values of F_{obs} were obtained from averages of the value of relative F^2_{hkl} but were scaled to an absolute basis.

The general agreement between observed and calculated structure factors is sufficiently good to indicate that this trial structure is a promising one which deserves an exhaustive examination.

Subsequent examination of these results with structure factor maps by Dr. Corey and Dr. Werner Nowacki has revealed that there is a promising degree of consistency among the shifts in atomic parameters that are indicated by the structure factors and the interatomic distances. A small shift in the carboxyl oxygen atoms that is not inconsistent with the Patterson distribution has materially reduced the disagreement in many of the planes. There is yet some uncertainty regarding the positions of C_{IV} and O_{III} and we feel that these atoms are responsible for much of the disagreement.

Preparations are now being made for more extensive work with structure factors, and for a Fourier projection to be made with the hk0 data with the signs established by structure-factor comparisons.

15. Conclusions

On the basis of the evidence provided by the Patterson distribution, structure factors, cleavage, and the plausible modes of packing and hydrogen bonding, we feel that the approximate structure shown in Figure 9 is with high probability fairly close to the correct structure, and that it can serve as the starting point for the precise evaluation of the parameters by the usual refinement procedures. We may state our conclusions more specifically as follows.

(a) It is hard to see how the conclusion regarding the relative orientation of the two oxygen atoms of the carboxyl group can be wrong.

(b) The proposed approximate parameters for the oxygen atoms are well supported not only by the agreement between the positions of expected and observed Harker and non-Harker peaks that we suppose to be due to these atoms, but also by the good agreement observed for their interactions with at least one other atom, and the fair agreement observed with additional atoms.

(c) The position of at least one atom in addition to the carboxyl oxygen atoms is probably established. As we have been able to devise a plausible structure only by identifying this atom as C_{III} in the same molecule, this identification appears very probable.

(d) Evidence from structure factors indicates that the general orientation of the molecule in space cannot be radically different from that proposed.

(e) The simultaneous satisfaction of the more obvious requirements of cleavage, hydrogen bonding, and packing that occurred when a structure conforming to all of the above restrictions was constructed is impressive and would seem to be very far from accidental.

(f) Comparisons of observed and calculated structure factors and of observed and calculated Patterson distributions showed promising systematic tendencies toward agreement and revealed no disagreement that appears impossible of resolution.

The work may be carried on from its present point with considerable confidence in the intensity data, which I believe are as good as can be expected with the use of the visual method, and probably as good as any that have been obtained by this method in this laboratory.

The present investigation has also shown that the complete three-dimensional representation of the Patterson function can be an extremely useful tool in the determination of crystal structures. We have also shown that the calculation of the function by punched card methods does not require an excessive amount of labor.

I would expect the three-dimensional Patterson method to be very useful in the determination of the structure of *dl*-serine.

There is some question as to whether the modification function that we used materially aided the interpretation of the Patterson function. The presence of a negative region associated with each peak has complicated the interpretation considerably. The integrated amplitude of a group of unresolved or partially resolved peaks is difficult to interpret in terms of the number of interatomic interactions involved. Preparations are now being made for a new Patterson calculation in which an artificial temperature factor may be used instead of the modification function described above.

16. Acknowledgements

I am very deeply indebted to Dr. Robert B. Corey for his generous and valuable contributions of advice, encouragement, and friendly cooperation. I am also very grateful to Professor Schomaker for his collaboration in the calculation and interpretation of the three-dimensional Patterson distribution, and for many valuable discussions. I believe that all crystal structure workers are indebted to him for suggesting the use of a three-dimensional Patterson distribution and for demonstrating the feasibility of calculating such a distribution by punched card methods.

I wish to thank Dr. Werner Nowacki for taking over the problem at its present stage.

I am grateful to Professor Carl Niemann and Mr. H. B. Milne for making available to us samples of threonine for this work, and to Professor J. H. Sturdivant for his cooperation and suggestions with regard to the use of the facilities of the x-ray laboratory and of the machine shops of the Department.

I wish to express my thanks for the large contributions made by Miss Lillian Casler in the preparation of gnomonic projections, in the calculation of Lorentz and polarization factors, in the preparation of structure factor maps, and in assisting with a large amount of other calculation work. I am indebted to Miss Jean Dewey for considerable assistance of this kind, particularly in the calculation of structure factors, to Miss Maryellen Kates for preparation of some of the figures and for other graphical work, and to Miss Virginia Berry for assistance with some of the punched card work. I also wish to thank Heinz G. Pfeiffer for assistance in the calibration of one of the cameras.

As also in connection with my work on other topics, I am grateful to Professor Linus Pauling for his valuable suggestions.

* * * *

The cost of this work was supported in part by a grant made by the National Foundation for Infantile Paralysis to the California Institute of Technology. I also wish to acknowledge the financial aid given to me by the National Research Council, of which I have been a Predoctoral Fellow since March 1946.

References

1. S. B. Schryver and H. W. Buston, *Proc. Roy. Soc. London.* B99, 476 (1925-26).

2. R. H. McCoy, C. E. Meyer, and W. C. Rose, *J. Biol. Chem.* 112, 283 (1935-36).

3. C. E. Meyer and W. C. Rose, *J. Biol. Chem.* 115, 723 (1936).

4. H. D. West and H. E. Carter, *J. Biol. Chem.* 119, 103, (1937).

5. H. D. West and H. E. Carter, *J. Biol. Chem.* 119, 109, (1937).

6. H. D. West and H. E. Carter, *J. Biol. Chem.* 122, 611, (1937-38).

7. H. B. Milne, unpublished work.

8. E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides". ACS Monograph No. 90. New York: Reinhold Publishing Corp., 1943. pp 358, 368.

9. Erwin Brand, *Ann. New York Acad. Sci.* 47 (Art.2), 187 (1946).

10. G. Albrecht, G. W. Schnakenberg, M. S. Dunn, and J. D. McCullough, *J. Phys. Chem.* 47, 24 (1943).

11. C. L. A. Schmidt, "The Chemistry of Amino Acids and Proteins", Second Edition. Springfield, Ill.: Charles Thomas, 1944. p. 1177.

12. Internationale Tabellen zur Bestimmung von Kristallstructuren. Berlin: Gebrüder Borntraeger, 1935.
    a. Vol.II, p. 584
    b. Vol. I, p. 120

13. J. J. deLange, J. M. Robertson, and I. Woodward, *Proc. Roy. Soc.* (London), A171, 398 (1939).

14. W. H. Zachariasen, "Theory of X-Ray Diffraction in Crystals". New York: John Wiley and Sons, 1945. p. 108.

15. G. Tunell, *Am. Mineralogist* 24, 448 (1939).

16. Herman E. Seemann, private communication.

17. W. H. Zachariasen, *op. cit.*, p. 110

18. G. Albrecht and R. B. Corey, *J. Am. Chem. Soc.* 61, 1087 (1939).

References (continued)

19. A. L. Patterson, Z. Krist. 90, 517 (1935).

20. David Harker, J. Chem. Phys. 4, 381 (1936).

21. P. A. Schaffer, Jr., Verner Schomaker, and Linus Pauling, J. Chem. Phys. 14, 648, 659 (1946).

22. D. M. Crowfoot and B. W. Rogers - Low, a Monograph on penicillin, to be published.

23. V. Schomaker, unpublished investigation.

24. G. A. Campbell and R. M. Foster, "Fourier Integrals for Practical Applications". Bell Telephone System Monograph B-584, 1931. p. 39. See also E. C. Titchmarsh, "Introduction to the Theory of Fourier Integrals", Oxford, 1937, p. 51. (Note that the form of the theorem given by these authors is different from that given in the text of this thesis; the form given here is obtained by a change of variables.)

25. Jurg Waser, Doctoral Thesis, California Institute of Technology, 1944, p. 70; Jurg Waser and Verner Schomaker, unpublished work.

26. L. L. van Reijen, Physica 9, 461 (1942).

27. H. A. Levy and R. B. Corey, J. Am. Chem. Soc. 63, 2095 (1941).

SUMMARY

An attempt has been made to prepare antibodies to histamine *in vitro* by partially denaturing serum globulin in the presence of histamine. Controlled tests with ileic strips showed, however, that this attempt was unsuccessful.

A study was made of conditions suitable for the growth of large crystals of aluminum orthophosphate. Crystals up to 8 mm. in length were prepared and the method that was tested appeared to be promising for the growth of larger crystals, but the work was abandoned in favor of war work of greater urgency.

The space group and lattice constants of crystals of *dl*-serine were redetermined. The results of this study confirmed those of previous investigators.

A more comprehensive x-ray study of ℓ-threonine was undertaken. The space group and lattice constants were determined and intensity data were obtained. An analysis of the intensity data by means of a three-dimensional representation of the Patterson function led to an approximate crystal structure for ℓ-threonine. Observed and calculated structure factors for this structure show promising general agreement. Work toward the refinement of this structure is in progress.

APPENDIX

THREE-DIMENSIONAL REPRESENTATION OF PATTERSON FUNCTION FOR *l*-THREONINE

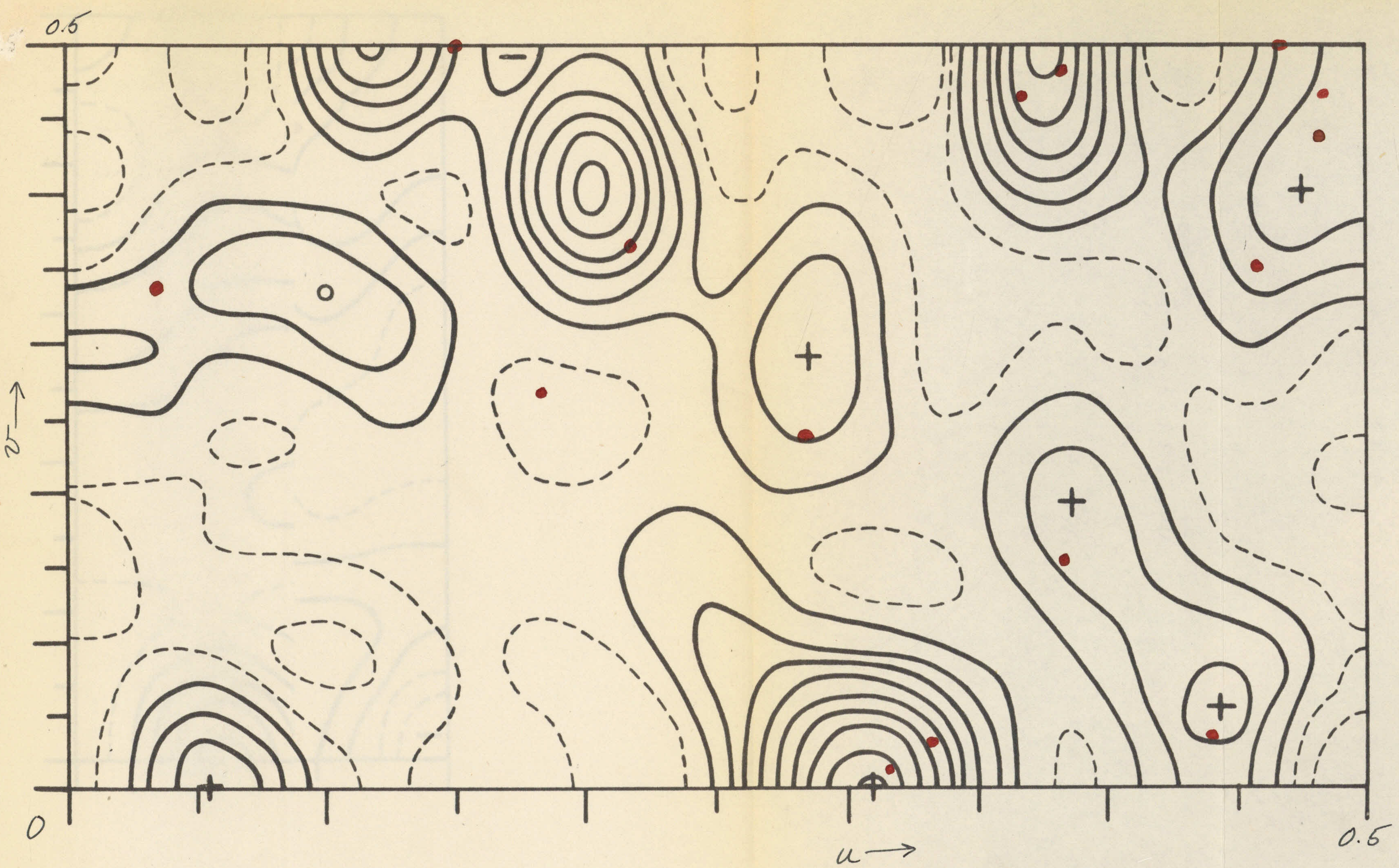
On the following pages are given eleven sections of the three-dimensional Patterson distribution for *l*-threonine. These are given at intervals of 0.05 in the w-parameter and cover the range w = 0.0 to w = 0.5 inclusive. On each section the origin of u and v coordinates is taken in the lower left hand corner. The coordinate u runs to the right from 0.0 to 0.5, and the coordinate v runs upward from 0.0 to 0.5. The eleven sections constitute an octant of the Patterson cell. The other octants are equivalent to this one by mirror planes at the surfaces, two-fold axes at the edges, and centers of symmetry at the corners of the octant. If a parameter is given a negative value in the text, its sign may be changed; if its value is greater than 0.5, it may be subtracted from 1.0.

Also given are the sections u = 0, u = 0.5, v = 0, and v = 0.5.

The unit of amplitude is that which resulted from arbitrary scaling of the coefficients to magnitudes convenient for the punched card work. In terms of this unit the contour interval is 50. Zero and positive contours are indicated by solid lines, negative contours are indicated by dashed lines. Minus signs are placed in some shallow negative regions in order to avoid confusion.

Expected positions of Patterson peaks for the probable approximate structure are plotted in red. Red dots indicate that the w parameters of the peaks are equal to those of the respective sections; red circles indicate that they are greater by about 0.025 (i. e. half way to the next section).

All of these sections are plotted to a scale of four centimeters per Ångström unit.



Harker section

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

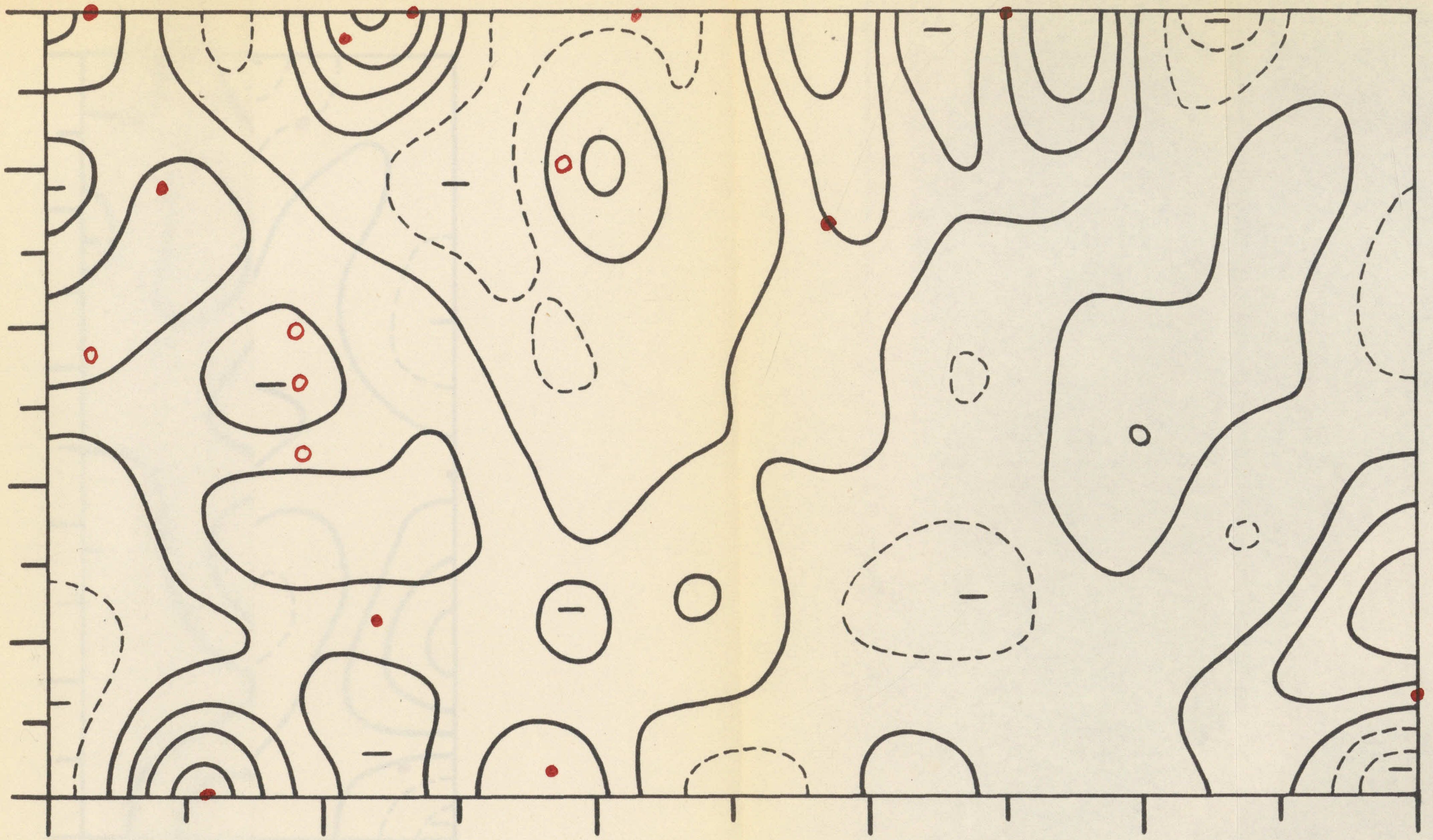
w= 0.50

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

$w = 0.45$

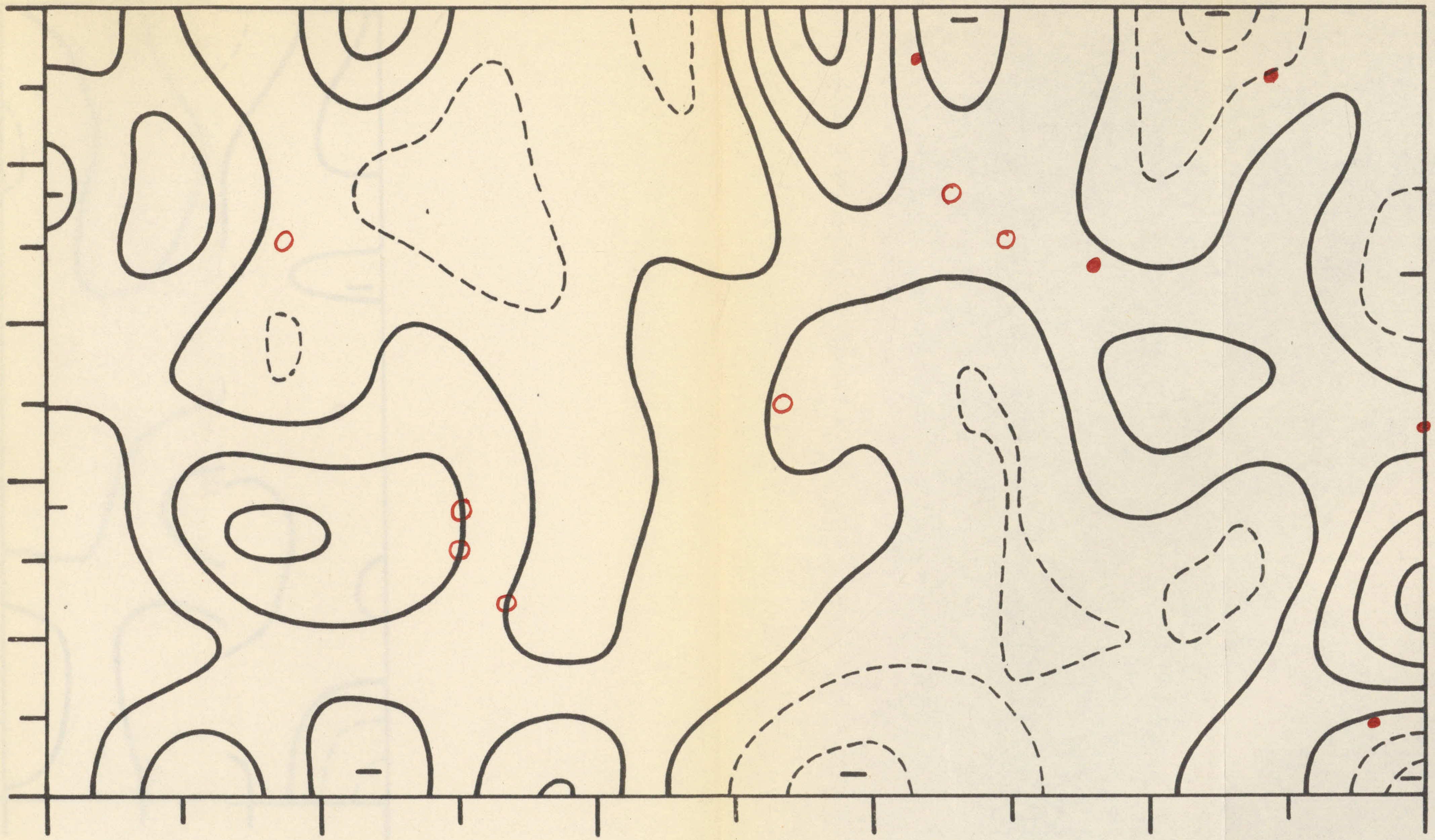


LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

w = 0.40



LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

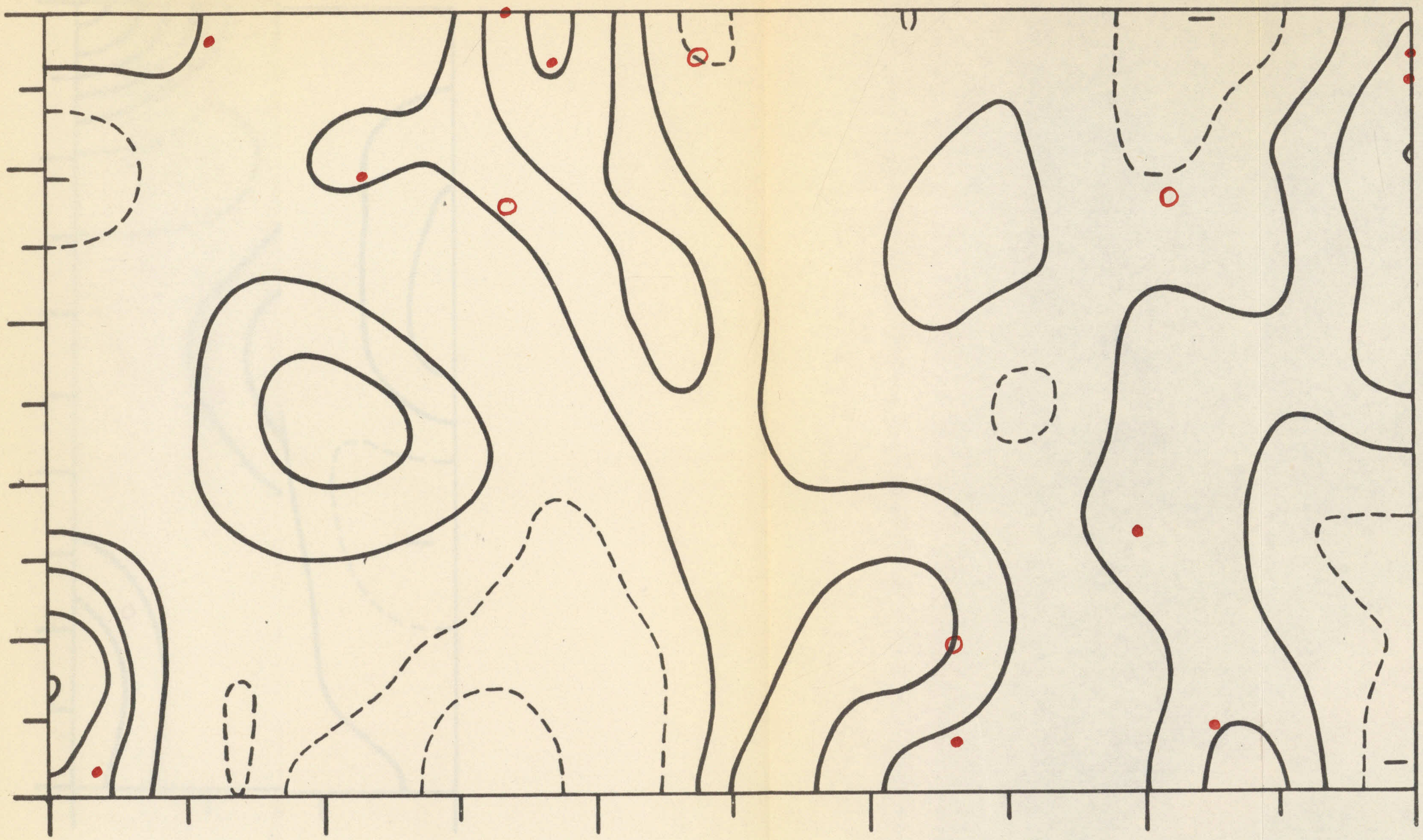
$\omega = 0.35$

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH	REF.	FILE

w = 0.30



LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY — DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

$w = 0.25$

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.
APP.
JOB
CH.
REF.
FILE

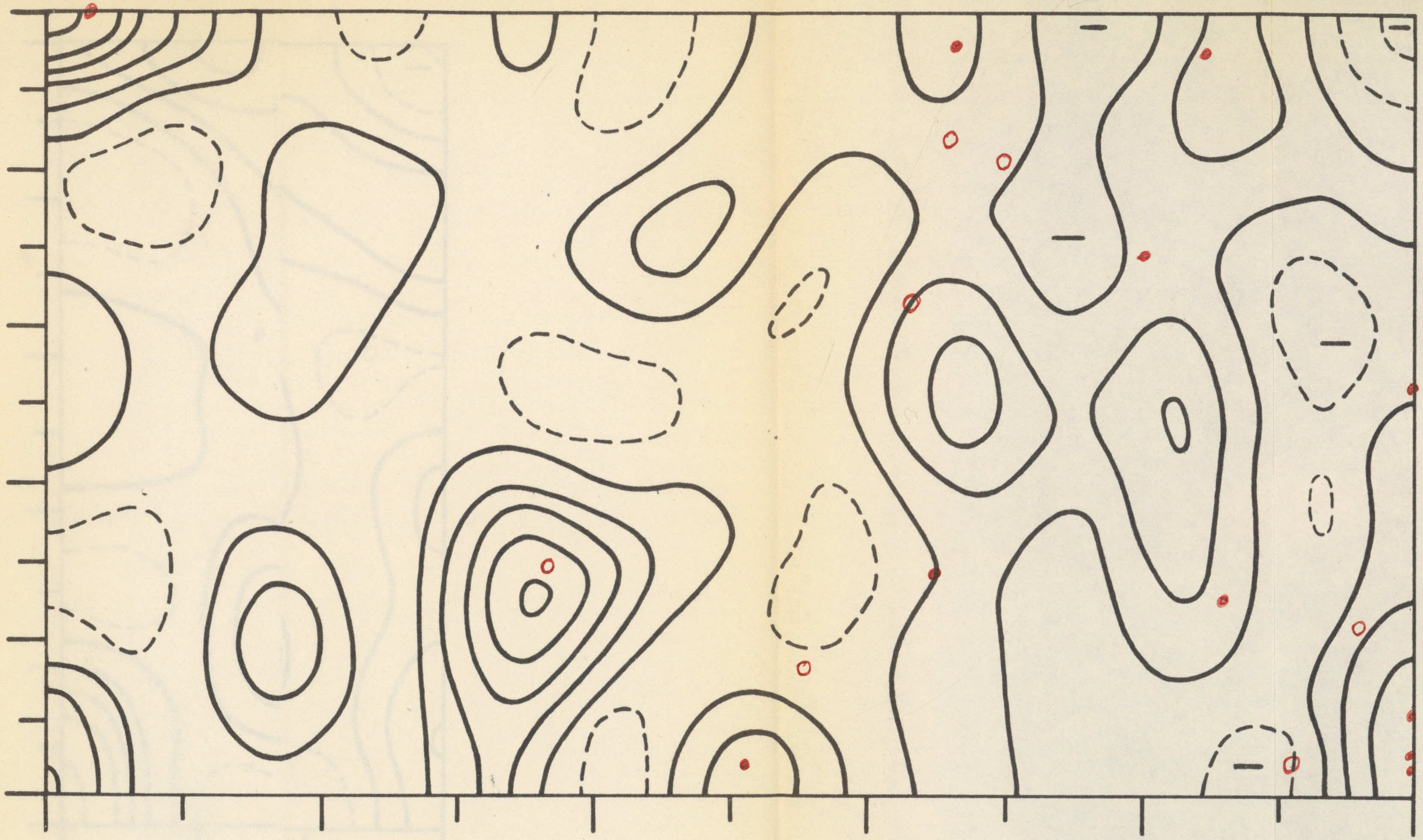
$w = 0.20$

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY — DEPARTMENT CH ISTRY

DR.	APP.	JOB
CH.	REF.	FILE

ω = 0.15



LTR.	CHANGE	DATE	BY	CH.

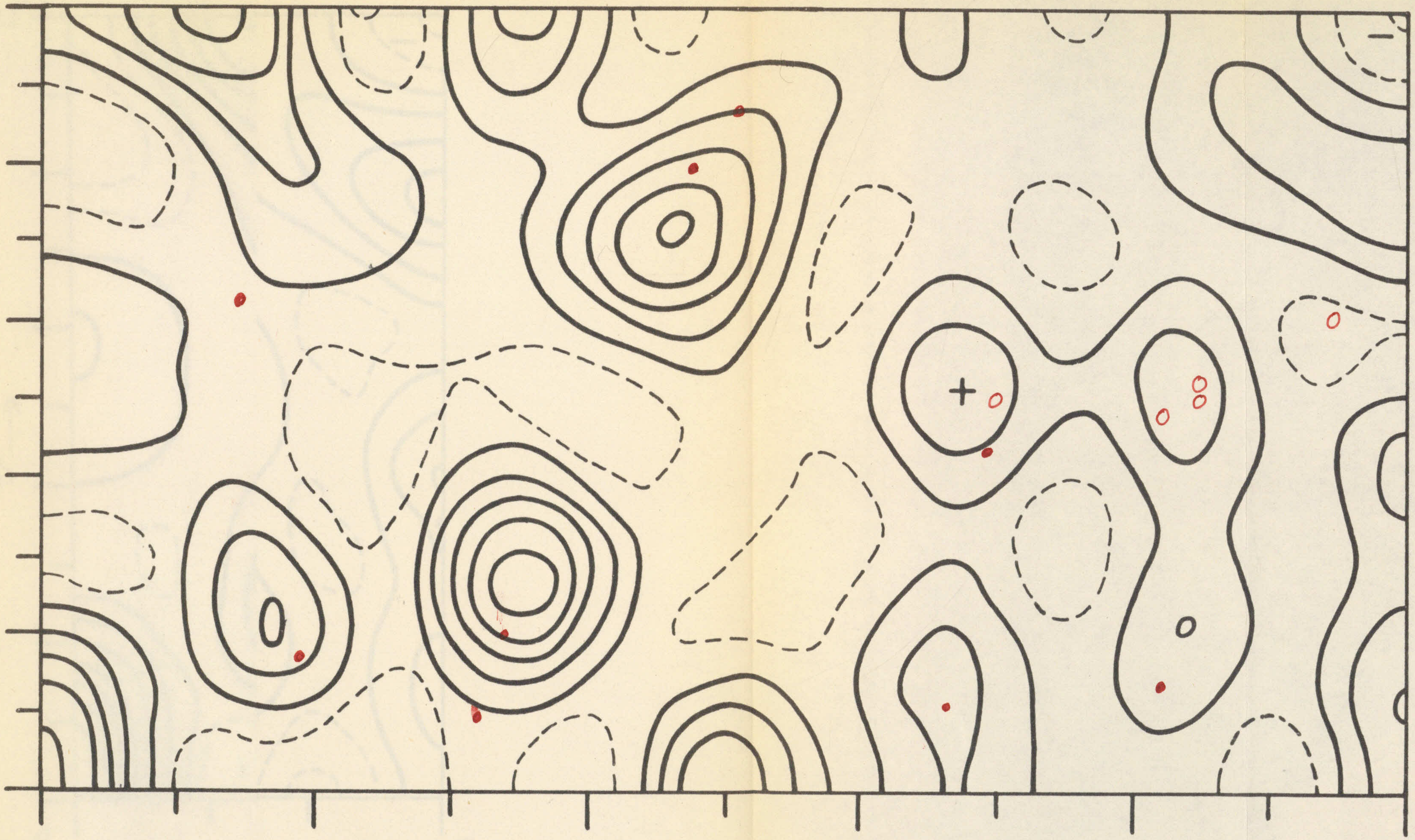
CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB	
CH	REF.	FILE	

$w = 0.10$

CLEARPRINT

FORM CL-8

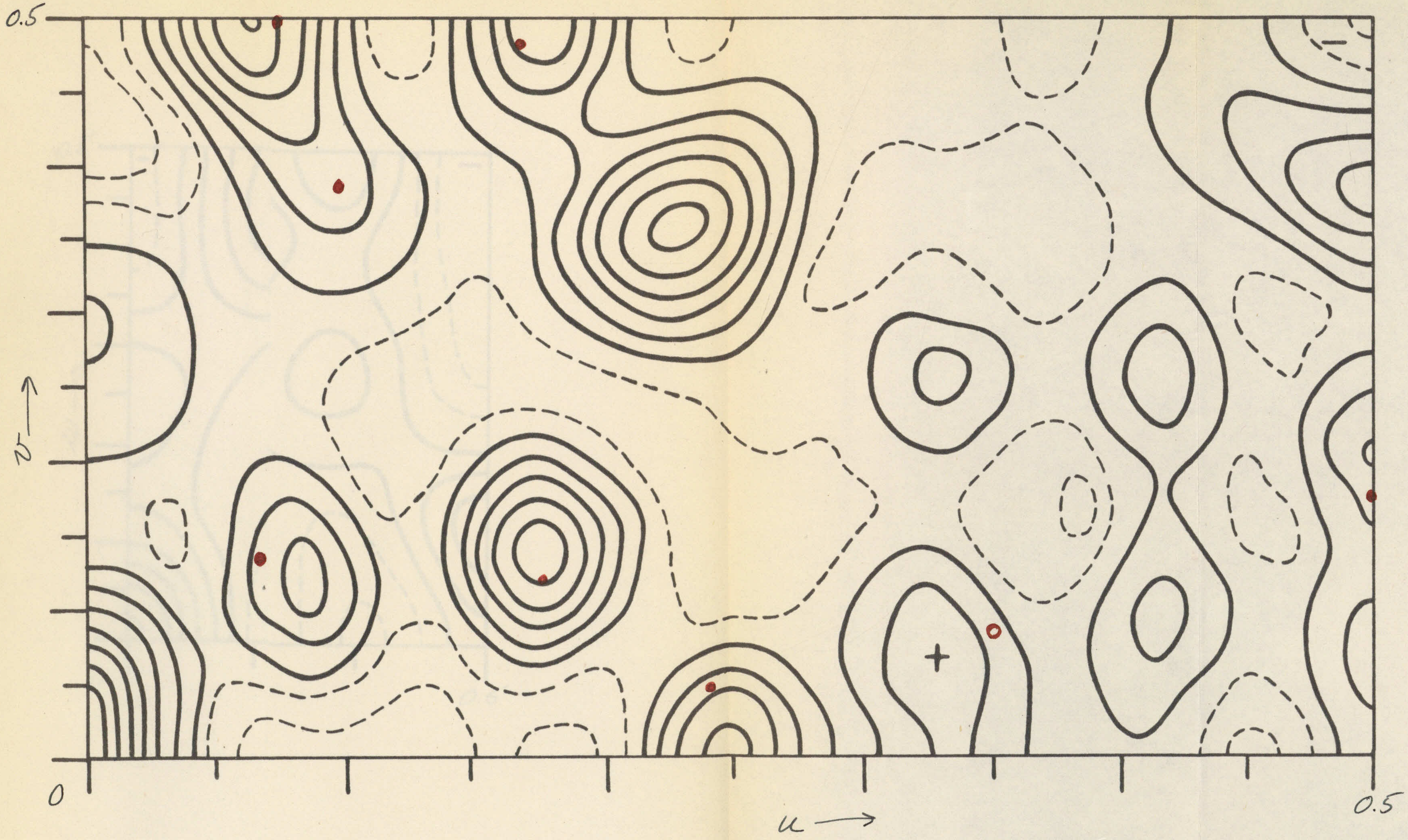


LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH	REF.	FILE

$\omega = 0.05$

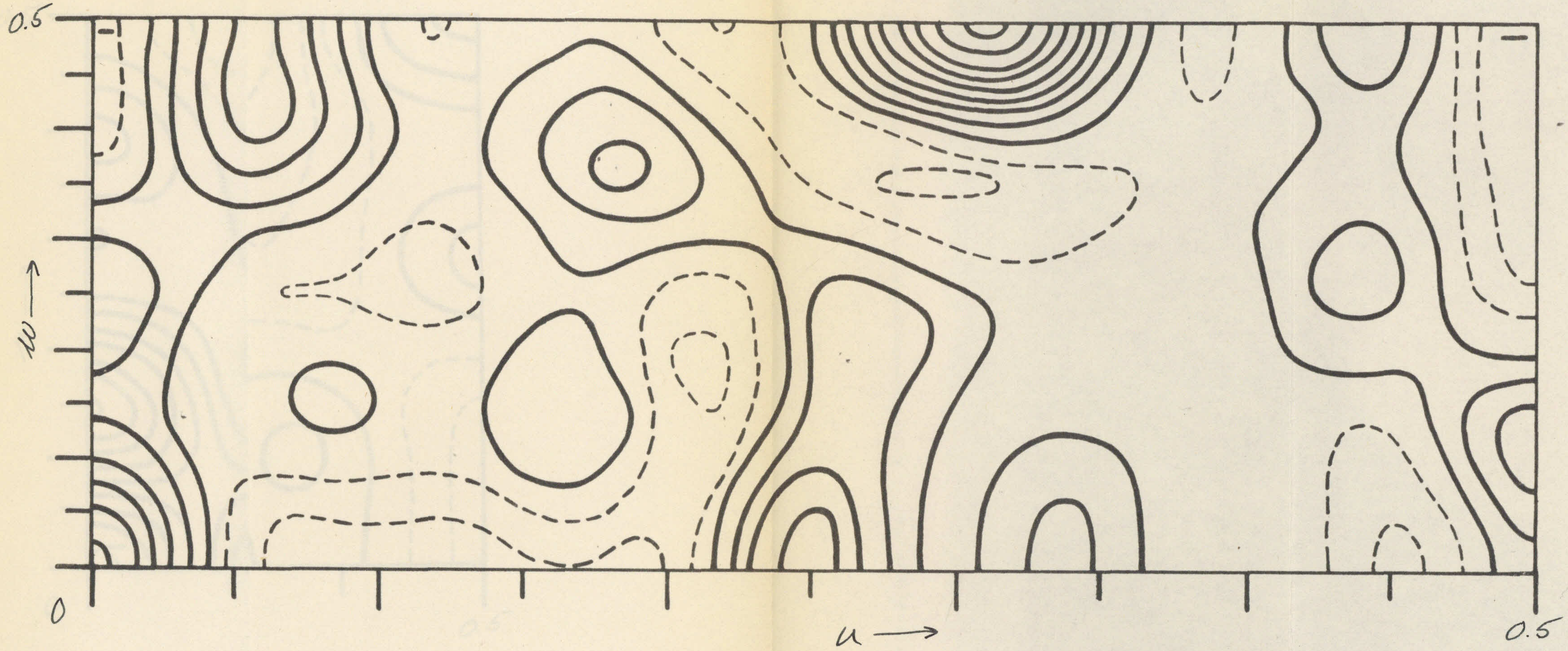


LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY — DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

w = 0.00

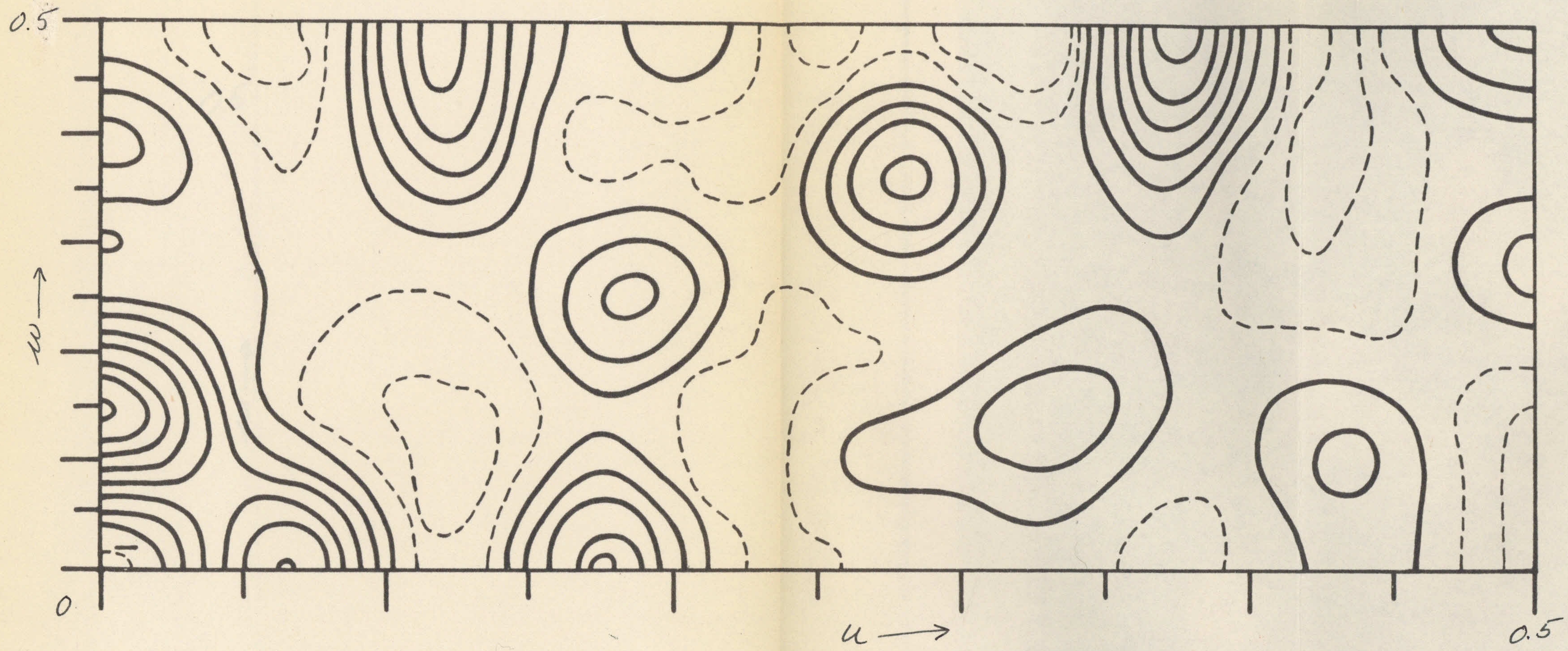


LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

$v = 0.00$

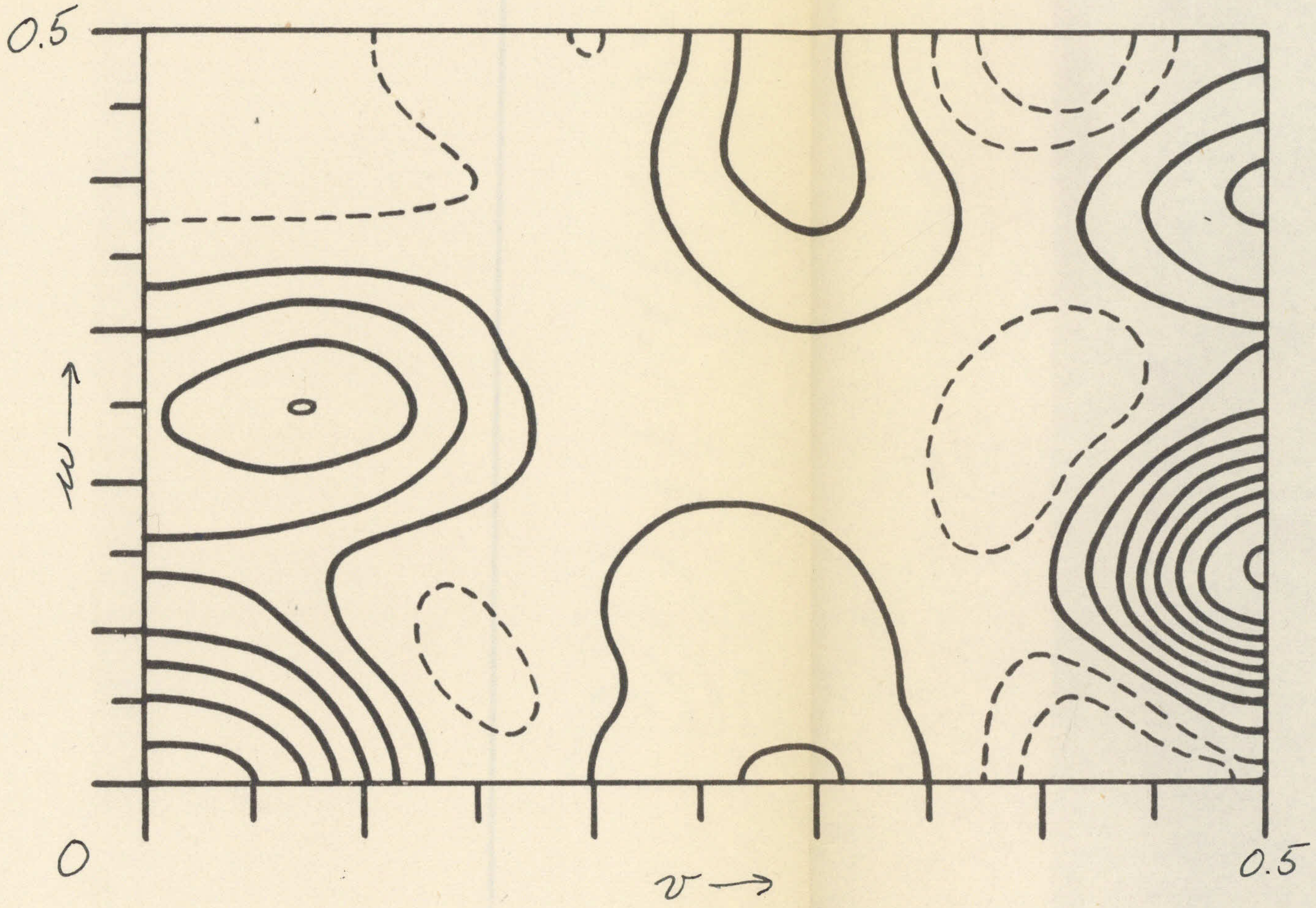


Harker section

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY — DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB	
CH.	REF.	FILE	
	v= 0.50		



LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY — DEPARTMENT OF CHEMISTRY

DR.	APP.	JOB
CH.	REF.	FILE

u = 0.00

Harker section

LTR.	CHANGE	DATE	BY	CH.

CALIFORNIA INSTITUTE OF TECHNOLOGY—DEPARTMENT OF CHEMISTRY

DR.
APP.
JOB
CH.
REF.
FILE

$u = 0.50$

PROPOSITIONS

1. (a) In the assignment of space groups to crystals prepared from racemic mixtures of enantiomorphic compounds, care should be exercised in rejecting space groups applicable to enantiomorphic structures, as the separation of isomers on crystallization from a solution of the racemic mixture is not a rare occurrence.

(b) Any indication that an enantiomorphic ~~compound~~ mixture crystallizes to a structure containing mirror planes should be looked upon with considerable suspicion unless additional molecular or ionic species are present.

(c) The erroneous assignment of the space group C_{2v}^1 - Pmm to threonine by Albrecht and coworkers[1] is an example of non-recognition of these principles.

(d) The validity of the space groups reported by Albrecht and coworkers[1] for dl-methionine (C_2^1 - P2, Z = 4) and dl-valine (C_2^2 - P2_1, Z = 4) should be checked by examination of crystals prepared from the individual isomers.

2. It has been proposed[2] that the critical tension (tensile strength) of a liquid is determined by the size of the smallest cavity in which vapor can exist in equilibrium with the liquid, and that such a cavity has a diameter equal to the average distance between molecules of the vapor at the vapor pressure of the liquid. Although this hypothesis appears to conform to a (rather uncertain) experimental observation[3] that the critical tension rises with the temperature to a maximum and then decreases, the assumptions involved in the hypothesis are not necessarily justified.

Even if dust particles or other nuclei are absent and if absorption of high energy radiation is excluded as a cause of rupture, the conditions for the incipient rupture of a liquid must be treated by statistical methods without undue regard for the equilibrium concepts of surface tension and vapor pressure.

3. (a) A striking feature of the phenomenon of superconductivity is its apparent absence in the Group VIII and nearby transition elements and in the rare earth elements.[4] On the basis of the known effects of external magnetic fields on superconductors and theoretical limitations on internal magnetic fields I propose that perturbations due to the resultant magnetic moments of unfilled non-bonding atomic orbitals[5] may inhibit superconductivity in these elements.

(b) The method of valence-bond wave functions should be applied to the investigation of the electronic structures of metals.

4. (a) In the visual method of estimating the intensities of x-ray reflections with multiple-film sets, a convenient scale for intensity interpolation is a logarithmic one, with the film factor taken as the base of logarithms.

(b) A routine procedure for measuring the film factor should be developed and applied to the testing of all x-ray film used in intensity work.

5. A practical method for placing observed structure factors on the correct absolute scale is proposed. In this method, the structure factors

are so scaled that they satisfy the equation

$$\sum_{h=-\infty}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l=-\infty}^{\infty} F_{hkl}^{2} = \sum_{i=1}^{N} 4\pi v_a \int_0^{\infty} f_i'^{2}(H)\, H^2\, dH,$$

where

$$H = \frac{2\sin\vartheta}{\lambda}.$$

The summation on the right is taken over all of the atoms in the unit cell. The primed form factor contains an approximate temperature factor, which is assumed to be isotropic. The treatment may be modified to take account of anisotropy in the temperature factor if necessary.

6. (a) In the estimation of viscosities of concentrated solutions of nitrocelluloses and other high polymers in volatile solvents with a capillary-type viscometer, errors resulting from evaporation of solvent may be practically eliminated by use of a viscometer which can be attached to the container in which the solution is prepared. A viscometer which can be used in this way has been described.[6]

(b) In the argentometric titration of chloride with fluorescein or eosin as indicator, the silver chloride suspension may be stabilized near the end point by the addition of a small amount of a suitable wetting agent, such as Aerosol OT.

7. In the treatment of quantum mechanical problems by the perturbation theory[7], it is sometimes found that the first-order treatment fails to remove the degeneracy of a level; it has been recognized[8] that caution should be exercised in proceeding with the second-order treatment in this case. If by the first-order treatment zero-order wave functions

can be found for which all non-diagonal matrix elements of the perturbation vanish, I propose that a second-order secular equation

$$\det \| H'_{mn} - \delta_{mn} W''_{n} \| = 0 ,$$

where

$$H''_{mn} = \sum_{\alpha}{}' \frac{H'_{m\alpha} H'_{\alpha n}}{W^{0}_{n} - W^{0}_{\alpha}} ,$$

be used to obtain the energy corrections to second order.

8. Martin and Synge[9] have proposed a theory of chromatography for linear absorption isotherms which takes into account the effects of diffusion and non-attainment of equilibrium by a treatment analogous to the treatment of fractionating columns with the concept of theoretical plates. This treatment may be generalized to non-linear isotherms and arbitrary initial adsorbate distribution by a method similar to that of DeVault[10] to obtain the differential equation

$$\left(\frac{\partial c}{\partial x}\right)_{v} + \frac{h}{2}\left(\frac{\partial^{2} c}{\partial x^{2}}\right)_{v} + \left\{\alpha + m f'(c)\right\}\left(\frac{\partial c}{\partial v}\right)_{x} = 0$$

in which *h* is analogous to the HETP of a fractionating column and other symbols are those used by DeVault.

9. (a) The three-dimensional representation of the Patterson function shows great promise of being a very useful tool in the determination of crystal structures of moderate difficulty.

(b) The inner symmetry of the Patterson distribution should be systematized at least for the more commonly encountered space groups.

(c) I propose that the three-dimensional Patterson function be applied to the determination of the crystal structure of *dl*-serine at the earliest opportunity.

10. (a) A paramagnetic oxygen analyzer which has been recently developed[11] depends for its operation on the transport of heat from a wire by magnetic convection in a non-homogeneous magnetic field. It should be pointed out that the response of this analyzer probably depends appreciably on factors other than the paramagnetic susceptibility of the gas mixture, among the most important of which are the viscosity and the heat capacity of the mixture. This analyzer should not be expected to have the general applicability of the Pauling Oxygen Meter.

(b) A modification of the Pauling Oxygen Meter in which the test body is brought to a null position by rotating the fork on which the fiber supporting the test body is mounted should be tested. An instrument constructed in this way should give a strictly linear response and should be capable of high precision. It may be an especially appropriate instrument for determining the magnetic susceptibilities of diamagnetic gases.

11. I propose that the method used by Stokes[12] for the preparation of a composite lens for the fly's eye camera can be considerably simplified. A possible simplification would be to make a direct impression of a sheet of plastic against an array of ball bearings, fill the depressions in the plastic sheet with a liquid of higher index of refraction, and cover the composite of liquid lenses with a glass plate. A hexagonal array of ball bearings would be easier to obtain than a suitable square array and may give a composite lens almost as useful.

References

1. G. Albrecht, G. W. Schnakenberg, M. S. Dunn, and J. D. McCullough, *J. Phys. Chem.* *47*, 24 (1943).

2. Allen King, *Phys. Rev.* *66*, 38 (1944).

3. D. P. Shoemaker, Thesis, Reed College, 1942. p. 39.

4. E. F. Burton, H. G. Smith, and J. O. Wilhelm, "Phenomena at the Temperature of Liquid Helium". New York: Reinhold Publishing Corp., 1940. p.92.

5. Linus Pauling, *Phys. Rev.* *54*, 899 (1938)

6. D. P. Shoemaker, Earl Hoerger, R. M. Noyes, and R. H. Blaker, *Anal. Chem.* *19*, 131 (1947).

7. Linus Pauling and E. B. Wilson, Jr., "Introduction to Quantum Mechanics". New York: McGraw-Hill, 1935. Chapter VI.

8. Jurg Waser, Doctoral Thesis, California Institute of Technology, 1944. p. 106.

9. A. J. P. Martin and R. L. M. Synge, *Biochem. J.* *35*, 1358 (1941).

10. Don DeVault, *J. Am. Chem. Soc.* *65*, 533 (1943).

11. F. Klauer, E. Turowski, and T. v. Wolff, *Angew. Chem.* 54, 494 (1941).

12. A. R. Stokes, *Proc. Phys. Soc.* *58*, 306 (1946).