

The Crystal and Molecular Structure of Acetyl Choline Bromide

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The crystal and molecular structure of acetyl choline bromide has been determined by the aid of X-ray methods. The crystals are monoclinic with $a = 11.10 \text{ \AA}$, $b = 13.67 \text{ \AA}$, $c = 7.18 \text{ \AA}$ and $\beta = 110^\circ$. The space group is $P2_1$ and there are four molecules per unit cell. It is shown that the acetyl choline ion is present in the structure in two distinct structural forms, one of which is the previously assumed extended form, the other being a sort of "ring" structure, where the choline radical, including the oxygen, forms a roughly planar "ring" with a distance from the ester oxygen to the methyl group in the "ring" plane of $\sim 3 \text{ \AA}$. Shortened intermolecular distances of $\sim 3 \text{ \AA}$ link the molecules together to form double strings of molecules running in the direction of the crystallographic a -axis. Atomic coordinates, interatomic distances and bond angles are given. It is suggested that the ability of acetyl choline to exist in two different structural forms may be of primary importance for its behaviour at lipoid-water interfaces, possibly also for its specific action in the transfer of nerve impulses.

Choline, $(\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{N}(\text{CH}_3)_3)^+\text{OH}^-$, is the parent compound of a series of physiologically active derivatives, widely distributed in plants and animals. The most important one of these derivatives may be acetyl choline, to which is ascribed the formula $(\text{CH}_3\cdot\text{CO}\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{N}(\text{CH}_3)_3)^+\text{OH}^-$, and which is known to play a specific role in the cholinergic nerve system, besides other physiological activities.

The choline derivatives or "cholines" form, along with the acid and dipolar derivatives named "betaines", an interesting type of compounds characterized by the tetra-substituted ammonium group. Accurate determinations of the structures of such compounds are rare in the literature. X-Ray investigations on the crystal structures of the iodides and bromides of palmityl and stearyl cholines have been reported by Stora¹, but accurate atomic positions were not derived in these works. Jelinek² has recently reported a structure determination of muscarine iodide, a substance which is related to acetyl choline bromide.

The understanding of the specific actions of acetyl choline is apparently closely connected with a detailed knowledge of the structure of the acetyl choline ion. The present structure determination was therefore undertaken to provide as complete a description of the acetyl choline ion and of the molecular packing as possible, and it is intended to carry out structure determinations for several characteristic compounds of related type.

EXPERIMENTAL

Crystallographic data of acetyl choline bromide have been given in a preliminary account of this work³. Repeating here the values of the lattice constants, correcting a misprint in a and choosing the obtuse angle we obtain: $a = 11.10 \text{ \AA}$, $b = 13.67 \text{ \AA}$, $c = 7.18 \text{ \AA}$, $\beta = 110.0^\circ$. There are four molecules per unit cell. The space group is $P2_1$.

Relative intensities of the $hk0$ -reflexions were obtained by visual estimation from multiple film Weissenberg photographs, taken with $\text{CuK}\alpha$ -radiation. As the specimen used was very small and of fairly spherical form, no correction has been applied for absorption. After corrections for Lorentz and polarization factors the intensities were brought to an approximately absolute scale by the method of Wilson⁴. The scale factor thus found was later on by comparison with calculated intensities found to be correct within 4%. Intensities of the reflexions on the equatorial layer lines with rotation around the $[010]$ - and the $[100]$ -axis, respectively, and on the first seven layer lines around the $[010]$ -axis were recorded in the same way. These intensities were transferred directly to an absolute scale by comparison with calculated intensities.

The X-ray photographs show subsidiary reflexions³, indicating some sort of long range modulation of the structure amplitude of the pseudo-cell. It has, however, been assumed that the structure of the pseudo-cell, representing an average structure, could be derived from the intensities of the main reflexions, disregarding those of the subsidiary reflexions. This "anomaly" has greatly complicated the derivation of the structure, and also impeded an ultimate refinement of the atomic coordinates. On the other hand, it may well be that the actual structure found in this work may reveal characteristic and important features of the acetyl choline ion, which might have been lost if a "normal" crystal, showing no subsidiary X-ray reflexions, had been obtained by crystallization under different conditions. In the continued work on this problem it is intended to investigate if this is possible.

The extrapolation graph for determination of the scale factor indicates rather strong thermal vibrations, the derived constant in the temperature factor being about 5 \AA^2 . This temperature factor has not been used in preparing the data for the Fourier syntheses, but has been introduced in comparing observed and calculated structure factors and amplitudes. Atomic scattering factors are taken from *Internationale Tabellen* 1935 and from Viervoll and Ögrim⁵.

DETERMINATION OF THE STRUCTURE PROJECTED ON (001)

A Patterson projection on (001) is shown in Fig. 1. There is only one high and sharp maximum with coordinates u , $b/2$ and this maximum must therefore correspond to interaction between bromine atoms connected by the screw axis. Since there are four molecules in the unit cell, and the space group has twofold general positions, there must be two independent molecules in the asymmetric unit. The only maximum which may be assumed to correspond to interactions between independent bromine atoms is the apparent double maximum around the position $a/2, b/4$ in the Patterson projection. These

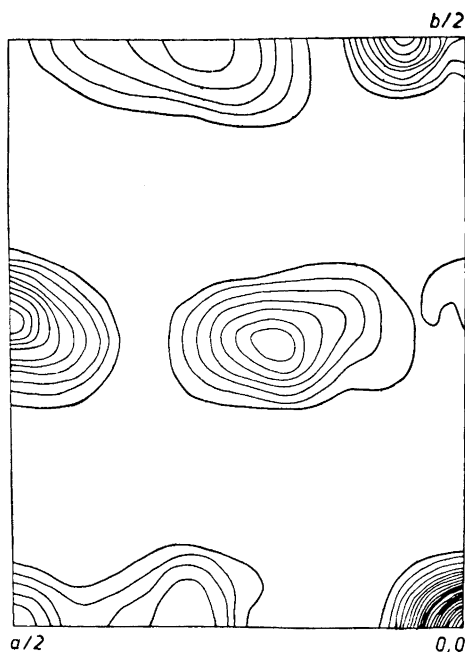


Fig. 1. Asymmetric part of the Patterson projection on (001) of acetyl choline bromide.

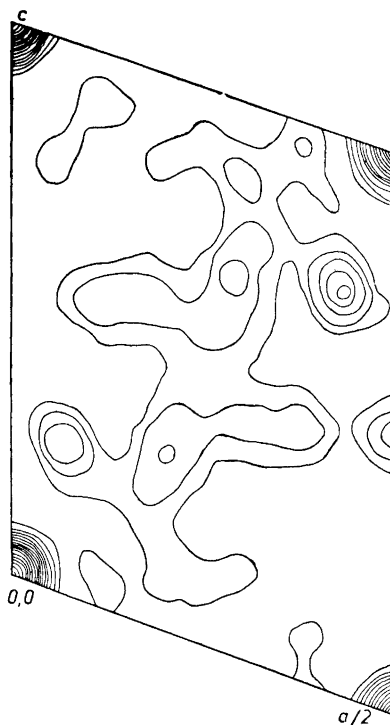


Fig. 2. Patterson projection on (010) of acetyl choline bromide.

maxima indicate the following approximate coordinates for the bromine atoms: $x_1 = \pm 0.036$, $y_1 = 0$ and $x_2 = 0.5 \pm 0.036$, $y_2 = 0.25 \pm 0.015$. The number of possible sign combinations is reduced by the choice of origin, and calculation of some structure amplitudes enabled a choice between the remaining possibilities; thus, the coordinates $x_1 = 0.036$, $y_1 = 0$ and $x_2 = 0.536$, $y_2 = 0.265$ were derived for the independent bromine atoms.

Derivation of coordinates of the light atoms from the Patterson projection would be rather fortuitous, but the projection clearly suggests that the molecules are arranged in layers parallel to (010) and interspaced approximately $b/4$. The square of the bromine scattering factor is about 1.6 times the sum of the squares of the scattering factors of the lighter atoms, and it might thus be expected that the knowledge of the bromine positions would lead to a recognizable structure of the acetyl choline ion in a Fourier synthesis calculated on this basis, although in this case the heavy atom may have a somewhat too strong influence. Only the (010)-projection, however, is centrosymmetric, and in this projection the overlapping of four molecules would be a serious obstacle for a straight-forward derivation of the structure.

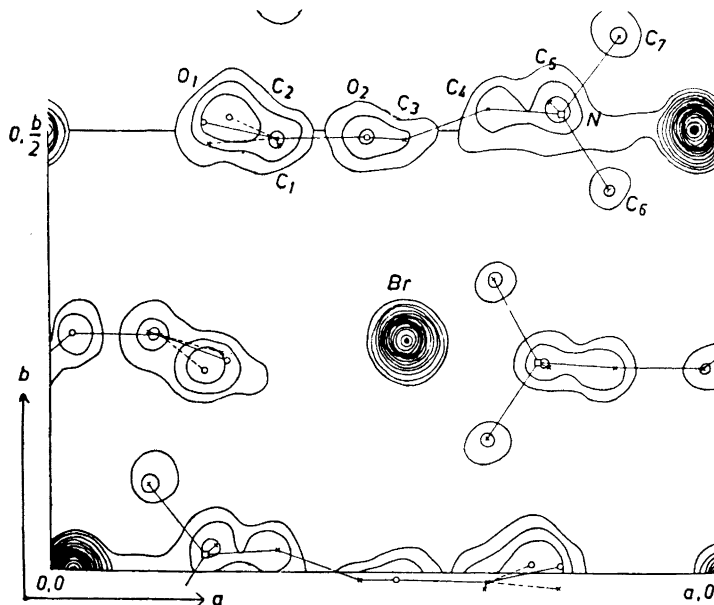


Fig. 3. Projection of electron density on (001) of acetyl choline bromide. Contour lines are drawn at equal but arbitrary intervals. Full circles, open circles, squares and crosses indicate bromine, oxygen, nitrogen and carbon atoms, respectively. Dashed lines indicate an alternative orientation of the acetyl groups.

The proposed layer structure and spatial considerations suggest that the independent acetyl choline ions were to be placed between bromine atoms in the direction of the a -axis, and having their long directions running in the same direction. Tentative coordinates of the lighter atoms, except hydrogen, were derived in this way and were included in the calculation of the first complete set of structure amplitudes and phases of the $hk0$ -reflexions, leading to a value of R of 0.32, which is only slightly lower than the value of R (0.35) when the bromine atoms only are included in the calculations. Two ambiguities had to be reckoned with in the derivation of the tentative coordinates, *first*, the direction of the molecules with respect to the positive a -axis direction, and *secondly*, whether independent molecules should run in the same or in opposite direction.

The first Fourier projection gave a fairly clear picture of the structure, but maxima corresponding to the two methyl groups, lying outside the approximate plane of the rest of the molecule, indicated that the directions of both molecules should be reversed. This feature is noteworthy as it indicates that the influence of the bromine atoms is strong enough to lead to a correct structure, even if some of the lighter atoms were included with wrong positions. The coordinates derived from the first Fourier projection led to a marked drop in the value of R , from 0.32 to 0.23, and the second Fourier projection gave a

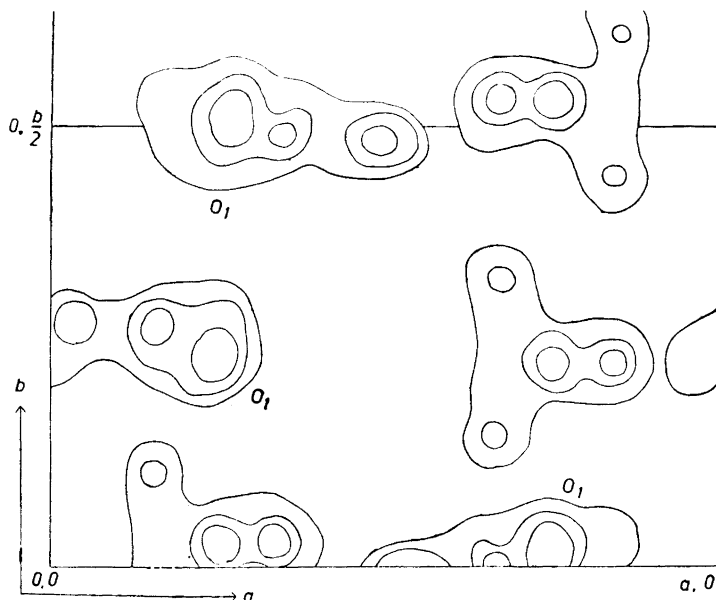


Fig. 4. Projection of electron density on (001) of acetyl choline bromide after subtraction of the bromine peaks. Contour lines at arbitrary intervals. The maxima corresponding to the O_1 -positions are broadened considerably in the direction of the b -axis.

substantially better picture of the molecular structure. In solving this projection the independent acetyl choline ions were, in the first stage, assumed to have approximately similar structures. This assumption had later on to be abandoned, though the corrections for this projection proved to be rather small (see next section). A map of electron density after subtraction of the bromine peaks has been calculated in order to correct for diffraction effects of the heavy bromine atoms. Four cycles of Fourier synthesis — structure factor calculation were carried out, and the resulting values of R are given in Table 1. Non-observed reflexions were included in evaluating R as far as the calculated structure amplitude exceeds the minimum value observed.

The projection of electron density on (001) is shown in Fig. 3, and the same projection, after subtraction of the bromine peaks, is shown in Fig. 4. Calculated structure amplitudes are compared with the observed ones in Table 4.

DETERMINATION OF THE STRUCTURE PROJECTED ON (010)

The projection of electron density on (001) in Fig. 3 shows that very pronounced overlapping of atoms of the individual molecules must be expected in a projection on (100), and overlapping of four different molecules in a projection on (010). The latter one, has, however, the advantage of being centrosymmetrical, and offers, furthermore, the possibility of improving the resolu-

tion by sectional syntheses along the b -axis, such that the projections of single molecules could be obtained. The projection on (010) was therefore chosen for the next stage in deriving the structure. The strong overlapping of the molecules in this projection impedes taking the full advantage of the heavy atom method. On the other hand the sectional syntheses involve too many tedious calculations to be used as a method of refinement. This projection was therefore, in the first stage, solved from the Patterson projection on (010) together with trial and error methods, using structure factor graphs, refined in the next stage as far as possible by the method of least squares, and finally three different sectional syntheses were computed as a check on the proposed structure. The Patterson projection on (010) is shown in Fig. 2. Since the x -coordinates of the bromine atoms were known from the (010)-projection, the maxima corresponding to Br-Br interactions were easily located, indicating z -coordinates of approximately 0.14 for both of the independent bromine atoms. The maximum at $1/2, 0, 0$ is only slightly lower than the origin peak as (010) is an *approximate* glide plane with translation $a/2$. The rest of the Patterson projection shows strongly overlapping maxima, and could not give any detailed informations as to the position of the acetyl choline ion. In the first attempts to derive the structure in this projection an extended model of the molecule was assumed, in accordance with the generally accepted point of view, the direction of the molecules being approximately parallel to the [101]-direction (see next section). This assumption could in effect lead to a reasonable packing of the molecules. Calculation of structure factors revealed, however, very serious contradictions for some low order reflexions. By turning the part of the molecule containing the acetyl group about 180° around the C_4-C_3 -bond, such that $C_5 \cdot N \cdot C_4 \cdot C_3 \cdot O_2$ form an approximately planar ring with a distance of about 3 Å from C_5 to O_2 , reasonably good agreement for all $h0l$ -reflexions could be obtained. A difficulty in deriving the structure lies in the small but significant difference between the two independent molecules, which are to a rough approximation related by a glide-plane of symmetry. The intensities of, *e.g.* the $h0l$ -reflexions with h odd (the values of h deviate slightly from odd integral values³⁾, show that such differences exist, but do not give very much clue to the real nature of these differences.

THE SECTIONAL SYNTHESSES

The projection of the structure on (001) clearly shows that a projected section between the limits $-b/8$ and $b/8$ could be expected to yield the structure of one of the molecules without serious overlapping. Similarly, a section between the limits $b/8$ and $3b/8$ would give the structure of the other molecule. Because of the screw axis, however, a calculation of, *e.g.*, the first of these sections, would also include the section between the limits $3b/8$ to $5b/8$, whereas the latter would also comprise the section between $5b/8$ to $7b/8$. This makes, however, no difference as the first molecule in either case is placed at such a distance from the screw axis that it does not overlap with the equivalent molecule in the projected section, which comes out as centrosymmetrical. In order to reduce the overlapping of the methyl groups

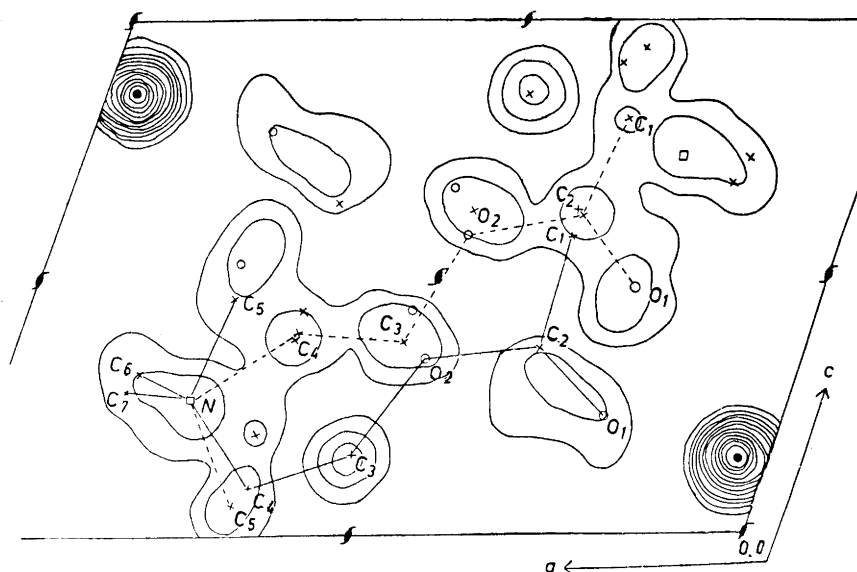


Fig. 5. Projected section of electron density on (010) between the limits $-b/16$ to $+b/16$ with the equivalent section between the limits $7b/16$ to $9b/16$. The calculation of this section is based exclusively on the bromine positions. Filled circles, open circles, squares and crosses indicate bromine, oxygen, nitrogen and carbon atoms respectively. An acetyl choline ion, lying roughly in the plane at $y = 0$, is indicated by dashed lines for the extended form, and with full lines for the "ring" form. The maxima of both forms will appear in this projection (see text). The maxima corresponding to C_3 -positions are too high, probably because of bromine atoms lying just above in the adjacent section. Atomic positions for the equivalent section are shown, but are not connected by lines.

a section was also calculated for the limits $-b/16$ to $b/16$ with its equivalent section form $7b/16$ to $9b/16$. The reflexions on the equatorial layer line and on the seven lowest layer lines around the [010]-axis were used for these sectional syntheses, comprising about 1 200 reflexions.

These sections, which appeared as very similar except for a translation of $a/2$ of the second section, showed maxima as expected for the assumed structure, but in addition there appeared some maxima which could not be very well accounted for. Some improvement could be obtained by assuming two possible orientations of the acetyl groups. More serious difficulties were encountered in calculation of interatomic distances between atoms of adjacent molecules, showing that a methyl group of one molecule would approach the acetyl group of another molecule to a distance of about 2.5 Å, which seems to be unreasonably short. To check the proposed structure once more, the section between the limits $-b/16$ to $b/16$ with the equivalent section from $7b/16$ to $9b/16$ was recalculated with amplitudes based exclusively on the bromine positions. This new section is closely similar to the section previously calculated, but it might be noticed that the heights of the additional maxima were slightly increased when using Br-positions only. This similarity is not surprising,

considering the strong influence of the bromine atoms, for example, the bromine atoms determine the signs of all the $h0l$ -reflexions (with h even) except for one extremely weak term.

It may be seen from this section, which is reproduced in Fig. 5, that the presence of two different structures of the acetyl choline ion, from consideration of peak heights both being of the same probability, would be necessary to account satisfactorily for the maxima in this section, *i.e.* the "ring" form, as previously assumed, and an extended form having its long direction approximately parallel to the crystallographic [101]-direction. The arguments for assuming such a mixed structure may be summarized as follows:

1. $h0l$ -reflexions with h close to an odd integral value are present in the X-ray photographs, but these reflexions are rather weak. This shows that the actual structure deviates from an "ideal" structure with a glide-plane parallel to (010) and translation $a/2$, the observed intensities of these reflexions being a measure of this deviation. $F(h0l)$'s with h odd, calculated on the assumption of "ring" form only, or extended form only, show in both cases calculated values which, on the average, are only about one tenth of the observed average value. If a structure with the independent molecules being of the "ring" form and the extended form, respectively, is assumed, calculated $F(h0l)$'s with h odd are of the same magnitude (or somewhat higher) as the observed ones. Some agreement between observed and calculated values is also obtained, the R -factor being 0.42 for these weak reflexions, which is not too bad, considering that the bromine atoms contribute practically nothing to these reflexions, and that their intensities may be more or less influenced by the apparent long range modulation of the structure.

2. As mentioned above the maxima of both forms show up in a section along the [010]-axis based exclusively on the bromine positions. Both forms are likely to appear in this section, since weak reflexions such as the $h0l$ -reflexions with h close to an odd integral value, and which would differentiate between the two forms, were not included in the calculation of the section.

3. The agreement between observed and calculated structure factors and amplitudes is considerably improved by assuming the presence of the two forms, both for an ordered structure of the two forms and for a disordered structure of the same forms.

4. All the interatomic distances lie within reasonable limits for a structure consisting of the two forms of the acetyl choline ion, whereas some unreasonably short distances are found in the case of "ring" form only or extended form only.

The coordinates of the atoms for an ordered structure of the two forms of the acetyl choline ion are given in Table 2, and interatomic distances within each ion may be found in Fig. 6 together with the bond angles. Observed and calculated structure factors of the $h0l$ -reflexions are compared in Table 3. The reliability index R is surveyed in Table 1.

Table 1. Survey of the values of the reliability index R for different sets of reflexions and for different assumed structures of acetyl choline bromide. The structure with the acetyl choline ion of the "ring" form is designated by I, and the structure with the extended form of the acetyl choline ion is designated by II. The value of R , when the Br atoms only are included in the calculations, is included for comparison.

| | I "ring" form | II extended form | I + II disordered | I + II ordered | Br only |
|-------|------------------|---------------------|----------------------|-------------------|------------|
| $h0l$ | 0.187 | 0.212 | 0.146 | 0.135 | 0.353 |
| $hk0$ | 0.169 | 0.173 | 0.151 | 0.163 | 0.344 |
| hkl | 0.223 | 0.204 | 0.182 | | 0.303 |

DESCRIPTION AND DISCUSSION OF THE STRUCTURE

i) *The acetyl choline ion.* A drawing of the acetyl choline ion, as resulting from the present determination, is shown in Figs. 6 *a* and 6 *b* for the "ring" form and the extended form, respectively. The atoms of the ion lie in both cases approximately in a plane, except for those of the methyl groups C_6 and C_7 . In contradiction to the previously accepted point of view of an extended form only, the acetyl choline ion is consequently able to exist also in a sort of "ring" structure with the atoms C_5 , N, C_4 , C_3 and O_2 lying approximately in a roughly planar ring, the distance between C_5 and O_2 being close to 3 Å. It is then likely that both of these forms exist in solutions in polar solvents. While preparing this article a paper appeared on the structure of the related molecule of muscarine iodide², for which a closely similar "ring" structure was found. The deviation from a tetrahedral arrangement around the nitrogen

Table 2. Atomic coordinates of the two independent molecules of acetyl choline bromide, assuming that the first molecule corresponds to the extended form and the second molecule corresponds to the "ring" form, *i. e.* for an ordered structure.

| First molecule | | | | Second molecule | | | |
|-----------------|--------|--------|-------|-----------------|-------|-------|-------|
| Atom | x/a | y/b | z/c | Atom | x/a | y/b | z/c |
| Br ₁ | 0.0367 | 0 | 0.142 | Br ₂ | 0.535 | 0.264 | 0.142 |
| N | 0.250 | 0.014 | 0.731 | N | 0.733 | 0.239 | 0.742 |
| O ₁ | 0.750 | 0.008 | 0.533 | O ₁ | 0.269 | 0.283 | 0.792 |
| O ₂ | 0.517 | -0.008 | 0.417 | O ₂ | 0.025 | 0.283 | 0.667 |
| C ₁ | 0.672 | -0.017 | 0.194 | C ₁ | 0.150 | 0.261 | 0.431 |
| C ₂ | 0.653 | -0.017 | 0.394 | C ₂ | 0.164 | 0.294 | 0.644 |
| C ₃ | 0.478 | -0.014 | 0.611 | C ₃ | 0.978 | 0.244 | 0.842 |
| C ₄ | 0.342 | 0.025 | 0.611 | C ₄ | 0.856 | 0.228 | 0.906 |
| C ₅ | 0.339 | 0.017 | 0.939 | C ₅ | 0.744 | 0.222 | 0.536 |
| C ₆ | 0.167 | -0.081 | 0.700 | C ₆ | 0.658 | 0.153 | 0.739 |
| C ₇ | 0.150 | 0.089 | 0.733 | C ₇ | 0.667 | 0.333 | 0.722 |

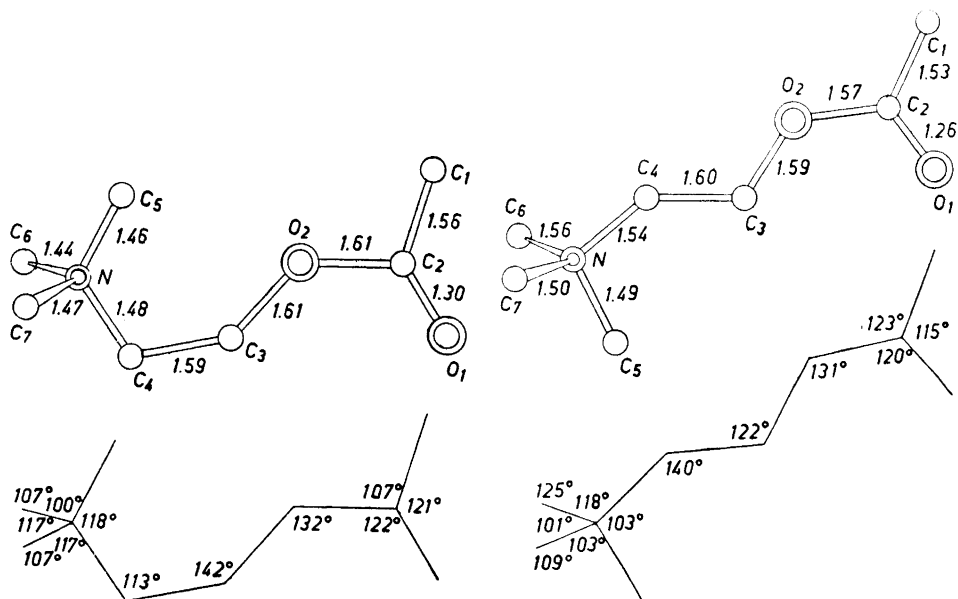


Fig. 6. a. Schematic drawings of the "ring" form of the acetyl choline ion showing interatomic distances and bond angles. Interatomic distances are given in Å.

b. Schematic drawings of the extended form of the acetyl choline ion with interatomic distances in Å and bond angles.

atom may be slightly more pronounced in the structure of the acetyl choline bromide than in muscarine iodide, and this may possibly be a consequence of a tighter packing due to the smaller size of the bromine atom. The distance in muscarine iodide corresponding to the C₅-O₂ distance in acetyl choline is reported by Jellinek to be 2.87 Å, a value close to that quoted above. The interatomic distances and bond angles within the "onium" group and the first bond in the chain show normal values within the limits of accuracy, though the values of the bond angles are spread from about 100° to about 120°. The shortest distances between non-bonded atoms belonging to the same molecule lie in the range 2.3—2.5 Å.

The bond lengths from the O₂-atoms are considerably longer than usually found for C—O single bonds, thus C₃-O₂ is 1.59 Å and 1.61 Å, C₂-O₂ 1.57 Å and 1.61 Å for the extended form and the "ring" form, respectively. The anomaly in these bond lengths may to some extent be due to inaccuracies in the derivation of the atomic coordinates, but it seems rather unlikely that the anomaly should be solely due to inaccuracies. The observed bond lengths from the ester oxygens would therefore suggest that these bonds were in some way affected by the neighbouring charged groups and ions. The bond angles also show some anomalous values, thus in the extended form the angle C₃C₄N is 140° and the angle C₃O₂C₂ is 131°, in the "ring" form the angle C₄C₃O₂ is

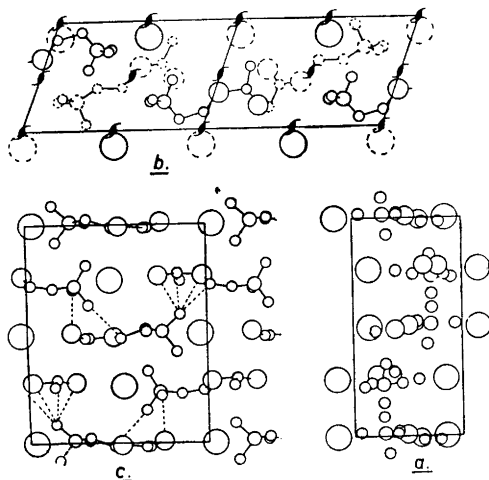


Fig. 7. The packing of the molecules of acetyl choline bromide as viewed along the a -axis direction (a), the b -axis direction (b) and along the c -axis direction (c). Bromine, oxygen, nitrogen and carbon atoms are represented by circles, the radius of which decreases from bromine to carbon, and in b dashed circles are used to indicate atoms of a molecule lying close $y = 0$ or $y = 1/2$ (in the unit cell on the right side) whereas circles in full lines indicate the atoms of molecules close to the plane through $y = 1/4$ or $y = 3/4$ (in the top left corner). Bonded atoms are connected by full lines. Some relatively short interatomic distances between neighbouring molecules, the lengths of which are about 3 Å, are indicated by dashed lines in c . These shortened interatomic distances link the molecules together to form endless double strings running in the direction of the a -axis.

142° and angle $C_3O_2C_2$ is 132°. It is likely that the high values of these bond angles originate from the same reasons as the large values of the bond lengths. It may be noted that bond angles of approximately the same magnitude have been found in other molecules showing Zwitter-ion character, *e.g.*, in cysteyl-glycine⁶. The structures of the acetyl groups are normal within the limits of accuracy.

ii) *The packing of the molecules.* The packing of the molecules in the crystal lattice is indicated in Figs. 7 a , b and c as viewed in the direction of the a -, b - and the c -axis, respectively. The Br_1 -ion has 9 nearest neighbours within a distance below 4 Å, these are two O_2' -atoms at distances 3.36 and 3.43, two C_3' -atoms at 3.51 and 3.92, one C_4' -atom at 3.96, one C_7' -atom at 3.85, one C_4 -atom at 3.91, one C_6 -atom at 3.94 and one C_7 -atom at 3.76, a prime denoting that the atom belongs to an adjacent molecule. Two N-atoms are at distances 4.11 and 4.37 and two N'-atoms at distances 4.30 and 4.88. There are five more nearest neighbours in the range from 4 to 4.5 Å, thus C_1 , C_5 , C_6 , C_7 and O_1' .

The Br_2 -ion has eight nearest neighbours within a distance of 4 Å, thus C_3' at 3.54, C_4' at 4.00, C_5' at 3.83, C_6' at 3.76, O_1 at 3.22, C_7 at 3.88, O_6 at 3.89 and C_5 at a distance of 3.05. Two N-atoms are found at a distance of 4.17 and 4.14, and two more N'-atoms at distances of 4.08 and 4.89. The

Table 3. Observed and calculated structure factors $F(h0l)$ for acetyl choline bromide, the "ring" form being designated by I and the extended form by II. I + II is the ordered structure of the two forms.

| hkl | $F(hkl)$ obs. | $F(hkl)$ calculated | | | hkl | $F(hkl)$ obs. | $F(hkl)$ calculated | | |
|---------------|------------------|---------------------|------|--------|-------|------------------|---------------------|-----|--------|
| | | I | II | I + II | | | I | II | I + II |
| 001 | 22 | 45 | 3 | 25 | 1004 | 22 | 3 | -7 | 16 |
| 002 | 81 | -88 | -36 | -61 | 1204 | 48 | 57 | 47 | 46 |
| 003 | 74 | -89 | -86 | -89 | 204 | 82 | -73 | -77 | -75 |
| 004 | 84 | -84 | -87 | -84 | 404 | 24 | -16 | -18 | -16 |
| 005 | 13 | -31 | -7 | -19 | 604 | - | 6 | 7 | -2 |
| 006 | 23 | 36 | 9 | 27 | 804 | 64 | 63 | 63 | 68 |
| 007 | 85 | 79 | 77 | 80 | 205 | 62 | -59 | -44 | -46 |
| 008 | 52 | 47 | 51 | 46 | 405 | 75 | -77 | -72 | -77 |
| 201 | 124 | 113 | 133 | 134 | 605 | 96 | -99 | -51 | -78 |
| 401 | 108 | 106 | 75 | 96 | 805 | 63 | -67 | -83 | -66 |
| 601 | 96 | 85 | 99 | 91 | 1005 | 54 | -37 | -28 | -45 |
| 801 | 48 | 30 | 40 | 36 | 1205 | - | -12 | 1 | -5 |
| 1001 | 30 | 36 | 28 | 32 | 205 | 36 | 13 | 47 | 34 |
| 1201 | 19 | -34 | -20 | -24 | 405 | 46 | 54 | 40 | 41 |
| 20 $\bar{1}$ | 44 | 64 | 12 | 57 | 605 | 90 | 59 | 83 | 78 |
| 40 $\bar{1}$ | 55 | -43 | -61 | -65 | 805 | 68 | 58 | 68 | 57 |
| 60 $\bar{1}$ | 51 | -64 | -74 | -52 | 206 | 71 | 48 | 47 | 47 |
| 80 $\bar{1}$ | 96 | -63 | -108 | -94 | 406 | 62 | -18 | -57 | -32 |
| 100 $\bar{1}$ | 55 | -73 | -66 | -62 | 606 | 34 | -40 | -71 | -54 |
| 120 $\bar{1}$ | 74 | -74 | -73 | -72 | 806 | 51 | -84 | -46 | -68 |
| 202 | 85 | 88 | 47 | 83 | 1006 | 63 | -50 | -75 | -59 |
| 402 | 54 | 45 | 81 | 74 | 1206 | 57 | -73 | -75 | -70 |
| 602 | 87 | 104 | 73 | 81 | 206 | 51 | 91 | 28 | 60 |
| 802 | 99 | 104 | 83 | 103 | 406 | 98 | 97 | 89 | 94 |
| 1002 | 72 | 89 | 60 | 68 | 606 | 68 | 57 | 46 | 51 |
| 1202 | 36 | 36 | 27 | 35 | 207 | 44 | 63 | 68 | 57 |
| 20 $\bar{2}$ | 43 | -82 | -43 | -79 | 407 | 37 | 34 | 47 | 46 |
| 40 $\bar{2}$ | 75 | -107 | -59 | -71 | 607 | - | 19 | 3 | 7 |
| 60 $\bar{2}$ | 110 | -108 | -88 | -108 | 807 | 25 | -30 | -21 | -19 |
| 80 $\bar{2}$ | 73 | -93 | -49 | -68 | 1007 | 60 | -53 | -55 | -59 |
| 100 $\bar{2}$ | 44 | -51 | -20 | -40 | 1207 | 43 | -56 | -62 | -55 |
| 203 | - | -16 | -12 | -14 | 207 | 65 | 65 | 71 | 62 |
| 403 | 69 | -57 | -41 | -59 | 407 | 53 | 39 | 58 | 53 |
| 603 | 67 | 41 | 51 | 50 | 208 | 60 | 53 | 57 | 61 |
| 803 | 76 | 72 | 98 | 78 | 408 | 84 | 80 | 82 | 78 |
| 1003 | 60 | 68 | 68 | 71 | 608 | 58 | 65 | 63 | 66 |
| 1203 | 65 | 61 | 71 | 67 | 808 | 39 | 50 | 51 | 44 |
| 20 $\bar{3}$ | 121 | -118 | -131 | -123 | 2010 | 30 | -25 | -30 | -24 |
| 40 $\bar{3}$ | 88 | -80 | -86 | -84 | 4010 | 44 | -43 | -16 | -29 |
| 60 $\bar{3}$ | 31 | -18 | -34 | -30 | 200 | 24 | 58 | 64 | 62 |
| 803 | 55 | -50 | -56 | -54 | 400 | 98 | 102 | 95 | 99 |
| 204 | 153 | -165 | -156 | -159 | 600 | 48 | 46 | 71 | 52 |
| 404 | 48 | -60 | -72 | -71 | 800 | 9 | 8 | 1 | 7 |
| 604 | 96 | -104 | -95 | -93 | 1000 | 93 | -92 | -91 | -90 |
| 804 | 25 | -25 | -24 | -38 | 1200 | 34 | -35 | -14 | -26 |

Table 4. Observed and calculated structure amplitudes and phases for the hkl -reflexions of acetyl choline bromide. $1/2(I + II)$ is the average of structure amplitudes calculated for the "ring" form (I) and the extended form (II). Both structure amplitudes and phases are given for the ordered structure of the two forms of acetyl choline bromide.

| hkl | $F(hkl)$ obs. | $F(hkl)$ calc. | | $a(hkl)$ | hkl | $F(hkl)$ obs. | $F(hkl)$ calc. | | $a(hkl)$ |
|-------|------------------|---------------------------|-----------|----------|-------|------------------|---------------------------|-----------|----------|
| | | $\frac{1}{2}$ (I + II) | ord. str. | | | | $\frac{1}{2}$ (I + II) | ord. str. | |
| 020 | 15 | 5 | 19 | -74.0 | 110 | 24 | 31 | 27 | 39.2 |
| 040 | 209 | 235 | 234 | 10.9 | 130 | 17 | 26 | 36 | 127.2 |
| 060 | 31 | 27 | 48 | -60.5 | 150 | 24 | 13 | 22 | 256.5 |
| 080 | 143 | 150 | 139 | 19.1 | 170 | 11 | 14 | 29 | 138.3 |
| 0100 | — | 24 | 45 | -39.8 | 190 | 14 | 8 | 11 | -56.6 |
| 0120 | 59 | 97 | 89 | 21.1 | 1110 | 29 | 31 | 40 | 144.8 |
| 0140 | 35 | 43 | 49 | -32.0 | 1130 | 18 | 10 | 10 | 150.8 |
| 0160 | 32 | 39 | 37 | 41.5 | 1150 | 9 | 8 | 12 | 175.1 |
| 120 | 66 | 61 | 63 | 7.2 | 210 | 62 | 55 | 62 | 141.8 |
| 140 | 19 | 17 | 9 | 212.4 | 230 | 63 | 62 | 60 | 49.1 |
| 160 | 57 | 57 | 57 | 23.6 | 250 | 15 | 9 | 7 | 256.1 |
| 180 | 26 | 25 | 20 | 267.2 | 270 | 66 | 58 | 50 | 58.6 |
| 1100 | 71 | 67 | 72 | 27.8 | 290 | 8 | 8 | 5 | 145.1 |
| 1120 | 19 | 24 | 26 | -63.2 | 2110 | 39 | 56 | 50 | 60.4 |
| 1140 | 60 | 58 | 63 | 42.2 | 2130 | — | 4 | 7 | 70.8 |
| 1160 | 33 | 35 | 40 | -51.4 | 2150 | 47 | 48 | 39 | 71.2 |
| 220 | 34 | 26 | 20 | -84.2 | 310 | 55 | 57 | 57 | 57.2 |
| 240 | 59 | 81 | 84 | 8.7 | 330 | 51 | 47 | 41 | 138.4 |
| 260 | 67 | 47 | 41 | -88.0 | 350 | 49 | 56 | 59 | 67.6 |
| 280 | 61 | 69 | 71 | 17.8 | 370 | 26 | 30 | 21 | 161.8 |
| 2100 | 72 | 62 | 54 | -79.5 | 390 | 51 | 53 | 59 | 73.0 |
| 2120 | 61 | 55 | 55 | 34.7 | 3110 | 31 | 22 | 15 | 167.7 |
| 2140 | 52 | 52 | 53 | -72.7 | 3130 | 40 | 45 | 49 | 81.8 |
| 2160 | 51 | 48 | 49 | 29.0 | 3150 | 19 | 10 | 6 | 180.0 |
| 320 | 94 | 92 | 89 | 6.8 | 3170 | 37 | 42 | 40 | 87.1 |
| 340 | 16 | 10 | 14 | -36.0 | 410 | 50 | 48 | 50 | 137.9 |
| 360 | 91 | 94 | 94 | 12.5 | 430 | 61 | 60 | 65 | 59.7 |
| 380 | 14 | 21 | 16 | -44.6 | 450 | 62 | 59 | 59 | 151.0 |
| 3100 | 51 | 49 | 51 | 34.6 | 470 | 58 | 63 | 69 | 71.4 |
| 3120 | 41 | 39 | 27 | -66.4 | 490 | 26 | 29 | 27 | 170.6 |
| 3140 | 27 | 35 | 41 | 43.6 | 4110 | 55 | 53 | 56 | 91.2 |
| 3160 | 23 | 24 | 21 | -51.0 | 4130 | 17 | 17 | 15 | 201.4 |
| 420 | 10 | 6 | 12 | 250.0 | 4150 | 29 | 42 | 49 | 99.0 |
| 440 | 105 | 108 | 107 | 5.5 | 510 | 45 | 65 | 60 | 39.4 |
| 460 | 19 | 6 | 20 | -68.4 | 530 | 45 | 57 | 56 | 140.5 |
| 480 | 73 | 78 | 73 | 17.9 | 550 | 74 | 86 | 83 | 43.4 |
| 4100 | 16 | 16 | 28 | -45.4 | 570 | 49 | 45 | 49 | 138.4 |
| 4120 | 37 | 48 | 42 | 30.8 | 590 | 70 | 75 | 70 | 52.5 |
| 4140 | 16 | 19 | 30 | -35.2 | 5110 | — | 4 | 7 | 159.2 |
| 520 | 19 | 25 | 26 | 3.8 | 5130 | 73 | 69 | 66 | 59.3 |
| 540 | 28 | 4 | 16 | 196.1 | 5150 | — | 2 | 9 | 139.2 |
| 560 | 17 | 21 | 28 | 20.8 | 610 | 67 | 66 | 66 | 138.0 |
| 580 | 19 | 5 | 11 | 165.8 | 630 | 84 | 80 | 80 | 51.9 |
| 5100 | 22 | 25 | 28 | 33.4 | 650 | 59 | 52 | 57 | 142.3 |
| 5120 | 16 | 4 | 8 | -83.5 | 670 | 70 | 73 | 71 | 64.3 |
| 5140 | 26 | 27 | 26 | 46.9 | 690 | 29 | 32 | 35 | 150.4 |
| 5160 | 17 | 11 | 17 | 45.0 | 6110 | 63 | 67 | 63 | 77.0 |
| 620 | 9 | 9 | 10 | -48.6 | 6130 | — | 15 | 13 | 154.0 |

Table 4 (continued).

| <i>hkl</i> | <i>F(hkl)</i> obs. | <i>F(hkl)</i> calc. | | <i>a(hkl)</i> | <i>hkl</i> | <i>F(hkl)</i> obs. | <i>F(hkl)</i> calc. | | <i>a(hkl)</i> |
|------------|-----------------------|---------------------------|-----------|---------------|------------|-----------------------|---------------------------|-----------|---------------|
| | | $\frac{1}{2}$ (I + II) | ord. str. | | | | $\frac{1}{2}$ (I + II) | ord. str. | |
| 640 | 14 | 21 | 22 | 18.6 | 6150 | 55 | 61 | 57 | 85.8 |
| 660 | 35 | 28 | 24 | -74.5 | 710 | 58 | 65 | 67 | 52.1 |
| 680 | 10 | 17 | 17 | 16.3 | 730 | 52 | 51 | 50 | 135.0 |
| 6100 | 24 | 21 | 19 | 81.1 | 750 | 57 | 57 | 62 | 65.6 |
| 6120 | — | 18 | 22 | 0 | 770 | 46 | 38 | 40 | 154.9 |
| 6140 | 33 | 27 | 20 | -81.2 | 790 | 52 | 57 | 67 | 70.7 |
| 720 | — | 42 | 39 | 184.0 | 7110 | 42 | 37 | 40 | 165.5 |
| 740 | — | 10 | 10 | 94.8 | 7130 | 57 | 58 | 69 | 77.9 |
| 760 | 28 | 40 | 35 | 184.6 | 7150 | 18 | 16 | 17 | 198.6 |
| 780 | 8 | 7 | 8 | 167.5 | 810 | 59 | 65 | 64 | 143.0 |
| 7100 | 14 | 11 | 5 | 180.0 | 830 | 73 | 78 | 77 | 53.9 |
| 7120 | — | 6 | 7 | 143.9 | 850 | — | 20 | 17 | 180.0 |
| 7140 | 21 | 7 | 10 | 90.0 | 870 | 87 | 87 | 83 | 67.2 |
| 820 | — | 9 | 8 | 57.9 | 890 | — | 20 | 10 | 204.3 |
| 840 | 12 | 7 | 8 | -20.5 | 8110 | 83 | 87 | 83 | 72.8 |
| 860 | — | 15 | 14 | 51.6 | 910 | 55 | 55 | 52 | 44.3 |
| 920 | 24 | 21 | 21 | 5.0 | 930 | 48 | 50 | 54 | 139.4 |
| 940 | — | 8 | 9 | 151.3 | 950 | 50 | 46 | 45 | 46.5 |
| 960 | 15 | 9 | 12 | 190.3 | 970 | 23 | 35 | 40 | 143.8 |
| 980 | — | 12 | 9 | 101.7 | 990 | 47 | 47 | 47 | 46.8 |
| 9100 | 34 | 28 | 34 | 191.1 | 1010 | 49 | 35 | 39 | 137.2 |
| 1020 | — | 4 | 4 | 189.7 | 1030 | 46 | 42 | 41 | 56.1 |
| 1040 | 67 | 68 | 67 | -12.6 | 1050 | 48 | 38 | 45 | 148.6 |
| 1060 | 13 | 8 | 11 | 138.4 | 1070 | 50 | 48 | 49 | 61.8 |
| 1080 | 63 | 58 | 56 | 202.0 | 1090 | 25 | 21 | 25 | 161.6 |
| 10100 | 9 | 8 | 14 | 166.4 | 10110 | 46 | 45 | 44 | 80.9 |
| 1120 | 55 | 63 | 62 | 192.2 | 1110 | 26 | 26 | 26 | 43.6 |
| 1140 | 7 | 7 | 7 | 114.1 | 1130 | 13 | 20 | 19 | 152.8 |
| 1160 | 58 | 59 | 57 | 194.7 | 1150 | 47 | 47 | 49 | 50.2 |
| 1180 | — | 17 | 26 | 112.6 | 1170 | — | 13 | 14 | 151.4 |
| 11100 | 55 | 50 | 46 | 202.8 | 1190 | 55 | 49 | 50 | 54.1 |
| 1220 | — | 9 | 10 | 19.5 | 1210 | — | 13 | 13 | 140.2 |
| 1240 | 52 | 44 | 47 | 189.6 | 1230 | 33 | 27 | 28 | 60.2 |
| 1260 | 22 | 17 | 20 | 62.3 | 1250 | — | 20 | 13 | 157.3 |
| 1280 | 52 | 42 | 45 | 197.7 | 1270 | 29 | 24 | 27 | 90.0 |
| 1320 | 67 | 66 | 66 | 185.2 | 1310 | 26 | 14 | 15 | 21.4 |
| | | | | | 1330 | 17 | 9 | 10 | 133.2 |

Br₂-ion has seven neighbours within distances lying in the range from 4.0 to 4.5 Å, thus C₄, C₆, C₇, O₂', C₅', 2C₁'. There are thus four "onium"-groups around each Br-atom, and the arrangement of these groups are approximately tetrahedral with the Br-atoms in the space center of the tetrahedra.

The methyl group C₇ of one molecule and the methyl group C₆ of the other molecule approach the acetyl group, C₃ and O₂ of an adjacent molecule to distances, which are somewhat below the normal van der Waals distances. The shortest of these distances lie between 2.9 and 3.0 Å, and it may be seen from Fig. 7 c, where they are indicated by dashed lines, that these short intermolecular distances connect the molecules to form double strings of

molecules in the direction of the a -axis. The methyl group C_5 of the extended form is also at a distance of 3 Å from the O_1 -atom of the "ring" form.

All the distances between atoms or groups of molecules belonging to different double strings are about or above the normal van der Waals distances, thus, for example, the distance O_1-O_1' is 3.8 Å.

The origin of the subsidiary reflexions, with non-integral values of the indices, has not as yet been investigated in detail. The existence of the acetyl choline ion in two different forms in the structure may perhaps offer an explanation, which might serve as a possible working hypothesis. In the calculation of the structure factors for an ordered structure of the two forms, it was assumed that the extended form is located about the position x from 0 to 0.7, $y \simeq 0$, whereas the "ring" form is located about the position x from 0.5 to 1.2 and $y \simeq 0.25$. The y -coordinates of the C_3 -groups give a slight preference for this arrangement as it leads to slightly more reasonable interatomic distances, else the positions of the different forms could just as well be interchanged. It is then reasonable to suggest that stacking faults in the sequence extended form — "ring" form produce a long range modulation of the structure, which gives rise to the observed subsidiary reflexions. Such a partially disordered structure would be in accordance with the observation that the averages of the structure factors for the two forms agree just as well with the observed values as those calculated for the ordered structure, and appreciably better than for either of the forms separately. Another observation pointing in the same direction is the marked broadening of the maxima of the O_1 -atoms in the direction of the b -axis. This broadening is especially pronounced in the difference map, reproduced in Fig. 4. Such a broadening might be expected for a disordered structure as the y -coordinates for the O_1 -atom would differ considerably for the two forms of the acetyl choline ion.

It is not unlikely that the ability of the acetyl choline ion to exist in two distinct structural forms may account for some of its specific activities. The extended form would be likely to prevail in solution in polar media, whereas the "ring" form might be the prevalent form in unpolar media. Such an ability of the acetyl choline ion would evidently have a strong bearing on its characteristic behaviour at lipoid-water interfaces, and possibly also on its specific action in the transfer of nerve impulses.

The present work was begun in the Department of Chemistry at *Forsvarets Forskningsinstitutt*, Kjeller, where the experimental part and the first stages of the structure determination were carried out. The work has been continued at *Fysisk Institutt, Norges Tekniske Høgskole*, with financial support from *Forsvarets Forskningsinstitutt*, which is gratefully acknowledged.

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Received November 3, 1958.