THE EFFECTS OF ELECTRON BOMBARDMENT
ON AMINO ACIDS

by

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INTRODUCTION

The effect of ionizing radiation on amino acids has been studied by Maxwell, et.al., (6), and Dale, et.al., (1). These investigators studied the effects of x-rays and high energy electrons on aqueous solutions of amino acids by identifying the reaction products arising as a result of exposure to these two types of radiation. Dale investigated the action of x-rays on amino acids in general while Maxwell, using both x-rays and electrons, limited his investigation to glycine. Some of the many reaction products identified by these workers are ammonia, methylamine, acetic acid and carbon dioxide to name only a few. The other reaction products identified were also volatile substances.

Although the work mentioned above has no direct bearing on the research discussed herein, it is cited for two reasons. First, the author wishes to call attention to the many reaction products that are possible when an amino acid is exposed to ionizing radiation, and secondly, a review of the many scientific journals indicated that the above mentioned work is as similar to the research discussed in this thesis as any that could be found.

When an amino acid is placed in an electron microscope and examined two things are observed to occur. First, as a result of the electron bombardment the amino acid appears to decompose. This apparent decomposition, as it appears on the viewing screen, is characterized by the rapid formation and bursting

of bubbles as well as a general agitation within the sample. The occurrence of the bubbles is the possible result of the formation of some of the volatile substances identified by Maxwell. However, in the present case, since the reaction is taking place in a vacuum, these reaction products would be in the form of gases and would be removed from the microscope. This first reaction lasts but a short time.

As a result of the violent agitation within the sample during the initial reaction the residue that remains is left with many long narrow projections coming off the more massive portions. In the case of some amino acids these projections appear to be charged as is indicated by their distorted appearance on the viewing screen. The second thing that is observed to occur usually begins on these long, charged projections. When one of these projections is moved into the electron beam of the microscope and bombarded for an extended period of time a dendritic type structure is observed to develope. This structure is shown in the micrographs exhibited on Plate I. The micrographs on Plate II show how the structure will develope over a period of time.

This growth was first observed on wheat gluten during studies relating to the basic reasons for the variation in flour protein quality[1]. Since wheat gluten has all the known amino acids in it, a limited investigation was made to determine

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[1]It should be pointed out that the growth does not occur of the substance put into the microscope but on the residue that is left after the initial reaction has ceased.

Micrographs of the branches as they occur on whole wheat gluten
and various amino acids.

Fig. 1. Whole wheat gluten.

Fig. 2. Glycine.

Fig. 3. Alanine.

Fig. 4. Histidine. (early stage of developement)
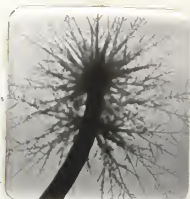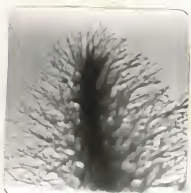
PLATE I

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

EXPLANATION OF PLATE II

A series of micrographs showing the developement of the branches
over a period of time. Magnification 18 X 10^3.

> Fig. 1. Micrograph taken before branches
> started to develope.
>
> Fig. 2. The way the branches appeared after
> eight minutes of developement.
>
> Fig. 3. After 14 min.
>
> Fig. 4. After 25 min.
>
> Fig. 5. After 33 min.
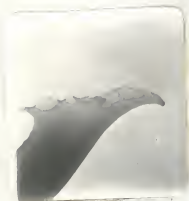
PLATE II

Fig. 1.
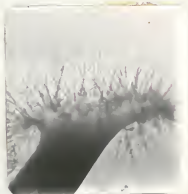
Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

if the growth was due to any one particular amino acid. It was
soon found that several of the amino acids were capable of pro-
ducing the growth. Besides the wheat gluten, the amino acids
that grew included: dl-alpha alanine, asparagine, glycine, his-
tidine and dl-tryptophan. Glulathione and glycl-glycl-glycline
also produced similar growths. The amino acids which did not
grow branches included: glutamic acid, cystine, proline, arginine
monohydrochloride and isoleucine. There was no apparent dif-
ference in the branches produced by the various substances.
This being the case, glycine, the simplest of all the amino
acids, was used for all subsequent work.

The purpose of the research reported here was to inves-
tigate the nature of this tree-like growth with particular
emphasis placed on determining the composition of the branches.

EXPERIMENTAL PROCEDURE

To realize the purpose of this research, techniques in
electron microscopy and electron diffraction were employed.
The instrument used was a R. C. A., EMU-2A electron micro-
scope with a fixed 50 KV accelerating potential. The micro-
scope was adapted with a selected area diffraction unit that
enabled the operator to obtain micrographs and electron dif-
fraction pictures from the same area of the sample.

Samples were prepared on standard electron microscope
specimen screens of the stainless steel variety, however, on
occasion copper screens were also used. The amino acid, in
the form of a powder, was made to adhere to the sample screen

by wetting the screen with distilled water and dipping it into
the amino acid.

After a sample was dry it was placed in the microscope
and upon reaching an operating vacuum, about .1 micron of Hg.,
the electron beam was brought to its maximum intensity. There
upon the apparent decomposition already referred to would take
place. The rate at which this process took place depended
upon the intensity of the beam. In general it was not difficult
to find regions of the sample that would support the growth.
However, in order to have enough material in the electron beam
to produce a suitable diffraction pattern it was necessary to
develope the branches for a considerable period of time. On
the average this took from one to three hours of continuous
operation.

Calibration of the microscope for diffraction purposes
was achieved from measurements made of an aluminum diffraction
pattern. The aluminum sample was prepared by evaporation
techniques. The resulting calibration curve is shown on Plate
III.

ANALYSIS BY ELECTRON DIFFRACTION

In the thirty years since Davisson and Germer performed
their now classical experiment that proved De Broglie's ear-
lier hypothesis, that all particles should exhibit a wave as
well as a particle like character, the method of electron
diffraction has been developed as a means of investigating
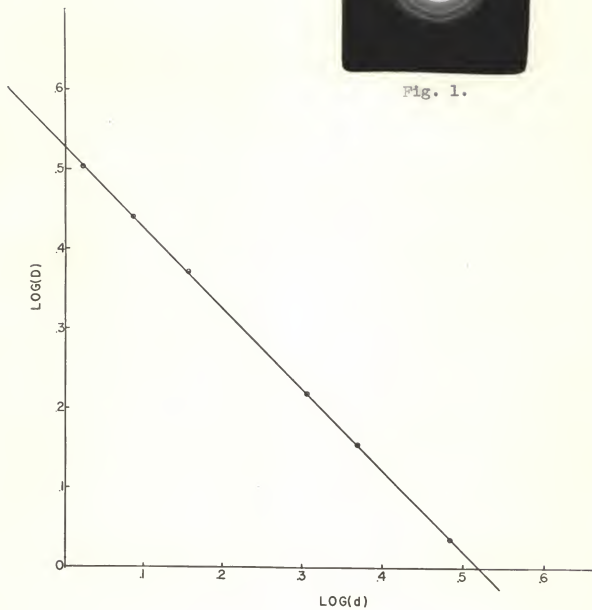the atomic structure of matter in surface layers, in thin

Calibration curve relating the logarithm of the diameter of the
diffraction rings, measured in centimeters, to the logarithm of
the distance between atomic planes measured in angstroms.

Fig. 1. The aluminum diffraction pattern from which
calibration measurements were made.

PLATE III

Fig. 1.

films and in gases, just as x-rays have been used to study the
structure of massive crystals. The pattern of electrons scat-
tered from a monochromatic primary electron beam by a perfect
crystal theoretically consists of very sharp and intense co-
herent scattering maxima at discrete angles satisfying Bragg's
law, the maxima being superimposed on a diffuse background of
incoherently scattered electrons. Amorphous solids, which have
no extended orderly geometrical arrangement of their atoms, dis-
play the diffuse background just mentioned, but the Bragg max-
ima degenerate into a series of very broad, ill-defined maxima,
which might be called bands or diffuse rings. The positions of
the diffuse rings are not determined by the Bragg relation but
instead are found from Wierl's formula (Thomson and Cochrane,9).

The Wierl formula is an expression for the scattering
cross section for the coherently scattered electrons that are
scattered by the molecules of an amorphous substance. It is
given by the relation

$$I_m = \sum_{i} E_i E_j \quad \frac{\sin(s\ r_{ij})}{s\ r_{ij}} \qquad i \neq j$$

where the sum is carried out over all the various atoms that form
the molecular make-up of the substance; r_{ij} is the distance be-
tween the ith and jth atoms; s is given by, $s = \frac{4\pi}{\lambda} \sin (\alpha/2)$
where α is the angle of scattering, i.e., the angle between the
forward direction of the primary beam and the forward direction
of the scattered beam. E_{ij} is the electron analog of the x-ray
atomic structure factor.

The Wierl formula is derived on the assumption that the distance r_{ij} is a fixed distance; this is equivalent to saying that a molecule has a rigid structure. However, since there is some motion of the nuclei in all molecules the Wierl formula should more properly be written (Karle and Karle,3)

$$I_m = \sum_{ij} E_i E_j \int_0^\infty P_{ij}(p) \; \frac{\sin(sp)}{sp} \, dp \qquad i \neq j$$

where $P_{ij}(p)dp$ is the probability that the distance between the ith and jth atoms has a value between p and p + dp. A Fourier transform may be made of this expression by multiplying both sides by s sin(sr) and integrating with respect to s as follows:

$$D(r) = \int_0^\infty sI_m(s) \; \sin(sr)ds = \sum_{ij} \int_0^\infty \frac{P_{ij}(p)}{p} \, dp \int_0^\infty E_i E_j \sin(sr) \, \sin(sp)ds$$

Without too much error, except at very small scattering angles, $E_i E_j$ may be replaced by a constant c_{ij}. The equations may then be written

$$D(r) = \int_0^\infty sI_m(s) \; \sin(sr)ds = \sum_{ij} c_{ij} \int_0^\infty \frac{P_{ij}(p)}{p} \, dp \int_0^\infty \sin(sr) \; \sin(sp)ds$$

$$= \sum_{ij} c_{ij} \int_0^\infty \frac{P_{ij}(p)}{p} \, \delta(p,r)dp = \sum_{ij} c_{ij} \frac{P_{ij}(r)}{r}$$

D(r) is called the radial distribution function and represents the probability of the occurrence of the internuclear distance, r, in the molecule.

In order to evaluate the above integral the molecular scattering data must be known to values of s equal to infinity.

In practice only a limited amount of data are available. Nevertheless, this limited data uniquely determines the structure of the molecule (Karle and Karle,3). The procedure used is to compute the following modified function,

$$f(r) = \int_{0}^{S_{max}} sI_m(s) \exp(-as^2) \sin(sr)ds$$

where s bounds the range of scattering data and a can be so chosen that the integrand of the above equation makes no essential contribution beyond s_{max}. The computed $f(r)$ curve, therefore, is the same as that which would have been obtained if s_{max} approached infinity. The damping factor was introduced by Degard (Karle and Karle,3) in order to improve radial distribution calculations.

The actual evaluation of $f(r)$ is achieved by the methods of numerical integration by writing the above integral as a sum as follows:

$$f(r) = \sum_{n=0}^{n=S_{max}} s_n I_m(s_n) \exp(-as_n^2) \sin(s_n r) \Delta s$$

s_n is found from the geometry of the electron diffraction camera and the associated electron wave length or else it is obtained from calibration measurements of a known crystalline substance. $I_m(s_n)$ is known from the experimental intensity curve, the other terms in the sum are readily found for any value of r and a given value of a in such sources as prepared by the Federal Works Agency, Work Projects Administration (4), (5). Δs is a measure of the accuracy of the approximation.

The total intensity of scattering is composed of a back-

ground that falls off rapidly with increasing scattering angle
and is due to atomic scattering upon which the molecular scat-
tering, $I_m(s_n)$ is superimposed. Theoretically the background
is given by (Mott and Massy,7),

$$I_a(s) = K\left[\sum_{\zeta} E_{\zeta}^2 - 4/s^4 \sum_{\zeta} S_i\right]$$

where S_i is the inelastic scattering function. However, as
pointed out by Karle (3) there are serious discrepancies be-
tween the calculated background and the observed background[1].
There are therefore uncertainties in what constitutes back-
ground with resulting uncertainties in the experimentally
determined molecular scattering curve. When these uncertain-
ties are computed into the radial distribution curve they effect
mainly the shapes but not the positions of the maxima of this
function (3). Hence, fairly reliable equilibrium distances
in a molecule can be obtained from the first computation of a
radial distribution curve. The best background is one that
gives rise to a distribution curve that is entirely positive (3).

The above procedure is referred to as the 'radial distri-
bution method' of deducing interatomic distances from dif-
fraction photographs. In applying this analysis to a known
substance a total intensity curve is first obtained. A smooth
background is drawn through the oscillations of this experimental

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[1]The theoretical expression for the background accounts
for the intensity caused by electrons that have been scat-
tered once. There is also a background due to electrons that
have undergone multiple scattering for which no analytic ex-
pression has been derived.

curve and the molecular scattering curve is found by taking the difference between the two curves. From the molecular scattering curve a radial distribution curve is computed. A radial distribution curve with negative values and/or extraneous maxima near the origin indicates a correction is needed in the background. The background is corrected until it is evident no additional improvement in the distribution is possible. At the same time the best molecular scattering curve is also obtained. Using interatomic distances obtained from the distribution curve molecular scattering curves are computed for various molecular models. The model that gives rise to a computed curve that agrees with the experimental curve being adopted. This analysis gives results that are in good agreement with other methods of determining molecular structure (Karle and Karle,3).

INTERPRETATION OF THE DIFFRACTION PATTERN

The diffraction pattern, a sample of which is shown on Plate IV, consisted of a strong central spot, characteristic of all electron diffraction patterns, and two somewhat diffuse rings. The question that naturally arose was what type of diffraction this was, molecular or crystalline? Crystalline substances as a rule give rise to a diffraction pattern consisting of many sharply defined rings like the aluminum pattern shown on Plate III. However, if the crystals are very small the rings have a tendency to lose definition. This of course was a possible explanation for the diffuseness of the rings in this

EXPLANATION OF PLATE IV

A series of pictures showing the diffraction pattern was due to
the branches.

Fig. 1. The appearance of the sample before the developement of
the branches. Magnification 18 x 10^3.

Fig. 2. Diffraction pattern before the developement of the
branches.

Fig. 3. Same area of sample as in Fig. 1. showing the branches,
Taken 84 min. after Fig. 1. and the magnification is
13 x 10^3.

Fig. 4. Diffraction pattern after the branches developed.
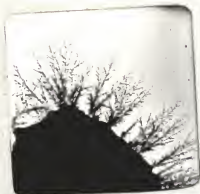
PLATE IV

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

case. If the branches were crystals they were certainly minute crystals and the series of pictures on Plate IV leaves little doubt that the branches were what gave rise to the diffraction pattern. Left unanswered, however was the appearance of only two rings; furthermore the rings showed no evidence of crystal orientation. The very nature of crystal growth would tend to prohibit the branches from being aggregates of smaller crystals, instead it would be expected that they were small crystals themselves. The diffraction pattern from such crystals would surely show some orientation effects.

Even though the rings were somewhat diffuse they were not as broad as would be expected from molecular diffraction. Nevertheless it was felt that the rings were the result of this type of scattering. This conclusion was reached primarily because of the similarity between this pattern and a pattern obtained by Murison (8) during some electron diffraction studies he conducted on long chain, hydrocarbon compounds. The particular pattern referred to also consisted of two diffuse rings and was obtained by reflecting electrons from thin films of oil spread over metal blocks.

The possibility of the branches being such a long chain compound was easy to accept. All the chemical components of such a compound were present in the amino acid and whatever the branches might be it was logical to suppose their composition was limited by the composition of the amino acid. In order to investigate this possibility further, it was decided to obtain a diffraction pattern from a known hydrocarbon

compound and compare it with the pattern produced by the branches.
Such a compound was readily available in the form of Parlodion;
a material often used in electron microscopy. The diffraction
pattern obtained from the Parlodion compared very favorably
with the pattern produced by the branches. Not only were there
two maxima but the diameters of corresponding rings were very
nearly the same. This in spite of the fact that the rings pro-
duced by the Parlodion were somewhat broader than the ones due
to the branches.

Since the rings were broad and diffuse it was difficult
to measure them with ordinary measuring devices. Therefore
comparison of the two patterns was carried out by comparing
densitometer traces obtained from the photographic plates for
the two cases. The degree of similarity between the two dif-
fraction patterns is shown by the curves on Plate V.

To obtain data for these curves it was necessary to use a
non-recording, non-scanning densitometer. In order to know
the position of the photographic plate with respect to the
densitometer a traveling microscope stage was fitted with a
guide, along which the plate would follow, and a bar that would
push the plate along with the stage. Starting at the center
of a diffraction pattern the plate was moved across the pin-
hole aperture of the densitometer with readings being taken
every .1 mm.

A complete analysis of the branch diffraction pattern
could not be made since the chemical composition of the branches
was not known. However, it was thought that the interatomic

EXPLANATION OF PLATE V

Densitometer traces of diffraction patterns produced by the branches (A) and the Parlodion (B).

PLATE V
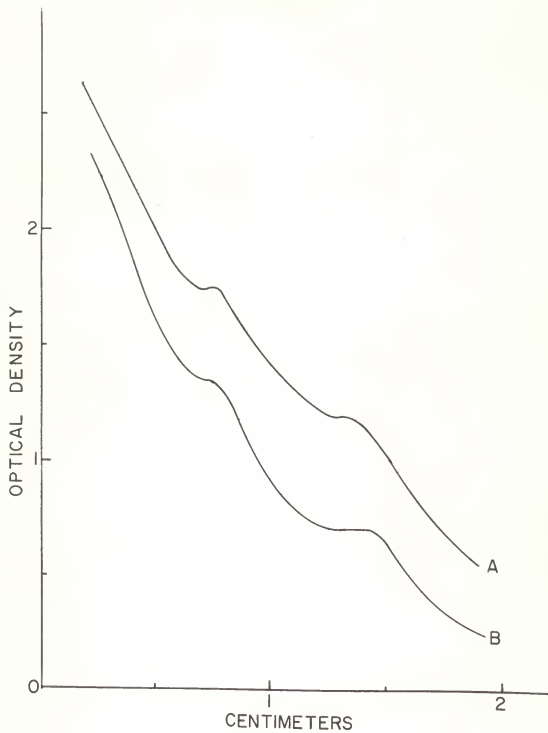
distances indicated from a radical distribution curve might give some indication of the atoms making up the molecules. The basis for this possibility rested upon the fact that the distances separating given atoms between which a given bond exists is somewhat standardized. For example the most probable distance between two single bonded carbon atoms is 1.5A where as one would expect a distance of 1.4A between carbon and oxygen atoms with a single bond between them (Gray,2).

The calculation of the radial distribution curve followed the procedure already outlined except the equation was computed in terms of the distance, measured in centimeters, from the center of the diffraction pattern. The relation between this distance, called x, and s was obtained from the aluminum calibration pattern through the Bragg relation and was found by $x = .266s$. The value of a', where a' $= a/(.266)^2$, was chosen to be 1.35. This value of a' gave the damping factor a value of .015 at the largest value of x. Increments in x of .02 were used.

A knowledge of the film characteristic for electrons was necessary before density measurements could be converted into intensities. The relationship between these two quantities was obtained through a series of ten exposures taken with the same intensity and varying the time. Since the reciprocity law holds for fast electrons (Thomson and Cochrane,9) it is equally valid to think of the resulting exposures as being obtained by varying the intensity and holding the time constant. In this work the time was taken to be unity and the intensity, in

arbitrary units, was read directly. A typical characteristic curve is shown on Plate VI.

The total intensity curve shown on Plate VII is the result of changing densities into intensities and multiplying each point of the intensity curve by its radial coordinate. Multiplying by the coordinate had the effect of integrating the measured intensity around a ring of radius x and gave an indication of the number of electrons scattered per unit solid angle. Values of $I_m(x_n)$ were taken as the difference between the background and the total intensity.

The numerical integration of $f(r)$ was carried out for values of r from zero to 3.7A in steps of one tenth. A plot of the distribution curve obtained from this calculation is shown on Plate VIII and indicates a single distance of 2.59A. The negative part of the curve indicates the background was not quite correct but as the position of the maxima is not appreciably affected by this no correction was made.

The one interatomic distance obtained from the distribution curve is probably correct but it is doubtful that it is the only distance between the various atoms forming the molecular make-up of the branches. A likely explanation for only one distance being calculated is that the range of the diffraction pattern was not great enough to give any other distance. Electron diffraction units designed to investigate amorphous substances will produce patterns over which the value of s will range from zero to as high as 35 (Karle and Karle,3). The intensity curves obtained from such patterns have many maxima

EXPLANATION OF PLATE VI

A typical characteristic curve relating optical density to ex-
posure for the particular photographic plates and developement
procedures used in this work.

PLATE VI
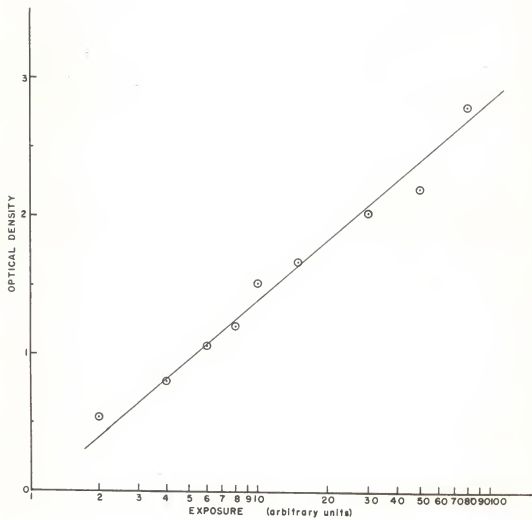
EXPLANATION OF PLATE VII

The solid line is the experimentally determined scattering curve
obtained by changing optical density into intensity and multi-
plying by the distance, measured in centimeters, from the center
of the pattern.
The broken line is the background used to compute the radial
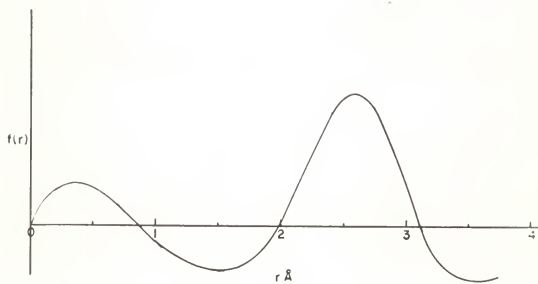distribution curve.

PLATE VII
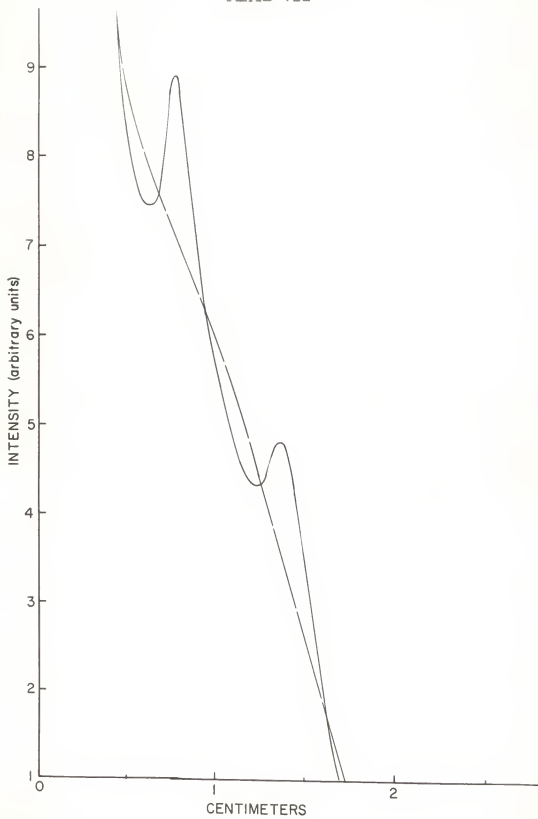
EXPLANATION OF PLATE VIII

The radial distribution curve obtained from the branch diffraction pattern.

PLATE VIII

f(r)

r Å

and contain much more information than do the curves obtained
from the unit used in this work. It is not possible with this
unit to investigate scattering for s values greater than eight.
With this limited range it is not possible to obtain what might
be referred to as the second order maxima for the smaller in-
teratomic distances and it would appear that at least two orders
of diffraction for each distance is needed before all distances
are uniquely determined by this method of analysis.

Fig. 1. General structure of long chain,
hydrocarbon compounds.

In spite of these limitations the calculation of the dis-
tribution curve added further evidence that the branches were
long chain, hydrocarbon compounds. The general form of these
compounds is indicated in Fig. 1. (Thomson and Cochrane,9).
The carbon atoms forming the zigzag chain lie in a plane and
it is seen that the 2.59A distance obtained from the distribu-
tion curve agrees fairly well with the 2.54A indicated in the
figure.

The evidence supporting the long chain type structure,
i.e., the calculated distance of 2.59A and the similarity be-
tween the diffraction pattern produced by the branches and that
produced by the Parlodion, is admittedly not conclusive. How-
ever, the evidence would seem to establish this structure as the
most probable.

GENERAL OBSERVATIONS

In watching the branches develope and in studying the micrographs the following things were noted. First, the rate of growth was some how dependent upon the intensity of the electron beam. It was difficult to obtain a quantitative relationship between these two quantities because the electron beam current and the mass per unit area of the sample were unknown. However, it is known that the rate of growth will vary from sample to sample and even between different regions of the same sample.

One of the many pecularities associated with the branches was their apparent instability. If the region upon which the branches were developing was moved out of the field of vision, i.e., out of the electron beam, for a period of two or three minutes, re-examination of the region revealed that the branches had fused together, curled and grown blunt. In most cases no further branch developement would occur on such a region.

In studying the micrographs it was observed that the branches were somewhat transparent to the electrons and that they grew nearly perpendicular to the electron beam. The latter being based upon the fact that the depth of focus of the microscope is probably less than a micron and none of the branches were observed to be greatly out of focus. Additional evidence of this was given by stereoscopic examination of stereomicrographs of the branches. Other than this the examination of the stereomicrographs gave the impression that the growth of the branches was strictly a random process. No other orderly arrangement of

any kind was observed. The nearest thing to order was the occurrence of a maximum angle of branching of about 50 degrees.

A PROPOSED MECHANISM FOR THE DEVELOPEMENT OF THE BRANCHES

As already indicated the growth usually started on long narrow projections that appeared to be charged. The charge was probably due to the absorption of electrons from the beam. The field due to this charge could be quite high at places on these projections which were sharply pointed. The proposed mechanism is based on the attractive force this field would have on positive ionized particles and on a possible sublimation process resulting from localized high temperatures.

One of the many effects the electron beam has upon a sample in an electron microscope is to set up temperature gradients between regions transparent to the electrons and regions that absorb electrons. The regions absorbing the electrons of course being at the higher temperatures. Watson (10) claimed to have observed temperatures as high as 1600°C. in a microscope, basing his claim on the melting of titamium dioxide crystals.

At temperatures as high as this it is not unreasonable to suppose that the material making up the branches is created by sublimation processes and coming from regions of the sample with high electron absorption. After this material has been freed from such regions it is assumed that it is ionized by collisions with the electron in the beam resulting in the necessary positive charge for it to be attracted to the negatively

charged projections where it forms a stable chemical bond. The
repetitive nature of such a process could easily result in the
formation of the branches.

Such a mechanism as this would explain why the branches
grow in a plane nearly perpendicular to the beam. If a branch
started to grow parallel to the beam it would soon get to the
point where it would absorb many electrons with a resulting rise
in its own temperature. At the higher temperatures the competi-
tive process of sublimation would begin and the branches would
stop growing. There should be therefore a maximum length a
branch could grow in this direction. Although there was evi-
dence of such branches it was not possible to measure their
length.

CONCLUSIONS

Considering the chemical composition of the amino acid the
calculated interatomic distance of 2.59A is probably the dis-
tance between carbon atoms since no other combination of atoms
present in the acid will, ordinarily, lead to such a spacing.
Furthermore, this spacing would indicate that one of the main
features of the branches is a carbon chain similar to that found
in some of the hydrocarbons. This supposition is further sup-
ported by the similarity between the branch diffraction pattern
and the Parlodion diffraction pattern.

Although the diffraction portion of the microscope used in
this work is suitable for studying crystalline substances it has
certain limitations that make it inapplicable for studying amor-

phous materials. The most serious limitation is the small range of s over which the scattering can be investigated. If further work on this problem is contemplated and since the branches are too small to be studied in conventional electron diffraction equipment some means must be found to overcome this limitation. One way to do this would be to increase the accelerating voltage. This would decrease the associated electron wave length and would increase the range of s for the same maximum angle of scattering.

ACKNOWLEDGMENTS

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When an amino acid is placed in an electron microscope and examined two things are observed to occur. First, as a result of the electron bombardment the amino acid appears to decompose. The second thing that is observed to occur is the formation of a dendritic type structure that developes on certain regions of the residue that is left after the initial decomposition has stopped. The region upon which this growth developed would in general appear to be charged.

The purpose of this research was to investigate the nature of this tree-like growth with emphasis placed on determining the composition of the branches.

The instrument used throughout this work was a R. C. A. EMU-2A electron microscope with a fixed 50 KV accelerating potential. The instrument was adapted with a selected area diffraction unit that enabled the operator to obtain micrographs and electron diffraction pictures from the same area of the sample.

Analysis of the branches was attempted by means of electron diffraction. The diffraction pattern produced by the branches consisted of two diffuse rings on a steeply falling background. This type of pattern indicated an amorphous type structure and the pattern was analyzed by the 'radial distribution method'. In this method of analysis a radial distribution curve that represents the probability of the occurrence of various interatomic distances was computed from the formula

$$f(r) = \sum_{n=b}^{n=S_{max}} s_n I_m(s_n) \exp(-a s_n^2) \sin(s_n r) \Delta s$$

Evaluation of this function indicated a single interatomic distance of 2.59A. This information along with the similarity between the diffraction pattern produced by a known long chain, hydrocarbon compound led to the belief that the branches were also some form of long chain, hydrocarbon compound.

The examination of the micrographs, both regular and stereoscopic, indicated the branches grew mostly in a plane perpendicular to the electron beam. Furthermore the impression was given that the branches grew in a very random fashion. No orderly arrangement of any kind was observed.

The proposed mechanism for the developement of the branches is based upon a possible sublimation process resulting from localized high temperatures that occur in an electron microscope and upon the ionizing effects and the fields produced by the electron beam.