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<td>N is the number of developing nuclei at time t.</td>
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<td>v_m is the volume of a single molecule B.</td>
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<td>x = \frac{k_s}{K} \left[e^{Kt} - 1\right]</td>
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<td>Fig 1,</td>
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<td>n(A/B) = n_B \left(\frac{aZ}{1+az}\right)^m.</td>
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<td>P = 1 - \exp \int_0^t \frac{1}{x} , dt</td>
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<td>Z = x/y the mole ratio</td>
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KINETICS OF SOME SOLID-SOLID REACTIONS
IN VOLVING SUGARS AND AMINO ACIDS

A Thesis presented to the University of Surrey
for the Degree of Doctor of Philosophy

by

SYRUS NOVPARAST

C.W. Davies Laboratories,
Department of Chemistry,
University of Surrey,
Guildford, Surrey

February 1971.
The glucose/valine reaction was investigated as an example of a reaction between organic solids. The reactions between glucose and other amino acids were studied in less detail. Both, the kinetics of the reaction and the chemical composition of the products were investigated.

Contrary to previous results, dry crystals of glucose and valine reacted readily at a temperature below the melting point of either reactant. Increase of humidity appeared to decrease the rate of reaction. Water, CO$_2$ and isobutyraldehyde were the major gaseous products. The rate of reaction was followed by measurement of pressure change on a vacuum line and also by measurement of weight loss on a thermogravimetric balance. The kinetic curves obtained on both systems were of sigmoid shape and seemed to be similar. The reaction did not go to completion.

The activation energy obtained was 34 Kcal/mole and did not vary with particle size. The approximate "orders" of reaction were 0.5 and 1.0 with respect to valine and glucose. Observation by optical microscope and electron microscope suggested the reaction might occur on the surface of glucose and photographs taken showed a build up of inert product which hinders further reaction.

Analysis of reaction products showed that α-amino acids and glucose react to give water, CO$_2$ and the aldehyde containing one fewer carbon atoms than the amino acid. Carbon-14 labelling showed the CO$_2$ to arise from the carboxyl group of the amino acid. A number of minor products were partly or wholly identified. There
was some evidence that the glucose was being degraded as well as reacting with valine.

Efforts were made to find out the kinetic rules for the reactions of this kind, and the data were fitted with varying degrees of success to equations suggested by Avrami and Erofeev and others. A mechanism of reaction involving the Strecker degradation was outlined.

The mass spectra of several hexoses and some pentoses and disaccharides were obtained and analysed. No molecular ion peaks were obtained and the most abundant ions generally appeared at mass numbers 73, 60, and 31. The differences between the sugars were insufficient for this to be an acceptable method of analysis.
ACKNOWLEDGEMENTS

The work described in this thesis was carried out in the Chemistry Department, University of Surrey, under the supervision of Dr. B.G. Reuben.

The author wishes to express his most sincere and deep appreciation for the invaluable guidance and constant help he has received from Dr. B.G. Reuben during the course of this work.

Also the author would like to thank members of the staff of the University of Surrey, particularly Dr. J.R. Jones, for assistance with the $^{14}$C labelling work, Mr. J. Delderfield for his assistance with the mass spectrometry, Dr. R.U. Koenigsberger for her help with the chromatography, and Dr. T.M. Poole for advice and discussions.

I am also indebted to Mr. Hillman and Mr. Reed of the Woolwich Arsenal for their advice on the problem of estimating water by Karl Fischer method, and also to Dr. T. Rohan for bringing some of the problems to our notice.
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INTRODUCTION
The purpose of this work is to study some aspects of the reactions between certain solid organic compounds and to investigate the kind of kinetic rules involved.

Previous investigations of reactions in solids have have been carried out mostly on the decomposition of single organic compounds. The latter normally took places at high temperatures over a prolonged period. Inorganic systems are usually simpler than organic ones in that no decomposition occurs and the products are simple and well defined. Very little information is thus available on solid-solid reactions in organic systems.

It was noted by Rohan, that crystalline sugars and amino acids appeared to undergo reaction in the dry state. This seemed to be an interesting system for study and in the following thesis, the kinetics of a number of reactions of this type are investigated. Initial investigations showed that substantial quantities of gases were evolved and it was therefore decided to carry out the reaction in a vacuum line and to follow the pressure change. Some measurement of the rate of weight loss was also made using a thermogravimetric balance.

Previous workers have examined the relative rate of reaction of a few amino acids and glucose in solution by means of calorimetry and measurement of fluorescence increase at low temperatures. Colorimetry was chosen because the reaction normally gives a strong brown colour. A very large number of products have been found and the coloured materials called melanoidins are apparently the result of a long series of
consecutive reactions. The relation between this brown colour and the sugar/amino acid reaction is obscure and the adoption of a vacuum line system seems more likely to lead to data on the primary steps of the reaction. The investigation of reaction in a vacuum line has other advantages:

1. The gaseous products are carried away from the reaction site, thus reducing the possibilities for concurrent and consecutive reactions involving them.

2. The possibility of prolonged pumping on the reactants ensures that they are as dry as possible at the start of the reaction.

Most of this work is concentrated on the reaction between glucose and valine, but the relative rates of reaction of some other homologous α amino acids as well as β and γ amino acids with glucose are also examined. Some chemical analysis of the gaseous and solid product is carried out, and an attempt made to identify the major and some of the minor products.

The early results led to the employment of other techniques. Some experiments were carried out with the reactants in a mass spectrometer ion source and this led to investigation of the mass spectra of sugars. Other work was carried out using optical and electron microscopy.
SURVEY OF PREVIOUS WORK

(I) Chemical Reaction of Sugar and Amino Acids.

I) Chemical reaction of sugar and amino acids.

The reaction between glucose and amino acids is part of the general reaction between reducing sugars, or aldehydes and ketones and the amino group of amines, amino acids and peptides. This reaction which is an example of a non-enzymatic reaction is called browning reaction or the melanoidin reaction. It has been of interest to the scientists especially food manufacturers, for many years, because it occurs frequently in foodstuffs, and fruits containing these two entities.

1) The beneficial aspects of this reaction are that it produces a pleasant aroma and flavour, in the dried food, but it is not always the case since different amino acids give different aromas.

2) A deleterious aspect of the amino acid/sugar reaction is, that in some dried food it produces an unpleasant stale taste. It also extends the development of brown discoloration, and the loss of protein solubility leading to a deterioration. There is an increased tendency to foam and froth, and the foam often develops the property of fluorescence under the ultra violet light. However, there are also some individual uses of the browning reaction, e.g. browning and production of malt, or gravy colouring.

The reaction was first described by the French chemist, Maillard \(^1,2\) (1912 a,b). Then over the next few years Maillard \(^3,4\) (1916, 1917) made a comprehensive study of
this interaction, and established that only reducing sugars are reactive. He produced a standard mixture of glucose/glycine in a concentrated form (4 parts of glucose to one part of glycine, and 4 parts of water) and followed the reaction at different temperatures ranging from 34°C to 150°C by means of Colorimetric methods. He followed the change of colour, from a faint yellow to the darkest brown, and found the rate of colour change was different, and faster at the higher temperatures. When the reaction had reacted to a certain point, evolution of carbon dioxide commenced. It was established that atmospheric oxidation did not take part, and by analytical experiments Maillard found a hydrogen/oxygen ratio similar to that in water. He postulated that carbon dioxide resulted from decarboxylation of the amino acid, and the water came from dehydration of polyhydroxylic compounds.

A review of this topic was written by Hodge (1953). In this he put forward a reaction scheme, which on the basis of the processes then studied, suggested the route leading to the formation of Melanoidin.

The carbonyl-amino reaction between α-amino acids and reducing sugars apparently starts with a simple condensation to give a Schiff's base.

\[
R_1\text{-CHO} + H_2\text{N} + \text{C}=\text{COOH} \xrightarrow{\text{heat}} R_1\text{-C}=\text{N} + \text{COOH}
\]
If one tries to isolate this condensation product, it frequently disappears, being hydrolysed back to its constituents.

Lea\textsuperscript{6} (1950) and Lea and Hannan\textsuperscript{7,8,9} (1949-1950 a,b) believe that below a moisture content of 13\% or relative atmospheric humidity of 65-70\% the reaction is halted. Bull\textsuperscript{10} (1944) on the basis of the Brunauer, Emmett and Teller\textsuperscript{11} theory (1938) suggests that reaction between glucose and the amino group of proteins takes place most readily in a monomolecular film of water adsorbed on the protein. This is supported by the idea of mixing protein with 3:1 glucose and no more, which abundance of glucose represents a unimolecular layer on the protein surface. Different behaviour of the reaction under different conditions has been reported.

(a) The effect of moisture.
A complete dehydration of reaction at any stage brings the reaction to a halt\textsuperscript{7,8,9}.

(b) The effect of pH
The alkaline condition favours the reaction\textsuperscript{2}. The rate of reaction increases between 7.8 - 11.0 and during the reaction the pH falls.

(c) The effect of temperature
The rate of reaction increases with increasing temperature. The relative rate of reaction of a number of amino acids and sugar derivatives has been described\textsuperscript{12,13} but in the majority of cases, the development of the brown colour has
been used as the measure of the extent of reaction. The reaction occurs most readily in concentrated aqueous solutions and as reported above, is apparently favoured by increased pH and temperature.

Few studies of the reaction appear to have been made at temperatures in excess of 100°C, presumably because experiments were carried out in aqueous solution, although Akabori's original description of the reaction was the result of studies at 130°C in glycerol solution. Neutral and basic conditions having been employed by most workers. It has been suggested that a somewhat different course may be followed under acidic conditions. These are discussed in further pages.

The Chemical Nature of the Sugar/Amino Acid Reaction

The simplest mechanism which has been suggested is the reaction between glucose and a primary amine.

\[
\begin{align*}
\text{Glucose I} & \quad \text{Schiff base II} \quad \text{N-Glucoside III} \\
H-C=O & \quad H-C=NR & \quad H-C-NH-R \\
H-OH & \quad H-OH & \quad H-OH \\
HO-C-H & \quad H-OH & \quad H-OH \\
H-C-OH & \quad H-C-OH & \quad H-C-OH \\
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH}
\end{align*}
\]

The Schiff's base exists in equilibrium with a cyclic N-Glucoside similar to O-Glucoside. The compound formed
between glucose and amino-acids has not the same reducing power as glucose. Lea and Hannan⁹ suggest that this compound then undergoes Amadori rearrangement (discussed further below) to give a substituted iso-glucosamine.

\[
\begin{align*}
\text{H-C-NH-R} & \quad \text{H} & \text{H} & \text{O} & \text{H} & \text{H-C-OH} & \text{H-C-OH} & \text{HO-C-H} & \text{CH}_2\text{OH} \\
\text{Amadori rearrangement} & \quad \text{H}_2\text{C-NHR} & \text{H} & \text{H} & \text{O} & \text{H} & \text{H-C-OH} & \text{H-C-OH} & \text{HO-C-H} & \text{CH}_2\text{OH}
\end{align*}
\]

N-Glucoside III \quad \text{Substituted iso-Glucose amine IV}

Amadori rearrangement was suggested and elucidated by Weygand ⁵ (1940). This is the isomerisation of the N-substituted aldose amines to N-substituted 1 amino, 1 deoxy, 2 ketoses. This reaction requires an acid catalyst. The possible mechanism of the reaction is shown as below. In this scheme the nitrogen of the aldosylamine (I) accepts a proton from the acid catalyst to form the ammonium ion II, which is proposed to be in equilibrium with cation III, though the contribution of this cation to the equilibrium mixture will be only small. A shift of electrons as indicated in IV accompanied by the release of a proton from C₂ yields the eneaminol V which will establish equilibrium with the keto form and ring forms of the fructose derivatives Gottchalk ¹⁶ (1952).
The mechanism for the Amadori rearranged was elaborated later by Isbell and Frush (1958) (Figure I). The acidic catalyst adds onto the ring oxygen of the aldaminine I. Then a flow of electrons from the nitrogen atom of (II) promotes the formation of a Schiff's base III. This step is inhibited with very weak bases by the strong attraction of substituents (RR') for the electrons of the nitrogen atom. The electronic shifts indicated in III lead to the formation of an enolic amine (IV). This step is difficult with strong bases, because the substituents (RR') have little attraction for the electrons of the nitrogen atoms. However good yields of Amadori products were obtained from strong bases in the
Figure 1.

Figure 2.

5-Hydroxy methyl Furfural (V)
presence of molar proportions of organic acids. To explain this exceptionally strong catalytic effect, Isboll and Frush suggested that the carboxylate ion and the iminium ion VI combined to form a transient intermediate VII; an intramolecular decomposition, as shown, would liberate the carboxylic acid, and produce the enolic amine IVa. By a tautomeric shift as indicated the enolic amine IV gives ketoseamine IVb.

Gottschalk and Partridge suggested a mechanism involving 5-hydroxymethyl furfural, and pointed out that furfural and other aldehydic sugar decomposition products can also replace reducing sugars in the Maillard browning reaction, and these may be formed by sugar decomposition under acid conditions.

Gottschalk and Partridge, established the reaction scheme for glucose and lysine as shown in Figure II. They supported their scheme by duplicating the aromatic N-Glucosides when aniline D-Glucoside, or p-toluidine D-Glucoside and p-tolyl iso-D-Glucosamine were heated with 2 N acetic acid for 60 minutes at 100°C. The assays turned black within a short time and yielded 5-hydroxymethyl furfural (5H,M.F) which was detected by thin layer chromatography. In the case of lysine the spots of the same mobility were reproducible, and the authors reasoned that such a spot could only be obtained from the N-Glucoside, or from a mixture of 5-H,M.F with the appropriate base.
The 5-H.M.F (V) is formed from aliphatic or aromatic N-Glycosides, (IV) upon mild acid treatment. Most of the 5-H.M.F is derived from this source\textsuperscript{19,20}. Structure II has been invoked to account for the ready conversion of N-Glycosides of amino acids formed from their components at pH 8.5, to structure V upon mild acid treatment. Structure II is substantiated by the positive enediol test with O-dinitrobenzene, and alkali in the cold.

For N-Glycosides of primary aromatic amines it has been established that dilute acids catalyse isomerization to ketose derivatives (Amadori rearrangement) which in their cyclic form VI by loss of water will give intermediate III in the same way as D-fructofuranose yields V upon mild acid treatment.

Another approach to the reaction was made by Anet\textsuperscript{21-25} who studied non-enzymatic browning in freeze dried fruits. After prolonged storage at low temperatures, \( \downarrow \) (N amino) 1-deoxy fructoses and glucose (the Amadori rearrangement products of amino acid - sugar reaction) were isolated by column chromatography\textsuperscript{21}. Those compounds were slowly decomposed by acids to give amino acids and H.M.F and other unidentified products, but no free sugars. It was also shown that di-keto/amino acids could be formed under similar conditions, although those were decomposed readily by heating to give mono-keto derivatives\textsuperscript{22}.

Anet also studied the mechanism of acid catalysed sugar decomposition, and obtained evidence that this could
It was suggested that the compounds II to IV could exist in equilibrium with their ring forms. The intermediate III and cis form of the osazone of IV were isolated. As a result of these investigations an alternative type of reaction in amino acid/sugar mixtures may be postulated. Although amino acids have relatively high $P K_a$ values, under certain conditions they may be efficient proton exchangers in acid catalysed reactions, by virtue of their polar nature, and their abilities to form a series of ions with protons. This could promote acid catalysed reactions of the type demonstrated by Anet in amino-acid-reducing sugar reactions, and the reductones so formed (e.g. H.M.F) might participate in conventional Strecker degradation of the amino acids. (The Strecker degradation will be discussed later in this section).

The Strecker degradation of amino acids by reducing sugars was first suggested by Akabori in 1933 but it is only quite recently that interest in this reaction as a potential source of aromatic material has been revived. Schönberg and Moubacher suggested that only carbonyl compounds of structure $\text{C}-(\text{C}=\text{O})_n-\text{C}=\text{O}$ (where $n$ may be zero) can initiate the reaction. Most monosaccharides when heated alone give rise to numerous products some of which are volatile. This type of decomposition however usually requires temperature in excess of 250°C, or prolonged heating at lower temperature. Spontaneous sugar decomposition
Figure 3.

\[
\begin{align*}
R-\text{CHO} & \quad \text{H}_2\text{N}-\text{CH}-\text{R}^1 \\
\text{COOH} & \quad \text{COOH}
\end{align*}
\]

Figure 4.
is unlikely, at low temperatures but amino-acids may
catalyse sugar degradation in accordance with the above
mechanism.

Recent studies$^{28-29}$ into flavour and aroma development
in foodstuffs prove that the products of the Strecker
degradation react further e.g. aldol condensation, and
aldehyde/amino reactions of the Strecker products. There
are recent indications that heterocyclic compounds are
formed under fairly moderate conditions, and substituted
pyrazines have been named as likely primary aroma components.

The most recent publication on the Browning reaction
is by Holtermann, $^{31}$ (1969) . He performed the reaction
in acid and alkaline media pH=2.3 and 9.6, and found that
the reaction occurs most readily in alkaline solution.
They found that in acidic solution the amino acids are very
stable; there is typically less than 1% decomposition in
the course of 200 hours, and the reducing sugars were also
stable, and only a trace of decomposition products could be
observed on the paper chromatogram. Meanwhile in alkaline
solution, though the amino-acids were almost as stable as
before (1% decomposition matter of 100 hours) the reducing
sugars underwent great changes during the same period.

He observed the course of reaction by following the
colour change and the reducing power of the sugar, during
the reaction. They also observed liberation of carbon
dioxide and aldehyde.
He suggested a mechanism which had originally been put forward by Harbst and Engle\(^1\) (1946) and later by Schönberg and Moubacher\(^2\) in which a transamination reaction takes place. This involves the shift of a hydrogen atom after the loss of CO\(_2\), resulting in a shift of the double bond. This is quickly accompanied by hydrolysis of the product. The mechanism is represented in Figure 4.

Further work has been done on the identification of more products and the elucidation of the mechanism. It was reported, when glucose and glycine or phenyl alanine were heated together, 2:5 dimethyl pyrazine\(^1\) was detected among the products. Dawes and Edwards\(^3\) suggested that pyruvaldehyde participated in a Strecker degradation forming an amino carbonyl compound that condensed to 2:5 dimethyl 3, 6 dihydropyrazine (II).

\[
\begin{align*}
\text{(I)} & \quad \text{2:5 dimethyl pyrazine} \\
\text{(II)} & \quad \text{Effect of oxidation}
\end{align*}
\]

It is believed that some of the compounds in non-enzymic browning are promoted by oxidation. Dawes and Edward (1966) suggested that 2:5 dimethyl pyrazine was formed by oxidation of the dihydropyrazine.

The effect of oxygen on non-enzymic browning in foods is linked with both water content of the system and the
temperature of storage. When the water content of potatoes exceeded 25%, the rate of browning in air was greater than the rate in vacuum; the difference between these rates increased as the water content increased from 25% to 79%, although the rate of browning decreased. (Hendel et al. 1955). The maximum rate of browning occurred at 13-17% water content, and oxygen had little effect.

These experiments were carried out at 65°C.

The effect of oxygen on sugar-amine interactions is an example of an unsolved problem in the field, which is currently attracting much attention. It has received little attention in the past and illustrates some effects of water content and temperature. However, the oxygen may make the degradation more severe which may open up additional reaction paths.

**Brown Pigments**

The brown pigments isolated from sugar/amine, or aldehyde/amine interaction contain nitrogen. Reynolds did several experiments on identification of brown pigments from aldehyde and simple amines. He separated some of them by dialysis and characterised some basic groups, produced from glucose and glycine and citrate (8:1:1 mol, pH 3.6 and 6.1). The ratio of glucose to glycine residues increased from 3:1 to 6:1 as the time of reaction increased. The ratio of non-dialysable to dialysable pigments also increased. Infra-red spectra of pigments isolated from the model system containing glucose and glycine showed the presence of the following groups: \(-\text{OH}, =\text{CH}_2, \text{C}=\text{O} \text{H-H-C-C-O}, \text{H} \text{H} \text{H} \text{H}
\text{-CH=O-CH=CH-and COO}^-\text{.}
\text{H} \text{H} \text{H} \text{H} \text{H}
II Kinetics of reactions in solids

The vast majority of studies of chemical kinetics have involved reactions in the liquid or gaseous phases. The reasons for this are clear. Reactions of this kind take place in homogenous phase and give results which are fairly easily reproducible, and which can be analysed on the basis of simple theories.

Reactions between solids, by contrast, are essentially heterogeneous. Adequate theories of heterogeneous catalysis (i.e. reactions of liquids and gases on the surface of solids) are still lacking and very little work indeed has been carried out on reactions in solids.

It is believed that almost all chemical reactions between solids giving solid products are exothermic. However, a few endothermic cases are also reported which are mainly dehydrations of inorganic crystal hydrate salts as CuSO$_4$·5H$_2$O by Topley$^{35}$ or MnC$_2$O$_4$·2H$_2$O by Smith and Topley$^{36}$. Several cases of carbonate decompositions are also reported e.g. AgCO$_3$ by Spencer$^{37}$ and Topley and the semi-quantitative works on the kinetics of decomposition by Zawadski$^{38}$. The exothermic character follows from the fact that the overall driving force of these reactions is the difference between the Gibbs Free Energy of the crystalline reactants and the products.
Experimental Methods for the study of Solid-Solid Reactions.

1) Use of Differential Thermal Analysis.

In most cases of solid reactions (excluding the beginning of reactions between extremely fine solids) one may easily impose isothermal conditions due to the low reaction rate in the solid state.

The heat produced in a solid state reaction causes an appreciable temperature rise. This method has evolved into a technique of differential thermal analysis (D.T.A.). The basic principle of D.T.A. is the measurement of a temperature difference between a sample and a reference material as they are simultaneously heated at a uniform rate. A solid state reaction will cause an evolution of heat which will be shown as a temperature difference ($\Delta T$) between the sample and the reference material. As this heat is dissipated to the surroundings, $\Delta T$ reduces to zero again. Measurement of temperature and $\Delta T$ over a suitable range will give a thermogram, characteristic of the reaction which has occurred. Kissinger has developed a method to work out the order of reaction by this technique.

2) X-ray powder technique.

X-ray diffraction represents an ideal technique for the identification of what is occurring if the reaction involves a solid phase. It has been demonstrated that given a micro-crystalline powder, it is uniquely characterised by a Debye-Scherrer diffraction pattern. Two compounds even with an identical lattice structure will have different lattice parameters and will be characterised by different angular
distributions. An atlas of the powder diagrams containing 10,000 entries has been published by A.s.T.M.\textsuperscript{40}, representing a good means of identifying the substance and its composition in the case of a mixture. This technique is also useful in assessing the dimensions of a crystal in a polycrystalline sample\textsuperscript{41a}.

The outstanding disadvantage of this technique in solid reactions is its dependence of the crystallinity of the material; many intermediate products in solid reactions are formed transiently and in a poorly crystallised condition and they are consequently difficult to detect by means of X-rays.

3) The study of variation in mass: thermogravimetry.

When the reaction proceeds with an increase or decrease in the mass of the solid phase (because one of the products or reactants is gaseous) a thermobalance will enable one to obtain the variation in mass as a function of time and therefore to evaluate the degree of progress (extent of reaction) \( \alpha \) of the reaction, the curve \( \alpha = f(t) \) is traced by a device automatically. The first aim with thermobalance analysis is to know the nature of product and reactants to work out the kinetics of reaction. This technique is frequently used to analyse the kinetics of dehydration of crystal hydrates.

4) Optical Microscopy and Electron Microscopy.

When reactants or products are in the solid phase direct observation is sometimes helpful in identifying the more obvious cases of heterogeneity although simple visual
examination is not usually sufficient for the observation of interesting details. As with an optical microscope, the electron microscope is an instrument which gives an enlarged image of an object and several details invisible to the naked eye. The basic difference between the two instruments is the source employed: in optical microscopy the image of an object is made by a light beam while in electron microscopy an electron beam is used and the advantage of the electron microscope to the former is a higher resolving power and therefore it produces a magnified image of the order of 500 times greater than the optical microscope.

Theories of Reactions in Solids.

1) Prout-Tompkins equation and Namples Law.

The decomposition of solids and the reaction between solid inorganic compounds have been discussed by several workers who will be mentioned below. They discuss the kinetics mainly in terms of potential reaction sites (nuclei) and rates of nucleation. According to different factors, such as geometry of the particles and size and kind of nucleation development, equations have been derived to explain the various reactions.

Two of these theories are relevant to this work:

a) Chain nucleation with overlapping of the nuclei and ingestion of the potential sites of nucleation

In this case (Prout and Tompkins 1942) the reaction is assumed to take place via the existence of certain
potential reaction sites or nuclei. When the reaction starts these nuclei develop various shapes. The boundary areas of the nuclei increase so that initially there is apparently a rapid auto-catalytic increase in the rate of reaction. When the nuclei get so large that adjacent nuclei are touching their growth is interrupted at the surface of contact and the increase in their total volume becomes less than the value calculated on a sample model. Simultaneously there is ingestion of potential sites of further nucleation by the nuclei already formed. This reduces the effective rate of nucleation. Prout and Tompkins developed an equation for this kind of mechanism which is called Prout and Tompkins law.

\[ \ln \frac{\alpha}{1 - \frac{\alpha}{2\alpha_1}} = k_\beta t \]

where: \( \alpha \) is the degree of progress of the reaction at a time \( t \), and \( \alpha_1 \) is the degree of progress of reaction when it has the maximum speed, and \( k_\beta \) is a constant representing the average number of nuclei generated by a single overlapping nucleus. It is called the branching coefficient.

This equation could be written in the form below if the pressure of gases evolved is assumed to be proportional

\[ \ln \frac{2P_{f_1}}{P_f(2P_i - P_f)} = k_\beta t \]

where: \( \alpha = \frac{P_i}{P_f} \) and \( \alpha_1 = \frac{P_i}{P_f} \). \( P_f \) is the final pressure at the
end of the reaction and $P_1$ is the pressure when the rate is at the maximum.

If the reaction curve is sigmoid and symmetrical,

$$P_i = \frac{1}{4}P_f$$

b) Rapid growth of nuclei followed by ingestion of sites.

Chain nucleation is a mechanism capable of accounting for the progress of certain heterogenous reactions. The hypothesis is based on a) rapid nucleation b) numerous potential nuclei and c) ingestion of a significant quantity of potential nuclei by the developing nuclei. These also form the basis of Humpel's law\textsuperscript{43} which is a generalized relationship established on the basis of a) The parameters of the progress of a heterogenous reaction dependent on numerical potential sites b) The rapid growth of nuclei c) A progressive ingestion of the potential sites. The overall rate equation is represented below:

$$-\ln(1-\alpha) = A \int_0^t (t-t_0)^3 e^{-k_1 t} dt_0$$

where: $\alpha$ is considered as the degree of progress of the reaction, $aA(\text{Solid}) \rightarrow bB(\text{Solid}) + cC(\text{Gas})$

t is time,

t\text{\textsubscript{0}} is the time for $t\text{=0}$

$(t-t_0)^3$ depends on the growth of nuclei,

$k_1$ is a constant, at one of the steps,

$$A = \sigma k_1 N_o k_2 3aM \frac{N}{Wm}$$
where \( \delta \) is a constant depending on the geometrical shape of the particle e.g. for a sphere it is \( 4/3 \pi \),
\( N_0 \) is the number of potential sites,
\( M \) is the molecular weight of \( A \),
\( W_0 \) is the initial mass of \( A \),
\( N \) is the number of particles,
\( v_m \) is the critical volume of nuclei.

However the integrated form of this equation is:

\[
-l \ln (1 - \alpha) = A \left[ e^{-k_1 t} - e^{-k_2 t} - \frac{(k_1 t)^2}{2} + \frac{(k_2 t)^3}{6} \right]
\]

when the reaction is fairly advanced and has already progressed for a certain time it is considered that all the terms in the bracket on the right hand side of the preceding equation are negligible in comparison to the \( t^3 \) term.

The equation then simplifies to:

\[
\alpha = 1 - e^{-At^3}
\]

Rearranging:

\[
1 - \alpha = e^{-At^3}
\]

\[
-l \ln (1 - \alpha) = -At^3
\]

\[
\frac{3}{l} \ln (1 - \alpha) = 3\sqrt{At}
\]

Prout and Tompkins\(^{42}\) found that their experimental results on potassium permanganate were well fitted in the range of
0.1 < \omega < 0.9 in their equation and for lead oxalate and nickel formate in the range 0.08 < \omega < 0.8. Vaughan and Phillips applied this equation to the decomposition of mercury fulminate and obtained two straight lines inclined at an angle by plotting \log \frac{P}{P_0} against time.

Vaughan and Phillips also applied the equation to thermal decomposition of mono and dinitro-benzene-diazo oxide. They again obtained two straight lines at an angle to each other with two different k's. Yoffe studied the decomposition of the covalent azide, trinitro triazidobenzene. Below the melting point, reaction was very slow, but above the melting point rapid decomposition occurred. Several other cases of organic decomposition have been reported. In some of them the initial decomposition gave a product which formed a eutectic with the reactant and a subsequent reaction took place in the liquid phase at a greatly increased rate.

Farmer, Hinshelwood, Bawn and Pollard examined the thermal stability of 2,4,6 trinitro phenyl methyl nitramine (tetrayl) extensively. The initial decomposition was shown to give a product which formed a eutectic with tetryl and it is this liquid fraction which gives rise to the acceleration and velocity of decomposition. The solid organic decomposition has been treated kinetically according to the character of the compounds.

1) Decomposition without melting leads to the Prout-Tomkins equation which has already been discussed.
2) Decomposition with partial liquefaction can occur when the reaction takes place near the melting point of the reactant if the decomposition gives rise to a product which lowers the melting point of the initial substance. This results in a pressure-time (p-t) curve of sigmoid shape.

If the reaction is represented as below:

\[ A \xrightarrow{(1-x) x} B + \text{gaseous product} \]

and A is soluble in the product B, then if the molecular solubility of the initial material in the product is S, the fraction of A in the liquid phase is \( xS \) and in the solid phase is \((1 - x - xS)\). The rate constants for the decomposition of A in the solid and liquid phases respectively are \( k_s \) and \( k_l \), then the rate of total decomposition of A is given by:

\[ \frac{dx}{dt} = k_s (1 - x - xS) + k_l (xS) \]

where \( K = k_l - k_s - k_s \)

Integrating the equation and applying the condition \( t = 0 \) when \( x = 0 \) gives

\[ x = \frac{k_s}{K} e^{Kt} - 1 \]

This is similar but not identical with the answer obtained by Bawn\(^{49}\).

2) The kinetics of solid-solid reactions have been investigated by a number of workers. Several equations and methods have been suggested for the analysis of this kind of reaction and to work out a possible reaction rate constant. The first method was suggested in 1927 by Jander\(^{50}\) for the kinetics of
reactions between inorganic compounds reacting at high temperatures. The gas product of this reaction was a single component, CO\textsubscript{2} and the graphs he obtained were parabolic. His equation is based on the idea of a diffusion controlled reaction in a sphere and has the form,

\[ \left[ 1 - (1-\alpha)^{\frac{1}{3}} \right]^2 = (k/r^2)t \]

where \( \alpha \) is the weight loss or the quantity of one of the reactants consumed and \( k \) is a constant; \( r \) is the radius of the particles and by plotting the left hand part of the equation against time, Jamder obtained a straight line through the origin. This equation has been modified by others for a two dimensional diffusion controlled reaction into a cylinder of radius \( r \):

\[ (1-\alpha) \ln(1-\alpha) + \alpha = (k/r^2)t \]

A more complete analysis given by Valensi\textsuperscript{52,53} reduces to the above equation when the product volume is the same as that of the original material.

Ginstling and Brounshtein\textsuperscript{54,55} suggested an equation for a diffusion controlled reaction starting on the exterior of a spherical particle of radius \( r \):

\[ 1 - \frac{3}{3} \alpha - (1-\alpha)^{\frac{3}{3}} = (k/r^2)t \]

The kinetics of reactions in solids have sometimes been treated on the basis that product growth is controlled by nuclei growth. This approach considers the nucleation of the product phases at active sites and the rate at which the nucleated particles grow. The general form of the kinetic equation for the nuclei growth model is\textsuperscript{55-57}
\[ \text{lin}(1-\alpha) = -(kt)^m \]

where \( m \) is a parameter which is a function of (a) reaction mechanism (b) number of nuclei present (c) composition of parent and product phases and (d) geometry of the nuclei. If a solid state reaction can be represented by a nuclei growth model according to the above equation, a plot of \( \text{lin} \ln_1/(1-\alpha) \) vs. \( \text{lin} \ t \) should yield a straight line with slope \( m \) and intercept \( \text{lin} \ ln \). This happens to fit certain reactions e.g. between zinc oxide and barium carbonate at 1075 °C⁵⁸.

Avrami-Erofeev equations.

The most adaptable equation suggested for graphs of sigmoid type has been suggested by Avrami⁵⁵ and EroFeYeV⁵⁹ who have given the following equation:

\[ 2\sqrt{-\text{lin}(1-\alpha)} = t \frac{2}{\sqrt{k}} \quad (1) \]
\[ 3\sqrt{-\text{lin}(1-\alpha)} = t \frac{3}{\sqrt{k}} \quad (2) \]

where \( \alpha \) is the degree of progress of reaction, \( k \) is a constant and \( t \) is time.

The mechanism which is based on a phase boundary controlled reaction assumes that the nucleation step occurs virtually instantaneously, so that the surface of each particle is covered with a layer of product. Production of nuclei, however, may be a random process, not followed by surface growth. As nuclei grow larger, they must eventually impinge on one another so that the growth ceases where they touch. These are considered in the above equations.

The equation 2 is remarkably similar to the Nample
equation derived previously with some minor differences in definition.

**Activation Energy of Solid Reactions.**

These are measured in various ways, from the temperature coefficients of (a) the rate of growth of nuclei (b) the induction periods of solid reaction (c) the overall rate of reaction and, where checks can be made between the various methods, the results are usually in agreement.

The induction period prior to explosion in solids was investigated by Ubbelohde and he reported that it depended on a number of factors such as temperature, and quantity of material used. The relation between the length of the induction period and the ignition temperature has been derived on the basis of both the thermal theory of explosion and the chain theory,

\[ \log \tau = B + \frac{E}{RT} \]

where \( B \) is a constant and \( E \) is the activation energy, and \( \tau \) the induction period. The activation energies so far measured range from 0-60 kcal/gram mole and in general those changes which occur at measurable speeds at low temperatures have lower activation energies than those occurring at higher temperatures. Jander reported that activation energy varies with particle size in solid-solid reactions.
EXPERIMENTAL

The purpose of the present work is to follow the kinetics of the amino acid/glucose reaction in the dry state. It was hoped that the products of the reactions in the solid phase would be simpler than those in the liquid phase. T. Rohan of the British Food Manufacturing Industries Research Association observed that when dried glucose is mixed with a dried amino acid, and the mixture heated to about 130°C a reaction takes place to give an unspecified mixture of products. This reaction is unexpected because one does not expect chemical reactions between dried solids. Furthermore, Maillard has claimed that this particular group of reactions is halted by dessication. This is important since the technique of freeze drying used in the food industry is dependent on the preservative effect of drying.

We propose to investigate the reaction from the following angles:

1) Kinetics of the reaction; e.g. order of reaction, dependence of rate on temperature and particle size, comparison of rate data with theories of solid-solid reactions.

2) The mechanism of the reaction. i.e. where it takes place, and what happens at the interface, and the products produced at different stages of reaction.

3) Nature of the products.

Work on systems of this kind in the liquid phase and also work on the thermal decomposition of solid glucose has resulted in the formation of a very large number of
products. We hoped to avoid a multiplicity of products:
a) By operating under vacuum and not in the atmosphere and
b) By using sensitive analytical methods which permit
experiments to be carried out at relatively low temperatures.

The majority of experiments were performed on a con-
ventional vacuum line. This was designed and built on a
Dexion framework. The line as shown in Figure (I) consists
of two parts, a reaction vessel and a sampling and pumping
system linked by two three-way taps. The two parts were
connected to manometers.

The line was connected via a liquid air cold trap to
a speedivac vapour diffusion pump, using Apiezon B as the
working fluid. The diffusion pump was backed by a Speedivac
SD39 rotary oil pump. A satisfactory vacuum could if
necessary be maintained with the rotary pump and liquid air
trap alone. The vacuum was checked by a Genevac thermocouple
gauge type TCG5. A vacuum of up to $10^{-4}$ torr could be
obtained.

The reaction vessel was as shown in Figure (I) and was
immersed in a thermostat bath. Four millimetre diameter
pellets were made from the reaction mixture using a pellet
press and were placed in the spoon and weighed. This was
then inserted into the ground glass side arm of the reaction
vessel. The spoon held the compound suspended a distance
above the thermostat bath so they were not initially heated
to the temperature at which reaction began. The system was
then evacuated. After the materials had degassed the spoon
was turned over, and the pellets were allowed to drop on the bottom of the reaction vessel where reaction took place. The top part of the reaction vessel had a finger dipping down in the container. This could be filled with solid carbon dioxide and acetone, or ice and acetone, and used to condense the less volatile products of the reaction. The pressure change from the gases evolved could be measured on one of the manometers.

The heating system used was an automatic thermostat liquid bath, type HB-Grant instrument, with a stirrer to keep the temperature uniform. A polyalcohol detergent was used as the bath liquid. It could be used at temperatures up to 200°C with very little evaporation. The taps were all greased with Apiezon T, which is recommended for high temperature work.
RESULTS

I) Kinetic runs on gas line

II) Kinetic runs on thermogravimetric balance

III) Electron Microscope studies

IV) Chemical analysis and identification of products

V) Mass spectrometric investigations
1) The Kinetic Runs on Gas Line.

The solid-solid reaction which was studied in greatest detail was the reaction between glucose and valine. A programme was planned to investigate the following phenomena:

1) Typical reaction curves and the effect of temperature.
2) The effect of particle size on the rate of reaction and on the activation energy.
3) The effect of composition (using A + variable B, or B + variable A).
4) Effect of humidity.

1) Typical Reaction Curves and the effect of temperature.

Glucose and valine were powdered finely with an agate pestle and mortar, and were sieved through a 200 mesh number sieve, each separately. Mesh number 200 has openings 0.0029". The mesh number indicates the number of holes per linear inch.

A 1:1 molecular mixture of the two reactants was prepared and pellets were made up from the mixture. A constant quantity of the mixture was taken each time for the experiment. The reaction was carried out under vacuum at constant temperature and the pressure change was read on the manometer and recorded as a function of time. Typical results are shown in graphs (1),(2),(3),(4) for arbitrarily chosen temperatures of 119°C, 124°C, 129°C and 134°C. The graphs generally obtained are of the sigmoid type and, as is shown, consist of three stages. The first is shallow and usually
Mesh 200
1:1 Mixture
\( t = 119^\circ C \)

**FIG. 1.**

Time in minutes

\[ 0 \quad 50 \quad 100 \quad 150 \quad 200 \quad 250 \]

\[ 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \]
Mesh 200
1:1 Mixture
t = 124°C

Fig. 2.
Fig. 3.

Mesh 200
1:1 Mixture
\( t = 129^\circ\text{C} \)
Fig. 4.

Mesh 200
1:1 Mixture
$t = 134^\circ C$
does not take more than four or five minutes. This period could be taken as the induction period. No colour change could be seen in this period, and no pressure change was noticed. This stage was followed by an abrupt increase of pressure over a short period of time and produced over \( \frac{3}{4} \) of the total pressure change. It appeared that the major reaction product was evolved in this stage. The third stage was slow and led to 'completion' of reaction. As shown, it took about \( \frac{3}{4} \) of the total period needed for the reaction, for gas evolution to come to a halt, within the limits of detection.

The time for 'completion' of reaction is not exact, and depends on different factors, which will be discussed in the other sections. The reason for the sigmoid rate curves could be autocatalysis. This will be discussed in the second section of chapter 5.

As is shown in graphs 1, 2, 3 and 4, the reaction is temperature dependent. The reaction goes faster at the higher temperatures, and this is shown by a change in slopes of the reaction curves, and also the induction period, and time of 'completion' of the reaction.

At lower temperatures, the induction period is longer and the curve is shallower than at higher temperatures. The total gas produced increases significantly for each temperature increase. It will thus be seen that the reaction goes more nearly to completion at higher temperatures. This was accompanied by the development of a brown colour on the
pellets. It was interesting to note that the solid residue after experiment at low temperatures did not change in appearance during the reaction. Meanwhile, the pellets which reacted at higher temperatures were uniformly brown, and deformed, and appeared to have a porous and brittle structure. It was shown that the reaction curves were reproducible at lower temperatures but less reproducible at higher temperatures.

The reaction did not go to completion at any of the temperatures studied. The solid residue of the reaction was analysed and it was found that a certain amount of valine was left unreacted (see section on chemical analysis). A number of reasons could be advanced to account for this:

a) Poor contact between the pellets and the reaction vessel, and poor conduction of heat through individual pellets and between them could result in the centre of the pellets not reacting in the time span of the experiment. This is unlikely because prolonged heating did not result in more extensive reaction.

b) Adequate mixing of amino-acid and glucose crystals might not occur. i.e. there may be many crystals of one reactant which are not in physical contact with a crystal of the other reactant, and which do not therefore react. There is a theoretical interpretation of this which will be discussed later.

c) Build up of inert products at crystal interfaces where reaction takes place,
The Effect of Particle Size and Temperature on the Rate of Reaction.

To investigate the effect of particle size, glucose and valine were powdered separately and were sieved through a series of different sieves of mesh numbers 100, 150, and 200. Mesh 100 has openings 0.0058". Mesh 100 pellets were made up from different mixtures of different particle sizes. The experiments for each mixture of each particle size were performed at different temperatures i.e. 119°, 124°, 129°, and 134°C. The results are shown on graphs 5, 6, 7, and 8 for mesh number 100 at the four above-mentioned temperatures. Graphs 9, 10, 11, and 12 show the reaction rate for particles of mesh number 150 at the same temperature; and graphs 1, 2, 3, 4 show graphs for the finest particle size, mesh number 200 at the same temperature.

The total pressure of gases produced ultimately for each particle size at the same temperature is different. As the particles become finer the total ultimate pressure increases.

It has been suggested by Jander that the activation energy of a solid-solid reaction, in general, changes with particle size. To test this hypothesis, on this particular organic reaction, and to compare this system with inorganic systems, Arrhenius plots for each particle size were performed. The rates were taken as proportional to the slopes of the straight parts of the sigmoid curves. It will be seen that a plot of log(slope) vs. reciprocal absolute temperature
Mesh 100
1:1 Mixture
$t = 12\frac{1}{2}^\circ C$

Fig. 6.
Mesh 100
1:1 Mixture
$\theta = 129^\circ C$

Fig. 7.
Fig. 9.

Mesh 150
1:1 Mixture
$t = 119^\circ C$
Mesh 150
$t = 129^\circ C$

Fig. 11.
Mesh 150
1:1 Mixture

Fig. 12.
Fig. 13.

Mesh 100

1:1 Mixture

$log$ slopes
$\text{cm Hg min}^{-1}$

$\frac{1}{T} \times 10^{-3}$

Fig. 13.
log slopes.

\[ \text{C}_\text{O}_2\text{Hg min}^{-1} \]

Mesh 150

1:1 Mixture

\( \frac{1}{T} \times 10^{-3} \)

Fig. 14
gives a reasonable straight line, using the conventional Arrhenius equation $k = A e^{-E/RT}$. The activation energies were obtained from the slopes of each graph for different particle sizes as shown in Figures 13, 14, 15. The activation energies are shown in the table below:

<table>
<thead>
<tr>
<th>Mesh 100</th>
<th>35.7 Kcal per mole.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesh 150</td>
<td>34.3 Kcal per mole.</td>
</tr>
<tr>
<td>Mesh 200</td>
<td>34.3 Kcal per mole.</td>
</tr>
</tbody>
</table>

The data obtained for the particle sizes are shown in Appendix I. The average activation energy for each particle size does not vary significantly with the change of particle size. This is contrary to the Jander and Komatsu theories of the dependence of activation energy on particle size based on studies of inorganic reactions at high temperatures. Their idea is that more energy is required to remove a molecule from a surface at low curvature than from one of higher curvature. Therefore, the activation energy should increase with particle size. This factor must in some way be modified in our system since the expected effect is not observed.

At any given temperature, the rate varies with particle size as shown for a constant temperature. The reaction curves at other temperatures for varying particle size may be obtained from graphs 1 to 12.

The slopes found from the gradient of straight-line sections of reaction curves of previous figures 1-12 are shown overleaf:
Table II

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Mesh 100&lt;sup&gt;®&lt;/sup&gt;</th>
<th>Mesh 150&lt;sup&gt;®&lt;/sup&gt;</th>
<th>Mesh 200&lt;sup&gt;®&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>0.869</td>
<td>1.442</td>
<td>1.856</td>
</tr>
<tr>
<td>124</td>
<td>1.592</td>
<td>2.222</td>
<td>3.703</td>
</tr>
<tr>
<td>129</td>
<td>3.258</td>
<td>4.9</td>
<td>6.1034</td>
</tr>
<tr>
<td>134</td>
<td>5.717</td>
<td>7.045</td>
<td>9.4736</td>
</tr>
</tbody>
</table>

<sup>®</sup>cm·Hg·min<sup>-1</sup>

It might have been expected that the rate would be proportional to the surface area of the reactants. If so, it would presumably increase inversely with the square of their diameter. The above data show that this is not so in the above case. To a first approximation, the rate is directly proportional to the mesh number, i.e., inversely proportional to the diameter.

Summarizing the data, therefore, it appears that the activation energy is about 34 Kcal and independent of temperature, and the reaction increases in rate with decreasing particle size.

3) The Effect of Composition.

An attempt was made to investigate the effect of composition, and to see whether the increase in proportion of one of the reactants had any effect on the rate of reaction. Maillard and the previous workers used glucose in excess of amino acid (e.g. 4:1 mixtures).

To investigate this, experiments were carried out,
Fig. 16.

$\Delta P$ (cmHg)

$T = 134^\circ C$

- 1:1 valine/glucose
- 2:1 valine/glucose
- 3:1 valine/glucose

Time in minutes

Fig. 16.
$T = 134^\circ C$

![Graph](image)

**Fig. 17.**
Fig. 18.

\[ \frac{\Delta P}{\text{cmHg}} \]

\[ t = 134^\circ \text{C} \]

1:1 glucose/valine

glucose/valine

1.3:0.75

Time in minutes
starting with mixtures of three parts of valine to one part of glucose, and two parts of valine to one part of glucose, as well as a 1:1 mixture. The results are shown and compared in Figure 16. It was hoped to see if more glucose would react assuming that part of the unreacted valine comes into more contact with glucose in glucose rich mixtures. The pressure changes and the slopes obtained were relatively lower than the rate obtained for the 1:1 mixture, at the same temperature. The slope of the straight part of the pressure change versus time graph was plotted against the molar ratio of glucose to valine. A straight line was obtained with a positive slope, indicating that, as the proportion of valine decreases towards the 1:1 mixture, the rate gradually increases. Also a few experiments were carried out keeping the quantity of valine constant, and increasing the quantity of glucose with respect to valine. Using glucose/valine ratios of 3:1, 2:1, 1.3:1, 1:1 mixtures, Figures 17, 18 were obtained. Plotting the slope of each reaction rate versus the molar ratio of valine to glucose gives a straight line as is shown in Figure 19 and 20. There is clearly no simple relationship between composition and rate but the order of reaction is positive with respect to both reactants. These are to be analysed and verified in the discussion later.

The quantity of each reactant in the mixtures was worked out in terms of weight, moles and volume taking the densities of glucose $\rho = 1.562$ and valine $\rho = 1.316$. The results are in Table III below:
<table>
<thead>
<tr>
<th>Relative Rate</th>
<th>V:G Ratio</th>
<th>V</th>
<th>G</th>
<th>Weights (mg.)</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>3:1</td>
<td>1.695</td>
<td>0.565</td>
<td>198.3</td>
<td>101.7</td>
</tr>
<tr>
<td>12</td>
<td>2:1</td>
<td>1.449</td>
<td>0.725</td>
<td>169.6</td>
<td>130.4</td>
</tr>
<tr>
<td>15</td>
<td>1:1</td>
<td>1.010</td>
<td>0.010</td>
<td>118.2</td>
<td>181.6</td>
</tr>
<tr>
<td>13.5</td>
<td>3:4</td>
<td>0.840</td>
<td>1.120</td>
<td>98.3</td>
<td>201.7</td>
</tr>
<tr>
<td>12</td>
<td>1:2</td>
<td>0.629</td>
<td>1.258</td>
<td>73.6</td>
<td>226.4</td>
</tr>
<tr>
<td>11</td>
<td>1:3</td>
<td>0.457</td>
<td>1.370</td>
<td>53.4</td>
<td>246.6</td>
</tr>
</tbody>
</table>

Table III

\((v = \text{valine}) \quad (G = \text{glucose})\)

4.) Humidity

Maillard\(^3\) found that the amino acid/glucose reaction was halted by dehydration of the reactants mixture inside an oven. Lea\(^6\) and Lea and Hannan\(^7,8,9\) found that in an atmosphere containing less than 13% of moisture (relative humidity 65 - 70%), the reaction was halted. There was an optimum level of humidity for the most rapid reaction.

The whole of the experimental work in this thesis contradicts these results. On all occasions the reactants were dried before being mixed and pelletized, and pellets were subsequently left under vacuum for a few hours until a vacuum of 10\(^{-3}\) torr was obtained. There was thus no chance of
an appreciable amount of water being left in the system. Nonetheless, an experiment was performed to examine the effect of particularly intensive drying. Powders of amino acid and glucose were placed in the same reaction vessel but separated into two small containers. The two powders were heated for a few hours under a vacuum of $10^{-3}$ torr. Then the powders were mixed under vacuum and heated again. The reaction occurred smoothly at the usual temperature.

It is clear therefore, that under conditions used in this work, the glucose valine reaction will take place in the absence of detectable quantities of water. In view of the results of the other workers, a few runs were performed in the presence of small amounts of moisture. Water was placed in a small container on one of the entries to the gas line and allowed to diffuse into the reaction vessel before a run was performed. Figures 21 and 22 show the results obtained at two different temperatures $129^\circ C$ and $134^\circ C$. The rates apparently drop as the amount of moisture increases, a result again in contradiction to those of previous workers.
The effect of humidity on the reaction
The effect of humidity on the reaction

Fig. 22.

The effect of humidity on the reaction
Several workers have chosen to follow the kinetics of solid-solid reactions by weight loss. This has been on the basis that the product was an identified gas, therefore the weight loss was directly equal to the amount of gas evolved. In order to confirm the significance of the kinetic runs on the gas line, and in order to correlate the weight loss on the thermogravimetric balance (T.G.B.) with the pressure produced in the gas line, a few runs were performed at temperatures of 120, 125, and 134°C. Samples of 150 mgms of the mixture were weighed in a platinum crucible, and were placed successively in the furnace of the thermogravimetric balance. The temperature was adjusted to the desired value. The gaseous products were carried away by a stream of nitrogen. The sample used in most of the thermogravimetric experiments was only half the weight of that used in the gas line experiments, because of lack of space in the platinum crucible. The kinetic curves run at the above temperatures are compared in Figure 23. An attempt was made to make a correlation between the kinetic runs obtained on the gas line and the ones on the T.G.B.. The rate of weight loss obtained on the T.G.B. was doubled, to make it comparable with the 300 mgm samples used on the gas line. The final weight loss on the T.G.B. curves was taken to be equivalent to the final pressure on the gas line at corresponding times, then the weight loss at different time intervals was converted to equivalent cm. Hg pressure on the
gas line.

The reaction curves obtained by both systems were compared by the above method. 106mgm weight loss was found to be equivalent to 17.8 cm. Hg pressure after 80 minutes. Figure 24 shows the reaction curves at 134°C superimposed. The agreement between them is excellent at longer times, but there are deviations in the early stages of the reaction. The validity of this comparison is confirmed by the observation that at 134°C, the weight lost by the 150 mgm sample on the T.G.B. was 52.8 mgm, while that lost by the 300 mgm gas line sample was approximately double i.e. 106 mgm. The difference of 54 mgms could be due to the gas line sample being weighed after two hours while the T.G.B. sample was finally weighed after 80 minutes. The reaction curves obtained on the T.G.B. seemed to rise slightly faster than the gas line curves and this effect was also noted in the runs at lower temperatures. Possible reasons for this include:

a) Catalytic effect of the crucible metal (platinum). The catalytic effect of metals has been noted by Rohan.⁶¹

b) Heat transfer was more efficient inside T.G.B., because metal conducts better than glass, and the T.G.B. sample was surrounded by a flow of nitrogen instead of being in a vacuum.

c) If the composition of products changes with time, the two curves would not coincide even in theory e.g. there is evidence elsewhere in this work that water continues to
log relative slopes.

![Graph showing weight loss in mgms vs time in minutes at different temperatures.](74.)

**Fig. 23.**

**Fig. 25.**

\[ \frac{1}{T} \times 10^{-3} \]
Fig. 24.
come off after iso-butyraldehyde. Therefore if the early product mixture is richer in iso-butyraldehyde and carbon dioxide than the later mixture, then the T.G.B. curve would be expected to rise faster anyway.

The second method used to correlate the significance of the kinetic runs on the T.G.B. with those on the gas line was by comparing the activation energy obtained by both systems. The logarithm of the slopes of the straight parts of the reaction curves were plotted against the reciprocal of absolute temperatures for each reaction. A straight line was obtained through the only three available points, and an activation energy of 31 Kcal was determined. The graph is as shown in Figure 25. This was in good agreement with the value of 34 Kcal obtained on the gas line.

The data obtained are given in the table below:

<table>
<thead>
<tr>
<th>t°C</th>
<th>1/T x 10⁻¹⁴</th>
<th>slopes of the straight part</th>
<th>log slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>25.499</td>
<td>1.07 mg·min⁻¹</td>
<td>0.02938</td>
</tr>
<tr>
<td>124</td>
<td>25.178</td>
<td>2.00 &quot;</td>
<td>0.3010</td>
</tr>
<tr>
<td>134</td>
<td>24.567</td>
<td>4.63 &quot;</td>
<td>0.66558</td>
</tr>
</tbody>
</table>

For the reasons given, the gas line and T.G.B. runs could not be expected to correlate exactly. The extent of the agreement between them suggests that similar phenomena are being observed in each case and that there are no serious systematic errors in either method.
III Electron Microscope Studies.

An electron microscopic technique was used to follow the reaction between the two sets of crystals. A method was first established to permit observation of particles reacting under the electron beam. The sample must be spread on a special fine copper grid of a total width of half a centimetre, having on it a network of a certain mesh number, as shown:

![Copper Grid Diagram]

The thickness of the layer should not exceed more than 300 Å, otherwise the beam does not pass through the layer. The particles of mesh size 200 of glucose and valine were suspended separately in acetone. (Acetone was chosen as the medium because neither components were greatly soluble in it and it is easy to evaporate). The electron beam heats up the system and thus initiates the reaction. It may also cause some ionisation, involving damage to the crystal lattice structure. In order to protect the crystal as far as possible from the latter effect (which occurs almost instantaneously) a technique was employed in which the crystals were covered with a film of carbon. This is highly recommended for high resolution work.

The carbon films were prepared on glass plates maintained under vacuum. High voltage sparks were passed between two graphite poles to create the film.
The resultant carbon film could then be grooved with a needle creating a small square the size of the copper grid. The glass plate was then slid into water to detach the film which was floated in the water surface in square pieces. A carbon film square was transformed to a number of grids.

The suspension of the particles of reactants were sprayed on to the grid with an atomizer, and the acetone allowed to evaporate. The resulting grids were put on a glass plate in vacuo and the arc struck again. The grids were thus covered by another layer of carbon film so that the crystals were protected by two layers in the form of sandwich. The samples were placed in the electron microscope. It was hoped that the heating caused by the electron microscope would promote the reaction. The photographs (1), (2), (3), and (4) as shown were taken at a magnification of 2500 times.

Crystals of glucose normally exist as long rods and cubes. Picture (1) shows a crystal of glucose as a long rod, cubic crystals were not observed in this experiment. The crystals of valine were much smaller in size and had no consistent shape. The sample of reactants when exposed to the electron beam, started to react immediately. This is probably due to the heating of the sample by the electron beam of the microscope. When the reaction started, the gases evolved could well have fractured the carbon film and the tears can be seen on the corner of photograph (3).

The photographs of the completed reaction show solid material surrounding the position in which the glucose
crystals had been. The centre of the crystal, however, has become less dense and may have disappeared completely. What appears to have happened is that the valine and glucose have reacted on the surface of the glucose to give a layer of inert product. Reaction has continued until the layer was too thick for glucose to diffuse efficiently from the centre of the crystal into the reacting zone and at this stage reaction ceased.

Study of the reaction on the heated sub-stage of an optical microscope confirmed this picture and suggested a degree of liquefaction inside the glucose crystal, the liquid being retained by the layer of inert product and the gaseous products escaping through gaps in it.
IV Chemical Analysis and Identification of Products.

Analysis of the product has been the focus of previous workers, who were primarily interested in the reaction in solution. We wished to know the mechanism and the products of the reaction in the dried state.

The products of the reaction were collected in three stages:

1) The gaseous products.
2) The solid residue which was left behind.
3) The products which sublimed on the walls of the reaction container.

However, traces of products from one stage often were detected in the other stages.

1) The gaseous product.

The first products that came to attention were the gaseous products. Initial experiments showed that carbon dioxide was a major constituent. Figure (26) shows an experiment done to determine the rate of evolution of carbon dioxide. A cold finger was used to freeze out the other volatile organic compounds so that only the carbon dioxide pressure was recorded on the manometer. The cold finger was cooled by solid carbon dioxide and acetone.

The volatile organic compounds were found to constitute half the total gases produced. They were difficult to collect in a sizeable quantity for analysis. The gaseous products were liquefied by liquid air in a specially designed trap. Primary analysis was done by mass-spectrometry. The most
Fig. 26. Rate of evolution of CO$_2$ at 134°C using Cardice to freeze the volatile compounds.
abundant peaks were observed at masses 18, 43, 44, and 72. Other major peaks were observed at mass numbers 28, 29, 39, 41, 43, 55, 56, 57, 60, and minor peaks at higher mass numbers 122 and 144. A diagram of the spectrum is shown in Figure 27. When only gaseous products were submitted to the mass spectrometer only 72, 60 were seen with other fragment ions at lower mass numbers.

Gas liquid chromatography (G.L.C.) was used to detect the number of components in the gaseous products. For this purpose, two columns were used, one containing Poropak Q (commercial name) at temperature 150°C, and the other dinonylphthalate (D.N.P.) at temperature 70°C. The gas samples were collected in evacuated sample bottles with taps and were introduced by a gas tight syringe into the G.L.C. system. Four components were detected, of which one was much more abundant than the others, i.e. the peaks on the D.N.P. column were in the ratios 10:18:0.6:0.5. The compound corresponding to the most abundant peak was presumably responsible for the large peak at m/e = 72 in the mass spectrum. Other aspects of the mass spectrum obtained e.g. the peak at mass 43, suggested a compound with a carbonyl group. The peak could be due to the hydrocarbon fragment $\text{C}_3\text{H}_7^+$ or to the oxygenated ion $\text{CH}_2\text{CO}^+$ though the latter does not arise from the higher aldehydes.

To investigate this, a few aldehydes and ketones were used as reference compounds on the G.L.C. column to try and identify the major reaction products. Isobutyraldehyde
Fig. 27. Mass-spectra of Condensed Gaseous Products from the Glucose/Valine Reaction
gave a split shallow peak on Poropak Q, which had virtually the same retention time as the highest peak given by the reaction products. This gave some reason to assume that mass 72 is the isobutyraldehyde. The fragmentation of isobutyraldehyde is known and the major peaks (in order of abundance) are 43 (C\(_3\)H\(_7\)^+), 41 (C\(_3\)H\(_5\)^+), 72 (parent ion), 27 (C\(_2\)H\(_3\)^+), 29 (CHO or C\(_2\)H\(_5\)^+), 39 (C\(_3\)H\(_4\)^+), 42, 28, 44, and 57. The most significant fragmentations are apparently 1) \(\text{CH}_3\text{--CH}\text{--CHO}\) and 2) \(\text{CH}_3\text{--CH}\text{--CHO}\) by loss of either methyl group.

In order to confirm this result, further investigations were carried out by Nuclear Magnetic Resonance and later by Infra-red techniques. The vapours liquefied over a series of runs were collected, and the liquid was mixed with carbon tetrachloride (CCl\(_4\)). The major component was extracted weakly in solvent, leaving a layer of water which was also presumably produced in the reaction. The carbon tetrachloride layer was used for N.M.R. purposes.

The infra-red spectrum was obtained using a silver chloride liquid cell, of path length 0.5 mm. since the components could not be freed of water easily. The CCl\(_4\) solution containing the extract was injected into the liquid cell.

The spectrum showed a sharp peak at 1705 wave-numbers, characteristic of carbonyl group absorption, and
also a peak at 2995 wave numbers indicated the C-H stretching. A series of peaks of medium intensity also occurred at wave numbers below 1600 which may be associated with H-C vibrations of the $\text{CH}_3>\text{CH}$ group.

Analysis by N.M.R. confirmed this result. The solvent $\text{C}_4\text{H}_8$ has no proton to appear in the spectrum and therefore was ideal. The N.M.R. spectrum of the compound showed two doublets appearing as one cluster at chemical shift $\delta = 1.2$ showing the two methyl groups joined to the neighbouring group, and seven shallow peaks at $\delta = 2.3$ showing $\text{CH}$ methyne group and also a doublet due to the proton in the aldehyde group CHO at $\delta = 9.6$.

We conclude therefore that one of the main volatile products of the glucose/valine reaction is isobutyraldehyde.

Further work was carried out to identify the minor components of the gaseous product. The mass spectrum of the gaseous product showed two minor peaks at m/e 114 and 122 as well as a peak at m/e 60. It was suspected that mass 60 could be acetic acid and the appearance of a fragment ion at m/e = 45 supported this. The gas was examined by G.L.C. fitted with a katherometer. A peak appeared at the same retention time as for the acetic acid used as reference. The production of acetic acid has been reported by Simon and Heubach$^{64}$ (1965) from the reaction between glucose and basic secondary amines, as well as by Hodge and Fisher and Nelson$^{65}$ (1963). Mass 122 was only observed as a shallow
peak and it is considered as a minor product. This compound is suspected to be dimethyl pyrazine identified by Dawes and Edwards (1966) from the volatile products of glucose and glycine. Mass 144 is a trace compound from the second stage which is discussed below.

2) Sublimed products.

After an hour's heating of a valine/glucose sample at 134°C, it was seen that part of the product coated the glass wall of the reaction vessel in the form of a brown colour as well as a white coating. This was washed carefully from the vessel with methanol and the solution left to dry. In spite of its probably being a mixture, a mass spectrum of the residue was run. The sample was introduced on a direct insertion probe into the ion source which was at 100°C. A reasonable spectrum was observed, showing a large peak at m/e 144 which appeared to be a parent peak.

The fragmentation pattern suggested that this compound could be the alleged reductone intermediate thought to be produced from the reaction between secondary amines and reducing sugars. The mass spectrum shown in Figure 28 suggests that the fragment at m/e 101 is obtained from the parent peak at 144 by loss of CH₃CO⁺ (43). Other peaks observed at lower mass numbers were at 84, 72, and 60. The peak at 72 could be a trace of isobutyraldehyde left in the compound, and the peak at m/e = 60 is probably acetic acid, as reported earlier in this section. The peaks at lower mass numbers such as 57, 55, 45, 44, 43, 42, 41, 39, and 31 were
also observed as shown in Figure 28. These were assumed to be partly the fragment ions from mass 72 i.e. 57, 55, 39, 41. The $P_{M+1}/P_M$ ratio of the compound of m/e was 14% was found to be 6.904%. The nearest ratio found to the above figure was at $P_{M+1}/P_M = 6.762\%$, corresponding to the empirical formula $C_9H_8O_4$. Evidence of functional groups in this compound was obtained by the use of an infra-red technique (Unicam SP200). Nulls of the impure compound were made in Nujol and hexachlorobutadiene. Fairly shallow peaks appeared (Nujol mull) at wave numbers 1610 and 1740. The former was characteristic of an alkene double bond conjugated to a keto group, and the latter probably corresponded to a diketone absorption, normally appearing in the region 1710-1730 wave numbers.

The second spectrum (hexachlorobutadiene mull) showed a fairly wide peak at 3400 wave numbers corresponding to a hydroxyl group, which normally appears in this region and another sharp, moderate peak at 2950 wave numbers characteristic of the C-H stretching mode.

The above information seemed to add more convincing evidence of the alleged compound $^{65}$:

$$\begin{align*}
\text{CH}_3 & \text{C} \equiv \text{C} \equiv \text{C} \equiv \text{C} \equiv \text{C} \equiv \text{CH}_3 \\
\text{O} & \text{OH} \text{OH} \text{OH}
\end{align*}$$

On general chemical grounds this would be expected to be unstable and may well exist in the form:

$$\begin{align*}
\text{CH}_3 & \text{C} \equiv \text{C} \equiv \text{C} \equiv \text{C} \equiv \text{C} \equiv \text{CH}_3 \\
\text{O} & \text{OH} \text{OH} \text{OH}
\end{align*}$$

A second sample of the sublimed product was prepared
Fig. 28. Compound at Molecular Weight - 144
and treated with 2:4 dinitrophenylhydrazine. A precipitate was obtained, which was filtered off and dried. The mass spectrum of the compound showed no mass number corresponding to the dinitrophenylhydrazone of the above compounds. The spectrum obtained showed a parent peak at m/e = 264, corresponding to a carbonyl compad of mass 84. This was not identified at this stage.

The filtrate was evaporated to dryness, and an orange yellow compound was crystallised. This compound decomposed at near 100°C. Its mass spectrum showed what was apparently a parent peak at m/e = 183. This could indicate the presence of an odd number of nitrogen atoms. The M+1/M ratio was of the magnitude 9.4%. The nearest figure found in the standard tables was 9.344%, suggesting a formula of C_7H_11N_4O_2. The infra-red spectra of the compound were run on Nujol and hexachlorobutadiene as the media. A doublet was obtained with maxima at 1585 and 1620 wave numbers. The former could be N-H bonding and the latter which was sharper could be due to C=N group.

Peaks were also appeared at 1340 wave numbers (medium) and 1450 wave numbers (shallow) and 1520 wave numbers (medium). The one at 1410 indicates O-H bending, and the one at 1520 indicates a secondary amide in open chain form.

A medium wide doublet also appeared (in hexachlorobutadiene mull) at 3300-3445 wave numbers. This is characteristic of amines and secondary amines, though the latter absorb only weakly. However, the results also are consistent with
the absorbance peaks for secondary amides: \( \overset{\text{O}}{\text{C-N-H}} \)

which have a similar \( \overset{\text{p}}{\text{NH}} \) group. The characteristic of the secondary amido in solid form is a small additional band at 3070-3100 wave numbers. This was found in the spectrum and supports the existence of the CO-N-H grouping in the compound.

Several other compounds were sublimed onto the cold finger, and onto the wall of the glass container. Samples were taken from different parts of the container and were introduced into the mass spectrometer. The various samples gave large peaks at 207, 142, and 74 respectively, but little further information was obtained.

3) Analysis of Solid Products.

Analysis of the solid reaction product is the most difficult part of these experiments. Some workers have suggested over thirty compounds are formed in the liquid phase. Relatively small amounts of product were obtained in the present work, so detailed investigation was impossible but a number of experiments were carried out.

The major solid product of the glucose/valine reaction was a brown solid residue. The sample used here was obtained after two hours of heating at 134°C. Attempts were made to find out about individual products to be separated. The residue was largely soluble in methanol at room temperature. A small amount of black material was filtered out, and a clear dark brown solution evaporated to dryness at room temperature. A considerable amount of unreacted valine was precipitated when the product was treated with ether. This
The filtered solution was dried out but the residue was a brown sticky tar which suggested a mixture of heavy compounds. An attempt was made to separate the mixture by thin layer chromatography (T.L.C.). To do this a method of trial and error was adopted. The spot of the mixture was mobile on a silica gel plate using a solvent of 9:1 carbon tetrachloride and acetone. The mobility of the spot increased with increasing polarity of the solvent i.e. by addition of a small amount of methanol.

Three reasonably clear spots were obtained, two brown and one yellow. The spots were extracted from the silica gel with methanol which was then allowed to evaporate. The residues were subjected to mass spectrometric analysis.

The "yellow" spot gave what appeared to be a parent peak at m/e = 207 but the amount of material was so small that a detailed cracking pattern could not be obtained.

The "brown" spots gave what appeared to be parent peaks at 256 and 225 and both gave a major fragment peak at m/e = 43 with insignificant peaks at 39 and 41. This suggests that they were carbonyl compounds. In no case was the spectrum clear enough for P+1:P ratios to be measured.

T.L.C. techniques were probably not ideal for the separation of this kind of mixture and it is possible that the spots themselves were also mixtures.

The apparent presence of four carbonyl compounds suggested that it might be worthwhile to try to obtain their separation of valine was repeated several times.
phenylhydrazones. 2:4 dinitrophenylhydrazine was added to a methanol solution of the brown sticky tar which had been used for the T.I.C. experiments. The solution was left for about 24 hours and a heavy precipitate was obtained, which was filtered off. An attempt was made to purify this by recrystallization. Some crystals were formed, but when left in the dessicator at room temperature to dry they decomposed to a dark brown liquid after a few days. Mass spectrometric analysis of these crystals gave a peak at m/e = 361. Assuming this was a 2:4 D.N.P.H. adduct, the spectrum would indicate a carbonyl compound of molecular weight 181. This will be discussed later. Evaporation of the filtrate from 2:4 D.N.P.H. treatment gave a precipitate most of which was 2:4 dinitrophenylhydrazine. A compound of m/e = 264 was also obtained which might have been the phenylhydrazone of a carbonyl compound of m/e = 84. This was presumably the same as the compound of m/e = 84 which appeared in the sublimed product.

Source of Carbon Dioxide.

It has been observed that CO₂ gas is evolved in the glucose/valine reaction. This has also been confirmed by others. The source of the carbon dioxide has not been established though it seems possible that it is produced from the carboxylic group of the amino-acid. To test this hypothesis, radio-active valine containing C¹⁴ on the carboxylic group was used. 0.00036 gm of valine was obtained of activity 0.1 milli curie. This was dissolved in 5mls. of
distilled water, of which 0.12 ml. was taken and added to 1.5 grams of valine dissolved in hot ethanol. This solution in diluted form was freeze dried under vacuum to give the labelled valine. A 1:1 mixture of the glucose and the dilute labelled valine was prepared, of which 400mgm was taken in the form of pellets to be used for each experiment. This was arranged so that the product would give of the order of $10^5$ counts per minute for each sample provided the whole of the valine reacted. It was arranged to collect the liberated $CO_2$ in the form of $BaCO_3$. Therefore a solution of $(H_2O)_2Ba$, barium hydroxide, which is slightly soluble in water, was made in hot boiling water, free from atmospheric $CO_2$. It was filtered when hot to give a clear solution of barium hydroxide, and this was immediately placed in a container, designed to be connected to the vacuum line. The $(OH)_2Ba$ solution was first frozen in liquid air and degassed, and then put under vacuum.

The container is as shown:

![Diagram](image)

The labelled valine and glucose were then allowed to react. The gaseous products were condensed above the frozen solution by liquid air. The tap was then closed and the container disconnected from the vacuum line. The iced solution was left to melt. The solution then was shaken vigorously so that the trapped $CO_2$ would dissolve and react with $(OH)_2Ba$.
to form a Ba CO$_3$ precipitate. The precipitate was filtered rapidly in a previously weighed glass sintered filter, with nitrogen blowing over the filter, to push the atmospheric CO$_2$ away from reacting with excess of (OH)$_2$Ba.

The filtered precipitate was then washed out carefully with methanol and hot water to remove possibly precipitated Ba(OH)$_2$ and organic compounds. The weights of barium carbonate found in four runs were 110, 109.6, 111.2, and 109.4 mgm, (average of 110 mgm) and these seemed reasonably reproducible. A suspension gel of 3.14 gm per litre was made with thixotropic gel powder (CAB-O-SIL, Packard made) and 20 mls of 2:5 dinitrophenyloxazole and toluene. This was placed in the counting bottle. The labelled precipitate was then transferred to the counting bottle, and this was suspended in the presence of 157.7 mgm of unlabelled valine. This was counted with reference to the 157.7 mgm of pure labelled valine in the presence of 110 mgms of unlabelled BaCO$_3$ in the same suspended form. The counts were done by LS100 Beckmann liquid scintillation counter. It is known that each 400 mgms of 1:1 mixture contains 157.7 mgms of valine. Therefore 157.7 mgm of labelled valine was suspended as above, and was counted in the presence of BaCO$_3$ to obtain a calibration. Each labelled compound was counted in the presence of the other unlabelled compound so as to provide the same environment in each case. The intensity of $\beta$-rays produced by BaC$^{14}$O$_3$ is reduced by unlabelled BaCO$_3$ and the same applies to the labelled
valine. Therefore adding unlabelled BaCO₃ and valine respectively to labelled valine and labelled BaCO₃ respectively ensure the same counting efficiency in each case. Each sample was counted several times and an average count was obtained for each one.

<table>
<thead>
<tr>
<th>Average C.P.M. (sample 1)</th>
<th>Average C.P.M. (sample 2)</th>
<th>Reference Valine (C¹⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41,728</td>
<td>32,500</td>
<td>101,040 C.P.M.</td>
</tr>
</tbody>
</table>

The fact that radioactive barium carbonate is obtained at all shows that some of the carbon dioxide must have come from the labelled carboxyl group of the valine. The proportion which does so can be calculated as follows:

Assuming all the carbon dioxide comes from valine, 117 gms of valine would give 44 gms of carbon dioxide which in turn would give 197 gms of barium carbonate. Hence 157.7 mgm of valine would give 265.2 mgm of barium carbonate and these two amounts would be expected to give the same count rate. Thus the quantity x of BaC¹⁴O₃ obtained for each run, on the above assumption could be worked out as follows:

Sample (1):

\[
\frac{\text{BaC}^{14}\text{O}_3}{x} = \frac{265,192}{41,700} \quad (\text{C.P.M. reference})
\]

where \( x = 109.45 \text{ mgm} \), the quantity of \( \text{BaC}^{14}\text{O}_3 \) expected. In fact 109.6 mgm of \( \text{BaC}^{14}\text{O}_3 \) was delivered to the sample bottle. The similarity of these figures shows that 99.8% of
the carbon dioxide produced comes from carboxyl group of the valine.

Considering sample (2):

The quantity of BaC\(^{14}\)O\(_3\) expected was obtained as above

\[
\frac{265.192}{x} = \frac{101.040}{32,000} \quad (C.P.M. \text{ reference})
\]

\[
x = 84.0 \text{ mgm of BaC}^{14}\text{O}_3
\]

Since 110 mgm of BaC\(^{14}\)O\(_3\) was delivered to the sample bottle, then

The percentage of CO\(_2\) from the carboxyl group is

\[
\frac{84 \times 100}{110} = 76.36\%
\]

In spite of the discrepancy in the second experiment, there is little doubt that the carbon dioxide comes entirely from the carboxyl group of the valine.

It is also possible to carry out a rough calculation of the percentage of valine which has reacted in these two experiments. Since one molecule of CO\(_2\) is evolved from one molecule of valine:

\[
\text{BaCO}_3 \rightarrow \text{CO}_2 \rightarrow \text{CH}_3\text{CH}=(\text{CH})\text{COOH}
\]

therefore, \(0.110 \times 117 = 0.065\) gram of valine reacted in 0.4 gm of mixture. This is a mere 41% of the whole valine consumed.

Water Content

It was noticed by G.L.C. that a large proportion of the product was water. An attempt was made to assess this
quantitatively, using a Karl-Fisher titration. Experience showed that this is a much more difficult method to operate successfully than is implied by Vogel. The Karl-Fisher reagent is prepared by the action of sulphur dioxide upon iodine dissolved in pyridine and methyl alcohol. This proceeds through a two step reaction. The first step is the oxidation of sulphur dioxide by iodine, which takes place only in the presence of an oxygenated molecule. This leads to the intermediate compound "pyridine-sulphur trioxide", which is the inner salt of pyridine-N-sulphonic acid. The second step is the formation of pyridinium methyl sulphate which prevents the pyridine complex from reacting with another molecule of water or other active hydrogen compound.

\[
i) \quad 3C_5H_5N + I_2 + SO_2 + H_2O \rightarrow 2C_5H_5NH^+I^- + C_5H_5\left[SO_2\right]_2^0 \]

\[
ii) \quad C_5H_5N^+SO_2 + CH_3OH \rightarrow C_5H_5N\left[OSO_2CH_3\right] \]

Hence one molecule of iodine is equivalent to one molecule of water. The reagent is usually specific for water but some compounds like aldehydes and ketones interfere with the standard technique and hinder the determination of the end point. This complicates the technique and further precautions and experimental details are given by Mitchel and others 69, 70, 71, 72.

A Karl-Fisher reagent was prepared and was standardized against a standard solution of water in dried methanol.
5.45 mgm of water corresponded to a titre of 1 ml. Karl-Fisher reagent. The product sample was diluted in 2 methoxy methanol (methyl cellosolve).

Because aldehydes were known to be present in the samples, two modifications were made to the standard technique. As recommended by Mitchell and Smith, the solutions were made up in 1:3 methanol/pyridine mixture, to facilitate the determination of the end point. Also the precision of the end point was improved by adding 1 ml of Karl-Fisher catalyst (N-ethyl piperidine obtained from H & W laboratories) to each sample. An experiment was carried out in which the products from 300 mgm of dry equimolar mixture of glucose and valine were collected in liquid air. The reaction was allowed to proceed for 120 minutes at 134°C.

The product was dissolved in 2-methoxy methanol and titrated by the Karl-Fisher method. It contained 47.7 mgm of water. Weight loss experiments showed that the total weight of product was about 106 mgm.

An attempt also was made to determine the kinetics of water production by collecting the products from the reaction at equal intervals of time each (20 minutes). Six samples were collected over a period of 110 minutes. The quantities of water produced in each 20 minutes are shown in Table IV:

<table>
<thead>
<tr>
<th>Time (mins.)</th>
<th>0-20</th>
<th>20-40</th>
<th>40-60</th>
<th>60-80</th>
<th>80-100</th>
<th>100-120</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water produced (mgm)</td>
<td>24.65</td>
<td>6.621</td>
<td>3.815</td>
<td>3.951</td>
<td>3.95</td>
<td>3.815</td>
<td>46.65</td>
</tr>
</tbody>
</table>
The final figure is in excellent agreement with the earlier measurement. As is shown, the largest quantity of water is produced during the first 20 minutes, where the overall rate of reaction is fastest. The second largest portion of water is produced in the second 20 minutes interval as the reaction advances further. To find the source of water production a calculation was attempted:

The quantity of valine reacting had been shown by the radioactive tracer experiment to be 41% i.e. 49 mgm if 117 mgm of valine are in the original sample. This quantity should react assuming the initial reaction is between one molecule of valine and one of glucose. If one molecule of valine plus one molecule of glucose gave one molecule of water, then 49 mgm valine plus 75.38 mgm glucose would give $0.049 \times \frac{18}{117} = 0.0754$ mgm water. In fact, about six times as much water is produced.

**Summary.**

A large number of compounds, many of them unidentified, result from the reaction of glucose with valine. The main products, however, are water, isobutyraldehyde and carbon dioxide and the amounts of the others are insignificant. This conclusion was suggested by the original G.L.C runs and is confirmed by weight loss calculations viz:

Tracer experiments showed that in a 300 mgm sample, 49 mgm valine reacted to give carbon dioxide. It is extremely likely that the residue of the valine molecule appeared as
isobutyraldehyde. Hence 49 mgm valine should give 18.4 mgm carbon dioxide and 30.15 isobutyraldehyde. 47.7 mgm water were also produced, giving these three products a combined weight of 96.3 mgm. The total weight loss, determined by an experiment on the gas line and again on the thermogravimetric balance was 106 mgm. Thus, the other volatile organic products could only have accounted for about 9% of the yield.
A series of homologous α-amino acids were selected for reaction with glucose, to investigate and compare their rates. The selected amino-acids were nor-valine, 2 amino-butyric acid, α-alanine, glycine and phenyl β alanine.

The reaction between glucose and each of these amino acids was carried out at a temperature of 124°C. Figures 29, 30, 31 and 32 show the reaction curves for each of the above mentioned amino acids except phenyl β alanine. Figure 33 shows the relative rates of all the above reactions on the same graph. It can be seen that the maximum slopes of all the reaction curves are similar. The total final pressure on the other hand increases as the number of carbon atoms in the amino acid increases. The induction period is also generally longer in the case of a higher number of carbon atoms in the amino acid.

The higher final pressures cannot be an artefact due to condensation of products because in that case one would expect the the highest pressures from the lowest molecular weight starting materials.

It should be noted that all experiments were carried out with 300 mgm samples of equimolecular mixtures of glucose and amino acids. In the case of the lower molecular weight amino acids, the total moles present in the sample will thus be slightly higher.
1:1 Mixture of glucose and nor-valine

$T = 124^\circ C$

Indications for each kinetic run are:

Fig. 29.
1:1 Mixture of glucose and 2-aminobutyricacid

\[ t = 124^\circ C \]

Indications for each kinetic run are:

Fig. 30
1:1 mixture of glucose and α alanine

Indications for each kinetic run are:

Fig. 31.

Time in minutes

1:1 mixture of glycine and glucose

Indications for each kinetic run are:

Fig. 32.

Time in minutes
Glucose/norvaline reaction

The kinetics of glucose/norvaline reaction were investigated, and the main products identified. The reaction curves are shown in Figure 29 which illustrates six runs and gives some idea of the reproducibility of the experiments. A sample of the gaseous products from the reaction was submitted to mass spectrometric analysis. The spectrum showed outstanding peaks at mass numbers 72, 57, 44, 43, 41, 39, 29 and 28. Mass 72 could be the molecular ion of butyraldehyde, expected from the reaction by analogy with valine. The fragmentation of this could lead to some of the other ion peaks as shown:

\[
\text{CH}_3\text{CH}_2\text{CHO} \quad \text{or} \quad \text{CH}_3\text{C=O} + \text{CH}_3
\]

Peaks at 41 and 39 lend some support to the idea that mass 43 is \(\text{C}_3\text{H}_7^+\). Mass number 28 could be a C=O fragment from the carbonyl group. The peak at 44 is of course due to carbon dioxide and some of the mass 28 will arise from this source. Some of the gaseous product was condensed and submitted to mass-spectrometric analysis, better sensitivity being obtained in this way. This time an extra peak at \(m/e = 60\) also appeared plus another peak at 45 as well as mass 43. These peaks could be indicative of acetic acid as reported also in the valine reaction.

The products also were studied by N.M.R. and I.R. techniques. The spectrum obtained by N.M.R. using \(\text{D}_2\text{O}\) as a solvent showed peaks at various chemical shifts:

1. A triplet at \(\delta = 0.91\) indicative of a methyl group,
(2) a sextet at $\delta = 1.55$ (3) a triplet at $\delta = 2.50$ indicative of another CH$_2$ group adjacent to the former CH$_2$ group (4) a triplet at $\delta = 9.69$ the region characteristic of the aldehyde group. The observations above were consistent with the major product being butyraldehyde $\text{CH}_3\text{CH}_2\text{CH} = \text{CHO}$. Other peaks at chemical shifts $\delta = 2.1$, $\delta = 2.2$, $\delta = 3.33$ and $\delta = 3.70$ suggested another olefinic compound, which could not be identified. Analysis by an infra-red technique was carried out using AgCl window cells and a liquid cell. The spectrum showed a peak at 1640 wave numbers. This peak was believed to belong to CHO absorption which would normally appear as 1700 cm$^{-1}$. The shift could be attributed to the solvent $\text{D}_2\text{O}$, and this was confirmed using a sample of butyraldehyde in heavy water.
Glucose and 2-amino-butyric acid reaction

The rate curve for glucose and 2 amino butyric acid is shown in Figure 30. Reproducibility runs are also shown. The reaction was carried out at 124°C under vacuum. Preliminary analysis was carried out by mass-spectrometer, and the spectrum of the liquified products obtained. Major peaks were found at mass numbers 15, 18, 27, 28, 29, 39, 41, 43, 44, 45, 55, 57, 58, 69, 84, and 98. The most abundant peaks observed were respectively 29, 58, 28, 27, 57, 41, and 39. These are known to arise in the mass-spectrum of propanal, \( \text{CH}_3\text{-CH}_2\text{-CHO} \). Mass 44 was intensified when the gaseous product was examined, this was due to \( \text{CO}_2 \) produced in the reaction.

Mass 84 is believed to be the carbonyl compound, which also was obtained in the glucose/valine product. Mass 98 could be the product of an aldol condensation between the two molecules of propanal, with loss of one molecule of water. The peak at m/e = 60 was fairly small, but there were also peaks at 43, 44 and 45, suggesting the existence of a small amount of acetic acid.

The condensed products from the reaction were extracted in CDCl\(_3\), for analysis by N.M.R and I.R techniques. The N.M.R spectrum showed a triplet at a chemical shift of \( \delta = 1.0 \) indicating of a CH\(_3\) group. There was also a cluster of four fairly shallow peaks at \( \delta = 2.4 \) indicating of CH\(_2\) group, and a triplet at \( \delta = 9.8 \) characteristic of aldehyde groups. A peak at \( \delta = 4.65 \) showed some trace of
water. The above data was consistent with the structure \( \text{CH}_3\text{-CH}_2\text{-CHO} \) propanal.

A further triplet appeared at \( \delta = 9.4 \) indicative of a second aldehyde possibly belonging to the aldol at \( m/e = 98 \). The extracted products in CDCl\(_3\) were also used for I.R analysis. A liquid cell with AgCl windows was used to contain the solution. The spectrum obtained showed a sharp peak at 1718 wave numbers characteristic of carbonyl absorption, as well as a sharp peak at 2920 indicative of H-C stretching. A medium peak also appeared at 1665 wave numbers, which could be due to the carbonyl group of an \( \alpha \) or \( \beta \) unsaturated carbonyl compound. This is further evidence for the occurrence of an aldol condensation to give \( \text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CHO} \) (3 Hexen, 1, al).
Glucose and α-alanine

The reaction between glucose and α-alanine was carried out at 124°C on the gas line and the rate of pressure change was obtained versus time as shown in Figure 31. Reproducibility runs are again shown. A sample of gaseous products, collected under vacuum was separated by G.L.C. Five peaks appeared close to one another, one being much larger than the others. They were in the ratio 11.0:1:0.5:0.3:0.25. The largest peak was thought to be acetaldehyde and it appeared at the same retention time as a reference sample of acetaldehyde. A sample of liquified gaseous products was introduced in mass-spectrometer. The spectrum obtained showed peaks at mass numbers 96, 95, 92, 91, 78, 58, 45, 44, 43, 42, 41, 39, 30, 28 and 27. The standard mass spectrum of acetaldehyde shows peaks at 29, 44, 43, 15, 42, 41, 28, 27, 45 and 30. Among the peaks not due to acetaldehyde were two at masses 96 and 95 of almost the same height. They could possibly be due to furfural as a minor compound in the mixture, since these are the biggest peaks in its spectrum. The peaks at masses 92, 91, 78, and 58 were not assigned.

The gaseous products were liquified and extracted in deuterated chloroform CDCl₃ and examined by the N.M.R technique. The spectrum showed only a doublet at chemical shift of δ = 1.9 indicating a methyl group and a quartet at δ = 9.74 indicating a CHO group. The solution was also used for I.R purposes. A peak appeared at 1710 wave numbers...
indicative of a carbonyl compound, and a sharp peak at 2905 wave numbers appeared due to H-C stretching. The above data confirm that the major component was acetaldehyde.

On one mass spectrum, a small peak was found at mass 70 which could have been due to the aldol condensation product CH$_3$.CH=CH.CH0 (crotonaldehyde) but this was not reproducible and there was no sign of the material in the I.R and N.M.R spectra.
The reaction between glucose and glycine was carried out at 124°C. The reaction curves and reproducibility runs are shown in Figure 32. The curves flattened at a much shorter time, than those of the other amino acids. The final pressure was also smaller. The gaseous products were collected under vacuum as usual and were separated on G.L.C. system with a D.N.P. column at 60°C. Four peaks appeared at the abundance ratios of 9:3:1:0.5. By analogy with the other amino acids formaldehyde was expected to be a major product, and a sample was used as reference. No formaldehyde was identified in the products.

A sample of the gaseous products was submitted to mass spectrometric analysis. The spectrum obtained showed peaks at mass numbers, 96, 95, 72, 58, 57, 45, 44, 43, 42, and 29. The peaks at 41 and 39 were smaller than the one at 43. The most abundant peak observed was at m/e = 44 presumably indicative of carbon dioxide. Masses 96 and 95 were of almost the same height and may be due to furfural. The characteristic furfural ion peaks were also present at masses 39 and 29 but many other compounds could also give peaks at those masses. The second highest peak was at m/e = 43, presumably indicative of a carbonyl compound.

The N.M.R. spectrum showed a single small peak at $\delta = 2.1$ which could be due to acetone which is certainly a carbonyl compound and which gives mass spectral peaks at 29, 58, 28, 43, 18, 57, 41, 39 and 15 in order of abundance.
Masses 72, 57 and 29 could arise from butanone but no confirmatory evidence was found and most of the products were not identified.
The reaction between glucose and 3-aminobutyric acid (which is not an \(\alpha\) amino acid) was carried out under vacuum at 124°C. The reaction curves obtained are shown in Figure 34. The reaction rates were not reproducible and the reaction seemed to be in two stages. The reaction started immediately without a noticeable induction period, though the reaction temperature was 10°C lower than in the glucose/valine reaction.

A sample of the gaseous products was submitted to G.L.C. for separation. The column used was a Poropak Q at 157°C. Two major peaks were observed close to one another, one being 4 times as abundant as the others. The minor compound was thought to be acetone, since the reaction time of a reference sample was almost identical. The gaseous sample of the product was submitted to mass spectrometric analysis. The spectrum showed peaks at 86, 84, 60, 58, 45, 44, 43, 39, 41, 29 and 22. Peaks at masses 44 and 22 showed good evidence for presence of \(\text{CO}_2\). A second sample of the condensed products was extracted in \(\text{D}_2\text{O}\) and was analysed mass spectrometrically. The spectrum showed peaks at 137, 119, 91, 87, 86, 69, 68, 60, 61, 58, 46, 45, 44, 43, 41, 39, 28. The relative abundant peak at 61 was thought to be the deuterated acetic acid at mass 60. This was supported by the relative increase of mass 46 to mass 45. Mass 58 was thought to be the acetone as reported above, and mass 87 may possibly be the deuterated 86.
1:1 Mixture of glucose and 3-aminobutyric acid

\[ t = 124^\circ C \]

Indications for each run are:

Fig. 34.
A third mass spectrum was run on a white sublimate which coated the top of the sample bottle. A molecular ion peak appeared at mass 152 but the compound could not be identified.

The main products were extracted in CDCl$_3$ and a few samples were prepared for N.M.R. analysis. One of them seemed to have one of the components in abundance and no significant peaks of other components were observed. The spectrum showed two doublets at chemical shifts of $\delta = 1.8$ and $\delta = 1.90$ and a singlet at $\delta = 2.10$. A doublet of coupling constant $J = 16.8$ $\text{Hz}$ and a quartet appeared at lower field. These are characteristic of allylic hydrogen couplings. The doublet was at $\delta = 5.78$ and the quartet $\delta = 7.0$.

The singlet was identified as an isolated methyl group adjacent to a non protonated group e.g. C=O. The two doublets at higher field were identified as a methyl group adjacent to a ethene group CH=CH. Each of the hydrogens of the group couples separately with the methyl group and this results in two doublets. In return the methyl group couples with each of the hydrogens of the ethene group at lower fields. The above information suggests the structure CH$_3$-CO-CH=CH-CH$_3$, 3-penten-2-one. An extra singlet of relative intensity of less than half appeared at $\delta = 2.02$, which was thought to be the acetone identified by G.L.C. The extracted solution of CDCl$_3$ was used for I.R. analysis. The spectrum obtained showed a strong peak
at 1686 wave numbers characteristic of unsaturated keto compounds and two other peaks at 1596 and 1640 wave numbers presumably due to the presence of a double bond. This peak usually appears weaker at wave numbers 1590-640. The peak due to H-C stretching appeared at 2930 wave numbers.

The two major reaction products detected by G.L.C. thus appear to be acetone and 3-penten-2-one. The latter contains five carbon atoms compared with the amino-acid's four, and almost certainly arises from the glucose. It is tempting to suggest that the acetone arises from simultaneous decarboxylation and deamination of the amino acid, analogous to the reaction of an α-amino-acid to give an aldehyde. The occurrence of carbon dioxide supports this view. Acetone was, however, detected as a reaction product in most of the other experiments and probably comes from the glucose. This conclusion is supported by the reaction curves which do not resemble the smooth sigmoid curves obtained from glucose and α-amino acids.
The reaction between 4-amino butyric acid and glucose was carried out at \( T = 124^\circ \text{C} \) under vacuum. The rate of pressure change was plotted against time. Figure 35 shows the reaction curves obtained. There was practically no induction period and the reaction was faster than with the \( \alpha \) and \( \beta \) amino acids. This is shown by the slope of the reaction curve obtained. The mass-spectrum of the gaseous products showed peaks at mass numbers 92, 91, 74, 72, 57, 44 and 43 and shallow peaks at 41 and 39. A sample of the liquefied products was also introduced into the mass-spectrometer, this giving greater sensitivity and more ion peaks.

The nearest ion was at \( m/e = 166 \) with a \( P+1 \) peak at 167. The ratio \( (P_{m+1}) \times 100/P_m = 0.63 \times 100/4.63 = 13.6 \) suggesting the empirical formula \( C_{12}H_{18}N \) of the nearest ratio 13.4776. Other peaks observed were at 149, 107, 77, 61, 58, 56, 44, 43, 4, and 39. A further sample of the liquefied gases was introduced directly into the mass-spectrometer. The spectrum obtained was quite different from the previous ones. It showed an ion peak at \( m/o = 118 \) and further peaks at masses 86, 76, 74, 71, 69, 61, 55, 44, 43, 41, 39, 29, and 28. Further mass spectra were inconsistent and reliable results were not obtained. This is frequently the case with the mass-spectra of pure sugars (see page 156). A G.L.C. of the gaseous compounds was carried out. Four components appeared at different retention times, of which one had a high
abundance and the others were small peaks. The masses at 58, 72, and 86 as were shown in the mass spectrum suggested the possible occurrence of some homologous ketones such as acetone, ethyl methyl ketone and methyl n-propyl ketone. The liquefied product therefore was run on a D.N.P. column at a temperature of 70°C, with reference to the above mentioned ketones. Three peaks appeared having identical retention times with the reference compounds. An attempt was made to separate the hydrazones of the above carbonyl compounds by means of thin layer chromatography. 2:4 D.N.P.H. was added to the liquefied mixture, a precipitate was obtained and filtered, and then dissolved in chloroform. A mixture of carbon tetrachloride, hexane and ethyl acetate in the ratio 10:2:1 was made as the mobile medium (recommended for the separation of homologous ketones). The hydrazone solution was then mounted on a silica gel plate, and after drying, was separated in the above medium. A few lines were separated of which three had higher $R_f$ values than the others, and were scraped off the plate and the compounds contained in them were washed out with absolute ethanol from the silica gel. The ethanol solutions were evaporated to dryness over a water bath around 70°C. Visible crystals were not obtained due to the paucity of material, but the compounds were placed on the insertion probe of the mass spectrometer and allowed to dry. Though trace hydrocarbons present in the hexane had contaminated the compounds, one of the samples appeared to have a molecular weight of 252 and the second a molecular
weight of 266. These correspond to carbonyl compounds of molecular weight 72 and 86 i.e. presumably methyl n-propyl ketone and methyl ethyl ketone.

The compound giving the third line was abundant enough for a few mgms of crystals to be obtained. The mass-spectrometric analysis showed a parent ion peak at m/e = 276, indicating a carbonyl compound of molecular weight 96, which had not been detected in the mass-spectra of the gas samples. The melting point of the crystals was determined to be 228°C. This corresponded most closely to the published value of 230°C for the 2:4 dinitrophenylhydrazone of furfural, which also has a molecular weight of 96.

This identification was confirmed by making a reference hydrazone of furfural and comparing the infra-red spectra of both compounds. Solvents were avoided and KBr discs of both compounds were made. Analytical anhydrous KBr crystals were powdered and sieved finely to mesh 200. One mgm of the compound was dissolved in methanol and then added to 200 mgm of the powdered KBr gently. The mixture was dried in an oven at 130°C for two days. Then a disc of thickness 3mm and diameter of almost 1.5cm. was made in a disc maker under 10-12 atmospheres pressure. A second KBr disc of the reference hydrazone was also made. The two discs were reasonably transparent to ordinary light. The I.R. Spectra obtained were totally identical in each single peak, which proves that the compound is furfuraldehyde. A shallow broad peak at 3450 wave numbers appeared on both spectra indicating
that some molecular water remained in the KBr crystals. Furfural is well known to result from dehydiation of pentoses (oat hulls etc.) but dehydiation of hexoses normally yields 5-hydroxymethyl furfural. The above experiments reflect the necessity of proving conclusively that the less expected product was the one which actually occurred.
1:1 Mixture

4-aminobutyricacid and glucose
\[ t = 124^\circ C \]

Number of runs

1:1 Mixture

β alanine and glucose
\[ t = 124^\circ C \]

Fig. 35

Time in minutes

Fig. 36.

Time in minutes
Glucose and β-alanine.

Reaction between glucose and β-alanine was carried out at 124°C. The reaction curves are shown in figure 36. The reaction started immediately with no noticeable induction period. A sample of the gaseous product was submitted to G.L.C. using a D.N.P. column. Four peaks in the ratio 3.9:0.6:0.2:0.2 appeared at different retention times close to one another. Mass-spectrometric analysis showed a large amount of CO2 produced. A set of gaseous products were liquefied and collected and again submitted to mass-spectrometric analysis. The spectrum obtained showed peaks at mass numbers 18, 19, 28, 29, 43, 45, 55, 60, 72, 117 and 119. An attempt was made to obtain an I.R. spectrum of the condensed products but they seemed to be insoluble in CCl4 and when dissolved in D2O the only recognizable peaks were those due to C=O and C-H stretching.

The N.I.R. spectrum obtained from the products extracted in CDCl3 showed a few sharp peaks which were not identified but the significant point was that no pattern due to an aldehyde group was observed. A possible conclusion from the above information was the presence of a ketone possibly of mass 72. This might be methyl ethyl ketone which would also give a fragment peak at 43 with no significant peaks at 39 and 41. The evidence however is not conclusive.
Glucose and phenyl \( \beta \) alanine

The reaction between glucose and phenyl\( \beta \)alanine was carried out to see the effect of an aromatic ring on the reaction. This reaction was carried out at 124°C and the gaseous products were collected in a sample bottle.

The mass spectrum of the products did not show a conspicuous peak at m/e = 120 which would correspond to phenyl acetaldehyde, possibly because of its low volatility. On the other hand, when some of the mixture was placed on the direct insertion probe inside the ion source, a large peak at m/e = 120 was obtained. This could have been a fragment ion \( C_6H_5\cdotCH_2\cdotNH_2^+ \), from phenylalanine but other ions of this molecule were of low abundance and it seems likely that the observed peak was indeed due to phenylacetaldehyde.

Peaks observed in the mass spectrum of the condensed gaseous products included 281, 207, 96, 95, 58, 45, 44, 43, 41, 39, 38, 29, 28. When another sample was submitted to the mass-spectrometer, an almost identical spectrum was obtained lacking 281 and 207. The mass-spectrum obtained for the gaseous products showed the peak at 44 as the most abundant and this was assumed to be CO\(_2\). Peaks at 96 and 95 were of almost equal abundance, and further presence of 38 and 39 corresponded to the spectrum of a standard sample of furfural. The peak at 58 had a relatively high abundance and possibly could be due to acetone as was suggested by evidence obtained by N.M.R and I.R.
The condensed products were extracted in CDCl$_3$, to be analysed by I.R. and N.M.R. techniques. The N.M.R. spectrum showed a triplet at chemical shift $\delta = 9.8$ characteristic of the aldehyde group, a broad peak at $\delta = 7.30$ identified as the phenyl group and a doublet at $\delta = 3.75$ due to the methylene group. Peaks were also observed at $\delta = 1.18$ and 1.89 which were not identified. Another sample prepared showed an extra peak at $\delta = 2.22$, this was suspected to be due to acetone, normally appearing as a singlet ion in this position.

The infra-red spectrum of the product in CDCl$_3$ obtained using a liquid cell showed peaks at wave numbers 1710 and 2950, due to carbonyl group absorption and C-H stretching.
v) Mass Spectrometric Investigations

Introduction

Previous work on the degradation of sugars, and on the glucose/amino acid reaction had all been carried out either in solution or at relatively high temperature. The possibility arises that the reaction might appear much simpler if carried out with very slow heating, and with a very sensitive method available for detecting products. The acquisition by the University of Surrey Chemical department of a mass spectrometer with a direct insertion probe, made it possible to carry out the reaction within the ion source of the machine. The interpretation of the results of such experiments required a knowledge of the mass spectra of the reactants. The mass spectra of amino acids are fairly well known but there appears to be little information on sugar.

Mass spectra of Monosaccharides

The mass spectra of pure carbohydrates have not been unduly investigated because of the difficulty of obtaining and interpreting fragmentation pattern. Mono, oligo and polysaccharides are thermally unstable and practically involatile.

Mass spectral studies therefore have been performed on their more volatile derivatives, such as methyl ethers acetates, and alkaldene derivatives. The characteristic feature of almost all mass spectra of carbohydrate derivatives is the absence of a peak due to the molecular ion.
The cyclic pyranoid and furanoid forms have been the most extensively studied among the derivatives of mono saccharides. The mass spectra of these compounds have been interpreted on the basis of numerous analyses using labelled analogues. The compounds investigated in the greatest detail are probably the methyl ethers and the acetates, but the most recent work done on mono methylated glucose has been done by Heyns. We shall only consider the monomethylated compound here as its fragmentation pattern is likely to be closer to that of glucose, the compound in which we are primarily interested.

Heyns obtained the mass spectrum of methyl D-gluco pyranoside and interpreted it as follows. The parent ion breaks down immediately eliminating the \(-\text{CH}_2\text{OH}\) or \(-\text{OCH}_3\) fragments as shown in Figure 37 (1a).

The most abundant peak obtained was at \(m/e = 60\) (taken as 100) and the next with an abundance of 50 was at \(m/e = 73\). There were also significant peaks at masses 57 and 43. Further fragmentation for \(m/e = 60\) is suggested in Figure 37 (1b) and \(m/e = 73\) is interpreted as shown in Figure 37 (1c). Here the mass number 73 is assumed to arise from carbons 2, 3, and 4. Heyns also obtained the mass spectrum of ribose as shown in Figure 38, but this was not interpreted. Ramnus and Samuelson obtained the mass spectrum of 6-O-(2 hydroxyl ethyl) D-glucose. The prominent peaks observed were \(m/e = 204\) (base peak) and 73. A peak at \(m/e = 103\) was observed, as is frequently the case with
Fig. 37.
Fig. 38. D-Ribose
other sugar derivatives, but there was no peak at \( m/e = 60 \). This could be taken to show that the \( C_6 \) carbon was responsible for the ion at mass 60.

Virtually no work appears to have been done on the mass spectra of pure mono saccharides. Chizov claimed it was generally not possible because the materials were too involatile, but Henys et al have published the electron impact mass spectrum of D-Ribose (reproduced in Figure 33) almost without comment. The spectrum using a field emission ion source is also shown and leads to a parent peak at \( m/e = 150 \) plus a large \( M+1 \) peak due to a protonated parent peak.
EXPERIMENTAL

The work was carried out on an AEI MS 12 single focussing mass spectrometer with a 12" radius tube. A direct insertion probe was available with a quartz tip of 2 mm with a hole 2 mm in the end in which the sample was inserted as a fine powder.

When inserted into the source through a vacuum lock, the sample was heated by conduction down the quartz tip and by radiation from the source block. The thermal conductivity of quartz is fairly low so heating is mainly by radiation and the sample warms up very slowly. The mass spectrum could be scanned up to six times per second, though this was much faster than was normally required. It could also be observed on a cathode ray tube, so that only worthwhile spectra need be recorded on photographic paper. The temperatures of the source block, which rose steadily during each run because it was heated by the filament which gave rise to the electron beam, was noted though it was only a very crude guide to the temperature of the probe.

Mass spectra of pure substances was obtained in the conventional manner.
Mass spectra were obtained for glucose, 2-deoxyglucose, mannose, rhamnose, galactose, fructose, sorbose, arabinose, lyxose, ribose, and some disaccharides such as lactose and sucrose. The mass spectra obtained are shown in Figures 39-50.

The problem of involatility mentioned by Chizov, did not appear to be a serious one. Mass spectra were obtained without the source being anywhere near the temperature, at which for example, glucose degrades. Parent peaks were not obtained, except for lyxose where very small peaks at 151 (PM+1) were observed. This was followed by another peak at (PM-1) at 149 mass numbers. This was also the case with ribose repeated again, a very small peak at PM-1 149 was observed, which was not reported by Heyns. The absence of parent peaks was not surprising, and there is no reason to support that mass spectra obtained are not genuine fragmentation pattern.

The abundance of the fragment ion peaks fluctuated from run to run. The glucose spectrum shown in Figure 39 was the average of six runs but the spectra of the other sugars are the results of single runs. Though the masses of fragment ions were reproducible, too much significance should not be attached to their abundances. The mass spectra obtained will be discussed later.

The reaction between dried glucose and valine was carried out, on the insertion probe of the mass spectrometer.
Fig. 40.
Mass 60 is the base peak taken as 100

![Diagram of Rhamnose molecule]

**Figure 42. Mass Numbers**
Fig. 43. Mass Numbers

Galactose

CH₂OH

H H H OH OH H H

Mass Numbers
Fig. 45. Mass Numbers

Sorbose
Base peak at 31 shown with a broken line is originally 1/3 more abundant than what is shown.
Peak at 73 is the base peak and originally is $3/5$ more abundant than what is shown.
Fig. 49. Mass Numbers

Lactose
Sucrose

![Sucrose structure diagram]

Fig. 50.

abundance

Mass Numbers

57 60 73

43

31

20 40 60 80 100 120 140 160
Solid glucose/valine reaction on mass spectrometer
The percent abundance of acetic acid

Fig. 52.
The materials were placed on the probe and spectra were obtained at increasing temperatures of almost 10°C intervals between 112°C - 207°C. These temperatures were recorded on a thermocouple attached to the ion source block. The source itself is believed to be about 27°C hotter than this, but on the other hand, the sample is being warmed only by radiation and only by conduction along the ceramic tip, so its temperature will lag behind that of the source.

Some ion peaks were observed which corresponded in mass numbers to the identified products obtained on the gas line. One of the spectra carried out at t=130°C is shown in Figure (51). The significant peaks observed were at mass numbers 256, 172, 144, 98, 91, 84, 74, 73, 72, 60, 58, 57, 46, 44, 43, 41, 39 and 29, (the numbers below 40 are not represented). As shown mass 72 was the most abundant peak indicative of isobutyraldehyde, this was followed by the other abundant peaks characteristic of iso-butyraldehyde fragmentation at 57, 55, 28, 43. The second abundant peak was $m/e = 74$, which was reported in the sublimed products. The peak at mass 44 was relatively abundant and could be assumed to be CO$_2$.

The other familiar peaks observed were at masses 144 and 84. The spectrum of mass 144 has been represented independently earlier, and mass 84 was the carbonyl compound separated as a 2:4D,N.P.H. adduct. The above-mentioned molecular ion peaks did not vary significantly with the
temperature increase. The only important ion peak which varied significantly was of mass 60. This compound was identified in earlier work as acetic acid. The variation in abundance of the peak was plotted against the varying temperature, and is shown in Figure 52. The abundance of the peak rise steadily to a 25% height at 162°C and falls sharply as the temperature is further increased. It could be inferred that temperature has a great affect on the degradation of glucose leading to the production of acetic acid.
DISCUSSION

(I) Kinetics of Solid-Solid Reaction.

(II) The Products and Mechanism of the Glucose/Aminoacid Reaction.

(III) Analysis of Sugar Spectra.
(1) **Kinetics of Solid-Solid Reaction**

Several methods of analysis were applied to the data obtained.

(1) The rate for each mixture versus the mole ratio for each mixture was plotted and these were shown in figures 19 and 20, Chapter 4. Two straight lines of equal and positive slopes were obtained for each set of mixtures. As the mole ratio increases from either direction, the rate increases. The reaction rate reaches a maximum at equimolar proportions.

(2) In a mixture of solids it is possible that the reaction rate is proportional to the area of contact between particles of glucose and valine. To analyse this the following treatment was carried out.

In a mixture containing equal volumes of glucose and valine, of equal particle size assuming the mixing is good, each particle of glucose will be in contact with an equal number of particles of glucose and valine. Similarly in a 2:1 valine-glucose mixture by volume each glucose particle will be in contact with twice as many particles of valine as of glucose and so on.

Suppose we have a mixture of \(x\) moles of valine and \(y\) moles of glucose, then the weights of reactants in a 0.3 gm. pellet will be

\[
\frac{117x}{117x + 180y} \times 0.3\ \text{gm. valine}
\]

and

\[
\frac{180y}{117x + 180y} \times 0.3\ \text{gm. glucose}
\]
and the volumes occupied by these amounts will be:

\[
\frac{117x \times 0.3}{(117x + 180y)1.316} \text{ cc valine}
\]

and

\[
\frac{180y \times 0.3}{(117x + 180y)1.562} \text{ cc glucose}
\]

A given particle of glucose will thus be in contact with other particles of valine and glucose in the ratio

\[
\frac{117x \times 0.3}{(117x + 180y)1.316} : \frac{180y \times 0.3}{(117x + 180y)1.562}
\]

\[
= \frac{117x \times 1.562}{180y \times 1.316} : 1 = 0.77x/y : 1
\]

e.g. in an equimolar mixture any given particle of glucose will be in contact with 0.77 particles of valine for every particle of glucose. (This is also the ratio of the number of particles of valine to those of glucose)

The rate of reaction of the given particle might be expected to be proportional to the amount of valine contact with it, i.e.

\[
\frac{0.77x/y}{1 + 0.77x/y}
\]

This is also equal to the ratio of the volume of valine to the total volumes of glucose and valine. But the number of particles of glucose available to react in this way is proportional to the volume of glucose, i.e.

\[
\frac{180y \times 0.3}{(117x + 180y)1.562}
\]
The overall rate of reaction should be proportional to the product of these, i.e.

\[
\frac{0.77x/y}{1 + 0.77x/y} \cdot \frac{180y \times 0.3}{(117x + 180y)1.562}
\]

The results obtained for the glucose and valine reaction with different compositions were analysed, by the above method. The data obtained are represented in Tables I and II. The data obtained for each mixture in terms of x/y or y/x are, of course identical. The above equation could be further simplified in a more generalised version, i.e.

\[
\text{Rate } \propto \frac{aZ}{1 + aZ} \cdot V_g
\]

The volume ratio of valine to glucose could be equal

\[
\frac{M_v}{M_g} \cdot \frac{\rho_g}{\rho_v} \cdot \frac{x}{y} = aZ
\]

where \( a \) is the ratio of volumes of valine to glucose and \( Z = x/y \) or the weight ratio, and \( V_g \) is the volume of glucose at different compositions. The results plotted for glucose in excess and valine in excess against relative rates are shown in figure 1. It shows a rising curve for glucose in excess, rather than a straight line. If we assume it to be approximately exponential, we can attempt to fit the data to curves of the type

\[
\text{Rate} = \frac{dP}{dt} = \text{C} \exp \left( \frac{aZ}{1 + aZ} \right) \cdot V_g
\]

A straight line was obtained, when log(relative rates) were plotted against the data obtained as shown in figure 2.

\[\text{N.B.} \]

The equation for the glucose to (glucose and valine) ratio
Fig. 1. $\frac{aZ}{1 + aZ} \cdot V_g$ (µ litre)

Fig. 2. $\frac{aZ}{1 + aZ} \cdot V_g$
### Table I.

<table>
<thead>
<tr>
<th>x:y</th>
<th>( \frac{aZ}{1 + aZ} ) = ( \frac{0.77x/y}{1 + aZ} )</th>
<th>( \frac{aZ}{1 + aZ} )</th>
<th>y:x</th>
<th>( \frac{a'Z'}{1 + aZ'} )</th>
<th>( \frac{a'Z'}{1 + aZ'} )</th>
<th>Relative rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.G.</td>
<td>G.V.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3:1</td>
<td>2.31</td>
<td>0.699</td>
<td>0.334:1</td>
<td>4.34</td>
<td>0.303</td>
<td>11</td>
</tr>
<tr>
<td>2:1</td>
<td>1.54</td>
<td>0.606</td>
<td>0.5:1</td>
<td>0.65</td>
<td>0.394</td>
<td>12</td>
</tr>
<tr>
<td>1:1</td>
<td>0.77</td>
<td>0.435</td>
<td>1:1</td>
<td>1.3</td>
<td>0.565</td>
<td>15</td>
</tr>
<tr>
<td>0.75:1</td>
<td>0.5775</td>
<td>0.366</td>
<td>1.33:1</td>
<td>1.73</td>
<td>0.634</td>
<td>13.5</td>
</tr>
<tr>
<td>0.5:1</td>
<td>0.385</td>
<td>0.278</td>
<td>2:1</td>
<td>2.6</td>
<td>0.720</td>
<td>12</td>
</tr>
<tr>
<td>0.33:1</td>
<td>0.257</td>
<td>0.205</td>
<td>3:1</td>
<td>3.9</td>
<td>0.796</td>
<td>11</td>
</tr>
</tbody>
</table>

\[
\frac{aZ}{1 + aZ} = \text{proportion of valine by volume.}
\]

\[
\frac{a'Z'}{1 + aZ'} = \text{proportion of glucose by volume.}
\]

### Table II.

<table>
<thead>
<tr>
<th>Mole ratio x:y</th>
<th>Volume of glucose ( V ) ((\mu \text{ litre}))</th>
<th>( \frac{aZ}{1 + aZ} ) ( V )</th>
<th>( \frac{aZ}{1 + aZ} ) ( V )</th>
<th>Volume of valine ( V ) ((\mu \text{ litre}))</th>
<th>( \frac{a'Z'}{1 + aZ'} ) ( V )</th>
<th>( \frac{a'Z'}{1 + aZ'} ) ( V )</th>
<th>Relative Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.G.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3:1</td>
<td>65.1</td>
<td>0.690</td>
<td>44.9</td>
<td>150.7</td>
<td>0.310</td>
<td>45.6</td>
<td>11</td>
</tr>
<tr>
<td>2:1</td>
<td>83.5</td>
<td>0.606</td>
<td>50.6</td>
<td>128.9</td>
<td>0.394</td>
<td>50.78</td>
<td>12</td>
</tr>
<tr>
<td>1:1</td>
<td>116.1</td>
<td>0.435</td>
<td>50.5</td>
<td>89.8</td>
<td>0.565</td>
<td>50.73</td>
<td>15</td>
</tr>
<tr>
<td>3:4</td>
<td>129.1</td>
<td>0.366</td>
<td>47.25</td>
<td>74.7</td>
<td>0.634</td>
<td>47.36</td>
<td>13.5</td>
</tr>
<tr>
<td>1:2</td>
<td>144.9</td>
<td>0.278</td>
<td>40.28</td>
<td>55.9</td>
<td>0.72</td>
<td>40.24</td>
<td>12</td>
</tr>
<tr>
<td>1:3</td>
<td>157.9</td>
<td>0.204</td>
<td>32.21</td>
<td>40.6</td>
<td>0.796</td>
<td>32.21</td>
<td>11</td>
</tr>
</tbody>
</table>
could be represented by an equation analogous to the above which proceeded at the contact points. The number of contact points is introduced

\[ \frac{dP}{dt} = C \exp \left( \frac{a'Z'}{1 + a'Z'} \right) \times V_v \]

where \( Z' = \frac{v}{x} \) and \( a' = \frac{\rho_v}{M_v} \times \frac{\rho_g}{\rho_g} \) and \( V_v \) is the volume of valine at different compositions.

3. A graph was plotted of the relative rates versus the product of the number of moles of valine and glucose, but the results (figure 3) showed little regularity.

Manipulation of the data showed that a graph of relative rates against \([\text{volume of valine}]^{2/3} \times \text{volume of glucose}\) gives a smooth curve, but there is no obvious explanation why this should be so. The graph is shown in figure 4.

4. Komatsu, (62) carried out experiments on inorganic solids such as CaCO\(_3\) - MoO\(_3\) and BaCO\(_3\) - SiO\(_2\). He treated the data in a different manner, and assumed that in a mixture of fine powders, the number of contact points between two components had an important effect on the rate of reaction, which proceeded

The number of contact points is introduced into the rate of reaction as a function of the ratio of the components by weight, \( Z \), ratio of (radius x density) of the two components, \( a \), and a parameter \( m \), which describes the packing state of fine powders.

The equation thus obtained predicts that the rate constant and the activation energy obtained directly from the experimental results should be a function of \( T \), \( a \), and \( Z \).

\[ \frac{n_A}{n_F} = n_B \left( \frac{aZ}{1 + aZ} \right)^m \]
Fig. 3. Mole products of glucose and valine

Fig. 4. \([V_g][V_v]\) in \(\mu\) litre
Fig. 5. \( \frac{aZ}{1 + aZ} \)

Fig. 6. \( \frac{a'Z'}{1 + a'Z'} \)
Where \( n(A/B) \) is the number of contact points and \( n_B \) is the total number of the particles of A and B surrounding the central one. Assuming a constant number of contact points, Komatsu reduced the above equation to the Jander equation and the latter's rate constant \( k \) was given as a product of the function \( a \) and \( Z \) and the function of the temperature \( k^0(T) \) viz.

\[
k(T,a,Z) = k^0(T) \left( \frac{aZ}{1 + aZ} \right)^m
\]

where \( k^0(T) = 2k' n_B/\tau_B^2 \)

The data here was treated almost in the same manner, and the corresponding results were obtained in form \( \frac{aZ}{aZ + 1} \) as shown in Table I. A graph can be plotted of relative rates versus the values for \( \frac{aZ}{1 + aZ} \) for each mixture. The graphs in figures 5 and 6 show two straight lines of opposite slopes for mixtures of \( \frac{aZ}{1 + aZ} \) and \( \frac{eZ'}{1 + eZ'} \). Komatsu's results also showed two straight lines but of opposite slopes, i.e., the overall graph was V shaped rather than A shaped.

The value of \( m \) was not estimated. Komatsu states it was determined experimentally in his work and is temperature dependant. His method of determination is not clear from the published work and answers to questions at symposia were of little help.\( (62) \)

The method seemed to be related to the variation of activation energy with particle size, which he observed. As this effect was absent from our work we were unable even to attempt to deduce \( m \).

(4) **Order of Reaction**

The results in the previous experiments only gave
a very rough idea of the order of reaction with respect to the two reactants. It should be stressed at this stage that the term "order of reaction" does not have the same significance in solid/solid reactions as in homogeneous gas or liquid phase reactions and we use it simply as a convenient way of expressing the variation of rate with concentration.

An attempt was made to find the order of reaction with respect to the individual reactants using the "isolation method". Samples of mixtures were made, keeping the quantity of one of the reactants constant at one gram, and varying the other. Small quantities, of the order of tens of milligrams of the second component were used. Samples were prepared but with excess glucose and excess valine, and the reaction was carried out using approximately 850 mgm samples of the mixtures. Small quantities of the second reactant were taken, so that the total volume of mixture would not vary greatly. Figures 7, 8, 9, and 10, show graphs of pressure change for 10, 20, 30 and 40 mgms of glucose mixed with one gram of valine and figures 11, 12 and 13 show graphs of rates for the same variable quantities of valine mixed with one gram of glucose. Log(relative slopes) of the straight part of the reaction curve for each set was plotted against log(varied weight) in mgms, and a straight line was obtained for each set of data, as shown in figures 14 and 15. The order with respect to each reactant was worked out from the positive slopes. It was $n = 0.96$ with respect to glucose and $n = 0.5$ for valine. The data are shown in Tables 3 and 4.
1000 mgms of valine with varying glucose

**Fig. 7.**

**Fig. 8.**

**Fig. 9.**

**Fig. 10.**
1000 glucose with varying valine

Fig. 11.

Fig. 12.

Fig. 13.
1000 mgm of valine vs variable glucose

1000 mgm of glucose vs variable valine

\[ \log \frac{dP}{dt} + 2 \]

\[ \log [M \text{ glucose}] \quad M = \text{weight in mgm} \]

Fig. 14

\[ \log [M \text{ valine}] \]

Fig. 15
Table III.

Rate data for reaction between 1000 mgm. of valine vs varying small quantities of glucose.

<table>
<thead>
<tr>
<th>Varying glucose weight in mgm.</th>
<th>log weight glucose</th>
<th>Pressure in mmHg</th>
<th>Time in seconds</th>
<th>Slope $\frac{\text{mmHg}}{\text{sec}}$</th>
<th>$2 + \log \frac{\text{dp}}{\text{dt}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1.602</td>
<td>120</td>
<td>960</td>
<td>0.125</td>
<td>1.0969</td>
</tr>
<tr>
<td>30</td>
<td>1.477</td>
<td>84</td>
<td>960</td>
<td>0.0875</td>
<td>0.942</td>
</tr>
<tr>
<td>20</td>
<td>1.301</td>
<td>74</td>
<td>960</td>
<td>0.07708</td>
<td>0.8865</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>40</td>
<td>1200</td>
<td>0.0333</td>
<td>0.5244</td>
</tr>
</tbody>
</table>

Table IV

Rate data for reaction between 1000 mgm. of glucose vs varying small quantities of valine.

<table>
<thead>
<tr>
<th>Varying valine weight in mgm.</th>
<th>log weight valine</th>
<th>Pressure in mmHg</th>
<th>Time in seconds</th>
<th>Slope $\frac{\text{mmHg}}{\text{sec}}$</th>
<th>$2 + \log \frac{\text{dp}}{\text{dt}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.477</td>
<td>100</td>
<td>1020</td>
<td>0.098</td>
<td>0.9912</td>
</tr>
<tr>
<td>20</td>
<td>1.301</td>
<td>75</td>
<td>900</td>
<td>0.0833</td>
<td>0.9207</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>53</td>
<td>960</td>
<td>0.0552</td>
<td>0.7427</td>
</tr>
</tbody>
</table>
Vant Hoff Isothermal Analysis

\[ \log \left( \frac{d(1-\alpha)}{dt} \right) + 3 \]

kinetic run on the gas line

at \( t = 134^\circ C \)

(300 mgm sample)

Slope = 1.43

Fig. 16.

\[ \log \left( \frac{d(1-\alpha)}{dt} \right) + 3 \]

kinetic run on T.G.B.

\( t = 134^\circ C \)

(150 mgm sample)

Slope = 1.3

Fig. 17.
Not only is the order with respect to glucose higher than that with respect to valine, but more gas is produced in the reaction, so that the reaction is prolonged. It is possible that the apparent low order with respect to valine is due to the catalytic decomposition of glucose which may be proceeding simultaneously. This aspect of the reaction in the presence of excess glucose also could be observed in reaction curves 16 and 17, Chapter 4.

The overall order of reaction can be calculated from the earlier results by the Van't Hoff differential method. This is done by plotting \( \log \frac{d(1-x)}{dt} \) against \( \log(1-x) \) for reaction curves obtained for 300 mgms of 1:1 mixture on the gas line as well as the ones obtained by T.G.B. for 150 mgms of mixture. \([\alpha]\) is the fraction of weight loss to the total weight loss at different times (mentioned in detail in further pages). Figures 16 and 17 show the kind of graphs obtained. Both graphs are linear over a wide range, the only deviation being at the very beginning and the end of the reaction. Similar kinds of graphs were obtained by Hupbert and Klawitter\(^{(58)}\) for the isothermal reaction between \(\text{BaCO}_3\) and \(\text{ZnO}\) containing 0.171 mole \% \(\text{Cr}_2\text{O}_3\).

The overall orders obtained from the gas line and T.G.B. results were respectively 1.43 and 1.3. These are in good agreement with the individual orders of 0.96 and 0.5 for glucose and valine respectively.
Further treatment of data using suggested equations by previous workers such as Jander,\(^5\) and modifications of them was attempted. These equations were mostly applicable to parabolic reaction curves and none of them gave satisfactory results for our sigmoid curves. Two equations were thought to be possibly applicable in this situation:

(a) Prout and Tompkin's equation and Mample law.

(b) Avramie and Erofeyev equation.

In order to obtain suitable curves to be applied conveniently by the above mentioned equations, the symmetrical parts of the curves belonging to kinetic runs on the gas line and T.G.B. were taken. Then by assuming the pressure at the end of symmetrical part as the final pressure \(P_f\), the degree of progress of reaction \(\alpha = \frac{P_1}{P_f}\) was worked out for \(P_1\) at different times. Similarly this was done for \(\alpha = \frac{\alpha_f}{\alpha_f}\) for kinetic runs on T.G.B. Figures 18 and 19 show plot of \(\alpha\) versus time obtained for both systems. The neglect of the final sections of the reaction curves was justified by the doubts about possible condensation of gaseous products at high pressures and the evidence that the main product towards the end of the reaction is water from the catalytic decomposition of glucose.

(a) The data obtained from the symmetrical sections of the reaction curves on the gas line were treated by Prout and
Symmetrical kinetic curves obtained on the gas line at different temperatures

Fig. 18.

Symmetrical kinetic curves obtained on T.G.B. at three different temperatures

Fig. 19.
Tompkin's equation as it was represented in Chapter 2 (p. 28).

$$\ln \frac{\alpha}{1 - \frac{\alpha}{2\alpha_i}} = k_3 t$$

Setting $\alpha_i = \frac{1}{2}$, the following form was obtained:

$$\ln \frac{\alpha}{1 - \alpha} = k_3 t$$

$\ln \frac{\alpha}{1 - \alpha}$ was worked out for the kinetic curves obtained on the gas line at the experimental temperatures 119, 124, 129, 134°C and was plotted against time. The graphs obtained are shown in figure 20. Straight lines were obtained at angles to one another. Vaughan and Phillips also obtained two straight lines at an angle for the decomposition of a single organic compound.

Nonetheless, the segmented straight lines showed no obvious trend and it was felt that few useful conclusions could be drawn from these results, and that the simple Prout and Tompkin's relationship was not obeyed.

Mample's law as mentioned in Chapter 2 has a general form:

$$\sqrt[\frac{3}{\alpha}]{\ln(1-\alpha)} = \sqrt[\frac{3}{\alpha}]{t}$$

This equation is identical with Erofeyev's second equation which was derived on different assumption for the decomposition of a single compound. This will be dealt with in Section b.

(b) The Avrami and Erofeyev equation was suggested to explain the decomposition of both single compounds and pairs of reactants. The mechanism which leads to a phase boundary
controlled reaction assumes that the nucleation step occurs virtually instantaneously, so that the surface of each particle is covered with a layer of product. Nucleation of the reactants, however, may be a random process, not followed by rapid surface growth. As nuclei grow larger, they must eventually impinge on one another, so that growth ceases where they touch. This process has been considered by Avrami and Erofeyev who have given the following generalised kinetics equations:

\[ 2 \sqrt{-\ln(1-\alpha)} = 2 \sqrt{kt} \quad (1) \]

\[ 3 \sqrt{-\ln(1-\alpha)} = 3 \sqrt{kt} \quad (2) \]

The first equation applies to the formation of flat nuclei, the second to the formation of cylindrical nuclei where their centres of formation are edges or surface cracks. It is said that these equations describe the kinetics of homogeneous and heterogenous processes. Erofeyev divides time into \( n \) intervals and considers the probability that the \( i \)th molecule reacts in the \( k \)th time interval. If \( P_i \) is the mean probability that an individual molecule has reacted by time \( t \), he shows that

\[ P = 1 - \exp \int_0^t \] pdt. \quad (3) \]

where \( P_i \) is the mean probability that the \( i \)th molecule reacts in the \( k \)th interval.

This is a generalised kinetic equation in which no assumption is made regarding the properties of the reacting system.
For isothermal unimolecular processes, as well as radio-active
disintegration, we assume in the above formula that $p = k = \text{Const}$,
whence $\alpha = \exp(-kt)$

For isothermal bimolecular reactions the initial
concentrations of the components A and B are assumed to be equal
to $a$, and $b$, respectively. The probability of the reaction of an
individual molecule of A is

$$p = \frac{k}{v(b-\alpha a)}$$

Here $\alpha$ is the fraction of A which has reacted, $k/v$ is not defined
in the original paper, but is apparently the rate constant for
unit volume of reactants.

Combining 5 and 7 we get

$$\frac{d\alpha}{dt} = \frac{k}{v(b-\alpha a)}(1-\alpha)$$

Integration by the method of partial fractions gives

$$\frac{kt}{v} = \frac{1}{b-a} \left[ \ln \frac{b-a\alpha}{b-b\alpha} \right]$$

which is identical with usual integrated form of the second order
rate equation if $x$, the concentration of A which has reacted after
time $t$ is taken as $\alpha a$.

Rearranging we get:

$$\alpha = 1 - \frac{a - b}{\alpha - b \exp \frac{k}{v(a-b)t}}$$
This result is similar to that of Erofeyev, but is expressed more simply.

Mott considers the electrons are responsible for the formation of nuclei in the case of decomposition of barium azide, and he suggests a theory which later was used in the Avrami Erofeyev equation. He suggests that the number of \( n \) electrons per unit of volume in the crystal increases linearly with the time. In a crystal of volume \( V \) and surface \( S \) he suggested the equation

\[
\frac{dn}{dt} = \frac{SQ}{V}
\]

where \( Q \) is a constant at a given temperature, and he supposes that \( Q \) is temperature dependent according to the law.

\[
Q = Q_0 e^{-q/RT}
\]

where \( q \) is the work required to remove an electron into the conduction band from a surface ion.

Integrating equation (1) he obtained

\[
n = \frac{SQ}{V} t.
\]

He considers the formation of a nucleus is structure sensitive (barium azide) and suggests the existence of an electron trap on the surface makes the formation of a nucleus possible.

The probability per unit time that an electron is trapped at a given sensitive point is proportional to \( n \). The probability that a second electron comes along before the first escapes is also proportional to \( n \). If 5 electrons are necessary to form a stable nucleus, the probability that a nucleus is formed in a
given time interval is proportional to \( n^\delta \). Thus if \( N \) is the number of nuclei at time \( t \)

\[
\frac{dN}{dt} = \left( \frac{SQ}{V} \right)^\delta \cdot \text{Const.} \tag{4}
\]

or on integration,

\[
N = \left( \frac{SQ}{V} \right)^{\delta+1} \cdot \text{Const.} \tag{5}
\]

He found at 100°C for barium azide \( \text{Ba(N}_3\text{)}_2 \), that \( N \) was proportional to \( t^3 \) and therefore deduced \( \delta = 2 \).

Erofeyev used this argument in his own equation. The rate of formation of nuclei is as equation (4)

\[
\frac{dN}{dt} = (n)^\delta \cdot \text{Const.}
\]

where \( n \) is the concentration of electrons in the conductivity band and \( \delta \) is the number of electrons necessary for setting up a stable centre of a reaction nucleus. The dependence of \( n \) upon time has the form of equation (3). On the assumption that conductivity electrons appear (in a number equivalent to interlattice cations) as a result of a surface reaction proceeding at a constant rate, the Mott case here corresponds to a limiting case: i.e., the reverse process of transition of conductivity electrons to the radicals absorbed on the crystal surface is ignored.

The second limiting case corresponds to steady state, i.e., when as a result of the established equilibrium of the surface reaction of dissociation of anion into radicals and electrons, we have \( n = \text{Const.} \)

For the Mott case, \( \frac{dN}{dt} = t^\delta \cdot \text{Const.} \)
and in the second limiting case \( \frac{dN}{dt} = \text{Const.} \)

For these cases Erofeyev deduces equations 1 and 2 for cylindrical nuclei and flat nuclei respectively.

The results obtained on the gas line were treated by the Avrami and Erofeyev equations (1) and (2). Equation (1) gave straight lines passing through the origin as shown in figure 21. The activation energy associated with these straight lines was determined by plotting \( \log k \) from these graphs against reciprocal absolute temperatures. The values of \( k \) are shown in Table V and the activation energy plot is shown in figure 22.

<table>
<thead>
<tr>
<th>( T^\circ C )</th>
<th>( T_{\text{absolute}} )</th>
<th>( \frac{k}{\text{min}}^{-1} )</th>
<th>( \log k )</th>
<th>( \frac{1}{T} \times 10^{-4} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>392.16</td>
<td>0.01888</td>
<td>2.276</td>
<td>25.5</td>
</tr>
<tr>
<td>124</td>
<td>397.16</td>
<td>0.3093</td>
<td>2.4903</td>
<td>25.18</td>
</tr>
<tr>
<td>129</td>
<td>402.16</td>
<td>0.50789</td>
<td>2.7057</td>
<td>24.86</td>
</tr>
<tr>
<td>134</td>
<td>407.16</td>
<td>0.06628</td>
<td>2.8214</td>
<td>24.57</td>
</tr>
</tbody>
</table>

The value obtained was 32 Kcal which was in good agreement with the values obtained by other methods of data treatment. The above equation (1) also was applied to the graphs obtained by T.G.B. Straight lines with different slopes were obtained which did not pass through the origin, but intercepted the time axis at 10, 7 and 3 minutes for temperature 120, 124, 134\(^\circ C\) as shown in figure 23. Equation (2) applied to the results obtained on the gas line did not give a reasonably straight line, but the results obtained from T.G.B. passing through the
\[ \sqrt{\frac{2}{-\ln(1-\alpha)}} \]

Gas line

Fig. 21. Time in minutes
Fig. 22. $\frac{1}{T} \times 10^{-4}$
Fig. 23.
$$\sqrt[3]{\text{lin}(1-\alpha)}$$

**Fig. 24**

Time in minutes.
origin. These are shown in figure 24.

The reasons why the gas-line graphs (figure 21) pass through the origin while the T.G.B. graphs (figure 23) are not clear. It is presumably related to the factors mentioned in Chapter 4.2, to explain the slightly different shapes of the gas line and T.G.B. reaction curves.
In Chapter 2 previous work on the reaction between reducing sugars and aminoacids in solution was reviewed. In contrast, in this work, all the experiments were carried out under vacuum, with the reactants carefully dried. Oxygen was of course absent. This might make the system simpler and reduce the number of products. It certainly cannot be expected that the solid phase reaction will lead to products directly comparable with those of the liquid phase reaction. The identification of minor products was not a basic aim of this work, but the nature of some products was investigated in the hope that it would elucidate the mechanism. A summary of the products found is given in the table below.

<table>
<thead>
<tr>
<th>Amino-acids</th>
<th>Major gaseous products identified</th>
<th>Minor gaseous products</th>
<th>Sublimed products</th>
<th>Solid product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>Iso-butyraldehyde; CO₂ + H₂O</td>
<td>Acetic acid carbonyl compound at mass 84</td>
<td>C₆H₈O₄</td>
<td>End product reductone at mass 181 (C₁₀H₁₇O₃N)</td>
</tr>
<tr>
<td>Norvaline</td>
<td>Butraldehyde; CO₂ + H₂O</td>
<td>Acetic acid + unknowns</td>
<td>C₁₇H₁₁N₄</td>
<td></td>
</tr>
<tr>
<td>2-amino-butyric-acid</td>
<td>Propionaldehyde, CO₂ + H₂O</td>
<td>Acetic acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

/contd...
<table>
<thead>
<tr>
<th>Amino-acids</th>
<th>Major gaseous products identified</th>
<th>Minor gaseous products</th>
<th>Sublimed products</th>
<th>Solid product</th>
</tr>
</thead>
<tbody>
<tr>
<td>α alanine</td>
<td>Acetaldehyde, CO₂ + H₂O</td>
<td>Furfural, aldol at mass 70, mass at 58.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>CO₂ + H₂O</td>
<td>Furfural, acetone mass at 72.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenyl β-alanine</td>
<td>Phenylacetaldehyde CO₂ + H₂O</td>
<td>Acetic acid, acetone</td>
<td>Compound at mass 281.</td>
<td></td>
</tr>
<tr>
<td>3-Amino-butyracic acid</td>
<td>CO₂ + H₂O</td>
<td>Acetic acid, 3-Penten-2-one acetone.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Amino-butyracic acid</td>
<td>CO₂ + H₂O</td>
<td>Furfural</td>
<td>Methyl ethyl ketone, methyl n-propyl ketone acetone.</td>
<td></td>
</tr>
<tr>
<td>β-alanine</td>
<td>CO₂ + H₂O</td>
<td>Aketo-compound.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) The major gaseous products from the reaction between glucose and α amino-acids are water vapour; carbon dioxide and the aldehyde one place lower in the homologous series than the original amino acid, i.e. the amino acid is deaminated and decarboxylated. The only exception to this rule was with the
lowest amino acid, glycine. This did not give rise to formaldehyde but produced CO₂ and water. It is possible that after the Schiff base is formed it does not undergo the transamination suggested below but undergoes further polymerisation.30

The reaction between glucose and other α amino-acids might well happen via the Strecker degradation, i.e., the glucose and α amino acid react together to give a Schiff's base. This decarboxylates readily and after transamination takes place the product can be hydrolysed by the water formed at the beginning of the reaction.

\[
\begin{align*}
R - CH - COOH + OHC - R' & \rightarrow R - CH - N = HCR' + H_2O \\
& \text{N tension sugar COOH}
\end{align*}
\]

\[\text{α amino acids} \]

\[\text{RCHO + H}_2\text{N} - CH_2 - R' \]

This mechanism accounts for the result of the \(^{14}\text{C}\) labelling experiments since CO₂ comes entirely from the carboxyl group of the amino acid. The transamination reaction has been postulated previously by Herbst and Engel.\(^{31}\) The reaction is said to be acid catalysed and perhaps the amino acid itself fulfils this role. There is no evidence whether or not an Amadori rearrangement is involved (see Chapter 2, p. 15).
(2) Production of acetic acid has not yet been reported in the reaction between glucose and \( \alpha \) amino acids, though it has been observed by Simon and Heubach\(^64\) and Hodge, Fisher and Nelson\(^65\) in the reaction between a keto-hexose and an amine salt. It was shown by running the reaction on the mass spectrometer direct insertion probe, that the amount of acetic acid goes through a peak as the temperature increases. Knowing that the other workers speeded the reaction by adding acid to the glucose/amine reaction mixture, we suggest that acetic acid plays an autocatalytic role in the reaction.

This effect together with the warming up of the reaction crystals by the heat produced in the reaction and the necessity to have some water available for the hydrolysis of the Schiff base might account for the induction period associated with the reaction.

(3) Production of water

Almost half of the weight loss from the glucose/valine reaction was shown to be as water. The calculation in Chapter 4, Section 4, showed only \( \frac{1}{7} \)th of the total water could come from the known reaction forming the Schiff base. Previous workers did not estimate the amount of water formed since most of them carried the reaction in aqueous solution. The additional water could arise from the degradation of glucose catalysed by amino acid and later by acetic acid produced. Degradation of glucose might occur, for example via elimination of cis and trans hydrogen and hydroxyl groups. If this is so, then the amount of
water involved should result in the degradation of virtually all the glucose present. This was indeed found to be so. While quantities of unreacted valine were found on several occasions, the 2:4 dinitrophenylhydrazone of glucose which is a particularly easy derivative to prepare was never found.

This conclusion is supported by the reaction curves obtained with glucose in excess which continue to rise after the main reaction has ceased.

(4) Degradation of glucose

Degradation of glucose may well accompany the glucose/amino acid reaction. Other workers have reported many different degradation products from reaction between sugars and amines, e.g. pyruvaldehyde and 5-H,M,F. The major degradation product from aldohexoses and ketohexose is 5-H,M,F. Furfural is the degradation product when pentoses are heated with acid. In this work no 5-H,M,F. was ever identified, when α amino acids were involved.

(5) Mechanism

The results in this work appear to fit the mechanism proposed by Hodge, Fisher and Nelson (1963) for the reaction between secondary amine salts and hexoses. (Secondary amine salts were used because less melanoidin was formed in reaction mixtures than when primary was used.) They heated reducing sugars with one or two mole of secondary amine salts in basic alcoholic media for 24 hours at 70°C-80°C. Aldo and keto hexoses
were tried (including glucose) and crystalline reductones were obtained with structure (I) as shown:

\[
\begin{align*}
R & \quad R \\
\text{N-} & \quad \Phi \\
\text{O} & \quad \text{OH} \\
\text{CH}_3 & \quad \text{OH}
\end{align*}
\]

Reducing sugars were also reacted with secondary amines in a methodical way to determine the precursors of isolated compounds. When glacial acetic acid was added to the reaction mixtures browning occurred much more rapidly.

They suggested that the reductone is formed through the followig intermediate 6 carbon reductone compound which undergoes a reaction with RR'NH as shown below.

\[
\begin{align*}
\text{CH}_3 & \quad \text{C} = \text{O} \\
\text{C} & \quad \text{O} \\
\text{C} & \quad \text{O} \\
\text{C} & \quad \text{O} \\
\text{CH}_3 & \quad \text{C} = \text{O}
\end{align*}
\]

The above formulation shows how the amino hexose reductone III would be formed by a double intramolecular aldol condensation in which both carbonyl groups and methyl groups participate. They
also suggested the formation of a 4 carbon fragment as below:

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
| & | & | \\
C - 0 & | & C - 0 \\
| & | & | \\
C - OH & \leftrightarrow & C - OH \\
| & | & | \\
C - OH & | & C - OH \\
| & | & | \\
C = 0 & | & C = 0 \\
| & | & | \\
\text{CH}_3 & | & \text{CH}_3 \\
\end{align*}
\]

Simon and Heubach (1965) used labelled C\(^{14}\) glucose, prepared the labelled reductones and showed that 25% of C methyl of (III) is derived from C - 1 of glucose and 72% from C - 6. They suggested a different mechanism but started with the intermediate I and finished with the same compounds (IIIa) and (IIIb).

In Chapter 4, Section 4, the presence of a compound of mass 144 and a empirical formulae \(C_6H_{10}O_4\) was reported. This could well have been the reductone (I).

The above compound provides a side reaction which might have produced acetic acid. Unfortunately no trace of a 4 carbon reductone or derivative of it was ever observed.

One of the 2:4 dinitrophenylhydrazones mentioned in Chapter 4 showed the existence of a compound with a molecular weight of 181. This is the same as Compound (III) above. Considering the glucose/valine reaction we suggest that it might take the following path which does not involve transamination.


\[ RCHO + H_2N - CH - R' \xrightarrow{-H_2O} RCH = N - CH - R' \]

\[ \text{glucose} \quad \text{valine} \xrightarrow{-CO_2} \text{Schiff base} \]

\[ R' - CH_2 - N = CHR \xrightarrow{+H_2O} R'CH_2 - NH_2 + RCHO \]

A second mechanism also is possible involving a reaction between the unreacted valine and the intermediate formed from glucose degradation.

\[ R'CH - NH_2 + COOH \quad \xrightarrow{+H_2O} \quad \xrightarrow{-2H_2O} \quad \xrightarrow{-CO_2} \]

The above compound after losing carbon dioxide leads to Compound (III).

\[ \text{CH}_3 \]

\[ \text{HO} - \text{C} - \text{CH} \]

\[ \text{HO} - \text{C} - \text{NH}_2 \]

\[ \text{HO} - \text{C} - \text{NCH}_2R' \]
5H.M.F. would not take the place of reductone in this reaction, though it has the same molecular weight. After the single carbonyl group in it had reacted, none would be left to give a phenylhydrazone.

(6) Glucose and other α amino acids

The reaction curves obtained from the reaction of glucose with α amino acids at 124° C were compared in Figure 33, Chapter 4. They are almost of equal slope. As the molar quantities of glucose and amino acid present in each case were similar, it appears that the rate of reaction is independent of the number of carbon atoms, though the total final pressure change varies. It has been suggested that the amine-glucose reaction is catalysed by acids. It is possible that the same applies to the α amino-acid-glucose reaction in which case the constancy of the rate might be related to the fact that all of the α-amino acids involved in this work have similar Pk values. Some of the Pk values of α-amino acids and β and γ amino acid are represented in the table below.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Pk_{1}^{(COOH)}</th>
<th>Pk_{2}^{(NH^+)}</th>
<th>Isoelectric Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>2.34</td>
<td>9.60</td>
<td>5.97</td>
</tr>
<tr>
<td>α-alanine</td>
<td>2.34</td>
<td>9.69</td>
<td>6.0</td>
</tr>
<tr>
<td>α-aminobutyric acid</td>
<td>2.55</td>
<td>9.60</td>
<td>6.08</td>
</tr>
<tr>
<td>Nor-valine</td>
<td>2.36</td>
<td>9.72</td>
<td>6.04</td>
</tr>
<tr>
<td>Valine</td>
<td>2.32</td>
<td>9.62</td>
<td>5.96</td>
</tr>
<tr>
<td>β-alanine</td>
<td>3.60</td>
<td>10.19</td>
<td>6.90</td>
</tr>
<tr>
<td>γ-aminobutyric acid</td>
<td>4.031</td>
<td>10.556</td>
<td>7.29</td>
</tr>
</tbody>
</table>
The reaction of glucose with $\beta$ and $\gamma$ amino acids was faster than with $\alpha$ amino acids, though $\beta$ aminobutyric acid appeared to react in two stages. This was shown for the liquid phase by Gottshalk and Partridge.\(^\text{18}\)

It may well be significant that $\beta$-alanine and $\gamma$-amino- butyric acid react faster than the $\alpha$-amino acids (e.g. $\beta$-alanine 0.8; $\alpha$-alanine 0.4 in arbitrary units) by an amount related to the hydrogen ion concentrations to which they give rise.

Quantitative estimates of minor products were not made, but it would appear that the amount of furfural increases and acetic acid decreases as one descends the homologous series.

(7) Thermochemistry

It is claimed by Tamman,\(^\text{83}\) and Hedvall\(^\text{84}\) that reactions between ionic inorganic crystals are exothermic. No experimental measurements of exothermicity were made in this work, but a crude estimate was made as follows. Assuming the overall process:

\[
\begin{align*}
\text{CH}_3\text{CH} - \text{CH} - \text{NH}_2 + \text{OHC} &\rightarrow (\text{CH}_3)_2 = \text{CH}_2 \text{CH}_2 \text{N} = \text{CH} \\
\text{valine} &\rightarrow \text{glucose} \quad \text{Schiff base} \\
\text{C}_5\text{H}_{11}\text{O}_5 &\rightarrow \text{CH}_2 - \text{NH}_2 + \text{CO}_2 + \text{CH}_3\text{CHO} \\
\text{glucose amine} &\rightarrow \text{isobutyraldehyde}
\end{align*}
\]

The heats of formation of some of the reactants and products can be found from the standard tables.\(^\text{85}\)
\[ \Delta H_f \text{ at } 25^\circ C \]

- **L-valine (crystal)**: \(-147.68\) Kcal/mole
- **\(CO_2\)**: \(-94.052\) Kcal/mole

The difference between the heat of formation of the glucoseamine and glucose can be worked out approximately from the difference between the heat of formation of the following compounds, since the aldehyde oxygen in glucose is replaced by \(NH_3\).

The heat of formation of few homologous amines and their corresponding aldehydes at \(25^\circ C\) are tabulated in the table below:

<table>
<thead>
<tr>
<th>Homologue amines</th>
<th>(\Delta H_f) (gas)</th>
<th>(\Delta H_f) (liq.)</th>
<th>Homologue aldehydes</th>
<th>(\Delta H_f) (gas)</th>
<th>(\Delta H_f) (liq.)</th>
<th>(\Delta H_f) (gas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylamine</td>
<td>-5.5</td>
<td>-11.3</td>
<td>Formaldehyde</td>
<td>-25.95</td>
<td>-40.0</td>
<td>20.45</td>
</tr>
<tr>
<td>Ethylamine</td>
<td>-11.35</td>
<td>-17.71</td>
<td>Acetaldehyde</td>
<td>-39.73</td>
<td>-45.88</td>
<td>28.38</td>
</tr>
<tr>
<td>Propylamine</td>
<td>-16.77</td>
<td>-24.26</td>
<td>Propionaldehyde</td>
<td>-45.45</td>
<td>-52.54</td>
<td>28.68</td>
</tr>
<tr>
<td>n-butylamine</td>
<td>-22.7</td>
<td>-30.5</td>
<td>Butyraldehyde</td>
<td>-48.94</td>
<td>-56.99</td>
<td>26.24</td>
</tr>
<tr>
<td>s-butylamine</td>
<td>-25.4</td>
<td>-32.86</td>
<td>Iso-butyraldehyde</td>
<td>-52.25</td>
<td>-59.79</td>
<td>26.85</td>
</tr>
</tbody>
</table>

Ignoring the \(\Delta(\Delta H_f)\) for formaldehyde and methylamine an average difference of \(27.3\) Kcal was obtained. The overall heat of reaction could be obtained by subtracting the heat of formation of product from the heat of formation of reactants.
\[ \Delta H_{\text{reaction}} = (-94.051 - 52.25) - (147.68) + 27.53 \]

approx. equal to \(-29\) Kcal per mole

The above calculations suggest that the first stages of the reaction are exothermic.
(III) Analysis of Mass Spectra of Sugars

The relative abundance of peaks obtained from the spectra of sugars and the possible fragment ion formula for the major peaks and significant peaks are tabulated in order of mass numbers. The most abundant peak for each spectrum is taken as 100.

(1) Glucose

The mass spectrum of glucose was obtained at 17 eV. It was observed that glucose does not give the parent molecular ion peak. The same phenomenon has been observed in the spectra of partially substituted derivatives of monosaccharides. The base peak for glucose was at m/e = 73 (see Fig. 4.39). The second highest peak was at mass 60, and the third at 43. The peaks observed in order of abundance were as follows:- 73, 60, 43, 31, 29, 57, 71, 15, 19, 59, 97, 55, and significant smaller peaks were at masses 101, 102, 103, 131, 149 and 163. A fragmentation pattern of the spectrum is suggested in Figures 23 and 24, corresponding to the above mass numbers observed.

Figure 23 shows the possible break down pattern of the open chain glucose through an intermediate to the mass numbers 101, 102 and 103 as well as mass 60. Figure 24 shows the possible fragmentation pattern leading to the peaks at mass numbers 73, 71, 60 and 43. There is some evidence that glucose (I) goes into the gas phase in a protonated form. On electron impact it could ionize to give Ia which could lose the OH on C - 6 to give Ib which could fragment to masses 60 and 43 (Ic). In another possible route glucose first loses CH₂OH to give (IIa)
Glucose

$M/e = 180$

\[ \begin{align*}
\text{Ia.} & \quad \text{M/e = 163} \\
\text{IIa.} & \quad \text{M/e = 149} \\
\text{IIIa.} & \quad \text{M/e = 163} \\
\text{IIIb.} & \quad \text{M/e = 131} \\
\text{IIIc.} & \quad \text{M/e = 73}
\end{align*} \]

\[ \begin{align*}
\text{Ib.} & \quad \text{CH}_2\text{CHO} \\
\text{M} & = 43 \\
\text{Ic.} & \quad \text{M/= 60} \\
\text{IIb.} & \quad \text{M/= 71} \\
\text{IIc.} & \quad \text{M/= 60}
\end{align*} \]

Fig. 25.
Fig. 26.
at 149, then this by losing a water molecule gives the peak at 131 (IIb). Straight fragmentation of this ion leads to masses 71 and 60 (IIc). In a third possible fragmentation glucose, by losing an OH group from either C₂ and C₃ (IIIa and IIIb) positions could break up to m/e = 73 (IIIc). (See Figures 25 and 26.)

(2) 2-Deoxyglucose

2-Deoxyglucose is similar to glucose, but one hydroxyl is absent on the C - 2. The spectrum was shown in Figure 40, Chapter 4. The highest mass numbers observed were small peaks at 147, 146, and 133. Other significant peaks observed in order of abundance were:

60, 57, 43, 74, 45, 86, 71, 56, 61, 29, 73, 44, 28, 55, 27, 41.

One main difference between glucose and 2-deoxyglucose is that the latter gives a base peak at 60 instead of 73, suggesting that the second carbon carbon atom is involved in the formation of the dihydroxyallyl ion. On the other hand, 73 is still a significant peak and presumably some dihydroxyallyl ions arise from other sources. Peaks at 73 and 71 are related to the allyl radical and would be stabilized by delocalization of electrons and by the electronegativity of the oxygenation (cf. glucose). A possible fragmentation pattern is suggested in Figures 27 and 28.
2-Deoxy glucose

\[
\text{M/e=147} \quad \text{M/e=164} \quad \text{M/e=133}
\]

Fig. 28.
2-Deoxy glucose

Fig. 27.
(3) **Mannose**

Mannose is a hexose and an optical isomer of glucose, the hydroxyl being inverted on C-2. The spectrum of this compound is shown in Figure 4.41. The major peaks (as represented in the table) observed in order of abundance were 73, 60, 31, 43, 29, 57, 71, 61, 42, 18, 44. On a second occasion the same peaks appeared with different abundances. The major peaks in order of abundance were 31, 73, 60, 29, 43, 61, 42, 44, 57, 56, 32 and 28. The breakdown pattern of these spectra is thought to be similar to that of glucose, since there are no major differences between this spectra and that of glucose, if the level of reproducibility is taken into account. The base peak at 31 in the latter is possibly due to loss of CH$_2$OH at C-6 or fragment CH$_3$CH$	ext{OH}$ ion.

Note: peaks at mass 18 were found with many of the sugars. They may have to due to water background in the mass spectrometer, to water absorbed on the sugar crystals, to water lost by thermal degradation of the sugar, or to elimination of the H$_2$O$^+$ ion from heavier ions. As the ionization potential of water is 13 eV which value is much higher than that of most organic compounds, and as the charge tends to stay with the fragment of lowest ionization potential, the last possibility is the least likely.

(4) **Rhamnose**

Rhamnose is glucose lacking an OH group on C-6. The spectrum of this compound was shown in Figure 4.42. The data
Fig. 29.
obtained are shown in the Table. The only peak at a high mass number appeared at 146, showing loss of a molecule of water. The most abundant peak appeared at mass 18, but it is unlikely that this is primarily due to fragmentation of the sugar ion (see previous paragraph). The second major peak was therefore taken as the base peak. The major peaks observed in order of abundance were 60, 73, 29, 31, 45, 58, 71, 42, 57, 27, 56, 28, 41. The presence of peaks at 73 and 60 indicates that C-6 is not always involved in the formation of these ions. On the other hand there is still a significant peak at mass 31. This was previously thought to arise from the hydroxyl group involving the C-6 carbon of the aldohexoses. There must be in fact another source, perhaps involving the elimination of CHO from the CH—\( ^{14} \text{OH} \) ion. A fragmentation pattern is suggested in Figure 29.

(5) D-Galactose

Galactose is an optical isomer of glucose, having an OH group inverted at C-4. The mass spectrum of this compound was shown in Figure 4.38. The major peaks observed in order of abundance were 71, 43, 61, 60, 73, 18, 57, 31, 29, 44, 5, 42, 56, 55, 19, 17, 41, 72, 74, 101, 102, 69, 91, 102 and 103.

The appearance of the base peak at mass 71 gives a distinct difference between the spectra of mannose and glucose, and on the other hand galactose. A possible suggestion to account for this could be the trans positions of the hydroxyl group and hydrogen on carbons 4 and 5 of galactose differing from those of
Fig. 30.
Fructose

![Chemical diagram showing the reaction pathways of fructose](image)

Fig. 32.
glucose and mannose which are in the cis positions. This may facilitate the loss of a molecule of water from the ion at M/e = 149, leading to a base peak at 71. (See Figure 30.)

(6) **D-fructose**

Fructose is a keto hexose having the aldehyde group replaced by a keto group on carbon 2. The spectrum of this compound was shown in Figure 4.44. The major peaks observed in order of abundance were at 73, 18, 43, 31, 57, 60, 61, 29, 71, 86, 45, 103, 55, 56. The spectrum obtained is surprisingly similar to that of glucose though the peak at 60 is of lower abundance. A fragmentation pattern of this compound is suggested in Figure 31.

(7) **Sorbose**

Sorbose is a keto hexose and an optical isomer of fructose having an OH group inverted on C-5. The spectrum of this compound was shown in Figure 4.45. The major peaks observed in order of abundance were at masses 31, 43, 29, 61, 73, 18, 60, 44, 42, 45, 57, 72, 32, 71, 27, 55. The base peak at 31 gives a distinctive difference between this compound and fructose. Though mass 73 is of lower abundance, it is thought that the fragmentation pattern of this compound is likely to be similar to the one of fructose.

(8) **Arabinose**

Arabinose is an aldopentose and its spectrum was shown in Figure 4.46. The molecular ion peak did not appear, but a
Arabinose

\[
\text{Fig. 31.}
\]
very small peak appeared at $P_{M-1} = 149$, which may correspond to the loss of a proton, though this seems an unlikely fragmentation.

The major peaks observed from spectra of arabinose, in order of abundance were at 31, 43, 29, 60, 73, 61, 42, 44, 72, 57 and the others are shown in the Table. A fragmentation pattern of this compound is suggested in Figure 32.

(9) Lyxose

Lyxose is another aldopentose, having an OH group inverted on C-2. The spectrum of this compound as represented in Figure 4.47 showed a small peak at $P_{M+1} = 151$, which may well correspond to the protonated ion.

The major peaks observed in order of abundance were at masses 73, 60, 43, 31, 29, 61, 42, 71, 57, 45, 44, 27, 19 and 86. The spectrum of this compound is similar to that of ribose in terms of the first three peaks, but differs from that of arabinose.

(10) Ribose

Ribose is an aldopentose and an optical isomer of arabinose and lyxose. The spectrum of this compound is represented in Figure 4.48 along with the spectrum obtained by Heyns, Figure 4.38. The spectrum is similar in view of the reproducibility level, with that of lyxose. The highest mass number appeared as a small peak at mass 149. Heyns obtained a peak at 150.

(11) Lactose

Lactose is a disaccharide formed of molecules of galactose and glucose. The spectrum of this compound was shown in Figure 4.49.
Two high mass peaks of uncertain origin appeared at mass numbers 173 and 191. No routes by which these could arise from the sugar could be visualised and they may be due to degradation products. Otherwise the spectrum obtained corresponded to the spectrum of the two monosaccharides.

(12) Sucrose

Sucrose is another disaccharide formed from a molecule of glucose and a molecule of fructose. The spectrum of this compound was shown in Figure 4.50 and corresponded to the spectrum of a monosaccharide, since the highest mass number observed was at m/e = 163.

It is probable that all disaccharides fragment on electron impact and give fragmentation patterns characteristic of their constituent monosaccharides.

(13) General Comments

It is clearly difficult if not impossible to use mass-spectrometry to distinguish the various pentoses or the various hexoses from each other, probably the ionization process destroys the conformation.

No sugars appear to give a molecular ion peak. It is possible that pentose ions are slightly more stable in that Heyns obtained a small peak at 150 in the mass spectrum of ribose by electron impact and a large peak at 151 by field emission mass spectrometry. The mass spectrum of ribose obtained by us showed no peaks at 150 and 151 only small peaks at masses 147, 149.
Disaccharide mass-spectra are not distinguishable either from each other or from monosaccharides. Presumably the oxygen bridge is rapidly destroyed on ionization leading to the observed peak at 163.

Some evidence from galactose suggested that trans elimination of water is easier than cis, also rhamnose readily loses a molecule of water from the parent molecule. Certain empirical differences between sugars could be observed but any attempt to use this as a method of analysis would be confused by instrumental factors and lack of reproducibility.
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CONCLUSIONS
1. Solid dried glucose will react with solid dried valine at a measurable rate at temperatures around 119°C - 134°C, and the rate of reaction seemed to reduce with increasing humidity.

2. The major gaseous products of this reaction are water, carbon dioxide and isobutyraldehyde. The carbon dioxide was shown by labelling experiments to arise from the carboxyl group of the valine.

3. The reaction occurred below the melting point of either reactant, e.g. at 119°C which is well below the melting points of glucose and valine. Electron microscopy as well as optical microscopy with a heated sub-stage showed that the reaction occurs on the surface of the glucose crystals. The reaction does not go to completion because a solid inert product is produced at the interface of the crystals and this inhibits the reaction.

   It is suggested that the reaction up to this stage proceeds via a Strecker degradation, producing the aldehyde, CO₂ and water. Further slow reaction might occur by diffusion of reactants through the layer of solid product, or probably by the straightforward thermal degradation of glucose.

4. The rate of reaction was followed both by measuring the weight loss by the reaction and by measuring the pressure of gases evolved. The results agree moderately well.

   Both sets of reaction curves are sigmoid in shape but levelling off does not correspond to the reaction going to
completion. If the reactants after reaction ceases, are ground up together, the reaction will start again, and analysis shows unreacted valine.

5. The activation energy of the reaction was about 34 kcal/mole and, contrary to the accepted theories of solid-solid reaction, did not vary with particle size. The heat of reaction was estimated from thermochemical data as about 29 kcal/mole exothermic.

6. The concept of order has little meaning in solid-solid reactions. Nonetheless some idea of the influence of amounts and concentrations of reactants on rate can be given by the statement that the reaction was roundabout first order for glucose and one half order for valine.

7. The kinetic curves showing the rates of reaction were analysed on the basis of a large number of models proposed in the literature (e.g. Jander, kinetic equations based on the concept of an order of reaction \( \ln(1-\alpha)^n = kt \), Ginstling-Brounshtein, Prout and Tompkins, \(^{42}\) Avrami and Erofeyev \(^{59}\)). Some of the results fitted well to the theories of Avrami and Erofeyev. In particular good results were obtained using the gas line results and the equation of Avrami and Erofeyev for the build up of flat nuclei on the crystal surface. This appears a reasonable model of the reaction.

On the other hand, the thermogravimetric balance results
fitted better to the model for cylindrical nuclei. It is clear that in so complex a reaction as this, it is not easy to distinguish between the various reaction mechanisms giving rise to sigmoid curves. Nonetheless, it appears likely that the reaction occurs via nuclei formed at points where the valine crystals come into contact with the surface of the glucose crystals and that these nuclei spread and ultimately merge.

8. The gaseous products from the reaction of other $\alpha$-amino acids with glucose correspond to those of glucose/valine except for glycine; that is the major products are water, carbon dioxide and the aldehyde obtained by decarboxylation and deamination of the amino acid. The rates of the above reactions were found to be similar to one another. It appears that the rate rises considerably for the reactions between glucose and the corresponding $\beta$ and $\gamma$ amino acids (e.g. $\beta$ alanine, and $\beta$ and $\gamma$ amino butyric acid). It is suggested that this could be due to their having higher $P_k$ acid values than the corresponding $\alpha$ amino acids. Traces of acetic acids, acetone and furfural were observed in the case of the lower amino acids.

9. Some investigation was carried out of the less volatile products of the glucose-valine reaction. Products found in the solid and sublimed product were carbonyl compounds of molecular weight 144 and 181. These suggested that the reaction might undergo a reaction analogous to that suggested by Simon and Heubach as well as Hodge, Nelson and Fisher for
the reaction between secondary amine salts and glucose in the
presence of an acid catalyst.

Minor products found in the glucose/valine reaction
were acetic acid, a nitrogen compound of mass 183 and a carbonyl
compound of mass 84, the amount of acetic acid went through a
peak as the temperature was raised. Other unidentified mass-
spectrometer peaks were found at mass 74, 174, 207, 256. Some
of these appear to be products of the thermal degradation of
glucose.

10. The main gaseous products from the reaction between
glucose, 3 and 4 amino acids were water and CO₂. No aldehydes
due to degradation of amino acid were obtained. Acetone and
3-penten-2-one were identified from the reaction between glucose
and 3-amino-butyric acid. These are probably glucose degradation
products.

Furfural was proved to be produced from the reaction
between glucose and 4-amino-butyric acid. Some keto compound
moities, such as acetone, ethylmethyl ketone, and methyl-n-propyl
ketone were identified from the above reaction.

There was some evidence that furfural occurred in small
quantities in the reaction of other amino acids. This was
surprising since hexose would be expected to lead 5-hydroxymethyl
furfural and not the lower molecular weight material.

11. The amount of water produced in the glucose/valine
reaction corresponded seven molecules of water for every molecule
of aldehyde produced. This proves what is suggested by the number
of minor products, that is that simultaneously with the decarboxy-
lation and deamination of the valine, the glucose is undergoing
thermal degradation probably catalysed by amino acid.

This is supported by the fact that in mixtures containing
excess glucose, some reaction continues for a long time after
valine appears to have stopped reacting.

12. Mass spectra were obtained of a number of hexoses,
pentoses and disaccharides. The disaccharide spectra were
undistinguishable from those of hexoses, and though hexoses and
pentoses could be distinguished, the various optical isomers could
not. Certain empirical differences between the sugars could
be observed, but any attempt to use them as a method of analysis
would be confused by instrumental factors and lack of reproducibility.

13. The main peaks obtained from all sugars occur at
m/e values of 73, 60, 31. These are presumably due to the
ions HO — CH = CH — CH = O³H, [HO — CH = CH — OH]⁺ and [CH₂OH]⁺
respectively. Other ions correspond to the loss of OH and
CH₂OH from the parent molecule ion, and the loss of a number of
molecules of water from the resulting fragments. Fragmentation
patterns were proposed to account for the main peaks.

The CH₂OH ion might be thought to arise from fission of the
hydroxymethyl group in the C-6 carbon. This was shown not
to be so because it is also a major ion in the spectrum of
rhamnose (which has a CH₃ in C-6 position). It has presumably
arisen by a number of fragmentations particularly of the ion at
mass 60.
(1) Some of the problems in the study of the kinetics, such as the areas of surface contact and the degree of mixing could be reduced if a standard pellet making apparatus was available. Other variables such as a uniform position for pellets to ensure constant heating of different samples could be employed.

More detailed studies of the evolution of water are clearly desirable, as is more comparative work on the thermogravimetric balance and gas line. It would also be useful to study the catalytic effect of other added solids which would alter the pH.

(2) More detailed studies using cine-photography in conjunction with optical microscopy could cast light on the role of the layer of inert product.

(3) The use of a mass spectrograph linked to a G.L.C. column could certainly give more detailed information as to the identity of the minor products.

(4) The building of the MIDAS computerization unit on to the departmental mass spectrometer should allow more detailed work by repetitive scanning on the somewhat irreproducible sugar spectra to see if significant differences exist between them.
(5) The use of the direct insertion probe on the mass spectrometer as a reaction chamber gave disappointing results. Given a water cooled ion block and a computerized output from the spectrometer, significant low temperature reaction products should be detectable.

(6) The major outstanding problem of the thesis is what happens to the nitrogen atom which remains in the largely unidentified solid products. Its location presents an interesting problem in organic chemistry.
APPENDIX I.

Data obtained from straight part of kinetic curves on the gas time to calculate activation energy for the mesh numbers 200, 150 and 100.

**Mesh 200**

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**Mesh 150**

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