STEREOCHEMICAL ENZYMIC SPECIFICITY

with special reference to

EXPONENTIAL ANALYSIS

and the

KINETICS

of

ASYMMETRIC EMULSIN-HYDROLYSIS

of

β-GLUCOSIDES

A THESIS

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PART. 1.

STEREOCHEMICAL ENZYMIC SPECIFICITY.

PART. 2.

EXPONENTIAL ANALYSIS and ASYMMETRIC EMULSIN-HYDROLYSIS.

PART. I.

STEREOCHEMICAL ENZYMIC SPECIFICITY.

A. INTRODUCTION.

While the general occurrence of natural products in optically active forms was early recognised by BIOT, the observation that natural agencies may differentiate in their action on optical isomers, is due to PASTEUR[1]. It was noted that the mould 'Pen. Glauc.' destroyed the d-component of ammonium racemate more quickly than the l-component, the latter being recovered in part from the solution.

This observation was gradually extended. Several racemic substrates were shown to give, with moulds, similar preferential assimilation of one component, and data accumulated mainly in the hands of LEWKOWITSCH and LE BEL. Instances of the selective action, on optical isomers, of animal metabolism and the growth of yeasts and bacteria generally were also recognised, and a stimulus to the correlation of these effects with molecular configuration was given by the VAN T'HOFF[2] - LE BEL[3] theory of the asymmetric carbon atom.

The biochemical importance of stereochemistry was emphasised by FISCHER[4] in his classic paper of 1898.

Prior to 1897, a distinction had been drawn between the long known soluble enzymes, diastase emulsin and trypsin, and the ferment agents of the living cells of yeasts or bacteria. With the isolation from the yeast cell, in 1897 by BUCHNER[5], of the enzyme-

complex 'zymase', which retained the sucroclastic power of the yeast, a unifying classification on a chemical basis was reached, and the concept of enzyme action as biochemical catalysis was revived from BERZELIUS[6]. The recent discovery, by KUHN[7] at Heidelberg, and by MITCHELL[8] in Glasgow, of the asymmetric photochemical decomposition of racemates by circularly polarised light, further directs attention to the physico-chemical aspects of the agencies involved in the asymmetry of natural compounds.

Owing to the diverse aims of enzyme study, botanical physiological or chemical, to the early insufficiency of criteria for identifying enzymes, and to the lack of recognition of the many factors controlling their action, much of the earlier data on stereochemical specificity is not easily correlated, is sometimes contradictory, and was frequently incidentally observed. By the early years of the century, however, the stereochemical specificity of enzyme action had already been thoroughly established in the broad fields of carbohydrates, proteins and esters, by FISCHER[9], FISCHER and ABDERHALDEN[10], and DAKIN[11].

Attention was directed to optical selectivity in enzymes by the chemical work of McKENZIE[12] on asymmetric synthesis, which found a biochemical counterpart in that of ROSENTHALER[13], and NEUBERG[14]. In addition the conception of the enzyme as a chemical individual whose preferential attack on optical isomers is conditioned by its own asymmetric nature, was furthered by the results of BREDIG and FAJANS[15] on the asymmetric catalytic effect of optically active alkaloids on the decomposition of d- and l-camphorcarboxylic acids, which formed a suggestive parallel, both in nature and degree

to the biochemical results of DAKIN with lipase on esters.

While results were periodically noted, mainly from the point of view of effecting biochemical resolutions, a really quantitative study of stereochemical specificity had to await the inspiration of the intensive study of enzymes begun by WILLSTÄTTER at Munich. With new methods of purification, quantitative comparison of the enzyme content in different preparates, and the extension under various noted conditions of the kinetic measurements initiated by O'SULLIVAN and THOMPSON[16], and developed by BROWN[17], HENRI[18] and ARMSTRONG[19], the factors involved were gradually delimited. A systematic study of the effect of esterases on optical isomers was begun. Not the least of the results of the application to enzyme kinetics of rigorous mass action principles, on the basis of the MICHAELIS equation[20] and its extensions, has been its recent success, with WILLSTÄTTER and BAMANN, and RONA and AMMON and also in England, in reconciling, particularly with the optical selectivity of esterases, many apparently contradictory results, and in placing stereochemical enzymic specificity on something approaching a fundamental quantitative basis.

B. OXIDATION and ASSIMILATION.

1. General.

In 1860, PASTEUR[1] proved that in a solution of NH_4-racemate, the mould 'Pen. Glauc.' destroyed the d-component. An increasing optical rotation was induced, and the l-component was recoverable. This observation was extended. Such preferential destruction of one antipode was found a common phenomenon with moulds and bacteria generally, and with the aim of biochemical resolution, similar experiments were made on racemic alcohols and hydroxy- and amino-acids. A summary of results prior to 1895 is given by WINTHER[2].

Table.1.

Fungus.	Isomer preferentially attacked.	Authority.
Pen. Glauc.	d-Me. Et. carbinol	LE BEL, COMBES.
" "	d-Me. n. Pr. "	"
Asp. Nig.	d-Me. n. Pr. "	"
'Mould Fungus'	d-Me. n. Pr. "	"
Pen. Glauc.	d-Me. n. But. "	COMBES and LE BEL
'Bacteria'	l-Me. n. Amyl "	LE BEL
Pen. Glauc.	l-Et. Pr. carbinol	COMBES and LE BEL
'Mould Fungus'	l-Me. Et. carbincarbinol	LE BEL
Cheese Bact.	d-Propylene glycol	LE BEL
Bact. Termo.	d-Propylene "	LE BEL
'Mould Fungus'	d-Propylene "	LE BEL
Pen. Glauc.	l-Ethoxysuccinic acid	PURDIE and WALKER
" "	d-Glyceric acid	LEWKOWITSCH
Bact. Ethacet.	l- " "	FRANKLAND and FREW
Pen. Glauc.	l-Lactic acid	LINOSSIER, LEWKOWITSCH
'Bacteria'	l- " "	FRANKLAND and McGREGOR
Pen. Glauc.	d-Tartaric acid	PASTEUR, LE BEL
Schizomycet.	l- " "	LEWKOWITSCH
'Mould Fungus'	l-Aspartic acid	ENGEL
Pen. Glauc.	d-Glutaminic acid	SCHULZE and BOSSHARD
Pen. Glauc.	d-Leucine	SCHULZE
Wine Lees, Schi.	d-Mandelic acid	LEWKOWITSCH
P. g., Asp. Muc.	l- " "	"
Bact. Termo.	l- " "	"
Yeast, Asp. Fumig.	l-β. Phenyldibromo-propionic acid	STAVENHAGEN and FINKENBEINER
'Mould Fungus'	d-Me. Et. Pr. isobut. -NH_4Cl	LE BEL

The mould species used were in not all cases rigorously isolated, and some of the results have been shown to be due to accidental atmospheric contamination. The type of isomer preferentially attacked depends both on the substrate and on the organism. An interesting distinction is the regularity of attack by 'Pen.Glauc.' on the d-components of the $CH_3 \cdot CHOH \cdot R$ alcohol series in contrast with that on the l-isomer of the $C_2H_5 \cdot CHOH \cdot C_3H_7$ member.

Systematic investigations with many pure cultures were made by PFEFFER[22] on dl.tartaric and dl.mandelic acids, and by PRINGSHEIM[23] on dl.leucine and dl.glutamic acid. Decomposition of each isomer at equal rates was observed in about 50 % of the experiments. Where preferential assimilation occurred, it was relative only, not absolute, but the degree varied with the organism and according as the substrate were attacked as acid, NH_4- or Na.-salt. The food nutriment was also shown to be not without effect. In PFEFFER's experiments in only one instance was d-acid recovered. With the amino-acids, unless decomposition ensued at equal speeds, invariably d-leucine and l-glutamic acid, the antipodes of the natural components, were recovered. Optical purity varied greatly.

An important complementary investigation was that of McKENZIE and HARDEN[24] on variation of substrate. The effect of a few pure cultures on many racemic acids was studied with a view to differentiate between absolute and relative specificity, and detect configurational and rotational relationships. The acids were examined mainly as Na, K, NH_4, or Ca salts.

The rotations of the salts recovered after digestion are given in Table.2. below.

Table.2.

Acid. used.	Culture.		
	P. glauc.	Asp. Nig.	Asp. Gris.
Racemic	l.	l.	l.
Dimethoxysuccinic	l.	l.	l.
Lactic	l.	l.	l.
α-Aminopropionic	l.	l.	l.
α-Ethoxypropionic	l.	i.	d.
α-Propoxypropionic	l.	-	-
α-Hydroxybutyric	l.	l.	l.
β-Hydroxybutyric	l.	l.	d.
Glyceric	l.	l.	l.
Malic	l.	-	-
Methoxysuccinic	l.	-	-
Ethoxysuccinic	l.	-	-
Propoxysuccinic	i.	-	-
Monobromosuccinic	d.	-	-
Mandelic	l.	l.	d.
Methoxyphenylacetic	l.	-	-
Ethoxyphenylacetic	l.	-	d.
Propoxyphenylacetic	l.	l.	-

It was found that the specificity was relative, and the method unsuitable for complete resolution, the extent of separation depending on the degree of selective attack. The regularity with which the two first cultures attack the d-components is very marked. Configurative relationship was indicated in the action of 'P. glauc.' on the tartaric malic alkyloxysuccinic and dimethoxysuccinic members, as also on the malic and monobromosuccinic acids.
The effect of space factors, previously noted by BUCHNER[25] and by EMMERLING[26], was seen with hydroxy- and alkyloxy-acids, substitution to give the second member retarding mould growth. Finally, the rotation obtained with 'Pen. Glauc.' from K. dl. lactate was repeated with the cell-free oxydase extract. In analogy with the chemical results of MARCKWALD and McKENZIE[27], the mechanism was suggested

of an optically active enzyme which formed addition compounds with both isomers, the resulting complexes decomposing, each at its own rate.

That asymmetry in the substrate is sometimes essential has been shown by HERZOG[28]. The action of various fungi was tested on some acid substrates, and the course of the action followed by the evolution of CO_2, oxidation not assimilation taking place. Practically no effect was observed on the symmetric series glycollic citric pyroracemic and hydroxyisobutyric acids, but with asymmetry existing, lactic racemic malic mandelic and β-hydroxybutyric acids were very easily attacked, though the remanent products were not strongly optically active.

The asymmetric decomposition of dl.Me.Et.pyruvic acid by carboxylase has been noted by NEUBERG and PETERSON[29], the d-component being preferentially attacked.

The preferential destruction, by yeast, of one component of a racemic mixture, has been successfully applied by EHRLICH[30] to the resolution of α-amino acids. With the use of a large quantity of pressed yeast and sugar, 60 - 75 % yields of optically pure l-alanine, l-valine, d-leucine, d-phenylalanine, d-serine, l-glutamic acid, d-histidine and l-isoleucine were obtained; phenylglycocoll, isovaline, and alanylglycine were recovered in large laevo-excess; but aspartic acid, tyrosine, proline and β-amino acids showed only symmetric decomposition. The preferential attack on the naturally occurring isomer is shown above in every possible case. Later[31], the resolution of d-tyrosine was also achieved with yeasts to which a little malt extract or yeast autolysate had

been added. The method has been used by ABDERHALDEN for the separation of a laevo-excess of α-amino-heptylic, -caprylic, -myristic acids.

While most work has been done with the complete organisms, the oxydases proper have also been used. The action of tyrosinase has been frequently noted. With dl.tyrosine, preferential assimilation of the l-component was early noted by WOHLGEMUTH in rabbit metabolism. No trace of asymmetric action in the enzyme from 'Russ.Quel.Fr.' was observed[32], in contrast with that from 'Russ.Del.' which oxidised the l-isomer more quickly.[33] With the enzyme from 'B.Prot.vulg.' and 'B.subt.', optically pure d-tyrosine was obtained by TSUDJI, while l-3.4.dihydroxyphenylalanine has been similarly prepared by RAPER[34].

Of many other observations on asymmetric action may be mentioned those of FISCHER on r-alanine, of PLOCH and MAYER on dl.phenyl.glycidic acid, of McKENZIE and of KIMURA on dl.lactic acid, and many of NEUBERG and coworkers on cystine, valine, glyceraldehyde, and aspartic and glutamic acids.

2. Sucroclastic.

Selective enzymic rupture of carbohydrates has been noted with bacteria, with living yeasts, and with the extracted enzyme-complex zymase.

In their classic paper in 1894, FISCHER and THIERFELDER[35] examined the effect of yeasts on a number of monoses, bioses, and glucosides. Only in the cases of d-glucose, d-fructose, d-mannose, and d-galactose, or with certain biose derivatives, (sucrose and

maltose), was fermentation observed. The effect was related to agreement in molecular structure, and the remarkable absolute specificity shown, was embodied in the famous lock-and-key analogy. Further, from racemised hexoses, FISCHER prepared by the action of beer yeast l-glucose, l-fructose, l-mannose, and l-galactose, the natural components being completely destroyed.

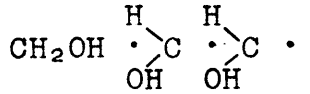
In similar experiments on racemised sugars, with all yeasts save one, a laevo-rotation was invariably developed. Sauterne wine yeast, however, destroyed the l-components preferentially, but, even here, the acetone-extracted enzyme also caused the normal type of rotation.

Later work served to corroborate this absolute specificity. Kinetic measurements showed that the fermentation mechanism of d-glucose, d-fructose, and d-mannose seems identical, but distinct from that of d-galactose. Fermentation with the latter is much slower, many yeasts decompose glucose which do not decompose galactose though no converse instance is known; further, no fermentation is found with d-talose or d- tagatose which are configurationally related to galactose as d-mannose and d-fructose are to d-glucose.

A remarkable type of optical selectivity was shown in experiments of WILLSTÄTTER and SOBOTKA[36] on the mutameric forms of d-glucose. Yeast-fermentation of equal weights of α-,β-glucoses gave equal rates, but in a mixture, the α-component was preferentially decomposed. Similar results have been found for glucose-galactose, and glucose-fructose mixtures. In the latter case, a rate-ratio of 1·78 / 1 has been independently reached by HOPKINS[37]

and IVEKOVICH[38].

The asymmetric decomposition of a racemic sugar in animal metabolism is well known. It has been shown by NEUBERG that with arabinose and mannose the d-isomer is preferentially assimilated. The oxidising action of certain bacteria on sugars is markedly specific. By the action of 'B.Xyl.', BERTRAND[39] converted aldoses and polyvalent alcohols to the corresponding acids and ketones. Oxidation was found to occur exactly where the substrate had the configuration shown.



Mannitol with two such chains was oxidised in one only to give fructose.

Lactic acid fermentation shows that other bacterial enzymes, however, are more general in their selective action, and the nature of the optically active products depends rather on the enzymes and the general conditions. In 1893, PÉRÉ[40] found that by the action of 'B.Typh.','B.Coli.1','B.Coli.d', and 'Microbe D', the lactic acid obtained from glucose was laevo-rotatory; if, however, in the food nutriment, peptonic nitrogen was substituted for ammoniacal, according to the organism used, an inversion in the optical nature of the products might take place. Similar results were obtained for galactose and mannose, and these have been extended by PÉRÉ, POTTEVIN[41], and HERZOG and HÖRTH[42]. The latter authors in a systematic examination of the lactic acid formed by many bacilli from sugars, found that -

(a). The acid is almost invariably optically active.

(b). The sign is dependent only on the bacillus and conditions.

(c). Dextrose, fructose, galactose, cane sugar, maltose and

lactose are extensively decomposed to give active acid in 30-70 % excess.

It seems probable that the production of active acid is really a biochemical asymmetric synthesis following an intermediate decomposition to symmetric compounds such as methyl-glyoxal. LEVENE and MEYER[43] obtained d-lactic acid from d-glucose, d-mannose and d-fructose by the action of leucocytes; with leucocytes and kidney tissue, d-lactic acid in excess was formed from methyl-glyoxal. It is also significant on this view of the mechanism, that in a study of the effect of additants on yeast fermentation of pure sugars, l-malic acid being obtained, the only substance found by DAKIN[44] to give a marked increase in yield, was sodium-fumarate, presumably by the asymmetric action of a yeast-contained hydrolase.

C. HYDROLYSIS.

1. Carbohydrates. (Emulsin, Maltase, Lactase, Invertase.)

With no class of compounds is enzymic stereochemical specificity so sharply defined as with the carbohydrates. This is particularly evident in hydrolysis. The enzymes emulsin, maltase and invertase have been most generally investigated.

It was early observed by FISCHER[45] that while emulsin hydrolysed β-Me.d.glucoside, it had no action on β-Me.l.glucoside; it had also no action on the α-Me.glucosides. Conversely, maltase hydrolysed α-Me.d.glucoside, but not the β-Me.glucosides nor α-Me.l.glucoside. This absolute specificity of maltase and emulsin for α-,β-d.glucosides respectively was found by FISCHER[4] to hold for some 30 glucosides, and has since been found general.

Similarly, lactase is specific for β-galactosides.

Considerable use has been made of this absolute stereochemical relation of enzyme to substrate. It has been extensively used by BOURQUELOT in emulsin-syntheses of alkyl-d.β-glucosides. It has formed a basis for classification and nomenclature of carbohydrases. It has been an instrument of value in elucidating the structure of higher sugars. For example, while β-Me.maltoside on hydrolysis with emulsin yields maltose and methyl alcohol by rupture of the β-glucosidic linkage, maltase gives glucose and β-Me.glucoside. The relation of substrate and enzyme is shown in Table.3.

It has very recently been reported by KÄRSTROM that the α-glucosidase of 'B.Coli.' which hydrolyses maltose, does not attack either sucrose or melicitose. The work is refuted by

WEIDENHAGEN, but again corroborated by VIRTÄNEN[46].

Table.3.

Enzyme.	Specific Substrates.
Emulsin	β-Glucosides, Gentiobiose, Cellobiose
Maltase	α-Glucosides, Maltose, Cane Sugar, Melicitose
Lactase	β-Galactosides, Lactose
Invertase	Fructosides, Cane Sugar, Raffinose, Gentianose

The configurational relation is further shown in the action of opt. active inhibitors, whether reaction products or separately added. The following table from ARMSTRONG[19], shows that the effect is particularly centred in the hexose residue of the substrate.

Table.4.

Enzyme	Hexose Effect on Hydrolysis Rate		
	Glucose	Galactose	Fructose
Lactase	------	Retards	------
Emulsin	Strongly Retards	Slightly Retards	------
Maltase	Retards	Slightly Retards	------
Invertase	------	------	Retards

The different inhibiting effects of α- and β-glucoses has been carefully noted by KUHN[47] on hydrolysis by invertase, maltase and emulsin. Additants did not alter the monomolecular course of the reactions, but varied the speed. α-glucose, independent of substrate tested, had practically no effect on invertase and emulsin. β-glucose inhibited considerably with both enzymes. With maltase hydrolysis of maltose, α- and β-glucoses both inhibited strongly and to the same degree exactly.

The explanation of these effects has led to many elaborate kinetic measurements by WILLSTÄTTER and KUHN, EULER and JACOBSEN,

NELSON and others. The theory of complex-equilibria in solution suggested by BROWN and by HENRI, and mathematically developed by MICHAELIS and others, has been found to explain the kinetics of sugar hydrolysis, particularly with invertase.

Maltase is more specific than emulsin. Change in any way in the α-glucosidic hexose residue completely inhibits maltase hydrolysis, but a slight variation is possible with emulsin. While no hydrolysis is attained with tetracetyl.β-Me.d.glucoside, slow hydrolysis occurs with the tetramethyl.compound; further, substitution in the terminal group is possible to the extent that β-Me.d-isorhamnoside is hydrolysed, though the intermediate β-Me.d-glucoside.6.bromohydrin is not attacked. Emulsin does not hydrolyse β-Me.d-xyloside.

While the hexose residue plays the important part in conditioning emulsin hydrolysis, the remaining part of the molecule is not without influence, and its effect was specially examined in FISCHER's last paper.[48] The rates of hydrolysis may be very slow even with some natural glucosides; aromatic derivatives are hydrolysed more quickly than aliphatic, and the glucosides of tertiary alcohols are very slowly attacked. Owing to pH considerations, the glucosides of acids may not be hydrolysed though the corresponding salts, amides, esters or nitriles be attacked.

Of particular interest is the action of emulsin on the β-glucosides of racemic compounds, a diastereoisomeric mixture being formed. A considerable preferential hydrolysis of one isomer has been found for the hydrolysis of such β-glucosides, separately or in the mixture, in a few instances. This forms a basis of consid-

-erable scope for biochemical resolution. An examination of this type of selective enzyme action, theoretical, experimental, and analytical is dealt with in PART. 2. of the present work.

2. Polypeptides. (Trypsin, Erepsin, Histozyme, Arginase.)

That configuration had an effect on the physiological activity of amino-acids was early recognised in 1886 by PIUTTI, who noted that d-asparagine has a sweet taste though the l-isomer is insipid. Similar taste differences have been found for the isomers of isoleucine (EHRLICH), leucine and valine (FISCHER), while l-adrenaline is ten times more active as a heart stimulant than the d-isomer.

The asymmetric nature of the enzymic hydrolysis of peptides was first established by FISCHER and BERGELL[49]. It was found that pancreatin selectively attacked the l-component of carbethoxy-glycyl.dl.leucine. Later, glycyl.l-tyrosine was hydrolysed, dl.leucyl.dl.alanine was selectively attacked to give an excess of d-alanine and l-leucine, while similar asymmetric hydrolysis was found with alanyl-leucine and leucyl-leucine. Systematic investigations with the 'trypsin' of pancreatic juice by FISCHER and ABDERHALDEN[50], for polypeptides generally, both with the separate isomers and the isomeric mixtures, led to the following conclusions -

(a). Stereochemical specificity of the enzyme was absolute.

(b). Where hydrolysis of an optically active polypeptide occurred it was confined to compounds built up completely from naturally

occurring amino-acid forms. This condition was necessary but not sufficient.

For example, of the alanyl-leucines, only (3) is attacked.

(1). d.alanyl.d.leucine. (3). d.alanyl.l.leucine.
(2). l.alanyl.l.leucine. (4). l.alanyl.d.leucine.

These results were later extended by ABDERHALDEN and SINGER, and by ABDERHALDEN[51] on tri-, tetra-, and penta-peptides, asymmetric hydrolyses being obtained with yeast maceration juice. The table below, based on the results of FISCHER, EHRLICH, and ABDERHALDEN with yeasts and trypsins, strikingly bears out FISCHER's rule, and shows the independence of hydrolysis on the length of the amino-acid chain, as such.

Table.5.

Hydrolysed	Unhydrolysed
d-Alanine	l-Alanine
d-Valine	l-Valine
l-Leucine	d-Leucine
l-Serine	d-Serine
d-Glutamic acid	l-Glutamic acid
l-Histidine	d-Histidine
d-Isoleucine	l-Isoleucine
l-Tyrosine	d-Tyrosine
d-Alanyl.glycine	l-Alanyl.glycine
d-Alanyl.d-alanine	d-Alanyl.l-alanine
l-Leucyl.l-leucine	l-Leucyl.d-leucine
d-Alanyl.l-leucine	d-Alanyl.d-leucine
l-Leucyl.d-alanine	l-Leucyl.l-alanine
d-Norvalyl.l-tyrosine	l-Norvalyl.l-tyrosine
Carbethoxyglycyl.l-leucine	Carbethoxyglycyl.d-leucine
d-Alanyl.glycyl.glycine	l-Alanyl.glycyl.glycine
l-Leucyl.glycyl.glycine	d-Leucyl.glycyl.glycine
Glycyl.l-leucyl.glycine	Glycyl.d-leucyl.glycine
l-Leucyl.glycyl.l-leucine	l-Leucyl.glycyl.d-leucine
l-Leucyl.glycyl.d-alanine	l-Leucyl.glycyl.l-alanine
d-Alanyl.diglycyl.glycine	l-Alanyl.diglycyl.glycine
l-Leucyl.triglycyl.glycine	d-Leucyl.triglycyl.glycine

It was recognised, however, that configuration was not the sole factor, as with glycine peptides asymmetry was not even necessary. The nature of the enzyme, the position of the amino-group, the number of amino-acids their structure and the order of their linking were all important conditioning elements.

Amino-acids of a high degree of optical purity were recovered in the above experiments. Asymmetric hydrolysis by pancreatin of Et. and Pr. esters of dl.leucine was applied by WARBURG[52] as a method of resolution, both components being recoverable. l-Leucine in 70 - 83 % yield with $(\alpha)_D$ = +15° - +15·5° in 20 % HCl was so obtained. Despite WARBURG's assurance to the contrary, it is still considered an open question whether the effect was not due really to pancreatic lipase.

The asymmetric action of the proteases found a parallel in that of the amino-acylases, arginase and histozyme. REISSER[53] hydrolysed inactive arginine with arginase. l-Arginine was unattacked, and the d-isomer gave d-ornithine as a product.
It was shown by MUTCH[54] in 1912 that the histozyme of minced kidney hydrolysed the Na.salt of dl.benzoyl.alanine with preferential attack on the d-isomer. No trace of a synthetic reverse action was observed. Further instances of selective action were given by SMORODINZEW[55] with histozyme from the muscles and kidney. Hippuric acid, l-leucine, d-benzoyl.alanine, l-benzoyl.leucine, d-α.benzoyl.aminobutyric acid, and d-α.benzamidobutyric acid were all hydrolysed, while l-α.benzoyl.aminobutyric acid, l-α.benzamidobutyric acid and β-amino.acids generally were unattacked. The source of the enzyme and the configuration of the substrate were

found to be important. This selective attack was utilised for the resolution of α-amino.acids. NEUBERG and LINHARDT[56] with the histozyme from Japanese taka-diastase obtained a 50 % cleavage of dl.benzoyl.alanine. HOPPERT[57] with that from taka-diastase, moulds and 'Asp.oryz.' prepared d-alanine and benzoyl.l-alanine respectively of 97 % , 94 % optical purity.

Further investigation has shown that FISCHER's rule that natural antipodes only are attacked, requires amendment. ABDERHALDEN and PRINGSHEIM[58] hydrolysed dl.alanyl.glycine and dl.leucyl.glycine with proteolytic enzymes from the juices of fungi whose specific nature is not so marked as that of the pancreas enzyme. Both components of the former racemic dipeptide were attacked at equal rates by the juice of 'Allesch.' or 'Asp.Nig.' Both components of the latter were attacked by the juice of 'Allesch.Gay.' and 'Rhiz.Tank.', that containing the naturally occurring l-leucine preferentially. Later, asymmetric hydrolysis of synthetic dipeptides from dl.amino.-heptylic, -caprilic, and -myristic acids was achieved by ABDERHALDEN and coworkers with yeast maceration juice.

Despite the large number of isomerides involved, the ease of racemisation, and the difficulties of separation of the products, modern work due to ABDERHALDEN and WALDSCHMIDT-LEITZ has brought out well marked distinctions in peptidases, and defined the problems more clearly. While the amino-acylases attack the C-N linkage, trypsin attacks the region of the free COOH group, and erepsin the free amino. group. The location of the attack seems to affect the asymmetry of the hydrolysis. To cite only one instance, it has been shown by WALDSCHMIDT-LEITZ and SCHLATTER[59] that dl.leucyl.

-glycyl.l-tyrosine and dl.bromoisocapronyl.glycyl.l-tyrosine are symmetrically attacked by trypsin to give complete tyrosine separation. With erepsin, however, attack is on one isomer only.

3. Esters. (Lipase, Phosphatase, Sulphatase.)

The stereochemical specificity of esterases, in contrast with that of proteases, is quantitative rather than absolute. It was first established in the fundamental investigations of DAKIN[11]. On hydrolysing ethyl dl.mandelate with a liver lipase, DAKIN found that according as the liberated acid was extracted at partial or complete hydrolysis, it was dextrorotatory or inactive. Thus a relative optical preference was exerted by the enzyme. The freed mandelic acid was easily fractionated to give the optically pure d-component. On analogy with the asymmetric esterification and hydrolysis of l-menthyl.dl.mandelates by MARCKWALD and McKENZIE[27], the mechanism was suggested of two complexes decomposing at different rates. From curves showing, for different degrees of hydrolysis, the specific rotation of the liberated acid, DAKIN estimated the ratio of hydrolysis rates as at least 2/1. Similar asymmetric hydrolysis was obtained with the methyl, isoamyl, and benzyl esters, the rate-ratios k_D/k_L having the respective values - 1·7, 1·4, 1·4. To determine whether a strong optically active alcohol group would influence the selectivity, the mandelates of l-menthol and d-borneol were prepared. Lipase, however, did not hydrolyse these. Differences in the optical specificity of horse and pig lipases were also observed.

Later[60], similar experiments on methoxy- and halo- phenyl. acetates related the optical selectivity of the enzyme to the configuration of the substrate. Further, asymmetry in the alcohol radicle of the ester was also found sufficient to give asymmetric hydrolysis with lipase, dl.phenyl.ethyl.carbinyl.acetate yielding products distinctly laevorotatory. An extension to aliphatic racemic esters was inconclusive owing to the lower rotations involved. The following table shows the results obtained.

Table.6.

Racemic Ester	Rotation of -	
	Libtd. Acid	Resid. Ester
Me.Mandelate	d.	l.
Et.Mandelate	d.	l.
Isoamyl Mandelate	d.	l.
Benzyl Mandelate	d.	l.
Me.Phenyl.chloroacetate	l.	d.
Me.Phenyl.bromoacetate	l. (?)	d.
Et.Phenyl.chloroacetate	l.	d.
Et.Phenyl.bromoacetate	l. (?)	d.
Me.Phenyl.methoxyacetate	d.	l.
Et.Phenyl.ethoxyacetate	d.	l.

In the following two decades, little attention was given to the asymmetric action of esterases. ABDERHALDEN SICKEL and UEDA[61] submitted r-tyrosine ethyl ester to the action of pancreatic lipase. Preferential hydrolysis of the l-component was observed.

Comparison with the previous results of WARBURG[52] with 'lipase-free' pancreatin on leucine esters, suggested an investigation of RONA and SPIEDEL[62]. Ethyl and propyl dl.leucine esters were tested with lipases and trypsins prepared and purified by the methods of WILLSTÄTTER. With lipase the l-leucinates were preferentially hydrolysed. By the tryptic enzyme, it is stated

that no decomposition was achieved, but the detail is not quite conclusive, and it is significant that no hydrolysis, by lipase, of ethyl esters of racemic polypeptides was found by ABDERHALDEN and ALKER[63].

With the asymmetric carbon atom in the alcohol residue, asymmetric hydrolysis of lecithin was achieved by MAYER[64]. The natural component was hydrolysed, d-glycerophosphoric acid being recovered as the Ba.-salt. l-Lecithin in 87 % optical purity remained. The parallel hydrolyses of monoacetin and monocaproin by ABDERHALDEN and WEIL[65] were symmetric only.

A noteworthy result was the recovery by NEUBERG and ROSENBERG[66] of d-dibromostearic acid from lipase hydrolysis of the triglyceride of the racemic acid. Here, the asymmetry was not centred in the α- C atom.

It was found by WILLSTATTER[67] in 1924 that hydrolysis of alkyl.dl.mandelate by pigs' pancreas lipase yielded a laevorotatory mandelic acid. A systematic continuation of the work founded by DAKIN was therefore initiated with the primary object of comparing lipases. Simple esters of racemic acids were hydrolysed under comparable conditions with liver and pancreatic lipases. The freed acid was recovered after not more than 20-30 % hydrolysis, and tested polarimetrically. In every case asymmetric action was found, as shown in Table.7. The values cover several concordant experiments. From the results it was evident that -

(a). Stereochemical specificity was more marked with pancreatic than hepatic lipase.

(b). The difference, between the enzymes, in nature of optical

preference did not extend to the halo- and amino- acids.

(c). Configurative relations were shown by both enzymes.

(d). In substitutive effect, $NH_2 >$ (OH or OMe.) $>$ Cl.

Table.7.

Racemic Substrate	Spec.Rotn. $(\alpha)_D$ of Lib. Acid	
	Pancr.Lipase	Liver Lipase
Et. Mandelate	-56° to -81°	+59° to +87°
Mandelic acid monoglyceride	-36°	+46°
Me. Phenylmethoxyacetate	-95° to -100°	+48° to +53°
Me. Phenylchloroacetate	-17° to -28°	-10° to -15°
Me. Phenylbromoacetate	+2·87° x	+2·96° x
Me. Tropate	+56° to +83°	0° to -17°
Pr. Phenylaminoacetate	+100°	+68°
Pr. Leucinate xx	+14·5°to+15·3°	+6° to +7°

x. Recovered ester. xx. In 20 % HCl - WARBURG.

Other esterases from various animal organs were similarly tested. Stereochemical selectivity with stomach lipases was low. Taka.esterase, however, was intermediate in properties to liver and pancreatic lipases. Other lipases were found later, by RONA and AMMON[68], to favour in various degrees some the d- others the l-component of Me.dl.mandelate.

That stereochemical specificity was an intimate property of the enzyme was shown by the effect of purity and additants. $CaCl_2$, Na.oleate, and albumin, which have a marked effect on absolute hydrolysis rate, had no effect on the rotation of the products obtained with pigs' stomach lipase. Enzymes of various degrees of purity were also tested on the same racemic substrate. In no case was any inversion of sign or marked change in magnitude observed. With pigs' stomach lipase, on Et.mandelate and Me.phenylchloroacetate, stereochemical specificity increased with purity.

Following these results, further data accumulated, particul-

-arly with reference to esters with the asymmetry in the acid radicle. The investigations, with lipase, of DAWSON PLATT and COHEN[69] on tartrates and substituted acetic acid esters, of MURACHI[70], and of RONA and ITELSOHN-SCHECHTER[71] on lactic esters, and of RONA, AMMON, and coworkers on mandelates appeared in quick succession. The range of esterases was extended by the discovery of phosphatase, specific for phosphoric esters, and of sulphatase which hydrolysed the sulphuric esters of aromatic phenols and naphthols. Similar asymmetric hydrolysis was in some cases obtained by FROMAGEST[72] with sulphatase, while NEUBERG and co-workers applied phosphatase-hydrolysis as a method for resolving racemic alcohols[73]. Some of the later results, as in Table.8., further show the extent of asymmetric esterase action on racemic substrates, and its dependence both on substrate and enzyme preparate.

The asymmetric action of esterases has also been shown in synthesis. It was found by RONA and AMMON[74] that when n.butyric acid was esterified in excess of dl.sec.butyl alcohol, the resulting ester was laevorotatory, and the recovered alcohol dextro-rotatory. Independent of esterase used, the same component was always the more quickly esterified, from a racemate, as was the more quickly hydrolysed. RONA and AMMON[75] found pigs' pancreas lipase formed the isoamyl ester of d-lactic acid more quickly than that of l-lactic acid, while similar asymmetric esterification was found by KUROYA[76] with phosphatase.

Physiological differences in geometric isomers have long been known, whether in taste, smell, or action on fungi.

A corresponding difference on enzymic esterification has been

recently observed by FABISCH[77]. Fumaric and maleic acids were

Table.8.

Substrate	Comp. Pref. Hydr.	Esterase	Authority
Et. Me.Et.acetate	d	Pancr.Lipase	D.P.and COHEN
Et. Me.But. ••	d	•• ••	••
Et. Et,But. ••	Sym.	•• ••	••
Et. Me.Benzyl.acetate	d	•• ••	••
Et. Et.Benzyl. ••	Sym.	•• ••	••
Et. Et.p.nitrobenzyl.acet.	Sym.	•• ••	••
Et. Et.Allylbenzyl ••	Sym.	•• ••	••
Di.Et.Racemate	l	•• ••	••
•• ••	d	Liver ••	••
Et.Lactate	d	Pig Liv.••	RONA and IT•SC.
Et. ••	Sym.	Cow Panc:•	MURACHI
Amyl Lactate	Sym.	•• •• ••	••
Capryl Lactate	Sym.	•• •• ••	••
Me. Mandelate	d	Asp.Oryz.	W.- LEITZ
•• ••	d	Pig Liv. Lip.	RONAandAMMON
•• ••	d	Liv.Tissue	•• ••
•• ••	d	Taka Lipase	•• ••
•• ••	l	Hum.Liv.Lip.	•• ••
•• ••	l	G.Pig Liv.Lip.	•• ••
•• ••	l	Pig Panc.Lip.	•• ••
Et. Mandelate	d	Asp.Oryz.	W.- LEITZ
•• ••	d	Pig Liv.Lip.	WILLST.,KUMAG.
•• ••	d	Taka Lipase	•• ••
•• ••	l	Pancr. ••	•• ••
•• ••	d	Pig Stomach L.	W.,BAMANN,W-GR.
•• ••	d	Sheep Liv.Lip.	BAMANN
•• ••	d	Dog •• ••	••
Me. Phenylchloroacetate	l	Asp.Oryz.	W.- LEITZ
•• ••	d	Pig Liv.Lip.	W.,BAMANN,W-GR.
•• ••	l	Pig Panc.••	•• •• ••
•• ••	d	Pig Stom.••	•• •• ••
•• ••	d	Dog •• ••	•• •• ••
Me. Phenylmethoxyacetate	d	Asp.Oryz.	W.- LEITZ
•• ••	l	Pig Stom.Lip.	W.,BAMANN,W-GR.
Me. Tropate	l	Asp.Oryz.	W.- LEITZ
•• ••	d	Pig Stom.Lip.	W.,BAMANN,W-GR.
Sec.Butyl n.Butyrate	l	Pig Panc.Lip.	RONA and AMMON
•• •• ••	l	•• Liv. ••	•• ••
Borneol Monophosphate	l-alc.	Takadiastase	NEUB.,W.,JACOB.
•• ••	l-alc.	Yeast Phosph.	KUROYA
•• ••	l-alc.	Kidn. ••	GUALDI
•• ••	l-alc.	Liv. ••	••
Sec.Amyl Phosphate	l-alc.	Plant ••	NEUB.,JACOBSOHN
p.Sec.Butyl.Phenol.K.Sulph.	d-alc.	Asp.Oryz.	FROMAGEST
•• •• •• ••	d-alc.	Pig Liv.Sul.	••
m.Me.Cyclohexyl p. phenol.K.sulphate	Sym.	Sulphatase	WEINMANN

esterified with a series of n.aliphatic alcohols under parallel conditions, liver, kidney, and pancreas lipases being used. Independent of enzyme or alcohol, the rate of esterification of maleic acid was twice that for fumaric acid. Similar experiments with the isomeric pairs, oleic and elaidic, and erucic and brassidic acids, gave rate-ratios of 1·2, 1·0 respectively, the 'trans' form having in the former pair the greater rate. Thus, increase in the length of the carbon chain reduces the difference between the rates for the 'cis' and 'trans' forms.

In the experiments of DAWSON PLATT and COHEN on di.Et.racemate the asymmetric hydrolysis had been directly corroborated from the separate isomers, the following relative speeds of hydrolysis being obtained -

Di.Et.d-tartrate	1·00
Di.Et.racemate	2·89
Di.Et.l-tartrate	4·77

$$\frac{1\cdot00 + 4\cdot77}{2} = 2\cdot89$$

It thus seemed as if the racemate acted merely as the sum of its components. In their comprehensive investigation on methyl d-,l-, and dl.mandelates with many lipase preparates, RONA and AMMON found, however, that frequently the rate of hydrolysis of the l-isomer was greater than that of the d-isomer, though in the racemate the d-component was preferentially attacked.

In a famous paper 'Asymmetric Ester Hydrolysis by Enzymes', WILLSTÄTTER KUHN and BAMANN[78], gave an explanation, to some degree quantitative, by applying the theory of complex-formation, as expressed by MICHAELIS and MENTEN[20]. On this basis it is assumed that the rate of enzymic hydrolysis of a substrate depends on two factors; (1). the affinity of the substrate for the enzyme, conditioning the amount of complex formed, and expressed by the

dissociation constant, (K), (2). the absolute rate of decomposition of the complex, (k).

Pigs' liver esterase, and ethyl d-, l-, and dl.mandelates were used. Activity - pS curves were determined for the separate isomers, and showed that while the d-isomer was the more quickly hydrolysed at very low concentrations of substrate, the curves crossed, and the l-isomer was the more quickly attacked at high concentration. From these curves, K_D, K_L, k_D, k_L were deduced in the usual manner. A repeat of DAKIN's curve for the specific rotation of the acid recovered from hydrolysis of the racemate was then made, with the substrate above 'saturation concentration' thus ensuring that the amounts of the complexes present, a_D, a_L, would be inversely proportional to their respective dissociation constants K_D, K_L. Thus, V_D, V_L being the rates of hydrolysis from the racemate, from the Activity - pS curves it was predicted that -

$$\frac{V_D}{V_L} = \frac{a_D \cdot k_D}{a_L \cdot k_L} = \frac{K_L \cdot k_D}{K_D \cdot k_L} = \frac{3{\cdot}2}{1} \cdot \frac{1}{1{\cdot}6} = 2{\cdot}0$$

As the recovered acid for a 20 - 30 % hydrolysis range had $(\alpha)_D$ = +60° to +70°, on the basis of the observed linear rate of hydrolysis, the real rate-ratio lay between -

$$\frac{156 + 60 \text{ to } 70}{156 - 60 \text{ to } 70} = 2{\cdot}3 \text{ to } 2{\cdot}6.$$

This was therefore satisfactory qualitative and quantitative agreement, and was in conformity with the estimate of 2·2 made by FAJANS on the original data of DAKIN.

It was shown, also, that the selectivity was independent of pH in the range considered.

Applications of the MICHAELIS theory with measurements of the above type, were exhaustively made on methyl and ethyl mandelates

by BAMANN with various lipases, and by RONA and AMMON and co-workers. Confirmation was good generally. In particular, very accurate measurements by WEBER and AMMON[79] with the micro-technique of RONA and LASNITSKI confirmed the results on both mandelates with pancreatic and liver lipases. With the former enzyme, a rate-ratio of 1·67 from measurements on Me.dl.mandelate was corroborated by the figure 1·70 from the separate Activity - pS curves. By varying concentration of substrate or enzyme, BAMANN[80], with lipases of low selectivity, obtained from Et.dl.mandelate, for one and the same enzyme, acid of specific rotations from -50° to $+50^{\circ}$.

Thus, on this theory, many apparently contradictory results on asymmetric enzyme action could be reconciled, as a basis of comparison older 'Quantitative specificity ratios' might be misleading as possibly compounded of four more fundamental constants, while finally a rational basis was given for the application, at least with small quantities, to directed biochemical resolution.

A striking analogy to the chemical work of BREDIG and FAJANS on optically ACTIVE catalysts was found with the action of optically active alkaloids on esterases. Some parallel features between alkaloids and organisms as resolving agents had been early stated by WINTHER. General effects of alkaloids on enzyme action were noted by SMORODINZEW and by RONA. No effect, with quinine and quinidine, on the stereochemical specificity of pigs' liver esterase on Me.dl.mandelate was detected by RONA and AMMON[68]. Recently, however, very striking stereochemical effects have been observed by BAMANN[81], the factors concerned being the alkaloid used, the esterase, the relative amounts of alkaloid and substrate and pH. Temperature, so far as tested, had no effect.

The experiments were carried out on Et.dl.mandelate with pig rabbit and human liver esterase, at pH 7 with phosphate buffer, the alkaloids being added as sulphates. On pigs' liver lipase little effect was observed with any of the alkaloids. With rabbit esterase, preferential hydrolysis of the d-component was increased, the d-acid recovered having its specific rotation increased by +17°, +3°, +20°, and +9° for strychnine, quinine, quinidine and cinchonine respectively.

With human liver esterase, the sign of the liberated acid was changed by a preferential hydrolysis of the l-type. With cinchonine and quinidine mandelic acid of $(\alpha)_D = -18°$ was obtained with the alkaloid vice $(\alpha)_D = +7°$ alone. Though brucine and morphine had little effect, with strychnine, $(\alpha)_D$ of the freed acid was between -70° and -100° showing that an initial symmetric hydrolysis had been converted to a highly preferential attack on the l-isomer. Further results with this esterase showed that the effect of strychnine was most marked in high relative concentration of the alkaloid, and decreased with fall in pH from 7·5 to 6·5.

Similar results with human liver esterase on Me.dl.mandelate have recently been given by AMMON and FISCHGOLD[82]. Further, Activity - pS curves for the separate isomers, with and without alkaloid addition, brought out the remarkable fact, that only on the l-isomer had the alkaloid any effect, K_D, k_D, being unchanged. For the l-isomer, K_L was still the same, but a 50 % increase in k_L accounted for the changed sign of the acid liberated.

The complex formed by alkaloid addition was found by BAMANN[83] to be dissociable, and the idea of the enzyme as accompanied by an

asymmetric activator was again brought into prominence. Instances of asymmetric adsorption from racemates by fibres, and by carbon from a diastereoisomeric mixture give support to this view. Though WILLSTÄTTER had found little difference in stereochemical specificity occasioned by purifying the enzyme, it was shown by BAMANN[84] that heat treatment and proteolytic degradation in acid solution had opposite effects on the stereochemical specificity of liver lipase, while addition of ethyl alcohol in the hydrolysis of ethyl dl.mandelate by human liver esterase favored the preferential hydrolysis of the d-ester.

The stereochemical specificity of esterases is further shown in the differing inhibiting or accelerating effects of foreign d-, and l-isomers whether the substrate be racemic or not. The many results of BAMANN and RONA and AMMON illustrate this with alkaloids. In one series it was found that l-quinine, l-adrenaline, d-suprarenine and l-hyoscyamine were of greater inhibiting power than their optical isomers. Cases of similar differences in the physiological activity of alkaloid d- and l-isomers are well known.

An interesting series of results with optically active alcohols, interpreted from the point of view of MICHAELIS kinetics was recently published from the Cambridge laboratories. MURRAY and KING[85] observed the inhibiting effects of d-, l-, and dl.sec.alcohols on the hydrolysis by sheep liver lipase of Me.n.butyrate. The racemate inhibited as the sum of its components, and independent of the alcohol used, the ratio of inhibiting powers for the d- and l-series was 5 / 1 for L / D. The aliphatic alcohol had the greatest absolute effect, and the influence of structure was stressed.

D. ASYMMETRIC SYNTHESIS.

The stereochemical specificity of enzymes has been shown on racemates or on the separate isomerides. The further possibility remained that from symmetric compounds, asymmetric syntheses - the term being used in the sense of MARCKWALD - might be achieved by the use of these biochemical catalysts. This has now been frequently observed, and indeed the asymmetric direction thus given to the reaction is frequently practically absolute, optical purity of more than 95 % having been attained in some instances. Asymmetric synthesis with enzymes is thus much more complete than with ordinary chemical methods. Classification of enzymes effecting asymmetric synthesis is not yet so well defined as that of hydrolytic enzymes, but the actions of oxynitrilases, hydrolases, and yeast reductase and carboligase are well established.

1. Oxynitrilase.

The emulsin of almonds, in addition to the hydrolytic enzyme β-glucosidase, also contains the enzyme oxynitrilase.
In 1908, ROSENTHALER[86], on shaking benzaldehyde with HCN in the presence of emulsin, obtained an optically active cyanohydrin which was invariably dextrorotatory.

$$C_6H_5 \cdot CHO + HCN \rightleftharpoons C_6H_5 \cdot \overset{\overset{OH}{\bullet}}{\underset{\times}{C}}H.CN$$

Suitable conditions gave a product which, on hydrolysis, yielded l-mandelic acid of $(\alpha)_D = -154^\circ$. The synthetic effect was attributed to a 'σ-emulsin' which, according to ROSENTHALER was separable from a δ hydrolysing component.

These results were not confirmed by BAYLISS.

It was later found by KRIEBLE[87] that an emulsin might give a laevorotatory nitrile, but ROSENTHALER, with some ten oxynitrilases including the emulsin of peach and cherry seeds, invariably obtained the d-nitrile. As a result of further work by ROSENTHALER KRIEBLE and KRIEBLE and WIELAND[88] the question was shown to be complicated by various other factors, but the following position now seems clear -

(a). The distinction between σ and δ emulsins has not been verified.

(b). The symmetric coexistent chemical synthesis is depressed at low pH, but is easily predominant at pH 7.

(c). Spontaneous racemisation of d-mandelonitrile is marked at pH 7.

By working at a low temperature and with a high acid content, KRIEBLE and WIELAND, using an extract of peach leaves, obtained one isomer in practically complete optical purity.

The reaction was extended with many aldehydes by ROSENTHALER[89]; and the results are tabulated as under.

Table. 9.

Aldehyde or Ketone	Nitr.	Acid	Aldehyde or Ketone	Nitr.	Acid
Acetaldehyde	d	?	Piperonal	d	?
Chloral	in.	in.	o.Nitrobenzaldehyde	d	l
Isobutyraldehyde	d	?	m. "	d	l
Heptylaldehyde	d	?	p. "	in.	in.
Octylaldehyde	d	?	Cinnamaldehyde	d	l
Citral	l	?	Phenylacetaldehyde	d	d
Furfurol	d	?	Protocatechuald.	in.	in.
Salicylaldehyde	in.	in.	o.Phthaldehyde	l	?
m.Hydroxybenzald.	in.	in.	Isophthalaldehyde	d	l
p.Hydroxybenzald.	in.	in.	Terephthalaldehyde	d	l
o.Methoxybenzald.	d	?	Me.Et.Ketone	in.	in.
Anisaldehyde	d	l	Hypnone	in.	in.
Cuminol	d	?	Benzaldehyde	d	l

The following results are obvious -

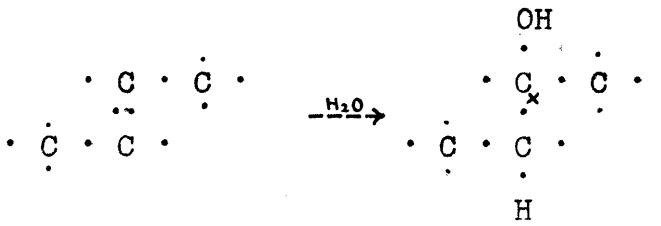
(a). In most cases, asymmetric synthesis is achieved.

(b). A phenol group, unless engaged, hinders optical activity.

(c). Especially with the aromatic derivatives, the majority of the nitriles are dextrorotatory.

Later, similar asymmetric syntheses were obtained for citronellal, isovaleraldehyde, p.tolylaldehyde, and chlorobenzaldehyde.

2. Fumarase.



The possibility of this asymmetric synthesis was observed in 1909 by DAKIN[90] who noted that NH_4·cinnamate injected in dog tissue, left an excess of l-phenyl.β.hydroxypropionic acid. Later[91] he observed that while muscle extract had practically no effect on maleic acid, with fumaric acid it gave a preponderance of l-malic acid. That a reverse action set in to give an equilibrium, was shown by the effect of the enzyme on i-malic acid; a positive rotation was gradually developed.

Similar asymmetric syntheses of l-malic acid were achieved by CHALLENGER and KLEIN[92] with 'Asp.Nig.' and by JACOBSON[93] with the enzymes of plant seedlings, peas, beans and yeasts. JACOBSON separated a malic acid with more than 90 % of the l-component. Alkaloids had no directive effect on the asymmetric synthesis.

3. Aspartase.

The addition of NH_3 to fumaric acid may be caused enzymically. Na.fumarate was digested by SUMIKI[94] with beer yeast in a buffered solution at pH 7·0 with a quantity of NH_4Cl added. The mixture

was maintained at 37° C. under reduced pressure. A 76 % yield of l-aspartic acid was obtained. The action is reversible.

4. Reductase, Ketoaldehydemutase, Carboligase.

It was discovered by DAKIN and DUDLEY[95] in 1913, that phenyl.glyoxal administered to rabbits, left an excess of l-mandelic acid. A repeat of the process with crude animal extracts showed the action to be enzymic, and the conversion of α-ketonic aldehydes to optically active hydroxy-acids was established, methyl.glyoxal and phenyl.glyoxal giving lactic and mandelic acids respectively with an excess of the laevorotatory component in each case. A similar result was found by NEUBERG[96].
The action may be attributed to the formation of additive complexes -

$$R \cdot CO \cdot CHO \rightarrow R \cdot \overset{\overset{OY}{|}}{\underset{X}{C}} \cdot CHO \quad \text{or} \quad R \cdot \overset{\overset{OX}{|}}{\underset{Y}{C}} \cdot CHO$$

and the sign directed by the optically active enzyme carrier.

The developement of such enzymic asymmetric syntheses is due almost entirely to NEUBERG and coworkers. In the fields of Phytochemical Reduction with reductase, Asymmetric Dismutation with ketoaldehydemutase, and Asymmetric Condensation with carboligase, which, in contrast with the zymase-complex, synthesises a carbon chain, many results are now known. Not all results are asymmetric, the rotations of the products depending in sign and degree on the enzyme used and the conditions. Many bacteria and green plants yield inactive products where moulds and yeasts give one isomer almost optically pure, while the striking difference between living yeast and the expressed juice has been shown by NEUBERG and KOBEL[97] in the asymmetric dismutation of methyl.glyoxal.

The table below gives typical results; including some not asymmetric syntheses, where a racemic substrate is similarly asymmetrically converted. The results have a considerable bearing on the problems of sugar fermentation.

Table.10. x = High degree opt. purity.

Substrate	Product		Enzyme
Benzoylformic acid	l-Mandelic acid		Yeast Red.
Acetoacetic "	d-β.Hydroxybutyric acid		Ferm.Yeast
Oxaloacetic ester	l-Malic acid		Yeast + Sugar
Me.Et. Ketone	d-Me.Et.CHOH		" "
Me.Pr. Ketone	d-Me.Pr.CHOH		" "
Me.Hexyl. "	d-Me.Hexyl.CHOH		" "
Me.Nonyl. "	d-Me.Nonyl.CHOH		" "
Me.Phenyl. "	l-Me.Phenyl.CHOH		" "
Hydroxyacetone	l-α.Propylene.glycol		Yeast
$CH_3CHO + CH_3CO.COOH$	l-Acetoin		Yeast Carbol.
$CH_3CHO + C_6H_5CHO$	l- $C_6H_5CHOH.CO.CH_3$		Carboligase
$CH_3CHO + CH_3CHO$	l- $CH_3CHOH.CO.CH_3$		"
Methylglyoxal	Inact. Lactic acid		B.Delbr.
"	" " "		B.Lact.
"	l-Lactic acid	x	Mucor.Jav.
"	l- " "	x	B.Fluoresc.
"	l- " "	x	Yeasts
"	Inact. Lactic acid		Yeast expr.Jce
Phenylglyoxal	l- Mandelic acid	x	B.Delbr.
"	d- " "	x	B.Lact.
"	l- " "	x	B.Ascendens
"	l- " "	x	B.Fluor.
"	d- " "	x	B.Lact.acet.dr
Me.glyoxalacetic acid	d-α.Hydroxyglutaric acid		B.Coli.
" " "	d- " "		Germ.Peas
" " "	d- "	x	Liv.Yeast
Th.glyoxal	l-α.Th.glycollic acid		Fresh Yeast
Acetaldol	d-β-Butylene.glycol		Yeast
"	d-β- " "	x	B.Ascendens
Butyroin	d-δ.ε.Octane.diol		Bot.Yeast
o.Me.Cyclohexanone	d-o.Me.Cyclohexanol		Starch-yeast
dl.Me.Et.Acetaldehyde	l-Prim.Amyl alcohol		Horse Liver
"	l- " "		Top Yeast
"	l- " "		B.Ascendens
"	d- " "		B.Past.
"	Inact. " " + i.valeric acid		B.Xyl.

PART. 2.

EXPONENTIAL ANALYSIS and ASYMMETRIC EMULSIN-HYDROLYSIS.

A. AIM and SCOPE of INVESTIGATION.

The effect of substrate configuration on enzymic stereochemical specificity has been frequently noted on separate d- , and l-isomers and diastereoisomers, and on racemic mixtures. Past practice with racemates tended to restrict the scope of the work to substrates which, either themselves or in their products, were of high specific rotation, or easily separable for polarimetric examination. With the separate isomers, kinetic measurements could be made without reference to polarimetry, but resolution was usually an indispensable preliminary.

The aim of the present work was an attempt to eliminate some disabilities of both methods -

(a). To explore the possibilities of applying curve-analysis to the enzymic decomposition of a mixture of isomers or diastereoisomers, in order to note any asymmetric action of the enzyme, on the components, without direct reference to the optical rotations involved.

(b). To use the method for a systematic examination, on one enzyme, of optically active groups of slightly varying nature, preferably where the use of other methods would be difficult. Accordingly, based on curve-analysis, a kinetic method of wide scope and some accuracy has been devised, and the effect of a

growing alkyl chain on selective emulsin-hydrolysis of dl.alkyl. d.β-glucosides, has now been examined.

Several considerations favored this choice.

(a). The stereochemically selective action of emulsin, even on the non-glucosidic radicle of dl.substrates, had been established by FISHER[98].

(b). If any systematic relation were to be found between stereochemical specificity and configuration, the most elementary variation seemed a simple extension of the n.alkyl chain.

(c). A general examination of the action of aliphatic dl.alcohols would involve either the tedious and costly process of preliminary resolution of the racemic alcohols, or, unless in strong concentration of substrate, the use of micro-polarimetry in the examination of alcohols of low specific rotation, and requiring separation from the aqueous liquid before examination.

(d). In particular, distinctive results with the separate d- and l-sec.octyl.d.β-glucosides had already been obtained by MITCHELL[99].

The possibility was thus considered of forming the d.β-glucosides of the racemic alcohol as a whole, following the hydrolysis of the diastereoisomeric mixture so formed, and analysing the resulting Decomposition - Time curves in the light of theory and previous experience.

Attention has been mainly restricted, here, to a series of new glucosides. The preparation of these glucosides, their hydrolysis with emulsin, and the analysis of the corresponding Hydrolysis - Time curves on the basis of the exponential equation-

$$y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t}$$

are fully detailed in the Experimental and Analytical Sections.

The theoretical basis for the method, and the practice of curve-analysis, are described in Section. B.

The scope and accuracy of the analytical method, and a survey of the results derived from its use, are discussed in the Final Section.

B. THEORETICAL.

1. Emulsin-Hydrolysis of Mixtures.

It has been established independently by COPPADORO[100], and V.HENRI[101], in the case of acid catalysis of Me.acetate hydrolysis and sucrose inversion, that for a given concentration of acid, the rates with the separate individuals are identical with those for a mixture in equal concentration. The results have been embodied in the 'Principle of Independence of Different Reactions'. A distinction was made by HENRI between 'pure' catalysis, (e.g. acid), and 'mediate' catalysis, (e.g. enzymic). It was found that in catalysis of mixtures of substrates by one enzyme, the total reaction was greater than the effect on either individual in similar concentration, but less than the combined separate effects.

On such experimental bases, the beginnings, on mass action principles, of an intermediate - complex theory of enzyme action was established by HENRI[18]. This view has now gained general acceptance.

Apart from agreement with theory, examination of data of HENRI, for example on the action of emulsin on a mixture of salicin and amygdalin[102], and on the action of trypsin on gelatine and casein[103], show that as the concentration of the substrate decreases, the effect on the mixture increasingly tends to the sum of the effects on the separate individuals. With dilute solutions, the results of a mixture would in many

cases be so close to those of 'pure' catalysis as to be legitimately analysed as the sum of the individual effects. This is the principle adopted in the elementary analysis which follows.

(a). Elementary Analysis.

Emulsin-hydrolysis of β-glucosides generally has been found by WILLSTÄTTER, KUHN and many others to follow, particularly in dilute solution, the course of a monomolecular reaction. In particular, the rates of hydrolysis, with emulsin, of the separate d- , l-sec.octyl.d.β-glucosides has been observed by MITCHELL. The Concentration of Glucoside - Time curves conformed to the usual monomolecular equation -

$$y = a \cdot e^{-kt}$$

Over a 90 % hydrolysis range, k_1 and k_2, the rates for the two components, did not vary from the mean value by more than ±1 %.

Consider the hydrolysis by emulsin of the dl.alkyl.glucoside mixture.

Let a_1, a_2, be at t=0, the resp. concentrations of d-,l-isomers.

Let x_1, x_2, be corr. conctns. of glucose produced at time t.

Then, $a = a_1 + a_2$ = total glucoside conctn. at t = 0, and

$x = x_1 + x_2$ = " glucose " at time t.

a and x are measured in the hydrolysis experiment.

Let k_1 and k_2 be the resp. rates of hydrolysis, under parallel conditions, of the separate components, then applying the mixture theory on the basis of dilute solutions,

$$\frac{dx_1}{dt} = k_1(a_1 - x_1) \qquad (t = 0,\ x_1 = 0 = x_2)$$

$$\frac{dx_2}{dt} = k_2(a_2 - x_2)$$

Integrating, $a_1 - x_1 = a_1 \cdot e^{-k_1 t}$, $a_2 - x_2 = a_2 \cdot e^{-k_2 t}$.

Adding, $a - x = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t}$

Thus, the Hydrolysis - Time curve for a diastereoisomeric mixture has an equation of the form -

$$y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t} \quad \ldots\ldots\ldots \quad (A).$$

where k_1 and k_2 are the resp. hydrolysis rates of the components and a_1 and a_2 their respective concentrations at the (arbitrarily selected) zero time.

The problem thus reduced to fitting the experimental curves with the four parameters a_1, a_2, k_1, k_2.

(b). Extended Theory.

It is believed that the concentrations of substrate used - 0·0054 moles/litre - are sufficiently dilute to justify the assumptions adopted, but it is of interest that a more rigorous application of mass action principles, forming in effect an extension of the MICHAELIS equation, yields for the curve an equation involving once more the sum of two exponential terms. A slight difference of interpretation is required for the index parameters. Such an expression is of a more fundamental nature, for the dissociation constants, whose reality has been corroborated by EULER for invertase-sugar, are independent of concentration of enzyme or substrate, and depend mainly on temperature.

Single Component. - Consider the enzymic hydrolysis of a substrate S (glucoside), into the products G (glucose) and A (alcohol).

Let E = total concentration of enzyme.

On the intermediate-complex theory, it is assumed that the following competitive equilibria are rapidly attained throughout

the reaction, the decomposition being immediately due to the monomolecular side-reaction for the complex (E.S).

Dissoctn.const. = K_S. $E + S \rightleftharpoons (E.S) \xrightarrow{k} E + A + G$.

.. .. = K_A. $E + A \rightleftharpoons (E.A)$.

.. .. = K_G. $E + G \rightleftharpoons (E.G)$.

Where k is the rate of decomposition of the complex, a the initial concentration of S, and a - x, x, x, the corresponding concentrations of S, A, G resp. at time t, the course of the reaction is given by -

$$\frac{k \cdot E}{K_S} = \frac{a}{t} \log. \frac{a}{a - x} \cdot \left[\frac{1}{K_A} \frac{1}{K_G} \frac{1}{a}\right] + \frac{x}{t} \cdot \left[\frac{1}{K_S} \frac{1}{K_A} \frac{1}{K_G}\right]$$

which constitutes the MICHAELIS equation.

If we have $\frac{1}{K_S} = \frac{1}{K_A} + \frac{1}{K_G}$, then the hydrolysis follows the course, as with emulsin-hydrolysis of a single β-glucoside, of a monomolecular reaction, and we get the simplified form -

$$y = a \cdot e^{-\frac{E}{1 + \frac{a}{K_S}} \cdot \frac{k}{K_S} \cdot t} \quad \ldots\ldots\ldots\ldots \text{(B)}.$$

cf. $y = a \cdot e^{-kt}$

The form of this constant in .....(B). indicates that the rate of decomposition is proportional to the total concentration E of enzyme, but that variation with respect to substrate concentration depends on K_S as well as on a. This is in accordance with experimental observation.

Two Components. - Consider a general extension of the above principles to the case of one enzyme (emulsin) hydrolysing a mixture of two substrates (dl.alkyl.d.β-glucosides) which separately give a monomolecular reaction, into three products (d.alcohol, l.alcohol, and glucose).

Let E = Total concentration of enzyme, free and bound.
" E_{S_1} = Concentration of enzyme - d. glucoside complex.
" E_{S_2} = " " - l. " "
" E_{A_1} = " " - d. alcohol "
" E_{A_2} = " " - l. " "
" E_G = " " - glucose "
" S_1 = " of free d. glucoside
" S_2 = " " " l. "
" A_1 = " " " d. alcohol
" A_2 = " " " l. "
" G = " " " glucose

Let a_1, a_2 be the initial concentrations of d- , l-glucosides respectively at $t = 0$. and $a_1 - x_1$, $a_2 - x_2$ the corresponding concentrations at time t, then, where k_1 and k_2 are the decomposition rates of the complexes E_{S_1} , E_{S_2} respectively, and the K's the dissociation constants of the enzyme-complexes, we have -

$$\left.\begin{array}{l} S_1 \cdot \\ S_2 \cdot \\ A_1 \cdot \\ A_2 \cdot \\ G \cdot \end{array}\right\} \left(E - E_{S_1} - E_{S_2} - E_{A_1} - E_{A_2} - E_G \right) = \left.\begin{array}{l} K_{S_1} \cdot E_{S_1} \ldots 1. \\ K_{S_2} \cdot E_{S_2} \ldots 2. \\ K_{A_1} \cdot E_{A_1} \ldots 3. \\ K_{A_2} \cdot E_{A_2} \ldots 4. \\ K_G \cdot E_G \ldots 5. \end{array}\right.$$

$$\frac{dx_1}{dt} = k_1 \cdot E_{S_1} \quad \ldots\ldots\ldots\ldots 6.$$

$$\frac{dx_2}{dt} = k_2 \cdot E_{S_2} \quad \ldots\ldots\ldots\ldots 7.$$

also, $A_1 = x_1$
$A_2 = x_2$
$G = x_1 + x_2 = x$
Total conctn. free glucoside $a = a_1 + a_2$ .....8.

By determinantal elimination of E_{S_1}, E_{S_2}, E_{A_1}, E_{A_2}, E_G from 1..5, 6.

$$\begin{vmatrix} K_{S_1} + S_1 & S_1 & S_1 & S_1 & S_1 & -E \cdot S_1 \\ S_2 & K_{S_2} + S_2 & S_2 & S_2 & S_2 & -E \cdot S_2 \\ A_1 & A_1 & K_{A_1} + A_1 & A_1 & A_1 & -E \cdot A_1 \\ A_2 & A_2 & A_2 & K_{A_2} + A_2 & A_2 & -E \cdot A_2 \\ G & G & G & G & K_G + G & -E \cdot G \\ k_1 & & & & & -\frac{dx_1}{dt} \end{vmatrix} = 0.$$

i.e. $(Z) \cdot \frac{dx_1}{dt} = - k_1 \cdot (X).$ .....9.

Similarly, $(Z) \cdot \frac{dx_2}{dt} = + k_2 \cdot (Y).$

where (X) , (Y) , and (Z) are given by the equations -

$$\begin{vmatrix} K_{S_1}+S_1 & S_1 & S_1 & S_1 & S_1 \\ S_2 & K_{S_2}+S_2 & S_2 & S_2 & S_2 \\ A_1 & A_1 & K_{A_1}+A_1 & A_1 & A_1 \\ A_2 & A_2 & A_2 & K_{A_2}+A_2 & A_2 \\ G & G & G & G & K_G+G \end{vmatrix} = (Z) = P \cdot (1+S).$$

$$\begin{vmatrix} S_1 & S_1 & S_1 & S_1 & -E\cdot S_1 \\ K_{S_2}+S_2 & S_2 & S_2 & S_2 & -E\cdot S_2 \\ A_1 & K_{A_1}+A_1 & A_1 & A_1 & -E\cdot A_1 \\ A_2 & A_2 & K_{A_2}+A_2 & A_2 & -E\cdot A_2 \\ G & G & G & K_G+G & -E\cdot G \end{vmatrix} = (X) = P \cdot -E \cdot \frac{S_1}{K_{S_1}}$$

$$\begin{vmatrix} K_{S_1}+S_1 & S_1 & S_1 & S_1 & -E\cdot S_1 \\ S_2 & S_2 & S_2 & S_2 & -E\cdot S_2 \\ A_1 & K_{A_1}+A_1 & A_1 & A_1 & -E\cdot A_1 \\ A_2 & A_2 & K_{A_2}+A_2 & A_2 & -E\cdot A_2 \\ G & G & G & K_G+G & -E\cdot G \end{vmatrix} = (Y) = P \cdot +E \cdot \frac{S_2}{K_{S_2}}$$

where $P = K_{S_1} \cdot K_{S_2} \cdot K_{A_1} \cdot K_{A_2} \cdot K_G$ .....10.

and $S = \frac{G}{K_G} + \frac{A_1}{K_{A_1}} + \frac{A_2}{K_{A_2}} + \frac{S_1}{K_{S_1}} + \frac{S_2}{K_{S_2}}$

Thus equations ....9. reduce to -

$$(1+S) \cdot \frac{dx_1}{dt} = E \cdot k_1 \cdot \frac{S_1}{K_{S_1}} \quad .....9a.$$

$$(1+S) \cdot \frac{dx_2}{dt} = E \cdot k_2 \cdot \frac{S_2}{K_{S_2}}$$

But for the separate components, the reactions follow a monomolecular course, hence, as before -

$$\frac{1}{K_{S_1}} = \frac{1}{K_{A_1}} + \frac{1}{K_G}, \qquad \frac{1}{K_{S_2}} = \frac{1}{K_{A_2}} + \frac{1}{K_G} \quad .....11.$$

Using equations ...8, ...10, ...11, we have -

$$S = \frac{G}{K_G} + \frac{A_1}{K_{A_1}} + \frac{A_2}{K_{A_2}} + \frac{S_1}{K_{S_1}} + \frac{S_2}{K_{S_2}}$$

$$= \frac{x_1+x_2}{K_G} + \frac{x_1}{K_{A_1}} + \frac{x_2}{K_{A_2}} + \frac{a_1-x_1}{K_{S_1}} + \frac{a_2-x_2}{K_{S_2}}$$

$$= x_1 \cdot \left[\frac{1}{K_G} + \frac{1}{K_{A_1}}\right] + x_2 \cdot \left[\frac{1}{K_G} + \frac{1}{K_{A_2}}\right] + \frac{a_1-x_1}{K_{S_1}} + \frac{a_2-x_2}{K_{S_2}}$$

$$= \frac{x_1}{K_{S_1}} + \frac{a_1-x_1}{K_{S_1}} + \frac{x_2}{K_{S_2}} + \frac{a_2-x_2}{K_{S_2}}$$

$$\text{i.e.} \qquad S = \frac{a_1}{K_{s_1}} + \frac{a_2}{K_{s_2}}$$

Let $\dfrac{E}{1 + \frac{a_1}{K_{s_1}} + \frac{a_2}{K_{s_2}}} = p$, $\dfrac{k_1}{K_{s_1}} = l_1$, and $\dfrac{k_2}{K_{s_2}} = l_2$,

from equations .....9a.

$$\frac{dx_1}{dt} = p \cdot l_1 \cdot (a_1 - x_1)$$

$$\frac{dx_2}{dt} = p \cdot l_2 \cdot (a_2 - x_2) \qquad (t = 0,\ x_1 = 0,\ x_2 = 0).$$

Integrating,

$$a_1 - x_1 = a_1 \cdot e^{-p \cdot l_1 \cdot t}$$

$$a_2 - x_2 = a_2 \cdot e^{-p \cdot l_2 \cdot t}$$

and adding, $a - x = a_1 \cdot e^{-p \cdot l_1 \cdot t} + a_2 \cdot e^{-p \cdot l_2 \cdot t}$

$y = a - x$, and t, are experimentally obtained, hence, resubstituting for p, l_1 and l_2, the equation for the Hydrolysis - Time curve for the diastereoisomeric mixture, has the form -

$$y = a - x = a_1 \cdot e^{\frac{-E}{1 + \frac{a_1}{K_{s_1}} + \frac{a_2}{K_{s_2}}} \cdot \frac{k_1}{K_{s_1}} t} + a_2 \cdot e^{\frac{-E}{1 + \frac{a_1}{K_{s_1}} + \frac{a_2}{K_{s_2}}} \cdot \frac{k_2}{K_{s_2}} t} \qquad .....(C).$$

cf. for a single component $y = a - x = a \cdot e^{\frac{-E}{1 + \frac{a}{K_s}} \cdot \frac{k}{K_s} t}$ .....(B).

Thus on the extended theory of competitive complex formation, the Concentration.of.Glucoside - Time curve for a mixture of two diastereoisomers is again, as in the elementary theory of approximate 'pure' catalysis, the sum of two exponential components, each of which has a simple formal analogy to that of equation ...(B), obtained on the MICHAELIS theory for a single member with a monomolecular reaction course.

On comparison with equation ....(A) of page 41, it is seen that a_1, a_2, have exactly the same significance as before.

On comparison with equation ....(B). it is seen that the ratio of hydrolysis rates of the separate components is equal or not to the ratio of rates in a mixture hydrolysed under parallel conditions, according or not as -

$$1 + \frac{a_1}{K_{s_1}} = , \neq , \quad 1 + \frac{a_2}{K_{s_2}} .$$

Hence, if any of the following hold -

$$\frac{a_1}{K_{s_1}} = \frac{a_2}{K_{s_2}} \quad \text{.....(a).}$$

K_{s_1} and K_{s_2} are LARGE .....(b).

a_1 and a_2 are SMALL .....(c).

- the rate-ratios are the same separately, as in the mixture.

It is believed that with the conditions adopted, at least .....(c). is fulfilled.

2. Practice of Curve Analysis.

(a). Exponential Analysis.

A method of exponential analysis, first published in simple form by WALSH[104], may be outlined as follows for the two components here in question.

To determine from the experimental curve of known form -

$$y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t} \quad \text{.....(1).}$$

the constants a_1, a_2, k_1, k_2.

Let y_0, y_1, y_2, y_3 be the values of y corresponding to the values 0 , δ , 2δ , 3δ of t, zero time and δ being arbitrarily chosen.

Then, where $e^{-k_1\delta} = \alpha_1$, $e^{-k_2\delta} = \alpha_2$ .....(2).

we have

$$\left.\begin{aligned} y_0 &= a_1 + a_2 \\ y_1 &= a_1\alpha_1 + a_2\alpha_2 \\ y_2 &= a_1\alpha_1^2 + a_2\alpha_2^2 \\ y_3 &= a_1\alpha_1^3 + a_2\alpha_2^3 \end{aligned}\right\} \quad \ldots\ldots(3).$$

Let p , q , be such that α_1 , α_2 , are the roots of

$F.(x). = x^2 + px + q = 0$

then, $y_2 + py_1 + qy_0 = a_1 \cdot F.(\alpha_1) + a_2 \cdot F.(\alpha_2) = 0$

and, $y_3 + py_2 + qy_1 = a_1\alpha_1 \cdot F.(\alpha_1) + a_2\alpha_2 \cdot F.(\alpha_2) = 0$

Eliminating p , q ,

$$\begin{vmatrix} x^2 & x & 1 \\ y_2 & y_1 & y_0 \\ y_3 & y_2 & y_1 \end{vmatrix} = 0.$$

α_1 , α_2 , the roots of this quadratic in **x**, are thus easily found. The values so obtained give k_1 and k_2 from ....(2). and a_1 and a_2 from any two of .....(3).

(b). Probability Correction.

This exponential analysis, being based only on four points from the mean curve, and being rather sensitive even to slight error in the chosen points, it was desirable to use the set of parameters a_1, a_2, k_1, k_2 so obtained, as a first approximation to the set giving an equation in closest agreement with all the available reliable data.

The following method was adopted.

Let $Y.(a_1, a_2, k_1, k_2) = y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t}$

where ...(1) of page 46 , is the equation best representing

the data of hydrolysis.

Let $\bar{a}_1$, $\bar{a}_2$, $\bar{k}_1$, $\bar{k}_2$, be the parameter set obtained by preliminary exponential analysis.

Let $a_1 = \bar{a}_1 \cdot (1+\beta_1)$, $a_2 = \bar{a}_2 \cdot (1+\beta_2)$, $k_1 = \bar{k}_1 \cdot (1+\lambda_1)$, $k_2 = \bar{k}_2 \cdot (1+\lambda_2)$ ..(4).

then, where β_1, β_2, λ_1, λ_2 are small,

$$\delta_n = Y.(a_1, a_2, k_1, k_2)_{t=t_n} - Y.(\bar{a}_1, \bar{a}_2, \bar{k}_1, \bar{k}_2)_{t=t_n}$$

$$= \left[\frac{\partial Y}{\partial \bar{a}_1} \cdot \bar{a}_1\beta_1 + \frac{\partial Y}{\partial \bar{a}_2} \cdot \bar{a}_2\beta_2 + \frac{\partial Y}{\partial \bar{k}_1} \cdot \bar{k}_1\lambda_1 + \frac{\partial Y}{\partial \bar{k}_2} \cdot \bar{k}_2\lambda_2 \right]_{t=t_n}$$

to first order,

Hence, $\delta_n = \beta_1 \cdot (\bar{a}_1 \cdot e^{-\bar{k}_1 t_n}) + \beta_2 \cdot (\bar{a}_2 \cdot e^{-\bar{k}_2 t_n})$

$+ \lambda_1 \cdot (-\bar{a}_1\bar{k}_1 t_n \cdot e^{-\bar{k}_1 t_n}) + \lambda_2 \cdot (-\bar{a}_2\bar{k}_2 t_n \cdot e^{-\bar{k}_2 t_n})$ .....(5).

$= h_{n1} \cdot \beta_1 + h_{n2} \cdot \beta_2 + h_{n3} \cdot \lambda_1 + h_{n4} \cdot \lambda_2$ say.

For each point $P_n(t_n, y_n)$ corresponding to a reliable reading, some 16 - 20 in all, an 'Equation of Condition' of type ...(5). in β_1, β_2, λ_1, λ_2 was thus formed, where t_n is calculated from P , the first of the four points selected for the preliminary exponential analysis, as zero time, and

$$\delta_n = y_n - \left[\bar{a}_1 \cdot e^{-\bar{k}_1 t_n} + \bar{a}_2 \cdot e^{-\bar{k}_2 t_n} \right]$$

From the equations of condition were formed, as usual in the 'Method of Least Squares' [105] , the four 'Normal Equations'

$$u_{r1} \cdot \beta_1 + u_{r2} \cdot \beta_2 + u_{r3} \cdot \lambda_1 + u_{r4} \cdot \lambda_2 = v_r \ (r=1,2,3,4) \ ..(6).$$

where $u_{rs} = \sum_{n=1}^{16-20} h_{nr} \cdot h_{ns}$, and $v_r = \sum_{n=1}^{16-20} h_{nr} \cdot \delta_n$.

Equations ....(6). were then solved for β_1, β_2, λ_1, λ_2, either as ratios of determinants, or by successive approximation. The values inserted in .....(4). gave the required best set of parameters a_1, a_2, k_1, k_2, whence equation .....(1).

This correction was of great service in establishing the agreement with the octyl member. Later experience showed that, so far as the rate-ratio was concerned, it might sometimes have been dispensed with. With the two lowest members of the series, it was found that circumstances did not justify what was, without a mechanical calculator, a somewhat laborious refinement.

Apart from theoretical considerations, a practical test of the validity of the method was available with dl.sec.octyl.d.β-glucoside, the rates for the separate isomers being already known.

By the use of an accurate analytical micro-method for the estimation of glucose, thus allowing a high dilution to be used, and by noting the course of the reaction at many intermediate stages over a full range of hydrolysis and an extended period of time, it was hoped to obtain a mean curve sufficiently accurate to allow of exponential analysis applying, to give some quantitative estimate of k_1 and k_2 .

C. EXPERIMENTAL. 1.

1. The Racemic Alcohols.

(a). Preparation of Ketones.

For the preparation of some of the secondary alcohols, the corresponding ketones were required. These were of the type -

$$CH_3 \cdot CO \cdot R ,$$

where R is n.amyl, n.heptyl, or n.octyl.
They were prepared by the catalytic method of SENDERENS[106] as modified by PICKARD and KENYON[107], from the corresponding normal caproic, caprylic and pelargonic acids, commercially obtained.

$$R \cdot COOH + CH_3 \cdot COOH \longrightarrow CH_3 \cdot CO \cdot R + CO_2 + H_2O$$

Pumice was thoroughly cleaned, granulated evenly, and used as support for the catalytic agent thoria, being impregnated with a strong solution of the nitrate, and then ignited. This was packed in a Pyrex glass tube of bore 1·5 cm. and length 50 cm. The tube was slightly inclined, and the upper end fitted with a dropping funnel and an inlet tube for CO_2.
100 gms. of the acid dissolved in 5-7 times its weight of glacial acetic acid was passed through from the dropping funnel at a constant rate of about 1 cc. per minute, a slight stream of CO_2 being maintained, and the catalytic chamber being kept at a temperature of 420 ± 10°C. by electric furnace and rheostat resistance, the temperature being noted by thermopile.
The effluent products were condensed and collected. Slight

charring occurred on the thoria, but activity was fully recovered on reignition. The products, usually slightly yellow and faintly acid, were salted out, dried and easily fractionated. While the lower ketones were distilled once only at atmospheric pressure, the others were twice fractionated at low pressure in a pear-shaped flask fitted with internal rod-and-spiral fractionating column. Close-boiling fractions were selected for the subsequent reductions.

Table.11.

Ketone	Yield	B.P. Final Fraction	Pressure
Methyl-n.amyl.	79 gms.	147·5 - 152·5 °C.	760 mm.
Methyl-n.heptyl.	49 gms.	75·5 - 77 °C.	12 mm.
Methyl-n.octyl.	60 gms.	91 - 92 °C.	10·5 mm.

(b). Preparation of Alcohols.

The racemic secondary alcohols used belong to the series -

$$CH_3 \cdot CHOH \cdot n.alkyl.$$

- and were made from the butyl to the decyl members. Me.n.Pr.carbinol was obtained commercially, and repeatedly fractionated before use. The remaining members were obtained from methyl-ethyl, methyl-n.amyl, methyl-n.heptyl, and methyl-n.octyl ketones by reduction, in the usual manner[108], with Na. - NaHO solution.

$$\dot{C} = O \xrightarrow{H} \dot{C}H.OH$$

The ethereal layers, separated, washed, and thoroughly dried with K_2CO_3, were carefully fractionated. As with the corresponding ketones, reduced pressure was used for the two higher members. Yield of final fraction - 65 to 75 %.

The secondary butyl member was prepared in considerable quantity.

Table.12.

Weight Ketone	Alcohol	B.P.Final Fraction	Pressure
Various	Me.Et.carbinol 70 %	98 - 99·5 °C	Atmos.
-----	Me.n.Pr.carbinol	118·8 - 119·3°C	··
79 gm.	Me.n.amylcarbinol 46 gm.	158 - 159 °C	··
49 gm.	Me.n.Non.carbinol 36 gm.	95 - 95·5 °C	15 mm.
60 gm.	Me.n.Oct.carbinol 40 gm.	104 - 105 °C	13-14 mm.

In addition, Me.Et.carbin-carbinol was prepared. Commercial 'active amyl alcohol' was repeatedly fractionated, and the distillate of B.P. 128 °C selected for use.

(c). Resolution of Sec. Butyl Alcohol.

For comparative purposes, the results, (to be described), of emulsin-hydrolysis of dl.sec.butyl.d.β-glucoside required confirmation, and the corresponding glucoside of at least one active form of the alcohol was required.

A resolution was therefore carried out, the procedure of PICKARD and KENYON[107] being followed.

1. 600 - 800 cc. of the alcohol were prepared as already described.

2. Theoretical quantities of alcohol and phthalic anhydride, 100 cc. and 162 gm. respectively, were heated for 15 hours on water bath. The resulting viscous oil, poured when cool into a dilute solution of Na_2CO_3 , dissolved slowly to give the sodium salt, unchanged alcohol and bye-products being removed by ethereal extraction. The required compound, precipitated with HCl, was extracted with $CHCl_3$. After removal of the solvent, the remaining syrup triturated with petroleum ether, gave a cryst-

-alline product of M.P. 57-58 °C. Yield. 200-210 gms. = 82-87 % . 1000 gms. were so prepared, a crystallisation from petroleum ether being taken if necessary, to give the required degree of purity.

3. 322 gms. of phthalate were dissolved in about 1·5 litres of acetone, and the theoretical quantity of anhydrous brucine (580 gms) slowly added to the boiling solution. The slight residue was filtered off, and on cooling, the solution gave a mass of white crystals. A second crop was recovered on concentrating the mother liquors. The salt was recrystallised a further three times from boiling acetone, considerable volumes being required.

After some 7-8 recrystallisations from methyl alcohol, isolation of the d-isomer was practically complete. The usual M.P. and Optical Rotation controls were noted throughout, and gave in the limit for the brucine-d-compound,

$(\alpha)^{20}_{5780} = -3{\cdot}9^{\circ}$, $(\alpha)^{20}_{5461} = -6{\cdot}0^{\circ}$ from 2·5 % soltn. in alcohol.

M.P. = 161 °C. (See Note 1.).

In all, 515 gms. of d-salt were obtained.

The earlier mother liquors were also concentrated and used for the recovery of partial l-alcohol.

4. 515 gms. of brucine-d-ester was dissolved in a minimum of hot alcohol and poured into dilute HCl, the precipitated acid phthalate being extracted with ether, washed, dried, and the solvent removed to give, on trituration with petroleum ether, 137 gms. of crystalline d-phthalate. Yield = 80 %. M.P.= 47°C. From rotations in 5 % alcohol solution,

$(\alpha)^{20}_{5461} = +45{\cdot}90^{\circ}$. $(\alpha)^{20}_{5780} = +39{\cdot}52^{\circ}$. $(\alpha)^{20}_{5892} = +38{\cdot}89^{\circ}$.

cf. $(\alpha)^{20}_{D} = +38{\cdot}44^{\circ}$, $+38{\cdot}97^{\circ}$ - PICKARD and KENYON[107].

From residues similarly treated, 251 gms. of l-dl.phthalate was obtained. M.P. = 53-54 °C.

$(\alpha)^{22}_{5461}$ = -17·47°. $(\alpha)^{22}_{5780}$ = -15·15°.

thus indicating a 69·1 - 30·9 % proportion of l- , to d-isomer.

5. The active phthalates were dissolved in excess of 25 % NaOH slightly warmed, and the resulting alcohol recovered by steam distillation, being easily salted out of the first portion of the distillate by addition of K_2CO_3 . The active alcohols were subjected to prolonged drying with freshly ignited K_2CO_3 , and then distilled.

l-dl.alcohol.- Yield of B.P. 97·5 - 99 °C = 50cc.

α^{20}_{5461} = -6·36° in 1 dm. tube. i.e. 69·7 % = l. 30·3 % = d.

cf. similar result from phthalate stage,

d-alcohol.- Yield of B.P. 97-99 °C = 30 cc.

α^{20}_{5461} = +26·04° in 2 dm. tube.

cf. α^{20}_{5461} = +13·02° in 1 dm. tube. (PICKARD and KENYON).

Hence for the d-component, complete resolution has been effected.

2. The d.β-Glucosides.

(a). Method.

Many alkyl.d.β-glucosides are now known, prepared either synthetically by FISCHER, or from the emulsin-syntheses of BOURQUELOT. The following series of d.β-glucosides of the racemic secondary alcohols of formula - $CH_3 \cdot CHOH \cdot$ n.alkyl , has not previously been prepared. The method adopted throughout, was essentially that of KÖNIGS and KNORR[109], as used by FISCHER[110]

in preparing a β-amyl.glucoside.

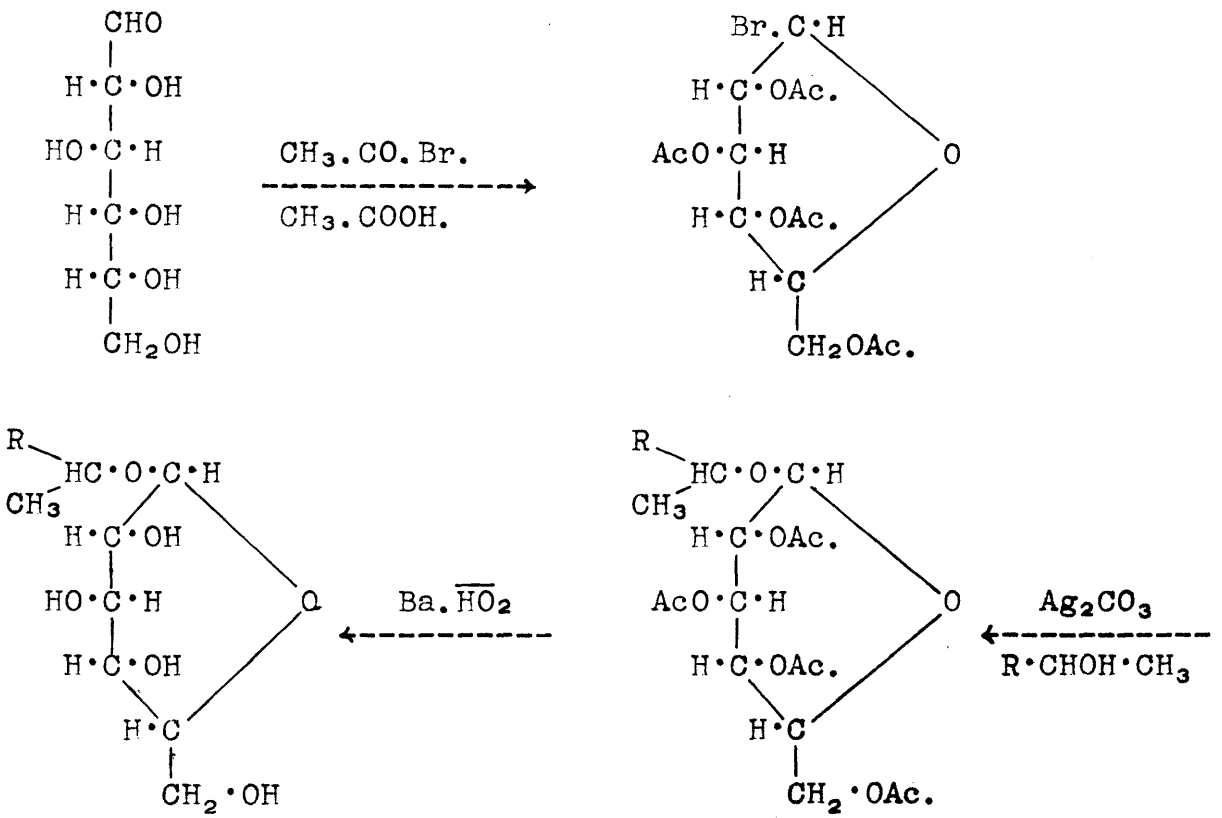
This method comprises three stages.

(a). Preparation of tetraacetylbromoglucose.

(b). Combined action of racemic alcohol + Ag_2CO_3 on (a). to give the tetraacetyl.d.β-glucoside.

(c). Removal of the acetyl groups by baryta hydrolysis to give the required β-glucoside.

The reactions are represented in the following sequence.



+ $(CH_3 \cdot COO)_2 \cdot Ba.$ +Ag.Br. + CO_2 + H_2O.

(b). Preparation of Tetraacetylbromoglucose.

The method of DALE[11], combining bromination and acetylation in one process, was used in preference to that of FISCHER[12], which requires the preliminary formation of the pentacetate.

The procedure has been applied generally to sugars, and with l-glucose, good yields have been obtained by KARRER and NÄGELI[113]. Detail variants were found expedient.

The bromo-acetylating mixture used was either a mixture of $CH_3CO.Br.$ and glacial CH_3COOH, (50 gm. - 30 gm.), or a saturated solution of HBr. in acetic anhydride, the HBr. being generated from bromine and boiling tetrahydronaphthalene in the manner described by HOUBEN[114], (Br.- 33 cc., $C_{10}H_{12}$ - 80 gm., acet.anhydr.- 100gm.). The gas was freed of bromine and dried with $CaCl_2$ before adsorption at ice temperature. The latter mixture gave a more vigorous reaction and greater yield, but not so pure an initial product.

25 gms. of oven-dried d-glucose were treated in a 500 cc. conical flask with 125 cc. of the bromo-acetylating mixture. Very vigorous action, requiring careful temperature control by ice-bath immersion, ensued to give a clear viscous straw-coloured syrup. This was extracted with chloroform, freed of acid by repeated washing with dilute $NaHCO_3$ and water, dried, concentrated, and the remanent syrup crystallised by trituration in ice-bath with low petroleum ether. Crude yields were obtained of 46 gm. = 80 % of theory.

Immediate recrystallisation was made from hot amyl alcohol, or hot ligroin (80-100 °C.B.P.), a preliminary crystallisation from dry ether being occasionally necessitated. The pure product was obtained in crystalline rosettes from amyl alcohol, and in long needles from ligroin, both of M.P. 88 °C. From the latter solvent, the product, if kept under vacuum in the dark over soda-lime, may be kept indefinitely.

Considerable quantities were so prepared, while some was also

obtained by purchase.

(c). Preparation of Tetracetyl.dl.Me.n.alkylcarbinyl.d.β-Glucosides.

dl.Sec.Butyl Member. - 150 cc. dl.sec.butyl alcohol + 50 cc. Na.-dried ether were placed in a brown stoppered bottle of 500 cc. capacity, and 7·5 gms. of acetobromglucose added. Three times the theoretical quantity of Ag_2CO_3, (7·5 gms.), freshly precipitated and dried with alcohol and ether, was then added. Vigorous evolution of CO_2 ensued, and the bottle was then shaken for 24 hours. On two further occasions at day intervals, 7·5 gms. each of acetobromoglucose and Ag_2CO_3 were added, followed by the usual shaking. The silver salts were then removed by filtration or centrifuging, and the alcohol recovered by low pressure distillation, leaving the crude product as a mass of crystalline rods. After crystallisation, once from hot alcohol, and once from 70 % alcohol, the compound was obtained in colorless rods of M.P. 115-116 °C. Yield. 13·5 gms. = 60 %. The silver residues, extracted twice with ether and ether-alcohol gave a further 1·25 gm., while from the mother liquors, a small quantity of much lower M.P. may be obtained. 70-80 gms. of this member was prepared.

The remaining tetracetyl.glucosides were prepared in a similar manner. In each case 22·5 gms. acetobromoglucose and 22·5 gms. Ag_2CO_3 were used in three separate additions. The alcohol quantities used ranged from 24-52 gms., no loss in yield being noted with the smaller volumes. 50-100 cc. ether was used in each case as solvent. With the lower members, the excess alcohol was recovered as before by low pressure distillation, but steam distillation was used with the nonyl and decyl members. With the

four higher members, the crude products were thick syrups instead of a crystalline mass.

The products of two recrystallisations from 80 % alcohol, giving either silky needles, needle clusters or prismatic rods, were used for the preparation of the corresponding glucosides.

The amyl member may be recrystallised from low petroleum ether in a fine micro-crystalline texture.

The tetracetyl-glucoside of the primary active amyl alcohol, β-Me.butanol, was also prepared.

All members were very soluble in hot alcohol, easily soluble in ether and hot aqueous alcohol (<30 % H_2O), and insoluble in water. The tetracetyl-glucosides prepared, with yields and physical and combustion data, are tabulated below. As the compounds form a mixture of diastereoisomers, the melting points were not very sharply defined. The calculated combustion figures are based on the formulae - $C_{18}H_{28}O_{10}$, $C_{19}H_{30}O_{10}$.....$C_{24}H_{40}O_{10}$.

Table.13.

Weight Alcohol used	Tetracetyl-d.β-glucoside of --- Alcohol		M.P. from aq.alc.	Combustion Data Found. % . C	H	Calcd. % . C	H
Various	dl.Me.Et.carbinol	13 g.	116°C	53·7	7·0	53·5	6·9
24 gm	d.·· ·· ··	13 g.	104°C	-----		------	
40 gm	l.dl·· ·· ··	13 g.	120°C	-----		------	
52 gm	dl.Me.n.Pr. ··	15 g.	82°C	54·6	7·35	54·5	7·2
--	dl.Me.n.But.··	x	99°C	55·8	7·3	55·6	7·4
46 gm	dl.Me.n.Am. ··	15 g.	89°C	56·6	7·8	56·5	7·7
--	dl.Me.n.Hex.··	x	95°C	57·2	7·7	57·4	7·8
36 gm	dl.Me.n.Hept··	14 g.	91°C	58·05	8·1	58·2	8·1
40 gm	dl.Me.n.Oct.··	19 g.	90°C	59·0	8·3	59·0	8·3
150 gm	dl.Prim.Act.Amyl.Al	14 g.	77°C	-----		------	

x. Already prepared.

Yields varied from 52 - 70 % .

(d). Preparation of dl.Me.n.alkylcarbinyl.d.β-Glucosides.

dl.Sec.Butyl Member. - 6 gms. of the finely powdered tetra-acetyl.compound (M.P. 116 °C.) was shaken for 24 hours at room temperature with an excess of baryta solution (24 gms. in 360 cc.) after which hydrolysis is complete. The excess baryta was removed as carbonate from the warmed solution, and twice extracted with hot water to remove adsorbed glucoside. The filtrates were taken to dryness, separated from the Ba.acetate in the residue by repeated extraction with alcohol, and the solvent finally removed. The glucoside, on trituration with petroleum ether in the cold, is obtained in the form of powdery crystals. Slight difficulty was experienced in obtaining a good crystalline product. The best results were obtained by drying the first product, in vacuo, over P_2O_5 and then recrystallising from perfectly dry ether, though a considerable volume of solvent was essential. One such recrystallisation gave the substance in small white rosettes. M.P. = 95 °C. with slight sintering at about 80 °C. Yield - 3·1 gm. = 85 % of theory. For combustion, one further recrystallisation was made. Unless kept over P_2O_5 , the glucoside absorbs water, and the M.P. falls to 78 °C.

The other glucosides were similarly prepared, 6 gms. of tetracetyl.compound being used in each case, and yields of 2·9 - 3·2 gms of powdery white crystals obtained after petroleum ether trituration. With the decyl member, a 50 % yield only was obtained. With the nonyl and decyl members, the precipitated $BaCO_3$ was extracted with alcohol also.

Owing to the comparative insolubility of the decyl glucoside in

water, it was precipitated in fine crystalline needles during the concentration of the aqueous solution of the mixture of glucoside and Ba.acetate, no further extraction increasing the yield. Two crops of crystals were so obtained, washed, and dried. The other glucosides were recrystallised as before from dry ether, the crystals being filtered quickly and kept in vacuo over P_2O_5. Particularly fine crystals, needles and rosettes respectively, were obtained for the d- and l-dl.butyl members.
The corresponding glucoside of β-Me.Butanol was also prepared.

The glucosides are all crystalline substances, extremely soluble in most solvents, slightly only in ether, and practically insoluble in petroleum ether. Solubility in water decreases markedly in the higher members. All members, on atmospheric exposure, become hydrated, and may even deliquesce.
Combustion and physical data as under, were obtained after drying the glucosides for ten days in vacuo over P_2O_5 at room temperature.

Table.14.

d.β-Glucoside of --- Alcohol	M.P. from Ether	Combustion Data: Found % C	Found % H	Calcd. % X C	Calcd. % X H	Calc.% X·H_2O C	Calc.% X·H_2O H
dl.Me.Et.carbinol	95 °C	50·6	8·5	50·8	8·5	-------	
d.·· ·· ··	118 °C	-------		-------		-------	
l.dl.·· ··	78 °C	-------		-------		-------	
dl.Me.n.Pr. ··	126 °C	52·4	9·1	52·7	8·9	-------	
dl.Me.n.But.·· x	115 °C	54·6	9·0	54·6	9·1	-------	
dl.Me.n.Am. ··	73 °C	55·3	9·4	56·1	9·4	52·7	9·5
dl.Me.n.Hex.··	96 °C	56·6	9·8	57·5	9·7	54·2	9·75
dl.Me.n.Hept··	83 °C	57·9	10·0	58·8	9·9	55·5	9·95
dl.Me.n.Oct.··	114 °C	59·4	10·0	59·9	10·1	-------	
dl.β-Me.Butanol z	82 °C	-------		-------		-------	

x Already prepared
z Very deliquescent

With some members, the M.P's were not sharp, considerable sintering taking place at a lower temperature. Such phenomena, in accordance with previous experience of glucosides, are to be expected from such diastereoisomeric mixtures, particularly where the presence of water is an added complication.
The combustion data for the heptyl to nonyl members indicate a low carbon content, due to tenacious retention of still about 1/3 mol. of water. It is also obvious, that the previously prepared octyl member, which when ordinarily dried had given a combustion indicating 1/2 mol. water, is not fully dehydrated either with the conditions employed.

For the hydrolyses, however, the presence of such traces of water is immaterial, the weight of glucoside originally taken acting merely as a check on the equally accurate value of original dry substance, calculated from the glucose set free at complete hydrolysis.

3. Emulsin Preparates.

During the course of the work, various emulsin specimens were used as under -

(a). Commercial 'emulsin'.

(b). Purifications of (a).

(c). A preparation by the method of WILLSTÄTTER[115] direct from sweet almonds.

(c). was prepared as follows - The almonds were skinned after immersion in warm water, and most of the oil pressed out. The

solid was repeatedly ground and extracted with ether to give a powder free of oil. From 200 gms. of this powder, the main part of the enzyme was dissolved by elution with dilute ammonia, and protein excess removed from the filtrate by precipitation with acetic acid. The centrifuged clear solution gave on addition of 5 vols. of alcohol a precipitate of creamy flakes, which were washed thoroughly in alcohol and ether, dried to a powder of very fine texture, and kept over P_2O_5 in vacuo.

This powder (No. 10.), was used for most of the enzymic hydrolyses, and was 7 times as active as any other preparate. One good commercial specimen (No. 2.) was also largely used. With one exception, commercial specimens, owing to the presence of oil and protein excess, were quite useless for kinetic measurements. If only a few months old, these, when purified as above by ammoniacal elution, could again be successfully used. Uniform results were obtained from a specimen over a period of a month or more, the lowering of the activity of the enzyme being slight. Later, however, a change in the course of the reaction became evident, a pronounced retardation being noted in the final stages of hydrolysis, due probably to the effect of enzyme decomposition products.

For hydrolysis curves used for exponential analysis, the enzyme specimens used gave practically complete aqueous solution, and were always tested by successful corroboration of previous results with the d-octyl or d-butyl glucosides.

In the Summary Tables for the hydrolysis experiments, the emulsins are numerically distinguished, some 10 preparates in all being used.

4. Analytical Method.

The course of hydrolysis throughout was followed by estimation of the glucose set free. The micro-method of MACLEAN[116] was used, the principle of which is as follows. -

An alkaline solution of $CuSO_4$, containing KIO_3 and KI , is prepared, and the free iodine content per cc. found by acidifying with a slight excess of HCl, the HIO_3 formed liberating the equivalent amount of iodine, which is estimated by titration with 0·01 N. thiosulphate. A micro-burette, 10.- 0·01cc. was used.

A known volume of the glucose-containing solution is allowed partially to reduce, under given conditions, a known volume of the standard solution, Cu_2O being formed. On acidification, part of the HIO_3 oxidises the Cu', and the difference in iodine set free is a direct measure of the glucose added.

Estimation is based on calibration from standard solutions of pure glucose, but the calibration curve being rectilinear in a range 0 - 2 mgm. glucose, the concentration of glucose, (or glucoside) may be expressed directly in equivalent ccs. of 0·01 N. thiosulphate. A sensitivity of 0·005 mgm. glucose is claimed.

A test of the method with pure glucose solutions gave a similar linear calibration curve, with 1 mgm. glucose = 3·60 cc. of 0·01 N. thiosulphate. Very careful standardisation of heating was found essential. The method is suitable for the hydrolysis of glucosides, as the freed alcohols gave no reduction, and the reducing effect of the enzyme is eliminated by difference, being constant throughout the experiment. In most cases the latter

effect was of the order of 0·01 - 0·02 cc. but with a few specimens in high concentration it amounted to 0·4 cc.

5. Emulsin-Hydrolysis of the d.β-Glucosides.

All the experiments were carried out with the emulsins described, at a temperature of 37 °C, and in unbuffered neutral solution. Just sufficient glucoside was used to utilise, at complete hydrolysis, the full linear range of the Glucose - Thiosulphate Calibration Graph, so that the hydrolyses, with a few noted exceptions, where the concentrations are double, are exactly comparable as regards molar substrate concentration. The latter is 0·0054 moles / litre.

In a typical experiment, a small quantity (c. 0·1 gm.) of glucoside was weighed out in a 50 cc. graduated flask, and an emulsin solution prepared and filtered. A drop of toluene was added to the emulsin solution, and the flasks were immersed overnight in a thermostat at 37 °C . The hydrolysis was begun by adding the emulsin solution up to the mark of the flask, and the progress noted over a period of 6 - 10 hours by estimating, by MACLEAN's micro-method, the glucose content in 1 or 2 cc. volumes periodically withdrawn. The progress of the decomposition was usually noted over 94-98 % of the full range. The α Readings, taken 24-48 hours later, agreed with those calculated for complete hydrolysis, from the weight of glucoside originally taken. Analytical difficulties, due to excessive frothing, were experienced with the nonyl and decyl members.

From the experimental data, corresponding values of Time -

Concentration. Glucoside (t, y.) were plotted, and the mean smooth curves accurately drawn.

In the Summary Table below, are given the experimental conditions of the various hydrolyses with the diastereoisomeric glucosides, together with reference to the Tables and Graphs in which the experimental data are detailed, and the corresponding Hydrolysis - Time curves shown.

Summary. Table. 15.

Emulsin Preparate	Weight Emulsin p. 100cc.	d.β-Glucoside Used	Weight Glucoside p. 50cc.	Table	Graph
No. 1.	0·1523 gm	dl.Me.Et.carbinyl.	0·1428 gm	16.a.	A.1.
No. 1.	0·0634 gm	d-Me.n.Hex.··	0·1964 gm	16.b.	A.2.
No. 2.	0·0435 gm	dl.Me.Et.carbinyl	0·0660 gm	16.c.	A.3.
No. 2.	0·2055 gm	dl.Me.Et.carbinyl	0·1324 gm	17.a.	B.1.
No. 2.	0·2280 gm	dl.Me.Et. ··	0·1289 gm	17.b.	B.2.
No. 10.	0·0090 gm	dl.Me.n.Pr. ··	0·0684 gm	18.a.	C.1.
No. 10.	0·0100 gm	dl.Me.n.Pr. ··	0·0682 gm	18.b.	C.2.
No. 2.	0·0393 gm	dl.Me.n.But.··	0·0701 gm	19.a.	D.1.
No. 2.	0·0504 gm	dl.Me.n.But.··	0·0701 gm	19.b.	D.2.
No. 2.	0·0961 gm	dl.Me.n.But.··	0·0708 gm	19.c.	D.3.
No. 10.	0·0066 gm	dl.Me.n.Am. ··	0·0761 gm	20.a.	E.1.
No. 10.	0·0119 gm	dl.Me.n.Am. ··	0·0766 gm	20.b.	E.2.
No. 2.	0·1000 gm	dl.Me.n.Hex.··	0·0818 gm	21.	F.
No. 10.	0·0066 gm	dl.Me.n.Hept··	0·0842 gm	22.	G.
No. 10.	0·0200 gm	dl.Me.n.Oct.··	0·0883 gm	23.	H.
No. 2.	0·1097 gm	dl.β-Me.Butanyl.	0·0717 gm	24.a.	I.1.
No. 2.	0·2031 gm	dl.β-Me.Butanyl.	0·0700 gm	24.b.	I.2.

The detailed tables are shown in the pages immediately following, and the corresponding graphs appended in the pages following Section. F.

Table. 16.

a. Time t. hrs. min.	a. Conctn. Glucoside. y.	b. Time t. hrs. min.	b. Conctn. Glucoside y.	c. Time. t. hrs. min.	c. Conctn. Glucoside y.
0 . 00.	7·84 cc.	0. 00	8·21 cc.	0. 00	7·04 cc.
0 . 4.	7·77	0. 3·5	8·165	0. 4·5	7·01
0 . 26.	7·18	0. 21·5	7·405	0. 23·5	6·975
0 . 47.	6·515	0. 41	6·665	0. 44	6·80
1 . 8	5·975	1. 0	6·06	1. 4	6·57
1 . 30·5	5·375	1. 20	5·56	1. 24·5	6·415
1 . 51·5	4·925	1. 40	5·025	2. 4	6·13
2 . 12·5	4·56	2. 1	4·615	2. 30·5	5·97
2 . 32·5	4·21	2. 21	4·245	3. 7	5·81
2 . 53	3·88	2. 41	4·065	4. 20	5·47
3 . 15	3·675	3. 1	3·86	5. 40	5·27
3 . 36	3·38	3. 23·5	3·685	22. 27·5	3·40
3 . 55·5	3·275	3. 43	3·485	72. 35	2·865
4 . 17	3·05	4. 8·5	3·325		
4 . 37·5	2·845	4. 41·5	3·165		
4 . 58·5	2·72	5. 15	3·005		
5 . 20	2·685	5. 53	2·94		
5 . 42	2·585	6. 24·5	2·82		
6 . 5	2·48	7. 11	2·715		
6 . 28	2·34	7. 58·5	2·665		
6 . 49	2·14	8. 57·5	2·585		
7 . 12	2·115	10. 3·5	2·52		
7 . 33	2·005	27. 10	2·04		
7 . 56	2·015				
8 . 25·5	1·875				
9 . 1	1·81				
9 . 37·5	1·755				
10 . 30	1·725				

See Graph. A.

These cases are cited to show the type of anomalous result that may be obtained with commercial emulsins, or by working with too dilute a concentration of enzyme.

Table. 17.a.

Time t. hrs.	min.	Conctn. Glucoside y
0	00	7·015 cc.
0	6·5	6·765
0	27	5·435
0	48	4·34
1	9·5	3·605
1	51·5	2·675
2	12·5	2·255
2	32·5	2·065
2	52·5	1·79
3	14·5	1·635
3	35	1·475
3	56·5	1·38
4	18	1·225
5	21	0·975
6	6	0·855
6	49	0·757
7	20·5	0·678
8	21	0·610
9	20	0·530
9	44	0·482
∝		0·00

Table. 17.b.

Time t hrs.	min.	Conctn. Glucoside y
0	00	6·845 cc.
0	5·5	6·63
0	27	5·085
0	48·5	4·055
1	8·5	3·33
1	29	2·82
1	50	2·48
2	11	2·082
2	32·5	1·885
2	53	1·693
3	14·5	1·555
3	35	1·40
3	55	1·28
4	40	1·065
5	0	0·935
5	51	0·805
6	12	0·720
6	56	0·63
7	19	0·565
7	43	0·570
8	5	0·535
8	43·5	0·495
∝		0·00

Table. 18.a.

Time. t. hrs.	min.	Conctn. Glucde. y
0	00	7·24 cc.
0	6	7·185
0	24·5	5·86
0	43	4·74
1	2	3·735
1	21	3·105
1	40·5	2·325
1	59	1·825
2	18·5	1·42
2	37·5	1·12
2	57	0·845
3	15·5	0·695
3	36	0·56
3	57	0·415
4	19	0·30
4	41	0·255
5	2·5	0·24
5	27·5	0·21
5	57	0·155
∝		0·00

Table. 18.b.

Time. t. hrs.	min.	Conctn. Glucde. y
0	00	6·985 cc.
0	6	6·935
0	24·5	5·60
0	43	4·515
1	1	3·57
1	19	2·85
1	37·5	2·225
1	55·5	1·695
2	13	1·38
2	30·5	1·035
2	48·5	0·85
3	6·5	0·67
3	24·5	0·54
4	1·5	0·315
4	23·5	0·245
4	48	0·20
5	18·5	0·11
6	0	0·05
7	2·5	0·005
∝		0·00

Table. 19.a.

Time t. hrs.	min.	Conctn. Glucoside y
0	00	6·96 cc.
0	6	6·945
0	27·5	6·16
0	51	5·48
1	14·5	4·895
1	36	4·405
1	58	3·915
2	20	3·535
2	41·5	3·175
3	1·5	2·885
3	24	2·63
3	47	2·365
4	16·5	2·015
5	19·5	1·59
5	51·5	1·35
6	51	1·065
7	41	0·88
8	43	0·685
9	47	0·595
10	43·5	0·515
∝		0·00

Table. 19.b.

Time t. hrs.	min.	Conctn. Glucoside y
0	00	7·08 cc.
0	6	6·90
0	28·5	5·77
0	49·5	4·93
1	11	4·185
1	32	3·535
1	53	2·99
2	14	2·60
2	35	2·165
2	59·5	1·775
3	24	1·48
3	46	1·275
4	16	1·015
5	5	0·74
5	36	0·555
6	16	0·43
6	59	0·345
7	40·5	0·235
9	35·5	0·105
∝		0·00

Table. 19.c.

Time t hrs.	min.	Conctn. Glucoside y
0	00	7·18 cc.
0	5·5	6·515
0	26	4·325
0	46·5	2·875
1	6·5	1·935
1	28	1·215
1	49·5	0·78
2	10·5	0·54
2	32	0·30
2	54	0·22
3	16	0·145
3	37	0·06
3	58	0·015
∝		0·00

Table. 20.a.

Time. t. hrs.	min.	Conctn. Glucde. y
0	00	6·89 cc.
0	6	6·605
0	24	5·195
0	42·5	4·190
1	1	3·485
1	19	3·055
1	38	2·69
1	56·5	2·435
2	15·5	2·17
2	33	2·100
2	51·5	1·945
3	10·5	1·93
3	34	1·71
3	55	1·675
4	20	1·57
4	49	1·43
5	18	1·28
5	48	1·23
6	20·5	1·115
6	56·5	1·015
∝		0·00

Table. 20.b.

Time. t. hrs.	min.	Conctn. Glucde. y
0	00	7·175 cc.
0	4·5	6·30
0	14·5	4·585
0	24	3·75
0	33·5	3·03
0	44	2·56
0	54·5	2·175
1	5·5	1·98
1	24	1·72
1	42·5	1·51
2	3	1·325
2	21	1·235
2	39·5	1·095
2	59·5	0·935
3	42	0·74
4	13	0·68
4	49	0·51
5	26	0·425
6	14·5	0·335
7	24	0·32
∝		0·00

Table. 21.

Time t. hrs.	min.	Conctn. Glucoside. y
0	00	6·98cc.
0	5	6·74
0	26	4·795
0	48·5	3·69
1	8·5	3·105
1	30·5	2·76
1	51	2·45
2	12	2·21
2	33	2·00
2	56	1·865
3	47·5	1·515
4	38	1·265
5	11	1·14
6	2	0·96
6	41	0·81
7	59	0·625
8	45	0·56
9	37	0·47
∝		0·00

Table. 22.

Time t hrs.	min.	Conctn. Glucoside y
0	00	6·825 cc
0	3·75	6·83
0	22	5·78
0	40·5	5·08
0	58·75	4·46
1	17·25	3·935
1	34·5	3·57
1	52·5	3·16
2	12	2·865
2	30·5	2·605
2	52	2·425
3	12·5	2·115
3	32	2·025
3	54	1·865
4	21	1·765
4	49	1·61
5	23	1·55
5	59·5	1·35
6	40	1·32
7	21	1·18
8	5	1·14
∝		0·00

Table. 23.

Time t hrs.	min.	Conctn. Glucoside y
0	00	6·89 cc.
0	4	6·865
0	14·75	5·46
0	22·5	4·713
0	33	3·70
0	43·5	2·95
0	54·5	2·485
1	6·5	1·918
1	23·5	1·415
1	41	1·265
1	59·5	0·95
2	17·5	0·772
2	35·5	0·638
2	53	0·565
3	18	0·52
4	4	0·305
4	24	0·33
5	6	0·16
∝		0·00

Table. 24.a.

Time. t. hrs.	min.	Conctn. Glucde. y
0	00	7·08 cc.
0	12	6·98
0	31	6·63
0	48	6·23
1	6	5·93
1	24	5·58
1	42	5·055
2	0	4·88
2	19	4·355
2	37	4·08
2	56	3·905
3	14	3·53
3	32	3·355
3	52	3·155
4	9	2·88
4	29	2·73
5	10	2·255
6	1	1·705
7	0	1·43
8	13	0·93
∝		0·00

Table. 24.b.

Time. t. hrs.	min.	Conctn. Glucde. y
0	00	7·39 cc.
0	5	7·08
0	25	6·28
0	44·8	5·46
1	5	4·735
1	25	4·065
1	44	3·525
2	4	3·09
2	24	2·705
2	44	2·375
3	3·5	2·105
3	28·8	1·74

For the data of Table. 24.a., I am indebted to Mr. Ian Sinclair B.Sc.

D. ANALYTICAL. 1.

1. General Features of Hydrolysis-Time Curves.

Consideration of the tables and corresponding curves, indicates the following -

The hydrolysis-curves for the diastereoisomers consist of two stages.

(a). A short linear period of induction. This is of the order of 2 - 5 minutes, and broadly speaking, independent of glucoside or emulsin specimen, is inversely proportional to the initial speed of hydrolysis, and conversely, proportional to the ratio of substrate to enzyme.

(b). A smooth curve indicating hydrolysis at a continuously decreasing absolute rate.

Some noteworthy anomalous features are seen in Graph.A., Table. 16. 1. Graph A.1. shows the action of an old commercial specimen of emulsin on dl.sec.butyl.glucoside. The series of sinuosities superposed on the curve in its later stages is very marked. These have been observed only with dl.glucosides, are an indication that the emulsin is nearing the end of its useful life for kinetic measurements, and may be removed by a single purification of the enzyme by WILLSTÄTTER's method. The effect is probably due to decomposition products of the emulsin affecting the normal equilibrium of the enzyme between its two substrates.

2. Graph A.2. shows the action of the same No.1. preparate on the d.sec.octyl member. The reaction is not monomolecular, and complete inhibition is reached after 80-90 % hydrolysis, but

no irregularities are observed. After purification, the enzyme, again tested, gave the required exponential curve.

3, Graph A.3. shows that, even when the emulsin specimen is satisfactory, a certain minimum enzyme concentration must be reached or the rate falls off very rapidly.

2. Analysis of Curves, Tables 17-24, Graphs B - I.

1. dl.Me.n.Hexylcarbinyl.d.β-glucoside. Table.21. Graph.F.

The validity of the method was first tested on this curve. The method of exponential analysis already described, was applied assuming the presence of two exponential components, i.e. that the curve has the equation -

$$y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t}$$

where a_1, a_2, k_1, k_2 are constants, t is time, and y is concentration of glucoside.

P, the initial point of the four, from which zero time was calculated, was chosen some little distance from the beginning of the curve to avoid possible initial irregularities. For the equations corresponding to different sets of points, the k values should be identical, but the a values depend on the initial point chosen. These also may be compared by extrapolation or interpolation to a common point. To eliminate fortuitous agreement of equation and experimental curve, the criterion was adopted of fair agreement in the actual values of k_1 and k_2 obtained from various analyses carried out from different sets of four points, with different time δ intervals, and covering different (large)

portions of the curve, before that equation agreeing best with the curve over its whole range was used as a basis for a further correction.

The preliminary analyses were found in all cases to be concordant unless otherwise mentioned.

From various equations giving a ratio for k_2/k_1 of 7·5 - 9·2, the four points (t , y) , (0 , 4·795) , (2·5 , 1·845) , (5 , 1·078) , (7·5 , 0·640) gave the equation -

$$y = 3{\cdot}05 \cdot e^{-0{\cdot}208\,t} + 1{\cdot}74 \cdot e^{-1{\cdot}610\,t}$$

which was used for the probability correction already described.

From the observed reliable experimental points (t , y), this equation gave the following 'equations of condition'

+3·054 β_1	+ 1·741 β_2	- 0·00 λ_1	- 0·00 λ_2	=	-0·00
+2·825	+ 0·952	- 0·221	- 0·575	=	-0·087
+2·635	+ 0·557	- 0·389	- 0·635	=	-0·087
+2·441	+ 0·308	- 0·547	- 0·534	=	+0·011
+2·274	+ 0·178	- 0·671	- 0·406	=	-0·002
+2·113	+ 0·101	- 0·778	- 0·288	=	-0·004
+1·964	+ 0·058	- 0·867	- 0·196	=	-0·022
+1·814	+ 0·031	- 0·945	- 0·125	=	+0·020
+1·517	+ 0·008	- 1·061	- 0·042	=	-0·010
+1·273	+ 0·002	- 1·114	- 0·014	=	-0·010
+1·135	+ 0·001	- 1·124	- 0·006	=	+0·004
+0·951	+ 0·00	- 1·081	- 0·001	=	+0·009
+0·830		- 1·081		=	-0·020
+0·633		- 0·997		=	-0·008
+0·540		- 0·935		=	+0·020
+0·451		- 0·862		=	+0·019

These gave the following 'normal equations'

+ 54·6916 β_1	+ 11·0296 β_2	- 17·3516 λ_1	- 6·8329 λ_2	= - 0·4829
+ 11·0296	+ 4·3877	- 0·8846	- 1·1813	= - 0·1288
- 17·3516	- 0·8846	+ 11·8405	+ 1·5209	= + 0·0528
- 6·8329	- 1·1813	+ 1·5209	+ 1·3213	= + 0·1032

Yielding $\beta_1 = +0{\cdot}026$, $\beta_2 = -0{\cdot}053$, $\lambda_1 = +0{\cdot}020$, $\lambda_2 = +0{\cdot}142$

and the corresponding best equation for the curve

$$y = 3{\cdot}13 \cdot e^{-0{\cdot}213\,t} + 1{\cdot}65 \cdot e^{-1{\cdot}840\,t} \quad \ldots\ldots(1).$$

This equation gives a ratio of hydrolysis-rates of $k_2/k_1 = 8{\cdot}6$. The value found for the separate isomers by MITCHELL was $k_D/k_L = 8{\cdot}4$. Table.25. shows the extent of agreement, at intervals over the full hydrolysis range, between y from the mean experimental curve of Graph.F., and that calculated from equation .....(1).

Table.25.

Time from P. hours	y.(Mean Curve). cc.Thios.	y. Calcd. cc.Thios.	Difference. cc.Thios.
- 0·1	5·21	5·18	+ 0·03
0	4·79	4·78	+ 0·01
+ 0·5	3·43	3·47	- 0·04
1·0	2·80	2·79	+ 0·01
1·5	2·39	2·39	----
2·0	2·08	2·08	----
2·5	1·85	1·86	- 0·01
3·0	1·64	1·66	- 0·02
4·0	1·32	1·33	- 0·01
5·0	1·07	1·08	- 0·01
6·0	0·87	0·87	----
7·0	0·70	0·70	----
8·0	0·58	0·57	+ 0·01
9·0	0·49	0·46	+ 0·03

The agreement is very satisfactory.

From the result, it would appear that the belief, based on previous theory and experience, that two exponential factors are present is justified. The two glucosides in the mixture, d- and l-Me.n. hexylcarbinyl.d.β-glucoside are hydrolysed at different rates, and the ratio of the reaction constants is in good agreement with that previously obtained for the separate diastereoisomers.

The results for the glucosides of the other racemic alcohols similarly analysed, are detailed below.

2. dl.Me.Et.carbinyl.d.β-glucoside. Table.17. Graph.B.

From 5 curves, some 16 sets of points gave a ratio for k_1 of 4·3 - 6·0. Two typical curves are instanced.

B.1. The point set (t, y), (0, 5·255), (2, 2·045), (4, 1·175), (6, 0·790), gave on analysis the equation -

$$y = 2{\cdot}15 \cdot e^{-0{\cdot}170\ t} + 3{\cdot}10 \cdot e^{-0{\cdot}899\ t}$$

which gave on application of the 'Least Squares' correction -

$$y = 2{\cdot}16 \cdot e^{-0{\cdot}166\ t} + 3{\cdot}08 \cdot e^{-0{\cdot}909\ t}$$

whence $k_2 / k_1 = 5{\cdot}5$.

B.2. The accuracy with which results may be repeated is instanced in this second curve, taken with a different specimen of glucoside.

The points, (0, 4·095), (2, 1·742), (4, 0·995), (6, 0·630) gave the preliminary equation -

$$y = 2{\cdot}291 \cdot e^{-0{\cdot}210\ t} + 2{\cdot}283 \cdot e^{-0{\cdot}924\ t}$$

which, on correction, yielded the final equation -

$$y = 2{\cdot}27 \cdot e^{-0{\cdot}197\ t} + 2{\cdot}34 \cdot e^{-1{\cdot}031\ t}$$

giving $k_2 / k_1 = 5{\cdot}2$. cf. result above.

Calculated and experimental curves agree as shown below.

Table. 26.a. Table. 26.b.

Time from P.	y curve B1. cc.	y Calcd. cc.	Diffce.	y curve B2. cc.	y Calcd. cc.	Diffce.
-0·2 hr.	----	-----	----	5·28	5·24	+0·04
-0·1	6·77	6·74	+0·03	----	----	----
0·00	5·25	5·24	+0·01	4·61	4·61	----
0·5	3·91	3·94	-0·03	3·46	3·46	----
1·0	3·07	3·07	----	2·70	2·70	----
1·5	2·46	2·47	-0·01	2·21	2·19	+0·02
2·0	2·05	2·05	----	1·85	1·83	+0·02
3·0	1·51	1·51	----	1·38	1·37	+0·01
4·0	1·17	1·19	-0·02	1·05	1·07	-0·02
5·0	0·95	0·98	-0·03	0·83	0·86	-0·03
6·0	0·79	0·81	-0·02	0·67	0·70	-0·03
7·0	0·68	0·68	----	0·56	0·57	-0·01
8·0	0·59	0·58	+0·01	0·50	0·47	+0·03

3. dl.Me.n.Pr.carbinyl.d.β-glucoside. Table.18. Graph.C.

Application of exponential analysis to these curves gave in most cases a single large exponential factor, with the second term reduced to a small correction. This anomaly seems due to the fact that, as might have been anticipated, the analysis could not readily distinguish as between approximate equations of the type

$$y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-(k_1 + n_1).t}$$

and $y = (a_1 + a_2 + n_3) \cdot e^{-(k_1 + n_2).t} \pm m.$ (n_1, n_2, n_3, $|m|$ small).

So far as the main object of the curve analysis was concerned, this was immaterial, for in either case, the only conclusion was that $k_2 / k_1 \doteq 1$. This was corroborated by the fact that the mean curves, analysed on the basis of a monomolecular reaction

$$k = \frac{2{\cdot}303}{t_n - t_1} \log. \frac{y_1}{y_n}$$

gave 'constants' deviating from the mean by less than ± 5 %.

The following tables show the values obtained.

Table. 27.a.

t hours.	y cc.	$k' = \frac{k}{4 \cdot 2{\cdot}303}$
0·00	7·185	---
0·25	6·08	0·0725
0·50	5·14	0·0727
0·75	4·315	0·0738
1·0	3·58	0·0756
1·25	2·99	0·0761
1·50	2·48	0·0769
1·75	2·035	0·0782
2·0	1·675	0·0790
2·25	1·375	0·0798
2·50	1·14	0·0799
2·75	0·945	0·0801
3·0	0·785	0·0801
3·5	0·555	0·0794
4·0	0·37	0·0805
4·5	0·275	0·0787

Table. 27.b.

y cc.	$k' = \frac{k}{4. 2{\cdot}303}$
6·935	---
5·80	0·0776
4·84	0·0781
4·055	0·0777
3·36	0·0787
2·765	0·0798
2·255	0·0813
1·82	0·0830
1·47	0·0839
1·195	0·0848
0·975	0·0852
0·805	0·0850
0·66	0·0851
0·45	0·0847
0·305	0·0848
0·21	0·0844

A feature of these curves is that the 'constants' obtained in this way, show a slight continuous rise, until at about 80 % hydrolysis they reach a steady maximum. This indicates that, if the isomeric components are present in equal amount or nearly so, the individuals must be deviating slightly from a monomolecular course. (See Note.2.). This anomaly, and its extent, is considered in detail later with the active butyl members.

4. dl.β-Me.Butanyl.d.β-glucoside. Table. 24. Graph.I.

For comparison with those for the sec.amyl member, the results with the primary amyl compound are here given. Again, the curves analysed only into the form -

$$y = (a_1 + a_2 + n_3) \cdot e^{-(k_1 + n_2).t} \pm m.$$

where n_2, n_3, and $|m|$ are small.

Again analysed as if only a single exponential component were present, curve I.1. gave values of k' rising continuously over a 75 % hydrolysis range from 0·103 to 0·106 , while curve I.2. gave similar constants from 0·179 to 0·186.

Thus for this member, again $k_2 / k_1 \doteq 1$.

Thus the amyl glucosides show general agreement, even to the slight continuous rise in k' , which however, is with the primary member, of very slight extent. The absolute rate for the primary member, referred to a standard concentration of enzyme, is considerably slower than that of the secondary member.

5. dl.Me.n.Butyl.carbinyl.d.β-glucoside. Table.19. Graph.D.

From curve D.2., the four points (0, 5·465), (2, 2·150), (4, 0·895), (6, 0·388) gave the equation -

$$y = 3{\cdot}52 \cdot e^{-0{\cdot}384\ t} + 1{\cdot}95 \cdot e^{-0{\cdot}662\ t}$$

which gave on correction the final equation

$$y = 3{\cdot}49 \cdot e^{-0{\cdot}398\ t} + 1{\cdot}97 \cdot e^{-0{\cdot}602\ t}$$

whence $k_2 / k_1 = 1{\cdot}5$.

The agreement between calculated and experimental values is as shown below.

Table. 28.b.

Time from P.	y curve D.2. cc.	y Calcd. cc.	Diffce.
-0·5 hr.	6·90	6·92	-0·02
0·0	5·43	5·46	-0·03
+0·5	4·33	4·32	+0·01
1·0	3·42	3·42	----
1·5	2·71	2·72	-0·01
2·0	2·15	2·16	-0·01
2·5	1·70	1·73	-0·03
3·0	1·37	1·38	-0·01
4·0	0·89	0·89	----
5·0	0·58	0·58	----
6·0	0·38	0·37	+0·01
7·0	0·25	0·24	+0·01
8·0	0·16	0·16	----
9·0	0·10	0·11	-0·01

Curves D.1. and D.3. and some tests of D.2. gave, as with the amyl members, the alternative type -

$$y = (a_1 + a_2 + n_3) \cdot e^{-(k_1 + n_2).t} \pm m.$$

Hence again, the real ratio k_2 / k_1 must be interpreted as intermediate to 1 / 1 and 1·5 / 1. , and exponential analysis is weak in distinguishing between rates of this order.

As with the amyl members, all three curves, analysed as if only one component were present, gave a series of constants varying only slightly from a mean value.

The ratio-of-rates for the remaining glucosides were rather of the order of those for the sec.octyl and sec.butyl members, and the calculated and experimental results were particularly concordant for the sec.heptyl and sec.nonyl members.

6. dl.Me.n.Amyl.carbinyl.d.β-glucoside. Table.20. Graph.E.

From Graph. E.1., the following set of points (0, 6·135), (1·75, 2·455), (3·5, 1·70), (5·25, 1·285) gave the equation

$$y = 2{\cdot}88 \cdot e^{-0{\cdot}154\ t} + 3{\cdot}26 \cdot e^{-1{\cdot}452\ t}$$

which on correction, gave the final equation -

$$y = 2{\cdot}82 \cdot e^{-0{\cdot}149\ t} + 3{\cdot}33 \cdot e^{-1{\cdot}430\ t}$$

whence $k_2 / k_1 = 9{\cdot}6.$

From Graph. E.2. was similarly obtained the equation -

$$y = 2{\cdot}43 \cdot e^{-0{\cdot}353\ t} + 1{\cdot}57 \cdot e^{-3{\cdot}23\ t}$$

giving $k_2 / k_1 = 9{\cdot}2.$

With both curves, agreement was excellent; the figures for the first curve are shown below.

Table. 29.a.

Time from P	y.curve.	y.calc.	Diffce.
0·00 hr.	6·135cc.	6·15cc.	-0·015cc.
0·25	5·04	5·05	-0·01
0·50	4·26	4·25	+0·01
0·75	3·660	3·66	----
1·0	3·23	3·23	----
1·5	2·64	2·65	-0·01
2·0	2·28	2·28	----
2·5	2·04	2·04	----
3·0	1·85	1·85	----
3·5	1·70	1·70	----
4·0	1·57	1·57	----
5·0	1·34	1·34	----
6·0	1·15	1·15	----
7·0	0·99	0·99	----

7. dlMe.n.Heptylcarbinyl.d.β-glucoside. Table.22.Graph. G.

No correction was carried out, a perfect fit being obtained from the preliminary analysis. The points (0, 6·35), (2, 2·865) (4, 1·79), (6, 1·35) gave the final equation -

$$y = 2{\cdot}17 \cdot e^{-0{\cdot}0876\ t} + 4{\cdot}18 \cdot e^{-0{\cdot}6935\ t}$$

giving $k_2 / k_1 = 7{\cdot}9$.

8. dl.Me.n.Octylcarbinyl.d.β-glucoside. Table.23.Graph. H.

Considerable experimental difficulty was found with this compound and as the data were of lesser accuracy, no correction was applied. The points (0, 5·75), (0·9, 1·95), (1·8, 0·94), (2·7, 0·56). yielded the final equation -

$$y = 1{\cdot}74 \cdot e^{-0{\cdot}440\ t} + 4{\cdot}01 \cdot e^{-1{\cdot}824\ t}$$

whence $k_2 / k_1 = 4{\cdot}2$.

The agreement for both nonyl and decyl glucosides was as shown -

Table.30. Sec.Nonyl.

Time from P. hrs.	y Curve cc.	y calcd. cc.	Diffce cc.
0·00	6·35	6·35	---
0·5	5·03	5·01	-0·02
1·0	4·08	4·08	---
1·5	3·38	3·38	---
2·0	2·865	2·865	---
2·5	2·47	2·47	---
3·0	2·17	2·19	-0·02
3·5	1·95	1·97	-0·02
4·0	1·79	1·79	---
4·5	1·65	1·65	---
5·0	1·54	1·53	+0·01
5·5	1·43	1·43	---
6·0	1·35	1·35	---
6·5	1·28	1·27	+0·01
7·0	1·21	1·21	---
7·5	1·15	1·15	---
8·0	1·10	1·09	+0·01

Table. 31. Sec.Decyl.

Time from P. hrs.	y Curve cc.	y calcd. cc.	Diffce cc.
-0·125	6·80	6·875	-0·075
0·00	5·75	5·75	-----
+0·25	4·15	4·10	+0·05
0·5	3·01	3·01	-----
0·75	2·26	2·27	-0·01
1·0	1·76	1·77	-0·01
1·25	1·42	1·42	-----
1·50	1·16	1·16	-----
1·75	0·97	0·98	-0·01
2·0	0·82	0·83	-0·01
2·5	0·62	0·62	-----
3·0	0·50	0·49	+0·01
4·0	0·305	0·30	+0·005
5·0	0·195	0·195	-----
6·5	0·12	0·10	+0·02

E. EXPERIMENTAL and ANALYTICAL. 2.

A comparative survey of the results for the series is reserved for later discussion, but the analytical detail brought out features that suggested further inquiry. Accordingly, a series of hydrolyses was made with the glucosides of the separate isomers, mainly of the butyl alcohols. The conditions of these supplementary experiments are summarised below.

Summary Table. 32

Emulsin Preparate	Weight Emulsin p. 100cc. gms.	d.β-Glucoside used	Weight Glucde. p.50cc. gms.	Remarks
No. 4.	0·0344	d.Me.n.Hex.carbinyl	0·0816	Tble.33 Gph.J
No. 5.	0·2016	d.Me.Et.carbinyl	0·0638	•• 34 ---
No. 10.	0·0300	••	0·0650	•• •• ---
No. 6.	0·3204	••	0·0630	•• •• ---
No. 9.	0·2416	••	0·0650	•• •• ---
No. 9.	0·1216	Do. + P.Ether.	0·0652	
No. 4.	0·2013	•• No Addition	·0318/25	Tble.35 Gph.K.
No. 4.	Same Sol.	•• + 0·04cc.d.alc.	·0318/25	•• ••
No. 4.	0·1380	d.Me.Et.carbinyl	0·0651	•• 36 •• L.
No. 4.	0·1380	l-dl. ••	0·0651	•• •• •• L.
No. 9.	0·1207	·0315gm.d.+ ·0335gm.l-dl. ••	0·0650	•• 37 •• L.

These experiments were made with three main objects, now discussed.

1. Inhibition by Lower Alcohols.

It had been observed in preliminary analyses of the dl.butyl member, that where the four selected points extended over the full range of hydrolysis, the ratios were most frequently of the order 4·9 - 5·4 , but, where the later portion of the range was

excluded, the ratios were more of the order 4·3 - 4·9. A probable explanation was that in the later stages, a falling off in the monomolecular rates ensued. This has been frequently observed in enzymic glucoside hydrolysis. Conversely, the continuous rise in the constants for the amyl members showed that some anomalous effect must occur, but here in the opposite direction.

Accordingly, d.sec.butyl.d.β-glucoside was prepared and separately hydrolysed.

It was found, that the Hydrolysis-Time curves for this isomer were accurately monomolecular to ± 1 % over the first 70% of the range, but that thereafter a marked falling off in speed ensued, amounting to some 10 - 15 % . This disturbing feature was examined in detail.

(a). In view of the results of Table 16. Graph A. the emulsin was first suspect. Accordingly, the d-butyl glucoside was hydrolysed at various speeds with different emulsin specimens. Table. 34. below shows the constants obtained by analysing three typical curves on the basis of the monomolecular reaction equation -

$$k = \frac{2\cdot303}{t_n - t_1} \log \frac{y_1}{y_n}$$

Table.34.

a.			b.			c.		
t.hrs.	y.cc.	k	t.hrs.	y.cc.	k	t.hrs.	y.cc.	k
0·oo	6·215	----	0·00	6·25	---	0·00	6·665	---
0·5	5·29	0·1398	0·25	5·545	0·0520	0·50	4·83	0·2798
1·0	4·49	0·1412	0·50	4·91	0·0524	1·0	3·495	0·2804
1·5	3·815	0·1412	0·75	4·35	0·0525	1·5	2·50	0·2839
2·0	3·245	0·1411	1·0	3·89	0·0515	2·0	1·808	0·2833x
2·5	2·765	0·1407	1·25	3·465	0·0512	2·5	1·36	0·2761
3·0	2·385	0·1386	1·50	3·09	0·0510	3·0	1·01	0·2732
3·5	2·045	0·1379	1·75	2·74	0·0512	3·5	0·743	0·2722
4·0	1·75	0·1376x	2·0	2·435	0·0512	4·0	0·555	0·2699
4·5	1·515	0·1362	2·25	2·165	0·0512x	4·5	0·427	0·2652
5·0	1·31	0·1352	2·50	1·94	0·0508	5·0	0·35	0·2560
5·5	1·145	0·1336	3·0	1·575	0·0499	6·0	0·23	0·2437
6·0	1·005	0·1319	4·0	1·045	0·0486			
x =75 % Hydrolysis			x =70 % Hydrolysis			x = 75 % Hydrolysis.		

It will be observed that in all cases, the even variation from the mean is ± 1 % up to the point x, marking about 70 % hydrolysis, but that thereafter a slight retardation ensues, independent of preparate used.

(b). In contrast, Table.33. Graph.J. relates to a typical curve obtained with the same specimens from d.sec.octyl.d.β-glucoside. The analysis is based, as above, on the monomolecular equation.

Table.33. Graph.J.

Time t. hrs. min.	Conctn. Glucoside y. cc.	From Point P t.hours.	y. cc.	Monomol.Const.k.
0 00	7·10			
0 5	7·015			
0 23·5	5·45			
0 42·5	4·325	0·0	5·645	-----
1 1	3·43	0·5	3·895	0·3514
1 20.5	2·59	1·0	2·565	0·3324
1 40	1·97	1·5	1·705	0·3444
1 59·5	1·565	2·0	1·145	0·3474
2 19	1·165	2·5	0·765	0·3470
2 39	0·90	3·0	0·515	0·3476
2 59	0·71	3·5	0·345	0·3470
3 18	0·555	4·0	0·230	0·3471
3 42	0·355	4·5	0·150	0·3477
4 1·5	0·295			
4 31	0·22			Mean = 0·346.
5 3	0·135			
5 39	0·05			
6 24	0·06			
7 6	0·02			
∝	0·00			

The uniformity of the constants from the mean curve, over the complete range and particularly in the final stages, is very striking, again shows that the emulsin specimen is not at fault, and confirms the previous results of MITCHELL. Curves of like accuracy have been repeatedly obtained with this glucoside, as criteria of the emulsin specimens used at various times.

Thus the end effect with the butyl member, though small, is

definite, and seemed rather dependent on inhibition by products of hydrolysis.

(c). The possibility of inhibition by the alcohol set free, was tested in two ways. An hydrolysis was made with a top layer of petroleum ether over the liquid in the graduated flask. No change from the phenomena of (a). was observed.

Finally, parallel simultaneous runs with the same emulsin solution and concentration of d-butyl substrate were made, a volume of 0·04 cc.d.sec.butyl alcohol being added to one of the flasks. The results are shown in Table.35. Graph.K.

Table. 35.a. + Alcohol

Time t. hrs.	min.	Conctn. Glucde. y. cc.
0	00	6·895
0	5	6·955
0	44	5·355
1	25	3·955
2	5	2·97
2	46·5	2·25
3	36·5	1·53
4	42	0·975
5	53	0·565
7	11	0·285
∝		0·00

Table. 35.b. Free.

Time. t. hrs.	min.	Conctn. Glucde. y cc.
0	00	6·77
0	25	6·05
1	6	4·41
1	46	3·185
2	27	2·445
3	10	1·68
4	9	1·18
5	16	0·70
6	33·5	0·365
7	57	0·24
∝		0·00

It was found that the initial hydrolysis rate for the glucoside with the added alcohol was slightly but distinctly less than that of the free solution, and also that no further inhibition set in in the later stages. The effect was quite small. Whether the effect is due to an alteration in the dispersity of the colloid, to the setting in of the reverse action even in the dilute solutions employed, or to simple destruction of the enzyme, was not observed. In favour of the last alternative, the destructive effect of C_2H_5OH on emulsin is well known, and has been

established in kinetic measurements on emulsin-hydrolysis of glucosides by JOSEPHSON[117].

These anomalous effects seem confined to the extreme members of the series, for frequent extrapolation outside the range of the point-set chosen for exponential analysis had already shown that -

1. Initial irregularities, if present, are, with all the glucosides tested, very small.

2. End effects do not ensue with the hexyl, heptyl, octyl, and nonyl members.

That sec.octyl alcohol does not inhibit destructively where lower alcohols do so, has also been shown by MURRAY[118], in an interesting test of HALDANE's theory to explain the lateral symmetry of some Activity - pS curves.

In any event, the difficulty is one, not of principle, but of degree of accuracy. The anomaly with the d-butyl member merely restricts, with the dl.butyl glucoside, the effective range of the analysis, or the accuracy of the results. Thus, if an inhibition occurs in the later stages of hydrolysis of a mixture, analysis over the full range will tend to give a maximum value for the ratio k_2 / k_1 .

Table. 37. Graph.L.2.

Time t hrs.	min.	Conctn.Glucde. y. cc.	Time. t. hrs.	min.	Conctn.Glucde. y. cc.
0	00	6·965	3	9	1·055
0	6·5	6·575	3	30	0·920
0	26	4·775	3	53	0·845
0	46	3·580	4	18·5	0·685
1	7·5	2·815	4	46	0·610
1	28·5	2·290	5	41·5	0·435
1	48·5	1·955	6	17	0·405
2	9·5	1·605	7	0	0·355
2	29·5	1·405	7	59	0·280
2	49·5	1·235	∝		0·00

Table.37. Graph.L2. accordingly shows the hydrolysis of a mixture of d- , l-dl., sec.butyl.d.β-glucosides in approximately equal amounts, which, by increasing the amount of the d-member in the mixture, allows of exponential analysis over a lesser range without any loss of accuracy. The equation from analysis was -

$$y = 3{\cdot}36 \cdot e^{-0{\cdot}382\ t} + 3{\cdot}22 \cdot e^{-1{\cdot}725\ t}$$

whence $k_2 / k_1 = 4{\cdot}5$.

Thus the more accurate figure, 4·5 is still in fair agreement with the values already obtained, 5·2 and 5·5 from analysis taken over a more complete range.

2. Determination of More Quickly Hydrolysed Component.

The results of exponential analysis for the series of dl. alkyl.glucosides suggested the query - Whether between the butyl and octyl members of the series there is a minimum value for -

$$\frac{\text{Rate of Hydrolysis of d.alkyl.component}}{\text{Rate of Hydrolysis of l.alkyl.component}}$$

or, a passage through the unit ratio of the amyl and hexyl members to a quicker hydrolysis of the l-isomer in the case of the butyl member? During the work on the d-butyl component, it had become evident that the latter alternative was the correct one.

From Table 36. Graph.L.1,3. taken for d- , l-dl., butyl glucosides, in equal substrate concentrations, and with identical weights of the same enzyme preparate, the quicker hydrolysis rate of the l-component is evident. While curve L.3. was not analysed owing to the low concentration of the d-component, it was clear from the initial speeds, that the ratio k_l / k_d is of the order

4 - 5 , again confirming previous conclusions.

Table. 36. Graph.L.1.

Time t hrs.	min.	Conctn. Glucoside y cc.
0	00	7·21
0	5	7·205
0	24	7·005
0	44	6·325
1	4	5·755
1	24	5·145
1	44	4·80
2	6	4·34
2	32	3·905
2	58	3·565
3	25	3·16
3	53	2·765
4	23	2·48
4	59	2·175
5	48	1·77
6	39	1·505
7	37·5	1·22
8	33	1·01
9	24	0·86
10	15	0·775
11	0	0·69
∝		0·00

Table. 36. Graph.L.3.

Time t hrs.	min.	Conctn. Glucoside. y cc.
0	00	7·21
0	5	7·115
0	24	5·185
0	43·5	3·84
1	4	2·905
1	24	2·31
1	45	1·84
2	6·5	1·625
2	26	1·23
2	46·5	1·125
3	6·5	0·865
3	28·5	0·805
3	54	0·575
4	19	0·60
4	55	0·42
5	37	0·46
6	7	0·31
6	43	0·355
7	24	0·26
8	7	0·245
∝		0·00

As will be shown, from micropolarimetric observations on the higher alcohols, which may be extracted with petroleum ether from the products of enzymic hydrolysis of the corresponding glucosides, it was found that with the dl.sec.heptyl, nonyl, and decyl members, the d-components were in all cases preferentially hydrolysed.

Thus for the series of glucosides, the isomer selectively attacked, and the degree of such stereochemical specificity of emulsin-hydrolysis had now been obtained.

In Graph.M., on the basis of the results of curve analysis, and those of the present Section, the relation is shown between the number of atoms in the alcohol part of the glucoside, and the ratio $k_{\rangle} / k_{\langle}$ or $k_{\langle} / k_{\rangle}$.

Further, on the assumption, which has been found justified in the present work, that absolute hydrolysis rate is proportional to concentration of total enzyme, the curves of Graph.N. were constructed to show the rates of hydrolysis of the separate d-alkyl and l-alkyl.series, referred to a standard concentration of enzyme content measured by activity per unit weight. The detail from which these curves were constructed is derived from -

(a). The detail of Tables 15 and 32.

(b). The corresponding Hydrolysis-Time curves and their analysis.

Most of the data refer to the enzyme specimens Nos. 2 and 10. While strict accuracy is not to be expected, it is believed that the order, at least, of any member is correctly reproduced, and that the graph substantially represents the facts.

3. Chemical Resolution of Racemic Alcohols with d-Glucose.

It has been already pointed out, that in the analytical equation,

$$y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t}$$

a_1 , a_2 represent the concentrations of the respective components at the arbitrarily selected zero time.

If the induction periods, a matter of a few minutes, do not differ much for the two isomers, an indication as to the original amounts of the respective components may be obtained by extrapolation to the end of the induction period.

Ordinarily, an equality of amounts would be expected, i.e. $a_1 = a_2$. While this was found so with the sec.octyl member, it was found that exponential analysis predicted, particularly with the decyl nonyl and butyl glucosides, a considerable excess of the more

quickly hydrolysed component. There were two possible explanations for this apparent inequality.

(a). A preferential formation or separation of one of the component diastereoisomers.

(b). A retarding effect on the hydrolysis, unallowed for, and therefore rendering exponential analysis not directly applicable.

In view of the equality of amounts of the sec.octyl components in the dl-alkyl mixture, it was at first thought that only the latter alternative was correct, but further investigation brought out the following facts.-

1. The curves for the nonyl and decyl members gave the same unequal initial amounts associated always with the same rate.

2. The initial a's of the heptyl curves, though the disparity was less marked, agreed with each other extremely well.

3. The analysis of the curve for the d-.l-dl. sec.butyl glucoside mixture indicated practically equal amounts of d-,l-, types as originally present, though an excess of d-component should have been ordinarily obtained. Hence analysis showed that the 70 % l- 30 % d-alcohol gave more than 70 % of l-butyl.glucoside, a fractionation that was in the same direction as that predicted from the ordinary dl.mixture.

Test of the dl.nonyl and dl.decyl alcohols from which the tetracetyl.glucosides had been formed showed no trace of optical activity, hence preferential formation of one component was excluded. Resolution of the alcohol, either as tetracetyl- , or as simple d.β-glucoside was thus indicated.

Various converging lines of evidence corroborating this conclusion for at least four of the alcohols, and particularly with the

tetracetyl.glucosides are summarised below.

(1). M.P. Data. The M.P's. of tetracetyl.d.sec.butyl.d.β-glucoside, and the corresponding compound from the 70 % l-alcohol were respectively 104 °c. , 120 °C. each after two recrystallisations. The M.P. obtained for the tetracetyl compound prepared from the racemic butyl alcohol varied from 103 °C to 125 °C. A specimen gave the following values for successive recrystallisations from alcohol - 114, 116. 117, 119, 122, and 125 °C. As the product was each time in crystalline rods, as combustion data were satisfactory, and as the M.P. of the d-isomer was steady at 104°C. after a single recrystallisation, these data led to the conclusion - There is a fractional separation from alcohol solution of one component; that component is the l-isomer and preponderates owing to its lesser solubility. Considerable variation was also found with different specimens of the decyl d.β-glucoside, but owing to the number of factors here operative, definite conclusions were not drawn.

(2). Rotation Data. Optical rotations were taken of 2·5 % alc. solutions of tetracetyl-d- , and tetracetyl-l-dl.sec.butylglucosides. d-Isomer. $(\alpha)_{5461} = -15{\cdot}0^{\circ}$, l-dl.isomer. $(\alpha)_{5461} = -40{\cdot}5^{\circ}$.

On the assumption that a fractionating effect takes place, tending to an accumulation of the l-component, it would follow that mixtures with M.P's. intermediate to 104 and 120 °C. would have rotations intermediate to those above, but that a fraction of M.P. over 120 °C. would presumably have a still higher value. This was found. for specimens from the racemic alcohol. Intermediate fractions gave $(\alpha)_{5461} = -27{\cdot}0^{\circ}$, $-35{\cdot}3^{\circ}$.

A fraction of M.P. 125°C. gave $(\alpha)_{5461} = -41{\cdot}3^{\circ}$.

(3). Solubility. Many observations showed the greater solubility in alcohol of the tetracetyl.d.butyl.glucoside. The following may be instanced.

1. During rotation experiments, with the fraction of M.P. 125°C. 2·5 % solutions tended to precipitate, whereas 5 % solutions of the d-alkyl component could easily be used.

2. In the preparation of the optically active butyl glucosides, while the ether washings of the silver salts gave no further yield of the tetracetyl-compound with the d-member, the yield from the dl.member was considerable, while with the l-dl.member, the amount of crystals caused precipitation from the filtrate.

(4). Confirmatory Micropolarimetric Observations.

With the d.β-glucosides of the lower alcohols, direct observation, from the hydrolysis of the mixture, as to which diastereoisomer is preferentially attacked, is scarcely feasible except on a large scale. With the higher homologues, however, owing to the increasing insolubility of the liberated alcohols, this has been obtained as follows -

Hydrolysis was carried through in the usual way, but with double the concentration of substrate. The action was stopped at an intermediate stage, usually at about 60 % hydrolysis, by immersion in ice, and the liberated alcohol extracted twice with 1 cc. petroleum ether, in which glucose and the glucosides, particularly with the competition of a large water content, are practically insoluble. The upper layer (A), drawn off and dried, was then polarimetrically examined. The graduated flask was then replaced on the thermostat, the hydrolysis taken to

completion, and the second fraction (B) of alcohol similarly separated and examined. The extent of hydrolysis reached at the stopping points was determined by the estimation of the glucose content in 1 cc. withdrawn. Finally the alcohol set free in a complete uninterrupted 100 % hydrolysis was similarly extracted and tested (C). By the use of a micro-tube of length 12 cm. and volume 1·2 cc. it was estimated that if the results were at all in accordance with the predictions of exponential analysis, absolute readings of the order of 0·20° were to be expected, and the sign at least should be unmistakable.

The following cases cover the various possibilities.

1. Extraction of alcohol complete.

a. Where the β-glucoside components were originally present in equal amounts in the mixture, the sign of rotation of (A) should indicate which type is the more quickly hydrolysed, (B) should give an equal rotation of opposite sign independent of the stage where hydrolysis was interrupted, and (C) should give no rotation.

b. If the diastereoisomers were originally in unequal amount, the sign of rotation of (A) should still indicate the component preferentially hydrolysed - except only where there is a very large preponderance of the slower component - while (B) is determined, in amount and sign, by the stage reached when the first extract was removed. The extract from complete hydrolysis (C) should indicate by its rotation, in kind and degree, the component originally in excess.

2. Incomplete extraction of alcohol.

The same deductions as before should hold for (A)

and for (C), but the rotation for (B) should be lowered in value - a and b - or even changed in sign - b only.

The following data were experimentally obtained.

Table. 38.

Sec.Hept.Glucde. Rotations.	Sec.Nonyl Glucoside Rotations	Sec.Nonyl Glucoside Rotations	Sec.Dec.Glucde. Rotations
(A).to 61 % Hydr. $\alpha = +0{\cdot}05^{\circ}$	(A).to 60% Hydr. $\alpha = +0{\cdot}285^{\circ}$	(A).to 66% Hydr. $\alpha = +0{\cdot}34^{\circ}$	(A).to 75% Hyd. $\alpha = +0{\cdot}235^{\circ}$
(B).61-100% H. $\alpha = +0{\cdot}01^{\circ}$?	(B).60-100% H. $\alpha = -0{\cdot}07^{\circ}$	(B).66-100% H. $\alpha = -0{\cdot}125^{\circ}$	(B). Not. Examined.
(C).100% Hydrol. $\alpha = +0{\cdot}025^{\circ}$	(C).100% Hydrol. $\alpha = +0{\cdot}11^{\circ}$	(C).100% Hydr. $\alpha = +0{\cdot}125^{\circ}$	(C).100% Hydr. $\alpha = +0{\cdot}165^{\circ}$

Owing to its comparative solubility in water, the results with the liberated heptyl alcohol belong to class 2. above, while those for the nonyl and decyl members give a fair approximation rather to class 1.b. The absence of glucose,(the only other substance of positive rotation in the aqueous solution), in the petroleum ether extract was proved by subsequent trial by the micro-method of MACLEAN. Glucose was never present to the extent of 0·01 mgm.

Consideration of these data in the light of the cases discussed, gave the following important conclusions -

With the heptyl, nonyl and decyl members, as with the sec. octyl glucosides, the d-alkyl component is also the more quickly hydrolysed.

The diastereoisomers, at least with the nonyl and decyl members, are not present in equal quantity in the mixture.

The excess in all cases is that of the more quickly hydrolysed component.

It is interesting, that the data for the nonyl member, for which two determinations were made, are even of some quantitative significance, for they consist remarkably well with the values to be expected by calculation from the equation of page.79.

Thus the resolutions predicted by the results of exponential analysis are consistently confirmed.

A large scale attempted resolution of dl.sec.butyl alcohol by fractional crystallisation of the tetracetyl.d.β-glucoside from alcohol was in progress. In view of the recent work of NEUBERG[119], however, of which we were unaware until very recently, the general principle of this method has now been well established.

But it confirms the validity of exponential analysis as a research instrument, that curve analysis of the type used should independently predict such an effect, show its extent in this series of homologous glucosides, and show that its magnitude with the nonyl and decyl members is considerable.

F. DISCUSSION OF RESULTS.

1. Scope and Accuracy of Exponential Analysis.

In chemical kinetics, many classes of reactions occur, whose representation involves exponential equations. Such theoretical classes comprise -

1. Two (or more) simultaneous monomolecular reactions.

$$A \xrightarrow{k_1} C_1 \quad , \quad B \xrightarrow{k_2} C_2$$

2. One (or more) consecutive reactions with two (or more) monomolecular intermediate stages.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

1.a. 2.a. Either of the above, with a simultaneous monomolecular side-reaction taking place in all or any of the reactants.

$$\begin{array}{l} \overset{k'}{\uparrow} \\ A \xrightarrow{k_1} C_1 \end{array} \quad , \quad \begin{array}{l} \overset{k''}{\uparrow} \\ B \xrightarrow{k_2} C_2 \end{array}$$

$$\begin{array}{l} \overset{k'}{\uparrow} \\ A \xrightarrow{k_1} B \xrightarrow{k_2} C \end{array}$$

3. Special complicated types.

$$\left.\begin{array}{l} E + A \rightleftharpoons A.E. \\ E + X \rightleftharpoons E.X. \\ E + Y \rightleftharpoons E.Y. \end{array}\right\} \xrightarrow{k_1} X + Y + E.$$

$$\left.\begin{array}{l} E + B \rightleftharpoons E.B. \\ E + X \rightleftharpoons E.X. \\ E + Z \rightleftharpoons E.Z. \end{array}\right\} \xrightarrow{k_2} X + Z + E.$$

4. The reduction of other order types to formal agreement with the above by the 'isolation method' of varying only one constituent, leaving the others in excess.

Where the stages or individuals can be followed or detected

independently, there is no gain in analysing results for the whole, but it frequently happens that the individuals are not separable or conveniently so, or that the analytical methods available have reference to the sum of the constituents or final products. Characteristic examples are the following -

(A). Decomposition of racemates or diastereoisomers.

e.g. dl.alkyl.glucosides $\xrightarrow{\text{acid enzyme}}$ alcohol + glucose

(B). Gentianose $\xrightarrow{\text{invertase}}$ gentiobiose $\xrightarrow{\text{emulsin}}$ glucose

(C). Measurements of all types on radioactive series.

(D) Estimated radicle present in two components in different state of oxidation. e.g. $K_2Mn_2O_8$ oxidation.

(E). Synthesis or hydrolysis of esters of polybasic acids (succinic alkyl esters) or of poly-alcohols (triglycerides).

In such cases, from the Decomposition - Time curves, which should be of the type -

$$y = S.\ a \cdot e^{-kt}$$

the individual effects might be derived by curve-fitting and the determination of the involved parameters to get maximum agreement with the experimental data.

Criticism of exponential analysis of experimental data is possible on two grounds -

a. Legitimacy of Application. b. Accuracy of Results.

It is at once admitted that the method has to be used with caution and that as it is subject to the disabilities common to numerical curve-fitting, no great accuracy is claimed, nor is numerical agreement necessarily a criterion of true representation of the facts. The application by MILLS[120] of a two-component exponential equation to the data of GLADSTONE[121] forms an example of accurate

fitting, yet on the basis of GLADSTONE's equation,

$$A.B. + C.D. \rightleftharpoons A.D. + C.B.$$

the curve is really hyperbolic. Further, in the present work, the method does not directly indicate which rate refers to which isomer.

But, it is rather sweeping to state with HUNTER[122] that - ' Fitting is no guarantee of correctness '. ' No theoretical deductions can be drawn from its use '. ' It is utterly useless as a method of extrapolation '. And, in short, that except as confirmatory evidence, the use of exponentials finds its legitimate sphere in empirical engineering practice.

On the contrary, in the classic paper by HARCOURT and ESSON[123] on the kinetics of the $K_2Mn_2O_8 - H_2SO_4 - (COOH)_2$ reaction, the progressive variation of one condition only gave, under certain conditions curves which fitted accurately equations with one or two components of the type -

$$a \cdot e^{-kt}$$

and which were interpretable from the scheme -

$$\overset{\uparrow \alpha}{A} \xrightarrow{\beta} M \xrightarrow{\delta} B$$

giving, where $y = A + M$, the equation -

$$y = \frac{a}{\alpha+\beta-\delta} \cdot \left[\beta \cdot e^{-\delta t} + (\alpha-\delta) \cdot e^{-(\alpha+\beta)\cdot t} \right]$$

As stated by the authors -

' What we can state with certainty is that the numbers are all satisfied by equations of the forms -

$$y = a_1 \cdot e^{-\alpha_1 x} - a_2 \cdot e^{-\alpha_2 x}$$

$$y = a \cdot e^{-\alpha . x}$$

$$y = a_1 \cdot e^{-\alpha_1 x} + a_2 \cdot e^{-\alpha_2 x}$$

and that successive (successive) sets of numbers obtained by varying one condition progressively, are satisfied by these successive sets of equations. These forms, and the order of their succession are accounted for by an hypothesis for which there is considerable experimental evidence, and it is thus highly probable that the results arrived at in the above discussion give a true account of the progress of the reaction.'

The widest field for application of exponential analysis is in the radioactive series, and the simple method of WALSH was devised with this application in view. The quantity of the n-th disintegration product in a series at time t is of the form -

$$\sum_{r=1}^{n} a_r \cdot e^{-\lambda_r t}$$

While in later work, owing to the methods of isolation, chemically physically or by the great disparity between some consecutive 'half-life periods', exponential analysis was rather confirmatory than determinative, yet in the pioneer work some striking results are also found.

In determinations of the β-ray activity of the products of radon, CURIE and DANNE[124] found the activity-time curves to be accurately expressible as the sum of two or three exponential components according to initial conditions, that the results were consistent on the disintegration theory of RUTHERFORD, and that in particular, from the experimental numbers, the 'half-life periods' were determined as 2·6, 21, 28 minutes for radium A,B, and C respectively. These values are in excellent agreement with the accepted figures of 3, 19·5, and 27 minutes.

Further examples may be cited in the experiments of BRONSON[125] or ANTANOFF[126], or in the work of SODDY and RUSSELL[127], where

for zinc absorption of uranium γ-rays, the equation -

$$\frac{I_T}{I_0} = A \cdot e^{-\lambda_1 T} + B \cdot e^{-\lambda_2 T}$$

gave components corresponding to the hard and soft rays.

Finally it is believed that in the present experiments, the analysis really represents the true facts.

1. That the separate curves are monomolecular, is found directly in two instances, and corroborated by previous experience of β-glucoside hydrolysis, especially the comparable results of MITCHELL.

2. That the diastereoisomeric mixture gives a sum of the two exponential components under the conditions used, has considerable theoretical support, either on the simple theory or on the more rigorous extension of the MICHAELIS equation.

3. Direct experimental confirmation of the reality of the interpretation is obtained with the octyl and butyl members.

4. The analysis, as required, generally fulfils the conditions that the indices must be -ve, and the coefficients +ve. In the exceptional cases, as might have been anticipated from experimental data, all occur where the simplest interpretation is that discrimination is difficult as between the equations -

(1). $y = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-(k_1 + n_1).t}$

(2). $y = (a_1 + a_2 + n_3) \cdot e^{-(k_1 + n_2).t} \pm m.$ $(n_1, n_2, n_3, |m| \text{ small})$.

In any event, the results from either form, so far as the main object of the analysis is concerned, are identical, as with either , $k_2 / k_1 \doteq 1$.

5. Internal evidence as to the reality of the exponential com-

-ponents is seen in the agreement of the preliminary analyses from various sets of points taken over different ranges of the curve, and in the possibility of extrapolating successfully even in the early stages of the reaction. In particular, with the butyl member, where systematic differences in k_2 / k_1, though slight, were found according to the range covered, the probable cause, a slowing of at least one component speed, was verified later on resolution, and analysis of a mixture composed to give no loss in accuracy if analysed over a lesser range, corroborated the slightly lower figure obtained originally by excluding the final stages of hydrolysis.

6. Finally the results from the other pair of constants a_1 and a_2 showed a divergence from the 50 - 50 % mixture to be ordinarily expected. These divergences have been consistently confirmed, in type and with the nonyl.glucoside even in degree, by various converging lines of evidence. Thus exponential analysis predicted the partial resolution of racemic alcohols with d-glucose, the principles of which, further confirming the present analysis, have been in the interim fully established by NEUBERG, even to one of the glucosides here used.

Objection on the score of accuracy is of more account. In the present work, the precautions taken to ensure this a maximum, have been -

a. The use of an accurate analytical micro-method of estimation.

b. The use of very dilute solutions.

c. The duplication of curves, and the taking of many readings over an extended peribd and full hydrolysis range, to give an accurate mean curve.

d. The application of a probability correction.

The accuracy attained may best be seen from consideration of the actual data and experimental curves. The best conditions seem to be those giving an equality of the two components, with a rate-ratio of a moderately high order.

In any event, in view of the number of reactions which may be made to follow a monomolecular course, and in view of the recent extension of work on stereochemical enzymic specificity, it is an advantage , where micro-polarimetric technique is difficult, to have a method which, as with emulsin-hydrolysis of dl.alkyl.d.β-glucosides, detects at least broad differences in optical specificity, which avoids the time and cost entailed in resolution, and which allows of the use of micro-technique quite independent of the magnitudes of the specific rotations involved.

2. The Optical Specificity of Emulsin.

The optical selectivity of the β-glucosidase of emulsin, apart from its distinction from maltase, was shown by FISCHER and BERGMANN. In FISCHER's last paper, the following results are summarised with many others.

d.Mandelonitrile.d.β-glucoside.	90 %	Hydrolysis in	24 hours.
l. " "	86 %	"	" 22 "
d.Mandelamide.d.β-glucoside	Nil	"	" 48 "
l. " "	100 %	"	" 20 "

The selective action with the amides, contrasts strongly with the symmetric action on the nitrile diastereoisomers.

Further, emulsin-hydrolysis of the d.β-glucoside of Na - dl.mandelate, gave free acid with $(\alpha)_D$ = -136° , though the glucosides of the pure acids gave no hydrolysis at all.

Later, kinetic measurements were made by MITCHELL on the separate d-,l-bornyl and d-,l-sec.octyl.d.β-glucosides, and differences again observed in the rates of hydrolysis.

Apart from the present work, some results by NEUBERG on dl.bornyl.d.β-glucoside confirm the results of MITCHELL with the separate isomers, agree with the present results on the sec.amyl member, and show a considerable difference in hydrolysis of the components of dl.menthyl.d.β-glucoside. The stereochemical specificity of the enzyme, with special reference to the non-glucosidic radicle, is quantitatively shown in the table below.

Table. 39.

Substrate. d.β-Glucoside of	Ratio of Hydrolysis Rates	Residue Type pref. hydrolysed	Reference.
dl.Me.Et.carbinol.	5·3	l.	Present Work
d-l.dl.Me.Et.carbinol.	4·5	l.	" "
dl.Me.n.Pr. "	c.1·0	---	" "
dl." " "	c.1·15	l.	NEUBERG
dl." n.Butyl "	1·3	Not Exd.	Present Work
dl.Me.n.Amyl "	9·4	d.	" "
d-,l-Me.n.Hexyl "	8·4	d.	MITCHELL
dl.Me.n.Hexyl "	8·6	d.	Present Work
dl.Me.n.Heptyl "	7·9	d.	" "
dl.Me.n.Octyl "	4·2	d.	" "
dl.β-Me.Butanol	c.1·0	---	" "
d-,l-Borneol	3·4	l.	MITCHELL
dl.Borneol	c.3·2	l.	NEUBERG
dl.Menthol	c.5·0	d.	"
d-,l-Mandelonitrile	c.1·0	---	FISCHER
d-,l-Mandelamide	Very Large	l.	"
Na.dl.Mandelate	>15·0	l.	"

From the results summarised in the above table, and from

the corresponding Graphs M.,N. a general survey of the effect of a growing alkyl chain can now be made. It is at once obvious that this is marked, not only in the absolute rates of hydrolysis for the separate d-alkyl and l-alkyl series, but also in the rate-ratios.

For the curves showing the speeds of the separate d-,l-series referred to a standard enzyme content, it is seen that the d-series curve, though with the greater range, is the more regular, rising uniformly to a maximum speed for the sec.heptyl member, and then decreasing in the higher members. The l-series curve again shows a steady decline as from the heptyl member, but the earlier speeds are comparatively high and erratic.

The ratios for the rates of hydrolysis give three types.

(a). Approximately 1/1. Amyl (Prim. and Sec.) and Sec.Hexyl.

(b). " 9/1. Sec.Heptyl, Octyl, and Nonyl members.

(c). " 4·5/1. Sec.Butyl and Decyl members.

It is evident that by emulsin-hydrolysis of the d.β-glucosides a considerable biochemical resolution of the racemic alcohols is possible with the (b). series.

No simple numerical relation correlating the rate-ratios with the number of carbon atoms in the alkyl chain, is obvious.

Of particular interest is the fact that not always is the d-component the more quickly hydrolysed. It would appear that the inversion of sign for the more quickly hydrolysed component occurs between the amyl and hexyl members of the series. For the adjacent butyl and heptyl members, the rate-ratios are large and while the heptyl, like the higher members, shows preferential

hydrolysis of the d-component, in the butyl and slightly in the amyl member (NEUBERG) the l-component is favored. Owing to the small amount of glucoside available, the difficulties of separating the liberated alcohol, and the small - if any - difference in the rates of hydrolysis of the isomers, no sign determination was made for the sec.hexyl member.

Sign inversions of the present type are not frequently met with in selective enzyme action. The ethyl, amyl, and capryl dl.lactates of MURACHI were all symmetrically hydrolysed, and the homologous alcohols of the present series were found by LE BEL to have invariably the d-isomer preferentially attacked by fungi. But it is noteworthy that an examination of the results of COHEN with esterases on racemic substituted acetic acid esters, shows that by replacing a methyl by an ethyl group, an asymmetric hydrolysis was converted to a symmetric one, while a similar replacement in sec.amyl alcohol yielded with 'Pen.glauc.' an inversion of sign, with LE BEL.

For comparison, it is hoped to carry out a parallel investigation on the α-glucosides of the above series of racemic alcohols with maltase. This enzyme exhibits a specificity even more marked than that of emulsin, and owing to the monomolecular course of α-glucoside hydrolysis by maltase, it is believed that the same analytical method would apply.

The alkyl chain has been studied from two aspects -

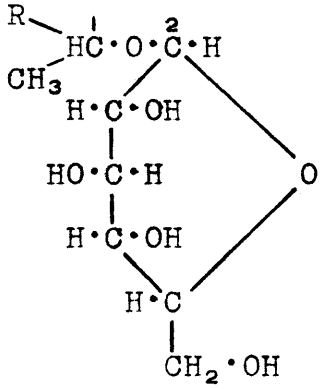
1. The X-ray investigations of MÜLLER and SHEARER.
2. The spiral theory based on the angle of 109° between the natural valency directions of the carbon atom.

If a tentative explanation of the results of the present work may

be suggested without reference to modern enzyme conceptions, it would seem to lie on the lines of 2. above.

In a summary of the evidence on the 'anomalous' effects shown by certain members of a series of compounds with long carbon chains, it was emphasised by PICKARD KENYON and HUNTER[128] that such members may be associated with the possession of a chain of 5 or 6 (or additional multiples of 5) carbon atoms, counted from the atom of a branch chain, or from the longest chain in the compound. In particular, the effect was well illustrated in optical rotation data for the present series of alcohols.

If we apply this in the present case, the carbon atoms marked 1 , 2 , are the significant members. For R = n.amyl, we get the completion of one spiral up to the first branch-chain asymmetric C_1 atom. The corresponding glucoside is the heptyl member, which in the d-series



separates the rising and falling parts of the speed curve, and in the l-series separates the higher erratic speeds of the earlier members and forms the first of the later group with low and more uniform rates of hydrolysis.

Counted from the C_2 atom, R = n.Propyl in sec.amyl glucoside gives almost one complete spiral turn, and brings the terminal C atom of the alkyl chain into the region of the glucose residue which has greatest effect in conditioning the stereochemical specificity of emulsin. R = n.Pr. or n.But. gives a spiral turn slightly under- or over-completed - with the corresponding

amyl and hexyl glucosides, the hydrolysis-rates of the components are practically equal and probably there is a change-over in the sign of the more quickly hydrolysed component (assuming the hexyl member is akin to the heptyl one). For the adjacent butyl and heptyl members, the spiral is by one CH_2 group the less or more complete respectively, and we get rate-ratios again of opposite sign, but of greater magnitude. This also accounts for the 1/1 ratio for the primary amyl glucoside.

It is interesting that only with the amyl members, where the spiral turn to C_2 is not quite complete, was an accelerating effect found superposed on the normal monomolecular rate. It has been observed by MURRAY that considerable differences of enzyme affinity existed between chain and ring compounds, and it may be that the completion of a spiral turn gives a lowering of optical selectivity, which, however, is again strongly marked, when, in the region of the C_2 atom, an additional CH_2 group is added or withdrawn. It is perhaps significant, that the decyl member, in which a second turn of the spiral from C_2 is near completion, again shows a lower rate-ratio. Similar anomalous effects of amyl chains in enzymic esterification of geometric isomers with a series of homologous alcohols are seen in the work of FABISCH. In every case, the rate for the amyl member was always a sharp maximum, or a sharp minimum.

But it is well to remember that on modern conceptions of complex formation, the rate-ratios above discussed would prove a figure compounded of four fundamental constants, and direct comparison would therefore be misleading. It has been shown by KUHN and SOBOTKA[129] that although β-phenyl.glucoside is hydrolysed

eight times more quickly than β-methyl.glucoside, yet a study of activity- pS curves shows that the difference may be interpreted, independent of enzyme preparate, as solely due to a difference in the dissociation constants of the enzyme-complexes, the fundamental rates of decomposition of these complexes being still equal. This result, however, is not borne out for the corresponding α-glucoside hydrolysis with maltase.

It is intended to determine activity - pS curves for the d-sec.butyl. and d-sec.octyl.glucosides of the present series. The values for K_{D_1}, K_{D_2}, k_{D_1}, k_{D_2} so deduced, should indicate whether there is to be obtained a more regular and fundamental interpretation of the effect of the alkyl chain on the stereochemical specificity of emulsin.

NOTES.

1. This figure is corroborated by Dr Buchanan (private communication) , who found 162 °C for a brucine salt yielding an alcohol giving $\alpha_{5461} = +12{\cdot}9^{\circ}$. (1 dm.). The figure given by Pickard and Kenyon is 155 °C.

2. It is sufficient to show that

$$x = a_1 \cdot e^{-k_1 t} + a_2 \cdot e^{-k_2 t} - (a_1 + a_2) \cdot e^{-\frac{a_1 k_1 + a_2 k_2}{a_1 + a_2} t}$$

cannot be negative for all values of t.

Case.A. $k_1 = k_2$. $x = 0$

Case.B. $a_1 = a_2$. and $k_2 > k_1 = k_1 + n$

Then,

$$x = a_1 \cdot e^{-k_1 t} + a_1 \cdot e^{-(k_1 + n)t} - 2a_1 \cdot e^{-\frac{2k_1 + n}{2} t}$$

$$= a_1 \cdot e^{-k_1 t} \left(1 - e^{-\frac{n}{2} t} \right)^2 = + \text{ve}.$$

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My indebtedness to Mr. I. Sinclair has already been mentioned.

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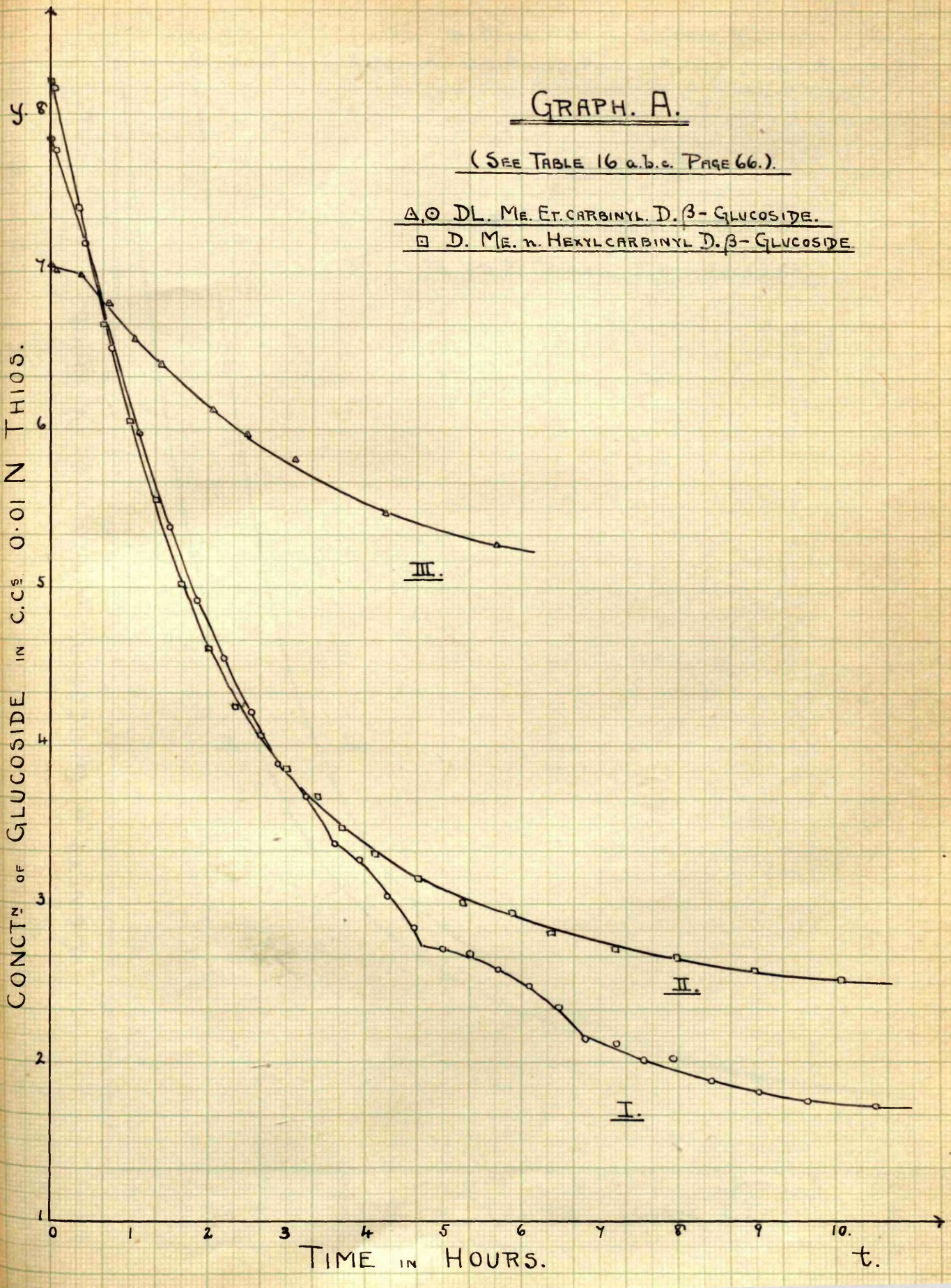
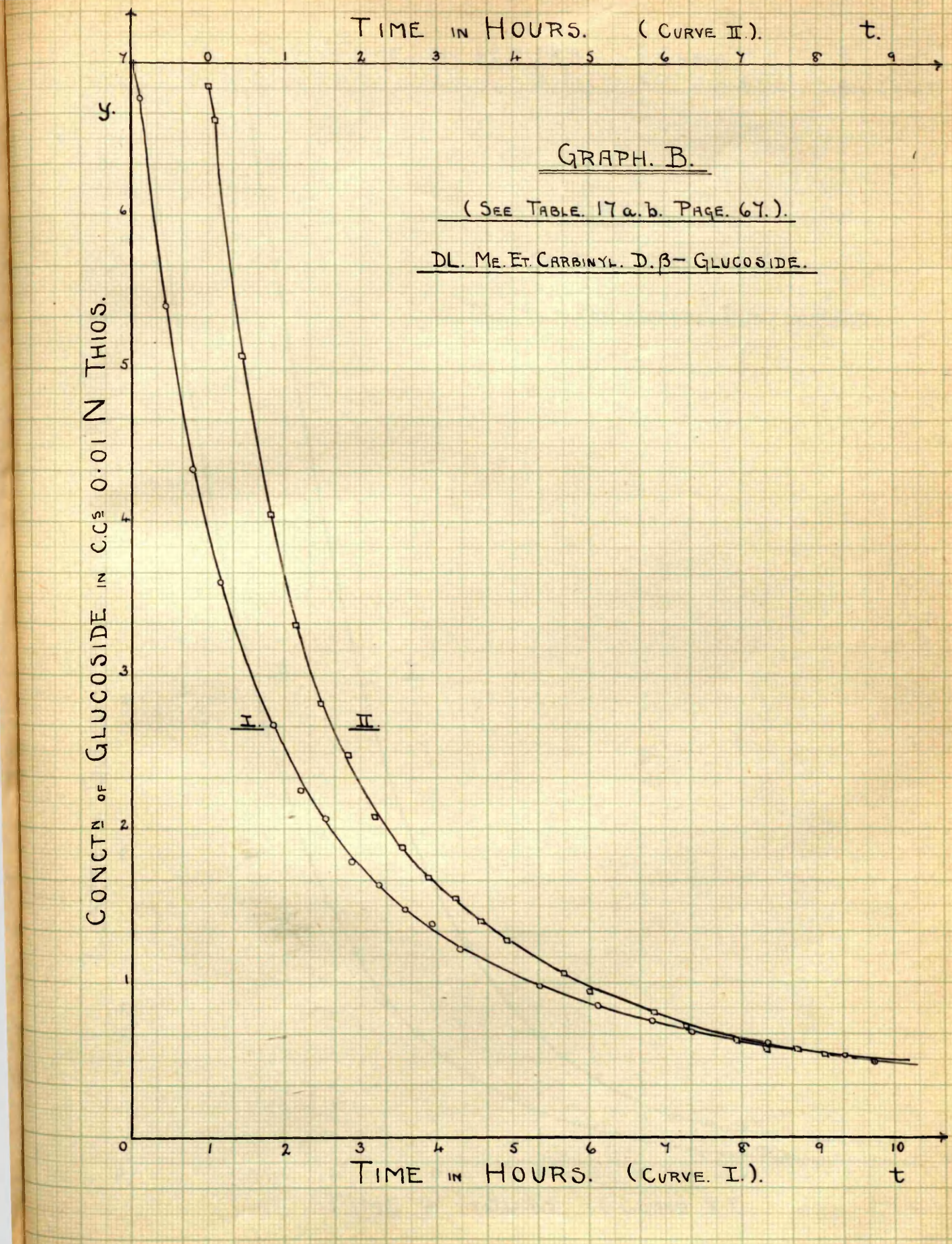
GRAPH. A.

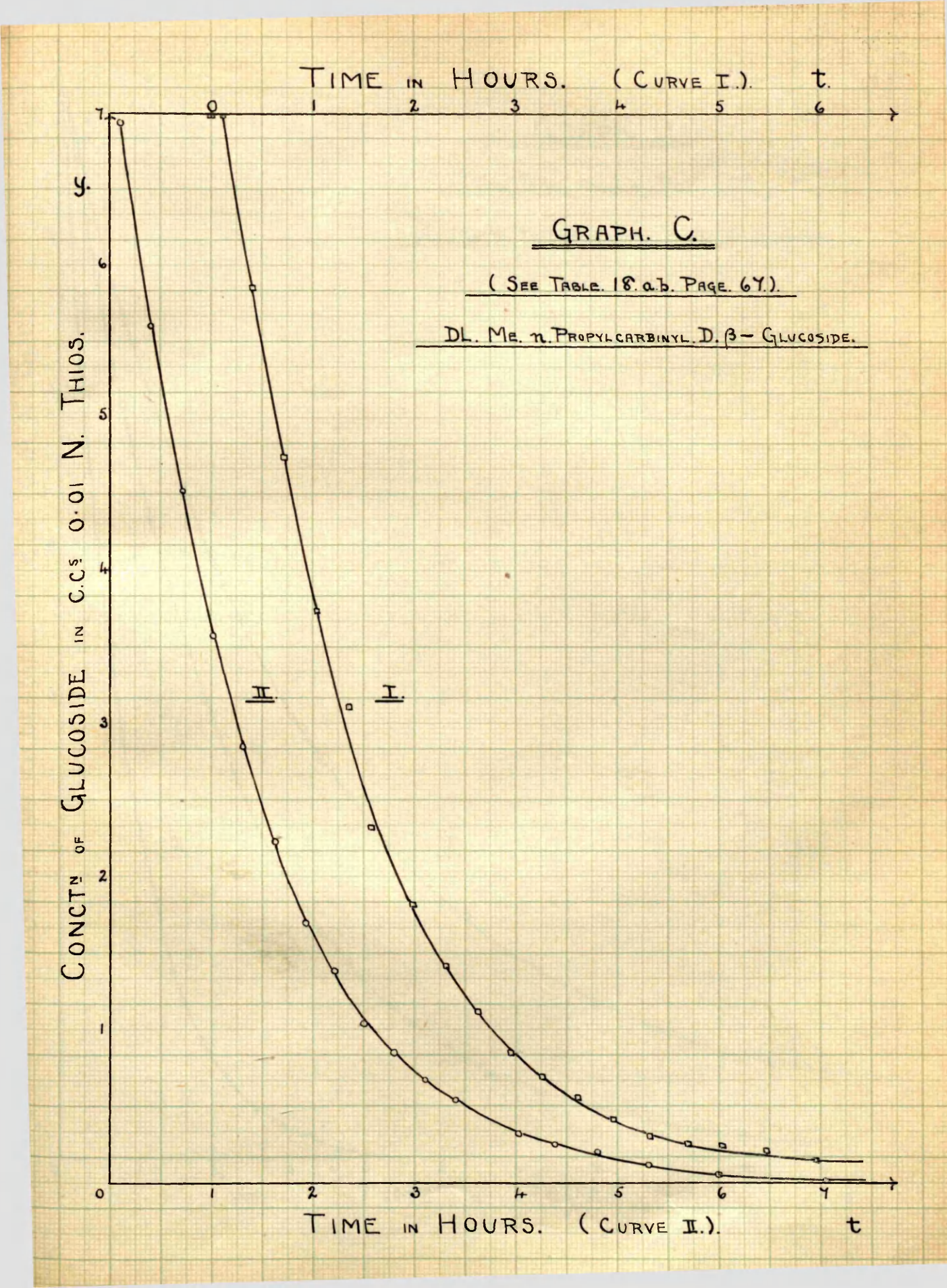
(See Table 16 a.b.c. Page 66.).

△,○ DL. Me. Et. carbinyl. D. β-Glucoside.

⊡ D. Me. n. Hexylcarbinyl D. β-Glucoside.




TIME IN HOURS. (CURVE II.) t.
y.
GRAPH. B.
(SEE TABLE. 17 a. b. PAGE. 67.).
DL. ME. ET. CARBINYL. D. β- GLUCOSIDE.
CONCTN OF GLUCOSIDE IN C.Cs 0·01 N THIOS.
I.
II.
TIME IN HOURS. (CURVE. I.). t



GRAPH. C.

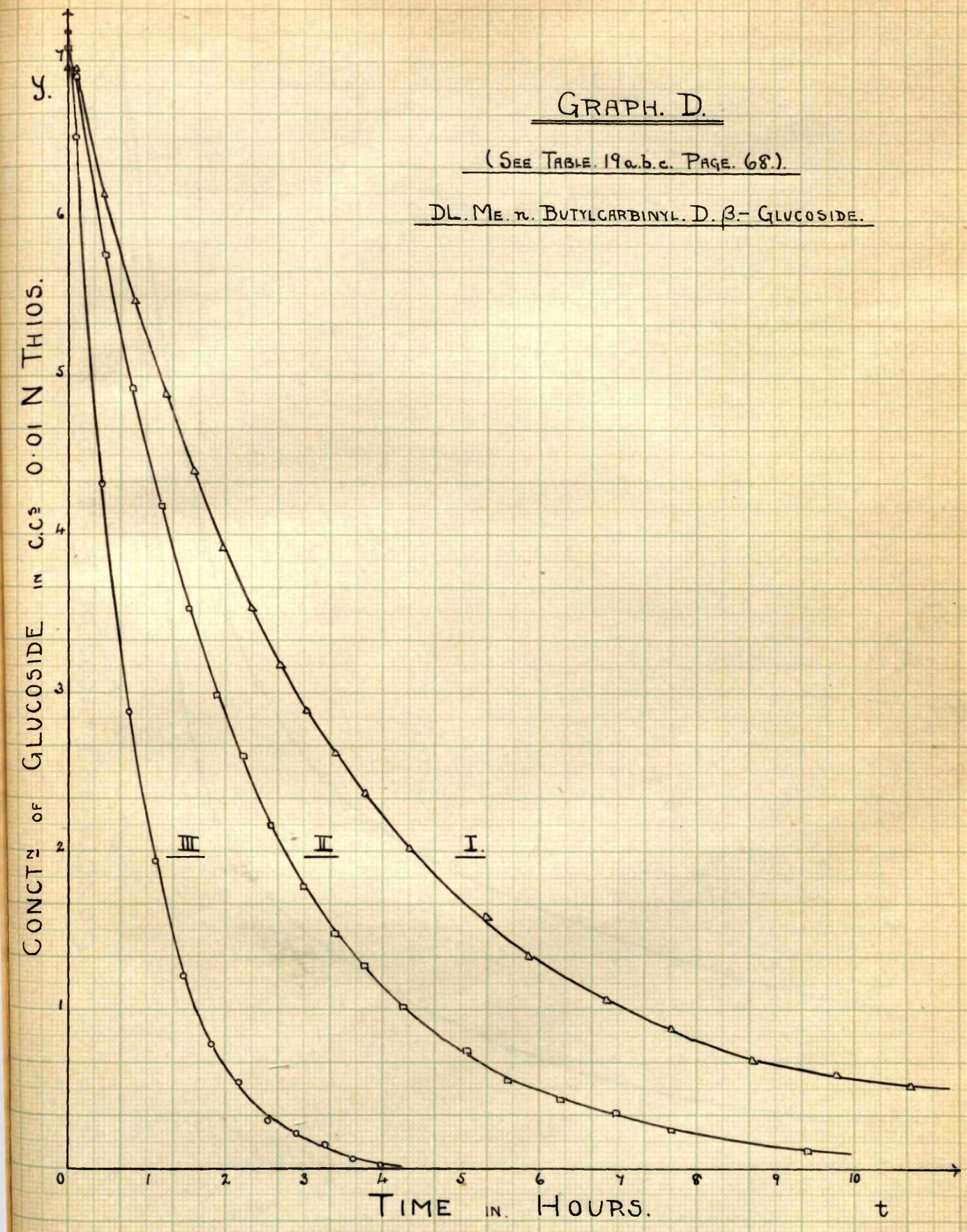
(See Table. 18. a.b. Page. 67.).

DL. Me. n. Propylcarbinyl. D. β – Glucoside.

GRAPH. D.

(See Table. 19a.b.c. Page. 68.).

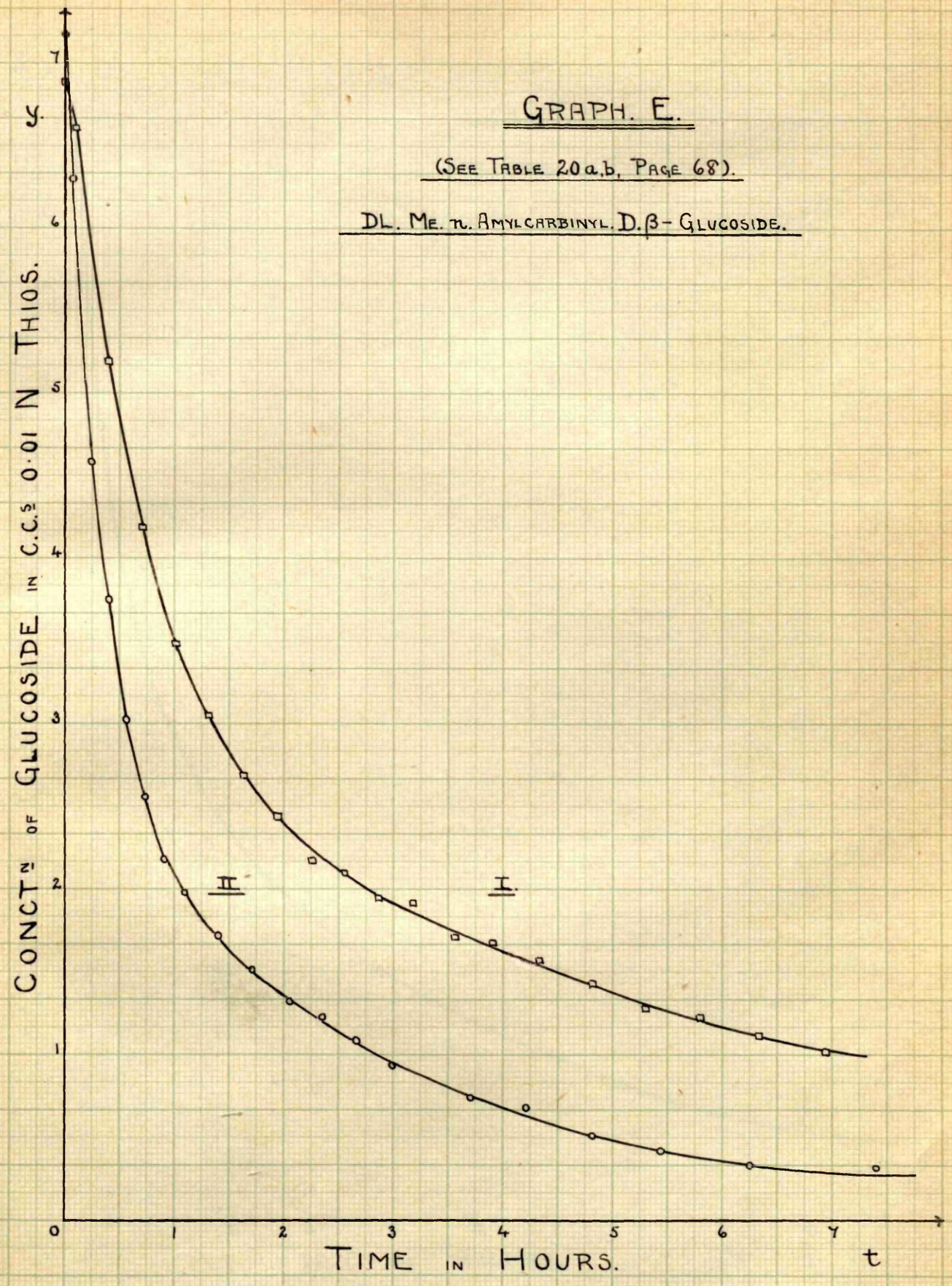
DL. Me. n. Butylcarbinyl. D. β.- Glucoside.



GRAPH. E.

(See Table 20a.b, Page 68).

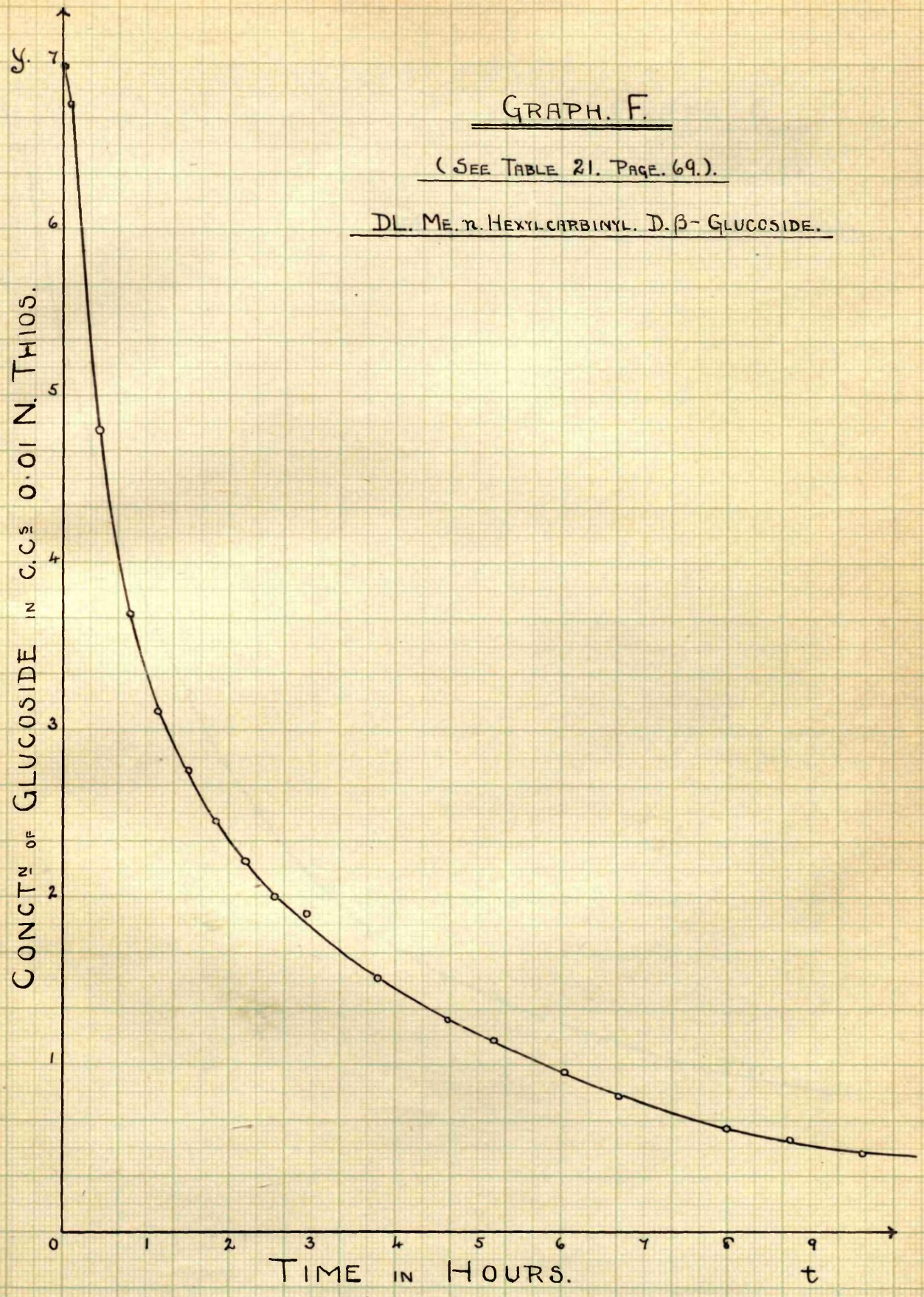
DL. Me. n. Amylcarbinyl. D.β-Glucoside.



GRAPH. F.

(See Table 21. Page. 69.).

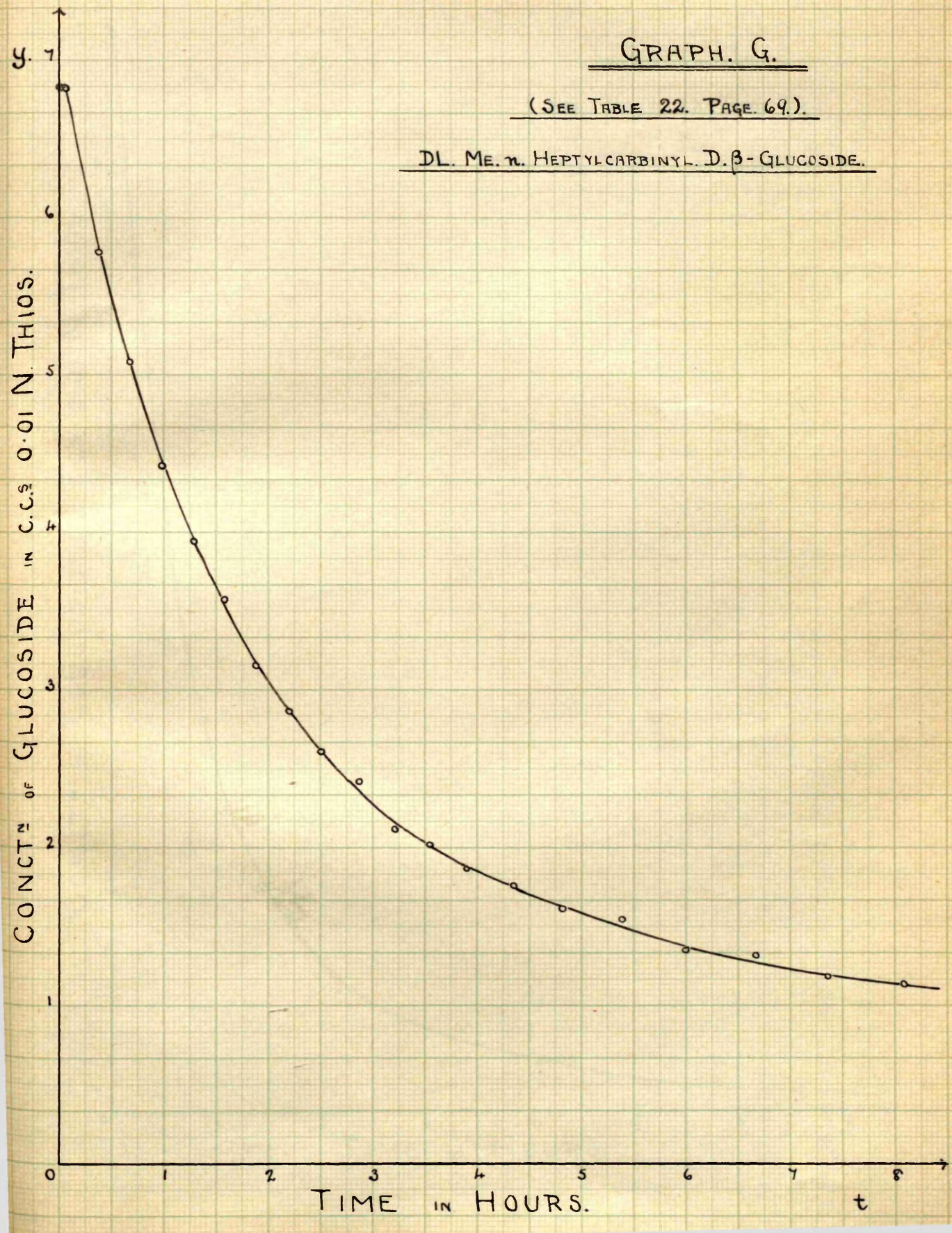
DL. Me. n. Hexyl carbinyl. D. β- Glucoside.



GRAPH. G.

(See Table 22. Page. 69.).

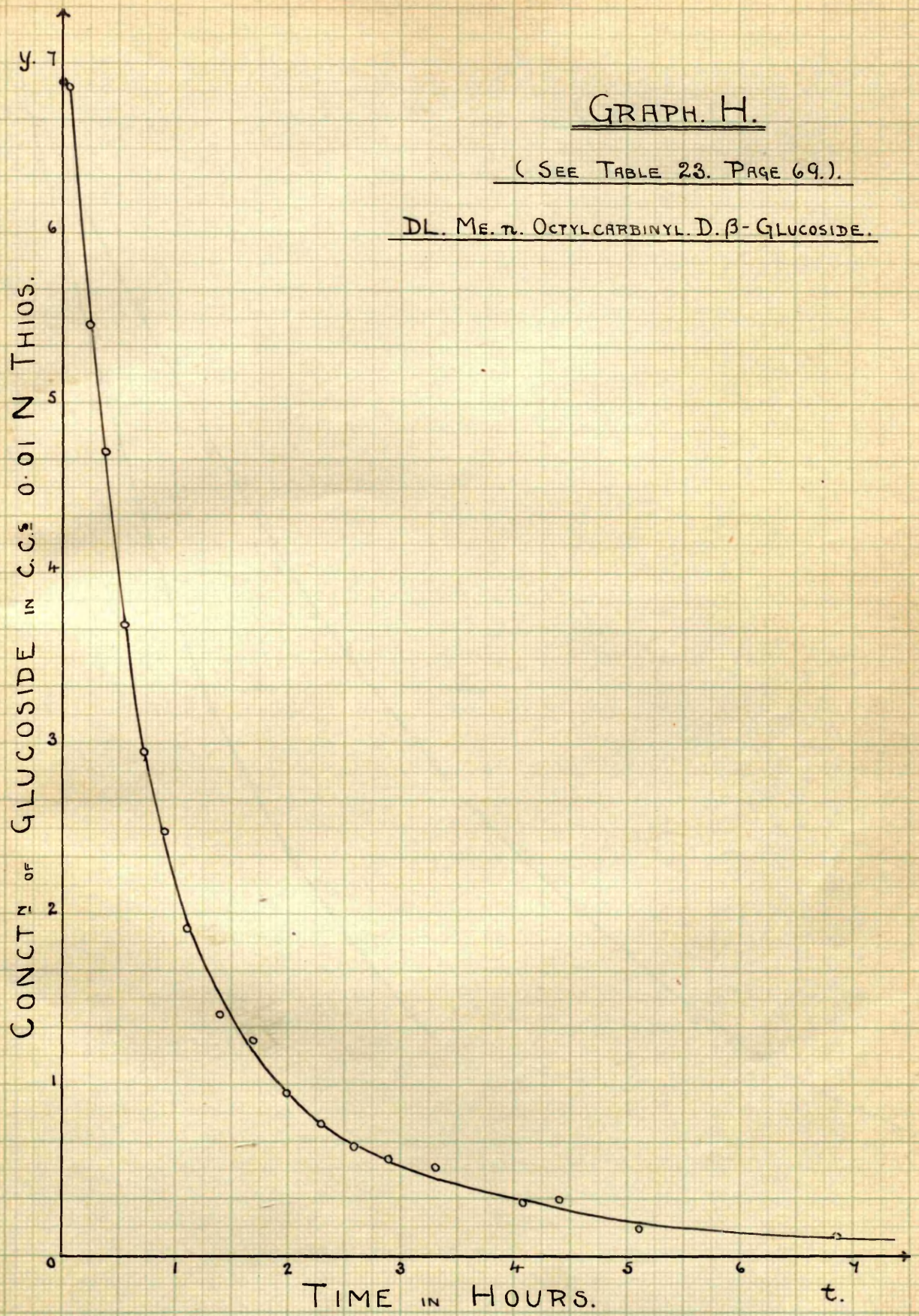
DL. Me. n. Heptylcarbinyl. D. β-Glucoside.



GRAPH. H.

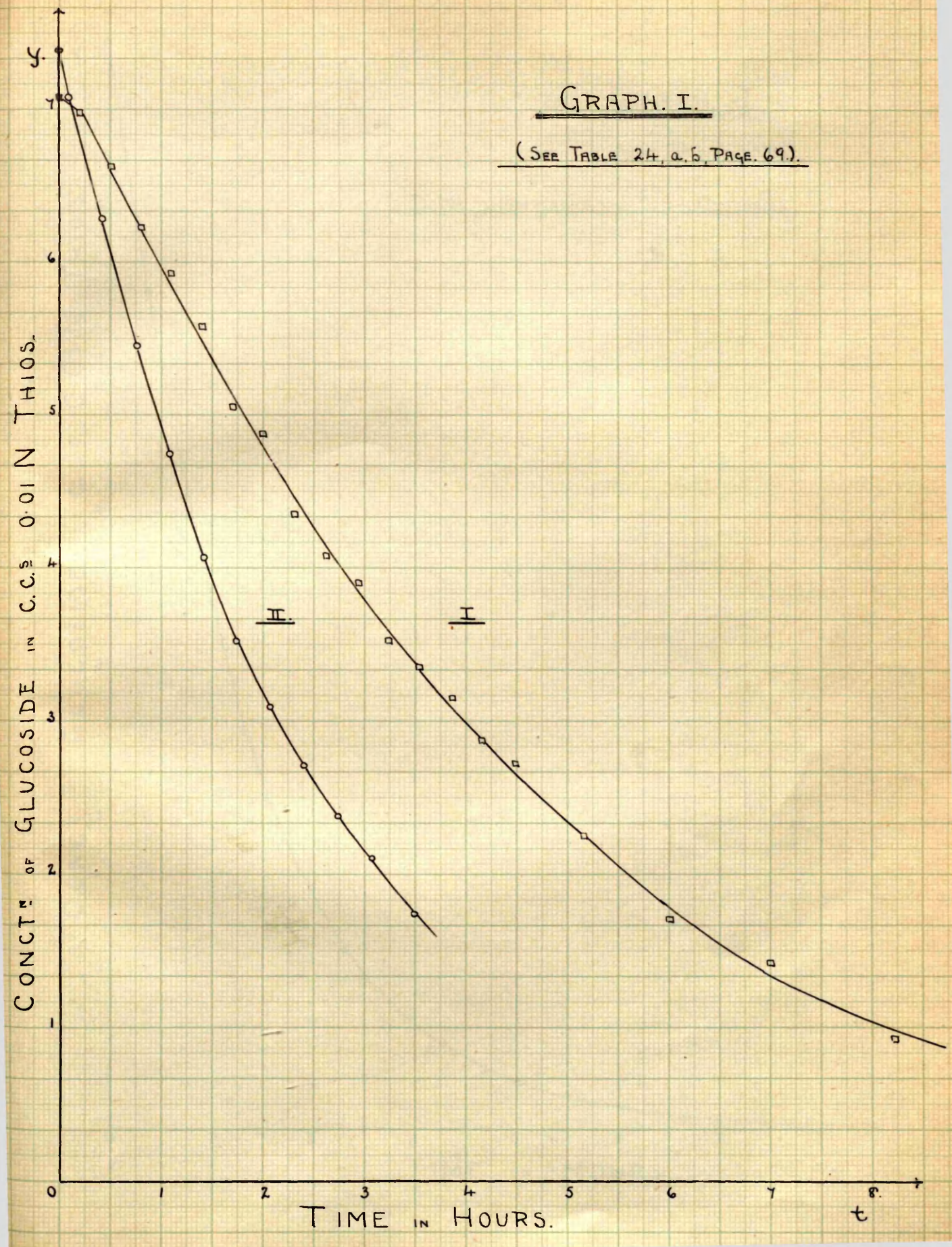
(SEE TABLE 23. PAGE 69.).

DL. ME. n. OCTYLCARBINYL. D. β-GLUCOSIDE.



GRAPH. I.

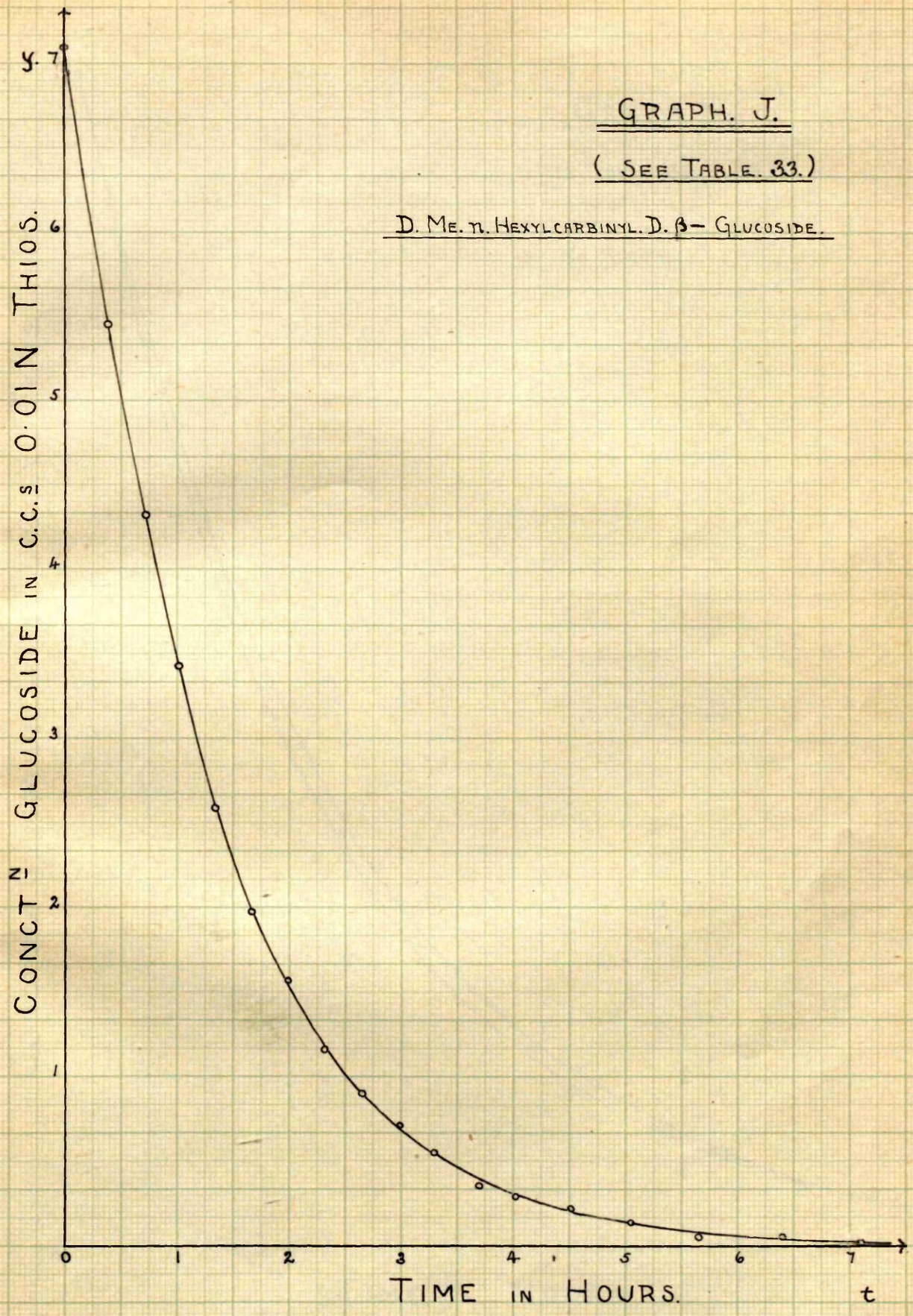
(See Table 24, a. b, Page. 69).

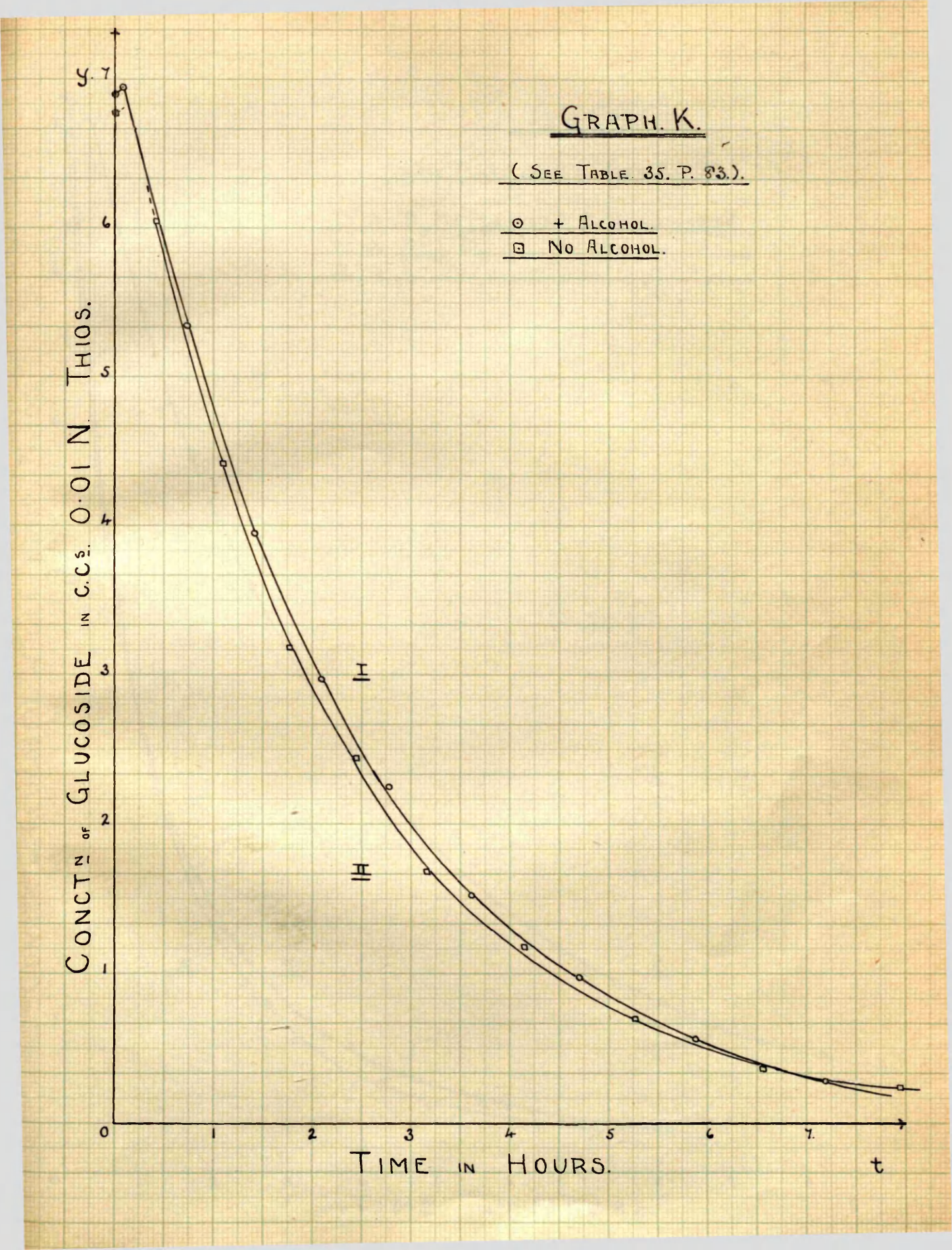


GRAPH. J.

(See Table. 33.)

D. Me. n. Hexylcarbinyl. D. β– Glucoside.

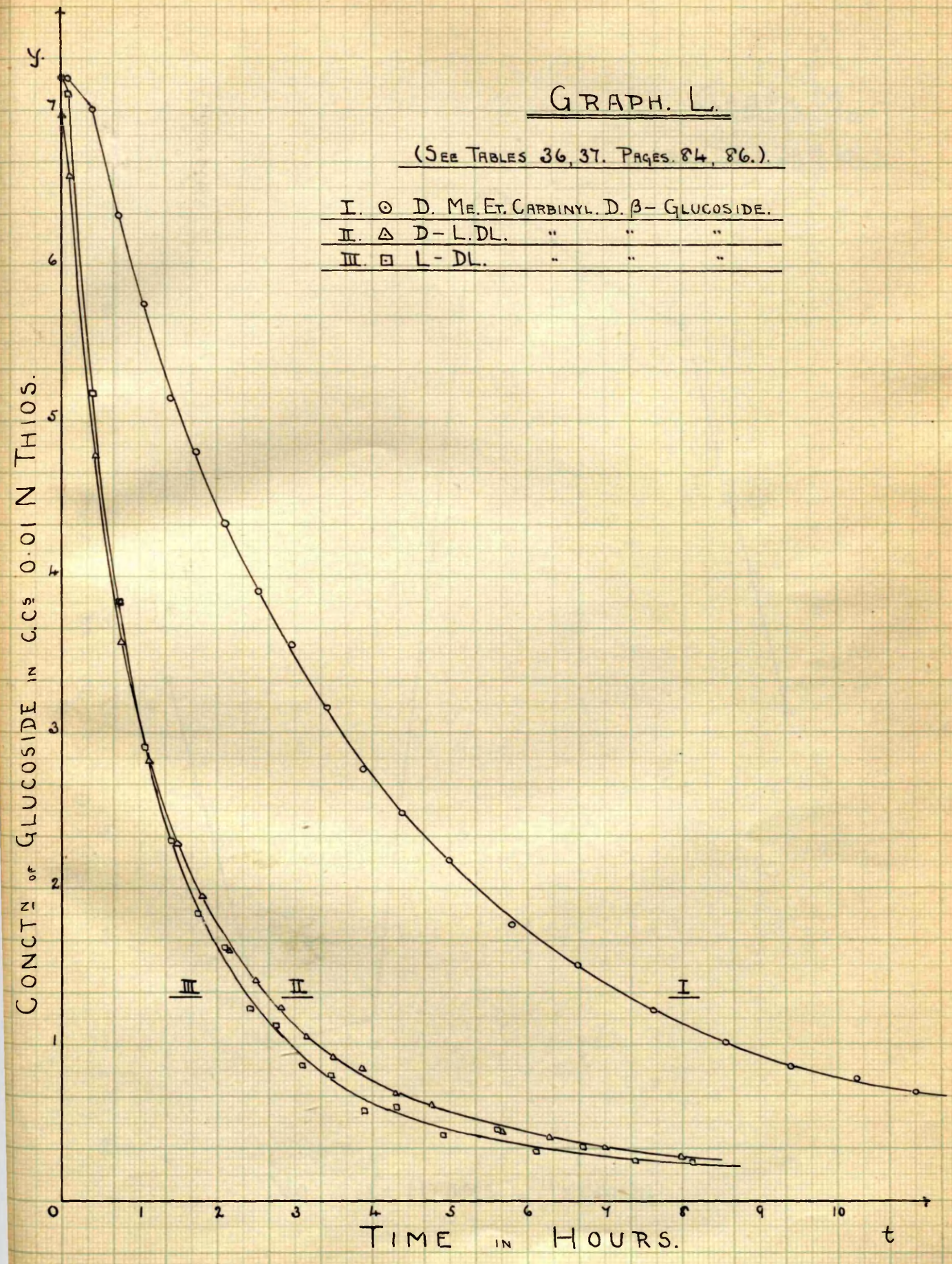



GRAPH. K.
(See Table. 35. P. 83.).
⊙ + Alcohol.
⊡ No Alcohol.
Conctn of Glucoside in c.cs. 0·01 N. Thios.
y.
7
6
5
4
3
2
1
0
I
II
1
2
3
4
5
6
7.
Time in Hours.
t

GRAPH. L.

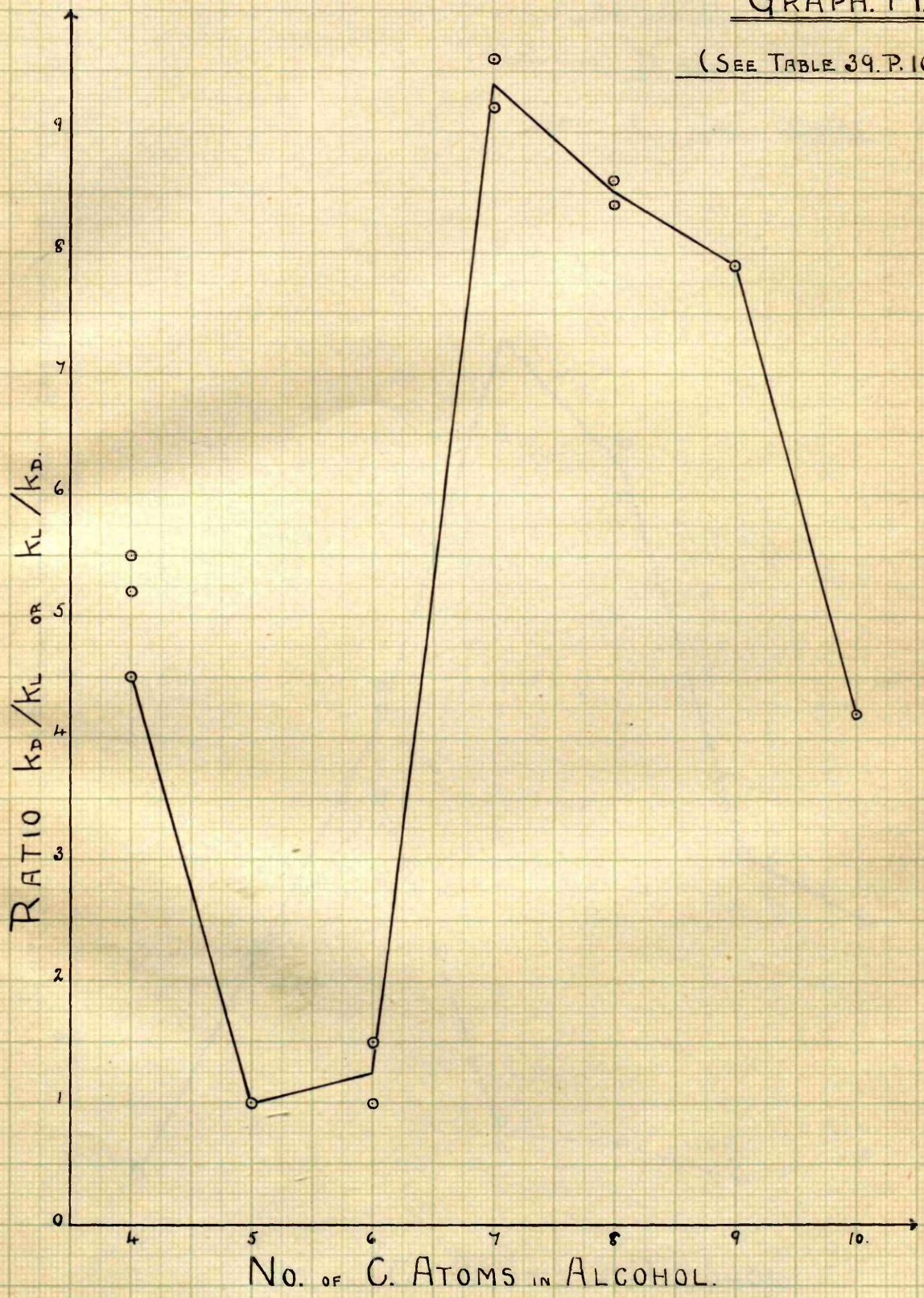
(See Tables 36, 37. Pages. 84, 86.).

I.	⊙	D. Me. Et. Carbinyl.	D. β-	Glucoside.
II.	△	D-L. DL.	"	"
III.	⊡	L-DL.	"	"

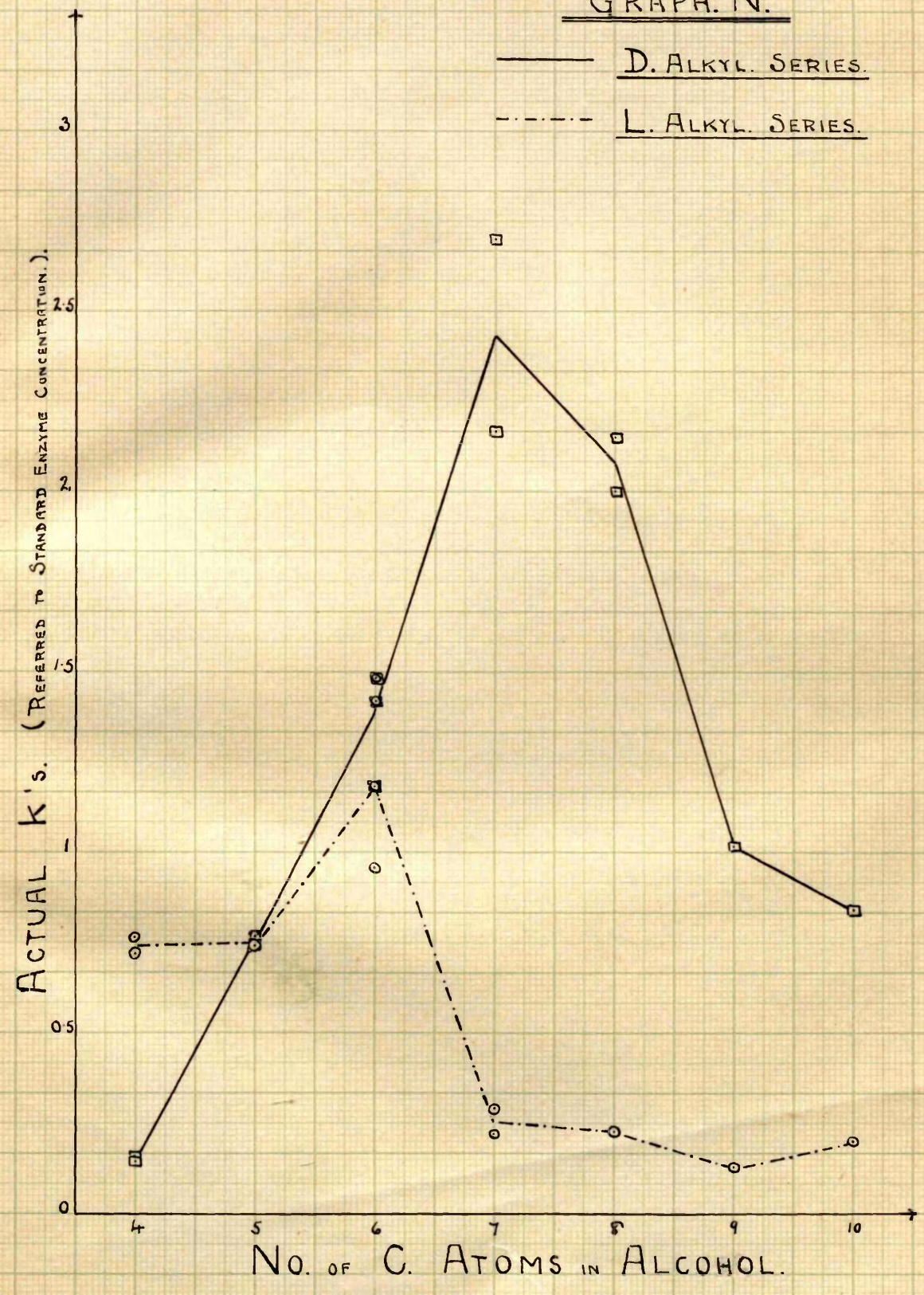


GRAPH. M.

(SEE TABLE 39. P. 101.)



GRAPH. N.


D. ALKYL. SERIES.
L. ALKYL. SERIES.
ACTUAL K's. (REFERRED TO STANDARD ENZYME CONCENTRATION.).
0
0.5
1
1.5
2
2.5
3
4
5
6
7
8
9
10
NO. OF C. ATOMS IN ALCOHOL.