THE ENZYMIC HYDROLYSIS OF STARCHES AND GLYCOGEN

by

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The course of hydrolysis of glycogen was also followed, using a perfused rat liver preparation and in addition the behaviour of the pig preparation in the presence of glycerol was studied.

The maltase activity of each liver preparation, and of the serum was determined and also the diastase activity of human and rat's blood.

In the case of the pig's liver preparation only, was there quantitative conversion to glucose and this was inhibited by the presence of glycerol.

Maltose was the sole end product with the cat, rabbit and perfused rat liver preparations. The unperfused rat liver preparations and the human liver preparations produced maltose alone in the early stages of hydrolysis, but this was gradually converted into glucose as digestion proceeded.

That the production of glucose by rat liver preparations could be due to the maltase content of the blood has been proved, since no glucose was produced by the perfused rat liver preparations.

The normal autolysis products of excised livers were also investigated. In no case was glucose the sole product, both maltose and glucose being produced. This is not in accord with the usually accepted view.

GENERAL INTRODUCTION.

Starches from different sources show very different rates of hydrolysis by amylases, which is presumably due to differences in the composition of the starch granules. Dietetically, however, starches are usually considered of equivalent food value, provided that they all undergo complete digestion.

Having found that potato and rice starches are hydrolysed at very different rates, as judged by the achromic point, and being unable to find in dietetical and chemical literature information concerning this, it was decided to investigate the rates of digestion and of the formation of the intermediate hydrolysis products of the starches which are incorporated in our diet. Glycogen was later included in the investigation.

In this way it was hoped to gain information concerning the differences in chemical constitution of the different starches and their relative digestibilities. Nägeli (1858) was the first worker to realise that starch consisted of two substances, one of which formed the envelope. Meyer (1895) called these two constituents and α-β amyloses. This priority in nomenclature of the two constituents is not generally realised and consequently most workers use the nomenclature introduced some time

later by Maquenne and Roux (1903-1906). These workers designated the inner and outer constituents of the starch granule amylose and amylopectin, respectively. They considered the amylopectin fraction alone to be concerned in paste formation. Amylose gives a blue colour with iodine whereas amylopectin gives no iodine coloration. The values obtained by different workers for the proportions of these two constituents vary considerably. Maquenne and Roux found starch to contain 15-20% amylopectin and 80-85% amylose. Tanret (1914) gives 67-79.5% for the amylopectin content of different starches. Samec and Haerdtl (1920) found that potato and arrowroot starches contained about 73% amylopectin content, wheat 60%, Maize 45% and rice 38%. Ling and Nanji (1923) state that the proportion of amylose to amylopectin is always in the proportion of 2 to 1. Taylor and Iddles (1926) who use the Meyer nomenclature consider the two constituents to be present in most starches in the proportion of 81-88% β amylose (amylose) to 12-19% α amylose (amylopectin), but in potato starch they find 97-98% β amylose and 1.8-2.9% α amylose. Baldwin (1930) gives values of 83-85% for amylose and 15-17% for amylopectin, whereas van Klinkenberg (1932) considers 36% of the starch granule to consist of amylopectin and 64% of amylose. Eckert

and Manzin (1932) find that amylopectin comprises 63-83% of the starch granule.

Maquenne (1908) considered that neither amylose nor amylopectin is homogeneous and that there is no sharp distinction between the least soluble amyloses and the least resistant amylopectins. Samec (1912) drew attention to the variability of amylopectins from different sources. Ling and Nanji (1923) consider amylose and amylopectin to exist partly in combination with one another. Hirst (1932) maintains that there is no sharp distinction between the two constituents and showed that they are probably identical in chemical structure.

Attempts have been made by various workers to separate these two constituents of the starch granule. Roux (1905) attempted to separate the two constituents by the action of diastase, but it is doubtful whether this was achieved. Gatin-Gruzewska (1908) disintegrated the wall of the starch granule by the action of NaOH, but this method only gives a 40% yield and is therefore unsatisfactory. Samec and Haerdtl (1920) separated the constituents by electrodialysis. Amylopectin migrates to the positive pole. Ling and Nanji (1923) found that on freezing a 5% starch solution, amylopectin separated out and could be filtered. Amylose was precipitated from the filtrate with alcohol. Sherman (1916) heated starch paste at 85° for 2-4 hours and then separated the amylopectin

by centrifuging. He obtained approximately 66% amylopectin, which is in agreement with the results of Samec.

Samec and Meyer (1921) found that amylose and amylopectin had specific rotations of $[\alpha]_D$189° and $[\alpha]_D$ 195-196°, respectively.

Samec and Isajevic (1923) found that just as on electrodialysis, starch was split up into a gel (P-containing amylopectin) and a sol (P-free amylose), so also could glycogen be split up. In the case of glycogen, however, only 20% separated into gel and 80% into sol. The P contents of the sol and gel from starch and glycogen are not identical. These workers give the following values:-

Potato starch	20% sol (amylose)	P_2O_5	0.0%
	80% gel (amylopectin)		0.175%
Glycogen	80% sol	P_2O_5	0.721%
	20% gel		0.135%

On phosphorylation, amylose forms a gel, but however strongly one phosphorylates glycogen does not.

Phosphoric acid constitutes the largest part of the non-carbohydrate constituents of the starch granule. A small amount of phosphoric acid can usually be extracted by acids, but the majority is in organic combination.

Samec and von Hoefft (1912) consider that the

gelatinisation property of starch, which was formerly attributed to amylopectin, is actually due to phosphoric acid in organic combination with amylopectin as a phosphoric ester.

As already quoted, Samec and Haerdtl (1920) separated many varieties of starch by electrodialysis into an amylopectin fraction characterised by a high viscosity, high electro-conductivity and high P content and an amylose fraction which was non-viscous, non-electroconducting and with a low P content. On heating amylopectin with water under pressure, phosphoric acid was freed and the resulting non-viscous carbohydrate was very similar to amylose. Similarly, Samec and Meyer (1922) found that amylose could be esterified with phosphoric acid to form a viscous product very similar to amylopectin. Pringsheim and Goldstein (1923) found that crystalline polyamyloses could be converted into gelatinising materials by the introduction of H_3PO_4.

Samec, Minaeff and Ronzin (1924) showed that amylopectins of different origins have varying viscosities, conductivities and P contents. Samec gives values of 0.185% P_2O_5 for amylopectin from potato starch and 0.007% P_2O_5 for amylose from the same source. He gave the following values for the P contents (expressed as P_2O_5) of whole starches; 0.112%, 0.105%, 0.034% and

0.039% for potato, wheat, maize and rice starches, respectively.

More recently, Hirst (1932) also working on potato starch, found that both amylose and amylopectin contained 0.20%P as P_2O_5.

Very few values have been given for the P content of glycogen. Reich (1932) purified glycogen by repeated electrodialysis and precipitation and obtained a final product containing less than 0.002% P (as P_2O_5). It had the same chemical and physical properties as impure glycogen, from which he concluded that P is not an essential part of the glycogen Molecule.

Certain starches, notably wheat starch, contain small amounts of protein. Samec considers this protein to be present in combination with amylopectin as protein-amylophosphoric acid.

Fatty acids are also incorporated in some starch molecules. Taylor and Nelson (1926) gives values of 0.61% and 0.83% fatty acids for maize and potato starch. Taylor and Lehrmann (1926) showed that these fatty acids cannot be extracted with fat solvents but are set free only after prolonged hydrolysis with acids or lipase-free amylases. These workers found that the combined fatty acid liberated on hydrolysis of maize starch consisted of 24% palmitic acid, 40% oleic acid and 36% linolic acid.

Ling and Nanji (1923) showed that many starch granules and particularly those of rice, maize, barley and wheat, contain hemicellulose, which they refer to as "amylohemicellulose". These workers give values of 14% for maize, 17% for rice and 15% for potato tuber; potato starch contains none since the hemicellulose probably remains as a constituent of the cell wall. Clayson and Schryver (1923) obtained much lower results and found in sago and maize up to 3.8%.

The identity of glycogens from different sources has not, until recently, been satisfactorily established. Cremer (1894) Clautriau (1895) and Gatin-Gruzewska (1904) found values for the specific rotation of yeast glycogen of +198.9°, +184.5° and +196-197°, respectively. Harden and Young (1902), using glycogen from oysters, rabbit liver and yeast obtained results varying from +191° to +203°. The iodine colour also varies with the source of glycogen. Claude Bernard (1857) found that muscle glycogen gave a pure violet colour with iodine, whereas liver glycogen gave a chestnut brown colour. Clautriau (1895) stated that yeast glycogen gave a violet iodine coloration. Norris (1913) found that the degree of opalescence and the iodine coloration varied with the source of glycogen. This, he thought, was probably attributable to variations in the colloidal state. This worker also found that different glycogens were hydrolysed at different rates by amylase prepared from pig's pancreas. He found that the relative rates of hydrolysis of yeast, rabbit liver, dog liver and oyster glycogen were 100 :94.3 : 86.7 : 83. Harden and Young (1912) followed the course of acid hydrolysis polarimetrically and found that there was no difference between rabbit, yeast and oyster glycogen. Daoud and Ling (1931) considered all glycogens to be fundamentally identical. They thought that variations

in the rotation, opalescence and iodine coloration could probably be accounted for by differences in the degree of polymerisation.

More recently, Bell and Young (1934), who determined the specific rotation, reducing power after acid hydrolysis, ash content, the iodine coloration and the polarimetric behaviour on acid hydrolysis, came to the conclusion that the liver glycogens from fasted and fed rabbits, fed rats and fish are not significantly different.

Concerning the chemical structure of starch and glycogen considerable work has been done. That of Samec, Ling and Nanji, Schardinger and Pringsheim all supports Maquenne and Roux's theory that the starch granule consists of two constituents, termed by these workers "amylopectin" and "amylose".

The work of Samec has already been referred to. According to this worker, the characteristic properties of amylopectin are due to the presence of phosphoric acid in organic combination as a phosphoric ester.

Ling and Nanji (1923) working on the hydrolysis of starch paste by barley diastase, found that amylose was converted quantitatively into maltose ~~and isomaltose~~, whereas amylopectin was not broken down further than $\alpha\beta$ hexa-amylose (a non-reducing polysaccharide). They concluded that all starches contain two basal units, α hexa-amylose and $\alpha\beta$ hexa-amylose. In the starch granule, these two units probably exist in polymerised forms, the degree of polymerisation varying in different starches. These polymerised forms are known as "amylose" and "amylopectin" respectively.

Schardinger (1904, 1909, 1911) obtained by the action of B. macerans on starch paste at 45°C for 3-4 days, two non-reducing, non-fermentable, crystalline dextrins which he called α and β dextrins. Both were soluble in

water, and precipitated by alcohol, ether or iodine solutions. Both formed addition compounds with iodine, α dextrin forming a compound which was blue when moist and greyish green when dry, and β dextrin a compound which was brownish moist and dry. Besides α and β dextrins, a third crystalline dextrin was also obtained, but in much smaller quantities.

Schardinger's work was continued by Pringsheim and co-workers. Pringsheim, Langhans and Eissler (1912) showed that Schardinger's three crystalline dextrins all had the empirical formula $C_6H_{10}O_5$ and all formed definite acetyl derivatives, which on hydrolysis gave crystalline dextrins isomeric with the original ones.

α dextrin $(C_6H_{10}O_5)_4$ after acetylation and hydrolysis gave $(C_6H_{10}O_5)_2$.

β dextrin $(C_6H_{10}O_5)_6$ gave after similar treatment $(C_6H_{10}O_5)_3$.

The "third dextrin" $(C_6H_{10}O_5)_6$ is structurally isomeric with β dextrin.

Pringsheim and Langhans divided these crystalline polyamyloses into two series, the α and β series. The α series give iodine addition compounds in the form of metallic green needles and comprise the following compounds:-

Hexa-amylose (Schardinger's "third dextrin") $\left[(C_6H_{10}O_5)_2\right]_3$

Tetra-amylose (Schardinger's α dextrin) $\left[(C_6H_{10}O_5)_2\right]_2$

Di-amylose $(C_6H_{10}O_5)_2$

The β series give brownish red iodine addition compounds and crystallising in prisms and comprise:-

Hexa-amylose (Schardinger's β dextrin) $\left[(C_6H_{10}O_5)_3\right]_2$

Tri-amylose $(C_6H_{10}O_5)_3$

The above formulae were confirmed by Pringsheim and Goldstein (1923) from the cryoscopic behaviour of their Me derivatives.

These workers realised that there was a striking resemblance between the properties of the "amylose" (of Maquenne and Roux) and of the α polyamyloses and between amylopectin and the β polyamyloses. Also, in fermentation of amylose and amylopectin by B. macerans, amylase gave a very large yield of α polyamyloses and amylopectin of β polyamyloses. It appeared, therefore, that amylose was built up of disaccharide units (diamylose of the α polyamylose series) and amylopectin of trisaccharide units (tri-amylose of the β series). This work was corroborated further by Pringsheim and Wolfsohn (1924), who found that on acetylating amylose and amylopectin, acetates of a disaccharide and a trisaccharide, respectively, were formed and on heating with glycerol, amylose and amylopectin gave a dihexosan $(C_6H_{10}O_5)_2$ and a trihexosan $(C_6H_{10}O_5)_3$ respectively. None of these was reducing.

On treating polyamyloses, amylose or dihexosan with cold concentrated HCl, a reducing disaccharide, "amylobiose", was formed, and on similar treatment of amylopectin and trihexosan, a reducing trisaccharide, "amylotriose", was formed.

Pringsheim regards glycogen as being identical with amylopectin, apart from the electrolyte content. Both substances yield the same depolymerisation products on treatment with HCl, and on heating with glycerine, and glycogen gives a brownish red colour with iodine which is also given with Pringsheim's β series of amyloses.

Klinkenberg (1932) also considers glycogen to consist almost entirely of α amylose (amylopectin).

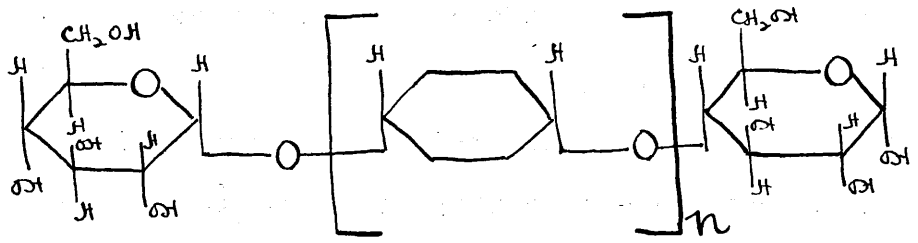
The view that natural starch is composed of amylose and amylopectin is, however, not generally accepted nowadays. These substances are reported to be formed by successive depolymerisation and repolymerisation of natural starch and their distinctive characteristics to arise from admixture with other substances. The work of Pictet and Karrer and more recently of Haworth and Hirst all supports the view that starch is homogeneous in structure.

Karrer and Nägeli (1921a) regard starch as polymeric maltose anhydride, and soluble starch and dextrin as lower polymers of the same substance. Karrer and

Nägeli (1921b) identify maltose anhydride with Pringsheim's α diamylose. The same workers (1921c) and Karrer (1921) consider glycogen to be very similar if not identical with starch, both being polymerisation products of maltose anhydride. They differ probably only in the degree of polymerisation.

Irvine and Macdonald (1922-1924) worked on the methylation of starch and the decomposition of methylated starch into methyl glucoses. The position of the Me group in the glucose should correspond to the position of the free OH groups in the starch molecule. These workers found that starch could be methylated in three stages, which are so definite that each stage must correspond to a distinct compound. They found that glycogen was also methylated in the same three stages. On hydrolysis of fully methylated starch, a 60-70% yield of 2:3:6 tri Me glucose was obtained. Fully methylated glycogen produced on hydrolysis 2:3:6: tri Me glucose as the sole product. They concluded that both starch and glycogen are built up chiefly, if not entirely, of glucose residues in which the OH groups attached to C atoms 2, 3 and 6 remain free in the polysaccharide molecule.

Haworth and Percival (1931) showed that starch and glycogen consist of maltose residues joined to form a chain of α glucopyranose units.



In order to ascertain the number of glucose units per molecule of polysaccharide, Haworth (1932) and (1935), Hirst et al. (1932) and Haworth and Hirst (1936) adopted the end group method. The percentage occurrence of the non-reducing terminal C_6 unit was in each case estimated after preliminary methylation of the polysaccharide followed by gravimetric assay of the tetra Me glucose. These workers found that in the case of methylated starches of potato, maize and waxy maize, "n" in the above formula was 23-28 (i.e. the starch molecule contains 26-30 glucose units) and in the case of glycogens of different origins "n" was 10-12 (i.e. the glycogen molecule contains 12-14 glucose units).

Estimates of molecular weight derived by different methods (e.g. from viscosimetric measurements, osmotic pressure, ultra-centrifuge data and the gravimetric chemical assay by the end group method) are frequently in variance with one another. These descrepancies, although not fully explained, indicate that a clear distinction must be made between the chemical molecule and the variable physical unit which is present in solutions. Thus the above workers found that viscosity

measurements indicated a chain length 5, 10 or 20 times that stated above. The amylopectin (α amylose) fraction showed a greater molecular size as compared with amylose (β amylose), whereas the end group method gave the same value for all specimens. After surface etching of starch grains with ethyl alcohol containing small quantities of hydrogen chloride, Haworth obtained a disaggregated starch which, in the form of its acetate and its methylated derivatives, gave the same value for the molecular weight both by the gravimetric assay of the end group method and by viscosity methods. Haworth believes that this simple disaggregated variety of starch is not degraded, but represents the chemical unit of starch. It is soluble in cold water, but when heated or dried or kept, reverts by re-aggregation and dehydration to physical assemblages, insoluble in water and of increasingly high viscosity corresponding to the original amylose or amylopectin of the starch granule. Haworth thus regards the chemical unit of starch to be limited in size, with an average molecular weight of 5000. These chemical units undergo aggregation to form physical units of much larger dimensions. (Haworth's method of molecular weight determination has been criticised by Irvine (1932) on the basis that methylation might produce degradation of the starch molecule).

Ultracentrifuge methods (Lamm, 1934) show that both amylose and amylopectin are polydisperse, the former consisting chiefly of particles of molecular weight of about 60,000 and the latter of about 300,000.

Using osmotic pressure measurements, Samec (1934) obtained molecular weight determinations of starch of 100,000 to 500,000 and of dog liver glycogen of 114,000. Bell and Young (1936) also using osmotic pressure measurements found that aqueous solutions of rabbit liver and muscle glycogen gave a molecular weight of 2×10^6, the same value being given by methylated glycogen in water.

PART I

THE ACTION OF SALIVARY, PANCREATIC AND MALT AMYLASES ON STARCHES AND GLYCOGEN.

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INTRODUCTION.

Most workers consider that the unrestricted action of maltase free amylases on starch leads to the formation of one sugar only, namely maltose, and that by the prolonged action of amylase there is at least 80% conversion into α glucosidic maltose.

Certain early workers, however, namely Lintner and Düll (1892 and 1893) considered that isomaltose is formed in addition to maltose. Isomaltose, as prepared by these workers by the action of malt diastase on potato starch, has a reducing power of 80% as compared with maltose, an $[\alpha]_D 140^\circ$, and forms an osazone of M.pt. 151°C, which crystallises in spherical groups of very fine light yellow needles and is very much less soluble in water and alcohol than maltosazone. Syniewski (1902) confirmed the above work and claimed to have isolated pure isomaltose (termed by this worker "dextrinose"). Serono and Cruto (1923) found that salivary amylase and dried glycerol-extracted pancreas formed isomaltose from starch and neither maltose nor glucose.

Brown and Morris (1895) showed that Lintner's isomaltose is not a separate chemical entity but is only a maltosazone altered in crystalline form and M.pt. by admixture with an amorphous osazone of a simple dextrin. Ling and Nanji (1923) also obtained isomaltose as a

product of the enzymatic hydrolysis of starch. Ling (1927) regards his isomaltose as ~~β~~ glucosido-~~β~~glucose, probably an isomer of maltose. He found that it could be converted into maltose by the action of amylases, by chemical means and even spontaneously. Pringsheim (1932) considers isomaltose to be identical with his amylobiose, prepared as previously mentioned by the action of cold concentrated HCl on amylose, etc. He considers that Ling's "isomaltose" should be regarded as a mixture of several disaccharides with amylobiose, since Ling's substance did not give quantitative conversion into maltose. (Amylobiose gives quantitative conversion). Pringsheim regards the role of amylobiose (isomaltose) in starch chemistry to be parallel with that of isocellobiose in cellulose chemistry. On acetylating cellulose, isocellobiose is formed before cellobiose and is converted into it after prolonged acetylation. Irvine and Black (1926) and Irvine (1927) regard isomaltose and isocellobiose as stereoisomers of maltose and cellobiose.

Pottevin (1899) interpreted the amylolytic hydrolysis of starch as due to partial hydrolysis of the starch granule by means of an enzyme "amylase", into a series of dextrins, ranging from amylodextrins, giving a blue colour with iodine and exhibiting slight Fehling's reducing properties, to achroo-dextrins or simple dextrins,

giving no colour with iodine and having high Fehling's reducing properties. A second enzyme "dextrinase" then converts these dextrins into maltose.

According to Chrzaszcz (1911), an amylase contains separate depolymerising, dextrinising and saccharifying enzymes, which view was later supported by Pringsheim (1924), but this assumption does not seem to be essential. Nishimura (1928) regards amyloses as being composed of one enzyme only, which saccharifies and depolymerises, the latter notably in the presence of a co-enzyme.

Olsson (1923) showed that the saccharifying enzyme is defferent from the liquefying enzyme because the addition of certain poisons reduced the liquefying action without affecting the saccharifying power.

Kendall and Sherman (1910) found that the liquefying action is predominant with pancreatic amylase and the saccharifying action with malt amylase. These two enzyme components have now been successfully isolated by van Klinkenberg (1934), Karrer (1921) considered diastatic action to consist of two phases, depolymerisation and hydration, the latter phase involving the hydrolysis of maltose anhydride to maltose.

The saccharification of depolymerised amylose and amylopectin does not proceed at the same rate.

Amylose smoothly and quickly gives rise to the theoretical amount of maltose; whereas the saccharification of amylopectin is slower and usually ceases when about 78% of the theoretical yield of maltose is produced. The remaining residual dextrin can, according to Pringsheim and Beiser (1924), be converted into maltose if a co-enzyme, present in yeast, is added. Pringsheim, Bondi and Thilo (1928) worked on the nature of the complement and found that it was a trypsin decomposition product of albumin. It is, therefore, presumably formed in yeast by autolysis of the yeast cells.

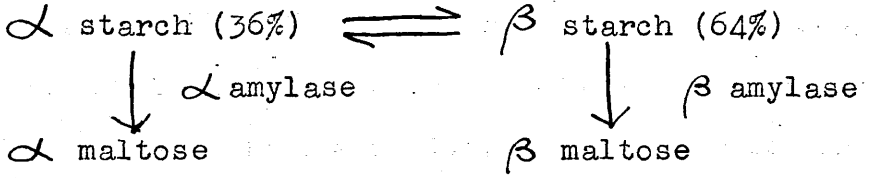
Kuhn (1924) divided amylases into two groups, the α and β amylases according to the point of attack of the starch molecule. α amylases produce α glucosidic maltose as the end product of starch degradation and β amylases produce β maltose initially. Amylases from animal sources and takadiastases are α amylases and vegetable amylases, with the exception of takadiastase, are β amylases. Kuhn concludes that in view of the behaviour of the starch granule towards plant and animal amylases, the starch granule consists of alternating and α-β glucosidic linkages. If both α and β glucosidic groupings occur in the same starch molecule, it should be possible to obtain glucose from starch if both α and β

amylases are made to act on starch paste. Pringsheim and Liebowitz (1925) showed this to be true.

Ohlsson (1930) showed that his "dextrinogen-amylase" from malt was identical with Kuhn's α amylase, and his "saccharogenamylase" with Kuhn's β amylase. As judged from the relative permanence of the starch iodine reaction, Ohlsson concluded that the former enzyme breaks down starch into progressively smaller molecules until maltose is produced, whereas the latter enzyme detaches maltose in successive stages. These conclusions of Ohlsson were later confirmed by Freeman and Hopkins (1936). These workers criticised Ohlsson's conclusions, which were based on the relative permanence of the starch iodine reaction, since it is not certain that the iodine colour is a function of the molecular dimensions, and they therefore studied in addition the formation of maltose.

Van Klinkenberg (1932 and 1934) did further work on α and β amylases. He showed that β amylase (isolated from malt) gave a 64% theoretical yield of β maltose from soluble starch, without the disappearance of the iodine reaction. The "Restprodukt" was non-reducing and was not attacked by β amylase. Glycogen was not a specific substrate for this enzyme. The liquefaction ran parallel with the saccharification. α amylases acting on soluble starch produced an approximately 36% yield of α maltose with great rapidity and with the disappearance of the

iodine reaction . Any further saccharification was very slow. Glycogen and the "Restprodukt" from the β amylose hydrolysis were attacked. The disappearance of the iodine reaction and saccharification ran parallel, whereas liquefaction was much quicker. (Both α and β amylases have liquefying action. Van Klinkenberg did not think justifiable the separation of liquefying and saccharifying enzymes). He concluded from these results that starch is a mixture of two components, α and β starch, which are present in the proportions of 36:64. α starch is saccharified by α amylases only and β starch by β amylases. α starch alone gives a colour with iodine. This theory is not in agreement with Kuhn, for whereas Kuhn regards the α and β linkages as alternating in the same molecule, van Klinkenberg regards the α and β glucosidic linkages as occurring in different molecules, in α and β starch, respectively. The following diagram represents van Klinkenberg's views:-



The interconversion of α and β starch has been confirmed by Nishimura (1931), who showed that achroodextrin, produced by the malt bydrolysis of starch at 50°, after standing for a long time with autolysed yeast, gave a violet blue iodine reaction.

It is not necessary to assume, as did Nishimura, the existance of a synthetic enzyme "amylosynthease" in autolysed yeast.

Glycogen is considered by van Klinkenberg to consist of α starch alone, which can only with difficulty be transferred into the β form.

Chraszcz and Janicki (1934) found that in germinated and ungerminated cereals there are natural paralysers of amylases, which they called "sistoamylases". Sistoamylases absorb plant amylases either in a state of solution or at the moment of being dissolved. Other substances, called "eleutoamylases", which counteract the action of sistoamylases, are also present in plants. A mixture of sistoamylase, eleutoamylase and amylase results in no inactivation of the amylase. If sistoamylase is first allowed to react with amylase and eleutoamylase added afterwards, there is partial or toal freeing of the amylase, according to the amount of eleutoamylase used. Using buckwheat malt, which is very rich in sistoamylase, these workers obtained a very quick inactivation of saliva and a similar increase in the activity after the addition of peptone. They concluded that only part of the amylase in saliva was present in an active form, the rest being inactive, presumably through the presence of sistoamylase. Thus, natural amylase paralysers occur together with amylases in both plants and animals. Lesser (1919 and

1921) showed that liver amylase is partly inactivated by absorption and that retardation of the hydrolysis of glycogen may take place not only in vivo, but may also be produced experimentally by the addition of absorbent. He showed that liver amylase can be freed by adrenaline, the active concentration of the enzyme increasing by the elution of the inhibitor.

Little work has been done on the rates of digestion of different starches and the results that are quoted are very discrepant. The chief reason for this discrepancy is that two different methods are used for determining the amount of digestion that has occurred. These two methods, the achromic point and a sugar estimation, are actually estimating different things. The achromic point determines when all the starch has been broken down at least as far as achroodextrin, although this does not necessarily mean that there is any sugar present. A sugar determination, on the other hand, estimates the maltose present and also any reducing dextrins, but there may be at the same time a considerable amount of unchanged polysaccharide left. In order to get an accurate conception of the amount of starch degradation that has taken place at any time, it is necessary to determine at least four factors; the achromic point, the unchanged polysaccharide, the total reducing power and the reducing power due to maltose alone.

O'Sullivan (1904) found that a much lower percentage of maltose was formed by the action of malt amylase on potato starch than on cereal starches.

Nagao (1911) found that wheat and rye starches were hydrolysed at equal rates by pancreatic amylase and that oat and barley starches were digested at approximately the same rate, but the rate of hydrolysis of the latter two was considerably higher than that of wheat and rye.

Sherman et al.(1919) showed that when similarly purified by washing with dilute alkali to extract any gluten, wheat, maize and rice starches were hydrolysed at equal rates. Potato starch generally showed a rate of hydrolysis equal to or slightly higher than the cereal starches. These experiments were performed with both pancreatic and salivary amylase. The percentage hydrolysis was determined in each case at the end of 30 minutes by estimating the reducing sugar by Fehling's method.

Concerning the breakdown of glycogen, Musculus and Mering (1879) showed that glycogen gave the same hydrolysis products as starch.

Cremer (1894) found that the amylolytic hydrolysis of glycogen was much slower than starch.

Tebb (1897) found that when glycogen was digested by ptyalin, amylopsin and malt diastase, both

maltose and isomaltose were formed.

Osborne and Zobel (1903) found that both pancreatic and malt amylases formed isomaltose alone as the end product, whereas both takadiastase and salivary amylase produced glucose in addition to isomaltose.

Philoche (1908) showed that there was a specific difference between animal and plant amylases. He examined the hydrolysis of soluble starch and glycogen by malt and pancreatic amylase. A known amount of malt produced much less maltose from glycogen than from starch, glycogen requiring 20 to 100 times as much malt as starch to form the same amount of maltose. This was not true of pancreatic amylase. He concluded that animal amylases have a special affinity for animal starch which plant amylases only possess to a much smaller degree.

Horton (1913) showed that the hydrolysis of glycogen by pancreatic amylase proceeded much faster in the early stages, but the subsequent breakdown of dextrin was very slow and there was seldom a quantitative conversion into maltose.

Barbour (1929) showed that with salivary amylase the chief end product was isomaltose, and there was also occasionally glucose present, but never any maltose. With pancreatic amylase, both isomaltose and glucose were formed. He found that there was a serial degradation of

of glycogen with pancreatic amylase, the increased reducing power corresponding with disappearance of glycogen, whereas with salivary amylase there was no such correspondence.

EXPERIMENTAL WORK

In view of these rather discordant results the present investigation was carried out using potato, maize, wheat and rice starches (all of these having been obtained from B.D.H.) and glycogen (Kahlbaum).

The ash and P content of all the samples were determined. As a further test of purity, nitrogen analyses were made, but the results were negative in all cases. The values obtained are given in the following table, all the estimations being performed on the dried material:-

Polysaccharide	% Ash	% Phosphorus
Maize starch	0.084	0.055
Wheat starch	0.185	0.142
Potato starch	0.285	0.119
Rice starch	0.284	0.068
Glycogen	1.100	0.298

The ash in each case gave a positive iron reaction. The P content was determined by Neumann's method using 0.5 to 2.0 g. of dried material. The results are of the same order as those obtained by Samec and Haerdtl (1920) and the higher value obtained for glycogen substantiates

the generally adopted view that glycogen has a higher electrolyte content than starches.

ACHROMIC POINT.

The achromic points were determined for each polysaccharide, using a 1% solution and hydrolysing it to turn with saliva, takadiastase, malt diastase and a solution of 'Holadin' (a pancreatic preparation particularly rich in amylopsin). Whichever amylase was used, the achromic points were always in the same order, this point being reached very much more quickly with potato starch and glycogen than with rice, wheat and maize starches, these latter three, however, all giving the achromic point at approximately the same time.

Amylase	Starch				
	Potato	Maize	Rice	Wheat	Glycogen
Salivary	20 secs	1½ hrs	1½ hrs	1½ hrs	Few secs
Pancreatic	5 secs	2¼ hrs	2¼ hrs	2¼ hrs	Immediate
Takadiastase	20 secs	4 hrs	4 hrs	4 hrs	Few secs
Malt	4 mins	3 hrs	3 hrs	3 hrs	2 mins

As will be seen from subsequent results, the achromic point serves as a very unreliable index of the amount of digestion that has taken place.

According to van Klinkenberg (1934) iodine

colouring atomic groups are present in α starch only, and the iodine reaction therefore only disappears by the action of α amylase.

RATE OF DEGRADATION OF THE DIFFERENT STARCHES AND OF GLYCOGEN.

The course of the enzymic hydrolysis of glycogen and of each of the starches was followed, using pancreatic amylase ('Holadin'). The salivary and malt digestions of wheat starch and glycogen were also followed.

The maltase activity of all the enzyme preparations was tested by incubating 2% maltose solutions with relatively large amounts of the enzyme and testing for glucose by an osazone test after 3 days. With the exception of the malt preparation, glucose was produced in every case. On diminshing the concentration of enzyme, however, no glucose was formed even with concentrations of enzyme ten times as great as those used in the subsequent hydrolysis. It is concluded that these enzyme preparations contain only traces of maltase which is ineffective in the low concentrations in which they were employed and would therefore by overlooked in most cases.

The following estimations were performed:-

(1) Estimation of unchanged polysaccharide

(2) Estimation of total reducing power using Wood Ost's method.

(3) Estimation of reducing power due to sugar alone using the same method.

In the later experiments Hane's method of sugar estimation was substituted for that of Wood Ost. From these data it is possible to calculate the amount of polysaccharide that has been broken down, the amounts of reducing dextrin and maltose formed, and by deduction, the amount of non-reducing dextrin.

These three estimations were performed on samples of the digest immediately after mixing and 15, 30, 60 or 90, 120, 180, 330 minutes, 24 or 72 hours after the beginning of the experiment.

An osazone test was also performed on samples from each digest, after alcohol precipitation of any residual starch and dextrins followed by evaporation of the alcohol from the filtrate. In no case was a typical maltosazone observed. Most frequently the osazone consisted of relatively large rosettes of very fine needles, but very often these needles were tufted at their free extremities. It seems impossible to place any reliance on the appearance of the osazone; either the typical crystalline form of maltose had become modified by admixture with another substance (perhaps dextrins which had not been completely removed by alcohol precipitation) or else maltose is not the sole product of hydrolysis.

Different methods were employed for the estimation of starch and glycogen.

The starch was estimated by a modification of the Th. von Fellenberg method (1916). This method depends on the principle that starch dissolves in concentrated $CaCl_2$ solution, from which it can be precipitated by iodine. Protein, which might be present in small amounts is insoluble in $CaCl_2$ solutions and dextrins are not precipated from $CaCl_2$ solutions by iodine.

The digestion mixture in the case of the pancreatic digestion consisted of 225 ml. of 1% starch solution in 0.5% NaCl, 70 ml. of borate buffer of pH 6.8 and 5 ml. of 0.1% solution of holadin in distilled water.(1) This mixture was left in a thermostat at 37°C and samples removed at the time intervals stated above.

The total reducing power was determined by Wood Ost's method, introducing 5 ml. of the digest into 20 ml. of the Wood Ost solution. This was performed in duplicate.

In order to determine the reducing power due to maltose alone, 10 ml. of the digest were removed, boiled to kill the enzyme, cooled and made up to 50 ml. in a graduated flask with absolute alcohol. This was left overnight, filtered, and 20 ml. portions of the filtrate used for the Wood Ost determination.

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(1) Phosphate buffers cannot be substituted for borate buffers, since it was found that boiling the starch and phosphate mixture with $CaCl_2$ led to the complete disappearance of the starch..

In many cases a further estimation was carried out. A portion of the alcohol filtrate, which should contain maltose alone, was heated on a water bath to drive off all the alcohol and the reducing power determined after acid hydrolysis. This should indicate whether maltose is the sole sugar formed.

Furthermore, in a few cases, polarimetric determinations were carried out on the alcohol filtrates, after the alcohol had been driven off and the subsequent solution considerably concentrated and the turbidity removed if necessary by the addition of a small amount of basic lead acetate.

At the end of each experiment the digest was tested for lactic acid by Uffelmann's reagent. This test was in all cases negative.

To estimate the residual starch, 10 ml. of digest was introduced into a boiling tube and 10 g. of granular $CaCl_2$ added. (Towards the end of the digestion 20 ml. of the digest was employed, in which case 20 g. $CaCl_2$ were added). This was boiled and the tube left in a boiling water bath for 10 to 15 minutes. It was cooled, diluted to 100 ml. and filtered. 20 ml. samples of the filtrate were introduced into centrifuge tubes (four estimations were done in each case) and 2 ml. of approximately N/10 iodine solution added. The tubes were left for 15 minutes or overnight and then centrifuged for

20 minutes. The supernatant fluid was poured off, the precipitate stirred up with 10 ml. of 60% alcohol and the tube recentrifuged. This process was repeated once. The alcohol was decanted, boiling distilled water added to the starch iodide precipitate and the resulting solution transferred into a 250 ml. Pyrex flask. The tube was washed out several times with boiling distilled water, so that the final volume was about 50 ml. This was boiled until all the iodine and alcohol were driven off and the boiling continued until the volume was reduced to about 10 ml. The flask was cooled and then 20 ml. conc. H_2SO_4 and 10 ml. of N/2 $K_2Cr_2O_7$ added and then boiled under a reflux condenser for 30 minutes. The excess $K_2Cr_2O_7$ was then determined.

Assuming that the starch is quantitatively converted into CO_2 and H_2O, one arrives at the factor:-

1 ml. N/2 $K_2Cr_2O_7$ ≡ 3.375 mg. starch

from which the starch content of the sample can be calculated.

The digestion mixture for the glycogen hydrolysis was the same as for the starches. The total reducing power and the reducing power due to sugar were determined in exactly the same way as described for starch. In the determination of the residual glycogen, the observations of Good, Kramer and Somogyi (1933), that the

optimal concentration of alcohol for the precipitation of glycogen is 50% and of Osterberg (1930) that this precipitation is assisted by the use of Na_2SO_4 which carries down mechanically the precipitated glycogen with the precipitate of Na_2SO_4, were made use of.

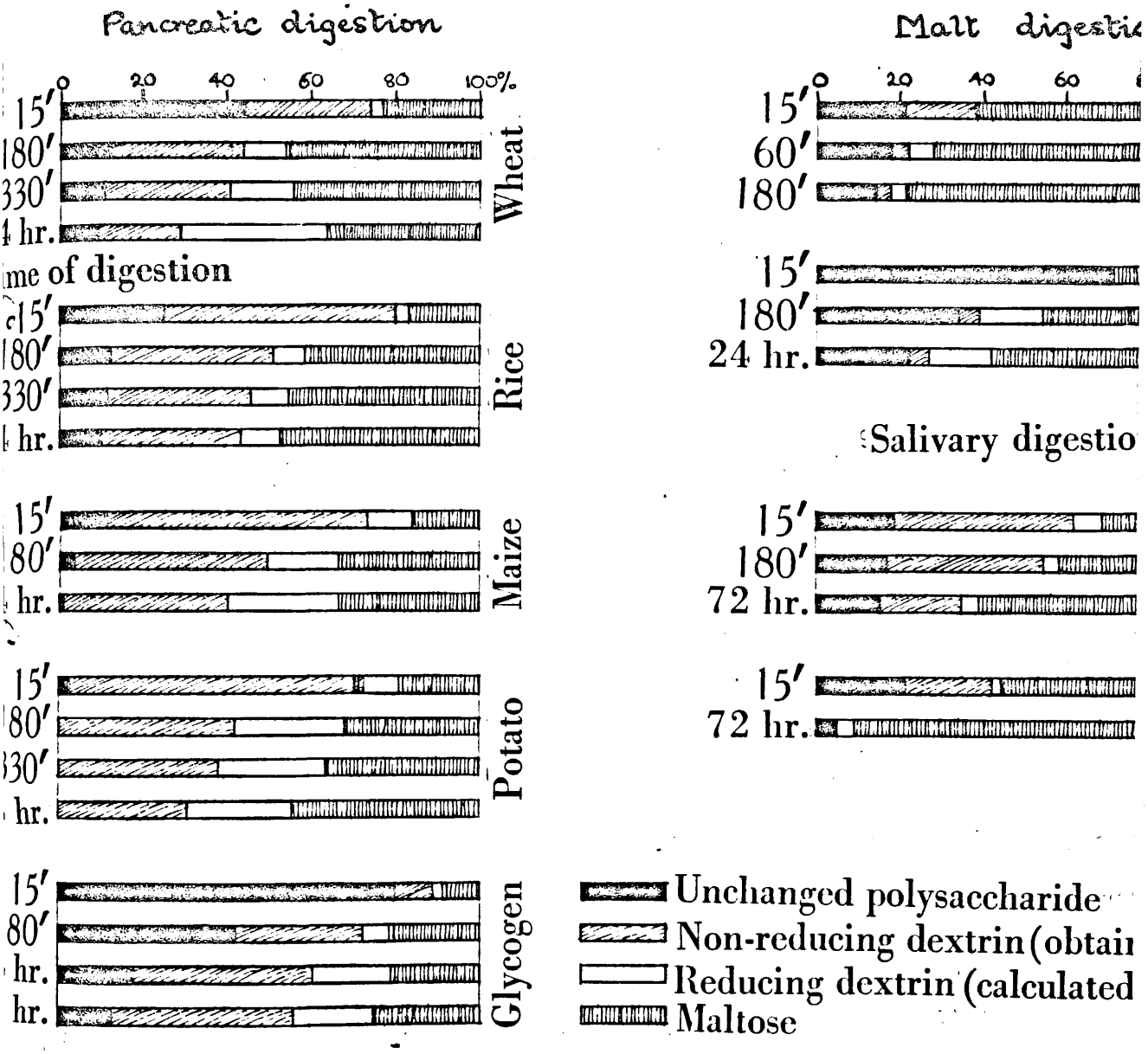
3 ml. of the digest were introduced into a centrifuge tube and 1 ml. of 5% Na_2SO_4 solution and 6 Ml. of absolute alcohol added, and the tube covered with a glass bulb. This was left for 10 minutes and then placed in a water bath until it just boiled, (this causes flocculation of the glycogen - Na_2SO_4 precipitate) removed from the water bath and left until absolutely cold. (This procedure probably reduced the concentration of alcohol to about 50-55%). The tube was centrifuged for 10-15 minutes, the supernatant fluid poured off and the tube heated in a boiling water bath until all the alcohol had evaporated. 5 ml. of N.HCl were added to the residue, the tube covered with a glass bulb and heated in a boiling water bath for 2 to $2\frac{1}{2}$ hours. The tube was removed and cooled, one drop of phenol red added as indicator and then solid Na_2CO_3 until the contents were just alkaline in reaction. This was transferred to a 50 ml. graduated flask, made up to the mark with distilled water and 5 ml. portions used for the estimation of glucose by Hane's (1929) method.

The results are represented diagramatically (Fig. I) and in addition ~~three~~ two graphs are given plotting respectively the % disappearance of polysaccharide, ~~the % formation of reducing dextrin and maltose~~, and the % formation of maltose against time. (Graphs I . II)

NATURE OF SUGAR FORMED ON HYDROLYSIS.

As has already been stated most observers agree that maltose is the sole sugar formed on hydrolysis of starch or glycogen by amylases. The present results are not entirely in agreement with this. The same sugar was formed from all the starches and from glycogen but as judged from the appearance of the osazone, the amount of glucose formed on acid hydrolysis and the optical rotation of the sugar, this sugar could not be identified with maltose. The appearance of the osazone has already been described. On acid hydrolysis of the digest, after precipitation of the unchanged polysaccharide and dextrins with alcohol, 120-140% yield of glucose was obtained (assuming that the sugar before hydrolysis was maltose). This means that the reducing power of the product of hydrolysis is only 70-85% that of maltose. In no case, as judged by the osazone was any glucose present, but if any were present this would mean that the other sugar would have a reducing power even further removed from

Figure I


Pancreatic digestion
Malt digestic
0 20 40 60 80 100%
15′
180′
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4 hr.
Wheat
me of digestion
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Rice
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15′
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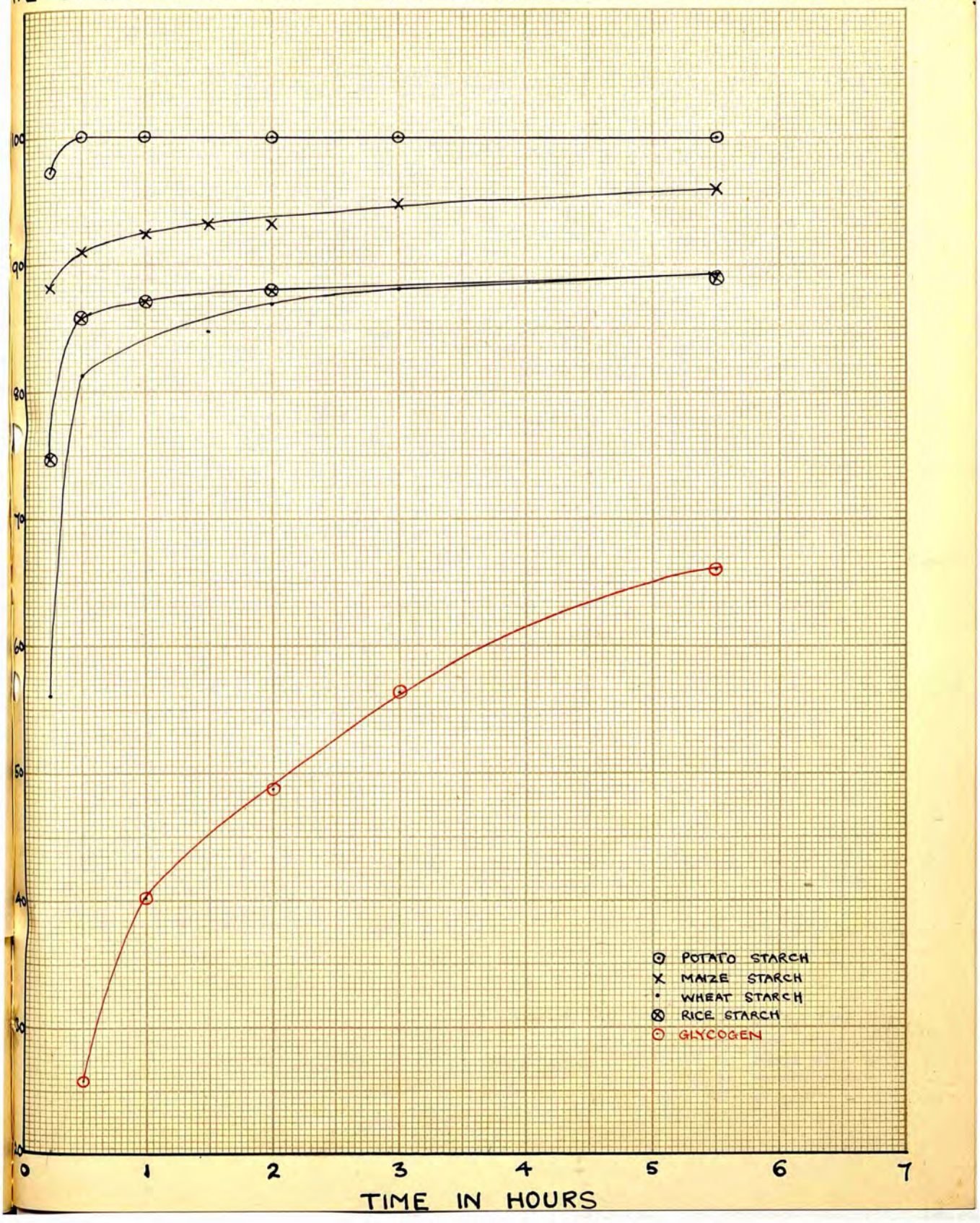
72 hr.
Maize
15′
180′
330′
hr.
Potato
15′
60′
180′
15′
180′
72 hr.
15′
80′
hr.
hr.
Glycogen
Unchanged polysaccharide
Non-reducing dextrin (obtai
Reducing dextrin (calculated
Maltose

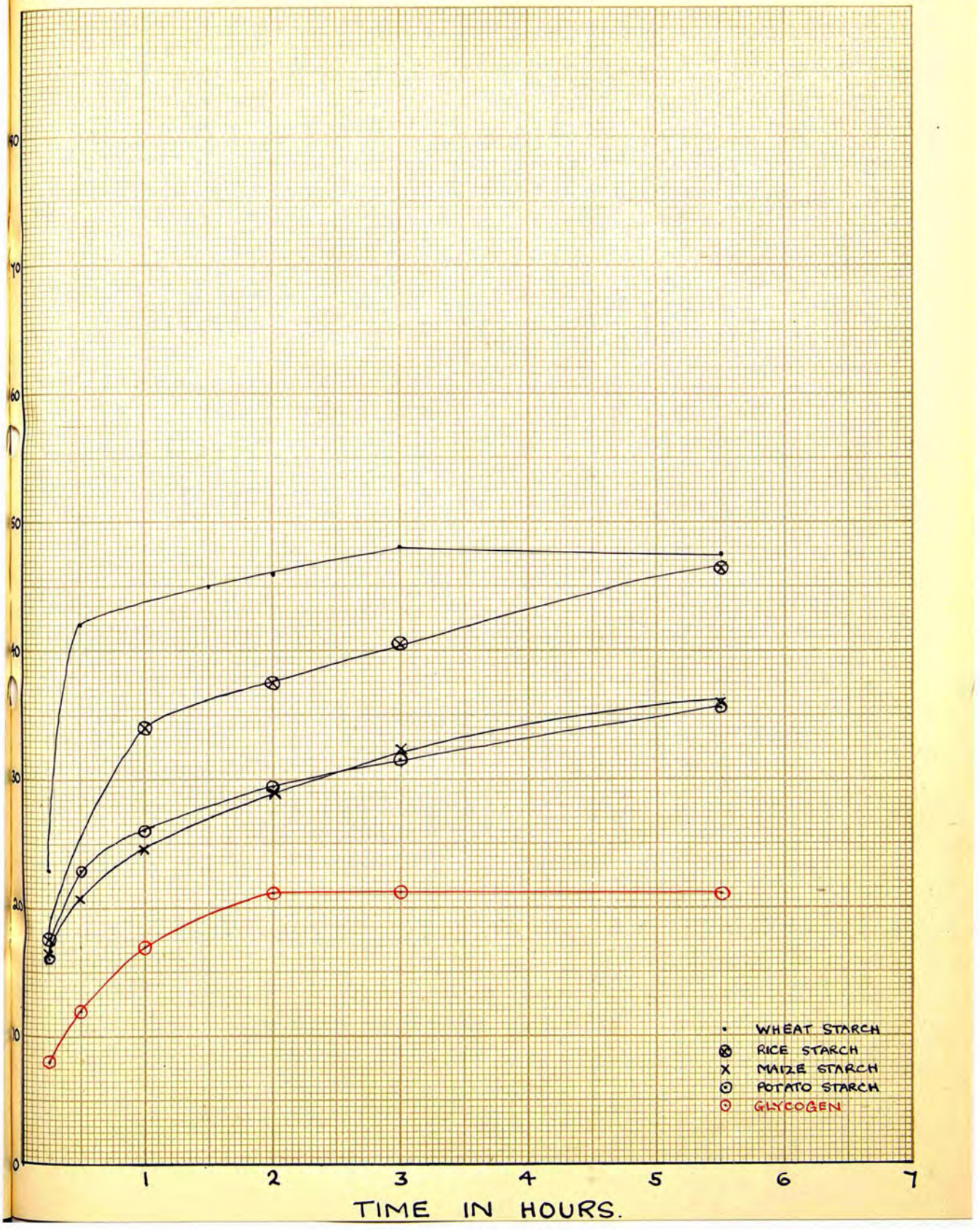
Graph I.

ATE OF DIGESTION OF POLYSACCHARIDE BY PANCREATIC AMYLASE



Graph II

RATE OF FORMATION OF MALTOSE BY PANCREATIC AMYLASE



TIME IN HOURS.

TABLE I

PANCREATIC DIGESTION OF POTATO STARCH

Time	0	15'	30'	60'	120'	180'	330'	24 hrs.
% starch left	82	2.7	0	0	0	0	0	0
% starch disappeared	18	97.3	100	100	100	100	100	100
Total reducing power as mg maltose/100ml digest		200	325	415	425	455	484	544
Mg maltose/100 ml digest		120	195	205	225	245	275	295
Reducing dextrin/100 ml expressed in mg maltose		80	130	210	200	210	209	209
% conversion of starch into reducing dextrin and maltose		25.9	41.2	52	53.6	57.6	61.2	68.4
% conversion of starch into maltose		17.1	22.8	26	29.4	31.5	35.6	42.8
% conversion of starch into reducing dextrin		8.6	18.4	26	24.2	26	25.6	25.6
% conversion into non-reducing dextrin (obtained by difference)		71.4	58.8	48	46.4	42.4	38.8	31.6

TABLE II

PANCREATIC DIGESTION OF WHEAT STARCH

Time	0 (30")	15'	30'	60'	120'	180'	330'	24 hrs.
% starch left	98	44	18.5	18.2	17.1	12	10.8	9.5
% starch disappeared	2	56	81.5	84.8	86.9	88	89.2	90.5
Total reducing power as mg maltose / 100ml digest	3.8	200	355	413	426	460	515	560
Mg maltose / 100ml digest		180	340	355	366	380	375	290
Reducing dextrin / 100ml expressed in mg maltose		20	14.8	60	60	80	140	270
% conversion of starch into reducing dextrin and maltose	0.5	25.3	44.9	52.4	53.7	58.1	65	70.6
% conversion of starch into maltose		22.8	42	44.8	46.1	48.2	47.5	36.6
% conversion of starch into reducing dextrin		2.5	2.9	7.6	7.6	9.9	17.5	34
% conversion into non-reducing dextrin (obtained by difference)		30.7	36.6	32.4	33.2	29.9	24.2	19.9

TABLE III

PANCREATIC DIGESTION OF MAIZE STARCH

Time	0' (25")	15'	30'	60'	120'	180'	330'	24 hrs.
% starch left	72.5	11.7	9.1	7.5	6.9	4.2	4	0.7
% starch disappeared	27.5	88.3	90.9	92.5	93.1	94.8	96	99.3
Total reducing power as mg maltose / 100ml digest	12	212	270	350	355	394	396	466
Mg maltose / 100ml digest		130	164	180	260	264	290	254
Reducing dextrin / 100ml expressed in mg maltose		82	106	170	95	130	106	202
% conversion of starch into reducing dextrin and maltose	1.5	26.7	34.1	44.1	44.7	49.7	49.9	59.2
% conversion of starch into maltose		16.4	20.7	24.7	28.8	32.3	35.7	32
% conversion of starch into reducing dextrin		10.3	13.4	19.4	15.9	17.4	14.2	27.2
% conversion into non-reducing dextrin (obtained by difference)		61.6	56.8	48.4	48.4	45.1	46.1	40.1

TABLE IV

PANCREATIC DIGESTION OF RICE STARCH

Time	0 (30")	15'	30'	60'	120'	180'	330'	24 hrs.
% starch left	97	25.3	14.3	13	12	12.5	12.5	10.8
% starch disappeared	3	74.7	85.7	87	88	87.5	87.5	89.2
Total reducing power as mg maltose / 100ml digest	7.5	160	216	320	328	360	404	450
Mg maltose / 100ml digest		140		270	272	302	368	380
Reducing dextrin / 100ml digest expressed in mg maltose		20		50	56	58	36	70
% conversion of starch into reducing dextrin and maltose	0.75	20	28.8	40.4	41.6	45.6	51.2	56.8
% conversion of starch into maltose		17.6		34	37.4	40.4	46.4	48
% conversion of starch into reducing dextrin		2.4		6.4	4.2	5.2	4.8	8.8
% conversion into non-reducing dextrin (obtained by difference)		54.7	56.9	46.6	46.4	41.9	36.3	32.4

TABLE V

PANCREATIC DIGESTION OF GLYCOGEN

Time	0	15'	30'	60'	120'	180'	330'	24 hrs.	72 hrs.
% Glycogen left	100	80.3	74.1	59.9	51.3	42.6	33.9	18.6	13
% Glycogen hydrolysed	0	19.8	25.9	40.1	48.7	56.4	66.1	81.4	87
Total reducing power as mg maltose / 100ml digest	0	70	105	142	186	214	254	314	347
Mg maltose / 100ml digest	0	64	95	132	166	164	166	166	194
Reducing dextrin /100 ml expressed in mg maltose	0	10	10	10	20	50	88	148	153
% conversion of glycogen into reducing dextrin and maltose	0	8.9	13.3	17.9	23.5	27	32.1	39.7	44
% conversion of glycogen into maltose	0	8.1	12	16.7	21	20.7	21	21	24.5
% conversion of glycogen into reducing dextrin	0	0.8	1.3	1.2	2.5	6.3	11.1	18.7	19.3
% conversion into non-reducing dextrin (obtained by difference)		10.9	12.6	22.2	25.2	29.4	34	41.7	43.2

TABLE VI

SALIVARY DIGESTION

	GLYCOGEN			WHEAT		
	15'	180'	72 hrs.	15'	180'	72 hrs.
% polysaccharide left	19.1	17.3	14.9	20.7		5.0
% digested	80.9	82.7	85.1	79.3		95
Total reducing power as mg maltose / 100ml digest	304	360	520	462	510	758
Mg maltose / 100ml digest	254	330	485	443	500	724
Reducing dextrin / 100ml expressed in mg maltose	50	30	35	14	10	34
% conversion polysacch. into maltose and reducing dextrin	38.4	45.5	65.5	58.4	64.5	95.4
% conversion polysacch. into maltose	32.1	41.7	61.2	56.7	63.1	91.5
% conversion into reducing dextrin	6.3	3.8	4.3	1.7	1.4	3.9
% conversion into non-reducing dextrin (obtained by difference)	42.5	37.2	19.6	20.9		0

TABLE VII

MALT DIGESTION OF GLYCOGEN

Time	50"	15'	30'	60'	120'	180'	330'	24 hrs.
% polysaccharide left	88.2	70.8	58.6	45.2	40.6	34.4	27.6	22.2
% polysaccharide disappeared	11.8	29.2	41.4	54.8	59.4	65.6	72.4	77.8
Total reducing power as mg maltose / 100ml digest	98	284	348	392	402	452	476	550
Mg maltose / 100ml digest		284	332	332	351	351	360	440
Reducing dextrin /100ml expressed in mg maltose		0	16	60	51	101	116	110
% conversion of glycogen into reducing dextrin and maltose	13.1	29.6	43.3	52.3	53.6	60.3	63.5	73.4
% conversion into maltose		29.6	41.3	44.3	46.8	46.8	48	58.7
% conversion into reducing dextrin		0	2.1	8.0	6.8	13.5	15.5	14.7
% conversion into non-reducing dextrin (obtained by difference)	0	0	0	2.5	5.8	5.3	8.9	4.4

TABLE VIII

MALT DIGESTION OF WHEAT STARCH

Time	15'	60'	180'
% starch left	20.8	18.2	14.6
% starch disappeared	79.2	81.8	85.4
Total reducing power as mg maltose / 100ml digest	466	586	605
Mg maltose / 100ml digest	466	542	584
Reducing dextrin / 100 ml expressed in mg maltose	0	44	21
% conversion of starch into reducing dextrin and maltose	62.2	78.1	80.7
% conversion into maltose	62.2	72.3	78.0
% conversion into reducing dextrin	0	5.8	2.7
% conversion into non-reducing dextrin (obtained by difference)	17.0	3.7	4.7

maltose than the value given above.

In a few cases only was the optical rotation determined but when this was done it was invariably found that there was lack of agreement between the optical rotation and the reducing power (assuming the sugar to be maltose only). The optical rotation was in each case 124.5% that of maltose. (This value is very similar to that given for α maltose, but this possibility is excluded because no mutarotation was observed after treatment with alkali.) The constancy of this figure obtained both from potato and wheat starches at different stages of hydrolysis indicates that only one sugar could be present. If a mixture of sugar were present it is extremely unlikely that they would be present in exactly the same proportions under different conditions. These results might be explained by the presence of reducing dextrins, but since the final concentration of alcohol before filtration of the unchanged polysaccharide was 80%, this seems improbable.

The appearance of the osazone and the reducing power correspond fairly closely with those of isomaltose as described by Lintner and Dull. The specific rotatory power, however, is different. The figure given for isomaltose is $[\alpha]_D 140^\circ$, which is only very slightly different from that given for maltose .

DISCUSSION OF RESULTS.

There are at least four different criteria of the rate of degradation of the polysaccharides:-

(1) Achromic point

(2) Rate of disappearance of polysaccharide.

(3) Rate of formation of maltose and reducing dextrin.

(4) Rate of formation of maltose.

The following table represents the order of these events, the polysaccharides being arranged in order of decreased of hydrolysis by pancreatic amylase as indicated by the different criteria.

Achromic Point	Rate of Disappearance of Polysaccharide	Rate of Formation of Reducing Dextrin and Maltose.	Rate of Formation of Maltose
Glycogen	Potato, Maize	Wheat, Potato	Wheat, Rice
Potato	Wheat, Rice	Maize, Rice	Potato
Maize, Wheat, Rice.	Glycogen	Glycogen	Maize
			Glycogen.

It is difficult to reconcile the very quick achromic point of glycogen with its slow rate of breakdown and formation of dextrin and maltose. It must be the figures for the achromic point which are at fault, since it was found very difficult to determine this value when very weak glycogen

solutions were used, the colour being only with difficulty distinguishable from that of a weak iodine solution.

It is interesting to note that in the case of glycogen, the rate of formation of maltose reached a steady state after two hours, although the formation of reducing dextrin and the disappearance of glycogen was still occurring. (The decrease in the 24 hours value for maltose seen in the results for wheat and maize starches is presumably due to fermentation).

In the case of the salivary digestion of glycogen and wheat starch, although the salivary amylase was obviously relatively very much stronger than the pancreatic amylase, the results are in the same order, the figures for glycogen being in every case smaller than those for wheat starch.

In the case of the digestion of glycogen and wheat starch by malt there is a slightly increased breakdown of glycogen than with pancreatic and salivary amylase. Malt contains chiefly β amylase, but this does not warrant the assumption that glycogen contains a preponderance of β linkages. The breakdown of glycogen by pancreatic, salivary and malt amylases is essentially similar in character.

These differences in the hydrolysis rates of the

different starches must be due to differences in the chemical constitution of the starch granules, since they cannot be attributed to differences in the enzyme system.

The rate of hydrolysis of all the starches is very much quicker than that of glycogen.

FACTORS WHICH COULD INFLUENCE THE RATE OF BREAKDOWN.

(1) Molecular Dimensions of the Polysaccharide. The smaller the molecular size, the quicker one would expect the breakdown to be. But the size of the glycogen molecule is, (according to Haworth), approximately half that of the starch molecule.

(2) Proportion of Amylopectin to Amylase. According to most modern workers the starch granule is homogeneous. But supposing that two constituents are present; the advocates of this theory agree with the original view of Maquenne and Roux (1906) that the viscosity of a starch solution is produced by the amylopectin. According to this view, potato starch, which gives the most viscous solution, should show the slowest rate of hydrolysis, since amylopectin is considered to be hydrolysed more slowly than amylose. This, however, is not the case, potato starch showing, if anything, a higher rate of hydrolysis in the initial stages than the other starches. The slower rate of hydrolysis of glycogen, however, could be accounted for if, as claimed by Pringsheim, glycogen

consists of amylopectin.

(3) Inorganic Content of Polysaccharide. According to Samec and Haerdtl (1920) a low rate of hydrolysis can be correlated with a high P content. Potato and wheat starches have much higher P contents than the other starches but their rates of hydrolysis are no slower. The lower rate of hydrolysis of glycogen, however, could be explained according to this theory by its high P content.

(4) Method of Breakdown. As has already been mentioned Ohlsson considers that α amylases break down starch into smaller and smaller molecules until maltose is finally produced whereas β amylases detach maltose successively. In the salivary and pancreatic digestions of glycogen and the various starches employed in this investigation, an early appearance of maltose was invariably observed. When malt, which contains β amylase in addition to α amylase, was used as the hydrolysing agent, no increased formation of maltose was observed. These results, therefore, do not substantiate the findings of Ohlsson. Moreover, Ohlsson's work was based on the relative permanence of the starch iodine reaction, and since it is not known with certainty that this colour is a function of the molecular dimensions, his conclusions seem rather unreliable.

In order to ascertain which of the above mentioned methods of breakdown was involved or which method was pre-

dominant, the following ratio was calculated for each digestion mixture throughout each hydrolysis.

$$\frac{\text{Polysaccharide destroyed in mg. per 100 ml.}}{\text{Maltose formed in mg. per 100 ml.}}$$

If this ratio remains constant throughout a hydrolysis it would indicate that maltose was liberated successively. If , on the other hand, the ratio decreases as digestion proceeds it would mean that maltose is formed only, or principally, in the final breakdown of the simple dextrins. These ratios, calculated from determinations made at 15 mins. from the beginning of each experiment at the intervals already mentioned, are tabulated below. The values cited represent values for this ratio obtained at the beginning, middle and end, respectively, of each experiment.

SUBSTRATE	PANCREATIC AMYLASE			MALT DIASTASE			SALIVARY AMYLASE		
Glycogen	2.16	2.46	3.15	1.24	1.40	1.50	2.52	1.98	1.39
Potato	4.00	3.52	2.78	-b	-	-	-	-	-
Wheat	1.87	1.83	1.86	1.27	1.13	1.09	1.39	-	1.04
Rice	2.60	2.56	1.89	-	-	-	-	-	-
Maize	4.38	2.89	2.70	-	-	-	-	-	-

The great variability of this ratio probably indicates that both methods of breakdown are occurring simultaneously. It is difficult to accept the deduction that the hydrolysis

of wheat starch by pancreatic amylase is the one instance amongst all those cited in which the former method of breakdown above occurs. An increase in this ratio as hydrolysis proceeds, observed with glycogen in two out of the three cases, seems very difficult to explain, especially as there was no glycogen resynthesis.

In the case of glycogen, the formation of maltose from dextrin appears to be very slow, which indicates that the hydrolysis is retarded at this stage, suggesting a reduced saccharification process. But since the same enzymes were used in the starch and glycogen digestions, this cannot be accounted for by the enzyme. Perhaps, as suggested by Pringsheim, a co-enzyme is necessary for the complete bydrolysis of glycogen.

(5) Nature of linkages in Polysaccharide. As discussed in the previous section, the slow rate of breakdown of glycogen cannot be explained by assuming a preponderance of β linkages in the glycogen molecule.

According to van Klinkenberg the iodine reaction is characteristic of α starch and only disappears by the action of α amylases. The very quick achromic point observed with glycogen appears at first sight to substantiate van Klinkenberg's view that glycogen is α starch. But this does not explain the slow breakdown of glycogen in comparison with the starches. If glycogen consisted

entirely of α starch, a very quick breakdown by α amylases would be expected.

It is thus seen that the theories of starch and glycogen structure so far enunciated do not explain satisfactorily the results of the amylolytic hydrolysis of these polysaccharides. There must be some difference in structure between the starch and glycogen molecules to account for the much lower rate of hydrolysis of the smaller glycogen molecule. There are also probably slight differences in structure between the different starches. It is also difficult to accept the identity of the starch and glycogen molecules as advocated by Haworth.

SUMMARY.

1. The course of digestion of potato, wheat, maize and rice starches and of glycogen by pancreatic amylase and in addition of wheat starch and glycogen by salivary amylase and malt diastase have been followed quantitatively.

2. The estimations included determinations of the achromic point, the residual polysaccharide, the total reducing power and the reducing power after the precipitation of the dextrins. Polarimetric observations were also made in a few cases.

3. The relative rates of formation of the various hydrolysis products were different with the different starches. The rate of hydrolysis of glycogen was in every case very much lower than that of the starches.

4. The same end product was formed from all the starches and from glycogen.

5. The properties of the reducing sugar formed as end product did not correspond with those of maltose, or, in all respects, with those of isomaltose.

6. Glucose was not formed in any of the above digestions, or, in the case of pancreatic amylase over the range pH 5,0 - 7.5. When, however, a very large amount of enzyme was employed, some glucose was produced.

7.. The bearing of these results on the chemical constitution of the starch and glycogen molecules and on the method of breakdown is discussed.

PART II

THE ACTION OF LIVER AMYLASES ON GLYCOGEN AND POTATO STARCH

Introduction

Great diversity of opinion exists as to the nature of the end products and the course of hydrolysis of glycogen by liver amylase.

Macleod (1926) stated that the final product was maltose which was, however, liable to be hydrolysed further to glucose. Osborne & Zobel (1903), working on muscle juice, found that maltose was the chief end-product together with a trace of glucose. Hollander (1934), using rat liver preparations and starch as substrate, found maltose as the sole end product. He arrived at the conclusion that the liver preparations contain two amylases comparable with α and β amylases. Tebb (1894-1897), using glycogen and a dried pig liver preparation, found glucose as the sole product. Preliminary saline perfusion of the liver through the portal vein, to remove all the blood, and subsequent drying provided a liver preparation which still had marked maltase activity. Hodgson (1936) using acetone-extracted and dried

rabbit liver preparations claimed, it appears without satisfactory experimental evidence, that glucose was the sole end-product and was unable to detect the presence of maltose at any stage of hydrolysis. These results are referred to again later. Barbour (1929) who used glycerol extracts of rabbit muscle claimed to have obtained a trisaccharide as the sole product of hydrolysis. This trisaccharide is stated to have a reducing power 30% of that of glucose (by Shaffer-Hartman method), a specific rotation $[\alpha]_D$ 181°, to be readily transformed into an anhydride and to form an osazone crystallising in small star-shaped aggregates of needles. This trisaccharide is not identifiable with Pringsheim's (1934) trisaccharide "amylotriose", obtained by the breakdown of glycogen and amylopectin with concentrated HCl. Lohmann (1926), using KCl extracts of frog muscle, similar to those used by Meyerhof (1926) studying simultaneously the production of lactic acid by glycogen, describes, in addition to glucose, the formation of a trisaccharide which he considers to be probably identical with Pringheim's amylotriose. Case (1931) without sufficient experimental data claims to have repeated and confirmed Barbour's results. Carruthers and Wei Yung-Lee (1935) also repeated Barbour's work, but

contrary to this worker found that maltose, and not a trisaccharide, was the chief product of hydrolysis. They also obtained evidence of the formation of some glucose. Carruthers (1935) explained Barbour's results as due to inhibition of the maltase activity of his preparations by the glycerol employed in the extraction.

EXPERIMENTAL WORK

In the present investigation liver preparations from man, the cat, rat, rabbit, pig, ox, guinea-pig and frog were employed. The preparations were made according to the method suggested to me by Prof. J. Mellanby and as used and described by Hodgson (1936).

The liver was cut up into small pieces, passed through a mincing machine and then shaken up with approximately twice its weight of acetone. The acetone was decanted off and this process repeated three or four times. The liver residue, which dried very rapidly in the air, was ground up finely in a mortar and sifted through very fine muslin, the resulting powder being used as the source of the glycogenase. Eadie (1927) pointed out the necessity for perfusing the liver to remove all blood, which contains some amylase, before testing the amylolytic activity of the liver preparation. Consequently a similar preparation was made from the liver of a rat which had been perfused until blood-free. In all cases the maltase activity of the dried liver preparations was tested by incubating 0.5g. of the liver preparation with 25 ml. 2% maltose solution, after addition of a few drops of toluene, for 24 hours at 37°. The reducing power of the digest was

determined by Hanes's method after suitable preliminary dilution and precipitation of proteins with "colloidal iron" and acetic acid.

An osazone was also made. In all the cases tested (the perfused liver preparation which was all employed in the glycogen hydrolysis was not tested) some glucose was formed, showing that all these preparations contain maltase. It must be emphasized, however, that the amount of liver preparation employed in this test was relatively very much greater than that used in the subsequent hydrolyses. The capacity of these preparations to form glucose from maltose might by due to the maltase activity of the liver tissue itself or to the maltase activity of the blood in the preparation. (A comparison of the hydrolysis by the unperfused and perfused rat liver preparations is of significance in this connection). The maltase activity of the serum of man, and of the rat, rabbit, cat and ox was tested in a similar manner to that described above, in this case employing 5 ml. serum. In the case of the rat serum, there was a quantitative conversion of maltose to glucose, an almost quantitative conversion with the ox serum, whereas human serum showed only very slight, and both cat and rabbit serum no maltase activity. These results are in general accord with those

of Barnes and Mackay (1936), who found that pig, rat, ox, dog, sheep and goat serum had marked maltase activity, cat only slight activity, whereas buman, monkey, rabbit, guinea pig and pigeon serum had negative activity. These results explain why neither maltose nor glycogen will relieve insulin hypoglycaemia in rabbits, why glycogen can overcome hypoglycaemia both from insulin and hepatectomy in dogs, and why maltose will alleviate insulin symptoms in mice.

Hynd and Macfarlane (1927) determining the maltase activity of whole blood, found that pig's blood alone gave positive results and obtained negative results with the blood of the mouse, rat, guinea pig, rabbit, kitten, ox and sheep. Compton (1921) found that the serum of the dog, goat, horse, pig, rat and sheep had positive maltase activity, whereas that of the cat, rabbit, guinea pig and man had negative activity. Tebb (1894) also found that pig serum had marked maltase activity.

The diastase activity of human and rat blood was also determined, according to the method of Baltzer (1936). 0.1 cc. blood was introduced into 1 cc. H_2O, and 1 cc. 0.9% NaCl, and 0.1 cc. saturated NaF and to this 9 cc. 0.3% glycogen solution was added. The whole was incubated at 37°C for 3 hours. A control experiment was set up which, after the addition of 5 cc. $ZnSO_4$ solution (Hagedorn and Jensen) and 1 cc. $\frac{N}{10}$ NaOH, was left in a

refrigerator for the same time. After 3 hours, the $ZnSO_4$ and NaOH was added to the incubated tube and both tubes heated for 3 minutes in boiling water, after which the sugar in each was determined by the Hagedorn and Jensen method, employing 6 cc. of the ferricyanide reagent.

The results showed that the human blood employed contained no diastase whereas rat blood contained a considerable amount.

COURSE OF DIGESTION OF GLYCOGEN BY LIVER AMYLASE.

Digestion mixtures were set up containing 1.0 g. glycogen, (Kahlbaum), in 160 ml. 0.1% NaCl, 40 ml. phosphate buffer pH 6.4, 1.0 g. liver preparation and 1 ml. toluene. A control mixture was set up omitting the glycogen. The hydrolysis of potato starch was also followed in some cases. The digestion mixtures were placed in an incubator at 37° and samples removed, boiled filtered and analysed at intervals up to 3 days. To test Carruthers' criticism of Barbour's work, 0.25 g. glycogen dissolved in 30 ml. 0.1% NaCl, 10 ml. glycerol, 10 ml. phosphate buffer pH 6.4 and 0.25 g. pig liver preparation were incubated for 17 hours.

The following estimations were performed:-

(1) Unchanged polysaccharide.

(2) Total reducing power.

(3) Reducing power due to sugar alone.

(4) " " after acid hydrolysis.

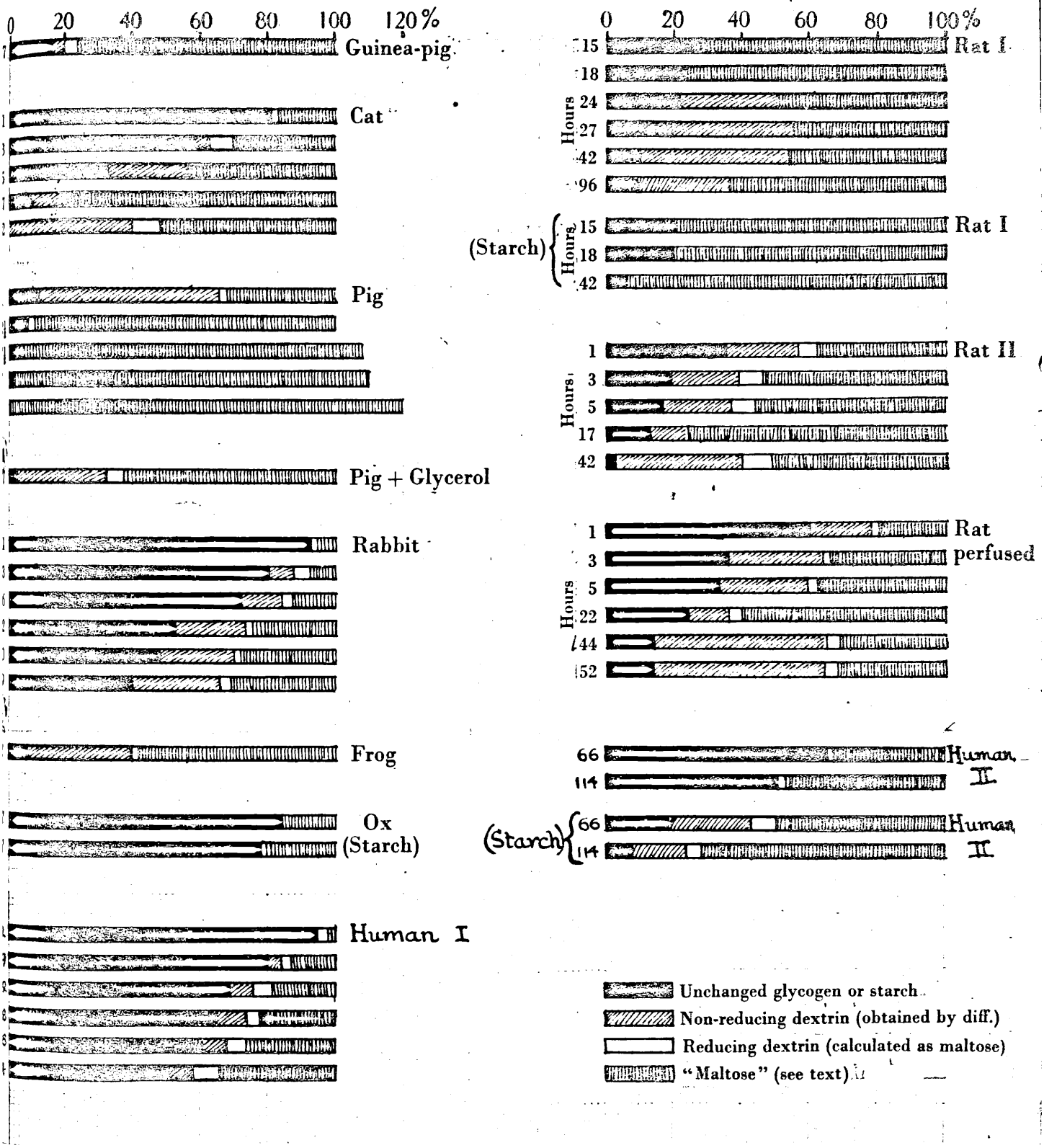
(5) Lactic acid.

The unchanged polysaccharide was estimated as described for glycogen in Part I (p. 36). The sugar determinations in each case were performed according to the ferricyanide method of Hanes (1929). The total reducing power was determined on 5 ml. samples of a 1 in 10 dilution of the digest. A further 10 ml. of the digest was diluted to 50 ml. with absolute alcohol, to precipitate any unchanged glycogen and dextrins, left for two to three hours or longer and filtered. Two 20 ml. portions of the filtrate were taken and the alcohol removed by evaporation. The reducing power of one portion was determined directly after suitable dilution and of the other after acid hydrolysis, followed by subsequent neutralization and dilution. 20 ml. of the digest were employed for the estimation of lactic acid, by the method of Friedemann and Graeser (1933). This 20 ml. of digest was introduced into a 100 ml. flask, treated with 10 ml. $CuSO_4$ solution and 10 ml. lime

suspension, and, after making up to the mark and leaving for ½ hour, centrifuged and the lactic acid determined in 20 ml. portions of the centrifugate. (Lactic acid determinations were not made in the early stages of hydrolysis). In each case an osazone was made after alcohol precipitation and subsequent evaporation of the alcohol. The results are given in Tables 9-17, and are represented diagrammatically in Fig. 2. The reducing sugar for convenience is calculated and expressed as "maltose" although in some cases the sugar was known to consist partly or entirely of glucose. In addition two graphs (Graphs 3 and 4) are given.

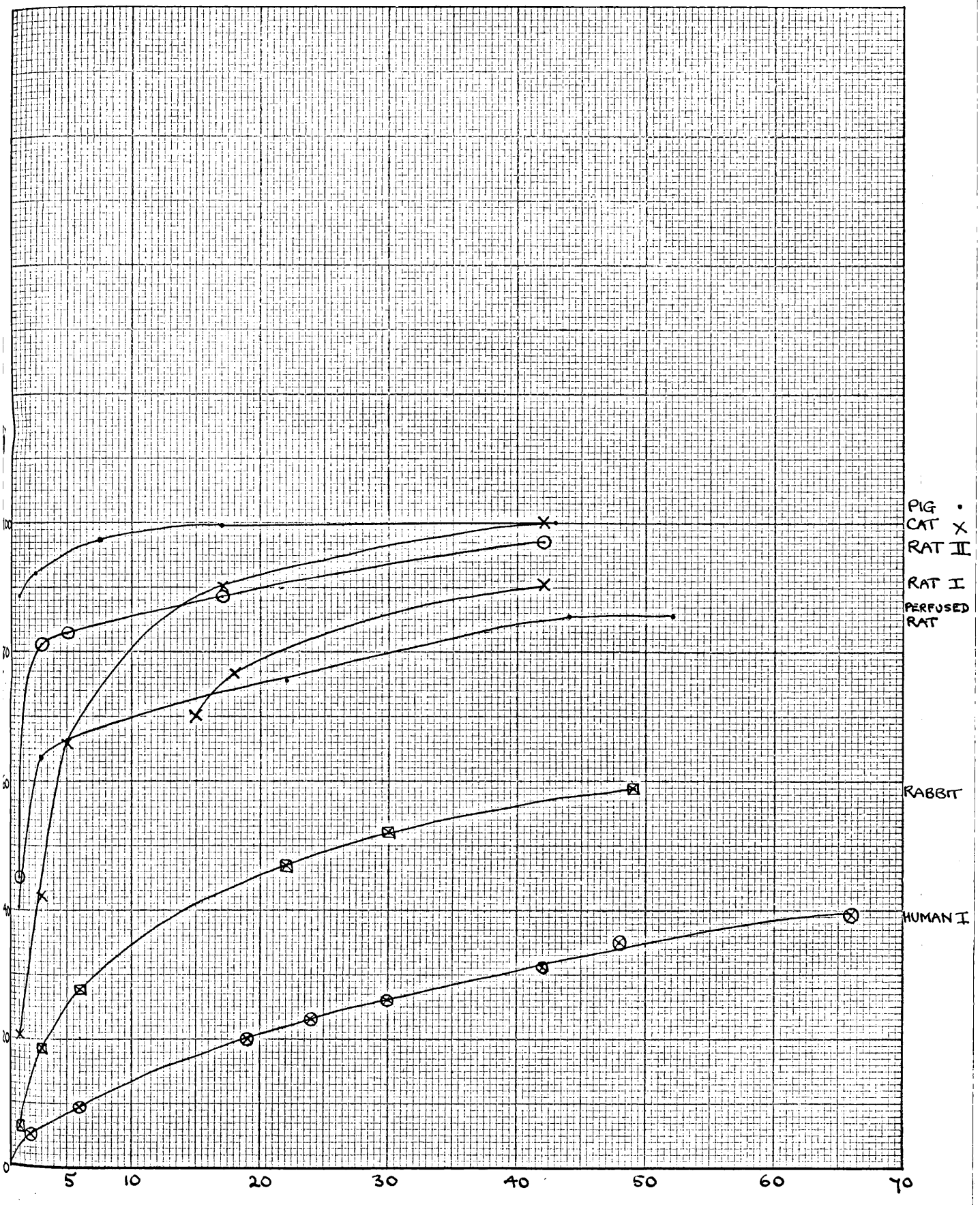
Figure II.

LIVER AMYLASE



Graph III

RATE OF DIGESTION OF GLYCOGEN BY LIVER AMYLASE.



Graph IV

RATE OF FORMATION OF MALTOSE BY LIVER AMYLASES.

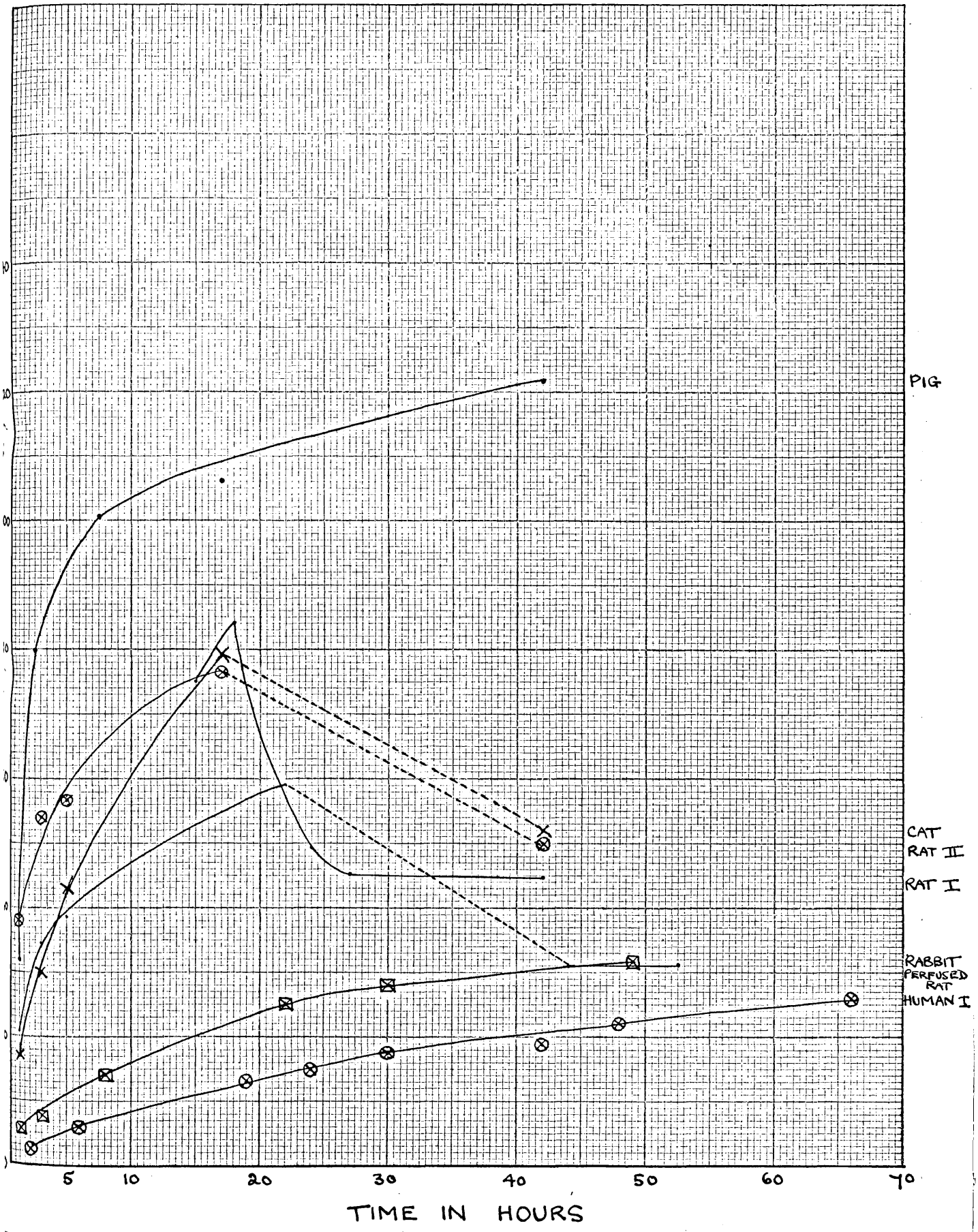


TABLE IX

Substrate: Glycogen

Enzyme: Human Liver Glycogenase I

Time in Hours	2	6	19	24	30	42	48	66	114
% Polysacch. gone	5.0	9.5	20	23	26	31.2	35	39.3	49.3
% Polysacch. left	95	91.5	80	77	74	68.8	65	60.7	50.7
% Formation of Total Reducing substances calcd as Maltose	3.4	6.4	13.5	17.4	18.1	20	23.3	28.4	36.4
% Formation Sugar calcd as Maltose	2.5	5.9	12.9	15	17.3	18.6	21.9	26	33.7
% Formation Glucose on hydrolysis calcd from Maltose					100		99	102	99
% Formation Lactic Acid							7.7		
Evidence for Glucose as given by Osazone	No	No	No	No	No	No	Some	Yes	Yes
Maltase Activity of Liver Preparation		Slight							
Maltase activity serum		Very slight							
Diastase Activity Blood		Negative							

TABLE X

Substrate — Glycogen and Potato Starch

Enzyme — Human liver glycogenase II

Time in Hours	66		114	
% Polysaccharide gone	36.66	81.61	48.93	91.84
% Polysaccharide left	63.34	18.39	51.07	8.16
% Formation of Total Reducing substances calcd. as Maltose	35.37	56.52	47.33	76.4
% Formation Sugar calcd. as Maltose	33.52	49.3	47.32	72
% Formation Glucose on hydrolysis calcd. from Maltose	98	97	100	96
Evidence for Glucose as given by Osazone			Some	Yes

TABLE XI

Substrate: Glycogen

Enzyme: Cat Liver Glycogenase

Time in Hours	1	3	5	17	42
% Polysaccharide gone	20.6	42.0	67.7	90.8	100
% Polysaccharide left	79.4	57.9	32.3	9.7	0
% Formation of Total reducing substances calcd as Maltose	18.3	37.5	48.1	81.3	61.2
% Formation Sugar calcd as Maltose	16.9	30.1	43.0	79.4	52.1
% Formation Glucose on acid hydrolysis calcd from Maltose		119		106	96
% Formation Lactic Acid					4.76
Evidence for Glucose as given by Osazone			None	None	None (All ferms)
Maltase activity serum	Negative				
Maltase activity of Liver preparation	Slight				

TABLE XII

Substrate: Glycogen

Enzyme: Pig Liver Glycogenase (+glycerol)

Time in Hours	1	2½	7½	17	42	17
% Polysaccharide gone	88.9	92.3	97.5	99.7	100	95.8
% Polysaccharide left	11.2	7.7	2.5	0.32	0	4.2
% Formation of Total Reducing substances calcd as Maltose	33.5	93.1	117	131 92.4	101	67.7
% Formation Sugar calcd as Maltose	31.8	89.8	106	100.5 92.12	121.5 101	62.2
% Formation Glucose on hydrolysis calcd from Maltose			98	100	100	129
% Formation Lactic Acid			1.43	1.44	1.53	
Evidence for Glucose as given by Osazone	No	No	Yes	Yes	Yes	No

N.B. Figures in red are calculated as glucose and not as maltose

TABLE XIII

Substrate: Glycogen

Enzyme: Rabbit Liver Glycogenase (Ler prep.)

Time in Hours	1	3	6	22	30	49
% Polysaccharide gone	6.3	18.6	27.5	46.6	51.9	59.1
% Polysaccharide left	93.7	81.4	72.5	53.4	48.1	40.9
% Formation of Total reducing substances calcd. as Maltose	5.9	10.4	16.3	26.8	29	34.2
% Formation Sugar calcd. as Maltose	5.59	7.45	15.9	25.1	27.9	31.6
% Formation Glucose on Acid Hydrolysis calcd. from Maltose	130	136	109			99.2
Evidence for Glucose as given by Osazone				No	No	No
Maltase activity rabbit serum	Negative					
Maltase activity liver preparation	Slight					

TABLE XIV

Substrate	Glycogen					Potato starch		
Enzyme	Rat Liver Glycogenase I					Rat Preparation I		
Time in Hours	15	18	24	27	42	15	18	42
% Polysacch.. gone	69.8	76.7	-	85.4	90.7	79.1	79.9	93.8
% Polysacch. left	30.2	23.3	-	14.6	9.3	20.9	20.1	6.2
% Formation Total reducing substances calcd as Maltose	77.6	86.7	50.4	47.1	45.3	79.0	80.0	95.1
% Formation Sugar calcd as Maltose	74.4	84.2	49.3	45.3	44.6	76.0	79.0	93.0
% Formation Glucose on acid hydrolysis of sugar	116	108			124	113	105	100

TABLE XV

Substrate: Glycogen

Enzyme: Rat Liver Glycogenase II

Time in Hours	1	3	5	17	42
% Polysaccharide gone	64.9	80.0	82.6	88.7	97.1
% Polysaccharide left	35.1	20.0	17.4	11.3	2.8
% Formation Total reducing substances calcd as Maltose	44.3	61.0	63.4	78.2	60.2
% Formation Sugar calcd as Maltose	37.7	54.3	56.4	76.3	50
% Formation Glucose on hydrolysis calcd from Maltose	111	117	99	97.6	93
% Formation Lactic Acid	-	-	1.12	1.48	1.64
Evidence for Glucose as given by osazone	No	No	Yes	Yes	Yes (½ Forms)
Maltase activity serum	Positive.	All maltose → glucose			
Diastase activity blood	Positive				

TABLE XVI

Substrate Glycogen

Enzyme Rat Liver Glycogenase (Perfused i.e. blood-free)

Time in Hours	1	3	5	22	44	52
% Polysaccharide gone	40.4	63.7	66.1	75.7	85.6	85.6
% Polysaccharide left	59.6	36.3	33.9	24.3	14.4	14.4
% Formation Total reducing substances calcd as Maltose	22.9	35.9	45.9	64.2	35.5	36.4
% Formation Sugar calcd as Maltose	20.7	34.5	38.4	59	31.1	31.5
% Formation Glucose on Acid Hydrolysis calcd from Maltose				102	100	99
Evidence for Glucose as given by Osazone				Trace only	No Usual	No Usual branched

TABLE XVII

Substrate	Glycogen	Glycogen	Potato	Glycogen
Source of liver preparation	Guinea pig	Ox	Ox	Frog
Time in Hours	17	17	17	17
% Polysaccharide gone	83.8	15.4	21.9	91.7
% Polysaccharide left	16.2	84.6	78.1	8.3
% Formation Total reducing substances calcd. as Maltose	80.7	15.1	20.6	60.5
% Formation Sugar calcd. as Maltose	76.2	14.6	19.7	58.4
% Formation Glucose on acid hydrolysis	105	99.6		
% Formation Lactic Acid	1.05	0.87		2.5
Maltase activity of serum	-	Positive	Positive	-

DISCUSSION OF RESULTS.

From Fig. 2 it appears that in the breakdown of glycogen to sugar there is no appreciable formation of reducing dextrin, since the reducing power of the digest after alcohol precipitaion of dextrins and unchanged glycogen is only very slightly different from that before precipitation. It would seem, therefore, that in the hydrolysis by liver amylase, sugar is chiefly split off successively from the glycogen molecule and is only formed to a much smaller degree from simple dextrins produced intermediately. In this respect the action of liver amylase is different from that of salivary, malt and pancreatic amylases.

From the results of the hydrolysis of potato starch by liver amylase it is seen that starch is broken down rather more easily than glycogen, but this difference between the rates of breakdown of the two polysaccharides by liver amylase is not nearly as marked as with salivary, pancreatic and malt amylases.

In all cases the formation of lactic acid was insignificant, the amount produced varying from 0.87% to 4.76%.

Three different types of osazone were observed.

The first was the "isomaltose" type consisting of relatively large rosettes of very fine needles, sometimes tufted at their free extremities. This type of osazone was observed in the early stages of hydrolysis with all the liver preparations and throughout the hydrolysis with the cat, rabbit, and perfused rat liver preparations, with the pig liver preparation in the presence of glycerol and generally in addition to glucosazone in the later stages of hydrolysis with the rat and human liver preparations. The second type of osazone (or hydrazone?) was one not previously observed. It consisted of large pale yellow fern-like crystals. Owing to their pale yellow colour and to the fact that they were relatively insoluble in hot water, it was considered possible that the crystals might be those of an hydrazone and not of an osazone. The osazone was generally found after 42 hours' hydrolysis with the cat and rat liver preparations. The third type of osazone, glucosazone, was the only product formed by the pig liver preparation after 17 hours and longer, and was also formed by the rat liver preparation, but in the latter case an additional osazone was always obtained.

The results of acid hydrolysis corroborate the view that glucose is the sole product of hydrolysis with

the pig liver preparation. The first type of osazone is probably produced by maltose, perhaps modified in crystalline form by admixture with unprecipitated dextrin, since the amount of glucose formed on acid hydrolysis in the later stages of hydrolysis was 100-110% of the theoretical yield.

If graphs are plotted of the % formation of sugar calculated as maltose against time it is seen that with the pig, human and rabbit liver preparations the formation of reducing sugar follows a normal hyperbola, whereas this is not true with the rat (perfused and unperfused) and cat liver preparations. In the latter cases there is a sudden fall in the reducing power which finally rises slowly after 42 hours. This type of hydrolysis curve would appear to be due to the reversible action of the liver amylase, the breakdown phase being predominant in the early stages of digestion and the synthetic phase in the later stages, probably initiated by accumulation of the hydrolysis products of the breakdown phase. The possible criticism that the decrease in the reducing power in the later stages might be due to the formation of hexose-phosphates is disproved by the fact that a similar type of curve was obtained using the rat liver preparation but replacing the phosphate buffer by a citrate buffer of the same pH. This decreased reducing

power also cannot be accounted for by increased lactic acid formation, for in no case was the percentage formation greater than 5.

It is interesting to note, in this connection, that the second type of osazone described above was only observed in the 42 hours' digest with the rat and cat preparations and is, therefore, probably correlated with the decreased reducing power. The breakdown of potato starch by rat liver preparations, however, follows a normal course.

The conversion of glycogen into glucose by liver preparations depends on the concentration of amylase and maltase. If both enzymes are very active, it is possible to get 100% conversion into glucose: this is the case with the pig liver preparation. If the concentration of either amylase or maltase is reduced, the yield of glucose will be less: this seems to be the case with the rat and human preparations. If the concentration of maltase is very small, the breakdown will stop at the maltose stage and will not proceed as far as glucose. It was shown, as already described, that all the preparations possessed maltase activity. In the case of the cat and rabbit preparations this activity must have been much less than that of the pig and rat preparations, so that in the amount of

preparation which was used in the glycogen hydrolysis, the maltase concentration was negligible. If the maltase activity of the preparation were due to the blood it contained, then one would suppose that after perfusion of the liver to render it blood-free, the breakdown of glycogen would not proceed further than the maltose stage. This was found to be true with the dried preparation from the perfused rat liver.

That the end product in this case was not glucose was further substantiated by the fact that after alcohol precipitation and subsequent evaporation of the alcohol, the liquid was not fermented by a yeast, known to be incapable of fermenting maltose. In the case of the rat, the maltase activity of the serum was found to be so great that the ability of the unperfused liver preparation to form glucose might easily be attributed to the maltase activity of the blood. It is doubtful, however, whether this is true of the pig liver preparation since in this case there was normally 100% conversion into glucose. (That perfused pig liver preparations still possess marked maltase activity has been shown by Tebb 1894). The maltase activity of human serum, though very slight, may be sufficient to account for the formation of glucose by the liver preparation, since

glucose was not formed until after 48 hours incubation. The breakdown of glycogen would also stop at the maltose stage if the maltase activity of the preparation were inhibited, for example by the method of preparation. The pig liver preparation, in the presence of glycerol, failed to produce any glucose after 17 hours. This also supports Carruthers' opinion of Barbour's work. In spite of the fact that glycerol in some way depresses enzymic activity, it is still extensively used. Winter (1937) recently used glycerol extracts of heart muscle to ascertain the breakdown products of glycogen by heart glycogenase, and obtained results similar to those of Barbour with muscle.

From the values of the reducing power before and after acid hydrolysis, the relative amounts of glucose and maltose at each stage of digestion can be calculated. At the end of 17 hours the sugar formed consisted in the case of the pig preparation of 16% maltose and 84% glucose and in the case of the rat, guinea-pig and ox liver preparations of 93% maltose and 7% glucose, 91% maltose and 9% glucose and 79% maltose and 21% glucose, respectively. After 42 hours the values were for the pig preparation 100% glucose, for the rabbit 100% maltose and for the rat 88% maltose and 12% glucose. In the case of the human liver preparation, there was 81% maltose and 19% glucose

after 66 hours and 32% maltose and 68% glucose after 114 hours. The necessity for estimating the reducing power both before and after acid hydrolysis in order to form an opinion as to the nature of the sugar produced is obvious when the results obtained with the pig preparation are considered: after 7½ hours only 15% of the sugar was glucose, after 17 hours 84%, whereas at 42 hours there was a 100% yield.

A final 100% yield of glucose does not necessarily mean that no maltose is produced intermediately. Hodgson (1936) claims that glucose is the sole product of hydrolysis of glycogen by rabbit liver preparations, without having performed any acid hydrolyses. It is obvious from the above results that such an assumption is unfounded.

The obvious criticism of these results is that the acetone extracted liver preparations employed in this investigation do not behave in the same way as intact liver. Consequently, further experiments were done to ascertain what sugars are normally formed on autolysis of fresh liver.

50 g of liver were ground up with sand and 100 cc. water, 1 ml. of toluene added and the whole incubated at 37°C for 2½ hours. This was then filtered through muslin and to 15 ml. of the filtrate were added 5 ml. of a cold

saturated solution of Na_2SO_4 in 2% acetic acid and 10 ml. of 20% colloidal iron. This was mixed and brought to the boil, allowed to stand for a few minutes and then filtered through a Whatman filter (No.30). Aliquot portions of the filtrate were used for the determinations of the reducing power before and after acid hydrolysis. An osazone was also made. Liver from the sheep, ox, cat and rabbit was employed. In all four cases both maltose and glucose were formed in different proportions. With the ox liver there was 72% maltose and 28% glucose and with the sheep liver, 23% maltose and 77% glucose. These figures with the ox liver agree very well with the corresponding figures obtained with the dried ox liver preparation (See Table 17). It has already been stated that all the dried liver preparations, when used in high concentrations, exhibited maltase activity and it is therefore not surprising that both glucose and maltose were formed on autolysis of the rabbit and cat livers, although no glucose was detected in the corresponding glycogen hydrolyses by the dried liver preparations, where much smaller quantities of the preparation were employed. Whether glucose or maltose is the chief hydrolysis product must depend on the relative proportions of amylase and maltase

in the liver preparation.

The results of these autolysis experiments are not in accord with the generally accepted view that liver glycogen is hydrolysed to glucose in the body without the intermediate formation of maltose, or that glucose is the sole end product of glycogen hydrolysis in an excised liver.

S U M M A R Y.

1. The hydrolysis of glycogen, and in a few cases of potato starch, by acetone-extracted and dried liver preparations of man and of the rat, cat, rabbit and pig in the presence of phosphate buffer of pH 6.4, was followed. The course of hydrolysis of glycogen was also followed using a perfused rat liver preparation and in addition the behaviour of the pig liver preparation in the presence of glycerol was studied.

2. The maltase activity of each liver preparation was tested, in all cases with a positive result. The maltase activity of the serum was also tested. That of the rat serum was found to be very great, that of the ox quite large, of human only very slight, whereas cat and rabbit serum possessed no maltase activity.

3. In the case of the pig liver preparation only, was there quantitative conversion to glucose. This formation of glucose is inhibited by the presence of glycerol.

4. Maltose was the sole end product with the cat, rabbit and perfused rat preparations.

5. The unperfused rat liver preparation and the human liver preparation produced maltose alone in the early stages

of hydrolysis, but this was gradually converted into glucose as digestion proceeded.

6. In the case of the rat (perfused and unperfused) and cat liver preparations the reducing power showed a steady decrease from 17 to 42 hours. Since there was no additional lactic acid formation, this phenomenon can only be explained by a reversal of the enzymic activity.

7. That the production of glucose by the rat liver preparation could be due to the maltase contained in the blood has been proved since no glucose was produced by the perfused rat liver preparation.

8. The normal autolysis products of the excised liver of the ox, sheep, rabbit and cat were investigated. In all cases glucose was not the sole product, both maltose and glucose being produced. This is not in accord with the usually accepted views.

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CXCVII. AN INVESTIGATION OF THE RATES OF DIGESTION OF STARCHES AND GLYCOGEN AND THE BEARING ON THE CHEMICAL CONSTITUTION.

I. ACTION OF AMYLASES ON STARCHES AND GLYCOGEN.

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STARCHES from different sources show very different rates of hydrolysis by amylases. This is presumably due to differences in the composition of the starch granules. Dietetically, however, starches are usually considered of equivalent food value, provided that they all undergo complete digestion. Having found that potato and rice starches are hydrolysed at very different rates, as judged by the achromic point, and being unable to find in dietetical and chemical literature information concerning this, we decided to investigate the rates of digestion and of the formation of the intermediate hydrolysis products of the starches which are incorporated in our diet. Glycogen was later included in the investigation. In this way it was hoped to gain information concerning the differences in chemical constitution of the different starches and their relative digestibilities.

Concerning the chemical structure of starch little need be said here. The work of Samec [1927], Ling & Nanji [1923], Schardinger [1909; 1911], Pringsheim & Wolfsohn [1924] all supports Maquenne & Roux's [1906] theory that the starch granule consists of two constituents (termed by these workers amylopectin and amylose). The work of Karrer & Nägeli [1921, 1, 2] and of Haworth [1931; 1932; 1935], Hirst *et al.* [1932], on the other hand, supports the now more generally adopted view that starch is homogeneous in structure. Amylose and amylopectin are reported to be formed by successive depolymerization and repolymerization of natural starch and their distinctive characteristics to arise from differences in physical character or from admixture with other substances.

Most workers consider that the unrestricted action of maltase-free amylases on starch leads to the formation of one sugar only, namely maltose, and that by the prolonged action of amylase there is probably at least 80% conversion into α-glucosidic maltose. Certain early workers, however, namely Lintner & Düll [1892; 1893] considered that *iso*maltose is formed in addition to maltose. *iso*-Maltose, as prepared by these workers by the action of malt diastase on potato starch, has a reducing power of 80% of that of maltose, $[\alpha]_D$ 140°, and forms an osazone of M.P. 151° which crystallizes in spherical groups of very fine light yellow needles and is very much less soluble in water and alcohol than maltosazone. Syniewski [1902] confirmed the above work and claimed to have isolated pure *iso*maltose (termed by this worker "dextrinose"). Brown & Morris [1895] showed that Lintner's *iso*maltosazone is not a separate chemical entity but is only maltosazone altered in crystalline form and M.P. by admixture with an amorphous osazone of a simple dextrin. Ling & Baker [1895] are also of this opinion. Pottevin [1899] interpreted the amylolytic hydrolysis of starch

as due to partial hydrolysis of the starch granule by means of an enzyme "amylase", into a series of dextrins, ranging from amylodextrins, giving a blue colour with iodine and exhibiting slight Fehling's reducing properties, to achroodextrins or simple dextrins, giving no colour with iodine and having high Fehling's reducing properties. A second enzyme "dextrinase" then converts these dextrins into maltose. According to Chrząszcz [1911], an amylase contains separate depolymerizing, dextrinizing and saccharifying enzymes, which view was later supported by Pringsheim [1924], but this assumption does not seem to be essential. Nishimura [1928] regards amylases as being composed of one enzyme only, which saccharifies and depolymerizes, the latter notably in the presence of a co-enzyme. Olsson [1923] showed that the saccharifying enzyme is different from the liquefying enzyme because the addition of certain poisons reduced the liquefying action without affecting the saccharifying power. Kendall & Sherman [1910] found that the liquefying action is predominant with pancreatic amylase and the saccharifying action with malt amylase. These two enzyme components have now been successfully separated by Van Klinkenberg [1934]. Karrer [1921] considered diastatic action to consist of two phases, depolymerization and hydration, the latter phase involving the hydrolysis of maltose anhydride to maltose. The saccharification of depolymerized amylose and amylopectin does not proceed at the same rate. Amylose smoothly and quickly gives rise to the theoretical amount of maltose, whereas the saccharification of amylopectin is slower and usually ceases when about 78 % of the theoretical yield of maltose is produced. The remaining residual dextrin can, according to Pringsheim & Beiser [1924] be converted into maltose if a co-enzyme (present in yeast) is added. Kuhn [1924] divided amylases into two groups, the α- and β-amylases according to the point of attack of the starch molecule. α-Amylases produce α-glucosidic maltose as the end-product of starch degradation and β-amylases produce β-glucosidic maltose initially. Amylases from animal sources and takadiastase are α-amylases and vegetable amylases, with the exception of takadiastase, are β-amylases. Kuhn concludes that in view of the behaviour of the starch granule towards plant and animal amylases, the starch molecule consists of alternating α- and β-glucosidic linkages. If both α- and β-glucosidic groupings occur in the starch molecule it should be possible to obtain glucose from starch if both α- and β-amylases are made to act on starch paste. Pringsheim & Liebowitz [1925] showed this to be true. Ohlsson [1930] showed that his "dextrinogenamylase" from malt was identical with Kuhn's α-amylase and his "saccharogenamylase" with Kuhn's β-amylase. As judged from the relative permanence of the starch-iodine reaction, Ohlsson concluded that the former enzyme breaks down starch into progressively smaller molecules until maltose is produced, whereas the latter enzyme detaches maltose in successive stages. Van Klinkenberg [1932], in opposition to Kuhn, regards the α- and β-glucosidic linkages as occurring not in the same molecule but in different molecules, in α- and β-starch, respectively.

Little work has been done on the rates of digestion of different starches and the results that are quoted are very discrepant. The chief reason for this discrepancy is that two different methods are used for determining the amount of digestion that has occurred. These two methods, the achromic point and a sugar determination, are actually estimating different things. The achromic point determines when all the starch has been broken down at least as far as achroodextrin, although this does not necessarily mean that there is any sugar present. A sugar determination, on the other hand, estimates the maltose present and also any reducing dextrins, but there may at the same time be a considerable

amount of unchanged polysaccharide left. In order to get an accurate conception of the amount of starch degradation that has taken place at any time it is necessary to determine at least four factors; the achromic point, the unchanged polysaccharide, the total reducing power and the reducing power due to maltose alone. O'Sullivan [1904] found that a much lower percentage of maltose was formed by the action of malt amylase on potato starch than on cereal starches. Stone [1904] found that potato starch was more readily digested by pancreatin than were cereal starches. Ford [1904] showed that when rice, barley, maize, wheat and potato starches were purified by the same method, they were digested equally by malt extracts. Nagao [1911] found that wheat and rye starches were hydrolysed at equal rates by pancreatic amylase and that oat and barley starches were digested at approximately the same rate, but that the rate of hydrolysis of the latter two was considerably higher than that of wheat and rye. Sherman *et al.* [1919] showed that, when similarly purified by washing with dilute alkali to extract any gluten, wheat, maize and rice starches were hydrolysed at equal rates. Potato starch generally showed a rate of hydrolysis equal to or slightly greater than the cereal starches. These experiments were performed with both pancreatic and salivary amylases. The percentage hydrolysis was determined in each case at the end of 30 min. by estimating the reducing sugar by Fehling's method.

Concerning the breakdown of glycogen, Musculus & Mering [1879] showed that glycogen gave the same hydrolysis products as starch. Cremer [1894] found that the amylolytic hydrolysis of glycogen was much slower than starch. Tebb [1897] found that when glycogen was digested by ptyalin, amylopsin and malt diastase, both maltose and *iso*maltose were formed. Osborne & Zobel [1903] found that both pancreatic and malt amylases formed *iso*maltose alone as the end-product, whereas both takadiastase and salivary amylase produced glucose in addition to *iso*maltose. Horton [1913] showed that the hydrolysis of glycogen by pancreatic amylase proceeded much faster in the early stages, but the subsequent breakdown of dextrin was very slow and there was seldom a quantitative conversion into maltose. Pringsheim's view that amylopectin and glycogen are identical was further substantiated by the work of Pringsheim & Beiser [1924] who showed that a co-enzyme was necessary for the complete hydrolysis of glycogen, just as in the case of amylopectin. Barbour [1929] showed that with salivary amylase the chief end-product was *iso*maltose, and there was also occasionally glucose present, but never any maltose. With pancreatic amylase, both *iso*maltose and glucose were formed. He found that there was a serial degradation of glycogen with pancreatic amylase, the increased reducing power corresponding with disappearance of glycogen, whereas with salivary amylase there was no such correspondence.

In view of these rather discordant results and conclusions the present investigation was carried out using potato, maize, wheat and rice starches (all of these having been obtained from B.D.H.) and glycogen (Kahlbaum).

The ash and P contents of all the samples were determined; as a further test of purity nitrogen analyses were performed but the results were negative in all cases. The values obtained are given in the following table, all the estimations being performed on the dried material.

Polysaccharide	% Ash	% P
Maize starch	0·084	0·055
Wheat starch	0·185	0·142
Potato starch	0·285	0·119
Rice starch	0·284	0·068
Glycogen	1·100	0·298

The ash in each case gave a positive iron reaction. The P was determined by Neumann's method using 0·5–2·0 g. of dried material. The results are of the same order as those obtained by Samec & Haerdtl [1920] and the higher value obtained for glycogen substantiates the generally adopted view that glycogen has a higher electrolyte content than starches.

Achromic point.

The achromic points were determined for each polysaccharide, using a 1% solution and hydrolysing it in turn with saliva, takadiastase, malt diastase and a solution of "Holadin" (a pancreatic preparation particularly rich in amylopsin) at the appropriate optimum *p*H. Whichever amylase was used, the achromic points were always in the same order, this point being reached very much more quickly with potato starch and glycogen than with rice, wheat and maize starches, these latter three, however, all giving the achromic point at approximately the same time.

	Starch				
Amylase	Potato sec.	Maize hours	Rice hours	Wheat hours	Glycogen
Salivary	20	1·5	1·5	1·5	Few sec.
Pancreatic	5	2·25	2·25	2·25	Immediate
Takadiastase	20	4	4	4	Few sec.
Malt	240	3	3	3	2 min.

As will be seen from the following results, the achromic point is a very unreliable index of the amount of digestion that has taken place.

According to Van Klinkenberg [1934] iodine-colouring atomic groups are present in α-starch only and the iodine reaction therefore only disappears by the action of α-amylase.

Rate of degradation of the different starches and of glycogen.

The course of the enzymic hydrolysis of glycogen and of each of the starches was followed using pancreatic amylase ("Holadin"). The salivary and malt digestions of wheat starch and glycogen were also followed.

The maltase activity of all the enzyme preparations was tested by incubating 2% maltose solutions with relatively large amounts of the enzyme and testing for glucose by an osazone test after 3 days. Except with the malt preparation, glucose was produced in every case. On diminishing the concentration of enzyme, however, no glucose was formed even with concentrations of enzyme ten times as great as those used in the subsequent hydrolyses. It is concluded that these enzyme preparations contain only traces of maltase which are ineffective in the low concentrations in which they were employed and would therefore be overlooked in most cases.

The following estimations were performed:

(1) estimation of unchanged polysaccharide;

(2) estimation of total reducing power using the Wood-Ost method;

(3) estimation of reducing power due to sugar alone using the same method.

In the later experiments Hanes's [1929] method of sugar estimation was substituted for that of Wood-Ost. From these data it is possible to calculate the amount of polysaccharide that has been broken down, the amounts of reducing dextrin and maltose formed, and, by deduction, the amount of non-reducing dextrin.

These three estimations were performed on samples of the digest immediately after mixing and 15, 30, 60 or 90, 120, 180, 330 min., 24 or 72 hours after the beginning of the experiment.

An osazone test was also performed on samples from each digest, after alcohol precipitation of any residual starch and dextrins followed by evaporation of the alcohol from the filtrate. In no case was a typical maltosazone observed. Most frequently the osazone consisted of relatively large rosettes of very fine needles but very often these needles were tufted at their free extremities. It seems impossible to place any reliance on the appearance of the osazone; either the crystalline form typical of maltosazone had become modified by admixture with another substance (perhaps dextrins which had not been completely removed by alcohol precipitation) or else maltose is not the sole product of hydrolysis.

Different methods were employed for the estimation of starch and glycogen.

The starch was estimated by a modification of the von Fellenberg method [1916]. This method depends on the principle that starch dissolves in concentrated $CaCl_2$ solution, from which it can be precipitated by iodine. Protein, which might be present in small amounts, is insoluble in $CaCl_2$ solutions and by dextrins are not precipitated from $CaCl_2$ solutions by iodine.

The digestion mixture in the case of the pancreatic digestion consisted of 225 ml. of 1 % starch solution in 0·5 % NaCl, 70 ml. of borate[1] buffer of *p*H 6·8 and 5 ml. of 0·1 % solution of holadin in distilled water. This mixture was left in a thermostat at 37° and samples were removed at the time intervals stated above.

The total reducing power was determined by the Wood-Ost method, introducing 5 ml. of the digest into 20 ml. of the Wood-Ost solution. This was performed in duplicate.

In order to determine the reducing power due to maltose alone, 10 ml. of the digest were removed, boiled to inactivate the enzyme, cooled and made up to 50 ml. in a graduated flask with absolute alcohol. This was left overnight, filtered and 20 ml. portions of the filtrate used for the Wood-Ost determination. In many cases a further estimation was carried out. A portion of the alcohol filtrate, which should contain maltose alone, was heated on a water-bath to drive off all the alcohol and the reducing power determined after acid hydrolysis. This should indicate whether maltose is the sole sugar formed.

Furthermore, in a few cases, polarimetric determinations were carried out on the alcohol filtrates, after the alcohol had been driven off and the subsequent solution considerably concentrated and the turbidity removed, if necessary, by the addition of a small amount of basic lead acetate.

At the end of each experiment the digest was tested for lactic acid by Uffelmann's reagent. This test was in all cases negative.

The digestion mixture for the glycogen hydrolysis was the same as for the starches. The total reducing power and the reducing power due to sugar were determined in exactly the same way as described for starch. In the determination of the residual glycogen, the observations of Good *et al.* [1933], that the optimum concentration of alcohol for the precipitation of glycogen is 50 % and of Osterberg [1930] that this precipitation is assisted by the use of Na_2SO_4, which carries down mechanically the precipitated glycogen with the precipitate of Na_2SO_4, were made use of. For the glycogen determinations 3 ml. samples of the digest were employed and the glucose, after acid hydrolysis was estimated by Hanes's method.

[1] Phosphate buffers cannot be substituted for borate buffers, since it was found that boiling the starch and phosphate mixture with $CaCl_2$ led to the complete disappearance of the starch.

The results are represented diagrammatically (Fig. 1) and in addition three graphs are given plotting respectively the percentage disappearance of polysaccharide, the percentage formation of reducing dextrin and maltose, and the

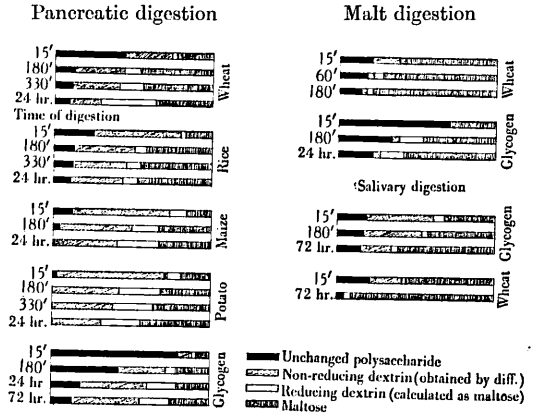

Fig. 1. Representing the formation of the products of digestion of starches and glycogen.

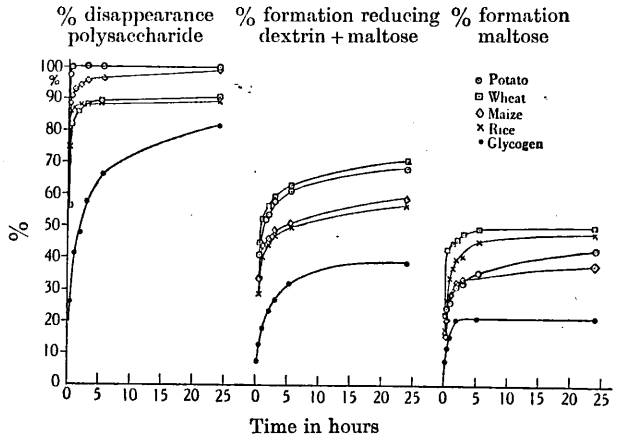

Fig. 2. Digestion of starches and glycogen by pancreatic amylase.

percentage formation of maltose against time. (The time axis is shortened for economy of space.)

Nature of sugar formed on hydrolysis.

As has already been stated most observers agree that maltose is the sole sugar formed on hydrolysis of starch or glycogen by amylases. The present results are not entirely in agreement with this. The same sugar was formed from all the starches and from glycogen but as judged from the appearance of the osazone, the amount of glucose formed on acid hydrolysis and the optical rotation of the sugar, this sugar could not be identified with maltose. The appearance of the osazone has already been described. On acid hydrolysis of the digest, after precipitation of the unchanged polysaccharide and dextrins with alcohol, 120–140% yield of glucose was obtained (assuming that the sugar before

hydrolysis was maltose). This means that the reducing power of the sugar before acid hydrolysis was only 70–85 % that of maltose. In no case, as judged by the osazone was any glucose present, but if any were present this would mean that the other sugar would have a reducing power even further removed from that of maltose than the value given above.

In a few cases only was the optical rotation determined, but when this was done it was invariably found that there was lack of agreement between the optical rotation and the reducing power (assuming the sugar to be maltose only). The optical rotation was in each case 124·5 % that of maltose. This value is very similar to that given for α-maltose, but this possibility is excluded because no mutarotation was observed after treatment with alkali. The constancy of this figure obtained both from potato and wheat starches at different stages of hydrolysis indicates that only one sugar could be present. If a mixture of sugars were present, it is extremely unlikely that they would be present in exactly the same proportions under different conditions. These results might be explained by the presence of reducing dextrins, but since the final concentration of alcohol before filtration of the unchanged polysaccharide was 80 % this seems improbable.

The appearance of the osazone and the reducing power corresponded fairly closely with those of *iso*maltose as described by Lintner & Düll. The specific rotatory power, however, was different. The figure given for *iso*maltose is $[\alpha]_D = 140°$, which is only very slightly different from that of maltose.

Discussion of results.

There are at least four different criteria of the rate of degradation of the polysaccharides:

(1) rate of disappearance of polysaccharide;
(2) rate of formation of maltose and reducing dextrin;
(3) rate of formation of maltose;
(4) achromic point.

The following table represents the order of these events, the polysaccharides being arranged in order of decreased speed of hydrolysis by pancreatic amylase as indicated by the different criteria.

Achromic point	Rate of disappearance of polysaccharide	Rate of formation of reducing dextrin and maltose	Rate of formation of maltose
Glycogen	Potato, maize	Wheat, potato	Wheat, rice
Potato	Wheat, rice	Maize, rice	Potato
Maize, wheat, rice	Glycogen	Glycogen	Maize
			Glycogen

It is difficult to reconcile the very quick achromic point of glycogen with its slow rate of breakdown and formation of dextrin and maltose. It must be the figures for the achromic point which are at fault, since it was found very difficult to determine this value when very weak glycogen solutions were used, the colour being only with difficulty distinguishable from that of a weak iodine solution.

It is interesting to note that in the case of glycogen, the rate of formation of maltose reached a steady state after 2 hours, although the formation of reducing dextrin and the disappearance of glycogen were still occurring. (The decrease in the 24 hours value for maltose seen in the results for wheat and maize starches is presumably due to fermentation.)

In the case of the salivary digestion of glycogen and wheat starch, although the salivary amylase was obviously relatively very much stronger than the

pancreatic amylase, the results are in the same order, the figures for glycogen being in every case smaller than those for wheat starch.

In the case of the digestion of glycogen and wheat starch with malt there is a slightly greater breakdown of glycogen than with pancreatic and salivary amylases. Malt contains chiefly β-amylase, but this does not warrant the assumption that glycogen contains a preponderance of β-linkages. The breakdown of glycogen by pancreatic, salivary and malt amylases is essentially similar in character.

These differences in the hydrolysis rates of the different starches must be due to differences in the chemical constitution of the starch granules, since they cannot be attributed to differences in the enzyme systems.

The rate of hydrolysis of all the starches is very much quicker than that of glycogen.

Factors which could influence the rate of breakdown.

(1) *Molecular dimensions of the polysaccharide.* The smaller the molecular size, the quicker one would expect the breakdown to be. But the size of the glycogen molecule is (according to Haworth) approximately half that of the starch molecule.

(2) *Proportion of amylopectin to amylose.* According to most modern workers the starch granule is homogeneous. But supposing that two constituents are present, the advocates of this theory agree with the original view of Maquenne & Roux [1906] that the viscosity of a starch solution is produced by the amylopectin. According to this view, potato starch, which gives the most viscous solution, should show the lowest rate of hydrolysis, since amylopectin is considered to be hydrolysed more slowly than amylose. This, however, is not the case, potato starch showing, if anything, a higher rate of hydrolysis in the initial stages than the other starches. The lower rate of hydrolysis of glycogen, however, could be accounted for if, as claimed by Pringsheim, glycogen consists of amylopectin.

(3) *Inorganic content of polysaccharide.* According to Samec & Haerdtl [1920] a low rate of hydrolysis can be correlated with a high P content. Potato and wheat starches have much higher P contents than the other starches, but their rates of hydrolysis are no lower. The lower rate of hydrolysis of glycogen, however, could be explained according to this theory by its high P content.

(4) *Method of breakdown.* As has already been mentioned Ohlsson considers that α-amylases break down starch into smaller and smaller molecules until maltose is finally produced whereas β-amylases detach maltose successively. In the salivary and pancreatic digestions of glycogen and the various starches employed in this investigation, an early appearance of maltose was invariably observed. When malt, which contains β-amylase in addition to α-amylase, was used as the hydrolysing agent, no increased formation of maltose was observed. These results therefore do not substantiate the findings of Ohlsson. Moreover, Ohlsson's work was based on the relative permanence of the starch-iodine reaction, and since it is not known with certainty that this colour is a function of the molecular dimensions, his conclusions seem rather unreliable.

In order to ascertain which of the above-mentioned methods of breakdown was involved or which method was predominant, the following ratio was calculated for each digestion mixture throughout each hydrolysis:

$$\frac{\text{Polysaccharide destroyed in mg./100 ml.}}{\text{Maltose formed in mg./100 ml.}}$$

If this ratio remains constant throughout a hydrolysis it would indicate that maltose was liberated successively. If, on the other hand, the ratio decreases as digestion proceeds it would mean that maltose is formed only, or principally, in the final breakdown of the simple dextrins. These ratios, calculated from determinations made at 15 min. from the beginning of each experiment and at the subsequent intervals already mentioned, are tabulated below. The values cited represent values for this ratio obtained at the beginning, middle and end, respectively, of each experiment:

Substrate	Pancreatic amylase			Malt diastase			Salivary amylase		
Glycogen	2·16	2·46	3·15	1·24	1·40	1·50	2·52	1·98	1·39
Potato	4·00	3·52	2·78	—	—	—	—	—	—
Wheat	1·87	1·83	1·86	1·27	1·13	1·09	1·39	—	1·04
Rice	2·60	2·56	1·89	—	—	—	—	—	—
Maize	4·38	2·89	2·70	—	—	—	—	—	—

The great variability of this ratio probably indicates that both methods of breakdown are occurring simultaneously. It is difficult to accept the deduction that the hydrolysis of wheat starch by pancreatic amylase is the one instance amongst all those cited in which the former method of breakdown alone occurs. An increase in this ratio as hydrolysis proceeds, observed with glycogen in two out of the three cases, seems very difficult to explain, especially as there was no glycogen resynthesis.

In the case of glycogen, the formation of maltose from dextrin appears to be very slow, which indicates that the hydrolysis is retarded at this stage, suggesting a reduced saccharification process. But since the same enzymes were used in the starch and glycogen digestions, this cannot be accounted for by the enzyme. Perhaps, as suggested by Pringsheim, a co-enzyme is necessary for the complete hydrolysis of glycogen.

(5) *Nature of linkages in polysaccharide.* As discussed in the previous section, the slow rate of breakdown of glycogen cannot be explained by assuming a preponderance of β-linkages in the glycogen molecule.

According to Van Klinkenberg the iodine reaction is characteristic of α-starch and only disappears by the action of α-amylases. The very rapid attainment of the achromic point observed with glycogen appears at first sight to substantiate Van Klinkenberg's view that glycogen is α-starch. But this does not explain the slow breakdown of glycogen in comparison with the starches. If glycogen consisted entirely of α-starch, a very quick breakdown by α-amylases would be expected.

It is thus seen that the theories of starch and glycogen structure so far enunciated do not explain satisfactorily the results of the amylolytic hydrolysis of these polysaccharides. There must be some difference in structure between the starch and glycogen molecules to account for the much lower rate of hydrolysis of the smaller glycogen molecule. There are also probably slight differences in structure between the different starches. It is also difficult to accept the identity of the starch and glycogen molecules as advocated by Haworth.

Summary.

1. The course of digestion of potato, wheat, maize and rice starches and of glycogen by pancreatic amylase and in addition of wheat starch and glycogen by salivary amylase and malt diastase have been followed quantitatively.

2. The estimations included determinations of the achromic point, the residual polysaccharide, the total reducing power and the reducing power after the precipitation of the dextrins. Polarimetric observations were also made in a few cases.

3. The relative rates of formation of the various hydrolysis products were different with the different starches. The rate of hydrolysis of glycogen was in every case very much lower than that of the starches.

4. The same end-product was formed from all the starches and from glycogen.

5. The properties of the reducing sugar formed as end-product did not correspond with those of maltose, or, in all respects, with those of *iso*maltose.

6. Glucose was not formed in any of the above digestions, or in the case of pancreatic amylase over the range *p*H 5·0–7·5. When, however, a very large amount of enzyme was employed, some glucose was produced.

7. The bearing of these results on the chemical constitution of the starch and glycogen molecules and on the method of breakdown is discussed.

I wish to thank Miss M. M. Murray for suggesting this work to me and for her helpful advice throughout the investigation.

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CCCXXI. AN INVESTIGATION OF THE RATES OF DIGESTION OF STARCHES AND GLYCOGEN AND THE BEARING ON THE CHEMICAL CONSTITUTION.

II. LIVER AMYLASE.

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Great diversity of opinion exists as to the nature of the end-products and the course of hydrolysis of glycogen by liver amylase.

Macleod [1926] stated that the final product was maltose which was, however, liable to be hydrolysed further to glucose. Osborne & Zobel [1903], working on muscle juice, found that maltose was the chief end-product together with a trace of glucose. Hollander [1934], using rat liver preparations and starch as substrate, found maltose as the sole end-product. He arrived at the conclusion that the liver preparations contain two amylases comparable with α- and β-plant amylases. Tebb [1894; 1897], using glycogen and a dried pig liver preparation, found glucose as the sole product. Preliminary saline perfusion of the liver through the portal vein, to remove all the blood, and subsequent drying provided a liver preparation which still had marked maltase activity. Hodgson [1936], using acetone-extracted and dried rabbit liver preparations, claimed, it appears without satisfactory experimental evidence, that glucose was the sole end-product and was unable to detect the presence of maltose at any stage of hydrolysis. These results are referred to again later. Barbour [1929], who used glycerol extracts of rabbit muscle, claimed to have obtained a trisaccharide as the sole product of hydrolysis. This trisaccharide is stated to have a reducing power 30% of that of glucose (by Shaffer-Hartman method), a specific rotation $[\alpha]_D$ 181°, to be readily transformed into an anhydride and to form an osazone crystallizing in small star-shaped aggregates of needles. This trisaccharide is not identifiable with Pringsheim's [1924] amylotriose, obtained by the breakdown of glycogen and amylopectin with concentrated HCl. Lohmann [1926], using KCl extracts of frog muscle, similar to those used by Meyerhof [1926] studying simultaneously the production of lactic acid by glycogen, describes, in addition to glucose, the formation of a trisaccharide which he considers to be probably identical with Pringsheim's amylotriose. Case [1931], without sufficient experimental data, claims to have repeated and confirmed Barbour's results. Carruthers & Wei Yung-Lee [1935] also repeated Barbour's work, but contrary to this worker found that maltose, and not a trisaccharide, was the chief product of hydrolysis. They also obtained evidence of the formation of some glucose. Carruthers [1935] explained Barbour's results as due to inhibition of the maltase activity of his preparations by the glycerol employed in the extraction.

In the present investigation liver preparations from the cat, rat, rabbit, pig, ox, guinea-pig and frog were employed. The preparations were made according to the method suggested to me by Prof. J. Mellanby and as used and described

by Hodgson [1936]. Eadie [1927] pointed out the necessity for perfusing the liver to remove all blood, which contains some amylase, before testing the amylolytic activity of the liver preparation. Consequently a similar preparation was made from the liver of a rat which had been perfused until blood-free. In all cases the maltase activity of the dried liver preparations was tested by incubating 0·5 g. of the liver preparation with 25 ml. 2 % maltose solution, after addition of a few drops of toluene, for 24 hours at 37°. The reducing power of the digest was determined by Hanes's method after suitable preliminary dilution and precipitation of proteins with "colloidal iron" and acetic acid.

An osazone was also made. In all the cases tested (the perfused liver preparation which was all employed in the glycogen hydrolysis was not tested) a considerable amount of glucose was formed, showing that all these preparations contain maltase. It must be emphasized, however, that the amount of liver preparation employed in this test was relatively very much greater than that used in the subsequent hydrolyses. The capacity of these preparations to form glucose from maltose might be due to the maltase activity of the liver tissue itself or to the maltase activity of the blood in the preparation. (A comparison of the hydrolysis by the unperfused and perfused rat liver preparations is of significance in this connexion.) The maltase activity of the serum of the rat, rabbit, cat and ox was tested in a similar manner to that described above, in this case employing 5 ml. serum. In the case of the rat serum, there was a quantitative conversion of maltose into glucose, an almost quantitative conversion with the ox serum, whereas both cat and rabbit serum showed no maltase activity. These results are in general accord with those of Barnes & Mackay [1936]. Hynd & Macfarlane [1927], determining the maltase activity of whole blood, found that pig's blood alone gave positive results and obtained negative results with the blood of the mouse, rat, guinea-pig, rabbit, kitten, ox and sheep. Tebb [1894] also found that pig serum had marked maltase activity.

Course of digestion of glycogen by liver amylase.

Digestion mixtures were set up containing 1·0 g. glycogen (Kahlbaum) in 160 ml. 0·1 % NaCl, 40 ml. phosphate buffer *p*H 6·4, 1·0 g. liver preparation and 1 ml. toluene.

A control mixture was set up omitting the glycogen. The hydrolysis of potato starch was also followed in some cases. The digestion mixtures were placed in an incubator at 37° and samples removed, boiled, filtered and analysed at intervals up to 3 days. To test Carruthers's criticism of Barbour's work, 0·25 g. glycogen dissolved in 30 ml. 0·1 % NaCl, 10 ml. glycerol, 10 ml. phosphate buffer *p*H 6·4 and 0·25 g. pig liver preparation were incubated for 17 hours.

The following estimations were performed:

(1) Unchanged polysaccharide.
(2) Total reducing power.
(3) Reducing power due to sugar alone.
(4) Reducing power after acid hydrolysis.
(5) Lactic acid.

The unchanged polysaccharide was estimated as described for glycogen in a previous paper [Glock, 1936]. The sugar determinations in each case were performed according to the ferricyanide method of Hanes [1929]. The total reducing power was determined on 5 ml. samples of a 1 in 10 dilution of the digest.

A further 10 ml. of the digest was diluted to 50 ml. with absolute alcohol, to precipitate any unchanged glycogen and dextrins, left for 2–3 hours or longer and

filtered. Two 20 ml. portions of the filtrate were taken and the alcohol removed by evaporation. The reducing power of one portion was determined directly after suitable dilution and of the other after acid hydrolysis followed by subsequent neutralization and dilution. 20 ml. of the digest were employed for the estimation of lactic acid, by the method of Friedemann & Graeser [1933]. Lactic acid determinations were not made in the early stages of hydrolysis. In each case an osazone was made after alcohol precipitation and subsequent evaporation of the alcohol. The results are represented diagrammatically in Fig. 1. The reducing sugar for convenience is calculated and expressed as "maltose" although in some cases the sugar was known to consist partly or entirely of glucose.

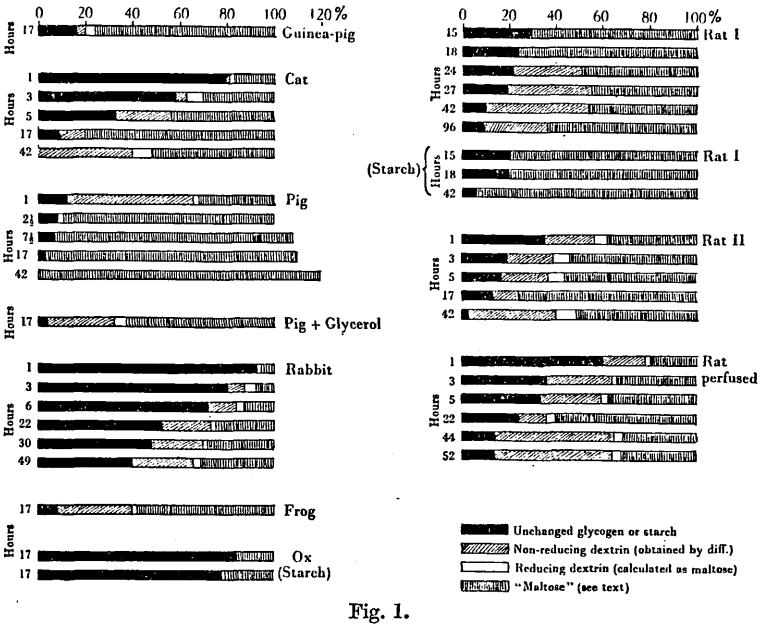

Fig. 1.

Discussion of results.

From Fig. 1 it appears that in the breakdown of glycogen to sugar there is no appreciable formation of reducing dextrin, since the reducing power of the digest after alcohol precipitation of dextrins and unchanged glycogen is only very slightly different from that before precipitation. It would seem, therefore, that in the hydrolysis by liver amylase, sugar is chiefly split off successively from the glycogen molecule and is only formed to a much smaller degree from simple dextrins produced intermediately. In this respect the action of liver amylase is different from those of salivary, malt and pancreatic amylases.

From the results of the hydrolysis of potato starch by liver amylase it is seen that starch is broken down rather more easily than glycogen, but this difference between the rates of breakdown of the two polysaccharides by liver amylase is not nearly as marked as with salivary, pancreatic and malt amylases.

In all cases the formation of lactic acid was insignificant, the amount produced varying from 0·87 to 4·76%.

Three different types of osazone were observed. The first was the "*iso*-maltose" type consisting of relatively large rosettes of very fine needles, sometimes tufted at their free extremities. This type of osazone was observed in the early stages of hydrolysis with all the liver preparations and throughout the hydrolysis with the cat, rabbit and perfused rat liver preparations, with the pig liver preparation in the presence of glycerol and generally in addition to glucosazone in the later stages of hydrolysis with the rat liver preparation. The second type of osazone (or hydrazone?) was one not previously observed. It consisted of large pale yellow fern-like crystals. Owing to their pale yellow colour and to the fact that they were relatively insoluble in hot water, it was considered possible that the crystals might be those of a hydrazone and not of an osazone. This osazone was generally found after 42 hours' hydrolysis with the cat and rat liver preparations. The third type of osazone, glucosazone, was the only product formed by the pig liver preparation after 17 hours and longer and was also formed by the rat liver preparation, but in the latter case an additional osazone was always obtained.

The results of acid hydrolysis corroborate the view that glucose is the sole product of hydrolysis with the pig liver preparation. The first type of osazone is probably produced by maltose, perhaps modified in crystalline form by admixture with unprecipitated dextrin, since the amount of glucose formed on acid hydrolysis in the later stages of hydrolysis was 100–110 % of the theoretical yield.

If graphs are plotted of the percentage formation of sugar calculated as maltose against time it is seen that with the pig and rabbit liver preparations the formation of reducing sugar follows a normal hyperbola, whereas this is not true with the rat (perfused and unperfused) and cat liver preparations. In the latter cases there is a sudden fall in the reducing power which finally rises slowly after 42 hours. This type of hydrolysis curve would appear to be due to the reversible action of the liver amylase, the breakdown phase being predominant in the early stages of digestion and the synthetic phase in the later stages, probably initiated by accumulation of the hydrolysis products of the breakdown phase. The possible criticism that the decrease in the reducing power in the later stages might be due to the formation of hexosephosphates is disproved by the fact that a similar type of curve was obtained using the rat liver preparation but replacing the phosphate buffer by a citrate buffer of the same *p*H. This decreased reducing power also cannot be accounted for by increased lactic acid formation, for in no case was the percentage formation greater than 5.

It is interesting to note, in this connexion, that the third type of osazone described above was only observed in the 42 hours' digest with the rat and cat preparations and is, therefore, probably correlated with the decreased reducing power. The breakdown of potato starch by rat liver preparations, however, follows a normal course.

The conversion of glycogen into glucose by liver preparations depends on the concentration of amylase and maltase. If both enzymes are very active, it is possible to get 100 % conversion into glucose: this is the case with the pig liver preparation. If the concentration of either amylase or maltase is reduced, the yield of glucose will be less: this seems to be the case with the rat preparation. If the concentration of maltase is very small, the breakdown will stop at the maltose stage and will not proceed as far as glucose. It was shown, as already described, that all the preparations possessed maltase activity. In the case of the cat and rabbit preparations this activity must have been much less than that of the pig and rat preparations, so that in the amount of preparation which

was used in the glycogen hydrolysis, the maltase concentration was negligible. If the maltase activity of the preparation were due to the blood it contained, then one would suppose that after perfusion of the liver to render it blood-free, the breakdown of glycogen would not proceed further than the maltose stage. This was found to be true with the dried preparation from the perfused rat liver.

That the end-product in this case was not glucose was further substantiated by the fact that after alcohol precipitation and subsequent evaporation of the alcohol, the liquid was not fermented by a yeast known to be incapable of fermenting maltose. In the case of the rat, the maltase activity of the serum was found to be so great that the ability of the unperfused liver preparation to form glucose might easily be attributed to the maltase activity of the blood. It is doubtful, however, whether this is true of the pig liver preparation since in this case there was normally 100 % conversion into glucose. (That perfused pig liver preparations still possess marked maltase activity has been shown by Tebb [1894].) The breakdown of glycogen would also stop at the maltose stage if the maltase activity of the preparation were inhibited, for example, by the method of preparation. The pig liver preparation, in the presence of glycerol, failed to produce any glucose after 17 hours. This also supports Carruthers's opinion of Barbour's work.

From the values of the reducing power before and after acid hydrolysis, the relative amounts of glucose and maltose at each stage of digestion can be calculated. At the end of 17 hours the sugar formed consisted in the case of the pig preparation of 16 % maltose and 84 % glucose and in the cases of the rat, guinea-pig and ox liver preparations of 93 % maltose and 7 % glucose, 91 % maltose and 9 % glucose and 79 % maltose and 21 % glucose, respectively. After 42 hours the values were for the pig preparation 100 % glucose, for the rabbit 100 % maltose and for the rat 88 % maltose and 12 % glucose. The necessity for estimating the reducing power both before and after acid hydrolysis in order to form an opinion as to the nature of the sugar produced is obvious when the results obtained with the pig preparation are considered: after $7\frac{1}{2}$ hours only 15 % of the sugar was glucose, after 17 hours 84 %, whereas at 42 hours there was a 100 % yield.

A final 100 % yield of glucose does not necessarily mean that no maltose is produced intermediately. Hodgson [1936], without having performed any acid hydrolyses, claims that glucose is the sole product of hydrolysis of glycogen by rabbit liver preparations. It is obvious from the above results that such an assumption is unfounded.

Summary.

1. The hydrolysis of glycogen, and in a few cases of potato starch, by acetone-extracted and dried liver preparations of the rat, cat, rabbit and pig in the presence of phosphate buffer of *p*H 6·4, was followed. The course of hydrolysis of glycogen was also followed using a perfused rat liver preparation and in addition the behaviour of the pig liver preparation in the presence of glycerol was studied.

2. The maltase activity of each liver preparation was tested, in all cases with a positive result. The maltase activity of the serum was also tested. That of the rat serum was found to be very great, that of the ox quite large, whereas cat and rabbit sera possessed no maltase activity.

3. In the case of the pig liver preparation only was there quantitative conversion into glucose. This formation of glucose is inhibited by the presence of glycerol.

4. Maltose was the sole end-product with the cat, rabbit and perfused rat preparations.

5. The unperfused rat liver preparation produced maltose alone in the early stages of hydrolysis, but this was gradually converted into glucose as digestion proceeded.

6. In the cases of the rat (perfused and unperfused) and cat liver preparations the reducing power showed a steady decrease from 17 to 42 hours. This can only be explained by a reversal of the enzymic activity.

8. That the production of glucose by the rat liver preparation could be due to the maltase contained in the blood has been proved since no glucose was produced by the perfused rat liver preparation.

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AN INVESTIGATION OF THE EFFECTS OF ADVERSE ATMOSPHERIC CONDITIONS SUCH AS ARE ENCOUNTERED IN VARIOUS INDUSTRIES ON MENTAL AND MUSCULAR EFFICIENCY

BY

GERTRUDE E. GLOCK

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By GERTRUDE E. GLOCK

From the Department of Physiology, Bedford College

(With 1 Graph)

Adverse atmospheric conditions, such as occur in mines and various industries, have been shown by numerous workers to have profound effects on both the health and efficiency of the industrial worker.

In this country, Leonard Hill's katathermometer is now extensively used to determine the suitability or non-suitability of the atmospheric conditions in factories, since the cooling power (c.p.) serves as a fairly reliable index of bodily comfort. But, as will be shown later, the katathermometer is not strictly comparable to the human body. Vernon (1926–8) and Bedford, Vernon and Warner (1926) attempted the very tedious task of calibrating the katathermometer in terms of human sensation. Workers in factories were asked if they found the atmospheric conditions "comfortable," "too cold" or "too hot." Taking the average of these observations, these workers found that a comfortable sensation of warmth was experienced in winter at a c.p. of 6·6 and in summer at a c.p. of 6·4. Hence the sensation of warmth is not directly proportional to the c.p., but varies with the season. Acclimatisation is also a very important factor.

In using the c.p. as a standard for atmospheric conditions in industry, Leonard Hill suggests that:

A dry c.p. of 6 and a wet c.p. of 18 is suitable for sedentary workers,
A dry c.p. of 8 and a wet c.p. of 25 is suitable for light manual work, and
A dry c.p. of 10 and a wet c.p. of 30 is suitable for heavy manual work.

The c.p. standard, however, is only used in England and her colonies. In America a different standard, the "effective temperature" (e.t.) has been resorted to. This depends on the fact that the same feeling of warmth is given by very different combinations of temperature, air movement and humidity. Atmospheric conditions producing the same subjective sensations are known as "equivalent conditions," and these equivalent conditions have the same "effective temperature." (An atmospheric condition has an e.t. of, for example, 65, if it is comparable to an air condition of 65° F., saturated with

moisture and with no air movement.) An E.T. chart has been constructed whereby it is possible to convert any combination of the above three atmospheric conditions into an E.T. It is improbable, however, that the E.T. standard will ever attain more than a very restricted use in industry, since the wet- and dry-bulb temperatures and the air velocity must all be determined, whereas if the katathermometer is used only one reading need be taken.

The adverse effects of high temperatures and humidities on the efficiency of the industrial worker are most marked when the work is of a strenuous physical character.

Orenstein and Ireland (1921–3) made a number of experimental observations on the relation between atmospheric conditions and the production of fatigue in mine labourers in the Rand Mines, and in 1923 Sayers and Harrington also made extensive observations on subjects at rest in deep metal mines.

Campbell and Hill (1922) found that during muscular exertion a definite relationship can ordinarily be demonstrated between the pulse rate (P.R.) and the dry C.P. If the load is kept constant, then the P.R. varies inversely as the dry C.P. These workers also showed that the efficiency of a trained man on a bicycle ergometer was not altered by raising the dry kata C.P. from 3·9 to 11·2. It is probable, however, that this is not true of the untrained individual.

Bauer and co-workers (1931), in their study of men working on a bicycle ergometer in a cold (54° F.) and a hot (93° F.) room, found that the O_2 usage was only very slightly modified. They conclude that the mechanical efficiency does not vary significantly with alterations in temperature between 54 and 93° F.

Amongst the most trying atmospheric conditions in England are those encountered in certain textile industries, such as the weaving of cotton and linen goods, in which it is important to have a hot and humid atmosphere, otherwise breakages of the yarn are very frequent and the quality of the woven material is depreciated. In addition to the textile industries, where a high temperature is desirable in the interests of production, there are other industries, such as laundry work, in which the incidental heat can scarcely be avoided.

The relation of sex to susceptibility to abnormal atmospheric conditions seems to have been given very little consideration, although statements are frequently made that women are more susceptible than men to variations in temperature, air movement and humidity. Since women are principally employed in textile industries and in laundries, and since it is in these industries that the most trying atmospheric conditions are encountered, it seems of importance to obtain some quantitative data as to the effect of abnormal atmospheric conditions on women. Consequently, the present investigation was carried out, all the subjects being women varying in age between 18 and 23 years.

The observations were made in the air conditioning hut at the Home Office Industrial Museum, which can be adjusted to practically any atmospheric condition. This consists of a central corridor open at both ends and separated

on either side by a metal partition from an outer corridor which extends right round the inner part. The walls of the central corridor are provided with four radiant heating panels of different intensities, and each side corridor has two convected heating panels. Near one end of the central corridor is a large electric fan screened by a wire meshwork on the outside and by a metal perforated screen in front, composed of separate metal partitions riveted together so that the air currents set up are unidirectional and parallel. The air can thus be circulated down the central corridor and back by the outer corridors and hence recirculated. The air can also be artificially humidified by steam or water jets. (In all the following experiments convected heat alone was used.) The following physical measurements were made:

(1) *Wet- and dry-bulb temperatures* by means of a sling psychrometer.

(2) *Percentage relative humidity* by means of a recording hygrometer.

(3) *Air movement* by means of a vane anemometer. The air velocity was adjusted from 100 to 400 ft. per min. An air velocity of 200 ft. per min. was the highest to be used in the later experiments, a higher value being considered incomparable to any indoor atmospheric condition.

(4) *Cooling power* (C.P.). The dry C.P. and in some cases the wet C.P., in addition, was determined. A self-recording katathermometer (Schuster's modification) was used whenever possible, but since this katathermometer does not register C.P.'s below 3·0, the ordinary alcohol katathermometer of Leonard Hill was employed at low C.P.'s. At air temperatures of 90° F., the high-temperature katathermometer was, in addition, used, since the great time taken for the ordinary kata to cool at this temperature renders it inaccurate. The high-temperature kata cools from 130° to 125° F. The C.P.'s obtained with the two instruments are not, however, comparable.

The C.P. exerted by the air on the kata is estimated at what is approximately the mean body temperature (100–95° F.), and it is sometimes supposed that its readings can be applied directly to the cooling action which the air exerts on the human body. But this direct comparison cannot hold for several reasons. The kata, by reason of its small bulk, exposes a relatively much larger surface to the air than the human body; it exposes an uncovered surface, whilst most portions of the human body are usually covered with clothing, which causes it to react differently to atmospheric conditions at different times and seasons. Under a few conditions only may the rate of cooling of the kata between 100 and 95° F. be taken as an index of the cooling of the body itself. The rate of cooling of the naked body when the skin is covered with moisture is directly comparable to the wet katathermometer, and when the skin is dry the rate of cooling is to some extent comparable to the dry katathermometer, but not wholly so, as there is always a considerable loss of heat at the body surface from insensible perspiration. But when the body is clothed it is practically impossible to obtain closely comparable conditions of cooling in the katathermometer. In spite of this, the dry kata readings are of extreme importance and form a fair index of comfort for ordinary atmospheric conditions.

Great difficulties arise in using the C.P. as a standard for the comparison of atmospheric conditions. The katathermometer tends to overestimate air movement and to underestimate humidity. The dry kata C.P. is, in fact, practically unaltered by increased humidity and according to Leonard Hill is entirely uninfluenced. Thus, in attempting to plot various physiological factors against C.P.'s, it is obvious that many discrepancies will arise, and these discrepancies are particularly emphasised at high humidities. The C.P. does, in fact, afford a much better qualitative than quantitative index of physiological reactions, but unfortunately there is no other single index, apart perhaps from the effective temperature, which is even as good as this.

The duration of each experiment at any one given atmospheric condition was 2 hours. During this time four blood samples were taken at intervals of 30 min. and three samples of expired air, the subject breathing into a Douglas bag for 15-min. periods, during which time the respiration rate and P.R. were also taken. The blood pressure, mouth temperature and skin temperature were taken at 40-min. intervals and also the mental efficiency tested for 5-min. periods at the same time intervals. The mouth and skin temperatures showed very small and insignificant variations, and hence these results are not tabulated.

After arriving at the hut in the morning the subject rested for about 20 min. and then a normal blood sample was taken and the normal mental efficiency on that day tested, as will be described later. Except when performing work for the determination of muscular efficiency, the subject rested during the whole of the 2-hour experimental period.

The profound effect of clothing on the physiological responses of a subject to any air condition has long been recognised, and hence in order to eliminate this factor as far as possible a standard uniform was worn by all the subjects. This consisted of a navy blue woollen stockinette pyjama suit.

The diet of the subject was also partly controlled, no breakfast being taken apart from a drink, and the subject neither ate nor drank during the whole period of the day's experiment.

The following results were obtained with two of the subjects. Subject A preferred hot to cool atmospheres, and subject B disliked both hot and humid atmospheres profoundly. It will be seen from the results presented that there are very definite reasons for these likes and dislikes.

PULSE RATE

The P.R. was taken over 5-min. periods every 40 min., while the subject was breathing into the Douglas bag and the average value taken (see Tables I and II). The results obtained with both subjects show that:

(1) The P.R. is approximately inversely proportional to the C.P., increased C.P. resulting in a decrease in the P.R. and decreased C.P. in an increase in the P.R.

(2) Air movement produces a decrease in the P.R. except at high air temperatures, when in the case of subject A, air movement at 90° F. produced no alteration in the P.R., and in the case of B at 90° F. and 100 per cent. saturation, air movement even increased the P.R. (Subject B found that under these conditions wind increased rather than decreased the discomfort experienced.)

(3) Increased humidity may or may not increase the P.R. In the case of A, the P.R. was unaffected by increased humidity, whereas with B increased humidity resulted in a marked increase in the P.R.

The variations in the P.R., as well as in most of the other physiological reactions investigated, were found to be connected very closely with the subjective sensations experienced. Subject A, who was not incapacitated either by high temperatures or humidities, only showed a range of P.R. from 63 to 80 over a C.P. range 1 to 10, whereas subject B, who was very susceptible to both increased air temperatures and particularly to increased humidity, showed a much wider range of P.R. under the same air conditions, namely from 65 to 108.

Table I. *Subject A. Registration rate, pulse rate and blood pressure*

	Air condition						Blood pressure		
Date	° F.	Percentage relative humidity	Air velocity in feet per min.	Cooling power	Respiration rate	Pulse rate	Systolic	Diastolic	Pulse
Nov. 16	50	71	0	8·0	12–13	63	106	73	33
Nov. 12	60	61	0	6·2	13	68	104	72	32
Nov. 11	60	60	100	8·3	12	67	109	67	42
Nov. 19	60	63	200	10·0	12	63–64	110	69	41
Nov. 12	70	53	0	4·2	14–15	68	103	70	33
Nov. 19	70	48	200	8·3	13–14	66–67	103	70	33
Nov. 30	75	45	0	3·0	14	74	98	65	33
	75	40	100	5·5	13–14	69	100	67	33
Jan. 11	75	44	200	6·9	12–13	69	107	68	39
Oct. 29	80	50	0	2·6	14–15	75	96	68	28
Nov. 4	80	50	200	5·2	14	72–73	99	68	31
Nov. 26	80	42	400	8·5	13–14	69–70	103	70	33
Nov. 5	80	100	0	1·8	16–17	76	94	62	32
Dec. 10	80	80	0	2·6	13	73	97	63	34
	80	63	200	6·0	13	69	102	65	37
Dec. 2	90	30	0	1·1	14–15	77	94	61	33
	90	26	100	1·6	14	80	98	63	35
Nov. 26	90	31	200	2·0	14–15	79	100	66	34

Blood pressure

Both systolic and diastolic blood pressures were measured every 40 min. by the auscultatory method (for results see Tables I and II). The results showed that:

(1) Both systolic and diastolic blood pressure (B.P.) are roughly proportional to the C.P. (excluding air conditions with high humidities in the case of B), both increasing slightly with an increase in the dry C.P.

High temperatures decrease the B.P. probably chiefly because of dilation of the cutaneous vessels, and low temperatures increase it because of vasoconstriction of the skin vessels, increased output of adrenaline from the suprarenals and also, as has been shown by Barcroft, because of an increase in the minute volume and systolic output of the heart.

(2) Increased humidity produced no noticeable effect on the B.P. in the case of A. With B, increased humidity resulted in a fall of B.P. except at high temperatures (90° F.). But all the values of the B.P. at 90° F. with B seem to be discrepant and can only be attributed to the individual idiosyncracies of the subject.

(3) The pulse pressure remains approximately constant.

Table II. *Subject B. Respiration rate, pulse rate and blood pressure*

	Air condition						Blood pressure		
Date	° F.	Percentage relative humidity	Air velocity in feet per min.	Cooling power	Respiration rate	Pulse rate	Systolic	Diastolic	Pulse
Jan. 25	50	61	0	7·7	9–10	68	113	70	43
	50	58	100	12·7	8–9	68	117	75	42
Feb. 15	50	52	200	16·5	9–10	74	119	75	44
Feb. 1	60	53	0	6·5	10	67–68	110	68	42
Feb. 15	60	50	0	6·4	10	68	110	68	42
Feb. 1	60	51	200	11·2	8–9	65	115	72	43
Feb. 29	60	100	0	5·9	13	72	102	72	34
Feb. 4	70	55	0	4·7	10–11	77	108	66	42
	70	50	200	9·4	9	74–75	115	72	43
Feb. 11	75	37	0	3·4	11	72	107	63	42
	75	33	200	7·0	10	72	111	68	43
Feb. 8	75	100	0	3·2	14	83	102	64	48
	75	100	200	5·9	11–12	82	110	66	44
Feb. 25	90	45	0	1·3	42	92	94	60	34
	90	35	200	2·7	24	92	116	70	46
				(Cheyne-Stokes' breathing)					
Mar. 14	90	100	0	1·0	44	98	115	82	33
	90	100	200	1·9	24	108	98	62	36

BLOOD SUGAR

A normal blood sample was taken soon after the subject arrived at the hut in the morning and four others at half-hourly intervals during the 2-hour experimental period, the first sample being taken at the end of the first half-hour. All the samples were placed on ice immediately and the blood-sugar estimations done on the same day.

The blood sugar was estimated by the method of Hagedorn and Jensen, using 0·1 c.c. of blood for each estimation. Each estimation was done in triplicate. (For results see Tables III and IV.) The results obtained show that:

(1) The blood sugar has a minimum value at some air condition (in the case of A at a C.P. of 3·4, which is equivalent to an atmosphere of 75° F. still). In the case of B, this minimum value was at a C.P. of 2·5–4·2 (*i.e.* between 70 and 80° F. still) and increases at both higher and lower C.P.'s.

The increase in blood sugar on exposure to cold has long been recognised and is attributed to an increased output of adrenaline and a consequently increased glycogenolysis in the liver.

Table III. *Subject A. Blood glucose*

Date	Air condition: ° F.	Air condition: Percentage relative humidity	Air condition: Air velocity in feet per min.	Cooling power	Initial blood glucose mg. %	Deviations of blood glucose in mg. % from initial level, measured at the following time intervals from the beginning of the experiment: 30	60	90	120
Nov. 16	50	71	0	8·0	89	+10	+ 4	+ 2	+ 1
Oct. 28	60	61	0	6·5	100	− 2	+ 1	− 2	− 1
Jan. 11	60	60	100	8·3	101	− 3	− 1	± 0	− 3
Nov. 19	60	63	200	10·0	100	+ 8	+ 3	+ 2	− 1
Nov. 12	70	53	0	4·2	104	− 9	−11	−13	− 6
Nov. 19	70	48	200	7·8	91	+ 9	+ 6	*	*
Nov. 26	75	45	0	3·3	110	± 0	− 5	−10	− 3
	75	40	100	5·2	110	− 2	− 2	− 3	− 6
Jan. 11	75	44	200	6·9	101	− 3	± 0	+ 5	+ 2
Nov. 9	80	50	0	2·5	98	−13	−11	+ 2	− 2
Nov. 24	80	50	200	5·0	100	− 5	− 6	− 9	+ 1
Nov. 26	80	42	400	8·5	102	− 3	− 2	− 3	− 3
Nov. 5	80	100	0	1·8	105	− 6	− 6	− 4	− 3
Nov. 10	80	78	0	2·6	107	− 8	−16	−17	−17
	80	63	200	6·0	107	−14	−12	− 8	− 3
Dec. 2	90	30	0	1·1	105	− 5	+ 5	+ 6	− 5
	90	26	100	1·6	105	+ 4	+ 4	+ 4	− 1
Nov. 26	90	31	200	2·0	102	− 7	−19	−16	− 5

* Results discrepant; omitted because of psychological disturbance due to visitor.

Table IV. *Subject B. Blood glucose*

Date	Air condition: ° F.	Air condition: Percentage relative humidity	Air condition: Air velocity in feet per min.	Cooling power	Initial blood glucose mg. %	Deviations of blood glucose in mg. % from initial level, measured at the following time intervals from the beginning of the experiment: 30	60	90	120
Jan. 25	50	61	0	7·7	108	+ 9	+12	+ 4	+ 5
	50	58	100	12·7	108	+12	+16	+ 5	+ 5
Feb. 15	50	52	200	16·5	115	+13	+17	± 0	+ 2
Feb. 1	60	53	0	6·5	110	+ 2	+ 2	± 0	+ 3
Feb. 18	60	50	0	6·4	121	− 1	− 2	± 0	+ 1
Feb. 1	60	51	200	11·4	110	+12	+15	+ 5	+ 2
Feb. 29	60	100	0	5·9	118	−10	− 9	+10	+ 2
Feb. 4	70	55	0	4·6	115	−13	− 9	− 3	± 0
	70	40	200	9·4	115	−15	−15	−12	+ 1
Feb. 8	75	37	0	3·4	114	− 1	−17	− 5	− 8
	75	33	200	7·0	114	− 9	− 9	± 0	− 2
Feb. 11	75	100	200	3·2	115	−13	−17	± 0	+ 2
	75	100	200	5·9	115	± 0	− 3	− 5	− 5
Feb. 25	90	45	0	1·3	114	+ 6	+14	+ 9	+ 7
	90	35	200	2·7	114	+ 1	+ 2	+ 1	± 0
Mar. 14	90	100	0	1·0	116	− 9	+13	+ 8	+16
	90	100	200	1·9	116	− 5	+ 2	+ 5	+ 2

Flinn (1924–5) subjected dogs to very high temperatures and found a similar increase in the blood sugar which he showed could not be attributed to an increased concentration of the blood solids. The increased blood sugar at high temperatures is probably due to an emergency mechanism which mobilises sugar.

(2) At and above the C.P. at which the blood sugar is at a minimum, air movement increases the blood sugar. At lower C.P.'s (*i.e.* at 90° F.) wind decreases the blood sugar, that is, it tends to restore it to its normal level. The beneficial effect of air movement is seen particularly well in the case of subject B. At high temperatures and humidities (*i.e.* at 90° F. and 100 per cent. saturation still) the blood-sugar level is still considerably above normal even after 2 hours' exposure, whereas when air movement is employed the blood sugar has returned practically to normal after 2 hours.

(3) In the case of subject B, increased humidity increases the blood sugar. This increase was in most cases preceded by an initial fall. The results obtained with subject A seem to be rather discrepant in connection with increased humidity.

RESPIRATION RATE

The respiration rate was taken while the subject was breathing into the Douglas bag, and hence those observed at high temperatures were probably higher than those that would occur normally. The results (see Tables I and II) show that:

(1) The respiration rate (R.R.) does not alter very considerably except under very hot air conditions. With subject A, the R.R. only altered from 12 to 16 within a range of C.P. of 1 to 11, but in the case of B it rose from 8 to 44. All the high values, however, were obtained at 90° F., at which temperature the subject experienced great discomfort.

(2) High temperatures result in an increased rate of respiration but also in a decreased depth, so that the ventilation of the lungs is probably not increased.

(3) Air movement produces a decrease in the R.R. This is most marked with B, especially at high temperatures and humidities, in fact at 90° F., a wind of 200 ft. per min. almost halved the R.R.

(4) Increased humidity produces an increase in the R.R. with B, but no noticeable effect with A. Cheyne Stokes' breathing was noticed with subject B at 90° F. and 100 per cent. saturation, but this only occurred when breathing into the Douglas bag. The discomfort experienced by B under these conditions was accentuated when breathing against such a resistance. The very quick and shallow breathing probably results in a deficient O_2 supply to the respiratory centre.

Respiratory quotient

Marked variations in the R.Q. were noticed (Tables V and VI), but there is no relation between the R.Q. and the C.P. or between the R.Q. and the temperature and humidity. The American workers McConnell and Yagloglou (1924, 1926 *b*), however, found that there was a direct relationship between the R.Q.

Table V. *Subject A. Metabolic rate*

Weight=70·37 kg. Height=164·6 cm. Surface area=1·79 sq. m.

Air condition	Expired air % CO_2	Expired air % O_2	Expired air % N_2	Vol. of expired air in 15 min. (in litres)	Vol. of expired air in 1 min. at N.T.P.	c.c. O_2 retained per min.	c.c. CO_2 per min.	R.Q.	Metabolic rate	Cooling power
50° F. still	4·121	16·37	79·509	87·2	5·504	276·1	224·5	0·770	44·98	8·0
60° F. still	4·011	16·62	79·367	87·2	5·504	258·36	209·9	0·821	41·18	6·1
60° F. 200 ft. per min.	4·135	15·935	79·93	89·45	5·963	309·5	225·0	0·728	48·87	11·0
70° F. still	3·556	16·62	79·824	91·5	5·666	242·4	177·0	0·731	38·3	4·2
70° F. 200 ft. per min.	4·232	16·17	79·598	85·7	5·713	275·7	220·0	0·798	44·9	7·9
75° F. still	4·115	16·43	79·455	76·0	4·670	238·2	177·1	0·808	37·8	3·0
75° F. 100 ft. per min.	4·086	16·43	79·484	81·3	4·964	254·9	182·7	0·809	40·19	5·5
80° F. still	3·556	16·62	79·824	91·5	5·05	242·4	177·0	0·731	38·30	2·8
80° F. 200 ft. per min.	4·129	16·80	79·794	87·0	5·15	242·6	191·4	0·868	38·93	4·9
80° F. still, 100 % saturation	3·593	16·79	79·617	92·83	5·513	259·4	193·9	0·748	41·21	1·8
80° F. (dry bulb), 75° F. (wet bulb)	3·574	17·19	79·236	90·5	5·375	233·9	195·4	0·762	37·61	2·7
80° F. (dry bulb), 75° F. (wet bulb), 200 ft. per min.	3·899	16·33	79·771	89·3	5·304	275·8	204·3	0·742	43·92	5·8
90° F. still	4·737	16·43	78·833	88·2	5·88	277·0	253·6	0·919	46·1	1·1
90° F. 100 ft. per min.	4·027	16·37	79·603	85·1	5·055	258·1	201·4	0·780	41·30	1·6
90° F. 200 ft. per min.	4·328	16·36	79·312	86·85	5·037	251·9	195·0	0·857	41·06	2·0

Table VI. *Subject B. Metabolic rate*

Weight=75·42 kg. Height=168 cm. Surface area=1·85 sq. m.

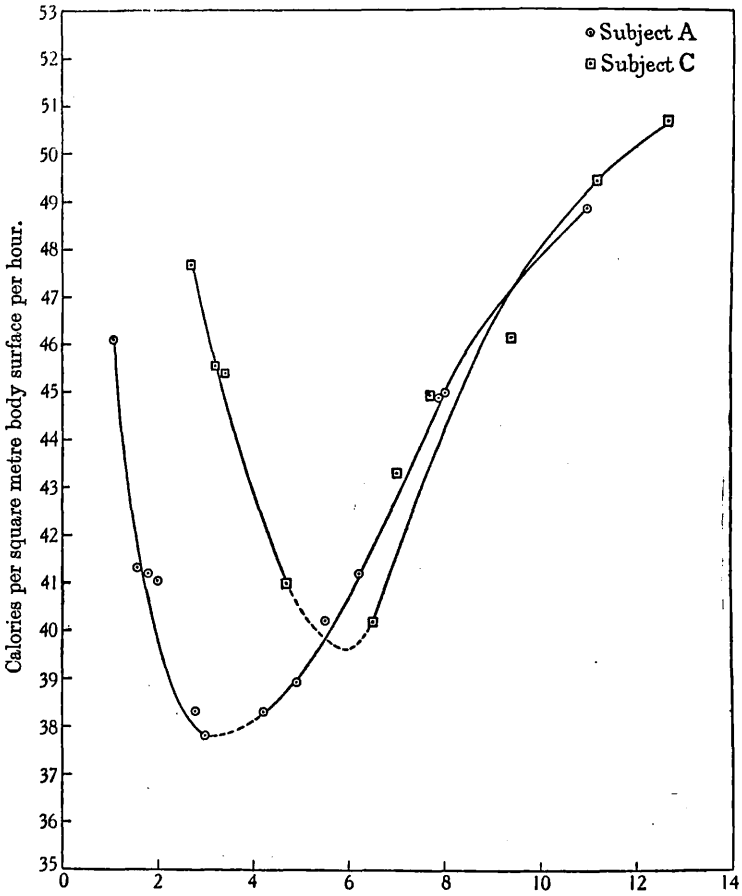
Air condition	Expired air % CO_2	Expired air % O_2	Expired air % N_2	Vol. of expired air in 15 min. (in litres)	Vol. of expired air in 1 min. at N.T.P.	c.c. O_2 retained per min.	c.c. CO_2 per min.	R.Q.	Metabolic rate	Cooling power
50° F. still	4·236	16·24	79·624	90·83	5·875	283·3	248·4	0·8813	44·93	7·7
50° F. 100 ft. per min.	3·551	16·783	79·706	116·5	7·534	325·4	263·3	0·8085	50·70	12·7
50° F. 200 ft. per min.	3·198	17·49	79·312	154·0	9·850	345·7	314·6	0·9102	55·32	16·5
60° F. still	3·974	16·562	79·464	88·05	5·746	252·5	222·5	0·893	40·21	6·5
60° F. 200 ft. per min.	3·450	16·995	79·555	124·2	7·685	313·9	265·3	0·848	49·45	11·2
60° F. still, 100 % saturation	3·506	16·82	79·674	108·4	6·842	292·8	239·8	0·8181	45·96	5·9
70° F. still	3·943	16·28	79·77	89·43	5·514	266·4	217·4	0·8244	41·00	4·7
70° F. 200 ft. per min.	3·874	16·17	79·95	96·9	5·974	298·7	231·2	0·774	46·15	9·4
75° F. still	3·799	16·38	79·821	100·9	6·141	291·7	233·0	0·799	45·40	3·4
75° F. 200 ft. per min.	3·924	15·90	80·176	88·87	5·407	283·3	211·9	0·732	43·31	7·0
75° F. still, 100 % saturation	3·395	17·23	77·375	126·0	7·410	286·7	251·2	0·876	45·55	3·2
75° F. wind, 100 % saturation	3·326	16·78	79·894	113·5	6·673	292·3	221·7	0·758	44·95	5·9
90° F. still	3·096	17·24	79·664	124·85	7·547	292·1	233·5	0·799	45·18	1·3
90° F. 200 ft. per min.	3·494	16·53	79·976	113·2	6·798	316·8	237·2	0·749	47·66	2·7
90° F. still, 100 % saturation	3·061	17·45	79·480	105·7	6·160	222·4	188·3	0·847	35·04	1·0
90° F. wind, 100 % saturation	2·886	17·81	79·304	130·1	7·505	247·2	209·7	0·912	38·95	1·9

and the E.T., the R.Q. increasing with an increasing E.T. Since the R.Q. is mainly a combustion quotient, it would mean that the E.T. could alter the fuel that was being used. This is very unlikely.

METABOLIC RATE

Three metabolic rate determinations were made during each 2-hour experimental period. The subject breathed into a Douglas bag for 15-min. periods at 40-min. intervals. The average of the three metabolic rates was taken for each air condition. The results (Tables V and VI) show:

(1) The metabolic rate is at its minimum at a certain air condition, in the case of A, at a C.P. of 2·7 (*i.e.* at 80° F. still) and in the case of B, at a C.P. of



Graph 1.

6·5 (*i.e.* 60° F. still) and increases at both higher and lower C.P.'s. The results obtained with B at high humidities seem to be very discrepant and hence are not discussed here nor are they plotted on Graph 1 above.

Increased metabolic rate at low temperatures is connected with increased muscular movement, including shivering, increased output of adrenaline, increased activity of the thyroid and probably also with variations in muscular tone.

Increased metabolic rate at high temperatures shows that under such conditions the regulative functions of the body have ceased to function, since the body is then behaving like a chemical reaction, increased temperature resulting in an increased velocity of reaction in the tissues. Under such air conditions there must be considerable unnecessary energy production, chiefly in the form of heat, and this under conditions in which it is difficult to dissipate the heat. The consequent necessity for increased energy intake in the form of food under such adverse air conditions is obviously of great importance to the industrial worker.

(2) With subject A the metabolic rate was absolutely unaffected by increased humidity, whereas in the case of B, at 60° F. and 100 per cent. saturation, an increase in the metabolic rate was produced, at 75° F. there was no effect and at 90° F. there was a fall. Theoretically, of course, one would expect a fall in the metabolic rate on exposure to high humidities, since there is reduced loss of heat by evaporation.

Mental efficiency

The mental efficiency of the subject was tested by the Woodworth and Wells efficiency tests. These involved adding up four single-integer figures at a time, which were read out to the subject fairly rapidly and as soon as an answer was received four others given. The number of groups of figures (each group thus comprising four figures) added correctly per minute and also the number incorrectly added were noted.

The normal mental efficiency was tested each day soon after the subject arrived at the hut in the morning, in the main building of the Museum, which is kept at a practically constant temperature of 65° F. The subject added up for two 5-min. periods and the average was taken. The mental efficiency was tested in this way three times at 40-min. intervals over each 2-hour experimental period (for results see Tables VII and VIII).

The results of these tests seem to follow extraordinarily closely the subjective sensations experienced.

Only a few observations were made with subject A, but these show that her mental efficiency decreases under cold atmospheric conditions and increases under hot conditions and especially in hot and humid atmospheres. Subject A dislikes cold intensely and enjoys heat and increased humidity and did, in fact, find an atmospheric condition of 90° F. and 100 per cent. saturation extraordinarily pleasant!

In the case of subject B, it was found that her maximum efficiency was at 65° F. still, the normal atmospheric condition in the Museum. It decreased very slightly under colder conditions but considerably under hotter conditions. Humid conditions, even at 60° F., produced a marked diminution in her mental capacity, and at 75° F. and 100 per cent. saturation this decrease was even greater than that observed at 90° F. still. Subject B is very averse to hot atmospheric conditions and found hot and humid atmospheres almost unbearable.

It is interesting to note that the mental efficiency seems to run almost parallel with the muscular efficiency, as will be seen in the next section.

Table VII. *Subject A. Mental efficiency*

	Air condition					Normal number added per 5 min. correctly	Normal number added wrongly	% deviation from normal
Date	° F.	Percentage relative humidity	Air velocity in feet per min.	Number added per 5 min. correctly	Number added wrongly			
Nov. 30	60	61	100	35	2	38	5	− 8
Nov. 30	75	45	0	34	2	37	3	− 8
	75	40	100	32	2	37	3	−13
Nov. 11	75	44	200	35	2	38	5	− 8
Dec. 12	80	78	0	42	1	39	1	+ 7·7
	80	63	200	39	1	39	1	± 0
Dec. 2	90	30	0	40	3	35	2	+14
	90	26	100	42	2	35	2	+20

Table VIII. *Subject B. Mental efficiency*

	Air condition					Normal number added per 5 min. correctly	Normal number added wrongly	% deviation from normal
Date	° F.	Percentage relative humidity	Air velocity in feet per min.	Number added per 5 min. correctly	Number added wrongly			
Mar. 16	50	64	0	74	1–2	76	0	− 3
„	50	62	200	67	4	68	0	− 1½
Mar. 20	60	50	0	74	2–3	73	2	− 1½
„	60	48	200	67	1	68	0	− 1½
„	60	100	0	74	2	79	1	− 6
Mar. 23	75	34	0	69	2–3	73	2	− 5½
„	75	31	200	72	1	73	2	− 1½
„	75	100	0	66	2	79	1	−16
Mar. 27	90	45	0	65	4	75	1	−14
„	90	35	200	67	1	72	1	− 8

MUSCULAR EFFICIENCY

The muscular efficiency of the subject was tested on a bicycle ergometer. Since muscular fatigue is due principally to some kind of circulatory failure, it was thought permissible to take the P.R., or rather the pulse index, as a criterion of muscular efficiency. The pulse index was taken as:

$$\frac{\text{P.R. for 2 min. immediately after exercise}}{\text{P.R. for 2 min. before exercise}}.$$

At the beginning of the experiment the subject sat in the resting position on the bicycle for about 10 min. and the P.R. was taken until it remained constant. Three-minute periods of work were done and the P.R. counted for each ¼ min. immediately after the exercise until it returned to normal. The work was varied by altering both the load and the speed of the exercise, so that about six to ten different degrees of work were performed at each atmospheric condition. Graphs were drawn plotting the pulse index against the work done in kilogram-metres. (This method was used by Barcroft in connection with the effect of

high altitudes on muscular efficiency.) From these graphs, the work done in kilogram-metres to give a pulse index of 1·2, 1·3, 1·4, 1·5, 1·6 and 1·7 was read off and tabulated.

In this way it was possible to compare the efficiency at different air conditions, *i.e.* to find how much work has to be done to produce a certain pulse index at each different atmospheric condition.

These results (see Table IX) show that the optimum temperature for the performance of muscular work is at 60° F. At higher temperatures, the same amount of work produces a higher pulse index. At lower temperatures, when the work is not of a severe character (*i.e.* consider the work necessary to produce

Table IX. *Subject B. Muscular efficiency*

	Air condition				Work done in kilogram-metres in 3 min. to give a pulse index of				
Date	° F.	Percentage relative humidity	Air velocity in feet per min.	Cooling power	1·3	1·4	1·5	1·6	1·7
Mar. 16	50	61	0	7·8	400	455	505	580	670
„	50	62	200	13·8	490	540	590	626	670
Mar. 20	60	63	0	6·2	500	515	535	580	640
„	60	50	200	11·2	500	530	555	600	650
„	60	100	0	5·9	385	425	455	486	520
„	60	100	200	11·0	510	570	600	606	615
Mar. 23	75	37	0	3·4	350	470	516	540	600
„	75	33	200	6·0	493	530	566	586	610
„	75	100	0	3·3	407	430	450	468	480
„	75	100	200	4·9	405	445	500	570	660
Mar. 27	90	45	0	1·3	353	455	506	555	610
„	90	35	200	2·7	345	370	435	525	645
„	90	100	0	1·0	340	390	420	430	440
„	90	100	200	2·4	255	335	415	500	580

a pulse index of 1·3 to 1·5), the heat first produced in the performance of muscular work is utilised to overcome the internal friction (or viscosity) of the muscles.

Taking the work done to produce a pulse index of 1·7 as a criterion of the efficiency, it is seen that the efficiency is very slightly increased at high C.P.'s. There is a decrease in the efficiency at 75° F. and about the same decrease at 90° F. The efficiency at these dry atmospheric conditions is practically uninfluenced by air movement, although there is a slight increase in the efficiency at 90° F. when there is a wind.

The efficiency is greatly reduced by increased humidity even at temperatures as low as 60° F., and in every case the efficiency is increased by air movement of 200 ft. per min. and restored practically to normal.

Conclusion

It is seen that different subjects react differently to varying atmospheric conditions, and hence it is impossible to lay down any hard and fast rules as to the effects which will be produced by any one atmospheric factor, such as increased humidity or air movement.

Some subjects are entirely uninfluenced by increased humidity of the air, whereas others are greatly incapacitated by it. The majority of people, indeed, find that air which is saturated or nearly saturated with moisture is very oppressive. This is probably due to interference with the loss of heat by evaporation from the body surface.

In all cases, the physiological reactions produced by alterations in the atmospheric conditions follow very closely the subjective sensations experienced. Thus, subject A, who liked hot-air conditions and was in no way incapacitated by atmospheres saturated by moisture, showed minimum values for the pulse rate, respiration rate, metabolic rate and blood sugar, etc., at a cooling power of 3·5 ($\equiv$75° F. still). Subject B, however, who preferred cooler air conditions, showed minimum values at a cooling power of 6·5 ($\equiv$60° F. still). Moreover, the range of the variations of these factors with B was much greater than with A, probably because A experienced no discomfort at any atmospheric condition to which she was subjected, whereas B found all hot and humid atmospheres most disagreeable.

Also, when the subject was subjectively uninfluenced by humidity, the physiological factors were also generally uninfluenced. Thus increased humidity had no effect on the pulse rate, respiration rate, blood pressure, metabolic rate and blood sugar, etc., of A, whereas these were markedly influenced in subject B.

Whenever discomfort was experienced, whether due to increased air temperature or to increased humidity, this was always mitigated by air movement (except in the case of subject B at high temperatures and humidities, *i.e.* at 90° F. and 100 per cent. saturation).

It has long been realised that a consideration of atmospheric conditions is of great importance in connection with industry. In the air conditions such as would be encountered in an unventilated cotton mill, for example, most people would show an increased pulse rate, respiration rate and metabolic rate; decreased blood pressure and decreased muscular and mental efficiency, whereas if the mill were ventilated by a wind of 200 ft. per min. (although this amount of air movement is rather more than could be borne with comfort by most people) all of these factors would return almost to the normal level.

Furthermore, since in the interests of efficiency workers are often grouped according to their suitability for certain tasks as shown by their performance in vocational tests, it would seem that a simple physiological test could be devised, based on the pulse index, which would assist in determining a worker's optimum working conditions or his or her suitability for work under air conditions of low- or high-cooling powers.

I am greatly indebted to the Home Office for allowing me to carry out this work in the air-conditioning hut of the Home Office Industrial Museum and also to Mr E. Murray for his valuable help in connection with the physical measurements.

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