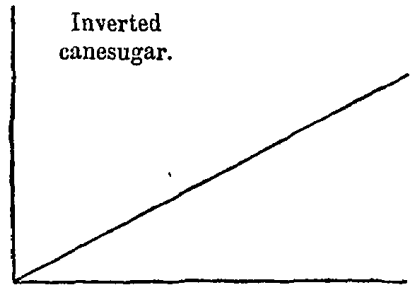
Chemistry. -- *"Enzyme Action."* By Dr. H. P. BARENDRECHT. (Communicated by Prof. J. M. VAN BEMMELEN).

(Communicated in the meeting of April 23, 1904.)

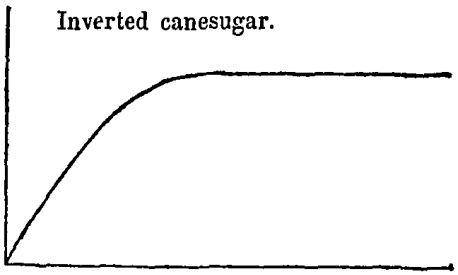
The following is a preliminary communication of the writer's researches on enzyme actions during the last two years.

From the commencement it has been the writer's object to ascertain in how far a continued research of simple enzyme actions might confirm the hypothesis that the enzymes exert their catalytic action by radiation. This hypothesis originated in the peculiarity of the action of the enzymes which distinguishes this action so sharply from that of the acids. A graphic representation of the action of the same quantity of acid or enzyme in the same time on sugar solutions of different concentrations renders this difference very perceptible.



Initial concentration of the canesugar.
Fig. 1.

Fig. 1 gives a scheme of the inversion by acids. The line which remains straight indicates that the quantity of inverted canesugar remains proportionate to the initial concentration.



Initial concentration of the canesugar.
Fig. 2.

In the case of enzymes the general course is represented by fig. 2. In the inversion of canesugar for instance, the line remains straight up to an initial concentration of 0.1%; it then inflects towards the x axis and runs henceforth parallel to this.

This characteristic behaviour of the enzymes is now at once explained by the radiation theory which will be further developed.

Let us, for the sake of convenience, confine ourselves to the action of invertin and let us suppose we have two solutions containing, respectively, 20% and 10% of canesugar. In the 20% solution the radiation from each enzyme particle will be comparatively soon absorbed by the surrounding molecules of canesugar; in the 10% solution the sphere to which the enzyme action can extend will be larger. So long as the solution is sufficiently concentrated to finally absorb by a sugar molecule all radiation emanating from an

enzyme particle, before the distance has become so great that the radiation fails to cause inversion, each enzyme particle is bound to exert the same action. One might compare an enzyme particle in concentrated sugar solutions with a source of light in a fog of varying density; the denser the fog the smaller the region around the source of light which absorbs all the light.

If, however, canesugar absorbs the radiation from invertin, we must expect the same to a greater or smaller extent from the products of inversion. On account of this power of absorbing the active rays these products must retard the inversion.

The result of my often-repeated experiments showed that the inversion of canesugar by invertin prepared from carefully dried yeast (we shall see, presently, that the method of preparing the invertin is of the greatest importance) is retarded equally by glucose, laevulose and invert sugar.

For instance, the same amount of yeast-extract inverted under the same conditions [1]) from

10% canesugar	49.3%
10% canesugar + 5% glucose	38.5%
10% canesugar + 5% laevulose	38.3%
10% canesugar + 5% invert sugar	38.3%

From the similarity of the last three figures it is already evident that we are not dealing here with a retardation due to a reversed reaction.

It was further ascertained that the other hexoses cause exactly twice as much retardation as glucose or laevulose:

8% canesugar	43.6% inverted
8% canesugar + 2% galactose	35.5% „
8% canesugar + 2% mannose	36.1% „
8% canesugar + 4% glucose	36.1% „

From these results it is evident that the inversion phenomena behave as if there are emitted by an invertin particle two radiations in equal quantity which we may call, provisionally, glucose and laevulose radiations. Each radiation by itself is capable of inverting a canesugar molecule; the glucose radiation is not absorbed by the glucose but by the laevulose; the laevulose behaves, conversely, in the same way. In accordance with this both radiations are absorbed by any other hexose. We may, therefore, regard invertin

[1]) All sugar determinations have been made by KJELDAHL's accurate gravimetric process.

as being, probably, a proteid containing glucose and laevulose groups in a peculiar radiating condition.

At all events, the radiation hypothesis will be found to lead to further quantitative research, to explain results already obtained and to predict future results.

Let us take a sufficiently concentrated solution of a grams of canesugar in 100 c.c. of water. A given amount of invertin then yields in the first minute a quantity of invert sugar m, independent of a. As soon, however, as a little invert sugar has been formed, the condition changes. The enzyme rays are now not only absorbed by the canesugar but also by the invert sugar. If we call n the absorption power of the invert sugar in regard to that of canesugar, then after the lapse of a time t when x is the remaining canesugar, the inversion velocity $-dx$ will be no longer m, but

$$-dx = m\frac{x}{x+n(a-x)}dt.$$

If we substitute $\frac{a-x}{a} = y$ and integrate, we obtain the equation

$$l\left(\frac{1}{1-y}\right) + \frac{1-n}{n}y = \frac{m}{na}t$$

or, using ordinary logarithms

$$log\frac{1}{1-y} + \frac{1-n}{n}0{,}434\,y = \frac{m}{na}0{,}434\,t.$$

In this equation two constants occur. The first m may be at once determined experimentally from the initial velocity. If we take, for instance the experiments of A. J. BROWN [1]), then $\frac{m}{a}$ or the fraction inverted per minute at the commencement is $\frac{0{,}130}{30}$. If in our equation we substitute this value of m, we find during the whole series of BROWN's figures a value for x of about 0.5.

It is, therefore, evident that the absorption powers of the canesugar and glucose, or laevulose molecules are in the proportion of about 2 to 1, that is to say in the proportion of their masses or, perhaps, surfaces.

Then we found the retarding influence of glucose and laevulose to be equal to that of invert sugar. Per unit of weight the number of molecules in glucose for instance and in canesugar are in the

[1]) Journ. Chem. Soc. 1902 pag. 377.

proportion of $\frac{342}{180}$. If now the absorption power of a molecule of glucose stands to that of a molecule of canesugar in the proportion of 180 : 342 and if we consider that glucose transmits without hindrance 50% of the total invertin radiation, the proportion between the absorption power of one part of glucose and that of one part of canesugar becomes:

$$\frac{342}{180} \cdot \frac{180}{342} \cdot \frac{1}{2} = \frac{1}{2}.$$

After inversion, one part of canesugar yields $\frac{360}{342}$ parts of invert sugar. Therefore n, the relative absorption power of the products of inversion of one part of canesugar, becomes $\frac{360}{342} \cdot \frac{1}{2} = 0{,}525$.

The formula for the inversion velocity thus becomes

$$log \frac{1}{1-y} + 0{,}393\, y = 0{,}827 \frac{m}{a} t.$$

BROWN's experiments conform still better to this formula than to HENRI's empirical formula $2k_1 = \frac{1}{t} log \frac{1+x}{1-x}$.

The correctness of our deduction may further be proved experimentally in the following way.

If in addition to the a grams of canesugar b grams of glucose, or laevulose are dissolved per 100 cc., the inversion velocity will be represented by:

$$-dx = \frac{x}{x + n(a-x) + \frac{1}{2}b} dt.$$

By again substituting $\frac{a-x}{a} = y$ we obtain, when using ordinary logarithms and calling $\frac{m}{na} 0{,}434 = k$:

$$log \frac{1}{1-y} + 0{,}393 \frac{1}{1 + \frac{b}{2na}} y = \frac{1}{1 + \frac{b}{2na}} kt$$

A same enzyme quantity acting under the same conditions in a solution containing canesugar only and in one containing canesugar plus glucose or laevulose gave the following figures:

10% canesugar.			10% canesugar + 5% laevulose.		
t	y	$k=\frac{\log\frac{1}{1-y}+0{,}393y}{t}$	t	y	$k'=\frac{\log\frac{1}{1-y}+0{,}393\frac{1}{1+\frac{b}{2na}}y}{t}$
91	0.412	0.00432	85	0.297	0.00273
191	0 72	0.00437	181	0.555	0.00276
			251½	0.72	0.00296
			306	0.763	0.0027
			360	0.824	0.00271

According to our formula we have:

$$k' = \frac{1}{1+\frac{b}{2na}}\, k = 0.68\, k = 0.00295$$

10% canesugar.			10% canesugar + 10% glucose.		
t	y	k	t	y	k'
57	0.191	0.00293	57	0.124	0.00145
118	0.364	0.00287	116	0.237	0.00142
242	0.642	0 00288	237	0.435	0.00142
			295	0.522	0.00144
			358	0.604	0.00146

$$\text{calculated } k' = \frac{1}{1+\frac{b}{2na}}\, k = 0.00149.$$

A further control is given by the determination of the initial velocity after previous addition of glucose or laevulose.

From

$$a\,dy = m\,\frac{a(1-y)}{a(1-y)+n\,a\,y+\frac{1}{2}b}\,dt$$

follows that the initial velocity in a solution of a canesugar + b glucose, or laevulose is:

$$\left(\frac{dy}{dt}\right)_{y=0} = \frac{m}{a}\,\frac{1}{1+\frac{1}{2}\frac{b}{a}}$$

whereas without such addition $\left(\frac{dy}{dt}\right)_{y=0} = \frac{m}{a}$.

These are the experimental results:

10% canesugar	10% canesugar + 2.5% laevulose	
inverted 14%	12.5%	$\frac{1}{1+\frac{1}{2}\frac{b}{a}}14 = 12.4$
10% canesugar	10% canesugar + 5% glucose	
inverted 12.4%	9.7%	$\frac{1}{1+\frac{1}{2}\frac{b}{a}}12.4 = 9.9$
10% canesugar	10% canesugar + 10% glucose	
inverted 19.1%	12.4%	$\frac{1}{1+\frac{1}{2}\frac{b}{a}}19.1 = 12.7$
8% canesugar	8% canesugar + 16% glucose	
inverted 16.6%	8.2%	$\frac{1}{1+\frac{1}{2}\frac{b}{a}}16.6 = 8.3$

It was now to be expected that many other neutral substances would also retard the inversion according to their capacity of absorbing the enzyme radiation.

These are some of the figures obtained:

Under the same conditions the same enzyme-quantities inverted of

10% canesugar	38.5%
10% canesugar + 5% urea	28.5%
10% canesugar + 5% mannitol	33.—%
10% canesugar + 5% erythrite	28.—%
10% canesugar + 5% glucose	29.8%

In another series:

10% canesugar	58.1%
10% canesugar + 5% dulcitol	50.4%
10% canesugar + 5% glucose	47.8%

There seems to be some kind of relation between the asymmetric carbon atoms and the absorption.

In the case of inversion of more diluted solutions of canesugar the above-mentioned simple relations will no longer exist. If we diminish the initial concentration, a dilution will soon be reached where a part of the radiation does not reach a sugar molecule in time, but is either finally absorbed by the water or when arriving has, in any case, become too weakened to cause inversion. The

quantity of canesugar inverted by a given amount of invertin will, therefore, go on decreasing. In the end, however, we shall arrive at an initial concentration where, within the sphere of action of an enzyme particle, two canesugar or invert sugar molecules can no longer shade each other. From this point, the inversion caused by the given enzyme-quantity will be just proportionate to the canesugar concentration. Then, during the whole of the process, the reaction velocity merely depends on the average number of canesugar molecules present within the active radiation sphere of an enzyme particle.

The following are some of the figures obtained which always exhibited the same regularity.

Concentration canesugar in grms. per 100 cc.	Inverted in grms. per 100 cc.	Inversion in %.
0.05	0.022	44.—%
0.1	0.0448	44.8%
0.125	0.0545	43.8%
0.25	0.097	39.—%
0.5	0.174	34.7%
1.—	0.240	24.– %
2.—	0.317	15.9%

Another series gave

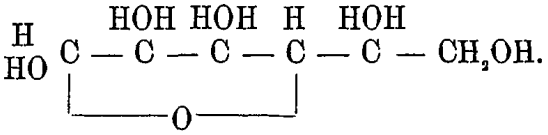
Concentration of canesugar in gr. per 100 cc.	Inverted in grms. per 100 cc.
3	0.86
4	0.95
5	0.96
7	0.93

The fact that, in very dilute solutions, the enzyme action really proceeds as a unimolecular reaction according to the formula $k = \frac{1}{t} log \frac{1}{1-y}$ was further again confirmed by experimenting with a solution containing 0,096 % of canesugar.

Up to the present we have for the sake of convenience disregarded the synthetical action of the enzyme rays. Light, being a catalyzer, can act either as a synthetical or decomposing agent, so we must expect the same from the enzyme rays. That we often do not notice such action is due in the first place to the secondary change of the

decomposition products, at least in the case of invertin. It has already been stated by O'SULLIVAN and THOMPSON [1]) that invertin separates glucose from canesugar in a birotatory condition. TANRET [2]) and SIMON [3]) have afterwards elucidated this birotation question.

The birotatory α-glucose is the sugar of the α-glucosides and the semi-rotatory γ-glucose that of the β-glucosides; according to the said authors they are the stereoisomeric lactones:



The form which in solution is stable, the β-glucose conforms to the aldehyde-formula $CH_2OH\,(CHOH)_4COH$.

This conclusion is opposed by other investigators such as ÅRMSTRONG [4]) and LOWRY [5]), who look upon the stable form not as an aldehyde but as a condition of equilibrium between α- and γ-glucose. My investigation goes in favour of the first opinion.

Invertin is, generally speaking, the enzyme of the α-glucosides. Canesugar may also be considered as an α-glucoside in accordance with the fact that on inversion, the glucose is always separated in the α-modification. This α-glucose is now, however, gradually converted into β-glucose and, therefore, prevents the reconversion into canesugar. Owing to this, all the canesugar is always finally inverted by invertin.

A continued research, however, showed that there may be still another reason for the non-appearance of reversal phenomena. The method of preparing the invertin, that is of the yeast extract was found to greatly affect the properties of the enzyme.

At first, I used for the preparation of a powerful invertin a yeast cultivated in a solution of canesugar. This yeast after being mixed with "kieselguhr", was first dried in vacuum at a low temperature and then for half an hour at 100° in an ordinary oven.

The addition of "kieselguhr" facilitates very much the subsequent extraction and filtration. The above experiments have been made each time with a freshly prepared filtrate.

Afterwards it was found that ordinary yeast also gives an enzyme with the same properties, provided it has not been dried at too high

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[1]) Journ. Chem. Soc. 1890 pag. 861.
[2]) Zie Dictionnaire de Chimie de Würtz. 2e suppl. p. 764.
[3]) C. R. 1901 pag. 487.
[4]) Journ. Chem. Soc. 1903 pag. 1305.
[5]) Journ. Chem. Soc. 1903 pag. 1314.

a temperature. Ordinary laevulose in solution is as a rule less stable than glucose. In invertin a difference in the same direction is also usually revealed. Drying at too high a temperature, heating the "kieselguhr" mixture above 100°, or precipitation with alcohol and redrying the precipitated enzyme, repeatedly gave invertin, the action of which is retarded considerably more by laevulose than by glucose. Active laevulose therefore generally becomes inert sooner than active glucose.

This explains the difference between my results and those of VICTOR HENRI [1]) who states:

"Pour l'addition d'une même quantité de sucre interverti, le ralentissement est d'autant plus faible que la concentration en saccharose est plus grande. Ce ralentissement est produit presque uniquement par le lévulose contenu dans le sucre interverti."

Probably, HENRI has obtained his invertin from yeast dried at more elevated temperatures or has used commercial invertin, prepared by precipitation with alcohol. That the retardation of a same quantity of invert sugar becomes smaller when the canesugar concentration becomes greater is quite in harmony with the radiation theory. The fact that the laevulose contributed most to that retardation was only a pathological phenomenon of the invertin.

We must further bear in mind the possibility that, owing to those harmful actions, the radiation gets so weakened that the reversion can no longer take place, or that the radiating α-glucose has been converted into radiating β-glucose and also that only the latter is capable of inverting. It is certainly to be expected that, if only active glucose or active laevulose is left behind, the power of causing reversion has either decreased or been destroyed.

In order to counteract the first cause of the non-appearance of the reversal, namely, the secondary conversion of α-glucose into β-glucose, we may apply much enzyme and so accelerate the conversion. A larger quantity of extract of the above yeast, which had been finally dried for half an hour at 100°, caused indeed a slower inversion of the last remaining percentages of canesugar.

The reversed action was afterwards noticed more distinctly with ordinary yeast, merely dried in vacuum at about 30°. We will first give a mathematical formulation of the phenomena to be expected.

Let us imagine an aqueous solution of invert sugar, liable to reversal and consequently containing the glucose in the α-form, in

[1]) C. R. 1902 Nov. 24. 917.

the presence of invertin particles, which render this invert sugar active. The velocity of synthesis will then be proportionate first to the product of the concentration of glucose and laevulose and further to the extent of the active radiation sphere surrounding each enzyme particle. The latter is inversely proportionate to the joint concentration of the invert sugar and the other dissolved matters eventually present, each with their own absorption coefficient.

The synthetical action of invertin in a solution containing $(a—x)$ grams of invert sugar and x grams of canesugar in 100 cc. is therefore:

$$dx = mp \frac{\left(\frac{a-x}{2}\right)^2}{x+n(a-x)} dt.$$

The complete formula for the inversion velocity of canesugar, in case the original products of inversion suffered no change, would then be [1]):

$$-dx = m \left\{ \frac{x}{x+n(a-x)} - \frac{1}{4} p \frac{(a-x)^2}{x+n(a-x)} \right\} dt.$$

The point of equilibrium would then be determined by the equation:

$$x - \frac{1}{4} p (a - x)^2 = 0.$$

Or returning to relative fractions by substituting $\frac{a-x}{a} = y$:

$$1 - y - \frac{1}{4} pa y^2 = 0.$$

If now we have introduced into the solution such a quantity of enzyme that this equilibrium point is attained before the birotatory glucose has been converted to any great extent into ordinary glucose, the inversion will not actually come to a standstill, but the line, indicating its progressive course, will exhibit a characteristic peculiarity in that place. Then, starting from that point, the formation of fresh α-glucose by inversion will be dominated by the velocity of the inversion of the total α-glucose present into β-glucose. This velocity is proportionate to the concentration of the α-glucose. If this is continually replenished by fresh formation of α-glucose from canesugar, both the said velocity and the inversion velocity will be

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[1]) The small increase in weight when $C_{12} H_{22} O_{11}$ changes into $2\ C_6 H_{12} O_6$ is here neglected; we might also suppose that it is taken into account in the coefficient p. By substituting the variable y this factor in p would in any case disappear again.

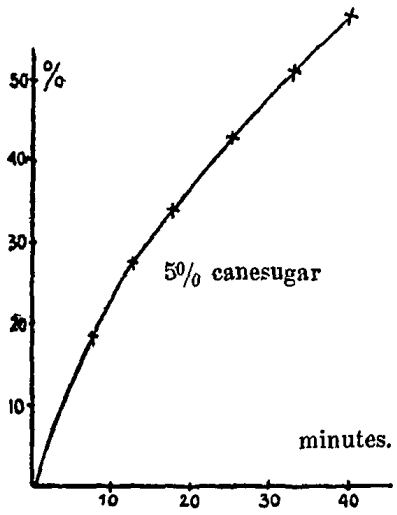

Fig. 3.

constant. From this point the line, representing the inversion as a function of the time, will of course be a straight one until the concentration of the canesugar has become too small to make the inversion keep pace with the glucose transformation.

With 5% canesugar (see fig. 3) the line commences its straight course at about $y = 0{,}35$. This substituted into the equation

$$1 - y - \frac{1}{4} p a y^2 = 0$$

gives $p = \frac{0{,}65}{0{,}153}$ or, practically $= 4$.

In the 10% solution (fig. 4) the equilibrium must then become

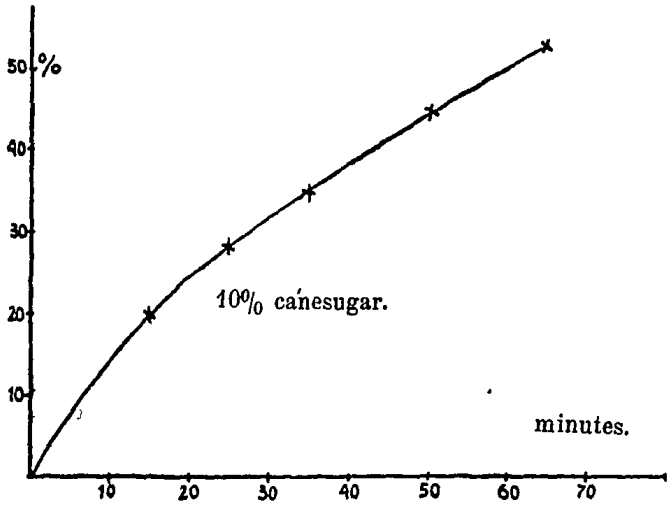

Fig. 4.

perceptible at the value of y to be calculated from

$$1 - y - 10y^2 = 0$$

therefore, at $y = 0{,}27$.

Those equilibria phenomena are observed more readily in the *inversion of maltose by yeast-extract.*

The enzyme which converts maltose into glucose is generally called maltase so as to distinguish it from invertin. It seems to me that there is no valid reason for making such a distinction. A yeast-extract, which inverts maltose, has always been found to also behave actively

towards canesugar [1]), but not the reverse. This is in harmony with the radiation theory. Maltose, like canesugar, is an α-glucoside. The connecting point of the laevulose in the canesugar molecule with the α-glucose is the C of the carbonyl group of the laevulose. In maltose the α-glucose is attached to the CH_2 of the otherwise uncombined molecule of glucose. Canesugar is also much more readily inverted by acids than maltose. Both radiating α-glucose and laevulose (probably also radiating β-glucose [2])) are liable to invert canesugar. Maltose is only converted by active α-glucose, but as may be expected from its behaviour towards acids and its constitutional formula it requires a more powerful radiation than canesugar. If, therefore, yeast-extract has been weakened by elevation of temperature or by precipitation, its power of inverting maltose may have been lost or much diminished, although canesugar is still fairly rapidly inverted.

The preparation of a yeast-extract with a powerful inverting action on maltose proved to me no more difficult than when inversion of canesugar was intended. The above-described yeast, derived from a canesugar solution, and which had been actually heated at 100° for half an hour, yielded after a year and a half an extract which readily inverted maltose. In this case, I used, of course, by preference a yeast which had been mixed with "kieselguhr" and dried at a low temperature. On extracting the dried mixture, the solution never contains zymase as experiment repeatedly showed.

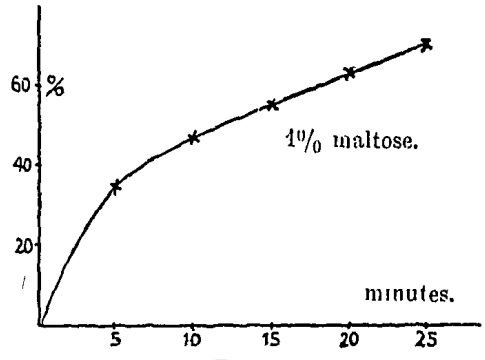

Fig. 5

Figures 5, 6, 7, 8 and 9 now clearly show the phenomena of equilibrium. If α-glucose in solution were stable, only 15% of the total might be inverted by yeast-extract in a 10% maltose solution.

The fact that maltose is decomposed by yeast-extract so much slower than canesugar is partly due to the circumstance that the point of equilibrium is reached so much earlier. The further decomposition then again merely keeps pace with the transformation of α-glucose into β-glucose.

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[1]) Potievin, Annales Inst. Pasteur 1903. p. 31.

[2]) Separate experiments showed that glucose, previously heated and therefore in the β-form, and glucose, dissolved immediately before adding the enzyme and therefore in the α-form, both retard the canesugar inversion to the same extent. β-Glucose therefore transmits the glucose rays (then perhaps converted into β-glucose rays) quite as well as the α-glucose.

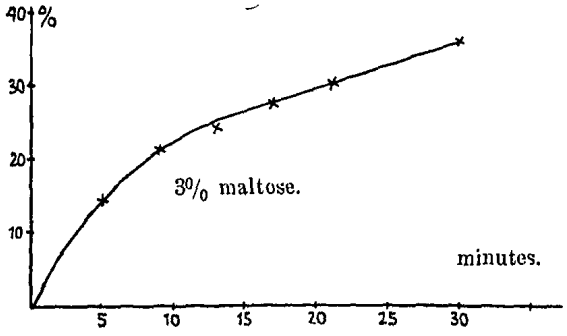

Fig. 6.

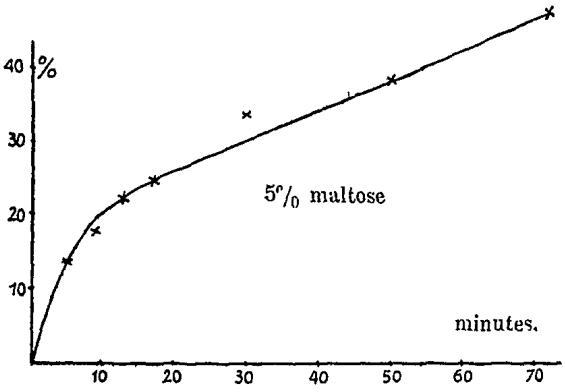

Fig. 7.

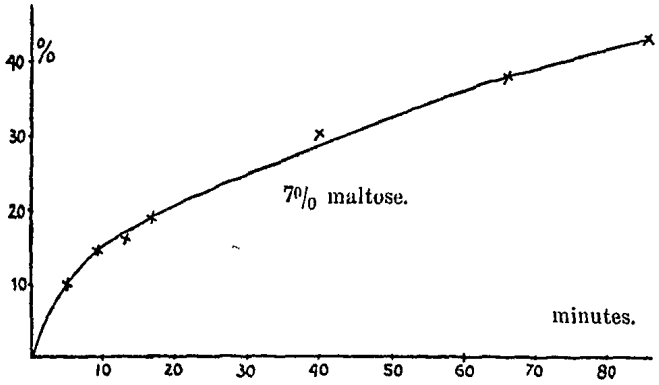

Fig. 8.

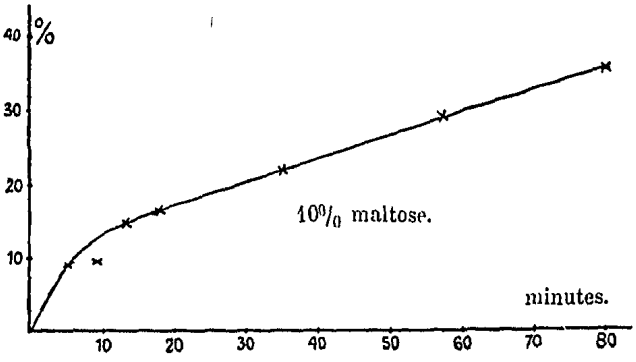

Fig. 9.

The points where the transformation lines commence to run straight, so the points of equilibrium, also conform in this case to an equation similar to that used for canesugar. Glucose is here the only product of inversion; we might, therefore, expect that the undisturbed transformation velocity would be here:

$$-dx = m\left\{\frac{x}{x+n(a-x)} - q\frac{(a-x)^2}{x+n(a-x)}\right\}dt$$

and the equilibrium equation, therefore:

$$1 - y - q\,a\,y^2 = 0.$$

For 10 grams of maltose in 100 cc. we experimentally found $y = 0.15$.

This gives $q = \frac{0.85}{0.225}$ or, practically, 4, therefore the same coefficient as for canesugar [1]).

If in the above equation we substitute $q = 4$; or

$$1 - y - 4\,a\,y^2 = 0,$$

the calculated points of equilibrium become for

a	y
10	0.146
7	0.172
5	0.20
3	0.25
1	0.39

This is therefore in accordance with the experiment.

The well-known researches of CROFT HILL [2]) on the reversal of maltose gave points of equilibrium which were situated at a more advanced transformation; for instance in a 10% solution $y = 0.945$. These equilibria were attained only after days and weeks; the above cited after a few minutes.

Afterwards [3]) HILL himself demonstrated that the resulting biose was not maltose but an isomer, which he called revertose. In HILL's numerous experiments, all the glucose was no doubt in the β-form. The synthesis found by HILL was therefore a combination of two molecules of β-glucose to a new biose, isomeric with maltose [4]).

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[1]) The diffusion velocities on which depends the velocity of meeting of two molecules, cannot differ much for glucose and laevulose.

[2]) Journ. Chem. Soc. 1898 p. 634.

[3]) Journ. Chem. Soc. 1903 p. 578. EMMERLING (Ber. 34, p. 600) had found isomaltose as reversal product.

[4]) Most of the natural glucosides appear to be compounds of bi- or semi-rotatory hexoses. When endeavouring to prepare lactose from galactose and glucose by

The retarding action of another added hexose is not studied so readily in the case of maltose inversion as in the transformation of canesugar, on account of the immediately occurring reversion. Still it was found that both laevulose and galactose cause the same retarding action, as might be expected from our theory now we are dealing with glucose-radiation only.

For instance, a same amount of yeast-extract gave under the same conditions and in the same time:

in :	inversion
6% maltose	18.9%
6% maltose + 1.5% galactose	15.5%
6% maltose + 1.5% laevulose	15.5%

Another series gave

in :	inversion
6% maltose	26.8%
6% maltose + 1.5% laevulose	24.8%
6% maltose + 1.5% galactose	25.–%
6% maltose + 1.5% glucose	13.5%

This last figure, verified by other experiments, requires a further explanation. This 1.5% glucose was undoubtedly β-glucose. Before mixing it with the maltose, the glucose was dissolved separately and completely converted into the stable form [2]) by placing the flask for some time in boiling water. The observed order of retardation shows that the β-glucose also takes part in the process of reversion. Now it is possible that in the maltose molecule the glucose with the still free carbonyl group is present in the β-modification and it is even probable that this free glucose group, when in solution, will be converted into the same stable form as glucose itself. In HILL's investigations, yeast-extract appeared capable of uniting two molecules of β-glucose; so, probably, also two molecules of α-glucose. The glucose formed in the enzyme-inversion of maltose may, therefore be called homogeneous. Each molecule of that glucose can unite itself under the influence of the enzyme radiation with any other molecule of that glucose to a biose. Therefore, the equation of equilibrium was here

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means of lactase, EMIL FISCHER and FRANKLAND ARMSTRONG only obtained an isolactose.

The synthesis of canesugar has not yet succeeded because we can only add β-glucose and not α-glucose to laevulose.

[2]) Separate experiments showed that unheated glucose causes the same retardation as heated, α-glucose therefore the same as β-glucose.

$$1 - y - 4\,a\,y^2 = 0.$$

It should also be mentioned that the inverting enzyme of yeast appears to be always the same whether canesugar or maltose has been present as a carbohydrate food. In an ordinary cereal extract a little canesugar occurs along with the maltose. A pure yeast-culture, cultivated by myself in a solution of pure maltose (plus the necessary salts and nitrogenous food) gave an enzyme extract which was retarded in its action equally much by glucose and laevulose, and twice as much by galactose. In the enzyme formation, therefore a partial conversion of glucose into laevulose seems to take place. LOBRY DE BRUYN and ALBERDA VAN EKENSTEIN [1]) have shown that these two hexoses may be converted into each other in an alkaline solution.

The investigations of O'SULLIVAN and THOMPSON [2]) have rendered it probable that the invertin-molecule (if we may use this expression) contains a carbohydrate group. These investigators have attempted to purify invertin and found that a constant component of the resulting proteid-complex, their so-called η-invertan, contained 18 parts of carbohydrate to one part of albuminoid.

A further development of the electron theory will probably elucidate the nature of those enzyme radiations. As LODGE [3]) observed, it is not the occurrence of radiations in matter which need cause astonishment but rather the fact that not a great many more radiation phenomena have already been discovered.

Many other catalytic phenomena such as the action of hydrogen-ions and those of BREDIG's anorganic ferments may, after all, be due to radiations. For hydrogen-ions, carriers of loose electrons and dispersed platinum cathodes probably also emit radiations owing to the motion of the electrons in or around the material particle. During the course of a same reaction, BREDIG often noticed an increase of the constant $k = \frac{1}{t} log \frac{1}{1-y}$ just as that shown by the invertin action. A retardation of the catalysis by indifferent matters has also been frequently noticed, for instance, by KNOEVENAGEL and TOMACSZEWSKI [4]) in the action of finely divided palladium or platinum on benzoin.

If the statements of the French investigators on the physiological

[1]) Rec. Trav. Chim. 1895 p. 201.

[2]) Journ. Chem. Soc. 1890 p. 834.

[3]) "On Electrons" Journ. Electr. Engineers 1903 Vol. 32 p. 45.

[4]) Ber. 1903. 2829.

n-rays should be promoted to objective truth, our hypothesis would receive a direct experimental support.

At all events, the above has demonstrated that the principal measurable phenomena, noticed in the enzyme action are in harmony with our hypothesis.

Meteorology. — "*On a twenty-six-day period in daily means of the barometric height.*" By Dr. J. P. VAN DER STOK.

1. A few years ago [1]) Prof. A. SCHUSTER investigated the problem, how to detect the presence of a periodical oscillation, the amplitude of which is small in comparison with large superposed fluctuations which may be considered as fortuitous with respect to the purely periodical motion.

Starting from an analogy which may be seen between this question and the problem of disturbances by vibrations in the aether — a problem treated by Lord RAYLEIGH [2]) in 1880 — Prof. SCHUSTER has endeavoured to apply the theory of probability to the determination of the first couple of coefficients of a FOURIER series, and the method he arrives at, and strongly advocates, is applied to records of magnetic declination observed at Greenwich during a period of 25 years.

The choice of this material, in Prof. SCHUSTER's opinion not favourable for the discovery of small effects, is justified by the remark that "the only real pieces of evidence so far (1899) produced in favour of a period approximately coincident with that of solar rotation were derived from magnetic declination and the occurrence of thunderstorms."

In this and in an earlier paper [3]) the author emphasizes that, in inquiries of this kind, it is not at all sufficient to come to some result, but that it is necessary to apply a reliable criterion by which a judgment may be formed about the value to be attached to the result arrived at.

His mathematical investigation, however, does not, lead to an outcome which in every respect can be regarded as satisfactory, in so far that a method of determining the mean and probable error of the result from the series of observations themselves is not given and,

[1]) Trans. Cambr. Phil. Soc. Vol. XVIII. 1899.

[2]) Phil. Mag. Vol. X. II, 1880.

[3]) Terrestrial Magnetism Vol. III, 1898.