ENZYME SCIENCE AND ENGINEERING

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LECTURE – 6

KINETICS OF ENZYME CATALYSED REACTIONS

Having understood the chemical and functional nature of enzymes we will now move on to the kinetics of enzyme catalyzed reactions. The enzyme kinetics deals with a quantitative analysis of the effect of various parameters on the rate of enzyme catalyzed reactions.

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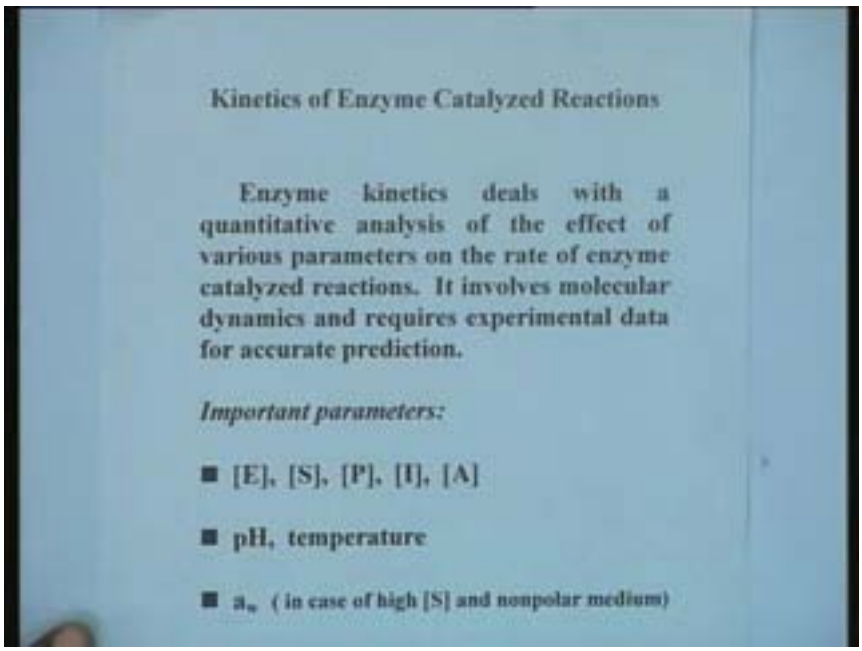

It involves molecular dynamics between the various species involved in the reaction. One of the principle species as you know is enzyme itself and the interaction of enzyme with all other chemical species that is the substrate, the product, if there is any inhibitor all those molecular species, dynamics of these inter relationship or interactions is involved in the study of enzyme kinetics.

One of the essential features for study of enzyme kinetics is the experimental data. To start with any kinetic analysis one needs to initiate with some basic experimental observations and to take some experimental observations the key parameters that are usually involved in the study are the enzyme concentration, substrate concentration, product concentration, concentration of inhibitor or activator if there is one where as enzyme, substrate and product are the universal chemical species involved in the case of an enzyme catalyzed reactions. Then you have a set of environmental parameters like hydrogen ion concentration reflected in pH, or the temperature of reaction.

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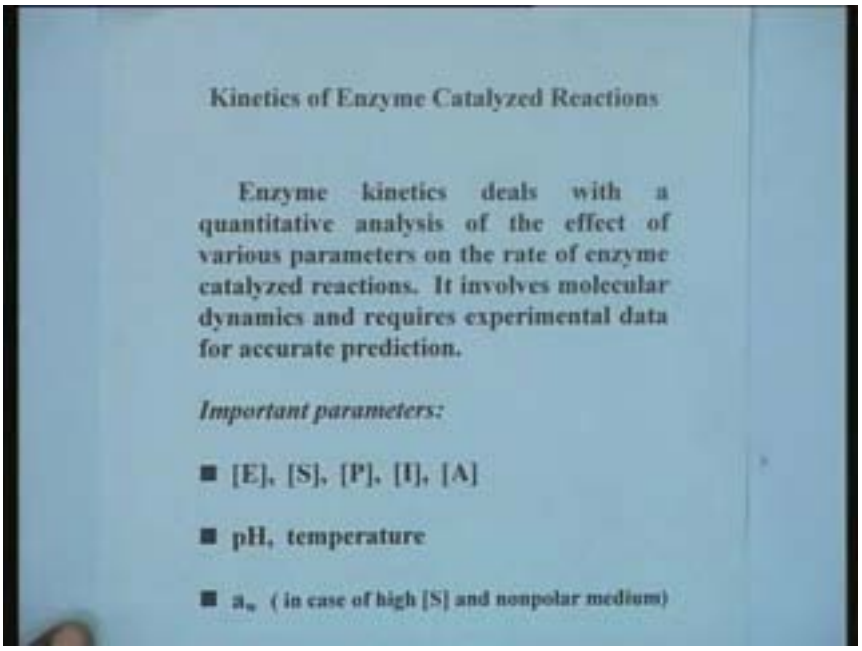

Then in most of the enzyme catalyzed reactions, we assume the water concentration to be very large almost in excess and for most of the analytical purposes it is ignored. But at least in two distinct cases where we are handling very high substrate concentrations of the order of say greater than 50 or 60% weight by volume or in cases where we are carrying out reactions in the non polar medium, water activity also becomes a key parameter for study under enzyme kinetics because then it controls the rate of enzymatic reaction and therefore it must be taken into account. So when we talk of enzyme kinetics we are looking into the molecular dynamics of various species that are involved in the reaction, their interconversions and the effect of those inter conversions on the rate of reactions as a function of different parameters that are listed here.

There are three major constituents in any kinetic analysis. As I mentioned earlier the first and probably the most important are the experimental data. With the start of experimental data one assume certain mechanistic hypothesis that means how various molecules involved in the reaction are interacting and you propose some kind of a mechanistic hypothesis or in other words you can say in a simplistic term the sequence of reaction steps that are involved in converting substrate into product and based on these

mechanistic hypothesis or reaction steps we arrive at a rate equation using mathematical analysis. This rate equation again has to be verified with more experimental data.

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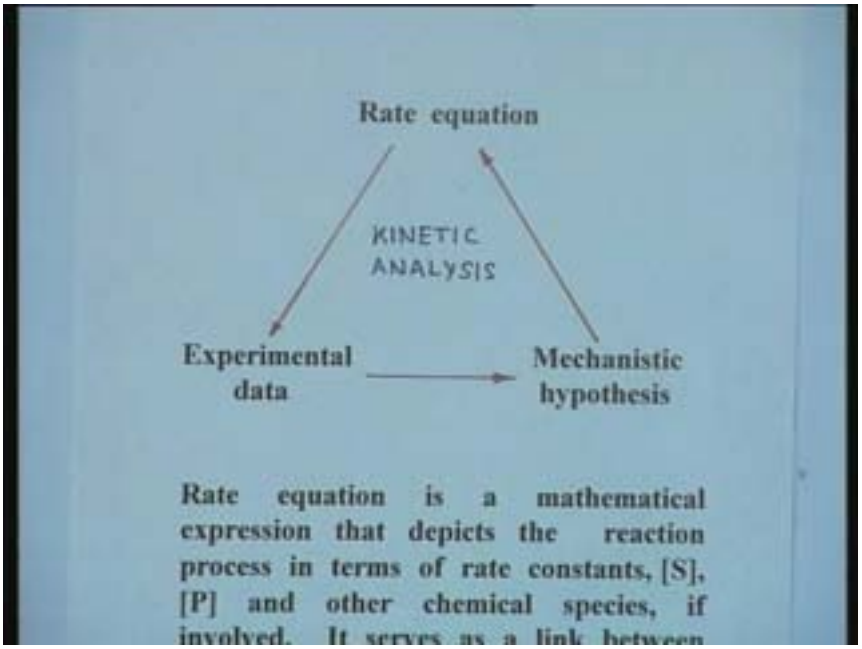

If it befits the experimental data, if it predicts or gives you a close relationship with experimental data it is acceptable, both the rate equation as well as mechanistic hypothesis we have assumed are acceptable. If not we again go back to the mechanistic hypothesis modify it, revise it, in the light of the deviations and then again go back to a new rate equation and therefore such an analytical sequence continues till we are able to match our rate expression with the experimentally observed data.

In a very simplistic term the rate equation is a mathematical expression that depicts the reaction process in terms of rate constants. I think we must appreciate that the rate equation has a very new dimension in terms of rate constants.

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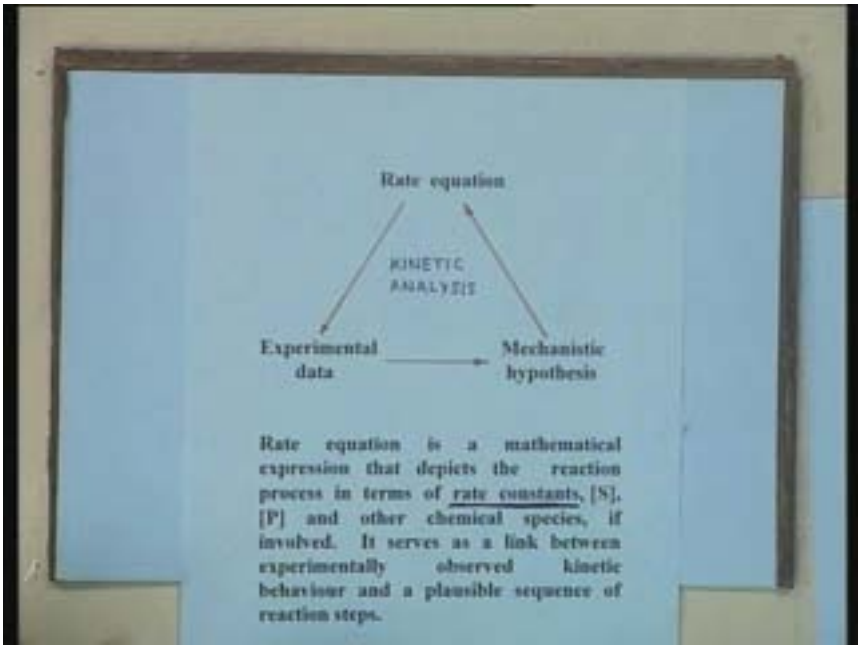

A large number of rate constants might be involved depending on the number of reaction sequence steps and substrate and product and other chemical species if involved. It also serves as a link between experimentally observed data and the possible reaction sequence steps. These three parameters or these three steps are the critical steps in study of any kinetic system and applies to also the enzyme kinetics.

The second question that might emerge in your mind is why do we study enzyme kinetics? What is the function? I think the first and foremost and probably the most trivial function that could be understood is to get a certain optimum set of parameters for carrying out the reaction. For example when we consider the optimum temperature of the reaction and the optimum pH for the reaction that is probably one of the primary objectives of establishing the whole kinetics analysis. As I mentioned you will always assume a mechanistic hypothesis to arrive at rate expression. It also gives a number of inferences about reaction mechanisms and the current accepted rate expressions for enzyme catalyzed reactions involve a hypothesis where enzyme substrate complex has to be formed. It gives you a very big inference when we look at the reaction mechanism also and that becomes a very important part of the study of reaction mechanism itself.

It can also give you lot of insight into the biological phenomena, the kind of reactions that the enzymes carry out inside the cell at molecular level, their intercellular concentrations, their physiology, directions and regulation. Lot of issues about the function of the enzymes within the cell can be understood and inferred from the kinetic analysis of the enzyme. Then from the point of view of biochemical engineers probably a very important requirement for enzyme kinetic study is to design enzyme reactors. The design of enzyme reactors, like in the case of chemical reactors is based on kinetic pattern. The kind of kinetics the reaction follows dictates very often the choice of reactor types, the design parameters for the reactor and they all involve enzyme kinetics and that becomes quite important tool for reactor design.

Finally from academic point of view I think the enzyme kinetics has also played a very important role in classification of the enzymes particularly when the same enzyme is obtained from different sources their kinetic characterization gives you a very good tool to distinguish between them and use them so that you can understand and identify the enzyme based on the kinetic characteristics. Also even as an analytical tool you can use when we want to determine many of the metabolite concentrations using enzymes, the basis of the design of experiment is kinetic parameters. We will probably talk when we will come to the actual kinetic aspects.

The third point which one must look at before studying the actual enzyme kinetics is some of the historical perspectives that have gone into the study of enzyme kinetics. The understanding in the mind of the scientists that there are some biological catalysts which catalyze a number of reactions particularly the key reaction which people have understood then thanks to the availability of alcoholic beverages that the yeast possesses some enzyme which hydrolysis sucrose into glucose. In those days the alcoholic fermentation enzyme was called as zymase although that kind of nomenclature is no longer valid.

The first enzyme in the whole reaction sequence was invertase which hydrolyses sucrose into glucose and fructose and that was essentially the a key enzyme which people were ready to study.

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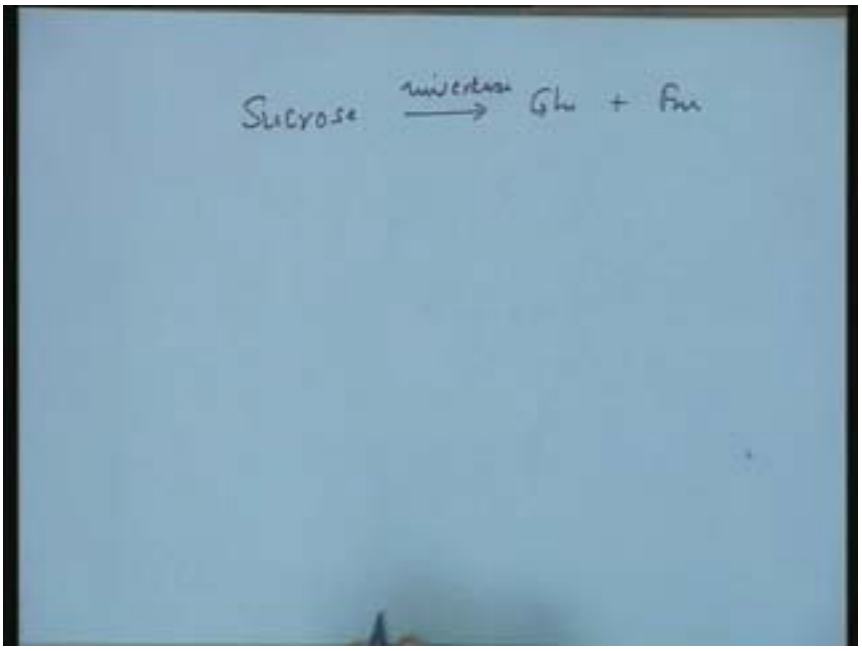

Many of the kinetic analysis in the early stages when I say early stages I am referring to the late 18th century or early 19th century when people tried to understand the kinetic

behavior and I think one of the earliest recorded interpretations or results of the kinetic analysis of enzyme was by O'sullivian and Tompson as early as 1890.

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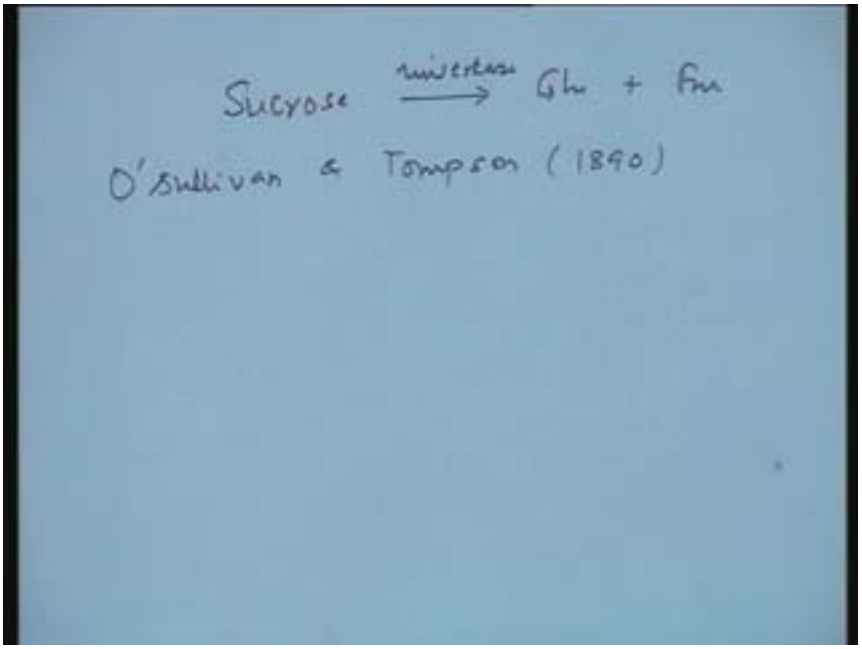

He also studied the same enzyme and probably concluded in a very broad fashion that the enzyme reaction rates are influenced by acidity and enzyme concentration. These are the two parameters which today we understand as pH and the temperature. He did not propose any basic hypothesis but suggested based on his experimental observations that with every ten degree centigrade rise in temperature the reaction rate doubles. As a rule of thumb it is still valid today.

He also gave a good clue which became a landmark in the applications of enzymes that enzymes are more stable in the presence of a substrate. In fact many of the enzyme preparations then could be stored in the presence of substrate and that was another conclusion or observation he gave way back in 1890. We store enzyme in the presence of substrate at low temperatures so that reaction rate is stopped during storage. The low temperature was always understood phenomena that at low temperatures the enzymes will have a much better stability. They didn't have any concept of enzyme substrate complex in those times and he only gave a hypothetical suggestion that one could store the enzyme at low temperature in the presence of substrate. His observations were all based on hydrolysis of sucrose by invertase.

Then the second major kinetic observations came from Brown, also in the early 19th century and he proposed for the first time the concept of an enzyme substrate complex.

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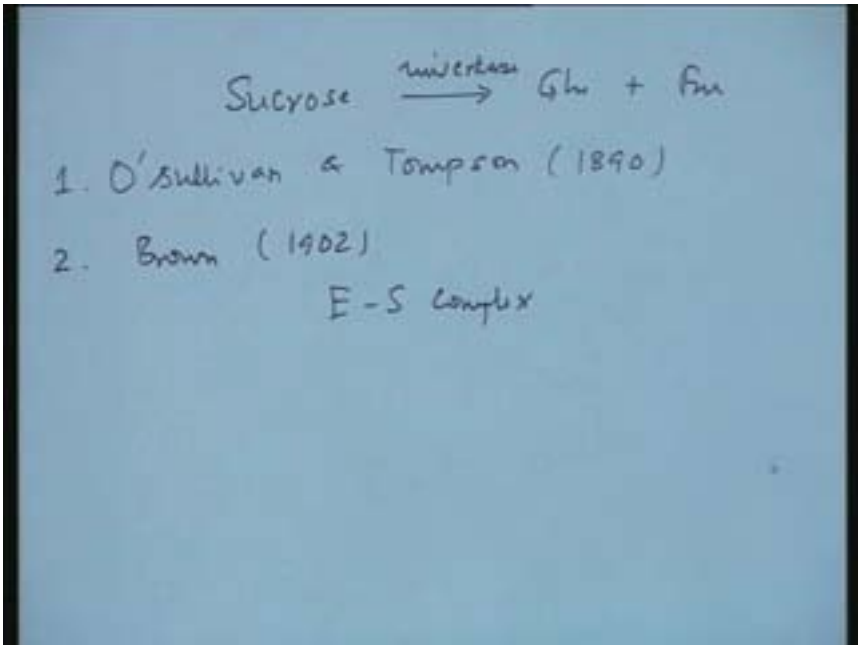

He also proposed based on experimental observations that the reaction rate for an enzyme catalyzed reaction that is conversion of substrate into product follows saturation kinetics. That means the rate increases initially and reaches to a saturation level. He did not give any experimental or any hypothesis or any sequence of steps but he was able to understand that the basic reaction kinetics follows a saturation behavior. The rate increases to a maximum level and then stops at that and beyond that there will be no increase in the reaction rate. At low substrate concentrations the enzyme reaction rate is proportional to substrate concentration. That is the phenomena we still understand, now with our detailed understanding of enzyme kinetics today. These are the broad observations by Brown.

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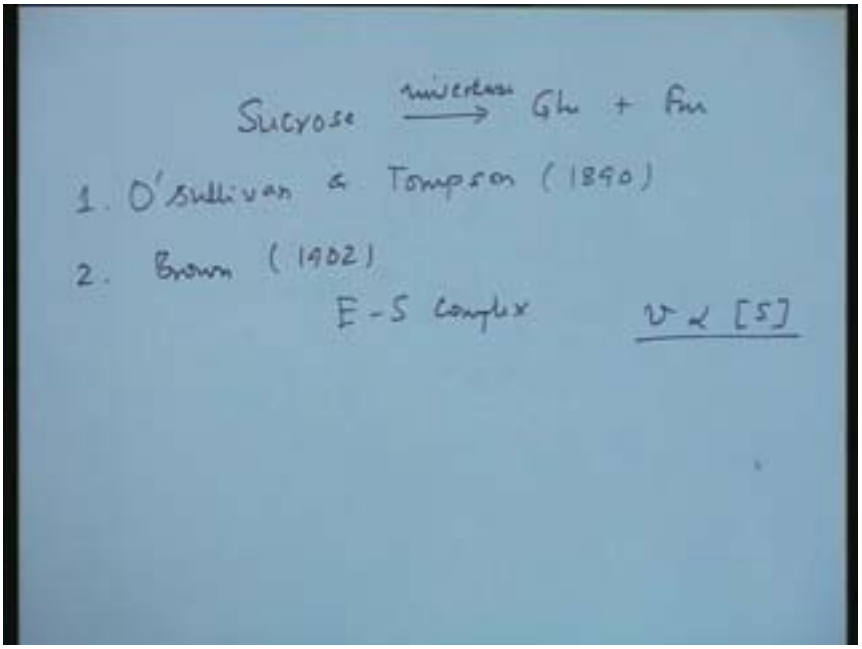

The major breakthrough in the enzyme kinetics was Michaelis and Menten as early as 1913. At that time we had great limitation of analytical methods.

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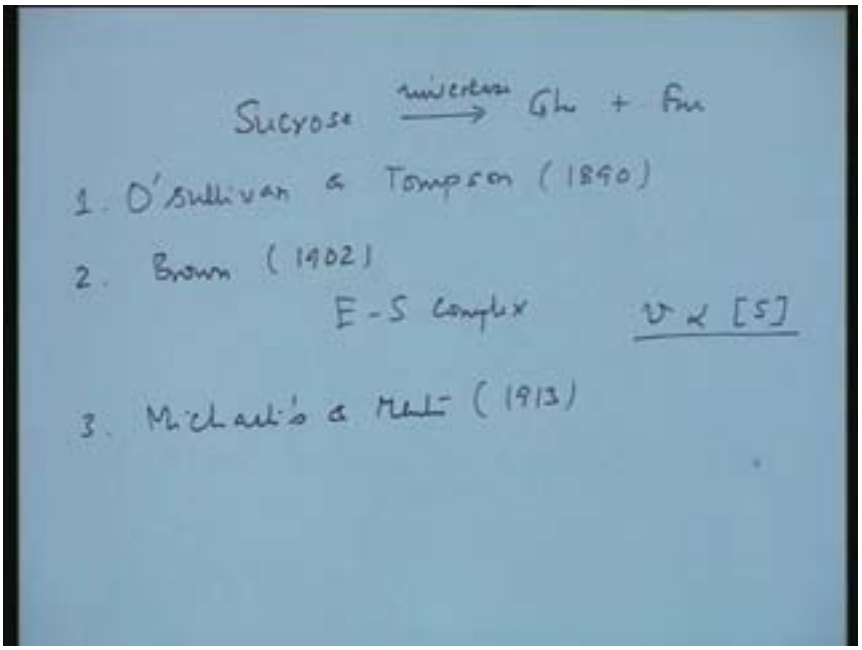

In most cases analytical procedures was not available to quantitatively measure the concentration of product. The concept of buffers was in a very primitive stage. Buffers were not a very common tool to be used in the kinetic analysis. Then they didn't have much understanding of the stability of the enzyme as well. Thirdly the enzymes they used were really not available in the highly purified state. What they were talking was with

crude enzymes preparations and so the observations they could get were very limited. One of the major contributions of Michaelis and Menten was that he confirmed Brown's observation that enzyme substrate interaction undergoes formation of enzymes substrate complex. That is

$$E + S \rightleftharpoons E\text{-}S$$

He also proposed that this interaction is reversible in nature and he was the first person to propose a mechanistic hypothesis which is followed by enzyme catalyzed reactions. This enzyme substrate complex then breaks irreversibly to form the product and enzyme is released back.

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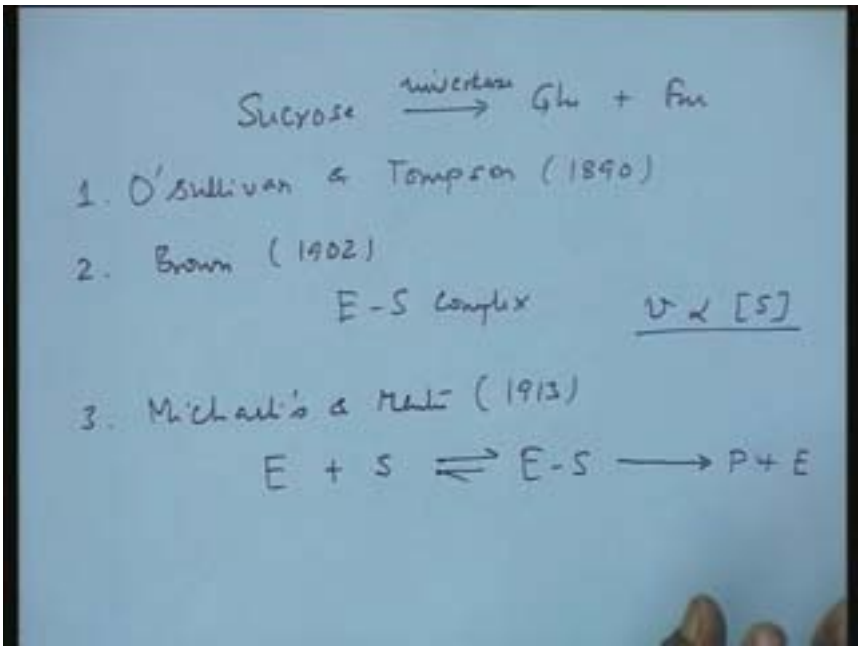

That was the hypothesis he proposed and probably is one of the landmark contributions in the study of enzyme kinetics even today. Secondly he could also generate data using buffered substrate. For the first time he carried out reactions under controlled pH. Then the third thing was that he introduced another concept. The first contribution was enzyme substrate complex, the second was buffered substrate.

The third and another important feature was that he said that when we talk of rate of reaction we must talk only of initial rate of reaction, v_o.

$$v_o = (dp/dt)_{t=0}$$

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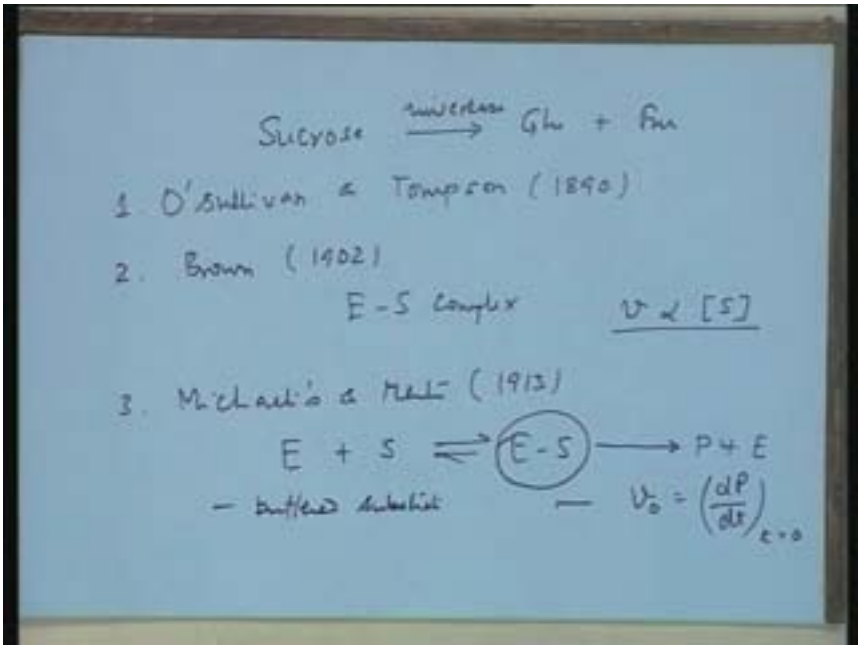

He said that once a significant quantity of product is formed the kinetic behavior changes. The enzyme follows a kinetic behavior which can only be understood at the initial reaction rate that means when you carry out the reaction the rate of product formation at time zero. The fourth thing was he confirmed the concept of enzyme substrate complex. He also mentioned that the interaction between enzyme and substrate to form enzyme substrate complex is in perfect equilibrium. It reaches equilibrium at a very short time and then the reaction proceeds further to form the product. Based on this hypothesis he developed his rate expression.

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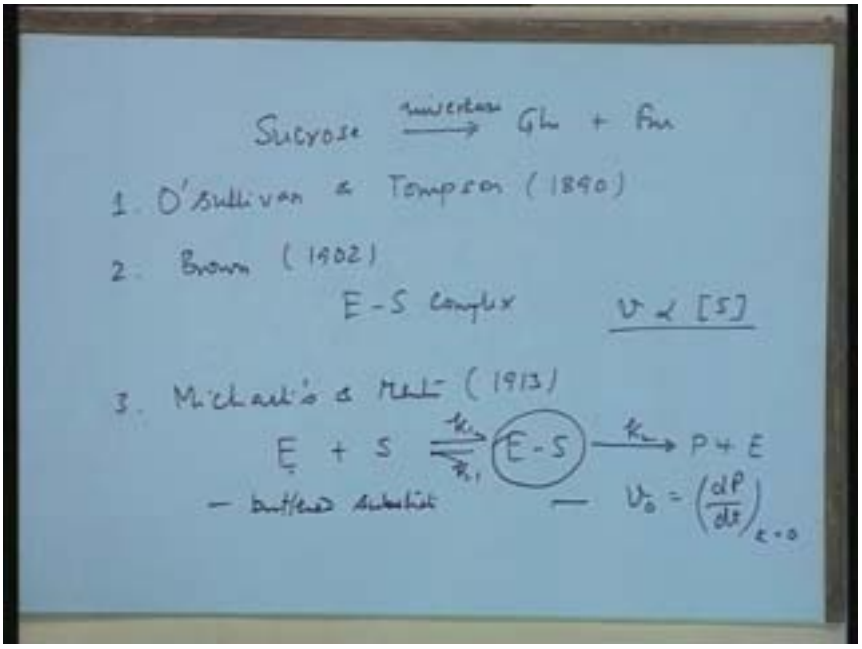

If you can write down the rate constant k_1, k_2 and k_{-1} for the three reaction steps that are involved in this case, one can write down the rate expression using equilibrium hypothesis. That means at the equilibrium

$$K_1[E]\,[S] = k_{-1}[ES]$$

and this by series of interpretations and using enzyme conservation equation

$$E_0 = [E] + [ES]$$

The total enzyme concentration at equilibrium is distributed between enzyme substrate complex and free enzyme. Substituting the value of ES from equilibrium analysis you can come down to a initial reaction rate

$$v_o = k_2\,[E\text{-}S]$$

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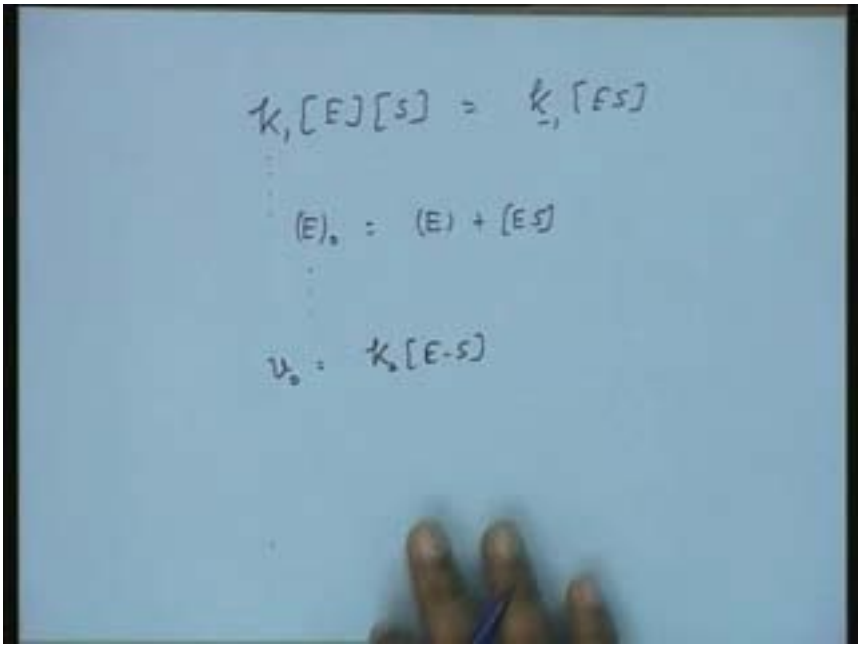

If you look at the original mechanistic hypothesis proposed by Michaelis and Menten, now you can write $k_2[E\text{-}S]$ will be the initial rate of reaction. Substituting the various parameters you get

$$v_o = k_2\,[E\text{-}S]$$
$$= k_2\,[E]_0\,[S]_0 \,/\, k_{-1}/k_1 + S$$

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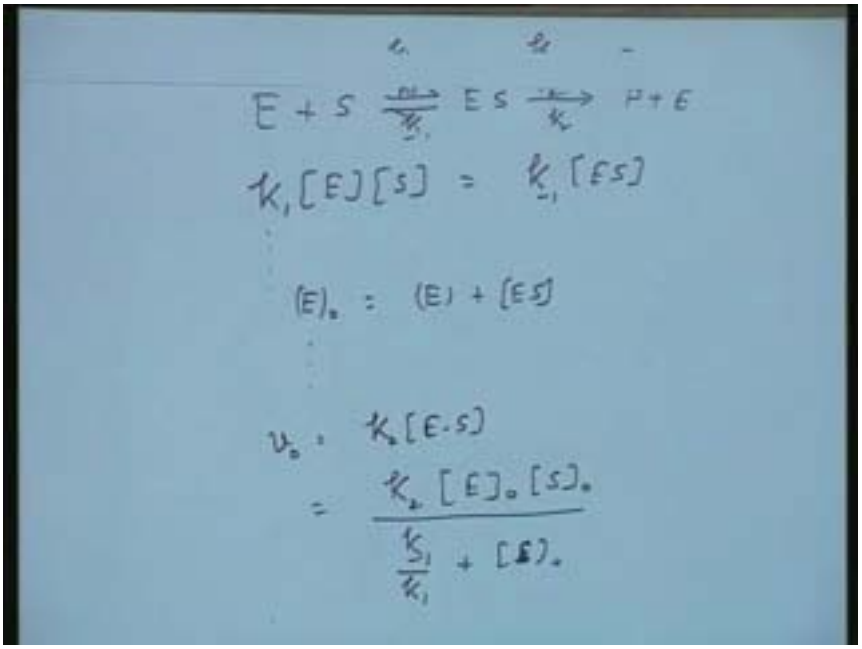

So you get initial rate expression where the initial reaction rate in terms of initial enzyme concentration and substrate concentration are given. The rate constant here as you notice is k_1, k_{-1} and k_2.

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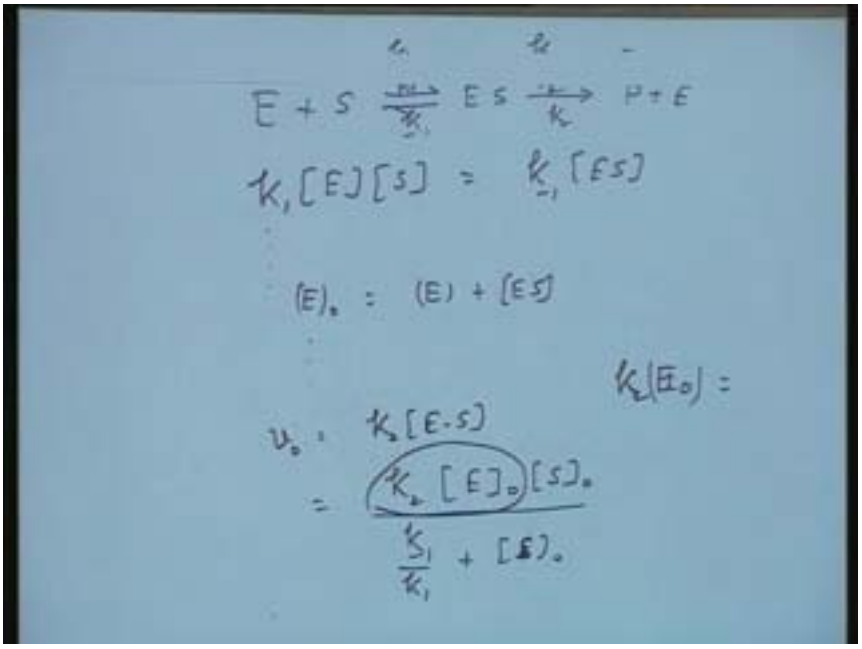

The term k_2E_0 was saturation reaction rate when all the enzymes were saturated with the substrate that is bound to substrate. This hypothetical situation will never emerge because there will always be equilibrium between enzyme, free enzyme, substrate and the enzyme substrate complex. But hypothetical situation is that if all the enzyme is say saturated

with the substrate then you have a maximum reaction rate which is called V_m. Also k_{-1}/k_2 ratio of two rate constants k_2 and k_{-1}that means the ratio of two rate constants which is involved in the break down of the ES complex, either ES complex can go to product formation or it can go back to substrate complex. The ratio of the two was considered as K_d, the dissociation constant of ES. At the time of Michaelis and Menten, there was no proposal of Michaelis and Menten constant.

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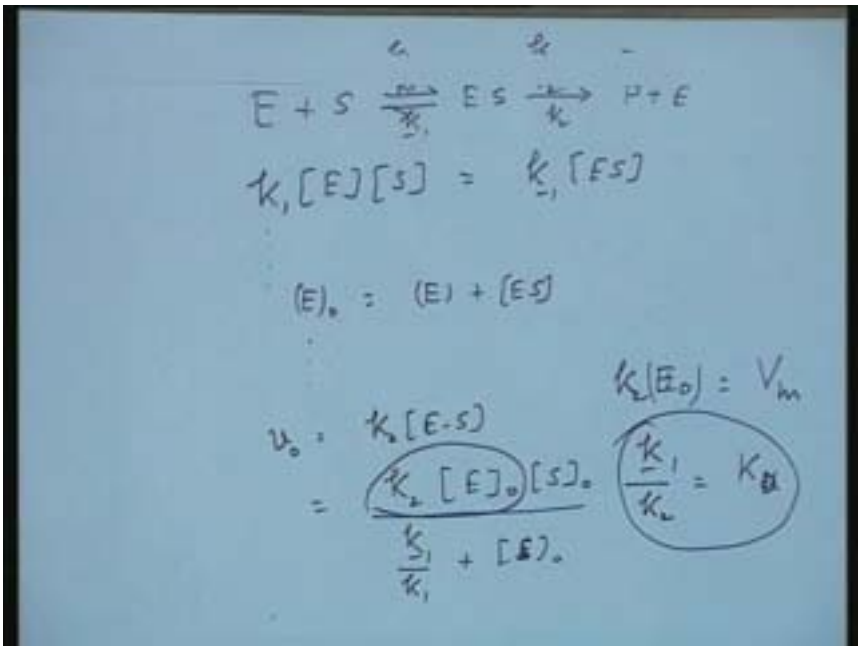

He only proposed the dissociation constant of enzyme substrate complex. The graphical representation of such reaction will be substrate concentration verses initial reaction rate. It will follow saturation behavior where the saturated reaction rate reaches to a value of V_m, which is nothing else but $k_2[E_0]$ and the reaction rate at half the value of maximum reaction rate was defined as K_d, dissociation constant.

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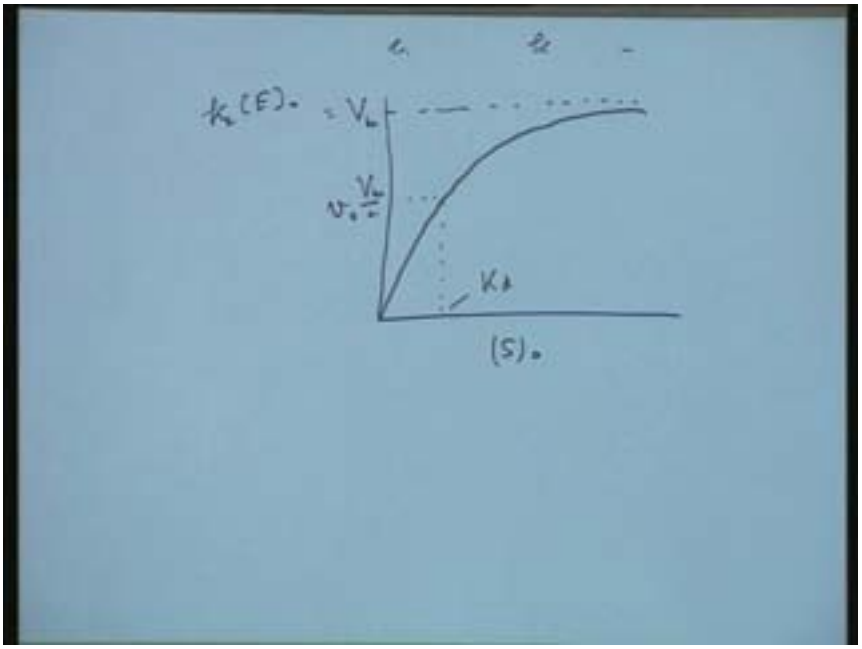

With that concept he was able to determine the dissociation constant of enzyme substrate complex formation. He also proposed that this substrate concentration at which the reaction rate reaches half the V_m value which is the dissociation constant also has direct implication with the affinity of enzyme to substrate. It is substrate concentration at which the reaction reaches half of the saturation behavior and therefore it directly implies the affinity behavior and is being represented in the terms of dissociation constant. This was the major contribution which for a long time was valid until the time another major contribution which came in the enzyme kinetics was by Briggs and Haldane in 1925.

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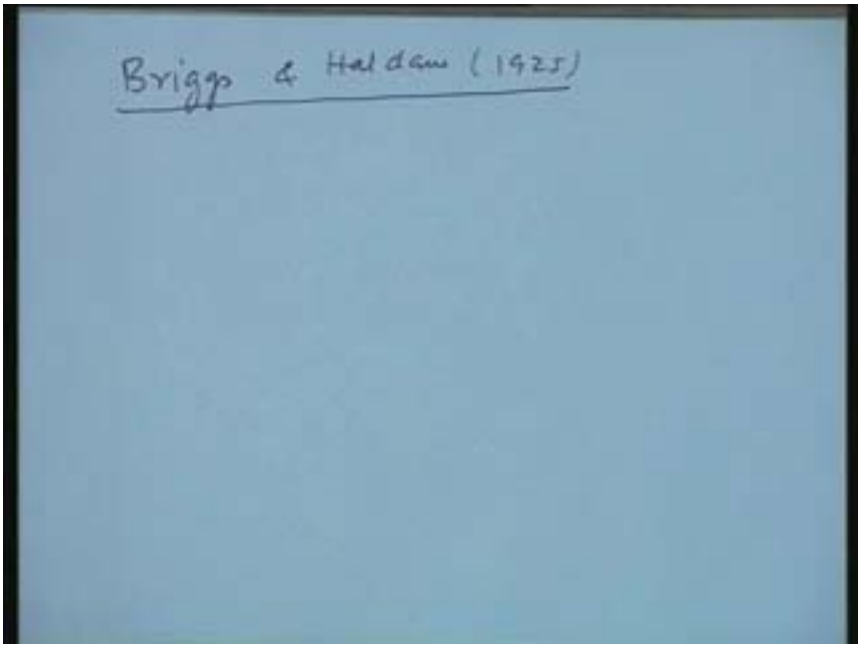

Mind it that J.B.S. Haldane was one of the scientist who did all of his work in India. They proposed a hypothesis that Michaelis-Menten hypothesis is very correct in most of the respect except that they did not agree that the interaction between enzyme and substrate is that of equilibrium behavior. Instead of equilibrium behavior they mentioned in the case of rate terms a steady state behavior. That means again they assumed the same thing

$$E + S \rightleftharpoons X \rightleftharpoons E + P$$

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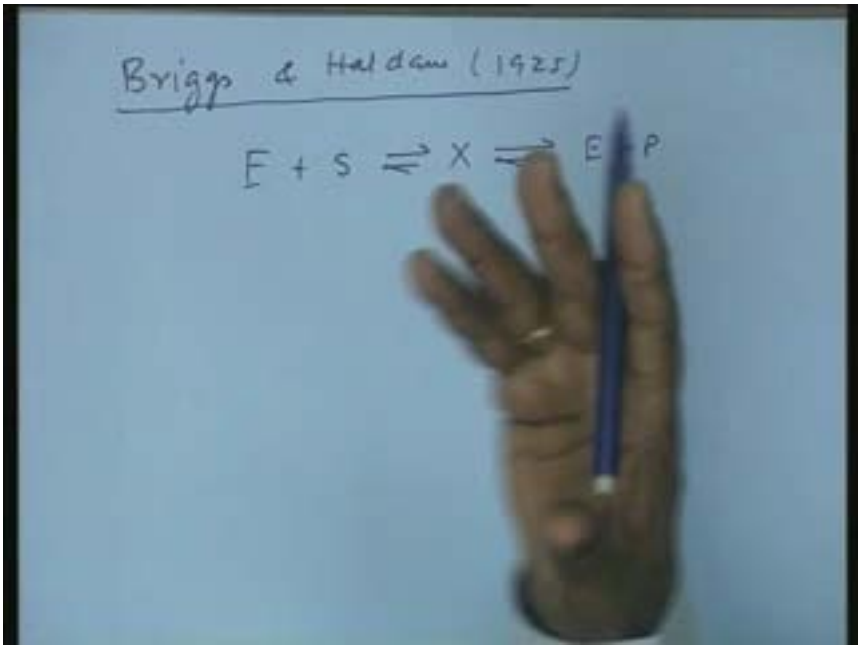

They also expanded the whole hypothesis. Instead of the enzyme substrate breaking down to irreversible step in many reactions which are known to be reversible in behavior, like the isomerization of glucose to fructose the true reversible enzyme reaction and there are many such examples where the reaction can be catalyzed in either direction. Therefore he also considered that the second step in the sequence is also reversible. We need not limit it to an irreversible behavior and he also mentioned that instead of having an equilibrium behavior here we have a steady state behavior which means the rate of change of x is zero with time.

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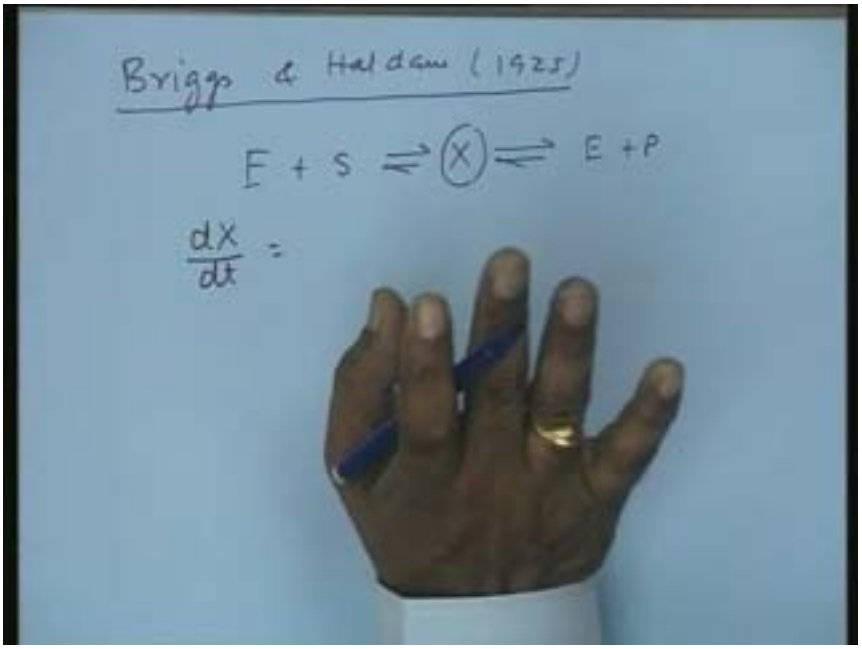

After a very short interval of time when the reaction starts concentration of enzyme and the enzyme substrate complex reaches a constant value and if you look at the time scale for the enzyme catalyzed reaction, this is concentration axis. Then enzyme concentration goes down, the enzyme substrate concentration builds up and this is the enzyme concentration and this is ES concentration. This is short time. When I show it in expanded view, this time scale is probably only for few seconds. This time scale up to here is only a few seconds which he named as induction period. You can call it transient state or free steady state period also but as soon as this transient period, which is very short time, passes both the enzyme substrate complex and rate of formation of enzyme substrate complex becomes a steady state and this will become zero.

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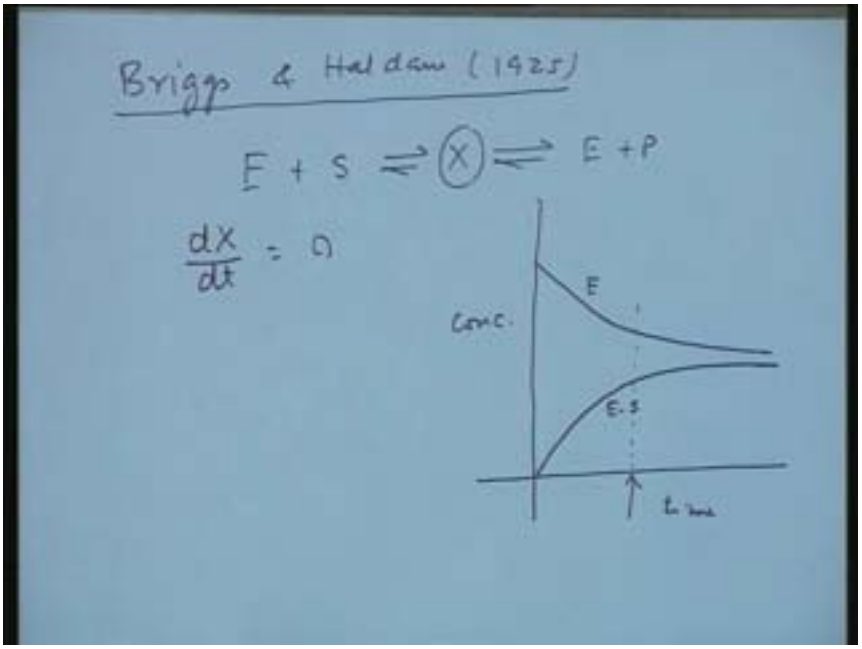

Based on that he wrote his rate expression and they are almost identical rate expression as Michaelis and Menten with slight difference which we will notice. You can write then with the assumption of

$$(dx/dt) = 0$$

$$(E_0) = (E) + (X)$$

X is enzyme substrate complex. You can also take the conservation equation of substrate which will be equal to the substrate concentration plus product concentration.

$$S_0 = (S) + (P) + (X)$$

If you write all the expressions in a rate term you will find the concentration of X will be

$$[X] = k_1E_0[S] + k_{-2}[E]_0\{P] / k_{-1} + k_2 + k_1[S] + k_{-2}[P]$$

But as a matter of fact one can always neglect X because it's too small a concentration compared to S. We assume in most of the analysis that S_0 is much, much greater than enzyme concentration.

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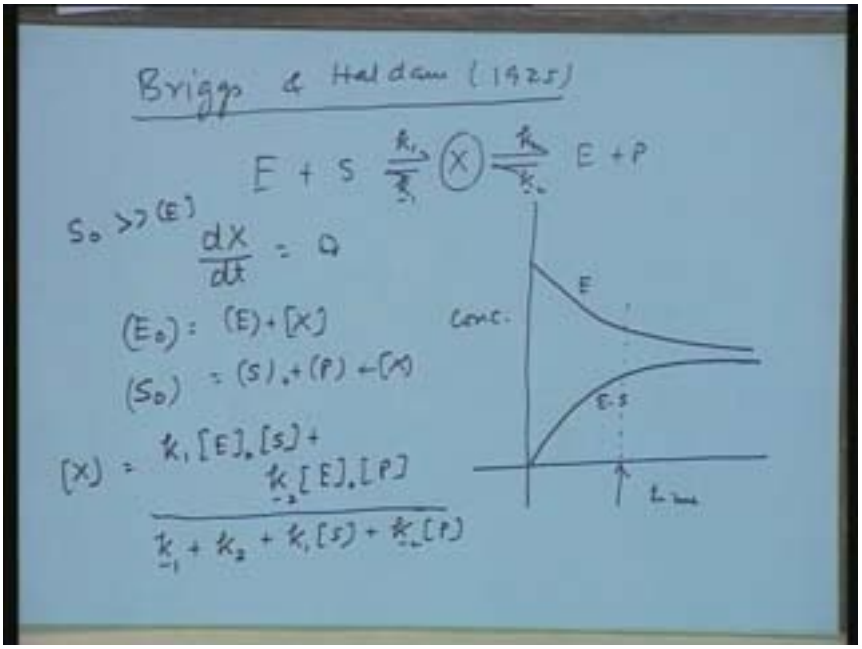

The concentration of substrate, being a small molecule usually is much, much larger compared to the enzyme concentration which is used as catalyst in very small concentration. Usually in any enzyme catalyzed reaction, the concentration of enzyme will be of the order of few milli molar or even lesser than that. So you get the concentration of enzyme substrate complex and based on this hypothesis you get the initial reaction rate. He also followed initial reaction rate concept as

$$V_0 = k_2 [E_0] [S]/(k_{-1} + k_2/k_1) + [S]$$

If you take a general reversible equation and put p equal to zero because at p equal to zero you are considering the forward reaction only where you start with the substrate. At the initial rate the product concentration is zero and in general expression if we write p equal to zero you will arrive at initial reaction rate.

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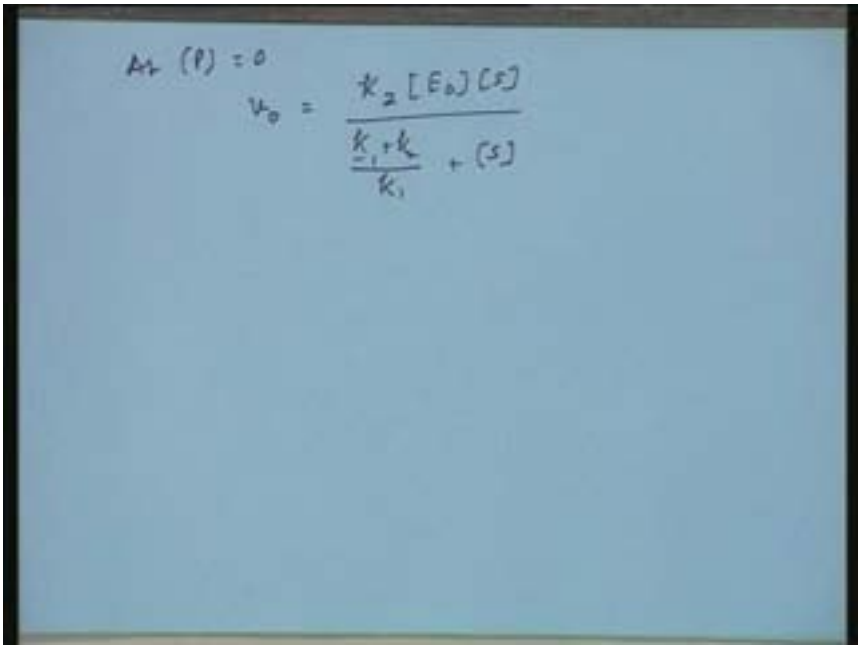

The difference between Michaelis and Menten kinetics and that proposed by Briggs and Haldane lies in the fact that in the case of Michaelis kinetics they proposed the dissociation constant

$$k_{-1}/k_1 \longrightarrow (k_{-1} + k_2 / k_1)$$

They named as this parameter ($k_{-1} + k_2 / k_1$) as Michaelis Menten constant in honour of Michaelis and Menten to recognize their contribution because bulk of the ideas they got in the whole analysis from the Michaelis and Menten assumptions.

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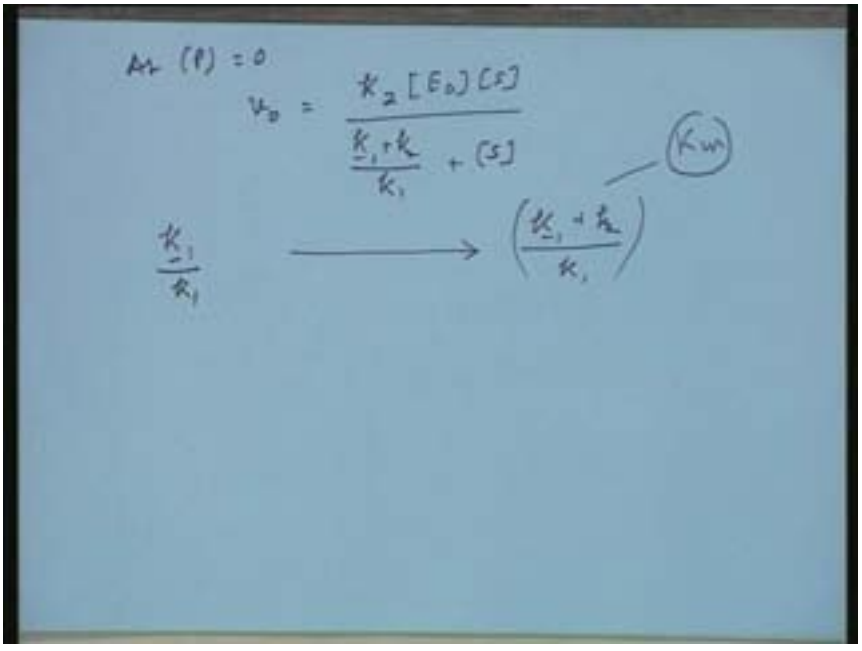

In fact Michaelis Menten himself never proposed or gave the name as Michaelis Menten constant. He always talked of dissociation constant which is a very fundamental parameter in the physical chemistry. The constant which Briggs and Haldane proposed was not developed by Michaelis and Menten. Briggs and Haldane's parameter of rate constant ($k_{-1} + k_2 / k_1$) was named as Michaelis and Menten constant which is analogous to dissociation constant. The difference between the two parameters is $k_2/k_1$and two parameters will become identical if we assume that the k_2 is much, much less than $k_{-1,}$ if the k_2 is assumed to be very, very small rate constant compared to k_{-1}.

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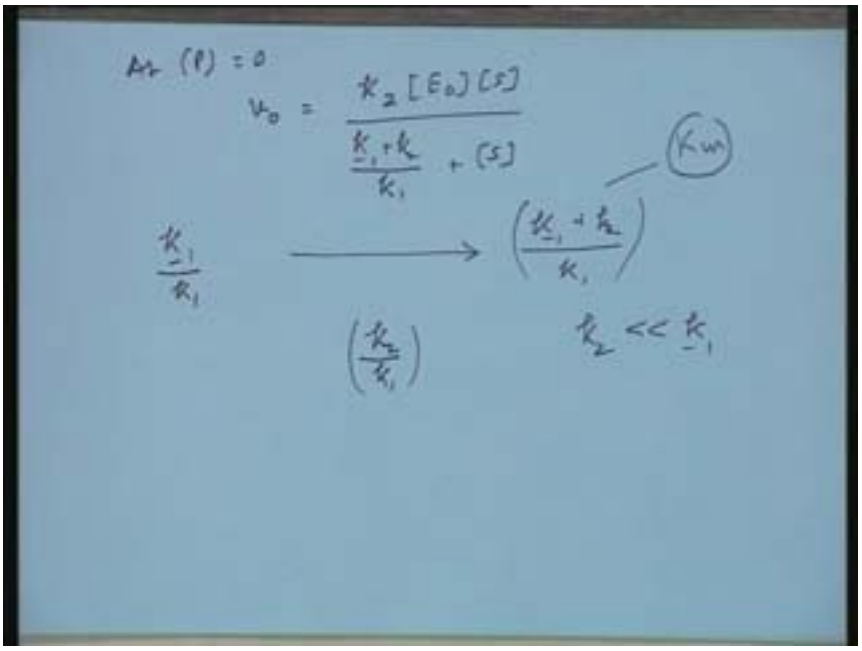

In most cases it is true and that is why even the dissociation constant concept is feasible. In most cases the rate of break down of enzyme substrate complex is the reason why many of the enzyme reaction are noted to be very slow in terms of the rate processes. The term k_2, the rate constant k_2 is usually in many cases it is fast also like for example in the case of invertase itself k_2 has significant magnitude. But in many enzyme catalyzed reactions the k_2 is very, very small compared to k_{-1}.

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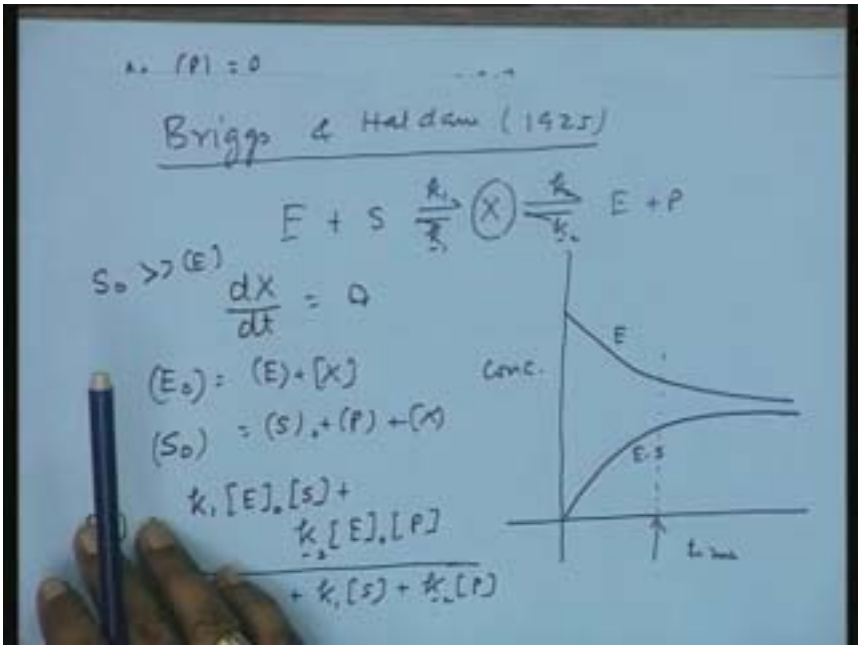

Therefore the two hypotheses provide you a very similar inference as far as the kinetic function of enzymes are concerned. The concept of Vm the maximum reaction velocity, the concept of Michaelis Menten constant, the physical significance of these two parameters which are the critical parameters also remain the same even with Briggs and Haldane.

To summarize the results of the Michaelis Menten kinetics and Briggs and Haldane kinetics, we have two kinetic parameters which can characterize any enzyme catalyzed reactions. One is the maximum reaction velocity which is

$$V_0 = k_2(E_0)$$
$$k_m = k_{-1} + k_2/k_1$$

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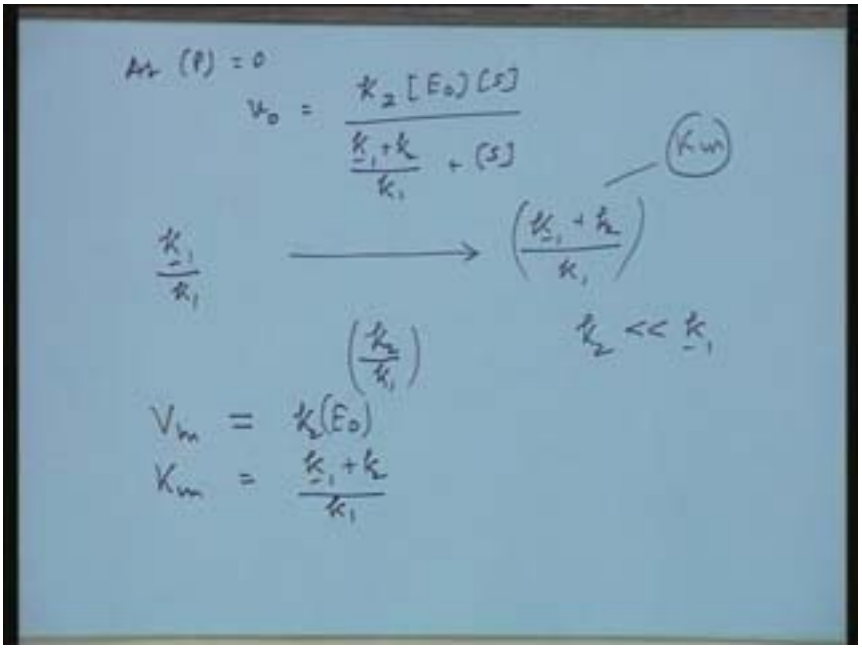

Another point I like to make it clear here is V_m is not a kinetic constant. V_m is a parameter and is not a kinetic constant. It is a function of enzyme concentration. If you increase enzyme concentration, the value of V_m increases. The kinetic parameter here is k_2, the rate constant which is responsible for break down of enzyme substrate complex into the product. Very often the k_2 is also called as turn over number which is the rate limiting step in the enzyme catalyzed reaction. The composite term the k_2 (E_0) characterizes an enzyme in terms of saturation. But that V_m value is at a particular enzyme concentration.

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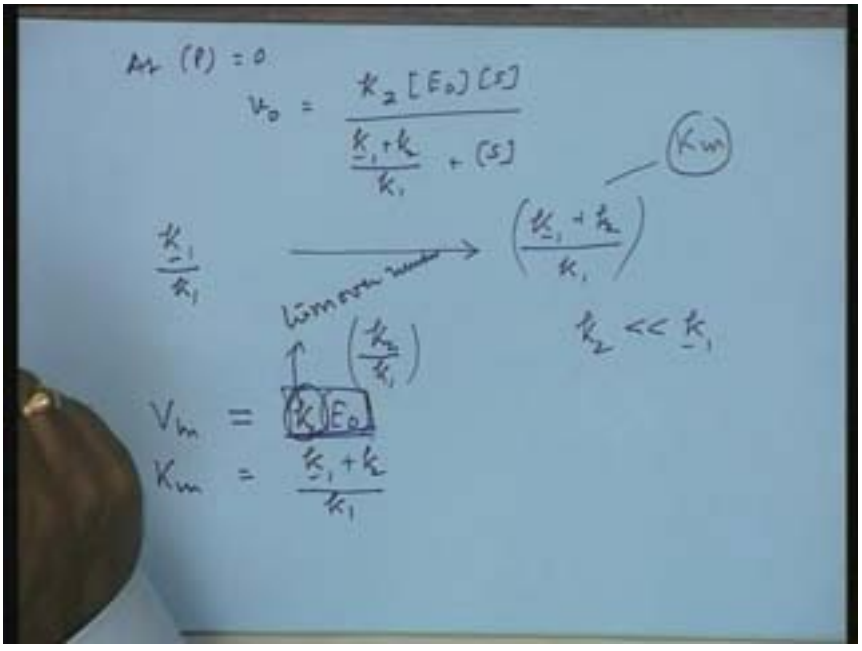

When you mention V_m as characteristic it refers to a particular enzyme concentration. K_m is a true kinetic parameter which is a ….. (33.35) parameter of the three rate constants and is typically characteristic of any enzyme function and very often K_m is used as a characterizing parameter for most of the enzymes that are available.

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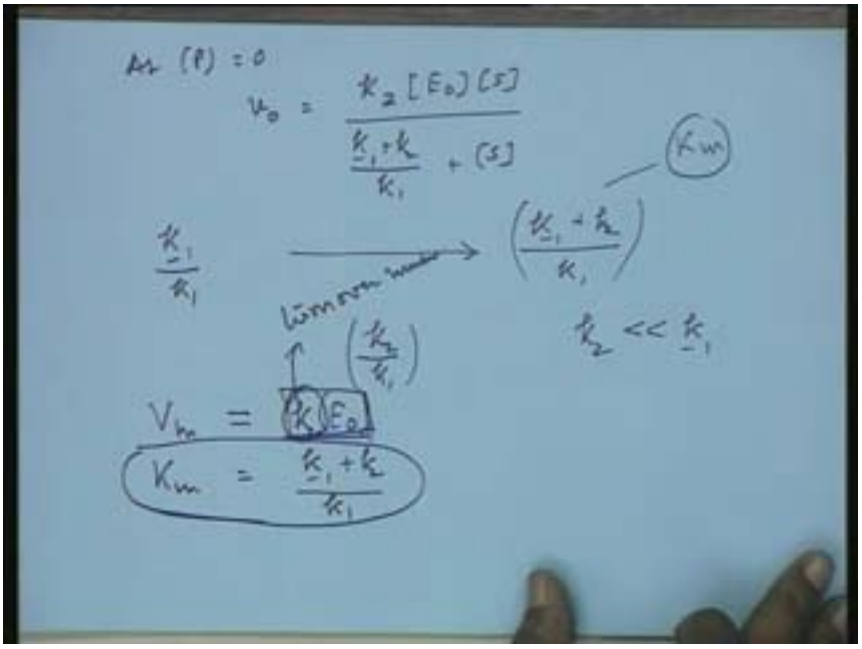

This kind of kinetics if you look at

$$V_0 = k_2[E]_0 [S] / Km + [S]$$

You will also notice a very significant trend if you look at a graphical representation, saturation kinetics, represented by a rectangular hyperbola this is K_m, and this is $V_m/2$. Unlike most of the chemical reactions you will notice that this represents a case where the order of reaction changes the substrate concentration.

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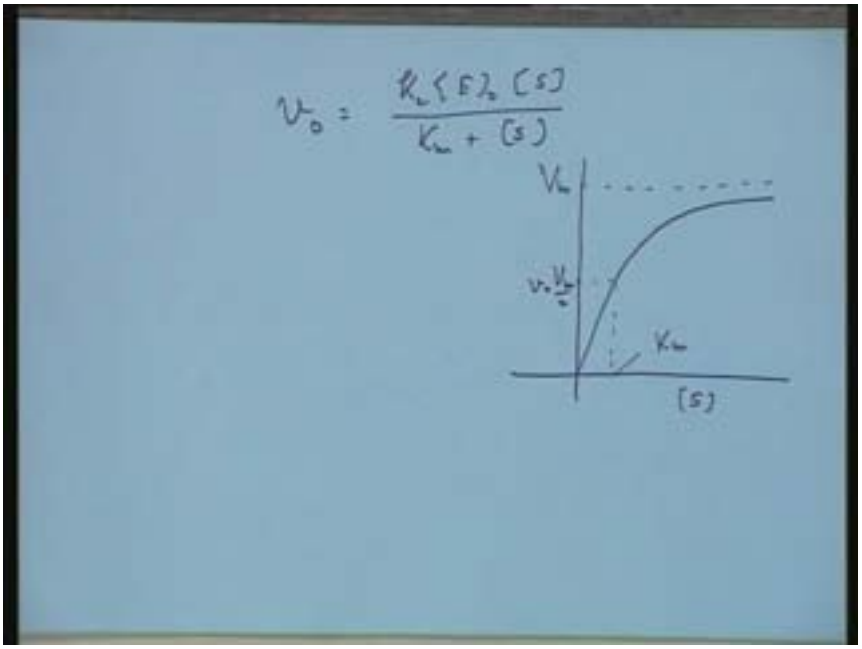

Most of the chemically catalyzed reaction or chemical reactions represent a particular order of reaction, zero order, first order or second order. In this case you will notice that at low substrate concentration, when substrate concentration is much, much smaller compared to K_m you can keep km as the sort of a …. (35.07). If it is very, very small compare to K_m, you can arrive at

$$v_0 = k_2[E_0]\,[S]$$

a simple first order kinetics and because $k_2[E_0]$ is a constant at a given enzyme concentration and substrate concentration is a variable, it becomes a first order kinetics and the portion represented by this area is first order kinetics.

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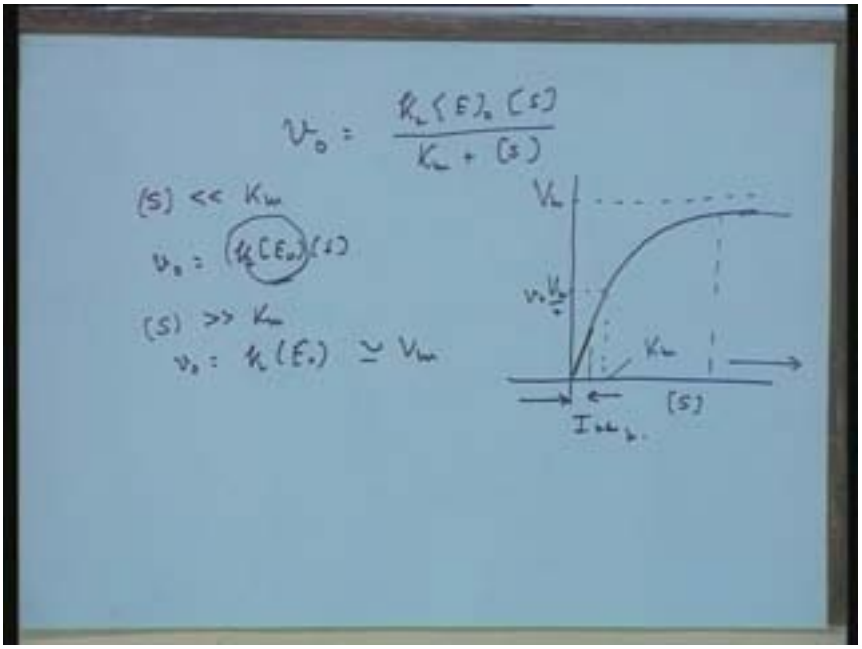

On the other extreme if you go to the concentration beyond this point when substrate concentration is much, much greater than K_m

$$v_0 = k_2[E_0] \sim v_m$$

which means a zero order kinetics. In between the two the enzymes follow some kind of a mixed kinetics and that is one very characteristic feature of enzyme catalyzed reactions that instead of following a fixed kinetic order it follows a changing order of reaction kinetics at the two extremes. This has very important feature. In most of the subsequent analysis of the kinetic parameters or the reaction rate or the reactor design to simplify the situation we often either go to the zero order design or go to the first order design very often because this expression is comparably much more complex to analyze rather than a zero order or first order design. For most of the analysis, to understand the system either we simplify it to a zero order design and experimental data can be collected either at a very low substrate concentration range or at very high substrate concentration. When we see the reaction rate these understanding can be used even for use of enzyme as analytical tool or the action of the enzyme itself.

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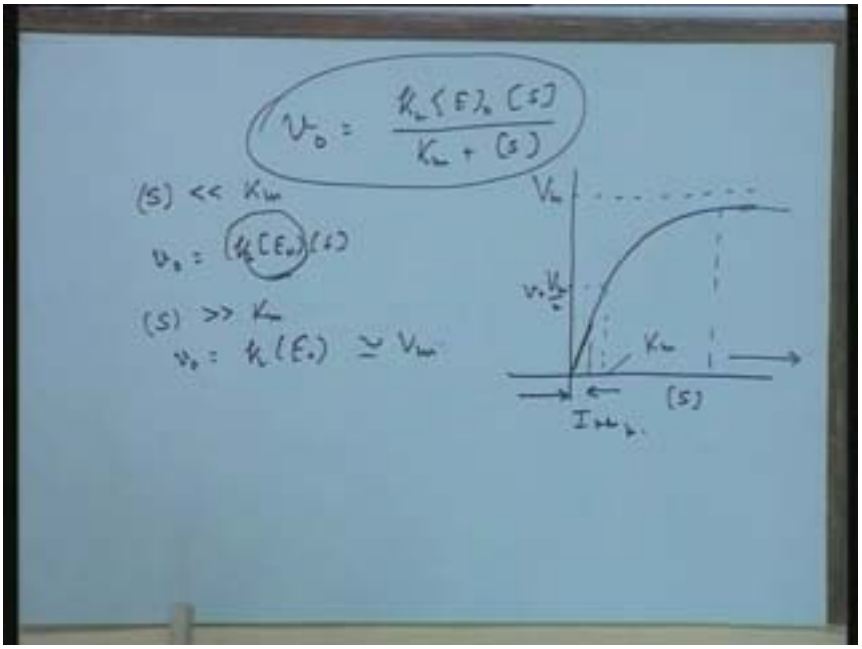

All these information can be used very significantly for those purposes. To expand further on the Briggs-Haldane's kinetics based on the reversible enzyme catalyzed reaction if you recall what we got

$$v = \{k_1k_2[S] - k_{-1}\, k_{-2}[P]\}E_0 / k_1[S] + k_{-2}\,[P] + k_{-1} + k_2$$

The original reaction rate expression was developed by Briggs and Haldane for reversible reaction that means you carry out the reaction either from the reverse direction or in the forward direction starting from s or starting from p and you can arrive at rate expression in terms of substrate and product concentration.

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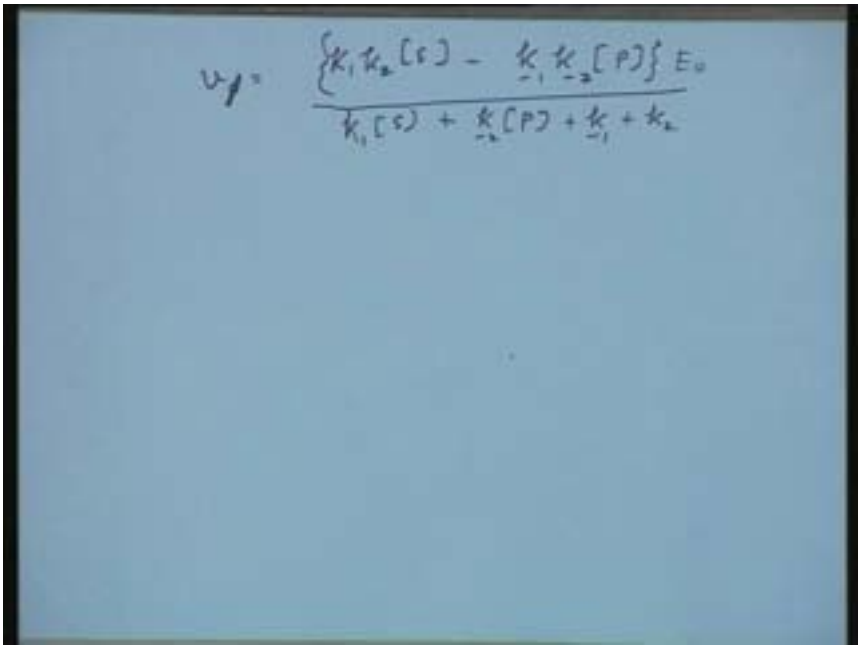

From this expression you can determine the equilibrium constant for the reaction. That means equilibrium constant is the one where the rate of forward reaction and the backward reaction are identical and the equilibrium constant the value of the concentration of product upon substrate at v = 0 will be

$$K_{eq} = [P]_{eq} / [S]_{eq} = k_1 k_2 / k_{-1} k_{-2}$$

or in terms of the basic kinetic parameters

$$K_{eq} = [P]_{eq} / [S]_{eq} = k_1 k_2 / k_{-1} k_{-2} = v_s.k_p / k_s.v_p$$

The expression can be arrived from the main rate expression which will give you the equilibrium constant either in terms of the rate constant. The equilibrium constant can be monitored experimentally and that can also give you an idea of measuring or determining the individual kinetic parameters experimentally.

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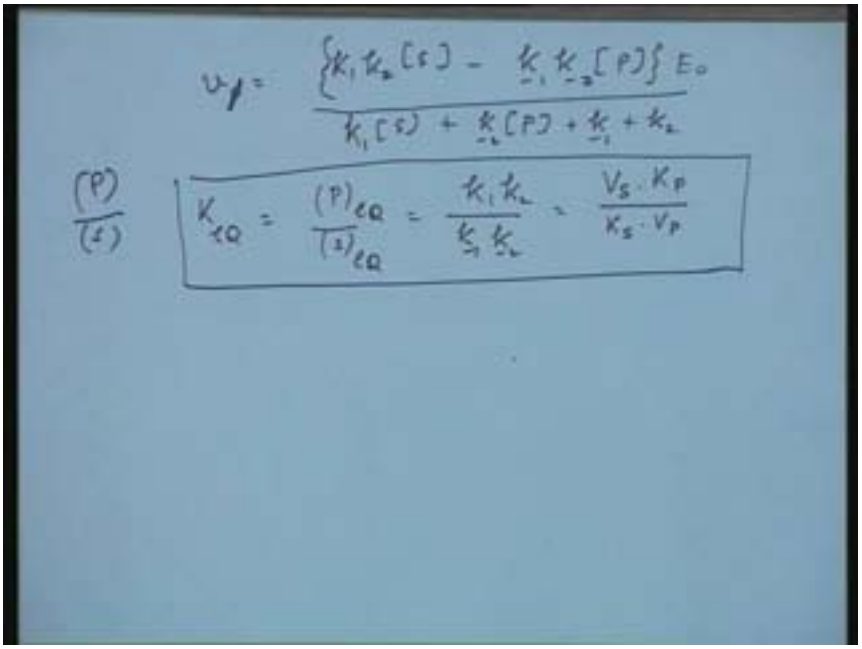

This you can arrive at if you put v = 0 and solve it and determine the value of P_{eq}. At equilibrium, reaction rate will be zero. I will leave it to you to develop this. I don't think it will be very difficult task to do that. V_s is the maximum reaction velocity corresponding to substrate. That means if you carry out the reaction with substrate the maximum reaction rate velocity it reaches is $V_{s.}$ Similarly the k_s is the Michaelis Menton constant corresponding to substrate s and p corresponds to the product when you start the reaction. These data v_s, k_s, k_p and v_p will be determined experimentally on the basis of initial reaction rates and not as a general reaction. These parameters will be determined on the basis of initial reaction rate corresponding to either s or either p. You can write as

$$K_{eq} = [P]_{eq} / [S]_{eq} = k_1 k_2 / k_{-1} k_{-2} = v^m{}_s . k_p / k_s . v^m{}_p$$

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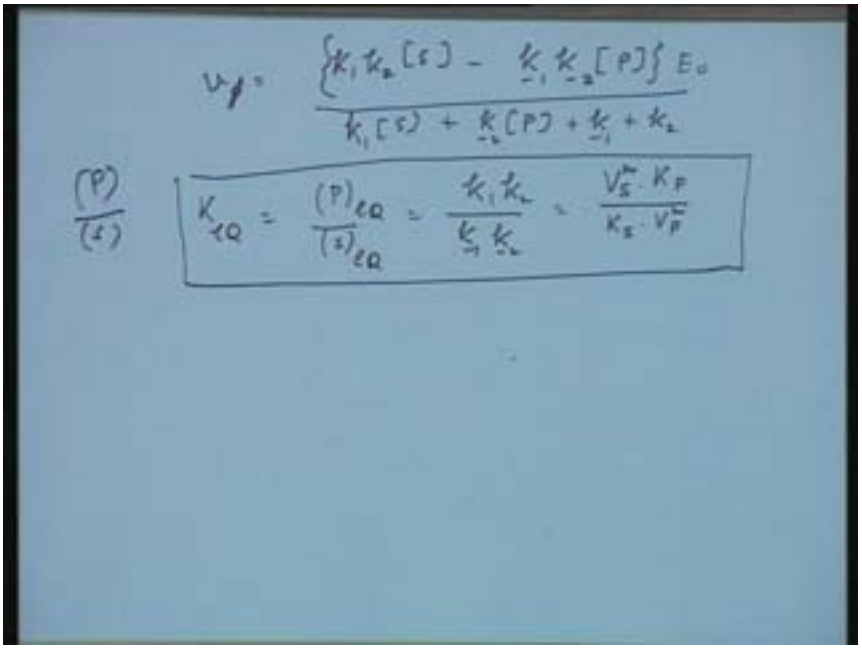

One would like to understand the enzyme kinetics on the basis of the experimental data. How to make a link between experimental data and the kinetic rate expression that was obtained by these two scientists separately? Today Briggs and Haldane's kinetics has become a universally accepted reaction kinetics which is followed all over the world to understand the enzyme catalyzed reactions.

The major issue is how to use those data primarily for determining the k_m value or the v_m value corresponding to the particular enzyme concentration. Experimentally you cannot determine the v_m value because it is a saturation rate asymptotic to the axis and therefore you can never reach that value experimentally. You may reach closer to it as you increase the substrate concentration. So an attempt was made for the treatment of experimental data so as to test or verify the hypothesis that has been proposed by Michaelis Menten initially and verified and approved or wetted by Briggs and Haldane.

The simplest way to do that was that these non linear equations if they could be converted by some means, by some modification, into a straight line relationship life will be much easier to analyze experimental data and the first attempt for that was given by Lineweaver and Burk.

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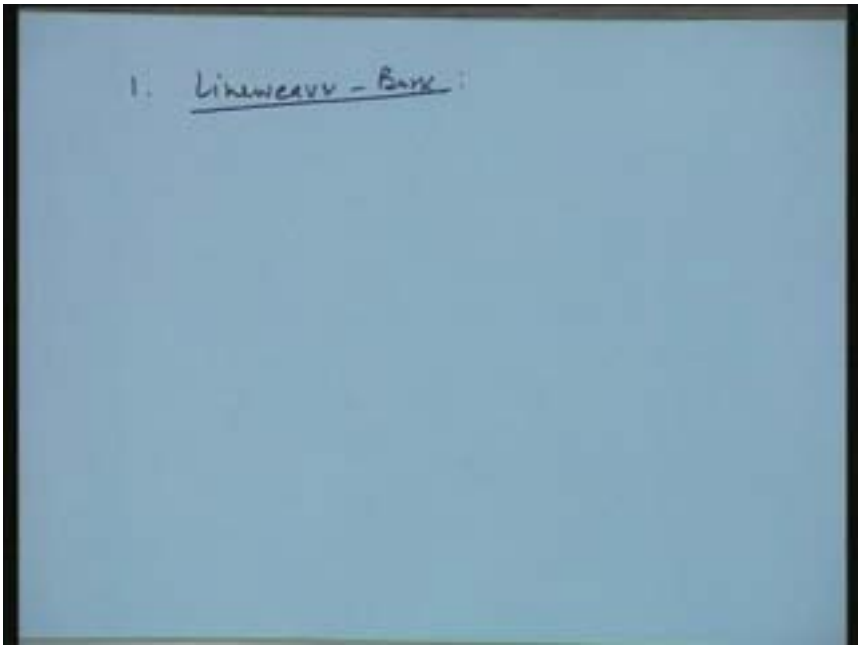

Lineweaver and Burk made a very simple approach.

$$v_0 = k_2[E_0]\,[S] / k_m + [S]$$

Both these sides you take reciprocal and you can just write

$$1/ v_0 = k_m/[S].\ 1/k_2[E_0] + 1/k_m[E_0]$$

Therefore this represents a straight line relationship between $1/v_0$ and $1/[S]$. That means when you plot $1/v_0$ and $1/[S]$ you get a straight line relationship with a slope equal to $k_m/k_2[E_0]$ with intercept $1/v_m$. On the x axis also this will intersect which will be $-1/k_m$.

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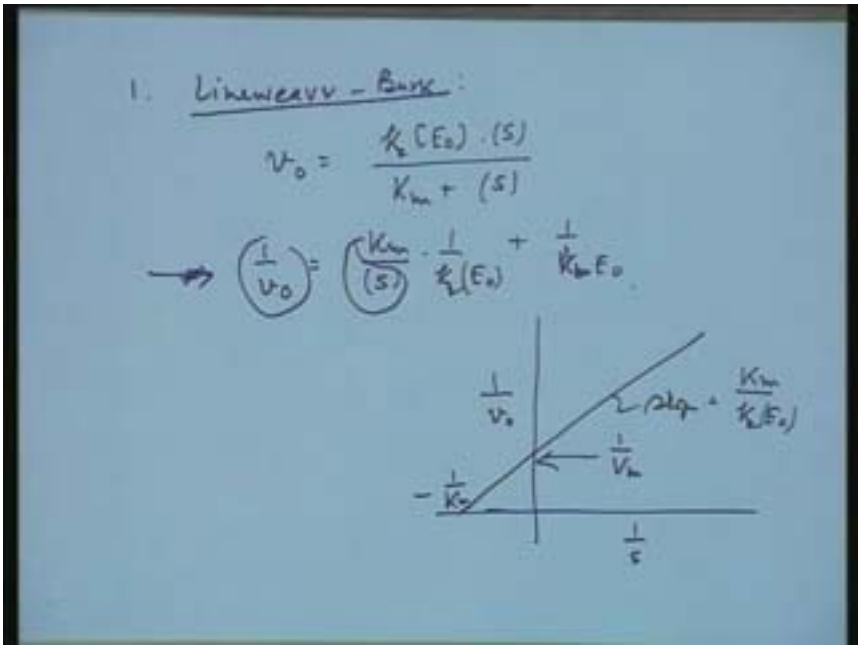

So in a very simplistic way if you can take a double reciprocal plot one can analyze the experimental data that are obtained on the rate of reaction as a function of substrate concentration and verify the hypothesis.

For most of the cases such a hypothesis has been verified and found to be proven under certain limited conditions which have to be in line with the assumptions made by the scientists. While Michaelis-Menten kinetics is the most accepted kinetic pattern of the enzyme catalyzed reaction, most of the enzyme catalyzed reactions in practice deviate from the basic assumptions and only under certain set of conditions they obey the enzyme kinetics proposed by Michaelis-Menten or Briggs and Haldane. I think with that we will stop at this point.