Fischer's ideas became particularly fruitful. When physical methods such as kinetics and X-ray crystallography were applied to them, he himself was scornful of such methods: Wilhelm Ostwald suggested to Fischer that physical methods would be helpful in organic Chemistry, but Fischer replied bluntly "We have no use for your methods!"

The important step in enzyme kinetics was made by the German biochemist Leonor Michaelis and his Canadian assistant Maud Leonora Menten (Michaelis and Menten, 1913). They had observed that the effect noted by Brown — That the rate of reaction is independent of the concentration of the substrate — is only observed at higher concentrations of substrate. At lesser concentrations the rate becomes proportional to the concentration of substrate. To explain this they considered the kinetic consequences of the idea that an enzyme–substrate complex is formed.

This process can be represented as

$$E + S \rightleftharpoons ES \longrightarrow E + \text{products}.$$

Where E is the enzyme molecule, S the substrate molecule, and ES the complex. The idea of Michaelis and Menten was that an equilibrium is established between E, S and ES, and that the slow step is the breakdown of ES. Since usually there are many more molecules of substrate present than of enzyme (on account of the high molecular mass of enzyme) the enzyme becomes saturated with substrate at higher substrate concentrations. The concentration of ES will therefore remain the same, and the rate will remain the same, as the concentration of substrate is varied. At low substrate concentrations on the other hand, the enzyme will not be saturated, and the concentration of ES, and therefore the rate of reaction, will be proportional to the concentration of S

This reasoning led to the well-known Michaelis–Menten Equation was given by George Edward Briggs and the British geneticist John Burdon Sanderson Haldane (Briggs and Haldane, 1925). They pointed out the Michaelis assumption that an equilibrium exists between E, S and ES is not always justified, and should be replaced by the assumption that ES is present not necessarily at equilibrium but in a Steady State. The resulting equation is of the same form, but the

Michaelis and his Colleagues also made important contributions to our understanding of the way in which the rate of an enzyme catalysed reactions is affected by the ~~the~~ pH of the solution. For sometime it had been noticed in many enzyme system that the rate is low if the pH is high or low, and passes through a maximum at some intermediate value, which is usually not far from neutrality (pH 7). In 1911, two years before the formulation of the Michaelis–Menten equation, Michaelis and Davidsohn had conducted from this pH behaviour that the catalytic ~~Center~~ Centres of enzymes must involve two ionizing groups (Michaelis and Davidsohn, 1911) for effective catalytic action one of these must be in the form in which it is capable of accepting a proton, while the other must be in ~~the form in which~~ a position to donate a proton after sometimes. This basic idea was extended, particularly to take into account the ionization of the enzyme–substrate complex as well as that of the enzyme. (Michaelis and Rothstein, 1920, von Euler, Josephson and Myrtack, 1924; Walby, 1953; Laidler, 1955). A strong body of evidence shows that the powerful catalytic activity of enzymes is due in part to the fact that they function by being simultaneously able to donate a proton to, and accept another proton from, the substrate molecule. This has been referred ~~to~~ as "push-pull mechanism". The basic idea that an enzyme reaction involves an enzyme–substrate complex as an intermediate is important, but requires much extension and elaboration. There is now a considerable body of evidence suggesting that in many cases there are two or more intermediates.

A schematic representation of that reaction is as:

$$E + S \rightleftarrows ES' \longrightarrow E + \text{products}$$

Where ES' is the second intermediate the example for such a mechanism has been obtained for the hydrolysis of acetylcholine by the enzyme acetylcholinesterase (Krupka and Laidler, 1961) In this system the rate goes through a maximum with increasing substrate concentration, and the evidence suggests that this is due to attachment of substrate molecules to the second intermediate ES'.

With the purification and crystallization of proteins in the nineteen twenties, Enzyme kinetics was able to enter a new phase. It became possible to study in much more detail the interaction between enzyme molecules and their substrates. This branch of Enzyme kinetics has been referred to as molecular kinetics (Butler, 1941).

Contd.