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REFUTATION OF THE SCHÜTZ LAW AND ITS ANALOGOUS EQUATIONS IN THE KINETICS OF ENZYMES

BY

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Introduction

Though there have been various equations expressing enzymatic reactions, and numerous equations obtained from experimental results have been introduced, the author has scarcely been able to find among them a completely applicable equation. The SCHÜTZ law is one of the most acceptable of these equations, and it seems to be the very first one, whenever one wishes to discuss the kinetics of any enzyme action. When the author made some experiments on the decomposition of various proteins with some proteolytic enzymes, he applied the SCHÜTZ law to his results,

but he was not satisfied with the outcome. The SCHÜTZ law seems to the author to have many defects, and not to mean anything in enzymatic reactions. Therefore, in this paper he proposes to examine these defects and give reasons for refuting the SCHÜTZ law. The following equations of various authors as, $\frac{x}{E^{\frac{1}{2}}}=K$, PALLADIN (35), $\frac{x}{t}=K$, ARRHENIUS (7), ABDERHALDEN and FODOR (1), SMORODINTZEV (44), $\frac{x}{\sqrt[3]{t}}=K$, MATSUYAMA and NAKAMURA (29), are considered analogous to the SCHÜTZ law. Anything that can be said of the latter law, can also be applied to these analogous equations. So this paper mainly discusses the SCHÜTZ law.

Discussion

I. The SCHÜTZ law and its origin

According to the Schütz law, in enzyme action, if x be taken as the quantity decomposed, E as the quantity of enzyme, t as the time of decomposition, the equations, $\frac{x}{\sqrt{E}}=K$, $\frac{x}{\sqrt{t}}=K$ or combining the two, $\frac{x}{\sqrt{E \cdot t}}=K$ are formed. It is generally considered that this law was brought forward by Emil Schütz in 1885, and many books and papers on enzymes refer to his thesis, "Zeit. physiol. Chem., 9, 577-590 (1885)", as its origin. According to this thesis, one finds that SCHÜTZ took pepsin from the stomach of a pig and decomposed egg-white with different amounts of enzyme, and from the degree of rotatory power, he concluded that an equation, $\frac{x}{\sqrt{E}}=K$, can be regarded as very trustworthy in peptic digestion. This thesis gave three experimental results, but there was no experiment which took time as a variable, nor had a suggestion for applying the equation, $\frac{x}{\sqrt{t}}=K$, to enzymatic reactions. Six years after, in 1891, BORISSOW (11) came to the same conclusion as SCHÜTZ; so the SCHÜTZ law, $\frac{x}{\sqrt{E}}=K$, is often called the SCHÜTZ-BORISSOW law. In 1893, SAMOJLOFF (38) maintained that the applicability of the equation, $\frac{x}{\sqrt{E}}=K$, could not be recognized. In the following year, METT (31) reported that the equation, $\frac{x}{\sqrt{E}}=K$, was valid. But the author has not been able to secure any copy of SAMOJLOFF and METT's papers. In 1900, SCHÜTZ, with HUPPERT, undertook many ex-

periments on protein decomposition. They took time or the quantity of pepsin as a variable and calculated their results according to the equations, $\frac{x}{\sqrt{t}}=K$ or $\frac{x}{\sqrt{E}}=K$. They affirmed that their results followed the original "SCHÜTZ law". The experimental basis of the SCHÜTZ law that takes time as a variable can be said to have originated from this investigation. Therefore, the equation, $\frac{x}{\sqrt{E \cdot t}}=K$, is often called the SCHÜTZ-HUPPERT law. Almost simultaneously Julius SCHÜTZ reported that although the SCHÜTZ law was applicable to the results obtained with dilute solutions of pepsin, it was not with concentrated solutions. From these facts one can infer that the SCHÜTZ law was already recognized at the time of those scholars. It is not clear, however, who had first called the equation, $\frac{x}{\sqrt{E}}=K$, in enzyme action the SCHÜTZ law, and who had first quoted SCHÜTZ's thesis in *Zeit. physiol. Chem.*, 9, (1885) as its origin. Nevertheless these decisions must probably have been due to the law of mass-action,* which remarkably influenced the chemical-world at that time. E. SCHÜTZ's first investigation which was supposed as the origin of his law was made in 1885, and as soon as the equation, $\frac{x}{\sqrt{E}}=K$, was recognized in peptic digestion, it may have been judged unconsciously that it was immediately able to induce the equation, $\frac{x}{\sqrt{t}}=K$. Moreover, many experiments have been made the results of which gave comparatively uniform values of $\frac{x}{\sqrt{t}}=K$. Such cases made one carelessly believe in the applicability of the SCHÜTZ law. Thus the law has been taken up to-day as one of the most important laws in the kinetics of enzymes.

For the sake of convenience of discussion, the SCHÜTZ law is divided into two equations, $\frac{x}{\sqrt{E}}=K$ and $\frac{x}{\sqrt{t}}=K$.

II. Defects of the equation, $\frac{x}{\sqrt{E}}=K$

In order to make clear how the equation, $\frac{x}{\sqrt{E}}=K$, can be applied

*Though there were studies of BERTHOLLET in 1801, and of WILHELMY in 1850, the law of mass-action was first investigated by GULDBERG and WAAGE in 1868; then HOSTMANN investigated it in the following year. Though T. W. GIBBS made a theoretical study of this subject completely, yet it was not known the world over then as at the present time. In 1877, VAN'T HOFF proved this law of mass-action quite independently, and it was become universally known by his publication of "Etude du dynamique chimique" in 1886.

to enzyme action, the principal results of investigations hitherto made to elucidate the quantitative relation between enzyme and substrate decomposed have been examined. From these, the names of enzymes, equations, the applicability of the law to each enzyme action and authors are tabulated as follows:—

Table I

Kind of enzyme	Equation.	Applicability of the Schütz law	Author
Lipase	castor bean $\frac{x}{E} = K$	bad	JALANDER (23).
	liver $\frac{x}{E} \neq K$?	KASTLE and LOEVENHART (26).
	stomach $\frac{x}{\sqrt{E}} = K$	good	VOLHARD (52); STADE (46).
	pancreas $\frac{x}{\sqrt{E \cdot t}} = K$	"	ENGEL (13).
	papain $\frac{x}{\sqrt{E}} = K$ (within certain extent)	pretty good	SANDBERG and BRAND (39).
"	$E \cdot t = K$	bad	" "
Amylase	$\frac{x}{E} = K$ (within earlier range)	"	EVANS (16).
	$\frac{x}{E \cdot t} = K$	"	WILLSTÄTTER, WALDSCHMIDT-LEITZ and HESSE (58).
Maltase	$\frac{x}{\sqrt{E}} = K$	good	HERZOG, BECKER and KASARNOWSKI (21).
	$\frac{x}{E \cdot t} = K$ ($E \cdot t = \text{const.}$)	bad	WILLSTÄTTER, OPPENHEIMER and STEIBELT (57).
α -Methylglucosidase	" "	"	"
Emulsin	$E \cdot t = K$	"	WILLSTÄTTER and CSÁNYI (54).
	$\frac{x}{E} = K$ (within 25 %)	"	TAMMANN (48).
Invertase	$\frac{x}{E} = K$	"	O'SULLIVAN and TOMPSON (34)
	$\frac{x}{E \cdot t} = K$	"	HUDSON (22) EULER and SVANBERG (15).
Lactase	$\frac{x}{E \sqrt{t}} = K$	"	ARMSTRONG (4).
Pepsin	$\frac{x}{\sqrt{E}} = K$	good	E. SCHÜTZ (40); BORISSOW (11); METT (31).
	$\frac{x}{\sqrt{E}} = K$ ($E = \text{dil.}$)	"	J. SCHÜTZ (42).
	$\frac{x}{\sqrt[3]{E}} = K$	bad	SAMOJLOFF (38) (calcd. by E. SCHÜTZ (41)).
	$\frac{x}{\sqrt{E}} \neq K$ ($E = \text{conc.}$)	"	J. SCHÜTZ (42).
	$E \cdot t = K$	"	SJÖQVIST (43).
	$\frac{a-x}{\sqrt{E \cdot t}} = K$	"	" (calcd. by ARRHENIUS).

Trypsin	{	$\frac{a}{t} \ln \frac{a}{a-x} - \frac{x}{t} = E \cdot K$	„	J. SCHÜTZ (42) („)
		$F \cdot t^2 = K$	„	SPRIGGS (45).
		$\frac{x}{E \cdot t} = K$	„	MADSEN and WALBUM (30).
		$\frac{x}{\sqrt{E \cdot t}} = K$	good	MORI (32).
		$E \cdot t = K$	bad	HEDIN (18); HENRI (19).
Peptidase	{	$E \cdot t = K$ (E: dil.)	„	BAYLISS (10).
		$E \cdot t = K$ (E: conc.)	„	„
		$E \cdot t = K$	„	EULER (14).
		$\frac{i}{E \cdot t} \log \frac{a}{a-x} = K$	„	WALDSCHMIDT-LEITZ and SCHÄFFNER (53).
		$\frac{x}{\sqrt{E}} \neq K$	„	EULER (14).
Urease	{	$\frac{x}{\sqrt{E}} = K$	good	EULER (14).
		$\log \frac{a}{a-x} + \varepsilon \cdot x = K \cdot t$	bad	ABDERHALDEN and MICHAELIS (2).
		$\frac{x}{E \cdot t} = K$ (E, t: const.)	bad	VAN SLYKE and CULLEN (51); YAMAZAKI (60).
		$\frac{x}{E} = K$	„	MORI (32).
		$E \cdot t = K$	„	REICHEL and SPIRO (37).
Lab	{	$E \cdot t = K$	„	HAMMARSTEN (17).
		(when E is dil. more time is required than calculated t)		
Yeast	{	$\frac{x}{E} = K$	„	TAMMANN (47).
		$\frac{\log K_1 - \log K_2}{\log C_1 - \log C_2} = n$	„	HERZOG (20).
(K: enzyme quantity; c: velocity)				
Phenyl- β -glucosidase		$\frac{x}{E} = K$ (under 30 % x)	„	TAMMANN (49).
β -Glucosidase		$\frac{x}{E} = K$	„	JOSEPHSON (24).
Zymase		„	„	BOYSEN-JENSEN (12).
Peroxidase		„	„	UCKO and BANSI (50); BANSI (9).

As shown in the above table, the cases of satisfactory application of the equation, $\frac{x}{\sqrt{E}} = K$ are very few. As for one kind of enzyme such as pepsin for instance, it is seen that several equations are proposed, so the SCHÜTZ law is not the only one even for peptic reaction. The present author wishes to examine further whether the equation, $\frac{x}{\sqrt{E}} = K$, is correct or incorrect in the kinetics of these enzymes.

In the VOLHARD-STADE method (52) (46) for lipase, to egg-yolk, either gastric or pancreatic juice is added, so as to let lipase act on the yolk. Then the digested substances are extracted with a mixed solution of alco-

hol and ether, and the quantity of decomposed fat is determined by titration. ENGEL (13) said that his results by this method followed the SCHÜTZ law as in the following table.

Tabel II

Pancreatin (c. c.)		0.04	0.09	0.16
After 4 hours	exp.	17.6	20.9	35.2
	cal.	16.8	24.5	31.6
	$\frac{x}{\sqrt{E \cdot t}}$	4.4	3.5	4.4
After 9 hours	exp.	18.4	36.3	48.4
	cal.	24.5	35.0	44.6
	$\frac{x}{\sqrt{E \cdot t}}$	3.1	4.0	4.0
After 25 hours	exp.	35.0	58.2	72.1
	cal.	38.2	53.0	65.0
	$\frac{x}{\sqrt{E \cdot t}}$	3.5	3.8	3.6

From the above table, the differences between the experimental and calculated values are tabulated by the present author as follows:—

Tabel III

Pancreatin (c. c.)	0.04	0.09	0.16	Sum of differences
After 4 hours	+0.8	-3.6	+3.6	+ 4.4 - 3.6 = +0.8
After 9 hours	-6.1	+1.3	+3.8	+ 5.1 - 6.1 = -1.0
After 25 hours	-3.2	+5.2	+7.1	+ 12.3 - 3.2 = +9.1

As shown, after 4 hours, the difference is -3.6 in the case with 0.09 c. c. of enzyme, while with 0.16 c. c. of enzyme it is +3.6. After 9 hours, in the case with 0.04 c. c. of enzyme, the difference is -6.1, which corresponds absolutely to as much as $\frac{1}{3}$ of the quantity decomposed—18.4%. After decomposition for 25 hours, the absolute differences sum up to 15.5, the larger part of which is positive. In the author's opinion the above data do not follow the SCHÜTZ law, though it contradicts ENGEL's conclusion.

The result of STADE's experiments (46) on the lipase of gastric juice as is clear in Table IV, shows values nearer to $\frac{x}{\sqrt{E}} = K$ than the pre-

Table IV

x	4.7	8.7	15.0	19.5	24.5
E	4.0	8.0	12.0	16.0	24.0
K	1.18	1.06	1.25	1.23	1.02

ceeding example from ENGEL. However, slight changes of the value of K are observed in that the value corresponding to 19.5 of x is 1.23, while it decreases to 1.02 when corresponding to 24.5 of x . In the case of the decomposition of protein with dilute peptic solution, the SCHÜTZ law holds. This will be discussed later. In the case of gastric lipase, the law may possibly be considered valid as in the dilute peptic solution. Yet it is very unsafe to deduce the conclusion from only the few data above cited.

SANDBERG (39) decomposed olive oil with papain. He concluded that his results proved the SCHÜTZ law to be true in a certain extent. One of his results is shown in Table V.

Table V

Papain; 2.5 mg olive oil, 5 c. c. H_2O , 1 c. c. 0.5 N NH_4Cl buffer PH. 8.9; 20 mg $CaCl_2$, 60 min.; 30° C.

papain (mg) e	x	$\frac{x}{e} = K$	$\frac{x}{\sqrt{e}} = K$
10	1.0	0.100	0.32
20	2.0	0.100	0.45
40	3.2	0.080	0.51
80	6.1	0.076	0.68
100	7.1	0.071	0.71
160	10.3	0.064	0.81
320	15.4	0.048	0.86

The author can not agree with his conclusion, for in the value of $\frac{x}{\sqrt{e}}$ there is a gradual change from 0.32 to 0.86, the ratio of which is 1 : 2.5. It may be better to conclude, "The value of K increases with the time, and the result does not follow the SCHÜTZ law."

There are three experimental results from which E. SCHÜTZ proposed the equation, $\frac{x}{\sqrt{E}} = K$. One of them which has the largest range of the quantity of pepsin, is taken, for example, as follows:—

Table VI

Enzyme quant.	1	4	9	16	25	36	49	64	Sum of differences
Exp. value	9.4	20.61	32.33	45.35	55.21	64.96	75.97	85.25	
Cal. value	10.8	21.6	32.4	43.2	54.1	64.9	75.7	86.5	
Difference	-1.4	-0.99	-0.07	+2.15	+1.11	+0.06	+0.27	-1.25	+3.59-3.71=-0.12

The numerals here show the degree of specific rotatory power of the products of decomposition. Seeing that the experimental data agree well with the calculated values, the above result may be taken to support the accuracy of the SCHÜTZ law.

As the author, to his great regret, has not been able to secure a copy of SAMOJLOFF's paper; he can not learn the details of that student's experiments. E. SCHÜTZ and HUPPERT opposed SAMOJLOFF's conclusion at the end of their paper. They said that the reason why SAMOJLOFF's results did not follow the SCHÜTZ law was the incompleteness of his experimental method and that if the length of protein pieces had been taken proportionally to the square root or to the cube root of the concentration of pepsin, and if gastric juice had been carefully treated, the results would have followed the SCHÜTZ law. They calculated two results of SAMOJLOFF and said that the data were, in these cases, proportional to the cube root of the concentration of pepsin as follows:—

Tabel VII

1. Pepsin quant.	64	32	16	8	4	2	1
[m.m. verdaut]	6.68	5.12	3.98	3.08	2.32	1.75	1.37
Square root	7.81	5.32	3.90	2.76	1.94	1.38	0.98
Cube root	6.25	4.98	3.92	3.12	2.42	1.97	1.56
2. Pepsin quant.		32	16	8	4	2	1
[m.m. verdaut]		6.72	5.33	4.20	3.07	2.01	1.69
Square root		7.08	5.45	3.84	2.72	1.92	1.39
Cube root		6.36	5.04	4.00	3.18	2.52	2.00

It seems rather strange that although E. SCHÜTZ and HUPPERT had already proposed the equation, $\frac{x}{\sqrt{E \cdot t}} = K$, here they recognized that SAMOJLOFF's data were proportional to the cube root of the concentration of pepsin. This contradiction of the two facts must be the evidence of the incompleteness of the SCHÜTZ law itself.

Their thesis was published on June 30th, 1900; while in August a paper written independently by Julius SCHÜTZ (42), as if to criticize E. SCHÜTZ's work, was published. J. SCHÜTZ treated protein with pepsin and from the change of the quantity of the residual coagulable protein, tried to ascertain the applicability of the SCHÜTZ law. He found that the law was suitable within certain limits of dilution of peptic solution, but when more concentrated solutions were used, their results did not agree with the law. By METT's method, he obtained a result as follows:—

Table VIII

Peptic soln. (c. c. in 100)	1	4	9	16	25	36	49	64
Exp. value (m. m.)	0.45	0.99	1.25	1.87	2.08	2.14	2.25	2.64
Cal. value (m. m.)	0.46	0.91	1.37	1.82	2.28	2.74	3.19	3.65

J. SCHÜTZ, taking the hint from HELMHOLZ, said that the SCHÜTZ law seemed to be a "Dissociationsformel" in dilute solution as is governed by the following principle; "The quantity of dissociated molecules in dilute solution at constant temperature and constant pressure, is proportional to the square root of entire concentration." When E. SCHÜTZ's results are examined from the standpoint of J. SCHÜTZ's idea, it is evident that his peptic solution obtained by dialysis must have been very diluted. And J. SCHÜTZ's own result as shown in Table VIII seems to agree with the SCHÜTZ law until the quantity of pepsin increases to 16. This concentration seems to be the limit of the applicability of J. SCHÜTZ's "Dissociationsformel." ABDERHALDEN and STEINBECK (3) used undiluted gastric juice on muscle. Their results did not follow the equation, $\frac{x}{\sqrt{E}} = K$. Therefore J. SCHÜTZ's opinion seems to be sound.

The results according to the reaction velocity of maltase obtained by LINTNER and KRÖBER (27), ARMSTRONG (5), HERZOG and his associates (21) do not agree one another. Among these results, only HERZOG's data were said by himself to follow the equation, $\frac{x}{\sqrt{E}} = K$. The applicability, however, can be seen within the limits of the quantity of enzyme from 1 to 4, while if increases to 8, the value of K decreases suddenly as is shown here below.

Table IX

Relative quantity of enzyme E	1	2	4	8
Velocity V	54	76	107	138
K=V: E	54	54	54	49

One result of LINTNER's experiments is taken here as an example. This result does not follow the equation, $\frac{x}{\sqrt{E}} = K$.

Table X

Quantity of yeast E	Dextrose	$\frac{x}{\sqrt{E}} = K$
1/2	1.654	7.397
1	3.048	3.048
2	3.940	2.789
4	4.783	2.391

From these facts, the coincidence of one of HERZOG's results with this law on maltase may be judged as special, just as a straight line coincides nearly or almost exactly with a curve in a special case or within the narrow limit of length.

Next the SCHÜTZ law will be examined in the action of peptidase. EULER's paper (14) described how he decomposed α -alanyl-glycin with pressed juice of yeast with following results, where F represents the relative quantity of enzyme. The value of each K has been calculated and compared by the present author.

Table XI

t=20 min.				t=30 min.			
$\frac{a}{11}$	1	1.72	1.0	$\frac{a}{15}$	1	1.73	2.07
$\sqrt{\frac{a}{F}}$	1	1.73	2.0	$\sqrt{\frac{a}{F}}$	1	1.73	2.0
K	11.00	10.94	10.45	K	15.0	15.0	15.5

The relative quantity of enzyme taken in this example is narrowly limited as from 1 to 4 which may be easily calculated from the above data. EULER's result conforms to the law, but he did not investigate with a greater or lesser quantity of enzyme. Next another one of his results will be seen to conform to the equation, $\frac{x}{\sqrt{E}} = K$ as follows:—

Table XII

0.1 N glycyl-glycin; 0.04 N NaOH; 5 g erepsin prep. in 100 c.c.

E	1000 K
5	6.5
4	5.4
3	4.3
2	2.8

When Abderhalden and MICHAELIS (2) investigated the decomposition of d-alanyl-d-alanin with the pressed juice of yeast, they got an experimental equation, $\ln \frac{a}{a-x} + \epsilon x = Kt$, where "a" represents the initial concentration, ϵ and K are constants, each of which having a definite value according to the quantity of enzyme.

As above noticed, there are papers which proposed different equations for peptidase; so it is certain that only one result of EULER as above shown is not sufficient to prove that the equation, $\frac{x}{\sqrt{E}} = K$, is generally acceptable for peptidase reaction.

From the author's examination of many results obtained with various kinds of enzymes, the equation, $\frac{x}{\sqrt{E}} = K$, may be criticized as follows:—

1. As J. SCHÜTZ already stated, in the case of decomposing protein with dilute peptic solution, the equation, $\frac{x}{\sqrt{E}} = K$, is sound within the wide limits of enzyme quantity. If it is concentrated, the equation can not be applied.

2. As for the action of another enzyme, the present author has found no paper which illustrates the propriety of the equation. If one finds such papers as affirm the fitness of the equation, they describe only the experiments within a narrow range of enzyme quantity.

3. There are, however, many papers which misjudge their results in applying the SCHÜTZ law.

Therefore the equation, $\frac{x}{\sqrt{E}} = K$, must not be recognized as an applicable equation in enzymatic reaction except in the sole case of dilute peptic solution.

III. Examination of the equation, $\frac{x}{\sqrt{t}} = K$

(1) On the theses which support the SCHÜTZ law

There is the ARRHENIUS law (8) which supports the SCHÜTZ law with theoretical explanation; NORTHROP's theory also supports it, giving theoretical and experimental explanations. These two theses will be discussed below.

(i) The ARRHENIUS law and its defects

An experimental equation which is widely applicable with exactness

must generally have a theoretical basis, whether its explanation is given or not. At the same time even though a theoretical explanation may support an equation, if it is not applicable in actual cases, the explanation must have some defects or insufficiency. The SCHÜTZ law is an equation derived from experiments. Therefore if it is true, it must have a theoretical foundation. As such a theoretical explanation we have the ARRHENIUS law, according to which the velocity of digestion is primarily proportional to pepsin concentration p , secondly, proportional to the quantity yet undigested $(a-x)$, and thirdly, inversely proportional to the quantity of protein digested x , as may be shown in the following equations.

$$\frac{\delta x}{\delta t} = K \cdot P,$$

$$\frac{\delta x}{\delta t} = K (a-x),$$

$$\frac{\delta x}{\delta t} = K \left(\frac{1}{x} \right).$$

Combining these three equations, one has

$$\frac{\delta x}{\delta t} = K \cdot P \left(\frac{a-x}{x} \right).$$

Integrating, one gets

$$K = \frac{1}{P \cdot t} \left(a \cdot \ln \frac{a}{a-x} - x \right)$$

which is the ARRHENIUS law.

As can easily be seen from the above equation, if the quantity $(a-x)$ is taken as constant when x is very small at the beginning of the reaction, then one has

$$\frac{\delta x}{\delta t} = K \cdot P \frac{1}{x},$$

which by integration, results

$$K = \frac{x}{\sqrt{P \cdot t}}.$$

This equation is the SCHÜTZ law itself. Therefore the ARRHENIUS equation explains the SCHÜTZ law theoretically, at the beginning of enzymatic reaction. Nevertheless, the present author believes that ARRHENIUS's theory does not agree with the results of experiments in many cases. For instance, as before stated, E. SCHÜTZ and HUPPERT got a result whose values of K are almost constant within such rather a large range up to 40 % decomposition. E. SCHÜTZ also got a similar result even within 60 % decomposition (calculated from the degree of rotation). These facts do not agree with the equation, $K = \frac{x}{\sqrt{P \cdot t}}$, introduced by ARRHENIUS. In many investi-

tions, directed to affirm the fitness of the SCHÜTZ law [cf. examples in (2) which will be illustrated afterwards], K can not be constant at the beginning of the reaction, while at the middle of the reaction, the values of K can be seen to be nearly constant (however the present author does not recognize them *constant*). These facts are quite against the theoretical explanation of ARRHENIUS. Therefore his theory can not be said to support the SCHÜTZ law theoretically. It also shows many defects in the results of actual experiments. Of course the disproving of the ARRHENIUS law does not mean at the same time the disproving of the SCHÜTZ law, but the refutation of the former, which supports the latter, is very clear.

(ii) NORTHROP's theory refuted

According to NORTHROP (33), at the beginning of enzymatic reaction in the presence of much substrate the quantity of active enzyme is small, hence the reaction velocity is also small. Furthermore at the end of the reaction, owing to the retarding effect of decomposed products, the velocity is also small, and only at the middle of the reaction, is the activity of enzyme so great that the sufficient application of the SCHÜTZ law can be seen. The results of many experiments which the SCHÜTZ law is supposed to be applicable do not give constant values of K really at the beginning as well as at the end of the reaction. But only at the middle of the reaction, they are comparatively near, which, however, in the opinion of the present author, are not constant in a strict sense. NORTHROP's explanation seems to have been widely recognized. In order to avoid complexity, the author does not discuss his theory here separately. By refuting the SCHÜTZ law hereafter (3), it will be naturally be refuted as well.

(2) Examples to which the SCHÜTZ law is taken to be applicable and some defects of the law

In order to examine the SCHÜTZ law, let the author first review several experimental results, to which the investigators took the SCHÜTZ law as applicable.

Lipase

The result obtained by WILLSTÄTTER and MEMMEN (55) in the decomposition of triacetin with pancreas lipase was calculated by Oppenheimer and said to follow the SCHÜTZ law. Here the experimental values are compared with those calculated by the present author.

Table XIII

Time (min.)	% decomposed	$K = \frac{x}{\sqrt{t}}$	Cal. value of x	Difference
16	3.2	0.80	3.6	-0.4
25	4.5	0.90	4.5	0.0
36	6.5	0.93	5.4	+1.1
49	6.7	0.96	6.3	+0.4
64	7.6	0.96	7.2	+0.3
81	8.4	0.93	8.1	+0.3
100	8.9	0.89	9.1	-0.2
		mean 0.905	+2.2-0.6 = +1.6	

As is shown above, the value of K increases gradually from 0.80 to 0.96, and then decreases. The difference between the maximum and the minimum values of K is 0.16 which seems apparently very small and the value of K seems almost constant, but the range of decomposition is also very small viz., $8.9 - 3.2 = 5.7\%$. Even though the difference between the maximum and the minimum K values (0.16) and the sum of differences (1.6) are apparently very small, they are pretty large compared with the mean value of K , 0.905 and the 5.7% decomposition respectively. Therefore it seems to the writer too hasty to say that the result follows the SCHÜTZ law. Moreover, if the reaction follows the law fundamentally, the absolute values of the positive and negative differences must each be about equal, and if the differences are experimental alone the positive or negative differences must occur at random. But the SCHÜTZ K actually contradicts these suppositions. These defects are very common in most experimental results which are mistakenly supposed to follow the SCHÜTZ law.

WILLSTÄTER and his coworker (59) decomposed olive oil with castor bean lipase to examine the relation between the time required and the quantity decomposed. From two results, they concluded that the value of K of the monomolecular equation decreased constantly while that of the SCHÜTZ law was uniform within the range of about 20-50% of decomposition, and that at the beginning, below about 6%, the quantity decomposed was proportional to the time required. One of their results is as follows:—

Table XIV

0.360 g cream of lipase, 2.50 g oil, 2.00 c. c. acetate buffer, $\text{PH} = 4.7$; 20° .

Time (min.)	Decomposed %	$K = \frac{1}{t} \log \frac{a}{a-x}$	$K = \frac{x}{\sqrt{t}}$	$K = \frac{x}{t}$
2.5	3.1	0.0055	2.1	1.2
5	6.0	0.0054	2.7	1.2
10	9.6	0.0044	3.0	1.0
20	15.7	0.0037	3.5	0.8
40	26.3	0.0033	4.2	0.7
80	40.9	0.0029	4.6	0.5
160	57.0	0.0023	4.5	0.4
320	66.6	0.0015	3.7	0.2

The opinion of WILLSTÄTTER about the applicability of the SCHÜTZ law to the result seems, to the present author, scarcely deducible from their two results.

There are many other available studies on the action of lipase, most of which were taken to follow the SCHÜTZ law but not the monomolecular equation, comparing the values of K with each other. In all such results, there are found the same defects as have been illustrated in the above examples.

Lichenase

The results obtained by KARRER, JOOS and STAUB (25) (Table XV and XVI) and also by PRINGSHEIM and SEIFERT (36) (Table XVII) are examined. The values of $\frac{x}{\sqrt{t}}$ of KARRER's results were calculated by OPPENHEIMER.

Table XV

Time (min.)	x %	$\frac{10^6}{t} \log \frac{a}{a-x}$	$\frac{x}{\sqrt{t}} \cdot 10^4$
105	9.3	403	36
225	18.0	383	48
345	24.4	353	53
465	30.6	342	56
1440	53.4	231	56

Table XVI

x %	$\frac{10^6}{t} \log \frac{a}{a-x}$	$\frac{x}{\sqrt{t}} \cdot 10^4$
13.5	600	53
29.5	679	79
40.0	636	86
46.8	590	87
71.3	377	76

Tabel XVII

Time (min.)	x %	$\frac{10^5}{t} \log \frac{a}{a-x}$	$\frac{x}{\sqrt{t}} \cdot 10^4$
60	11.06	84.8	75
150	24.71	86.0	110
240	39.57	88.1	131
345	45.9	77.3	130
465	53.5	71.5	131
585	59.66	67.4	129

However examining these results, the author unexpectedly found out mistakes of their calculations. The values of $\frac{x}{\sqrt{t}}$ calculated by him are as follows:—

Tabele XVIII

(1) Table XV			(2) Table XVI		(3) Table XVII		
Time	x %	$\frac{x}{\sqrt{t}}$	x %	$\frac{x}{\sqrt{t}}$	Time	x %	$\frac{x}{\sqrt{t}}$
105	9.3	0.65	13.5	0.94	60	11.06	1.43
225	18.0	1.20	29.5	1.97	150	24.71	2.02
345	24.4	1.31	40.0	2.15	240	38.57	2.49
465	30.6	1.42	46.8	2.17	345	45.9	2.47
1440	53.4	1.41	71.3	1.88	465	53.5	2.46
					585	59.66	2.44

The change of the K value in each example has the same tendency as in the previous examples. The equation, $\frac{x}{\sqrt{t}}=K$, can not be called suitable for (1) and (2), while it can be applicable to (3) within the range from 38.57 to 59.66 for x. This application is recognized only within this narrow range. Such "application" will be examined afterwards [cf. (3) (ii)].

Lactase

The result obtained by ARMSTRONG (5) is as follows:—

Table XIX

Time	0.5	1.0	2.0	3.0	4.5	6.0	23	29	48	53	144
x %	3.2	4.8	6.4	7.6	9.0	10.0	19.7	22.0	29.0	30.7	62.2
$\frac{x}{\sqrt{t}}=K$	4.5	4.8	4.5	4.4	4.2	4.1	4.1	4.1	4.2	4.2	5.2

The value of K first increases, and then it decreases. When the values calculated from the mean value of K are compared with experimental values (Table XX), they seem scarcely to disprove the equation in respect to the sum of differences which corresponds to $5.2-6.7=-1.5$, while within the time from 0.5 to 53, the sum of differences becomes $0.7-6.7=-6.0$ which leans strongly to the negative to disprove the equation. So it is rather to be considered that the result dose not follow the law. It seems proper to say, considering the above data, that the SCHÜTZ law is

applicable within the range of decomposition from 9.0 to 30.7 %.

Table XX

Time	0.5	1.0	2.0	3.0	4.5	6.0	23	29	48	53	144	Sum of differences
Exp. value	3.2	4.8	6.4	7.6	9.0	10.0	19.7	22.0	29.0	30.7	62.2	
Cal. value	3.1	4.4	6.2	7.6	9.3	10.7	21.1	23.6	30.4	32.0	57.7	
Difference	+0.1	+0.4	+0.2	0.0	-0.3	-0.7	-1.4	-1.6	-1.4	-1.3	+4.5	+5.2-6.7=1.5

Trypsin

ARRHENIUS, estimating the change of electric conductivity in the hydrolysis of gelatin and casein, found his results consistent with the SCHÜTZ law.

Table XXI

4 % gelatin; 44.3°

Time (min.)	Change of elec. cond.	$x=7.37\sqrt{t}$	Diff.
10	27.3	29.3	-2.0
20	44.0	41.5	+2.5
30	53.0	50.0	+3.0
40	58.7	58.7	0.0
55	65.7	68.8	-3.1
			+5.5-5.1=+0.4

Table XXII

Casein in 2 % NaHCO₃

Time (min.)	Change of elec. cond.	$x=7.59\sqrt{t}$	Diff.
10	24	24	0.0
20	36	34	+2.0
30	41	42	-1.0
40	42	48	-6.0
50	44	54	-10.0
			+2-17=-15

If the sum of differences is examined, it seems recognizable that the former result agrees with the SCHÜTZ law, but when one considers the same defective tendency in the occurrence of positive and negative differences as in the previous examples, it does not seem at the same time to be a strict application of the law. The present author can not recognize its application to Table XXII.

Pepsin

E. SCHÜTZ and HUPPERT (41) asserted the applicability of their own law by many results of their experiments. One of these is as follows:—

Table XXIII

Time	1	4	9	16	25	Sum of differences
I Albumose exp.	(0.0718)	0.0938	0.1254	0.1787	0.2530	
cal.	0.0463	0.0926	0.1389	0.1852	0.2315	
Difference		+0.0012	-0.0135	-0.0065	+0.0215	+0.0227-0.0200=
						+0.0027

Time	1	4	9	16	25	Sum of differences
II Albumose exp.	0.2206	0.5583	0.6887	0.9594	0.9451	
cal.	0.2427	0.4854	0.7283	0.9708		
Difference	-0.0221	+0.0729	-0.0396	-0.0114	+0.0729	-0.0731 = -0.0002

The differences between experimental and calculated values are not always satisfactorily small, when their ratio to the corresponding experimental values are considered. But the law may be taken to be applicable to these results. However in both cases, the range of decomposition is very narrow.

Next, one of the analogous equations of the SCHÜTZ law will be considered. MATSUYAMA and NAKAMURA (29) said that, in their digestive experiments on egg-white, during the time from 1 to 6 hours at 30°–62° C., the equation $\frac{x}{\sqrt[3]{t}} = K$ was most satisfactory in comparison with such equations as $\frac{x}{t} = K$ or $\frac{x}{\sqrt{t}} = K$. The values of K nearly coincided within the limits of 1 to 4 hours at 30°–37° (Table XXIV). E. SCHÜTZ and HUPPERT persisted in applying the equation, $\frac{x}{\sqrt[3]{E}} = K$, to SAMOJLOFF's results obtained with different amounts of pepsin. From these two equations of MATSUYAMA and E. SCHÜTZ, the author considers it possible to derive the equation, $\frac{x}{\sqrt[3]{E \cdot t}} = K$, in peptic digestion, which, however, is not correct for the same reasons as already stated in II and as will be stated in (3).

Table XXIV

PH. 1.75		1	2	3	4	5	6
30°	Exp. value %	0.0426	0.0547	0.0608	0.0679	0.0720	0.0751
	$K = \frac{x}{\sqrt[3]{t}}$	0.0426	0.0434	0.0422	0.0428	0.0421	0.0413
33°	Exp. value	0.0454	0.0606	0.0678	0.0743	0.0786	0.0818
	$K = \frac{x}{\sqrt[3]{t}}$	0.0454	0.0481	0.0470	0.0468	0.0459	0.0450
37°	Exp. value	0.0560	0.0720	0.0812	0.0882	0.0918	0.0956
	$K = \frac{x}{\sqrt[3]{t}}$	0.0560	0.0571	0.0563	0.0555	0.0536	0.0526

One can see also in these results that the value of K changes with the same defective tendency as in preceding examples.

Many examples of the application of the SCHÜTZ law has been ex-

aminated. However, some of them are the results obtained with no care regarding the hydrogen ion concentration of a solution. The same defective tendencies of the law can be seen in respect to any results which were obtained at a definite PH value. Here one of the present author's results is taken.

Glycinin was prepared by the method of OSBORNE. 0.6 g of the dried substance was dissolved in 100 c.c. of 1/100 N NaOH solution and then treated as follows:—

10 c.c. of the filtrate + 6.5 c.c. H₂O + 12.5 c.c. of CLARK and LUB's buffer solution of PH value 1.2 + 1 c.c. of 1/200 water solution of MERCK's pepsin; 40°C; + 3 c.c. of 1/5 N NaOH + 7 c.c. of 20% CCl₃COOH.

Five c.c. of the filtrate of the above solution were treated by the micro-Kjeldahl method.

Table XXV

t (min.)	x %	$\frac{x}{\sqrt{t}} = K$	Cal. value t=15-300 K=3.73	Diff.	Cal. value t=60-300 K=3.97	Diff.	Cal. value t=120-300 K=4.09	Diff.
15	9.82	2.54	14.45	-4.63				
30	18.50	3.38	20.43	-1.93				
60	29.29	3.78	28.89	+0.40	30.83	-1.54		
90	35.57	3.75	35.39	+0.18	37.76	-2.19		
120	45.41	4.15	40.86	+4.55	43.60	+1.81	44.80	+0.61
180	55.30	4.12	50.05	+5.25	53.40	+1.90	54.87	+0.43
240	63.43	4.10	57.79	+5.64	61.67	+1.76	63.37	+0.06
300	69.26	4.00	64.60	+4.66	68.93	+0.33	70.84	-1.58
			+20.68-6.56= +14.12		+5.80-3.73= +2.07		+1.10-1.58= -0.48	

In respect to the above data, one can point out the same defects of the law as has been illustrated already.

Many scholars concluded that the SCHÜTZ law was applicable to their results of experiments which were made with various kinds of enzyme. The author has picked out above several examples and showed many defects of the law. The reasons for refuting the law may be stated as follows.

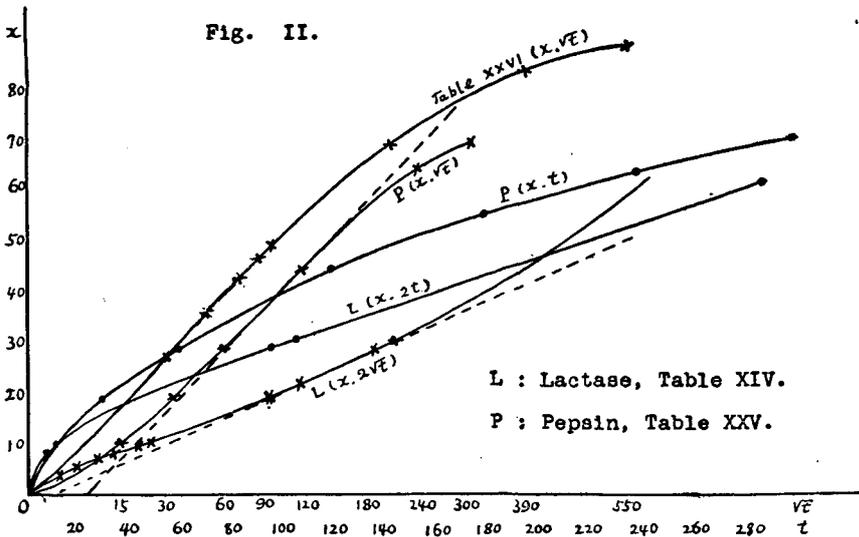
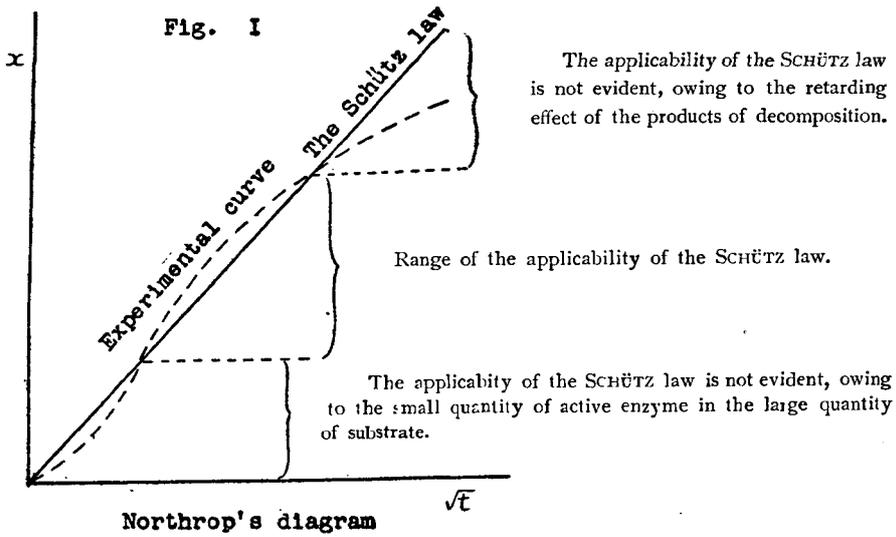
(3) Reasons for refuting the SCHÜTZ law, $\frac{x}{\sqrt{t}} = K$

(i) Applicable range and characteristic tendencies of the equation,
 $\frac{x}{\sqrt{t}} = K$

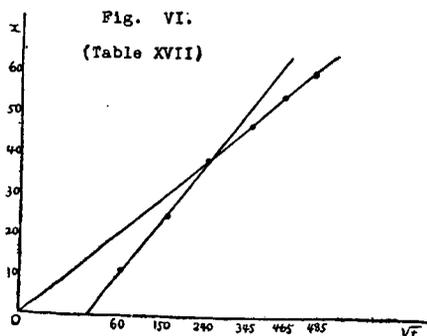
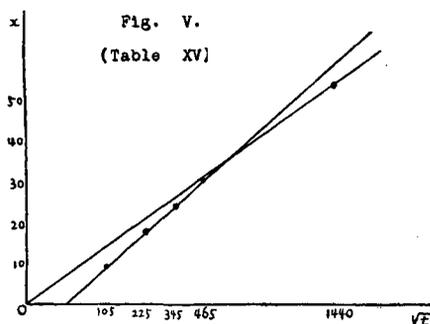
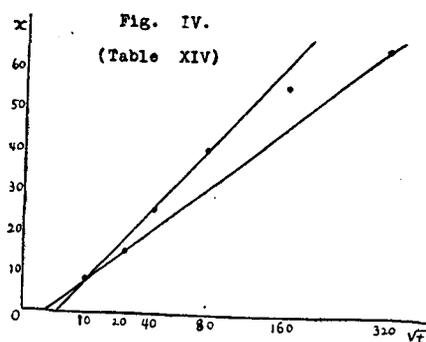
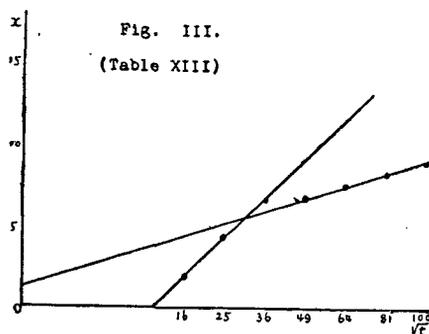
If one regards the applicable limits of the equation, $\frac{x}{\sqrt{t}}=K$, in the range of enzymatic decomposition, it is usually so narrow as to be under 20 % or 30 % at most, of the decomposition of substrate, for instance from 10 to 30 % or from 25 to 55 %. One may obtain comparatively uniform values of K within such limits, but at both extremes, the SCHÜTZ K value increases or decreases suddenly in all cases. In the majority of experiments, the value of K is usually small at the beginning, but gradually increases and then decreases again when the experiments are continued. In the preceding example of lactase, this tendency is reversed. One may seldom find such a tendency of K , when the interval of reaction time is wide, having an experimental curve of small inclination, as seen in Fig. II. Yet one finds in all results, that the range to which the law is applicable is very narrow, and that although the values of K are approximately near, they are not so near as to make it possible to regard them as *constant*. Though Northrop explained the SCHÜTZ law, he supported it not from an experimental basis. The author wishes to discuss NORTHROP's theory again later on. Putting aside his theory, if one examines the SCHÜTZ K with its tendency to increase or decrease, it is not really *constant*. So long as there is no experimental proof allowing such a tendency to SCHÜTZ's K , one can not recognize it as *constant*. As have been stated above, J. SCHÜTZ recognized the equation, $\frac{x}{\sqrt{E}}=K$, to be applicable but not in all cases of peptic digestion. But his investigation did not awake people's attention. On the contrary, the equation, $\frac{x}{\sqrt{E}}=K$, has been recognized by many people without any censure as applicable to all enzymatic reactions. The origin and the first recognition of the SCHÜTZ law, $\frac{x}{\sqrt{t}}=K$, are very vague, and yet it has been recognized as a definite law. As there have been many cases whose apparently near values of $\frac{x}{\sqrt{t}}=K$ made one misbelieve the equation, it has been accepted the more firmly as one of the widely recognized equations. At the earlier period of the introduction of the law, the reason mentioned above, would surely have been enough to refute the SCHÜTZ law, but in these days, this simple reasoning may not be sufficient, as the law has been accepted for many years. Below are two or three more reasons to refute the law.

(ii) Experimental results must not be discussed on the co-ordinates (x, \sqrt{t})

If one squares $\frac{x}{\sqrt{t}} = K$, $x^2 = t \cdot K^2$ results. Then if the equation is graphed on the co-ordinates (x, t) , a parabola results, but if it be taken

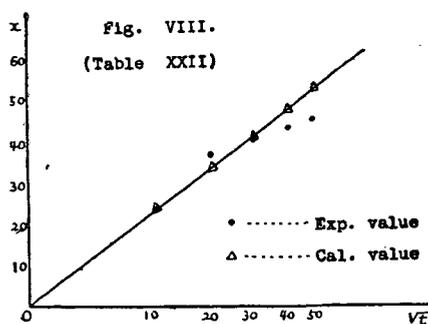
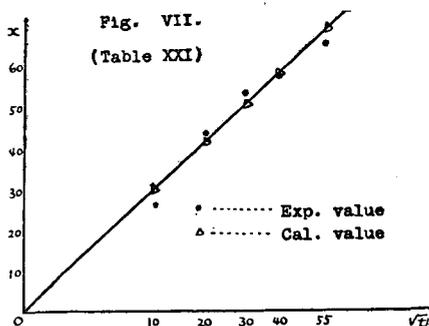


on the co-ordinates (x, \sqrt{t}) , a straight line passing through zero point is obtained. On the latter co-ordinates (x, \sqrt{t}) , most experimental results show curves as NORTHROP stated, which are concave at first and then change into convex. Yet it is a great mistake to take this curvature to represent the decomposition velocity as weak during the first half, owing to the small quantity of free enzyme, and also to be weak during the latter half, owing to the retarding effect of decomposition products. It is the present author's opinion that it is not suitable to express the experimental result of the enzymatic decomposition on the co-ordinates (x, \sqrt{t}) , because on the co-ordinates (x, t) , whatever units of time are taken, an experimental curve shows generally such a curvature as that of general theoretical equations of chemical kinetics, $K = \frac{1}{t} \log \frac{a}{a-x}$ or $K = \frac{1}{t} \frac{x}{a(a-x)}$, while on the co-ordinates (x, \sqrt{t}) , it shows generally a curvature concave at first and then convex, which is caused by the difference of the degree of decrease of the square root of t at the earlier and at the later part of the reaction time. Therefore the curvature of a graph of the experimental data on the co-ordinates (x, \sqrt{t}) makes one misbelieve that the activity



of enzyme is weak at both ends, as in NORTHROP's explanation. The activity of lactase is retarded by the decomposition products of the substrate, glucose and galactose, as was made very clear by ARMOSTRONG (3), yet one of his results, for instance, is diagrammed concavely on the co-ordinates (x, \sqrt{t}) as already stated which possibly makes one mistakenly suppose that lactase activity is accelerated. The diagram on the co-ordinates (x, t) does not show such contradiction. It is clear therefore that one must not employ the co-ordinates (x, \sqrt{t}) to indicate experimental results.

These facts as stated above, mean the inapplicability of the SCHÜTZ law at the same time. In other words, if the law holds, an experimental result should have graphed as a straight line on the co-ordinates (x, \sqrt{t}) as previously stated. In order to illustrate this, let one diagram several results and compare them on the co-ordinates (x, \sqrt{t}) and (x, t) . If one compares straight lines which pass through corresponding points of SCHÜTZ's K, or of calculated as well as experimental values, it is not difficult to understand how the SCHÜTZ law can not be depended upon (Fig. II-VIII).



Briefly stating, the problem becomes: If the SCHÜTZ law holds, the curve of a graphed experimental result must be a part of a parabola, $x^2 = t.K^2$, on the co-ordinates (x, t) . One can not find such an experimental result. If one finds such a result as is satisfied by this equation within narrow limit, it is correct to consider that this result is shown by an equation which coincides nearly within that narrow limit, though it belongs to another kind of equation than the perfectly represented equation of the result, as for example when any kind of curve, such as a parabola or hyperbola, if its little part be taken, may possibly be represented by a linear or some other kind of equation. But, in such a case, there is no coincidence except only on a restricted part. It is a matter of fact that

the values of the SCHÜTZ K are not constant or the values of tangents of the curve do not give nearly a definite value if the result is diagrammed on the co-ordinates (x, \sqrt{t}) , notwithstanding that the experimental result does not show such a curve as $x^2 = t.K^2$.

(iii) Examination of the relation between the quantity decomposed and the time required

If the enzymatic reaction is carried on according to the SCHÜTZ law and if neither a retarding nor an accelerating side reaction occurs, then the time which is required for the perfect decomposition, is theoretically four times that of decomposing half of the substrate. It is shown as follows:—

Square the equation, $x = \sqrt{t.K}$, one gets

$$x^2 = t.K^2.$$

Substituting $1/2$ and 1 for x , one gets

$$(1/2)^2 = t_1 K^2 \dots\dots\dots (1),$$

$$(1)^2 = t_2 K^2 \dots\dots\dots (2).$$

Dividing (2) by (1), there results

$$\frac{t_2}{t_1} = 4.$$

If the decomposition follows according to the monomolecular equation, $\frac{1}{t} \log \frac{a}{a-x} = K$, and if there occurs no side reaction, then the decomposition will be completed in about ten times the time which is required to decompose one half of the substrate. This is shown in the following way. Let $a = 1000$ and let the decomposition of $999/1000$ of the entire substrate be taken as the perfect decomposition, one gets, by substituting 500 and 999 for x ,

$$\frac{1}{t_1} \log 2 = K \dots\dots\dots (1)$$

$$\frac{1}{t_2} \log 1000 = K \dots\dots\dots (2)$$

Dividing (2) by (1), one gets

$$\frac{t_2}{t_1} = 10$$

The ratio between any two different times can be calculated similarly as above illustrated. In order to examine some experimental results, let one take a result of amygdalase obtained by WILLSTÄTTER and CSÁNYI (54)

(Table XXV) and a result of lactase by WILLSTÄTTER and OPPENHEIMER (56) (Table XXVI).

Table XXV

1 % amygdalin; 2.5 mg emulsin 20 c.c.; PH=6.0; 30°.

Time (min.)	Quantity decomposed x (%)	$\frac{10^5}{t} \log \frac{a}{a-x} = K$	$\frac{x}{\sqrt{t}} = K$
12	6.68	575	1.85
24	10.95	483	2.23
40	14.50	492	2.29
60	22.03	414	2.84
90	31.84	425	3.36
120	39.24	415	3.57
180	51.75	404	3.86
1200	96.00	257	2.79
		$575 \div 257 = 2.24$	$3.86 \div 1.83 = 2.11$

Table XXVI

5 % lactose; PH=7; 30°

Time (min.)	Quantity decomposed x (%)	$\frac{10^5}{t} \log \frac{a}{a-x} = K$	$\frac{x}{\sqrt{t}} = K$
30	28	476	5.11
52	37	386	5.13
60	40	370	5.16
70	43	349	5.14
80	47	345	5.25
90	49	325	5.17
110	55	315	5.24
135	62	311	5.34
172	65	265	4.96
210	69	243	4.76
270	73.5	214	4.47
390	84.0	204	4.30
550	87.5	182	3.73
∞	(100.0)	$476 \div 182 = 2.61$	$5.34 \div 3.73 = 1.43$

From these data, the authors concluded that their results did not follow the equation, $\frac{1}{t} \log \frac{a}{a-x} = K$, for the value of K decreases with the time. There are many authors who concluded that their results followed the SCHÜTZ law much better comparing only the values of K in both equations, $\frac{x}{\sqrt{t}} = K$ and $\frac{1}{t} \log \frac{a}{a-x} = K$. It is the present author's opinion that it

is not correct to determine the most applicable equation comparing only the values of K of different kinds of equations. To illustrate this opinion, each SCHÜTZ's K of the above two results is calculated and compared with that obtained by using monomolecular equation respectively. The ratio of time at about the middle and the end points of the reaction of Table XXV according to the SCHÜTZ law and the monomolecular equation will be calculated and compared. It required 180 minutes to decompose about one half of the substrate, 51.75. As above stated the time required for the entire decomposition according to the two equations is calculated as follows:—

$$\frac{1}{t} \log \frac{a}{a-x} = K_m \quad 180 \times 10 = 1800$$

$$\frac{1}{\sqrt{t}} = K_s \quad 180 \times 4 = 720$$

Therefore if the reaction follows the latter theoretically it must be completed in 720 minutes, but the experimental data show that the decomposition of 96 % required all of 1200 minutes.

In respect to the result of Table XXVI, the time required for entire decomposition according to the two equations calculated as follows:—

$$\frac{1}{t} \log \frac{a}{a-x} = K_m \quad 90 \times 10 = 900$$

$$\frac{a}{\sqrt{t}} = K_s \quad 90 \times 4 = 360$$

In fact, only 84 % was decomposed in 390 minutes. It seems that the above two results follow the SCHÜTZ law, if the comparison of K_m and K_s only is taken, but it is certain that $\frac{1}{t} \log \frac{a}{a-x} = K$ is much more applicable than $\frac{x}{\sqrt{t}} = K$ as is above illustrated. At the present time, one can not know how much the retarding or accelerating side reaction occurs in enzymatic reactions, but it is now made clear that one must not determine the applicability of an equation only by the comparison of the degree of nearness of K values of different kinds of equations. So, in respect to such results above illustrated, it is better and more reasonable to say "The enzymatic reaction followed fundamentally such an equation as $\frac{1}{t} \log \frac{a}{a-x} = K$, but through some kind of unknown factor, uniform values of K were not obtained," than to say "The enzymatic reaction followed fundamentally the SCHÜTZ law, but such experimental curves as

NORTHROP secured, resulted from the influence of the substrate and the retarding effect of decomposition products."

(iv) Discussion on the curvature of graphs of experimental results

It is generally known that in enzymatic reaction, some products of decomposition retard, while other products accelerate the reaction. In such decomposition which is accompanied by some positive autocatalytic reaction, if the principal reaction follows the SCHÜTZ law fundamentally, the experimental curve must be concave on the co-ordinates (x, \sqrt{t}) . However, one can not find such experimental results. On the contrary, the fact that one of ARMSTROMG's results with lactase, whose activity is retarded by the decomposition products of the substrate as stated above, such a concave, on the co-ordinates (x, \sqrt{t}) as illustrated on Fig. II shows the impropriety of using the co-ordinates (x, \sqrt{t}) to diagram the experimental result, and at the same time shows that the reaction did not follow the the SCHÜTZ law fundamentally.

The first reason for refuting the SCHÜTZ law, is that it is not trustworthy as a standard equation in enzymatic kinetics, because of its meaningless constant. The author does not absolutely insist upon this first reason to refute the SCHÜTZ law, as some insist upon overlooking such a defect, taking the law as an experimental equation.

The second reason is absolute, i. e. there occurs an unreconcilable contradiction between the actual fact and the SCHÜTZ law if the latter holds.

The third reason may not be absolute, but it points to the fact that it is a mistake to determine the applicability of an equation by comparing only the values of K of different kinds of equations with each other. The SCHÜTZ law has often been supported by such a mistaken reasoning.

The fourth reason, the writer believes, is one of the principal reasons as is the second, which has been deduced from many experimental results.

One has been so far many data, to which the SCHÜTZ law is said to be applicable, but one has not been able to see any discussion which examines the law itself and affirms the propriety of it, except those of ARRHENIUS and NORTHROP previously discussed.

As this paper illustrated, the equation, $\frac{x}{\sqrt{t}}=K$, must not be taken as an equation in the kinetics of enzymes, even though some cases are seen where the values of K of the experimental results are near.

Conclusion

From the reasons explained above, the author concludes that the SCHÜTZ law and its analogous equations do not have any worth in the kinetics of enzymes except for the application of the equation, $\frac{x}{\sqrt{E}}=K$, to the results obtained with diluted peptic solutions. The author has frankly criticized also the ARRHENIUS law and the NORTHROP theory which supported the SCHÜTZ law.

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