ON THE DIFFERENCE BETWEEN β-GLUCOSIDASE AND β-GALACTOSIDASE

by

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The question of enzyme specifity has frequently been discussed. Among the glycosidases HELFERICH and his co-workers (for literature, see VEIBEL [1]) have particularly investigated almond emulsin from sweet almonds in order to find out how many different glycosidases this product contains. From his experiments HELFERICH has concluded that the catalysis of the hydrolysis of β-glucosides and of β-galactosides is brought about by one and the same enzyme, as the treatment of the almond emulsin with different purifying or damaging agents causes identical procentual alterations in the β-glucosidatic and the β-galactosidatic faculties of the enzyme preparation. Continuing these experiments HELFERICH and his collaborators found in alfalfa seed emulsin and in coffee emulsin (HELFERICH and VORSATZ [2], HILL [3]) enzymes which were able to bring about the hydrolysis of β-galactosides but not of β-glucosides, and HELFERICH concluded from this that two different β-galactosidases must exist, one present in almond emulsin being identical with β-glucosidase, whereas the other, present in alfalfa seed emulsin, is different from this enzyme.

On the other hand, other investigators (ANTONIANI [4], VEIBEL, MØLLER, and WANGEL [5]) found enzyme preparations which were able to hydrolyse β-glucosides but not β-galactosides. This necessitates either the adoption of two different β-glucosidases as well as two β-galactosidases, or the assumption that β-glucosidase and β-galactosidase are in reality two different enzymes but that they are so closely related that the action of purifying or damaging operations on the two enzymes is parallel.

This latter explanation seems to us the most natural as it, with the assumption of only two different enzymes, makes comprehensible that the relation between the velocities of hydrolysis of β-glucosides and of β-galactosides may vary within very broad limits with emulsin preparations of different origin, the limits being extended by the experiments just mentioned to cover the range from ∞ to 0.

The velocities regarded in this discussion are exclusively defined as the monomolecular velocity constants of hydrolysis, k_{obs}, calculated directly from the experimental results. VEIBEL and LILLELUND [6, 7] have, however, called attention to the fact that a more correct picture is obtained by comparing not the k_{obs}-values but the k_3-values, the velocity constants of fission of the enzyme-substrate-compound formed according to the MICHAELIS-MENTEN theory [8]. In order to calculate the k_3-values it is necessary to determine the „affinity constants" K_m, K_{m1} and K_{m2}, which are the dissociation constants of the complexes formed by the enzyme with the glucoside (K_m), the carbohydrate (K_{m1}) or the aglycone (K_{m2}) respectively. k_3 is then, according to VEIBEL and LILLELUND, calculated from the expression

$$k_3 = k_{obs}\,(K_m + c + K_m/K_{m_h}\cdot c_h + (K_m/K_{m1} + K_m/K_{m2} - 1)x)/e \text{ (sal. f.)}$$

The term K_m/K_{m_h} accounts for the influence on the k_{obs}-values of the presence of an inhibiting substance h with affinity constant K_{m_h} in the concentration c_h, provided the inhibition is competitive. k_{obs} has to be calculated from point to point, and

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x is the mean value of the molar concentration of the products of hydrolysis in the two points used for the determination of k_{obs}. *e* as usual means quantity of enzyme (in g) in 50 ml solution, and sal. f. is used to express the force of the enzyme preparation, determined either with salicin as substrate (JOSEPHSON [9], WEIDENHAGEN[10]) or with o-cresol-ß-d-glucoside as substrate (VEIBEL and LILLELUND [11]).

Obviously this expression for the enzymic force has no real significance for β-galactosidase if this enzyme is different from β-glucosidase, and especially it does not allow the comparison of the β-galactosidatic action of enzyme preparations of different origin. HELFERICH has, however, as mentioned above, shown that the relation between the β-glucosidatic and the β-galactosidatic action of almond emulsin is independent of the degree of purity of the enzyme preparation, and consequently the sal. f.-value may for these preparations be used also for a comparison of the β-galactosidatic actions.

By comparing the k_3-values instead of the k_{obs}-values two advantages are gained, as (1) the glycoside concentration may be chosen at will, k_3 being independent of the glycoside concentration, whereas k_{obs}, according to the expression (I), is dependent on this concentration in a rather complicated way; and (2) the possibility of being independent of the colloidal carrier, seat of the prostetic group which may be regarded as the real hydrolyzing agent, is augmented. It seems natural to presume that the „affinity" between substrate and enzyme is dependent on the structure both of the prostetic group and of the colloidal carrier, and whereas it appears likely that the prostetic group of a certain glycosidase is independent of the source of the enzyme, it is quite as likely that the colloidal carrier is different from plant to plant. In this way the K_m-values for enzymes of different origin become different and as K_m is used for the calculation of k_3, this latter constant may become independent of the source of the enzyme.

In a previous paper VEIBEL and LILLELUND [7] have examined the β-glucosidatic properties of almond emulsin and the variation of these properties with p_H and with the composition of the buffer solution. As substrate o-cresol-β-d-glucoside was used. It was found that the p_H-optimum was not the same in an acetate buffer as in a phosphate-citrate buffer. The affinity constant, as expected, was dependent on p_H and also on the composition of the buffer solution. One peculiarity was noted, namely that at p_H-optimum the k_{obs}-value is greater in phosphate-citrate-buffer than in acetate buffer, whereas the k_3-value, on the contrary, is greater in acetate buffer than in phosphate-citrate buffer. It does not seem as if the different actions of the buffer solutions are caused by differences in salt concentration or in ionic strength of the buffer solutions.

In this paper we describe a repetition of these experiments using o-cresol-β-d-galactoside as substrate instead of o-cresol-β-d-glucoside. The results are to a certain degree analogous with the results obtained in the previous investigation. Below the mean values are given in tables and curves and, to facilitate a comparison, some of the curves previously found are also reproduced.

EXPERIMENTAL PART

Substrates. o-Cresol-β-d-galactoside was prepared as indicated by HELFERICH and SCHMITZ-HILLEBRECHT [12]. It is very essential not to omit the purification of the galactoside-tetracetate by dissolving it in ether and reprecipitating it with petrol ether. By this procedure a small quantity of o-cresol-α-d-galactoside-tetracetate, which cannot be separated

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from the β-galactoside-tetracetate by simple recrystallisation from alcohol, remains in the liquid phase. HELFERICH and SCHMITZ-HILLEBRECHT indicate M.P. 113-114°, $[\alpha]_D$ = —4° ($CHCl_3$). We find M.P. 116-117°, $[\alpha]_D^{20°}$ = —5.9° ($CHCl_3$, c = 2.215, l = 2, α = —0.26°).

The entacetylation was carried out by the method of ZEMPLEN and PASCU [13]. HELFERICH and SCHMITZ-HILLEBRECHT indicate for the galactoside M.P. 193-195° and $[\alpha]_D$ = —43.3° (H_2O). We find the same M.P. but $[\alpha]_D^{20°}$ = —40.5° (H_2O, c = 3.542, l = 2, α = —2.87°). The galactoside seems to retain one molecule of water very tenaciously. 193-195° is the M.P. of the anhydrous galactoside, — 43.3° its specific rotation, whereas —40.5° is the specific rotation of the galactoside with 1 mol H_2O.

o-Cresol-β-d-glucoside was prepared by current methods and showed the constants indicated in the literature.

Enzyme. The emulsin used was a preparation of almond emulsin (MERCK), identical with the preparation used in the previous investigation of o-cresol-β-d-glucoside.

Buffer-solutions. The acetate buffers were mixtures of 0.1 n acetic acid solution and 0.1 n sodium acetate solution, the phospahte- citrate buffers were mixtures of 0.2 m disodium phosphate solution and 0.1 m citric acid solution as indicated by Mc. ILVAINE [11].

Method. As usual, samples to 5 ml were taken, the enzymic action in the samples was suppressed by mixing with 1 ml 20% K_2CO_3-solution and the rotation determined after 2-3 hours (Mutarotation). The specific rotation of the galactoside and of galactose at p_H 10.5-10.6 (the p_H of the samples after mixing them with K_2CO_3-solution) were determined in separate experiments.

1. *Determination of p_H-optimum.*

The results are given in Table I and Fig. 1. In all experiments a galactoside concentration 0.0400 m was used.

TABLE I

p_H		3.6	4.0	4.4	4.8	5.2	5.6
$10^2.k_{obs}$/e (sal. f.)	Acetate	66.2	72.9	81.5	92.7	98.0	94.1
	Phosphate	62.2	88.2	104	108	101	89.6

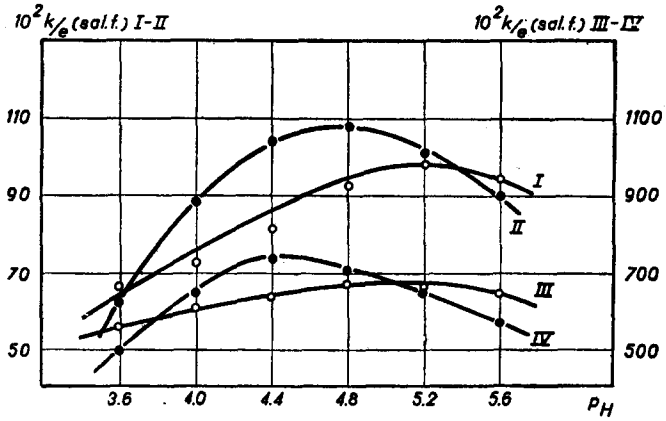

Fig. 1.
I: o-Cresol-ß-d-galactoside.
II: o-Cresol-ß-d-galactoside.
III: o-cresol-ß-d-glucoside.
IV: o-Cresol-ß-d-glucoside.
o Acetate buffer.
● Phosphate-citrate buffer.

As seen from Fig. 1 the p_H-optimum is displaced a little towards higher p_H-values when compared with the values valid for β-glucosidase. This is the case in the acetate buffer as well as in the phosphate-citrate buffer. No significant difference in the shape of the curves for the two glycosidases seems to exist. The value of k_{obs}/e for the galactoside is about 1/7 of the value of k_{obs}/e for an ocresol-β-d-glucoside solution of the same concentration.

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2. *Determination of K_m.*

K_m was determined at 3 different p_H-values, in acetate buffer at p_H 3.6, 5.2 and 5.6, in phosphate-critate buffer at p_H 3.6, 4.8 and 5.6. Table II and Fig. 2 show the results. (Determination according to VEIBEL and LILLELUND [6]).

TABLE II

DETERMINATION OF K_m

$c_{Galactoside}$	Acetate e (sal. f.)k'_{obs}			Phosphate-citrate e (sal. f.)/k'_{obs}			
	pH 3.6	pH 5.2	pH 5.6	pH 3.6	pH 4.8	pH 5.6	
0.0100 m	1.06	0.75	0.66	0.82	0.61	0.79	
0.0200 m	1.09	0.84	0.75	1.08	0.76	0.94	
0.0400 m	1.35	1.04	1.03	1.35	0.93	1.08	
0.0600 m	1.57	1.22	1.18	1.61	1.11	1.37	
0.0800 m	1.80	1.49	1.41	1.80	1.34	1.76	(0.0944 m)
0.1000 m	2.02	1.61	1.59	—	1.47	1.86	
K_m	0.077	0.063	0.059	0.055	0.053	0.056	

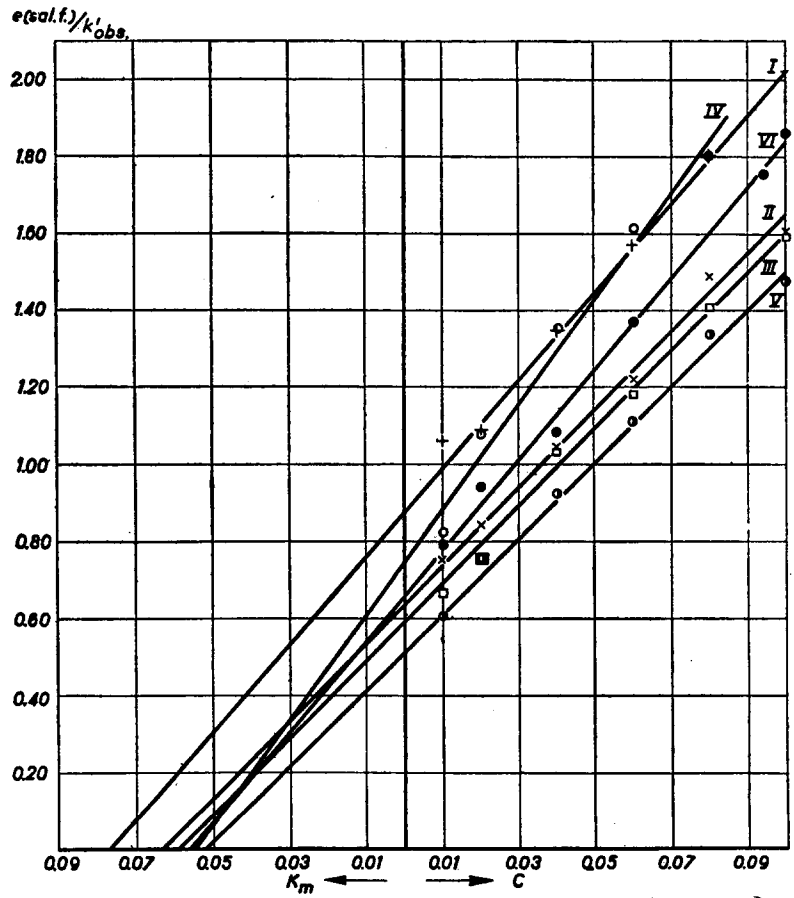

Fig. 2. o-Cresol-ß-d-galactoside. K_m-values.

I + Acetate buffer p_H 3,6 IV ○ Phosphate-citrate buffer p_H 3,6
II × Acetate buffer p_H 5,2 V ◑ Phosphate-citrate buffer p_H 4,8
III □ Acetate buffer p_H 5,6 VI ● Phosphate-citrate buffer p_H 5,6

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Fig. 3 shows the corresponding values for o-cresol-β-d-glucoside (VEIBEL and LILLELUND [1]).

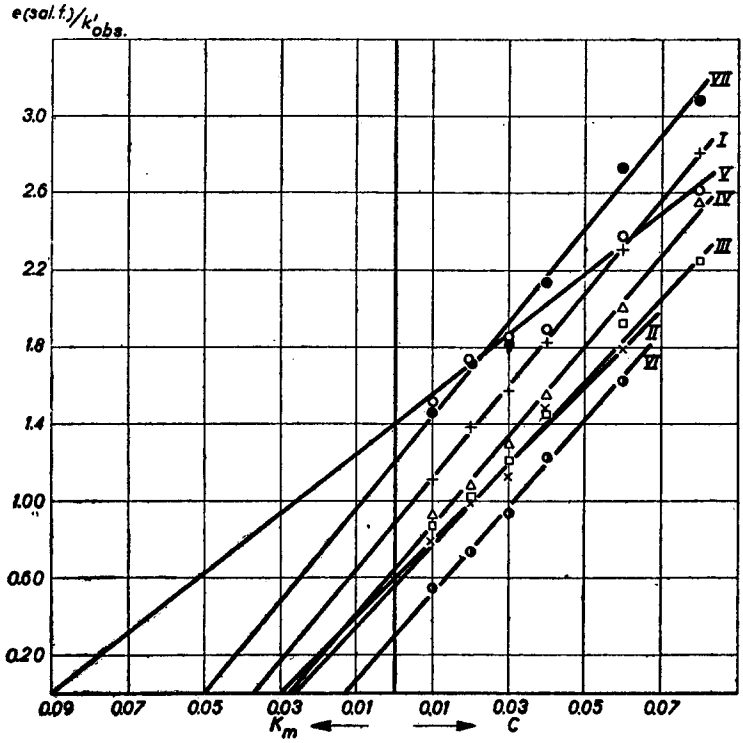

Fig. 3. o-Cresol-ß-d-glucoside K_m-values.

I + Acetate buffer pH 3,6 V o Phosphate-citrate buffer pH 3,6
II × Acetate buffer pH 4,4 VI ⊙ Phosphate-citrate buffer pH 4,4
III □ Acetate buffer pH 5,0 VII ● Phosphate-citrate buffer pH 5,6
IV △ Acetate buffer pH 5,6

3. *Determination of* K_{m_1}.

As usual, K_{m_1} was determined by investigation of the velocity of hydrolysis of solutions of the galactoside which besides galactoside contains galactose in increasing concentration. The expression (II) allows then the calculation of K_{m_h}, h being the inhiting substance present in the concentration c_h.

$$K_{m_h} = \frac{K_m . c_h}{(K_m + c)\,(k'_{obs}/k'_{obsh} - 1)} \qquad (II)$$

Table III gives the result at 3 different pH-values.

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TABLE III

DETERMINATION OF K_{m1}

$c_{Galactoside}$	k'_{obs}	$\frac{k/k_h-1}{c_{Galact.}}$	K_{m1}	k'_{obs}	$\frac{k/k_h-1}{c_{Galact.}}$	K_{m1}	k'_{obs}	$\frac{k/k_h-1}{c_{Galact.}}$	K_{m1}
Acetate buffer. Substrate o-Cresol-β-d-galactoside 0.0400 m									
p_H 3.6 K_m = 0.077				p_H 5.2. K_m = 0.063			p_H 5.6. K_m = 0.059		
0.00	71.9	—		94.0	—		81.4	—	
0.01	72.3	-0.6		98.5	—		78.6	2.8	
0.02	68.4	2.6		96.5	—		76.3	3.5	
0.04	68.4	1.3	0.41	92.1	—	0.87	75.2	2.1	0.26
0.08	62.8	1.8		94.0	—		68.5	2.4	
0.12	55.9	2.4		87.1	0.7		77.2	0.5	
average		1.5			0.7			2.3	
Phosphate-citrate buffer. Substrate o-Cresol-β-d-galactoside 0.0400 m									
p_H 3.6. K_m = 0.054				p_H 4.8. K_m = 0.054			p_H 5.6. K_m = 0.054		
0.00	62.3	—		100.1	—		79.1	—	
0.01	59.5	4.7		98.2	2.0		71.5	11.0	
0.02	54.6	7.1		94.6	2.9		79.5	-0.3	
0.04	58.8	1.5	0.21	93.4	1.8	0.44	63.2	6.3	0.15
0.08	59.6	0.6		95.0	0.7		78.5	0.1	
0.12	63.7	-0.2		111.9	-0.9		64.3	1.9	
average		2.7			1.3			3.8	

The precision of the determination of the k'_{obs}-values is not as great as in the corresponding experiments with o-cresol-β-d-glucoside as substrate, the solutions not being clear enough to allow the readings in 2 dm tubes, but only in 1 dm tubes. The cause is partly that, in order to get a velocity of hydrolysis sufficient to carry the experiments through in one day, it is necessary to use some 5 times so much emulsin as in the glucoside-experiments, and partly that the galactoside preparation available did not yield a perfectly clear solution. The impurity could not be eliminated by recrystallisation. The inaccuracy is unmistakeable especially at the p_H-optimum where the inhibition is minimum, being in acetate buffer so great that the velocity constants are identical within the limits of error up to 0.08 m galactose. In this instance we have, therefore, calculated the K_{m1}-values from the velocity-constants in the solution without galactose and in the solution 0.12 m with regard to galactose. Much credit cannot be ascribed to a value found in this way, but the experiment shows clearly that the affinity between the glycosidase and galactose is very slight.

At the other p_H-values examined the affinity is greater and for the calculation of the mean value of k/k_h-1 we have made use of all the experimentally found values, even if some of them, on account of experimental error, are found to be negative.

The main result of the investigation is that at all events galactose does not inhibit the hydrolysis of β-galactosides as much as the hydrolysis of β-glucosides is inhibited by glucose. Not only the K_{m1}-values are different in the two cases, but also their p_H-dependence.

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4. *Determination of* K_{m2}.

TABLE IV

DETERMINATION OF K_{m2}

c_{Cresol}	k'_{obs}	$\frac{k/k_h - 1}{c_{Cr.}}$	K_{m2}	k'_{obs}	$\frac{k/k_h - 1}{c_{Cr.}}$	K_{m2}	k'_{obs}	$\frac{k/k_h - 1}{c_{Cr.}}$	K_{m2}
Acetate buffer. Substrate o-Cresol-β-d-galactoside 0.0400 m.									
pH 3.6. K_m = 0.077				pH 5.2. K_m = 0.063			pH 5.6. K_m = 0.059		
0.00	75.9	—		90.5	—		82.6	—	
0.01	67.8	12.1		75.9	20.0		77.1	7.2	
0.02	58.9	14.6		65.2	19.4		65.0	13.2	
0.04	40.7	21.7	0.036	52.5	18.1	0032	56.4	11.6	0.045
0.08	28.7	20.6		38.2	17.2		37.6	15.0	
0.12	19.8	23.7		25.1	21.8		25.2	19.0	
average		18.5			19.3			13.2	
Phosphate-citrate buffer. Substrate o-Cresol-β-d-galactoside 0.0400 m.									
pH 3.6. K_m = 0.054				pH 4.8. K_m = 0.054			pH 5.6. K_m = 0.054		
0.00	61.1	—		104.8	—		80.1	—	
0.01	57.2	6.7		81.9	27.6		65.2	21.8	
0.02	47.4	14.3		78.0	17.2		61.6	15.0	
0.04	41.2	12.1	0.051	63.6	16.2	0.035	53.2	12.6	0.033
0.08	36.4	8.5		55.9	10.9		30.4	20.5	
0.12	21.6	15.2		52.9	10.3		24.0	19.5	
average		11.4			16.4			17.9	

The determination of K_{m2} is quite analogous to that of K_{m1}, the inhibiting substance being o-cresol instead of galactose. Table IV contains the material for the calculation of K_{m2}.

The dependence on the composition of the buffer solution and on p_H is much like that found for o-cresol-β-d-glucoside. The inhibition is very strong and seems to be to the greatest extent competitive. Only in acetate buffer at p_H 3.6 can the question of a non-competitive inhibition arise.

TABLE V

K_{mx}-VALUES FOR O-CRESOL-β-D-GALACTOSIDE

pH	3.6	4.0	4.4	4.8	5.2	5.6
			Acetate-buffer			
K_m	0.077	0.074	0.070	0.066	0.063	0.059
K_{m1}	0.41	0.65	0.87	0.97	0.87	0.26
K_{m2}	0.036	0.028	0.026	0.027	0.032	0.045
K_m/K_{m1}	0.19	0.11	0.08	0.07	0.07	0.23
K_m/K_{m2}	2.14	2.64	2.69	2.44	1.97	1.31
$K_m/K_{m1} + K_m/K_{m2} - 1$	1.33	1.75	1.77	1.51	1.04	0.54
			Phosphate-citrate-buffer			
K_m	0.054	0.054	0.054	0.054	0.054	0.054
K_{m1}	0.21	0.28	0.36	0.44	0.42	0.15
K_{m2}	0.051	0.046	0.041	0.035	0.031	0.033
K_m/K_{m1}	0.26	0.19	0.15	0.12	0.13	0.37
K_m/K_{m2}	1.08	1.17	1.37	1.51	1.74	1.68
$K_m/K_{m1} + K_m/K_{m2} - 1$	0.34	0.36	0.47	0.63	0.87	1.05

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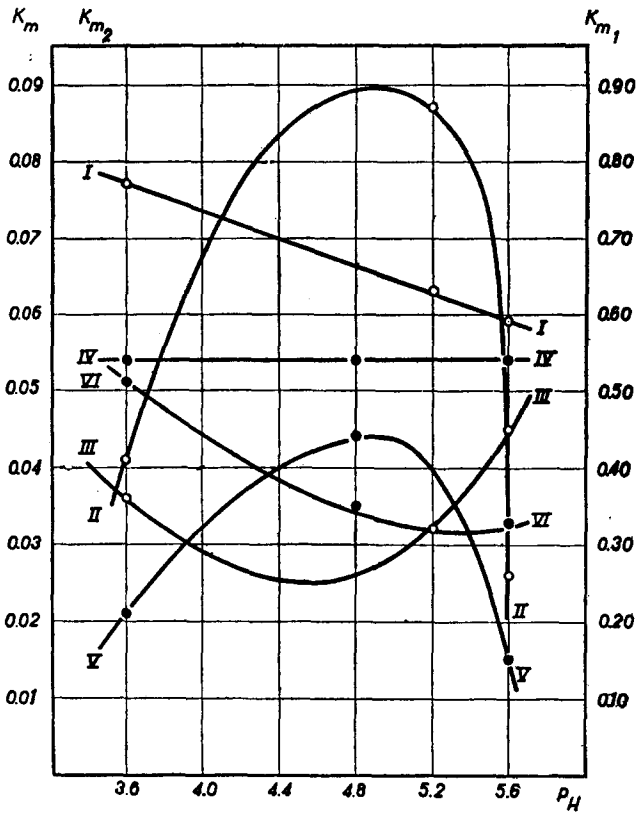

Fig. 4. o-Cresol-β-d-galactoside. K_{m_x} values.

I K_m	Acetate buffer	IV K_m	Phosphate-citrate buffer
II K_{m_1}	,, ,,	V K_{m_1}	,, ,, ,,
III K_{m_2}	,, ,,	VI K_{m_2}	,, ,, ,,

In Table V and Fig. 4 we have collected all the results of these investigations. Table V also contains the value of the correction-term in the expression (1) at different p_H. The K_{m_x}-values at such p_H-values where no experimental determination exists have been found by graphical interpolation. They are, consequently, approximate, as a curve determined by only 3 experimental points must necessarily be very uncertain.

In Fig. 5 we have reproduced the corresponding curves for o-cresol-β-d-glucoside (VEIBEL and LILLELUND [7]).

In Table VI we have finally collected all results from experiments at p_H 3.6, 4.8 (or 5.2) and 5.6, indicating the k_{obs} as well as the k_3-values. It is seen clearly that whereas the k_{obs}-values are strongly dependent on the concentration, the k_3-values are, allowing for a tolerable uncertainty, constant. The experiments are carried through by 3 different experimenters within a period of 2 years. The constancy of k_3 is not, therefore, dependent on the subjective readings of one single experimenter.

In the table the k_{obs}-values in experiments with 0.0400 m galactoside solutions without inhibiting substances are underlined and the mean values of these constants are given as well.

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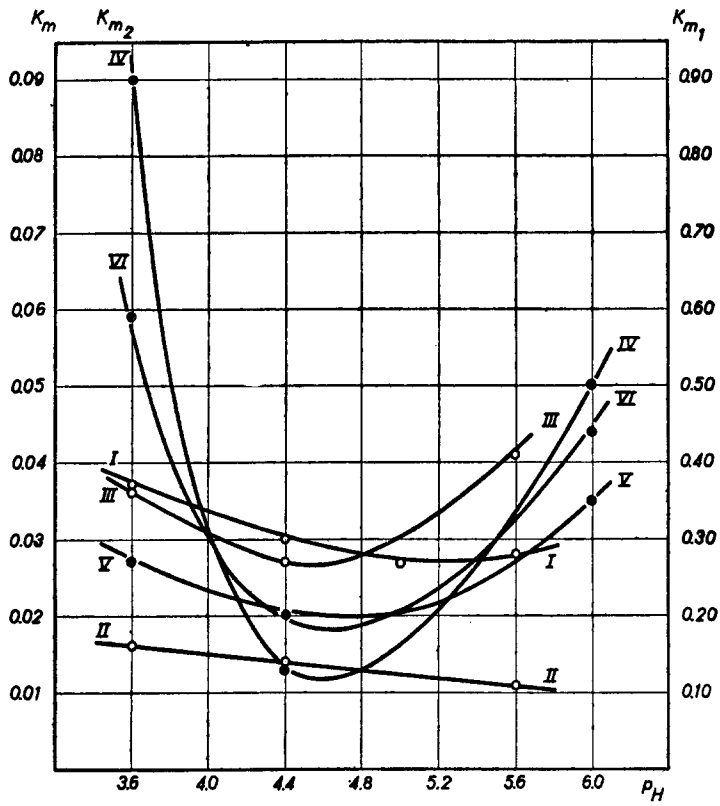

Fig. 5. o-Cresol-β-d-glucoside. K_{m_x}-values.

I	K_m	Acetate buffer	IV	K_m	Phosphate-citrate buffer
II	K_{m_1}	,, ,,	V	K_{m_1}	,, ,, ,,
III	K_{m_2}	,, ,,	VI	K_{m_2}	,, ,, ,,

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TABLE VI

O-CRESOL-β-D-GALACTOSIDE. K_{obs}-AND K_3-VALUES.

Acetate-buffer.

			pH 3.6		pH 5.2		pH 5.6	
$c_{Galactose}$	$c_{Galactose}$	c_{Cresol}	k_{obs}	k_3	k_{obs}	k_3	k_{obs}	k_3
0.0400	0.00	0.00	**66**	8.8	**98**	11.8	**94**	10.1
0.0100	0.00	0.00	92	8.2	139	10.4	155	10.9
0.0200	0.00	0.00	88	9.1	127	11.1	132	10.8
0.0400	0.00	0.00	**73**	9.1	**98**	11.0	**101**	10.3
0.0600	0.00	0.00	61	9.0	84	11.0	85	10.5
0.0800	0.00	0.00	55	9.1	68	10.4	71	10.3
0.1000	0.00	0.00	47	8.8	60	10.7	62	10.2
0.0400	0.00	0.00	**74**	9.2	**96**	10.5	**89**	9.0
0.0400	0.01	0.00	71	8.9	100	11.0	85	8.9
0.0400	0.02	0.00	65	8.3	98	10.9	78	8.3
0.0400	0.04	0.00	65	8.5	92	10.3	78	8.7
0.0400	0.08	0.00	65	9.0	95	10.9	71	8.5
0.0400	0.12	0.00	58	8.5	82	9.7	75	9.6
0.0400	0.00	0.00	**79**	9.7	**91**	9.8	**87**	8.8
0.0400	0.00	0.01	68	9.7	75	9.6	81	9.3
0.0400	0.00	0.02	57	9.4	66	9.6	66	8.4
0.0400	0.00	0.04	44	9.0	57	10.6	57	8.7
0.0400	0.00	0.08	27	8.0	40	10.5	42	8.6
0.0400	0.00	0.12	19	7.1	29	9.8	31	7.9
	average		**73**	8.8	**96**	10.5	**93**	9.4

Phosphate-citrate buffer

			pH 3.6		pH 4.8		pH 5.6		
0.0400	0.00	0.00	**62**	6.1	**108**	11.2	**90**	9.7	
0.0100	0.00	0.00	111	7.3	162	10.6	120	8.2	
0.0200	0.00	0.00	88	6.7	130	10.0	111	8.7	
0.0400	0.00	0.00	**71**	6.9	**110**	10.9	**89**	9.0	
0.0600	0.00	0.00	62	7.3	89	10.7	72	8.8	
0.0800	0.00	0.00	55	7.5	74	10.4	(55	8.7	0.0944 m)
0.1000	0.00	0.00	—	—	71	11.6	52	8.5	
0.0400	0.00	0.00	**62**	5.9	**106**	10.4	**84**	8.4	
0.0400	0.01	0.00	58	5.7	101	9.9	79	8.0	
0.0400	0.02	0.00	61	6.2	102	10.2	87	9.2	
0.0400	0.04	0.00	61	6.4	99	10.0	66	7.4	
0.0400	0.08	0.00	61	7.1	97	10.3	73	9.4	
0.0400	0.12	0.00	64	8.1	107	12.0	73	10.3	
0.0400	0.00	0.00	**64**	6.1	**100**	9.8	**75**	7.5	
0.0400	0.00	0.01	60	6.3	87	9.7	66	7.6	
0.0400	0.00	0.02	47	5.5	82	10.4	64	8.5	
0.0400	0.00	0.04	43	5.8	60	9.4	53	8.8	
0.0400	0.00	0.08	30	5.3	48	10.3	33	7.6	
0.0400	0.00	0.12	25	5.5	34	9.3	29	8.5	
	average		**65**	6.4	**106**	10.4	**85**	8.6	

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DISCUSSION

From the material given in this and the previous paper (VEIBEL and LILLELUND [7]) describing experiments with o-cresol-β-d-glucoside it seems possible to discuss the question of the identity or the non-identity of β-glucosidase and β-galactosidase. For instance, the variation of the K_{m_x}-values with p_H may be compared (see Fig. 4 and Fig. 5). It is seen that a difference exists, but it is by no means excluded that it is caused either by inexactitude in the experiments or by differences in the colloidal carriers of the two glycosidases, the affinity between the enzyme and the substrate (or its components, carbohydrate and aglycone) being at all events partly determined by the carrier. The p_H-dependence alone, therefore, does not permit a definite answer.

If the k_{obs}-values for 0.0400 m solutions of the two glycosides (the average values underlined in Table VI) are compared, the following values (Table VII) are found :

TABLE VII

$\frac{k_{obs\ Glucoside}}{k_{obs\ Galactoside}}$	pH	3.6	4.0	4.4	4.8	5.2	5.6
	Acetate	8.4	8.3	7.8	7.2	6.8	6.8
	Phosphate-citrate	7.9	7.4	7.1	6.5	6.4	6.3

It will be seen that the hydrolysis of the galactoside is favoured relatively at increasing pH-values both in acetate- and in phosphate-citrate-buffers, in accordance with the results in Table I, showing a pH-optimum for the β-galactosidase at a little higher value than for β-glucosidase. This may be due to different colloidal carriers in the two glycosidases. An assumption of two different enzymes is not required.

On the other hand, if the k_3-values are compared instead of the k_{obs}-values, a result as indicated in Table VIII is obtained.

TABLE VIII

$\frac{k_{3\ Glucoside}}{k_{3\ Galactoside}}$	pH	3.6	4.0	4.4	4.8	5.2	5.6
	Acetate	4.9	—	—	—	4.2	4.5
	Phosphate-citrate	10.5	—	—	3.6	—	4.8

Here the relation $k_{3\ gluc.}/k_{3\ gal.}$ for experiments in acetate buffer is rather independent of pH. The deviations may be explained in the same manner as the variations in Table VII. For experiments in phosphate-citrate buffer, however, the variations of the relation with p_H are too great to be described as uncertainty. The experiments are better understandable on the assumption of two different enzymes, causing the hydrolysis of the glucoside and the galactoside respectively. The two enzymes are not identical, but very closely related. As mentioned previously this assumption allows a very simple explanation of the fact that some enzyme preparations may catalyse the hydrolysis of β-glucosides but not of β-galactosides, whereas other catalyses the hydrolysis of β-galactosides but not of β-glucosides. The two enzymes are usually found together in the enzyme preparations, but in a few cases, some of which have been mentioned above, only one or the other of the enzymes are found and consequently the hydrolysis of only one of the two β-glycosides takes place.

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Further elucidation of the problem might possibly be obtained in examining the hydrolysis of a β-glucoside in the presence of galactose and the hydrolysis of a β-galactoside in the presence of glucose. If the two glycosidases are identical, the K_{m_1}-value for glucose, determined with a β-galactoside as a substrate, should be identical with the value found with a β-glucoside as substrate, and the $K_{m_1\ galactose}$-value should be independent of the use of a β-glucoside or a β-galactoside as substrate.

Table IX shows the results of such determinations.

TABLE IX

DETERMINATION OF $K_{m1\ galactose}$ FOR β-GLUCOSIDASE AND $K_{m1\ glucose}$ FOR β-GALACTOSIDASE

Substrate o-cresol-β-d-glucoside. Acetate buffer. pH 3.6. K_m = 0.037				Substrate o-cresol-β-d-galactoside. Phosphate-citrate buffer. pH 4.8. K_m = 0.053			
$c_{Galactose}$	k'_{obs}	$\frac{k/k_H-1}{c_{Galact.}}$	K_{m1}	$c_{glusoce}$	k'_{obs}	$\frac{k/k_H-1}{c_{Gluc.}}$	K_{m1}
0.00	462	—		00.0	106	—	
0.01	486	-4.7		21.0	105	0.6	
0.02	476	-1.5		80.0	103	1.4	
0.04	467	-0.2	∞	40.0	98	2.1	0.41
0.08	397	2.1		20.0	95	1.4	
0.12	416	1.0		10.0	88	1.7	
	average	-0.7				1.4	

$K_{m_1\ galactose}$, using glucoside as substrate, is ∞ or at all events so great that the value cannot be determined, whereas with a β-galactoside as substrate a value 0.41 was found (see Table III). Galactose practically does not inhibit the β-glucosidase, whereas it noticeably inhibits the β-galactosidase.

$K_{m_1\ glucose}$, using a β-galactoside as substrate, is 0.41. With a β-glucoside as substrate a value 0.21 was found (VEIBEL and LILLELUND [7]). Glucose inhibits consequently the β-glucosidase to a somewhat greater extent than it inhibits the β-galactosidase.

If from the experiments described in Table IX k_3-values are calculated, once with identical K_{m_1}-values for glucose and galactose and then with the individual values found here, a result as listed in Table X is obtained. These results too, justify the assumption of two different enzymes.

TABLE X

HYDROLYSIS OF O-CRESOL-β-D-GLUCOSIDE, ADDITION OF GALACTOSE		HYDROLYSIS OF O-CRESOL-β-D-GALACTOSIDE, ADDITION OF GLUCOSE	
K_{m1} = ∞	K_{m1} = 0.41	K_{m1} = 0.41	K_{m1} = 0.21
k_3 41.3	45.7	10.4	10.8
Average value previously found 42.7		10.4	

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SUMMARY

The β-glucosidase and the β-galactosidase of almond emulsin have been compared. The results obtained, especially the variation of the relation $k_{3\ glucoside} / k_{3\ galactoside}$ with p_H (phosphate-citrate buffer) are most easily explained on the basis of two different glycosidases. The inhibiting action of glucose and of galactose on the hydrolysis of β-glucosides and of β-galactosides catalysed by almond emulsin also suggests that the two β-glycosidases are different.

RÉSUMÉ

La β-glucosidase et la β-galactosidase de l'émulsine d'amandes ont été comparées. Les résultats obtenus et spécialement la variation de la relation $k_{3\ glucoside} / k_{3\ galactoside}$ avec le p_H (tampon phosphate-citrate) s'expliquent facilement si on suppose qu' il existe deux glycosidases différentes. L'action inhibitrice du glucose et du galactose sur l'hydrolyse de β-glucosides et de β-galactosides catalysée par l'émulsine d'amandes suggère aussi que les deux β-glycosidases sont différentes.

ZUSAMMENFASSUNG

Die β-Glucosidase und die β-Galaktosidase des Mandel-Emulsins werden mit einander verglichen.

Die Resultate, insbesondere die Aenderung der Relation $k_{3\ Glukoside} / k_{3Galaktoside}$ mit p_H (Phosphat - Zitrat - Puffer), können ohne Schwierigkeiten erklärt werden, wenn man zwei verschiedene Glycosidasen annimmt.

Die hemmende Wirkung, die durch Glucose und Galaktose auf die durch Mandelemulsin katalysierte Hydrolyse von β-Glucosiden und β-Galaktosiden ausgeübt wird, weist ebenfalls darauf hin, dass die beiden β-Glycosidasen verschieden sind.

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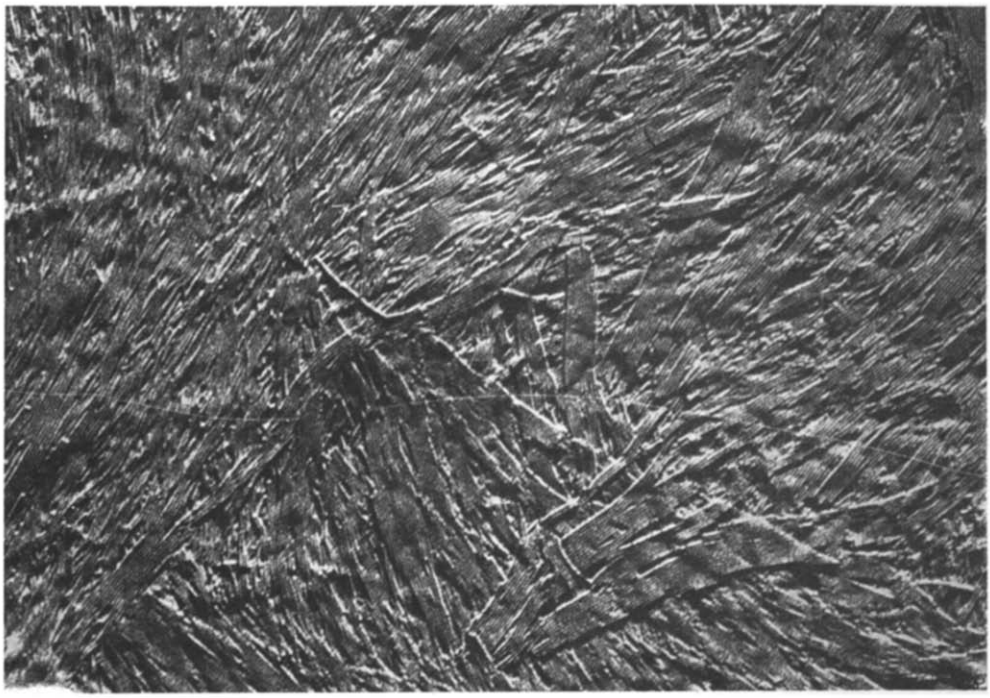

Figure 1. Electron micrograph of a gold shadowed replica of a moderately concentrated solution of the tobacco mosaic virus protein dried on glass. Magnification 23,000x.

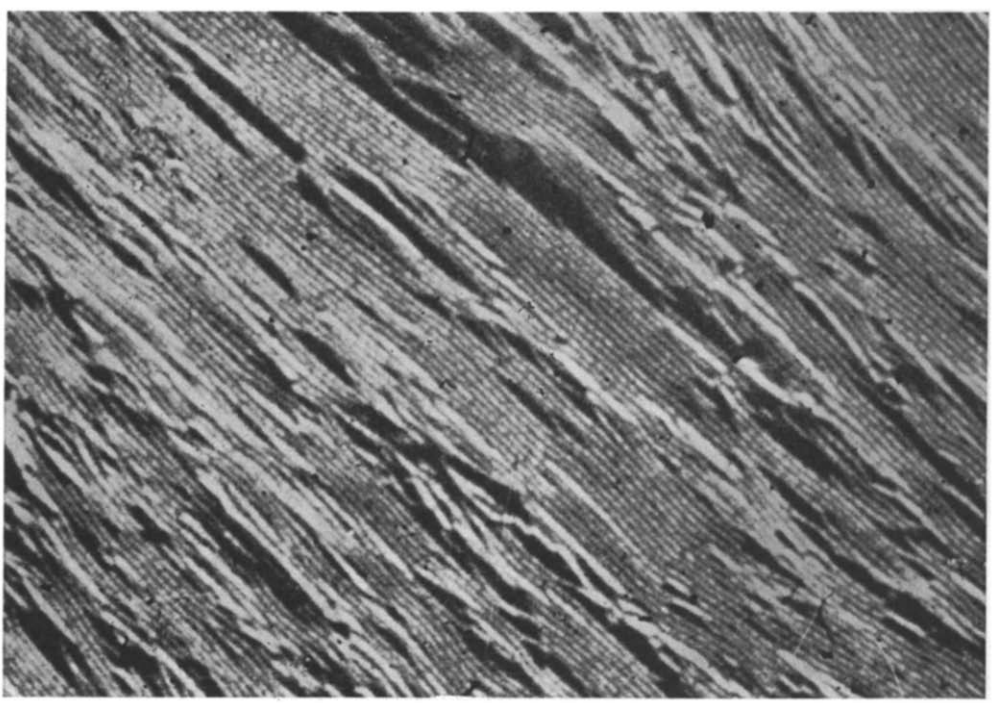

Figure 2. Micrograph of a replica of a region showing tobacco mosaic rods in parallel alignment. Magnification 50,000x.

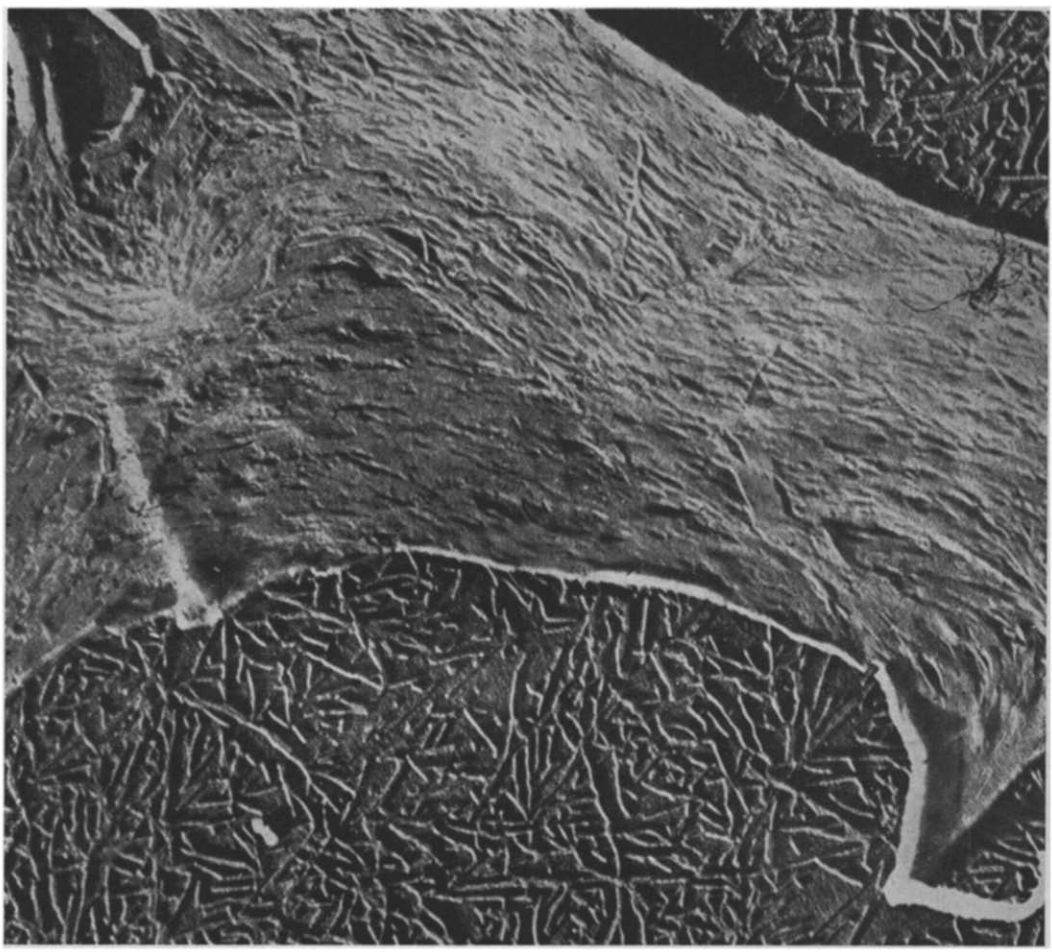

Figure 3. Micrograph of a shadowed frozen-dried solution showing an extended sheet of tobacco mosaic protein. Magnification 23,000x.

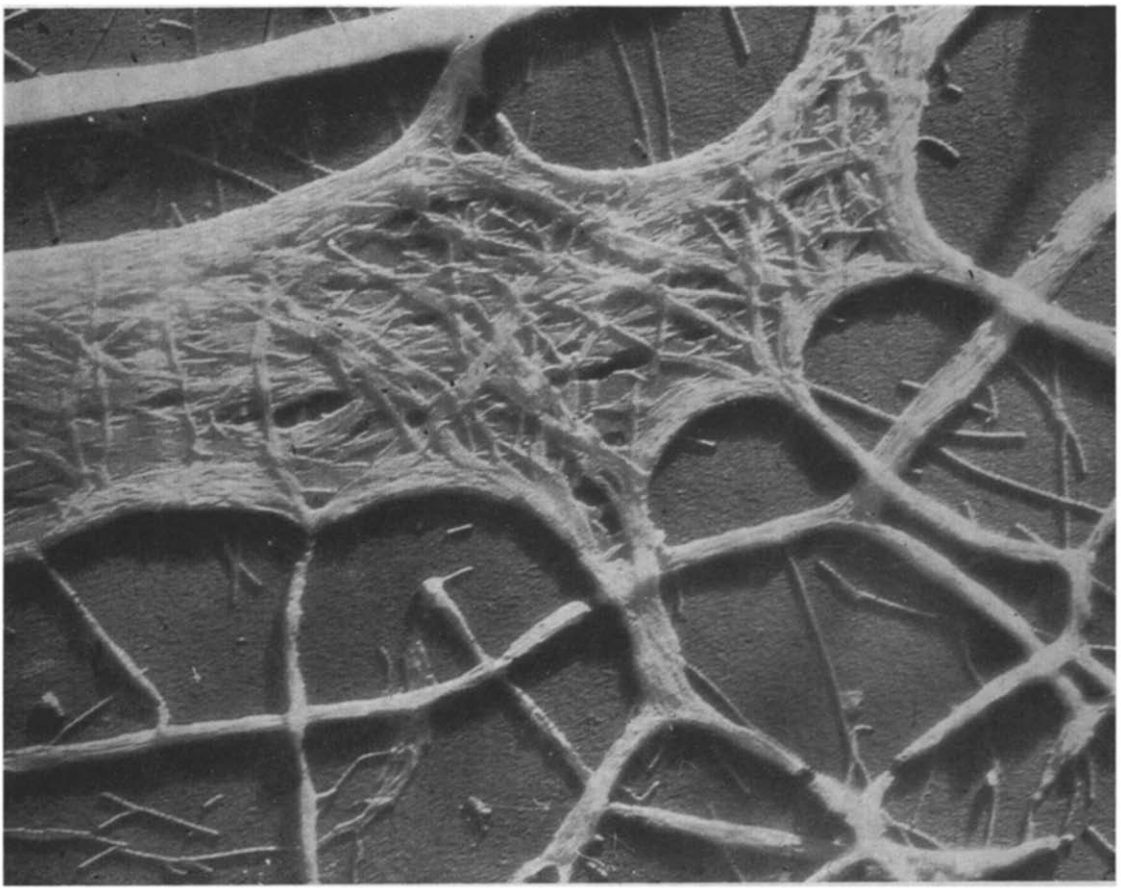

Figure 4. Micrograph of a frozen-dried tobacco mosaic preparation containing a network of thick bundles of particles. The formation of these bundles by the curling up of fragments of a sheet can be watched under the microscope. The fact that some bundles overlie others is evidence that the sheets from which they were derived were at different levels. Magnification 23,000x.

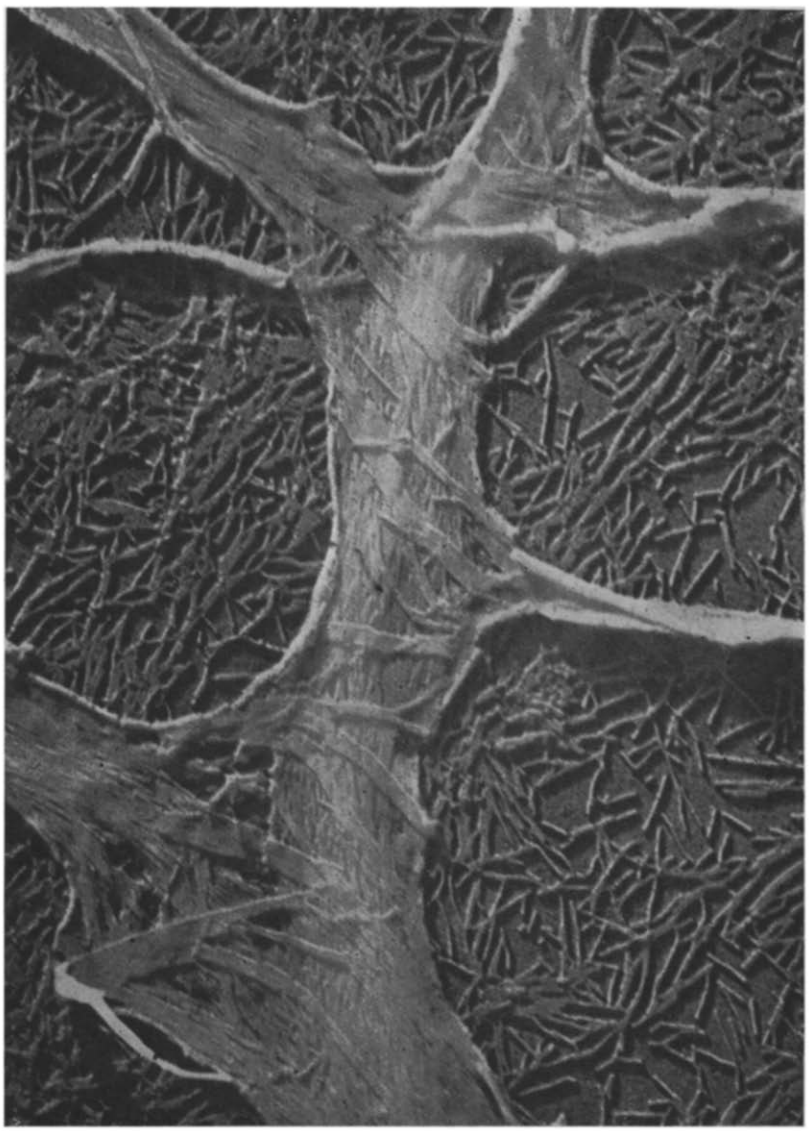

Figure 5. Micrograph of part of a sheet of tobacco mosaic protein having the apparent form of a negative tactoid. Tobacco mosaic rods on the collodion substrate are more numerous in this than in the two preceding photographs. Magnification 23,000x.