CELL PERMEABILITY AND DECARBOXYLATION OF α-KETO ACIDS BY INTACT YEAST*

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INTRODUCTION

The effect of cell permeability on the metabolic functions of microorganisms has been repeatedly discussed in the literature, but little direct evidence has been presented. As a barrier inhibiting contact with the enzyme systems inside the cell, however, the cell membrane may exert an extremely important effect on the relations to substrates and inhibitors[1–4]. On the basis of the limited number of observations in this field it can be concluded that penetration of substances through the cell membrane of a microbe follows the general rules for cell permeability[5]. Thus, it has been found that the lower normal fatty acids penetrate into the yeast cell by virtue of their fat solubility: the longer the carbon chain, the faster the fatty acids penetrate into the yeast cell[6,7]. Earlier investigations have further revealed that the fatty acids penetrate the yeast cell readily as compared with the di- and tricarboxylic acids[4,7–9]. The significance of the permeability of the cell membrane as a factor influencing the metabolism of the keto acids by yeast cells was first indicated in HÄGGLUND's investigation on pyruvic acid[10], and confirmed in later work[11–13]. The results corroborate the view of COLLANDER[14] that only an undissociated acid molecule can penetrate without difficulty into the intact cell, and be metabolized.

The power of yeast carboxylase to split different α-keto acids has been made familiar by the investigations of NEUBERG and co-workers (*cf.* [15]). Later investigations have shown that the power of yeast carboxylase to decarboxylate straight-chain α-keto acids decreases as the carbon chain increases in length, up to α-ketocaproic acid[16–18]. Dry yeast or disintegrated yeast in which the cell membrane is damaged should function as a decarboxylating agent like a raw carboxylase preparation, *i.e.* it should decarboxylate long-chain α-keto acids more slowly than short-chain ones. According to prevailing opinion, the carboxylase enzyme in intact yeast is located inside the cell membrane. The keto acids have to pass the membrane barrier, which they should penetrate according to their lipoid solubility as do the fatty acids. The longer the carbon chain, the faster should be the penetration. Since the barrier constituted by the cell membrane is presumably greatest for short-chain acid molecules, it seems logical to assume that the short-chain α-keto acids are decarboxylated by intact yeast less rapidly than would be expected from the enzymic decarboxylating order.

* A preliminary report of the results has been presented at the meeting of the Scandinavian biochemists in Copenhagen, June 3rd, 1957[22].

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EXPERIMENTAL

Material

The baker's yeast used was a commercial brand produced by the Rajamäki Factories of the State Alcohol Monopoly, Rajamäki. The brewer's yeast originated from the brewery of Oy Mallasjuoma, Lahti. The commercial baker's yeast obtained from the factory was used as such, while the yeast-cream samples of brewer's yeast were washed and sucked on a Büchner funnel until they contained about 25% dry matter. All experimental results are expressed on a dry weight basis.

The dry yeast preparations were made by pressing fresh yeast through a narrow-meshed wire net (8 meshes per cm) and collecting the thin threads on filter paper. The yeast was then air-dried at room temperature. The acetone-dried yeasts and the Lebedew extract of baker's yeast were prepared in the usual manner (*cf.* [18]). The disintegrated yeast was prepared by freezing fresh yeast in a test tube submerged in a mixture of dry ice and ethanol and thawing the frozen yeast in a lukewarm water-bath. The treatment was repeated 3 to 4 times.

Pyruvic acid (Merck) was purified before use by vacuum distillation. The other keto acids were of Fluka's pure grade, and were used without further purification. The sodium salts of the keto acids were prepared by adding sufficient NaOH solution to neutralize a weighed amount of the keto acid. The sodium pyruvate used was, however, obtained from Merck. All other chemicals used were of pure or C.P. grade.

Experimental procedure

The decarboxylating power of the yeasts and of the yeast preparations was determined in a Warburg apparatus by measuring the amount of CO_2 split from the keto acids per unit time. The details of the procedure are as follows: The decarboxylating power in an acid medium was measured by pipetting the yeast suspension, 0.1 ml of a 0.2 *N* succinic acid solution, and a sufficient quantity of water into the main chamber of the Warburg flask. 0.5 ml of a generally 0.2 *N* keto acid solution was added to the side arm. The final volume was 2.5 ml. The decarboxylating power of the yeast and of the yeast preparations in a more neutral medium was measured by replacing the keto acid by its sodium salt, the succinic acid being replaced by 1.0 ml of a 0.1 *M* citrate–phosphate McIlvaine buffer, pH 5.8. This pH value equals the intracellular acidity of the yeast cell[19] and is near the optimum of the yeast carboxylase[10,20,21]. The air was removed from the flasks by nitrogen ventilation for 15 to 20 min. The measurements were made at a temperature of 30°, and the first reading was taken 3 min after addition of the substrate. The results are generally expressed as μl CO_2/3 min/40 mg yeast (dry weight).

The velocity of penetration of various keto acids into the yeast cell was measured by adding 0.3 *N* keto acid solution to a heavy yeast suspension and measuring the change of pH reflecting the penetration of the keto acid into the cell as a function of time during the first 60 sec after addition of the acid[6].

RESULTS AND DISCUSSION

In order to confirm earlier observations, the decarboxylating power of intact commercial baker's yeast and of intact brewer's yeast was compared with that of different yeast preparations in which the permeation barrier of the cell membrane had been destroyed either by drying or by dissolving with lipoid solvents or by freeze-thawing. The comparative tests were performed at pH 2, obtained by addition of succinic acid, and at pH 5.8, obtained with McIlvaine's citrate-phosphate buffer. The results are given in Table I.

The results obtained with fresh yeast confirm earlier observations that the pyruvate ion scarcely penetrates at all into intact baker's yeast cells and only slowly into brewer's yeast cells[4,10]. When yeast preparations were used, however, the pyruvate ion made contact with the carboxylase. The increased permeability is shown by the decrease in the decarboxylating power at the strongly acid pH 2.0. The results obtained with dry baker's yeast seem to indicate that its plasma membrane still functions as a partial barrier (*cf.* NILSSON's "intakte Trockenhefe"[18]).

In order to compare the speed of decarboxylation of various keto acids with yeast preparations, the saturation point of carboxylase with different substrates had

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TABLE I

THE DECARBOXYLATION OF PYRUVIC ACID BY INTACT BAKER'S YEAST, INTACT BREWER'S YEAST AND BY VARIOUS YEAST PREPARATIONS AT pH 2.0 AND pH 5.8

The amount of pyruvic acid used was sufficient almost to saturate the carboxylase in the yeast samples studied. The results are expressed as μl CO_2/3 min/40 mg yeast (dry weight).

Substrate	*Baker's yeast, µl CO_2*				*Brewer's yeast, µl CO_2*			
	Intact	*Dry*	*Acetone-dried*	*Disintegrated*	*Intact*	*Dry*	*Acetone-dried*	*Disintegrated*
Pyruvic acid, pH 2.0	148	53	12	6	212	19	—	0
Pyruvic acid, pH 5.8	4	50	36	52	32	660	365	720

to be estimated. The experiments were performed with four keto acids in a citrate-phosphate buffered medium at pH 5.8, using dry baker's or brewer's yeast as the decarboxylating agent. The results obtained with brewer's yeast are seen in Fig. 1; the results using baker's yeast were similar.

It is interesting to note the more intense decarboxylation of α-ketobutyric acid at concentrations below 0.01 *N* as compared with that of pyruvic acid.

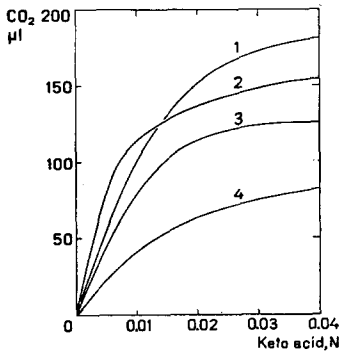

Fig. 1. The decarboxylation of various α-keto acids by dry brewer's yeast at pH 5.8, as a function of the concentration of the keto acid. The results are expressed as μl CO_2/3 min/10 mg yeast (dry weight). 1. Pyruvic acid, 2. α-ketobutyric acid, 3. α-ketovaleric acid, 4. α-ketocaproic acid.

Estimation of the decarboxylation velocity of various α-keto acids with baker's and brewer's yeast preparations in a citrate–phosphate buffered medium of pH 5.8, gave the results seen in Table II.

TABLE II

THE DECARBOXYLATION OF VARIOUS α-KETO ACIDS BY PREPARATIONS OF BAKER'S AND BREWER'S YEAST AT pH 5.8

The keto acid concentration was 0.04 *N*, which was sufficient almost to saturate the carboxylase in the samples. The results are expressed as μl CO_2/3 min/40 mg yeast (dry weight).

Substrate	*Baker's yeast, µl CO_2*			*Brewer's yeast, µl CO_2*	
	Dry	*Acetone-dried*	*Disintegrated*	*Dry*	*Disintegrated*
Pyruvic acid	72	29	75	768	772
α-Ketobutyric acid	66	35	89	575	640
α-Ketovaleric acid	49	27	58	488	506
α-Ketocaproic acid	49	23	52	232	302
α-Ketooenanthic acid	32	—	12	132	190
α-Ketocaprylic acid	16	5	18	112	168
α-Ketoisovaleric acid	11	11	20	203	280

For baker's yeast, the values obtained in the time interval from 3 to 6 min are given. If, as for brewer's yeast, the time interval between 0 and 3 min is taken as a basis, the corresponding readings for baker's yeast would be: 69, 33, 144, 98, 103, 125 and 56 μl CO_2/3 min/40 mg yeast (dry weight). On the other hand, in the time interval from 6 to 9 min the trend is the same as between 3 and 6 min, giving the readings: 75, 64, 51, 48, 34, 13, 15.

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The formation of carbon dioxide took place at a steady rate when brewer's yeast preparations were used. The baker's yeast preparations, however, showed widely varying results at 3 min intervals, at 0–3 min, at 3–6 min and at 6–9 min, as seen in Table II and as shown in the renewed series of similar tests. The Lebedew extract prepared from baker's yeast gave similarly varying results, although the activity of this preparation was rather low. In general, we can say that the results with the carboxylase incorporated in brewer's yeast fully confirm the earlier reports on the decrease of the decarboxylating power of yeast carboxylase as the straight carbon chain of the α-keto acid becomes longer[15–17]. In the case of baker's yeast, the results obtained correspond to the earlier reports, provided the experimental sources of error are taken into consideration. The branched α-ketoisovaleric acid, on the other hand, was decarboxylated less rapidly by brewer's yeast preparations than was the α-ketocaproic acid, although the latter exceeds the former by two atoms in the carbon chain. The decarboxylation of α-ketoisovaleric acid by baker's yeast preparations was perhaps still slower.

When the decarboxylating power of fresh intact yeasts was studied, the data obtained on the speed of decarboxylation of various α-keto acids at first seemed to be very unexpected, but when the speed was estimated as a function of the concentration of the keto acid the results became clearly understandable. As the pyruvate ion does not penetrate into the intact yeast cell under anaerobic conditions (*cf.*[11], and Table I), the determinations were performed in a solution acidified with succinic acid, the pH of the solution being 2.0 after addition of the keto acid. Figs. 2 and 3 show the results obtained in two test series using intact commercial baker's and brewer's yeast.

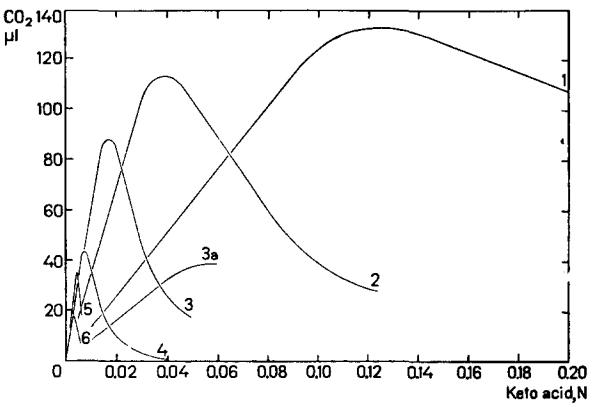

Fig. 2. The decarboxylation of various α-keto acids by intact baker's yeast at pH 2.0, as a function of the keto acid concentration. The results are expressed as μl CO_2/3 min/30 mg yeast (dry weight). 1. Pyruvic acid, 2. α-ketobutyric acid, 3. α-ketovaleric acid, 3a. α-ketoisovaleric acid, 4. α-ketocaproic acid, 5. α-ketooenanthic acid, 6. α-ketocaprylic acid.

The results using intact yeasts differ completely from those obtained with the dried yeast preparations (*cf.* Fig. 1), being opposite with respect to the decarboxylation order at weak concentrations of the keto acids. The ability of intact baker's and brewer's yeast to decarboxylate various α-keto acids at low concentrations is directly proportional to the length of the carbon chain of the acid (Table III). The only branched α-keto acid tested, α-ketoisovaleric acid, was decarboxylated more slowly than α-ketobutyric acid with the same carbon-chain length. There exists a maximum activity of decarboxylation at a certain concentration for all the keto acids tested; with increasing concentration the decarboxylation activity decreases

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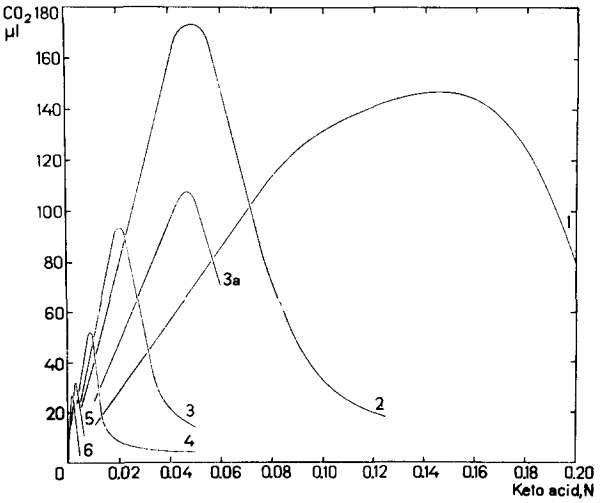

Fig. 3. The decarboxylation of various α-keto acids by intact brewer's yeast at pH 2.0, as a function of the keto acid concentration. The results are expressed as µl CO_2/3 min/20 mg yeast (dry weight). 1. Pyruvic acid, 2. α-ketobutyric acid, 3. α-ketovaleric acid, 3a. α-ketoisovaleric acid, 4. α-ketocaproic acid, 5. α-ketooenanthic acid, 6. α-ketocaprylic acid.

sharply. The longer the carbon chain of the keto acid, the lower the substrate concentration at which the point of maximum activity is reached.

The rapid decrease of the decarboxylating power of intact yeast occurring with increasing substrate concentration cannot be regarded as being due to inhibition by the aldehyde formed as a result of the decarboxylation process, especially in the case of the long-chained keto acids. This becomes clear from the following. When pyruvic acid is decarboxylated, the acetaldehyde concentration during the above experimental conditions can increase up to at most 0.016%. According to WETZEL[23] (*cf.* also [20,21]), an acetaldehyde concentration of this strength acts by depressing somewhat the decarboxylation of pyruvic acid: the carbon dioxide formed is only 90% of that formed in an acetaldehyde-free solution. Of the higher aldehydes, WETZEL mentions only butyraldehyde, which, however, has a less depressing effect than acetaldehyde.

TABLE III

THE DECARBOXYLATION OF THE VARIOUS α-KETO ACIDS BY INTACT BAKER'S AND BREWER'S YEAST AT pH 2.0 IN TWO DIFFERENT SUBSTRATE CONCENTRATIONS

The results indicating inactivation are in parentheses. The results are expressed as µl CO_2/3 min/40 mg yeast (dry weight).

Substrate	*Baker's yeast, µl CO_2*		*Brewer's yeast, µl CO_2*	
	concentration of keto acid		*concentration of keto acid*	
	0.003 N	*0.0075 N*	*0.0015 N*	*0.0075 N*
Pyruvic acid	5	13	4	22
α-Ketobutyric acid	13	35	14	62
α-Ketovaleric acid	24	61	16	76
α-Ketocaproic acid	27	57	22	102
α-Ketooenanthic acid	33	(17)	44	(14)
α-Ketocaprylic acid	(23)	(4)	54	(0)
α-Ketoisovaleric acid	3	8	7	36

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Hence, there is no reason to believe that the higher aldehydes have any marked inhibitory effect on the decarboxylation of the keto acids.

Nevertheless, the possible effect of aldehyde was studied. When dimedon was added to the reacting solution to bind the aldehyde (*cf.* [24]) no change in the course of the decarboxylation of pyruvic acid and α-ketovaleric acid was observed. It was further found that the decarboxylation of α-ketovaleric acid was the same in a solution containing 0.05% valeraldehyde as in aldehyde-free solution. An inhibitory effect, of 25% after 3 min and of 50% after 9 min, was first observed at a valeraldehyde concentration of 0.15%. When carbon dioxide formation was followed over a longer period of time with different α-keto acids as substrates and with baker's yeast as the decarboxylating agent, it was found (Table IV) that at least during the first 9 min no depression in the rate of evolution occurred. Hence, it is clear that the aldehyde formed during this time did not depress the decarboxylation of the α-keto acids.

TABLE IV

THE DECARBOXYLATION OF α-KETO ACIDS BY INTACT BAKER'S YEAST IN A MEDIUM ACIDIFIED WITH SUCCINIC ACID TO pH 2.0, RECORDED AT INTERVALS OF 3 min

The results are expressed as $\mu l\ CO_2$/3 min/40 mg yeast (dry weight).

Recording time min	*Pyruvic acid 0.02 N* $\mu l\ CO_2$	*α-Ketobutyric acid 0.02 N* $\mu l\ CO_2$	*α-Ketovaleric acid 0.02 N* $\mu l\ CO_2$	*α-Ketocaproic acid 0.0075 N* $\mu l\ CO_2$
0–3	34.0	85.0	49.0	7.5
3–6	33.2	98.0	49.0	6.8
6–9	31.5	102.0	54.8	7.1
9–12	28.0	102.0	32.2	6.2
12–15	28.5	88.4	17.5	5.2
15–18	24.2	81.8	15.4	5.1
18–21	23.2	74.5	14.0	5.1

When, on the other hand, a lower fatty acid such as caproic acid was added to the reaction solution in sufficient amounts, a distinct decrease was observed in the decarboxylation velocity of the keto acid. Small amounts of caproic acid do, it is true, have an activating effect on the decarboxylation of pyruvic acid, but larger amounts depress it. This depression is similar to the inhibition appearing in the decarboxylation of α-ketocaproic acid as a function of substrate concentration (Fig. 4).

The observations on the decarboxylation of α-keto acids by intact yeasts are easily explained on the assumption presented as the working hypothesis, that the penetration of various α-keto acids into the yeast cell is a function of the length of the carbon chain of the acid. The ascending portions of the curves in Figs. 2 and 3, representing the speed of decarboxylation as a function of the keto acid concentration, illustrate the situation when the penetration of the acid acts as a factor limiting its decarboxylation. The increase in the keto acid concentration in the solution surrounding the cell will result in an increase in the amount penetrating into the cell per unit time, and it will also cause an increase in the decarboxylation proportional to the permeability. The long-chain keto acid molecules penetrate the cell membrane more readily than do the short-chain ones, and accordingly the ascending portion of the curve will be steeper with the long-chain acids than with the short-chain ones.

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As the keto acid concentration is further increased, a situation will be reached when the breakdown of the acid by decarboxylation equals its penetration speed. If the concentration is further increased, the speed of penetration will exceed that of breakdown. As a result of this, the acid will start to accumulate in the cell, causing a decrease in the decarboxylating power by a lowering of the pH or by inhibition.

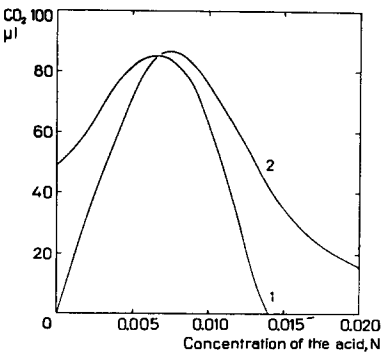

Fig. 4. The power of intact baker's yeast (1) to decarboxylate pyruvic acid in solutions containing various concentrations of caproic acid, and (2) to decarboxylate α-ketocaproic acid as a function of its concentration. The results are expressed (1) as µl CO_2/3 min/120 mg fresh yeast and (2) as µl CO_2/3 min/240 mg fresh yeast.

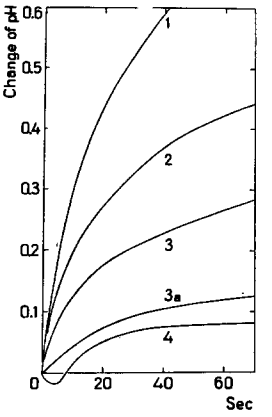

Fig. 5. The order of penetration of α-keto acids into intact baker's yeast cells. The change of pH measured potentiometrically in a heavy baker's yeast suspension after addition of various keto acids, compared with the change produced by pyruvic acid. 1. α-ketocaprylic acid, 2. α-ketocaproic acid, 3. α-ketovaleric acid, 3a. α-ketoisovaleric acid, 4. α-ketobutyric acid.

In the curves representing the decarboxylation of different α-keto acids by fresh yeasts, the keto acid concentration required to achieve the maximum activity is considerably lower with long-chain than with short-chain acids. This is obviously the concentration needed completely to saturate the carboxylase inside the yeast cell.

It was finally possible by direct potentiometric measurement of the pH change in a heavy baker's yeast suspension, reflecting the keto acid penetration, to demonstrate that the keto acids actually do penetrate into the intact yeast cell at different speeds, and that the order of penetration corresponds to the speed of their decarboxylation by intact yeasts. Fig. 5 depicts the relative penetration velocities of different keto acids when the penetration of pyruvic acid is taken as a basis. It can be observed that the penetration is proportional to the length of the carbon chain and that the cell membrane offers the greatest barrier to the short-chain pyruvic acid.

SUMMARY

Straight-chain α-keto acids containing up to at least eight carbon atoms are decarboxylated by baker's and brewer's yeast preparations in which the cell membrane has been destroyed by various methods. The decarboxylation order is similar to that occurring when yeast carboxylase is used, the velocity being the slower, the longer the carbon chain of the keto acid.

Straight-chain α-keto acids containing up to eight carbon atoms are decarboxylated at low substrate concentrations by intact baker's or brewer's yeast, the speed of the process increasing with the chain length of the acid. With all the keto acids investigated, a maximum decarboxylating activity occurred at a certain concentration. When the substrate concentration exceeded this

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level, the decarboxylation showed a sharp decrease. The maximum activity was reached at a weaker concentration with long-chain acids. The depression of decarboxylation at concentrations exceeding the maximum is not due to aldehyde formation, but to penetration of the keto acid at a rate exceeding the decarboxylation velocity, and its consequent accumulation in the yeast cell.

The reverse behaviour of straight-chain α-keto acids with yeast preparations and with intact yeast can only be explained by assuming that the cell membrane of intact yeast cells offers a barrier particularly to the penetration of short-chain keto acids. This assumption has been confirmed experimentally by potentiometric measurement of the penetration speeds.

The only branched α-keto acid investigated, α-ketoisovaleric acid, was decarboxylated slower by yeast preparations than were the next straight-chain keto acids. With intact yeast, α-ketoisovaleric acid was decarboxylated less rapidly than α-ketobutyric acid.

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GLUCOSONE

II. INHIBITION OF YEAST METABOLISM, YEAST HEXOKINASE ACTIVITY AND TISSUE GLYCOLYSIS*

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D-Glucosone has been reported[1,2,3] to inhibit the anaerobic fermentation of glucose by baker's yeast, but the reports were not in agreement as to the amount of glucosone required to produce inhibition. There was also lack of agreement as to whether glucosone does or does not inhibit respiration of yeast cells[2,3]. These variances,

* Taken in part from a thesis submitted by MARIE T. HUDSON to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science, June 1956.

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