ON THE SYNTHESIS AND DECOMPOSITION OF ANEURIN-PYROPHOSPHATE BY LIVING YEAST

by

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I. INTRODUCTION

Yeast is probably the microorganism with the most intensively studied metabolism. Its aneurin content has also been the subject of a great number of investigations, especially after it had been shown that the pyrophosphoric acid ester of this vitamin is identical with cocarboxylase, the coenzyme of the enzyme carboxylase. This enzyme catalyses one of the intermediate stages of alcoholic fermentation, the decarboxylation of pyruvic acid. By far the greatest part of the total amount of aneurin is present in the form of aneurinpyrophosphate [1], which we shall further abridge to APP in this paper. Now it has been shown [2, 3, 4] that the APP content of yeast can be very considerably increased by incubating the yeast with large amounts of aneurin. Apart from its practical importance regarding the significance of yeast as a foodstuff, this observation was very interesting from a theoretical point of view. For it seemed possible that this augmentation of the content of a coenzyme might influence the whole metabolism of the yeast.

Shortly after we had started an investigation on this subject a very interesting note by SPERBER appeared [3], in which he utilized some observations on dried yeast, made by two of the present authors together with VAN DORP and GRUBER [5], to arrive at certain conclusions concerning his studies on living yeast. SPERBER's note prompted us to publish a preliminary communication [6] in which his main conclusion, viz. the identity of the enzyme responsible for APP synthesis and the yeast phosphatase, studied by us, was refuted.

In our opinion the main result of our work is the demonstration that both processes, the decomposition of added APP by the yeast phosphatase and the synthesis of APP by the living yeast cells are not merely the reverse of one another. It is not free APP that is synthesized, but probably an APP-protein symplex, not identical with the catalytically active APP-protein symplex known as carboxylase.

We could further determine the conditions under which a certain amount of added aneurin is completely converted into APP by the yeast. And as APP can be determined by a manometric method, based upon its function as the coenzyme of the enzyme by which pyruvic acid is split into acetaldehyde and CO_2, we have also succeeded in determining aneurin by a manometric technique. We believe our method to be more sensitive than OCHOA and PETERS' manometric determination of aneurin, based on its inhibition of the decomposition of APP by yeast phosphatase [7, 8].

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In this paper we shall commence by treating the extent of synthesis of APP from aneurin, as influenced by various factors as time, temperature, p_H and respiration substrates. This will be followed by some considerations on the manometric determination of aneurin and by chapters dealing with the condition of the formed APP and the reaction mechanism of its formation.

2. THE SYNTHESIS OF ANEURINPYROPHOSPHATE FROM ANEURIN

a. Methodical.

Previous to discussing various examples of APP synthesis the general method followed in these investigations may be given.

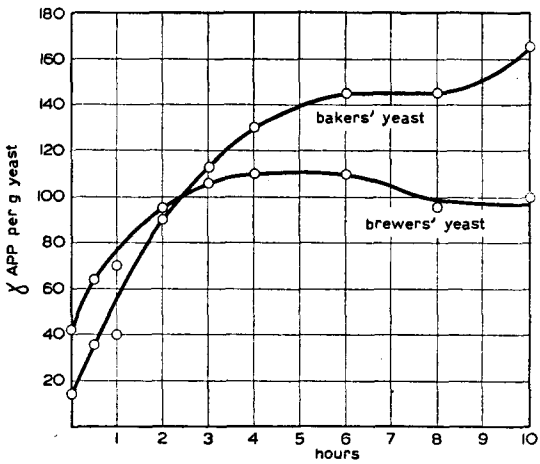

Fig. 1. Synthesis of aneurinpyrophosphate from aneurin by bakers' yeast and brewers' yeast at p_H 3.8 and 27.5° C.

The fresh, pressed yeast was suspended in 0.1 mol succinate buffer or 0.1 mol phosphate-citrate buffer. The desired amount of aneurin was added and the mixture was shaken in a waterbath of constant temperature. The total volume was 10 ml per g of yeast. The p_H was generally 3.7, the optimal p_H for the reaction velocity according to SPERBER, except when our object demanded another p_H-value.

At various times 1 or 2 ml samples were drawn off and brought into 5 or 10 ml boiling 0.05 N HCl, containing about 50 times as much aneurin as the amount of APP expected to be present in the sample. The mixture was brought to the boil again as rapidly as possible and kept boiling for 1 minute. After cooling the p_H

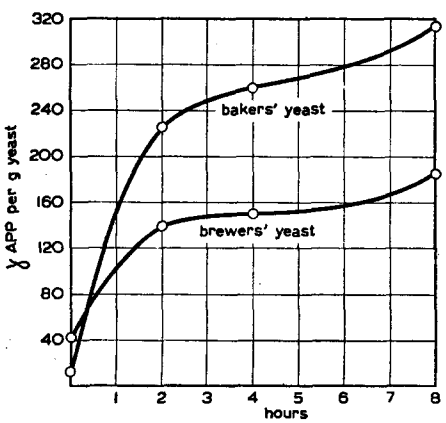

Fig. 2. Synthesis of aneurinpyrophosphate from aneurin by bakers' yeast and brewers' yeast in the presence of 5% glucose at p_H 3.8 and 27.5° C.

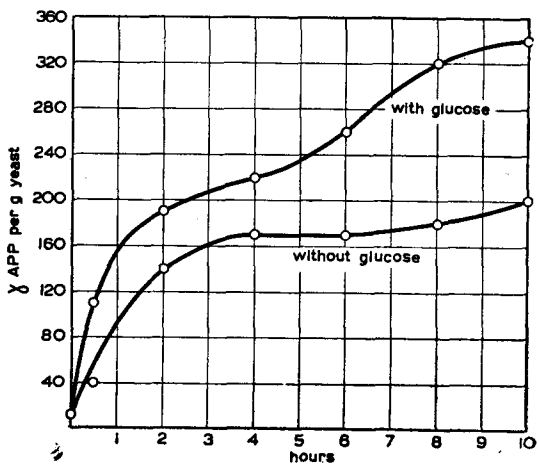

Fig. 3. Influence of 5% glucose on the synthesis of aneurinpyrophosphate from aneurin by bakers' yeast at p_H 3.8 and 27.5° C.

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was adjusted to 6.2, whereupon so much of a 0.1 mol phosphate buffer, p_H 6.2, was added that the APP concentration was suitable for manometric determination in the WARBURG apparatus. For particulars regarding this determination we refer to previous papers [1].

When the yeast is boiled with 0.05 N HCl the presence of excess aneurin is necessary to inhibit the yeast phosphatase, which might partly decompose the APP in the short time between the killing of the yeast and the inactivation of the phosphatase by the heat [5].

b. Form of the time-synthesis curves. Bakers' and brewers' yeast.

Fig. 1 to 3 serve to illustrate our numerous preliminary synthesizing experiments. They all give the amount of APP formed as a function of the time. The curves of each separate figure represent the results of experiments carried out simultaneously. Though these curves could not be reproduced with great accuracy, some conclusions could be drawn from them, which formed the foundation of further work. In all these experiments the p_H was 3.7. They were not continued during more than 10 hours.

All curves are of the same type: after a rapid rise they run nearly horizontally, roughly between 4 and 8 hours' incubation time, again followed by a slow rise. We do not know the meaning of this peculiar shape.

Experiments carried out simultaneously with bakers' yeast und brewers' yeast, of which Fig. 1 and 2 show examples, have proved that more APP is synthesized by bakers' yeast than by brewers' yeast. Moreover bakers' yeast is more adequate for these experiments because the brewers' yeast is more easily killed, causing the APP to be lost again (the APP formed is stored in the cells and does not accumulate in the medium *). Therefore nearly all other experiments were carried out with bakers' yeast.

c. Temperature.

As for the influence of the temperature on the synthesis of APP, we have received the impression from several experiments that the velocity of the synthesis as well as the amount of APP formed increases with increasing temperature from 27.5° to 42° C. But these results were not always reproducible. This is probably due to different sensitivities of various samples of yeast to higher temperatures. Indeed in cases where no synthesis or a gradual decrease of the amount of formed APP could be observed, the growth test showed that all yeast cells had been killed by their failure to propagate themselves.

Brewers' yeast is much more sensitive to higher temperatures than bakers' yeast; at temperatures where the latter gives a large synthesis no APP is formed by the former.

The fact that the formed APP as well as that originally present disappears when the yeast cells die off indicates that only living cells are capable of synthesis and that the formed APP is only stable inside the living cells.

For practical use we concluded that when yeasts with a high APP content are to be prepared the temperature should not exceed 27.5°, as the increase of APP at higher temperature is not so large as to outweigh the risk of the cells dying off.

d. p_H; respiration substrates.

SPERBER and RENVALL have shown 1st that the maximal velocity of APP synthesis is found at p_H 3.7 and 2nd that this velocity is greatly increased by adding respiration substrates as glucose or alcohol to the yeast suspension.

*) See preliminary commanication [6], experiment 4.

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We wished to know whether these factors also influence the maximal amount of APP the yeast is capable of synthesizing. For glucose, Fig. 3 and Fig. 2, as compared to Fig. 1, show that the amount of APP formed from 1 mg aneurin by 1 g yeast is considerably increased when the medium contains 5% glucose. The same appears from Fig. 4, giving the p_H-synthesis curves after 7 hours' incubation time, in the presence and in the absence of 5% glucose.

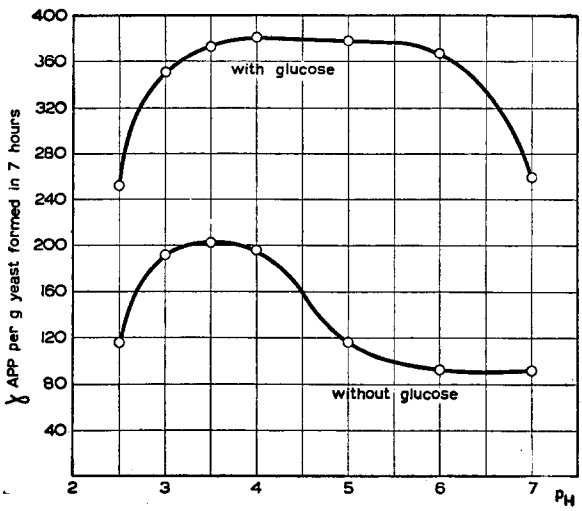

Fig. 4. pH-activity curves for the synthesis of aneurinpyrophosphate from aneurin in the absence and in the presence of 5% glucose at 27.5° C.

The time-synthesis curves show that synthesis is not quite complete after 7 hours, but as smaller experiments have later demonstrated that the general shape fo the p_H-synthesis curves, constructed after 7 hours, is really the same as that of the curves for maximal synthesis, we feel justified in employing the shorter reaction time prompted by practical considerations (see also Table II).

In the absence of glucose the optimum for synthesis is situated at about the same p_H at which SPERBER and RENVALL found the optimum of the reaction velocity. In the presence of glucose the synthesis is much higher at all p_H's and then the curve is practically horizontal from p_H 3 to p_H 6. Hence glucose has a much greater influence at p_H 6 than at p_H 3.7. As may be seen from Fig. 4, at p_H 3.7 the amount of APP formed in the presence of glucose is twice as large as the amount formed in the absence of glucose, while at p_H 6.0 the ratio of these amounts is 4.

The following experiments confirm these interesting results:

1. 4 samples of 1 g of yeast in 10 ml phosphate-citrate buffer were incubated with 1 mg aneurin for 6 hours at 27.5° C, at p_H 3.7 and p_H 6.0, in the absence and in the presence of glucose. Particulars and results are to be found in Table I. The ratios c : a = 2 and d : b = 4 (see last column of Table I) fully confirm our conclusions regarding the ratios of APP formed in the absence and the presence of glucose, drawn from Fig. 6.

TABLE I

INFLUENCE OF 5% GLUCOSE ON THE SYNTHESIS OF APP IN 7 HOURS AT p_H 3.8 AND 6.0

γ APP formed per g yeast				ratios
without glucose		with glucose		
a p_H 3.8	b p_H 6.0	c p_H 3.8	d p_H 6.0	
160	80	320	360	c : a = 2 d : b = 4

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2. 4 samples of 1 g yeast were incubated under the same conditions as above for 24 hours. 2 ml samples were drawn off with intervals of 8 hours. Particulars and results are given by Table II.

TABLE II

INFLUENCE OF REACTION TIME AND OF GLUCOSE ON APP SYNTHESIS AT p_H 3.8 AND 6.0

reaction time in hours	γ APP formed per g yeast				ratios
	without glucose		with glucose		
	a p_H 3.8	b p_H 6.0	c p_H 3.8	d p_H 6.0	
8	156	78	315	285	c : a = 2; d : b = 4
16	188	90	345	383	c : a = 2; d : b = 4
24	188	100	345	383	c : a = 2; d · b = 4

The last experiment shows 1st that from 8 to 16 hours the increase of APP is only small as compared to the increase in the first 8 hours, 2nd that in 16 hours the synthesis is complete, 3rd that there is no essential difference in the influence of the p_H on the amount of APP formed whether determined after 8 hours or 16 hours, and 4th that also in case of complete synthesis the ratio of the amounts of APP formed in the presence and in the absence of glucose is 2 at p_H 3.7 and 4 at p_H 6.0.

Hence we can definitely conclude that the shape of the curves in figure 4 is really the shape of the curves, indicating the amount of APP which the yeast is capable of synthesizing as a function of the p_H, in the presence and in the absence of glucose.

e. Amount of aneurin.

We have next envisaged the question of the quantity of APP synthesized as a function of the amount of aneurin incubated with a certain amount of yeast. We have seen above that the yeast can store enormous quantities of aneurin in the form of APP and this fact logically raised the question whether the yeast is capable of completely binding certain quantities of added aneurin. If this were the case, a method for the determination of aneurin might be founded upon this property.

We have carried out such experiments at p_H 3.7 with incubation times of $\frac{1}{2}$ hour and 6 hours. Fig. 5a and 6a and 5b and 6b give the results. The reasons for working with the amounts of yeast and aneurin indicated in the diagrams will be discussed below. We first want to call attention to the shape of the curves of Fig. 5a and 6a. The curves for $\frac{1}{2}$ hour's incubation time show that the amount of APP formed only increases with increasing amounts of aneurin for the smallest aneurin concentrations. When either 200 or 300 mg yeast are employed, no increase of APP is found for amounts of aneurin from 20 γ upwards. Regarding the curves for 6 hours' incubation time we see that the increase of APP is extended to higher aneurin concentrations, the curve only running horizontally at an aneurin level of 100 to 200 γ.

Fig. 5b and 6b show the percentages of the amounts of aneurin added to the yeast bound in the form of APP. These figures as well as the two preceding ones indicate that the larger the amount of aneurin, the smaller the percentage that is bound, whether the reaction time is short or long. For small amounts of aneurin the percentage that

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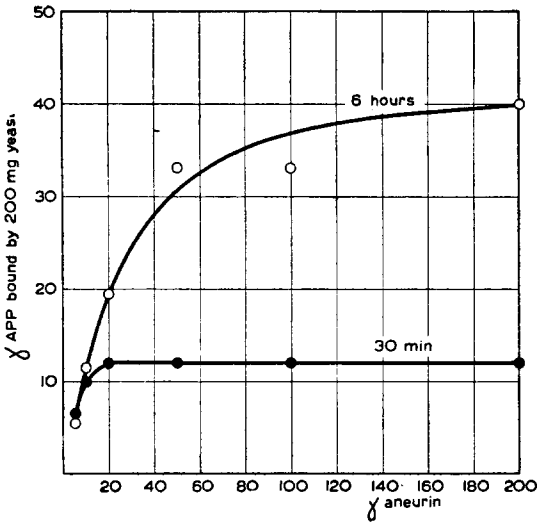

Fig. 5a. Amount of aneurinpyrophosphate formed in half an hour and in 6 hours by 200 mg yeast from various amounts of aneurin at pH 3.8 and 27.5° C.

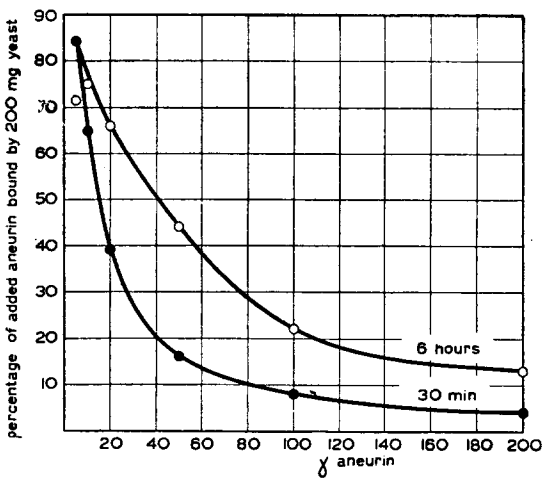

Fig. 5b. Percentages of various amounts of aneurin transformed into aneurinpyrophosphate by 200 mg yeast in half an hour and in 6 hours at pH 3.8 and 27.5° C.

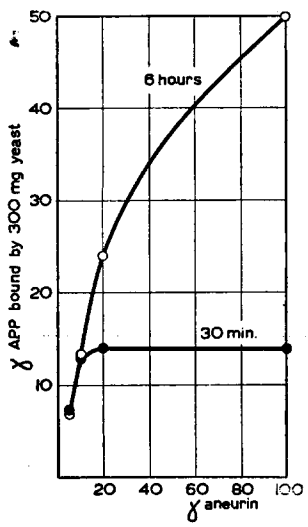

Fig. 6a. Amount of aneurinpyrophosphate formed in half an hour and in 6 hours by 300 mg yeast from various amounts of aneurin at pH 3.8 and 27.5° C.

is converted into APP is very high. When 5 γ aneurin are added to 200 mg yeast, 84% is bound in the first half hour. This figure increases to 93% when 300 mg yeast are employed. Taking the experimental inaccuracy into account we may say that in the latter case 100% APP is formed from the added aneurin (see also Table IV).

We have seen above that the amount of APP synthesized in 6 hours or in a longer time from a relatively large amount of aneurin (1 mg aneurin per g yeast) is very markedly increased by the presence of 5% glucose. We have been unable, however, to detect any influence of glucose on the amount of APP synthesized by 100 mg yeast from 5 γ aneurin. We have carried out several experiments on this question as the result was important in view of designing a method for determining aneurin. When such a method is based on the quantitative conversion

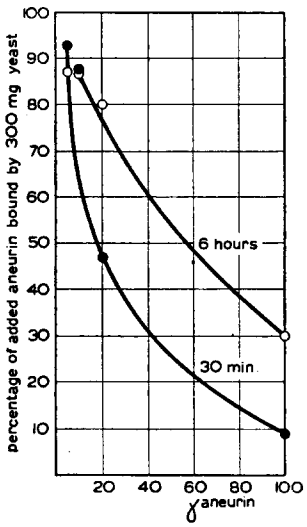

Fig. 6b. Percentages of various amounts of aneurin transformed into aneurinpyrophosphate by 300 mg yeast in half an hour and in 6 hours at pH 3.8 and 27.5° C.

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of aneurin into APP it is imperative to perform this with the smallest possible amount of yeast, to increase the accuracy by keeping the amount of APP in the yeast proper low. This problem will be more fully discussed in the next chapter.

Tables III and IV give the results of the experiments on the influence of glucose, employing half an hour's reaction time. The slight increase observed with glucose in the first experiments of Table III is probably caused by experimental errors. In any case it is not comparable with the glucose effect shown in Tables I and II. In the experiment described by Table IV an effect of glucose was only observed in the case of 100 γ aneurin, the APP synthesis with lower aneurin concentrations is quite unaffected by the glucose.

TABLE III

γ APP FORMED FROM 5 γ ANEURIN, INCUBATED WITH 100 MG YEAST

Exp. no.	γ APP formed			
	in ½ hour		in 2 hours	
	without glucose	with glucose	without glucose	with glucose
1	4.4	5.4	—	—
2	5.0	4.5	—	—
3	6.0	5.5	6.0	5.0

TABLE IV

γ APP FORMED FROM VARIOUS AMOUNTS OF ANEURIN, INCUBATED FOR 30 MINUTES AT 27.5° C WITH 200 MG YEAST IN THE ABSENCE AND IN THE PRESENCE OF 5 % GLUCOSE

γ aneurin	γ APP formed	
	with glucose	without glucose
5	6.8	6.8
10	10.8	9.8
20	14	14
100	14	21

TABLE V

γ APP FORMED FROM VARIOUS AMOUNTS OF ANEURIN, INCUBATED WITH 200 MG YEAST DURING VARYING TIMES AT 27.5° C

	γ APP formed							
γ aneurin added	20 γ		50 γ		100 γ		200 γ	
5 % glucose	—	+	—	+	—	+	—	+
½ hour	13	15	13	20	13	23	13	26
1 hour	20	15	19	20	20	29	20	40
3 hours	20	15	25	26	26	35	26	45
6 hours	22	15	24	28	30	40	34	55

Table V gives the results of an experiment with various amounts of aneurin and varying reaction times, with and without glucose. Though the figures of this table

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show a number of irregularities (synthesis from 20 γ aneurin wit glucose from 1 to 6 hours lower than synthesis in the absence of glucose ; with 50 γ aneurin positive effect of glucose in half an hour, while the effect is negligible during longer reaction times), they allow for the following conclusions:

1) If the reaction time is not too long, the APP synthesized in the absence of glucose does not increase with increasing amounts of aneurin.

2) For amounts of aneurin from 100 γ per 200 mg yeast upwards glucose has a pronounced effect upon the synthesis in half an hour ; the maximal effect is reached during the first hour and does not increase when the reaction time is prolonged (see also Fig. 7).

These peculiarities concerning the effect of glucose on the APP synthesis have puzzled us for a long time. Our insight into the complicated mechanism of the living yeast cell has not yet advanced so far, however, as to give an adequate explanation.

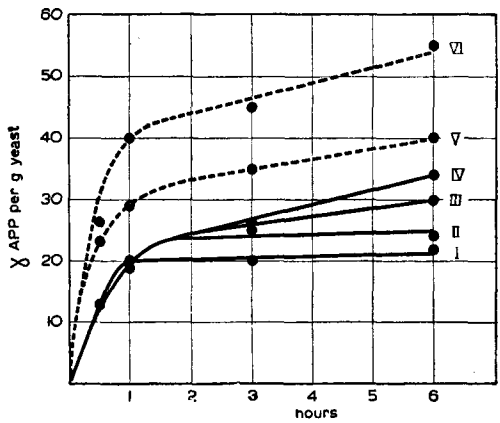

Fig. 7. Synthesis of aneurinpyrophosphate from various amounts of aneurin by 200 mg yeast at pH 3.8 and 27.5° C. Influence of glucose.

I. 20 γ aneurin
II. 50 γ aneurin
III. 100 γ aneurin
IV. 200 γ aneurin
V. 100 γ aneurin + 5% glucose
VI. 200 γ aneurin + 5% glucose

3. MANOMETRIC DETERMINATION OF ANEURIN

The principle of the method for determining free aneurin, based on the properties of the yeast described above, is very simple : conversion of aneurin into APP, preferably in not too long a reaction time, followed by manometric APP determination. However, there are some points which must be carefully considered once more, though they will already be clear to the reader of the preceding chapters.

a. The aneurin to be determined must be converted into APP as completely as possible. This may be done better by greater than by smaller amounts of yeast. Now the yeast already contains some APP (about 14 γ per g in the fresh bakers' yeast employed by us), and the amount of APP synthesized from added aneurin is found by subtracting the original APP content from the total amount of APP found after synthesis. So the use of large amounts of yeast has the great disadvantage that it increases the error of the APP determination according as the ratio original APP/ synthesized APP increases, for the percentual error of the APP derermination is always the same. It is therefore important to use the smallest possible amount of yeast.

Hence we have repeatedly tried to determine the smallest amount of yeast that can completely take up 5 γ aneurin. 100 mg yeast always appeared to be insufficient ; during a period of several weeks 200 mg were found to be sufficient, later on we were compelled to use 300 mg. The results of these experiments are listed in Table VI. They show that the accuracy of such an aneurin determination will not be less than that of practically all other methods for determining vitamins. The following example shows the importance of choosing a suitable amount of yeast.

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TABLE VI

γ APP FORMED BY INCUBATING VARIOUS AMOUNTS OF FRESH YEAST WITH 5 γ ANEURIN DURING 30′ AT 27.5° C (100 % CONVERSION GIVES 7.5 γ APP)

100 mg yeast		200 mg yeast		300 mg yeast	
Date	γ APP	Date	γ APP	Date	γ APP
13. 8.'43	5.5	13. 7.'43	7.8	17. 3.'44	7.9
18.—.—	5.0	16.—.—	8.4	29.—.—	7.0
26. 9.—	4.4	16. 8.—	8.6		
8.11.—	6.0	17.—.—	6.5		
		18.—.—	8.0		
		19.—.—	7.0		
		20.—.—	7.0		
		24.—.—	6.7		
		25. 1.'44	6.3		
		2. 2.—	5.2		
		5.—.—	6.4		
		14. 3.—	6.5		

Example.

5 γ aneurin to be determined. Theoretically 7.5 γ APP can be formed. 1 g yeast contains about 14 γ APP. The error of the determination is about 10 %. When 1 g yeast is used in the determination the uncertainty arising from the APP determination in the yeast itself is 1.4 γ, when 300 mg are used only 0.4 γ.

If there is no indication regarding the amount of aneurin to be determined, several trials may be necessary before finding the required amount of yeast. As we have seen in the preceding chapter this amount cannot be reduced by adding glucose to the reaction mixture.

b. In mixtures of aneurin and APP the sum of both substances is determined. As has been described in the preliminary communication on this work, added APP is decomposed by living yeast and the resulting aneurin is resynthesized again to APP. We will revert to this matter in the next chapter of this paper. Hence there is no difference in conduct between added APP and added aneurin. To find the amount of free aneurin the amount of APP must be determined separately in another sample of the mixture and subtracted from the sum of aneurin and APP.

The reader will not find precise directions for determining aneurin in all possible conditions in this chapter. But with the aid of what has been described above and in Chapter 2 (Methodical), he will be able to adapt the method to his own particular requirements.

Application of the method to urine.

We have tried to apply the method to urine. Urine contains free aneurin, but no APP. The aneurin is usually determined by the thiochrome method. It would be desirable to be able to control the results obtained with the thiochrome method by some other method, the more so as the results obtained with various modifications of the thiochrome method in several cases are markedly at variance with each other (see e.g. [10]). Our method, however, proved to be inadequate for the determination of

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aneurin in urine. The urine appears to contain one or more substances inhibiting the synthesis of APP by the yeast.

This may be illustrated by the description of some of the experiments carried out.

A. We compared :

1. 25 ml urine,
2. 25 ml urine, to which 5 γ aneurin had been added,
3. an aqueous solution of 5 γ pure aneurin.

To each of these solutions 500 mg yeast were added. After $\frac{1}{2}$ hour's incubation at p_H 3.7 and 27° C the synthesized APP was determined. The results were :

1. synthesized from aneurin in urine . . . . . 5.4 γ
2. ,, ,, ,, ,, ,, + added aneurin . . . . . 8.4 γ
3. ,, ,, pure aneurin . . . . . 8.4 γ

Assuming that the amount of 5.4 γ APP synthesized from aneurin present in the urine indicates the true aneurin content of the urine 13.8 γ APP should have been synthesized in reaction 2, instead of 8.4 γ, as found. So it seems as if the APP synthesis is depressed in both mixtures containing urine.

Or, to reason the other way round : the amount of APP synthesized from urine + 5 γ aneurin is not larger than the amount formed from 5 γ aneurin alone, while reaction 1 indicates that some APP is formed from the aneurin contained in urine. Which brings us to the same conclusion regarding partial inhibition of APP synthesis by urine.

This experiment has been repeated twice, always with substantially the same result. It is only by a coincidence that the amounts of APP formed in reactions 2 and 3 are equal.

However, the results of these experiments can still be explained in another way. Apart from the possibility that the aneurin uptake and/or the succeeding APP synthesis are inhibited by urine it seems possible that the CO_2 output in the manometric reaction might be lower than corresponds with the veritable amount of APP, when the decarboxylation of pyruvic acid takes place in the presence of urine. We did not positively exclude the latter possibility, but only proved the correctness of the former assumption by the next experiment (B).

B. 25 ml urine were brought to p_H 3.7 and incubated for $\frac{1}{2}$ hour at 27° C with 0.5 g yeast (1). After centrifuging the centrifugate was incubated again with 0.5 g fresh yeast (2), and so on. After having thus treated the urine with 5 samples of 0.5 g fresh yeast successively, 5 γ aneurin were added. Then the fluid was incubated once more with the same amount of yeast (6).

All yeast samples appeared to have synthesized aneurin, viz. sample 1 : 4.5 γ, sample 2 : 4.5 γ; sample 3 : 4.5 γ; sample 4 : 3.5 γ; sample 5 : 3.0 γ; sample 6 : 5.0 γ.

These figures show : 1st that the yeast is only capable of taking up a very restricted amount of aneurin from urine, 2nd that even by treating the urine 4 times with fresh yeast not all the aneurin is extracted, 3rd that the inhibiting substances are not removed by treatment with yeast, for 5 γ added to the 5th centrifugate is only partly recovered by ensuing incubation with yeast. (We know from the preceding sections that 0.5 g yeast is more than sufficient to take up 5 γ aneurin from a pure aqueous solution).

We have treated another sample of urine 10 times with yeast in the same way. Only the tenth time no APP was formed.

So our method is not suitable for the determination of aneurin in urine, without some previous treatment of the urine to remove the disturbing substances. Various attempts to do this, viz. treatment of the urine with lead acetate and adsorption of the aneurin in fuller's earth with subsequent elution by alkali, were unsuccessful. Hence we do not think it necessary to describe these experiments in detail.

4. CONDITION OF THE APP FORMED

We shall now pass on to the question of the state in which the APP synthesized in our experiments occurs in the yeast cell. It is very improbable that it is free APP, for APP added to a suspension of fresh yeast is immediately decomposed into

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aneurin and phosphoric acid. This is done by the yeast cell and not by some enzyme formed and excreted by the cell and then working in the medium. When yeast is suspended in buffer of p_H 3.7, and is removed again by centrifuging, the clear filtrate also has a strong decomposing action. This phosphatase, present in the medium, probably originates from damaged cells. For after repeating the washing of the yeast a few times the third washing fluid is inactive, while a suspension of the washed cells is again very active (see Table VII).

So we must conclude that the APP has to penetrate into the cells in order to be decomposed, or that the surface of the yeast cells possesses phosphatase activity. In the latter case free APP could conceivably exist in the innermost regions of the cell, where it would not be assailable for the phosphatase, but this assumption seems so fantastical that we believe we may safely conclude from the above-mentioned observations that free APP cannot exist in the living yeast cell *.

TABLE VII

PHOSPHATASE ACTION OF UNWASHED YEAST, WASHED YEAST AND WASHING FLUIDS

APP determined in :	γ APP	
	per g yeast	per 5 ml fluid
1. fresh yeast	13	
2. a suspension of 1 g yeast in 5 ml of 0.1 mol primary phosphate (p_H 4.5), which had been left standing at 15° C for 30 min after the addition of 50 γ APP	13	
3. the centrifugates, obtained from repeatedly washing 3 g yeast with 15 ml primary phosphate, 30 min after the addition of 50 γ APP to 5 ml of centrifugate (15° C),		
1st centrifugate		0
2nd ,,		35
3rd ,,		60
4th ,,		60
4. the washed yeast cells, suspended in 15 ml primary phosphate, 30 min. after the addition of 50 γ APP per 5 ml suspension (15° C)	13	

In the normal yeast cell APP is for a great part present in the form of carboxylase, therefore linked to a protein. In this form it is resistant to the action of the phosphatase. Hence it seems reasonable to suppose that the APP synthesized from added aneurin is also bound as carboxylase. But of course it may also be bound to some other protein. The APP-protein symplex may be identical with some other normally occurring APP compound with a physiological significance, e.g. pyruvodehydrogenase, better known from investigations on animal tissues, or it may be a storage form of aneurin. However, we can also imagine that this form of APP never occurs under normal conditions, but that the yeast is compelled to syn-

* The aneurin formed from APP after its penetration into the yeast cell does not accumulate here, but leaves the cell again, as is shown by the following experiment: 1 g yeast was washed 4 times with 5 ml of primary phosphate. The washed cells were suspended in 5 ml of this solution and 50 γ APP were added. After standing for 5 minutes at 15° C. the suspension was centrifuged and aneurin was determined by the thiochrome method in cells and centrifugate separately. The cells contained 2 γ and the centrifugate 25 γ free aneurin.

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thesize it when brought into a medium with an abnormally high aneurin concentration.

We first examined whether the complex formed was identical with carboxylase. This was proved not to be the case*. As Table VIII, taken from our preliminary communication, shows, the carboxylase activity of a dried sample ** of aneurin-treated yeast, containing 560 γ APP per g, was rather lower than higher than that of a dried non-treated sample of the same yeast, containing only 34 γ APP per g.

TABLE VIII

CARBOXYLASE ACTIVITY OF NORMAL AND ANEURIN-TREATED YEAST

4 mg aneurin were added to 14 g yeast, suspended in 126 ml succinate buffer, p_H 3.7. The suspension was shaken for 16 hours at 27°.5 C. The yeast was separated by centrifuging, washed 3 times with water and dried at room temperature in a thin layer on a glassplate (yeast 1). Another sample of the same yeast, non-treated with aneurin, was dried in the same way (yeast 2).

Carboxylase determinations were carried out in the following way: the main compartments of WARBURG-bottles contained a few mg dried yeast, suspended in 1.5 ml 0.1 mol phosphate buffer, p_H 6.2. 5 mg Na pyruvate, dissolved in 0.2 ml of the same buffer, were tipped in from the side bulb.

	yeast 1 (500 γ/g)		Yeast 2 (34 γ/g)	
	2.5 mg	5 mg	2.5 mg	5 mg
CO_2 production in 28 min (μl)	86	133	95	182

We next investigated whether the complex was identical with pyruvodehydrogenase by measuring the oxygen uptake in the absence of a respiration substrate or in the presence of pyruvic acid or glucose, of a sample of normal yeast and of a sample of the same yeast, previously treated with aneurin.

This experiment was carried out as follows:

2 samples of 1 g fresh yeast, A and B, were suspended in succinate buffer, p_H 3.7, to a volume of 9 ml. 1 mg aneurin was added to A. Both suspensions were shaken for a couple of hours at 27.5° C. Then 1 cm^3 was drawn off for APP determination.

Sample A contained 156 γ APP per g and sample B 14 γ per g.

The p_H of the remainder of the suspensions was brought to 6.2 and the volume made up with 0.1 mol. phosphate buffer, p_H 6.2, to 50 ml. 1 ml samples of these suspensions were used for the determination of the oxygen uptake by the WARBURG method under the conditions already mentioned above, viz. in the absence of a respiration substrate or in the presence of 5 mg pyruvic acid or 18 mg glucose. All measurements were carried out in duplicate.

The results of this experiment can be found in Fig. 8, which shows that the synthesized APP has nothing to do with the respiration system either.

So we did not succeed in finding the physiological meaning of this APP compound. We do not even know with certainty whether small amounts of it occur in yeasts which have not been treated with abnormally high amounts of aneurin.

We believe to have demonstrated in a previous investigation [12]) that yeasts,

* The conclusions drawn by SPERBER and RENVALL from a paper by WEIL-MALHERBE[11] in connection with their own work are not to the point, as WEIL-MALHERBE really synthesized carboxylase by allowing aneurin, phosphoric acid and Mg to react with an apocarboxylase preparation, obtained from LEBEDEW juice.

** As the living yeast cell is impermeable to pyruvic acid, the yeast must be dried or plasmolyzed before its carboxylase content can be determined.

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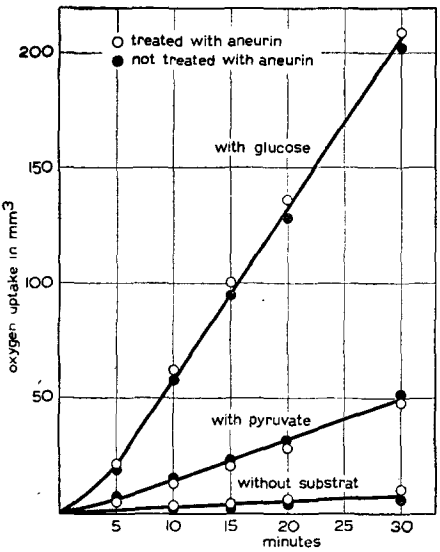

Fig. 8. Influence of previous treatment with aneurin upon the oxygen uptake of bakers' yeast in the absence of a respiration substrate and in the presence of glucose and sodium pyruvate.

dried at room temperature, contain more than one APP compound. This was done by determining what we have called the activity quotient ("A.Q."), i.e. the carboxylase activity of an amount of yeast containing 0.1 γ APP. The A.Q.'s of different yeasts are largely at variance with each other. The lower the A.Q., the more APP must be present in a form different from carboxylase.

The variance of the A.Q. can certainly not be completely ascribed to the occurrence of varying amounts of the APP complex, postulated in this paper. For after treatment with phosphate buffer of p_H 6.2, the A.Q. of some yeasts changes, that of other yeasts is unchanged, but great differences between the various yeasts remain.

Now when a yeast, which has been enriched with APP by incubation with aneurin, is dried — drying alone does not affect the APP content — and subsequently suspended at p_H 6.2, the synthesized APP rapidly disappears. The same happens upon plasmolysis of the yeast, brought about by freezing with liquid air or by ether. The rapid loss of APP indicates that the linkage with APP is broken up in these cases, whereupon the APP can be decomposed by the yeast phosphatase. So, however stable, this APP-protein symplex may be in the living yeast cell, it is rapidly decomposed by treatments as described above which involve the death of the cells.

It is clear that yeasts which have been "loaded" with APP are subject to great changes in A.Q. upon washing the dried yeast at p_H 6.2. But if small amounts of this compound would be the sole cause of differences in the A.Q. of normal yeasts, we should expect these differences to be obliterated by treatment with buffer of p_H 6.2, instead of remaining. Moreover the A.Q. of bakers' yeasts does not change by washing at p_H 6.2, and these are just the yeasts which possess the faculty of transforming aneurin into APP to such a high degree. And in the cases in which the A.Q. of a yeast is altered by this treatment this may quite as well be explained by the removal of free APP which might have been formed during the drying process. Indeed free APP cannot occur in the living yeast, but it might be stable in the partly or wholly dried yeast, for we know nothing about the activity of the phosphatase in yeasts that are in a more or less dried condition.

So besides having established which physiological functions the new APP compound does not possess, our knowledge is restricted to its mode of formation and its lack of stability under certain conditions.

All these experiments, designed to throw light on the nature of the APP formed from aneurin by living yeast, were carried out with yeast incubated for a relatively short time with aneurin in a medium in which scarcely any multiplication could take place. As we have seen, this results in the formation of an apparently unphysiological

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APP-protein symplex. But it is conceivable that when the yeast is given the chance to grow in a medium that is extra rich in aneurin, it will respond by forming more of the physiological APP compound carboxylase. To examine this we have cultivated yeast in the presence of a relatively high amount of aneurin. But also under these circumstances a large quantity of the new APP compound was formed, while the carboxylase content of the yeast had hardly increased.

Two flasks with 200 ml malt extract, each containing 35 γ aneurin per 100 ml, were inoculated with the same bakers' yeast employed in all other experiments. To the contents of one of the flasks 2 mg aneurin were added. Both were incubated for 3 days at 30° C under vigorous aeration. After washing with water, yeast 1 (cultivated in the presence of added aneurin ; output 2.67 g) contained 230 γ APP per g, yeast 2 (cultivated in the absence of added aneurin ; output 2.37 g) contained 9 γ APP per g. After drying the yeasts in the usual way in a thin layer at room temperature, yeast 1 appeared to possess a carboxylase activity only slightly higher than that of yeast 2. This is shown by Table IX. Thus, though there is some increase of carboxylase activity, in contrast to the yeast not cultivated but only incubated with a high amount of aneurin, there is no proportionality at all between the increase of the APP content and the increase of the carboxylase content.

TABLE IX

CARBOXYLASE ACTIVITY OF YEAST CULTIVATED IN MALT EXTRACT WITH AND WITHOUT 1 mg OF ADDED ANEURIN PER 100 ml EXTRACT

	yeast 1 (230 γ APP/g)		yeast 2 (9 γ APP/g)	
	2.5 mg	5 mg	2.5 mg	5 mg
CO_2 production (mm^3) in 10' : . .	41	81	36	65
in 30' : . .	107	197	94	165

5. REACTION MECHANISM OF SYNTHESIS AND AGENTS INVOLVED

a. Is the phosphatase involved in the synthesis?

Considering the problem of the reaction mechanism of APP synthesis by living yeast and the agents playing a part in this process, we shall have to discuss the opinion of SPERBER and RENVALL on this subject. Obviously these authors consider the synthetical process as essentially the reverse of the decomposition of added free APP. We are convinced, however, that we have to do with the synthesis of some APP-protein symplex whose function in the yeast is still unknown.

Though according to their own observation exhausted yeast does not synthesize APP, while its phosphatase content is not diminished, SPERBER and RENVALL seem to consider the phosphatase (= phosphatese) as the only agent in the APP synthesis. Their arguments are the following :

1. The decomposition of APP by yeast phosphatase and the APP synthesis by living yeast have the same p_H optimum (3.7) ;

2. both processes are inhibited by „pyrimidyl" (2-methyl-4-aminopyrimidyl-5-methyl-aminodihydrochloride).

Neither of these arguments is convincing :

1. Our experiments in which the p_H-optimum of the decomposition of APP by yeast phosphatase was determined were carried out with suspensions of dried yeast,

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hence the p_H measured was indeed the p_H of the reaction medium. When, on the contrary, living yeast cells are suspended in a medium of p_H 3.7 we do not know the p_H of the interior of the cell, in which the reaction takes place. Dr SPERBER (private comm.) thinks it possible that the phosphatase is situated in the outmost layers of the cell, so that it can exert its action in the medium and it would not be necessary for the APP molecules to penetrate into the yeast cell in order to be cleaved. The p_H measured would also in this case be the p_H at which the phosphatase exerts its action. We do not know if Dr SPERBER has any proofs for this hypothesis, but should this not be the case, we cannot ascribe any importance to this argument. Moreover the p_H-optimum is no sharp characteristic of an enzyme, as it depends upon the state of the substrate and the medium (degree of purification).

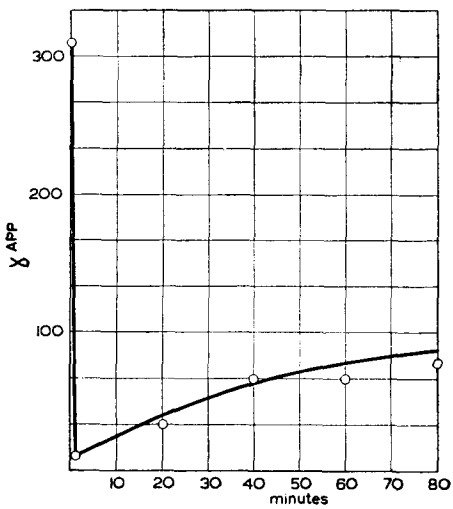

Fig. 9. Decomposition and ensuing reformation of aneurinpyrophosphate by bakers' yeast at p_H 3.7 and 27.5° C.

2. The inhibition of the APP synthesis by "pyrimidyl" is no more a specific property of the enzyme system than the p_H-optimum, for it must be considered a case of competitive inhibition.

The decomposition of APP by yeast phosphatase is inhibited by aneurin and by "pyrimidyl". The only possible explanation of these effects seems to be the assumption that the place otherwise occupied by the APP molecules on the surface of the phosphatase particles can be taken in by aneurin or "pyrimidyl" molecules. Competitive inhibition of enzyme reactions is often found with substances structurally related to the substrate. Hence it is quite logical that not only an enzyme decomposing APP, but also another enzyme, playing a part in the synthesis of APP from aneurin, will be inhibited by "pyrimidyl".

The most convincing argument for the correctness of our assumption that the synthesis of APP under the conditions prevailing in our experiments is not a simple reversal of its decomposition, brought about by one and the same enzyme, acting as a phosphatese or a phosphatase, is given by the following experiment.

4 g of yeast were incubated in 36 ml 0.1 mol succinate buffer, p_H 3.7, at 27.5° C. 300 γ APP were added and samples were pipetted at 30", 20', 40', 60' and 90' after the addition of the APP. APP determinations gave the following results:

Time:	30"	20'	40'	60'	90'
γ APP per g yeast:	4	34	68	68	78

So we see that the APP added is completely decomposed within 30". Thereupon a much slower synthesizing process sets in (see also Fig. 9). Would the enzyme only establish the simple equilibrium, described by the equation.

$$\text{aneurin} + 2\ H_3PO_4 \rightleftarrows \text{APP} + 2\ H_2O,$$

we could not understand why all added APP is first decomposed, instead of only being decomposed up to an amount corresponding with the situation of the equilibrium.

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b. Synthesis of free APP as the initial stage of the formation of the APP-protein symplex.

Having abandoned SPERBER's and RENVALL's notion of the transformation of aneurin into APP being brought about by the yeast phosphatase, now acting as phosphatase, we might of course suppose that the free APP would yet primarily be formed by some other agent, and would thereupon be coupled to the protein.

The formation of the APP-protein symplex would then be the result of the competition of two reactions, the linking of APP to protein and the decomposition of APP by the phosphatase, the amount of the symplex formed ultimately depending upon their relative velocities. As the yeast phosphatase is exceedingly active towards APP, we think it very improbable that any symplex is formed in this way. Yet we have thought it worth while to investigate this point as closely as possible. For decomposition of added APP by the phosphatase need not neccessarily lead to the presumption that the same fate awaits APP formed from aneurin in the interior of the yeast cell, especially if one assumes with SPERBER that the phosphatase only exercises its activity in the outermost regions of the cell.

We have therefore examined whether the APP-protein symplex could be formed from APP, added to the yeast, when the decomposition of the APP was prevented by "pyrimidyl". We had to use "pyrimidyl" as inhibitor, for aneurin, the most effective inhibitor, would itself give rise to the formation of the APP-protein symplex, which would greatly confuse the issue. However, we did not succeed in inhibiting the phosphatase action of living yeast to such an extent (see next section), that no decomposition of APP could be detected during a time which must certainly be considered sufficient for the synthesis.

So the only experiment which might possibly give an answer to the question whether preformed APP can be directly bound to the yeast protein or that it must first be split into aneurin and phosphoric acid, had to be carried out as follows:

A sample of yeast was incubated with aneurin, "pyrimidyl" and APP. An identical sample was incubated with aneurin and "pyrimidyl" only, APP being omitted in this case. "Pyrimidyl" was present to prevent the formation of the symplex from aneurin; aneurin and "pyrimidyl" together had to inhibit the phosphatase action as far as possible (the added amount of aneurin was so large that should any aneurin nevertheless be formed from APP it would be of no account). We should expect to find more APP synthesized by the yeast sample which had been incubated with APP than by the control sample.

This experiment was performed under various conditions, viz. 1st very short reaction time (2 min), in which no decomposition of APP could be detected, but which was possibly also too short to detect any synthesis of the symplex; 2nd. long reaction time (1 hour), during which a great part of the APP was certainly hydrolyzed. We think it superfluous to describe these experiments in detail; suffice it to give the general result, viz. that we never obtained any indication that a higher synthesis of the symplex takes place in the presence of added APP.

So these experiments did not render it probable that APP synthesis is the first stage in the formation of the APP-protein symplex; however, we may not conclude that this is impossible, especially in view of the possibility that just the coupling of APP to the protein might be inhibited by "pyrimidyl".

So the extreme rapidity with which added APP is decomposed as compared

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to the slowness with which the symplex is synthesized remains the main ground of our conviction that the formation of APP is not the initial stage of the synthesis of the symplex, though this argument is also not quite unchallengeable (phosphatase possibly not active in cell interior).

c. Inhibition of phosphatase action and of synthesis of APP by "pyrimidyl".

In the course of the above-mentioned attempts to inhibit the phosphatase activity of living yeast by "pyrimidyl" we observed the interesting fact that much smaller amounts of "pyrimidyl" are required to inhibit the synthesis of the APP-protein symplex than to inhibit the phosphatase action.

We were inclined to adopt the amount of "pyrimidyl" necessary for inhibition of the phosphatase from our earlier experiments on dried yeast [3]. In one of these experiments, for instance, no decomposition of APP could be detected after 32 minutes' incubation of 20 mg dried yeast with 100 γ APP and 2 mg aneurin (= 20 × the APP concentration). To obtain the same result with "pyrimidyl" instead of aneurin about ten times as much of the former compound (i.e. 20 mg) had to be added.

In the case of fresh yeast, however, 4000 γ aneurin (80 × the APP concentration) were required to, inhibit the phosphatase action of 1 g yeast on 50 γ APP to such an extent that it could not be detected after 2 minutes' reaction time.

With an aneurin/APP ratio of 20, as employed in the above-cited experiment with dried yeast, 30 γ APP of 50 γ added were decomposed in 2 minutes. The complete results as well as particulars about this experiment are to be found in Table X.

TABLE X

DECOMPOSITION OF APP BY LIVING YEAST IN THE PRESENCE OF INCREASING AMOUNTS OF ANEURIN

Reaction mixture: 1 g fresh yeast, 50 γ APP, various amounts of aneurin; volume 10 cm^3, p_H 3.7. APP determined after 2 minutes' incubation. 1 g yeast contained 13 γ APP.

γ aneurin added	0	50	500	1000	2000	4000
γ APP found after 2 minutes	19	20	23	33	46	62
γ APP decomposed	44	43	40	30	17	1

In the case in which no decomposition of APP could be detected after 2 minutes (Aneurin/APP ratio = 80), inhibition will be no means have been practically complete (100% competitive inhibition is theoretically impossible). For when the reaction time was prolonged to an hour only a very partial inhibition was observed, though the amount of aneurin was increased to 530 times the APP concentration. This is to be seen in Table XI. That some inhibition of the phosphatase action does occur is clear when the figures in this table are compared to those in the first column of Table X.

TABLE XI

INFLUENCE OF THE REACTION TIME ON THE DECOMPOSITION OF APP BY LIVING YEAST IN THE PRESENCE OF ANEURIN

Reaction mixture: 1 g yeast, 32 mg aneurin, 60 γ APP. Volume 10 ml, p_H 3.7. At times indicated below 1 ml was drawn off, centrifuged, and APP was determined in the supernatant liquid.

Reaction times	2′	5′	10′	20′	40′	60′
γ APP in centrifugate	57	52	48	42	31	20

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However, we must not conclude upon comparing the results obtained with dry and with fresh yeast that more aneurin is necessary to inhibit the phosphatase of yeast in the latter condition. The amounts of yeast employed in both experiments were not identical, certainly the amount of fresh yeast was larger. Hence, though we could not detect any decomposition of APP by 20 mg dry yeast after 32 min the phosphatase action might possibly have been very distinct with 200 mg an amount comparable with 1 g fresh yeast. But on the other hand it is not improbable that larger amounts of the inhibitors would be necessary for fresh yeast, as we know nothing about their resulting concentrations within the cell. So the phosphatase in the living cell might be checked be the same relatively small amount of inhibitor as the phosphatase of dried yeast.

As could be expected from the observations made when employing dried yeast, still much larger amounts of "pyrimidyl" would be necessary to inhibit the phosphatase of fresh yeast. Indeed no inhibition was observed in a 45 minutes' experiment, in which 75 mg "pyrimidyl" were added to 1 g yeast and 60 γ APP.

"Pyrimidyl", however, in confirmation of SPERBER's and RENVALL's experiments, appeared to have a very marked inhibitory effect on the APP synthesis from aneurin. For example, 2 samples of 100 mg yeast were incubated with 800 γ aneurin and with 800 γ aneurin + 16000 γ "pyrimidyl" respectively. With aneurin alone, 72 γ APP were synthesized, and with aneurin + "pyrimidyl" 24 γ APP were formed in 75 minutes.

The following experiment shows that with a "pyrimidyl" concentration by which the APP synthesis is completely inhibited all added APP is decomposed.

1 g fresh yeast was incubated with 60 γ APP and 75000 γ "pyrimidyl" at 27.5° C (volume 9 ml, p_H 3.7). After 0, 40 and 80 minutes 2 ml were drawn off and centrifuged. The APP contents of the centrifugate and of the washed residue were determined. The results are shown in Table XII.

TABLE XII

SYNTHESIS AND DECOMPOSITION OF APP IN THE PRESENCE OF "PYRIMIDYL"

time	γ APP in residue (per g)	γ APP in centrifugate (per 9 ml)
0′	15	57
40′	16	0
80′	17	0

We see that after 40 minutes all added APP is decomposed. In the next 40 minutes a considerable part of the formed aneurin could have been employed for the synthesis of the APP-protein symplex, which would have accumulated in the cell mass. No increase of APP was found, however, which must be due to the presence of "pyrimidyl". The amount of "pyrimidyl" used in these experiments was very high. We know, however, from SPERBER's experiments that inhibition of the synthesis can be brought about by much smaller amounts of "pyrimidyl" (1 g yeast, 50 γ aneurin, 1000 γ "pyrimidyl", volume 8.5 ml).

Of course the difference in sensitivity of the synthesizing system and the phosphatase towards the action of "pyrimidyl" is no argument against SPERBER's view that the synthesizing enzyme is identical with the phosphatase For in the case

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of the synthesis there is a competition between aneurin and "pyrimidyl", in the case of the phosphatase action a competition between APP and "pyrimidyl". We know that the affinity of APP towards the phosphatase is much larger than the affinity of aneurin towards the phosphatase. So a reaction, catalyzed by a certain enzyme, will be more easily inhibited by "pyrimidyl" when aneurin is the substrate, than another reaction will be, catalyzed by the same enzyme, but now with APP as the substrate.

SUMMARY

1. Great amounts of aneurinpyrophosphate (APP), up to a few hundred γ per g, accumulate in the yeast cells when living yeast (normal content about 10 γ per g) is incubated with aneurin.

2. Bakers' yeast has a higher synthesizing capacity than brewers' yeast.

3. The best temperature for this reaction is about 30° C. At higher temperature the process takes place more rapidly, but then the yeast dies off sooner, and consequently loses the synthesized APP as well as the APP originally present.

4. The maximal amount of APP is formed at p_H 3.7, and this is also the optimum for the velocity of the reaction.

5. When the yeast is incubated with high amounts of aneurin, e.g. 1 mg per g yeast, the presence of 5% glucose increases the synthesis by about twice the amount at p_H 3.7 and by about four times the amount at p_H 6.0. With glucose the p_H-activity curve for the synthesis has a much broader optimum than when glucose is omitted. The lower the amount of aneurin, the smaller is the effect of glucose; it is zero for about 50 γ per g yeast.

6. With the bakers' yeast as employed by us, 5 γ aneurin is practically completely bound within 30 minutes by 200 or 300 mg yeast. With higher amounts of aneurin a little more is bound, but from about 20 γ upwards no further increase was found during this time. With longer reaction times the increase in APP with increasing aneurin continues up to amounts of aneurin of about 100 or 200 γ with the above mentioned amount of yeast.

7. A manometric method for aneurin determination could be based on the property of yeast to bind small amounts of aneurin completely in the form of APP.

8. This method proved to be unsuitable for the determination of aneurin in urine, which appears to contain one or more substances inhibiting the synthesis of APP by yeast.

9. It was proved that the APP synthesized must be linked to some compound of the yeast cell, probably a protein, for APP added to a suspension of repeatedly washed yeast is very rapidly decomposed by the yeast phosphatase still present in the washed cells. This presumed APP-protein symplex was proved not to play any part in the respiratory system of glucose or pyruvic acid, nor is it identical with carboxylase. Its physiological meaning, if it has any, is still unknown. The APP present in this form is resistant to drying the yeast at room temperature, but it rapidly disappears when the dried yeast is suspended in a solution of p_H 6.2. It is equally decomposed by plasmolysis of the yeast, either by ether or by freezing.

10. The synthesis of APP by the yeast cell is not a simple reversal of its decomposition into aneurin, Mg and phosphate, both processes thus being catalyzed by the same enzyme, the phosphatase. The authors think it improbable that the synthesis of free APP is the initial stage in the formation of the postulated APP-protein symplex.

11. The synthesis of the APP-protein symplex is inhibited by much smaller concentrations of 2-methyl-4-aminopyrimidyl-5-methylaminodihydrochloride than is the decomposition of free APP by the yeast phosphatase.

RÉSUMÉ

1. De grandes quantités de pyrophosphate d'aneurine (APP) jusqu'à concurrence de quelques centaines de γ par g s'accumulent dans les cellules de levure quand on procède à l'incubation de la levure vivante (teneur normale 10 γ par g environ) avec de l'aneurine.

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2. La levure de boulanger possède un plus grand pouvoir synthétisant que la levure de bière.

3. La température qui convient le mieux à cette réaction est 30° C environ. A des températures plus élevées, le processus s'effectue plus rapidement, mais alors la levure meurt plus tôt et, par conséquent, elle perd le APP synthétisé ainsi que le APP présent à l'origine.

4. La quantité maximale d' APP est formée à p_H 3.7, lequel est également l'optimum de la vitesse de la réaction.

5. Lorsque l'incubation de la levure a lieu avec de grandes quantités d'aneurine, p. ex. 1 mg par g de levure, la présence de 5% de glucose fait doubler la synthèse à p_H 3.7 et elle la fait quadrupler à p_H 6.0. Avec le glucose, la courbe d'activité du p_H pour la synthèse a un optimum beaucoup plus large que lorsque l'on opère sans glucose. Plus la quantité d'aneurine est petite, plus l'effet du glucose est faible, il est nul pour 50 γ par g de levure environ.

6. Avec la levure de boulanger employée par nous, 5 γ d'aneurine sont pratiquement complètement liés en 30 min par 200 ou 300 mg de levure. Avec de plus grandes quantités d'aneurine, la quantité liée est un peu plus forte, mais au-dessus de 20 γ, aucune nouvelle augmentation n'a été constatée pendant cette durée. Lorsque les durées de réaction sont plus longues, l'augmentation en APP avec des quantités plus grandes d'aneurine continue jusqu'à concurrence de 100 ou 200 γ d'aneurine avec la quantité de levure mentionnée plus haut.

7. Une méthode manométrique pour la détermination de l'aneurine pourrait être basée sur la propriété que possède la levure de pouvoir lier complètement de faibles quantités d'aneurine sous la forme de APP.

8. Cette méthode s'est averée inadéquate pour la détermination de l'aneurine dans l'urine ; celle-ci semble contenir une ou plusieurs substances inhibant la synthèse de APP par la levure.

9. Il a été prouvé que l'APP synthétisé doit être lié à quelque composé de la cellule de levure, probablement à une protéine, puisque l'APP ajouté à une suspension de levure lavée à plusieurs reprises est très rapidement décomposée par la phosphatase de levure encore présente dans les cellules lavées. La preuve a été faite que ce simplex APP-protéine ne joue aucun rôle dans le système respiratoire du glucose ou de l'acide pyruvique, et qu'il n'est pas identique à la carboxylase. Sa signification physiologique, s'il en a une, n'est pas encore connue. L'APP présent sous cette forme résiste à la dessication de la levure à la température intérieure, mais il disparaît rapidement quand la levure sèche est en suspension dans une solution de 6.2 p_H .Il est également décomposé par la plasmolyse de la levure, soit par l'éther, soit par congélation.

10. La synthèse de l'APP par la cellule de levure n'est pas une simple inversion de sa décomposition en aneurine, en Mg et en phosphate, les deux processus étant ainsi catalysés par la même enzyme, la phosphatase. Les auteurs croient qu'il est improbable que la synthèse de l'APP libre soit l'échelon initial de la formation du simplex APP-protéine présupposé.

11. La synthèse du simplex APP-protéine est inhibée par des concentrations beaucoup plus faibles de 2-méthyle-4-aminopyrimidyle-5-méthylaminodihydrochloride que ne l'est la décomposition d'APP libre par la phosphatase de levure.

ZUSAMMENFASSUNG

1. Grosse Mengen von Aneurinpyrophosphat (APP) bis zu einigen Hundert γ pro g häufen sich in den Hefezellen auf, wenn lebende Hefe (Normalgehalt ca 10 γ pro g) mit Aneurin inkubiert wird.

2. Bäckerhefe besitzt ein höheres Synthese-Vermögen als Bierhefe.

3. Die geeignetste Temperatur für diese Reaktion ist ca 30° C. Bei höherer Temperatur vollzieht sich der Prozess schneller, aber die Hefe stirbt dann auch schneller ab, und verliert infolgedessen sowohl das synthetisierte APP als auch das ursprünglich vorhandene APP.

4. Die Maximalmenge von APP wird bei p_H 3.7 gebildet ; dieser p_H ist zugleich der optimale Wert für die Reaktionsgeschwindigkeit.

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5. Wird die Hefe mit grösseren Mengen Aneurin behandelt, z.B. mit 1 mg pro g Hefe, dann erhöht das Vorhandensein von 5% Glukose die Synthese auf ca das doppelte bei p_H 3.7 und auf ca das vierfache bei p_H 6.0. Mit Glukose besitzt die p_H-Aktivitätskurve für die Synthese ein viel breiteres Optimum als bei Weglassen von Glukose. Je kleiner die Aneurinmenge ist, desto geringer ist die Glukosewirkung; sie verschwindet für ca 50 γ pro g Hefe.

6. Bei der von uns verwendeten Bäckerhefe, werden 5 γ Aneurin innerhalb von 30 min durch 200 bzw. 300 mg Hefe praktisch vollständig gebunden. Bei grösseren Aneurinmengen wird eine etwas grössere Menge gebunden, von ca 20 γ aufwärts wurde jedoch keine weitere Erhöhung während dieser Zeitdauer festgestellt. Bei längeren Reaktionszeiten dauert die Zunahme von APP durch Erhöhung der Aneurinmengen fort bis zu ca 100 bzw. 200 γ Aneurin bei vorerwähnter Hefemenge.

7. Eine manometrische Methode zur Aneurinbestimmung konnte auf der Eigenschaft der Hefe, kleine Aneurinmengen vollständig in der Form von APP zu binden, basiert werden.

8. Diese Methode erwies sich als unbrauchbar für die Bestimmung von Aneurin in Harn; dieser scheint eine oder mehrere Substanzen zu enthalten, welche die APP-Synthese durch Hefe hemmen.

9. Es wurde bewiesen, dass das synthetisierte APP an irgendeinen Bestandteil der Hefezelle, wahrscheinlich an ein Protein, gebunden sein muss, da das APP, welches zu einer Suspension von wiederholt gewaschener Hefe hinzugefügt wird, durch die in den gewaschenen Zellen noch vorhandene Hefephosphatase sehr rasch gespalten wird. Es wurde bewiesen, dasz dieses vermutete APP-Proteinsymplex keine Rolle im Veratmungssystem der Glukose oder Brenztraubensäure spielt, und auch nicht mit Carboxylase identisch ist. Seine physiologische Bedeutung, falls es eine hat, ist noch unbekannt. Das in dieser Form vorhandene APP widersteht der Hefetrocknung bei Zimmertemperatur, verschwindet aber schnell, wenn die getrocknete Hefe in einer Lösung von p_H 6.2 suspendiert wird. Es wird ebenfalls durch Plasmolyse der Hefe, entweder mit Aether oder durch Gefrieren, zerlegt.

10. Die APP.-Synthese durch die Hefezelle ist nicht eine einfache Umkehrung seines Abbaus in Aneurin, Mg und Phosphat, wobei beide Prozesse durch dasselbe Enzym, die Phosphatase, katalysiert werden. Die Verfasser betrachten es als unwahrscheinlich, dasz die Synthese von freiem APP die erste Stufe des postulierten APP-Proteinsymplex ist.

11. Die Synthese des APP-Proteinsymplex wird durch viel kleinere Konzentrationen von 2-Methyl-4-Aminopyrimidyl-5-Methylaminodihydrochlorid gehemmt als die Zerlegung von freiem APP durch die Hefephosphatase.

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