

The Control Mechanism of Thiamine Biosynthesis

A MODEL FOR THE STUDY OF CONTROL OF CONVERGING PATHWAYS

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1. Thiamine or the pyrimidine moiety of thiamine added in excess to a growing culture of *Salmonella typhimurium* LT2 repressed subsequent thiamine synthesis in non-growing organisms. 2. A mutant unable to convert added pyrimidine moiety into thiamine was not repressible by the pyrimidine, showing that thiamine, not the pyrimidine, was the repressor. 3. Thiamine repression occurred at 40 μg . of thiamine/mg. dry wt. or above and de-repression occurred at 30 μg . of thiamine/mg. dry wt. or below. 4. Thiamine controlled the pyrimidine and thiazole pathways at the same concentration and to the same extent. 5. Biosynthesis of the thiazole moiety had, in contrast with biosynthesis of the pyrimidine moiety, an additional feedback inhibition control that allowed utilization of the exogenous thiazole. 6. The enzymes joining the pyrimidine and thiazole moieties were repressible by high concentrations of thiamine. 7. Thiamine was rapidly converted into thiamine pyrophosphate and this appeared to be the active repressor. 8. Theoretical aspects of control of converging pathways are discussed.

Studies described in the preceding paper (Newell & Tucker, 1966) with *Salmonella typhimurium* LT2 revealed that growth of cells in the presence of adenosine lowered the cellular thiamine concentration, which led to enhanced synthesis of thiamine. This suggested that this biosynthesis was normally controlled by thiamine repression. The technique of adenosine preincubation, by freeing thiamine biosynthesis from such repression, allowed study of the control mechanism to be made, and by applying this technique to thiazole and pyrimidine auxotrophic mutants the separate control over the formation of the two halves of the molecule could be distinguished and studied. This dual control is of particular importance in view of the problems associated with control of converging pathways of which thiamine biosynthesis presents a good example.

MATERIALS

Thiamine pyrophosphate (co-carboxylase) and triphenyltetrazolium chloride were obtained from British Drug Houses Ltd., Poole, Dorset. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks.

The following auxotrophs of *S. typhimurium* were used: mutant thi-1 (pyrimidine of thiamine auxotroph); mutant thi-10 (thiazole of thiamine auxotroph); mutant thi-28 (auxotrophic specifically for thiamine or thiamine pyrophosphate); mutant T (a tryptophan auxotroph). Mutants thi-1, thi-10 and thi-28 were kindly provided by Dr J. Childs from the collection of Dr M. Demerec. The thiamine-

tryptophan double auxotrophs thi-1/T, thi-10/T and thi-28/T were obtained by mutation of the above auxotrophs and selection of strains requiring tryptophan in addition to their original requirement for thiamine or precursors. Mutation was performed with the chemical mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (100 μg ./ml.) by the method of Adelberg, Mandel & Chen (1965).

All other materials were obtained as described by Newell & Tucker (1966).

METHODS

The thiamine assay with *Lactobacillus fermenti* 36, the pyrimidine assay, the method of adenosine preincubation and the preparation of washed-cell suspensions were as described by Newell & Tucker (1966).

Assay of the thiazole moiety of thiamine. This was a microbiological assay with the *S. typhimurium* auxotroph thi-10, which responded to thiamine or the thiazole moiety of thiamine. The organism was maintained on Oxoid nutrient agar slopes, and the cultures for use as inocula were grown from these for 18 hr. in minimal medium supplemented with 20 μg . of thiazole/ml. They were then washed three times in sterile water, diluted 1:50, and 0.1 ml. of this suspension was used per assay tube. Extraction of the thiazole from the cells and treatment with Takadiastase were carried out as described for thiamine assay with *L. fermenti* 36 (Newell & Tucker, 1966).

The assay was performed in plugged sterile tubes containing 6.5 ml. of minimal medium with assayed material, and the organism was grown aerobically in tilted tubes on a reciprocating shaker at 37° for 16 hr. Growth was measured in an EEL colorimeter (Evans Electroselenium Ltd., Halstead, Essex) with a neutral-density filter. The standard

curve used ranged from 0.2 to 6 $\mu\text{g.}/\text{tube}$. Over this range the response to the thiazole was linear. The response to thiamine was linear down to about 1 $\mu\text{g.}/\text{tube}$, below which thiamine gave scarcely any response. Since mutant thi-10 responded to either the thiazole or thiamine a correction had to be made for any thiamine present in the assayed material. Thus material containing thiamine was also assayed by a thiamine-specific assay (*L. fermenti* 36 or *S. typhimurium* thi-28) and the response of mutant thi-10 due to the thiamine present was deducted from the total response observed. Because of the very low concentrations of the thiazole present relative to the large amounts of thiamine found in cells, this assay could only be used where cells had been depleted of thiamine or the concentration of the thiazole artificially raised.

Thiamine assay with *S. typhimurium* thi-28. *S. typhimurium* thi-28 needs specifically thiamine or thiamine pyrophosphate for growth. The organism was used in addition to *L. fermenti* 36 to assay thiamine when the thiazole or pyrimidine moiety was also being assayed as it was slightly more responsive to low thiamine concentrations than was *L. fermenti* 36. Conditions for assay were the same as for mutant thi-10.

Bioautography. Bioautography (Winsten & Eigen, 1948) was used to estimate the extent and rate of phosphorylation of thiamine. A 400 ml. portion of minimal medium containing 1.75% (w/v) of agar was sterilized by autoclaving and equilibrated in a water bath at 50° for 0.5 hr. Triphenyltetrazolium chloride (60 mg. in 5 ml.) was also autoclaved and added to the medium while still warm. About 10 mg. dry wt. of bacteria as a 3 ml. aqueous suspension was rapidly warmed to 50° and inoculated into the agar, which was then poured into a sterile tray measuring 30 cm. \times 40 cm. When set a paper chromatogram was carefully placed on the agar, and the plate covered and incubated at 37° for 16 hr. The spots of growth caused by growth factors on the chromatogram were indicated by the reduction of the triphenyltetrazolium chloride to the red formazan (Usdin, Shockman & Toennies, 1954). Depletion of the organisms before use was essential, and was achieved by growing the mutants on limited amounts of thiamine until they ceased to grow, followed by washing in sterile water. Bioautography is very sensitive; about 2–5 $\mu\text{g.}$ of thiamine or its phosphorylated derivatives gave the best results. The solvent normally used for developing the chromatograms was propan-1-ol-3-methylbutan-1-ol-water-isobutyric acid-aq. 28% (w/v) ammonia (72:25:75:120:2, by vol.) (Akagi & Kumaoka, 1963).

Rapid sampling of cultures by using Millipore filters. The normal method of sampling cultures for thiamine analysis was by removal of 10 ml. from the culture, chilling in icy water and centrifuging off the cells. This was too slow where rapid events had to be measured, so, instead of centrifuging, a 5 ml. sample was filtered through a 25 mm. diam. Millipore membrane filter (type DA; V. A. Howe and Co. Ltd., London, W. 11) and the membrane rapidly transferred to a measured volume of 0.05M-sodium acetate buffer, pH 5.0, in a boiling-water bath, to stop the biosynthesis and extract the thiamine. The membrane and debris were later centrifuged off. To ensure a rapid filtration a rotary pump was used in preference to a water pump. The time from taking the sample to its being placed in boiling buffer was about 30 sec. and this was allowed for in the calculation.

RESULTS

Repression of thiamine biosynthesis by thiamine and the pyrimidine moiety

Studies with mutant T of *S. typhimurium* LT2 showed that lowering the thiamine concentration by preincubation with adenosine caused de-repression of thiamine biosynthesis in washed-cell suspensions (Newell & Tucker, 1966). This suggested that addition of excess of thiamine during such preincubation should prevent the de-repression.

The thiamine synthesized by non-growing washed-cell suspensions was determined after preincubation under various conditions (Fig. 1). The presence of added thiamine or the pyrimidine moiety during the adenosine preincubation stage prevented the subsequent synthesis of thiamine by non-growing washed-cell suspensions but the thiazole moiety did not have this effect. This

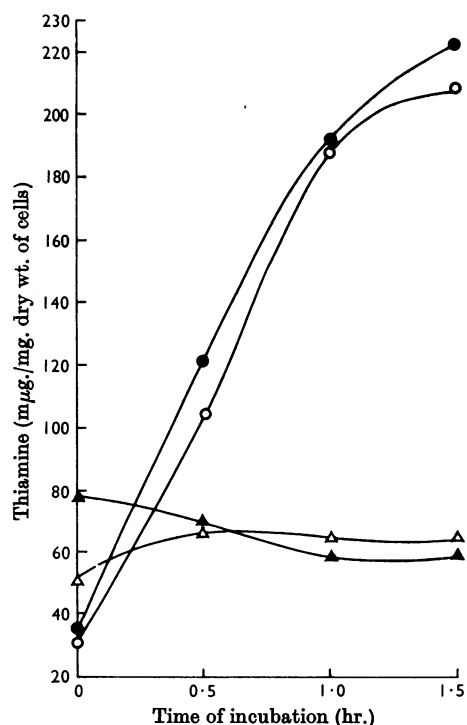


Fig. 1. Synthesis of thiamine by non-growing washed-cell suspensions of mutant T that had been preincubated for 2.5 hr. in minimal growth medium with tryptophan and the following supplements: ●, adenosine (300 $\mu\text{g.}/\text{ml.}$); ▲, adenosine (300 $\mu\text{g.}/\text{ml.}$) + thiamine (8 $\text{m}\mu\text{g.}/\text{ml.}$); △, adenosine (300 $\mu\text{g.}/\text{ml.}$) + pyrimidine moiety (8 $\text{m}\mu\text{g.}/\text{ml.}$); ○, adenosine (300 $\mu\text{g.}/\text{ml.}$) + thiazole moiety (8 $\text{m}\mu\text{g.}/\text{ml.}$).

action of thiamine and its pyrimidine appeared to be due to repression of thiamine biosynthesis rather than to inhibition of enzyme action, since if they were added during the non-growing stage they did not affect the formation of thiamine.

The point at which synthesis stopped in washed-cell suspensions was governed by the length of time elapsing since growth was arrested. The enzyme system appeared to be unstable, activity being completely lost within 2.5–3 hr.

Effect of the pyrimidine moiety. The pyrimidine, unlike the thiazole, appeared to repress thiamine biosynthesis, but this might result from one of two distinct mechanisms. The pyrimidine could either repress independently of thiamine or it could act by being converted into thiamine, the latter being the actual repressing substance. To decide between these possibilities, use was made of mutant thi-10/T, which required the thiazole moiety of thiamine as well as tryptophan for growth. This organism could not therefore convert added pyrimidine moiety into thiamine and so should indicate whether or not the pyrimidine could repress of its own accord.

The effect of adenosine preincubation on mutant thi-10/T was to cause accumulation of large amounts of the pyrimidine of thiamine by washed-cell suspensions in a similar way to the thiamine accumulation by mutant T. Addition of thiamine to the adenosine preincubation mixture prevented the accumulation of the pyrimidine by washed cells (Fig. 2). In contrast, addition of the pyrimidine had no such effect even at several times the concentration that repressed mutant T. It is concluded that the effect of the pyrimidine found with mutant T must have been indirect by conversion of the pyrimidine into thiamine and that the pyrimidine

itself has no controlling action. Adenosine inhibits the formation of the pyrimidine, so that addition of the latter during preincubation would overcome this block and allow synthesis of the normal concentration of thiamine, giving the appearance of repression by the added pyrimidine moiety.

Concentration of thiamine causing repression. In a growing culture of *S. typhimurium* thiamine biosynthesis is strictly controlled. Thiamine is not excreted into the medium and the cells normally contain about 40 $\mu\text{g./mg.}$ dry wt.

If for any reason the concentration of thiamine is lowered, as occurs for example in cells that have been at stationary phase for several hours, then when growth is restarted in fresh medium the concentration of thiamine rises to about 40 $\mu\text{g./mg.}$ dry wt. and then slowly oscillates about this value (Fig. 3, lower curve). Control must therefore operate so that biosynthesis is repressed at about 40 $\mu\text{g./mg.}$ dry wt.

The concentration at which repression is removed was found by adding a large dose of thiamine to resting cells and allowing them to grow and deplete themselves of thiamine (Fig. 3). For 2.75 hr. scarcely any thiamine was synthesized (Fig. 4). When the cellular thiamine concentration reached 30 $\mu\text{g./mg.}$ dry wt. synthesis suddenly started and the concentration rose to just over 40 $\mu\text{g./mg.}$ dry wt. (Fig. 3).

Control is thus precise and appears to operate so that the repressed and de-repressed states are between 40 and 30 $\mu\text{g.}$ of thiamine/mg. dry wt.

Control of biosynthesis of the pyrimidine and thiazole moieties by thiamine. The above experiments measure the effect of thiamine in repressing its own complete pathway. This action might,

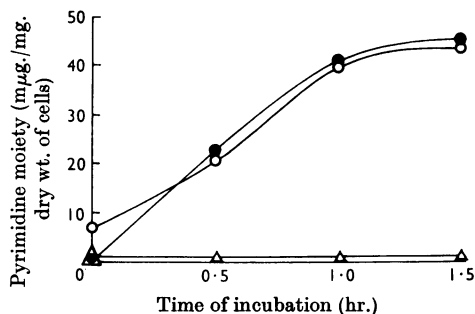


Fig. 2. Synthesis of pyrimidine moiety by non-growing washed-cell suspensions of mutant thi-10/T that had been preincubated for 2.5 hr. in minimal growth medium with tryptophan and the following supplements: ●, adenosine (300 $\mu\text{g./ml.}$); △, adenosine (300 $\mu\text{g./ml.}$) + thiamine (8 $\text{m}\mu\text{g./ml.}$); ○, adenosine (300 $\mu\text{g./ml.}$) + pyrimidine moiety (8 $\text{m}\mu\text{g./ml.}$).

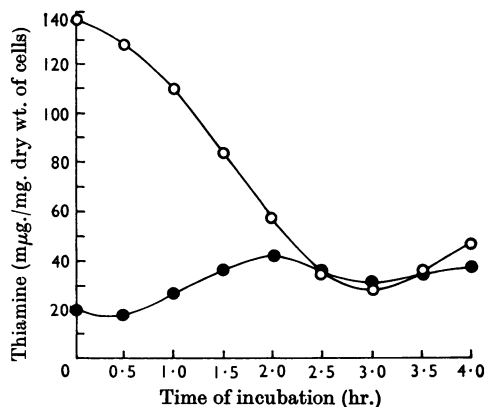


Fig. 3. Thiamine concentration of growing cultures of mutant T: ●, without additions (control); ○, with thiamine (4 $\text{m}\mu\text{g./ml.}$) added initially. The inocula were from glucose-depleted stationary-phase cultures.

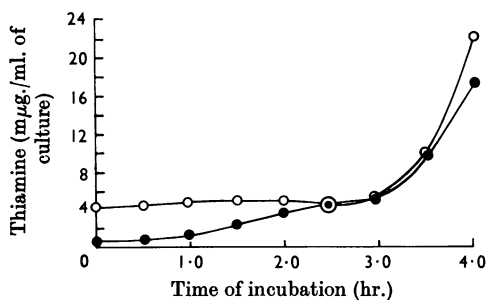


Fig. 4. Total thiamine content per ml. in growing cultures of mutant T: ●, without additions (control); ○, with thiamine (4 $\mu\text{g./ml.}$) added initially. (Results are from the same culture flasks as for Fig. 3.)

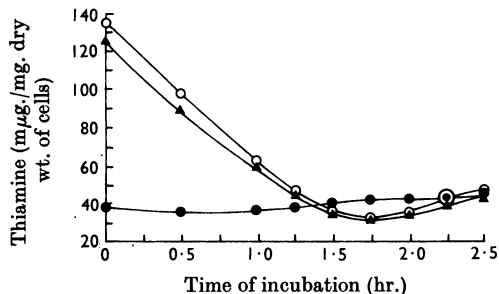


Fig. 5. Thiamine content of growing cultures of mutant T: ●, with no additions (control); ○, with thiamine (8 $\mu\text{g./ml.}$) + thiazole moiety (15 $\mu\text{g./ml.}$) added initially; ▲, with thiamine (8 $\mu\text{g./ml.}$) + pyrimidine moiety (15 $\mu\text{g./ml.}$) added initially. The inocula were exponential-phase cultures.

however, occur through only one of the two converging pathways; thus the biosynthesis of either the pyrimidine moiety or the thiazole moiety might be rate-limiting.

To test such a hypothesis an experiment was performed similar to that just described except that 300 $\mu\text{g.}$ of either the pyrimidine or the thiazole/mg. dry wt. was added in addition to the large amount of thiamine. If the two pathways were controlled at different concentrations or if one pathway were uncontrolled then the repression of thiamine biosynthesis should be released at different cellular thiamine concentrations with either the pyrimidine or the thiazole present. The results (Fig. 5) show that addition of the pyrimidine or the thiazole makes no difference at all to the system and release from repression occurs in both cases at 30 $\mu\text{g.}$ of thiamine/mg. dry wt. This implies that thiamine controls both the pyrimidine and thiazole pathways at precisely the same concentration. This was checked with adenosine-preincubated washed cells. Here again addition of either the pyrimidine or the thiazole to the washed-cell suspensions gave no alteration in the concentration of thiamine needed during preincubation to show repression of its own pathway. When sufficient thiamine was added so that at the end of the adenosine preincubation stage its concentration was just above 40 $\mu\text{g./mg.}$ dry wt., no synthesis occurred in washed-cell suspensions prepared subsequently even when the pyrimidine or the thiazole was added to them. In both types of experiment, on the other hand, addition of both the pyrimidine and the thiazole gave rapid synthesis of thiamine, showing that the reactions from the pyrimidine and the thiazole to thiamine were operating and that the two moieties were available to the cells.

If the above reasoning is correct and both the pyrimidine and thiazole pathways become de-

repressed at precisely the same thiamine concentration, then one might deduce that addition of either the pyrimidine or the thiazole to a normal growing culture would not in either case raise the concentration of thiamine. This would occur since, if only one moiety were supplied, thiamine could control its biosynthesis by means of the other moiety. Experimentally this was shown to be correct; addition of the pyrimidine or the thiazole to a growing culture did not affect the thiamine concentration.

Inhibition by the thiazole moiety of its biosynthetic pathway

When mutant thi-10, which is auxotrophic for the thiazole moiety of thiamine, was grown on a limited supply of thiamine, it produced large amounts of the pyrimidine moiety, which accumulated in the cells and in the medium. However, in a similar experiment, mutant thi-1, which is auxotrophic for the pyrimidine moiety, did not accumulate the thiazole and the concentration of this substance remained very low (about 1 $\mu\text{g./mg.}$ dry wt.), which was difficult to measure accurately. With mutant thi-28, which is auxotrophic for thiamine or thiamine pyrophosphate specifically, and which might have been expected to form both the pyrimidine and the thiazole, the pyrimidine again accumulated but not the thiazole. Moyed (1964) did not detect accumulation of any thiazole when the biosynthesis of the pyrimidine moiety was inhibited by adenosine in *Aerobacter aerogenes*.

Since the thiazole does not repress thiamine formation its ability to control its own biosynthesis is presumably due to feedback inhibition. If this is correct then the enzymes for synthesis of the thiazole should still be present even under condi-

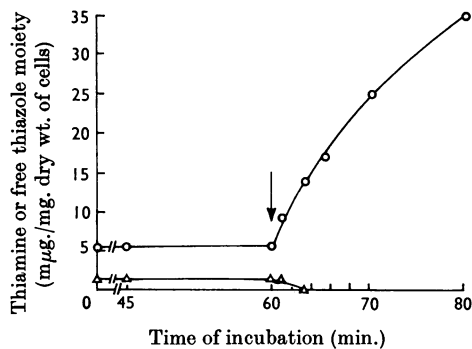


Fig. 6. Concentrations of thiamine (○) and the free thiazole moiety (Δ) in thiamine-depleted non-growing washed-cell suspensions of mutant thi-1/T. Pyrimidine moiety (100 μg./ml.) was added after 60 min. incubation as indicated by the arrow.

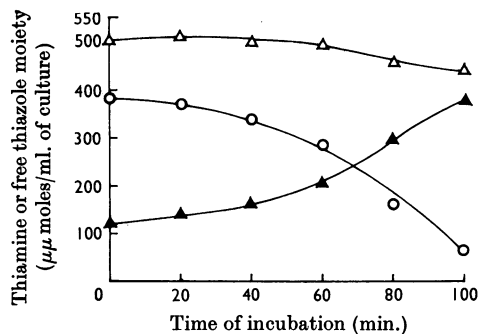


Fig. 7. Concentrations of thiamine and the free thiazole moiety in a growing culture of mutant T after adding thiazole moiety (7 mμg./ml.) to the culture medium initially. ○, Concn. of free thiazole moiety; ▲, concn. of thiamine; Δ, concn. of free thiazole moiety + thiamine.

tions where the thiazole failed to be accumulated. To confirm this, use was made of mutant thi-1/T, which is auxotrophic for tryptophan and the pyrimidine moiety of thiamine. A culture was grown until lack of the pyrimidine prevented further growth. Washed-cell suspensions were then prepared and the cells incubated in minimal medium. During the first hour the concentrations of thiamine and the thiazole did not alter (Fig. 6). After 1 hr. 100 mμg. of the pyrimidine/ml. was added to the flask and organisms were taken at short intervals thereafter for assay of thiamine and the thiazole, by using the rapid sampling technique with Millipore filters (see the Methods section). The concentration of thiamine rose immediately at maximum rate and that of the thiazole fell to zero. Since synthesis started immediately after adding the pyrimidine there was no time for synthesis of the enzymes required for making the thiazole moiety and they must have been present in an inhibited state before addition of the pyrimidine.

With such a feedback inhibition mechanism organisms should be able to incorporate the thiazole added to the culture medium and inhibit endogenous synthesis. To test this a large amount of the thiazole (70 mμg./mg. dry wt.) was added to a growing culture of mutant T with added tryptophan for growth. The results (Fig. 7) show that the thiazole concentration in the medium plus cells fell as the thiamine concentration rose, owing to growth of the culture. (The concentration of thiamine in the cells stayed roughly constant at about 40 mμg./mg. dry wt.) Expressed as μμ moles the total concentration of thiamine plus the thiazole stayed constant within experimental error. This stoichiometry indicates that the exogenous thiazole can stop further endogenous synthesis, and

supports the hypothesis that the thiazole possesses its own feedback inhibition mechanism, as without such a system the other repressive controls by thiamine would not stop endogenous synthesis in favour of the exogenous thiazole.

Lack of a feedback inhibition in biosynthesis of the pyrimidine moiety. Since the pyrimidine accumulated freely in thiazole mutants it was assumed that pyrimidine possessed no feedback inhibition system. As a result no sparing of endogenous synthesis should occur when excess of the pyrimidine was added to the growth medium. In an experiment performed concurrently with that just described for incorporation of the thiazole and under analogous conditions, but with the pyrimidine instead of the thiazole, about 80% of the added pyrimidine could be accounted for as free pyrimidine moiety at the end. Moreover, the strongly upward trend of the curve of the pyrimidine plus thiamine showed that endogenous synthesis of the pyrimidine had been only slightly inhibited by the added pyrimidine. This result, which contrasts with the results obtained with the added thiazole, confirms that biosynthesis of the pyrimidine does not possess a powerful feedback inhibition mechanism as does biosynthesis of the thiazole.

Control of the synthetase system

When the thiazole and the pyrimidine were added simultaneously to a growing culture or to a non-growing washed-cell suspension thiamine was very rapidly synthesized. Preincubation of the washed-cell suspension with adenosine gave very little or sometimes no increase in activity. It was deduced that normally in minimal media the

reactions leading from the thiazole and the pyrimidine to thiamine (referred to here as the synthetase system) are uncontrolled.

If thiamine was added to the preincubation flasks repression could be demonstrated although much larger amounts were necessary than for control of formation of the pyrimidine and thiazole moieties (Fig. 8). The control was also shown to be much less precise in the concentration of thiamine required than for control of the two moieties. Thus when the experiment described above, in which a large amount of thiamine was added to a growing culture (see Fig. 5), was performed with the thiazole and the pyrimidine also present in high concentration in the medium, no precise point was ever reached at which release of repression by thiamine could be observed. Instead a slow synthesis was noted when the thiamine concentration reached approx. $100\text{m}\mu\text{g./mg. dry wt.}$, which maintained the thiamine concentration at this level until all the pyrimidine and the thiazole had been exhausted. This occurred even though care had been taken to repress the culture thoroughly with thiamine beforehand. Moreover, the repression sometimes did not appear to be absolutely complete even at $150\text{--}200\text{m}\mu\text{g.}$ of thiamine/mg. dry wt. The rate of synthesis from the two moieties by

uncontrolled organisms is very rapid (Fig. 8) and is over twice the rate of new synthesis by de-repressed organisms. Normally the cellular thiamine concentration in minimal media does not rise much above $40\text{m}\mu\text{g./mg. dry wt.}$, so that this control mechanism would not normally be operative. However, if thiamine were present in the medium it could function to repress further endogenous synthesis.

Thiamine pyrophosphate as the active repressor

The end product of the thiamine biosynthetic pathway is thiamine pyrophosphate. Throughout this work, however, thiamine was originally used to study the control mechanism as it was considered that permeability effects might hamper any work carried out with the pyrophosphate form. Bioautography of normal growing cells showed, on the other hand, that thiamine was present wholly as the pyrophosphate. We have since repeated the above experiments (in which thiamine showed repression) with thiamine pyrophosphate, and shown that it behaves exactly like thiamine, and mole for mole has the same repressive ability. Rather surprisingly a study of the uptake of thiamine and thiamine pyrophosphate into *S. typhimurium* from the medium revealed that the rates were identical and very rapid. When $150\text{m}\mu\text{g.}$ of thiamine or thiamine pyrophosphate/mg. dry wt. of cells was added to a washed-cell suspension it was absorbed completely within 10 min. Moreover, the technique of bioautography showed that within a further 10 min. all the thiamine that was present in the cells had been converted into thiamine pyrophosphate plus a trace of thiamine monophosphate, and a few minutes later only the pyrophosphate could be found. Even in experiments in which large quantities of thiamine were added to cultures, it must soon all have been present as the pyrophosphate. Clearly thiamine pyrophosphate is the stable form of thiamine in these cells and this must be the actual repressing substance.

DISCUSSION

The thiamine biosynthetic pathway is unusual in that it possesses two converging pathways synthesizing products that do not normally accumulate, and that (as far as is known) are not required for other pathways. This presents a problem of control in addition to that of controlling output of substances required only in minute amounts.

The four main types of solution to the problem are outlined in Scheme 1. In all types the control may be repressive or inhibitive. With Scheme 1(a) each of the two pathways controls its own synthesis

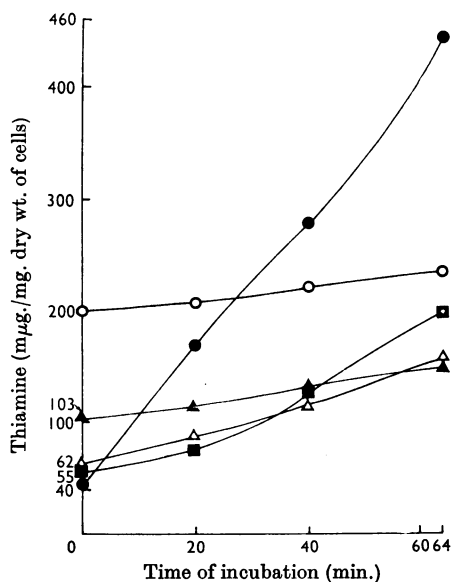
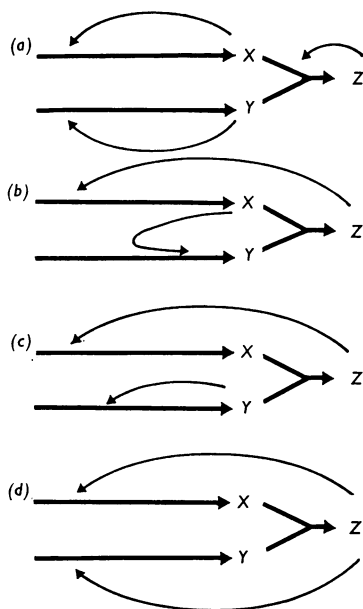


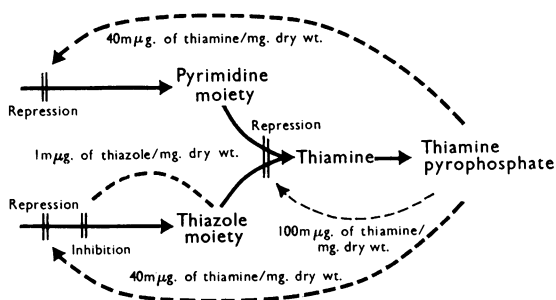
Fig. 8. Thiamine synthesized from added thiazole moiety plus pyrimidine moiety by non-growing washed-cell suspensions of mutant T, which had been preincubated for 2.5 hr. with the following supplements: ●, no supplements (control); ■, $5\text{m}\mu\text{g.}$ of thiamine/ml.; △, $10\text{m}\mu\text{g.}$ of thiamine/ml.; ▲, $15\text{m}\mu\text{g.}$ of thiamine/ml.; ○, $25\text{m}\mu\text{g.}$ of thiamine/ml.



Scheme 1. Theoretical schemes for the control of converging pathways. The thin arrowed lines indicate repression or inhibition by the intermediate products X and Y , or by the final product Z over these schematic biosynthetic pathways (thick lines). The mechanism of control by intermediate X over intermediate Y in (b) is induction or activation of the enzymes for the biosynthesis of intermediate Y .

and each creates a pool of the intermediates X and Y . Compound Z could then control the joining of intermediates X and Y and the latter would at no time accumulate more than the regulated amount. With Scheme 1(b), compound Z controls intermediate X directly and the formation of intermediate Y is dependent on induction or activation of its biosynthetic enzymes by intermediate X . Scheme 1(c) is like Scheme 1(b) in that compound Z controls intermediate X directly but intermediate Y is now self-regulating. In both Schemes 1(b) and 1(c) the end product controls only one of the converging pathways directly. With Scheme 1(d) the end product Z controls both pathways directly and if intermediates X and Y are not to accumulate the control must operate at precisely the same concentration of compound Z and to the same extent.

With the control of the thiamine pathway Scheme 1(a) cannot operate as the pyrimidine possesses little or no control of its own pathway. The experiment with mutant thi-28/T, in which large amounts of the pyrimidine accumulated while the thiazole remained controlled, showed that Scheme 1(b) cannot be functional as this mechanism



Scheme 2. Summary of the control mechanisms found for thiamine biosynthesis. The thick continuous lines represent the biosynthetic pathway. The thick broken lines represent control mechanisms operating in minimal media, and the thin broken line represents an additional control operating in thiamine-rich media.

requires that if one moiety accumulates so should the other. The actual control (summarized in Scheme 2) appears to be a combination of Schemes 1(c) and 1(d). Although the thiazole possesses its own control as in Scheme 1(c), thiamine pyrophosphate can control both pathways as in Scheme 1(d). Normally the latter would be the controlling system and the thiazole control could only operate under conditions in which the pyrimidine/thiazole ratio was altered, as for example would occur if biosynthesis of the pyrimidine were preferentially inhibited or if the thiazole were present in the growth medium. Since *S. typhimurium* normally inhabits the gut it is possible that in this environment it receives a supply of the thiazole as a thiamine breakdown product under conditions in which the pyrimidine moiety is degraded.

A problem that the organism has somehow overcome is the mechanism of one compound controlling two independent pathways at precisely the same concentration and to the same extent. One mechanism that could be employed would be to have the genes for the rate-determining enzymes of the pyrimidine and thiazole pathways in the same operon, thus allowing co-ordinate control. Such a possibility can be tested when genetic mapping is carried out with the pyrimidine and thiazole mutants.

It was noted above that the enzymes for synthesizing thiamine appear to be labile and activity is completely lost after 3hr. if growth is stopped. This effect is due to the lability of the pyrimidine- and thiazole-forming system and it is these pathways that are controlled by thiamine. If such a repression-controlled system had very stable enzymes, then the end product would fluctuate enormously in concentration. With labile enzymes a much finer control is achieved and this may be an important part of the control mechanism.

The control of the synthetase system that joins the pyrimidine and thiazole moieties differed from all the others since it could only operate where thiamine was present in the medium. The gut is an environment whose thiamine content would be expected to vary greatly according to the type of food ingested, but would inevitably contain some thiamine to support life of the host. With the powerful ability that the organism possesses for absorbing and concentrating the vitamin its cellular content could easily rise to the concentration necessary for this control to operate.

Two types of control can thus be deduced. In minimal media the thiamine pyrophosphate concentration acts as a master repressor over biosynthesis of the pyrimidine and thiazole moieties, the latter also possessing a self-regulating device in case the pyrimidine/thiazole ratio is altered in favour of excess of the thiazole. In thiamine-rich media a further control operates to prevent formation of

the now unnecessary enzymes for joining the pyrimidine and thiazole moieties.

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