THE EFFECT OF HEME ON TETRAPYRROL SYNTHESIS IN A HEME REQUIRING STAPHYLOCOCCUS AUREUS.*

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Received June 4, 1962

Staphylococcus aureus JT/52, a mutant strain derived from S. aureus Sg 511, cannot synthesize heme (Jensen and Thofern, 1953;1954). In spite of this, JT/52 grows quite well in the absence of heme. Cells grown in this fashion lack the various hematin enzymes which the parent strain possesses and which are synthesized by the mutant when heme is added to the medium. The heme requirement is quite specific: only ironporphyrins closely related to ironprotoporphyrin are utilized (Paul and Thofern 1960), free porphyrins, even protoporphyrin in the presence of ferrous iron, are not (Jensen and Thofern 1953; Lascelles 1956). Though this was taken as indirect evidence for the mutant's inability to incorporate iron into the tetrapyrrol nucleus, the synthesis of porphyrins by this strain or other heme requiring bacteria was never investigated.

JT/52 produces only minute amounts of porphyrins if grown in the absence of heme; however, in the presence of heme and under otherwise suitable conditions, porphyrins may accumulate in such quantities that the culture becomes "rusty" in color. This indicates that heme, the end product, exerts a profound influence on the biosynthesis of porphyrins by this organism. It is the purpose of this communication to demonstrate this influence and to arouse interest in heme as a possible regulator in tetrapyrrol synthesis.

* Supported by PHS research grant C-3165, National Cancer Institute.

METHODS: 30 ml brain heart infusion broth (BBL) were inoculated with strain JT/52 and incubated in 125 ml flasks in a water bath shaker. Incubation time and temperature were varied: 2-24 hrs and 31 - 39 °C. After centrifugation the supernatant fluids and the sediments were analysed for porphyrins. Representative samples of the resuspended sediments were dried to constant weight; parallel samples were used for catalase, and oxygen-uptake measurements. The porphyrins were extracted and purified according to well-established procedures (Schwartz et al.1960). Cyclohexanone was used for the extraction of uroporphyrin (URO) (Dresel et al. 1956). URO was estimated according to the method of Rimington and Sveinsson 1950; and Rimington 1960. Paperchromatography, absorptionspectra, and HCl numbers served to identify the isolated porphyrins; A - measurements at the Soret peaks were used for quantitative estimations.

RESULTS: Under "standard conditions" that is, 16 hrs incubation at 37°C in broth supplemented with 2.0 µg heme* and 2.5 mg δ - amino levulinic acid - HCl (ALA) per 30.0 ml of medium, the following distribution of porphyrins is found: with the exception of small amounts of URO** (2-5% of the total), only coproporphyrin III (COPRO) is detectable in the supernatant fluid. 70 - 80% of COPRO accumulates as Zn-complex, the remaining 20-30%, as free COPRO. In one batch of the medium approximately 50% of the metal-COPRO was not fluorescent, not extractable with concentrated HCl from ethyl acetate, and was later identified as Cu-COPRO III. Subsequently, trace amounts of this complex were always found. The reason for its greatly increased proportional occurrence in the one batch of medium has not been elucidated as yet. Coproporphyrinogen as estimated by the differential iodine method is found in small and variable amounts (up to 1%). Porphobilinogen, 2-carbox-

* as hemin from a frozen stock solution with constant biological activity (Jensen 1961).

** Isomere type of URO not yet established.

ylporphyrins, and iron-porphyrin complexes could not be detected under any of the conditions investigated.

After 8-10 hrs incubation, rapidly increasing amounts of porphyrins can be extracted from the cells. In the sediment the proportion is reversed: 90% of the porphyrin is URO, 10% COPRO III.

The data summarized in Table I show: 1) small amounts of COPRO are produced in the absence of heme, 2) in the absence of heme ALA does not affect porphyrin synthesis, 3) heme enhances the synthesis significantly, and 4) in the presence of heme, ALA greatly stimulates porphyrin-formation. Furthermore, protoporphyrin is completely inactive in the presence and in the absence of ALA.

Table I

Effect of heme (H) and/or ALA on COPRO III accumulation in supernatant fluid of 16 hr JT/52 cultures. Unless otherwise stated, incubation temperature: 37°C. For growth conditions, see text.

Addition to 30 ml broth	Remarks	COPRO III produced (μg) Total	per mg bacteria	Total bacterial dry weight (mg)
2.0μg H + 2.5mg ALA	not inoculated	0.00	0.00	0.0
NONE		0.51	0.03	19.3 (1)
2.5mg ALA		0.60	0.03	21.1
2.0μg H		7.70	0.12	65.0 (2)
2.0μg H + 2.5mg ALA		33.60	0.52	65.0 (3)
2.0μg protoporphyrin		0.63	0.03	20.0
2.0μg proto + 2.5mg ALA		0.68	0.03	21.5
2.0μg H + 2.5mg ALA	31°C	2.80	0.05	60.5
same	33	28.40	0.38	75.0 (4)
same	37	52.50	0.66	79.5 (4)
same	39	62.00	0.80	78.0 (4)

(1), (2), (3) average values of 11, 16, and 21 experiments respectively, all others of at least 5

(4) parallel runs under identical conditions, except incubation temperature. A new batch of medium was used, giving higher porphyrin- and dry weight yields.

The importance of the incubation temperature, as suggested in Table I, is more apparent in Fig. 1. The significant differences in porphyrin synthesis can hardly be explained by the slight difference in growth rates at the two temperatures. The temperature seems to have a direct effect upon porphyrin synthesis. Preliminary experiments with temperatures higher than 37°C which result in still higher yields (Table I) appear to substantiate this assumption.

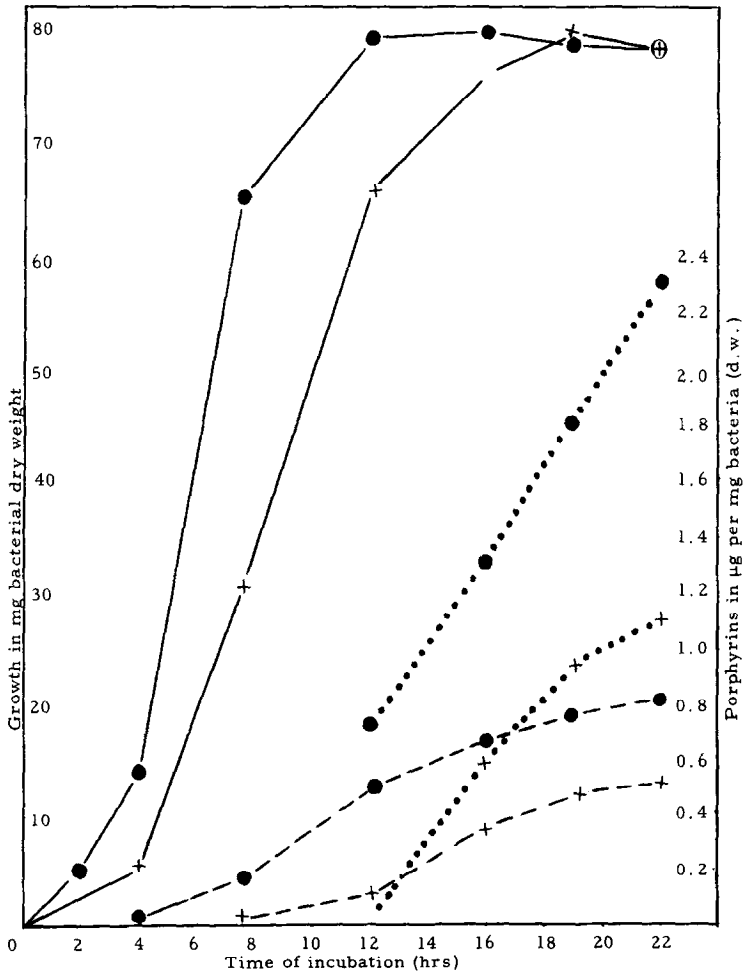

Fig. 1. Growth curves of JT/52 in aerated brain heart infusion broth, and corresponding porphyrin production; - ● - at 37°C; - + - at 33°C. Solid line: growth; dashed line: COPRO III in supernatant fluid; dotted line: URO in sediment.

As the heme concentration is increased to 2.0 μg per 30 ml broth, an enhancement in total growth and in porphyrin synthesis (per mg bacteria) is observed. Higher heme concentrations, however, have little effect on growth, enhance O_2-uptake, but appreciably depress porphrin synthesis. A representative experiment (Table II) illustrates this relationship.

Table II

COPRO III (supernate) and QO_2 in JT/52 after 7 hrs aerobic incubation at 37°C. (a) One initial addition of 2.0μg heme; (b) One initial addition of 4.0μg and three subsequent additions of 2.0μg heme each in two hr. intervals.

	COPRO III (μg/mg bact.)	QO_2(1)	QO_2(2)	Total bact. (mg)
a)	0.21	56	114	69
b)	0.13	97	95	73

(1) Based on O_2 consumption of washed cells in buffer-glucose
(2) Same as (1) but buffer-glucose supplemented with excess heme (2.0μg/ml)

Exact data regarding the concentration-dependence are difficult to procure since the concentration of available heme is constantly changing in a growing culture. Further investigations with the aid of a "chemostat" are planned.

DISCUSSION: The biological system under consideration is extremely complex. Individual enzymes of the synthetic sequence have not been isolated, and attempts to design an adequate synthetic medium have been unsuccessful so far. Therefore, an interpretation of these preliminary results is bound to be speculative. The stimulating effect of heme on porphyrin synthesis is not incompatible with its role in a negative feedback control mechanism (Lascelles 1960; Burnham 1962). The endproduct inhibition found by these authors concerns ALA synthetase, whereas the stimulation obviously occurs later in the biosynthetic chain, at a level beyond ALA synthesis. Since small amounts of porphyrins are formed in complete absence of heme, it seems unlikely that one of the enzymes in the chain is a heme enzyme. Furthermore, isolated

enzymes of the sequence (Granick 1958; Granick and Mauzerall 1958; Mauzerall and Granick 1958) do not seem to exhibit the characteristics of heme enzymes. Serious objections can be raised against an inducer mechanism. It seems to be quite possible, however, that the heme effect is essentially non-specific; that it is due to the pronounced change in metabolic possibilities following the incorporation of heme. Experimental evidence (Table II) is not in favor of this view since it indicates that optimum stimulation is observed under conditions of relative heme starvation.

These considerations resulted in the following working hypothesis: The heme apoenzymes, synthesized by the mutant in the absence of heme (Jensen and Thofern 1953), act as inhibitors at the level of uroporphyrinogen synthesis (competing for porphobilinogen?). As heme combines with the apoenzyme in a terminal synthetic step (Jensen 1957) the inhibition is released and more porphyrin (and heme) is produced. Heme supplied (or synthesized) in excess of apoenzyme combining sites would then depress porphyrin synthesis via end-product inhibition operative at the same level and/or the level of ALA synthetase.

Whether or not this working hypothesis proves successful, it is obvious that in the system under consideration tetrapyrrol synthesis is closely regulated by its endproduct. Any one of the many possible disturbances of this regulation must result in dysfunction of the biosynthetic sequence.

ACKNOWLEDGMENT: I wish to thank Dr. Samuel Schwartz and his coworkers and Drs. S. Granick and D. Mauzerall for their advice and the liberal donations of reference materials.

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