cillin it is apparent that 2 or 3 or possibly more injections would be required to maintain a comparable blood concentration over the same period.

Fig. 2 shows the units of penicillin per ml of blood

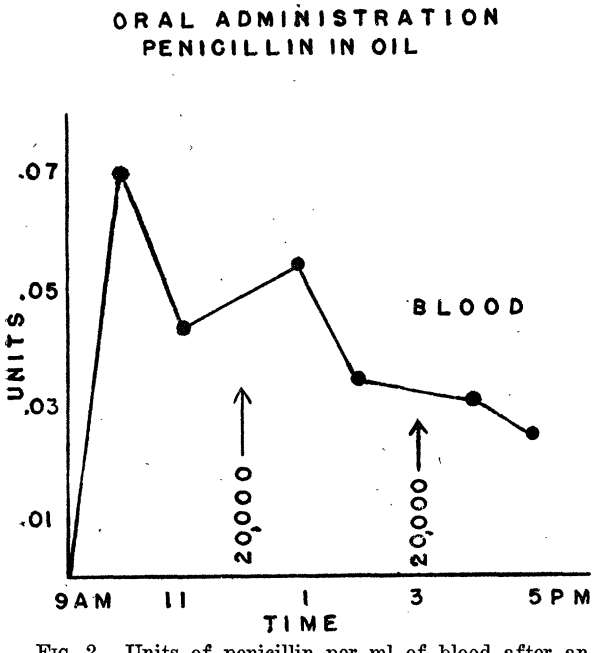

FIG. 2. Units of penicillin per ml of blood after an initial oral dose of 90,000 units and two subsequent doses of 20,000 units of penicillin in oil given three and six hours later.

obtained after the oral administration of 90,000 units of penicillin in oil and two subsequent doses of 20,000 units each given 3 and 6 hours later to a human being. As indicated, a therapeutic blood level was maintained for a period of at least seven hours and only slightly less than a therapeutic level as previously defined was found after eight hours.

It is apparent that even with the oral administration of penicillin in oil a portion is inactivated probably by the gastric acidity, so that one would expect that optimum blood levels would be obtained if the dose were given on an empty stomach. This has been found to be true in dogs and is illustrated in a human in Fig. 2. The first subsequent dose of 20,000 units was given on an empty stomach with a consequent 25 per cent. increase in the penicillin blood level. The second dose of 20,000 units was given approximately one hour after a heavy meal, no apparent increase in the penicillin blood level being observed.

It should be noted that although these preliminary results indicate that a greater amount of penicillin might be required if administered orally than if by intramuscular injection, this increased use of penicillin will probably be offset by several factors. In this regard certainly the greater ease of administration both from the point of view of the doctor as well as the patient must be considered. Furthermore, for oral use less highly refined penicillin should be entirely satisfactory, thus simplifying the present procedures for the production of suitable material.

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RIBOFLAVIN PRODUCTION BY CANDIDA SPECIES

THE synthesis of relatively large quantities of riboflavin (vitamin B_2) by the yeast *Candida guilliermondia* (Northern Regional Research Laboratory strain No. 488) during cultivation in a synthetic medium has been reported by Burkholder.[1] In a second communication[2] the same investigator has described the influence on riboflavin formation of such factors as the concentration and type of carbohydrate, nitrogen and inorganic salts in the medium. For example, it was noted that whereas a riboflavin yield of 75 μg per ml was obtained in the basal medium containing 2 per cent. glucose, the use of maltose, xylose or galactose in place of glucose resulted in a marked inhibition of riboflavin synthesis, although growth was abundant. Similarly, it was found that urea, when employed at a concentration of 0.5 g per liter, enhanced riboflavin production, while in larger concentrations (2.0 to 4.0 g per liter) it was inhibitory to the synthesis of this vitamin. Yeast extract also was shown to promote growth while inhibiting riboflavin production.

These results were confirmed in our laboratory, where it was found that riboflavin formation was extremely variable, ranging from 2 to 80 μg per ml under seemingly identical conditions; whereupon a thorough study was undertaken of the influence of each of the constituents of the medium upon vitamin synthesis. From these studies it was learned that riboflavin production was greater and that higher yields were obtained more consistently, when the trace elements (B, Mn, Zn, Cu, Mo and Fe) were omitted from Burkholder's medium. When the medium was treated with 8-hydroxyquinoline in the manner described by Waring and Werkman[3] to remove Fe, Cu, Zn, Mn, etc., higher riboflavin yields resulted. However, when each of the latter elements was added to a medium extracted with 8-hydroxyquinoline, only

[1] P. R. Burkholder, *Proc. Nat. Acad. Sci.*, 29: 166, 1943.
[2] *Idem., Arch. Biochem.*, 4: 217, 1944.
[3] W. S. Waring and C. H. Werkman, *Arch. Biochem.*, 1: 303, 1943.

Fe inhibited riboflavin production. The striking effect of small concentrations of iron is demonstrated in the following experiment.

The medium was prepared by dissolving in 200 ml of distilled water 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2.0 g urea, 2.0 g aspargine and 40 g glucose. About 10 mg of 8-hydroxyquinoline dissolved in 2 ml of chloroform was added to the medium, and the mixture was shaken vigorously in a separatory funnel. The funnel was allowed to stand, and the chloroform which settled out was withdrawn. Then the medium was treated similarly with chloroform alone. This alternate treatment with 8-hydroxyquinoline in chloroform and chloroform alone was repeated until the chloroform layer was colorless. This is essentially the method of Waring and Werkman, whereby they reduced the iron content of media to 0.07 to 0.3μg per 100 ml. Twenty ml portions of the extracted medium, adjusted to pH 5.0, were placed in 500 ml Erlenmeyer flasks previously cleaned with aqua regia and then 80 ml of water, triply-distilled to remove the iron, was added to each flask. One tenth of one μg of biotin was added to each flask and $FeSO_4 \cdot 7H_2O$ was added to give various concentrations up to 50 μg of iron per 100 ml of medium. After being sterilized at 126° C. for 15 minutes, the flasks were cooled and inoculated with a suspension of yeast cells which had been washed by centrifugation from triply distilled water. The weight of cells in the fermented medium and the potency of the riboflavin in the cell-free liquor were determined after an incubation period of seven days at 30° C., during which the cultures were shaken continuously. The effect of iron on growth and riboflavin formation by two strains of *Candida guilliermondia*[4] and one strain of *Candida flareri*[4] is shown in Table 1. Riboflavin was determined by spectrophotometer. Results on culture filtrates by this method were found to be in good agreement with those obtained by fluorometric and microbiological procedures.

The results show that, when the medium was practically free of iron, both cell proliferation and riboflavin synthesis were retarded. However, the iron concentration required for maximum riboflavin formation was much lower than that needed for maximum growth. When the basal medium was supplemented with iron to raise the concentration 0.5 to 1.0 μg per 100 ml, the highest riboflavin yields were obtained. At this level, the cell crop attained by *C. flareri* was approximately 40 per cent. of that formed at the highest iron level, and that attained by *C. guilliermondia* was 80 per cent. of that formed at the highest iron level. The addition of 10 μg of iron per 100 ml sharply reduced the riboflavin synthesis, while it enhanced the yeast growth. *C. flareri*, although equally sensitive to iron, synthesized considerably more vitamin than did either of the *C. guilliermondia* strains. Although riboflavin formed by *Clostridium acetobutylicum* in cereal grain mashes is stimulated by the presence of small quantities of iron[5] and inhibited by larger quantities,[6] the concentrations involved are much greater than those described here. From experiments performed in this laboratory, the optimum iron concentration for synthesis of riboflavin by *Cl. acetobutylicum* has been found to be approximately one μg per ml, which is 100 times greater than the optimum for the Candida species.

TABLE 1

EFFECT OF IRON CONCENTRATION UPON GROWTH AND RIBOFLAVIN SYNTHESIS BY CANDIDA SPECIES

Culture	Iron added μg Fe/100 ml	Dry wt. of yeast g/100 ml	Dry wt of cell-free residue g/100 ml	Riboflavin synthesized μg/ml	Riboflavin synthesized μg/g of dry cells	Riboflavin synthesized μg/g of dry cell-free residue
Candida guilliermondia NRRL 488	0.0	0.27	0.45	108.0	40,000	24,000
	0.5	0.57	0.67	123.0	21,570	18,350
	1.0	0.66	0.56	120.0	18,180	21,420
	10.0	0.89	0.26	7.2	810	2,770
	50.0	0.92	0.19	3.2	350	1,670
Candida guilliermondia NRRL 324	0.0	0.21	0.37	107.0	50,950	28,700
	0.5	0.67	0.33	125.0	18,660	37,900
	1.0	0.61	0.33	157.0	25,740	47,600
	10.0	0.89	0.35	16.5	1,850	4,720
	50.0	0.82	0.19	10.6	1,290	5,580
Candida flareri NRRL 245	0.0	0.42	1.30	195.0	44,430	15,000
	0.5	0.49	0.75	216.0	44,080	28,880
	1.0	0.55	0.72	216.0	39,270	30,100
	10.0	1.12	0.28	8.9	740	3,180
	50.0	1.31	0.52	4.1	310	788

Although the above experiment was performed with a synthetic medium similar to that described by Burkholder, it has been found that various sugars and many organic and inorganic nitrogen compounds reported to inhibit vitamin synthesis[1,2] can be employed in media for the production of riboflavin by Candida species if proper adjustment is made of the iron level of the medium.

These studies, as well as the investigation of various procedures for the regulation of iron concentration in semi-pilot plant fermentations, will be reported in detail elsewhere.

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[4] These cultures were obtained from Dr. L. J. Wickerham, Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill.

[5] A. Saunders and L. S. McClung, *Jour. Bact.*, 46: 575, 1943.

[6] C. F. Arzberger, U. S. Patent 2,326,425, 1943.

[7] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.