3. The synergistic effect of choline and inositol on liver lipids has been confirmed.

4. No evidence of any effect of choline, inositol or biotin upon the absolute amount of phospholipin or of free cholesterol in the liver has been obtained in this series of experiments, nor has any effect upon kidney lipids been observed.

5. The absence of a specific effect of biotin on the deposition of liver fat necessitates a revision of certain theories and deductions, two of which have been discussed. It is recommended that the term 'biotin fatty liver' be abandoned.

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The Antagonism of some Antimalarial Drugs by Riboflavin

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An approach to the chemotherapy of malaria from the point of view of antagonism of essential growth factors presents the difficulty that the requirements of essential factors by the malaria parasites are unknown. However, as a large number of drugs has been tested for antimalarial activity, it was thought that a study of the essential metabolites antagonized by them, with reference to an organism more easily handled *in vitro* than the malaria parasite, might throw some light on the mode of action of antimalarial drugs.

Riboflavin was selected for initial work as bearing some structural resemblance to the acridine antimalarials (Fig. 1), and *Lactobacillus casei* ϵ as the test organism because it requires an extraneous supply of riboflavin for its growth. Of a number of substances tested as riboflavin inhibitors, only those which are antimalarials have now been shown to be antagonized by riboflavin.

EXPERIMENTAL

Organism. *Lactobacillus casei* ϵ is known to require an extraneous source of riboflavin for its growth and was used as test organism.

Medium. The medium used was essentially that described by Snell & Strong (1939) for the quantita-

tative assay of riboflavin. It contains: photolyzed NaOH-treated peptone 0.5%, glucose 1%, Na acetate 0.6%, cystine 0.01%, inorganic salts and a riboflavin-free yeast supplement.

In the present work Evans's peptone (Evans, Sons, Lescher and Webb) was used instead of Difco Bactopeptone, and the riboflavin-free yeast supplement prepared as follows: Marmite (100 g.) in water (150 ml.) was treated with basic Pb acetate (250 ml. 10%) and filtered. The clear filtrate was made alkaline (pH 10) with ammonia and the resulting precipitate discarded. The Pb salts were removed with H₂S, the PbS filtered and the H₂S removed by a current of air. Five ml. of this clear solution were used for each 250 ml. medium.

The medium prepared in this way produces a very slight, but definite, growth of *L. casei*. Addition of riboflavin up to 0.05 μ g./ml. caused an increase of growth, with no further increase with larger quantities of riboflavin. The growth was measured turbidimetrically.

Method for observing the effect of riboflavin on the growth inhibitory action of some drugs

Double-strength medium, prepared according to Snell & Strong (1939), was adjusted to pH 6.8. Two parts of this medium were mixed with one part of

riboflavin solution (100 µg./ml. in 0.02M acetic acid). Two further parts of the original medium were mixed with one part of a more dilute solution of riboflavin (1 µg./ml.). After sterilizing in the autoclave, both media were bulk inoculated with a 24 hr. old culture of *L. casei* (0.1 ml./1000 ml.) in a medium containing 2.5 µg./ml. riboflavin. Such cultures for inoculum were prepared weekly from stock cultures of *L. casei* which were carried in a stab culture in agar yeast water glucose medium. Both stock and inoculum cultures were kept in the refrigerator.

Suitable consecutive falling twofold dilutions of the drug to be assayed (in 0.5 ml.) were placed in parallel series of tubes. To one of these series 1.5 ml. of the bulk inoculated medium, rich in riboflavin, was added. The other received the same amount of the medium with less riboflavin. After incubation for 18–24 hr. at 37° an inspection for whether or not growth had taken place was made. In dubious cases an experiment was repeated, but instead of twofold dilutions of the drug closer dilutions were used, usually the concentration differing by a factor of 0.7.

RESULTS

Table 1 shows a typical experiment using Mepacrine and *dl*-pantoyltaurine as antagonists of growth promoted by riboflavin.

Table 1. *Typical experiment in which Mepacrine and dl-pantoyltaurine were compared as riboflavin antagonists*

	μg./ml. riboflavin	Conc. of drug (mg./ml.)				
		0.32	0.16	0.08	0.04	0.02
		Growth				
Mepacrine	2.5	—	+	+	+	+
	0.25	—	—	—	+	+
<i>dl</i> -Pantoyltaurine	2.5	—	—	tr.	tr.	+
	0.25	—	—	tr.	tr.	+

+ Denotes visible growth; — no detectable growth; tr. just enough growth to produce a sediment which, when dispersed in the medium, did not render it appreciably turbid.

Table 2 shows the concentration (mg./ml.) in the last tubes to show growth in media containing 2.5 or 0.25 µg./ml. of riboflavin.

To observe the effect of concentrations of riboflavin between 0.25 and 2.5 µg./ml., media containing suitable amounts of this substance were bulk inoculated and added to dilutions of the drug in question, as previously described. Table 3 shows the results obtained when the drug was 3349. With quinine, Mepacrine, 2666 and 3502 the results obtained were similar, differing only in the concentration of drug required to suppress the growth.

Table 2. *Antagonism of some drugs by riboflavin*

	Maximal concentration of substance (mg./ml.) at which growth of <i>L. casei</i> was observed. Concentration of riboflavin (µg./ml.)	
	2.5	0.25
Quinine	1.75	0.6
Mepacrine (Atebrine)	0.25	0.06
Propamidine	0.1	0.02
2666	1.22	0.29
3349 } See Fig. 1	2.5	0.42
3502	0.06	0.02
Methylene blue	0.01	0.0034
Proflavine	0.012	0.012
5-Aminoacridine	0.024	0.024
Crystal violet	0.16	0.16
Chloramine T	0.25	0.25
Formalin	0.001	0.001
Salicylic acid	1.25	1.25
Mercuric chloride	0.004	0.004
Penicillin (units/ml.)	1.2	1.2
Sodium fluoride	0.06	0.06

Table 3. *Antagonism of 3349 (see Fig. 1) by riboflavin*

3349 (mg./ml.)	Riboflavin (µg./ml.)									
	25	12.5	6.2	3.1	1.5	0.7	0.36	0.18	Nil	
0.12	+	—	—	—	—	—	—	—	—	
0.096	+	—	—	—	—	—	—	—	—	
0.076	+	—	—	—	—	—	—	—	—	
0.058	+	—	—	—	—	—	—	—	—	
0.047	+	+	+	—	—	—	—	—	—	
0.038	+	+	+	tr.	—	—	—	—	—	
0.030	+	+	+	+	+	+	+	+	tr.	

Signs have same significance as in Table 1.

DISCUSSION

Lack of success in the *in vitro* culture of the malaria parasites makes it very difficult to know its nutritional requirements and biochemical make-up. However, it appears a reasonable assumption that in these metabolic processes the same coenzymes are involved as in the metabolism of other organisms. Which of the components of these coenzymes are synthesized by the parasites themselves and on which they are dependent of an external supply is not known. The antagonism by some chemotherapeutic agents of essential metabolites which may play a role in enzyme systems has been given as an explanation of the mode of action of these drugs. The similarity of the shape of the molecule of the antagonizing agent to the metabolite appears in some cases to be the reason for the action of the drug. The similarity of the formal structure of riboflavin with that of some antimalarial drugs, such as Mepacrine (atebrine), 2666, 3349 and 3502 (Fig. 1) has already been pointed out (Curd, Davey & Rose, 1944).

The examination of these and other antimalarial drugs as riboflavin antagonists using *L. casei* as a

test organism shows (Table 2) that the growth inhibitory action of these drugs is, to some extent, counteracted by riboflavin. The effect is much more marked at a relatively high concentration of ribo-

whether the action of drugs which antagonize riboflavin is in preventing the synthesis of a coenzyme or a competition with the coenzyme for the apoenzyme.

Riboflavin

Mepacrine

2666

3502

3349

Fig. 1. Formulae of some of the chemical substances mentioned.

flavin. This is different from the sulphanilyl drugs—*p*-aminobenzoic acid and the pantoyletaurine—pantothenic acid antagonisms, where the antagonistic drug exerts its action over a wide range of concentrations of the essential factors.

It is difficult to picture the precise mode of the antagonism of the antimalarial drugs by riboflavin. Riboflavin has to combine with other low molecular substances before becoming a coenzyme. At the moment there is not enough evidence to decide

SUMMARY

Of a number of substances tested as riboflavin inhibitors, only those which are antimalarials were antagonized by riboflavin, using *L. casei* ϵ as test organism.

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