# LETTERS TO THE EDITORS

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## New Components of the Vitamin B Complex

AN attempt to prepare a concentrate of folic acid from liver by the method of Hutchings *et al.*[1] resulted, as already noted in a recent review[2], in the preparation of a fraction probably containing folic acid, together with another fraction soluble in chloroform, which likewise stimulated the growth of certain micro-organisms. A second chloroform-soluble fraction with similar properties has now been obtained. All three of these fractions stimulate the growth of *Lactobacillus helveticus* and *Streptococcus lactis* R 8082, but not that of *Lactobacillus arabinosus* 17/5. Although the growth-promoting properties of the chloroform-insoluble fraction are probably due to folic acid, the chloroform-soluble fractions are believed to contain new factors not previously described.

At first, an alcohol precipitate obtained as a by-product in the manufacture of commercial liver extracts was used as the starting material for the preparation of these fractions; but later, it was found more convenient to prepare the chloroform-soluble factors from the liver extract ('Examen') itself, manufactured according to the method of Laland and Klem[3]. This was subjected to the following fractionation procedure: extraction with chloroform at *p*H 3, concentration of the washed extract *in vacuo* to dryness; solution of the residue in water; adsorption on 'Decalso' at *p*H 4·5; elution with hot 10 per cent sodium chloride solution; extraction of the eluate with phenol at *p*H 3; and transference of the activity to water by addition of ether.

The eluate and the combined 'Decalso' filtrate and washings were each concentrated *in vacuo* to the original volume of the 'Examen'. In this way, two fractions were separated, an eluate and a filtrate factor.

Both concentrates stimulated the growth of *L. helveticus* and *S. lactis* R when added in place of a folic acid concentrate, but in different ways. With *L. helveticus*, the presence of both factors produced a synergistic effect, whereas with *S. lactis* R, the effect was additive. The concentrates were added at levels of 0·5, 1·0 and 2·0 and occasionally at 4 ml./10 ml. to a basal medium which was a modification of the basal medium described by Landy and Dicken[4]. At levels of 2–4 ml./10 ml. of medium, the filtrate factor alone appeared to be a complete substitute for folic acid for both organisms, but a mixture of the filtrate and eluate factors could replace folic acid at much lower levels (0·1–0·5 ml./10 ml. of medium). In the case of *S. lactis* R the effect appeared to be purely additive, but with *L. helveticus* the effect was greater than the sum of the growth-stimulating effect of each. Two typical results are given below, expressed as the amount of standard alkali necessary to neutralize the acid produced:

*L. helveticus*. Incubation for 48 hr. at 37°.

Chloroform-soluble filtrate factor

| ml. concentrate in 10 ml. medium | Blank | 0·5 | 1·0 | 2·0 |
|---|---|---|---|---|
| ml. 0·1 *N* NaOH .. .. | 0·1 | 2·1 | 4·5 | 6·8 |

Chloroform-soluble eluate factor

| ml. concentrate in 10 ml. medium | Blank | 0·5 | 1·0 | 2·0 |
|---|---|---|---|---|
| ml. 0·1 *N* NaOH .. .. | 0·1 | 0·9 | 0·9 | 0·9 |

Chloroform-soluble filtrate + eluate factors

| ml. concentrates in 10 ml. medium | Blank | 0·1 + 0·1 | 0·5 + 0·5 |
|---|---|---|---|
| ml. 0·1 *N* NaOH .. .. | 0·1 | 2·6 | 4·5 |
| | | 1·0 + 1·0 | 2·0 + 2·0 |
| | | 8·3 | 9·9 |

The synergistic effect of the two factors together is clear from these results.

*S. lactis* R incubated for 72 hr. at 30°.

Chloroform-soluble filtrate factor

| ml. concentrate in 10 ml. medium | Blank | 0·5 | 1·0 | 2·0 |
|---|---|---|---|---|
| ml. 0·05 *N* NaOH .. .. | 0·1 | 2·0 | 2·8 | 5·4 |

Chloroform-soluble eluate factor

| ml. concentrate in 10 ml. medium | Blank | 0·5 | 1·0 | 2·0 |
|---|---|---|---|---|
| ml. 0·05 *N* NaOH .. .. | 0·1 | 1·4 | 1·9 | 2·7 |

Chloroform-soluble filtrate + eluate factors

| ml. concentrates in 10 ml. medium | Blank | 0·1 + 0·1 | 0·5 + 0·5 |
|---|---|---|---|
| ml. 0·05 *N* NaOH .. .. | 0·1 | 2·1 | 3·2 |
| | | 1·0 + 1·0 | 2·0 + 2·0 |
| | | 6·3 | 8·2 |

Here the influence of the two factors is apparently additive.

Neither of the chloroform-soluble factors was destroyed by nitrous acid, by acetylation with acetic anhydride in sodium hydroxide solution (at room temperature), or by benzoylation with benzoyl chloride and sodium hydroxide solution.

The chloroform-soluble factors appear to be different from folic acid and indeed from other similar factors described in the literature, which are all reported to be insoluble in the common organic solvents, except glacial acetic acid.

The full experimental evidence will be published in due course.

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June 13.

[1] *J. Biol. Chem.*, **141**, 521 (1941).
[2] NATURE, **153**, 478 (1944).
[3] *Acta Med. Scand.*, **88**, 620 (1936).
[4] *J. Lab. Clin. Med.*, **27**, 1086 (1942).

## An 'Incomplete' Antibody in Human Serum

A STUDY of the properties of mixtures of different types of human anti-*Rh* sera has led to the recognition of what appears to be an incomplete antibody. The research arose out of a suggestion by Prof. R. A. Fisher that this technique might throw some light on the problem of antibody absorption.

Human anti-*Rh* serum of the type called by Wiener "standard" agglutinates red cells of the gene $Rh_1$ and also those of $Rh_2$. 'Anti-$Rh_1$' serum agglutinates the former cells but not the latter[1,2]. If, however —and this was the observation that started the present work—cells of the genotype $Rh_2Rh_2$ or $Rh_2rh$ are added to a mixture of these two sera, the expected agglutination due to the standard anti-*Rh* serum does not occur. It was then found that the sera need not be mixed, for if the $Rh_2$ cells are suspended in anti-$Rh_1$ serum—which causes no agglutination—and after a few minutes are separated from the serum, washed and re-suspended in saline, then these treated cells can no longer be agglutinated by standard anti-*Rh* serum.