and packed cell volume began on the first day of B_{12}–L-glutamic acid treatment. In our opinion, this rise occurred too early to have been an actual response to B_{12}–L-glutamic acid treatment. It appears more likely to have been an effect of the crystalline vitamin B_{12} therapy given during the preceding control period. On the other hand, we admit that the second reticulocyte response following B_{12}–L-glutamic acid treatment indicated that L-glutamic acid promotes the absorption of vitamin B_{12} to some extent at least.

Our results with Schilling tests, however, indicate that L-glutamic acid does not in general increase the absorption of vitamin B_{12}, or at least not to anywhere near the same extent as the intrinsic factor preparation. Accordingly, we do not find ourselves in agreement with Heathcote and Mooney and we cannot deny the existence of an "intrinsic factor".

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[1] Mooney, F. S., and Heathcote, J. G., *Brit. Med. J.*, i, 232 (1961).
[2] Heathcote, J. G., and Mooney, F. S., *Second European Symp.*, Hamburg, 1961, 540 (Enke, Stuttgart, 1962).
[3] Heathcote, J. G., and Mooney, F. S., *Nature*, **193**, 380 (1962).

The authors of the preceding communication state that "According to them [Heathcote and Mooney], vitamin B_{12} is absorbed as either small peptide or amino-acid units". We trust that this misinterpretation of our actual beliefs is due to lingual difficulty and does not imply that we believe in the peptide or amino-acid nature of vitamin B_{12} itself.

Dr. Heinivaara and Dr. Palva state further that the Schilling test is "suitable for investigation of the supposed promoting effect of L-glutamic acid on the absorption of vitamin B_{12}". It is unfortunate that they do not give their reasons for this statement because the significance of their own work depends entirely on the validity of this assumption. We should particularly like to know why they think that this test—which actually measures the urinary excretion of radioactive vitamin B_{12}—necessarily gives a true measure of the amount absorbed? Can they demonstrate—as the Schilling test presumes—that all forms of vitamin B_{12} when absorbed into the blood stream are incorporated into the stores and blood-forming tissues at an equal rate? Furthermore, can the "flushing dose"—assuming that it dislodges some radioactive B_{12} from the serum—also release that which has been actively incorporated into the liver and blood-forming tissues? Unless they can produce such evidence, their own results can be equally well interpreted simply as demonstrating an improved retention or an increased "resistance to flushing" of radioactive B_{12} in the presence of glutamic acid. Do they also believe that the Schilling test is a better index of therapeutic efficiency than the type of test which we have used, whereby hæmatological progress is assessed? Has it in fact any physiological merit at all?

With reference to our work, Dr. Heinivaara and Dr. Palva "admit that the second reticulocyte response following B_{12}–L-glutamic acid treatment indicated that L-glutamic acid promotes the absorption of vitamin B_{12} to some extent at least". They go on to state that "In both their cases the rise in hæmoglobin and packed cell volume began on the first day of B_{12}–L-glutamic acid treatment. In our opinion, this rise occurred too early to have been an actual response". Examination of the figures in our paper will show, however, that in case 49 (Fig. 1) neither the hæmoglobin nor the packed cell volume was estimated during the first four days of treatment with B_{12}–L-glutamic acid mixture, and that in case 52 (Fig. 2) a fall in both hæmoglobin and packed cell volume was recorded in the first estimation performed after the commencement of B_{12}–L-glutamic acid treatment.

Finally, it may be of some interest to mention, briefly, the present state of health of the two patients who formed the basis of our communication. Both are still on the same form of therapy, are well, and have no neurological symptoms. Case 49 has now been maintained for 973 days on an average daily dose of 28 μg of the mixture (expressed as B_{12} content). At present his hæmoglobin is 91 per cent and packed cell volume 40 per cent. Case 52 has been maintained for 885 days on an average daily dose of 24 μg of B_{12}–glutamic acid mixture. His hæmoglobin is 107 per cent and packed cell volume 44 per cent. A third patient (case 62) has also been treated with B_{12}–glutamic acid, since our earlier publication. He is a man aged seventy-five who had, on admission, a hæmoglobin of 52 per cent and packed cell volume of 25 per cent. He has been treated for 639 days with an average daily dose of 16 μg of the mixture. At present his hæmoglobin is 95 per cent and packed cell volume 40 per cent.

In conclusion, although we have no particular desire to undermine any faith which they may hold in "intrinsic factor", might we suggest to Dr. Heinivaara and Dr. Palva that a critical re-appraisal of the value of the Schilling test, as a basis for their belief, might not be entirely without profit?

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Increase of Trypsin Inhibitor in Serum during Pregnancy

In normal pregnancy increased quantities of trypsin inhibitor are excreted in the urine, especially during the second and third trimester, followed by a rapid decrease to normal after parturition[1].

Excretion of trypsin inhibitor seems a response to stress, since it is increased after various forms of stress and after administration of ACTH or glucocorticoid hormones[2,3]. The amount excreted appears to be regulated by the concentration of glucocorticoids (endogenous or exogenous) in the body[3,4]. Hence, it is also believed that the increased trypsin inhibitor excretion observed in pregnancy is produced by an increase in glucocorticoid production.

The physiological mechanism of the excretion of trypsin inhibitor is unknown, but we have found that in certain forms of stress (surgical) the increase in trypsin inhibitor excretion parallels an increase in trypsin inhibitor concentration in blood serum, suggesting that variations in trypsin inhibitor excretions reflect variations in serum concentration of the same inhibitor[5]. In order to substantiate this concept we wish to report an increase of trypsin inhibitor concentration in serum during pregnancy, corresponding in pattern to the variations in urinary trypsin inhibitor excretion[1] and in serum concentration of glucocorticoid hormones[6].

The estimation of trypsin inhibitor in serum was performed as before[5]. One trypsin inhibitor unit represents the amount which inhibits 1 μg of a preparation of crystalline, pure trypsin containing 24·4 Anson units per g (kindly supplied by the NOVO Laboratories, Copenhagen).

Blood samples were obtained from 11 normal women and from 47 women with normal pregnancies of different durations. Blood was centrifuged immediately after spontaneous coagulation, and serum was stored at −8° C until analysed.

The results (Table 1) show great individual variations in the serum concentration of trypsin inhibitor. This was also encountered in the amount excreted in the urine during 24 h (ref. 1). Thus, in the non-pregnant group the serum concentration ranged from 300 to 1,965 trypsin inhibitor units per ml. with an average value of 1,140 units per ml. (*S.E.*, 134). In the pregnant groups the deviations tended to be slightly less. The difference between the average concentration in the first trimester