A preparation of the acetylated nucleotide was carried out using TDP-[^{14}C]-glucosamine. Residual TDP-glucosamine and the mixture of TDP-*N*-acetylglucosamine and TDP-*N*-acetylgalactosamine were isolated by column chromatography. The sugar from the TDP-glucosamine was chromatographed by the method of GARDELL and found to contain no galactosamine. The sugars from the TDP-*N*-acetylamino-sugar peak, after deacetylation, were chromatographed in the same way. The radioactivity was located in 2 peaks corresponding to galactosamine and glucosamine; the ratio of glucosamine:galactosamine was 7:3.

The acetylating system in the crude sonic extract will acetylate glucosamine-1-*P* at a rate 60% of that of TDP glucosamine. Glucosamine, galactosamine and glucosamine-6-*P* are not acetylated. Thus, the enzyme differs in specificity from the glucosamine-6-phosphate-*N*-acetylase of baker's yeast[8]. We have been unable to detect the formation of TDP-acetylglucosamine from acetylglucosamine-1-*P* and TTP.

The problem of whether the reaction of TTP with glucosamine-1-*P* is catalyzed by the same enzyme as the reaction of TTP with glucose-1-*P* is also being studied. Fractionation of the enzyme with $(NH_4)_2SO_4$ followed by adsorption and elution from calcium phosphate gel yielded fractions in which the ratio of activities of TDP-glucose pyrophosphorylase to TDP-glucosamine pyrophosphorylase varied from 10:1 to 2:1 suggesting that two separate enzymes are involved.

Work is now in progress to purify and separate the enzymes involved in these reactions. The finding of amino-sugars linked to deoxyribosylthymine provides an alternate series of nucleotides which should be considered in addition to the well known uridine series as possible precursors of amino-sugar-containing polysaccharides.

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The effect of fluoroacetate on cyanocobalamin absorption in the rat

There seems little doubt that cyanocobalamin, when administered in physiological amounts, is absorbed by some process involving active transport. Evidence has already been advanced that an enzyme reaction is probably concerned[1]. With this in view, it seems reasonable to assume that energy is required for absorption of the

vitamin, and that this could in some way depend upon the availability of ATP. We have, therefore, administered sodium fluoroacetate to test animals with results that seem conclusive.

Female rats of the Scott–Russ strain, 3-months old and weighing approx. 200 g, were used throughout. The test and control groups each consisted of 14 animals. 160 μg of sodium fluoroacetate dissolved in 0.4 ml of 0.9% NaCl were administered intraperitoneally to each of the test animals, and 0.4 ml of the saline alone was administered by the same route to each of the controls. 2 h after the injection each animal was given 15 mμg of radioactive cyanocobalamin dissolved in 1.0 ml of distilled water. The dose was administered by means of a stomach tube at approx. 11.30 a.m.; the animals had received no food or drink after 6 p.m. on the previous day. 24 h after the administration of the vitamin, each animal was killed by a blow on the back of the neck, and its intestinal tract completely removed after previous clamping at both ends. Care was taken that bleeding occurred into the carcass. The carcass was now dissolved as completely as possible in 100 ml conc. HNO_3 with heating, and the total volume then reduced by evaporation to approx. 80 ml. The material was quantitatively transferred to a counting bottle, the volume made up to 120 ml with water and after thorough mixing, its radioactivity was determined in the scintillation counter. By comparison with the radioactivity of a standard solution of radioactive cyanocobalamin it was possible to determine the percentage of the original dose which was now present in the carcass and which could therefore be considered as a measure of absorption of the vitamin. The test animals gave a mean percentage absorption of 21.6 ± 2.5 and the control animals 30.6 ± 1.98. The difference was statistically significant; Student's t test gave a P value somewhat less than 0.01. The fluoroacetate must have significantly depressed absorption.

Since it was possible that the effect could have been due to interference with gastro-intestinal motility, three further control animals and three test animals were treated in a similar fashion to the above, but the stomach, the small intestine and large intestine were separately removed after previously clamping their ends so that the contents could not escape. The results demonstrated that relatively small quantities of the vitamin remained in the stomach and small intestine after 24 h in either group and that there was no significant difference. There was somewhat more cyanocobalamin remaining in the large intestine in the test animals than in the controls (mean values 22% and 13%, respectively), but the difference was not significant.

There still remained the possibility that the fluoroacetate reduced absorption by some effect on the mucosal cells themselves. This could be due to such reasons as toxicity or local circulatory phenomena. We have therefore undertaken studies related to the uptake of cyanocobalamin by the four different quarters of the intestine. The technique employed was essentially that already described. The animals were, however, slaughtered at various times after administration of the vitamin. The small intestine was removed, and cut into four equal segments. Each of these was cut open along its whole length, washed several times with 0.9% saline and then homogenised in 10 ml saline for determination of radioactivity, from which measurement the percentage of the original dose of the vitamin now present in the mucosa of each segment could be determined. Our results are illustrated in Fig. 1, in which each point represents the mean of at least four observations. No difference in uptake has been found in relation to the first, second and fourth quarters, but a significant de-

pression of uptake by fluoroacetate has been observed in relation to the third quarter. It would be very strange for a toxic or circulatory phenomenon to affect this part of the intestine and not the remainder. There seems little doubt also that it is the third quarter of the small intestine in the rat which is most actively concerned with

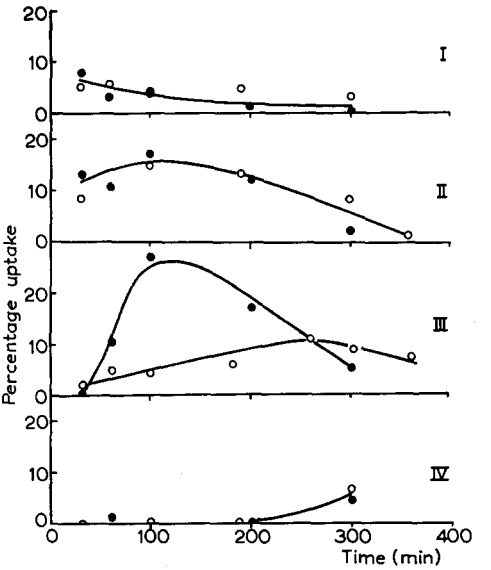

Fig. 1. Percentage uptake of cyanocobalamin by each of the four segments of the small intestine in both test (○) and control (●) animals.

cyanocobalamin absorption[2,3], and here again the activity of fluoroacetate would seem to be selective. It seemed unnecessary to prolong our studies for a period of time longer than 6 h, since at this stage cyanocobalamin uptake of the third portion of the small intestine of the fluoroacetate-treated animals had also already reached a maximum. It is interesting to note that in both test animals and the control group the sum of the intestinal mucosal uptakes of the second and third quarters approximated quite closely to the amounts of cyanocobalamin absorbed in the 24-h experiment.

We can only conclude, therefore, that there is a strong possibility that the absorption of cyanocobalamin in the rat is in some way linked with the formation of ATP or some other nucleoside triphosphate. This could be involved at the level of mucosal uptake from the intestine or during the release from the mucosa to the circulation. That the former is a possibility is indicated in Fig. 1, whether or not the latter is involved awaits further investigation.

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