a kinetic current. Full analysis[8], using methods very similar to those of MEITES AND MOROS[9], will be described in a separate publication.

The evidence given above proves conclusively that there is no hydride of cobalamin at neutral pH. This is consistent with the absence (unpublished observations) of a metal hydride resonance in the NMR spectrum of B_{12s} solutions. Thus the reactions of B_{12s} should be written as those of a strong nucleophil, cobalt(I). We cannot prove that in a biological system where solvent conditions may be very different from those in aqueous solution, the hydride is not formed by addition of a proton. However, this point is easily checked by reference to the spectrum of B_{12s} which is unchanged from pH 5 to pH 14 in water. Below pH 5 the cobalt might be protonated but the product is not stable since the reaction $B_{12s} + H^+ \rightarrow B_{12r} + \frac{1}{2} H_2$ is observed. We have found that controlled potential reduction of B_{12a} in *N,N*[1]-dimethylformamide, an aprotic solvent, does give B_{12s} having the same spectra as that in water.

The fact that the spectrum of B_{12s} is so very unlike the spectra of other Co(III) derivatives of vitamin B_{12} can now be explained. Complications in the spectra due to charge transfer transitions from cobalt(I) to the ring could well arise. It is known[10] that cobalt(I) gives rise to extremely low-energy charge-transfer transitions in dimethylglyoxime and phthalocyanine complexes.

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1 R. BONNETT, *Chem. Rev.*, 63 (1963) 573.
2 K. BERNHAUER, O. MÜLLER AND F. WAGNER, *Angew. Chem. Intern. Ed. Engl.*, 3 (1964) 200.
3 R. BURNETT, H. P. C. HOGENKAMP AND R. H. ABELES, *J. Biol. Chem.*, 241 (1966) 1483.
4 S. L. TACKETT, J. W. COLLAT AND J. C. ABBOTT, *Biochemistry*, 2 (1963) 919.
5 H. A. O. HILL, J. M. PRATT AND R. J. P. WILLIAMS, *Chem. Ind. London*, (1964) 197.
6 J. W. COLLAT AND J. C. ABBOTT, *J. Am. Chem. Soc.*, 86 (1964) 2308.
7 G. C. HAYWARD, H. A. O. HILL, J. M. PRATT, N. J. VANSTON AND R. J. P. WILLIAMS, *J. Chem. Soc.*, (1965) 6485.
8 P. K. DAS, H. A. O. HILL, J. M. PRATT AND R. J. P. WILLIAMS, in preparation.
9 L. MEITES AND S. A. MOROS, *Anal. Chem.*, 31 (1959) 23.
10 P. DAY, G. SCREGG AND R. J. P. WILLIAMS, *Biopolymers Symp.*, 1 (1964) 271.

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Synthesis of α-L-fucose-1-phosphate and guanosine diphosphate-α-L-fucose

During the course of experiments designed to study red blood cell metabolism, it became desirable to have available GDP-β-L-fucose. Although attempts to synthesize this compound were not successful, the synthesis of the α anomer was accomplished. This communication describes the synthesis of α-L-fucose-1-phosphate by

slight modifications of MACDONALD's[1] procedure and the synthesis of GDP-α-L-fucose using a micro modification[2] of the procedure described by ROSEMAN *et al.*[3]. During the course of these experiments, the authors became aware that LEABACK, HEATH AND ROSEMAN[4] previously reported having synthesized α-L-fucose-1-phosphate, however, the synthesis of GDP-α-L-fucose appears not to have previously been reported.

Synthesis of α-L-fucose-1-phosphate. The synthesis of α-L-fucose-1-phosphate was performed by reacting α-L-fucose tetraacetate[5] (14.5 mmoles) with crystalline phosphoric acid (65 mmoles) (MATHESON, COLEMAN AND BELL) at 50° for 8 h *in vacuo*. The reaction mixture was dissolved in 30 ml of tetrahydrofuran and this solution was added to 260 ml of cold 1 M LiOH solution. The mixture was allowed to come to room temperature, stirred overnight and filtered. The amount of fucose-1-phosphate in the filtrate was assumed to be equivalent to the amount of acid hydrolyzable phosphate (0.5 M H_2SO_4, 100°, 10 min). Acid hydrolyzable phosphate was determined by subtracting the amount of inorganic phosphate measured by the LOWRY AND LOPEZ[6] procedure (without prior acid hydrolysis) from the amount of inorganic phosphate measured by the FISKE–SUBBAROW procedure[7] after acid hydrolysis*. The difference in the amount of phosphate in the filtrate obtained by these two assays indicated that there was a 30 % yield of crude fucose-1-phosphate. The filtrate was passed through a column containing the cyclohexylammonium form of Dowex 50-X8 resin to remove lithium and to convert fucose-1-phosphate to the cyclohexylammonium salt. After washing the column with water until all of the acid-labile phosphate (0.5 M H_2SO_4, 100°, 10 min) was eluted, the eluate was lyophilized. The dry residue was extracted with isopropylalcohol (about 200 ml) and the residue was dissolved in a minimum volume of water. Crystallization of the dicyclohexylammonium salt of fucose-1-phosphate was induced by the addition of absolute alcohol. It was recrystallized twice from approx. 80 % alcohol. The product (1.47 g) was obtained in a 20 % yield. 2 % of the phosphate content of the product was inorganic phosphate. To remove most of the inorganic phosphate, 0.66 g of the product was treated with ammoniacal magnesium acetate[1]. The mixture was filtered and the filtrate was passed through a column of the cyclohexylammonium form of Dowex 50-X8 as described above. The combined eluate and water wash was concentrated to a syrup *in vacuo* and crystallized twice from 80 % alcohol. The product (0.275 mg of white needlelike crystals) contained only 0.12 % inorganic phosphate. This highly purified preparation of fucose-1-phosphate was used in further characterization experiments.

Elemental analysis on the dicyclohexylammonium salt of fucose-1-phosphate performed by the Galbraith Laboratories showed, in %, C, 48.69; H, 9.08; N, 6.13; P, 6.80 compared to the calculated values of C, 48.86; H, 8.88; N, 6.33; P, 7.00. The ratio of fucose[8] to acid-labile phosphate to reducing sugar[9] was 1.02:1.00:0.93. The molar rotation of a 3.99 % (w/v) aqueous solution of the product was determined at 25° using a Rudolph Model 80 polarimeter and was found to be −33156°. This value agrees favorably with the value −34400° reported by LEABACK, HEATH AND

* Under the highly acidic conditions of the FISKE–SUBBAROW[7] inorganic phosphate assay procedure fucose-1-phosphate undergoes hydrolysis to fucose and inorganic phosphate at such a rate that the estimation of inorganic phosphate in the presence of L-fucose-1-phosphate would be difficult or impossible. Under the milder conditions of the LOWRY AND LOPEZ procedure[6] (pH 4) fucose-1-phosphate is stable, therefore, this procedure can be used to assay inorganic phosphate in the presence of fucose-1-phosphate.

ROSEMAN[4] for α-L-fucose-1-phosphate. In addition, NMR analysis of a sample of our preparation of fucose-1-phosphate and of a sample of α-L-fucose-1-phosphate kindly supplied by Dr. ROSEMAN were identical. The NMR analyses were conducted on the lithium salt of fucose-1-phosphate in ^2H_2O. The anomeric proton absorption was at 5.89 ppm relative to an external standard of tetramethylsilane. The anomeric proton absorption of biosynthetically prepared β-L-fucose-1-phosphate has been reported to be 5.23 ppm (ref. 10). From the polarimetric and NMR data it was concluded that the product was the α anomer of the dicyclohexylammonium salt of fucose 1-phosphate.

O'BRIEN[11] has reported a modification of MACDONALD's synthesis in which a mixture of the α and β anomers of *N*-acetylglucosamine-1-phosphate was obtained. By increasing the ratio of crystalline phosphoric acid to pentaacetyl-α-D-glucosamine to 8:1 and the reaction temperature from 50° to 83° he obtained a maximum yield (33 %) of a 2:3 mixture of α and β anomers of acetylglucosamine-1-phosphate. In our hands, reaction of crystalline phosphoric acid and the α-tetraacetate[5] of fucose in a 4:1 ratio at 83° *in vacuo* for 45 min, followed by manipulation of the sample as described above (except that treatment with ammoniacal magnesium acetate was omitted) resulted in a 38 % yield of dicyclohexylammonium fucose-1-phosphate. The molar rotation of the product at 20° was −33, 319° which indicated that the product was the α anomer. Had an appreciable amount of the β anomer been formed, a less negative rotation would have been expected.

Synthesis of GDP-α-L-fucose. GDP-α-L-fucose was synthesized on a micro scale[2] by condensing GMP morpholidate[12] with the tri-*n*-butyl-ammonium salt of α-L-fucose-1-phosphate. The morpholidate (139 μmoles) and sugar phosphate (65 μmoles), contained in separate arms of the reaction vessel, were dried as previously described[2], that is, by alternately condensing dry pyridine in each arm of the vessel, followed by evaporating the pyridine *in vacuo* five times. Approx. 2 ml of dry pyridine were introduced into each arm of the reaction vessel. The solution of the sugar phosphate and suspension of the morpholidate were thoroughly mixed and then the volume of the solvent was reduced *in vacuo* to approx. 2 ml. The reaction vessel, still under vacuum, was sealed and the reaction was allowed to proceed at 37° for 3 days. The vessel was opened and its contents were suspended in water and filtered. The filtrate was applied to Whatman 3MM chromatography paper in a narrow band and developed with ethanol–1 M ammonium acetate pH 7.5 (7:3)[13]. The ultraviolet-absorbing band (254 mμ) containing GDP-fucose (R_{GMP} 1.7) was well separated from the ultraviolet-absorbing compounds having the mobility of GMP morpholidate (R_{GMP} 4.1) and an unidentified compound which moved slower than GMP. In some preparations a compound with a migration rate slightly greater than GDP fucose (R_{GMP} 1.9) was observed. This could be completely removed from GDP-fucose by rechromatography in the same solvent. After elution of the band containing GDP fucose with water, its concentration was estimated spectrophotometrically assuming it has the same molar absorption coefficient as GMP (ref. 13). It was obtained in a final yield of 18 to 26 % (three experiments). In a similar experiment except that GMP morpholidate and the tri-*N*-butylammonium α-fucose-1-phosphate were reacted in dry pyridine for 10 days at 37°, GDP fucose was obtained in only 5 % yield.

Several properties of GDP-α-L-fucose were examined. It exhibited an absorption spectrum typical of guanosine nucleotides at pH 1, 7 and 11. The ratio of guanosine:

acid labile phosphate (0.5 M H_2SO_4, 100°, 10 min):total phosphate[7]:fucose[8] in the product was 1.05:0.87:1.92:1.00. The synthetic GDP-α-L-fucose co-chromatographed with GDP-β-L-fucose (prepared enzymatically from GDP-mannose by the procedures of GINSBURG[14]) on thin-layer cellulose chromatography using isobutyric acid:1 M NH_4OH (10:6) as solvent. Examination of the ultraviolet-absorbing (254 mμ) acid hydrolysis products by thin-layer chromatography using polyethyleneimine-impregnated cellulose[15] and 1 M LiCl as solvent yielded the following results: (a) hydrolysis at pH 2, 100°, 10 min completely destroyed the GDP-fucose and yielded primarily GDP and some GMP; (b) continued heating at pH 2 for 30, 60 and 90 min resulted in a progressive increase in the amount of GMP and progressive decrease in the amount of GDP; (c) hydrolysis in 1 M HCl, 100°, 10 min yielded primarily guanine.

The method of synthesis as well as the analytical data and acid hydrolysis products indicate that the product is GDP-α-L-fucose.

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1 D. L. MACDONALD, *J. Org. Chem.*, 27 (1962) 1107.
2 J. H. NORDIN, W. L. SALO, R. D. BEVILL AND S. KIRKWOOD, *Anal. Biochem.*, 13 (1965) 405.
3 S. ROSEMAN, J. J. DISTLER, J. G. MOFFATT AND H. G. KHORANA, *J. Am. Chem. Soc.*, 83 (1961) 659.
4 D. H. LEABACK, E. C. HEATH AND S. ROSEMAN, *Abstr. 147th Meeting Am. Chem. Soc., 1964*, p. 25D.
5 G. A. LEVVY AND A. MCALLAN, *Biochem. J.*, 80 (1961) 433.
6 O. H. LOWRY AND J. A. LOPEZ, *J. Biol. Chem.*, 81 (1946) 629.
7 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
8 Z. DISCHE AND L. B. SHETTLES, *J. Biol. Chem.*, 175 (1948) 595.
9 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
10 H. ISHIHARA, D. J. MASSARO AND E. C. HEATH, *Federation Proc.*, 25 (1966) 526.
11 P. J. O'BRIEN, *Biochim. Biophys. Acta*, 86 (1964) 628.
12 J. G. MOFFATT AND H. G. KHORANA, *J. Am. Chem. Soc.*, 83 (1961) 649.
13 *Circular OR-10 P-L Biochemicals*, Milwaukee, 1956.
14 V. GINSBURG, *J. Biol. Chem.*, 235 (1960) 2196.
15 K. RANDERATH AND E. RANDERATH, *J. Chromatogr.*, 16 (1964) 111.

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