potassium by another cation, possibly in combination with a different solvent, may overcome this problem.

The synthesis of the internucleotide bond by this new method is fast and specific. Pyrophosphate formation under these conditions has not been detected, and the absence of protecting groups for the amino groups in the three bases will simplify purification of intermediates in a stepwise synthesis. Starting components for the synthesis of oligodeoxyribonucleotides can be prepared by existing procedures or modifications of these. We hope that the synthesis of oligodeoxyribonucleotides in strong anhydrous base will be a good alternative for the existing methods in nucleic acid chemistry.

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28 October 1969

Fabry's Disease: Alpha-Galactosidase Deficiency

Abstract. *The leukocytes of male patients with Fabry's disease are deficient in α-galactosidase. The α-galactosidase activity in the leukocytes of female carriers of the disease is 15 to 40 percent of the amount present in normal leukocytes. The activities of β-galactosidase, β-acetylgalactosaminidase, and β-acetylglucosaminidase in the leukocytes of affected individuals are normal.*

The metabolic defect in Fabry's disease (*1*) is a deficiency of the enzyme ceramide trihexosidase, which catalyzes the cleavage of galactose from ceramide trihexoside. Consequently, the latter substance accumulates in various tissues. Generalized G_{M1} gangliosidosis is due to a β-galactosidase (E.C. 3.2.1.23) deficiency (*2*, *3*) which results in the accumulation of a ganglioside with a terminal β-D-galactose moiety. While in generalized gangliosidosis the deficiency can be demonstrated with a chromogenic aryl substrate, no such substrate appeared suitable for the detection of the enzyme defect in Fabry's disease. Indeed, it has been suggested (*4*) that the only substrate that can be hydrolyzed by this enzyme is the ceramide trihexoside itself. Many related β-galactosides have no inhibitory effect on the ceramide trihexosidase assay (*4*), and the trihexoside did not influence the brain methylumbelliferyl-β-galactosidase activity (*5*). My data indicate that leukocytes from patients with Fabry's disease are totally deficient in the nonspecific α-galactosidase (E.C. 3.2.1.22) activity. The enzymic defect can now be revealed with chromogenic aryl substrates in almost every other sphingolipidosis, especially in Gaucher's disease (*6*), in generalized gangliosidosis (*2*, *3*), in the usual (*7*) and the exceptional (*8*) form of Tay-Sachs disease, in metachromatic leucodystrophy (*9*), and in fucosidosis (*3*).

The finding (*10*) that a ceramide trihexoside with a terminal α-D-galactose occurs in cancer tissue led to the idea that this substance might also be present in normal tissues and that the glycolipid accumulation in Fabry's disease might be due to the absence of a nonspecific α-galactosidase. Because human leukocytes contain a number of hydrolytic enzymes it was of interest to determine the activity of acid-α-galactosidase in the leukocytes of normal individuals and of patients with Fabry's disease.

Homogenates of purified leukocytes were prepared by the method of Kampine *et al.* (*11*). The activity of β-galactosidase, β-acetylgalactosaminidase, and β-acetylglucosaminidase (E.C. 3.2.1.30) were assayed with fluorogenic methylumbelliferyl substrates; α-galactosidase activity was measured colorimetrically with *p*-nitrophenyl-α-galactoside and fluorimetrically with methylumbelliferyl-α-galactoside as substrate. Both methods gave similar results. The incubation mixtures for the different enzyme assays included: (i) for β-galactosidase, 0.2 μmole of methylumbelliferyl-β-D-galactoside, 6 μmole of acetate buffer (*p*H 4.0), and 20 μl of leukocyte extract in a final volume of 200 μl; (ii) for β-galactosaminidase, 0.6 μmole of methylumbelliferyl-2-acetamido-2-deoxy-β-D-galactoside, 15 μmole of acetate buffer (*p*H 4.0), and 20 μl of leukocyte extract in a final volume of 200 μl; (iii) for β-glucosaminidase, 0.5 μmole of methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucoside, 15 μmole of acetate buffer (*p*H 5.0), and 10 μl of leukocyte extract

Table 1. Enzyme activities in leukocytes of control subjects and of patients with Fabry's disease. The values expressed are nanomoles of substrate hydrolyzed per hour per 10^6 cells. Numbers in parentheses indicate the range of values.

Subjects	Kinship	Age (yr)	β-Galactosidase (mean)	β-Acetylglucosaminidase (mean)	β-Acetylgalactosaminidase (mean)	α-Galactosidase	
						Colorimetric (mean)	Fluorimetric (mean)
		Normal males (n = 15)					
		15–33	76 (27–153)	295 (139–446)	71 (45–128)	13 (6–25)	12 (7–17)
		Fabry's disease, affected males					
L. G.		31	110	433	105	< 0.5	< 0.2
Mo. A.		16	71	485	114	< 0.5	< 0.2
Mu. A.	Brother of Mo. A	9	122	1220	173	< 0.5	< 0.2
		Fabry's disease, female carriers					
L. D.	Sister of L. G.	26	60	346	50	2.4	1.8
R. C.	Mother of L. G.	53	68	378	54	5.6	5.0
Fa. A.	Sister of Mo. A.	11	48	526	96	4.2	3.9
Na. A.	Sister of Mo. A.	6	56	1000	112	3.8	4.9

in a final volume of 100 μl; (iv) for α-galactosidase: colorimetrically, 6 μmole of *p*-nitrophenyl-α-D-galactoside, 20 μmole of acetate buffer (*p*H 4.5), and leukocyte enzyme extract in a final volume of 500 μl; and fluorometrically, 1 μmole of methylumbelliferyl-α-D-galactoside, 15 μmole of acetate buffer (*p*H 5.0), and leukocyte extract in a final volume of 200 μl. All assays were done at 37°C. At the end of the incubation time (2 hours for α-galactosidase, 30 minutes for the other three enzymes), the mixtures were diluted to 3 ml with 0.1*M* diaminoethane buffer (*p*H 10.5). The extinction of the *p*-nitrophenol at 410 nm and the fluorescence of the methylumbelliferone (excitation, 360 nm; fluorescence 450 nm) could then be measured. The activity of the four enzymes from leukocytes of 15 normal males and of patients (from two families) with Fabry's disease is shown in Table 1.

The α-galactosidase activity was absent from the male patients' white cells, whereas the activity of the other three lysosomal enzymes was normal or higher than normal. Mixing a leukocyte homogenate of a control with that of a patient showed that an inhibitor was not responsible for the diminished activity in the patients' white cells. These findings support the assumption that the accumulated glycolipid in Fabry's disease has a terminal α-galactose residue. In fact, the exact conformation—α or β—of the glycosidic bonds in the accumulated ceramide trihexoside is difficult to establish and, to our knowledge, has not been determined.

Because the disease is transmitted by a sex-linked recessive gene, the α-galactosidase activity was measured in the leukocytes of other members of both families. Four females, presumed to be carriers, showed reduced enzyme activities (Table 1). This assay should therefore provide a simple and reliable method for the diagnosis of Fabry's disease in the clinical and in the carrier state.

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3 October 1969; revised 19 December 1969

Transformation of Tetramitus Amebae into Flagellates

Abstract. *Methods have been developed for controlling the transformation of* Tetramitus rostratus, *a protozoan able to reproduce either as an ameba or as a flagellate. An essential condition for transformation is a low concentration of oxygen in the environment.*

The amebo-flagellate *Tetramitus rostratus* can reproduce stably in either of two distinct phenotypes. For 70 years after the organism was discovered in 1852 (*1*), it was known only as a flagellate. Flagellates of *T. rostratus* have four anteriorly directed flagella, and a characteristic shape which includes a cytostomal groove and a beaklike rostrum. The flagellates, which reproduce by binary fission, have been unhesitatingly classified among the Mastigophora. Then in 1922 Bunting (*2*) found an ameba, whose appearance and movement was similar to many small (10 to 20 μm) amebae, which reproduced by binary fission, and which would be placed in the class Sarcodina. Bunting's amebas, however, could transform into flagellates that were indistinguishable from *T. rostratus*. Although the ability of this organism to alternate between two very different but stable phenotypes offers numerous opportunities for study, *T. rostratus* remains little known. One reason for this is that it has been impossible to control the phenotypic changes.

There have been four previous studies of conditions influencing the ameba-to-flagellate transformation. Three used standing cultures in complex media, with bacteria present (*2–4*). In the most recent of these, Outka (*4*) found that a threshold population density of amebae was necessary for transformation. He observed no flagellates until 24 hours after inoculation, but eventually obtained up to 80 percent flagellates. Since flagellates can reproduce, it is difficult in such long-term experiments to determine what proportion of the flagellates result from transformation and what proportion from differential growth of transformed cells. Brent (*5*) made the only study of transformation in nonnutrient buffer. He observed flagellates within 2 hours, to a maximum of 3 percent by 7 hours. Brent found more flagellates at higher population densities, which indicated as did Outka's experiments that an interaction of amebae is important for transformation. Brent sought, but did not find, a "flagellating substance."

In our initial attempts to control *Tetramitus* transformation, we could obtain only about 1 percent flagellates in buffer, or up to 50 percent in nutrient media, where some of the flagellates undoubtedly came from growth (*6*). We confirmed that higher population densities of amebae favor transformation. Although at suitable population densities, standing, undisturbed cultures would transform, agitated cultures would not. The requirement for a stagnant environment was unfortunate, since transformation could best be studied with cells in homogeneous suspension, so that the environment around each cell would be uniform and so that random samples could be removed to measure the phenotypic change (compare *7*). Efforts were made to find conditions for transformation of agitated cultures. Agitation prevented transformation only when it resulted in continuous exchange of gases between culture and environment. Sealed liquid cultures—for example, in syringes—transformed if the population density was sufficient regardless of whether or not they were agitated. This suggested that the flagellating substance was volatile. All attempts to collect the substance from one transforming culture and introduce it into another, using Loomis' "air bridge" method (*8*), were unsuccessful. The failure to find a volatile substance led to the hypothesis that the flagellating substance might be something taken from the environment rather than something added to it. Oxygen seemed a likely candidate. Oxygen would be expected to disappear from standing cultures, or sealed agitated cultures, at a rate proportional to the population density of amebae. When this hypothesis was tested, using agitated suspensions of amebae in low-oxygen atmospheres, most cells transformed fairly rapidly.

The best conditions available at present give transformation comparable to