The data from the two pedigrees (Fig. 1) are entirely compatible with sex-linked inheritance. Mothers of all affected males are heterozygous, and the D− band appears in all the daughters but not in the sons of affected males.

Thus far most surveys for G-6-PD variants have been directed toward Negro and other populations indigenous to tropical regions while similar studies of unselected Caucasian subjects have been limited almost entirely to several Mediterranean populations (*1*). The discovery of two individuals with the D− variant of G-6-PD, in a rather small sample of Caucasians of non-Mediterranean origin, suggests that it may be a characteristic G-6-PD variant in populations of northern European origin.

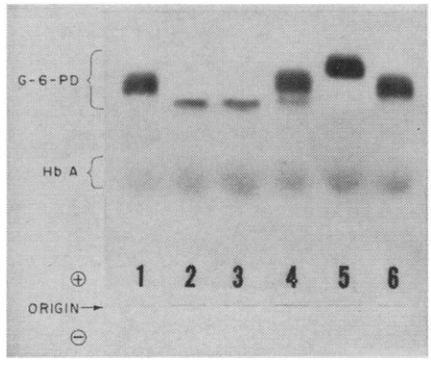

Fig. 2. Phenotypes of erythrocytic glucose-6-phosphate dehydrogenase after starch gel electrophoresis. *1*, Caucasian male, type B+; *2*, deficient male of Irish descent, type D−; *3*, deficient male of German ancestry, type D−; *4*, heterozygous mother of 3, type B+D−; *5*, Negro male, type A+; *6*, Negro male, type B+. Distance from origin to leading edge of A band is 9 cm. Vertical electrophoresis (4 to 5 v/cm) was carried out for 16 hours at 2° to 4°C in gel buffer containing 0.021*M* tris, 0.02*M* boric acid, and $5.4 \times 10^{-4}M$ EDTA. Five milliliters of $2.5 \times 10^{-3}M$ triphosphopyridine nucleotide (TPN) was added to the molten gel before degassing. The gel was prepared as described by Smithies (*12*). The bridge buffer solution contained 0.21*M* tris, 0.15*M* boric acid, and $4.7 \times 10^{-3}M$ EDTA adjusted to *p*H 8.0 with HCl. The cathode tray next to the gel contained $3.4 \times 10^{-5}M$ TPN. Whole blood was collected in ACD (acid-citrate-dextrose) solution, and stromata-free hemolysates were prepared by lysing washed red cells with one volume of distilled water and extracting with four-tenths volume of toluene, the toluene layer being discarded. Hemolysates were then centrifuged at 30,900*g* for 20 minutes, and about 0.025 ml of hemolysate was placed in each gel slot. After electrophoresis, the gels were incubated from 3 to 5 hours at 32°C in a staining solution composed of 10 ml of 0.05*M* tris-HCl, *p*H 8.0, $6 \times 10^{-4}M$ glucose-6-phosphate, 1 to $2 \times 10^{-4}M$ TPN, 10 mg nitro blue tetrazolium, $2 \times 10^{-4}M$ phenazine methosulfate, and $5 \times 10^{-3}M$ $MgCl_2$ made up to 100 ml with distilled water.

Kirkman *et al.* (*2*, *7*) have recently reported a variant (Seattle I) which seems to fit into this same category. They describe a male of Scotch-Welsh ancestry whose red cells have 8 to 15 percent of normal G-6-PD activity, and on electrophoresis this enzyme migrates 10 percent more slowly than normal. Although this variant has not been tested in our laboratory as yet, it is possible that the variant reported here and the Seattle mutant are identical.

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14. Supported in part by grants 2 TI GM-76, RF-9252, AM 07752-01 HEM, and A-946 from the USPHS, and project 1798 from the Psychiatric Training and Research Fund of the Illinois Department of Mental Health.

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Thymidine Incorporation by Mitochondria in Physarum polycephalum

Abstract: *Cytoplasmic particles of the slime-mold,* Physarum polycephalum, *incorporate tritiated thymidine in a form which is insoluble in acid and in ribonuclease, and soluble in deoxyribonuclease. Evidence for the mitochondrial nature of these thymidine-incorporating particles is based on their distribution, size, and staining behavior.*

Extranuclear DNA has been reported to occur in mitochondria (*1*), in chloroplasts (*2*), and in other cytoplasmic particles (*3*, *4*) which as yet are unidentified. We report here on the incorporation of tritiated thymidine into the mitochondria of the slime-mold, *Physarum polycephalum*, in a form which is insoluble in acids and can be extracted with deoxyribonuclease but not with ribonuclease.

Mitotically synchronized plasmodia of *P. polycephalum* were prepared, by fusion, from microplasmodia growing submersed in agitated culture (*5*), as described previously (*6*). The nuclei of such plasmodia divide in synchrony until the plasmodia reach a size of approximately 5 cm (*6*). At different times between the second and the third mitosis after fusion, the plasmodia were exposed for 3-hour periods to a growth medium (*5*) containing tritiated thymidine (*7*); smear preparations were then fixed in duplicate on cover slips. Fixation was in 95 percent ethanol, followed by ethanol–acetic acid (3 : 1 by volume).

After all the smears had been incubated with ribonuclease (*8*) according to the method of Plaut and Sagan (*3*), one slide from each set of preparations was treated with deoxyribonuclease (*3*, *9*), while the duplicate slide from each set was incubated with a portion of the buffer used as the solvent for the deoxyribonuclease and thus served as a control. The preparations were then covered with Kodak AR-10 stripping film, and after exposure for 4 weeks the autoradiographs were developed in Kodak D-19 developer (10 minutes at 20°C).

During all phases of the mitotic cycle, thymidine-H^3 was incorporated in considerable quantities by numerous, evenly distributed particles having diameters of approximately 2 μ in the thinnest areas of the preparation. The

autoradiographs *A* and *B* in Fig. 1 were obtained from a plasmodium that was incubated for 3 hours before the beginning of prophase. During this time less than 5 percent of the nuclei incorporated thymidine-H^3 (*10*).

Degenerating nuclei could not have accounted for the presence of the numerous labeled particles observed, because in smear preparations obtained from growing plasmodia such nuclear forms accounted for less than 0.5 percent of the total number of nuclei. Similarly, contaminating bacteria could be ruled out as a possible site of thymidine incorporation since the cultures were maintained under rigidly controlled, axenic conditions. We believe therefore that the labeled particles were cytoplasmic particles.

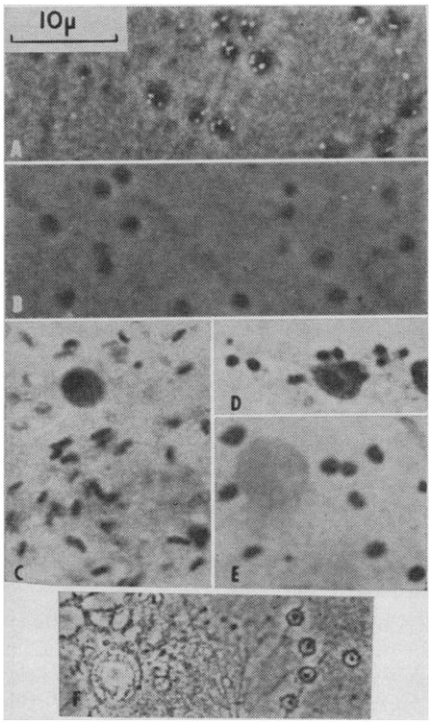

Fig. 1. (*A*) and (*B*) Autoradiographs of unstained smear preparations from a plasmodium of *Physarum polycephalum*. Smears were fixed in ethanol (95 percent) followed by ethanol–acetic acid (3 : 1); phase-contrast microscopy. The plasmodium was exposed to thymidine-H^3 during late interphase. Prior to the application of the film emulsion, both preparations were treated with ribonuclease. This treatment was followed in (*A*) by McIlwain buffer, and in (*B*) by the same buffer containing deoxyribonuclease. The mitochondria in (*B*) are slightly out of focus so that the few silver grains can be seen. (*C*) Section through a plasmodium during late interphase. The mitochondria appear as rod-shaped bodies (thickness of section, 3.0μ). Fixed with Champy's liquid; stained with acid fuchsin by Altmann's procedure. (*D*) and (*E*) Two areas of different thickness (*D*, "normal"; E, extremely thin) in the same smear preparation from a plasmodium during mid-interphase, fixed with Champy's liquid, and stained with acid fuchsin (Altmann). Nucleus and mitochondria in (*D*) appear small in comparison with the structures shown in (*E*). (*F*) Thin area in a smear preparation showing mitochondrial ghosts. Fixed with ethanol followed by ethanol–acetic acid; phase-contrast microscopy.

Cytological evidence for our assumption that the comparatively large labeled particles were mitochondria is presented in Fig. 1, *C* to *F*. In sections from explants fixed with Champy's liquid and stained with acid fuchsin according to Altmann's procedure, the mitochondria (Fig. 1*C*) appeared as rod-like elements with a maximum length of approximately 2 μ. In smear preparations fixed and stained as above (Fig. 1, *D* and *E*), the mitochondria appeared as small globules which varied in diameter within the same smear preparation from approximately 1 μ in the thicker parts (Fig. 1*D*) to 2 μ in extremely thin parts (Fig. 1*E*). There was a continuous transition between these extremes in sizes. After fixation of smear preparations with ethanol (95 percent) followed by ethanol-acetic acid (3 : 1 by volume), the mitochondria were preserved as ghosts (Fig. 1*F*). The autoradiographs in Fig. 1, *A* and *B*, were taken from areas similar to those shown in Fig. 1, *E* and *F*.

In addition to mitochondria, *P. Polycephalum* contains only two other groups of cytoplasmic particles which can be distinguished with the light microscope, either in stained preparations or by phase contrast. These particles are the pigment granules and polyphosphate granules (see *6*, Figs. 8, 10). Neither of them is similar, in size or distribution, to the granules shown in Fig. 1. The pigment granules are of irregular size, and their diameters vary from 0.2 μ to 2 μ. In smear preparations they are distributed in irregular clusters. The diameters of the polyphosphate granules are less than 0.2 μ in smear preparations.

The identity of the thymidine-incorporating material with DNA must be established by more direct procedures. The large amount of thymidine-H^3 incorporated by the mitochondria should make it possible to study the kinetics of this process in relation to the mitotic cycle of the plasmodia.

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Incorporation of Uridine-H^3 into Nuclei of Virus-Infected Tobacco

Abstract. *Tritiated uridine is selectively incorporated into the nucleus and nucleolus of tobacco leaf discs infected with tobacco mosaic virus that has been treated with actinomycin D. Such incorporation did not occur in noninfected tobacco treated with actinomycin D. Nontreated, infected, and healthy tobacco incorporated substantially more tritiated uridine.*

Actinomycin D has been shown by Sänger and Knight (*1*) to inhibit the incorporation of uracil-2-C^{14} into the ribonucleic acid (RNA) of healthy tobacco but not to interfere with the incorporation into the RNA of tobacco infected by tobacco mosaic virus (TMV). Both the radioactivity and the infectivity were associated with the same RNA fraction. Actinomycin D has also been shown to have little effect on the multiplication of RNA-containing animal viruses (*2*, *3*). These observations suggested that autoradiography of infected tissues treated with actinomycin D and fed labeled uridine might provide evidence of the cellular localization of TMV-RNA synthesis.

In the present study uridine-H^3 was selectively incorporated into the nucleus of leaf discs treated with actinomycin D when the leaf discs had been infected with tobacco mosaic virus, but the uridine was not incorporated into