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*ALTERATIONS OF A MATERNALLY INHERITED MITOCHONDRIAL STRUCTURAL PROTEIN IN RESPIRATORY-DEFICIENT STRAINS OF NEUROSPORA**

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One approach to an understanding of the nature of extrachromosomal heredity, subcellular morphology, and nucleocytoplasmic relationships is through the investigation of the genetics of enzymes and proteins of mitochondria. Genetic information for the primary structure of some mitochondrial-localized enzymes resides in nuclear DNA;[1, 2] no direct demonstration of a gene-protein relationship involving mitochondrial DNA has been previously reported.

Concepts developed in several areas of independent investigation led to the working hypothesis of this study. First, although concepts of extrachromosomal heredity have been evolving for many years, only one class of extrachromosomal mutations affecting mitochondria are well known; these are the respiratory-deficient mutants of yeast and *Neurospora.*[3] Extensive analyses of such mutants have not produced results interpretable in terms of either the classical one gene–one enzyme hypothesis or related corollaries regarding pathways of intermediary metabolism. Rather, several of the components of the electron transport chain are found to occur in either excess or deficit in those mutants. In addition, Green and associates[4] have proposed that polymacromolecular assemblies called elementary particles, with a fixed set of proteins in invariant proportions, are associated with the mitochondrial inner membrane. Among the principal components of those particles are the enzymes of the electron transport system and the mitochondrial structural protein. The importance of the structural protein is emphasized not only by its quantitative preponderance in the mitochondria, but also by its critical role in the organization and assembly of the enzymes of the particles. Moreover, recent investigations indicate that mitochrondria from all organisms contain DNA.[5] That this DNA may play a hereditary role is indicated by observations that *Neurospora* mitochondria replicate by division of pre-existing mitochondria[6] and exhibit genetic continuity after interhyphal transplantation.[7]

In this communication, the results of experiments stimulated by the foregoing concepts support the hypothesis that two different extrachromosomal mutations of (presumably) mitochondrial DNA lead to alterations of the primary structure

and function of the principal protein of the mitochondria, a structural protein. Secondarily, we propose that such mutations are expressed as pleiotropic respiratory deficiencies because of the critical role of the structural protein in the organization and assembly of the electron transport chain. In a later paper,[8] the hypothesis developed here will be extended to an analysis of a nucleocytoplasmic relationship. The function and association of a nuclear-determined enzyme, malate dehydrogenase, with mitochondria, mitochondrial membranes, and the structural protein will be described.

Materials and Methods.—All of the *Neurospora* stocks, with the exception of "stopper," were obtained from the Fungal Genetics Stock Center, Dartmouth, N.H. The "stopper" mutant[9] is a maternally inherited strain characterized by irregular growth cycles but is not known to have any respiratory deficiency; it was kindly furnished by Dr. Thad Pittenger. Samples of beef heart and yeast mitochondria and beef heart MSP were generously furnished by Dr. Richard Criddle.

Methodology and analysis of experiments involving gel electrophoresis, inhibition of MDH, and immunological tests are described elsewhere.[2]

Amino acid analyses were carried out on a Beckman amino acid analyzer, model 120. Samples of approximately 1 mg of protein were hydrolyzed at 110° for 36 hr in 1 ml of constant-boiling HCl *in vacuo*, by the method of Spackman, Moore, and Stein.[10, 11]

Results.—Purification of Neurospora mitochondrial structural protein: *Neurospora* mitochondrial structural protein (MSP) was purified by a method essentially identical to that described by Criddle *et al.*[12] for beef heart MSP. Occasionally this method yielded a protein that was visibly yellow. However, a colorless preparation could be obtained by additional washing with the lauryl sulfate, cholate, deoxycholate solution, and a second wash with a 20 per cent butanol, 20 per cent ammonium sulfate solution. Purified MSP was dialyzed exhaustively against distilled water, lyophilized, and stored in a desiccator over $CaCl_2$ at room temperature. Some preparations, although not readily soluble in water or at pH 8, were dissolved in 0.1 *N* NaOH and 8 *M* urea. The protein was then dialyzed against 0.01 *M* Tris-Cl, pH 8.0, and lyophilized. Such preparations were soluble in dilute buffers near pH 8. The solubility of MSP in 50 mM Tris-Cl, pH 8, at 4° was considerably less than the solubility at 25°.

Mitochondria were isolated from *Neurospora* mycelia by differential centrifugation of cell extracts as described by Luck.[5] Fresh moist mycelia were suspended in 0.7 *M* sucrose at 4°, homogenized at low speed in a Waring Blendor for 1 min, and ground in a stone ball mill with a glass beads for 1 hr at 4°. On the average, 35 gm of fresh mycelia yielded 1 gm of total mitochondrial protein. One gram of total mitochondrial protein generally yields about 200 mg of structural protein.

Criteria of purity of MSP: Several criteria of purity have been applied to various preparations of MSP. A single symmetrical Schlieren peak was observed after centrifugation at 59,780 rpm in a model E analytical ultracentrifuge. The 2.1*S* value observed is similar to values reported for mitochondrial structural proteins from other sources.[12] A single protein peak was observed after sedimentation in a sucrose density gradient for which a 2.2*S* value was calculated.

After electrophoresis in a polyacrylamide gel, a single band was observed; the band was diffuse, presumably due to rapid diffusion and/or different states of aggregation. A similar diffuse band was observed on Ouchterlony plates in the form of a precipitin band at the junction of the diffusion products of MSP and anti-MSP antibody preparations.

Molecular weight analyses: The molecular weight of *Neurospora* mitochondrial structural protein was estimated by three methods: sedimentation equilibrium,[13] titrations to determine minimal combining weight with NADH or ATP[2], and nearest-integer calculations from amino acid composition data.[14] All three methods yield a molecular weight of 23,000 for the smallest species of subunit. The molecular weight of beef MSP was reported to be 22,000.[12]

Functional properties of MSP: Measurements of the functional properties of *Neurospora* MSP are based upon its association with either coenzyme nucleotides, ATP or NADH, or other proteins such as MDH or myoglobin.[2] Association of MSP with MDH is revealed by fluorescence polarization titration, inhibition of MDH activity, and alteration of Michaelis constants for malate.[2, 8] Association of MSP with myoglobin is demonstrated by density gradient centrifugation, electrophoresis, and immunological methods.[2] The association of beef heart MSP with myoglobin, cytochromes, phospholipid, and coenzyme nucleotides has been described by others.[12, 15]

TABLE 1

AMINO ACID COMPOSITION OF MITOCHONDRIAL STRUCTURAL PROTEIN

Amino acid	μMoles Amino Acid per 100 mg Hydrolyzed Protein* *Neurospora* (74A)	Beef	Yeast
Lys	52	53	58
His	19	11	14
Arg	44	42	33
Asp	73	65	74
Thr	44	41	40
Ser	48	46	48
Glu	82	89	77
Pro	34	37	33
Gly	71	72	41
Ala	84	79	59
Val	58	57	50
Met	17	18	13
Ileu	44	43	43
Leu	71	76	68
Tyr	23	26	24
Phe	32	38	34
Tryp†	11	8‡	8‡
½ Cys†	15	20‡	40
No. analyses	24	1	2

* Underlined values differ significantly from *Neurospora* at the 95% confidence level where the coefficient of variation is taken as 4%.

† *Tryp* and ½ *cys* of *Neurospora* were determined by several methods (Table 5). For beef and yeast, *tryp* was determined by the method of Goodwin and Morton[16] and ½ *cys* was determined by Boyer's method.[18]

‡ Do not differ from *Neurospora mi*-1 MSP (see Table 5).

Amino acid composition of MSP from Neurospora, beef heart, and yeast: Amino acid compositions of MSP from wild-type *Neurospora*, beef heart, and yeast are summarized in Table 1. The beef and *Neurospora* proteins differ significantly only in *his, phe, tryp,* and ½ *cys,* whereas yeast and *Neurospora* differ in *his, arg, gly, ala, met, tryp,* and ½ *cys.* The similarity in amino acid composition of beef and *Neurospora* is paralleled by their similarity in functional properties (see above) and immunological properties. Anti-*Neurospora* MSP rabbit globulin precipitates with either yeast or beef MSP.[2] In addition, mitochondrial structural proteins from those sources are also similar in solubility properties, e.g., soluble in 0.01 *N* alkali and only slightly soluble in water or at pH 6. It is interesting to note that MSP from mutant *mi*-1 differs from beef heart MSP only in *his* and *phe.*

Comparative properties of wild-type and mutant MSP of Neurospora: Analyses of the associative properties of MSP indicate that extrachromosomal respiratory-deficient mutants, *mi*-1 and *mi*-3, differ from wild-type (Table 2). The apparent dissociation constants of MSP-NADH and MSP-ATP complexes, determined by fluorimetric titration, are summarized in Table 2, together with the inhibition constants determined by titration of wild-type MDH with MSP. By all of those parameters, the data indicate that MSP of either mutant, *mi*-1 or *mi*-3, associates to a lesser degree than wild-type MSP with NADH, ATP (except *mi*-3), and MDH.

TABLE 2

ASSOCIATION PROPERTIES OF MITOCHONDRIAL STRUCTURAL PROTEIN OF WILD-TYPE AND TWO CYTOPLASMIC MUTANTS OF *Neurospora*

Protein	Compound Bound* NADH K'_d ($M \times 10^7$)	ATP K'_d ($M \times 10^7$)	MDH K_i ($M \times 10^7$)
Wild type (ST-A4)	6	4	9
mi-1	20	14	36
mi-3	160	6	31

* Apparent dissociation constants (K'_d) were determined by fluorescence titration at pH 7.4 and 25°. The inhibition constant (K_i) for MDH-MSP association was determined at pH 7.4 and 35° in the reverse reaction. (See ref. 2 for experimental analyses.)

In another series of experiments related to those mentioned above,[8] it was found that the association of MSP from either *mi*-1 or *mi*-3 with wild-type MDH leads to alteration of the Michaelis constant of MDH for malate. Conversely, purified mutant MDH's exhibit a wide range of Michaelis constants for malate when combined with MSP of either *mi*-1 or *mi*-3. These interrelationships further indicate that the associative properties of *mi*-1 and *mi*-3 MSP differ from those of ST-A4 MSP.

Amino acid composition: Amino acid composition analyses of MSP from six strains of *Neurospora* are summarized in Table 3. Significant differences in amino acid composition of MSP from two strains were found, involving tryptophan and cysteine. Tryptophan was determined by absorbancy, fluorescence, and colorimetric methods. The absorbance extinction and fluorescence emission coefficients are summarized in Table 4. Analyses of tyrosine and tryptophan are summarized in Table 5. MSP from various preparations of six strains was analyzed by several methods for tyrosine and tryptophan. Mutants *mi*-1 and *mi*-3 each contain one less tryptophan residue per mole of MSP compared to MSP from the other four strains. Analyses of cysteine by mercuribenzoate and DDPM titrations are also summarized in Table 5. There were no significant differences among five of the six strains, but both methods of determination indicate that *mi*-1 MSP contains an additional cysteine residue compared to the five other strains. In addition, a strain of the genotype, *mi*-1 *f*, with a nuclear gene, (*f*), that suppresses the slow-growing character of *mi*-1, also produces MSP with one less tryptophan and an additional cysteine.

Peptide analyses: The number and pattern of tryptic peptides of wild-type and mutant structural proteins have been examined by the "fingerprint" procedure employed by Helinski and Yanofsky.[20] Under conditions yielding nearly complete proteolysis, as determined by titration with NaOH in a recording pH-stat, 27 ninhydrin spots were observed. This number is in reasonable agreement with the expected number, i.e., 25. based upon the amino acid composition (Table 3). Mutant proteins *mi*-1 and *mi*-3 yield two tryptophan-containing peptides, as evidenced by fluorescence and color reaction with Ehrlich's reagent, whereas wild type contains three such peptides, thus confirming the compositional analyses (Table 5). Tryptic digests of proteins treated with the yellow sulfhydryl reagent, DDPM, yield four yellow peptides from wild-type and *mi*-3 MSP, whereas an additional fifth orange peptide is found in digests of *mi*-1 MSP, thus confirming compositional data for cysteine (Table 5).

Discussion.—The results of this investigation indicate that two different extrachromosomal mutations of (presumably) mitochondrial DNA of *Neurospora* lead to alterations of the structure and function of the principal protein of the mitochondria,

TABLE 3

AMINO ACID COMPOSITION OF MITOCHONDRIAL STRUCTURAL PROTEIN OF WILD-TYPE AND RESPIRATORY-DEFICIENT MUTANTS OF *Neurospora*

	Residues Amino Acid per Mole Protein									
	Protein Source									
Amino acid	Wild type (ST-A4)	Cytoplasmic Mutants *mi*-1	*mi*-3	*st*	Nuclear Mutants *cyt*-1	*cyt*-2	x_w*	s_w†	N.i.‡	C.v.§
Lys	13.0	13.0	13.4	13.0	13.2	13.3	13.1	0.2	13	1.5
His	3.94	3.53	3.78	4.06	3.44	3.98	3.85	0.2	4	5.2
Arg	10.5	10.6	10.4	10.8	10.9	11.3	10.6	0.3	11	2.8
Asp	18.6	18.8	18.8	18.6	18.6	18.5	18.6	0.1	19	0.5
Thr	11.2	10.7	11.9	11.1	11.2	11.4	11.2	0.4	11	3.6
Ser	12.1	11.8	12.7	12.2	11.7	12.2	12.2	0.3	12	2.5
Glu	19.7	19.5	21.1	20.6	21.9	20.8	20.6	0.8	21	3.9
Pro	9.2	8.9	9.2	8.8	9.3	8.7	9.0	0.2	9	2.2
Gly	21.0	20.4	19.9	19.4	19.5	19.3	20.1	0.7	20	3.5
Ala	22.2	23.7	23.3	22.6	23.7	22.4	22.8	0.6	23	2.6
Val	15.4	16.1	16.0	15.3	16.3	15.5	15.7	0.4	16	2.6
Met	4.11	3.97	4.00	3.34	3.62	3.86	3.90	0.2	4	5.1
Ileu	11.7	10.9	11.0	10.5	11.3	11.1	11.2	0.3	11	2.6
Leu	19.4	17.3	18.5	18.2	17.4	18.1	18.4	0.8	18	4.3
Tyr	6.44	5.93	6.40	6.03	5.5	6.04	6.17	0.3	6	4.8
Phe	8.8	8.1	8.5	8.0	8.4	8.0	8.4	0.4	8	4.8
Tryp**	3.1	1.8	2.1	3.4	2.9	3.2	3.1	0.1	3	4.5
½ Cys**	4.1	5.0	3.9	4.1	3.7	4.1	4.0	0.2	4	5.0
							Total residues		213	
							Total weight		23,130	
No. analyses	12	5	6	4	3	6				
Except: Tryp	5	3	3	1	1	2				
½ Cys	3	2	1	1	1	1				

* Weighted mean of 36 analyses of 36 hr acid hydrolysates. Not included in the average is *tryp* for *mi*-1 and *mi*-3 and ½ *cys* for *mi*-1.
† Weighted standard deviation.
‡ Nearest integer.
§ Coefficient of variation: $(s_w/\bar{x}_w) \times 100$.
** Determined by other methods (see Table 5).

TABLE 4

ABSORBANCY AND FLUORESCENCE PROPERTIES OF *Neurospora* MITOCHONDRIAL STRUCTURAL PROTEIN

	Molar Extinction Coefficient*		Molar Emission Coefficient†		Moles Amino Acid per Mole Protein‡	
Protein source	Obsd.	Calc.	Obsd.	Calc.	*Tyr*	*Tryp*
Wild type (ST-A4)	4.15	4.27	3.51	3.40	6	3
Cytoplasmic mutant:						
mi-1			1.94	2.28	6	2
mi-3			2.16	2.28	6	2
Tryp	1.27		1.12			
Tyr	0.078		0.006			

* $E_M \times 10^{-4}$, 280 mμ, pH 7.4 in 50 mM potassium phosphate buffer. The observed weight extinction coefficient, E(cm²/mg) at 280 mμ, was 1.77 for wild type (calculated, 1.85).
† $E'_M \times 10^{-5}$, activation, 280 mμ; emission, 340 mμ. Protein in 50 mM potassium phosphate buffer, pH 7.4. Average of 3 analyses.
‡ See Table 5.

a structural protein. One mutant protein (*mi*-1) is characterized by the amino acid replacement, *tryp* → *cys*, a replacement that is consistent with the presently known genetic code[21] assuming single base substitutions. The other mutant protein (*mi*-3) also lacks one of the three tryptophan residues but does not have an extra cysteine residue. The amino acid replacing tryptophan in *mi*-3 is not yet known; however, on the basis of known codons, *leu*, *arg*, *gly*, or *ser* are permissible replacements for *tryp*.[21] On the other hand, the amino acid composition of MSP from three other strains is indistinguishable from wild-type MSP. Two of the strains (mutants *cyt*-1 and *cyt*-2) with "wild-type" MSP are also respiratory-deficient but are altered

TABLE 5

SELECTED AMINO ACID COMPOSITION OF MITOCHONDRIAL STRUCTURAL PROTEIN OF WILD-TYPE AND RESPIRATORY-DEFICIENT MUTANTS OF *Neurospora*

		Moles Amino Acid per Mole Protein*						
		Tyr Method†		Tryp Method†			½ Cys Method†	
Protein source	Prep. no.	A	B	A	C	D	E	F
Wild type (ST-A4)	1	6.5		3.0			4.1	
	2	6.1		2.8	3.1		4.3	4.0
	3	6.9	6.2‡	3.0		3.4		
Cytoplasmic mutants:								
mi-1(po)	1	5.8	5.9‡	2.1	1.7		5.1	
	2					1.6		4.9
mi-3	1	7.2	6.4§	2.3	1.9		3.9	
	2					2.2		
st			5.6			3.4	4.1	
Nuclear mutants:								
cyt-2		7.0	5.6**	3.2		3.0	4.1	
cyt-1			5.7‡	2.9			3.7	

* Mol wt 23,000.
† Method: A, spectrophotometric (Goodwin and Morton);[16] B, amino acid analyzer (Spackman, Moore, and Stein);[10, 11] C, fluorimetric (Table 4); D, colorimetric (Spies and Chambers);[17] E, HMB titration (Boyer);[18] F, DDPM titration (Gold and Segal).[19]
‡ Av. of five analyses.
§ Av. of ten analyses.
** Av. of ten analyses.

in nuclear genes. Still a third mutant (*st*) with "wild-type" MSP is *not* respiratory-deficient, but its mutant characteristic (sporadic cessation of growth) is maternally inherited.[9] No mitochondrial function is known to be affected in this mutant.

The amino acid replacement in either mutant protein, *mi*-1 or *mi*-3, causes the protein to differ from the wild-type protein in associative properties with either coenzyme nucleotides or the enzyme, malate dehydrogenase. Such functional alterations may be due to the replacement of the hydrophobic *tryp* residue by a hydrophilic residue such as cysteine in *mi*-1.

The mitochondrial structural protein plays a critical role in the organization and assembly of the electron transport chain.[4, 12] Hence, genetic structural alterations leading to decreased affinity of the protein for small molecules such as coenzyme nucleotides or other proteins that are directly or indirectly associated with the assembly may disrupt the organization and assembly of this system. Indeed, among the several quantitative differences in components of the electron transport chain found in mutants *mi*-1 and *mi*-3, cytochrome *c* accumulates by 16-fold in a nonparticulate cell fraction, riboflavin occurs in a twofold excess, and a 15-fold excess of long-chain unsaturated fatty acids occurs. In addition, deficiencies in cytochrome *a*, cytochrome *b*, and cytochrome oxidase have been observed in *mi*-1.[3]

Since the respiratory system of mitochondria contains at least ten or more enzyme proteins in addition to the structural protein, genetically predicated structural alteration of any one enzyme may be expected to provoke alterations in the assembly and organization of the respiratory system. Indeed, the respiratory-deficient nuclear mutations, *cyt*-1 and *cyt*-2, resemble the extrachromosomal mutants *mi*-1 and *mi*-3 in that a variety of quantitative alterations of components of the respiratory assembly occur.[22] This pleiotropic effect may, in analogy to comparable mutants of yeast,[23] be a consequence of alteration of the structure or regulation of cytochrome *c* synthesis. Genetic alteration of the structure of other mitochondrial proteins normally associated with MSP *in vivo* may be expected to lead to deficiencies in respiration or electron transport. Certainly the structural and functional integrity

of the mitochondrial structural protein is important in determining the precise conformational and functional properties of malate dehydrogenase.[8]

Direct demonstration of a structural gene function associated with mitochondrial DNA remains to be carried out. Classical methods of genetic analysis involving recombination or transformation have not been applied to the problem of mitochondrial heredity. Nevertheless, indirect evidence based on preliminary data with isolated *Neurospora* mitochondria,[24] and on data with mitochondria isolated from other sources,[25, 26] indicate that C^{14} leucine is incorporated into an insoluble protein when incubated under conditions favorable for protein synthesis. This may indicate that the gene controlling the primary structure of MSP is located in the DNA of the mitochondrion. Luck,[6] in addition, has identified a DNA-dependent RNA polymerase in *Neurospora* mitochondria. On the basis of the amount of DNA present in mitochondria,[5] there should be sufficient information to specify many proteins. By analyzing labeled amino acid incorporation during protein synthesis in isolated mitochondria, it should be possible to identify such proteins if this is indeed the case.

Multimeric proteins of various types have been described in which the activity of one or more subunits is sensitive to interactions with another protein. Crawford and Ito[27] demonstrated serine deaminase activity in the B protein of tryptophan synthetase; this activity is lost when the B protein is complexed with A protein. Conversely, the A protein alone is capable of converting InGP to indole, but this reaction is increased greatly in the AB complex.

Another striking example of the effect of binding between two proteins on the activity of one of them was reported by Gerhart[24] in a study of aspartic transcarbamylase.

The phenomenon of interallelic complementation appears to involve a similar sensitivity based on protein-protein interactions between differentially defective monomeric subunits.[29]

The suggested mechanism for the behavior of the mutants described in this paper is clearly not a novel type of interaction. It is simply being applied to a new system in which some of the interacting protein components are controlled by nuclear genes and at least one protein is specified by a cytoplasmic gene. Interactions such as those described between MSP and MDH are in fact nucleocytoplasmic interactions. Similar investigations have been carried out in yeast[30] and *Neurospora*[31] in unsuccessful attempts to detect altered proteins in respiratory-deficient mutants.

Despite the fact that a number of questions concerning cytoplasmic inheritance are answered by studies with this gene-protein system, it does not provide a clear answer to certain other questions. For example, if each mitochondrion contains DNA, each would be expected to be independent of other mitochondria in the same cell. This of course raises the question of how it would be possible for a mutation in mitochondrial DNA to be expressed unless it were a dominant mutation. If recessive, in order to isolate a mutant such as *mi*-1 or *mi*-3, it would be necessary to prevent replication of all the mitochondrial DNA except one mutant type; otherwise, a great deal of heterogeneity among mitochondrial populations would be expected; such was not observed in our experiments. An obvious alternative is that one type of mitochondrion might overproduce and thus eventually outgrow and replace the slower type.

An explanation which invokes a master DNA template in either the nucleus or in only one mitochondrion is not compatible with Luck's[32, 33] experiments on the replication of mitochondria. This system should, in any event, be useful in studying these and other related questions concerning mitochondrial structure and function, such as how proteins controlled by nuclear genes get into mitochondria, and how both nuclear and cytoplasmic suppressor mutations might affect MSP structure.

Summary.—The mitochondrial structural protein (MSP) from two maternally inherited, respiratory-deficient mutants of *Neurospora* (*mi*-1 and *mi*-3) differ from wild-type MSP. The MSP from *mi*-1 has one less tryptophan residue per mole of MSP and one more cysteine residue than does wild-type MSP. The MSP from *mi*-3 also has one less tryptophan residue, but no other differences have yet been detected in amino acid composition. The mutations in *mi*-1 and *mi*-3 appear to result from alterations in mitochondrial DNA which in turn result in single amino acid replacements. Based on the nucleocytoplasmic interactions that are described, which involve protein-protein interactions between malate dehydrogenase and mitochondrial structural protein, an explanation for the pleiotropic phenotype of *mi*-1 and *mi*-3 is offered.

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Abbreviations used: DDPM, N-(4-dimethylamino 3,5-dinitrophenyl) maleimide; MSP, mitochondrial structural protein; MDH, malate dehydrogenase; NADH, nicotinamide adenine dinucleotide (reduced); ATP, adenosine triphosphate.

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*STIMULATION OF FATTY ACID BIOSYNTHESIS BY PHOSPHORYLATED SUGARS**

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The synthesis of long-chain fatty acids (palmitic acid) from acetyl CoA and malonyl CoA has been shown to be catalyzed by a complex of six or more enzymes and a small molecular weight protein known as acyl carrier protein (ACP).[1, 2] The enzyme-complex was isolated intact from animal tissue[3] and yeast[4] but was readily dissociable into its various components in extracts of *E. coli*,[1, 2] from which at least eight individual enzymes were isolated and characterized. The enzymes were found to be specific for acyl ACP derivatives, thus establishing a synthetic pathway for fatty acids different from the classical β-oxidation pathway, although some of the *acyl* intermediates, such as acetyl, β-ketoacyl, α-β-unsaturated acyl, and saturated acyl groups are common intermediates in both sequences.

The level and activity of the fatty acid synthesizing system appears to be closely controlled by the nutritional state of the cell. The availability of carbohydrate substrates appears to affect both the level and/or activity of the enzyme protein.[3–7] Citrate, which is the metabolic precursor of extramitochondrial acetyl CoA,[8, 9] causes a substantial increase in malonyl CoA formation due to an allosteric stimulation of acetyl CoA carboxylase.[10, 11]

Following is a report of our data, which show that phosphorylated sugars increase palmitic acid synthesis from acetyl CoA and malonyl CoA by stimulating the activity of the fatty acid synthesizing system. These data also indicated that of the materials tested fructose 1,6-diphosphate caused the greatest stimulation of this system; even at higher concentrations glucose 1-phosphate, glucose 6-phosphate, α-glyceryl phosphate, pyrophosphate, and orthophosphate caused lesser degrees of stimulation of fatty acid synthesis. This effect was demonstrated with the fatty acid synthesizing systems both of animal tissues and of *E. coli*.

Experimental Procedure.—Preparation of the fatty acid synthesizing enzymes: The fatty acid synthesizing system of animal tissues was prepared from pigeon livers by a procedure similar to that described by Bressler and Wakil[3] except for the following modifications. The pigeons used in these studies were of the white Carneau variety obtained from Palmetto Pigeon Plant, Sumter, S. C. Usually, 12 pigeons, 8–10 weeks old, were used for each enzyme preparation. The pigeons were first starved for 48 hr, refed a diet of Purina Pigeon Chow for 24 hr, and then sacrificed. The livers were removed and homogenized in a Waring Blendor and fractionated as described by