seriously affected clinically as a homozygote. The clinical and urinary findings in G.F., E.M., J.P., B.B. and their respective families are compatible with this formulation and provide the first direct evidence that the three known mutations responsible for the genetic heterogeneity in cystinuria are allelic. Additional families must be studied to test this hypothesis. The failure of others (*1, 2, 5*) to identify pedigrees with more than a single type of heterozygote may reflect either small sample size (*5*), or, as suggested by Harris (*7*), differences in newer methods of amino acid analysis.

Previous results of the intestinal transport and absorption studies indicate that cystinuric subjects with a proposed III–III genotype retained the ability to transport dibasic amino acids by the intestine, in contrast to subjects with a I–I genotype. Thus, it was not surprising that G.F. and E.M., whose genotypes appear to be I–III, responded to an oral cystine tolerance test with findings intermediate between those observed with I–I and III–III subjects. The transport studies in vitro, however, failed to distinguish G.F. and E.M. from type I homozygotes, an indication either that our techniques were not sensitive enough to detect such subtle differences or that the polypeptides coded by the involved loci are subunits of more complex protein molecules whose configuration is altered sufficiently by the presence of type I polypeptide to be essentially completely inactivated. Resolution of this most important question will demand identification and characterization of the postulated peptide carriers or enzyme molecules.

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Anaphase Delay after Inhibition of Protein Synthesis between Late Prophase and Prometaphase

Abstract. *The relationship between protein synthesis and mitosis was studied in* Physarum polycephalum, *a plasmodial slime mold whose nuclear divisions are synchronous. Results of studies with actidione (cycloheximide), an inhibitor of protein synthesis, indicated that the essential structural proteins for mitosis and nuclear reconstruction were completed prior to the dissolution of the nucleolus in prophase. Proteins that determine the duration of the transition from metaphase to nuclear reconstruction were synthesized from late prophase to prometaphase. It is proposed that these proteins are concerned with the transformation of chemical energy into the mechanical work of mitosis.*

In a study concerning the effect of actidione [cycloheximide, an inhibitor of protein synthesis (*1*)] on mitosis (*2*) it was shown that proteins necessary for mitosis were synthesized until late prophase, which is the time of nucleolar dissolution, but after that time morphologically normal mitosis and nuclear reconstruction occurred in the absence of protein synthesis. Most mitoses and nuclear reconstructions proceeded at or near the normal rate during inhibition of protein synthesis, but a significant proportion of the cultures were delayed in their transition from metaphase to nuclear reconstruction. We have tried to establish the relationship between the time of treatment with actidione and this delay in anaphase.

We have designed a procedure for critically timing metabolic events relative to the highly synchronous metaphase of the plasmodial slime mold *Physarum polycephalum*. Cultivation of *Physarum* and preparation of synchronous cultures were as described by Mittermayer, Braun, and Rusch (*3*). We began each experiment with a compact, vigorously growing plasmodium to insure good synchrony in all parts of the plasmodium. When late prophase was detected (*4*), the plasmodia were cut into quarters, and at set intervals three of the quarters were transferred to petri dishes and treated with 30 μg of actidione per milliliter; the fourth quarter served as an untreated control. Treatment with actidione preceded metaphase in every case. Smear preparations were made from all of the quarters at different intervals, and these smears were stored for microscopical observation and scoring at the end of the experiment. The scoring procedure permitted determination of the precise time of metaphase in the control quarter; this value was used as a reference point for the treated quarters. When untreated plasmodial quarters from a single plasmodium were compared according to a procedure identical to the experimental one, the time of metaphase varied by no more than two minutes.

Our data (Table 1) confirm the earlier observations on prophase blockage (*2*) (note the last line of Table 1) and clarify variations in the speed of transition from metaphase to reconstruction. They also show that treatment with actidione during very late prophase causes a slow transition from metaphase to reconstruction, whereas actidione treatment during prometaphase has no effect on the duration of mitosis. In both cases, nevertheless, the reconstructed nuclei are morphologically identical to the nuclei in untreated plasmodia. Our observations (Table 1) also indicate that the effect of actidione on complete prophase blockage is expressed rapidly because treatment 13 minutes before metaphase (just before the nucleoli dissolved) totally blocks mitosis, while treatment 11 to 12 minutes before metaphase (the period

Table 1. The effect of treatment with actidione in prophase and prometaphase.

Start treatment (minutes before metaphase)	Plasmodia observed (No.)	Time between metaphase and early reconstruction* (min)
	Prometaphase	
1	2	5, 5
2	2	5, 5
3	1	5
4	2	5, 10
5	3	5, 5, 20
	Late prophase	
7	2	30–60, 60–120
8	1	60–120
10	1	60–120
11	1	60–120
12	4	60–120, 60–120, P†, P
	Prophase	
13	4	P, P, P, P

* The time of transition in untreated plasmodia is normally 5 minutes. † P represents prophase blockage.

in which nucleoli begin to dissolve) permits structurally normal mitosis; thus, treatment with actidione must take effect in somewhat less than 2 minutes.

The primary consideration, in the interpretation of these results, is whether or not this delay in anaphase was caused by the inhibition of protein synthesis during late prophase. Actidione might interact with spindle proteins to cause the delay, but this explanation is probably incorrect, since actinomycin D, which is a specific inhibitor of RNA synthesis and is chemically unrelated to cycloheximide, causes a similar delay in the transition from metaphase to reconstruction if treatment is initiated during interphase (*3*). It can also be argued that this delay is caused by a general and unspecific reduction in metabolism and that the effect is most pronounced during prophase, when spindle organization is most active. This argument cannot, however, explain the abrupt and permanent blockage in early prophase, before nucleolar dissolution, whereas mitosis and nuclear reconstruction go to completion in the absence of protein synthesis after nucleolar dissolution, nor can it explain why RNA synthesis is not drastically inhibited along with protein synthesis during interphase (*2*). Arguments that the delay in anaphase is caused by effects other than an inhibition of protein synthesis are, therefore, of secondary importance, and the most probable explanation of these data is that the effect is related to an inhibition of protein synthesis. The essential structural units for mitosis and nuclear reconstruction must, however, be completed prior to the total blockage of prophase because the entire process is completed and morphologically normal even though the duration of anaphase is extended. It is, therefore, reasonable to propose that the proteins that are synthesized between late prophase and prometaphase are involved in the transformation of chemical energy into the mechanical work of mitosis. These proteins must have a purely catalytic function, because they limit the duration of mitosis but are not necessary for its completion. An earlier investigation suggested that the messenger RNA molecules that encode the information for these proteins are more sensitive to inhibition by actinomycin D than are the messenger RNA molecules that determine the essential structural proteins for mitosis (*3*). All of these experiments point out the necessity for distinguishing between the structural factors required for completely normal mitosis and the factors that regulate the duration of the process.

In summary, the essential structural proteins for mitosis and nuclear reconstruction are completed before the dissolution of the nucleolus in prophase. Proteins which determine the duration of the transition from metaphase to nuclear reconstruction are synthesized from late prophase to prometaphase. It is proposed that these proteins are concerned with the transformation of chemical energy into the mechanical work of mitosis.

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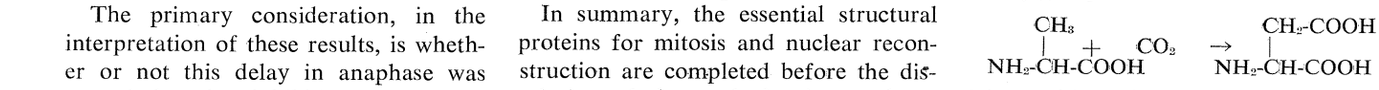
Synthesis of Amino Acid Residues with Reactive Side Chains under Simple Conditions

Abstract. *The side chains of amino acid residues can be converted from nonreactive to reactive species. Under the influence of electrical discharge, alanine is converted to aspartic acid in the presence of CO_2, for example. The possible implications of these findings to events taking place on primitive Earth are discussed.*

Amino acid residues, such as lysine and aspartic acid, with reactive side chains play an important role in biological processes. A study was undertaken to establish means by which amino acid residues with carboxyl and amino function in their side chains could be synthesized. The substitution of methyl hydrogens with more polar functions has been demonstrated by the sparking of an aqueous solution of acetic acid in the presence of nitrogen with the production of glycine (*1*). If a carboxyl group were added to the side chain of alanine (at β-methyl), aspartic acid would result.



The addition of an amino group to the β-methyl group of alanine would give α, β-diaminopropionic acid:

$$NH_2\,CH(CH_3)\,COOH + (NH_2) \rightarrow NH_2CH(CH_2\,NH_2)\,COOH + \tfrac{1}{2}H_2$$

The latter reaction is similar to the production of glycine from acetic acid already cited. A possible mechanism for this reaction has been discussed (*1*). Diaminopropionic acid is used here as a model compound for an amino acid containing an ω-amino group in its side chain.

To investigate the possibility of converting alanine to a residue with a reactive side chain, an aqueous solution of 0.01*M* L-alanine was prepared. The solution was "sparked" for 1 hour as described (*1*); longer periods of sparking led to decomposition. A mixture of carbon dioxide and nitrogen (1 : 1) was blown through the chamber during the process, and a transformer able to supply 9000 volts was used. The reaction vessel was cooled by a constant air stream blown across it. The solution was mixed by a magnetic stirrer. Portions of the product were chromatographed on Whatman No. 1 paper with appropriate standards in parallel; with separate sheets, the following solvent systems were used: a mixture of *n*-butanol, acetic acid, and water (4 : 1 : 5, by volume, organic phase); a mixture of *t*-butanol, 2-butanone, formic acid, and water (4 : 3 : 2 : 1, by volume); and a mixture of isopropanol and water (4 : 1, by volume). Products and standards were located by ninhydrin. The production of aspartic acid was definitely established. A trace of α,β-diaminopropionic acid was observed. Another portion of the product was chromatographed; a sample was eluted at the R_F of aspartic acid and analyzed by the ninhydrin colorimetric procedure (*2*). The yield was 5 percent.

When a solution of α-aminobutyric acid was similarly sparked, glutamic acid was produced, presumably by the addition of CO_2. A surprising result of this experiment was that aspartic acid was also produced, in about twice the yield of the glutamic acid. This could