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AMINO ACID DEPENDENT CONTROL OF DNA SYNTHESIS IN BACTERIA AND VEGETATIVE PHAGE

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In *Escherichia coli*, and other bacteria, the biosynthesis of both DNA and RNA has been found to depend on the presence of amino acids either synthesized endogenously or supplied in the medium. Amino acid auxotrophs, when starved for the required amino acid, generally cease to synthesize RNA and within a short time DNA synthesis also stops.[1-8] Several studies have been made of this relationship in the hope of establishing a dependence of nucleic acid synthesis on protein synthesis. The results have been conflicting. They have been clarified by the experiments of Stent and Brenner[9] who demonstrated that the dependence of RNA synthesis on the availability of amino acids was under the control of a genetic locus, distinct from the genes governing the amino acid requirement itself. They termed auxotrophic strains in which RNA could be synthesized in the absence of amino acids "relaxed strains" and others in which the synthesis depended on their presence "stringent strains."

Studies on the *in vitro* synthesis of RNA and DNA have demonstrated that the primer and template involved in the synthesis of both nucleic acids may be DNA.[10-14] The synthesis of both nucleic acids also involves common precursors in the form of riboside monophosphates.[15, 16] It seems pertinent therefore to inquire whether RNA and DNA synthesis could not be controlled by a common mechanism. An indication that this might be the case was afforded by the similar results of studies on the effect of amino acid starvation on, respectively, RNA or DNA synthesis.[3, 5] It was concluded that inhibition of synthesis might involve amino acid activated ribonucleotides[3] or deoxyribonucleotides.[5]

In the present paper we show that not only synthesis of bacterial DNA, but indeed also that of phage, is controlled in a stringent or relaxed fashion by the presence of amino acids. Moreover, as with the synthesis of RNA,[17, 18] DNA synthesis may be restored by chloramphenicol in amino acid starved auxotrophs or phage-

auxotroph complexes. The following properties of the phage system are useful: (1) Phage DNA continues to be produced when protein synthesis is completely inhibited by chloramphenicol added 8 min after infection.[19] (2) Adaptive enzyme systems of the cell are no longer inducible after infection. This property may be related to the destruction of genetic information contained in the bacterial nucleus.[20] By virtue of these properties it is easier to distinguish a direct dependence of DNA synthesis on amino acids from mechanisms depending on protein synthesis.

Materials and Methods.—Bacterial strains: Stringent auxotrophic strains of *E. coli* B (referred to as B in this paper) were derived from prototrophic strain 251 (obtained from Dr. W. Arber[21]). The amino acid requiring mutants had been derived by the modified penicillin method.[22]

As relaxed strain, we used the strain K12 HfrC, (K) which had been used also in the study of Stent and Brenner.[9] This strain is methionine requiring and was supplied by Dr. W. Hayes.

Phage strains: For the studies of phage replication we used $T4Dec_3$ (T4) supplied by Dr. G. Streisinger. This strain has the advantage that lysis can be completely controlled: (a) by cultivating at 37°, where no active lysozyme is produced;[23] (b) by superinfection at 7–8 min the r + function provides lysis inhibition. Unfortunately, in the course of the present work we discovered that T4 absorbs only very slowly on the strain K. To avoid this, we used phage T2H (T2) which absorbs equally well on both bacterial strains. Lysis inhibition by superinfection was applied.

Media: A minimal glucose-salt medium, M9,[24] was used in all the experiments. Amino acids were obtained from Sigma Chemical Co. Chloramphenicol was donated by Parke Davis Co.

Radioisotopes: Tritiated thymidine or uridine was obtained from Volk Radiochemical Co. The thymidine solution was mixed with nonradioactive thymidine to give a final specific activity of 270 mc/mM. The uridine solution was diluted with nonradioactive uridine to give a specific activity of 70 mc/mM. In experiments in which incorporation was allowed to proceed for only 1 min, thymidine was added to the medium at a final concentration of 10^{-3} μM/ml, uridine at 4×10^{-3} μM/ml. Final concentrations of 10^{-2} μM/ml or 4×10^{-2} μM/ml respectively were used in those experiments in which incorporation was followed for longer periods. All samples were counted in a Packard Tricarb scintillation counter by immersion of the membrane filter (see below) in an organic liquid scintillator.

Experimental Procedure.—Preparation of starved uninfected and infected cells of B or K: Auxotrophs of B or K were grown exponentially in minimal medium supplemented with the required amino acid. Experiments with uninfected cultures were carried out by collecting these cells on a membrane filter (Schleicher and Schuell A, coarse), washing with, and resuspending the cells in, prewarmed minimal medium. In general this procedure took 2 min. Such cultures could then be supplemented as desired. In experiments with phage infected bacteria, bacteria were infected with a multiplicity of 3–5 phage per bacterium. At 6 min such cultures were washed with iced minimal medium and resuspended in iced minimal medium containing phage, thus, giving superinfection by 3–5 phage per bacterium which produced lysis inhibition. This permitted us to carry out measurements over sufficiently long time periods without having to consider partial lysis of the culture. For the experiments, samples of the cultures were warmed to 37° for 10–15 min and then added to a mixture of thymidine plus such other supplements as might be of interest. The length of time that the culture was held at 0° did not influence the rate of incorporation (see Fig. 1*B*).

Incorporation of radioactive thymidine: When either B or K is incubated with tritiated thymidine, in cases where DNA synthesis occurs, incorporation, although rapid at first, soon ceases (Fig. 1*A*). This phenomenon has been noted previously by Rachmeler *et al.*[25] who demonstrated that an intracellular enzyme is induced, which destroys thymidine. Phage infected cells continue to incorporate thymidine for long periods of time (Fig. 1*B*). This is in agreement with the observation that enzyme induction is abolished after phage infection.

This particular property influenced the choice of our experimental procedure: (1) In experiments with uninfected bacteria, we measured the rate of DNA synthesis as the incorporation of a 1-min pulse of tritiated thymidine. 1-ml samples of the cultures to be tested for DNA synthesis were pipetted into tubes containing 0.1 ml of radioactive thymidine (to reach the final concentration given above) and incubated with aeration for 1 min. At the end of this time they were quickly poured into chilled tubes containing 0.3 ml of 17% TCA and 2.5 mg/ml nonradioactive

thymidine. (2) Infected cultures were added to radioactive thymidine (to reach the final concentration given above) at 37° and 0.2 ml samples were withdrawn at intervals and pipetted into chilled tubes containing 1 ml of 6.6% TCA and 750 μg/ml nonradioactive thymidine.

The cold acid insoluble material of the samples from both experiments was collected on membrane filters, washed with water, dried, and counted in the scintillation counter. The cold acid insoluble fraction contains both RNA and DNA. To determine the proportion of thymidine incorporated into DNA only, RNA was removed by alkaline hydrolysis (1 *N* NaOH at 37° for 24 hr). By this criterion 70–90% was incorporated into the DNA of growing or amino acid starved uninfected cells. 99% was incorporated into the DNA of phage infected cells.

Results.—Effect of amino acid starvation on DNA synthesis in uninfected cells: We have compared the 2 methionine requiring auxotrophs of B and K with respect

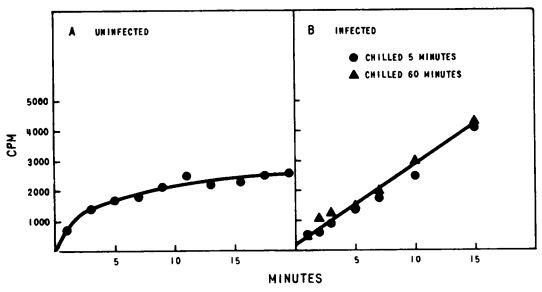

FIG. 1.—*Incorporation H^3 thymidine into the DNA of T4 infected and uninfected cells of E. coli B threonine⁻.* (*A*) Uninfected cells: at zero min H^3 thymidine was added to the culture whose density was 10^8 cells/ml growing in minimal medium plus threonine. (*B*) Infected cells: The culture was infected at a titer of 2×10^8 cells/ml with phage T4 at a multiplicity of 3. At 6 min, the cells were harvested by filtration, resuspended in minimal medium at 0°C and superinfected at a multiplicity of 3. After 5 min (●) or 60 min (▲) at 0° the culture was placed at 37° and incubated for 15 min. Threonine and H^3 thymidine were then added and incorporation into the cold TCA insoluble fraction measured. The number of infected cells was 10^8/ml. In uninfected cells 70–90% of the activity is alkali insoluble; in infected cells 99% is alkali insoluble.

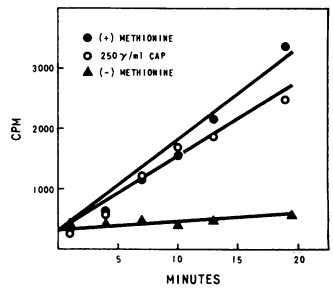

FIG. 2.—*The effect of methionine deprivation on the incorporation of H^3 uridine by methionine requiring auxotrophs of E. coli B.* Cells grown in minimal medium plus methionine were collected and washed by filtration and suspended in minimal medium at 37° and divided into three aliquots. Methionine was added to one (10 μg/ml), chloramphenicol to another (250 μg/ml), and nothing to the third. H^3 uridine, nonradioactive thymidine and fluorodeoxyuridine were added to all three aliquots and incorporation of uridine into the cold TCA insoluble fraction measured. Fluorodeoxyuridine (10^{-4} *M*) and thymidine (10^{-3} *M*) were added to insure incorporation of the uridine into RNA rather than conversion into thymidine and subsequent incorporation into DNA.

to their ability to synthesize DNA in the absence of amino acids. From Table 1 it can be seen that strain K synthesized DNA both in the presence and absence of the required amino acid. Strain B incorporates much less thymidine when methionine is absent. Hence, in strain B the amino acids exert a stringent control over the synthesis of DNA. Since B is different from the stringent strain used by Stent and Brenner, we have also tested the uptake of labeled uridine and found that the synthesis of RNA, as expected, is also under stringent control (Fig. 2). The decrease of the rate of DNA synthesis occurs extremely rapidly after removal of the amino

TABLE 1

RATE OF INCORPORATION OF LABELED THYMIDINE INTO THE ACID INSOLUBLE FRACTION (CPM)

t (min)	B Meth⁻ +M	B Meth⁻ −M	B Meth⁻ CAP	K Meth⁻ +M	K Meth⁻ −M	K Meth⁻ CAP
3	1,722	301	1,694	2,038	1,961	1,769
10	2,234	288	1,688	2,040	2,371	1,722
20	1,952	298	1,444	1,806	1,703	1,568
30	1,955	240	1,601	2,090	1,740	1,895
50	1,485	302	1,053	2,133	1,683	1,538
70	1,517	189	875	2,259	1,286	1,188
90	252*	202	565	1,866	1,118	652

* Cell division had ceased in this sample (see below) due to overcrowding.

INCORPORATION OF THYMIDINE UPON ADDITION OF METHIONINE OR OF CHLORAMPHENICOL AFTER DIFFERENT PERIODS OF STARVATION

Min starved	B Meth⁻ Recovery in Meth	B Meth⁻ Recovery in CAP	K Meth⁻ Recovery in Meth	K Meth⁻ Recovery in CAP
25	1,490	1,161	1,339	1,121
35	1,622	1,401	1,394	1,460
55	1,393	1,160	1,391	1,054
75	746	832	566	721
95	441	1,123	513	776

DNA synthesis by B Meth⁻ and K Meth⁻ in the absence and presence of methionine and in the presence of chloramphenicol: Cultures of B Meth⁻ or K Meth⁻ were grown with methionine to a titer of 2×10^8 cells/ml. Methionine was then removed by filtration as described in *Materials and Methods* and the cells suspended in minimal medium which was then supplemented with either methionine 10 μg/ml (+Meth) or chloramphenicol 250 μg/ml (CAP). A third aliquot received no supplements (−Meth). The aliquot containing methionine increased in titer from about 2×10^8 cells to 10^9 cells per ml. Cell division had ceased in the culture of B + Meth by 90 min, but was still observed in the K + Meth culture. Starved aliquots increased by a factor of 1.6 (60% of the cells divided) during the 90-min period. All aliquots were tested for DNA synthesis by measuring the incorporation of tritiated thymidine which occurred in 1 min (see *Materials and Methods*). The ability of starved cells to recover their capacity for DNA synthesis was tested by adding either Meth or CAP to aliquots of starved cells at the times indicated following the removal of methionine. After 8-min incubation with Meth (10 μg/ml) or chloramphenicol (250 μg/ml) these aliquots were further tested for their ability to incorporate thymidine for 1 min.

acid; after the 3-min period necessary for the removal of the amino acid, the reduction of the rate is essentially that obtained after prolonged starvation.

As with RNA synthesis,[17, 18] the inhibition of DNA synthesis brought about by amino acid deprivation can be removed by the addition of chloramphenicol. Moreover, either the specific amino acid or chloramphenicol alone can restore DNA synthesis when added to a starved auxotroph (Table 1). However, this recovery becomes more difficult if starvation has been prolonged up to 70–90 min. This is probably a secondary effect related to the prolonged inhibition of protein synthesis.

Identical results were obtained with a threonine requiring mutant of B.

Effect of amino acid deprivation on DNA synthesis in phage infected cells: In Figure 3 we see that threonine or methionine deprived auxotrophs of B infected with T4 cannot synthesize significant amounts of phage DNA. These T4 infected B-cells behave as if under stringent amino acid control of phage-DNA synthesis. As with uninfected cells, this stringent dependence of nucleic acid synthesis on amino acids can be removed by the addition of chloramphenicol. Unlike the synthesis of RNA,[17, 18] the concentration of chloramphenicol does not appear to be important, since both 25 and 250 μg/ml are equally effective.

Due to the slow rate of adsorption it is difficult to infect K with T4. However, rapid adsorption to both B and K can be obtained with T2. Figure 4 compares the effect of amino acid deprivation of T2 infected stringent and relaxed hosts. On B the synthesis of phage DNA is decreased when methionine is absent, showing a stringent control, as with T4. On K the control is relaxed. Thus the control mechanism of the host cell is maintained after the infection with phage.

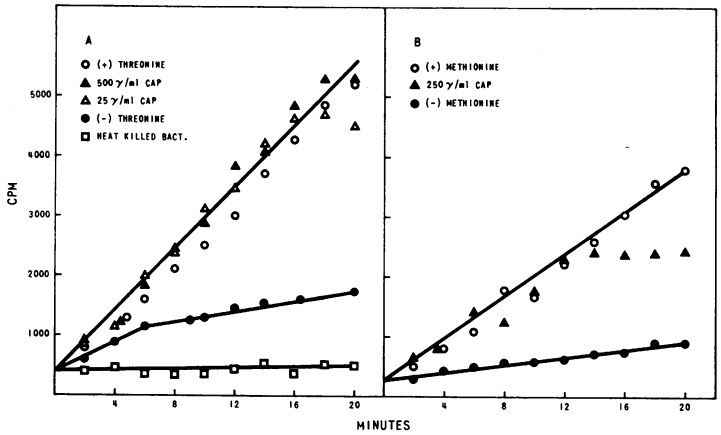

FIG. 3.—*The effect of amino acid deprivation on thymidine incorporation by T4 phage infected auxotrophs of E. coli B requiring methionine or threonine.* (*A*) A threonine requiring auxotroph of B was grown in the presence of threonine and infected with T4 at a multiplicity of 3. At 3 min, it was deprived of threonine, superinfected with phage and placed at 0°C. Aliquots were incubated at 37° for 15 min and then mixed with H^3 thymidine plus: only threonine (100 μg/ml); only chloramphenicol (500 μg/ml); only chloramphenicol (25 μg/ml); or only minimal medium (i.e., minus threonine). (*B*) A methionine requiring auxotroph of *E. coli* B251 was treated as in (*A*). Aliquots were mixed with H^3 thymidine plus: only methionine (10 μg/ml); only chloramphenicol (250 μg/ml); or only minimal medium (minus methionine).

Thymidine incorporation into the cold TCA insoluble fraction was measured.

Role of other amino acids in stringent control of DNA synthesis: Beside methionine and threonine we have tested 9 other amino acids on their corresponding, amino acid requiring, T4 infected, B strain. Table 2 gives the numerical data of this survey experiment. They show that the inhibition of DNA synthesis by the deprivation of the required amino acid occurs for all amino acids tested. The extent of inhibition is variable and may represent variation in the ability of these auxotrophs to synthesize phage DNA or a possible "leakiness" of some of the mutants.

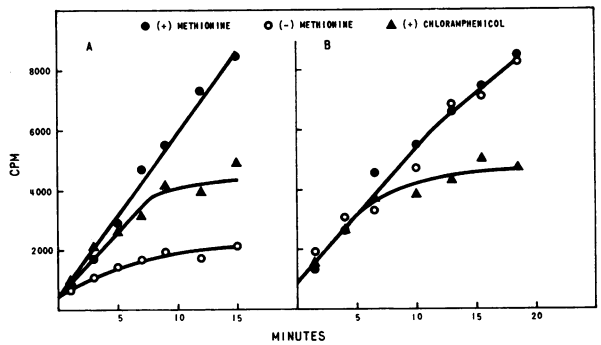

FIG. 4.—*The effect of amino acid deprivation on thymidine incorporation by stringent and relaxed auxotrophs of E. coli infected with T2.* (*A*) Methionine requiring stringent auxotroph of B infected and superinfected with T2 at a multiplicity of 4. Cells were collected and washed as before and incubated at 37° in minimal medium for 5 min before mixing aliquots with thymidine plus: Methionine (10 μg/ml); chloramphenicol (250 μg/ml); or minimal medium (minus methionine). (*B*) As in (*A*) but the methionine requiring relaxed auxotroph of *E. coli*, K was used, infected with a multiplicity of 6 T2/cell. Thymidine incorporation into the cold acid insoluble fraction was measured.

TABLE 2

Supplement	Cpm	Supplement	Cpm	Supplement	Cpm
Cysteine		Phenylalanine		Serine	
(5 μg/ml)	8,720	(5 μg/ml)	13,005	(200 μg/ml)	6,820
...	6,562	...	3,410	...	2,445
CAP	10,260	CAP	7,685	CAP	3,423
Arginine		Tryptophane		Histidine	
(20 μg/ml)	4,100	(5 μg/ml)	7,821	(5 μg/ml)	6,892
...	2,190	...	1,994	...	4,555
CAP	3,540	CAP	5,881	CAP	7,930
Proline		Leucine		Glycine	
(20 μg/ml)	10,675	(10 μg/ml)	9,062	(20 μg/ml)	2,326
...	3,672	...	3,299	...	1,160
CAP	8,542	CAP	7,860	CAP	2,160

The incorporation of H^3 thymidine into the cold TCA insoluble fraction of phage infected auxotrophs of E. coli B: Mutants of B required the amino acid supplement indicated. Absence of amino acid in the incubation mixture is indicated as (---). Cells were infected with T4, deprived of amino acid, superinfected and incubated at 37° for 15 min as described in Figure 3. Aliquots were then mixed with H^3 thymidine and immediately distributed into tubes containing either: the required amino acid (to give the concentration noted), only minimal medium (minus amino acid), or 250 μg/ml chloramphenicol. After 15-min incubation, the incorporation of H^3 thymidine was measured. The concentrations of amino acid used were between 5 and 10 times the amount necessary to provide optimal growth.
The amount incorporated represents the counts/min per 10^8 infected cells.

In most cases chloramphenicol largely overcomes the inhibition. An apparent exception in this respect is serine. This is true in uninfected cells as well as in the infected auxotroph. The results in Table 3 indicate that for both RNA and DNA synthesis chloramphenicol does not reverse the effect of serine deprivation.

Since certain amino acids act as precursors for the synthesis of the purine and pyrimidine bases, experiments were performed in which deoxyribosides and ribosides were supplemented to the medium. If serine acted as precursor for such a synthesis, the experiment might show the restoration of both RNA and DNA synthesis in the presence of chloramphenicol. This was not the case. We are tempted, therefore, to conclude that serine plays a supplementary, particular role, which is distinct from that of the other amino acids.

Discussion.—The experiments reported here are designed to demonstrate that in the bacterial strain B, the presence or absence of amino acids exercises a stringent control over the synthesis not only of RNA, as shown previously, but also of DNA. This is true of the host, as well as of vegetative bacteriophage T2 or T4, DNA's. The inhibition produced by the absence of a required amino acid is readily overcome by the addition of this amino acid or of chloramphenicol. Our experiments indicate that the amino acid enters the control mechanism in a simple form and not as a protein. This direct effect of the amino acids is accompanied by other probably indirect effects in both relaxed and stringent strains as soon as longer periods of starvation or longer periods of chloramphenicol treatment are studied. We tend to think that the late effects previously observed[1–8, 26] have to be reconsidered to see whether the system used is under stringent or relaxed control. Even intermediate degrees of amino acid dependence have been encountered.[7, 27]

TABLE 3

	Serine			Arginine			Leucine		
	200 μg/ml	--	CAP	20 μg/ml	none	CAP	10 μg/ml	none	CAP
H^3 Thymidine	424	208	135	369	150	305	343	117	348
H^3 Uridine	1,425	158	159	668	170	548	1,389	143	1,291

Ability of unsupplemented auxotrophs of E. coli B to incorporate H^3 thymidine or H^3 uridine in the presence of chloramphenicol: Auxotrophs were collected and washed by filtration and suspended in minimal medium at 37°. Aliquots were supplemented with *either* the required amino acid at the concentration given *or* 250 μg/ml chloramphenicol. Incorporation of thymidine or uridine was measured during a 1-min period. Counts/min per 10^8 cells are given.

It seems unlikely that DNA synthesis is directly dependent on a concomitant RNA synthesis since Okazaki and Okazaki[5] have demonstrated that RNA and protein synthesis can be stopped completely by uracil starvation, without affecting DNA synthesis. Under the same experimental conditions, however, amino acid starvation inhibited both DNA and RNA synthesis. One should also note that in phage infected cells ribosomal RNA synthesis has ceased.

As an alternative we propose that both RNA and DNA are under the same control. Further experiments with isogenic strains, in which only the RC marker is different,[28] could provide proof for such a hypothesis.

Such a common control can be imagined as acting on either of the following steps: (1) by regulating the supply of common precursors (the ribotide pool); (2) by inhibiting the ability of DNA to act as primer; (3) by inhibiting the polymerization of the nucleotides at the enzyme level.

It seems improbable that amino acid deprivation is curtailing the source of a common precursor, since: (A) All of the evidence indicates that RNA synthesis ceases[1-3, 30] whereas, as may be seen in Tables 1, 2, and 3, DNA synthesis proceeds at a slower rate, but does not stop. (See also ref. 3.) (B) Preliminary experiments on the deoxyriboside pool[29] show that the pool of the cold acid soluble deoxyribosides obtained in phage infected auxotrophs deprived of the required amino acid is not significantly depleted or exhausted. Previous experiments have demonstrated an increase in the acid soluble ribotide pool.[3]

Let us consider whether mechanisms connected with genetic regulation can provide an explanation. Stent and Brenner[9] and Kurland and Maaløe[30] describe in detail a hypothesis for the stringent-relaxed control of RNA, which is based on the regulatory mechanisms proposed by Monod and Jacob.[31] The relaxed control would correspond to the constitutive mutants in enzyme systems, and would imply that either no repressor or a "wrong" repressor is made, or that the operator is changed so as to become insensitive to the repressor. They came to the conclusion that the transfer RNA could represent this repressor.

If we apply the same concept to the regulation of DNA synthesis we are faced with the fact that the stringent or relaxed control of the host cell is maintained after infection with T2 or T4.[32] Other experiments indicate that the informational content of the host DNA is destroyed after infection.[20] To reconcile this observation with the genetic regulation model, one must assume (1) that the RC locus is not the operator, which implies that it (2) must control a repressor locus. Such a repressor, made by the host, must still be active against phage DNA. This would mean that the transfer RNA of a stringent strain acts as repressor on the sensitive substance of both host and vegetative phage. If we assume that DNA and RNA syntheses are under the genetic control of the same repressor locus it follows that the action spectrum of the repressor substance is uncommonly broad.

An alternative hypothesis is that the stringent and relaxed strains differ in the mechanism whereby newly synthesized RNA is removed from the DNA template. Under these conditions the DNA template is unavailable for further nucleic acid synthesis. Such an alternative would imply that amino acids may be required to participate in the RNA polymerizing system in stringent cells.

The astonishing effect of chloramphenicol needs to be further studied. Further experiments have to be designed to decide between a hypothesis of turnover

described previously[33] and others—i.e., chloramphenicol could also act by substitution for amino acids in their immediate role.

The special role of serine must be shortly emphasized. In this case, chloramphenicol is not able to reverse either RNA or DNA synthesis. Glycine does not show this effect. Thus the entry of serine in the pathway of purine synthesis has been excluded and we must search for other explanations. A possibility in view of the observation[34] that serine is not removed from DNA or RNA by careful purification procedures may be that serine has a special position on the structure of biologically active DNA.

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[32] When amino acids are removed later than six minutes after infection—i.e. 10 minutes—the effects observed persist. This conflicts with the results of Burton: *Biochem. J.*, **61**, 473–483 (1955). This conflict may be resolved if we assume that the strain employed by Burton was relaxed.

[33] Kurland and Maaløe[30] postulate that the effect is due to a constant small turnover of proteins. In the presence of chloramphenicol the degradation would be unimpaired, but since the rebuilding of proteins is stopped, the amino acid pool would be replenished. For this idea experimental evidence is given; indeed they showed that in bacteria which had been starved for the required amino acid, the RNA synthesis initiated by chloramphenicol recovers later than in that case where both an amino acid and chloramphenicol were used. However, in the latter case, a similar but shorter lag was also observed. They showed also that small concentrations of chloramphenicol are not effective to initiate the recovery in absence of the amino acid. In our experiments with vegetative phage DNA, we found no such lag, although the infective cells had been starved for 10–15 min. (We have also observed similar stringency and lack of a lag in phage infected bacteria starved for only 5 min.)

[34] Bendich, A., personal communication.

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*ELECTROPHORETIC HETEROGENEITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND ITS RELATIONSHIP TO ENZYME DEFICIENCY IN MAN**

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The hemolytic anemia induced by the antimalarial drug, primaquine, is associated with inherited,[1] X-linked deficiency of erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD). It is uncertain whether such deficiency is produced by an alteration in enzyme structure or by an aberration in a regulatory mechanism. Qualitative enzymatic abnormalities have been observed in certain uncommon individuals with an apparently X-linked form of congenital nonspherocytic hemolytic anemia.[2] Such abnormalities have not been detected among Negro and Mediterranean subjects with the more commonly occurring forms of G-6-PD deficiency.[2–4] Recently, Marks, Gross, and Banks[5] have described an Italian family in which several male members have intermediate levels of G-6-PD activity, altered enzymatic properties, and variant electrophoretic mobility of partially purified erythrocyte enzyme.

In a preliminary account,[6] we described electrophoretic variation in G-6-PD of crude hemolysates obtained from normal Negro subjects. This observation has been independently reported by Kirkman, who has adduced evidence for X-linkage of such variation.[7] Our preliminary data were consistent with autosomal inheritance; subsequently, inconsistencies to the autosomal hypothesis became apparent. These inconsistencies were resolved through the use of leukocytes as a source of G-6-PD. As will be indicated in this report, electrophoretic variation of G-6-PD is, in fact, X-linked, and the responsible structural locus appears to be either tightly linked or identical to the locus governing G-6-PD deficiency in Negroes.[8]

Materials and Methods.—Subjects: Blood samples were obtained from healthy individuals, chiefly hospital personnel, prisoners, or pre- and postpartum women and their families.

G-6-PD assay: Crude hemolysates were assayed by a modification of Glock and McLean's method[9] described by Zinkham.[10] Purified erythrocyte and leukocyte enzymes were assayed in the usual manner.[11] One unit of enzyme activity in either assay system was taken to represent