natural populations are largely mutational. The weight of the evidence rapidly accumulating in the literature indicates that both mutational or balanced loads are important both in natural and in experimental populations of Drosophila.

Summary.—Four experimental populations, derived originally from the same group of founders, and maintained in the laboratory for about 6 years, were used. Two of these populations were maintained at the near-optimum temperature of 16°, and the other two at the stress temperature of 27°C. Progenies obtained by brother-sister matings, and by matings of unrelated parents, have been compared for all the populations, at the same two temperatures, 16° and 27°. The estimates of the magnitudes of the genetic loads come out higher in the populations kept at 27°, and in the tests made at 27° than in those at 16°. The so-called B/A ratios are mostly below 2, but whether this indicates that the genetic loads are maintained predominantly in balanced state seems questionable.

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*THE UTILIZATION OF GLUCOSE 6-PHOSPHATE BY GLUCOKINASELESS AND WILD-TYPE STRAINS OF ESCHERICHIA COLI**

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It has been known for some time that certain bacterial species are able to utilize exogenous glucose 6-phosphate.[1] It has been generally thought that the first step in the utilization of such phosphorylated compounds is dephosphorylation,[2] and it has been shown in several laboratories that *Escherichia coli* will incorporate only the nucleoside portions of nucleoside monophosphates.[3–5] In this organism the utilization of yeast glucose-6-phosphate[6] and of D-α-glycerophosphate[7] as sources of carbon

TABLE 1

GROWTH RATES AND ENZYME ACTIVITIES

Strain	Hfr 3300	MM-6	GN-2	FR-1
Origin→	Wild type	Hfr 3300	MM-6	GN-2
Doubling times:*				
Galactose	87	84	98	82
Glucose	77	>600	∞	128
Fructose	117	∞	∞	78
Enzyme activities:†				
Glucokinase	67	69	0	0
"Fructokinase"	5	3	6	6
β-Galactosidase	864	4260	5770	3640

*Doubling time, in minutes, at 35° for cultures grown with aeration in medium 63[17] containing 4 mg per ml of the carbon source and thiamine, 5 μg per ml.

† Enzyme activities were measured with the supernatant fractions after sonication and centrifugation at 20,000 × *g*. Cells were grown in medium 63 containing nutrient broth, 10 mg per ml, and glucose, 4 mg per ml. Glucokinase was assayed spectrophotometrically,[12] the assay mixture including glucose, 2×10^{-4} *M* and ATP 2×10^{-3} *M*. The fructokinase assays contained fructose, 2×10^{-3} *M* instead of glucose. β-Galactosidase was measured by the method of Lederberg.[18] All activities are expressed as mμmoles per min per mg protein at room temperature. Strain Hfr 3300 has the galactosidase genotype i^- and all of its derivatives were constitutive for this enzyme. In other respects it is a typical "wild type."

is absolutely dependent on the presence of the enzyme alkaline phosphatase. Such a role for this enzyme has been supported by the finding that it is apparently localized at the periphery of the cell.[8]

As early as 1951, however, Roberts and Wolffe showed that when *E. coli* was grown on glucose 6-phosphate or fructose 6-phosphate as the sole carbon source, most of the organic phosphate of the cell came from the hexose phosphate substrate, despite the presence of excess inorganic phosphate.[9] This strongly suggested that utilization of hexose phosphates did not involve prior hydrolysis. Lin and his co-workers[7] have recently presented convincing evidence that *E. coli* utilizes L-α-glycerophosphate directly without hydrolysis. Wild-type cells use glycerol and L-α-glycerophosphate equally effectively as sole sources of carbon. A mutant lacking the enzyme glycerol kinase was unable to grow on glycerol, but used L-α-glycerophosphate as effectively as did the wild type. Thus, the entry of L-α-glycerophosphate could not have involved dephosphorylation. This conclusion was also supported by the results of experiments using a mutant lacking both glycerol kinase and the next enzyme in the pathway, L-α-glycerophosphate dehydrogenase.[10] This strain was found to take up from the medium a substantial concentration of L-α-glycerophosphate, and no exchange reactions were found between the accumulated material and free glycerol or inorganic phosphate.

We have been studying a mutant of *E. coli* which is unable to phosphorylate glucose. Our experiments have shown that glucose 6-phosphate must enter intact into the cells of this mutant and into the wild type, and that prior hydrolysis cannot be involved.

Hexose Phosphorylation in E. coli.—Our studies with a mutant of *Salmonella typhimurium* deficient in phosphoglucose isomerase have shown that the major pathway of glucose metabolism in *Enterobacteriaceae* is via glucose 6-phosphate.[11, 12] Whereas the ability of cell-free extracts of *E. coli* to phosphorylate glucose has often been demonstrated,[13, 14] fructose phosphorylation by these extracts was much less than glucose phosphorylation, although fructose was an excellent carbon source for growth. A glucokinase has been somewhat purified from *E. coli;* this enzyme is specific for glucose, and fructose is not a substrate.[15] To our knowledge it has not hitherto been possible to obtain a mutant strain of *E. coli* lacking glucose kinase, but

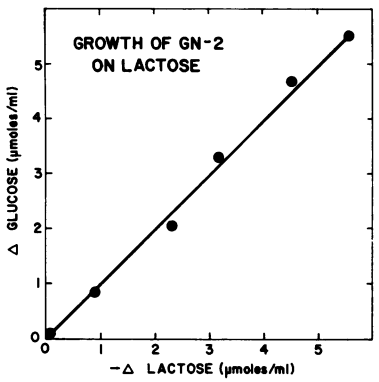

FIG. 1.—Growth of GN-2 on lactose. GN-2 was inoculated at a density of 0.02 mg dry weight per ml into minimal medium containing lactose, 2 mg per ml. The culture was incubated at 37° in a water bath shaker, and during growth 1-ml samples were removed, and the cells separated by filtration through S.&S. membrane filters, type B-6. The filtrates were assayed for lactose and glucose.[16]

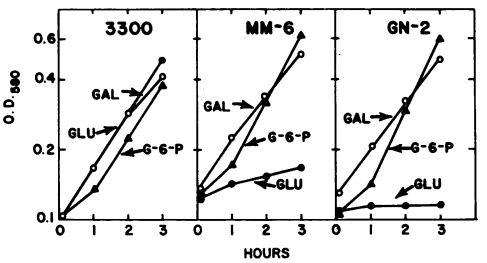

FIG. 2.—Growth on glucose, galactose, and glucose 6-phosphate. The three strains were inoculated after growth on galactose into minimal medium containing galactose, glucose, or glucose 6-phosphate, 4 mg per ml. Incubation was at 37° on a water bath shaker. Absorbancy measurements at 580 mμ were made with a Hitachi-Perkin-Elmer spectrophotometer.

able to grow on other sugars, such as galactose, although a strain, MM-6, with this phenotype has been studied in some detail.[16] Strain MM-6 grows adequately on lactose and galactose, but very slowly on glucose, and not at all on fructose (see Table 1). This organism and its parent strain have similar glucokinase activities (Table 1). Because of the failure to find an enzymatic deficiency in MM-6, its abnormal permeability to galactosides and other peculiarities of its phenotype (for example, inability to utilize succinate), it has been considered to be defective in a permeability character. However, many of its characteristics can be explained by another model in which the wild type is presumed to possess two glucose-phosphorylating activities, one specific for glucose and readily measured in extracts, the other a nonspecific hexokinase which acts on glucose, fructose, or mannose. The latter, for some reason, is difficult to measure in extracts. According to this model, MM-6 lacks the hexokinase and therefore does not grow on fructose or mannose. It grows slowly on glucose because it, like the wild type, has the specific glucokinase. It is noteworthy that the *in vitro* activity of the specific glucokinase is less than 0.1 μmole per mg protein per minute, which is to be compared with the *in vivo* rate of glucose utilization, about 0.5 μmole per mg protein per minute. In the same units, glycerol kinase in *E. coli* was found to have an activity of about 0.6,[7] and gluconokinase in *S. typhimurium* about 0.5.[12]

If the second model were correct, then one should be able to isolate from MM-6 an absolute glucose-negative strain which lacks both glucokinase and hexokinase. Such a strain, GN-2, was found after ethyl methane sulfonate mutagenesis[19] and penicillin selection. Like the parent, MM-6, it is capable of growth on lactose and galactose, but it is negative on both glucose and fructose and has no detectable glucokinase activity (Table 1). One would further predict that a fructose revertant selected from GN-2, which should have regained the nonspecific hexokinase, should

TABLE 2

SPECIFIC ACTIVITIES OF RIBONUCLEOTIDES AFTER GROWTH ON GLUCOSE 6-P^{31} IN AN EXCESS OF P_i^{32}

Nucleotide	Cpm/μmole	μMole P^{32}/μmole
UMP	4.09×10^3	0.057
GMP	3.73×10^3	0.052
AMP	3.96×10^3	0.055
CMP	4.02×10^3	0.056

Strain Hfr 3300 was grown in medium 63 containing P_i^{32} (86 μmoles per ml, 7.17×10^4 cpm per μmole), thiamine (2 μg per ml), and glucose 6-phosphate (10.2 μmoles per ml) as the sole carbon source. The cells were harvested in logarithmic growth phase at a density of 0.34 mg dry weight per ml and extracted once with perchloric acid, 0.5 *M*. The precipitate was hydrolyzed with KOH, 0.7 *N*, 37° for 15 hr.[22] The mixture was reacidified, and the ribonucleotides in the supernatant were absorbed on charcoal, eluted with ethanolic ammonia (50% EtOH containing 0.3% NH_3), separated by electrophoresis at pH 3.5 in ammonium formate,[23] and the specific activities determined. In a similar experiment with glucose as sole carbon source the ribonucleotides each contained 1 mole radioactive phosphate per mole.

TABLE 3

RATIO OF P^{32} TO C^{14} IN RIBONUCLEOTIDES AFTER GROWTH ON GLUCOSE-U-C^{14} 6-P^{32}

		Ratio*
Substrate:	Glucose 6-phosphate	1.98
Product:	UMP	1.62
	GMP	1.70
	AMP	1.80
	CMP	1.82

* P^{32}/C^{14} normalized to the number of carbon atoms in glucose 6-phosphate.

Strain Hfr 3300 was grown from a small inoculum in medium 63 (P_i concentration 80 μmoles per ml) containing thiamine, 2 μg per ml, and doubly labeled glucose 6-phosphate, 8.2 μmoles per ml, as sole carbon source, *ca.* 3.4×10^4 cpm in P^{32} and 8×10^4 cpm in C^{14} per μmole, counted at infinite thinness in a windowless Geiger counter before and after shielding the C^{14} with two layers of Scotch tape. This procedure eliminated all of the C^{14} counts and 10–12% of P^{32}. A correction was made for the latter. The doubly labeled substrate was a mixture of glucose-U-C^{14} 6-P^{31}, prepared from glucose-U-C^{14} 1-P^{31} (International Chemical and Nuclear Corp.) by the action of phosphoglucomutase, and glucose 6-P^{32} prepared from glucose and ATP^{32} (β-γ-labeled, kindly provided by Dr. J. Hurwitz) by the action of hexokinase and myokinase. The two radioactive species were mixed and recrystallized. The cells were harvested at the end of growth (*ca.* 0.46 mg dry weight per ml), and the pellet was suspended in 5 ml of dilute saline-citrate (0.015 *M* KCl, 0.0015 *M* citrate). Five ml of 80% phenol was added, the mixture shaken for 30 min at room temperature, and the aqueous phase, together with one wash, dialyzed against 4 l of 0.2 *M* NaCl. The preparation was then hydrolyzed for 40 hr in 0.3 *N* KOH at 37°, acidified to 5% TCA, the TCA removed by ether extraction, and the ribonucleotides absorbed on charcoal, eluted, and separated by electrophoresis as in Table 2.

also grow on glucose, but lack the specific glucokinase. Such a strain, FR-1, was found after ethyl methane sulfonate treatment of GN-2 and selection on fructose medium (Table 1). FR-1 is also a revertant for mannose and for succinate.

Glucose and Glucose 6-Phosphate Utilization by GN-2.—If strain GN-2 indeed lacks all glucose-phosphorylating activity, it should be useful for the demonstration that glucose 6-phosphate utilization does not involve hydrolysis. Proof of the proposed explanation of the MM-6 and GN-2 phenotypes depends on the demonstration of the postulated hexokinase in the wild type and on a genetic analysis of the strains. A mutant defective in glucose transport would also fail to utilize glucose, but grow normally on glucose 6-phosphate.[20] However, GN-2 is unable to utilize glucose even when it is inside the cell. When GN-2 was grown on lactose, glucose appeared in the medium in equimolar amount to the lactose utilized (Fig. 1). Thus, glucose presented to the cell internally, by the cleavage of lactose, was not utilized. This strongly supported our assumption that GN-2 is completely unable to phosphorylate glucose. On the other hand, the wild type, MM-6, and GN-2 all grew equally well on glucose 6-phosphate[21] (Fig. 2). By arguments formally analogous to those of Lin *et al.*,[7] this shows that glucose 6-phosphate must enter the cells without hydrolysis.

Glucose 6-Phosphate Utilization by the Wild Type.—Since wild-type and mutant strains grow at equal rates on glucose 6-phosphate, the conclusion that the mutant utilizes this compound directly must apply as well to the wild-type strain. The remaining experiments, therefore, were performed with the wild-type parental strain, Hfr 3300. Hayashi *et al.*[10] were able to demonstrate the accumulation of L-α-

TABLE 4

UTILIZATION OF GLUCOSE 6-PHOSPHATE BY GN-2 WHEN IT HAS ALKALINE PHOSPHATASE

Time (min)	Cell density*	Alkaline phosphatase†	Glucose 6-phosphate‡	Glucose‡	P_i‡
0	0.174	112	3.79	0.45	0.30
20	0.170	97	3.53	0.94	0.36
40	0.182	80	3.52	1.16	0.56
60	0.233	78	2.65	1.33	2.00
124	0.365	50	1.44	1.52	2.83
178	0.586	35	0.0	1.64	4.51
216	0.574	38	0.0	1.62	4.50

* Absorbancy at 580 mμ in a 1-cm cell.
† mμMoles p-nitrophenol liberated in Tris 1 *M*, pH 8 per min per mg cells.
‡ μMoles per ml medium.
Cells of GN-2 were inoculated after growth on triethanolamine (TEA) medium[8] containing galactose and 5×10^{-4} *M* phosphate to TEA medium containing glucose 6-phosphate, 4.2 μmoles per ml as sole source of carbon and phosphate. Incubation was at 37° with shaking. Growth was followed by turbidity, and at intervals 1-ml portions of the culture were removed and chilled. The cells were removed by centrifugation and assayed for alkaline phosphatase.[24] The medium was assayed for glucose 6-phosphate,[25] glucose,[26], and inorganic phosphate.[27]

glycerophosphate in mutants lacking L-α-glycerophosphate dehydrogenase, despite the apparent presence of another minor pathway of L-α-glycerophosphate metabolism. Similar experiments could not be performed with glucose 6-phosphate, for only a mutant lacking all three known enzymes of glucose 6-phosphate metabolism[12] could be expected to accumulate glucose 6-phosphate. Instead, we have studied the origin of phosphate in a metabolic product of glucose 6-phosphate, the ribonucleotides of RNA. If glucose 6-phosphate, as the sole carbon source, were to enter the cells without hydrolysis, then the phosphate groups of RNA would be derived primarily from glucose 6-phosphate rather than from inorganic phosphate. On the other hand, if glucose 6-phosphate were cleaved, its phosphate group would not be the major contributor to nucleic acid phosphate when excess inorganic phosphate was present. When strain Hfr 3300 was grown in medium 63 containing P^{32}-inorganic phosphate, with glucose 6-phosphate as the sole carbon source, the specific activity of phosphate in RNA was 5 per cent of the specific activity of the inorganic phosphate (Table 2). The inorganic phosphate concentration in the cells was shown to be equal to the outside concentration (*ca.* 0.1 *M*), with a specific activity 83 per cent of that of the inorganic phosphate in the medium. In accord with the results of Roberts and Wolffe,[9] glucose 6-phosphate must have entered pentose phosphate and nucleic acid without appreciable exchange with inorganic phosphate.

A similar experiment was carried out with doubly labeled glucose 6-phosphate in the presence of unlabeled inorganic phosphate (Table 3). The ribonucleotides were isolated after alkaline hydrolysis of RNA and the P^{32}:C^{14} ratios measured. As in the other experiment, there was little dilution of the phosphate from glucose 6-phosphate by inorganic phosphate. The small dilution observed may be due to the conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate and the conversion of this product to pentose phosphate by the action of transketolase.

Inducibility of Glucose 6-Phosphate Utilization.—When strain Hfr 3300 was incubated at 37° with aeration in medium 63 containing chloramphenicol, 50 μg per ml, and glucose 6-phosphate, 5 μmoles per ml, glucose 6-phosphate disappearance from the medium was at a constant rate equivalent to 0.72 μmoles per mg of dry weight per hour if the cells had been grown on glucose, and 5.95 μmoles per mg of dry weight per hour if the cells had been grown on glucose 6-phosphate. This sub-

stantial difference in rate is presumably due to the induction, by glucose 6-phosphate, of some factor necessary for its utilization. Since the metabolic enzymes of glucose 6-phosphate metabolism (isomerase, dehydrogenase, and mutase) are all present in glucose-grown cells, the inducible factor might be a transport system.[21]

The Role of Phosphatases in Glucose 6-Phosphate Utilization.—It has been suggested earlier[8] that alkaline phosphatase has an obligatory role in the utilization of glucose 6-phosphate by whole cells. The results reported in this paper, on the contrary, show that glucose 6-phosphate enters the cell without hydrolysis and that phosphatase is not involved. This apparent contradiction can be related to the fact that the earlier experiments all involved low concentrations of glucose 6-phosphate (5×10^{-4} *M*) and nongrowing cells which had not previously been exposed to glucose 6-phosphate, but which did contain alkaline phosphatase. Under these conditions we would expect alkaline phosphatase to play a role in the utilization of glucose 6-phosphate. The experiments reported thus far in this paper have involved growth in media containing high concentrations of phosphate (0.1 *M*). This phosphate concentration would both inhibit the activity of alkaline phosphatase and repress alkaline phosphatase synthesis.

One might ask whether in a low phosphate medium strain GN-2 could grow on glucose 6-phosphate, for if alkaline phosphatase were active, the glucose 6-phosphate would be hydrolyzed and glucose cannot be utilized by this strain. In fact, strain GN-2 grows normally from small inocula in a medium containing glucose 6-phosphate as sole source of carbon and phosphate. There is no alkaline phosphatase activity in such a culture, an expected result, since the phosphate requirement for growth is far less than the carbon requirement, and glucose 6-phosphate utilization *ultimately* involves release of most of this phosphate as inorganic phosphate in the medium. This result suggests that alkaline phosphatase is not a significant factor in glucose 6-phosphate entry, even in "low-phosphate" medium. This question has been checked in more detail (Table 4) in an experiment in which GN-2, after growth on phosphate-limited medium and containing alkaline phosphatase, was incubated in a medium containing glucose 6-phosphate as sole source of carbon and phosphorus. After a brief lag, when there was some accumulation of glucose in the medium, there was substantial growth, little further glucose accumulation, much phosphate accumulation, and complete utilization of the glucose 6-phosphate. Despite the fact that even at the end of growth the cells contained substantial alkaline phosphatase activity, most of the glucose 6-phosphate escaped the action of this enzyme and was able to support growth.

We conclude that wild-type *Escherichia coli* has a mechanism for the direct entry of glucose 6-phosphate. Under the appropriate conditions, glucose 6-phosphate may also be cleaved by alkaline phosphatase, but this cleavage does not appear to be necessarily related to its entry into the cell. Alkaline phosphatase does have an obligatory role in the utilization of certain phosphate esters and, as Hayashi *et al.*[10] point out, its physiological role may be mainly in the provision of phosphate, since it is repressed by inorganic phosphate. It is not known what enzymes in high phosphate media are responsible for nucleotide cleavage; Neu and Heppel[28] have recently described an extramembranal nucleotidase which might have this role. It is also not known how many phosphate esters can enter the cell without hydroly-

sis. At present, the list is restricted to L-α-glycerol phosphate, glucose 6-phosphate, and probably glucose 1-phosphate and fructose 6-phosphate.[9]

It is of considerable interest that a mutant (FR-1) can be isolated which is capable of growth on glucose despite the apparent absence of glucokinase activity. The existence of this mutant provides support for the hypothesis that *E. coli* and related strains contain two kinases, one specific for glucose and the other active with a number of sugars, including glucose, fructose, and mannose. Thus far, no kinase has been detected in these organisms which accounts for the phosphorylation of fructose or mannose;[15, 29] it is likely that when this enzyme is found, it will act with glucose, as well as fructose and mannose. The function of the specific (but less active) glucokinase remains to be determined.

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