ON THE RELATION BETWEEN EFFECTOR CONCENTRATION AND THE RATE OF INDUCED ENZYME SYNTHESIS

GAD YAGIL *and* EZRA YAGIL

From the Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel and the Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel

ABSTRACT The Jacob and Monod scheme for the regulation of enzyme formation leads to the following relation between the relative rate of enzyme synthesis α and cellular effector concentration E (the lower sign is for repressible systems):

$$\log\left(\frac{\alpha}{1-\alpha}-\alpha_b\right)=\pm n\log[E]+\log\alpha_b\mp\log K_1\,.$$

This equation permits linear plotting of experimental data and the evaluation of three quantities: n, the number of effector molecules combining with a repressor molecule, K_1, the dissociation constant of this interaction and K_2/R_t, the ratio of repressor-operator dissociation constant to total repressor concentration. Measurements on the repression of alkaline phosphatase in *Escherichia coli* as a function of phosphate concentration are reported and fit the proposed equation with $n = 1$, indicating that the binding of a single phosphate to the repressor species may be sufficient to cause repression. K_1 of this interaction was found to be $0.58 \pm 0.11 \times 10^{-3}$ M. The available data regarding the enzymes of the *lac* operon in a variety of *E. coli* strains, and several other enzymes are analyzed. It is confirmed that the *lac* repressor interacts with 2 isopropyl thiogalactoside (IPTG) molecules to relieve repression with a $K_1 = 50 \pm 20 \times 10^{-12}$ M^2. In some strains, separate binding constants for the first and second IPTG molecules can be evaluated.

INTRODUCTION

The model proposed by Jacob and Monod (1961) for the regulation of enzyme synthesis in bacteria is now generally accepted (for review see: Beckwith,1967; McFall and Mass, 1967; Richmond, 1968). The most direct evidence is the recent isolation of the repressor of the *lac* operon (Gilbert and Müller-Hill, 1966, 1967; Riggs, Bourgeois, Newby, and Cohn, 1968), and the demonstration that it can modulate the synthesis of the enzyme in a cell-free system (Zubay and Lederman, 1969). An aspect of the regulation process which has not yet been sufficiently clarified is the quantitative relationship between the components of the system in the

intact organism, in particular between the small effector and the repressor molecules. A number of workers proposed to obtain this information from measurements of the rate of enzyme induction as a function of effector concentration (Boezi and Cowie, 1961; Marr and Marcus, 1962; Sadler and Novick, 1965; Overath, 1968). Still, a convenient quantitative treatment based on the Jacob-Monod model is not available.

In this communication a simple relation connecting the rate of enzyme synthesis with effector concentration is formulated. This relation is based on the operon model and is suitable for evaluation of experimental results. Data obtained by several authors are utilized to estimate the number of effector molecules binding to the repressor molecule in vivo and to derive affinity constants between effector and repressor molecules. Measurements are reported in the alkaline phosphatase regulatory system indicating that a single effector (phosphate) molecule may be involved.

QUANTITATIVE CONSIDERATIONS

The two basic equilibria implicit in the model as formulated by Jacob and Monod (1961) for inducible systems are (several assumptions made here are discussed in later sections):

$$nE + R \overset{K_1}{\rightleftharpoons} RE_n, \tag{1}$$

$$O + R \overset{K_2}{\rightleftharpoons} OR. \tag{2}$$

E is an effector molecule (inducer or corepressor); R is an unbound repressor molecule in the cell; RE_n is a repressor molecule which binds n effector molecules; O is an operator free to be transcribed; OR is an operator which is binding a repressor; $[O_t]$ is the total number of operators in a cell:[1]

$$[O_t] = [O] + [OR]. \tag{3}$$

$[R_t]$ is the total concentration of repressor. We shall first consider the case where the fraction of repressor partially binding effector is small so that:

$$[R_t] = [R] + [RE_n] \tag{4}$$

(the single repressor, OR, possibly bound to the operator, is neglected).

---

[1] There are at most four operator sites in a cell and also not many repressor molcules (Gilbert and Müller-Hill, 1966), so that the question may be raised as to the meaning of concentration and the validity of thermodynamic treatment of these interactions. This question has been raised for many kinds of "small" systems, and was treated in detail (Hill, 1963). It has been pointed out that thermodynamic equations can be applied to small systems as long as a large ensemble of such systems is being measured. It may be best to view $[O]$ as the probability of an operator being free of repressors, etc.

The two equilibrium constants associated with equations 1 and 2 are:

$$K_1 = \frac{[R][E]^n}{[RE_n]}, \tag{5}$$

$$K_2 = \frac{[O][R]}{[OR]}. \tag{6}$$

Introducing R_t into equation 5, we obtain:

$$R = \frac{K_1[R_t]}{K_1 + [E]^n}. \tag{7}$$

Let α be the fraction of free operators in a population $\alpha = [O]/[O_t]$; similarly, $1 - \alpha = [OR]/[O_t]$. This, in combination with equations 6 and 7, leads to

$$\frac{\alpha}{1 - \alpha} = \frac{K_2}{K_1[R_t]}[E]^n + \frac{K_2}{[R_t]}. \tag{8}$$

When the concentration of effector $[E]$ equals zero (repression is maximal), enzyme is synthesized at basal rate, and equation 8 reduces to $\alpha/(1 - \alpha) = K_2/[R_t]$. Under these conditions, the majority of operators is bound, i.e. $\alpha \ll 1$, and α at basal level (α_b) is given by:

$$\alpha_b = \frac{K_2}{[R_t]}. \tag{9}$$

Basal enzyme synthesis is independent of the affinity of effector to repressor (K_1) and depends only on total repressor present (R_t) and its affinity (K_2) to the operator.

We can now rewrite equation 8:

$$\frac{\alpha}{1 - \alpha} = \frac{\alpha_b}{K_1}[E]^n + \alpha_b, \tag{10}$$

or in logarithmic form

$$\log\left(\frac{\alpha}{1 - \alpha} - \alpha_b\right) = n \log [E] + \log \frac{\alpha_b}{K_1}. \tag{11}$$

The left-hand expression of equation 11 can be plotted against the logarithm of effector concentration; a straight line should result if the model employed is correct. The slope of the straight line, n, gives the stoichiometry of the $E - R$ interaction and the intercept, $\log \alpha_b/K_1$, permits the calculation of K_1. We shall refer to such plots as induction plots.

α values are measured as the relative rate of enzyme synthesis assuming that the rate of enzyme synthesis is proportional to the number of operons free to be tran-

scribed. The experimental quantity generally employed to measure the rate of induced enzyme synthesis is the increase in enzyme relative to the increase in bacteria, $\Delta E/\Delta B$ (Monod, Pappenheimer, and Cohen-Bazire, 1952; Koch, 1967) (E—enzyme units per milliliter of culture, B—bacteria per milliliter of culture). This quantity is usually constant a few minutes after the inducing signal is given. Alternatively, E/B, the specific activity after several generations of growth, is sometimes employed (cf. Marr and Marcus, 1962, Appendix). Thus, we have for α:

$$\alpha = \frac{(\Delta E/\Delta B)}{(\Delta E/\Delta B)_{max}} \quad \text{or} \quad \alpha = \frac{(E/B)}{(E/B)_{max}} . \tag{12}$$

The subscript max denotes rate of enzyme synthesis at full induction. Values of α_b are obtained in a similar way by measurements in the absence of effector.

In systems where enzyme synthesis is *repressed* by an effector (for instance alkaline phosphatase), equilibrium equation 2 has a somewhat different form:

$$O + RE_n \overset{K_2}{\rightleftharpoons} ORE_n ,$$

$$K_2 = \frac{[O][RE_n]}{[ORE_n]} . \tag{13}$$

This leads to the following relation:

$$\frac{\alpha}{1-\alpha} = \frac{K_1 K_2}{[R_t]} \frac{1}{[E]^n} + \frac{K_2}{[R_t]} . \tag{14}$$

Here, the first term becomes negligible at *high* effector concentration, leaving us again with a basal rate of $\alpha_b = K_2/[R_t]$. The same log-log plot as before can be utilized to evaluate n and K_1:

$$\log\left(\frac{\alpha}{1-\alpha} - \alpha_b\right) = -n \log [E] + \log \alpha_b \cdot K_1 . \tag{15}$$

The slope in a repressible system is negative and the intercept has a form slightly different from that of an inducible system.

A case worth considering is with $n = 2$, where the affinity of the repressor molecule for the first effector molecule is not considerably lower than for the second one. In that case, each binding step has to be considered separately:

$$R + E \rightleftharpoons RE ; \quad K_{11} = \frac{[R][E]}{[RE]} \tag{16}$$

$$RE + E \rightleftharpoons RE_2 ; \quad K_{12} = \frac{[RE][E]}{[RE_2]} \tag{17}$$

$$K_1 = K_{11} \cdot K_{12} \tag{18}$$

and

$$[R_t] = [R] + [RE] + [RE_2]. \tag{19}$$

From these expressions one can derive, in the same way as before

$$\frac{\alpha}{1 - \alpha} = \frac{\alpha_b [E]^2}{K_{11} \cdot K_{12}} + \frac{\alpha_b [E]}{K_{11}} + \alpha_b \,. \tag{20}$$

At high E values, $E^2 > E$, the second term of the equation becomes negligible, and the expression reduces to the expression of equation 10 with $n = 2$. In this region, the induction plot will have a slope of 2 and $K_1 = K_{11} \cdot K_{12}$ can be calculated as before. At low E values, the first term of equation 20 becomes negligible, resulting in a simple linear relation; a suitable plot yields a value for K_{11}, the affinity constant of the first effector molecule. Division of K_1 by K_{11} gives a value for K_{12}, the affinity constant for binding the second effector molecule. Alternatively $((\alpha/[1 - \alpha]) - \alpha_b)/[E]$ can be plotted vs. $[E]$; a linear plot results, the intercept of which gives α_b/K_{11} and the slope $\alpha_b/K_{11} \cdot K_{12}$. Both constants can thus be evaluated.

THE EXPERIMENTAL EVIDENCE

The Lactose Operon

The most extensive measurements of the effect of inducer concentration on enzyme synthesis have been performed in the lactose operon of *E. coli*, and shall be discussed first. The results reported by several investigators are summarized in Table I. Values of log $((\alpha/[1 - \alpha]) - \alpha_b)$ were calculated for each set of data and plotted against the logarithm of inducer concentration, according to equation 11.[2]

Plots of the data reported by Boezi and Cowie (1961), Müller-Hill, Rickenberg, and Wallenfels (1964), Alpers and Tomkins (1966), as well as some of the extensive measurements recently published by Overath (1968) are shown in Figs. 1–6. Straight lines result in all cases where the maximal rate of synthesis is well established. This demonstrates that the Jacob-Monod model does indeed provide a quantitative explanation of the induction process. The slopes of the straight lines, which according to equation 11 indicate the stoichiometry of the effector-repressor interaction (n), are given in the figures and are listed in Table I. All the plots have a slope of either 2 or close to it, leading to the conclusion that two inducer molecules

[2] Some of the data utilized do not include values of α_b. Since the contribution of α_b to the ordinate is negligible except at the lowest effector concentrations, data can often be plotted without α_b, but a value of K_1 cannot be calculated. In some cases, once n is known, one can plot $\alpha/(1 - \alpha)$ vs. E^n according to equation 10 and obtain a value of α_b by extrapolation to zero; this value can be utilized to calculate K_1.

TABLE I

SUMMARY OF QUANTITATIVE INDUCTION MEASUREMENTS

Author	Organism and strain	Enzyme	Inducer*	$\alpha_b (= K_2/R_t)$	n	K_1‡	Remarks
						(μM^2)	
Herzenberg, 1959; Table 4	*E. coli* ML3 (y^-)	β-galactosidase	IPTG	—	2.4	—	—
Fig. 4	"	"	"	—	2.68	—	—
"	"	"	TMG	—	1.79	—	—
Boezi and Cowie, 1961; Figs. 2, 3	*E. coli* ML3(y^-)	β-galactosidase	IPTG	0.0004	1.91	40	See Fig. 1.
Pardee and Prestidge, 1961; Fig. 2	*E. coli* K12 C600-1(y^-)	β-galactosidase	IPTG	—	2.78	—	—
Müller-Hill et al., 1964; Table 4	*E. coli* ML3 (y^-)	β-galactosidase	MTF	0.00013	1.82	24,000	See Fig. 5.
Sadler and Novick, 1965; Figs. 4, 15	*E. coli* K12 W14 (y^-)	β-galactosidase	IPTG	0.000104	2.01	44	—
	W14 D ($y^-/F'y^-$)	"	"	0.000047	2.12	39	—
Alpers and Tomkins, 1966; Table 1	*E. coli* AT2322 (y^-)	β-galactosidase	IPTG	—	2.13	—	—
Gilbert and Müller-Hill, 1966; Fig. 1	*E. coli* W3102 (i^s y^-)	β-galactosidase	IPTG	0.0014	0.86; 1.97	1.53 (K_{11} = 0.67 μM; K_{12} = 2.28 μM)	—

Overath, 1968; Figs. 1, 2	*E. coli* B BB220 lac_5^- (y^-)	β-galactosidase	IPTG	0.000625	2.25	41.5	
	E. coli K12 2001c (y^-)	"	"	0.00179	2.27	35	See Fig. 2.
	E. coli 15 TAU lac_2^- (y^-)	"	"	0.0114	1.10; 2.19	71 (K_{11} = 11.6 μM; K_{12} = 16.4 μM)	See Fig. 3.
	E. coli K12 C600 o^c (y^-)	"	"	0.0654	1.12; 2.06	13 (K_{11} = 3.4 μM; K_{12} = 3.8 μM)	See Fig. 4.
Schlesinger and Magasanik, 1965; Table 1	*A. aerogenes* 35	Histidine-NH_3-lyase	Imidazole propionate	0.0384	2.04	60	5,400 μM²§ See Fig. 10.
Chasin and Magasanik, 1968	*B. subtilis* SH4 (histidase$^-$)	Urocanase	Histidine	0.01	2.30	0.23	2.3 μM²§ See Fig. 10.
Marr and Marcus, 1962; Fig. 4	*A. agilis*	Mannitol dehydrogenase	Ribitol	—	3.13	—	—
Nijkamp and De Haan, 1967; Table 4	*E. coli* K12 (*gua* A$^-$)	IMP dehydrogenase	Guanine	—	0.92	—	See Fig. 11.
	" " (*gua* B$^-$)	XMP aminase	"	—	0.63	—	—
This paper, Table 3	*E. coli* K10	Alkaline phosphatase	PO_4^{-3}	0.00018–0·00054	0.93	0.47–0.70 mM	See Fig. 9.

* IPTG: isopropyl thiogalactoside; MTF: methyl-1-thio-β-D-fucoside; TMG: thiomethyl galactoside.
‡ K_1 can be calculated only when a firm value of α_b is available. All K_1 values are based on external effector concentrations.
§ K_1 corrected for difference between external and internal concentrations, as explained in text.

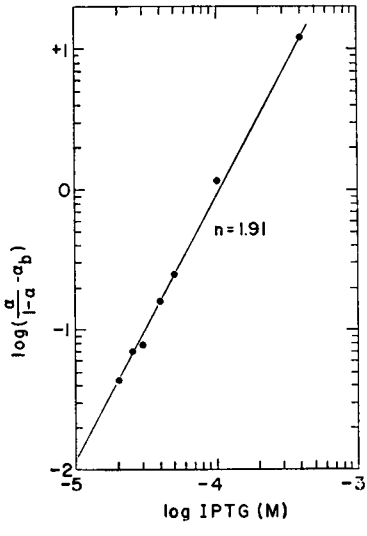

FIGURE 1

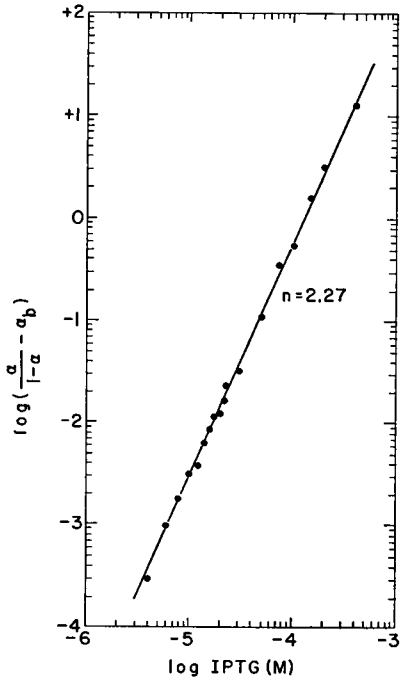

FIGURE 2

FIGURE 1 Induction of β-galactosidase by IPTG in *E. coli* strain ML3. The data of Boezi and Cowie (1961, Fig. 3) are plotted according to equation 11.

FIGURE 2 Induction of β-galactosidase by IPTG in *E. coli* K12 strain 2001c. The data of Overath (1968, Fig. 1) are plotted according to equation 11.

interact with a single repressor molecule to relieve the repressed state of the β-galactosidase gene. This conclusion has been reached by an indirect procedure by Sadler and Novick (1965; see legend to Fig. 15) and also by Boezi and Cowie (1961), employing, however, a scheme different from that of Jacob and Monod, which involves direct interaction between effector and an unstable product of the structural gene.

It is seen from equation 11 that in order to evaluate K_1, the binding constant of effector to repressor, firm values for the basal as well as maximal rates of synthesis are required. K_1 values were calculated for those cases where α_b values were available and are shown in Table I. Care was taken to read the intercept amidst the experimental points so as to minimize the effect of deviations of the slope from 2. Most data yield a value of K_1 for IPTG which is around 40×10^{-12} M^2. Exceptions are the value for the tight binding strain (i^t) reported by Gilbert and Müller-Hill (1966) and the operator constitutive strain (o^c) reported by Overath (1968). While the lower value of Gilbert and Müller-Hill is expected, the latter may be due to an experimental deviation (one should remember that any deviation will affect K_1 to the second power).

In some of the cases, e.g. Figs. 3 and 4, the slopes at the lowest inducer concentra-

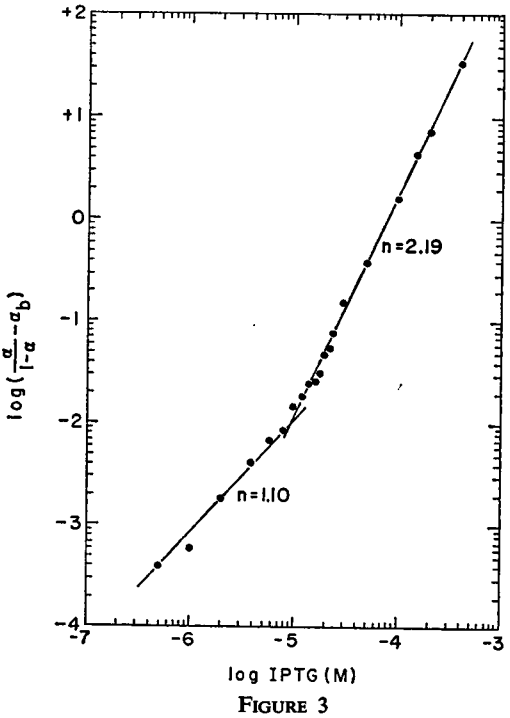

FIGURE 3

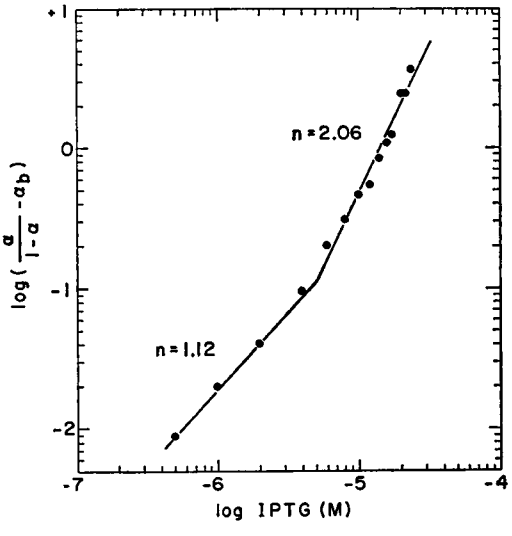

FIGURE 4

FIGURE 3 Induction of β-galactosidase by IPTG in *E. coli* 15 strain TAU lac_2^-. The data of Overath (1968, Fig. 1) are plotted according to equation 11.

FIGURE 4 Induction of β-galactosidase by IPTG in *E. coli* K12 operator constitutive strain C600 o^c y_1^-. The data of Overath (1968, Fig. 1) are plotted according to equation 11.

tion approach unity, indicating that in this range only one inducer molecule is bound. This linear relation is also demonstrated in Fig. 1 of Gilbert and Müller-Hill (1966) and in Fig. 2 of Overath (1968). Values of the individual binding constants K_{11} and K_{12} were evaluated using equation 20 and are shown in Table I. If the two binding sites were equivalent and completely independent, one would expect $K_{12} = 4K_{11}$ because of the statistical factor (the first molecule has two possibilities to get on the repressor and only one to get off, whereas for the second molecule, the opposite situation prevails). This is borne out by the values of Gilbert and Müller-Hill's i^s strain; for the other strains the values are closer than four, indicating that the two binding sites are interacting; the binding of the first molecule to the repressor somewhat strengthens the affinity of the second one. A certain degree of cooperativity is thus apparent even in these cases. It should be noted that the K_1 values reported are based on extracellular concentrations of the inducer. However, all strains listed are permease negative (y^-) and Kepes (1960) has shown in one of them (ML3) that the internal concentrations of effector are very close to the external ones.

In two cases, induction by effectors other than IPTG was evaluated. Both thio-methylgalactoside (TMG, Herzenberg, 1959) and methyl-1-thio-β-D-fucoside

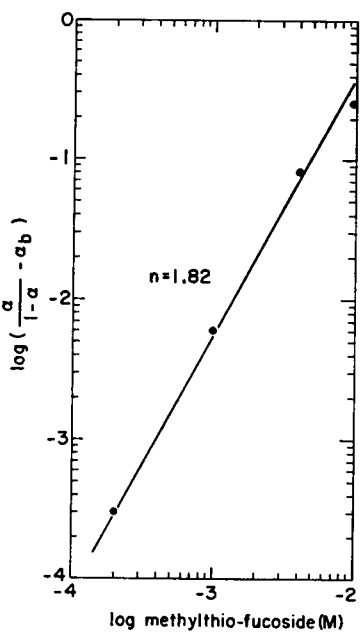

FIGURE 5

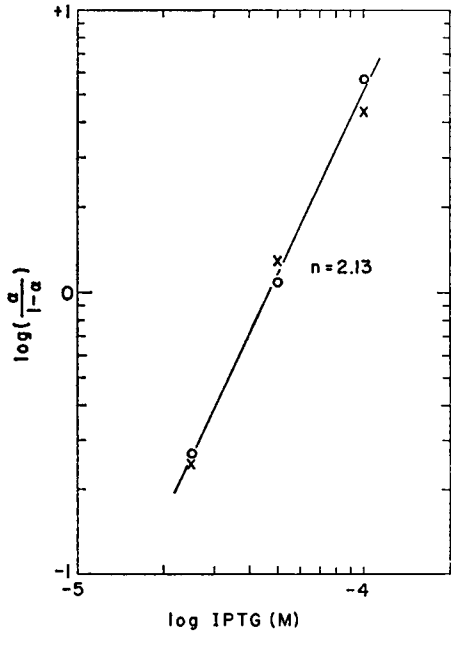

FIGURE 6

FIGURE 5 Induction of β-galactosidase by methyl-1-thio-β-D-fucoside in *E. coli* strain ML3. The data of Müller-Hill, Rickenberg, and Wallenfels (1964; Table 4) are plotted according to equation 11. $(E/B)_{max}$ was taken as 7000 (cf. their Table 3).

FIGURE 6 Induction of β-galactoside (○) and thiogalactoside-transacetylase (×) in *E. coli* strain AT2322. The data of Alpers and Tomkins (1966, Table I) are plotted according to equation 11. No data on α_b are given.

(MTF, Müller-Hill, Rickenberg and Wallenfels, 1964) yield linear induction plots with slopes close to 2 (Fig. 5). The data of Müller-Hill et al. contain a value of α_b, so that K_1 can be calculated. A value of $K_1 = 24{,}000 \times 10^{-12}$ M^2 is obtained, indicating that this inducer is bound 25 times weaker than IPTG.

The data reported by Alpers and Tomkins (1966) include measurements on thiogalactoside-transacetylase, a second enzyme of the lactose operon (Fig. 6). The induction plot for this enzyme is identical to that of β-galactosidase, as should be expected.

Alkaline Phosphatase

The synthesis of alkaline phosphatase is repressed by the orthophosphate ion (Torriani, 1960). So far, no operator site has been detected in this system; still, two regulatory genes are known, the products of which cooperate to form the active repressing species (Echols, Garen, Garen, and Torriani, 1961; Garen and Echols, 1962 *a*, *b*). The most pronounced derepression of this enzyme is observed with phosphate concentrations between 10^{-5} and 10^{-6} M. At these concentrations the growing

TABLE II

RATE OF ALKALINE PHOSPHATASE SYNTHESIS IN *E. coli*, AS FUNCTION OF PHOSPHATE CONCENTRATION IN THE MEDIUM

(For details see legend to Fig. 7.)

Phosphate in growth medium	Phosphate at end of experiment*	Generation time	$\Delta E/\Delta B$	α	$\alpha - \alpha_b$
(mM)	(mM)	(min)	$\left(10^3 \times \frac{units}{ml \cdot A_{540}}\right)$	($\times 10^4$)	($\times 10^4$)
>0.010	0	—	45,700	Unity	
0.4	0.25	67	25	5.47	2.73
0.8	0.8	67	19	4.18	1.44
1.5	1.8	68	16	3.51	0.78
5.0	4.4	69	14	3.00	0.32
10.0	9.0	72	12.6	2.76	
20.0	18.5	70	13.4	2.94	$\alpha_b = 2.76 \times 10^{-4}$
50.0	39.0	73	12.4	2.72	

* Determined by the method of Lowry, Roberts, Leiner, Wu, and Farr (1954).

bacteria rapidly consume the small amounts of phosphate present, and it is difficult to perform accurate measurements at fixed phosphate concentrations. The effect of higher phosphate concentrations on the low rates of enzyme synthesis can, however, be determined.

A series of measurements were carried out on the rate of alkaline phosphatase synthesis in exponentially growing wild type *E. coli* K10. A typical experiment, in which phosphate concentration varied over 100-fold ($0.4–50 \times 10^{-3}$ M) is shown in Table II. It is seen that only at the lowest concentration is an appreciable fraction of the phosphate in the growth medium consumed. It is also seen that the generation time is unaffected by the amount of phosphate present; this is important, since only under these conditions is the rate of enzyme synthesis per bacterium, $\Delta E/\Delta B$, a true measure of the fraction of genes free to synthesize enzyme (see Marr and Marcus, 1962, Appendix; Koch, 1967). Plots of enzyme units per ml against bacterial mass at the various phosphate concentrations are shown in Fig. 7. The slopes of the straight lines obtained give the values of $\Delta E/\Delta B$ shown in Table II. The rate of synthesis under maximal derepression (when phosphate is depleted from the medium) was found to be 45.7 units/ml. This value is used to calculate α values as shown in Table II. The values of $\Delta E/\Delta B$ are plotted against phosphate concentration in Fig. 8. It is seen that repression is maximal from 10 mM phosphate up and leads to $\alpha_b = 2.76 \times 10^{-4}$ for the relative rate of synthesis at basal level. In Fig. 9, $\log(\alpha - \alpha_b)$ is plotted against log phosphate concentration; data of two separate experiments are included. A straight line with a slope of -0.93 is obtained, suggesting that a single phosphate molecule interacts with a repressor species to cause repression.

In two further experiments the relative rate of synthesis was determined by measur-

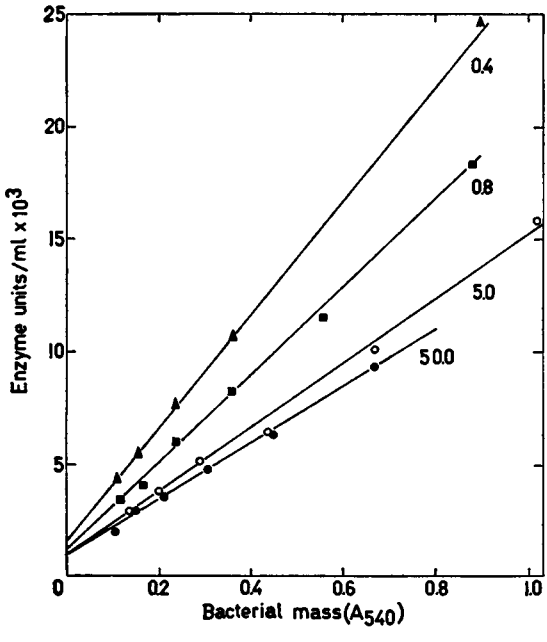

FIGURE 7

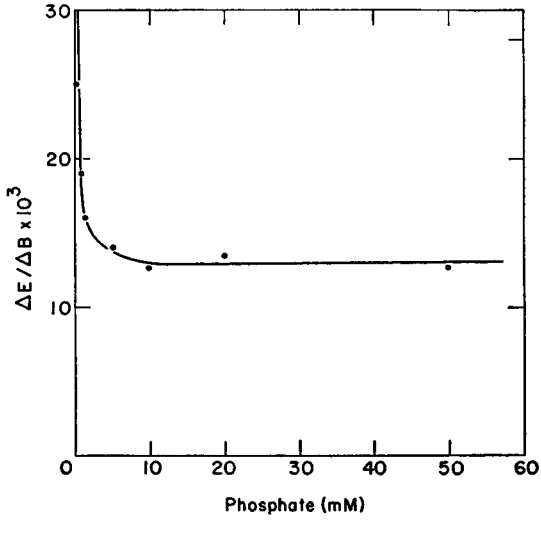

FIGURE 8

FIGURE 7 Alkaline phosphatase synthesis in *E. coli* K10 at various potassium phosphate concentrations. (▲): 0.4 mM; (■): 0.8 mM; (○): 5.0 mM; (●): 50.0 mM. *E. coli* cells (wild type strain K10) growing exponentially in TG medium (Echols et al., 1961) supplemented with 1.0 mM KH_2PO_4 were centrifuged, washed with unsupplemented TG medium, and resuspended in the same growth medium supplemented with KH_2PO_4 at the concentrations indicated. The cells were grown at 37°C under constant shaking. Bacterial growth was measured as A_{540} in a Zeiss PMQ II spectrophotometer (Carl Zeiss, Inc., N.Y.). Alkaline phosphatase was assayed as described by Rothman and Coleman (1968) except that the concentration of *p*-nitrophenylphosphate in the assay solution was 0.8 mg/ml, in 1 M Tris, pH 8.0. Concentration of enzyme in assay solution was the same as in the culture. Enzyme unit is defined as ΔA_{410} per min^{-1}.

FIGURE 8 Slopes of enzyme units vs. bacterial mass (ΔE/ΔB of Fig. 7 and Table II), plotted against phosphate concentration in the medium.

ing the specific activity (E/B) after approximately 10 generations of growth in various phosphate concentrations. From these specific activities α values were calculated according to equation 12. The resulting induction plots have also slopes around -1. The exact n values, as well as those of K_1 calculated from the intercepts are summarized in Table III. All four values of K_1 are close to 0.5×10^{-3} M. This figure indicates that the binding of phosphate to the alkaline phosphatase repressor is about two orders of magnitude weaker than the binding of IPTG to the *lac* repressor. However, it should be pointed out that we have no information as to whether phosphate concentration inside the cells is equal to the concentration in the medium. Although the linearity of the induction plots is consistent with the proposed treatment, the possibility of either a constant proportionality in phosphate concentra-

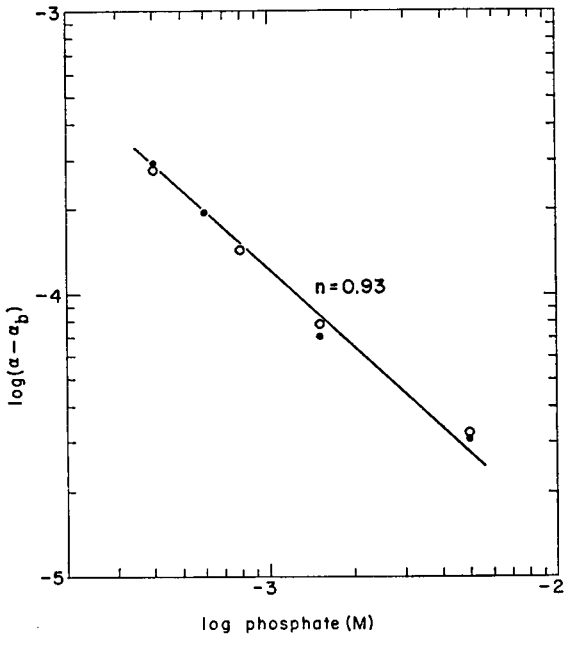

FIGURE 9 Alkaline phosphatase repression by orthophosphate plotted according to equation 15. At the concentrations of phosphate used $\alpha \ll 1$ and therefore $\alpha \approx \alpha/(1 - \alpha)$. Values of α were calculated from the $\Delta E/\Delta B$ values of Table II (○) and of experiment 2 in Table III (●).

TABLE III

ALKALINE PHOSPHATASE—SUMMARY OF REPRESSION EXPERIMENTS

No. of experiment	Method determining rate of enzyme synthesis	α_b	n	K_1
				(μM)
1	$\Delta E/\Delta B$	2.76×10^{-4}	0.93	467
2	"	1.79×10^{-4}	0.93	697
3	E/B	4.16×10^{-4}	1.26	467
4	"	5.4×10^{-4}	1.04	542

tion across the membrane, leading to an error in K_1 by a constant factor, or an induction process other than that proposed by Jacob and Monod cannot be excluded.

Other Regulatory Systems

Not many data on the effect of inducer concentration on other regulatory systems are available. Data reported for two enzymes of the histidine degradation path-

way, histidine ammonia lyase in *Aerobacter aerogenes* (Schlesinger and Magasanik, 1965) and urocanase in *Bacillus subtilis* (Chasin and Magasanik, 1968) were calculated according to equation 11 and the induction plots are shown in Fig. 10. The available points seem to fall on a straight line; the slopes of these lines are again close to 2 (Table I), indicating a stoichiometry of inducer-repressor interaction similar to that of the lactose operon. The values of K_1 calculated from the plots should, however, be taken carefully, because both inducers, histidine and imidazole-propionate, tend to accumulate within the cells. According to Hartwell and Magasanik (1964) *B. subtilis* concentrates histidine by a factor of 10 over a range of external histidine concentrations. According to Schlesinger and Magasanik (1965) imidazole propionate is concentrated 90-fold in *A. aerogenes*. Corrected values of K_1 were obtained using these factors and are shown in the last column of Table I.

Data on the effect of ribitol on the induction of mannitol dehydrogenase in *A. agilis* have been reported by Marr and Marcus (1962). Here also, a straight induction plot is obtained, with a slope close to 3, suggesting that if indeed induction in this system follows the Jacob-Monod scheme, then the interaction is of a higher order than in the preceding cases.

A set of data in a repressible system is reported by Nijkamp and De Haan (1967)

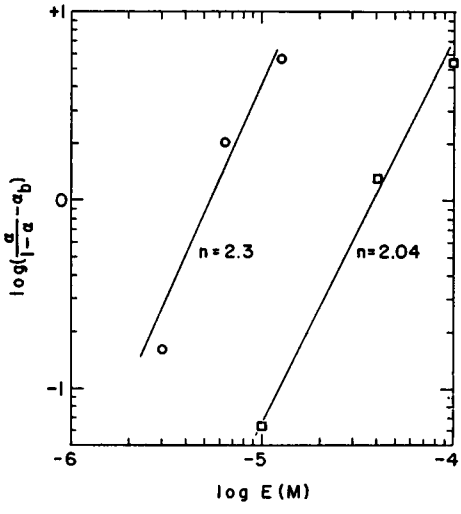

FIGURE 10

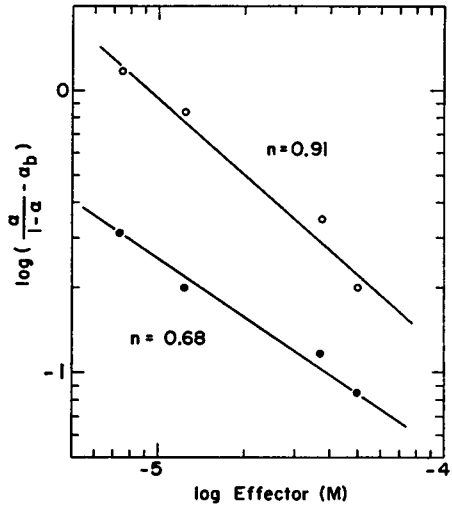

FIGURE 11

FIGURE 10 Induction plot of two enzymes in the histidine degrading operon. (□): hsitidine ammonia lyase induction by imidazole propionate in *Aerobacter aerogenes* strain 35; data of Schlesinger and Magasanik (1965, Table 1). (○): urocanase induction by histidine in *B. subtilis* strain SH4; data of Chasin and Magasanik (1968, Fig. 4].

FIGURE 11 Induction plot of two enzymes of the guanine operon in *E. coli* K12. The data of Nijkamp and De Haan (1967, Table 4) are plotted according to equation 15, with guanine as effector. $(E/B)_{max}$ values were obtained by extrapolating Fig. 3 to 16 hr. α_b was taken as 0. (○): inosine-monophosphate dehydrogenase, in a *gua* A^- strain; (●): xanthine-monophosphate aminase in a *gua* B^- strain.

concerning two enzymes of the guanine biosynthesis operon, inosine-monophosphate dehydrogenase and xanthine-monophosphate aminase. At least one of the two enzymes yields an induction plot similar to alkaline phosphatase, with $n = 1$ (Fig. 11).

DISCUSSION

The data analyzed lead to the conclusion that the relation between effector concentration and rate of induced enzyme synthesis in vivo can be described by an equation (equation 11) based on the Jacob-Monod model for the regulation of enzyme synthesis. The linearity of the plots based on this equation gives quantitative support to the basic assumptions of the model. From these plots one can derive numerical values for n, the number of effector molecules combining with a repressor molecule; for K_1, the affinity constant between effector and repressor molecules and for $K_2/[R_t]$, the ratio between represor affinity to the operator and repressor concentration.

Before discussing these three quantities some of the assumptions made in deriving and applying equation 11 should be restated.

(*a*) The rate of enzyme synthesis is proportional to the number of free operators.

(*b*) The effector concentration, when measured outside the cell, is correlated with the inside concentration in a known way.

(*c*) The amount of repressor which is only partly saturated with effector is negligible. When this is not the case, an equation such as equation 20 has to be applied.

(*d*) There are more repressor molecules than operators in the cells, so that $[OR] < [R_t]$. When this is not the case, more complicated equations have to be used, as carried out by Koch (1967).

(*e*) Rate factors do not play a role, i.e., equilibria I and II are reestablished and full rate of synthesis is resumed before rate of enzyme production is determined.

The data for the lactose (and histidine) operon indicate that two effector molecules interact with each repressor molecule to bring about full induction. This concurs with the recent findings in a cell-free system of β-galactosidase induction (Zubay and Lederman, 1969). There is evidence that the *lac* repressor is composed of more than two (possibly four) subunits (Sadler and Novik, 1965; Riggs and Bourgeois, 1968). It is possible that only two of the subunits have binding sites for IPTG or else additional IPTG molecules may bind to the repressor in a way which does not affect enzyme production.

In repressible systems, our data on alkaline phosphatase as well as the data on the guanine operon (Nijkamp and De Haan, 1967) fit the analogous equation for repressible systems (equation 15). In both cases, one rather than two effector molecules is bound to the repressor.

From the intercepts of the induction plots one can determine the value of K_1,

the association constant between effector and repressor. It is seen that in a large series of *E. coli* strains, a constant K_1 value of about 40×10^{-12} M^2 for the interaction of IPTG with the *lac* repressor is obtained. In some of the plots of β-galactosidase, it is possible to determine separately the constants associated with the first and second binding steps. The absolute values of K_{11} and K_{12} show that the binding of the two effector molecules is somewhat cooperative. The values of K_{11} and K_{12} obtained are in the range of $1–10 \times 10^{-6}$ M; this value is of the same order of magnitude as the binding of many coenzymes and substrates to enzymes. By in vitro binding studies, Zubay and Lederman (1969) estimate a K_1 of 1.8×10^{-6} M.

The third quantity obtained by the proposed treatment is $\alpha_b = K_2/[R_t]$ (equation 9), the ratio of the affinity constant between repressor and operator to total repressor concentration. It is not possible to obtain separate values for K_2 or $[R_t]$ unless one of them is already known. In this way, Gilbert and Müller-Hill (1966), employing a value of 10–20 repressor molecules per cell ($[R_t] = 1–2 \times 10^{-8}$ M), estimate that $K_2 = 1–2 \times 10^{-11}$ M.

Overath (1968) presents detailed measurements of β-galactosidase induction in a series of four strains, the basal enzyme level of which varies over 100-fold (Table I). As implied by equation 9, and as seen from the Table, α_b is independent of K_1. In the operator constitutive strain (C600 o^c), where the mutation is in the operator gene, the highly elevated basal activity can only be due to a change in K_2 and not in $[R_t]$. The relatively high α_b in the i^t strain of Gilbert and Müller-Hill (1966) (Table I) means either that its repressor concentration is low (which is unlikely since repressor was isolated from this strain) or that the affinity of its repressor to operator (K_2) is also affected by the i^t mutation. In strains diploid for the *lac* operon, α_b is reduced by a factor of two (see data of Sadler and Novick, 1965, in Table I; cf. also Table V of Overath, 1968). According to equation 9, the reason is the doubling of R_t in the diploid strains.

In conclusion, the treatment presented enables one to determine, by a relatively simple procedure, stoichiometric relationships and binding constants between the elements controlling the synthesis of specific proteins in the intact cell. Recently progress has been made in reconstituting effector-controlled enzyme synthesis in a cell-free system (Müller-Hill, Crapo, and Gilbert, 1968; Zubay and Lederman, 1969). It is thus becoming possible to obtain values for the quantities discussed by direct measurement, at least in the *lac* operon. The comparison of the values obtained in cell-free systems with those obtained by measurements in the intact cell may lead to further interesting results on the intact regulatory unit.

The technical assistance of Mrs. Nava Silberstein is acknowledged.

Supported by a grant to E. Yagil by the B. de Rothschild Foundation for the Advancement of Science in Israel.

Received for publication 17 March 1970.

REFERENCES

ALPERS, D. H., and G. M. TOMKINS. 1966. *J. Biol. Chem.* **241**:4434.
BECKWITH, J. R. 1967. *Science (Washington)*. **156**:597.
BOEZI, J. A., and D. B. COWIE. 1961. *Biophys. J.* **1**:639.
CHASIN, L. A., and B. MAGASANIK. 1968. *J. Biol. Chem.* **243**:5165.
ECHOLS, H., A. GAREN, S. GAREN, and A. TORRIANI. 1961. *J. Mol. Biol.* **3**:425.
GAREN, A., and H. ECHOLS. 1962 *a. J. Bacteriol.* **83**:297.
GAREN, A., and H. ECHOLS. 1962 *b. Proc. Nat. Acad. Sci. U.S.A.* **48**:1398.
GILBERT, W., and B. MÜLLER-HILL. 1966. *Proc. Nat. Acad. Sci. U.S.A.* **56**:1891.
GILBERT, W., and B. MÜLLER-HILL. 1967. *Proc. Nat. Acad. Sci. U.S.A.* **58**:2415.
HARTWELL, L. H., and B. MAGASANIK. 1964. *J. Mol. Biol.* **10**:105.
HERZENBERG, L. A. 1959. *Biochim. Biophys. Acta.* **31**:525.
HILL, T. L. 1963. Thermodynamics of Small Systems. W. A. Benjamin, Inc., New York.
JACOB, F., and J. MONOD. 1961. *J. Mol. Biol.* **3**:318.
KEPES, A. 1960. *Biochim. Biophys. Acta* **40**:70.
KOCH, A. L. 1967. *J. Theor. Biol.* **16**:166.
LOWRY, O. H., N. R. ROBERTS, K. Y. LEINER, M.-L. WU, and A. L. FARR. 1954. *J. Biol. Chem.* **207**:1.
MARR, A. G., and L. MARCUS. 1962. *Biochim. Biophys. Acta.* **64**:65.
MCFALL, E., and W. K. MAAS. 1967. *In* Molecular Genetics. J. H. Taylor, editor. Academic Press, Inc. Ltd., London. (Pt. II):255–321.
MONOD, J., A. M. PAPPENHEIMER, JR., and G. COHEN-BAZIRE. 1952. *Biochim. Biophys. Acta* **9**:648.
MÜLLER-HILL, B., L. CRAPO, and W. GILBERT. 1968. *Proc. Nat. Acad. Sci. U.S.A.* **59**:1259.
MÜLLER-HILL, B., H. V. RICKENBERG, and K. WALLENFELS. 1964. *J. Mol. Biol.* **10**:303.
NIJKAMP, H. J. J., and P. G. DE HAAN. 1967. *Biochim. Biophys. Acta.* **145**:31.
OVERATH, P. 1968. *Mol. Gen. Genet.* **101**:155.
PARDEE, A. B., and L. S. PRESTIDGE. 1961. *Biochim. Biophys. Acta.* **49**:77.
RICHMOND, M. H. 1968. *In* Essays in Biochemistry. P. N. Campbell and G. D. Greville, editors. Academic Press, Inc. Ltd., London. **4**:105–154.
RIGGS, A. D., and S. BOURGEOIS. 1968. *J. Mol. Biol.* **34**:361.
RIGGS, A. D., S. BOURGEOIS, R. F. NEWBY, and M. COHN. 1968. *J. Mol. Biol.* **34**:365.
ROTHMAN, F., and J. R. COLEMAN. 1968. *J. Mol. Biol.* **33**:527.
SADLER, J. R., and A. NOVICK. 1965. *J. Mol. Biol.* **12**:305.
SCHLESINGER, S., and B. MAGASANIK. 1965. *J. Biol. Chem.* **240**:4325.
TORRIANI, A. 1960. *Biochim. Biophys. Acta.* **38**:460.
ZUBAY, G., and M. LEDERMAN. 1969. *Proc. Nat. Acad. Sci. U.S.A.* **62**:550.