Sulfur Metabolism of *Aerobacter aerogenes*. I. A Repressible Sulfatase

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Received August 20, 1963

When *Aerobacter aerogenes* (American Type Culture Collection strain 9621) is grown on a synthetic medium containing a growth-limiting amount of sulfate or with methionine as its sole source of sulfur, sulfatase synthesis occurs. This enzyme is repressed by sulfate, thiosulfate, sulfite, and cysteine in normal growth concentrations. It is readily assayed in whole cells, either in liquid media or on agar plates, the assay consisting of spectrophotometrically following the liberation of *p*-nitrophenol from the enzymic hydrolysis of *p*-nitrophenylsulfate. Derepression of the enzyme as a function of various sulfur sources added to a sulfateless synthetic medium has been studied. Methionine and its analogs provide the highest amounts of enzyme, while substances such as cystine or lanthionine are less effective in derepressing enzyme synthesis. Phosphate inhibits the enzymic hydrolysis of *p*-nitrophenylsulfate, while sulfate has no effect. The optimum temperature for enzyme synthesis is in the region of 28°; higher or lower temperatures retard enzyme formation. Chloramphenicol prevents enzyme formation. Although the exact biological function of the enzyme is not known, it is assumed that it functions as an esterase, providing the cell with sulfate for growth. The ease of assay with whole cells and the inability to increase enzymatic activity by rupturing the cells suggest that this is another of a class of enzymes bound to the surface of the cell.

Sulfatase activity has been demonstrated in a variety of animal systems; however, its existence in bacteria appears to be limited (Dodgson and Spencer, 1956). We considered that one reason for the limited demonstration of a sulfatase system in bacteria could be related to the manner by which the biosynthesis of this enzyme is controlled: a metabolic surplus of sulfate in the bacterial growth medium could repress enzyme synthesis. Sulfur deprivation would be expected rapidly to effect protein synthesis (Roberts *et al.* 1957), this deprivation, possibly expressing itself in the increased synthesis of enzymes involved in the acquisition of sulfate or sulfur-containing substances. In order to demonstrate this type of enzymatic response we focused our attention on the possibility that sulfate limitation would elicit the synthesis of a sulfatase in a manner analogous to the alkaline phosphatase system of *E. coli* (Horiuchi *et al.*, 1959; Torriani, 1960). This paper reports the existence of a sulfatase of *Aerobacter aerogenes* synthesized 600–700 times more in sulfate-limiting media than in normal amounts of sulfate.

Methods and Materials

Culture.—*Aerobacter aerogenes* (American Type Culture Collection Strain 9621) was grown on a synthetic medium (R-1) containing sulfur in the form described for each experiment. The composition of this medium was as follows: NaCl, 0.1 M; NH_4Cl, 0.01 M; $MgCl_2$, 0.001 M; $CaCl_2$, 10^{-6} M; potassium phosphate buffer, *p*H 7.0, 0.1 M, and glycerol, 10 g/liter. Cultures were grown in test tubes (150 × 18 mm) containing 5 ml of R-1 with the added sulfur source. The cultures were agitated on a reciprocating shaker at the temperatures indicated for each experiment.

Assay for Sulfatase.—Bacteria were harvested by centrifugation at 8000 rpm, 5°, in a Servall refrigerated centrifuge, Model RC-2. The bacterial pellet was suspended in 2 ml of cold Tris-acetate buffer[1] (0.1 M, *p*H 7.1) and recentrifuged. This pellet was suspended in 1 ml of the same buffer and 0.5 ml of this suspension was incubated at 37° for 3 minutes. After this time, 0.5 ml of *p*-nitrophenylsulfate (0.04 M) in Tris-acetate (*p*H 7.1, 37°) was added. After 30 minutes the reaction was stopped with the addition of 2 N KOH (0.2 ml).

The cellular debris was removed by centrifugation and the optical density of the clear supernatant was determined at 420 mμ. The specific activity, k', is defined as the number of micromoles of *p*-nitrophenol ($\epsilon^{PH12} = 14 \times 10^3$) liberated per unit of optical density (650 mμ) of bacteria per minute. All optical density measurements were made on a Zeiss PMQ II spectrophotometer.

Chemicals.—All methionine analogs were obtained from General Biochemical Co. DL-Lanthionine plus mesolanthionine, L-homocystine, DL-mesohomocystine, and DL-cystathionine plus allocystathionine were obtained from California Corp. for Biochemical Research. Chloramphenicol was a Parke, Davis product. NPS was synthesized by the method described by Burkhardt and Lapworth (1926) and phenolphthaleindisulfate was prepared using the method of Whitehead *et al.* (1952). Polypeptone was a product of the Baltimore Biological Laboratory Co., and bactotryptone was obtained from the Difco Laboratories.

Results

Sulfatase Assay

The dependence of *p*-nitrophenylsulfatase activity on the amount of bacteria added to the standard assay medium is depicted in Figure 1A. The *p*-nitrophenol formed per unit time is shown to be directly proportional to the amount of bacteria added. In Figure 1B the rate of hydrolysis of *p*-nitrophenylsulfate is shown to be zero order with respect to substrate concentration over the 30-minute period used for the assay. The number of bacteria (the specific activity of which approached the upper limits found under any growth conditions) used in this assay was about twice the usual amount. The temperature dependence of the *p*-nitrophenylsulfatase activity is shown in Figure 1C. The highest specific activity was obtained

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[1] Abbreviations used in this work: Tris, tris(hydroxymethyl)aminomethane; NPS, *p*-nitrophenylsulfate.

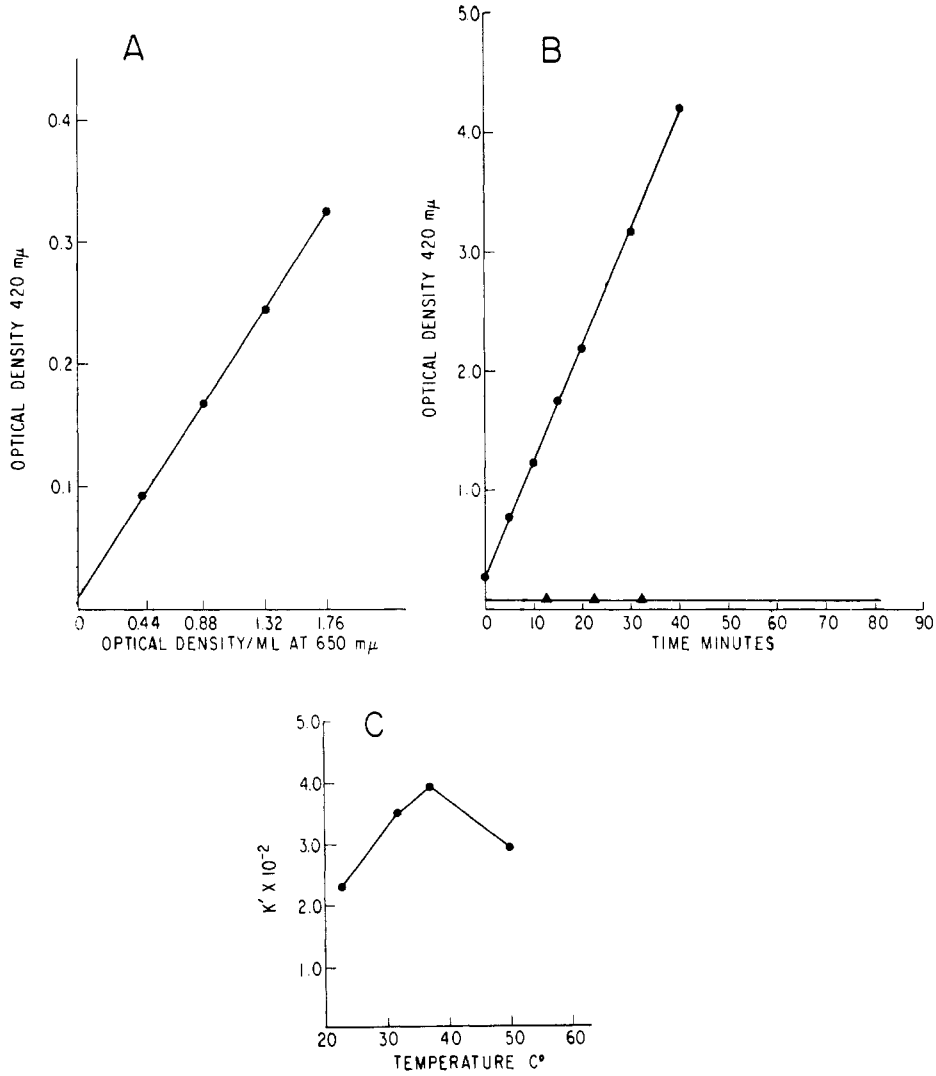

FIG. 1.—(A) The linear dependence of the hydrolysis of *p*-nitrophenylsulfate (optical density at 420 mμ) on the number of *A. aerogenes* (optical density 650 mμ) added to the standard assay system. Bacteria were grown on R-1 medium containing DL-methionine (2 mM) for 18 hours at 28°. (B) The linear dependence of the hydrolysis of *p*-nitrophenylsulfate in the standard assay medium as a function of time. The triangles (▲) represent the amount of *p*-nitrophenylsulfate hydrolyzed by bacteria grown on R-1 medium containing sulfate (2 mM), while the solid circles (●) represent the amount by bacteria grown on methionine as described in (A). (C) The specific sulfatase activity in the standard assay medium as a function of temperature. Bacteria were grown on methionine as described in (A).

around 37° and this temperature was routinely used for the assay. The *p*H optimum was found to be 7.1.

As shown in Table I, phosphate strongly inhibits *p*-nitrophenylsulfatase activity. It was found that consistently reproducible results could be obtained by a careful single washing of the bacteria in Tris-acetate buffer (0.1 M, *p*H 7.1) after the initial centrifugation.

Growth Requirements for Sulfatase Formation

Growth Medium.—A. aerogenes grown on enriched medium such as that described by Luria and Burrous (1957) or in R-1 medium containing 1% bactotryptone or 1% polypeptone failed to show significant enzymatic activity either with whole cells or cell-free extracts. R-1 media, which contains only trace amounts of sulfate,[2] provided low-growth titers of bacteria, but bacteria with demonstrable enzymatic activity. The growth response of *A. aerogenes* as a function of sulfate concentration is shown in Figure 2. At suboptimal concentrations of sulfate *p*-nitrophenylsulfatase activity is readily detectable, while at concentrations which support a constant growth rate up to the time of assay enzymatic activity is negligible.

In an effort to secure good growth conditions with concomitant maximal formation of the sulfatase, bacteria were grown in R-1 containing as the sole added sulfur source the substances listed in Table II. These substances were tested at several concentrations and the conditions which yielded the highest sulfatase specific activity with adequate growth (OD 650 mμ of over 1.0 for 18 hours of growth) were further studied with respect to growth rate, onset of enzyme synthesis, and the effect of temperature on enzyme synthesis.

The Dependence of Growth Rate and Enzyme Synthesis on the Sulfur Source.—The growth rate of *A. aerogenes* can be controlled not only by varying the total amount of sulfur available to the growing culture but also by varying the sulfur source. Thus, maximal growth rates are achieved when sulfur is supplied in the form of sulfate, thiosulfate, and sulfite, while diminished growth rates are found with substances such as cystine, methionine, and lanthionine. With substances which

[2] The concentration of sulfate introduced as trace contaminate in the other constituents of the medium is about 10^{-6} M.

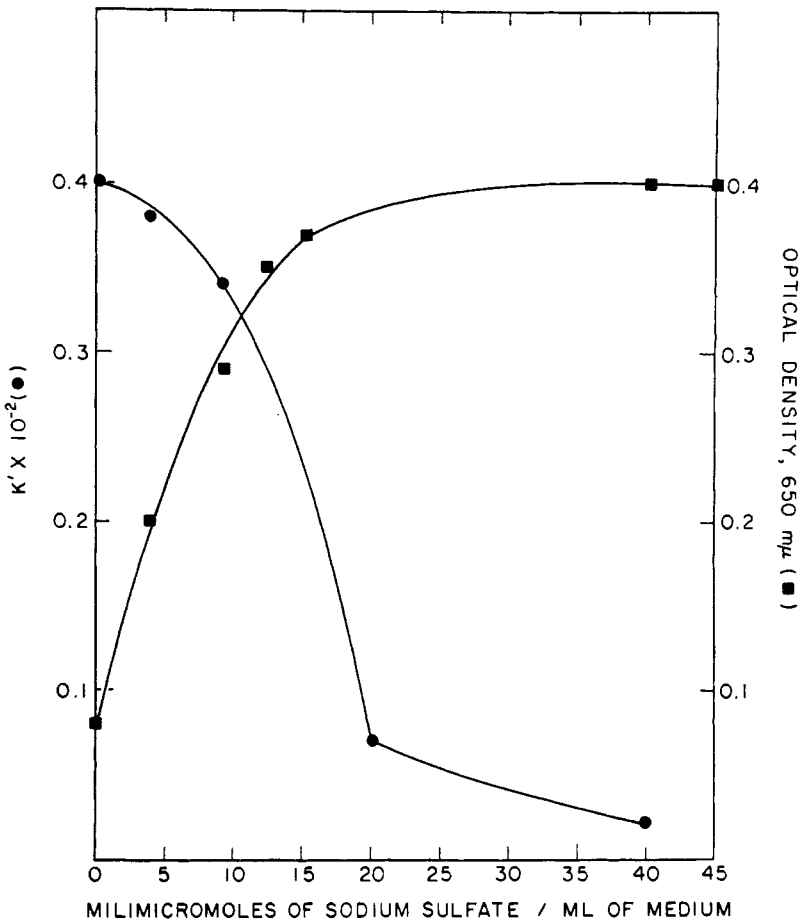

FIG. 2.—Specific sulfatase activity (●) and growth (■) of *A. aerogenes* cultured for 4.5 hours at 30° as a function of sodium sulfate concentration in R-1 medium.

TABLE I
PHOSPHATE INHIBITION OF SULFATASE ACTIVITY IN WHOLE CELLS[a]

Phosphate Molarity	$K' \times 10^{-2}$	Inhibition (%)
—	4.35	—
0.003	3.08	31.0
0.006	2.75	36.6
0.018	2.44	44.0
0.024	1.92	56.0
0.053	1.53	64.7
0.10	1.39	68.0
0.167	1.13	74.0
0.33	1.13	74.0

[a] Assays were carried out in the usual manner using washed cells in Tris-acetate buffer (0.167 mM, *p*H 7.1). Potassium phosphate (*p*H 7.1) was added to the assay medium to give the final concentration noted. *A. aerogenes* were grown at 28° on R-1 medium containing DL-methionine (8 mM) as the sole source of sulfur.

provide growth-limiting amounts of sulfur, sulfatase formation is observed. The growth rates expressed as generation time for several sulfur sources are listed in Table III. The numbers in the columns headed by the letters A and B correspond to the letters indicated in Figure 3. In every instance the initial generation time A corresponds closely to the exponential generation time of bacteria grown with sulfate as the sole source of sulfur. The duration of this slope depends upon the size of the initial inoculum as well as its source.[3] When bacteria are grown on sulfur sources which cannot be readily converted to cysteine, a second diminished exponential growth rate (slope B) is observed. Figure 3 compares the growth kinetics of *A. aerogenes* with L-cysteine, sulfate, and DL-methionine as the sole added sulfur sources. In the case of bacteria growing on DL-methionine, the initial generation time (A) is 80 minutes and the second generation time (B) is 185 minutes. *p*-Nitrophenylsulfatase activity always increases rapidly at the rate transition point (C). Specific activity reaches a maximum in less than a generation time and subsequently decreases. Similar kinetics have been observed with analogs of methionine and substances which can be converted into methionine *in vivo* (see Table II). On the other hand, bacteria growing with comparable concentrations of sulfate have a single exponential growth rate and show complete repression of sulfatase synthesis as illustrated in Figure 3. Bacteria growing on cysteine show a rate transition late in exponential growth with a concomitant small degree of sulfatase synthesis. Lanthionine, which is most likely converted to cysteine at a growth-limiting rate, yields an intermediate amount of the enzyme, enzyme formation again occurring just prior to the transition in exponential growth rate.

Sulfur Sources Which Repress Sulfatase Synthesis.—The exact biosynthetic pathway of cysteine in *A. aerogenes* is obscure; however, in *E. coli* (Roberts *et al.*, 1957) and in *Salmonella typhimurium* (Dreyfuss and Monty, 1963), its formation from sulfate proceeds via sulfite and thiosulfate. In *A. aerogenes* these

[3] Roberts *et al.* (1957) have indicated that in *E. coli* an initial rapid rate of growth which occurs with cells growing in sulfur-deficient medium most probably reflects the cell's use of its internal pools of cysteine and glutathionine.

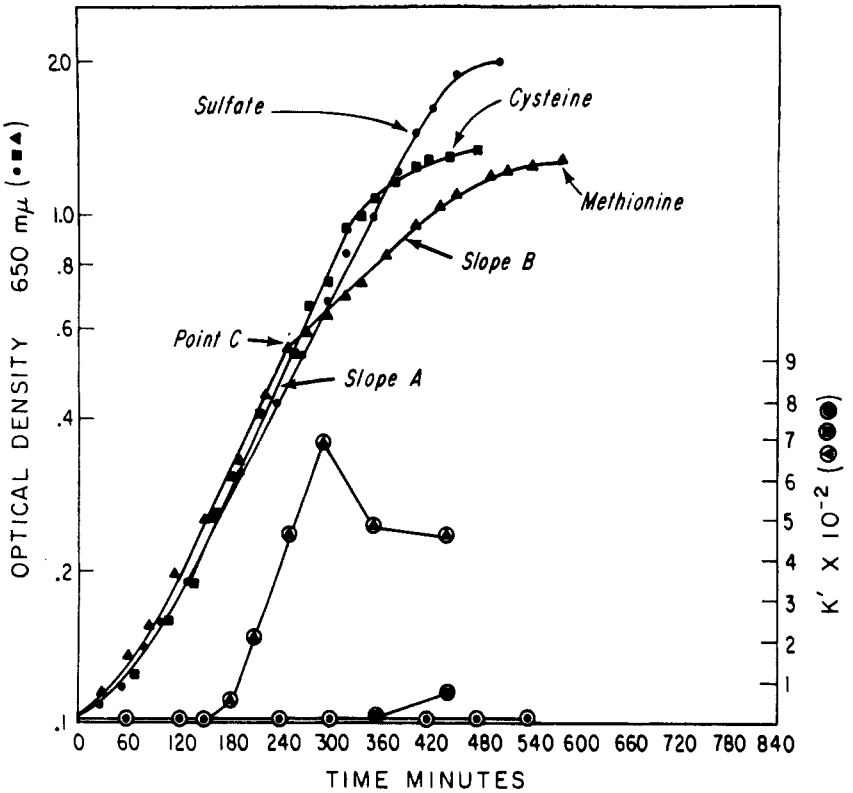

FIG. 3.—The growth rate and rate of sulfatase formation as function of the sulfur source. The solid circles (●) represent growth on sulfate (1.5 mM), the squares (■) on L-cysteine (0.5 mM), and the triangles (▲) on DL-methionine (1.0 mM). The circled markings represent specific sulfatase activity for the sulfur sources as described. Bacteria were grown on R-1 medium at 28° with the added sulfur sources. See text for definition of point and slope.

TABLE II

THE DEPENDENCE OF SULFATASE FORMATION ON THE SULFUR SOURCE[a]

Sulfur Source	Concn (mM)	Added Sulfate (mM)	Growth (OD 650 mμ)	$K' \times 10^{-2}$
Sodium sulfate	1.0	—	1.44	0.01
	4.0	—	1.68	0.03
DL-Methionine	2.7	—	1.74	6.5
	27.0	—	1.67	6.8
	2.7	1.0	1.70	0.3
L-Cysteine	5.0	—	1.71	0.02
DL-Methionine sulfoxide	2.4	—	1.72	2.9
DL-Methionine sulfone	2.4	—	0.38	2.2
DL-Ethionine	2.4	—	1.35	5.8
DL-Ethionine sulfoxide	2.4	—	1.0	3.9
DL-Ethionine sulfone	2.4	—	0.54	1.65
DL-Cystathionine plus allocystathionine	1.8	—	0.67	2.4
p-Nitrophenylsulfate	1.0	—	1.30	1.25
L-Homocystine	0.3[b]	—	1.08	4.56
Djenkolic acid	0.6	—	0.9	0.23
Taurine	0.6	—	0.825	2.2
L-Cystine	1.0[b]	—	1.20	1.8
DL-Lanthionine plus mesolanthionine	0.5[b]	—	0.73	6.0

[a] Cultures of *A. aerogenes* were grown overnight at 28°. Sulfate was added at 0 time. [b] Milliliters of saturated solution added to 5 ml of growth medium.

substances and cysteine each provide maximal growth rates, but represses sulfatase formation. Table IV shows the results obtained when bacteria are grown in the presence of these compounds in addition to methionine as the sulfur source. The bacteria were assayed after rapid sulfatase synthesis had begun in the control containing methionine alone. It is apparent that sulfatase formation is effectively repressed by sulfate, thiosulfate, sulfite, and cysteine.

TABLE III

DEPENDENCE OF GROWTH RATE AND ENZYME SYNTHESIS ON THE SULFUR SOURCE[a]

Sulfur Source	Concn (mM)	Generation Time (min) A	Generation Time (min) B
Sodium sulfate	1.5	90	—
DL-Methionine	8.0	80	135
	1.0	80	185
DL-Methionine sulfoxide	2.4	78	160
DL-Ethionine	2.4	80	170
L-Cysteine	0.5	80	—
DL-Lanthionine plus mesolanthionine	0.4[b]	80	100

[a] *A. aerogenes* were grown at 28° in R-1 medium containing the compounds listed as the sole sulfur sources. [b] Derived from an average of the solubility of meso- and DL-lanthionine.

Sulfate added to a culture of *A. aerogenes* rapidly synthesizing sulfatase at a linear rate immediately suppresses further enzyme synthesis. This effect is clearly demonstrated in the experiment depicted in Figure 4.

Localization and Specificity of Sulfatase Activity.—In order to determine whether the sulfatase enzyme is entirely localized in the cells of *A. aerogenes* or is partially excreted into the culture medium following synthesis, the bacteria were grown in a minimal salts–glycerol medium containing DL-methionine (1.0 mM)

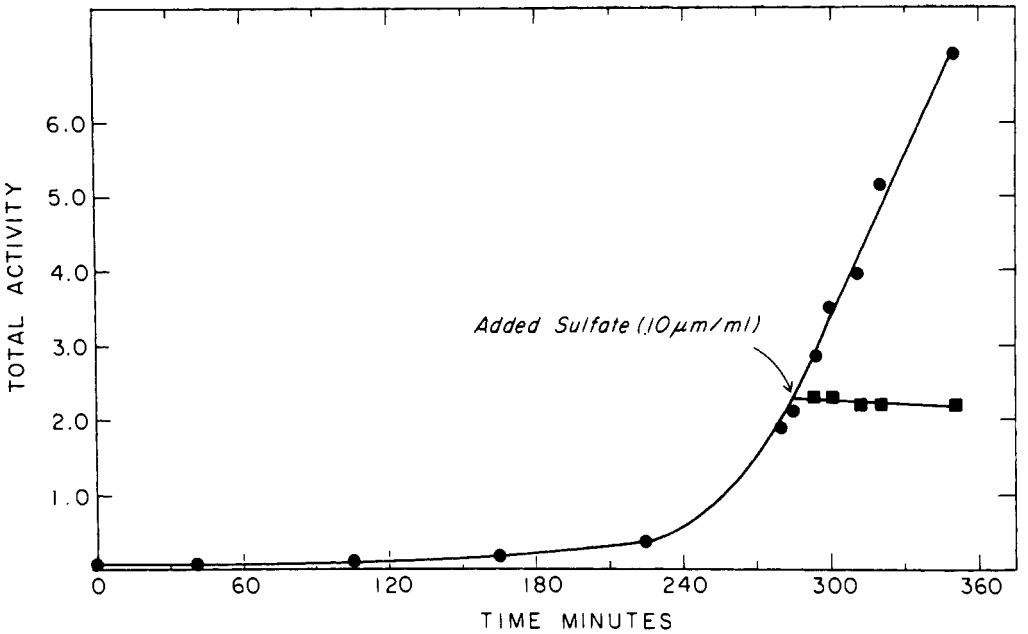

FIG. 4.—Sulfate repression of sulfatase synthesis. The solid circles (●) represent the rate of increase in total sulfatase activity. Bacteria were grown on R-1 medium at 28° with DL-methionine (1.0 mM) as the sole sulfur source. At 292 minutes, sulfate (final concn, 10 mM) was added. The squares (■) represent the total sulfatase activity after the addition of the sulfate.

TABLE IV
REPRESSION OF SULFATASE FORMATION[a]

Repressor	Concn (mM)	$K' \times 10^{-2}$
—	—	1.85
Sodium sulfate	2.0	0.02
Sodium thiosulfate	2.0	0.04
Sodium sulfite	2.0	0.06
L-Cysteine	2.0	0.03

[a] Repressors were added to cultures of *A. aerogenes* in R-1 medium containing DL-methionine (8 mM) at 0 time. Cultures were grown at 28° for 240 minutes and assayed for *p*-nitrophenylsulfatase activity.

buffered at *p*H 7.1 with Tris-chloride (0.1 M), and containing only 10^{-3} M phosphate. The bacteria were removed as described and the supernatant was assayed for enzymatic activity. No *p*-nitrophenylsulfatase activity could be detected. All the activity ($K' \times 10^{-2} \approx 2.8$) resided in the whole cells. Sonic disruption (Branson Instruments Incorp., Stamford, Conn.) or treatment of washed cells with toluene for various intervals of time did not increase the activity over that obtained with whole cells.

Sonic disruption releases sulfatase activity totally into the supernatant even when microscopic examination reveals no obvious disintegration of the majority of cells. When bacteria were grown on sulfate (2.0 mM) as the sole sulfur source, no enzymatic activity could be detected with whole or sonically disrupted cells.

No sulfatase activity could be detected with cells which hydrolyze *p*-nitrophenolsulfate when phenolphthaleindisulfate was used as a substrate (Whitehead *et al.*, 1952; Harada, 1957).

The Effect of Chloramphenicol on Sulfatase Synthesis.—Chloramphenicol has been shown to inhibit protein synthesis (Wisseman *et al.*, 1954). This substance was added to a culture of *A. aerogenes* growing on methionine as the sole sulfur source in an effort to determine whether *de novo* enzyme synthesis or the activation of preformed enzyme subunits occurs during the initial rapid rise in specific sulfatase activity. Chloramphenicol (10 and 100 μg/ml) was added at the onset of the rapid increase in *p*-nitrophenylsulfatase activity (see Fig. 3). Enzyme synthesis was stopped immediately and the total sulfatase activity did not change over 100 minutes. During this time, growth (OD 650 mμ) was restricted to about 20% of the nontreated control.

The Effect of Temperature on Sulfatase Synthesis.—Although there is a slight increase in the growth rate of *A. aerogenes* when grown at 37° as compared with 28–30°, there is a considerable difference in the specific activity of the sulfatase produced. In Figure 5 the specific activities of 16-hour cultures grown at several temperatures with DL-methionine as the added sulfur source are compared with those grown with sulfate at the same temperatures. It is apparent that the highest specific activity is obtained with cultures grown at approximately 28°.

DISCUSSION

The demonstration of specific metabolite mediated regulation of the rate of enzyme synthesis in bacteria is well documented (Pardee, 1961; Vogel, 1961). This type of regulation appears to be genetically controlled. The cytoplasmic product of a regulator gene, presumably acting in concert with a specific product of the metabolic sequence controlled by the regulator, forms the repressor. According to the theory proposed by Jacob and Monod (1961), the repressor effects its control on enzyme synthesis by regulating the expression of the structural genes which determine the enzymes to be synthesized.

Little is known of the chemical nature of the repressor. Cursory evidence suggests the involvement of proteins (Horiuchi and Novick, 1961); however, the possible involvement of nucleic acid (Pardee and Prestidge, 1959; Ames and Hartman, 1964) or nucleoprotein has not been eliminated. The primary aim of the experiments reported in this and the following publication (Fowler and Rammler, 1964) has been to establish an enzymatic system which we feel will be

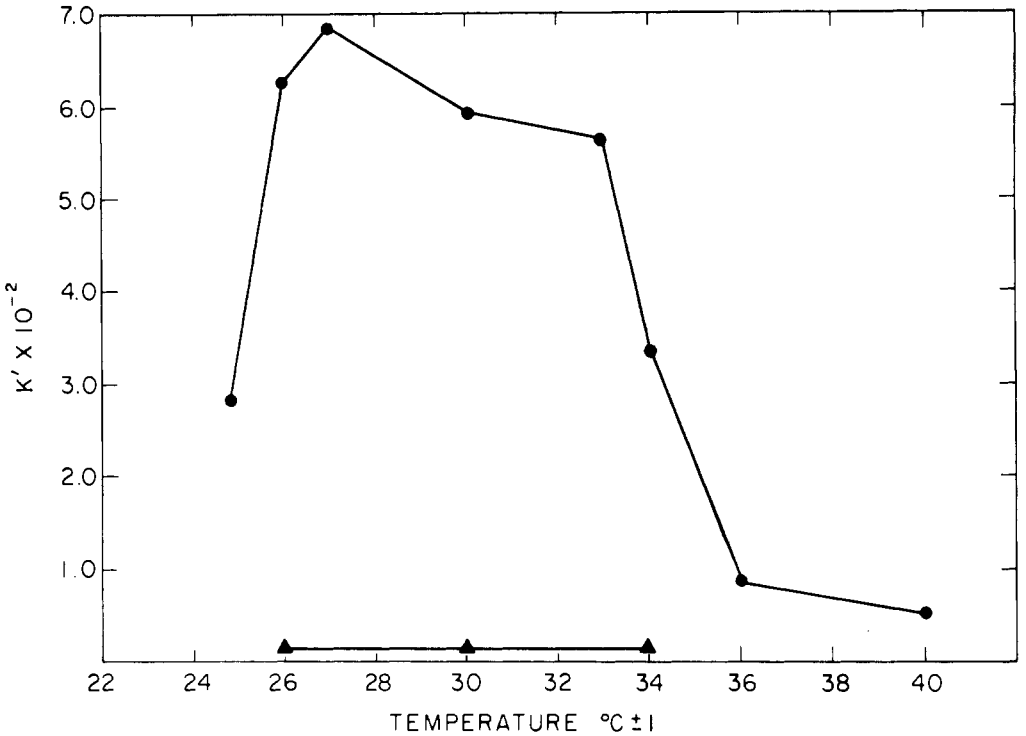

FIG. 5.—The specific sulfatase activity of *A. aerogenes* as a function of growth temperature. The solid circles (●) represent bacteria grown on R-1 medium containing DL-methionine (8 mM) and the triangles (▲) on sulfate (2.0 mM) as the sole source of sulfur.

amenable to our studies on the nature of the repressor. To this end we sought a repressible enzymatic system which will permit the use of a radioactive tracer normally not found in high concentrations in the bacterial cell and which is not a primary constituent of ribonucleic acid.

We have examined several species of bacteria for enzymes which hydrolyze sulfate esters when grown under conditions of sulfate limitation. The presence of this type of enzymatic activity was sought using *p*-nitrophenylsulfate as the substrate *p*-nitrophenol, the chromophoric hydrolysis product providing a facile optical assay for the activity of bacteria in liquid medium as well as on agar plates. Under various growth conditions we were unable to demonstrate significant sulfatase activity in several strains of *E. coli*, *Bacillus subtilis*, *Salmonella*, and *Proteus;* however, activity was obtained with *Aerobacter aerogenes* (Harada, 1957). In this organism, sulfatase synthesis appears to be a function of the availability of sulfur to the cells. When the amount of sulfur is made suboptimal for growth either by limiting sulfate or providing the growing culture with a less ready source of sulfur, the growth rate is markedly retarded after exhaustion of the internal sulfur pool (Roberts *et al.*, 1957). Sulfatase synthesis generally begins shortly before this rate change and increases rapidly during the onset of the slowed growth rate. In every instance where the growth rate of *A. aerogenes* is retarded because of sulfur deprivation compared with its growth rate on sulfate, sulfatase synthesis occurs (for an analogous situation with regard to the derepression of the enzymes in histidine biosynthesis, see Ames and Garry, 1959). The substances which most effectively permit sulfatase synthesis are methionine, its analogs, or substances which can be converted to methionine. Although the exact pathway of methionine metabolism in *A. aerogenes* is not known, it is not unreasonable to assume that, as in *E. coli* (Roberts *et al.*, 1957), there exists no direct method for converting methionine into cysteine. At least one-half the sulfur in *A. aerogenes*, like *E. coli* (Roberts *et al.*, 1957), is most probably in cysteine. For this reason, limitation of cysteine should be reflected not only in poor growth rates but also in the final total amount of bacteria obtained. It has been shown that the final growth titers obtained with methionine and its analogs approaches that obtained with sulfate (see Table II). This suggests that these substances, as well as sparing the glutathione pool (Roberts *et al.*, 1957) for exclusive conversion to cysteine and thus extending the duration of the initial rapid growth rate, are slowly degraded to sulfate. This sequence would always provide the cell with growth-limiting amounts of sulfate causing derepression of the sulfatase. The growth rate of *A. aerogenes* on cysteine is approximately the same as that obtained with sulfate; however, when lanthionine is the only source of sulfur, the growth rate is diminished and enzyme synthesis occurs. The stimulation of enzyme synthesis by lanthionine can be attributed to the possible slow rate of its conversion to cysteine.

Enzyme synthesis cannot be induced with substances which are structurally similar to *p*-nitrophenylsulfate, such as benzene sulfonic acid or dissimilar substances such as tyramine under the conditions described.[4]

Inorganic sulfur compounds of the cysteine biosynthetic pathway (Roberts *et al.*, 1957) repress sulfatase synthesis. It is known that in *E. coli* (Roberts *et al.*, 1957) cystine and lanthionine are rapidly assimilated and are precursors to cysteine, yet these substances permit sulfatase synthesis in *A. aerogenes*. Although the exact substance or substances responsible for repression of sulfatase synthesis are unknown, preliminary evidence obtained with cysteine auxotrophs[5]

[4] The induction of sulfatase activity in *A. aerogenes* by tyramine has been reported by Harada (1957). The nature of the tyramine effect is the subject of a forthcoming report from this laboratory.

[5] Unpublished results of C. Grado and D. H. Rammler.

suggests that inorganic sulfate is most directly involved.

The curious temperature effect on the rate of enzyme synthesis is most probably not the result of heat inactivation of the enzyme. This possibility seems to be excluded on the basis of the optimum assay temperature and the temperature stability of the purified enzyme (Fowler and Rammler, 1964). The high (38–40°) and low (24°) temperature inhibition need not be directly related, but can represent two dissimilar events both of which effect enzyme synthesis. Although the enzyme shows no cofactor requirements (Fowler and Rammler, 1964), the possibility of a temperature-sensitive controlling element exclusive of the enzyme-synthesizing system may be entertained.

An enzymatic system analogous to the sulfatase is alkaline phosphatase of *E. coli* (Horiuchi *et al.*, 1959; Torriani, 1960). There are, however, some differences. Sulfatase synthesis is repressed by sulfate but its catalytic activity is not effected, while alkaline phosphatase synthesis and catalytic activity are inhibited by phosphate (Stadtman, 1961). When *A. aerogenes* is disrupted and the cellular debris is removed, all enzymatic activity can be recovered in the supernatant solution. Although we have been unable to effect lysozyme digestion of the bacterial cell wall under a variety of conditions, it would appear that the sulfatase, like alkaline phosphatase (Malamy and Horecker, 1961) is neither extracellular in nature nor tightly associated with particular cellular constituents. This system, like the acid phosphatase of *Saccharomyces cerevisiae* (McLellan and Lampen, 1963), appears to be another of a class of enzymes found on the periphery of the cell. Although its exact biological role is unknown it most probably functions in this soil bacterium as an arylsulfatase providing the cell with sulfate from arylsulfate esters found in decaying vegetative matter.

ACKNOWLEDGMENT

The authors wish to acknowledge the helpful criticism of Ernst Freese and A. L. Taylor of this laboratory.

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Sulfur Metabolism of *Aerobactor aerogenes* II. The Purification and Some Properties of a Sulfatase

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Received August 20, 1963

An enzyme which catalyzes the hydrolysis of *p*-nitrophenylsulfate has been extensively purified from derepressed cultures of *Aerobacter aerogenes*, strain 9621 (American Type Culture Collection). The sulfatase activity of the organism is entirely an expression of this enzyme. The preparation is shown to be homogeneous in the ultracentrifuge and to have a molecular weight of approximately 41,000. A number of physical properties useful for the characterization of the enzyme have been determined. Novel properties of the sulfatase include an anomalous hydrolysis rate-temperature profile and a substrate-dependent inactivation by cyanide.

An enzyme having phenolsulfatase activity may be defined as one which catalyzes the hydrolytic cleavage of sulfate monoesters of phenols to the parent phenol and inorganic sulfate. Despite the fact that the biochemical literature contains many reports of studies on crude and purified preparations of phenolsulfatases from a variety of sources (for summarizing references see Gregory and Robbins, 1960; Dodgson and Spencer, 1956b), no clear understanding of the physiological role played by this family of enzymes has emerged. A major difficulty in this regard has been the unavailability of a homogeneous enzyme protein obtainable from a well-characterized organism such as a bacterium, in which the pathways of sulfur metabolism are amenable to investigation. Dodgson *et al.* (1955) have

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