Dynamics of Growth Processes

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II. EXPERIMENTS WITH THE CHEMOSTAT ON THE RATES OF AMINO ACID SYNTHESIS IN BACTERIA

BY AARON NOVICK AND LEO SZILARD[1]

INFORMATION is accumulating on the pathways of the biosynthesis of amino acids in microorganisms, but it is not known what regulates the rates of the individual steps in such syntheses. As a rule, a bacterium synthesizes from ammonia and some simple carbon source all of the amino acids that are contained in its proteins, and it produces of each amino acid no more than is needed. For instance, if a strain of coli that requires tryptophane grows in an arginine-free nutrient in which the tryptophane concentration is kept above a certain minimum level, it will grow rapidly, obviously synthesizing all the arginine contained in its proteins at a correspondingly high rate. If the tryptophane concentration is maintained at a low level, the strain will grow slowly, perhaps ten times more slowly than before, and we see from our experiments (unpublished) that there is no appreciable outpouring of arginine (or ornithine or citrulline) into the medium. This leads us to believe that the rate at which arginine is synthesized is also reduced tenfold. One may ask how such a regulation of the synthetic rates is accomplished.

We may speculate that perhaps when protein synthesis is slowed, by supplying the tryptophane at a low rate, the internal "free amino acid" level of all the other amino acids rises and that somehow the increased concentration of each amino acid depresses the rate of the individual steps of synthesis leading to the formation of that amino acid.

Other experiments of ours (unpublished) indicate that a tryptophane-requiring strain of coli growing in a nutrient in which a certain very low concentration of arginine (of the order of 1 γ/l) is maintained, will, with increasing concentration of arginine, synthesize a smaller fraction of the arginine contained in the bacterial proteins and will take up the rest from the medium. Again one may assume that the level of "free arginine" inside the bacterium regulates the biosynthetic steps leading to the formation of arginine, and the question arises as to how this is accomplished.

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The normal regulation of the rate of the individual steps along a biosynthetic pathway leading to the formation of an amino acid might, however, be absent for a certain class of mutants in which a *precursor* of the amino acid, instead of being converted into the amino acid, is converted into some slightly modified compound which is poured out into the nutrient medium. The production of this precursor may then, under certain circumstances, go on at a high rate, apparently removed from the normally operating controls and limited only by the synthetic capacity of the bacterium. Such might be the case for a mutant of coli that we used in experiments described in this paper. This mutant requires tryptophane, is unable to grow on indol, and when grown slowly at very low concentrations of tryptophane pours out into the medium at a high rate a compound which is not indol and yet has a U.V. absorption spectrum closely resembling that of tryptophane. The absorption of this compound in the U.V. makes it easy to determine the rate at which it is poured out in any given experiment, and this permits us to study how the rate of this biosynthesis is affected when we vary selected factors of interest.

In these experiments, as in all the other experiments reported here, we used a special technique that utilizes a bacterial population which is maintained in the growth phase in a Chemostat (Novick and Szilard, 1950a, 1950b, 1951; Monod, 1950). In this device the bacterial suspension is contained in a growth tube which is provided with an overflow. Fresh nutrient enters the growth tube continuously at a rate of w cc/sec. The contents of the growth tube are kept stirred by aeration, and the bacterial suspension leaves the growth tube at the same rate at which fresh nutrient enters it so that the volume V of the bacterial suspension in the growth tube remains constant. The fresh nutrient entering the growth tube contains in excess all the factors needed for the growth of the bacteria except one, which is called the *controlling growth factor.* In our experiments we used tryptophane as the controlling growth factor and a strain of coli that requires tryptophane for its growth. The nutrient consisted of an ammonium lactate medium (Friedlein) with a certain input concentration a of tryptophane, which was varied from experiment to experiment within the range of 1/2 to 1 mgm/l.

In the stationary state the tryptophane concentration c in the growth tube is very low, of the order of a microgram per liter, and at such low concentrations the growth rate α of the bacteria is an increasing function of the tryptophane concentration. Fig. 1 gives the growth rate

a as a function of the tryptophane concentration c. The growth rate a is defined as follows. In a nutrient medium in which the tryptophane concentration is maintained at a value c, the bacterial density n rises with time according to the formula $n = n_o e^{at}$. The coefficient a in the exponent is, by definition, the growth rate. The reciprocal of this value, $1/a = \tau$, is the generation time. The doubling time of the bacteria is obtained from the generation time τ by multiplying by $\ln 2 = 0.693$.

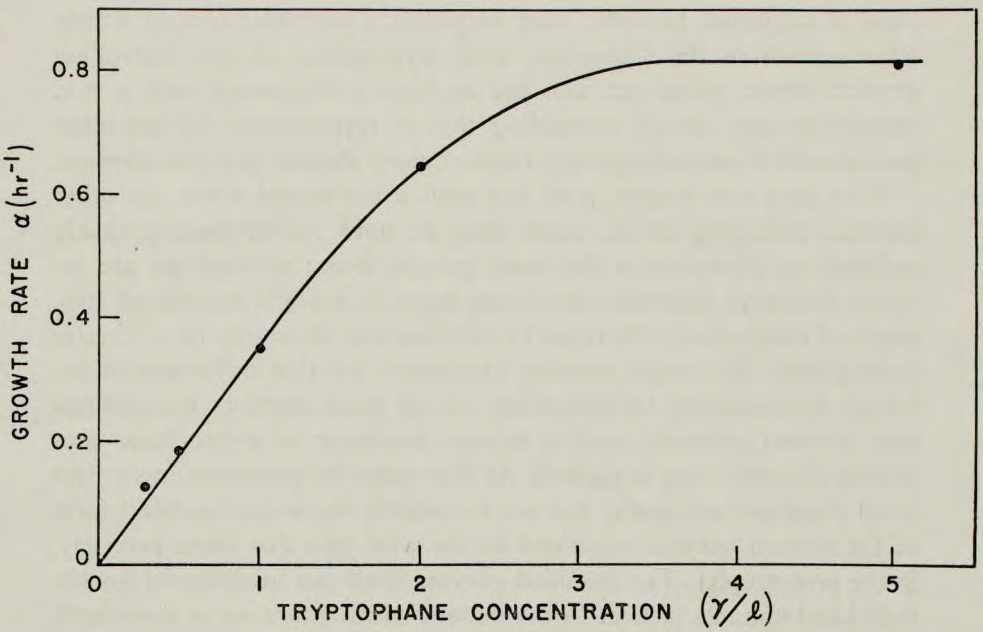

Fig. 1. Growth rate of *E. coli* B/lt as a function of tryptophane concentration.

Since in the stationary state the bacteria grow as fast as they are washed out, a tryptophane concentration c is automatically established for which we have: $a(c) = w/V$. The reciprocal of the washing out rate gives us the generation time τ of the bacteria ($\tau = V/w$).

If the concentration c of the controlling growth factor in the growth tube is small compared to the input concentration a, then in the stationary state a change in the flow rate w will affect the generation time only and leave the bacterial density n unaffected. Such is the case if our tryptophane-less mutant strain of coli is grown with tryptophane as the controlling growth factor under the conditions of our experiments. The bacterial density in the growth tube in the stationary state is then proportional to the concentration of the controlling growth factor in

the incoming fresh nutrient. For an input concentration of $a = 500$ γ/l we obtain a bacterial density of $n = 2 \times 10^8$/cc as determined by colony count, and the bacterial suspension has an optical density of 0.135 at 350 mμ as determined by a Beckman spectrophotometer.

The tryptophane-requiring mutant that we used was obtained by picking from the B strain of *E. coli* a mutant resistant to the bacterial virus T1 and unable to use indol as a substitute for tryptophane. About 20% of the mutants resistant to the virus T1 fall into this well-known class. It happened, however, that we picked a particular mutant which, when grown in the Chemostat with tryptophane as the controlling growth factor, pours out into the medium a compound with a U.V. absorption very closely resembling that of tryptophane. Of ten other mutants which we subsequently isolated, none showed this phenomenon.

Why does one mutant pour out such a compound when ten other mutants belonging to the same class do not? All of them probably suffered an alteration at the same genetic locus; at least we are inclined to believe this since all of them have, in a single mutational step, acquired resistance to the virus T1 and also lost the ability to synthesize tryptophane. We might attempt to account for this difference in behavior by assuming the following. In all these mutants biosynthesis may proceed normally until a certain precursor of tryptophane containing the indol ring is reached. At that stage the precursor encounters in all these mutants some, but not necessarily the same, modified form of the normal enzyme contained in the wild type (or more precisely, in the prototroph). The modified enzyme itself can be different for the individual mutants picked. In the mutant which pours out a compound, the modified enzyme reacts with the tryptophane precursor and diverts it from the normal synthetic pathway by transforming it into a compound which is poured out by the bacterium. In the other mutants the modified enzyme may leave the precursor unchanged, and the increased concentration of the precursor might depress the rate at which it is synthesized so that its concentration inside the bacterium does not reach the threshold at which it will be poured out. This model is presented here solely for illustration and without any claim that other models might not be equally good or even better.

For simplicity, we shall refer to the compound poured out by our mutant as the "Compound." The absorption spectrum of the "Compound" in aqueous solution at pH 7 is shown in Fig. 2. It has a maximum close to 280 mμ and the ratio of the absorption at 280 and 250 mμ is about 2.2. Assuming that the molar extinction coefficient of

this compound at 280 mμ is the same as that of tryptophane, we can determine the quantity of the "Compound" in the growth tube of the Chemostat by centrifuging off the bacteria and measuring the optical density of the supernatant against the optical density of the original nutrient at 280 mμ. In the absence of other absorbing microbial products, the optical density at 280 mμ may be taken as a measure of the quantity of the "Compound" present. In each particular case, however, we have

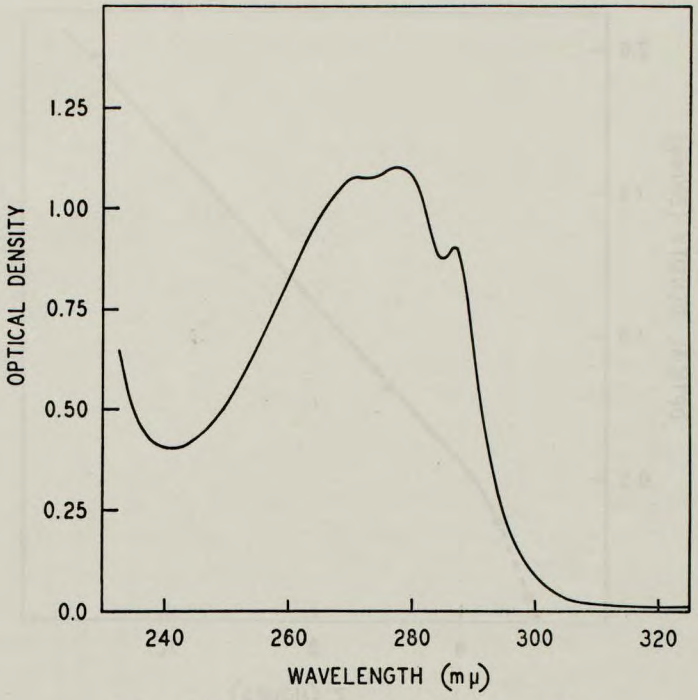

Fig. 2. Absorption spectrum of "Compound."

to check the spectrum between 250 and 280 mμ in order to see if the presence of other U.V.-absorbing bacterial products makes it necessary to apply a correction. A correction will be needed whenever the ratio of the absorption at 280 and 250 mμ falls considerably below the value of 2.2.

Fig. 3 shows the optical density of the supernatant at 280 mμ for our bacterial strain growing in the Chemostat in the stationary state with a tryptophane input concentration of $a = 500\,\gamma/l$ at different generation times τ. As the figure shows, the concentration of the "Compound" is proportional to the generation time between $\tau = 4$ hrs

and $\tau = 14$ hrs; i.e. between these limits the "Compound" is poured out at a constant rate corresponding to an increase in optical density of 0.141/hr. As the generation time is decreased below 3 hrs the rate of production of this "Compound" begins to fall off, and at a generation time of less than 2 hrs it is poured out at a rate of less than 0.040/hr.

By measuring the optical density of a known concentration of trypto-

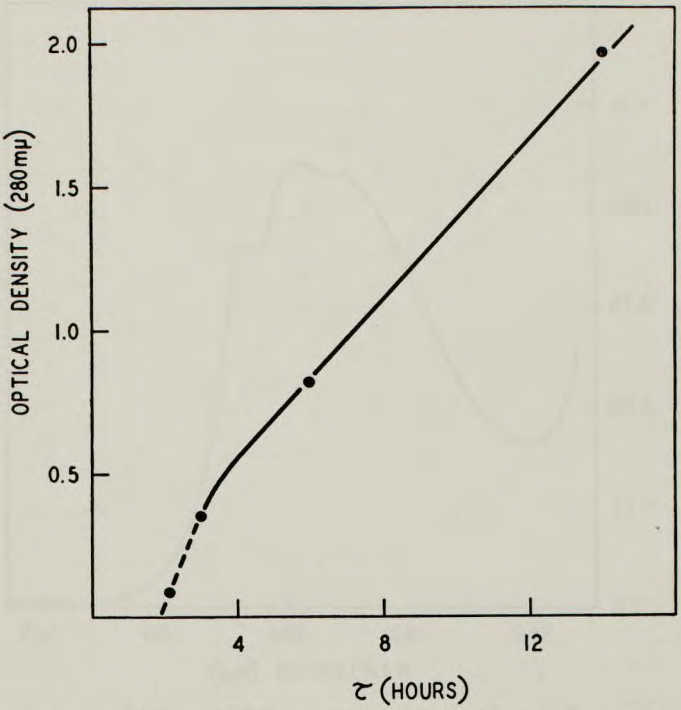

Fig. 3. Optical density of the supernatant at 280 mμ as a function of generation time.

phane at 280 mμ, we can compute on a mole-for-mole basis how much faster the "Compound" is poured out in a given experiment than tryptophane is taken up. One thus finds that at a generation of 14 hours the "Compound" is produced about 125 times faster than tryptophane is taken up. This furnishes a measure of the bacterium's capacity for tryptophane synthesis. A simple computation shows that at 37°C. tryptophane could be synthesized about four times as fast as the bacterium would need it if it grew—in the absence of tryptophane but otherwise under optimal nutritional conditions—at its maximum

growth rate, i.e. with a generation time of about 30 minutes (doubling time about 20 minutes).

It seemed of interest to find out whether our strain of bacteria continues to produce a compound even if we block protein synthesis by stopping the inflow of fresh nutrient into the growth tube of the Chemostat. In order to see this we first permitted a stationary state to establish itself at the generation time of about three hours with the bacterial population maintained at an optical density of 0.245 at 350 mμ, and then we stopped the inflow of nutrient. The small amount of tryptophane contained in the growth tube is then exhausted within a few seconds and so protein synthesis ceases almost instantaneously. As shown in Fig. 4, we found, when the inflow was stopped at zero time, that the

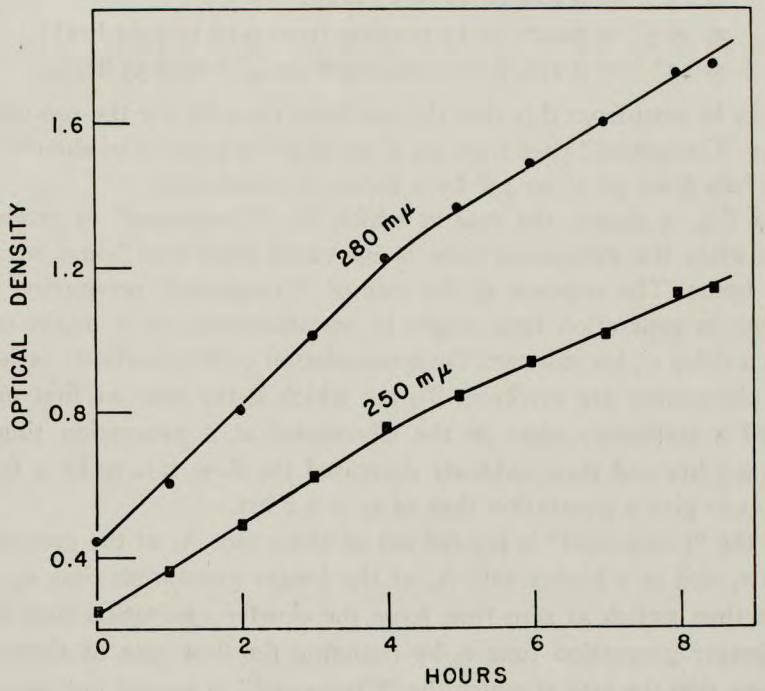

Fig. 4. "Compound" produced as a function of time after growth was stopped.

optical density at 280 mμ of the supernatant increased proportionately with time for about four hours at the rate of 0.19/hr; the rate then decreased to 0.12/hr and the "Compound" continued to pour out at this lower rate for another four hours, at which time the experiment was stopped. This means that even though a large number of steps and

a correspondingly large number of enzymes must be involved in the synthesis of the "Compound" from lactate and ammonia, its production is continued at a high rate for more than 8 hours after protein synthesis has stopped.

In order to see how the rate of production of the "Compound" depends on the temperature when the generation time is long enough to permit us to assume that "Compound" production is going on at the maximum rate, we recorded the optical density of the supernatant in the stationary state at 280 mμ at three different temperatures. With the bacterial population maintained at an optical density of 0.272 at 350 mμ, we found the following rates at which the "Compound" is poured out:

at 25° = 0.106/hr (τ = 13 hrs);
at 36.5° = 0.226/hr (τ ranging from 6.18 to 7.14 hrs);
at 43° = 0.170/hr (τ ranging from 4.18 to 8.55 hrs).

It may be seen from this that the synthetic capacity for the production of the "Compound" rises from 25.5° to 36.5° by a factor of about 2 and then falls from 36.5° to 43° by a factor of about 0.75.

As Fig. 3 shows, the rate at which the "Compound" is produced rises when the generation time is increased from two hours to, say, four hours. The response of the rate of "Compound" production to a change in generation time might be instantaneous, or it might occur with a delay if, for instance, the accumulation of intermediates or adaptive phenomena are involved. To see which is the case we first maintained a stationary state in the Chemostat at a generation time of $\tau_1 = 2.3$ hrs and then suddenly decreased the flow rate w by a factor of 8/3 to give a generation time of $\tau_2 = 6.2$ hrs.

If the "Compound" is poured out at some rate A_1 at the generation time τ_1 and at a higher rate A_2 at the longer generation time τ_2, and if we then switch at zero time from the shorter generation time τ_1 to the longer generation time τ_2 by changing the flow rate w, then—assuming that the rate at which the "Compound" is poured out responds instantaneously to the change in generation time—the optical density of the supernatant at 280 mμ should be given by the formula:

$$\sigma = A_1\tau_1 + (A_2\tau_2 - A_1\tau_1)(1 - e^{-t/\tau_2})$$

If this formula holds we should obtain a straight line when we plot the optical density of the supernatant at 280 mμ against $(1 - e^{-t/\tau_2})$, and this straight line should start at zero time with $\sigma = A_1\tau_1$.

Fig. 5 shows such a plot of the results. The dotted line indicates the straight line along which the optical density would increase if the rate of production of the "Compound" remained unchanged when the generation time increased, i.e. if $A_2 = A_1$. The observed points, with the exception of the first few, lie on a straight line which rises more

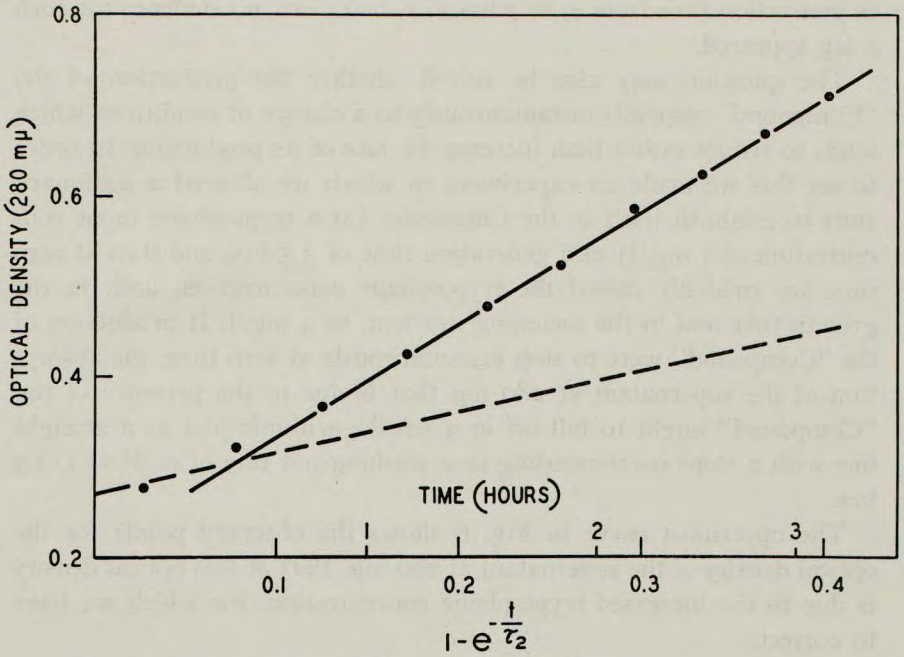

Fig. 5. Effect on the production rate of "Compound" brought about by a sudden decrease in growth rate.

steeply, corresponding to the increased rate of "Compound" production at the longer generation time. But this straight line does not start at zero time with $\sigma = A_1\tau_1$ as does the dotted line; rather, the two straight lines intersect at a time near 30 minutes, giving the impression that the increase in rate of production of the "Compound" sets in with a lag of about a half hour.

This impression might, however, be misleading because at the shorter generation time of $\tau = 2.3$ hrs there seems to be present a bacterial product (other than the "Compound") which absorbs at 280 mμ and which makes its presence manifest by a decreased ratio of the optical densities for 280 and 250 mμ. At the shorter generation time this ratio is 1.68, whereas after switching to the longer generation

time the ratio becomes 1.86, and under optimal conditions it may be as high as 2.2, corresponding to the absorption spectrum shown in Fig. 2. This extraneous absorption at the shorter generation time might simulate a lag when in fact there is no lag involved, and under the circumstances we can only say that if there is a lag, it is shorter than 30 minutes. When the experiment was repeated at 25°C. with a shift in generation time from $\tau_1 = 7$ hrs to $\tau_2 = 14$ hrs, no evidence for such a lag appeared.

The question may also be raised whether the production of the "Compound" responds instantaneously to a change of conditions which tends to reduce rather than increase the rate of its production. In order to see this we made an experiment in which we allowed a stationary state to establish itself in the Chemostat (at a tryptophane input concentration of 1 mg/l) at a generation time of 3.5 hrs, and then at zero time we suddenly raised the tryptophane concentration, both in the growth tube and in the incoming nutrient, to 4 mg/l. If production of the "Compound" were to stop instantaneously at zero time, the absorption of the supernatant at 280 mμ that is due to the presence of the "Compound" ought to fall off in a semilogarithmic plot as a straight line with a slope corresponding to a washing-out rate of $w/V = 1/3.5$ hrs.

The uppermost curve in Fig. 6 shows the observed points for the optical density of the supernatant at 280 mμ. Part of this optical density is due to the increased tryptophane concentration, for which we have to correct.

This correction is made as follows. When the tryptophane concentration is raised there is a corresponding increase in the absorption at 280 mμ. If tryptophane is converted into indol or some other compound containing the indol ring, there should be no change in the absorption at 280 mμ. However, the amount of free tryptophane present will be diminished by the amount of tryptophane which is bound in the protein of the bacteria. (We disregard as negligible the amount of free tryptophane that might be soaked up by the bacteria because we assume that it is not more than 1 mg/l.) From the excess of the bacterial population over the initial bacterial population we compute how much less free tryptophane is present at a given time than the amount that was added at zero time. The bacterial density is determined from the observed optical density of the bacterial suspension at 350 mμ. As Fig. 6 shows, this optical density follows in the semilogarithmic plot a straight line giving a bacterial growth rate of $\alpha = 1/2$ hrs.

The corrected curve for the absorption at 280 mμ shown in the figure turns out to be a straight line which falls with a slope corresponding to a washing-out rate of $w/V = 1/4$ hrs. This means that the "Compound" present in the growth tube at zero time is washed out almost as fast as the growth tube is washed out by the incoming nutrient (washing-out time 4 instead of 3.5 hrs). If the two rates were exactly equal, this would mean that after zero time no "Compound" is poured out by the bacteria. Within the limits of our experimental

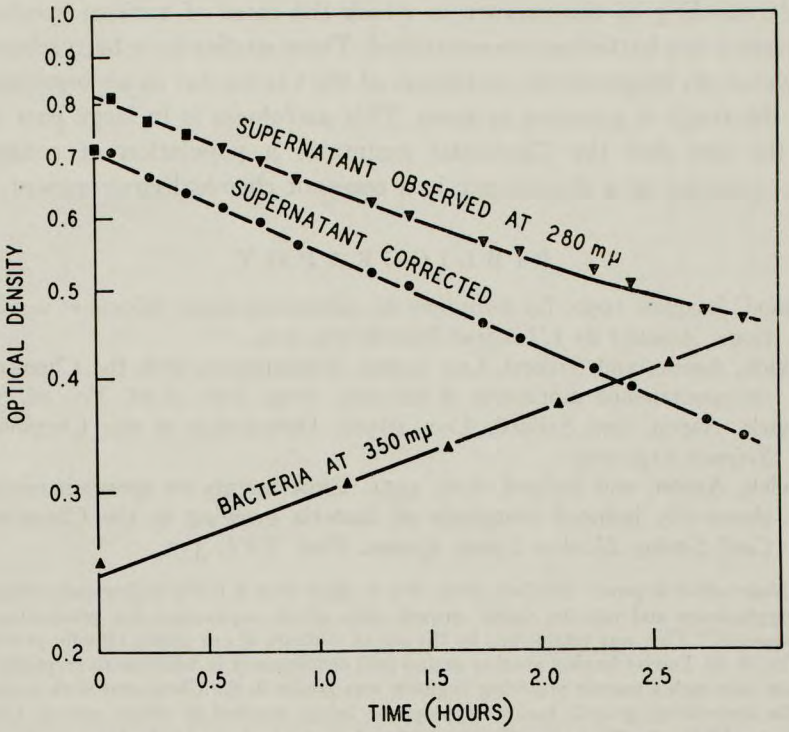

Fig. 6. Effect on the rate of production of "Compound" brought about by a sudden increase in tryptophane concentration.

accuracy we may only say that if any "Compound" is poured out after zero time the rate is less than one-third of the original rate. The corrected curve, taken at face value, shows that the decrease of the rate occurs without a lag, and within the limits of our experimental accuracy we may say that if there is a lag it is less than 10 minutes.

In all the experiments presented here, the rate at which the "Compound" is poured out is lower at generation times of about 2 hours than

at longer generation times. But in all these experiments using the Chemostat, bacteria growing with a generation time of 2 hours are of necessity growing at a tryptophane concentration c higher than those concentrations prevailing at longer generation times. For this reason we cannot say whether the reduced rate of the production of the "Compound" is due to a depressing effect of the higher tryptophane concentration on this rate or whether the faster growth rate itself precludes a high rate of "Compound" production.*

The experiments described in this paper only begin to provide some understanding of the manner in which the rates of various synthetic processes in a bacterium are controlled. These studies have been selected, however, to illustrate the usefulness of the Chemostat as an instrument for the study of growing systems. This usefulness is in large part due to the fact that the Chemostat maintains a population of constant size, growing at a chosen rate in a constant chemical environment.

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* *Note added in proof*: We have been able to show that it is the higher concentration of tryptophane and not the faster growth rate which suppresses the production of "Compound." This was established by the use of mutants of our strain (kindly provided by Dr. B. D. Davis) having another amino acid requirement in addition to tryptophane. In one case such a mutant requiring arginine was grown in the Chemostat with arginine as the controlling growth factor, tryptophane being supplied in slight excess. Under these conditions the "Compound" was produced at most at one-tenth the rate observed when the same strain was grown with tryptophane being limited and arginine being supplied in slight excess. Similar results were obtained with another mutant requiring histidine as well as tryptophane.

July 11, 1952

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Information is accumulating on the pathways of the biosynthesis of amino acids in microorganisms, but it is not known what regulates the rates of the individual steps in such syntheses. As a rule, a bacterium synthesizes from ammonia and some simple carbon source all of the amino acids that are contained in its proteins, and it produces of each amino acid no more than is needed. For instance, if a strain of coli that requires tryptophane grows in an arginine-free nutrient in which the tryptophane concentration is kept above a certain minimum level, it will grow rapidly, obviously synthesizing all the arginine contained in its proteins at a correspondingly high rate. If the tryptophane concentration is maintained at a low level, the strain will grow slowly, perhaps ten times more slowly than before, and we see from our experiments (unpublished) that there is no appreciable outpouring of arginine (or ornithine or citrulline) into the medium. This leads us to believe that the rate at which arginine is synthesized is also reduced tenfold. One may ask how such a regulation of the synthetic rates is accomplished.

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In the stationary state the tryptophane concentration c in the growth tube is very low, of the order of a microgram per liter, and at such low concentrations the growth rate α of the bacteria is an increasing function of the tryptophane concentration. Curve A in Fig. 1 gives the growth rate α as a function of the tryptophane concentration c. The growth rate α is defined as follows. In a nutrient medium in which the tryptophane concentration is maintained at a value c, the bacterial density n rises with time according to the formula $n = n_o e^{\alpha t}$. The coefficient α in the exponent is, by definition, the growth rate. The reciprocal of this value, $\frac{1}{\alpha} = \tau$, is the generation time. The doubling time of the bacteria is obtained from the generation time τ by multiplying by $\ln 2 = 0.693$.

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Fig. 3 shows the optical density of the supernatant at 280 mμ for our bacterial strain growing in the Chemostat in the stationary state with a

tryptophane input concentration of a = 500 γ/l at different generation times τ. As the figure shows, the concentration of the "Compound" is proportional to the generation time between τ = 4 hrs and τ = 14 hrs; i.e., between these limits the "Compound" is poured out at a constant rate corresponding to an increase in optical density of 0.141/hr. As the generation time is decreased below 3 hrs the rate of production of this "Compound" begins to fall off, and at a generation time of less than 2 hrs it is poured out at a rate of less than 0.040/hr.

By measuring the optical density of a known concentration of tryptophane at 280 mμ, we can compute on a mole-for-mole basis how much faster the "Compound" is poured out in a given experiment than tryptophane is taken up. One thus finds that at a generation of 14 hours the "Compound" is produced about 125 times faster than tryptophane is taken up. This furnishes a measure of the bacterium's capacity for tryptophane synthesis. A simple computation shows that at 37° C. tryptophane could be synthesized about 4 times as fast as the bacterium would need it if it grew—in the absence of tryptophane but otherwise under optimal nutritional conditions—at its maximum growth rate, i.e., with a generation time of about 30 minutes (doubling time about 20 minutes).

It seemed of interest to find out whether our strain of bacteria continues to produce a compound even if we block protein synthesis by stopping the inflow of fresh nutrient into the growth tube of the Chemostat. In order to see this we first permitted a stationary state to establish itself at the generation time of about 3 hours with the bacterial population maintained at an optical density of 0.245 at 350 mμ, and then we stopped the inflow of nutrient. The small amount of tryptophane contained in the growth tube is then exhausted within a few seconds and so protein synthesis ceases almost instantaneously. As shown in Fig. 4, we found, when the inflow was stopped

at zero time, that the optical density of the supernatant increased proportionately with time for about 4 hours at the rate of 0.19/hr; the rate then decreased to 0.12/hr and the "Compound" continued to pour out at this lower rate for another 4 hours, at which time the experiment was stopped. This means that even though a large number of steps and a correspondingly large number of enzymes must be involved in the synthesis of the "Compound" from lactate and ammonia, its production is continued at a high rate for more than 8 hours after protein synthesis has stopped.

In order to see how the rate of production of the "Compound" depends on the temperature when the generation time is long enough to permit us to assume that "Compound" production is going on at the maximum rate, we recorded the optical density of the supernatant in the stationary state at 280 mμ at three different temperatures. With the bacterial population maintained at an optical density of 0.272 at 350 mμ, we found the following rates at which the "Compound" is poured out:

at 25° = 0.106/hr (τ = 13 hrs);

at 36.5° = 0.226/hr (τ ranging from 6.18 to 7.14 hrs);

at 43° = 0.170/hr (τ ranging from 4.18 to 8.55 hrs).

It may be seen from this that the synthetic capacity for the production of the "Compound" rises from 25.5° to 36.5° by a factor of about 2 and then falls from 36.5° to 43° by a factor of about 0.75.

As Fig. 3 shows, the rate at which the "Compound" is produced rises when the generation time is increased from 2 hours to, say, 4 hours. The response of the rate of "Compound" production to a change in generation time might be instantaneous, or it might occur with a delay if, for instance, the accumulation of intermediates or adaptive phenomena are involved. To see which is the case we first maintained a stationary state in the Chemostat at a generation time of τ_1 = 2.3 hrs and then suddenly decreased the flow rate w

by a factor of $\frac{8}{3}$ to give a generation time of $\tau_2 = 6.2$ hrs.

If the "Compound" is poured out at some rate A_1 at the generation time τ_1 and at a higher rate A_2 at the longer generation time τ_2, and if we then switch at zero time from the shorter generation time τ_1 to the longer generation time τ_2 by changing the flow rate w, then—assuming that the rate at which the "Compound" is poured out responds instantaneously to the change in generation time— the optical density of the supernatant at 280 mμ should be given by the formula:

$$\sigma = A_1\tau_1 + (A_2\tau_2 - A_1\tau_1)(1 - e^{-t/\tau_2})$$

If this formula holds we should obtain a straight line when we plot the optical density of the supernatant at 280 mμ against $(1 - e^{-t/\tau_2})$, and this straight line should start out at zero time with $\sigma = A_1\tau_1$.

Fig. 5 shows such a plot of the results. The dotted line indicates the straight line along which the optical density would increase if the rate of production of the "Compound" remained unchanged when the generation time increased, i.e., if $A_2 = A_1$. The observed points—with the exception of the first few—lie on a straight line which rises more steeply, corresponding to the increased rate of "Compound" production at the longer generation time. But this straight line does not start off at zero time with $\sigma = A_1\tau_1$ as does the dotted line; rather, the two straight lines intersect at a time near 30 minutes, giving the impression that the increase in rate of production of the "Compound" sets in with a lag of about a half hour.

This impression might, however, be misleading because at the shorter generation time of $\tau = 2.3$ hrs there seems to be present a bacterial product (other than the "Compound") which absorbs at 280 mμ and which makes its presence manifest by a decreased ratio of the optical densities for 280 and 250 mμ. At the shorter generation time this ratio is 1.68, whereas after

switching to the longer generation time the ratio becomes 1.86, and under optimal conditions it may be as high as 2.2, corresponding to the absorption spectrum shown in Fig. 2. This extraneous absorption at the shorter generation time might simulate a lag when in fact there is no lag involved, and under the circumstances we can only say that if there is a lag, it is shorter than 30 minutes. When the experiment was repeated at 25° C. with a shift in generation time from $\tau_1 = 7$ hrs to $\tau_2 = 14$ hrs, no evidence for such a lag appeared.

The question may also be raised whether the production of the "Compound" responds instantaneously to a change of conditions which tends to reduce rather than increase the rate of its production. In order to see this we made an experiment in which we allowed a stationary state to establish itself in the Chemostat (at a tryptophane input concentration of 1 mg/l) at a generation time of 3.5 hrs, and then at zero time we suddenly raised the tryptophane concentration, both in the growth tube and in the incoming nutrient, to 4 mg/l. If production of the "Compound" were to stop instantaneously at zero time, the absorption of the supernatant at 280 mμ that is due to the presence of the "Compound" ought to fall off in a semilogarithmic plot as a straight line with a slope corresponding to a washing-out rate of $\frac{w}{V} = \frac{1}{3.5 \text{ hrs}}$.

The uppermost curve in Fig. 6 shows the observed points for the optical density of the supernatant at 280 mμ. Part of this optical density is due to the increased tryptophane concentration, for which we have to correct.

This correction is made as follows. When the tryptophane concentration is raised there is a corresponding increase in the absorption at 280 mμ. If tryptophane is converted into indol or some other compound containing the indol ring, there should be no change in the absorption at 280 mμ. However, the amount of free tryptophane present will be diminished by the amount of tryptophane

which is bound in the protein of the bacteria. (We disregard as negligible the amount of free tryptophane that might be soaked up by the bacteria because we assume that it is not more than 1 mg/l). From the excess of the bacterial population over the initial bacterial population we compute how much less free tryptophane is present at a given time than the amount that was added at zero time. The bacterial density is determined from the observed optical density of the bacterial suspension at 350 mμ. As Fig. 6 shows, this optical density follows in the semilogarithmic plot a straight line giving a bacterial growth rate of $\alpha = \frac{1}{2 \text{ hrs}}$.

The corrected curve for the absorption at 280 mμ shown in the figure turns out to be a straight line which falls with a slope corresponding to a washing-out rate of $\frac{w}{v} = \frac{1}{4 \text{ hrs}}$. This means that the "Compound" present in the growth tube at zero time is washed out almost as fast as the growth tube is washed out by the incoming nutrient (washing-out time 4 instead of 3.5 hrs). If the two rates were exactly equal, this would mean that after zero time no "Compound" is poured out by the bacteria. Within the limits of our experimental accuracy we may only say that if any "Compound" is poured out after zero time the rate is less than one third of the original rate. The corrected curve, taken at face value, shows that the decrease of the rate occurs without a lag, and within the limits of our experimental accuracy we may say that if there is a lag it is less than 10 minutes.

In all the experiments presented here, the rate at which the "Compound" is poured out is lower at generation times of about 2 hours than at longer generation times. But in all these experiments using the Chemostat, bacteria growing with a generation time of 2 hours are of necessity growing at a tryptophane concentration c higher than those concentrations prevailing at longer generation times. For this reason we cannot say whether the reduced rate of the production of the "Compound" is due to a depressing effect of the higher

tryptophane concentration on this rate or whether the faster growth rate itself precludes a high rate of "Compound" production.

The experiments described in this paper only begin to provide some understanding of the manner in which the rates of various synthetic processes in a bacterium are controlled. These studies have been selected, however, to illustrate the usefulness of the Chemostat as an instrument for the study of growing systems. This usefulness is in large part due to the fact that the Chemostat maintains a population of constant size, growing at a chosen rate in a constant chemical environment.

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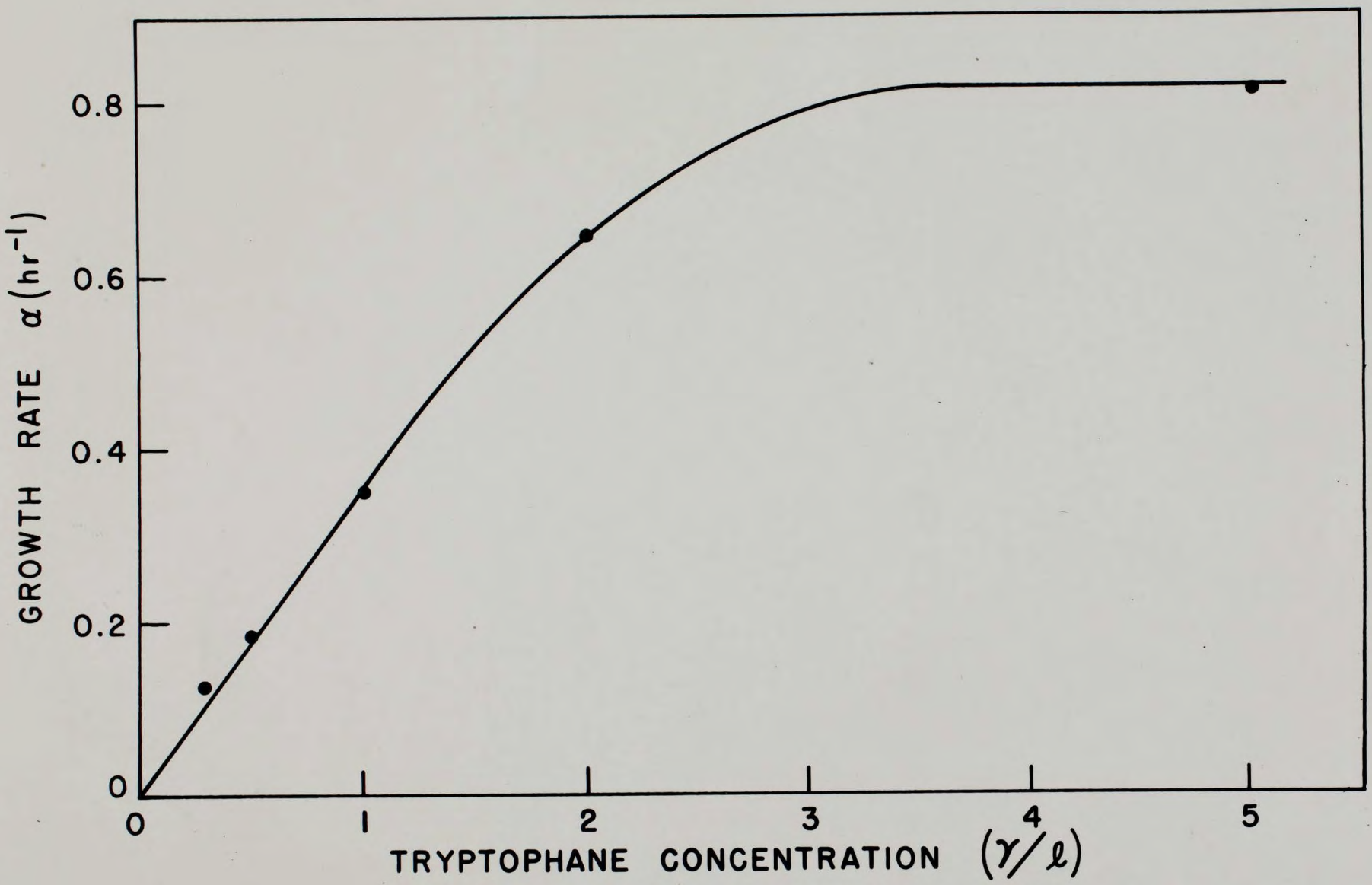
GROWTH RATE α (hr⁻¹)
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
TRYPTOPHANE CONCENTRATION (γ/l)

FIGURE 1

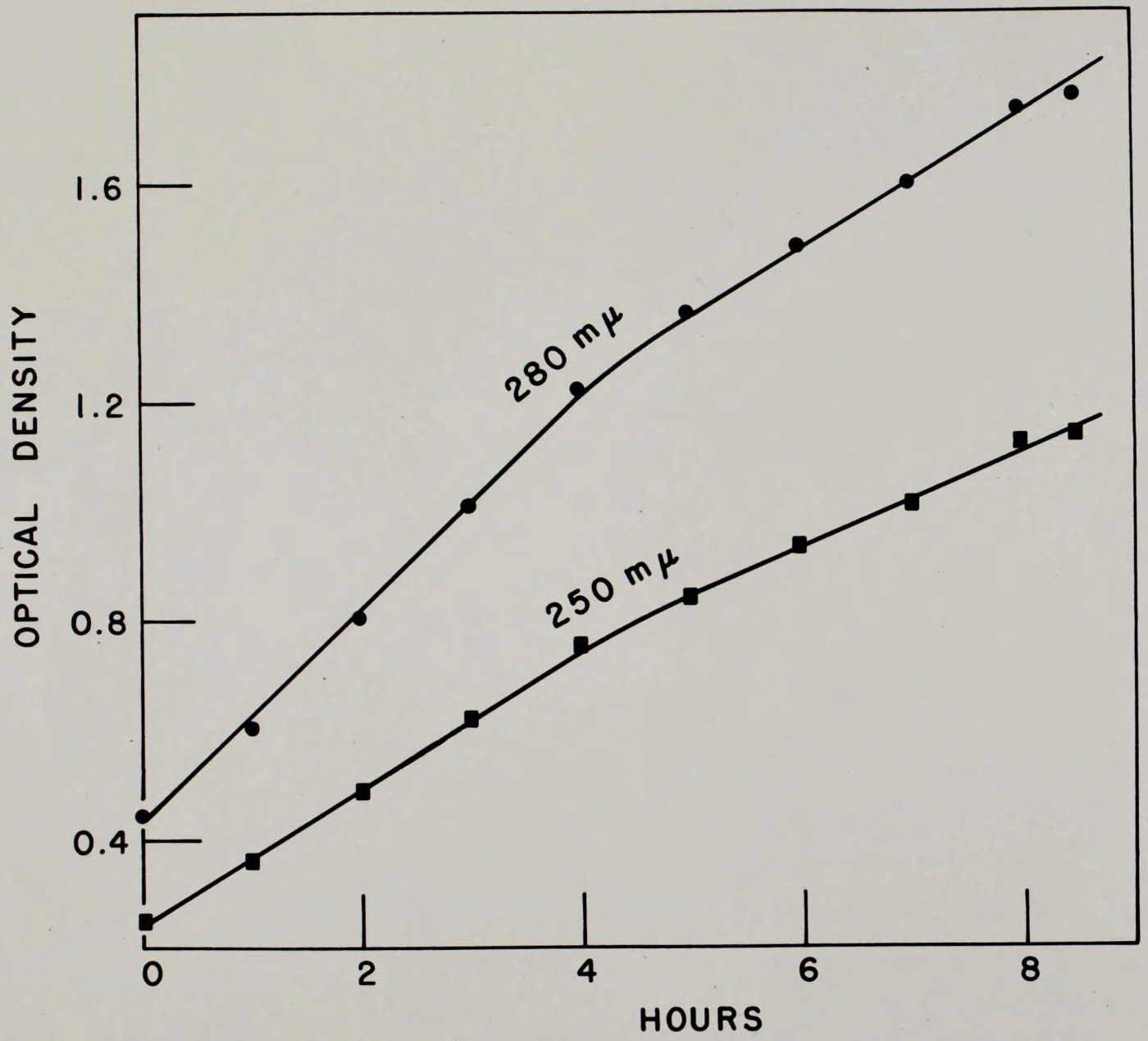

FIGURE 4

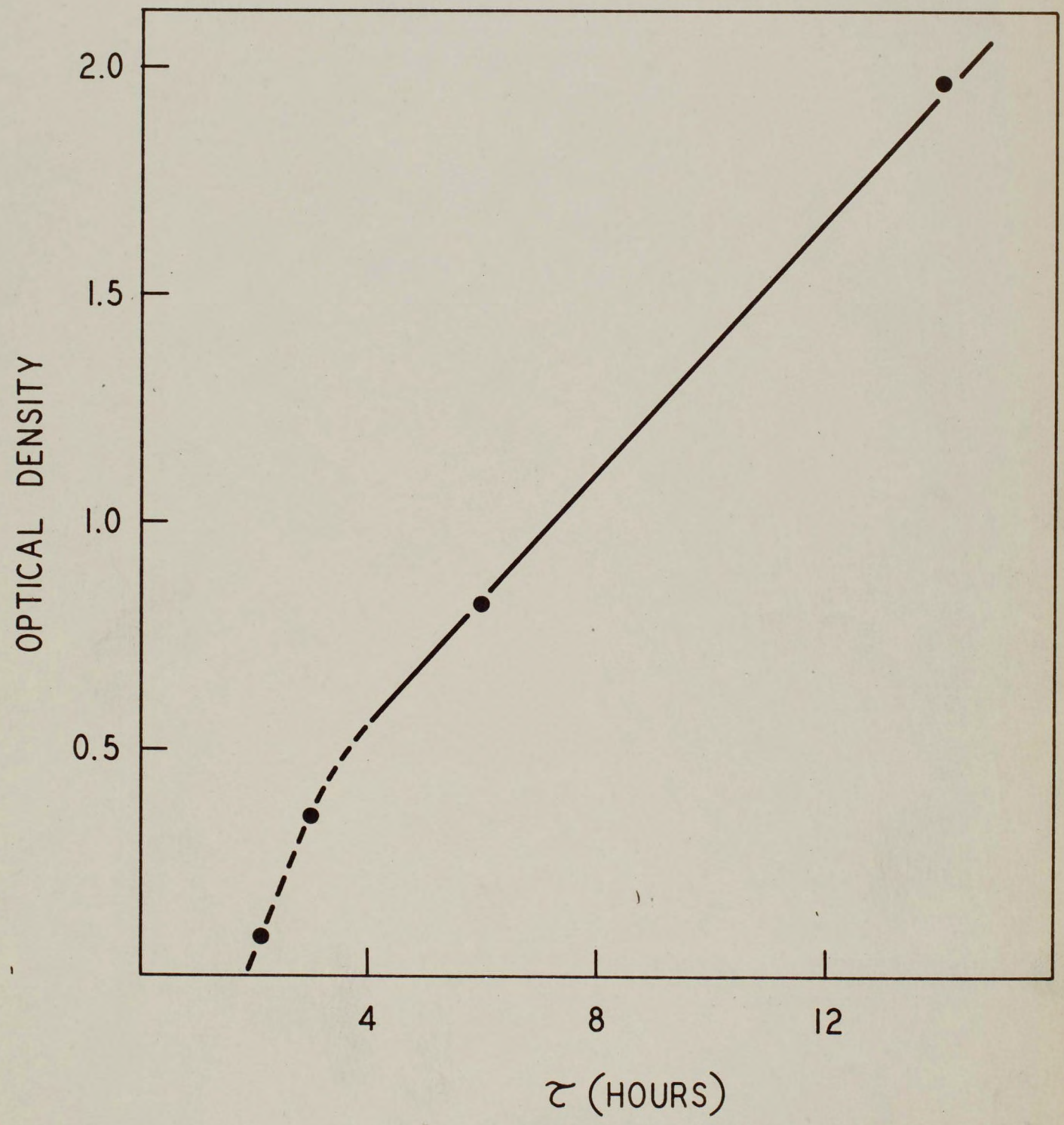

FIGURE 3

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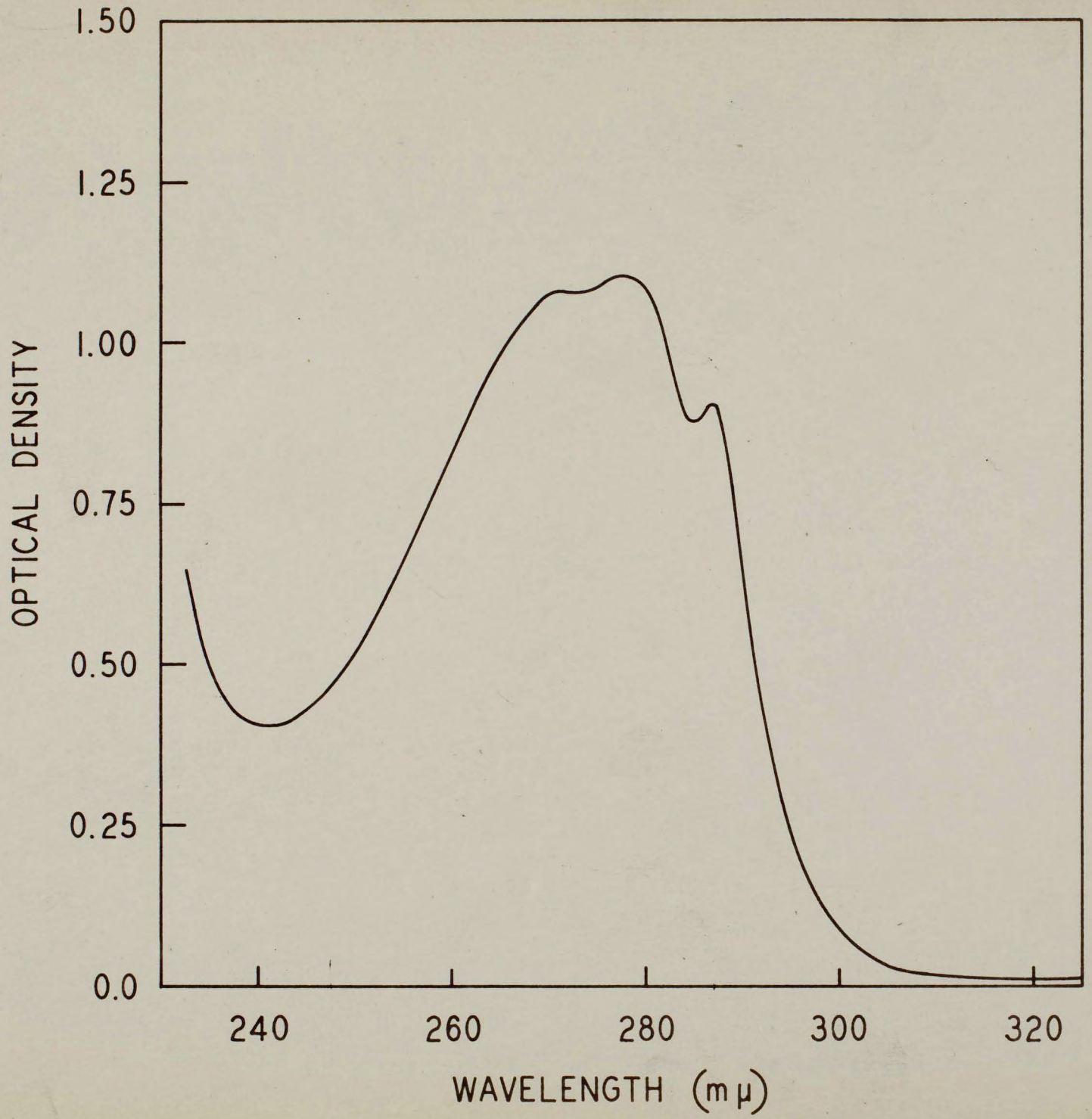

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FIGURE 2

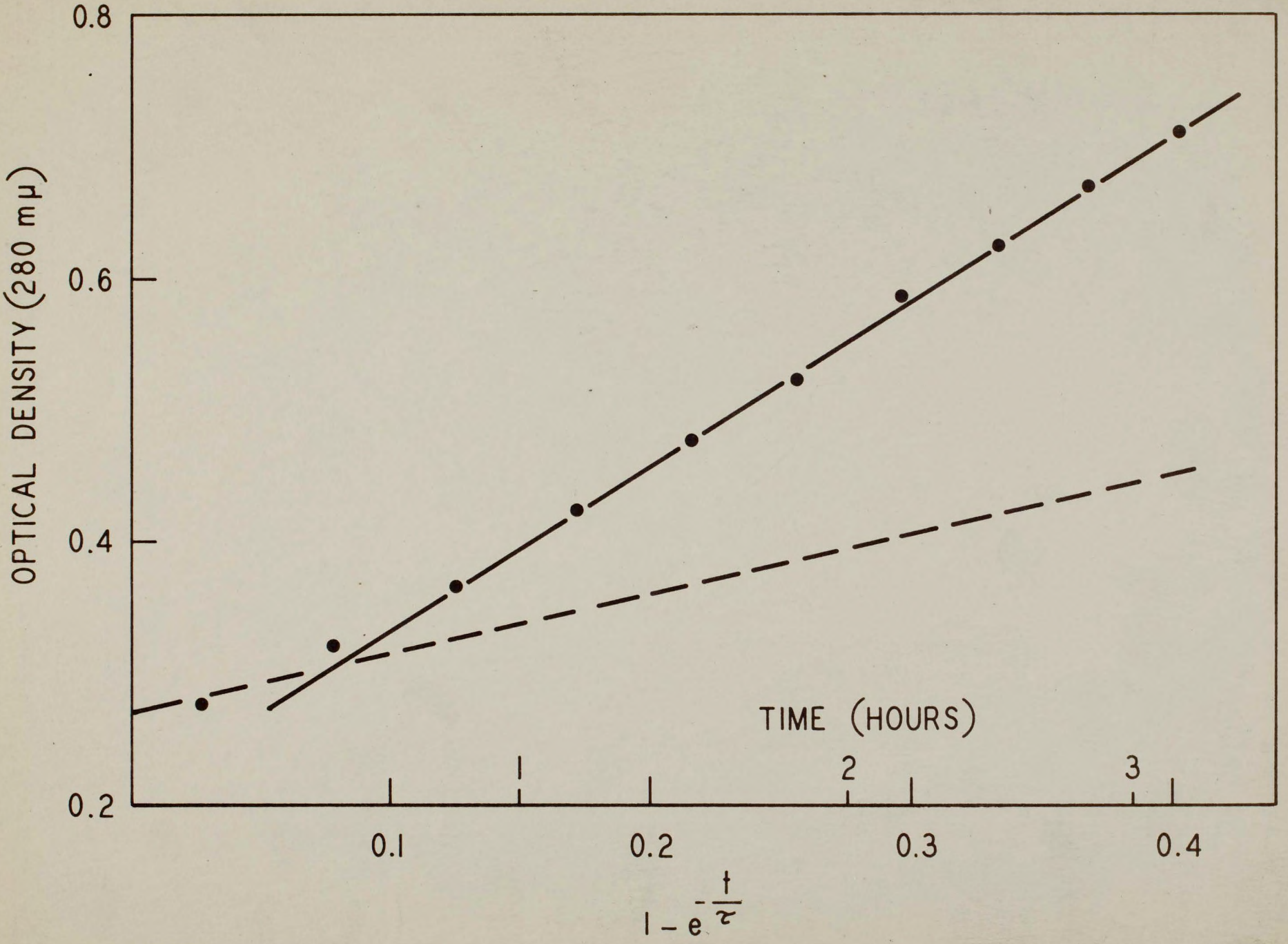

FIGURE 5

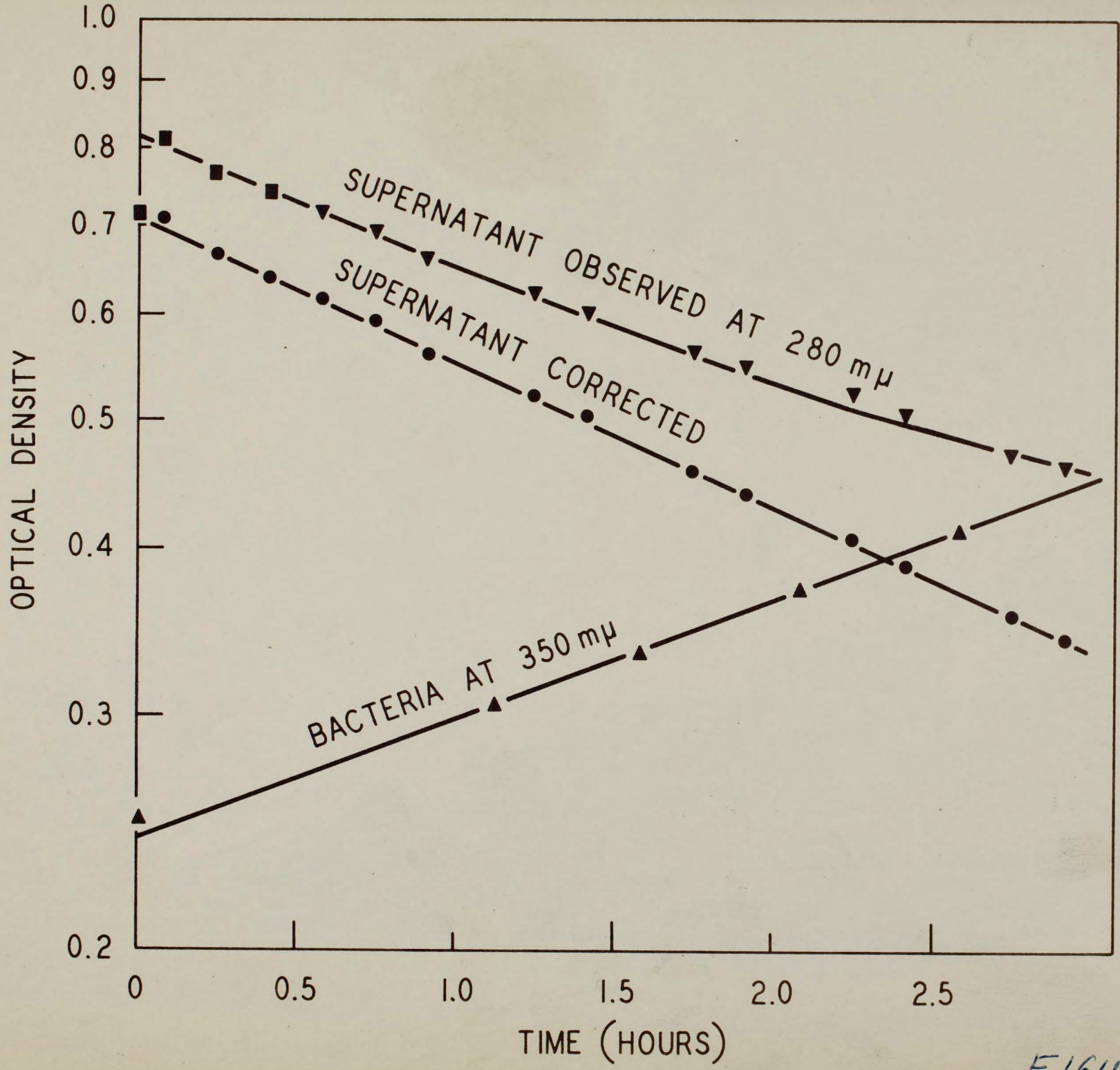

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FIGURE 6