physical description is that describing the experimental meaning of the quantities in the equation—or better, the way the equations are to be used in describing experimental observations. This being the case, perhaps the best way to proceed is to try to guess equations, and disregard physical models or descriptions. For example, McCullough guessed the correct equations for light propagation in a crystal long before his colleagues using elastic models could make head or tail of the phenomena, or again, Dirac obtained his equation for the description of the electron by an almost purely mathematical proposition. A simple physical view by which all the contents of this equation can be seen is still lacking.

Therefore, I think equation guessing might be the best method for proceeding to obtain the laws for the part of physics which is presently unknown. Yet, when I was much younger, I tried this equation guessing and I have seen many students try this, but it is very easy to go off in wildly incorrect and impossible directions. I think the problem is not to find the *best* or most efficient method for proceeding to a discovery, but to find any method at all. Physical reasoning does help some people to generate suggestions as to how the unknown may be related to the known. Theories of the known which are described by different physical ideas may be equivalent in all their predictions and hence scientifically indistinguishable. However, they are not psychologically identical when one is trying to move from that base into the unknown. For different views suggest different kinds of modifications which might be made and hence are not equivalent in the hypotheses one generates from them in one's attempt to understand what is not yet understood. I, therefore, think that a good theoretical physicist today might find it useful to have a wide range of physical viewpoints and mathematical expressions of the same theory (for example, of quantum electrodynamics) available to him. This may be asking too much of one man. Then new students should as a class have this. If every individual student follows the same current fashion in expressing and thinking about electrodynamics or field theory, then the variety of hypotheses being generated to understand strong interactions, say, is limited. Perhaps rightly so, for possibly the chance is high that the truth lies in the fashionable direction. But, on the off-chance that it is in another direction —a direction obvious from an unfashionable view of field theory—who will find it? Only someone who has sacrificed himself by teaching himself quantum electrodynamics from a peculiar and unusual point of view, one that he may have to invent for himself. I say sacrificed himself because he most likely will get nothing from it, because the truth may lie in another direction, perhaps even the fashionable one.

But, if my own experience is any guide, the sacrifice is really not great because if the peculiar viewpoint taken is truly experimentally equivalent to the usual in the realm of the known there is always a range of applications and problems in this realm for which the special viewpoint gives one a special power and clarity of thought, which is valuable in itself. Furthermore, in the search for new laws, you always have the psychological excitement of feeling that possibly nobody has yet thought of the crazy possibility you are looking at right now.

So what happened to the old theory that I fell in love with as a youth? Well, I would say it's become an old lady, who has very little that's attractive left in her, and the young today will not have their hearts pound when they look at her anymore. But, we can say the best we can for any old woman, that she has been a very good mother and has given birth to some very good children. And, I thank the Swedish Academy of Sciences for complimenting one of them. Thank you.

Chemotaxis in Bacteria

Motile *Escherichia coli* migrate in bands that are influenced by oxygen and organic nutrients.

Julius Adler

Chemotaxis is the movement of organisms toward or away from a chemical. This phenomenon has been observed in a wide variety of microorganisms, plants, and animals (*1, 2*). In bacteria chemotaxis has been known ever since the end of the 19th century, when Engelmann, Pfeffer, and other biologists discovered chemotaxis toward oxygen, minerals, and organic nutrients (for a review see Weibull, *3*). These workers demonstrated chemotaxis microscopically by observing whether bacteria in a suspension accumulated near or away from a gas bubble or a chemical introduced at one point.

In 1893 Beijernck (*4*) demonstrated chemotaxis toward oxygen macroscopically by showing that a variety of motile bacteria placed at the bottom of a test tube filled with water would form a sharp, easily visible band that rose until it came to a stop near the meniscus. The band would then descend if the air above the liquid was replaced by oxygen, and it would ascend if an atmosphere depleted in oxygen was used. Beijerinck interpreted this to mean that the bacteria seek a certain optimum concentration of oxygen. More recently, Sherris, Preston, and Shoesmith (*5*) and Baracchini and Sherris (*6*), using capillary tubes instead of test tubes, confirmed and extended these results.

Very little is understood about the mechanism of chemotaxis in bacteria. In order to learn about this, *Escherichia coli* was chosen for study because the vast knowledge of its biochemistry and genetics could be brought to bear on the problem. Many strains of *E. coli*

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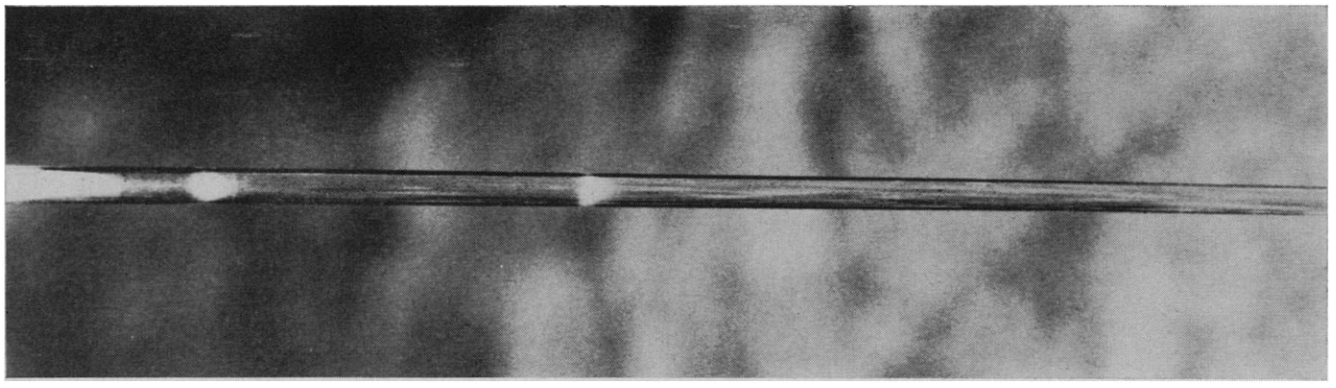


Fig. 1. Photograph showing bands of *E. coli* in a capillary tube. In all the experiments reported here, capillary tubes (*18*) were filled with a liquid medium (*19*), inoculated at one end with 2×10^5 to 2×10^6 bacteria (*20*), and then closed at the ends with plugs of agar and clay, all according to a procedure described in full elsewhere (*8*). The tubes were incubated horizontally at 37°C. The origin, which is turbid because of the bacteria that have not moved out, is visible at the left, then the second band of bacteria, then the first band. Plugs at ends are not shown. The concentration of galactose was 2.5×10^{-4} mole per liter.

are motile by virtue of several flagella distributed around the cell. Beijerinck (*4*) and Baracchini and Sherris (*6*) had already tested a large number of species and shown that *E. coli* are chemotactic toward oxygen.

To study chemotaxis in a medium containing only known chemicals, it was necessary first to devise a simple chemically defined medium that would still allow motility, and to determine the optimal conditions for motility by use of an assay developed for this purpose (*7*).

This article aims, first, to demonstrate that chemotaxis does occur in *E. coli*; second, to determine what kinds of substances elicit chemotaxis in *E. coli*; and, third, to discuss the mechanism of chemotaxis.

Demonstration of Bands

About a million motile cells of *E. coli* are placed at one end of a capillary tube filled with a solution containing 2.5×10^{-4} molar galactose as the energy source, and the ends of the tube are closed with plugs of agar and clay. Soon afterward, two sharp, easily visible bands of bacteria have moved out from the origin, and some bacteria still remain at the origin. These features are shown in Fig. 1.

The bands can be observed under the microscope. For undistorted viewing, flat capillary tubes are used according to the suggestion of Sherris, Preston, and Shoesmith (*5*). The two sharp bands are easily visible as highly crowded regions of bacteria whose motion is extremely rapid and jerky; in the first band the bacteria appear to be considerably more motile than in the second, and the bacteria left at the origin are not motile.

The bands can also be demonstrated by dividing the tube into compartments, plating the contents of each compartment, and counting colonies to determine the number of viable bacteria present throughout the tube (Fig. 2). Another method for demonstrating the bands and observing their rate of movement is to scan the tube with a recording microdensitometer (*9*) at various times (Fig. 3).

The easiest way to locate the bands is simply to measure their position with a ruler, and this is the method used in the work described in the remainder of this article. Figure 4 shows the location of the bands at several different times. The "first" band is not only faster, but it also forms first; it is visible after 5 minutes. The "second" band becomes

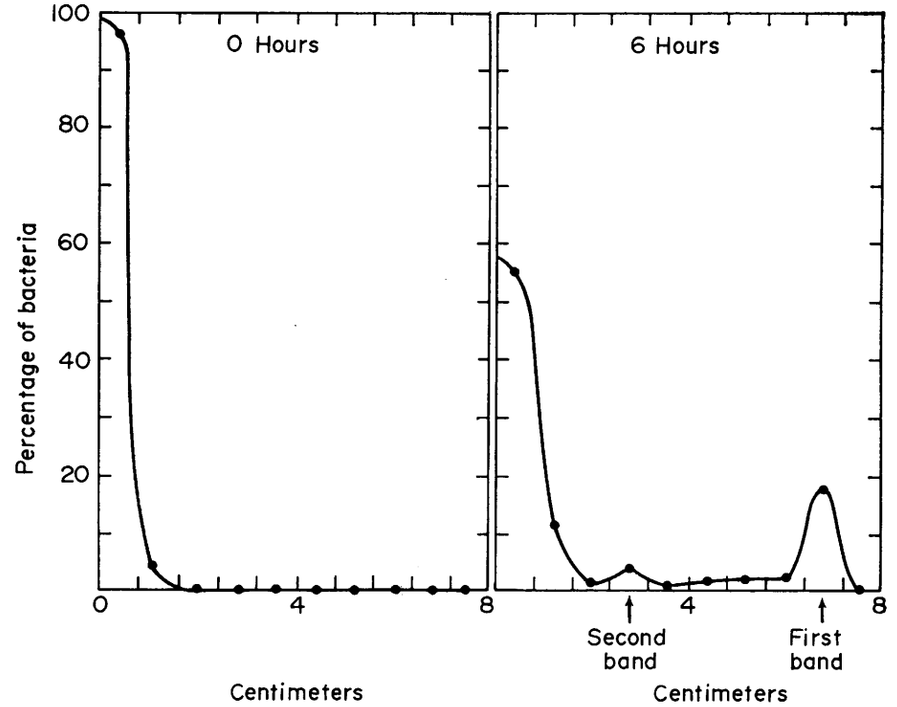


Fig. 2. Bands of bacteria shown by assay of viable bacteria. The tube contained $2.5 \times 10^{-4}M$ galactose and was incubated for 0 or 6 hours. At 6 hours the bands were visible where shown by the arrows. The tubes were fractionated into ten compartments, each 8 mm long, by breaking at one end of the column of liquid and withdrawing samples with a smaller capillary tube. A total of 9.8×10^5 viable bacteria were recovered at 0 hours and a total of 1.4×10^6 at 6 hours. The last eight compartments were free of any viable bacteria in the tube harvested at zero time. The procedure is described in full elsewhere (*8*).

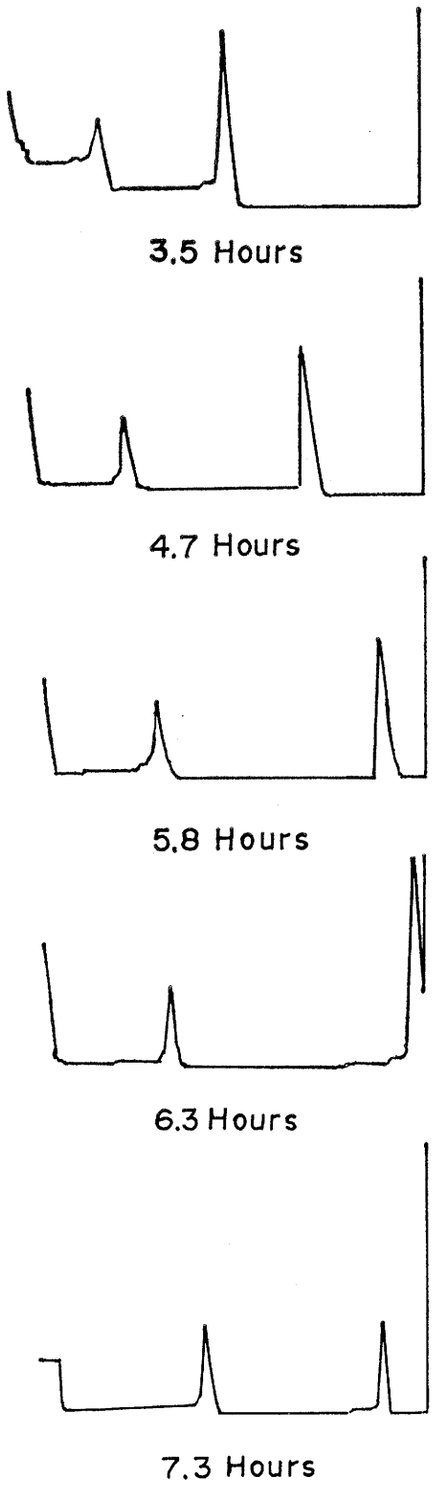


Fig. 3. Bands of bacteria shown by tracings from a recording microdensitometer. The tube, containing 2.5 × $10^{-4}M$ galactose, was incubated at 37°C for 3.5 hours and then kept at room temperature for measurements (*9*). The times indicate hours from the start of the experiment. From right to left may be seen a vertical line representing the edge of the plug at the right end of the tube, then the first band, then the second band, and at the left a line representing the bacteria remaining at the origin. The plug at the left end of the tube is not represented.

visible after 20 minutes. (These early time points are not shown in the figures.) Figures 3 and 4 show that when the first band reaches the top, part of it remains there and part of it retreats. This retreating fraction eventually fuses with the second band, and the fused bands then disappear.

Heterogeneity Excluded

It is possible to take out separately the first band, the second band, and the material left at the origin, and then immediately to use each of these over again in fresh tubes. (Actually, to get enough bacteria the first bands were pooled from 12 tubes, the second bands from 35 tubes, and the material at the origin from 12 tubes.) The result in each of the three cases is the same as the original result: each component forms two bands, and some bacteria remain at the origin. Single colony isolates also form two bands and leave residual bacteria at the origin. It may be concluded that how a bacterium behaves depends on where it finds itself when first placed into the tube, rather than on physiological or genetic heterogeneity among the bacteria.

Use of Oxygen and Galactose

The experiments described so far and those to be discussed next were carried out with a solution containing 2.5 × 10^{-4} moles of galactose per liter. The galactose is present in excess over the oxygen, since the concentration of oxygen in water saturated with air at 37°C is about 2.0 × 10^{-4} mole per liter and it takes six molecules of oxygen to fully oxidize a molecule of galactose.

The amount of oxygen remaining throughout the tube after the bands appeared was measured by inserting a polarographic needle electrode (*10*) into the capillary tube. Figure 5 shows that the first band consumes all or nearly all the oxygen and that the second band is in an anaerobic environment.

The amount of galactose remaining throughout the tube was measured by using C^{14}-galactose, dividing the tube into compartments, removing and chromatographing the contents of each compartment, and measuring the amount of radioactivity in the region of the chromatogram corresponding to galactose. Figure 6 shows that the first band uses a part of the galactose, and the second band uses all (or at least 99 percent) of the rest.

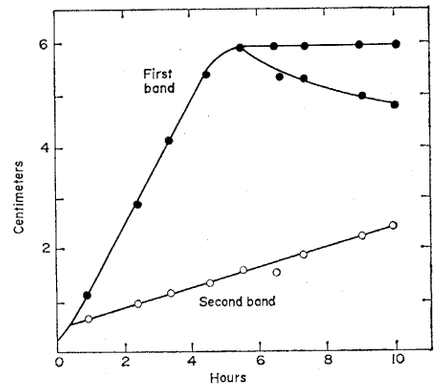


Fig. 4. Rate of movement of bands of bacteria. The concentration of galactose was 2.5 × 10^{-4} mole per liter. At 6 hours the first band has reached the end of the column of liquid. The location of bands in this and all later experiments was measured with a ruler.

It is clear from these data that the first band of bacteria travels along consuming all the oxygen to oxidize a part of the galactose, while the second band uses all the residual galactose anaerobically. When the first band reaches the end of the column of liquid, it begins to use up the galactose there (see Fig. 6, 8 hours), and a portion of the bacteria then retreats (as shown in Figs. 3 and 4) to consume the unused galactose anaerobically. Bacteria that remain where they were placed at the start of the experiment are no longer motile,

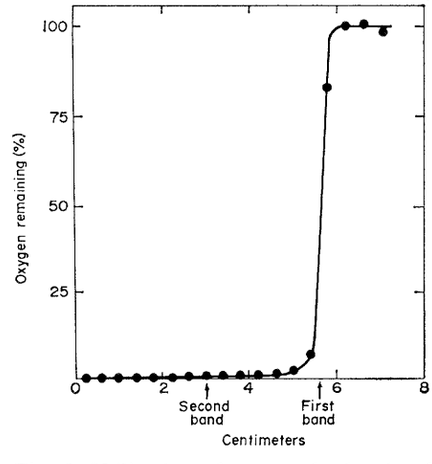


Fig. 5. Utilization of oxygen by bands of bacteria in 2.5 × $10^{-4}M$ galactose. Oxygen was measured polarographically by inserting an oxygen needle electrode (*10*) into the capillary tube in 4-mm steps when the first and second bands were visible where shown by the arrows.

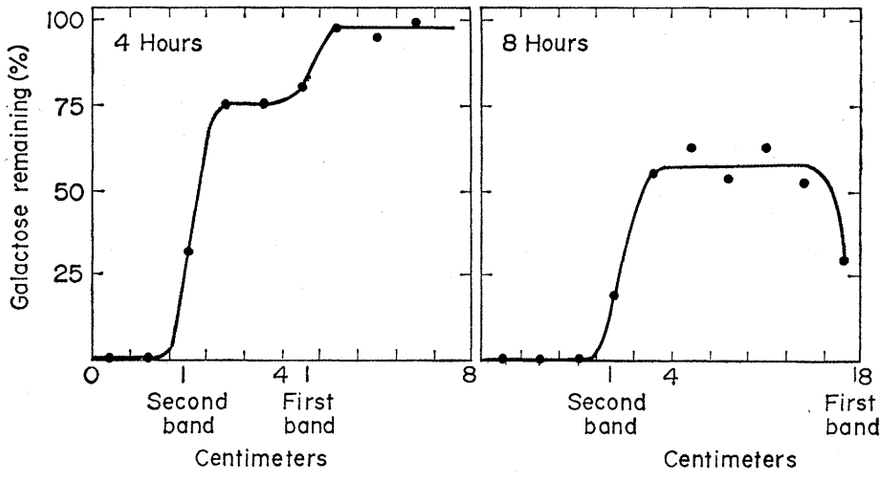


Fig. 6. Utilization of 2.5 × $10^{-4}M$ galactose. The tubes contained C^{14}-galactose (2.5 × 10^{-4} mole/liter and 1 × 10^{6} counts per minute per milliliter). At 4 or 8 hours, when the first and second bands were visible where shown by the arrows, the tube was fractionated into ten compartments, each 8 mm long, as in Fig. 2. The contents of each compartment were chromatographed on paper, with *n*-butanol, acetic acid, and water (12:3:5) as the solvent, and the radioactivity in the galactose region of the chromatogram was measured in a paper-strip counter.

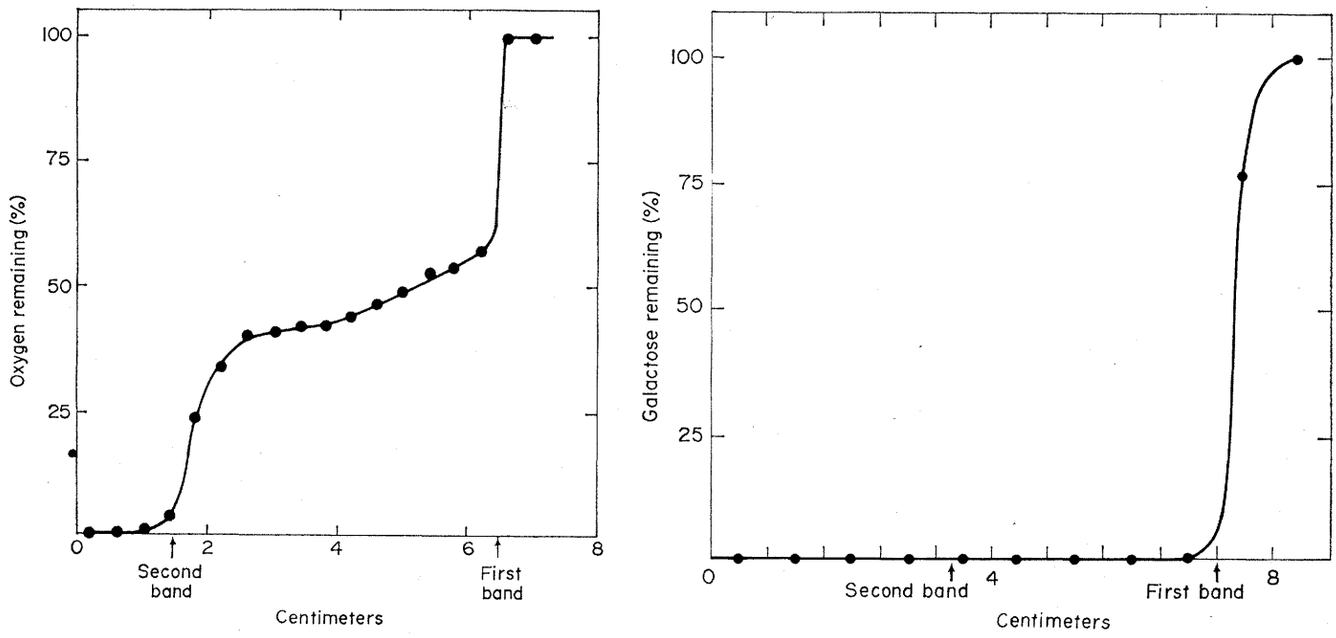
because they are left without any galactose as energy source and their endogenous energy source is not available anaerobically (*7*).

Experiments were also carried out with 5.0 × $10^{-5}M$ galactose, a concentration which is lower than that of the dissolved oxygen. Figure 7 shows that under these conditions the first band uses a part of the oxygen and the second band uses all the rest, and Fig. 8 shows that the first band consumes all the galactose while the second band is operating in the absence of galactose. These data show that when oxygen exceeds galactose the first band of bacteria aerobically consumes all the galactose as it travels along and leaves behind unused oxygen; the second band consumes all the residual oxygen to oxidize an endogenous energy source known (*7*) to be present.

Varying the Amounts of Galactose and Oxygen

When the concentration of galactose was varied systematically, results were obtained that are shown in Fig. 9. At about 1 × $10^{-4}M$ galactose only one band forms. One would predict that the "equivalence point" at which neither galactose nor oxygen is present in excess would occur at this concentration and that therefore only one band would form which would consume all the oxygen and all the galactose as it travels along. This prediction takes into account that only about half the galactose would be oxidized and the rest would serve as the source of carbon for the synthesis of cellular material.

As the galactose concentration is increased above 1 × 10^{-4} mole per liter, the second band travels less and less far in 4 hours. This is interpreted to mean that the band does not move ahead until it has used all or nearly all the galactose, and the more galactose there is present the longer it will take the band to use it. At the highest concentrations of galactose the second band does not form at all, because more galactose is present than can be used up in the time allowed; a microscopic examination of the tube shows that the bacteria everywhere are motile, so the failure to form a second band cannot be ascribed to lack of motility. The dis-



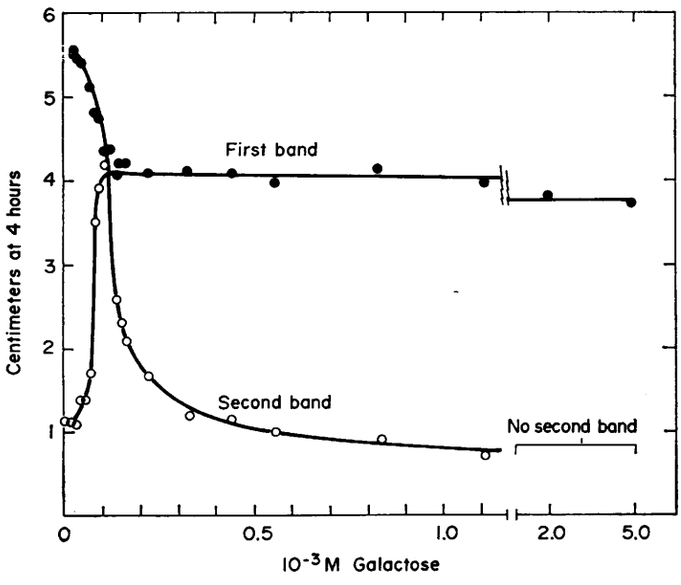
Figs. 7 and 8. Fig. 7 (left). Utilization of oxygen by bands of bacteria in 5.0 × $10^{-5}M$ galactose. Measurements were made as in Fig. 5. Fig. 8 (right). Utilization of 5.0 × $10^{-5}M$ galactose. The tube contained C^{14}-galactose (5.0 × 10^{-5} mole/liter and 2 × 10^{5} counts per minute per milliliter). At 4.75 hours when the first and second bands were visible where shown by the arrows, the tube was fractionated and chromatographed as described in Fig. 6.

tance moved by the first band, on the other hand, is independent of the galactose concentration above 1×10^{-4} mole per liter, presumably because at this concentration the rate of oxygen uptake has reached a maximum; that is, increasing the galactose concentration further does not increase the rate of oxygen consumption.

Below $1 \times 10^{-4}M$ galactose the first band moves less far in 4 hours as the galactose concentration is increased, presumably because it takes the bacteria longer to use up the galactose when more of it is present. The second band, on the other hand, moves farther in 4 hours as the galactose concentration is increased. This is explained by supposing that less oxygen is left behind by the first band when there is more galactose to be oxidized; the less oxygen there is present, the less time it takes for the second band to consume it and move on. When no galactose is added, one band still forms, as shown in Fig. 9. This band travels along consuming the oxygen (according to measurements with the oxygen electrode) to oxidize an endogenous energy source known (*7*) to be present.

The effect of varying the concentration of oxygen was studied at a concentration of galactose so high (5×10^{-3} mole per liter) that no second band appeared during the experiment. Figure 10 shows that this "first" band travels less and less far in 2 hours as the oxygen concentration is increased. This is interpreted to mean that the band does not move on until it has consumed all or nearly all the oxygen, and the more oxygen there is present, the longer it takes the band to use it up. Actually this effect is more pronounced than Fig. 10 indicates, since at high concentrations of oxygen the band contains more bacteria and therefore the oxygen is used faster. No band is formed in the absence of oxygen (see Fig. 10).

Baracchini and Sherris (*6*) had al-



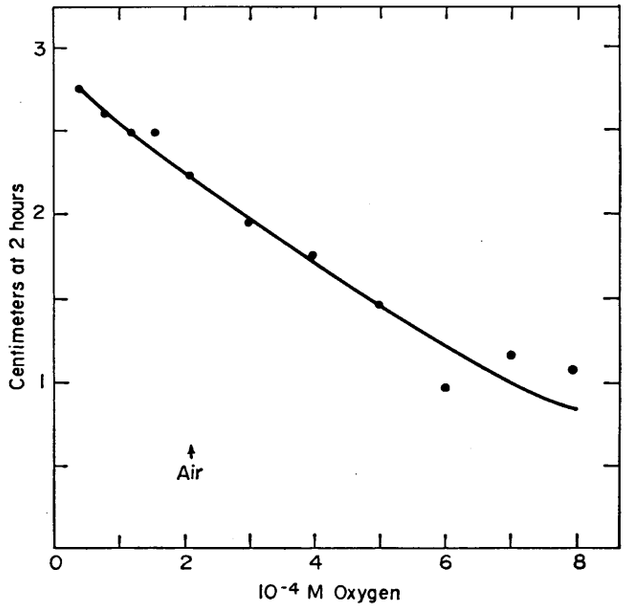


Fig. 9 (left above). The effect of varying the galactose concentration. A set of tubes, each containing a different concentration of galactose, was inoculated and incubated. The figure shows the location of the bands at 4 hours in each of the tubes.

Fig. 10 (above right). The effect of varying the oxygen concentration. A set of tubes, each containing a different concentration of oxygen, was prepared in this way: just before the medium was put into the capillary tube, its concentration of oxygen was adjusted to the desired concentration by bubbling nitrogen or oxygen through 30 ml of medium in a beaker containing the probe of a Beckman model 777 oxygen analyzer. Each tube was then inoculated and incubated. The figure shows the location of the band of bacteria at 2 hours in each of the tubes. The concentration of oxygen in medium saturated with air is taken as 2.1×10^{-4} mole per liter (shown by the arrow). Galactose was present at such a high concentration (5×10^{-3} mole/liter) that no second band appeared during the experiment.

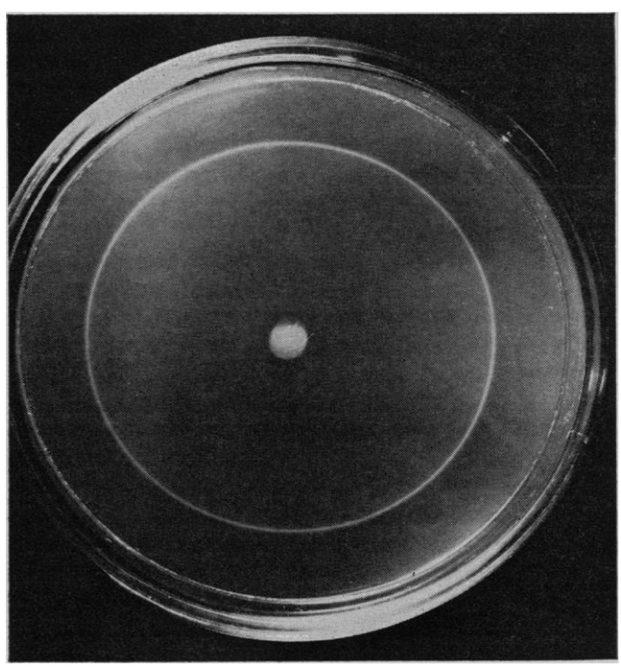


Fig. 11 (right). One ring of *Escherichia coli* in a galactose agar plate. About 10^8 motile cells were deposited at the center of a plate containing $1.8 \times 10^{-4}M$ galactose, 0.2 percent agar, and the medium described in *19*. The photograph was taken after 18 hours of incubation at 37°C.

ready noted that a band of *Pseudomonas viscosa* chemotactic toward oxygen would ascend more slowly if the peptone solution in which the bacteria were suspended was first treated with pure oxygen, and no band at all would form if the dissolved air was first driven off by boiling.

The bacteria migrate in the form of a ring (Fig. 11) when they are deposited on a 0.2-percent agar plate containing galactose plus the same medium that was used for filling the capillary tubes. The concentration of agar is so low that the bacteria can swim in this medium. The ring of bacteria originates from the deposit and then grows in diameter. This ring is not restricted to the surface of the agar, but rather it goes throughout the depth of the agar. Measurements of C^{14}-galactose have shown that the ring consumes all (> 99 percent) of the galactose as it travels along. Increasing the concentration of galactose brings about a decrease in the speed of the ring; these data look just like those presented in Fig. 9 for the band that consumes all the galactose.

The ring of bacteria depletes the oxygen by only about 20 percent, according to measurements with the oxygen electrode. Thus the supply of oxygen on the agar plate, unlike in a capillary tube, is never exhausted. This explains why only one ring forms on an agar plate, while in the same medium two bands form in a capillary tube.

Experiments with Glucose and Amino Acids

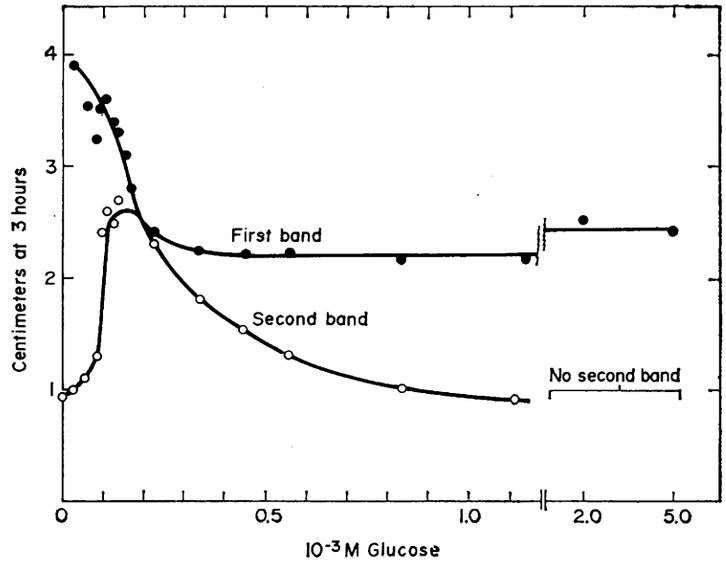
Entirely similar results are obtained when glucose rather than galactose serves as energy source. Varying the glucose concentration (Fig. 12) affects the two bands of bacteria similarly to varying the galactose concentration (Fig. 9). Measurements of oxygen and C^{14}-glucose have shown that at $3.0 \times 10^{-4}M$ glucose the first band consumes all the oxygen and part of the glucose, and the second band uses all the rest of the glucose anaerobically. Just as with galactose, increasing the concentration of oxygen slows the movement of the single band which forms at $5 \times 10^{-3}M$ glucose, and exclusion of oxygen prevents formation of the band.

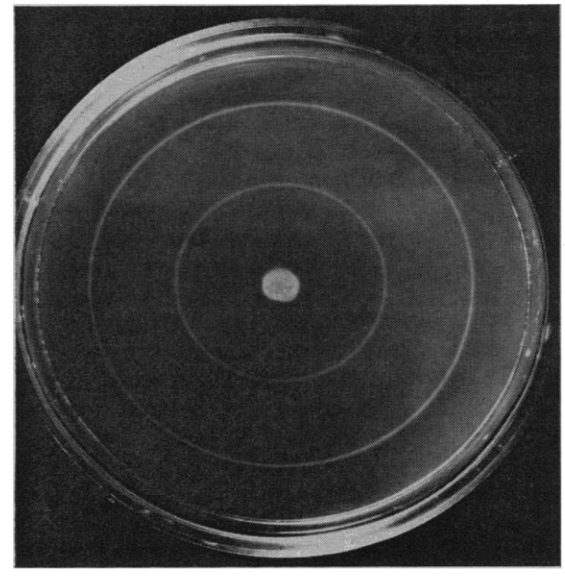
In an agar plate containing glucose as the energy source, one ring forms which consumes all (>99 percent) of the glucose as it travels along. When both glucose and galactose are present, two rings form in an agar plate (Fig. 13) and three bands appear in a capillary tube. Measurements of C^{14}-glucose and C^{14}-galactose (in separate experiments) have shown that the first ring consumes all the glucose and that the second ring consumes all the galactose. The area between the two rings contains 25 to 85 percent of the galactose, but no detectable glucose. The bacteria thus use glucose in preference to galactose.

When this strain of *E. coli* is grown aerobically on a mixture of the 20 amino acids commonly occurring in proteins, serine is found to be the only one of the amino acids that can be used both anaerobically and aerobically as an energy source, at least for motility (*7*). As expected, two bands of bacteria form on serine, and a study of these bands (*8*) gives results just like those presented for galactose and glucose.

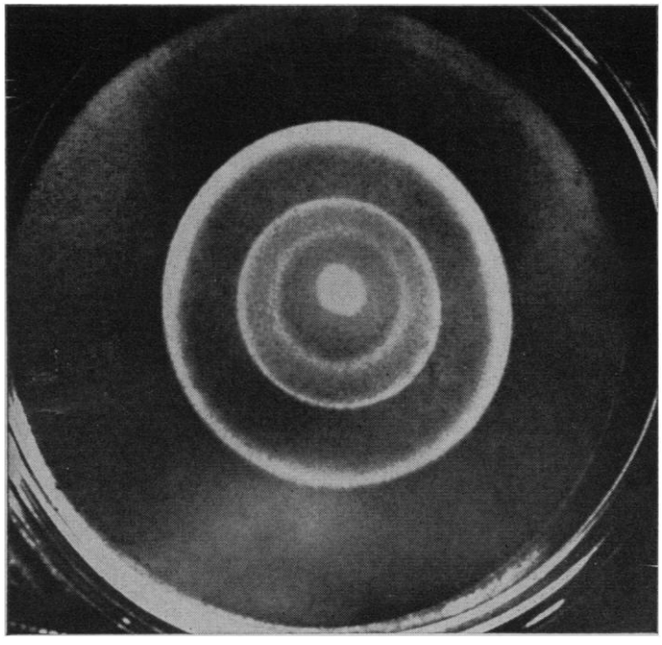
Proline, aspartic acid, alanine, threonine, tryptophan, glutamine, glutamic acid, glycine, and asparagine are oxidizable (in that decreasing order) but cannot be used anaerobically by these bacteria when they have been grown aerobically on a mixture of the 20 amino acids. Each of these oxidizable amino acids gives but a single band which consumes the oxygen as it travels along (*8*). This band moves three to eight times faster than a band which forms even without the addition of an energy source and which utilizes oxygen to oxidize an endogenous energy source. The remainder of the amino acids are not oxidized at an appreciable rate and give a band which moves at the same slow rate as the one formed when no energy source is added (*8*).

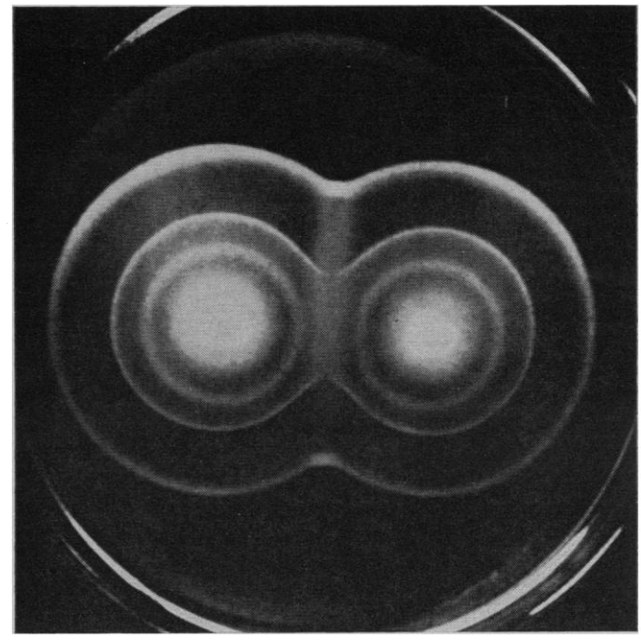
When a mixture of the 20 amino acids is used in the capillary tube, two bands of bacteria form. The first band consumes all the oxygen to oxidize a part of one or several of the amino acids, and the second band consumes





Figs. 12 and 13. Fig. 12 (left). The effect of varying the glucose concentration. A set of tubes, each containing a different concentration of glucose, was inoculated and incubated. The figure shows the location of bands at 3 hours in each of the tubes. Fig. 13 (right). Two rings of *Escherichia coli* in an agar plate containing glucose and galactose. Procedure was described in Fig. 11, except that both glucose ($1.8 \times 10^{-4}M$) and galactose ($5.4 \times 10^{-4}M$) were present. When glucose and galactose were each present at 1.8×10^{-4} mole per liter, two rings still formed, but they were then separated by only 1 or 2 mm; in that case, it was again the outer ring that consumed all the glucose.





Figs. 14 and 15. Fig. 14 (left). Three rings of *Escherichia coli* in a tryptone-agar plate after 5 hours of incubation at 37°C. About 10^6 motile cells were deposited at the center of a plate containing Difco tryptone (10 g/liter), sodium chloride (5 g/liter) and agar (2 g/liter). [Photograph by John L. Tschernitz] Fig. 15 (right). A suspension of motile *Escherichia coli* deposited at two places on the surface of a tryptone-agar plate swarms out in rings that stop when they meet. [Photograph by John L. Tschernitz]

all the residual serine anaerobically (see *8*).

Motile cells of *E. coli* deposited on a trytone, 0.2-percent agar plate swarm out in three (or sometimes more) rings (Fig. 14). Time-lapse movies, taken by J. L. Tschernitz, show that each ring originates from the deposit and remains intact as it moves over the plate. The amino acids of casein are the major constituents of tryptone. Amino acids labeled with carbon-14 were added one at a time to a tryptone agar plate, and remaining labeled amino acid was then determined after chromatography of samples taken from behind each ring.

The first ring consumes all (>99 percent) of the serine. It also uses 90 to 95 percent of the oxygen. The second ring, which is restricted to the top of the agar where oxygen is available, consumes 90 percent of the aspartic acid, an amino acid that can be used only aerobically. (Perhaps the remaining 10 percent is unavailable at the bottom of the agar plate, where conditions are anaerobic.) The third ring uses all (>99 percent) of the threonine. This ring occurs at the bottom of the agar; presumably a pathway that allows anaerobic use of threonine has been induced under these anaerobic conditions. The other five oxidizable amino acids were not used, or were used at most to the extent of 15 percent of the supply, by any of the three rings. (Asparagine and glutamine are heat-labile and therefore would not be present in this autoclaved medium.)

Just as these bacteria prefer glucose over galactose, so is there an order of preference among the amino acids. The mechanism by which this preference is expressed has not been studied here. The presence of one energy source somehow prevents the use of another, as has been found in the well-known diauxie phenomena.

When bacteria are deposited at two places on a tryptone-agar plate (Fig. 15), the corresponding rings from each deposit stop when they meet, presumably because the particular amino acid involved has been exhausted in the area already traversed by each ring.

Mechanism of Chemotaxis

In the studies reported here, the bacteria create a gradient of oxygen or of an energy source, and then they move preferentially in the direction of the higher concentration of the chemical.

How does a chemical influence the swimming of the bacteria to make them move toward the chemical, or away from it? By the end of the 19th century Engelmann, Pfeffer, Rothert, Jennings, and others had discovered the "avoiding reaction" (or "shock reaction") in bacteria (*1–3*). The avoiding reaction may be described as follows. Imagine a gradient between a low and a higher concentration of oxygen. A bacterium that happens to swim from the higher into the lower concentration suddenly stops or becomes apparently uncoordinated for an instant or jumps back, and then it goes off in a new, randomly chosen direction; in some species that can swim equally well forward or backward, the organism stops and then swims away in the reverse direction. If the new direction takes the bacterium further into the region of low oxygen concentration, the avoiding reaction is repeated, but if a higher concentration is encountered the bacterium continues to swim in the new direction. The net result is that the bacteria accumulate in the region of higher concentration. (Actually, very high concentrations are also avoided.) This taxis by avoidance of an unfavorable concentration is known as "phobotaxis" (*2, 3, 12*). More complex cells and organisms, for example certain sperm, algae, and animals, show another kind of taxis known as "topotaxis" (*2, 12*). In that case the organisms orient themselves in the direction of the source of stimulation and then move directly toward or away from the stimulus.

The very jerky motion of the bacteria in the bands studied here suggests that *E. coli* carries out the avoiding reaction

during chemotaxis. Other workers in recent years have also observed bacterial movements that resemble the avoiding reaction. Baracchini and Sherris (*6*) pointed out that their observations of *Pseudomonas viscosa* in a band chemotactic toward oxygen were in accord with earlier descriptions of avoiding reactions which cause the bacteria "to reverse the direction of their movement or to develop a complex and apparently uncoordinated series of movements." Lederberg (*11*) has reported "very rapid but jerky movements" in *Salmonella paratyphi* during chemotaxis away from phenol, and Clayton (*12*) has studied "reversals of swimming direction" in his extensive work on phototaxis and chemotaxis in *Rhodospirillum rubrum*.

Many questions remain unanswered. How is the stimulating chemical sensed? Links (*13*) has suggested that the decreasing concentration of the chemical is detected by means of a sudden decrease of the energy-supplying substance (probably adenosine triphosphate) in the motor apparatus. Another possibility is that there is a specific chemoreceptor for each kind of stimulating chemical. How are the sensed data translated into action? Is there a coordinating system that directs the flagella? Can bacteria "learn" to swim toward or away from a chemical?

One approach toward answering some of these questions is by the isolation and study of mutants that are defective in motility and chemotaxis. In the closely related genus *Salmonella*, mutants of the following types have already been described (*14*): nonflagellated bacteria; paralyzed bacteria, that is, bacteria with morphologically normal but nonfunctioning flagella; and bacteria with morphologically abnormal flagella, such as curly mutants. By picking from the center of a tryptone-agar plate such as the one shown in Fig. 14, we have now isolated all these types also in *E. coli*, and in addition we have found a novel type of mutant which is fully motile but fails to show any chemotaxis (*15*). These bacteria do not form bands or rings in any of the situations described in this article; they are generally nonchemotactic. Attempts are in process to isolate mutants which are specifically nonchemotactic, that is, bacteria which fail to show chemotaxis toward only one chemical or a small group of closely related chemicals.

Modern studies of biology have revealed a universality among living things. For example, all organisms have much in common when it comes to their metabolism and genetics. Is it not possible that all organisms also share common mechanisms for responding to stimuli by movement? Just as the higher organisms' machinery for metabolism and genetics appears to have evolved from processes already present in the lowest forms, so it is possible that the nervous system and behavior of higher organisms evolved from chemical reactions that can be found even in the most primitive living things. From this point of view one may hope that a knowledge of the mechanisms of motility and chemotaxis in bacteria might contribute to our understanding of neurobiology and psychology.

This is not a new idea. Binet expressed it in 1889 in his *Psychic Life of Micro-organisms* (*16*), Verworn in 1889 in *Psycho-physiologische Protisten-studien* (*17*), and Jennings in 1906 in *Behavior of the Lower Organisms* (*1*). In recent times Clayton has compared phototaxis in *R. rubrum* with excitability of muscle and nerve (*12*), and Delbrück, Dennison, Reichardt, Shropshire, and others have emphasized the importance of studies on the photoresponses of the fungus *Phycomyces* for an understanding of sensory physiology (*12a*).

Summary and Conclusion

Motile *Escherichia coli* placed at one end of a capillary tube containing an energy source and oxygen migrate out into the tube in one or two bands, which are clearly visible to the naked eye and can also be demonstrated by photography, microscopy, and densitometry and by assaying for bacteria throughout the tube. The formation of two bands is not due to heterogeneity among the bacteria, since the bacteria in each band, when reused, will form two more bands.

If an anaerobically utilizable energy source such as galactose is present in excess over the oxygen, the first band consumes all the oxygen and a part of the sugar and the second band uses the residual sugar anaerobically. On the other hand, if oxygen is present in excess over the sugar, the first band oxidizes all the sugar and leaves behind unused oxygen, and the second band uses up the residual oxygen to oxidize an endogenous energy source.

The essence of the matter is that the bacteria create a gradient of oxygen or of an energy source, and then they move preferentially in the direction of the higher concentration of the chemical. As a consequence, bands of bacteria (or rings of bacteria in the case of agar plates) form and move out. These results show that *E. coli* is chemotactic toward oxygen and energy sources such as galactose, glucose, aspartic acid, threonine, or serine. The full repertoire of chemotactic responses by *E. coli* is no doubt greater than this, and a more complete list remains to be compiled.

The studies reported here demonstrate that chemotaxis allows bacteria to find that environment which provides them with the greatest supply of energy. It is clearly an advantage for bacteria to be able to carry out chemotaxis, since by this means they can avoid unfavorable conditions and seek optimum surroundings.

Finally, it is necessary to acknowledge the pioneering work of Englemann, Pfeffer, and the other late-19th-century biologists who discovered chemotaxis in bacteria, and to point out that the studies reported here fully confirm the earlier reports of Beijerinck (*4*) and Sherris and his collaborators (*5, 6*) on a band of bacteria chemotactic toward oxygen. By using a chemically defined medium instead of a complex broth, it has been possible to study this band more closely and to demonstrate in addition the occurrence of a second band of bacteria chemotactic toward an energy source. Beijerinck (*4*) did, in fact, sometimes observe a second band, but he did not offer an explanation for it.

References and Notes

1. H. S. Jennings, *Behavior of the Lower Organisms* (1906; republished by Indiana Univ. Press, Bloomington, 1962); H. S. Jennings and J. H. Crosby, *Amer. J. Physiol.* **6**, 31 (1901).
2. G. S. Fraenkel and D. L. Gunn, *The Orientation of Animals* (Dover, New York, 1961), pp. 5–6, 270–291.
3. C. Weibull, *The Bacteria*, I. C. Gunsalus and R. Y. Stanier, Eds. (Academic Press, New York, 1960), vol. 1, p. 153.
4. M. W. Beyerinck, *Zentr. Bakteriol. Parasitenk.* **14**, 827 (1893).
5. J. C. Sherris, N. W. Preston, J. G. Shoesmith, *J. Gen. Microbiol.* **16**, 86 (1957).
6. O. Baracchini and J. C. Sherris, *J. Pathol. Bacteriol.* **77**, 565 (1959).
7. J. Adler and B. Templeton, *J. Gen. Microbiol.*, in press.
8. J. Adler, *J. Bacteriol.*, in press.
9. Joyce Chromoscan, Joyce-Loebl and Company, Gateshead, England. The instrument was fitted with a capillary-tube holder that has a slit just narrower than the capillary tube.
10. No. 17365 electrode, Instrumentation Laboratory Inc., Boston, Mass.

11. J. Lederberg, *Genetics* **41**, 845 (1956).
12. R. K. Clayton, *Arch. Mikrobiol.* **19**, 141 (1953); in *Encyclopedia of Plant Physiology*, W. Ruhland, Ed. (Springer, Berlin, 1959), vol. 17/1, p. 371; in *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1964), vol. 2, p. 51.
12a. W. Shropshire, Jr., *Physiol. Rev.* **43**, 38 (1963).
13. J. Links, thesis (1955), Leiden (in Dutch; summary in English).
14. T. Iino and J. Lederberg, *Monographiae Biol.* **13**, 111 (1964); T. M. Joys and B. A. D. Stocker, *J. Gen. Microbiol.* **41**, 47 (1965).
15. J. B. Armstrong, J. Adler, M. M. Dahl, manuscript in preparation.
16. A. Binet, *The Psychic Life of Micro-organisms* (Open Court, Chicago, 1889). Translated from the French.
17. M. Verworn, *Psycho-physiologische Protistenstudien* (Fischer, Jena, 1889).
18. The capillary tubes (10 cm long; internal diameter, about 1 mm) were Owens-Illinois Kimax No. 34502 melting point tubes; the somewhat wider No. 34500 tubes were used when oxygen was measured. The use of capillary tubes for studying chemotactic bands of bacteria was introduced by Sherris and collaborators (*5*, *6*).
19. The medium for filling the capillary tubes contained $1 \times 10^{-2}M$ potassium phosphate at *p*H 7.0, $1 \times 10^{-3}M$ $MgSO_4$, $1 \times 10^{-3}M$ $(NH_4)_2SO_4$, $1 \times 10^{-4}M$ ethylene-diamine-tetraacetic acid, and $3 \times 10^{-5}M$ leucine, methionine, and threonine. Galactose (Sigma "essentially glucose-free" grade) or another energy source was added at concentrations specified in the text. One milliliter of the medium was shaken in a test tube at 37°C for one half hour just before use, in order to standardize the amount of dissolved oxygen. The addition of phosphate is necessary for buffering, since the motility is *p*H-dependent (*7*). Concentrations of phosphate greater than 1×10^{-2} mole per liter, however, lower the rate of travel of the bands. The $MgSO_4$ and $(NH_4)_2SO_4$ are not required, but they stimulate the rate of travel of the bands 25 to 50 percent. Since the motility is very sensitive to inhibition by heavy-metal ions (*7*), ethylene-diamine-tetraacetic acid was always included and only water distilled in glass was used. When leucine, methionine, and threonine (which are required for growth) are omitted, bands do not form readily, even though the bacteria are still highly motile. Actually, only methionine must be added to get the bands to form and travel; leucine and threonine stimulate the rate of travel of the bands about 50 perecnt. Prototrophic strains form the two bands on galactose or glucose very well without the addition of any amino acid.
The effect of the inoculum size was studied. When fewer bacteria were put into the capillary tube, it took longer for the bands to appear. For example, at 1/10 the usual inoculum size, the first band had a lag period of ¾ hour and the second band 1¾ hours. Apparently the migration does not begin until the bacterial population has grown to a certain density. Once the bands have formed, they move at about the same speed, no matter what the initial concentration of bacteria. Temperatures between 23° and 37°C were optimal for the migration of the bands. There were no bands at 0° or at 48°C, where motility is inhibited (*7*).
20. The *E. coli* used is a strain of K12 which is here called B275. It was prepared from A. Garen's F3-W1-6, a derivative of the Lederberg strain W1. For growth, it has an absolute requirement for leucine and methionine and a partial requirement for threonine. For experiments in which amino acids were the energy source, the bacteria were grown in a medium which contained minerals and each of the 20 amino acids (0.25 g/liter) commonly occurring in proteins. A full description of this medium and details of growing and washing the bacteria will be presented elsewhere (*8*). For experiments in which galactose or glucose was the energy source, the 20 amino acids in the growth medium were replaced by galactose (5 g/liter) plus the three essential amino acids (0.02 g of each per liter). Glucose was not used in the growth medium because in this strain of *E. coli* it inhibits formation of flagella (*7*).
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A preliminary account, of some of this work appeared in *Cold Spring Harbor Symp. Quant. Biol.* **30**, 289 (1965).

Ethics for an International Health Profession

World health problems give rise to questions that are not answered by the physician's traditional code.

Carl E. Taylor

Increasing numbers of physicians are being attracted to service in international health. Many international assignments are carried out by short-term experts on leave from regular positions in their own countries, but a career corps is needed to maintain the administrative framework of international agencies, to ensure continuity of activities, and to provide imaginative exploration of the many new dimensions of this expanding field.

Any professional group tends to develop an ethic or binding matrix of values, which is often more important than knowledge or skills in setting that group apart from other groups (*1*). The general medical profession has with considerable pride made its ethic explicit. No other group has such a long and consistent tradition of trying to maintain an idealistic view of its function and role; its ethical principles were long ago embodied in the Oath of Hippocrates, the Oath of Maimonides, and in India, the oath prescribed by Susruta from traditional rituals which originated well before the 1st century A.D. The total milieu of medical education specifically provides for the inculcation of these distinctive values.

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To a remarkable degree the professional subculture of medicine cuts across national and other cultural boundaries. But new problems in international health pose questions—most conspicuously, but by no means solely, the question of the effect of disease prevention on population growth—which are not encompassed by an ethic based on the relation of individual physician to individual patient. Thus the ethic of the specialty remains implicit and undefined. The basic values are vaguely perceived and, at best, are acquired by imitation in loosely organized preceptorship assignments with "old hands" who have made many of the obvious mistakes but have not necessarily learned the needed answers. Urgently needed is a clearer definition of the ethical values which will shape professional decisions in the troubled days ahead.

Ethic of the Physician

Respect for the sanctity of human life is the underlying value of the medical ethic. The seriousness of the "life and death" responsibility has led society to attach a special aura to the physician. The patient turns over to him his pains and his fears. Even when, in fact, he can do little to help organic disease, the physician brings assurance by "laying on hands" (*2*).