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Technical Papers

Description of the Chemostat

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We have developed a device for keeping a bacterial population growing at a reduced rate over an indefinite period of time. In this device, which we shall refer to as the Chemostat, we have a vessel (which we shall call the growth tube) containing V ml of a suspension of bacteria. A steady stream of nutrient flows from a storage tank at the rate of w ml/sec into the tube. The contents of the tube are stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the tube at all times. An overflow sets the level of the liquid in the growth tube, and through that overflow the bacterial suspension leaves the tube at the same rate at which fresh nutrient enters it.

The chemical composition of the nutrient is such that it contains a high concentration of all growth factors required by the bacterium, with the exception of one, the controlling growth factor, the concentration of which is kept relatively low. The concentration of the controlling growth factor, a, in the storage tank will then determine the density, n, of the bacterial population in the growth tube in the stationary state, and it can be shown that, except for very low values of n, we have $n = \frac{a}{A}$, where A is the amount of the controlling growth factor needed for the production of one bacterium.

The growth rate $\alpha = \frac{1}{n}\frac{dn}{dt}$ of a strain of bacteria is a function of the concentration, c, of the controlling growth factor in the medium, and in general we may expect the growth rate, at low concentrations c, first to increase rapidly with increasing concentration and then slowly to approach its highest attainable value, α_{max}.

The Chemostat must be so operated that the washing-out time, $\frac{w}{V}$, should be lower than the growth rate α_{max} for high concentrations of the controlling growth factor. It can be shown that in that case a stationary state will become established in which the growth rate, α, will be just equal to the washing-out rate, $\frac{w}{V}$.

What happens is that n will increase until it becomes so large that the bacteria will take up the controlling growth factor from the tube just as fast as it is necessary in order to reduce c to the point where the growth rate $\alpha(c)$ becomes equal to the washing-out rate, $\frac{w}{V}$.

Using a tryptophane-requiring strain of coli and a simple lactate medium with tryptophane added, we have used both lactate and tryptophane as the controlling growth factor. Using tryptophane, we have kept bacterial populations growing over long periods of time at rates up to ten times lower than normal. We are thus able to force protein synthesis to proceed very slowly while certain other biochemical processes may continue at an undiminished rate.

A study of this slow-growth phase by means of the Chemostat promises to yield information of some value on metabolism, regulatory processes, adaptations, and mutations of microorganisms. A study of the spontaneous mutations of bacteria growing in the Chemostat has been made and is being published elsewhere.

Because for most investigations a number of such Chemostats will be needed, we attempted to perfect a simple yet adequate design. Of various possible designs, we eliminated those in which changes in the barometric pressure affect the rate of flow of the nutrient from the storage tank into the growth tube. We also discarded designs that permit growth of the bacteria on the inner walls of the growth tube, or permit growth of bacteria in the Chemostat anywhere except homogeneously dispersed in the liquid nutrient in the tube. After trying out several designs, we found the one shown in Fig. 1 satisfactory.

CHEMOSTAT

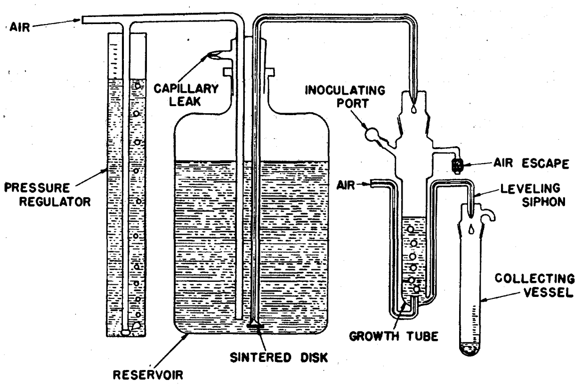

FIG. 1

A tube leading to the bottom of the storage tank is connected to a small air compressor (for example, an air pump such as is used for aerating aquaria). When the compressor is first started, the air rises rapidly in bubbles through the nutrient liquid in the storage tank and accumulates in the space above the liquid level until the pressure in the nutrient at the bottom of the tank becomes equal to the air pressure in the tube. The air space in the storage tank above the liquid level communicates through a narrow capillary with the outside air, and therefore the air will continue indefinitely to bubble through the nutrient liquid in the storage tank, but at a very slow rate (of perhaps one bubble per minute).

The pressure of the air entering the tube is regulated by a simple pressure regulator consisting of an air outlet located at the bottom of a glass cylinder filled with water up to a certain level. Above this level, the air communicates freely with the outside air. By changing the water level in the pressure regulator, the air pressure can be adjusted to any value required for the operation of the Chemostat.

In this arrangement, the pressure at the bottom of the storage tank will always be greater than the pressure of the outside air by the height of the water column in the pressure regulator, and hence will be independent of the height of the level of the nutrient liquid. This is important because the level of the nutrient will gradually fall during the operation of the Chemostat.

From the storage tank the nutrient liquid is forced through a sintered glass filter into the growth tube, where it is mixed drop by drop with the bacterial suspension. The content of the growth tube is continuously stirred by aeration.

The level of the liquid in the tube is set by a siphon, and the volume of the bacterial suspension is thus maintained constant. The nutrient liquid and the bacteria suspended in it leave the tube through the syphon at the same rate at which fresh nutrient enters. The air space above the nutrient liquid in the growth tube communicates with the outside air, hence the pressure which forces the nutrient liquid through the sintered disk is at all times equal to the height of the water column in the pressure regulator.

If, after the Chemostat has been in operation for some time, the barometric pressure falls very suddenly, the pressure of the air entering into the storage tank also falls suddenly, and the nutrient liquid will rise in the air pressure tube to a certain height. If this happens, the pressure at the bottom of the storage tank will no longer exceed the outside pressure by the height of the water column in the regulator, but rather by a greater amount, and the flow of the nutrient liquid into the growth tube increases. Because of the capillary communication between the air space above the nutrient liquid and the outside air, this condition will be quickly corrected. As air flows out of the storage tank through the capillary outlet, the pressure diminishes, and the liquid which had risen into the air pressure tube in the tank is pushed out. Thus, within a short period of time, the pressure at the bottom of the storage tank is restored to its former value.

In this manner the Chemostat keeps the rate of flow of the nutrient liquid into the growth tube constant, independent of changes in barometric pressure and in the liquid level in the tank. The flow rate can be changed as desired by changing the water level in the pressure regulator.

Sickling: A Property of All Red Blood Cells

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Spontaneous sickling of red blood cells has been considered a property of the blood of certain individuals in Negro families, and various theories have been proposed to account for the phenomenon. New light may be thrown on the problem by observing the effect of thick gelatin "solutions" (e.g., Le Page's glue) on red blood cells. When a drop of blood of a *normal* individual is stirred with a drop of Le Page's glue (fishskin gelatin)[1] on a glass slide, the red blood cells immediately assume the sickle shapes (*1*) (see Fig. 1). The same phenomenon is noted with the blood of patients with sickle cell anemia, individuals with the sickle cell trait, cats, dogs, chickens,

[1] Specimens of Le Page's glue, as well as the purest form (Le Page's Photoengraving Glue) were furnished by N. C. Phillips, of Le Page's, Inc.