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.

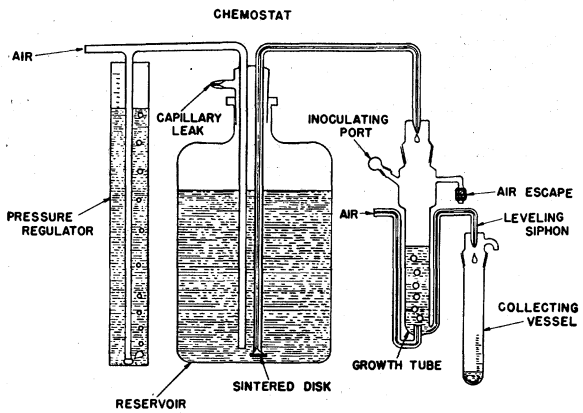

FIG. 1

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.

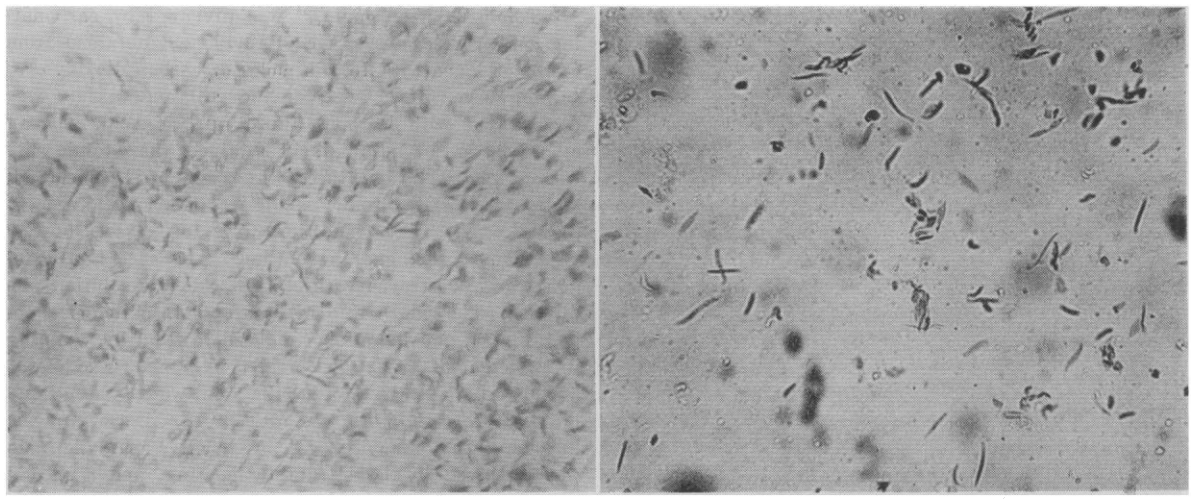

FIG. 1. Sickling of red blood cells of a normal white individual, on mixing with glue. (Microphotograph courtesy of George J. Anday.)

frogs, patients with spherocytosis, and human nucleated red blood cells. In blood with marked anisocytosis large and small sickled forms develop (Fig. 2). All the bizarre forms noted in sickle cell anemia are simulated (Fig. 3). A drop of Le Page's glue is placed on a glass slide, and a drop of blood (fresh, citrated, oxalated, or heparinized) is placed beside it. The two are then stirred together with a rod. Immediately, on examination, practically all the red blood cells will be found to have assumed the "sickle" shapes. Gelatin "solutions" of the same viscosity may be used.[2] These may be made by adding the smallest amount of hot water necessary to liquefy gelatin powder that contains urea as a liquefying reagent. If the gelatin is not viscous enough, the cells "sickle" for a few seconds and then become rounded.

In most preparations practically 100% of the red blood cells assume the sickle form. A permanent preparation may be made by rapidly drying thin films of the glue mixture, or by mixing the sickled cell suspension with formalin or with 1% osmic acid solution. If the freshly sickled cells are examined under a cover glass, it is seen that the cells eventually become spherical, fragment, and hemolyse. If a diluting agent (e.g., saline solution, plasma, albumin solution) is added to the glue, the cells do not sickle, and the phenomenon is not noted with mucilage or dilute gelatin "solutions." The sickling does not disappear if oxygen is bubbled through the suspension of sickled cells. If a diluting (saline) solution is added to the sickled cells, they become spherical or irregularly spheroid. The addition of concentrated glue to this mixture results in some of the cells resuming the sickle form.

The mechanism of the reaction is not clear, but two factors are evident at present: First, there is the mechanical factor of the cells being mixed with a very viscous solution and, second, the nature of the colloidal suspension.

[2] Mammalian gelatin for liquefication with urea was furnished through the kindness of Roy C. Newton, of Swift & Co.

The sickling with concentrated glue and gelatin solutions is similar to the reaction that is noted at times on the edge of a blood film of normal individuals. Frankly sickled forms, or red blood cells that are blunt at one end and elongated and narrow at the other end (the end pointing to the outside of the film), are evidently produced when the plasma dries slowly enough to allow for the concentration to affect the cells before they dry. The sickle forms that appear on the edge of the blood films of certain individuals who do not show the sickle cell trait are fairly constant for the individual, and their appearance varies with the humidity, with the rate at which the blood film dries, and possibly with other factors.

It was noted with mammalian gelatin "solutions" that some concentrations, more dilute than those that produce sickling, may cause all the red blood cells to assume

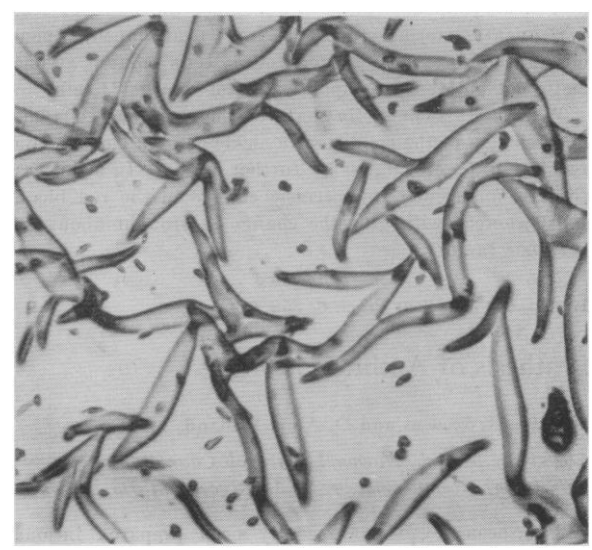

FIG. 2. Variation in size of "sickled" red cells produced in blood of a white man, with marked anisocytosis. (Osmic acid fixation.)

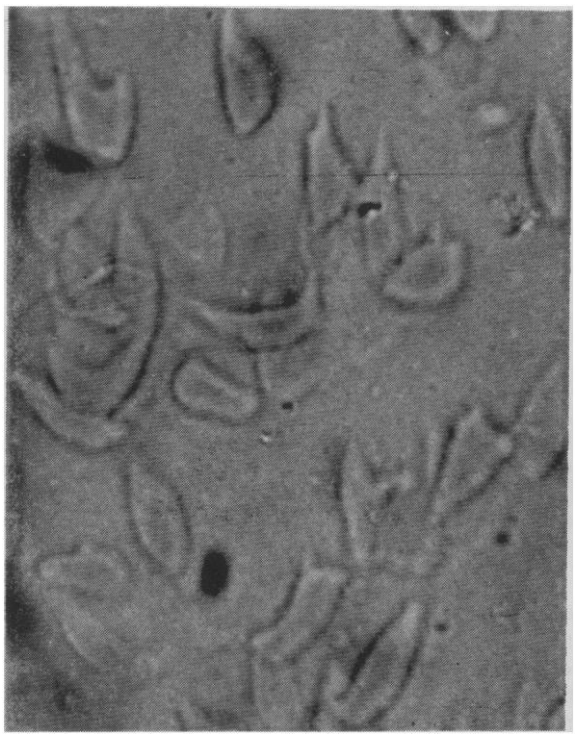

FIG. 3. Sickle and distorted forms of red blood cells produced with normal blood and glue solutions. (Microphotograph courtesy of George J. Anday.)

"pencil" shapes (elongated, with parallel sides, the length five or more times the width). Cells of this shape appear in a fractional percentage in blood of individuals who suffer from chronic hemorrhage.

This observation adds another form that normal red blood cells may characteristically assume under artificial conditions: spherical (including semispherical), crenated, and sickle-shaped. These differ from the more or less permanent abnormal forms, such as oval-shaped, "pencil" forms, grossly distorted forms, cells pointed at one end or at both ends (amphioxic), and "target" cells.

Whether this change in shape of the red blood cells has any relation to the condition in sickle cell trait or anemia remains to be elucidated by further study. In sicklemia there is apparently an intrinsic change in the red blood cells, whereas with glue the change is brought about by extrinsic factors.

Reference

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Synthesis of Anthracene-9-C_1^{14}

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Anthracene-9-C_1^{14} has recently been prepared in our laboratory for further experimental use as self-exciting crystal-counter material. Because the synthesis may be of interest to others, we wish to report the method used.

o-Toluic acid-carbonyl-C_1^{14} was prepared by carbonating a twofold excess of Grignard reagent obtained from *o*-bromotoluene with 10 millimoles of carbon dioxide containing 2 mc of C^{14}. After the reaction mixture was decomposed with ice and dilute sulfuric acid, and an ether solution of *o*-toluic acid obtained, the *o*-toluic acid was extracted into a threefold excess of 1 *N* sodium hydroxide. The *o*-toluic acid was then oxidized to phthalic acid by the addition of a 10% excess of 5% potassium permanganate solution. The excess permanganate was destroyed with ethanol, and the solution was filtered. The colorless filtrate was put in a small evaporating dish, and its volume reduced to 5 ml by warming in a current of air. Concentrated hydrochloric acid was added to precipitate the phthalic acid, which was filtered off. In order to obtain a more complete recovery of the labeled phthalic acid, ½ g of inactive phthalic acid was dissolved in the filtrate by heating, and a second quantity of phthalic acid was obtained on cooling. The combined acids were recrystallized from water and dried at 120° C. Yield was 1.9 g.

To convert the phthalic acid into anhydride, it was refluxed with twice its weight of thionyl chloride for 1 hr, after which 3 successive portions of benzene were distilled from the anhydride to free it from excess thionyl chloride. Benzoyl-benzoic acid was prepared from the anhydride by a Friedel-Crafts reaction using 10 ml of benzene and 3.8 g of aluminum chloride. The crude acid obtained was dissolved in dilute ammonium hydroxide, a small amount of diatomaceous earth was added, and the solution was filtered. Acidification with hydrochloric acid precipitated the benzoyl-benzoic acid as an oil, which crystallized on standing.

Anthraquinone was obtained from the benzoyl-benzoic acid according to the method of Dougherty and Gleason (*1*). The benzoyl-benzoic acid obtained was dissolved in 30 ml of 96% sulfuric acid and heated in an oil bath at 120° C for 1 hr. The reaction mixture was then poured into excess cold water, and the slurry digested on a hot plate. The anthraquinone was filtered, washed with warm dilute ammonium hydroxide, and dried at 120° C. Yield of anthraquinone was 2.1 g.

The anthraquinone was reduced to anthracene by a two-step reduction. Anthrone was prepared by the method of Meyer (*2*), using 50 ml of glacial acetic acid and 5 g of mossy tin. After being refluxed for 1½ hr, the solution was diluted with 25 ml of water, filtered hot, and allowed to stand overnight in a refrigerator. The anthrone, filtered and washed with water, was reduced to anthracene with copper-activated zinc and sodium hydroxide (*3*). To 10 g of zinc dust in a 200-ml, round-bottom flask, was added 10 ml of copper sulphate solution containing 0.04 g of $CuSO_4 \cdot 5H_2O$. After a few minutes, the solution was decanted, and the activated zinc was washed once with water by decantation. The anthrone from the preceding preparation, 80 ml of 2 *N* sodium hydroxide, and 20 ml of toluene were added. This mixture was refluxed for 24 hr. After cooling slightly, 20 ml of warm benzene was added, the liquid layers were decanted into a separating funnel, and the zinc was washed with another 20 ml of hot benzene.