of administration above 0.5 mg per minute concentration in the blood rises steeply because the rate of removal does not correspondingly increase. This question needs further study.

A comparison of the results of Table I and Table II explains why injudiciously fast intravenous administration of atabrine may readily lead to toxic effects upon the heart muscle in the isolated heart, not encountered when even large amounts are given by slow intravenous administration, and entirely unlikely in the intact animal or man when appropriate therapeutic doses are given orally.

Summary. In the isolated heart of the dog in the form of the heart-lung preparation atabrine has a negative inotropic action which appears in some cases at concentrations of 1 mg per liter of blood and is always observed at concentrations of 1.5 mg per liter or above. As a result, the work capacity of the heart is impaired. Slowing of the heart rate sometimes occurs. Irregularities of cardiac rhythm are seen only with high and acutely toxic doses.

Injection of 10-15 mg of atabrine within 1 minute into a total blood volume of 500 to 900 cc leading abruptly to blood levels of several milligrams per liter of blood causes a severe heart failure, while the continuous infusion of the same amount at a rate of 0.5 mg per minute will barely reach the toxic concentration in the blood.

A severe myocardial failure of the isolated heart, once established, does not appear to be reversible. Epinephrine hydrochloride has a transient positive inotropic and chronotropic action, while the cardiac glycosides effect a prompt and long-lasting improvement of the work capacity of the failing heart in doses which do not change the heart rate and do not lead to irregularities of heart rhythm.

15414

Control of pH in Roller Tube Cultures.

GEORGE H. PAFF.

From the Department of Anatomy, Long Island College of Medicine, Brooklyn, N. Y.

The purpose of these experiments was to determine in roller tube cultures the pH range of media which would support growth of fibroblasts. The study was undertaken as preliminary to the cultivation of bone *in vitro* under standardized conditions. It was prompted by the fact that the optimal reaction for the activity of alkaline bone phosphatase lies between pH 8 and pH 10.[1] Such values are distinctly beyond the range of pH considered desirable for tissue *in vitro*. In the case of chick tissue the accepted pH range is 7.2 to 7.8.

Various methods of controlling the pH range suggested themselves. The obvious was either to choose buffers operative in this alkaline range (8 to 10) or to use the ordinary solutions, such as Tyrodes, and control the amount of CO_2 in equilibration with the media. We chose the latter approach and devised an apparatus for continuous introduction of the gas.

Technic. The apparatus used to introduce gases into the roller tubes has been described.[2] The following gases were introduced into the tubes: air without CO_2; air; air with 1%, 2%, 4%, 8%, 16% and 32% CO_2. Air with no CO_2 was made by first drawing approximately 18 liters of air into a 5-gallon bottle and introducing a 10% NaOH wash bottle in the pipe line to the roller tubes. Air alone required no special treatment. All of the CO_2 mixtures were made with the aid of a Kipp generator. The gas displaced the proper amount of water and by siphoning

[1] Williams, H. L., *U. Western Ont. Med. J.*, 1940.

[2] Paff, G. H., and Samuelsen, G., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**,

TABLE I.
Hydrogen Ion Concentration of the Fluid Media at the Beginning and End of Air Flow Experiments.

Experiment	Number of cell colonies	pH of media at start	pH of media at end
CO_2 free air	40	7.57	9.17
Controls	40	7.57	7.63
Air alone	120	7.64	8.36
Controls	120	7.64	7.65
1% CO_2 in air	40	7.55	7.60
Controls	40	7.55	7.80
2% CO_2 in air	40	7.60	7.38
Controls	40	7.60	7.70
4% CO_2 in air	40	7.58	7.32
Controls	40	7.58	7.75
8% CO_2 in air	40	7.60	6.9
Controls	40	7.60	7.74
16% CO_2 in air	40	7.55	6.75
Controls	40	7.55	7.60
32% CO_2 in air	40	7.50	6.55
Controls	40	7.50	7.62

of additional water, the necessary amount of air was drawn into the bottle. Since CO_2 is highly soluble in H_2O the precaution was taken of adding 2 to 3 cc of concentrated H_2SO_4 to the water in contact with the gas.

All experiments were conducted over a 2-day period and the amount of gas delivered to each tube was 2 liters per day. For tests with each gas at least 4 roller tubes each containing 10 cultures were prepared. Controls were run simultaneously in the form of 4 completely sealed tubes each containing 10 cultures. All tests employed fibroblasts derived from the tips of ventricles from the hearts of 8-9-day chick embryos. A ventricle was excised and ½ to 1 mm cubes were cut. Care was taken to apportion fragments impartially between experimental and control tubes. After the fragments were set in the plasma lining the tube and clotting had occurred, each tube received 3 cc of 25% chick embryo extract of known pH.

At the end of 48 hours mineral oil was layered on the surface of the liquid media in the experimental tubes. This was done as the gas flowed through the tube. The fluid medium was then removed and a pH determination was made under oil using a Beckman pH meter. After determining the pH of both the experimental and control culture media the cultures were fixed with 10% formaldehyde. The amount of growth was determined by projection outline drawings of the cultures at a magnification of 55 ×. Both the original culture and the peripheral limits of growth (and migration) were outlined. The area of growth was computed by the paper weight technic. Controls were considered as showing 100% growth. The growth of the experimental cultures was figured in % relationship to the controls. Exclusive of many preliminary experiments our data is based on 800 colonies of fibroblasts.

Observations. Table I gives a list of the pH values for the liquid media at the start and after 48 hours for each experiment. It will be noticed that the pH of all media at the start was practically the same for all experiments. It ranged from 7.5 to 7.64. After 48 hours of cultivation the expected spread in pH became evident. It ranged from the noticeably acid 6.55 of the 32% CO_2 in air to the distinctly alkaline pH of 9.17 for CO_2 free air.

In Fig. 1 the amount of growth of each of the "air flow" tubes is represented. It

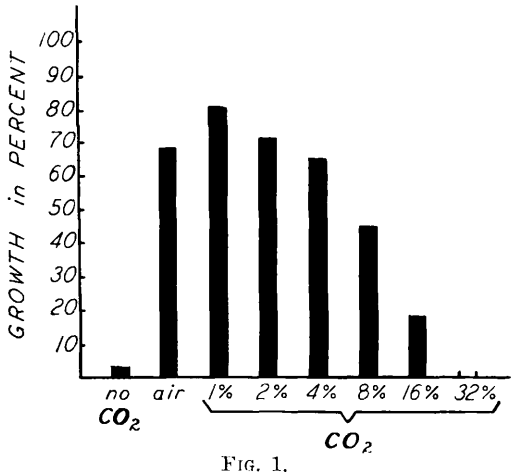

FIG. 1.

Graphic representation of extent of growth in air flow tubes. For explanation see text.

can be seen that in flow tubes the greatest amount of growth occurred with air, and 1%, 2% and 4% CO_2 in air. Only 3.4% growth was obtained with CO_2 free air. No growth occurred in 32% CO_2 in air.

The only obvious morphological difference observed among the cultures of different ionic concentration was that toward the acid side the cells showed more vacuoles than they did on the basic side. Fischer[3] made the same observation and Bauer[4] observed additional effects in drop cultures treated with high CO_2 tensions.

Discussion. These results indicate the range of pH possible in roller tube cultures by the simple procedure of changing the CO_2 content of the atmosphere. The technic has proven to be much simpler than making a series of solutions of varying pH. It is hoped that this method may lead to more studies of various tissues cultivated at different values of pH. Based solely on fibroblast studies the idea has become fixed that in tissue culture studies the media should have a pH in a rather fixed range. For chick fibroblasts obtained from certain sources this range (7.2 to 7.8) is optimal. One might well wonder if the same range of pH is optimal for gastric epithelium, or the deep lying cells in hyaline cartilage, or for renal epithelium. The blood pH is fixed within narrow limits but local economy in many parts of the body must operate under hydrogen ion concentrations well removed from that of the blood.

As to the results themselves, it will be noticed in Fig. 1 that growth is expressed in %. The amount of growth in the controls run simultaneously with experimental cultures was considered to be 100%. None of the values in the "air flow" tubes reached this value. The nearest approach to the large area of growth of control cultures was in the 1% CO_2 in air cultures. Here the growth was 81%. To go further, in our air flow experiments the optimal pH range based on extent of growth lay between 7.32 (pH of 4% CO_2 in air cultures) and 8.36 (pH of air alone cultures). This range is at variance with the findings of Fischer,[4] with solid media. On the basis of careful work Fischer found that optimal growth occurred between pH 7.0 and 7.8. We obtained very good growth in flow tubes in which the atmosphere was untreated air. This was not expected because of the relatively high alkalinity. The average of 12 pH determinations (all of which gave values above pH 8.0) was pH 8.36. There is no doubt that growth was good. The experiment with untreated air was repeated with comparable results. The average growth of 120 cell colonies was 69% of the control values. The control cultures themselves grew luxuriantly.

In drop cultures Lewis and Felton[5] obtained abundant growth in media having a hydrogen ion concentration from pH 6.0 to pH 9.0. These limits are broader than those obtained in roller tubes but the difference may be more apparent than real. We worked with CO_2 as our controlling instrument whereas Lewis and Felton (Fischer also) controlled their pH with media of variable composition. It is not easy to prevent drifts in pH determinations of solutions whose CO_2 tensions differ markedly from the atmosphere above them. If drifts occurred they were toward the pH value of the media existing

[3] Fischer, A., *Tissue Culture*, Levin and Munksgaard, Copenhagen, 1925, 71.

[4] Bauer, J. T., *Bull. J. Hopkins Hosp.*, 1925, **37**, 420.

[5] Lewis, M. R., and Felton, L. D., *Science, N. S.*, 1921, **54**, 636.

before CO_2 was added. We made our pH determinations under oil but our lower limit of 6.55 could well have been 6.3 or even lower. At the other extreme our 8.36 value could have been higher. Indeed, among the 12 determinations of which 8.36 is the average, there were readings of 8.5 and 8.6.

The pH of the control media became slightly more alkaline by the end of 48 hours (Table I). One might expect tissue metabolites to cause a shift toward the acid side. In roller tubes with 3 cc of a buffered fluid medium above small fragments of tissue growing in a thin lining of blood plasma, the acidifying effect of metabolites is negligible. The alkaline shift in the control media may have been due to the presence of alkali in the glass.

Summary and Conclusions. Employing a flowing atmosphere in roller tube cultures the range of hydrogen ion concentration permitting growth of fibroblasts was ascertained. The atmosphere was varied to produce the pH desired. The gases with the resulting pH of the media were as follows: air with no CO_2, pH 9.17; untreated air, pH 8.36; air with 1% CO_2, pH 7.6; with 2% CO_2, 7.38; with 4% CO_2, 7.32; with 8% CO_2, 6.9; with 16% CO_2, 6.75; and with 32% CO_2, pH 6.55. The best growth was obtained with untreated air and with air containing 1%, 2%, and 4% CO_2.

15415

An Apparatus for Permitting Control of the Atmosphere in Roller Tube Cultures.

GEORGE H. PAFF AND GEORGE S. SAMUELSEN.

From the Departments of Anatomy and Biochemistry, Long Island College of Medicine, Brooklyn, N. Y.

Various types of containers have been devised to permit control of the atmosphere overlying tissue cultivated in hanging drops[1] and in stationary flasks[2,3] *in vitro*. None of these are easily adaptable to roller tube cultures because of the constant rotation of the drum in which the tubes are placed. Ott, Tennant, and Liebow[4] suggested that a controlled atmosphere can be provided for roller tube cultures if a Novy jar is mounted on the drum. The difficulties and limitations of such a procedure are obvious.

Recently we have found it necessary to devise an efficient apparatus which would permit complete control of a flowing atmosphere at all times and eliminate both the handling of the tubes and the opening of the incubator. This apparatus has given us such satisfactory results that it seems worthy of description.

In order to insure a constant flow of air (or any desired gas) through the roller tubes, the gas is first made to displace water from a reservoir, such as a 5-gallon bottle. After all of the water is displaced, a fine steady stream of water is permitted to flow into the gas-filled bottle to displace the gas. The gas is then led into the incubator where it passes through the apparatus shown in Fig. 1. The gas enters tube "A" and passes successively through tubes "B," "C," "D" and "E" and then to the roller tubes "F." Tube "A" is fixed, tube "B" is movable and fits over tube "A": the mercury seal prevents the leakage of gas. "C" is a rubber tube which allows the movement of the horizontal shaft "D" to be transmitted to the vertical tube "B." Tube "D" is a part of the drum axle which is hollowed out to permit the gas to

[1] Bauer, J. T., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 420.

[2] Parker, R. T., *Medical Physics*, Year Book Publishers, Inc., 1944, 1578.

[3] Carrel, A., *Compt. rend. Soc. biol.*, 1934, **117**, 1144.

[4] Ott, L. H., Tennant, R., and Liebow, A. A., *Science*, 1940, **91**, 437.