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.

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

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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

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[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.

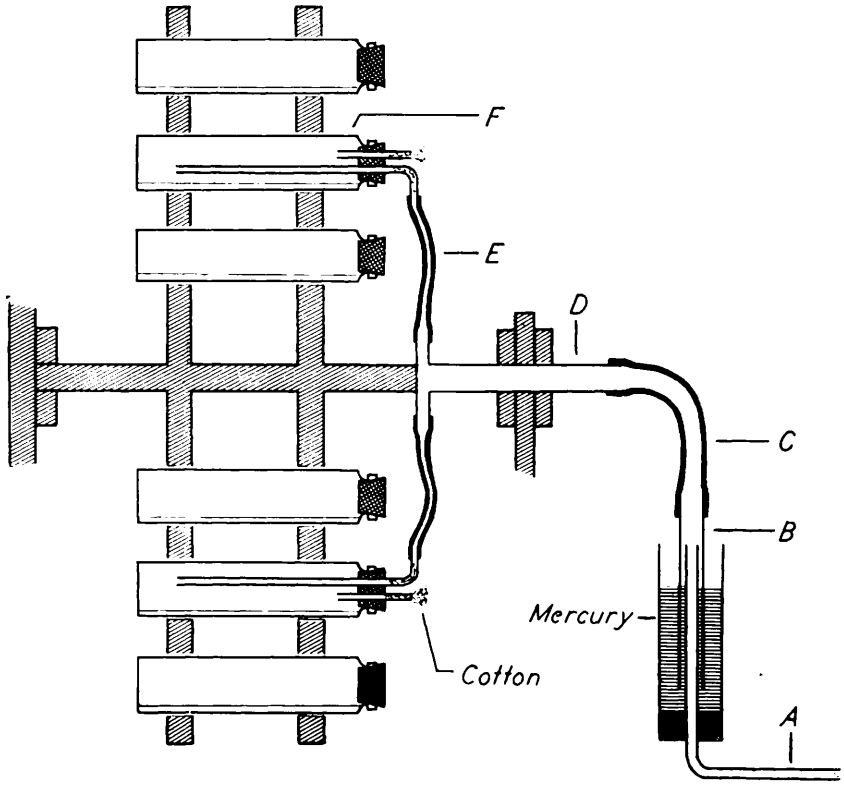

FIG. 1.
Sectional view of apparatus permitting control of the atmosphere in roller tube cultures. No attempt was made to diagram the driving mechanism of the drum. For description of remainder of apparatus see text.

flow through tubes "E" to the roller tubes "F" (in the sectional view of the apparatus only 2 "E" tubes are shown although 4 were used). The gas is led through a long fine bore tube to the bottom of the roller tube (this long tube extends into the roller tube only far enough to clear the fluid medium when the tube is set upright). From the roller tube the gas is allowed to escape into the atmosphere of the incubator. Small loose cotton filters are sufficient to prevent air borne contamination.

This apparatus has been used now with several hundred cultures in roller tubes and it has been found to have the following advantages: (1) the composition of the atmosphere above the cultures can be controlled; graded concentrations of CO_2 in air have been used thus far but other gases or mixtures of gases can be tested for effects on various types of tissue; (2) control of the pH of the media is excellent since there is good equilibration between atmosphere and media; (3) mechanically the apparatus is easy to operate.

Although no special effort has been made to determine the optimal volume of "air flow" in the tubes we have had good results with a daily volume of 2 liters of atmosphere per tube.