oxidized to cystine, which is an excellent carrier of oxygen.

It is possible that cysteine-cystine or a derivative is responsible for the subsurface growth. That oxidation-reduction is important seems likely, since when 0.001 per cent. methylene blue or 0.002 per cent. indigo carmine is added to the cysteine-cystine stab medium and autoclaved, directly on removal no color is apparent. Immediately on standing a deep blue dye line of about a millimeter is noted on the surface. Some color diffuses from the dye line into the medium to impart to it a light blue color. On standing overnight the dye line sinks into the medium about 1½ centimeters, the color below it disappears, and that above is a lighter blue. The color above the dye line fades apparently more rapidly than that of the dye line. In time color sinks below the dye line. There is some fading. If the concentration of the dye is increased the color is increased above and below the dye line. The organisms studied grew above this dye line, but when stabs were made to a depth below it none or very little growth was obtained (the dye was not added to the medium in which the organisms were grown). An extended study of the dye line will be the subject of a future paper.

I hope to correlate the above observations with disease and invasiveness of skin fungi and factors which make invasion of microorganisms and chronicity probable. That this is possible seems evident when we consider some of the factors of importance in inflammation. There is increase of acidity, splitting of moieties with some of the split products evidently in reduced form, and increase in the number of ions with resultant increase in osmotic pressure. The host attempts to neutralize the excess acidity and in so doing increases the activity of reduced forms as reducing substances. This decreases oxygen tension and may be responsible in part of increased oxygen carriage to the part. We know that sulphydryls are important constituents of living tissues and from the work of Hammett and co-workers, published in the recent issues of *Protoplasma,* that the reduced sulphydryls ("wound hormone") stimulate mitoses. It is probable that the reduced sulphydryl is one of the most active reducing agents in living tissue and is an important factor in the mitoses of inflammation. In chronic inflammation the above reactions probably occur in minor degree, dependent upon the immune reaction of the host. The importance of enzymes can not be denied, but there is danger of overemphasizing these entities, the nature of which is unknown, and thereby overlooking features otherwise evident. One can not deny that the trigger to the above reactions is the initial injury which by disrupting and destroying cells apparently liberates substances which catalyze reactions elaborating products which stimulate the compensatory mechanism of the host. In future work I hope to add light to the above subject.

JOHN W. WILLIAMS

GLUTATHIONE AND ASCORBIC ACID

THE reversibly oxidized form of ascorbic acid (Vitamin C) possesses the same antiscorbutic potency as the reduced form of the vitamin, whether administered *per os* or subcutaneously. Comparisons in feeding experiments with suboptimal doses of the vitamin showed that the antiscorbutic potency of a solution of the oxidized vitamin was equal to the quantity of oxidized ascorbic acid which can be converted to the reduced form by H_2S in acid solution.

From pH 5.5 upwards at 37° C. the reversibly oxidized form undergoes an irreversible change, whereby it loses its antiscorbutic potency and the property of convertibility to the reduced form by H_2S. This change is not an oxidation. It proceeds as quickly *in vacuo* as in contact with air.

Since this irreversible change proceeds quickly at pH 7.4, the antiscorbutic potency of the reversible oxidized form indicates that it is protected from this change *in vivo*. Experiments on the blood and urine and tissue slices of animals showed that this protective action consists in the reduction of the reversible oxidized vitamin.

This reduction can be effected rapidly by glutathione when it is present in sufficiently high concentration—100 to 200 mg per cent.—as it is in such animal tissues as the liver and adrenal. Thus at pH 7.0 and 37° C., in approximately equimolar concentrations of glutathione and ascorbic acid, only 2 per cent. of the reversibly oxidized ascorbic acid (initial concentration 4 mg per cent.) was reduced in 4 hours, a 7.5 fold concentration of glutathione effected a reduction of 40 per cent., a 12.5 fold concentration 65 per cent., and a 25 fold concentration 95 per cent. reduction in the same length of time.

However, the absolute concentration of glutathione rather than its relative excess over that of ascorbic acid is more important in the physiological range of ascorbic acid concentration. With 200 mg per cent. glutathione at pH 7.0, 50 per cent. of the ascorbic acid is reduced in five minutes, about 80 per cent. in fifteen minutes with ascorbic acid concentrations ranging from 4 to 25 mg per cent.

This reaction between glutathione and oxidized ascorbic acid possesses several interesting features. It reveals an important function of glutathione in animal tissues. It proceeds sufficiently rapidly not to require an enzyme—though an enzyme mechanism in addition is not excluded. It shows the great importance of the concentrations of the reactants on the rate of a biological oxidation.

These experiments with glutathione and ascorbic

acid were carried out with a vacuum technique. The failure of previous workers to observe any significant amount of reduction of ascorbic acid by glutathione is to be ascribed to their having worked at too low a pH level and without a sufficient excess of glutathione over ascorbic acid.

H. BORSOOK
C. E. P. JEFFREYS
CALIFORNIA INSTITUTE OF TECHNOLOGY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE COMBINATION OF CONTINUOUS EXTRACTOR AND EXTRACT-WASHER

IN organic and biochemical laboratories the extraction of liquids with lighter organic solvents and the subsequent washing of the extract with aqueous solutions are very common operations. Yet, continuous extractors for this purpose are used comparatively rarely, although several are on the market. We suspect the reason for this is the expense and complexity of the apparatus available at present. Automatic extract-washers are almost never used.

We have devised a simple automatic extractor-washer combination which can be made from the usual laboratory glassware with the ordinary glass-blowing equipment. All materials are of Pyrex glass and include a 500 cc Kjeldahl flask, a large test-tube (about 400 × 50 mm), a 250 cc Erlenmeyer flask and glass tubing. The assembled apparatus is illustrated in Fig. 1. *A* is the extraction flask—any size Kjeldahl flask may be used to which the side arm is sealed as indicated. This is filled to the neck with the solution which is to be extracted. *B* is the extract-washer, made from a large test-tube by sealing on the side arm. This tube is filled with the solution to be used for washing the organic solvent. *C* is the Erlenmeyer flask and contains the lighter solvent. *D* is a tin can lined with asbestos paper and contains a 75-watt bulb.

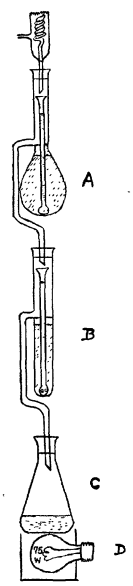

FIG. 1.

The small holes at the lower ends of the inner tubes which provide a fine spray of the solvent may be made readily from a strand of wire taken from a chromel wire gauze (A. H. Thomas Co.: Catalog No. 9993), which is sharpened to a fine point and held in a pliers or sealed into a glass rod. The glass bulb and pointed wire are heated with a fine pin-point blow-torch flame and, with a little practice, fine holes are easily punched. The upper ends of the inner tubes are flanged out with a carbon from an arc lamp.

The solvent head in the inner tube necessary to overcome the hydrostatic pressure of the aqueous solution may be calculated from the specific gravities of the respective liquids and the height of the aqueous solution.

We have used this apparatus for the extraction of oestrin from pregnancy urine with ether. However, any immiscible solvent may be used which is lighter than the liquid to be extracted. We have also used benzene, in which case the 75-watt bulb is replaced with an electric hot plate.

The great advantage of this apparatus is that it prevents completely the formation of emulsions which, a glance at the oestrin literature will show, are so common when urine is shaken with ether in a separatory funnel. The washer, as we use it, is filled with sodium carbonate solution and automatically removes acidic constituents from the ether extract. It is obvious that any type of washing solution and any number of washers may be inserted.

The apparatus is efficient, giving a very thorough extraction of oestrin from a 24-hour human pregnancy urine when allowed to run over night.

The apparatus can be made even more convenient, if it is desirable to use it very often, by replacing the cork connections with ground-glass joints.

H. BOYD WYLIE
M. J. SCHMULOVITZ,
Weaver Fellow in Biological Chemistry
UNIVERSITY OF MARYLAND
MEDICAL SCHOOL

BOOKS RECEIVED

COLLINS, FRANCIS A. *The Boys' Book of Model Aeroplanes.* Third edition, revised. Pp. vii + 262. Illustrated. Appleton-Century. $2.00.

DICKINSON, H. W. *James Watt, Craftsman and Engineer.* Pp. xiii + 207. 14 figures. 17 plates. Cambridge University Press, Macmillan. $4.00.

FOSTER, WILLIAM. *The Romance of Chemistry.* Second edition, revised. Pp. xviii + 497. Illustrated. Appleton-Century. $3.00.

LUGN, A. L. *The Pleistocene Geology of Nebraska.* Bulletin No. 10, Second Series, of the Nebraska Geological Survey, 1935. Pp. 223. 38 figures. The Survey.

MEYER, ARTHUR W. *An Analysis of the "De Generatione Animalium" of William Harvey.* Pp. xx + 167. Stanford University Press. $3.00.

Science Reports of the Tôhoku Imperial University, Sendai, Japan. Second Series (Geology). Vol. XVIII, No. 3. Pp. 44. 11 plates. Maruzen Company, Tokyo and Sendai.

VAN UVEN, M. J. *Mathematical Treatment of the Results of Agricultural and Other Experiments.* Pp. vi + 309. P. Noordhoff N. V. Groningen-Batavia.