investigation of this substance. Drs. B. L. Souther and Alice G. Renfrew and also Misses Mary Hostler and Mary A. Clapp have been active in this work.

In this department is a fellowship that is carrying on broad research on nutritional aspects of dental caries, under a grant from The Buhl Foundation of Pittsburgh. This extension of the study of the problem described last year is being conducted by Dr. G. J. Cox and two assistants. It is hoped that it will provide an answer to the interesting and important question, is there present in the diet during a critical period of tooth formation a factor that will aid in the construction of teeth resistant to decay?

For six years the institution has been supporting comprehensive research in the Institute of Pathology of the Western Pennsylvania Hospital in Pittsburgh, through arrangements made by Dr. C. B. Schildecker. This investigational work, which is being conducted by a group of scientists under the direction of Dr. R. R. Mellon, also relates in part to the treatment of pneumonia and allied pulmonary diseases and has resulted in the preparation of an apparently effective antipneumococcic serum. Late in 1935 members of this staff reported artificial breeding experiments with the tuberculosis bacillus that led to the discovery of a new form of this organism and shed new light on the disease. Then, too, progress has been made in an anti-streptococcic serum.

The new building of the institute, whose construction will soon be completed, is being gradually occupied. At present the department of research in pure chemistry and twelve industrial fellowships are located in this edifice.

W. A. HAMOR

MELLON INSTITUTE OF INDUSTRIAL RESEARCH

SPECIAL ARTICLES

INVASIVENESS OF SKIN INFECTIONS CAUSED BY PATHOGENIC FUNGI AND SUBSURFACE MYCELIUM[1]

THERE is little known with reference to the basic factors which determine chronicity and invasiveness of pathogenic fungi in skin infections. For this reason I have begun a study of the ability of pathogenic fungi to invade various solid mediums in an attempt to correlate these findings with what occurs in the body.

The invasiveness of pathogenic fungi for hair and skin has been amply demonstrated histologically. For this reason it seemed logical that a medium using as its source of nitrogen hydrolyzed hair and hydroylzed skin would be practicable. The Difco Laboratories prepared these products for me in the manner in which they prepare their peptone. The following mediums were used: 1 per cent. peptone, 4 per cent. dextrose, (Sabouraud's), 1½ per cent. agar; 1 per cent. hydrolyzed pig skin, 4 per cent. dextrose, 1½ per cent. agar; 1 per cent. hydrolyzed hair, 4 per cent. dextrose, 1½ per cent. agar. Plants were made using women's hydrolyzed hair, men's hydrolyzed hair and children's (before puberty) hydrolyzed hair. Children's hydrolyzed hair which was planted in duplicates was used for these descriptions. The pH was adjusted to 5.6. The tubes were incubated at room temperature in diffused light for a period of 30 days.

The following representative fungi and non-pathogenic fungus, *Scopulariopsis brevicaulis,* were planted in duplicate: *Achorion schoenleinii, Acladium castellani, Candida candida, Endodermophyton indicum, Endomyces capsulatus, E. dermatitidus, Epidermophyton cruris, E. inguinale, E. rubrum, Glenospora gammeli, Geotrichum bachmann, Indiella americana, Microsporon audouini, M. felineum, M. gypseum, Monosporum apiospermum, Monilia albicans, Oöspora humi, Sporotrichum schenkii, Trichophyton crateriforme, T. granulosum, T. gypseum, T. gypseum asteroides, T. gypseum lacticolor, T. interdigitale, T. louisiana, T. niveum, T. chophyton purpureum, T. sulfureum, Willia anomala.*

The following changes were noted. The growths in general became more subsurface on the skin medium and distinctly more subsurface on hair medium. In many instances there was complete loss of surface growth on hair medium. One could note the plate of growth (point of departure) successively deeper in skin and hair mediums. Coincidently the length and amount of surface mycelium and the roughness of the colony would become less or disappear. In most instances the plate of growth was distinct, but in a few this distinctness was lost so that the growth consisted only of mycelium, the breadth of which widened as the distance from the surface of the medium increased. The latter occurrence was noted only in hair medium.

The above findings suggested the possibility that cysteine might be a factor, since the amount of cysteine in hydrolyzed skin would be about one third to one fourth less than that in hydrolyzed hair. Cysteine hydrochloride was therefore substituted as the source of nitrogen, and it was found that the growths were very predominantly subsurface. This seemed surprising unless cysteine had to a considerable extent been

[1] Contribution No. 69 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

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