This hypothesis has been verified by the chromatographic identification in the products of the plasmalogen-mercuric chloride reaction, of a free fatty aldehyde containing mercury and lysolecithin. Also we have isolated in 21 per cent yield ß-chloromercuriacetaldehyde[6] from the reaction of the model system, butyl vinyl ether plus aqueous mercuric chloride.

This reaction with mercuric chloride has been developed for histochemical localization of plasmalogen (unpublished work).

This work was supported by U.S.P.H.S. Research Grant B1006(C4) and Interdisciplinary Grant 2M-6418(C2) N.I.M.H.

WILLIAM T. NORTON
Department of Medicine, Division of Neurology
and Department of Biochemistry,
Albert Einstein College of Medicine
of Yeshiva University,
New York 61, New York.
June 11.

[1] Rapport, M. M. and Franzl, R. E., *J. Neurochem.*, **1**, 303 (1957).
[2] Feulgen, R., and Voit, K., *Pflugers Arch. ges. Physiol.*, **206**, 389 (1924.)
[3] Gray, G. M., *Biochem. J.*, **70**, 425 (1958).
[4] Folch, J., Lees, M., and Sloane-Stanley, G. H., *J. Biol. Chem.*, **226**, 497 (1957).
[5] Chatt, J., *Chem. Revs.*, **48**, 7 (1951). Polgár, A., and Jungnickel, J. L., in "Organic Analysis", vol. 3, pp. 301–310 edit. by Mitchell, J., Kolthoff, I. M., Proskauer, E. S., and Weissberger, A (Interscience Publishers Inc., New York, 1956).
[6] Nesmayanov, A. N., Lutsenko, I. F., and Vereschagina, N. I., *Bull. Acad. Sci. U.R.S.S., Classe. sci. chim.*, 63 (1947). *Chem. Abstr.*, **42**, 4148i (1948).

Hydroxylation of Proline *in Vitro*

WHILE carrying out a study of hydroxyproline synthesis in biological media, by hydroxylation of proline or peptides containing proline, we have also investigated the possibility of hydroxylation of pyrrolidine ring *in vitro*. We commenced our study by ascertaining[1,2,3] whether or not cortisone inhibited the formation of free hydroxyproline in animal tissues during their embryonal development and thus interfered with the biosynthesis of collagen.

The possibility of incorporating free hydroxyproline[4,5] (and hydroxylysin in analogy[6,7]), into collagen proteins was denied in most papers. However, in a recent study by Mitoma *et al.*[8], on the same experimental material as in our case, proofs are presented that bound hydroxyproline also originates from free hydroxyproline. Furthermore the central significance of hydroxylation of proline for the synthesis of collagen arises in the papers of Robertson[9,10] and Gould[11] who have found proof for hydroxylation inhibition of proline in the case of ascorbic-acid deficiency. The possibility of hydroxylation of both aromatic[12] and sterol rings[13] has been proved by many authors.

Our experiments have shown that in the reactive medium containing ethylene diamine tetraacetic disodium salt, Fe^{++}, ascorbic acid, hydrogen peroxide and proline, a substance forms which can be determined by specific reaction on hydroxyproline[14]. By means of paper ionophoresis, partition chromatography, as well as by isolation of hydroxyproline in the form of reineckate and by measuring the absorption curves, we have found that the substance formed has properties inherent to hydroxyproline. Hydroxylation does not occur either in the absence of ascorbic acid or hydrogen peroxide; ethylene diamine tetraacetic disodium salt and Fe^{++} are not essential, but in their presence, however, hydroxylation becomes more intensive.

Hydroxylation is almost completed within three minutes; if the incubation lasts for more than 30 min. the amount of hydroxyproline formed decreases. The presence of pure oxygen in the reactive medium, instead of hydrogen peroxide, also brings about the formation of hydroxyproline; however, the reaction rate is slow and not intensive.

We have found that the optimal concentration of substances in the reactive medium and the optimal conditions of reaction are: 8×10^{-3} *M* ferrous sulphate, $2{\cdot}6 \times 10^{-3}$ *M* ethylene diamine tetraacetic disodium salt, 8×10^{-3}–1×10^{-2} *M* ascorbic acid, $4{\cdot}7 \times 10^{-2}$ *M* hydrogen peroxide, 0·1–0·15 *M* solution of phosphate buffer, *p*H in the range 4·5–5·6. There is a definite relationship between the temperature and degree of hydroxylation (studied up to 55°C.). The amount of hydroxyproline formed is related to the concentration of proline in the reactive medium and the degree of conversion is in the region of 2–4 per cent. We have also studied the possibility of hydroxylation of prolylglycine and prolylglutamylglycine and have found the same degree of conversion as in proline.

Thus we have been able to show that in the reactive medium, of the same composition as was used before by Udenfriend *et al.*[12] for the hydroxylation of the substituted aromatic ring, hydroxylation of proline also occurs.

Further experiments aiming at the biological utilization of these results are being carried out.

M. CHVAPIL
J. HURYCH
Institute of Industrial Hygiene and
Occupational Diseases,
Prague, Czechoslovakia.

[1] Roberts, E., Karnofsky, D. A., Frankel, S., *Proc. Soc. exp. Biol. (N.Y.)*, **76**, 289 (1951).
[2] Chvapil, M., *Physiol. Bohemoslovenica*, **7**, 391 (1958).
[3] Chvapil, M., *Physiol. Bohemoslovenica*, **8**, 186 (1959).
[4] Stetten, M. R., *J. Biol. Chem.*, **181**, 31 (1949).
[5] Smith, R. H., Fitton, Jackson, S., *Biochem. J.*, **64**, 80 (1956).
[6] Piez, K. A., Likins, R. C., *J. Biol. Chem.*, **229**, 101 (1957).
[7] Sinex, F. M., Van Slyke, D. D., Christman, D. R., *J. Biol. Chem.*, **234**, 918 (1959).
[8] Mitoma, Ch., Smith, T. E., Friedburg, F., Rayford, C. R., *J. Biol. Chem.*, **234**, 78 (1959).
[9] Robertson, W. van B., Hiwett, J., Herman, C., *J. Biol. Chem.*, **234**, 105 (1959).
[10] Robertson, W. van B., Schwartz, B., *J. Biol. Chem.*, **201**, 689 (1953).
[11] Gould, B. S., *J. Biol. Chem.*, **232**, 637 (1958).
[12] Udenfriend, S., Clark, C. T., Axelrod, J., Brodie, B. B., *J. Biol. Chem.*, **208**, 731, 741 (1954).
[13] Cier, A., Nofre, C., Revol, A., *C.R. Acad. Sci.*, **274**, 542 (1958).
[14] Stegeman, H., *Ztschr. physiol. Chem.*, **311**, 41 (1958). *Naturwissenschaften*, **45**, 264 (1958).

Microbial Degradation of Rutin

COMPARATIVELY little work on the metabolism of rutin has been published. 3,4-dihydroxyphenyl acetic acid and homovanillic acid are found in urine after oral administration of rutin to the rat[1,2], and protocatechuic acid accumulates in rat kidney homogenates in the presence of quercetin[3].

We have shown[4] that a fungus, *Pullularia fermentans* var. *candida*[5], forms phloroglucinol, protocatechuic acid and an unknown substance when cultivated in aqueous rutin solution. This unknown substance has now been identified as 2-protocatechuoyl phloroglucinol carboxylic acid.

The organism (about 50 mgm. wet-weight) was incubated with rutin (1 gm.) in 1 l. of 0·003 *M* phosphate buffer (*p*H 6·0) at 25°C. for 5 days, and the liquid was extracted with ether. After removal of ether, the remaining mass was dissolved in hot water (60°C.), and about 0·1 gm. substance was obtained in white needles after cooling in a refrigerator. When subjected to paper chromatography, the R_F value of this substance agreed well with that of the unknown