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This reaction with mercuric chloride has been developed for histochemical localization of plasmalogen (unpublished work).

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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.

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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

substance, as was reported previously, both in *n*-butanol/acetic acid/water (4:1:2) and in 80 per cent phenol.

This substance, after recrystallization from hot water, contained 2·5 mol. of water of crystallization and melted, effervescing at 174°C., and produced dark green dyes and red orange dyes with ferric chloride and with benzidine diazo reagents[6], respectively.

When hydrolyzed with 10 per cent potassium hydroxide, the substance gave phloroglucinol and protocatechuic acid and it dissolved in a sodium bicarbonate solution evolving carbon dioxide, suggesting the presence of a carboxylic group in its molecule. These facts suggest that this substance is identical with a protocatechuoyl phloroglucinol carboxylic acid (anal.: calc. for $C_{14}H_{10}O_8.2{\cdot}5H_2O$: C, 47·87; H, 4·30; found: C, 48·44; H, 4·39).

Since this substance easily loses the carboxyl group on heating to 100°C., it was methylated with an excess of diazomethane and the methyl ether methyl ester was obtained as colourless needles, which melted at 144°C. after recrystallization from absolute alcohol (anal: found: C, 60·91; H, 5·16). When admixed with 2-veratroyl 4, 6-dimethoxyphloroglucinol carboxylic acid methyl ester (anal.: calc. for $C_{19}H_{20}O_8$: C, 60, 63; H, 5·36; found: C, 60·69; H, 5·02), which had been synthesized from 2, 4-dimethoxyphloroglucinol carboxylic acid methyl ester and veratroyl chloride, this methyl ether methyl ester did not show any depression of melting point, suggesting the identity of these substances.

From these results it is evident that the substance produced by the fungus is identical with 2-protocatechuoyl phloroglucinol carboxylic acid.

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Chemical Nature of a Plant-Virus Inhibitor from Rice

The inhibition of tobacco mosaic virus infection of primary bean leaves (*Phaseolus vulgaris* L. var. Pinto) by extracts of various portions of rice plants has been described recently[1]. The inhibitor or inhibitors present in rice resemble those derived from other plants such as spinach (*Spinacea oleracea* L.)[2], poke weed (*Phytolacca acinosa* Roxb. var. *esculenta*)[3], New Zealand spinach (*Tetragonia expansa* Murr.)[4], and sweet william (*Dianthus barbatus* L.)[5], in that the infection of test plants is inhibited when the plant extract and virus inoculum are mixed and applied simultaneously. The inhibitor in rice extracts differs in that it protects bean leaves against tobacco mosaic virus infection even when applied to the leaves (which are then rinsed with water) 1–3 days prior to inoculum application. In so far as we are aware, the only other plant extracts that protected test plants against virus infection were derived from carnation (*Dianthus caryophyllus* L.)[6], but the time between inhibitor application and inoculation was only 1–3 hr.

Experiments were conducted to determine the general chemical nature of the inhibitor as a basis for subsequent more detailed chemical investigation. The source of the inhibitor used was rice polish, since the inhibitor is concentrated in this readily available by-product in the milling of rice. The antiviral activity of the various chemical fractions of rice polish was determined by the local-lesion bioassay described by Holmes[7]. In these assays one member of each pair of opposite primary bean leaves was rubbed with the preparation plus tobacco mosaic virus and the opposite member with a comparable untreated control inoculum.

The inhibitor could be extracted from the polish with water, but not with methanol, and addition of methanol to the water extract caused complete inactivation with the formation of a precipitate. Centrifugation of the cloudy aqueous extract at 15,000 r.p.m. for 30 min. gave a clear, slightly yellow solution retaining all its activity. Addition of 20 per cent trichloroacetic acid to a final acid concentration of 10 per cent in the extract completely destroyed the activity of the inhibitor, with the formation of a slight precipitate. Addition of cold saturated ammonium sulphate solution to cold rice-polish extract to 80 per cent saturation, followed by centrifugation, gave a precipitate that was found to be active. Very slow addition of cold absolute ethanol to cold rice-polish extract, to give a final ethanol concentration of 40 per cent, followed by centrifugation, gave an active precipitate. By mixing the aqueous extract with various powdered adsorbents, it was found that the inhibitor was adsorbed on alumina, magnesium oxide ('Sea Sorb'), and charcoal, but not on silica ('Celite'). The inhibitor failed to pass through a Visking membrane in 4 hr. after the extract was placed in a stainless steel ultrafiltration apparatus and 40 lb. of nitrogen per square inch was applied.

These preliminary experiments indicate that the virus inhibitor in rice polish is probably a protein, with a molecular weight greater than 13,000.

The aqueous extract of rice polish loses its activity slowly upon standing, even at 5–7° C., with the formation of a precipitate which may be denatured protein. The fresh extract is approximately neutral in reaction, but on standing, either in the cold or at room temperature, it becomes acidic, with consequent loss in activity.

Further investigation of this virus inhibitor is in progress.

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