As shown in Figure 3, a sample of DNA prepared according to GULLAND[8] belonged to the double-stranded type of WATSON-CRICK, whereas the heat-denatured DNA derived from GULLAND's DNA belonged to the single-stranded type. The same investigation was carried out on FEULGEN's DNA and b-nucleic acid, and clarified the fact that these acids were both single-stranded ones as indicated in Figure 3.

GULLAND's DNA was converted to b-acid more slowly than FEULGEN's DNA. Full particulars will be reported later elsewhere[9].

Zusammenfassung. FEULGEN fand, dass die Degradation der Desoxyribonukleinsäure durch Pankreatin keine Mononucleotide bildete, jedoch auf der Stufe der Oligonucleotide (b-Nukleinsäure) blieb. In dieser Mitteilung wird die b-Säure mit dem ersten Degradationsprodukt identifiziert, welches aus Nukleinsäure unter Einwirkung von DNase 1 gebildet wird, und der Verlauf der Nukleinsäure-Degradation wird ausführlich verfolgt.

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October 19, 1962.

[8] J. M. GULLAND, D. O. JORDAN, and C. J. THRELFALL, J. chem. Soc. *1947*, 1129.

[9] The authors are grateful to Dr. A. WADA, Ochanomizu University, to Dr. K. KURIHARA, Tokyo Institute of Technology, and to Dr. S. MATSUNAKA, National Institute of Agricultural Sciences for the estimation with light scattering, diffusion and sedimentation.

Influence of Cysteine on the Light Production Rate of the Crustacean Cypridina Luciferin-Luciferase System

Cysteine is a well known radiation protection substance. However, not much is known about its effect on the material it protects. The light emitting enzyme-substrate (luciferase-luciferin) system, obtained from the small ostracod crustacean Cypridina, provides a good media to study this effect.

The isolation of pure, crystalline luciferin from the Cypridina[1] and its structure[2] have been reported previously. Luciferin was dissolved in methyl alcohol (0.1 mg/ml) because it is relatively stable in that solution. The cysteine solution consisted of cysteine HCl, dissolved in 0.1 *M* phosphate buffer, to produce concentrations varying between 0 and 4.65 *M*.

A few minutes before the test for light emission, 25 λ of cysteine solution were added to 25 λ of the methanolic luciferin solution, and the mixture was brought to 10 ml with 0.1 *M* phosphate buffer making a final concentration of 0.25 μg/ml.

Luciferase was dissolved in 0.1 *M* phosphate buffer to produce a concentration of 0.1 mg/ml. 25 λ of cysteine solution were added to 25 λ of this enzyme solution. However, experiments showed that with cysteine concentrations above 0.16 *M*, the flash-height peak dropped very sharply to about zero, due to a change in pH which destroyed the enzyme. To avoid this enzyme destruction, 10 ml of phosphate buffer were added to 25 λ of buffered luciferase solution before adding the cysteine solution. This minimized the pH change.

In these experiments an RCA Type 6217 Phototube with a range of highest response from 3500 to 6000 Å and a Moseley X-Y Chart Recorder were used. The negative voltage to the phototube was obtained from a Baird-Atomic Model 312A Super-Stable High Voltage Supply. The phototube as well as a test tube with a fixed geometry relative to the phototube were in a light-tight box.

First, 10 ml of the buffered luciferin solution were poured into this test tube. The reaction was then initiated by injecting rapidly, with a hypodermic syringe, 10 ml of the 0.25 μg/ml buffered luciferase solution.

The flash-height peak distribution as a function of the cysteine concentration is shown in Figure 1. The peak heights increase with increasing cysteine concentrations. Using a 4.65 *M* cysteine solution, this increase is about 39% compared with the control. The peak height decreases markedly, however, if the pH of the luciferase solution is decreased by cysteine as shown in Figure 1 (broken line). The pH values of these solutions were measured grossly using Fisher Alkacid Tester Paper.

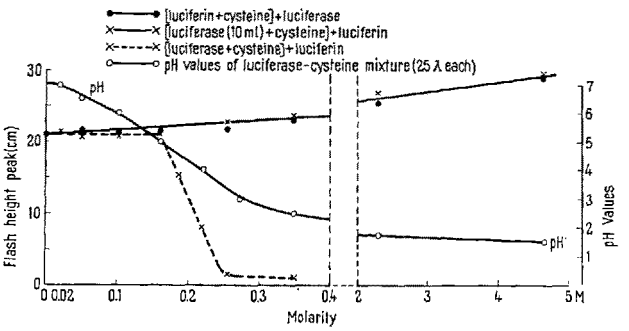

Fig. 1. Flash-height peaks of the Cypridina luciferase-luciferin system as a function of the cysteine concentration.

Similar results were obtained for the slope and the total light emitted (that is, area under the curve). The flash-height peak width remains constant; however, it increased tremendously (from about 1 to 18 cm) when 25 λ of cysteine (0.35 *M*) were added to 25 λ of luciferase solution as shown in Figure 2.

An increase in light intensity was observed recently by AIRTH et al.[3] after the addition of CoA to the reaction mixture of the firefly system (luciferase, luciferin, ATP, glycyl-glycine, and $MgSO_4$). This increase was directly proportional to the CoA concentration. However, the rise in the light intensity could be observed only when CoA was added to the reaction mixture after the inhibi-

[1] O. SHIMOMURA, T. GOTO, and Y. HIRATA, Bull. chem. Soc. Japan *30*, 929 (1957). – Y. HANEDA et al., J. cell. comp. Physiol. *57*, 55 (1961).

[2] Y. HIRATA, O. SHIMOMURA, and S. EGUCHI, Tetrahedron Letters No. 5, 4 (1959). – O. SHIMOMURA, Nippon Kagaku Zasshi *81*, 179 (1960).

[3] R. L. AIRTH, W. C. RHODES, and W. D. MCELROY, Biochim. biophys. Acta *27*, 519 (1958).

tion became prominent. There was no effect on the initial flash-height peak when CoA was added initially; the total light emitted, however, was greater in the presence of CoA. AIRTH et al.[3] suggested the following explanation for this effect: CoA removes the inhibitory effect of oxyluciferin by reacting with it and forming the compound oxyluciferyl-CoA. This compound reacts with cysteine, as could be shown, and forms oxyluciferyl-cysteine. There was, however, no stimulation of the light emission by this secondary addition of cysteine to the reaction mixture. Moreover, no cysteine derivative of oxyluciferin could be formed in the absence of CoA, indicating that cysteine alone has no stimulatory effect.

Quite different from this are the results obtained in the Cypridina luciferase-luciferin system. Not only does the addition of cysteine alone increase the total light emitted but, also, it increases the initial flash-height peak. This stimulated light emission is directly proportional to the cysteine concentration. This indicates the possibility that cysteine is capable of reacting directly with the oxyluciferin compound or with any other inhibitor—whatever it is—of the Cypridina system. The increase in the initial flash-height peak indicates either a release of luciferase from small amounts of impurities still present in the enzyme solution or some kind of energy transfer. This latter reaction, the electron transition, seems more than likely. There was no indication that autoxidation is the process responsible for this effect. No autoxidation could be observed during the time the measurements were made[4].

Zusammenfassung. Cysteinlösung (Cystein-HCl gelöst in 0,1 *M* Phosphatpuffer) von verschiedenen Konzentrationen (0–4,65 *M*) wurde zu Luziferinlösung (gereinigtes kristallines Luziferin gelöst in Methanol, 0,1 mg/ml) oder zu praktisch reiner, gepufferter Luziferaselösung (0,1 mg/ml) zugegeben. Mit zunehmenden Cystein-Konzentrationen ergibt sich eine Zunahme in der Höhe der Reaktionskurve.

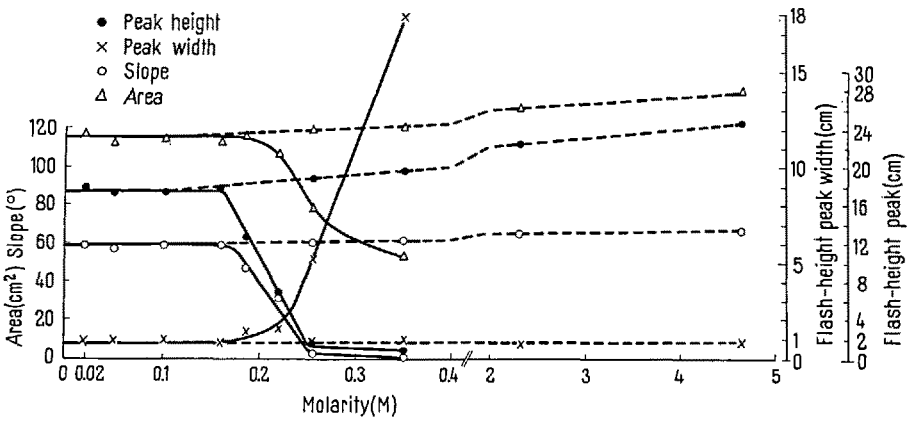

Fig. 2. Influence of cysteine concentration on the Cypridina luciferase-luciferin system. 25 λ of cysteine were added to the same quantity of luciferase in either 25 λ (solid line) or 10 ml (dotted line) of solution.

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Methyl 11-Methoxy-18-oxo-3-epialloyohimban-16α-carboxylate, a New Keto Ester Derived from Reserpine

Recent reports on the chemistry of methyl *neo*reserpate (V)[1] prompt us to describe some experiences with preparation of this compound. Our investigations lend additional experimental support for the mechanism proposed for formation of V from reserpine (I)[1b]. Extended reaction times (refluxing for 64 h with sodium methoxide) in the methanolysis of reserpine to methyl reserpate (II) resulted in the formation of the more stable methyl *neo*-reserpate (V)[1a]. We have found that an additional product is formed when the reaction is carried out in a glass-lined bomb at 100° for 6 h. Although a small amount of methyl *neo*reserpate was recovered from accumulated mother liquors by alumina chromatography, the major product, formulated as methyl 11-methoxy-18-oxo-3-epialloyohimban-16α-carboxylate (IV), appeared to be a keto ester from its infrared spectrum ($\nu_{\text{Nujol}}^{\text{max}}$ 1730 and 1700 cm^{-1}). This colorless crystalline substance, m.p. 114.5–117.5°, $[\alpha]_D^{25}+69°$ (*c*, 0.49 in pyridine) (Found: C, 67.0; H, 7.08; N, 7.45; $(O)CH_3$, 7.78; H_2O, 4.90. $C_{22}H_{26}N_2O_4 \cdot 3/4\,H_2O$ requires C, 66.7; H, 6.99; N, 7.07; $2(O)CH_3$, 7.58; H_2O, 3.41), gave a bright yellow crystalline 2,4-dinitrophenylhydrazone, m.p. 194–204°, $\nu_{\text{max}}^{\text{Nujol}}$ 1732 cm^{-1}, $\lambda_{\text{CHCl}_3}^{\text{max}}$ 360 mμ[2]. The loss of methoxyl suggested by the

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[2] For visible spectra of other saturated ketone dinitrophenylhydrazones see C. DJERASSI and E. RYAN, J. Amer. chem. Soc. *71*, 1000 (1949). – J. P. PHILLIPS, J. org. Chem. *27*, 1443 (1962).