thalamic mechanism and could therefore be attributed only to the cortex.

The facilitated transmission of the ascending impulses in the ventro-postero-lateral nucleus of the thalamus could be accounted for either by a functional depression of structures controlling the transmission of the centripetal volley during light sleep or by the intervention of mechanisms actively facilitating the transmission of the impulses in the thalamus during deep sleep.

Riassunto. Le risposte corticali evocate da stimoli somatici, sia periferici che centrali, aumentano di ampiezza durante il sonno profondo. Tale amplificazione è dovuta, almeno in parte, ad una facilitata trasmissione degli impulsi ascendenti a livello del nucleo ventro-postero-laterale del talamo.

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b-Nucleic Acid, and the Initial Step of Deoxyribonucleic Acid-Degradation by Deoxyribonuclease I

In 1935, FEULGEN[1] observed that the solution of DNA[2] prepared according to his own method[3] was strongly liquefied by the action of pancreatin without the liberation of purines and phosphates, and the reaction product, which was obtained in high yield, yet maintained nearly the same acid-precipitability as that of the parent DNA. He called this substance b-nucleic acid, and the enzyme concerned nucleogelase.

For some time we have also studied the nucleogelase reaction and obtained the following results. To 0.4 ml of 1% solution of Feulgen's DNA in $M/10$ acetate buffer (pH 6.0) was added 0.1 ml of enzyme solutions of various activities by extracting NBC's pancreatin with water, and the viscosity change of the reaction mixture was observed. The relative viscosity which was at first ca. 4, dropped to the final value of ca. 1.2 after various time passages, according to the concentrations of nucleogelase. An aliquot of the reaction mixture was poured into 30 vol of cold 0.25N HCl and the resultant precipitate was dissolved in $M/15$ Na_2HPO_4; then an aliquot of this solution was subjected to paper electrophoresis (200 V, 4 mA, the current flow lasting 2 h, the electrolyte solution used being $M/50$ acetate buffer of pH 5.0). After the finish the paper was observed with mineralight Model SL 2537. The original DNA of FEULGEN did not move from the starting line, while b-acid showed a circumscribed sharp shadow having a fairly large migration rate. In accordance with the drop in the viscosity of the reaction mixture the spot of the original DNA detectable by paper electrophoresis diminished rapidly, while that of b-acid increased quickly. When crystalline DNase 1 was used instead of pancreatin without Mg^{++}, the same electrophoretic pattern as above was observed, suggesting that FEULGEN's nucleogelase might be the same enzyme with DNase 1. Therefore, more detailed comparisons of the two enzymes were carried out as follows. A solution of DNase 1 and the nucleogelase solution prepared as described above were fractionated with ammonium sulphate, and every fraction of both enzymes was compared in DNA-liquefying activity. As indicated in Figure 1 (a), the enzyme activity in both cases was found to be concentrated on the fraction between 57 and 71% ammonium sulphate saturation. As the next step, the b-acid-decomposing activity of nucleogelase was compared with that of DNase.

To 1% solution of FEULGEN's DNA dissolved in $M/10$ acetate buffer of pH 6.0 was added each of the following solutions containing, in liquefying activity order, DNase 1(10 γ/ml) > pancreatin extract > DNase 1(5 γ/ml) respectively. After incubation, an aliquot was taken from each reaction mixture and poured into 30 vol of 0.25N HCl and centrifuged. The supernatant solution was investigated for its light absorbency at 260 mμ in order to estimate the degradation of b-acid. As indicated in Figure 1(b), no difference was seen between the b-acid decomposing activity of nucleogelase and that of DNase 1. Furthermore, the pH-activation curve of liquefying activity (production of b-acid) and that of b-acid decomposing were compared between these two enzymes. For this purpose, DNA or b-nucleic acid was dissolved to 1% in phosphate buffer of various pH's as indicated in Figure 1(c), and to this solution was added nucleogelase solution

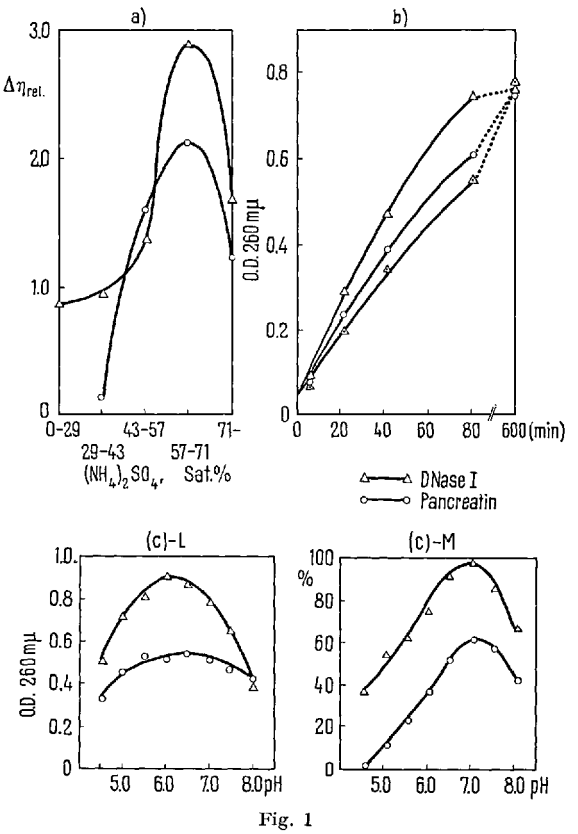

Fig. 1

[1] R. FEULGEN, Z. physiol. Chem. *237*, 261 (1935).

[2] Abbreviations: DNA, deoxyribonucleic acid; DNase 1, deoxyribonuclease 1; NBC, Nutritional Biochemicals Corporation, Cleveland, Ohio; OD, optical density; P, phosphate.

[3] R. FEULGEN, Z. physiol. Chem. *90*, 261 (1914).

(the fraction precipitated by 57–71% ammonium sulphate saturation) or DNase 1 solution. As the viscosity-lowering activity varied with the pH changes, it was expressed by the percentage of difference of the relative viscosities between 1% original DNA and 1% purified b-acid solution at various pH's (Figure 1 (c-M)).

As indicated in Figure 1 (c-M) the pH-optimum of the viscosity-lowering activity was 7 in both cases and that of the b-acid decomposing activity (Figure 1 (c-L)) existed at 6.0–6.5 in both cases.

Next, we pursued the process of DNA degradation by DNase 1 by determining the acid-soluble P, the acid-precipitable P, the residual DNA and the produced b-acid. To 1% DNA solution in $M/10$ acetate buffer (pH 6.0) was added $^1/_4$ vol of DNase 1 solution (final concentration of crystalline DNase was 2 γ/ml). At various intervals of incubation an aliquot was taken, and the acid-soluble and precipitable P were estimated by ALLEN's method[4]. The acid-precipitable part was subjected to paper electrophoresis as described above, and the resultant spots of DNA and b-acid were eluted by heating with $M/15$ Na_2HPO_4 solution and used for measuring their light absorbency at 260 mμ. As demonstrated in Figure 2, the original DNA decreased rapidly coincidentally with the

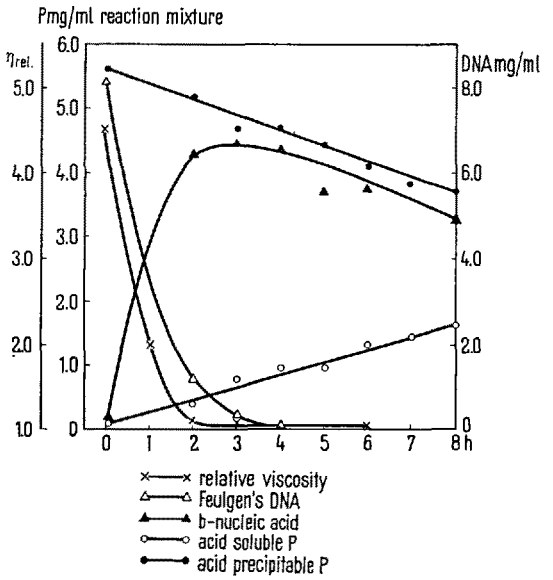

Fig. 2

prompt lowering of viscosity and disappeared after 2 h, while b-acid increased rapidly and then decreased very slowly. Concurrently with this phenomenon the acid-soluble P increased very slowly and the acid-precipitable P decreased at the same rate. Anyhow, it is due to the fact that the production of b-acid is rapid, whereas the degradation of b-acid is very slow, that the main product in both cases of pancreatin and DNase 1 is b-acid. When Mg^{++} was added to the reaction system the pattern of the DNA-degradation changed completely, the accumulation of b-acid being scarcely observed. This fact seemed to indicate that the effect of Mg^{++} on DNase 1 exists in a strong activation of b-acid degradation. In fact, it was confirmed that the enzymic hydrolysis of b-acid was strongly accelerated by the addition of Mg^{++}.

500 mg of FEULGEN's DNA were incubated with 250 γ of crystalline DNase 1 at 37°C under almost the same conditions as mentioned above, and precipitated by the addition of hydrochloric acid. The precipitate obtained was dissolved in 1% sodium acetate, filtered and again precipitated with alcohol; 320 mg of b-nucleic acid were obtained. The acid was shown to contain no more original DNA and could not be distinguished from b-acid prepared by degrading DNA with the extract of pancreatin, judging from the electrophoretic pattern and other tests.

In order to investigate the molecular weights and molecular sizes of b-acid and Feulgen's DNA, b-acid prepared from Feulgen's DNA by treating with pancreatin and Feulgen's DNA, after they were purified by Sevag's method, were subjected to ultracentrifugation, diffusion and light-scattering: b-acid So, 3.5×10^{-13} Do, 3.25×10^{-7}; DNA So, 28.8×10^{-13} Do, 0.2×10^{-7}. From these constants were calculated molecular weights for b-acid and FEULGEN's DNA to be 6×10^4 and 700×10^4 respectively. Based on the ultracentrifugal and diffusion patterns, it could be imagined that b-acid had a more uniform shape than the parent DNA. Molecular sizes (end to end distance) were 600 Å and 3000 Å respectively. From these facts it can be conceived that FEULGEN's DNA is degraded by the action of nucleogelase or DNase 1 into about 1/100 of the original DNA. Contrary to their relatively large molecular weights, their intrinsic viscosities were rather small, especially in the case of FEULGEN's DNA, that is, 0.26 and 2.5 respectively. HERMANNS[5] has found likewise a small intrinsic viscosity for a heat-denatured DNA derived from a high-molecular one having a large intrinsic viscosity. In order to know whether nucleic acid samples concerned in this study were double-stranded or single-stranded, they were dissolved in a solution containing $0.15M$ NaCl and $0.015M$ sodium citrate, and the light absorbency at 260mμ of the solutions at various temperatures (T) were measured to see the relation[6] of the increase in the ratio of $OD_T/OD_{25°C}$ to the temperature; and furthermore the increase[7] in $OD_{260m\mu}$ by the action of formaldehyde upon DNA were investigated.

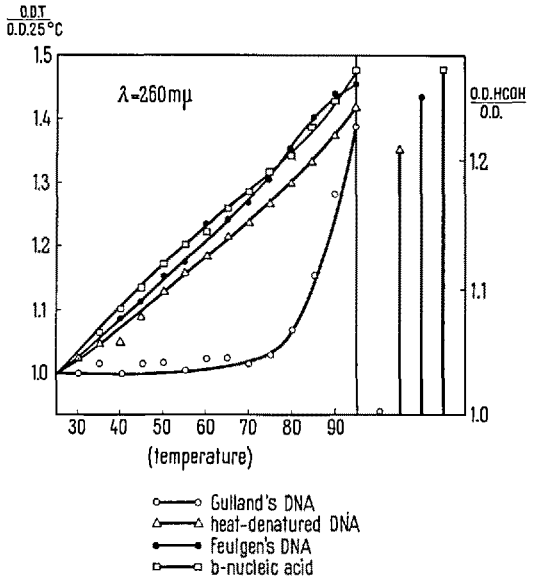

Fig. 3

[4] R. J. L. ALLEN, Biochem. J. *34*, 858 (1940).

[5] J. J. HERMANNS, *Solution Properties of Deoxyribonucleic Acid* (1958), p. 75.

[6] P. DOTY, H. BOEDTKER, J. R. FRESCO, R. HASELKORN, and M. LITT, Proc. nat. Acad. Sci. *45*, 482 (1959).

[7] H. FRAENKEL-CONRAT, Biochim. biophys. Acta *15*, 307 (1954).

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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Department of Biochemistry, Jikei University School of Medicine, Shiba, Minatoku, Tokyo (Japan),
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.

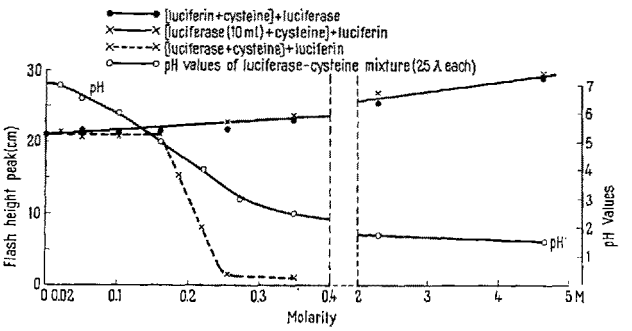

Fig. 1. Flash-height peaks of the Cypridina luciferase-luciferin system as a function of the cysteine concentration.

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).