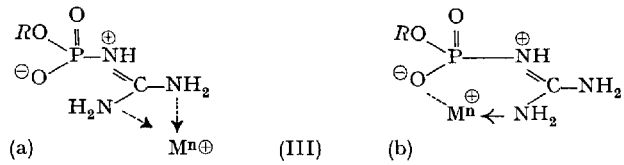
dass dabei die positive Ladung auf dem N-Atom der P–N-Bindung lokalisiert wird.

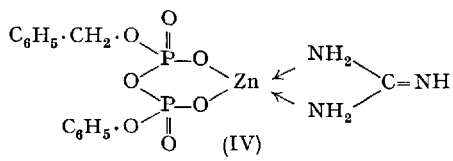


(a) (III) (b)

Wir möchten von Versuchen berichten, die eine solche Wirkung zeigen.

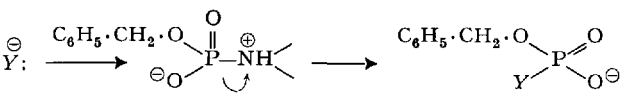
Monobenzyl-Phosphoroguanidid (II, $R = CH_2 \cdot C_6H_5$) bildet Komplexe mit den folgenden Ionen: Li^{I}, Mg^{II}, Cr^{II}, Mn^{II}, Fe^{III}, Co^{II}, Ni^{II}, Cu^{II} und Zn^{II}, und wir haben die Komplexe mit Mg^{II}, Mn^{II}, Co^{II} und Zn^{II} isoliert. In Gegenwart von Wasser können die Mn^{II}, Fe^{III}, Co^{II}, Cu^{II}, und Zn^{II} Komplexe hydrolisiert werden unter Bedingungen, in denen (II: $R = CH_2 \cdot C_6H_5$) stabil ist. Trotzdem sind die angewandten Bedingungen noch verhältnismässig intensiv (nach 12 h bei 80° wird der Zinkkomplex zu 50% hydrolysiert, während unter denselben Bedingungen der Magnesiumkomplex mehrere Tage lang stabil bleibt: der Mangankomplex ist beträchtlich labiler als der Zinkkomplex).

Die Reaktion des Zinkkomplexes (III: $R = CH_2 \cdot C_2H_5$, M = Zn) mit Monophenylphosphorsäure ergab P^1-Benzyl-P^2-Phenyl-Pyrophosphat, das als (IV) isoliert wurde.



(IV)

Sowohl diese Pyrophosphat-Bildung als auch die oben erwähnte Hydrolyse sind Beispiele eines nucleophilen Angriffes (z. B. von $Y^{\ominus}$) auf das Phosphoratom des Komplexes (III: $R = CH_2 \cdot C_6H_5$, M = Zn) mit gleichzeitigen Spaltung der P–N-Bindung.



Wir glauben, dass eine solche Metallionen-Katalyse für die *in-vivo*-Phosphorylierung von ADP durch Phosphagene verantwortlich sein könnte, obwohl man beachten muss, dass die *in-vivo*-Labilität von Phosphagenen viel grösser ist als jede, die man bisher in einem *in-vitro*-System beobachtet hat.

Summary. Phosphoroguanidates, a class of compounds which includes the enzymatically-active phosphagens, exhibit exceptional *in vitro* stability. This lack of reactivity implies an *in vivo* mode of activation, as yet undiscerned. A variety of metal ions catalyse the cleavage of the phosphorus-nitrogen bond with consequent phosphoryl-group transfer, but the enhancement of rate seems insufficient to account for the known activities of phosphoro-creatine and phosphoro-arginine. It is suggested that yet another mode of activation, chemical or biochemical, awaits discovery.

V. M. Clark und S. G. Warren

University Chemical Laboratory, Cambridge (England), May 24, 1963.

The Course of Depolymerization of DNA by DNase I

In the preceding report[1] we observed that thymus DNA, having a molecular weight of some 500×10^4, prepared according to Feulgen or Gulland, was depolymerized by DNase I in the absence of Mg^{++} into a small DNA called b nucleic acid[2] having a single-stranded structure, the molecular weight of which was about 5×10^4. DNA prepared by the method of Gulland[3] (Gulland's DNA) showed the properties of a double helical structure, whereas DNA prepared by the method of Feulgen[4] (Feulgen's DNA) showed ones of a double random coil[5].

Recently we have made an investigation of an intermediate through which a double helical DNA, for example Gulland's DNA, was degradated by DNase I into b nucleic acid, in order to decide what kind of molecular structures it has from amongst various possibilities, such as a, b, c and d shown in Figure 1; a showed a single-stranded DNA, b a double random coil, c a double helical strand constructed of two short polynucleotide chains and d a double helical strand cut short from some ends.

To 0.1% Gulland's DNA solution in water was added DNase I (final concentration 5 μg/ml) at 35° and the changes of viscosity and absorbancy at 260 mμ of the reaction mixture were observed. As shown in Figure 2, $OD_{25°}^{digest}/OD_{25°}^{origin}$ [6] hardly increased until the reaction mixture showed a decrease of as much as 80% in relative viscosity, but $OD_{70°}^{digest}/OD_{25°}^{digest}$ [6] increased from the beginning in accordance with the drop in the viscosity (dotted line in Figure 2). Then an aliquot of the same reaction mixture was taken at various intervals of incubation and injected into 5 vol of ethanol adjusted to pH 4.5 with 0.25*N* HCl, and the resultant precipitate was subjected to paper electrophoresis (200 V, 4 mA the current flow lasting 2h, the electrolyte solution used being *M*/50 acetate buffer of pH 5.0). Before the relative viscosity of the reaction mixture dropped to 20% of the original value, the spot of the original DNA detectable by UV light diminished and disappeared rapidly, while a new spot, having as large migration rate as b nucleic acid, appeared and increased quickly (Figure 3). These facts might show that this b acid-like intermediate produced at the initial step of the reaction had yet a considerable hypochromicity at 25° in contrast with b nucleic acid, but even this hypochromicity disappeared easily at 70° in contrast with Gulland's DNA.

10 mg of Gulland's DNA were incubated with 50 μg of DNase I at 35° under almost the same conditions as mentioned above, and when the relative viscosity of the reaction mixture dropped to 20% of original value, the reaction mixture was injected into 5 vol of ethanol adjusted

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[2] R. Feulgen, Z. physiol. Chem. *237*, 261 (1935).

[3] J. M. Gulland, D. O. Jordan, and C. J. Threlfall, J. chem. Soc. *1947*, 1129.

[4] R. Feulgen, Z. physiol. Chem. *90*, 261 (1914).

[5] M. Matsuda and K. Makino, unpublished data.

[6] See the legend under Figure 2 and Figure 5.

to pH 4.5 with 0.25N HCl. The sediment resulting from centrifugation was washed twice more with ethanol and finally ether, and dried in a vacuum desicator (yield 8.5 mg).

The intermediate obtained in this manner was compared by paper partition chromatography with the single-stranded DNA prepared by the method of MATSUWARA et al.[7] from Gulland's DNA, the double random coil DNA (Feulgen's DNA) and b nucleic acid. These substances were applied to TOYO Filter Paper No. 51 and developed with a solution of 1.0M NH_3 in 2M NaCl and 0.01M phosphate[8]. The substances were compared also by paper electrophoresis (condition as mentioned above). As indicated in Figure 4, the intermediate moved with the same migration rate as that of b nucleic acid, while the single-stranded or the double random coil DNA hardly moved at all.

In order to know the difference between the intermediate and b nucleic acid, the following experiment was carried out. The intermediate was dissolved in 0.15M NaCl containing 0.015M sodium citrate and heated at 98° for 10 min, followed by cooling rapidly to obtain a 'denatured' or 'single-stranded' intermediate[7]. And then the heat-treated intermediate, the original intermediate and b nucleic acid were dissolved respectively in M/15 phosphate buffer (pH 7.3) containing 2% formaldehyde and incubated at 35°, and the changes in absorbancy at 260 mμ by the action of formaldehyde were investigated[9]. As shown in Figure 5(a), OD^{HCHO}/OD^{no} of the intermediate increased only slowly, whereas that of the heat-treated intermediate or b nucleic acid increased at a tolerable rate. Subsequently the intermediate, the heat-treated intermediate and b nucleic acid were dissolved respectively in M/10 acetate buffer (pH 6.0) and the intrinsic viscosities of these substances were calculated from specific viscosities at various concentrations of these solutions. As seen from Figure 5(b), the specific viscosity of the intermediate solution and the dependence of this value upon concentration were greater than that of the heat-treated intermediate or b nucleic acid. These facts might indicate that the intermediate had an asymmetrical molecular form due to a double helical structure and was split by means of heat-treatment into b acid-like polynucleotides.

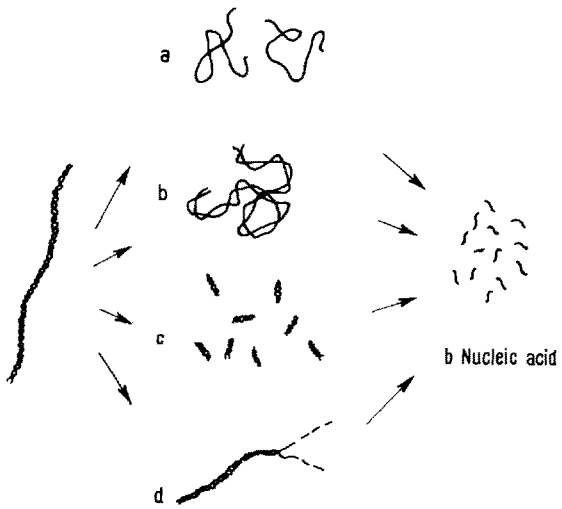

Fig. 1

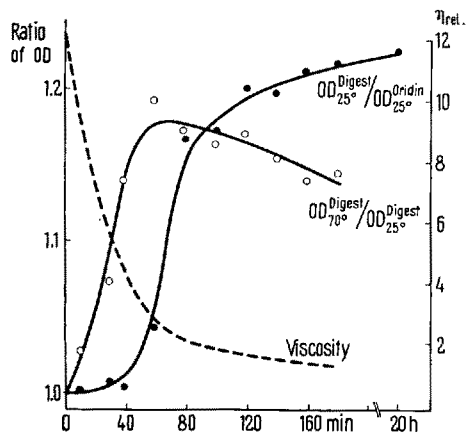

Fig. 2. $OD_{25°}^{digest}/OD_{25°}^{origin}$ means the ratio of optical densities at 25° of the digested DNA solution to the non-digested one. $OD_{70°}^{digest}/OD_{25°}^{digest}$ means, in digested DNA solution, the ratio of optical densities of the solution heated at 70° to the same solution maintained at 25°. The dotted line means the relative viscosity of the reaction mixture.

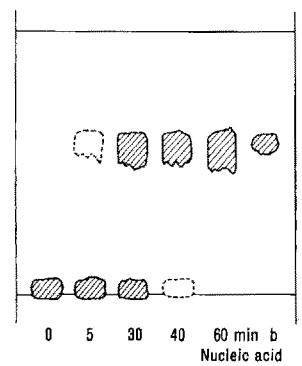

Fig. 3

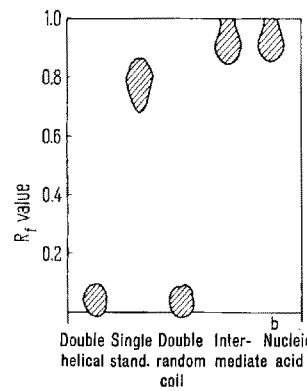

Fig. 4

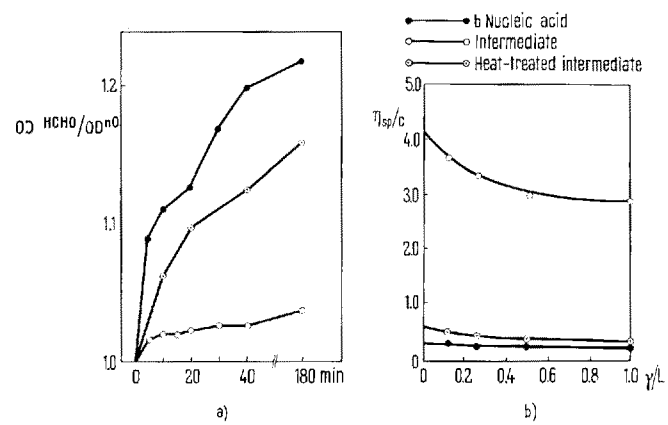

Fig. 5. OD^{HCHO}/OD^{no} means the ratio of optical densities of the DNA solution containing formaldehyde to the same solution not containing formaldehyde.

[7] K. MATSUWARA and Y. TAKAGI, Biochim. biophys. Acta *55*, 389 (1962).

[8] A. BENDICH and H. S. ROSENKRANZ, Arch. Biochem. Biophys. *94*, 417 (1961).

[9] H. FRANKEL-CONRAT, Biochim. biophys. Acta *15*, 306 (1954).

Furthermore, in order to ascertain this assumption, the intermediate, the heat-treated intermediate and b nucleic acid were dissolved respectively in 1% NaCl and the solutions obtained were analysed at 59,780 r.p.m. (259,700 × *g*) in a Hitachi model UCA-I type ultracentrifuge. The sedimentation constants obtained from the intermediate, the heat-treated intermediate and b nucleic acid were 6.2×10^{-13}, 4.2×10^{-13} and $3,9 \times 10^{-13}$ respectively. That is, by heat-treatment the sedimentation constant, 6.2×10^{-13} of the intermediate was altered into a value similar to that of b nucleic acid.

The results obtained above cannot be interpreted unless it is assumed that the intermediate is a small DNA constructed double-helically from two b nucleic acids. Accordingly, in the degradation of a large double helical DNA by DNase I, the DNA seems to be converted through c in Figure 1 into b nucleic acid.

Zusammenfassung. Es wird beobachtet, dass während der Depolymerisation von DNA mit DN-ase I zur kleinmolekularen DNA (b-Nukleinsäure), ein bemerkenswertes Zwischenprodukt mit physico-chemischen Eigenschaften der b-Nukleinsäure mit doppelter Helix auftritt.

M. Matsuda and K. Makino

Department of Biochemistry, Jikei University School of Medicine, Shiba, Minatoku, Tokyo (Japan),
June 13, 1963.

Observations on the Species-Nonspecificity of the Human Renal and Placental Basement Membrane Antigens

Antisera produced in the rabbit by heteroimmunization with rat or human kidney and placenta preparations are capable when labeled with fluorescein isothiocyanate of specifically 'staining' epithelial and mesenchymal basement membranes as well as other membranous and fibrillar structures of rat and human tissues, respectively[1-4]. It is thus apparent that these 'membrane antigens', of which probably six are present in the human kidney and placenta[4], are not specific to any one organ but rather are widespread in the body. In order to determine whether these antigens are also *heterogenic*[5], that is, 'widely distributed in nature without regard to degree of relationship', the fluorescent antibody technique of Coons and Kaplan[6] was utilized.

The preparation of anti-human kidney and anti-human placenta sera, their immunological examination and tagging with fluorescein isothiocyanate as well as the staining and control scheme are described in detail elsewhere[3,4]. The labeled antisera were twice absorbed with one tenth by volume of acetone extracted mouse liver powder and lyophilized human blood plasma. Kidney specimens of rabbits, guinea-pigs, rats, and mice were quick frozen within minutes after sacrifice, sectioned in a cryostat at 4 μ and incubated with one of the fluorescein labeled antisera. The incubation procedures used and the results obtained are summarized in the accompanying Table and compared with the results achieved by the same techniques on human tissue.

Fluorescence microscopical examination of kidney sections of the guinea-pig and mouse incubated with fluorescein labeled anti-human kidney or placenta serum revealed specific, bright greenish yellow fluorescence localized within the basement membranes of the glomerular capillaries, Bowman's capsules and tubules (Figure 1). In the control sections the glomeruli were barely discernible, whereas the tubules stood out due to their stronger nonspecific cytoplasmic fluorescence (Figure 2). It was noticed that a slight quantitative difference existed in the

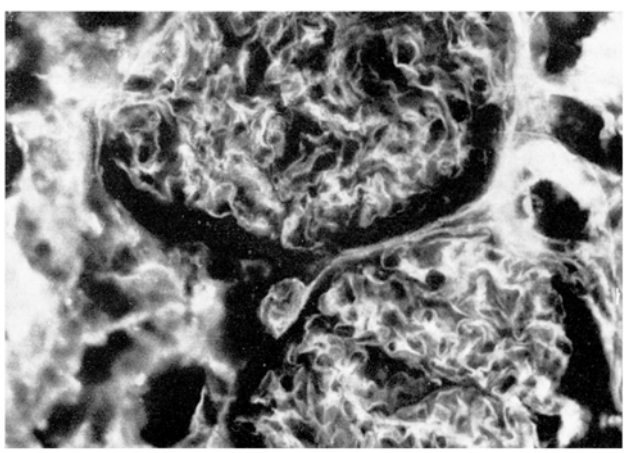

Fig. 1. Section of guinea-pig kidney incubated with fluorescein labeled anti-human kidney serum showing specific fluorescence within the basement membranes of the glomerular capillaries, Bowman's capsules and tubules. ×500.

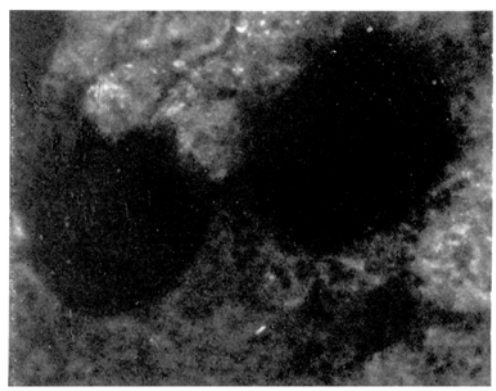

Fig. 2. Control section of guinea-pig kidney incubated with fluorescein labeled anti-human kidney antiserum absorbed with kidney. The two glomeruli are barely discernible and sharply contrast with the tubules, which stand out due to their pronounced nonspecific cytoplasmic fluorescence. × 125.

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[3] J. H. Boss, Lab. Invest. *12*, 332 (1963).
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[6] A. H. Coons and M. H. Kaplan, J. exp. Med. *91*, 1 (1950).