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.

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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

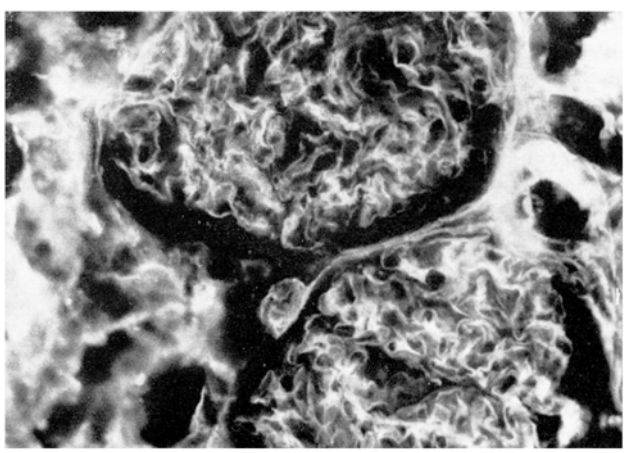

Fig. 1. Section of guinea-pig kidney incubated with fluorescein labeled anti-human kidney serum showing specific fluorescence within the basement membranes of the glomerular capillaries, Bowman's capsules and tubules. ×500.

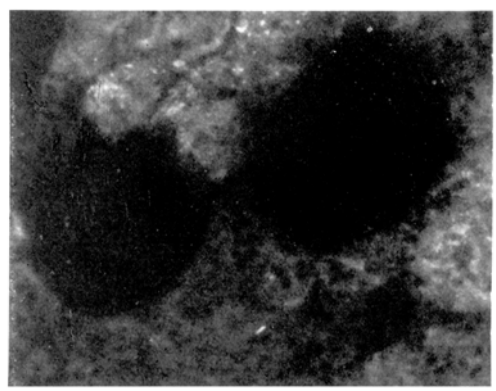

Fig. 2. Control section of guinea-pig kidney incubated with fluorescein labeled anti-human kidney antiserum absorbed with kidney. The two glomeruli are barely discernible and sharply contrast with the tubules, which stand out due to their pronounced nonspecific cytoplasmic fluorescence. × 125.

[1] B. Cruickshank and A. G. S. Hill, J. Path. Bact. *66*, 283 (1953).
[2] J. H. Boss and J. M. Craig, Amer. J. Path. *42*, 413 (1963).
[3] J. H. Boss, Lab. Invest. *12*, 332 (1963).
[4] J. H. Boss, Arch. Path., in press.
[5] E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry*, 2nd ed. (Charles C. Thomas, Springfield 1961).
[6] A. H. Coons and M. H. Kaplan, J. exp. Med. *91*, 1 (1950).

Results of staining and control procedures

Procedure	Human kidney	Rabbit kidney	Guinea-pig kidney	Rat kidney	Mouse kidney
Fluorescent anti-kidney serum	++	–	+	–	+
Fluorescent anti-placenta serum	++	–	+	–	+
Fluorescent anti-kidney serum[a] absorbed with kidney	–	–	–	–	–
Fluorescent anti-kidney serum[a] absorbed with placenta	–	–	–	–	–
Fluorescent anti-placenta serum[a] absorbed with placenta	–	–	–	–	–
Fluorescent anti-placenta serum[a] absorbed with kidney	–	–	–	–	–
Fluorescent anti-fibrinogen serum	–	–	–	–	–
Fluorescent anti-γ-globulin serum	–	–	–	–	–
Fluorescent normal rabbit serum	–	–	–	–	–
Untagged anti-kidney serum[b] and fluorescent anti-kidney serum	–	–	–	–	–
Untagged anti-kidney serum[b] and fluorescent anti-placenta serum	–	–	–	–	–
Untagged anti-placenta serum[b] and fluorescent anti-placenta serum	–	–	–	–	–
Untagged anti-placenta serum[b] and fluorescent anti-kidney serum	–	–	–	–	–
Untagged anti-fibrinogen serum[b] and fluorescent anti-kidney serum	++	–	+	–	+
Untagged anti-γ-globulin serum[b] and fluorescent anti-placenta serum	++	–	+	–	+

[a] In the absorption tests the tagged antiserum was absorbed with organ homogenate prior to its application.
[b] In the inhibition tests the sections were first incubated with untagged antiserum, washed in buffered saline and then incubated with fluorescent antiserum.

intensity of the specific fluorescence in so far as it was more pronounced in the human kidney than in either the guinea-pig or mouse kidney. The negative results of the control tests (*vide* Table) indicated that the specific fluorescence of the basement membranes of the guinea-pig and mouse kidneys was due to an immunological reaction. In order to further demonstrate the specificity of this reaction, the antisera were absorbed prior to incubation with either homogenized mouse kidney sediment (i.e. organ-homologous but species-heterologous material) or lyophilized rat liver powder (i.e. organ- and species-heterologous material). Whereas absorption with rat liver was without effect upon the resultant findings, absorption with mouse kidney brought about complete disappearance of all specific fluorescence. No specific fluorescence was observed in kidney sections of the rabbit and rat.

Antigens of animal material have been characterized by their organ, individual and species specificities[7]. However, the occurrence of cross-reactions with antigens from a number of unrelated species is well established[6]. BAXTER and GOODMAN[8] brought forth evidence for a cross-reaction between rat and mouse nephrotoxic serum antigens residing within the renal basement membranes. Further indication of the species-nonspecificity of the renal basement membrane antigens is furnished by STEBLAY and LEPPER[9] who produced nephritis in the dog by the injection of anti-human glomerular basement membrane serum. PIERCE et al.[10] in their paper on the histogenesis of basement membranes also reported on species cross-reactions of the mouse basement membrane antigens. Of interest in this connection are the findings of HENLE and CHAMBERS[11] who described in the 'heavy particle' material of homologous organs of many different species antigens which cross-reacted with several anti-organ sera. Our observations point out that one or more of the human renal and placental 'membrane antigens' is *heterogenic* in so far as in at least two other species the renal basement membranes are specifically stainable with fluorescent anti-human kidney and placenta serum. Apart from other considerations, the localization of the specific fluorescence in the basement membranes excludes the Forssman antigen as being responsible for this phenomenon[12]. Our failure to find specific fluorescence in the rabbit and rat kidneys does not rule out the presence of similar antigens in these species, particularly in view of the findings of BAXTER and GOODMAN[8] and HUNTER et al.[13]; it is quite possible that with the fluorescence microscope a positive reaction would be so weak as to be below the threshold of discernibility. The lesser intensity of specific fluorescence in the mouse and guinea-pig kidneys as compared to the human kidney may denote the existence of certain dissimilarities in the chemical structure of the homologous and heterologous antigens. This assumption is strengthened by the observations of HUNTER et al.[13] using Ouchterlony's agar gel diffusion technique. These investigators found that sera of rats isoimmunized with rat kidney antigen occasionally produce a precipitation band with rat, rabbit, and guinea-pig kidney antigens but without giving a reaction of identity; this indicates the existence of common as well as distinctive antigenic determinants in the kidneys of these species[14].

Zusammenfassung. Kaninchenantisera, mit Antikörpern gegen Basalmembranantigene menschlicher Niere und Plazenta, wurden, bei Anwendung der Coonsschen fluoreszierenden Antikörpermethode zur Bestimmung von heterogenen Antigenen in vier anderen Spezies, angewandt. Es wurde nachgewiesen, dass die glomerulären und tubulären Basalmembrane der Meerschweinchen- und Mausniere ein oder mehrere Antigene enthalten, welche mit Antihumannierenserum und Antihumanplazentaserum eine Kreuzreaktion zeigen.

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[7] E. L. TRIPLETT, J. Immunol. *89*, 505 (1962).
[8] J. H. BAXTER and H. C. GOODMAN, J. exp. Med. *104*, 467 (1956).
[9] R. W. STEBLAY and M. H. LEPPER, J. Immunol. *87*, 636 (1961).
[10] G. B. PIERCE, A. R. MIDGLEY JR., and J. SRI RAM, J. exp. Med. *117*, 339 (1963).
[11] W. HENLE and L. A. CHAMBERS, Nature *92*, 313 (1940).
[12] N. TANAKA and E. H. LEDUC, J. Immunol. *77*, 198 (1956).
[13] J. L. P. HUNTER, D. B. HACKEL, and W. HEYMANN, J. Immunol. *85*, 319 (1960).
[14] This investigation was supported by United States Public Health Service Grant No. H-6991.