Table I

Experiment No.	Dried on agar surface		Sprayed onto a collodion membrane	
	Phage-like %	Spherical %	Phage-like %	Spherical %
13	34.8 ± 6.1[a]	65.2 ± 6.1[a]	35.7 ± 5.9[a]	64.3 ± 5.9[a]
14	60.0 ± 6.2	40.0 ± 6.2	58.3 ± 6.2	41.7 ± 6.2
15	35.2 ± 5.8	64.8 ± 5.8	34.5 ± 5.7	65.5 ± 5.7

[a] Mean ± S.E.

Table II

Experiment No.	Phage-like %	Spherical %	Haematocrit values % L	% E
22 A	85.9 ± 3.8[a]	14.1 ± 3.8[a]	33.0	8.0
B	80.8 ± 2.1	19.2 ± 2.1	24.0	7.0
23 A	71.0 ± 0.9	29.0 ± 0.9	36.5	35.5
B	26.1 ± 12.0	73.9 ± 12.0	41.0	7.5
C	63.9 ± 8.7	36.1 ± 8.7	11.0	9.0
24 A	58.0 ± 11.0	42.0 ± 11.0	10.0	17.0
B	72.1 ± 3.8	27.9 ± 3.8	15.0	12.0

[a] Mean ± S.E.

membrane enveloping the whole particle (Figure 4) – the head as well as the tail – in contrast to the structure of the true tailed phages.

The shape of the virus particle should be considered as a function of the intimate relation of the virus to the functional state of the cell and its medium (the blood plasma).

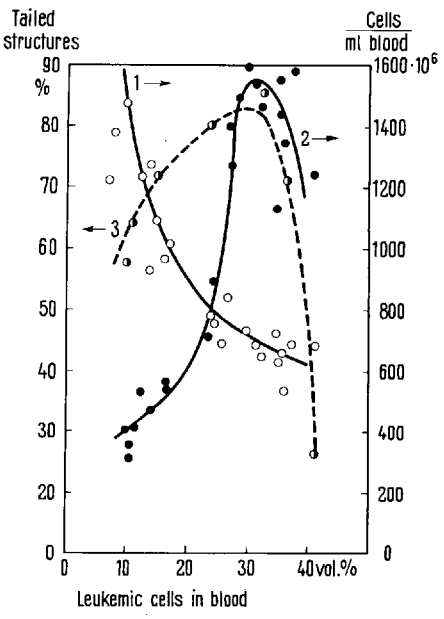

Fig. 3. Relation of the occurrence of phage-like forms in plasmas (curve 3–o) to the stage of the leukemic process. Curve 1–●: erythrocytes, curve 2–o: myeloblasts.

We are now investigating to what degree the phage-like structure, especially its tail, can be influenced *in vitro* under experimental conditions similar to those used in the study of contractile proteins and contractile biological structures.

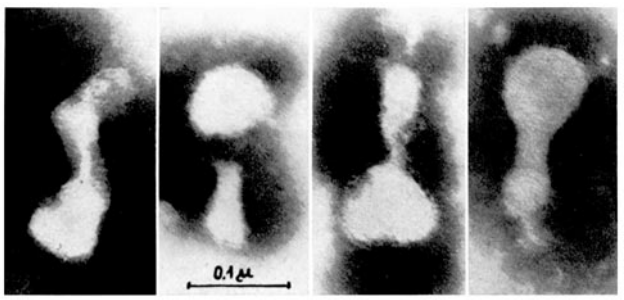

Fig. 4. Types of phage-like particles negatively stained.

Zusammenfassung. Für das Vorhandensein der spheroidalen und der phag-ähnlichen Form des Hühner-Leukosis-Virus (BAI, Stamm A) dürfte das Stadium des Leukämieprozesses, metabolische Eigenschaften der Myeloblasten sowie deren Milieu verantwortlich sein.

P. Bartl, J. Říman, and F. Šorm

Laboratory for Biochemical Investigation of Cancer, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague (Czechoslovakia), April 4, 1963.

Glomerular and Proximal Tubular Lesions Due to Inulin in Rabbits, as Seen by Electron Microscopy[1]

Different lines of evidence indicate that inulin represents the best substance used to measure glomerular filtration rate. The classical properties of this polysaccharide are extensively reviewed in Smith's fundamental monograph[2]. Among other characteristics inulin was found to be completely filtered out of the plasma, not handled by renal tubules and rapidly excreted.

However, Gayer[3], Frey[4], as well as Balint and Forgács[5], recently came to the conclusion that some storage of inulin occurs in dog's and rabbit's kidney. Our own investigations indicated that the substance is stored to a small extent by the renal parenchyma, at least under certain conditions[6].

Although inulin is said to be non-toxic and physiologically inert[2], the present experiments were designed to investigate whether this polysaccharide, in view of its physical chemical properties, might alter the ultrastructure of the nephron.

Material and Methods. Ten rabbits of both sexes were used, weighing 2.7 to 3.0 kg. Inulin, as a so-called 'purissimum', pyrogen-free solution, was administered in two ways:

(1) Five animals received 3 h intravenous perfusion, without anaesthesia, of a solution containing either 16 g/l or 34 g/l of inulin in saline. The infusion was preceded by a loading injection of 0.3 or 0.6 g inulin per kg body weight. The total amount of inulin thus administered varied from 1.5 to 2.5 g/kg body weight. The rate of infusion was less than 1 ml/mn and the total volume did not exceed 130 ml. Plasma concentrations of inulin were determined[7].

(2) Five other animals were given a daily intravenous injection of inulin, repeated for two weeks. The daily dose was either 0.5 g/kg body weight or 1.5 g/kg.

In three control rabbits, the effects of saline alone were observed. The following procedures were applied to kidneys removed 1 h after the end of perfusion for group 1 animals, and the day after the last injection for the second group of rabbits: for electron microscopy, specimens of tissue were fixed in buffered osmium tetroxide, with addition of 34 mg of sucrose per ml of fixative. The blocks were dehydrated in acetone and embedded in Vestopal W[8]. Phosphotungstic acid was used during dehydration to enhance contrast (1% PTA in 100% acetone). The ultrasections were done with a Porter-Blum ultramicrotome, and examined with a Zeiss EM 9 electron microscope.

Results. Standard histological examination of the kidneys did not reveal obvious lesions following administration of inulin.

With electron microscopy, the following changes were seen, which will be described more completely in a following publication[9]. The same type of lesion was observed in both groups of animals, although they were more pronounced in rabbits having received inulin for two weeks. There were first numerous macrophages in capillary loops, with vacuoles containing an electron dense, finely granular material. Endothelial cells were enlarged and showed disappearance of their pores; certain of these cells contained an increased number of organelles (Figure 1). Numerous blebs or 'balloons' of endothelial origin were seen in the capillary lumens; occasionally loops presented with clumps of platelets. There was no obvious alteration

[1] Aided by grants from J. R. Geigy AG, Basel, Switzerland, and from the Fonds National Suisse de la Recherche Scientifique (Crédit No. 2364).

[2] H. W. SMITH, *The Kidney, Structure and Function in Health and Disease* (Oxford University Press, New York 1951).

[3] J. GAYER, Klin. Wschr. *35*, 568 (1957).

[4] J. FREY, Klin. Wschr. *36*, 11 (1958).

[5] P. BÀLINT and I. FORGÀCS, Acta physiol. hung. *15*, 15 (1959).

[6] A. FALBRIARD, G. SCHALLER, G. SIMON, and R. ZENDER, to be published.

[7] J. H. ROE and N. P. GOLDSTEIN, J. biol. Chem. *178*, 839 (1949).

[8] A. RYTER and E. KELLENBERG, J. Ultrastruct. Res. *2*, 200 (1958).

[9] G. SIMON and A. FALBRIARD, to be published.

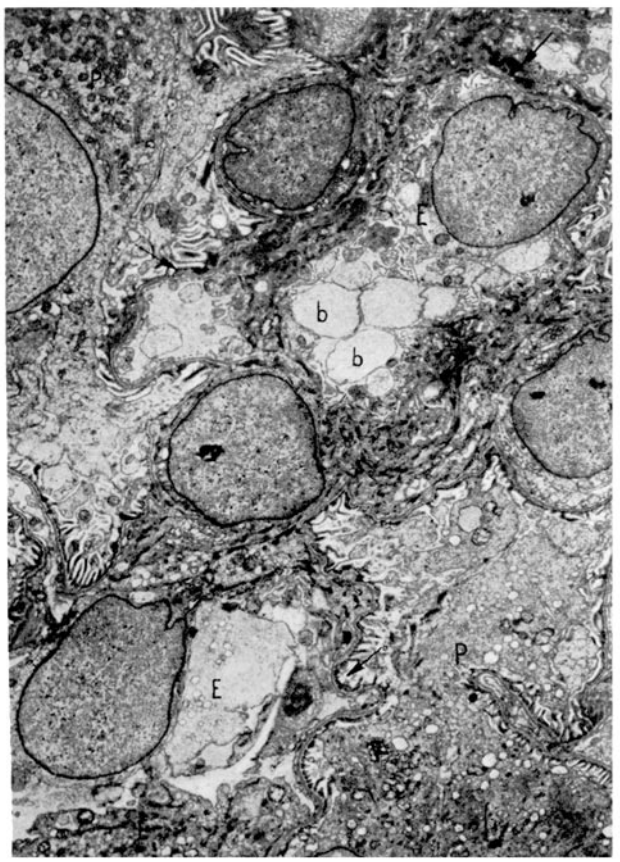

Fig. 1. Glomerulus. Rabbit from group II experiments: daily intravenous injection of 0.5 g/kg inulin for two weeks. Endothelial cells (E) are enlarged and contain many organelles; 'balloons' (b) of endothelial origin tend to obliterate the capillary lumen. Epithelium (P) is also enlarged and presents with numerous vacuoles. Foot-processes are fused in some areas (arrows). Magnification: ×5000.

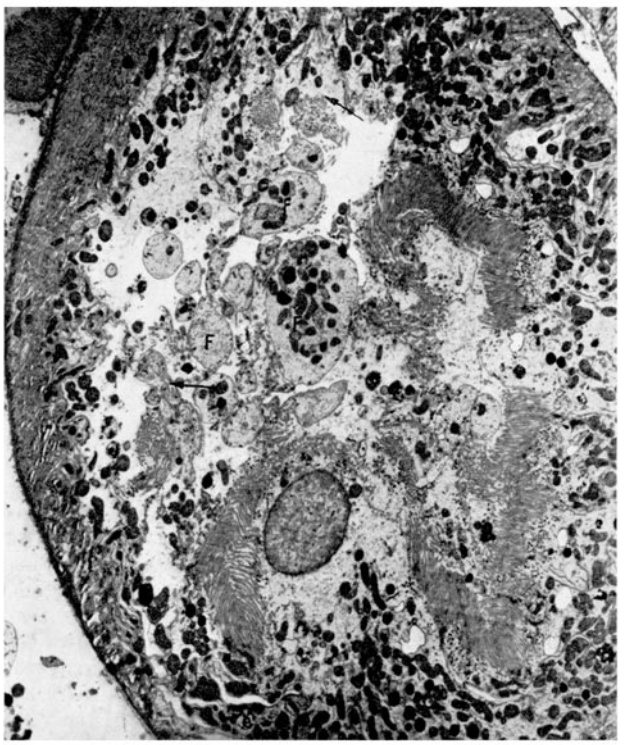

Fig. 2. Proximal tubule. Rabbit from group I experiments: a 3 h perfusion (total amount of inulin given in this case: 2.5 g/kg). Numerous cellular fragments (F) are seen in the tubular lumen, resulting from an apical necrosis (potocytosis). Note the disappearance of the brush border (arrows). ×5000.

of basement membrane in the first group of animals, at most a moderate widening of this structure in the second group. No basal-like, fibrine or fibrinoïd deposits were seen. The epithelial cells were enlarged and showed fusion of the foot processes (Figure 1); at these sites there was some densification of the cytoplasm. An increased number of organelles was encountered, as well as vacuoles containing an electron dense material.

In the proximal convoluted tubules, beside many abnormal, dense inclusions, necrotic cells were seen, with destruction of the brush border. There were numerous cellular fragments in the lumens, pointing to an apical necrosis (Figure 2).

Control rabbits did not show any definite lesions.

In the first group of animals, plasma concentrations of inulin ranged from 36 to 62 mg/100 ml.

Discussion. The present experiments show that inulin produces in rabbits definite alterations of glomerular as well as proximal tubular ultrastructure. The most striking of them were: presence of numerous macrophages in the capillary lumens, endothelial tumefaction and hyperactivity, enlargement of epithelial cells with fusion of foot processes; necrosis of the proximal convoluted tubules. When the substance was given daily for two weeks, the same type of lesion was encountered, but more pronounced.

It should be pointed out that these changes were produced by doses of inulin below the maximal amounts which have been given to man or animals. Plasma levels as large as 565 mg/100 ml have been reported in dog, and 400 mg/100 ml in man[2].

The pathogenesis of the ultrastructural lesions, shown in the present study, remains conjectural and it would be premature to attempt any comparison with other experimental or spontaneous alterations. A first approach to an understanding of changes due to inulin, however, should refer to the physical chemical properties of this polysaccharide. Inulin forms supersaturated solutions, liable to precipitate in certain conditions. Its molecular weight exceeds 5000, which results in low diffusibility. Moreover, the diffusion coefficient is considerably less than would be expected, due to an elongation of the molecule, and is equivalent to a molecular weight of approximatively 15,000.

The present findings indicate that the more or less complete filtrability of a substance does not exclude the possibility of glomerular as well as tubular alterations. The above-mentioned small intrarenal storage of inulin might bear some relationship to these alterations. The tendency to precipitate is favoured in the tubules by the process of urine concentration. However, one cannot rule out some transient storage of inulin in the glomeruli, where cellular reactions to the polysaccharide seem to occur. Further studies should definitely establish whether inulin administration is contraindicated.

Résumé. La microscopie électronique a permis d'observer des lésions du glomérule et du tube proximal, après administration intraveineuse d'inuline chez les lapins: nombreux macrophages dans les anses glomérulaires, tuméfaction endothéliale, tuméfaction épithéliale avec soudure des pédicelles; nécrose des cellules épithéliales du tube proximal.

La poursuite de cette étude devrait établir définitivement si l'emploi de l'inuline est contre-indiqué dans l'exploration fonctionnelle rénale.

A. Falbriard, G. Schaller, and G. Simon

Clinique Universitaire de Thérapeutique, Hôpital Cantonal Genève et Départements d'Histologie et de Pathologie de l'Université de Genève (Switzerland), September 16, 1963.

The Osmiophilic Granules of the Pineal Body in Rats

The presence of osmiophilic granules in the cells of the pineal body has been reported by some authors (Gusek and Santoro[1]; Pellegrino, De Iraldi, and De Robertis[2]; Clementi, Fraschini, Muller, Ornesi, and Zanoboni[3]) and has been considered as evidence of secretory activity. This paper reports the results of an histological and histochemical study performed on the pineal body of young and adult rats.

Material and Methods. 30 Wistar male rats were divided into two groups. The first was formed by animals 20–30 days old and the second one by rats 120–180 days old. The pineal bodies were removed.

Five glands of each group were fixed in osmium tetroxide buffered solution at pH 7.2 according to Palade[4] and embedded in a mixture 9:1 of *n*-butyl and *n*-ethyl methacrylate. Sections 700–2000 Å thick were cut on a Porter Blum type microtome equipped with a glass knife. Part of the sections was examined with a phase-contrast microscope according to the Fabbrini and Giacomelli technique[5], another part was stained with PASM[6] and observed with phase-contrast and light microscopes.

Five glands of each group were fixed in Bouin solution and embedded in paraffin and beeswax mixture. Sections 0.5–1 μ thick were stained with PASM, PAS, and hematoxiline and observed in a light microscope[9].

Five pineal bodies of each group were fixed in 10% calcium-formalin solution and embedded in a 9:1 *n*-butyl and *n*-ethyl methacrylate. The sections, 700–2000 Å thick, were examined in a phase-contrast microscope[10].

[1] W. Gusek and A. Santoro, Endokrinologie *41*, 105 (1961).
[2] A. Pellegrino, E. De Iraldi, and E. De Robertis, Exper. *17*, 122 (1961).
[3] F. Clementi, G. Fraschini, E. Muller, A. Ornesi, and A. Zanoboni, Atti Accad. Med. Lomb. *17*, 209 (1962).
[4] G. E. Palade, J. exper. Med. *95*, 285 (1952).
[5] A. Fabbrini and F. Giacomelli, Z. wiss. Mikrosk. *64*, 130 (1960).
[6] Silver methenamine staining (Jones' technique[7,8]).
[7] D. B. Jones, Amer. J. Path. *29*, 33 (1953).
[8] D. B. Jones, Amer. J. Path. *95*, 313 (1957).
[9] The light micrographs were taken with a Leitz-Ortolux microscope equipped with wide range lenses (objective: CPI, 40:1, 170/0,17; ocular, Periplan ×8).
[10] For the phase-contrast observation Leitz lenses were used (objective Pv Fl Oel, 70:1, 170/0,17; ocular, Periplan ×8; condenser for phase-contrast in black and white and colour).