

the electrophoretic pattern, a progressive loss in enzyme activity which is more pronounced for the fast component of the original pattern and for its duplicated form.

The differential liability of the individual electrophoretic component of RBC acid phosphatases to the inactivation following treatment with GSSG, is further substantiated by a series of experiments with 2-mercapto-ethanol. This reagent added to an hemolysate incubated with GSSG prevents or induces a reversion of the duplication of the original acid phosphatase pattern that GSSG produces when alone, but not of the fading of the faster component of the original pattern (see Figure 2)⁶.

Experiments on the incubation of erythrocytes with APH. With a series of earlier experiments⁴ we have shown that: (1) the incubation of erythrocytes with APH in the absence of glucose induces a modification of the electrophoretic pattern of RBC acid phosphatases very similar to the one obtained by incubating the hemolysates with GSSG; (2) no alteration of the electrophoretic pattern could be appreciated when the incubation was carried out in the presence of glucose.

However, we have later realized that the protective effect of glucose is not complete when the incubation is continued for a long time (± 5 h). In this case, while the slower component of the original pattern remains unchanged, the faster one disappears almost completely, as is shown in Figure 3.

We feel that this phenomenon is of the same nature as the one observed in the experiments on the incubation of hemolysates with GSSG; that is to say, that the slower components of acid phosphatases are more stable than the faster ones towards an oxidative agent such as APH.

The results of these 2 series of experiments suggest a different liability of the acid phosphatase components to treatment with GSSG or APH; the slow fractions of the various phenotypes appearing as if they were more stable than the faster ones.

It is tantalizing to evaluate these observations against the quantitative and qualitative polymorphism of RBC acid phosphatases. One wonders whether there is any connection between the observed higher stability of the slower acid phosphatase components and the differences reported by SPENCER et al.⁷ in the overall activity of the different acid phosphatase phenotypes.

From a more general standpoint, one wonders whether the differential liability of isoenzyme fractions towards toxic agents could result in a differential fitness in favour of the genotypes bearing the most stable combination of isoenzymes⁸.

Riassunto. Le frazioni che costituiscono il normale quadro elettroforetico delle fosfatasi acide eritrocitarie mostrano una differente resistenza al trattamento con glutazione ossidato o con acetilfenilidrazina: le frazioni lente dei genotipi studiati sono apparse infatti più stabili.

E. BOTTINI, G. MODIANO,
L. BUSINCO, and G. FILIPPI
with the technical assistance of
C. SANTOLAMAZZA

*Gruppo di Ricerca «Commissione per la Genetica» del
C.N.R., Istituto di Genetica e Clinica Pediatrica
dell'Università di Roma (Italy),
September 5, 1966.*

⁶ A more detailed account on the protective effect of 2-mercapto-ethanol on RBC acid phosphatases will be presented elsewhere.

⁷ N. SPENCER, D. A. HOPKINSON, and H. HARRIS, *Nature* 201, 299 (1964).

⁸ Acknowledgment: We wish to express our gratitude to Prof. M. SINISCALCO and to Prof. D. CAVALLINI for their helpful criticism and for having read the manuscript before publication.

Monoaminergic Innervation of the Kidney. Aorticorenal Ganglion - A Sympathetic, Monoaminergic Ganglion Supplying the Renal Vessels

The aorticorenal ganglion is a small ganglion situated in the angle between the aorta and the renal artery. MAILLET¹ has described various lesions of the renal parenchyma after the chemical destruction of this ganglion. Afferent fibres run from the greater splanchnic nerve; efferent ones supply the renal plexus surrounding the renal artery (MITCHELL²). The ganglion sends filaments also to the mesenteric plexus. In the dog this ganglion is situated under the lower border of the suprarenal gland. After the removal of the left renal vein, we may find the ganglion when preparing the branches of the renal plexus which surround the renal artery. The ganglion was extirpated from 8 dogs on the left side and examined histologically. 6-8 days after the operation, the dogs were killed and the vegetative abdominal plexus of each animal prepared anatomically. In 6 cases the connection of the greater splanchnic nerve with the renal plexus was destroyed entirely; in the remaining 2 cases (dogs Nos. 4 and 6) the situation was different: in the vicinity of the

examined ganglion a thin nerve branch was found which connected the mentioned vegetative nerves.

The left and right kidney of each dog were examined histologically. The silver impregnation techniques according to Bielschowski-Jabonero (after my own modification) and that of Bodian were used in all 8 cases. In 3 cases (dogs Nos. 6, 7 and 8) we also employed the histochemical fluorescence method according to FALCK³. A number of authors (FALCK⁴, MALMFORS⁵, and DAHLSTRÖM et al.⁶) have proved the high specificity of this method for catecholamines in ganglion cells in vegetative nerve terminals.

¹ M. MAILLET, *Acta neuroveg.* 20, 337 (1960).

² G. A. G. MITCHELL, *Cardiovascular Innervation* (E. S. Livingstone Ltd., London 1956).

³ B. FALCK and CH. OWMAN, *Acta univ. lund.*, Sect. II, 7, 1 (1965).

⁴ B. FALCK, *Acta physiol. scand.*, Suppl. 197, 56, 1 (1962).

⁵ T. MALMFORS, *Acta physiol. scand.*, Suppl. 246, 64, 1 (1965).

⁶ A. DAHLSTRÖM, K. FUXE, and N. A. HILLARP, *Acta pharmac. tox.* 22, 277 (1965).

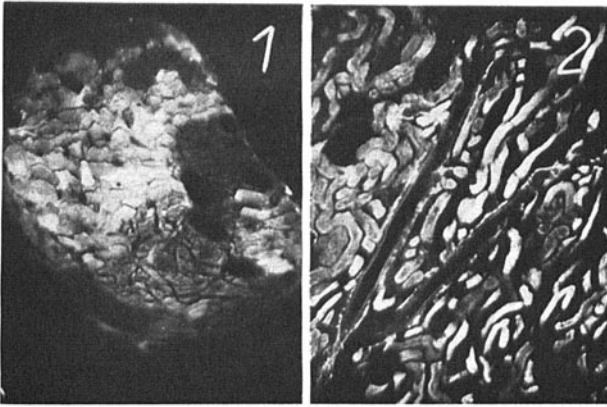


Fig. 1. Ganglion aorticorenale of the dog (fluorescence method).

Fig. 2. Normal kidney (dextra) of the dog (fluorescence method). Interlobular arteries with numerous monoaminergic nerve terminals.

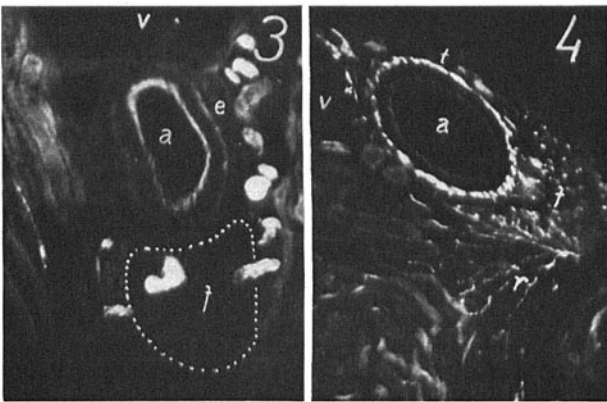


Fig. 3. Denervated kidney (sinistra) of the dog (fluorescence method). (a) = interlobar artery with the internal elastic layer; (e) = external elastic layer without nerve terminals; (f) = fibrous skeleton without nerve terminals; (v) = vein without nerve terminals.

Fig. 4. Normal kidney (dextra) of the dog (fluorescence method). (a) = interlobar artery with the internal elastic layer; (t) = nerve terminals on the surface of the media; (f) = fibrous skeleton with nerve terminals; (r) = vasa recta with nerve terminals; (v) = vein; (*) = nerve terminal in the venous wall.

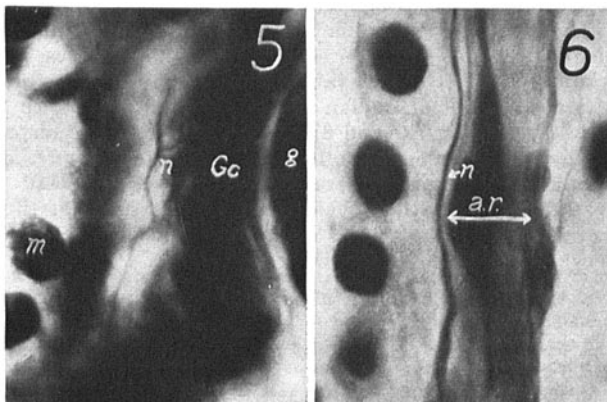


Fig. 5. Denervated kidney (sinistra) of the dog (Bielschowski-Jabonero). Vascular pole of the glomerulus with the nerve fibre. (g) = glomerulus; (Gc) = Goormaghtigh cells; (n) = nerve fibre; (m) = macula densa.

Fig. 6. Denervated kidney (sinistra) of the dog (Bielschowski-Jabonero). (ar) = arteria recta medullaris spuria; (n) nerve fibre.

The method of FALCK was performed according to the following schedule: The tissue was quenched in liquid propan, lyophilized, condensed with paraformaldehyde at 80°C, embedded in paraffin, and examined with a fluorescence microscope (mercury high-pressure bulb HBO 50, filters Schott BG.12/4, GG.11/1).

Results. With the fluorescence technique a great number of monoaminergic ganglion cells were found in the aorticorenal ganglion, which must therefore be regarded as a sympathetic prevertebral one (Figure 1).

Denervated left kidney: results obtained with the histochemical fluorescence method. Throughout the whole kidney the monoaminergic nerves terminating on the surface of the media of arteries, on the vasa recta, on the veins, in the fibrous skeleton of the kidney, and in the muscular part of the pelvic wall (DOLEŽEL^{7,8}) showed complete degeneration (Figures 2-4). Only a very small number of nerve terminals was found, especially on the arteries. In some parts of the kidney of dog No. 6, the

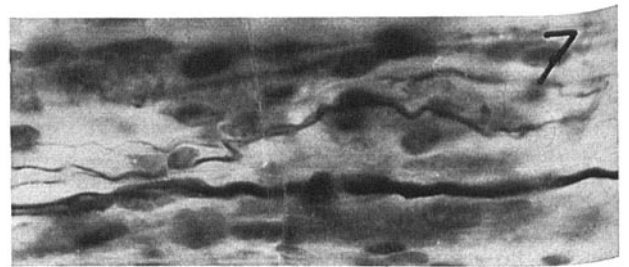


Fig. 7. Normal kidney (dextra) of the dog (Bielschowski-Jabonero). Bundle of the vasa recta with thick and thin nerves.

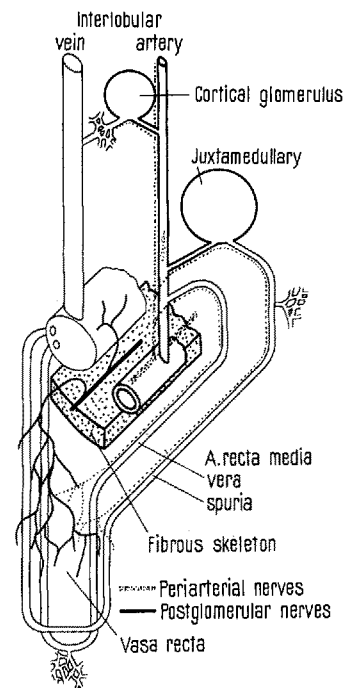


Fig. 8. Diagram of the innervation of the kidney.

⁷ S. DOLEŽEL, Čslká Morf. 2, 20 (1954).

⁸ S. DOLEŽEL, Folia morph., Praha 14, 168 (1966).

number of intact nerve fibres was considerably greater than in the kidneys of the other dogs.

Denervated left kidney: results obtained with impregnation techniques. Extensive reduction of periarterial nerves was found in all 8 cases. The motor plexus on the surface of media especially was nearly completely degenerated. Wrinkled Schwann cell nuclei with remnants of degenerated axons were found in the vicinity of the arterial wall. No degeneration was found in the myelinated fibres which are to be found in nerves surrounding large arteries. Only in dogs Nos. 4 and 6 (incomplete denervation) was the reduction of periarterial nerves less pronounced than in other cases. As to the efferent arteriole, no degeneration was observed on its fine nerve running from the afferent to the efferent arteriole through the juxtaglomerular apparatus. In the vicinity of the glomerulus this fibre is situated between the macula densa and the Goormaghtigh cells. The innervation of the efferent arteriole was found intact both in the cortical and in the juxtamedullary glomeruli (Figures 5, 6). In the latter, these fibres innervated the vasa recta. The fibres accompanying the efferent arterioles of the cortical zone ended not far from the glomeruli. Considerable reduction of all nerves was found in the fibrous skeleton of the kidney and in the vasa recta as well. Among the parallel fibres accompanying the vasa recta the thick ones showed complete and the thin irregular ones considerable degeneration. Only in case No. 4 was a small number of thick fibres found in the kidney (Figures 7 and 8).

Normal right kidney: results obtained with both methods. In the right kidney no reduction of nerves was

found either with the silver impregnation or with the fluorescence techniques. No monoaminergic nerve terminals were found in the efferent arteriole. According to the results obtained, the aorticorenal ganglion supplies the renal arteries, veins, vasa recta and the pelvic wall with sympathetic monoaminergic nerves⁹.

Zusammenfassung. Bei Hunden wurde das aorticorenale Ganglion der linken Seite herausgenommen und die Niereninnervation mit der Silberimprägnations- und der histochemischen Fluoreszenzmethode auf Monoamine nach FALCK untersucht. Auf Grund der Degenerationserscheinungen kann geschlossen werden, dass das Ganglion die Nierenarterien, das motorische Nervenengeflecht an der Oberfläche der Media, die vasa recta und Nierenbeckenwand mit adrenergischen sympathischen Nervenfasern versorgt.

S. DOLEŽEL

*Institute of Normal and Pathological Physiology,
Slovak Academy of Sciences, Bratislava (Czechoslovakia),
July 20, 1966.*

⁹ Thanks are due to Mrs. A. Svobodová for technical assistance, to Prof. J. VAŠKŮ, Head of the Institute of Pathological Physiology, University of Brno for permission to use the laboratory equipment, and to Dr. J. TOMČEK for help in the surgical treatment of dogs.

Ultrastructure of a Microorganism Associated With Bovine Platelets

Particles, presumably representing a previously undescribed microorganism, were discovered on the platelets of a splenectomized calf¹. The predominant form of the organism in Giemsa-stained smears was a delicate ring with a suggestion of light-microscopic resemblance to eperythrozoon. Temperature rise was the only clinical symptom associated with peak occurrence of the agent particles. Subsequently, the agent has been passaged in 3 splenectomized calves.

The present report describes the main features observed in the ultrastructure of the agent. Blood from one of the splenectomized calves at the height of the reaction provided the material. The blood sample was processed and electron microscopy carried out as described by TUOMI and BONSDORFF², with the exception that EDTA was used as anticoagulant.

Agent particles, often in large numbers, were observed on most of the platelets (Figure 1). The number of particles on individual platelets varied widely. They seemed to be loosely attached to the platelets in general. No indications of phagocytosis of the particles by platelets were observed.

The agent exhibited pleomorphism (Figures 1 and 2). Most frequently, they were basically round yet irregular forms up to 0.4 μ in diameter. Elongated forms, up to 1.5 μ in length, were also frequently seen. They had mostly one or several constrictions. Their shape appar-

ently reflects 2 modes of reproduction: division into 2 roughly equal parts, and chain division. Evidence of budding of smaller particles was also noted.

The agent particles had no cell wall; they were bounded only by a plasma membrane (Figure 2). The inner structure presented ribosome-like granules in a ground substance of lower density. No definite nucleoid areas were observed.

In several platelet vacuoles, roundish bodies, some of them presenting very high density, were observed (Figure 1). Part of the platelets showed signs of degeneration such as disappearance of granules and reduced density.

Obviously, no very definite standpoint can be taken as regards the taxonomic position of this agent associated with bovine platelets until the question has been elucidated by results from studies employing other methods, but some clues are offered by the present findings. The size of the agent particles, their lack of cell wall, and their disorganized mode of reproduction suggest some resemblance with mycoplasmas; the first 2 characteristics also speak in favour of relationship to *Eperythrozoon* and *Haemobartonella*³.

¹ J. TUOMI, *Experientia* 22, 458 (1966).

² J. TUOMI and C.-H. v. BONSDORFF, *J. Bact.*, in press.

³ H. TANAKA, W. T. HALL, J. B. SHEFFIELD, and D. H. MOORE, *J. Bact.* 90, 1735 (1965).