

containing several-week old males emitted the pleasant odour associated with the acetates those containing females of a similar age did not.

The results raise for consideration the possibility that the acetates supplied by the tubular glands are the precursors of the aldehydes stored in the median reservoir, the role of the accessory glands being to provide the necessary mechanism. It accords well with this suggestion that if the tubular glands are blocked or removed the median reservoir fails to fill up with secretion⁶. To explain the fact that the scent released by mature male insects contains an appreciable quantity of unmodified tubular gland scent it is necessary to suppose only that the tubular gland and median reservoirs are emptied simultaneously.

Working on a pentatomid bug, *Nezara viridula*, GILBY and WATERHOUSE¹¹ found that in the tubular gland extracts the concentration of decenyl acetate was much higher and decenal much lower than in the scent stored in the median reservoir. They suggested that the aldehydes are formed in the median reservoir. It has also been reported that the tubular scent glands of giant water bugs

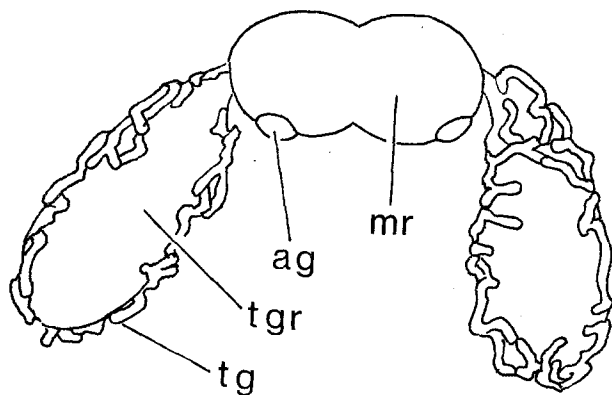
of the genus *Lethocerus* secrete largely hexenyl acetate¹²⁻¹⁴ and it is of considerable interest that as in *O. fasciatus* the glands are sexually dimorphic. In *Lethocerus* spp, however, the male glands are very much larger than the female and a separate reservoir with accessory glands is lacking.

It has been suggested that the sexual dimorphism in the tubular scent glands of Lygaeid bugs such as *O. fasciatus* is a physiological necessity somehow connected with the fact that the males are smaller than the females¹. It has also been suggested that because *O. fasciatus* is gregarious it has no need for specialized mechanisms for sound or scent production, or for courtship, for one sex to find the other¹⁵. We suggest that the male specific acetates have an as yet undiscovered role to play in the sexual activities of the adults¹⁶.

Zusammenfassung. Die Metathorakalduftdrüsen der Landwanze *Oncopeltus fasciatus* erzeugen als Hauptkomponenten ungesättigte aliphatische Acetate und Aldehyde. Die röhrenförmigen Duftdrüsen, die Acetate erzeugen, und das Vorkommen dieser Acetate im Duftsekret sind für die Männchen spezifisch.

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Scent gland complex of mature male *Oncopeltus fasciatus* (after JOHANSSON, 1957). ag, accessory gland; mr, median reservoir; tg, tubular gland; tgr, tubular gland reservoir.

¹¹ A. R. GILBY and D. F. WATERHOUSE, *Nature*, Lond. 216, 90 (1967).

¹² A. BUTENANDT and N. TAM, *Hoppe-Seyler's Z. physiol. Chem.* 308, 277 (1957).

¹³ G. PATTENDEN and B. W. STADDON, *Ann. ent. Soc. Am.* 63, 900 (1970).

¹⁴ B. W. STADDON, *J. ent. Soc. S. Engl. A*, 46, 69 (1971).

¹⁵ W. LOHER and H. T. GORDON, *Ann. ent. Soc. Am.* 61, 1566 (1968).

¹⁶ This work is supported by the Science Research Council. We thank Professor A. H. JACKSON for facilities and Dr. D. S. MILLINGTON for assistance with GC-MS.

Intracellular Enzymes in Renal Lymph as a Measure of Anoxic Injury of the Kidney

Anoxic damage to the renal tissue is a major problem in traumatic shock and is one of the difficulties encountered in kidney transplantation. It seemed reasonable to look for the signs of cellular injury ensuing after interruption of blood flow to the kidney in the intercellular fluid, or at least in the part which is accessible for collection and study, i.e. in the lymph. The present report describes the biochemical changes which occurred in the lymph collected in the renal hylum of dogs after a transient occlusion of the renal artery.

Material and methods. In dogs under pentobarbitone general anaesthesia a lymph vessel in the hylum of the left kidney was cannulated. Urine was collected through plastic catheters introduced into both ureters. After preliminary lymph collection, the renal arteries were clamped for 30 min or 2 h. Lymph and urine were again collected after the release of the clamp for 1 h.

The concentrations of the following 8 intracellular enzymes were estimated according to the methods listed in the references: lactate dehydrogenase (LDH)¹ maleic

acid dehydrogenase (MDH)² GOT and GPT³ glutamic acid dehydrogenase⁴ acid-⁵ and alkaline phosphatase⁶ and leucinarylamidase (AA)⁷. All results are expressed in international units, corresponding to 1 μ Mol of transformed substrate per min at 25 °C.

It is assumed that LDH, MDH and GOT are exclusively or mostly present in the cytoplasm, alkaline phosphatase,

¹ F. WROBLEVSKY and J. S. LA DUE, *Proc. Soc. exp. Biol. Med.* 90, 210 (1955).

² J. KING and M. B. MORRIS, *Archs Dis. Childh.* 36, 604 (1961).

³ W. W. UMBREIT, G. K. KINGSLEY, R. R. SCHAFFERT and H. SIPLET, *J. Lab. clin. Med.* 49, 454 (1957).

⁴ E. SCHMIDT and F. W. SCHMIDT, *Klin. Wschr.* 40, 962 (1962).

⁵ A. L. BABSON and G. E. PHILLIPS, *Clin. chim. Acta* 13, 264 (1962).

⁶ K. WALTER and C. SCHÜTT, in *Methoden der enzymatischen Analyse*, 2nd edn. (Ed. U. U. BERGMAYER; Akademie Verlag, Berlin 1970), vol. 1, p. 818.

⁷ C. A. BRATTON and E. K. MARSHALL, *J. biol. Chem.* 128, 537 (1939).

Table I. Biochemical composition of plasma and renal lymph 1 h after a 30 min ischaemia

	Plasma		Lymph	
	Control	After ischaemia	Control	After ischaemia
Protein (mg/ml)	55.5 \pm 1.70	56.5 \pm 1.8	32.7 \pm 1.0	33.3 \pm 1.2
K (maequ/l)	4.46 \pm 0.41	4.82 \pm 0.39	4.53 \pm 0.12	4.33 \pm 0.14
LDH (mU/ml)	75.2 \pm 20.0	148.5 ^a \pm 16.0	70.6 \pm 19.6	526.2 ^b \pm 41.2
MDH (mU/ml)	64.7 \pm 13.4	146.3 \pm 32.1	132.2 \pm 29.2	748.6 ^a \pm 68.6
GOT (mU/ml)	8.5 \pm 1.6	8.1 \pm 1.0	4.6 \pm 1.0	11.7 ^a \pm 2.8
GPT (mU/ml)	4.1 \pm 0.8	6.7 \pm 0.7	4.2 \pm 1.1	4.4 \pm 1.3
GDH (mU/ml)	3.4 \pm 0.5	2.6 \pm 0.7	2.7 \pm 0.5	3.3 \pm 0.6
AA (mU/ml)	9.4 \pm 1.1	8.8 \pm 1.2	3.6 \pm 1.1	3.5 \pm 1.1
alk P (mU/ml)	27.7 \pm 4.6	25.4 \pm 4.3	10.1 \pm 2.6	13.4 \pm 3.0
Ac P (mU/ml)	4.1 \pm 0.6	6.2 \pm 0.4	0.6 \pm 0.4	1.9 \pm 0.8
C _k	25.5 \pm 4.2	5.0 ^b \pm 1.7		

Averages of 13 experiments. ^a Significant change $p < 0.05$. ^b Significant change $p < 0.01$ – 0.001 .

GPT and GDH in the mitochondria, AA in the microsomes and acid phosphatase mainly in the lysosomes^{8–11}.

Protein concentration in lymph was estimated by the biuret method¹² and potassium by flame photometry. Glomerular filtration rate was calculated from endogenous creatinine clearance.

Results. The clamping of the renal artery for 30 min produced a profound acute decrease in urine flow and glomerular filtration. In renal lymph the concentrations of the cytoplasmic enzymes LDH, MDH and GOT increased considerably. LDH concentration increased significantly also in circulating (arterial blood) plasma.

No changes were observed in the activity levels of the enzymes associated with intracellular organelles (GPT, GDH, AA, alk. P and ac.P) (Table I). A 2 h renal ischaemia produced an even more marked increase of the level of the cytoplasmic enzymes in renal lymph. There was actually a 20fold increase of LDH-concentration and a 5fold increase of GOT-concentration. Plasma concentration of both enzymes rose also significantly. A marked (nearly 3fold) increase of the concentration of the mitochondrial enzyme alkaline phosphatase occurred in the renal lymph, and the lysosomal enzyme acid phosphatase, previously almost absent in renal lymph, appeared in well measurable concentration. There was a small, statistically not significant increase in the lymphatic concentration of leucine arylamidase, an enzyme commonly held to be localized in the cells of proximal tubuli and associated with the microsomal fraction (Table II).

Discussion. During acute injury there is an escape of intracellular enzymes from the injured tissue^{8,13,14}. It seems however that, at least as long as the injury is not too severe, the enzymes may escape from cells which are still functional, but the membrane permeability has been increased by the injury. A more severe injury obviously leads to the complete breakdown of the damaged cells.

After a renal ischaemia lasting 30 min in renal lymph there is a marked increase of cytoplasmic enzymes but practically no change in the concentration of the lysosomal or mitochondrial enzymes. Accordingly, this phase is probably characterized by protein leakage from the cells without a severe damage to their organelles. A more prolonged ischaemia, lasting 2 h, leads not only to a more pronounced rise of the cytoplasmic enzymes in lymph and plasma, but also to a significant increase in the lymphatic

⁸ B. HESS, *Enzymes in Blood Plasma* (Academic Press, London 1963).

⁹ G. H. HAGEBOM and W. C. SCHNEIDER, *J. biol. Chem.* **204**, 233 (1953).

¹⁰ H. HANSON, H. J. HÜTTER, H. G. MANNSELT, K. KRETSCHMER and C. SOHR, *Hoppe-Seyler's Z. physiol. Chem.* **348**, 680 (1967).

¹¹ P. J. WRIGHT, P. D. LEATHWOOD and D. T. PLUMMER, *Enzymologia* **42**, 459 (1972).

¹² G. R. KINGSLEY, *J. Lab. clin. Med.* **27**, 840 (1942).

¹³ J. S. LA DUE, F. WROBLEVSKY and A. KARMER, *Science* **120**, 497 (1954).

¹⁴ T. A. RUDOLPH, R. DUTTON and J. A. SCHAFER, *J. clin. Invest.* **34**, 940 (1955).

Table II. Biochemical composition of plasma and renal lymph 1 h after a 2 h ischaemia

	Plasma		Lymph	
	Control	After ischaemia	Control	After ischaemia
Protein (mg/ml)	63.5 \pm 0.8	62.2 \pm 0.5	42.1 \pm 2.0	47.1 \pm 1.7
LDH (mU/ml)	46.5 \pm 5.9	107.2 ^a \pm 10.1	60.3 \pm 15.4	1273.0 ^b \pm 70.3
GOT (mU/ml)	6.3 \pm 2.1	20.4 ^b \pm 3.5	6.2 \pm 1.6	36.8 ^b \pm 7.3
GPT (mU/ml)	6.6 \pm 1.6	8.7 \pm 1.5	5.7 \pm 1.2	11.0 ^b \pm 2.3
AA (mU/ml)	9.2 \pm 0.5	10.7 \pm 0.9	4.1 \pm 0.8	7.7 \pm 1.8
alk P (mU/ml)	17.3 \pm 3.5	22.3 \pm 3.5	5.8 \pm 2.1	16.2 ^a \pm 8.7
ac P (mU/ml)	3.8 \pm 0.7	5.3 \pm 0.7	1.0 \pm 0.6	4.5 ^a \pm 1.0
C _k (ml/min)	32.6 \pm 7.3	2.2 ^b \pm 1.1		

concentration of the mitochondrial and lysosomal enzymes. This observation points to the presence of severe cellular damage and breakdown. It should be mentioned that 2 h clamping of renal artery leads in anaesthetized dogs to severe but usually reversible renal insufficiency¹⁵, in unanaesthetized animals this injury is fatal¹⁶.

¹⁵ E. E. SELKURT, Am. J. Physiol. 144, 395 (1945).

¹⁶ P. BÁLINT, A. FEKETE and J. TARABA, Acta med. hung. 20, 421 (1964).

Zusammenfassung. Nachweis, dass 30-minütige Nierenischämie zum Austritt von Enzymen des Zellplasmas aus den Zellen führt. Nach 120 min Nierenischämie kommt es auch zum Austritt von lysosomalen und mitochondrialen Enzymen aus den Zellen.

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Importance of Reverse Transcriptase in Plant Tumour Tissue of Viral Origin Cultivated in vitro

The wound tumour virus is known to be the causative agent of *Rumex acetosa* tumour¹⁻³. GENTILE⁴ and RAYCHAUDHURI⁵ reported that the virus is not present in the tumour tissue of *R. acetosa* maintained in vitro over long periods. We report in this communication certain tumour-specific characteristics in the in vitro cultivated tissue of *Rumex acetosa*, and the possible role of the reverse transcriptase⁶ in their inheritability in tissue devoid of the original double stranded RNA virus.

Materials and methods. The normal and the tumour tissue was kindly supplied by Dr. S. K. SRIVASTAVA, M. S. University, Baroda, India, and was maintained under 12-hourly dark and illumination rhythms on the medium described by GENTILE⁷. Generally 15-day-old tissue was used for investigation.

The extracts of the tissue were prepared in each case by grinding 1 part by weight of fresh tissue with 1 part by volume of 0.9% sodium chloride solution in cold, and after centrifugation at about 1,100 g, the supernatants were examined after disc electrophoresis⁸ for NADP dependant isocitric dehydrogenase by the method of FINE and COSTELLO⁹ and peroxidase isozymes by the method of HIRSCHFELD¹⁰. NADP dependant isocitric dehydrogenase was estimated by the method of OCHOA¹¹, peroxidase by the method of LÜCK¹² and protein by the

method of LOWRY et al¹³. Subcellular fractions were prepared as described by PEACOCK and DINGMAN¹⁴.

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⁴ A. C. GENTILE, J. expl. Bot. 14, 412 (1963).

⁵ S. P. RAYCHAUDHURI, *Advances in virus Research* (Academic Press, Inc., New York 1966), vol. 12, p.175.

⁶ H. M. TEMIN and S. MIZUTANI, Nature Lond. 226, 1211 (1970).

⁷ A. C. GENTILE, Tissue Culture proceedings of the Seminar held in Baroda, India, under the auspices of the U. G. C. and the M. S. University, Baroda, India (Ed. C. V. RAMAKRISHNAN; Dr. W. Junk Publishers The Hague 1965), p. 358.

⁸ B. DAVIS, *Disc Electrophoresis*, part II (preprinted by Distillation Products, Industries Division of Eastman Kodak Company) 1961.

⁹ I. H. FINE and L. A. COSTELLO, *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1963), vol. 6, p. 958.

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¹¹ S. OCHOA, in *Methods in Enzymology*, (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 1, p. 699.

¹² H. LÜCK, in *Methods of Enzymatic Analysis* (Ed. H. U. BERGMAYER Academic Press, New York and London 1963), p. 895.

¹³ O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. 193, 265 (1961).

¹⁴ A. C. PEACOCK and C. W. DINGMAN, Biochemistry 6, 1818 (1967).

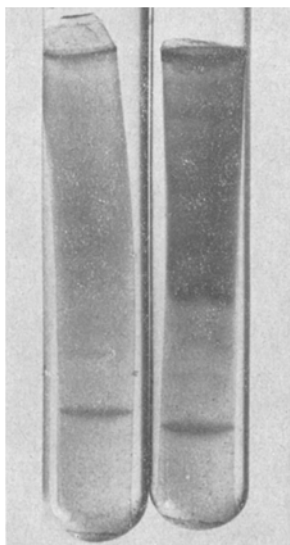


Fig. 1. Protein components in the supernatant fractions (20,000 g) of normal (1) and tumour (2) tissue of *R. acetosa* after disc electrophoresis under identical conditions.

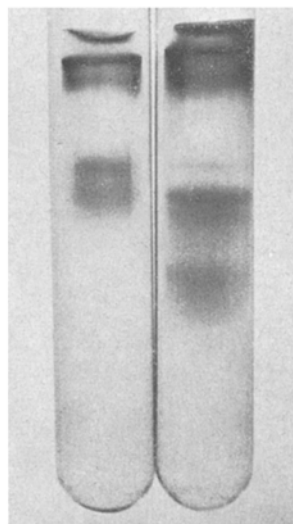


Fig. 2. Differences in peroxidase isozymes between normal (1) and tumour (2) tissue of *R. acetosa* after disc electrophoresis.