HISTOCHEMICAL EFFECTS OF PROSTAGLANDIN A_2 (PGA_2) ON THE RODENT KIDNEY

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ABSTRACT

Renal prostaglandins are synthesized *de novo*. PGA_2, originates from PGE_2 and could function as a circulatory hormone since prostaglandins of A series escape destruction in the lung. In the present investigation, attempt has been made to analyse the effect of PGA_2 histochemically on different renal constituents such as nucleic acids, lipids, amino acids and a few enzymes. PGA_2 inhibited reaction for DNA, alkaline and acid phosphatase, tyrosine and arginine accompanied with a histological injury.

INTRODUCTION

INTERSTITIAL cells of the renal medulla are considered to be the site of synthesis of renal prostaglandins. Moreover, changes in ultrastructure and granularity of interstitial cells in turn may be involved in the pathogenesis of hypertension and altered renal function[1]. Prostaglandin synthesis of rabbit's renal cortex is less than 10% of that of medulla[2]. Renal prostaglandins do not accumulate and are efficiently metabolized to 16-Keto derivatives[2,3], Prostaglandin A_2 which originates from the conversion of PGE_2, may function as a circulatory hormone since prostaglandins of A series escape destruction in the lung[4]. However, no data are available on the specific effects of PGS on renal morphology[5]. The present report concerns the histochemical analysis of squirrel's (*Funambulus pennanti*) kidney with regard to nucleic acids, neutral lipids, phospholipids, alkaline phosphatase, acid phosphatase, tyrosine and arginine contents under the influence of prostaglandin A_2. These studies are expected to be helpful in evaluating the direct and/or indirect renal effects of prostaglandins on renal morphology and biochemistry.

MATERIALS AND METHODS

Since major differences in the effects of prostaglandins depend on the dose and route of administration, a stock solution at 1 mg/ml in ethanol of prostaglandin A_2, supplied by UP-John Co. (U.S.A.) was prepared and stored at 4°C. It was diluted by adding 0·25 M phosphate buffer (pH 6·5) by adding 0·1 ml of the diluent. 0·2 ml of this diluted solution was injected (intramuscularly) to squirrels on each alternate day for 30 days. Thus the total dose of PGA_2 during this experiment was 13·0 mg. A group of ten adult (two months old) squirrels weighing 80 ± 10 g received this treatment, while the remaining ten of the same age and weight injected with 0·2 ml of olive oil served as controls. All the animals were fed on laboratory chow and tap water *ad libitum* during the experiment.

On 31st day, squirrels were killed by decapitation. Both the kidneys were removed, fixed in suitable fixatives recommended, sectioned and applied following specific histochemical tests. In addition to haematoxylin/eosin staining, Feulgen reaction[6], methyl green pyronin-y-method[7], Sudan Black-B propylene glycol method[8], acid hematin test[9], azo-dye method[10], lead nitrate method[11], Million's test[12] and Sakaguchi reaction[13] were also employed. Suitable controls to check the specificity of each reaction were employed simultaneously.

RESULTS

General health of the PGA_2 treated animals was found satisfactory except the loss in their body weight. There were no local inflammation, granuloma formation or lymphocyte infiltration.

Light microscopical study, was made to determine the nature of injury caused by PGA_2. Glomerular and tubular dilation could very well be observed. Ruptured epithelial cells in some of the proximal and distal tubules were seen. Necrotic spaces in the cortex were also prevalent. Nuclei of the medullary cells changed their shape and were noted escaping from the cell mass. Overall hardening of the capsule was also noticed (figure 1).

Histochemical staining of nucleic acids signify the interference of PGA_2 with DNA and RNA contents of the kidney. Very dull Feulgen reaction could be seen in the cortex and medulla. Capsular cells exhibited and enhanced positive reaction (figure 2). However, control kidney exhibited a strong positive reaction (figure 3). On the other hand stimulated activity for RNA was noticed after PGA_2 treatment. Nuclear part also showed a strong positive reaction (figures 4 and 5).

Histochemical study revealed more accumulation

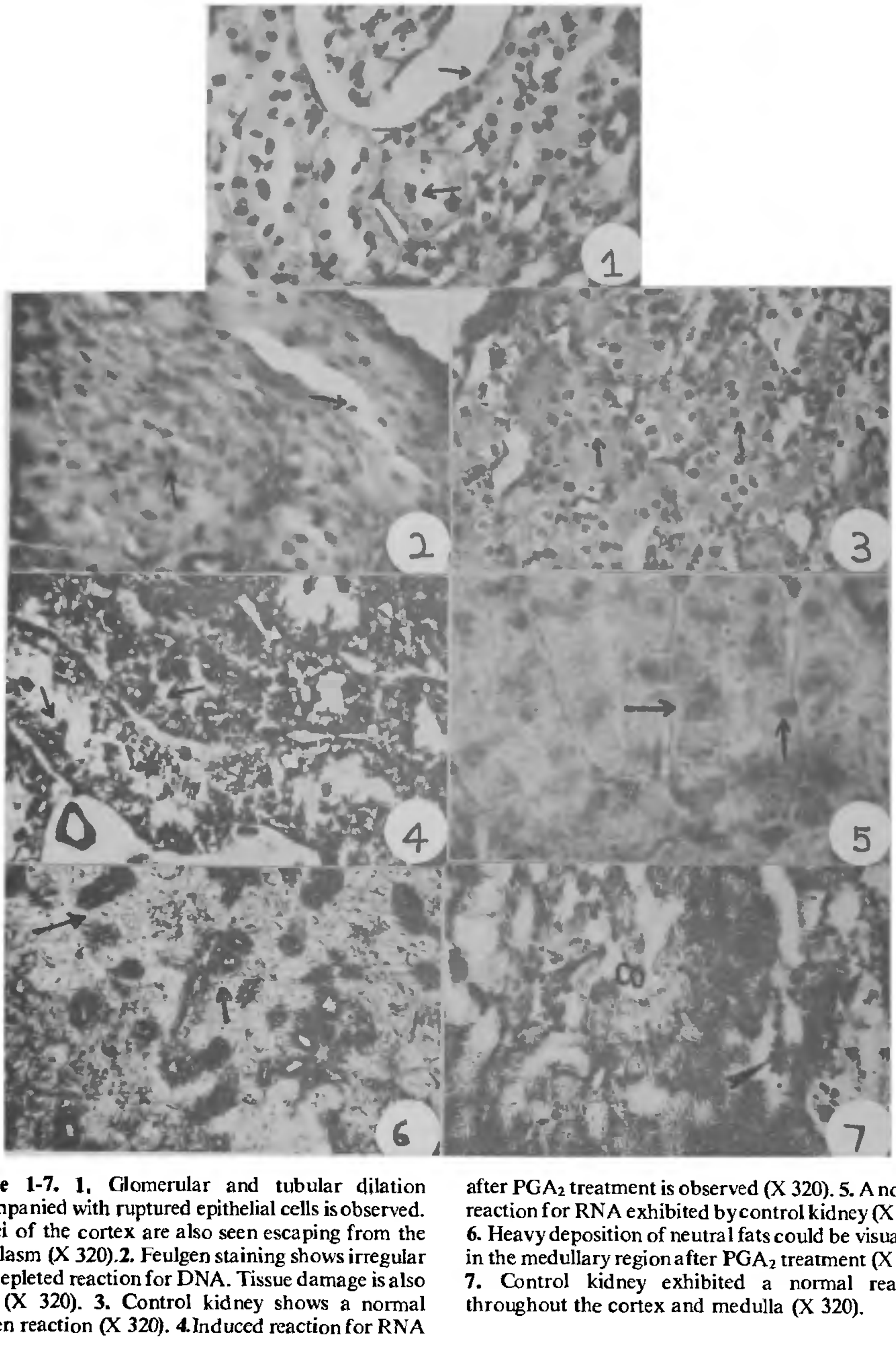
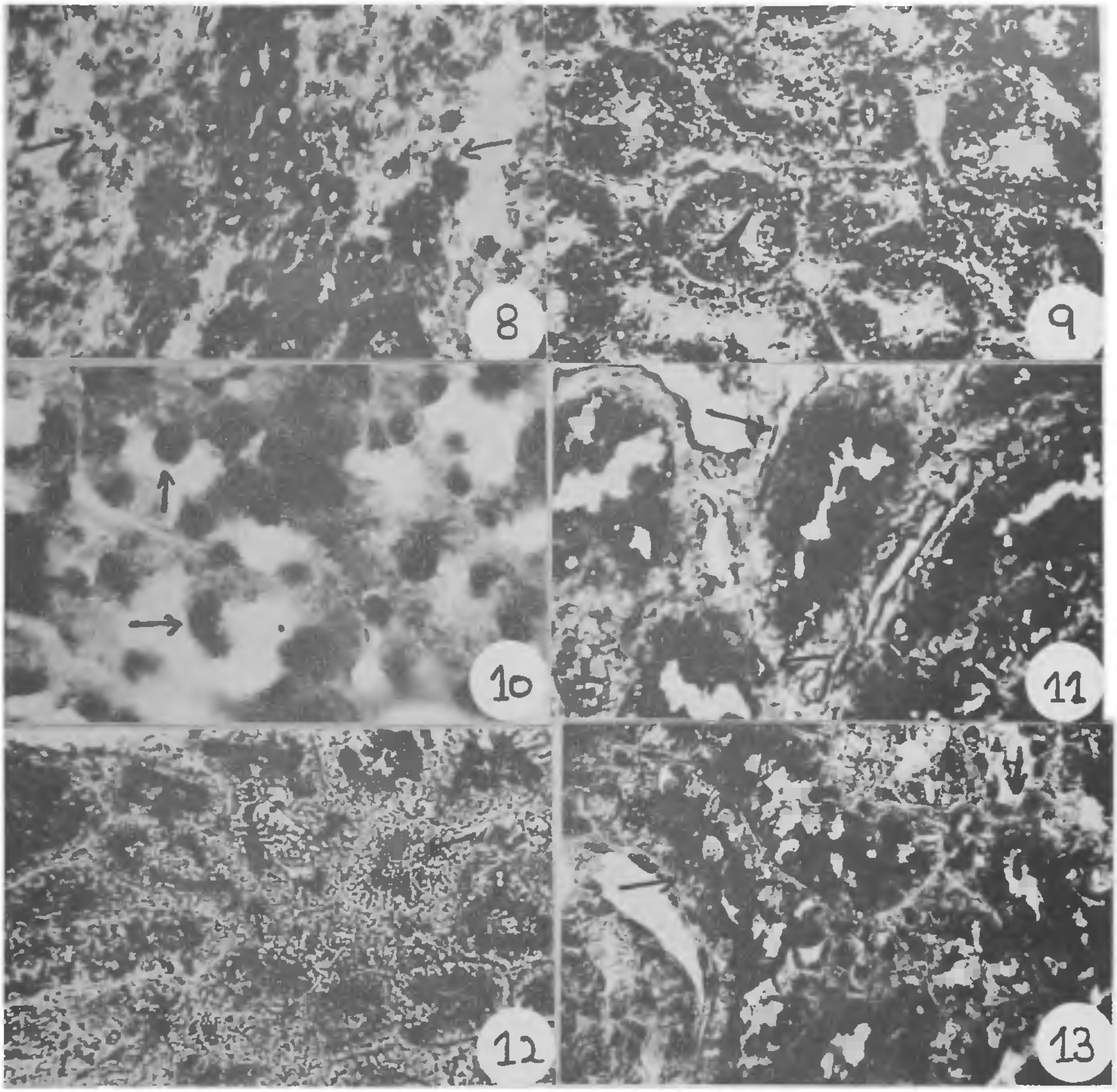


Figure 1-7. 1. Glomerular and tubular dilation accompanied with ruptured epithelial cells is observed. Nuclei of the cortex are also seen escaping from the cytoplasm (X 320). **2.** Feulgen staining shows irregular and depleted reaction for DNA. Tissue damage is also clear (X 320). **3.** Control kidney shows a normal feulgen reaction (X 320). **4.** Induced reaction for RNA after PGA_2 treatment is observed (X 320). **5.** A normal reaction for RNA exhibited by control kidney (X 500). **6.** Heavy deposition of neutral fats could be visualized in the medullary region after PGA_2 treatment (X 125), **7.** Control kidney exhibited a normal reaction throughout the cortex and medulla (X 320).



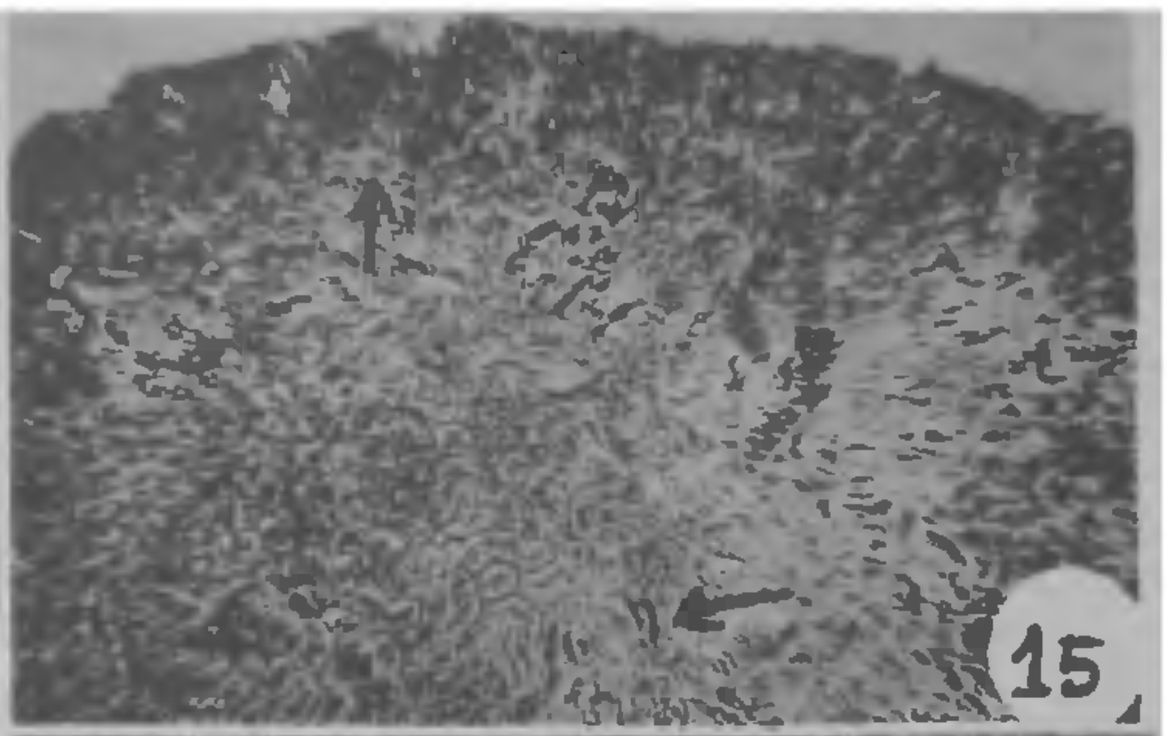
Figures 8-13. 8. Stimulated reaction for phospholipid is visible in the medullary cells (X 150). **9.** Control kidney showed a normal reaction throughout the tissue (X 320). **10.** PGA_2 treated kidney showed an intense reaction for AlPase only in the nuclei of the cortex cells (X 500). **11.** Control kidney shows AlPase in the cortex quite different from that of PGA_2 treated kidney (X 500). **12.** Brush border region of the proximal tubules which are just below the capsule only exhibit a positive reaction for AcPase (X 320). **13.** In control kidney, distal convoluted tubules show a positive reaction for AcPase (X 320).

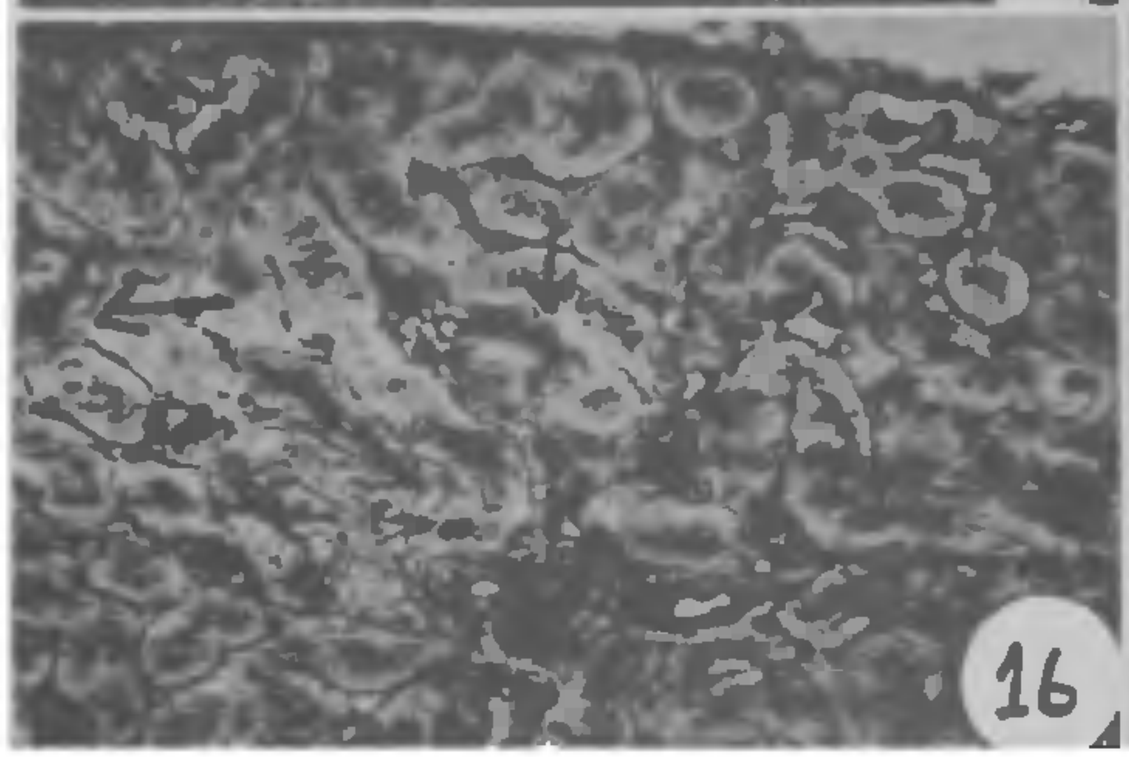
of neutral fats and phospholipids in the kidney in comparison to controls (figures 6 to 9).

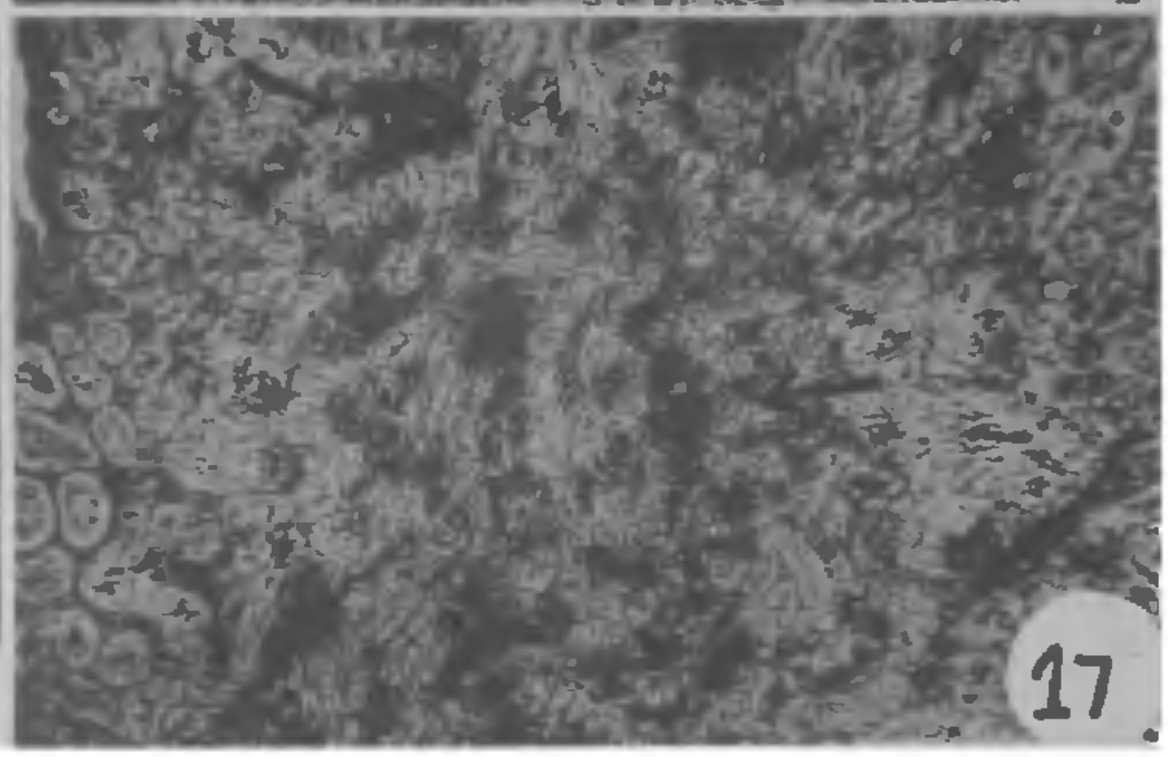
Studies on alkaline phosphatase showed that it is localized in the nuclei after PGA_2 treatment (figures 10 and 11). Acid phosphatase was also depleted (figures 12 and 13). These observations may mean that PGA_2 alters sub-cellular organelles like lysosomes and endoplasmic reticulum. Histochemical mapping indicated loss of tyrosine (figures 14 and 15) and arginine (figures 16 and 17) after PGA_2 treatment.

Discussion

The present investigation indicates that except for RNA, neutral fats and phospholipids and other constituents such as alkaline phosphatase, acid







Figures 14-17. 14. A dull reaction for tyrosine is observed in some of the cortical tubules after PGA_2 treatment (X 80). **15.** Intense reaction for tyrosine is visible in control kidney sections (X 80). **16.** Almost negligible reaction for arginine is visualized after PGA_2 treatment (X 150). **17.** Intense arginine reaction is exhibited by the proximal and distal tubules of control kidney (X 80).

phosphatase, arginine and tyrosine are depleted and/or inactivated by exogenous PGA_2 treatment *in vivo*. More important is the histological damage caused by it. PGA is known to cause renal cortical vasodilation which may explain the increased cellularity of glomerulii and proliferation of cortical and medullary cells. Excessive accumulation of lipids in renal tissue indicates probable specific affinity between lipases and prostaglandins. This assumption is supported by the hypothesis that interstitial cell's lipid droplets serve as renal prostaglandin precursors[14].

The changes observed in relation to DNA and RNA are rather interesting. It was proposed earlier[15,16] that PGS can bind to DNA and regulate gene action. It is likely that the effect of PGA_2 on RNA and DNA observed in this investigation may have relevance to these hypotheses . It is known that PGE can alter leukocyte alkaline phosphatase activity[17,18], which is believed to be due to the action of PGS on gene(s) coding on this enzyme. Thus, the reports obtained with alkaline and acid phosphatases in the present investigation can also be related to the action of PGA_2 on DNA. PGA_2 induced loss of proteins containing tyrosine and arginine. Mechanism of this action remains unknown. But this may have relevance to the variation in the enzymatic activity observed.

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ANNOUNCEMENTS

NATIONAL SYMPOSIUM ON BIOTECHNOLOGY

A National Symposium on Biotechnology will be held at Chandigarh during March 13-15, 1982. For further information contact: Dr. S. C. Jain, Organising Secretary, National Symposium on Biotechnology, Department of Chemical Engineering & Technology, Panjab University, Chandigarh 160014 (India).

RAMAN AWARD FOR G. N. RAMACHANDRAN

The Indian National Science Academy has awarded the C. V. Raman Medal for 1982 to Prof. G. N. Ramachandran for his outstanding work on molecular biophysics and crystallography.

Prof. Ramachandran has been distinguished fellow of the Academy since 1962. He is also a fellow of the Indian Academy of Sciences Bangalore and the Royal Society, London. He was Editor of *Current Science* during 1950-57.

The Raman medal is one of the three general medals of the academy awarded once in three years for outstanding work in the field of science.

MAHALANOBIS AWARD FOR NAYUDAMMA

Prof. Y. Nayudamma had been awarded the P.C. Mahalanobis Medal 1981 by the Indian National Science Academy. He is a fellow of the National Academy of Science, Allahabad, Indian Academy of Sciences, Bangalore, International Union of Leather Chemists Societies and American Leather Chemists Association.

HORA MEMORIAL MEDAL

The Indian Society of Ichthyologists has conferred the S. L. Hora Memorial Medal to Prof. B. I. Sunder Raj, Professor and Head of the Department of Zoology, Delhi University, for his contributions in the field of Ichthyologists. Dr. Sunder Raj is a past student of the Mysore University.