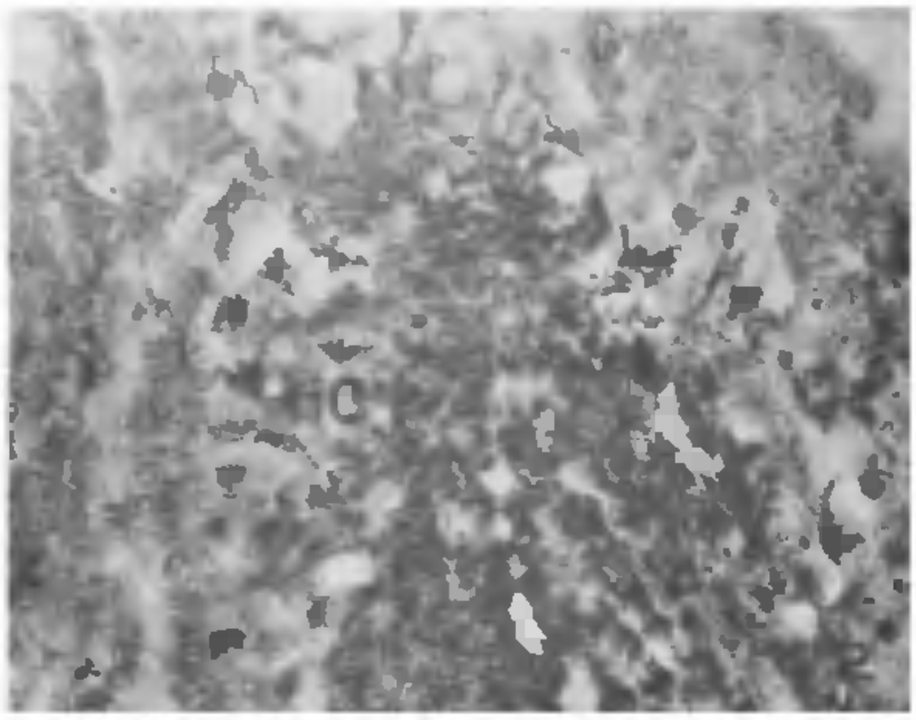

FIG. 2. T.S. liver of *L. guntea* showing zymogen bearing hepatocytes, × 450 (Bensley's zymogen).

Bensley's zymogen stain reveals two zones in the liver, the central, with deeply stained cells and the outer, with unstained cells (Fig. 2). The cytoplasm of the cells is eosinophilic. These observations indicate that the cells in the central zone of the liver of *L. guntea* bear zymogen granules.

According to earlier investigators[1,4,5], the zymogen granules contain pepsinogen stored in the pancreatic acinar cells and in the peptic cells of the stomach of the fishes in the form of inactive precursors and are activated outside the cells in an acidic medium. However, in the opinion of the present authors the unusual occurrence of zymogen-bearing cells in the liver of *L. guntea* seems to be a compensatory device for the loss of a functional stomach.

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STAINING OF DNA WITH AQUEOUS SOLUTION OF AN OXAZINE DYE, MELDOLA'S BLUE

IT was reported earlier that mammalian tissue sections following extraction of RNA through treatment with 0·1 N sodium hydroxide at 60° C for 15 min when stained with basic dyes reveal well-stained nuclei only[1-3]. Tissue sections, that are not hydrolysed in the alkali, reveal staining of both the nuclei and the cytoplasm when stained with these dyes. Treatment of sections with mild solution of sodium hydroxide at 60°C causes detachment of some sections at times from the slides and is, therefore, not quite a practical method for routine extraction of RNA. The author's[4] introduction of phosphoric acid extractions of RNA at 5° C has solved this difficulty and has opened up the possibility of using basic dyes in aqueous solution for the staining of DNA. This communication presents results of staining mammalian tissue sections with Meldola's blue after phosphorid acid extraction of RNA and also embodies the spectral characteristics of the dye as well as of the stainel nuclei.

Meldola's blue (Naphthol blue) (G. T. Gurr, London, C. 1. No. 51175), the first synthesized oxazine dye, was used as 1% aqueous solution. The dye solution after filtration revealed a pH of 4·7 to 4·8. This pH was adjusted to 3·0, 7·0, 9·0 and 10·0 with an aqueous solution of sodium hydroxide or hydrochloric acid as the case may be.

Paraffin sections (12 μm) of rat liver, kidney and testis fixed in 10% buffered neutral formalin as well as in acetic acid-alcohol (1:3) were used. RNA from the deparaffinised sections was extracted in concentrated phosphoric acid at 5° C for 20 min or in 75% acid at 5° C for 2 h[4]. Staining with an aqueous solution of Meldola's blue for 3–5 min was performed after a thorough washing of the sections in water. Sections were then dried between folds of filter paper and left in butanol for one minute, cleared in xylol and mounted in DPX. In some cases regular dehydration through grades of ethanol was also performed.

Absorption properties of 5 stained nuclei were recorded in a midrospectrophotometer[5,6] at a magnification of × 650. The method for determining the absorption characteristics of stained nuclei was as follows: Each of the 5 nuclei individually was focussed carefully under the microscope and light from the monochromator starting from 450 nm was allowed to pass through the central region of the nucleus and the galvanometer readings were taken at every 10 nm upto 630 nm (I_1). Then the nucleus was shifted slightly from its original position and the light from 630 nm upto 450 nm in the reverse order was allowed to pass through the blank (I_0) and the galvanometer readings noted at every 10 nm. The ratio of I_1/I_0 was obtained as transmission. Extinction values from these transmissions were obtainel from the table as given by Brode[7]. Absorption values of an aqueous solution of the dye were recorded in a Beckman DB spectrophotometer.

Staining of DNA with aqueous solution of Meldola's blue following phosphoric acid extraction of

RNA indicates highly satisfactory result (Fig. 1). Of the different pHs tried in this investigation, pH 7·0 seemed to be the best. It has been found that the dye works best only when used after fresh adjustment of its pH. The dye solution whose pH has been adjusted to 9·0 or 10·0 and left for hours on the table shows precipitation. It has also been noticed that the intensity of staining with acetic acid-alcohol fixed tissues is somewhat low as compared with tissues fixed in formalin (Fig. 2). Dehydration of sections in butanol is better than that of ethanol.

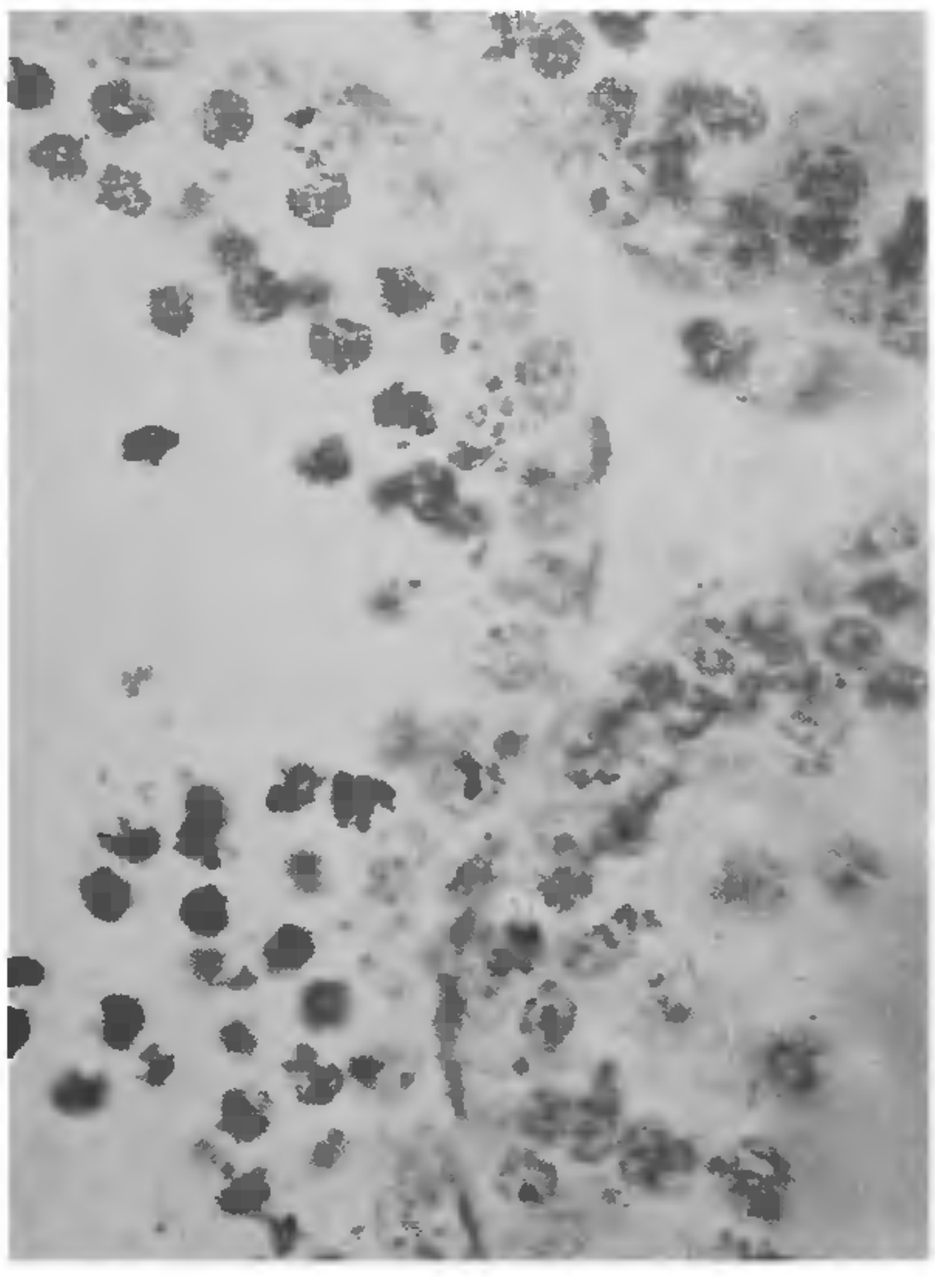

FIG. 1. Photomicrograph of rat testis stained with aqueous solutions of Meldola's blue at pH 7·0.

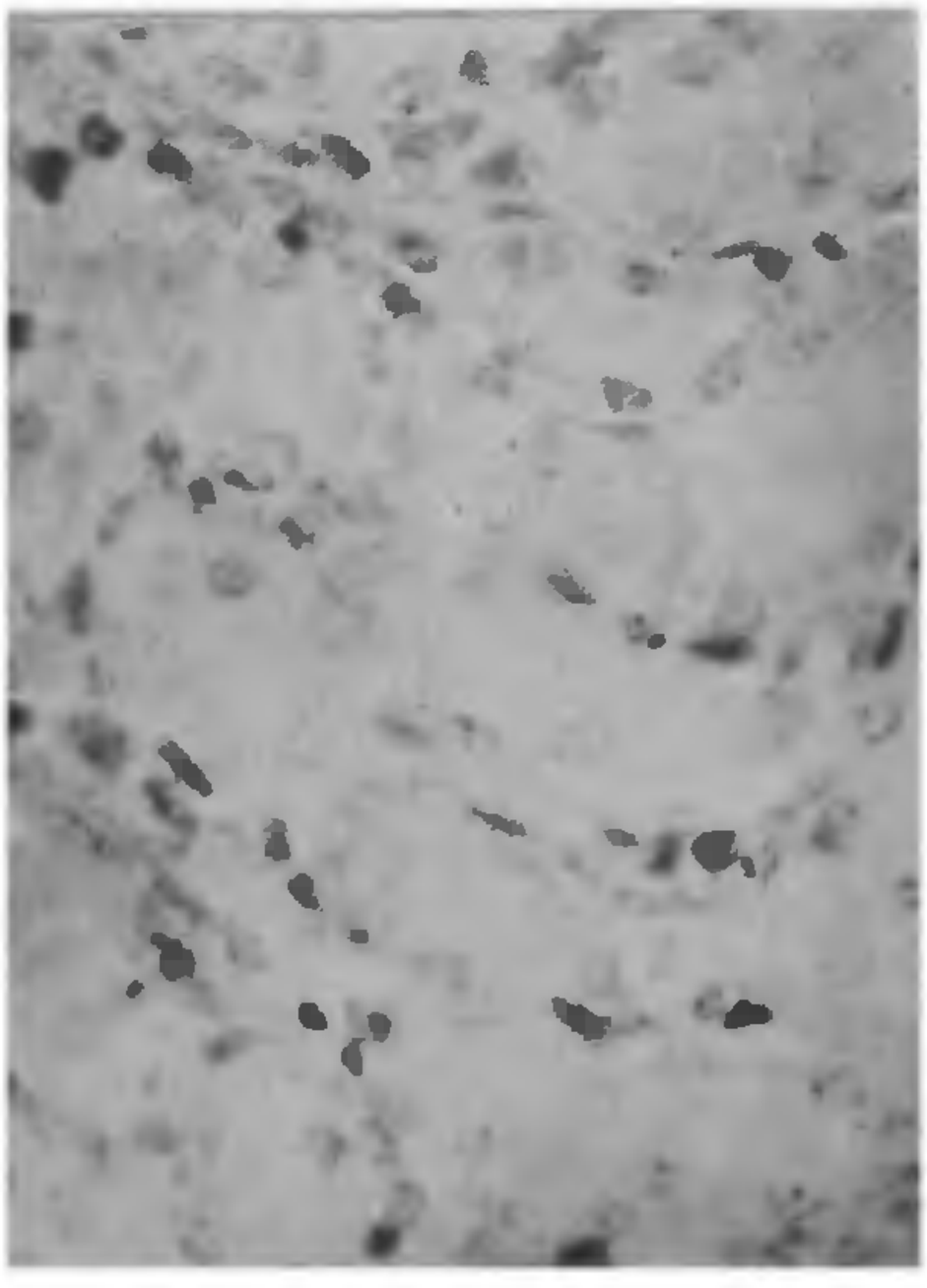

FIG. 2. Photomicrograph of acetic acid-alcohol fixed rat kidney stained with Meldola's blue.

The *in situ* absorption characteristics of stained nuclei indicate the typical bell-shaped curve with the peak of maximum absorption at 590 nm and a secondary shoulder at 560 nm (Fig. 3). The absorption properties of an aqueous solution of the dye shows peak at 575 nm. On the whole the shape of the two curves is identical (Fig. 3).

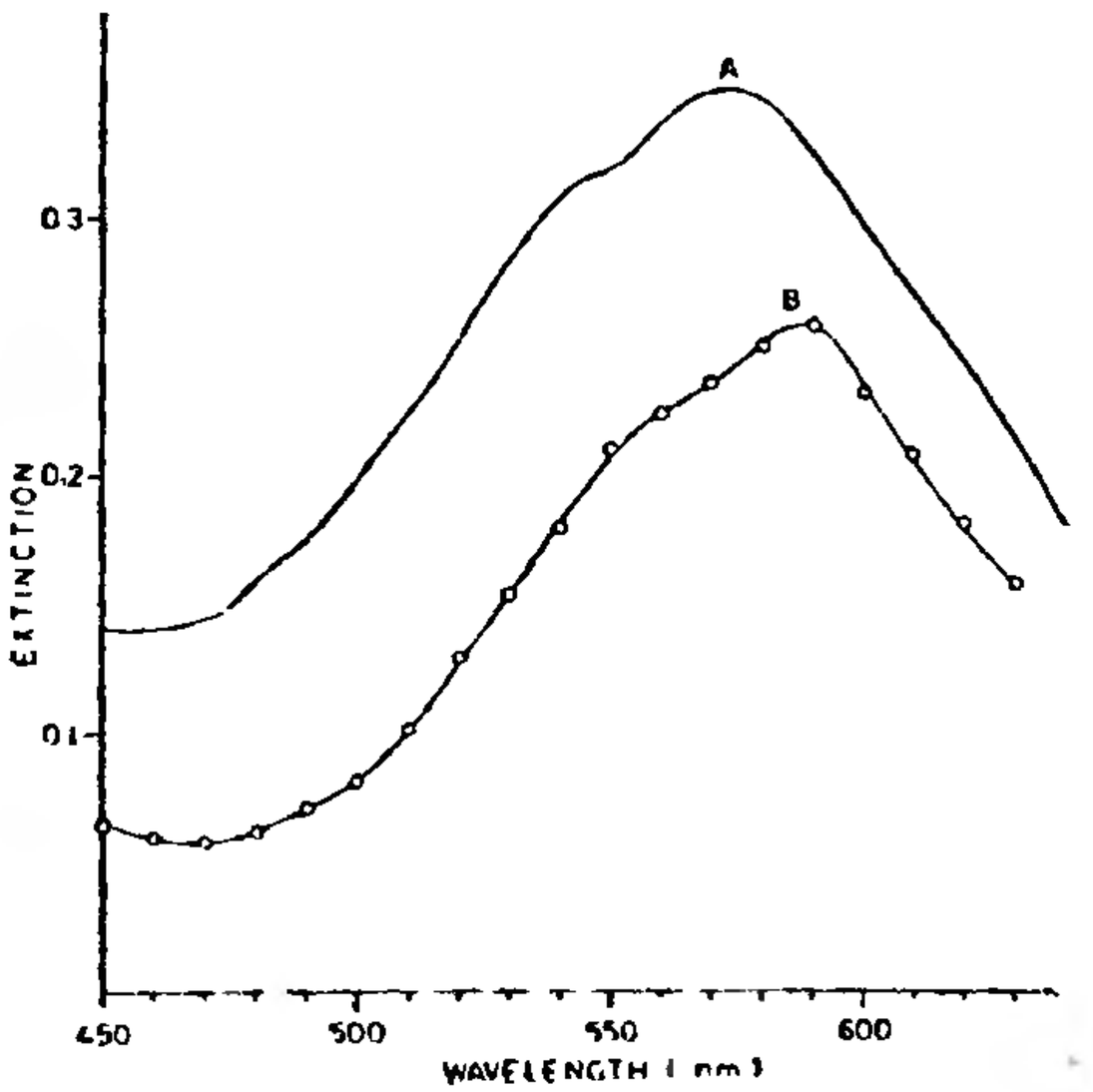

FIG. 3. Absorption characteristics of (A) an aqueous solution of Meldola's blue and (B) stained nuclei.

From the results presented above it is evident that staining of DNA after selective extraction of RNA through cold phosphoric acid is reliable and can be followed in routine staining. The method is rapid and the binding of the dye with DNA is perfect as is evident from the shift of the position of the peak of maximum absorption which is hypsochromic. The mechanism of binding is perhaps through the phosphate groups of DNA just as is the case of methyl green staining except for the fact that Meldola's blue binds with polymerised as well as depolymerised DNA whereas, methyl green binds with only the highly polymerised DNA.

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OCCURRENCE OF SYMBIOTIC BIOLUMINESCENT BACTERIA IN INDIAN LEIOGNATHIDS

Introduction

LUMINOUS bacteria occur freely in sea-water or in association with other organisms either as symbionts or as parasites or saprophytes[1–10]. The light organs of silver-bellies are 'donut' shaped encircling the distal end of the oesophagus and harbouring symbiotic bioluminescent bacteria[11–14]. Knowledge concerning bacterial luminescence is totally lacking from Indian Seas. The present study is first of its kind dealing with the occurrence of symbiotic bioluminescent bacteria in the light organs of 4 species of silverbellies belonging to 3 genera of the family Leiognathidae.

Material and Methods

Four species of silver-bellies, *viz.*, *Leiognathus splendens*, *Gazza* sp., *Secutor ruconius* and *S. insidiator* were obtained from shore-seine catches from Porto Novo waters (Lat. 11° 30′ N, Long. 79° 46′ E) in living condition and were sacrificed fori solation of bacteria. The 'sterile-technique' adopted by Reichelt *et al.*[14] was followed in the present study to remove the symbionts from the light organs for culturing them in nutrient-agar medium with 3 ml of glycerol per litre. Electrical micro-balance (Oertling) was made use of for taking the weight of light organ corrected upto 0·0001 g. The bacterial population was enumerated on wet weight basis of the luminescent organ.

Results and Discussion

Table I shows the numbers of viabl esymbiotic bioluminescent bacteria in different species of leiognathids. The bacterial population ranged from log 9·6284 to log 10·1367 per gram (wet weight) of the organ. The present observation on *Leiognathus splendens* and *Secutor ruconius* is almost similar to the report of Hastings and Mitchell[12] in the waters of New Guinea. Viable colonies per gram wet weight of the organ in *Gazza* sp. (log 9·6284) seemed to be lower than in *S. insidiator* (log 10·1206). Maximum bacterial population was found in two species of the genus *Secutor* where weight of the light organ is more in relation to body weight. Hastings and Mitchell[12] have also shown that major fraction of the weight of the light organ is mainly due to viable symbiotic luminescent bacteria.

TABLE I

Numbers of symbiotic bioluminescent bacteria in leiognathid light organs

Species	Total length of fish (mm)	Weight of the fish (g)	Weight of the organ (mg)	Log of total bacterial counts/organ	Log of bacterial counts/g of organ (wet weight)
Leiognathus splendens	104	22·5	42·1	8·2989	9·6749
Gazza sp.	133	40·6	194·4	8·9175	9·6284
Secutor ruconius	72	7·6	62·0	8·9294	10·1367
S. insidiator	92	13·4	77·6	9·0103	10·1206

All the bacterial colonies observed presently were luminescent (Figs. 1 and 2) which confirms the earlier findings of Hastings and Mitchell[12]. Figure 3 shows the luminescent colonies isolated from *Gazza* sp. Simple

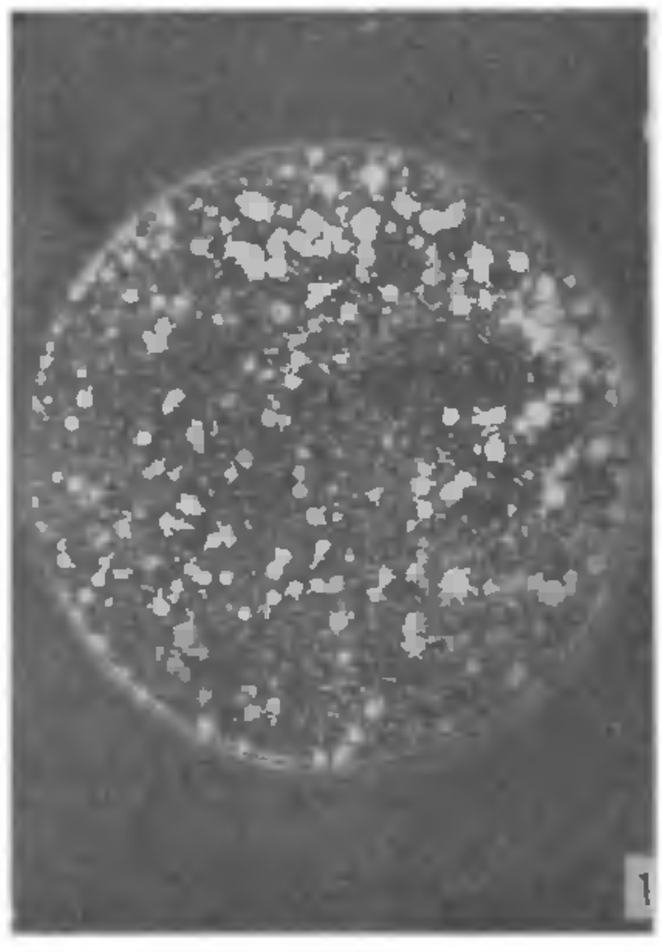

FIG. 1

tests on the contents of the luminous gland in 3% NaCl solution showed the presence of luminescence. The solution was luminescent at room temperature ($28 \pm 2°$ C) but not so at 4° C. In distilled water it failed to emit luminescence. When centrifuged only the residue maintained it. This confirms the earlier observations of Haneda[11] and Haneda andTs uji[13].

Beijerinck[15] proposed that all luminescent bacteria should be placed under a single genus *Photobacterium*. But Reichelt *et al.*[14] and Nealson and Hastings[16] grouped the luminescent bacteria under 2 genera