

## M A T E R I A L   A N D   M E T H O D S

Live specimens obtained from the common bile ducts and gall bladders of infected hosts (buffaloes) were first washed with normal saline and brought to the laboratory in Hedonfleig's solution (Clegg, 1957) in thermos containers. These were subsequently fixed in neutral formalin 10%, formalin 4%, Helly's fluid, Bouin's fluid and Carnoy's fixatives for histological and histochemical studies. The material for histochemical preparation for esterases was fixed in cold acetone (Gomori, 1952) and for toto preparations, indicating the enzyme, specimens were fixed in cold 10% neutral formalin. Paraffin processed material, sectioned at 5-8  $\mu$ m was stained with H & E, Mallory's triple stain and Heidenhain's Azan. Toto mounts were stained with Grenacher's Borax carmine. The specimens for toto mounts were stretched between two slides for the maximum display of the organ systems.

For qualitative histochemical studies various techniques, as given in Pearse (1960), Lillie (1965) and for standeradization of staining methods, those as cited in BDH manual (1972) were adopted. A schedule of histochemical techniques for relevant tests is given below (Table II).

TABLE - II

Substance investigated	Fixative	Technique employed
1. Glycogen and other polysaccharides	Carnoy's	PAS
2. "	"	PAS, after diastase digestion.
3. Glycogen	"	'Best's Carmine'
4. "	"	'Best's Carmine after diastase digestion.
5. Acid mucopoly saccharides	Bouin's	Alcian blue
6. Proteins	Carnoy's	Mercury bromophenol blue
7. Basic proteins	"	Acid solochrome Cyanine
8. RNA + DNA	"	Pyronine <u>Y</u> + Methyl green
9. Reticulin	Helly's	Silver impregnation method.
10. Lipids	Formalin 10%	Sudan Black B
11. Bound Lipids	Carnoy's	Acetone Sudan Black B
12. Non-specific esterases	10% Cold Neutral formalin and cold acetone	Indoxyl acetate
13. Acetylcholinesterase	10% Cold neutral formalin and cold acetone	Indoxyl acetates, after Eserine treatment
14. Acetyl cholinesterase	10% Cold neutral formalin and cold acetone	Acetylthiocholine iodide

The various histological, histochemical and in toto preparations were mounted in Canada balsam, DPX and glycerine jelly accordingly. For most of the morphological descriptions Camera lucida diagrams were drawn.