MATERIAL AND METHODS

The adult cockroach, Periplaneta americana served as the chief material for the present investigations. The brain and the thoracic ganglia were used as the representative ganglia of the central nervous system of this insect, as they are large enough to be handled easily in preparations.

A dose of 0.0025 μg/gm of malathion (O, O, dimethyl S-bis (carboethoxy)ethyl phosphorodithioate)—an organophosphorus insecticide, was injected in the thorax of each cockroach with the help of a syringe. With this dose, attainment of complete paralysis requires only about thirty to forty minutes, depending to a large extent upon the physical condition of the individual cockroach. The treated roaches were divided into four groups as follows:

1. Normal i.e., an untreated animal.
2. Freshly treated i.e., about ten to fifteen minutes after treatment when the animal shows hyperactivity.
3. Morbid i.e., when the animal shows loss of balance.
4. Paralysed i.e., when the animal shows complete inability to move voluntarily. Only those cockroaches were chosen that would still elicit some very minor response upon repeated stimulation with a camel hair brush.

Extreme caution has been exercised to prevent the inclusion of dead animals, so that the histopathological pictures here are those brought about by the insecticide, supposedly uncomplicated by post mortem degenerative changes.
The ganglia of the above said groups of cockroaches were dissected out and were put into four different groups of tubes containing various fixatives. The fixatives employed were Zenker, cadmium-formaldehyde, formol-calcium with postchroming, carnoy with chloroform, alcoholic Bouin, cold acetone, 10% neutral formalin and weak Bouin. They were then processed according to the fixing fluid used and were embedded in paraffin (52°C to 53°C and 60°C to 62°C) as well as in gelatin for the morphological and the various histochemical tests. The paraffin sections were cut at 5 μ and the gelatin sections at 10 μ.

For the detection of Golgi bodies, Aoyama silver technique (1930) was employed. In this technique the material was fixed in cadmium-formaldehyde mixture for 18 hours and treated for fifteen hours with 1.5% AgNO₃ solution followed by the treatment with reducing mixture (hydroquinone 2.0 gms., formalin 6.0 ml., sodium sulphite 0.15 gms. and distilled water 100 ml) for eight hours. The material was washed in running water, dehydrated and embedded in paraffin. The sections were cut at 3 μ.

Besides the above cytological technique, the following histochemical techniques were employed.

NUCLEIC ACIDS

Feulgen technique for DNA: This technique depends on the treatment of the fixed tissue by mild acid hydrolysis which could release aldehyde groups from the deoxypentose sugar of DNA. When transferred to Schiff's reagent, the exposed aldehyde groups
react to the stain and produce the purple colour. For the present purpose, Zenker, and Carnoy with chloroform fixed sections were hydrolysed in H-HCl for twenty five minutes respectively and then after washing were transferred to the Schiff's reagent prepared according to de Tomasi (1936). The DNA appeared in shades of reddish-purple (Feulgen and Rossenbeck, 1924; Swift, 1950; Pearse, 1968).

Modified Gallocyanin method for nucleic acids (de Boer and Sarnaker, 1956): Carnoy fixed sections were stained in gallocyanin-chromalum for 24 hours. The nucleic acids were stained deep blue.

Toluidine blue-methyl green-orange G method for differentiating DNA and RNA (Korson, 1951): The Carnoy fixed paraffin sections were immersed in 4% aqueous orange G, rinsed in distilled water and then immersed in 0.15% methyl green for fifteen minutes. DNA stained green, RNA blue and the cytoplasm ranged with variable amounts of blue staining (RNA) structures. The specificity of this stain for nucleic acids was confirmed by the extraction of RNA with 10% perchloric acid for eleven hours at 4°C.

CARBOHYDRATES

To demonstrate and characterize polysaccharides and mucopolysaccharides, the method was based on the combination of the sugar moiety with Schiff's reagent after oxidation with periodic acid and lead tetra-acetate (Gomori, 1952; Pearse, 1968).

Periodic acid-Schiff test: This technique was followed according
to Hotchkiss (1948) and Pearse (1968). The oxidation was usually carried out in 0.5% aqueous periodic acid for 3-5 minutes as recommended by Casselman (1959). This was followed by treatment with Schiff's reagent. To ascertain the PAS reactivity due to 1:2 glycol the test was performed in the conventional manner accompanied by its acetylation and KOH reversal (Mcmanus and Cason, 1950) control.

**Best's Carmine technique for glycogen:** For the study of glycogen this technique was employed as recommended by Best (1906). The material was fixed in alcoholic Bouin and after sectioning it was treated with Best carmine stain before and after malt diastase treatment. To avoid any loss of glycogen sections were coated with 1% celloidin.

**LIPIDS**

**Sudan black B:** Sudan black B is considered to be a specific and fairly sensitive lipid colourant (Baker, 1949, 1956; Cain, 1950; Pearse, 1968) depending upon its solubility in the fat itself. For the present investigations gelatin sections fixed in formaldehyde calcium with postchroming were treated with saturated Sudan black in 70% ethanol at room temperature and at 60°C (Baker, 1944, 1949, 1956). This colours the general lipids black.

**Acid haematein test for phospholipids:** The phospholipids combine readily with chromium salts and are thereby rendered insoluble and mordanted as well. On staining with haematein, the dye attaches itself to the chromium in blue and brown colours (Cain, 1947b). The acid haematein technique was carried out on the formaldehyde calcium fixed and postchromed gelatin sections.
The sections were then mordanted for one hour in dichromate calcium at 60°C, and treated with freshly prepared acid haematein solution for 5 hours at 37°C as recommended by Baker (1946). A positive reaction with acid haematein and negative after pyridine extraction shows the presence of phospholipids in them.

**Nile blue for neutral and acidic lipids:** This technique is based on the principle that neutral lipids will dissolve out the oxazine and free base (both red in colour) from the aqueous solution of Nile blue only, whereas the acidic lipids will dissolve the oxazine and combine with the free base to form blue lipid-soluble compounds (Cain, 1947a). This technique was employed on formaldehyde calcium/postchromed gelatin sections along with pyridine extraction control using 1% and 0.02% solution of the dyes at 60°C. The acidic lipids stain blue while the neutral lipids pink.

**The phosphomolybdic acid method for choline bound lipids** (Landing et al., 1952): Formalin fixed paraffin sections were immersed for 15 minutes in 1% phosphomolybdic acid in ethanol and chloroform (50:50) and then dipped in 1% stannous chloride in 3N HCl. Choline containing lipids developed the molybdenum blue colour with stannous chloride.

**ENZYMES**

**Myristoyl choline method for cholinesterases** (Gomori, 1952): Cold formalin and cold acetone fixed paraffin as well as gelatin sections were incubated for 2-24 hours at 37°C in the working solution of myristoyl choline. They were then washed in distilled water and immersed in a dilute solution of yellow ammonium
The sites of cholinesterase and probably some non-specific esterases appeared black.

**Calcium Cobalt method for alkaline phosphatase** (Gomori, 1952): The technique for demonstrating alkaline phosphatase depends on the liberation of phosphate ions from organic phosphate esters. Cold acetone paraffin sections and cold formalin gelatin sections were incubated in the medium containing 20 ml of 3% aqueous sodium glycerophosphate; 30 ml of 2% aqueous sodium diethyl barbiturate; 4 ml of 2% aqueous calcium chloride; 2 ml of 2% aqueous magnesium sulphate; 30 ml distilled water, for one-half to sixteen hours at 37°C. The sections were washed briefly in water and then immersed in a dilute solution of yellow ammonium sulphide. The presence of alkaline phosphatase was indicated by a dark brown to black precipitate of lead sulphide.

**Lead phosphate method for acid phosphatase** (Gomori, 1952): This technique depends, as does the alkaline phosphatase method, on the liberation of phosphatase ions from organic phosphate esters. The reaction is buffered at pH 5.0. Lead nitrate was used in the incubating solution and its phosphate is insoluble at pH 5.0. The precipitate lead phosphate is converted by means of yellow ammonium sulphide into the brown sulphide of lead. Sites of acid phosphatase activity appeared black.