IV. HISTOPATHOLOGY OF THE GILL, LIVER AND KIDNEY
OF THE FISH EXPOSED TO MALATHION AND CADMIUM

Insecticides as well as heavy metals cause injury
to the organisms in various ways and most of the pollutants
are known to induce histopathological lesions in various
organs of the body. Gills which are the respiratory
organs of many aquatic animals are among the most delicate
organs of the body which come in direct contact with the
external environment. As such the gills are the ones
which could be expected to be easily affected by the
toxicants. However, though the gills are in intimate
contact with the pollutants of the habitat, very little
work has been reported on the influence of toxic
substances on the gills. Of the meagre information
available in this regard, work that could be cited, is
that of Roche who in 1974 studied the histopathological
lesions on molluscan gills and observed the disappearance
of ciliated columnar cells at the tip of the gill lamellae
of the quahog clam (Mercenaria mercenaria) exposed to
0.25 mg/l of copper chloride for 96 hours. Couch (1977)
exposed marine adult pink shrimp to 763 µg/l of cadmium
for 15 days. He observed blackening of gills and cell
death followed by autolysis, necrosis and deposition of
electron opaque material in distal filament tissue.

Practically no literature is available on histopathological lesions induced by pesticides and heavy metals in the gills of fishes.

Lebot et al. in 1974 studied the toxic action of copper on gills of fresh water carp *Cyprinus carpio* and observed mucous cell depletion followed by inhibition of mucosecretion. Another author Gong et al. (1977) observed some particles around the gills in *Cyprinus carpio* and *Stenotomus chobara* exposed to 10 ppm of zinc and copper salts.

Histopathological lesions induced by toxicants in the liver which is major metabolically active organ and kidney responsible for osmoregulation and discharge of waste material have been investigated by many workers in the field. Quite a few workers have reported histopathological changes induced by pesticides in fishes. Amminikutty et al. (1977, 1978) studied the histopathological changes in various organs of widow tetra (*Gymnocorymbus ternetzi*) exposed to pesticides thioldan E.C. 35 and agalloi '3'. They observed destruction of hepatocytas and pyknosis of nuclei in the liver of exposed animals. They also reported high vacuolization of the kidney cells followed by the degeneration of glomerular
as well as tubular cells and hematopoietic tissue. Mima (1978) working on hepatic pathology in Channa punctatus exposed to sublethal and chronic levels of diosinon, methyl parathion and dimethoate revealed cytoplasmic vacuolization, granular cytoplasmic inclusions and damaged blood supply of the hepatic cells. During the same year Scosry and Sharma (1978) gave an account of histopathological lesions induced by endrin on the liver of Channa punctatus. Recently degenerative changes induced by malathion have been reported in the same species by Dubalo and Shah (1979).

As regards the histopathological lesions induced by heavy metals it may be stated here that Sangalang and O’Halloran (1973) reported lesions in testis of brook trout (Salvelinus fontinalis) exposed to water containing 10 and 25 ppb of cadmium. Later, Khanna and Shate in 1974 observed atrophy and degranulation of α-cells and β-cells of pancreatic islets of Clarias batrachus exposed to cobalt chloride. Mukherjee and Bhattacharya (1975) studied hepatopancreas of Catlioccephalus punctatus and Clarias batrachus exposed to various industrial pollutants which comprised phenol, ammonia, copper sulphate and sodium sulphide. Lesions observed by them were characterized by liver cord disarray, necrosis and cytoplasmic and nuclear disintegration of cells. Kendall
(1977) working on acute effects of methyl mercury toxicity in channel cat fish (Ictalurus punctatus) liver, reported necrosis of exocrine pancreatic and parenchymal cells and inflammation at the hepatic capsular surface. The same year Wong et al. reported severe histopathological changes in the liver of Cyprinus carpio and Osteochirysogodon idellus treated with zinc and copper salts. A preliminary account of the work done here on the damage done by cadmium nitrate on the liver of Cherne punctatus and its temporary recovery is also published in Experientia (1979).

Whatever work has been done so far on the histopathological lesions, throws an interesting side light on the damage done to the tissues of the exposed animals. Destruction of the tissues, vacuolization of the cells, pyenotic conditions of the nuclei, necrosis, etc., are some of the degenerative changes induced by the toxicants.

As seen from the survey, the literature available on the histopathological lesions induced by malathion in fish is too meagre. Likewise, though extensive work has been reported on the ill effect of cadmium on mammals not much is known about the damage caused to fresh water fishes.

In order to facilitate a better understanding of the injury caused to the piscine tissues, it is considered
desirable to give an account of the normal structures in
the fish before mentioning the destruction caused to
these organs by pollutants. The histological features of
the gill, liver and kidney in the normal fish are as
follows:—

1. HISTOLOGY OF THE GILL, LIVER AND KIDNEY OF NORMAL
   CYANOK F.UNCTATUS:

la. THE GILL:

   Each gill has a common basal region and two
   hemibranchs which arise from the base. Distally each
   hemibranch is further divided into a number of flat,
   tapered structures known as gill lamellae. The respiratory
   surface of each gill lamella gives off numerous folds
   known as secondary lamellae (Plate 1, Fig. 1).

   A transverse section of gill shows the presence of
   a single nerve in the middle region towards the base of
   the hemibranch. Arising on each lateral side of nerve is
   an afferent artery. Below and at the outer lateral side
   of each afferent artery is seen a well developed
   cartilaginous rod. An abductor muscle arises along the
   lateral and ventral border of each cartilage. An abductor
   muscle also arises from the lower border of this cartilage.
   In between the two abductor muscles of the lamella is
   located a single afferent artery (Plate 1, Fig. 2).
Plate I

Microphotographs of the transverse sections of normal gills of Chaetos interruptus showing:

Fig. 1. Entire gill structure (160 x) to reveal the arrangement of gill lamellae.

Fig. 2. Basal region of Fig. 1 (560 x). Nerve cord, afferent artery, cartilagenous rod, adductor muscle, adductor muscle and efferent artery are clearly visible.

Fig. 3. Secondary lamellae (560 x).

Fig. 4. Highly magnified secondary lamellae (1600 x) with epithelial cells and cartilagenous rod.

Fig. 5. Chloride cells in interlamellar region (560 x).

Abbreviations:

GL. - Gill lamellae;
SL. - Secondary lamellae;
NC. - Nerve cord;
AA. - Afferent artery;
EA. - Efferent artery;
AD. - Adductor muscle;
AA. - Adductor muscle;
CR. - Cartilagenous rod;
EC. - Epithelial cells;
SE. - Samaeous epithelium;
HC. - Hacous cells;
WL. - Wanderung leukocytes;
COC. - Chloride cells.
From each cartilage ossa supporting rod extends to each gill lamella along the outer midregion. The cartilagenous supporting rod gives off branches, each of which runs through the midregion of every secondary lamella.

The common afferent artery gives off one primary afferent branch to each gill lamella running along the outer border. The primary afferent branch gives off numerous minute capillaries running through the secondary lamellae. Finally the capillaries converge to form a primary afferent artery along the inner side. The primary afferent arteries join to form the single afferent artery situated at the base of hemibranch as described earlier.

The secondary lamellae are extremely thin and are principally comprised of epithelial tissue and central rod of cartilagenous supporting tissue. The epithelial tissue is composed of a layer of squamous epithelial cells followed by another layer of mucous cells and basal lamina. A few blood lacunae are also present in between the epithelial layer and central rod of cartilagenous tissue. In addition, wandering leukocytes are seen randomly distributed throughout the secondary lamellae. The central rod of cartilage is profusely provided with blood tissue supported by pilaster cells (Plate I, Figs. 3 and 4).
PLATE II

Microphotographs of the sections of normal liver of *Chauna punctatus* showing:

**Fig. 1.** Hepatic cord like arrangement (500 X)

**Fig. 2.** Hepatic cells (1600 X).

**Fig. 3.** Blood sinusoids (1600 X).

Abbreviations:

*BS*.- Blood sinusoid;
*BC*.- Bile canaliculi.
Chloride cells are present at the junction of two adjacent secondary lamellae (Plate I, Fig. 5).

1b. THE LIVER:

The liver of normal fish comprises a continuous mass of hepatic cells with cord-like formation (Plates II, Fig. 1). Unlike in the normal liver, in fish no clear division of hepatic cells into lobules is observed. The wall separating the neighbouring cords is found to be two cells thick. The hepatic cells are large in size, generally hexagonal in shape with more or less centrally placed nucleus and homogeneous cytoplasm (Plate II, Fig. 2). A large number of blood sinusoids is found in the hepatic mass of these cords (Plate II, Fig. 3). Very thin bile canaliculi are observed between the hepatic cells.

1c. THE KIDNEY:

The kidney of a normal fish comprises numerous functional excretory units termed as nephrons. Each nephron consists of a renal corpuscle, a coiled urineiferous tubule and interstitial hematopoietic tissue.

The renal corpuscle is made up of a glomerulus and Bowman's capsule. Glomerulus is a network of blood capillaries connecting afferent arterioles with efferent arterioles (Plate III, Figs. 1 and 2). Glomerular walls are very thin and consist principally of squamous
endothelial cells. Mesangial cells are also present in between the capillaries mainly towards the hilar region.

The Bowman's capsule is a cup shaped double walled structure, of which the inner one closely surrounds the glomerulus. This inner wall is composed of visceral epithelium and closely adheres to the capillaries of the glomerulus. The cells of the visceral epithelium are squamous in nature. The outer wall is known as parietal layer. The latter is thicker than the inner wall and is formed mainly of cuboidal cells. A space termed as Bowman's space separates the inner wall from the outer one.

Bowman's capsule is actually the proximal terminal end of the uriniferous tubule and is followed by a tubular neck. Other regions of the tubule are a proximal tubule, a distal tubule and a collecting tubule.

The neck segment is lined by a single layer of columnar epithelial cells with generally centrally placed nuclei. Long cilia arise from the inner margin of the cells so as to form a brush border (Plate III, Fig. 3). The proximal tubule is cytologically differentiated into an initial proximal tubule and a second proximal tubule. The initial proximal tubule is characterized by columnar cells with centrally or apically placed nuclei. A well developed brush border is located along the apex of the
PLATE III

Microphotographs of the sections of normal kidney of Channa punctatus showing:

Fig. 1. A renal corpuscle and interstitial hematopoietic tissue (960 X).

Fig. 2. Highly magnified glomerulus (1600 X); Visceral epithelium, capillaries cells and mesangial cells are seen in section.

Fig. 3. Neck segment (960 X).

Fig. 4. Proximal tubule and hematopoietic tissue (960 X).

Fig. 5. Proximal and distal tubule (960 X).

Fig. 6. Collecting tubule (960 X).

Abbreviations:

RC. - Renal corpuscle;
III. - Interstitial hematopoietic tissue;
G. - Glomerulus;
VE. - Visceral epithelium;
MC. - Mesangial cells;
CC. - Capillary cells;
N. - Neck segment;
P. - Proximal tubule;
D. - Distal tubule;
C. - Collecting tubule.
The cells of the second proximal tubule are also columnar with basal nuclei. However, the brush border is not very distinct. The lumen of the distal tubule is lined by tall, dome shaped cells as well as basophilic intercalated cells towards the base (Plate III, Fig. 5). The cells of the collecting tubules are cuboidal and possess centrally placed nuclei. The brush border is absent. Apical mucous granules are prominent in these cells (Plate III, Fig. 6).

The hematopoietic tissue occupies intertubular space. The cells are parenchymatous in nature, round to polygonal in shape with distinct nuclei in the centro (Plate III, Figs. 1 and 4). The tissue is characterised by the presence of numerous red blood corpuscles in different stages of formation.

The following observations were made during the present investigations carried out to study the injury caused to the tissues of murrel by malathion and cadmium.

2. **TOXICITY INDUCED BY MALATHION**

2.1. IN THE Gill:

2.1a. **Histology of the Gill exposed to 1.00 ppm of malathion:**

Upto 4 days of exposure to 1.00 ppm of malathion
the gills of the fishes appeared quite normal. By seventh day however the mucous cells of the epithelium were intensely stained indicating their enhanced activity. Besides, there was a slight increase in the number of wandering leukocytes throughout the gill lamellae (Plate IV, Fig. 1).

By eleventh day the precipitation of mucus was observed in most of the mucous cells and as a result less mucoid substance was seen spread along the gill surface. Moreover, the nuclear material in some of the wandering leukocytes started showing signs of shrinkage. By now the central cartilaginous rod which forms the main support of the gills was also affected. Its cells started losing their cell contents. Damage was also noticed in both cytoplasmic and nuclear contents of the chloride cells and the debris was seen in them as precipitated material (Plate IV, Fig. 2).

Some of the mucous cells of the gills of the fishes exposed for fourteen days contained less mucoid substance resulting in their partial vacuolization. The damage by now spread to a large number of wandering leukocytes. Most of them were now observed to have nuclear material in the precipitated form in the centro with shrunken border. The cells of the cartilagenous rod lost their identity and the entire structure appeared as tube filled with
PLATE IV

Microphotographs to show the histopathological lesions in the gills of fishes exposed to 1.60 ppm of malathion for:

Fig. 1 7 days (960 X).
Fig. 2 11 days (960 X).
Fig. 3 14 days (960 X).
Figs. 4-5 21 days (960 X).
Fig. 6 37 days (960 X).

Abbreviations:

DC.— Buccal cells;
WL.— Wandering leukocytes;
CR.— Cartilaginous rod;
CIL.— Chloride cells;
V.— Vacuole.
precipitated debris and intermittent empty spaces (Plate IV, Fig. 3). Most of the chloride cells had lost their cytoplasm resulting in the formation of empty spaces. In such damaged cells the nuclei were seen pushed towards the periphery.

By twenty-first day the process of disintegration was more pronounced. Some of the mucous cells started appearing empty. Most of the leukocytes were completely disintegrated. Their cytoplasm became less and nuclear content reduced to dark spots in the centre. By now the central rod of cartilage lost most of the precipitated material resulting in the formation of large number of empty spaces (Plate V, Fig. 4). The chloride cells were more or less completely damaged and were seen with empty spaces and cellular debris deposited as round shaped clusters (Plate IV, Fig. 5).

Twenty-seven days exposure resulted in a drastic reduction of cellular material in practically all the cell constituents of the gill lamellae. Likewise a large number of empty spaces appeared throughout the gill lamellae. In addition the number of leukocytes decreased considerably. The central cartilaginous rod was now seen practically as an empty tube with very little precipitated material. Interlamellar space of the gill filament became devoid of any cellular material. However some remnants of debris were observed (Plate IV, Fig. 6).
The cellular structure was more or less completely destroyed by fortieth day and complete necrosis of the epithelial cells was observed. There were hardly any leukocytes in the gill lamellae. However, a few of damaged leukocytes were represented as the remains of shrunken nuclei. The space occupied by the chloride cells became almost blank with few patches of debris spread in between (Plate V, Fig. 1).

2.1b. Histology of the gill exposed to 2.0 ppm and 3.0 ppm of maldichion:

With 2.00 and 3.00 ppm of the pollutant in the medium, the damage was detectable as early as after 24 hours of exposure. With both these concentrations the degenerative changes were similar except for the degree of destruction which became severe with higher dose.

By second day the mucous cells were densely packed with secretory material indicating their stimulated activity. Simultaneously, a large number of leukocytes were also seen to spread throughout the gill lamellae (Plate V, Fig. 2).

By fourth day a larger quantity of mucus was seen in the mucous cells. While the number of leukocytes more or less remained the same, some of them became devoid of cytoplasmic material and their nuclei were seen as darkly
stained structures. The cells of the central cartilaginous rod showed some precipitated material (Plate V, Fig. 3 and Plate VI, Fig. 1). The damage had also spread to the chloride cells.

By seventh day most of the cells of the gill lamellae were severely affected. Some of the mucous cells were lacking in mucus. Due to further damage of the cells quite a few vacuoles developed within the epithelial tissue. Some of the leukocytes were destroyed and were represented merely by remnants of nuclear material in precipitated form and with an irregular border. The central cartilaginous tissue appeared as an indistinct structure practically without any demarcation between adjacent cells (Plate V, Fig. 4 and Plate VI, Fig. 2). The chloride cells contained loss of cytoplasmic material deposited as precipitated case and partial vacuolization was quite visible in them.

By ninth day almost all the cells of epithelium were practically devoid of the cellular material resulting in the formation of a large number of empty spaces. However, some small patches of precipitated mucus were found in them. By now the number of leukocytes had decreased. Besides, the protoplasm of the central cartilaginous rod was damaged more or less completely
PLATE V

Microphotographs to show the histopathological lesions in the gills of fishes exposed to 1.00 ppm (Fig. 1) and 2.00 ppm (Figs. 2-6) of malathion for:

Fig. 1 40 days (960 X).
Fig. 2 2 days (960 X).
Fig. 3 4 days (960 X).
Fig. 4 7 days (960 X).
Fig. 5 9 days (960 X).
Fig. 6 12 days (960 X).

Abbreviations are same as in Plate IV.
with cellular content deposited as precipitated material. Consequently, the entire structure appeared as a long tube filled with debris and vacuoles in between (Plate V, Fig. 5 and Plate VI, Fig. 3). The interlamellar space appeared mostly as a vacuolated structure with little remains of cellular debris.

Most of the cells of the gill lamellae were completely necrotised by twelfth day. The remains of epithelial tissue appeared as large vacuoles and contained only negligible quantity of debris and ruptured leukocytes. The central cartilaginous rod appeared as an almost empty tube (Plate V, Fig. 6). Large empty spaces as well as some patches of damaged tissue were observed in the interlamellar region.

2.1c. histology of the gill exposed to 4.00 ppm of malathion:

The damage caused by 4.00 ppm of malathion was noticed within a very short period and became quite acute by the end of 24 hours. By this time even the secretory activity which was higher during the early stage of exposure to lower dosage of the toxicant was less and quite a few vacuoles were observed within the mucous cells. The leukocytes were also affected by now and the damage was evinced by loss of cytoplasm and precipitated nuclear material accumulated towards the centre. Moreover,
PLATE VI

Microphotographs to show the histopathological lesions in the gills of fish exposed to 3.00 ppm (Figs. 2-3) and 4.00 ppm (Fig. 4) of malathion for:

Fig. 1. 4 days (200 x).
Fig. 2. 7 days (200 x).
Fig. 3. 9 days (200 x).
Fig. 4. 1 day (960 x).

Abbreviations are same as in Plate IV.
PLATE VI
the central cartilaginous rod almost became a hollow tube filled with precipitated debris of cellular material. The damage had spread to the chloride cells also and the debris was deposited as precipitated mass (Plate VI, Fig. 4).

By second day, the damage was wide spread in the mucous cells and most of them had lost their secretory activity as indicated by formation of empty spaces devoid of mucous. Most of the leukocytes were destroyed and were seen without cytoplasm. The nuclear material was deposited as dark patches in the middle. Even the debris which was seen earlier in cartilage started becoming less by end of 24 hours. The tubular structure appeared quite hollow with few patches of debris in between (Plate VII, Fig. 1). A further damage of chloride cells resulted in the beginning of vacuolization (Plate VII, Fig. 2).

The cellular structure of the gill lamellae was more or less completely lost by the end of third day of exposure. The epithelial tissue was seen as diffused mucoid material. The leukocytes had almost disappeared and only a few remnants of the nuclear material were visible. The entire central cartilage appeared as a hollow tube (Plate VII, Fig. 3). The chloride cells developed large vacuoles with little remains of debris.

The damage become very acute by fifth day and by
PLATE VII

Microphotographs to show the histopathological lesions in the gills of fishes exposed to 4.00 ppm of melathion for:

Fig. 1-2. 2 days (960 x).
Fig. 3. 3 days (960 x).
Fig. 4. 5 days (960 x).

Abbreviations are same as in Plate IV.
this time the tissue organisation was completely
disintegrated (Plate VII, Fig. 4). Most of the fishes
died soon thereafter.

2.2. IN THE LIVER:

2.2a. Histology of the liver exposed to 1.00 ppm of
malathion:

One day exposure period marked the beginning of
the precipitation of both cytoplasmic and nuclear material
leading to partial vacuolization in some of the cells. In
such cells the nuclei were seen pressed towards the
periphery. Their nuclear material was congested in the
centre (Plate VIII, Fig. 1). The precipitation of
cytoplasmic material and vacuolization was noticed in some
more cells by the end of second day. Moreover, the bile
canaliculi appeared more distinct being packed with
precipitated material (Plate VIII, Fig. 2). Four days
exposure resulted in a complete vacuolization of some of
the cells (Plate VIII, Fig. 3). Besides, a reduction in
the nuclear material was also observed in them. By this
time the degenerative changes appeared in the blood cells
of some of the sinusoids.

By seventh day about one third of the hepatic cells
were either completely damaged or were in the process of
PLATE VIII

Microphotographs to show the histopathological lesions in the liver of fishes treated with 1.00 ppm of malathion for:

Fig. 1. 1 day (1600 X).
Fig. 2. 2 days (1600 X).
Fig. 3. 4 days (1600 X).
Fig. 4. 7 days (1600 X).
Fig. 5. 14 days (1600 X).
Fig. 6. 21 days (1600 X).

Abbreviations:

BS. - Blood sinusoid;
BC. - Bile canaliculi;
PV. - Partial vacuolisation;
SN. - Shrunken nuclei;
CV. - Coagulate vacuolisation.
degeneration. There was a marked reduction of nuclear material by this time (Plate VIII, Fig. 4). The degenerative changes became severe gradually up to eleven days. By fourteenth day all the cells practically became devoid of their normal structure and most of them were seen in damaged state. Highly damaged hepatic cells could be easily marked into two types, one type appearing as completely vacuolated with distinct borders and other one with diffused material and rather indistinct border (Plate VIII, Fig. 5).

A reduction in the remains of the diffused material was noticed by twenty-first day. Besides, cytoplasmic and nuclear degeneration was observed in blood cells of most of the sinusoids. A decrease in the number of blood cells was also noticed within the sinusoids. By this time associated blood capillaries were filled mostly with damaged coagulated blood cells (Plate VIII, Fig. 6). By twenty-seventh day most of the hepatic cells were necrotised and nuclei were seen at various stages of degeneration (Plate IX, Fig. 1). By fortieth day practically all the hepatic cells were destroyed and the damage was almost complete. Due to the accumulation of damaged material, the bile canaliculi appeared quite distinct. The sinusoids which are full of blood in the normal liver appeared now practically empty with the
remains of some damaged blood cells concentrated on one side (Plate IX, Fig. 2).

2.2b. **Histology of the liver exposed to 2.00 and 3.00 ppm of malathion:**

Both with 2.00 and 3.00 ppm of malathion the process of degeneration of the tissue was more or less similar except that the damage was slightly more severe with 3.00 ppm concentration of the pollutant in the medium.

One day exposure had already resulted in a complete vacuolization of quite a few hepatic cells. The precipitation of nuclear material also started simultaneously in some cells (Plate IX, Fig. 3). By second day a marked reduction in both cytoplasmic and nuclear material was observed in some more number of cells. Moreover, the bile canaliculi became distinct being packed with fluid debris. Cytoplasmic and nuclear degeneration of the cells of some sinusoids were also noticed.

By fourth day the destruction of protoplasmic material was found to be widespread. Further, the number of the blood cells in the sinusoids was reduced and those present had lost their compactness and showed distinct signs of degeneration (Plate IX, Fig. 4 and Plate X, Fig.1).
PLATE IX

Microphotographs to show the histopathological lesions in the liver of fishes exposed to 1.00 ppm (Figs. 1-2) and 2.00 ppm (Figs. 3-6) malathion for:

Fig. 1. 27 days (1600 X).
Fig. 2. 40 days (1600 X).
Fig. 3. 1 day (1600 X).
Fig. 4. 4 days (1600 X).
Fig. 5. 7 days (1600 X).
Fig. 6. 12 days (1600 X).

Abbreviations are same as in Plate VIII.
Seven days exposure resulted in the loss of border of some of the cells and consequently coagulate vasculisation was noticed in them. A further reduction in the content of nuclear material was observed (Plate II, Fig. 5 and Plate I, Fig. 2). By ninth day the damage of the tissue material became quite distinct in the cells bordering the periphery of the sinusoids. A significant reduction of the protoplasmic material was noticed in the rest of the cells. The nuclei were seen at various stages of degeneration. A further decrease in the number of blood cells was observed in most of the sinusoids. The damaged blood cells were spread quite apart in the sinusoids and as a result empty spaces appeared in them (Plate I, Fig. 3).

By twelfth day, most of the cells became practically devoid of cytoplasm. The nuclear material was further reduced and some of the nuclei lost most of their contents. More bile canaliculi were seen further packed with the debris of the decomposed tissue (Plate II, Fig. 6 and Plate I, Fig. 4). Fishes maintained in these concentrations of malathion viz., 2.00 and 3.00 ppm did not survive beyond twelve days of exposure.

2.2c. Histology of the liver exposed to 4.00 ppm of malathion:

Disintegration of the tissue was quicker and more severe
PLATE X

Microphotographs to show the histopathological lesions in the liver of fishes exposed to 3.00 ppm of malathion for:

Fig. 1. 4 days (1600 X).
Fig. 2. 7 days (1600 X).
Fig. 3. 9 days (1600 X).
Fig. 4. 12 days (1600 X).

Abbreviations are same as in Plate VIII.
with this concentration. Here the fish did not survive for more than five days. Even one day exposure brought about precipitation of cytoplasmic and nuclear material with consequent vacuolisation in most of the hepatic cells (Plate XI, Fig. 1). The damage of blood cells started in some of the sinusoids. By second day complete vacuolization and pushing of the nuclei outside the hepatic cells were noticed. In addition, there was a marked reduction in the content of nuclear material (Plate XI, Fig. 2). Moreover, bile canaliculi were seen fully packed with disintegrated material.

By third day of exposure almost all the cells were vacuolated and seen with negligible amount of cytoplasm located around the periphery. Further, quite a few of them were observed with indistinct border (Plate XI, Fig. 3). By now most of the sinusoids were seen with highly degenerated blood cells. The liver of the animals fixed immediately after the death on fifth day showed a complete destruction of the hepatic cells. Few remains of disintegrated cellular material was seen spread at random in them. Practically all the blood sinusoids were full of coagulated blood cells (Plate XI, Fig. 4).
PLATE XI

Microphotographs to show the histopathological lesions in the liver of fishes exposed to 4.00 ppm of malathion for:

Fig. 1. 1 day (1600 X).
Fig. 2. 2 days (1600 X).
Fig. 3. 3 days (1600 X).
Fig. 4. 5 days (1600 X).

Abbreviations are same as in Plate VIII.
PLATE XI
2.3. IN THE KIDNEY:

2.3a. Histology of the kidney exposed to 1.00 ppm of malathion:

No marked changes were observed up to four days of exposure with 1.00 ppm concentration. However, seven days exposure marked the beginning of the precipitation of cytoplasmic material in quite a few proximal tubular cells. A reduction in the content of nuclear material also started in these cells (Plate XII, Fig. 1). By eleven days the blood flowing through the glomeruli also showed signs of degeneration. Cytoplasmic content became reduced in some of its blood cells and the nuclear material showed the signs of shrinkage (Plate XII, Fig. 2). As regards the proximal tubules a large number of their cells were seen affected resulting in the precipitation of cytoplasmic as well as nuclear material. The damage was also spread to hematopoietic tissue. The precipitation of protoplasmic material was seen in some of its parenchymatous cells (Plate XII, Fig. 3).

The damage became severe by fourteen days and spread to all the tissues of the kidney. Quite a few empty spaces developed within the glomeruli due to the degeneration of blood contents as well as cell components of the capillaries (Plate XII, Fig. 4). It also resulted in the disappearance of the cytoplasmic and nuclear
content of the proximal tubular cells. The nuclei developed irregular shapes. The brush border lost its uniform structure and appeared as an irregular mass of cilia (Plate XII, Fig. 5). By this period the damage was also extended to the distal and collecting tubules (Plate XIII, Fig. 6). The damage of the hematopoietic tissue became severe and even the developing blood corpuscles started disintegrating. Empty spaces were seen in the hematopoietic tissue as a result of the damage to parenchymatous cells.

By twenty-first day the damage was widespread affecting practically all the glomeruli. As a consequence, the glomerular tissue developed empty spaces (Plate XIII, Fig. 1). Further some of the proximal tubular cells were completely damaged and appeared quite empty (Plate XIII, Fig. 2). The disintegration of the cellular mass of the distal and collecting tubules resulted in the formation of lumps of precipitated material (Plate XIII, Fig. 3). Moreover, the precipitation of cytoplasmic as well as nuclear material spread to many cells of hematopoietic tissue (Plate XIII, Fig. 4).

By twenty-seventh day the damage was widespread and a lesser number of blood corpuscles were seen within the glomerulii. The latter appeared vacuolated and quite indistinct with remnants of some blood cells and
Microphotographs of the kidney of fishes exposed to 1.00 ppm of malathion to show the damage caused to the:

Fig. 1. glomerulus (960 x). EP:— 21 days.
Fig. 2. proximal tubule (960 x). EP:— 21 days.
Fig. 3. collecting tubule (960 x). EP:— 21 days.
Fig. 4. hematopoietic tissue (960 x). EP:— 21 days.
Fig. 5. proximal tubule (960 x). EP:— 27 days.
Fig. 6. collecting tubule (960 x). EP:— 27 days.

EP:— Exposure period.

Abbreviations are same as in Plate XIII.
degenerating cell components of the capillaries.

Destruction of cells was also rampant in the various regions of uriniferous tubules. The damage was characterized by the loosening of cell borders and vacuolization of cytoplasmic material. The tubular cells possessed very little material mostly in the diffused form with lot of empty spaces. The brush border was interrupted at many places (Plate XIII, Figs. 5 and 6). The hematopoietic tissue was also very severely affected and more vacuoles appeared indicating more or less a complete destruction of some of the tissue material.

By fortieth day the number of vacuoles had increased in the glomerular region. Some of the tissue cells of the capillaries became loose and could be seen as separate entities. The number of blood cells was drastically reduced and were seen with shrunken nuclei (Plate XIV, Fig. 1).

The protoplasmic material of almost all the proximal tubular cells was practically reduced to vacant spaces (Plate XIV, Fig. 2). Further, the cells of the distal and collecting tubules had very little material mostly in a diffused form (Plate XIV, Fig. 3). The lumen of a few collecting tubules became irregular in shape and was filled with fluid debris. The hematopoietic tissue was considerably damaged and was seen as detached loose cells with distinct empty spaces. Their protoplasmic material appeared like a diffused mass
PLATE XIV

Microphotographs of the kidney of fishes exposed to 1.00 ppm (Figs. 1-3) and 2.00 ppm (Figs. 4-6) of malathion to show the degeneration caused to the:

Fig. 1. glomerulus (960 X). EP.- 40 days.
Fig. 2. proximal tubule (960 X). EP.- 40 days.
Fig. 3. distal tubule (960 X). EP.- 40 days.
Fig. 4. proximal tubule (960 X). EP.- 2 days.
Fig. 5. glomerulus (960 X). EP.- 4 days.
Fig. 6. proximal tubule (960 X). EP.- 4 days.

EP.- Exposure period.

Abbreviations are same as in Plate XIII.
with more or less indistinguishable cytoplasm and nucleus.

2.3b. **Histology of the kidney exposed to 2.00 and 3.00 ppm of malathion:**

With 2.00 and 3.00 ppm of malathion the damage to the kidney started earlier than the one observed with lower concentration. Fishes exposed to these toxic levels did not survive beyond twelve days. The damage induced by both these concentrations was generally similar except for some variation in the degree of injury caused proportional to the dosages.

No marked damage was observed in various cell constituents of the kidney up to 24 hours of exposure. However, after this period some of the proximal tubular cells started showing signs of decomposition. Precipitation of cytoplasmic as well as nuclear material was noticed in these cells. Parenchymatous cellular material of the hematopoietic tissue started coagulating (Plate XIV, Fig. 4). By fourth day the blood cells flowing through the glomeruli were affected and started showing signs of disintegration. The cytoplasmic material gradually started getting reduced and nuclei were shrunk (Plate XIV, Fig. 5). The cells of proximal tubules were similarly damaged (Plate XIV, Fig. 6). There was a marked reduction in the protoplasmic material of some parenchymatous cells of hematopoietic tissue and
some of them appeared more or less empty with shrunken nuclei in the centre.

By seventh day the injury was wide spread and practically all the tissues of the kidney were severely affected. The blood corpuscles passing through the capillaries were in a highly damaged state and little cellular material was left in them. The injury was spread to the capillaries also. Most of the proximal tubular cells lost their entity and appeared empty. The brush border became irregular. Besides, the protoplasm of both the distal and collecting tubular cells were seen with precipitated material strung in patches (Plate XV, Fig. 1). The hematopoietic tissue lost its homogenous structure and was seen with diffused material spread in between empty spaces (Plate XV, Fig. 2).

Nine days exposure resulted in the formation of diffused structure of all the cell components of the kidney. As a result of the further damage, the glomeruli lost their typical structure and empty spaces developed in them. The remnants of degenerating cells of capillaries were seen as patches of diffused material (Plate XV, Fig. 3). The condition of the proximal tubular cells appeared similar to the one seen on the seventh day of exposure except for the brush border which had almost
Microphotographs of the kidney of fishes exposed to 2.00 ppm of malathion to show the degeneration caused to the:

Fig. 1. proximal and collecting tubules (960 x). EP. = 7 days.

Fig. 2. hematopoietic tissue (960 x). EP. = 7 days.

Fig. 3. glomerulus (960 x). EP. = 9 days.

Fig. 4. proximal and collecting tubules (960 x). EP. = 9 days.

Fig. 5. glomerulus (960 x). EP. = 12 days.

Fig. 6. proximal tubule (960 x). EP. = 12 days.

EP. = Exposure period.

Abbreviations are same as in Plate XII.
disappeared. Further, the cells of both distal as well as collecting tubules showed some remnants of cellular material in diffused form with empty spaces in between (Plate XV, Fig. 4). The lumen of quite a few tubules became indistinct. The hematopoietic tissue was severely affected and most of the parenchymatous cells had disappeared completely. The spaces were however filled with some developing blood cells in a damaged condition.

On twelfth day the glomeruli appeared as highly vacuolated structures, with formation of clusters of disintegrated material caused by the disintegration of the blood cells and capillaries (Plate XV, Fig. 5). Almost all the proximal tubules were completely necrotised. Their cell membrane had ruptured and the debris of material had spread at random. The brush border was completely obliterated. The lumen of some of the tubules appeared irregular and filled with decomposed material (Plate XV, Fig. 6). Few remains of the cell content of the distal as well as collecting tubules were observed as diffused patches with large empty spaces. Hematopoietic tissue appeared as a vacuolated structure with highly decomposed remains of the developing blood cells (Plate XVI, Fig. 1).
2.3e. Histology of the kidney exposed to 4.00 ppm of malathion:

The toxic effect of 4.00 ppm of malathion was very severe and the injury was marked even in the kidney of 24 hours treated animals. The precipitation of both cytoplasmic and nuclear material had already started in most of the proximal tubular cells. Besides, coagulation of cytoplasm was also noted in the parenchymatous cells of hematopoietic tissue (Plate XVI, Fig. 2). By the end of second day all parts of the kidney were affected. Even the blood passing through the glomeruli started losing their cellular structure. Further, the cells of the glomerular capillaries also showed signs of degeneration (Plate XVI, Fig. 3). Some empty spaces were seen inside the proximal tubular cells with cellular debris spread along the border (Plate XVI, Fig. 4). The cells of the distal as well as collecting tubules were similarly affected. Precipitated cytoplasm was seen in the form of distinct patches in collecting tubular cells and to some extent in the distal tubules also (Plate XVI, Fig. 5). The nuclear material of these tubular cells become less and was pressed towards the basal end. Some of the parenchymatous cells were completely damaged and the cellular components of the remaining ones were seen as diffused mass with empty spaces in between. The damage
PLATE XVI

Microphotographs of the kidney of fishes exposed to 2.00 ppm (Fig. 1) and 4.00 ppm (Figs. 2-6) of malathion to show the damage caused to the:

Fig. 1. hematopoietic tissue (960 X). EP.— 12 days.
Fig. 2. proximal tubule (960 X). EP.— 1 day.
Fig. 3. glomerulus (960 X). EP.— 2 days.
Fig. 4. proximal tubule (960 X). EP.— 2 days.
Fig. 5. collecting tubule (960 X). EP. 2 days.
Fig. 6. hematopoietic tissue (960 X). EP.— 2 days.

EP.— Exposure period.

Abbreviations are same as in Plate XII.
also spread to the developing blood cells and a reduction in the cytoplasmic content and shrinkage of the nuclear material was noted (Plate XVI, Fig. 6).

Third day exposure resulted in the development of quite a number of empty spaces in glomeruli, as a result of complete destruction of the blood as well as cell constituents of the capillaries (Plate XVII, Fig. 1). By now the proximal tubular cells became practically devoid of cytoplasmic material. However, remains of nuclear material were seen towards the base. The brush border became indistinct. The collecting tubules were completely damaged. Their cellular material was seen in the form of large clusters of homogenous material and empty spaces surrounding them (Plate XVII, Fig. 2). The cellular content of the parenchymatous cells had more or less completely disappeared and was seen as patches of damaged material and empty spaces in the hematopoietic tissue.

The glomerular structure was more or less completely damaged and ruptured at the end of 5 days of exposure. The glomerulus now appeared as a cluster of damaged cytoplasmic material and shrunken nuclei (Plate XVII, Fig. 3). Most of the proximal tubular cells had lost their cell membranes and coagulate vasculisation was noted. The remains of cellular content were seen as debris spread at random. Very little cytoplasm was left in the cells of the distal
PLATE XVII

Microphotographs of the kidney of fishes exposed to 4.00 ppm of malathion to show the damage caused to the:

Fig. 1. Glomerulus (960 X). EP. - 3 days.
Fig. 2. Collecting tubule (960 X). EP. - 3 days.
Fig. 3. Glomerulus and proximal tubule (960 X). EP. - 5 days.
Fig. 4. Distal tubule (960 X). EP. - 5 days.

EP. - Exposure period.

Abbreviations are same as in Plate XIII.
and collecting tubules and large empty spaces were seen in them (Plate XVII, Fig. 4). The hematopoietic tissue cells were destroyed more or less completely with the exception of a few blood cells which were observed with reduced cytoplasm and shrunken nuclei in the centre.

3. TOXICITY INDUCED BY CADMIUM

3.1. IN THE GILL:

3.1a. Histology of the gill exposed to 0.01 ppm of cadmium:

No marked changes were observed up to 10 days of exposure with 0.01 ppm of cadmium. However, by 13th day a large number of wandering leukocytes were seen throughout the gill lamellae. The mucous cells became more active as evidenced by their staining intensity of the mucus (Plate XVIII, Figs. 1 and 2). By 17th day the degenerative changes were well marked within the epithelial tissue. The intensity of the staining of the secretory cells became lighter indicating a slow activity of the mucous cells. By now central cartilaginous tissue rod was also affected (Plate XVIII, Fig. 3).

By 26th day the damage became widespread and affected all the cells of the gill lamellae. Some of the mucous cells stopped secreting the mucus and vasculoles were
**PLATE XVIII**

Microphotographs to show the histopathological lesions caused to the gills of the fishes exposed to 0.01 ppm of cadmium for:

- **Fig. 1.** 13 days (960 x).
- **Fig. 2.** 13 days (960 x).
- **Fig. 3.** 17 days (960 x).
- **Figs. 4-5.** 28 days (960 x).
- **Fig. 6.** 40 days (960 x).

**Abbreviations:**

- MC. = Buccous cells;
- WL. = Wandering leukocytes;
- CR. = Cartilaginous rod;
- CHO. = Chloride cells;
- V. = Vacuole.
seen in them. Degenerative changes were observed inside the blood vessels passing through the central cartilagenous rod (Plate XVIII, Fig. 4). The blood cells lost some of their cytoplasm and the nuclear material was seen in the precipitated form in midregion. The cytoplasmic material inside the chloride cells got precipitated and vasoconstriction was observed in them (Plate XVIII, Fig. 5).

By 40th day a drastic reduction of mucus was seen in almost all the mucous cells resulting in the formation of an increased number of empty spaces. Cytoplasmic and nuclear destruction was spread to large number of wandering leukocytes. Quite a few of them were completely disintegrated and were represented by specks of precipitated nuclear material. The protoplasm of the central cartilagenous rod was in disarray and the debris was precipitated within the cells (Plate XVIII, Fig. 6). The destruction of blood cells flowing through the rod and partial degeneration of the protoplasmic material resulted in the formation of empty spaces.

By 47th day most of the protoplasm of all the constituents of the gill lamellae was destroyed resulting in the formation of numerous empty spaces. The nuclear remains of the damaged leukocytes were seen as round or oval structures. The cellular boundaries almost disappeared and a few patches of debris were seen spread
at random (Plate XIX, Fig. 1). Likewise the chloride cells were represented by blank spaces with intermittent clusters of debris.

By 51st day the tissue organization was completely damaged. The entire gill lamellae was seen with remnants of diffused cytoplasmic material. Even the remnants of the nuclear remains were destroyed more or less completely. The central cartilage as well as surrounding tissue appeared as a hollow longitudinal tube with some debris filled inside. The chloride cells were destroyed completely and were seen with a few patches of decomposed material (Plate XIX, Fig. 2).

3.1b. Histology of the gill exposed to 0.03 ppm of cadmium:

Degenerative changes were observed only at the end of 10 days of exposure with 0.03 ppm of the toxicant. The secretory material was deeply stained in the mucous cells indicating its increased activity. Simultaneously, a large number of wandering leukocytes were seen to spread throughout the gill lamellae (Plate XIX, Fig. 3).

By 17th day the activity of mucous cells had decreased and less mucous was found in mucous cells. Some wandering leukocytes were observed in the process of degeneration as marked by the loss of some protoplasmic material. The degenerating cellular material of central
Microphotographs to show the damage caused to the gills of fishes when exposed to 0.01 ppm (Figs. 1-2) and 0.03 ppm (Figs. 3-6) of cadmium for:

Fig. 1. 47 days (960 X).
Fig. 2. 51 days (960 X).
Fig. 3. 10 days (960 X).
Fig. 4. 17 days (960 X).
Figs. 5-6. 20 days (960 X).

Abbreviations are same as in Plate XVIII.
PLATE XIX
cartilaginous rod showed some precipitation (Plate XIX, Fig. 4). The damage had spread to chloride cells also.

By 20th day of exposure the secretory activity of some mucous cells had ceased and empty spaces were observed in the epithelial tissue. The nuclear material of some of the wandering leukocytes had precipitated in the midregion and the number of cells were reduced and assumed abnormal shapes. The cells of the central cartilaginous tissue lost their identity and the entire rod appeared like a hollow tube filled with precipitated debris and some empty spaces (Plate XIX, Fig. 5). The injury caused to the chloride cells induced the formation of the precipitation and consequent vacuolization of the protoplasmic material (Plate XIX, Fig. 6).

By 28th day the process of disintegration was well marked in all the cells of gill lamellae. As a result there was a further reduction of mucoid substance with empty spaces formed in the mucous cells. Most of the wandering leukocytes were represented by dots of nuclear material with some negligible cytoplasm. By now even the debris became less in the central cartilaginous rod and consequently more number of empty spaces were formed in them (Plate XX, Fig. 1). Chloride cells were by now reduced to empty spaces with debris in between.
By 40th day the entire epithelial tissue appeared as a patch of empty spaces along with a few clusters of diffused debris of the cytoplasm. Most of the leukocytes were damaged and the rest were in the process of disintegration (Plate XX, Fig. 2). The interlamellar region also appeared empty.

By 47th day of exposure there was hardly any distinction between the epithelial tissue, central cartilagenous rod and the chloride cells. The entire lamella appeared as full of vacuoles along with debris of the protoplasm in the diffused form and a few remains of some leukocytes (Plate XX, Fig. 3).

By 51st day the entire lamella appeared as an empty space having distinct border and few patches of damaged protoplasmic material within (Plate XX, Fig. 4).

3.1c. Histology of the gill exposed to 0.05 ppm of cadmium:

The damage caused by 0.05 ppm of cadmium was detectable by the end of 6th day of exposure. Initially the mucous cells became more active as evinced by the deeply stained mucus. In addition, an increase in the number of wandering leukocytes was also noticed (Plate Xa, Fig. 5).
Microphotographs to show the histopathological lesions in the gills of fishes exposed to 0.03 ppm (Figs. 1-4) and 0.05 ppm (Figs. 5-6) of cadmium for:

Fig. 1. 28 days (960 X).
Fig. 2. 40 days (960 X).
Fig. 3. 47 days (960 X).
Fig. 4. 51 days (960 X).
Fig. 5. 6 days (960 X).
Fig. 6. 10 days (960 X).

Abbreviations are same as in Plate XVIII.
By 10th day the degeneration was wide spread affecting all the cells of the lamella. The secretory activity of some of the mucus cells was declined. The leukocytes were also affected and some of them were seen with little cytoplasm and precipitated nuclear material in the centre. The cells of the cartilage lost their cytoplasm and debris of precipitated nuclear material was seen. The protoplasm was seen in precipitated form in some of the chloride cells resulting in the formation of some empty spaces (Plate XI, Fig. 6).

By 20th day most of the mucous cells became devoid of mucus and as a consequence vacuoles appeared in the epithelium. The number of wandering leukocytes decreased throughout the gill lamellae and were seen at various stages of protoplasmic disintegration. The cytoplasm and central cartilagenous rod was reduced and was represented as debris of precipitated cellular material and intermittent empty spaces (Plate XI, Fig. 1). Almost all the chloride cells were affected by now and a large number of empty spaces were observed with some patches of precipitated protoplasm inbetween (Plate XI, Fig. 2).

By 29th day the cellular structure of gill epithelium was completely destroyed. The mucus cells lost their secretory activity. Leukocytes were damaged and reduced to specks of shrunken nuclear material. The entire
PLATE XII

Microphotographs to show the histopathological lesions in the gills of the fishes exposed to 0.05 ppm of cadmium for:

Figs. 1-2. 20 days (960 X).
Fig. 3. 29 days (960 X).
Fig. 4. 40 days (960 X).

Abbreviations are same as in Plate XVIII.
cartilagenous rod was filled with diffused debris along with a few remnants of damaged nucleus (Plate XXI, Fig. 3). The interlamellar region was mostly empty except for some negligible remains of debris.

40th day of exposure resulted in complete necrosis of most of the cells of the gill lamellae. The entire gill lamella was reduced to a disintegrated cartilagenous rod full of debris and large empty spaces bounded by distinct border (Plate XXI, Fig. 4). The chloride cells were also completely destroyed and were seen as blank spaces.

3.2. IN THE LIVER:

3.2a. Histology of the liver exposed to 0.01 ppm of cadmium:

Within 24 hours of exposure the precipitation of the cytoplasmic material and partial vacuolisation had started in a few hepatic cells. In such cells the nuclear material was deposited in the form of dense granular structure (Plate XXII, Fig. 1). By second day the damage spread to a larger number of cells and precipitation of cellular material and vacuolisation was noticed in them. By 4th day the degenerating protoplasmic material started disappearing. Besides, it also resulted in the shrinkage of nuclei, which started moving towards the border of cells (Plate XXII, Fig. 2). By 13th day the cytoplasm in most
Microphotographs to show the histopathological lesions in the liver of the fishes exposed to 0.01 ppm of cadmium for:

Fig. 1. 1 day (1600 X).
Fig. 2. 4 days (1600 X).
Fig. 3. 13 days (1600 X).
Fig. 4. 17 days (1600 X).
Fig. 5. 28 days (1600 X).
Fig. 6. 47 days (1600 X).

Abbreviations:

BS. - Blood sinusoid;
BO. - Bile canaliculi;
PV. - Partial vasculisation;
3K. - Shrunken nuclei;
CV. - Coagulate vasculisation.
of the hepatic cells was drastically reduced and
vasculisation was more or less complete. The nuclei were
observed at various stages of degeneration (Plate XXII, Fig. 3).
The damaged nuclear material was seen in the form of
precipitation deposited eccentrically. The nuclei were
closely pressed towards the border of cells.

A very interesting phenomenon was noticed thereafter.
Instead of further damage to the tissue, actually the
regeneration of protoplasmic material was observed by
17th day of exposure. The cells started regaining the lost
protoplasm (Plate XXII, Fig. 4). As a result both the
cytoplasm and the nuclear material of most of the hepatic
cells became normal by 28th day (Plate XXII, Fig. 5).

However, by 40th day the protoplasm started
degenerating once again, as evinced by the precipitation
of cytoplasmic material. The nuclear material also started
getting reduced. By 47th day the cytoplasm became less
and as a consequence vascuolisation was marked once again.
By now, most of the nuclei had shrunk (Plate XXII, Fig. 6).
The blood cells of sinusoids were also affected as evinced
by the loss of cytoplasmic as well as nuclear material.
The latter was seen as a dense precipitated mass. By 51st
day almost all the hepatic cells were completely destroyed
with debris of the cytoplasm spread at random. Besides,
the shrunken nuclear material was reduced to a small speck
in the centre (Plate XXIII, Fig. 1). The blood cells in most of the sinusoids were also damaged indicating more or less complete necrosis of all the cell components of the tissue. The experiment was discontinued after 51 days.

3.2b. **Histology of the liver exposed to 0.03 ppm of cadmium:**

One day exposure brought about the precipitation of both the cytoplasmic and nuclear material. As a consequence partial vacuolization was observed in many of the hepatic cells. In such damaged cells the nuclei were seen closely pressed towards the border (Plate XXIII, Fig. 2). By fourth day the degeneration was widespread affecting almost all the hepatic cells. Precipitation of cytoplasmic material leading to vacuolization of the cells was observed. Besides, some of the nuclei started shrinking (Plate XXIII, Fig. 3). Ten days exposure resulted in a loss of protoplasmic material and by 17th day most of the hepatic cells became devoid of cytoplasmic material. The cells were completely vacuolised and nuclei lost most of their contents (Plate XXIII, Fig. 4).

However, by 20th day the hepatic cells started showing signs of recovery and gradually the cells became normal with the synthesis of more protoplasmic substance (Plate XXIII, Fig. 5). The nuclei also assumed normal size and shape. The process of regeneration was rather
Plate XXIII

Microphotographs to show the histopathological lesions in the liver of the fishes to 0.01 ppm (Fig. 1) and 0.03 ppm (Figs. 2-6) of cadmium for:

Fig. 1. 51 days (1600 X).
Fig. 2. 1 day (1600 X).
Fig. 3. 4 days (1600 X).
Fig. 4. 17 days (1600 X).
Fig. 5. 20 days (1600 X).
Fig. 6. 28 days (1600 X).

Abbreviations are same in Plate XXII.
slow with 0.03 ppm and it took about 20 days more before the hepatic cells appeared normal once again (Plate XXIII, Fig. 6 and Plate XXIV, Fig. 1). Soon thereafter, the damage started once again and by 47th day the destruction of protoplasm with consequent formation of precipitation and vacuolization was observed at least in some cells (Plate XXIV, Fig. 2). The damage also spread to the blood cells passing through sinusoids and was marked by loss of their cytoplasmic material and precipitation of nuclear material in the centre. By 51st day the disintegration of the tissue became more pronounced and practically all the cells were completely necrotized (Plate XXIV, Fig. 3). The blood sinusoids were also destroyed and the blood cells within them were seen at various stages of cytoplasmic and nuclear disintegration. Nonetheless, the animals continued to survive at least up to 51 days after which the experiment was discontinued.

3.20. Histology of the liver exposed to 0.05 ppm of cadmium:

The histopathological lesions induced by 0.05 ppm of cadmium were similar to those observed with 0.03 ppm during early period of exposure (Plate XXIV, Fig. 4). By ten days of exposure the tissue was marked by the loss of cytoplasm and nearly one half of the hepatic cells were completely vacuolated and the nuclei pushed towards the periphery. In the remaining cells however, only
Microphotographs to show the histopathological lesions in the liver of the fishes exposed to 0.03 ppm (Figs. 1-3) and 0.05 ppm (Fig. 4) of cadmium for:

Fig. 1. 40 days (1600 X).
Fig. 2. 47 days (1600 X).
Fig. 3. 51 days (1600 X).
Fig. 4. 1 day (1600 X).

Abbreviation are same as in Plate XXIII.
precipitation of cytoplasmic and nuclear material was seen (Plate XXIV, Fig. 1). By 20th day practically all the cells became devoid of cytoplasm and vacuolization was more or less complete. Their nuclei were also disintegrated and had shrunk (Plate XXV, Fig. 2). The blood cells of sinusoids started degenerating. Their cytoplasm became less and nuclear material was concentrated in dense precipitated form. By 29th day a few of the hepatic cells lost their borders and coagulate vacuolization was observed. Consequently large vacuolated structures were seen with two or more nuclei in them (Plate XXV, Fig. 3). Most of the blood sinusoids were severely damaged. No regeneration of protoplasmic substance was seen except for some negligible quantity of cytoplasm in a few cells by 40th day. Most of the fish started dying soon thereafter and the experiment was discontinued (Plate XXV, Fig. 4).

3.3. IN THE KIDNEY:

3.3a. Histo!ogy of the kidney exposed to 0.01 ppm of cadmium:

No marked changes were observed up to 10 days of exposure with 0.01 ppm of cadmium. The proximal tubular cells were the first to be affected. By 13th day the cytoplasmic and nuclear material in some proximal tubular cells showed precipitation (Plate XXVI, Fig. 1). By 17th day some of the blood cells flowing through glomeruli
PLATE XXV

Microphotographs to show the damage caused to the liver of fishes, when exposed to 0.05 ppm of cadmium for:

Fig. 1. 10 day (1600 X).
Fig. 2. 20 days (1600 X).
Fig. 3. 29 days (1600 X).
Fig. 4. 40 days (1600 X).

Abbreviations are same as in Plate XIII
started degenerating. Their cytoplasm became less and nuclei showed signs of shrinkage (Plate XXVI, Fig. 2).
The cytoplasm started disappearing in the proximal tubular cells (Plate XXVI, Fig. 3). By now the hematopoietic tissue was also affected and precipitation of cytoplasmic and nuclear material was seen in some of its parenchymatous cells.

Twenty-eight days of exposure brought about disintegration of some blood cells and cell components of capillaries resulting in the formation of vacuoles within the glomeruli. The protoplasmic content was reduced in the proximal tubular cells. Their brush border became irregular at some places (Plate XXVI, Fig. 4). The damage also spread to distal and collecting tubular cells and protoplasmic substance precipitated in quite a few of them (Plate XXVI, Fig. 5). Further, some parenchymatous cells of hematopoietic tissue were destroyed and as a consequence empty spaces were developed within the tissue.

By 40th day the damage became very severe. As a result of gradual disintegration of blood and capillaries, the glomeruli appeared mostly as large empty spaces (Plate XXVI, Fig. 6). The cells of the proximal tubules were more or less completely destroyed and contained remnants of cytoplasm and shrunken nuclei. The brush border became indistinct (Plate XXVII, Fig. 1).
PLATE  XXVI

Microphotographs of the kidney of fishes exposed to the 0.01 ppm of cadmium to show the damage caused to the:

Fig. 1. proximal tubule (960 X). EP.- 13 days.
Fig. 2. glomerulus (960 X). EP.- 17 days.
Fig. 3. proximal tubules (960 X). EP.- 17 days.
Fig. 4. proximal tubules (960 X). EP.- 26 days.
Fig. 5. distal and collecting tubule (960 X). EP.- 28 days.
Fig. 6. glomerulus (960 X). EP.- 40 days.

EP.- Exposure period.

Abbreviations:

G.- Glomerulus;
P.- Proximal tubule;
D.- Distal tubule;
C.- Collecting tubule;
HT- Hematopoietic tissue;
V.- Vessel;
SN- Shrunken nuclei.
By now empty spaces were formed in the distal as well as collecting tubular cells also (Plate XXVII, Fig. 2). The parenchymatous cells of the hematopoietic tissue were severely affected. Most of them were completely destroyed and the rest were in the process of destruction. Their cellular material was seen in the precipitated form with empty spaces in between. The developing blood cells were also affected as evinced by their precipitated cellular material.

Forty-seven days of exposure resulted in complete destruction of practically all the tissues of the kidney. The glomeruli became quite indistinct. Most of their protoplasmic material was deposited as debris resulting in the formation of large sized vacuoles inside. Due to the loss of cytoplasmic material empty spaces were seen within proximal tubular cells also. By now the nuclear membrane of some nuclei became indistinct (Plate XXVII, Fig. 3). Due to the further degeneration of protoplasmic substance large number of empty spaces appeared within the cells of distal and collecting tubules. Nuclei of some of these cells were practically devoid of any nuclear material (Plate XXVII, Fig. 4). The hematopoietic tissue was also severely affected and represented as large empty spaces along with debris of both parenchymatous and developing blood cells. The latter had little cytoplasm
Microphotographs of the kidney of fishes exposed to 0.01 ppm of cadmium to show the damage caused to the:

*Fig. 1.* proximal tubule (960 X). EP.– 40 days.
*Fig. 2.* collecting tubule (960 X). EP.– 40 days.
*Fig. 3.* proximal tubule (960 X) EP.– 47 days.
*Fig. 4.* collecting tubule (960 X). EP.– 47 days.
*Fig. 5.* glomerulus (960 X). EP.– 51 days.
*Fig. 6.* proximal tubule (960 X). EP.– 51 days.

EP.– Exposure period.

Abbreviation are same as in Plate XXVI.
left in them and nuclear material appeared as a small speck.

By 51st day the cells of glomeruli lost their structural organisation. Clusters of diffused debris and empty spaces were seen in them. The number of blood cells was reduced drastically (Plate XXVII, Fig. 5). The proximal tubular cells were completely necrotised and quite a few of them had ruptured (Plate XXVII, Fig. 6). Distal and collecting tubular cells were practically devoid of protoplasmic substance. Their nuclei were seen at various stages of disintegration (Plate XXVIII, Fig. 1). The hematopoietic tissue was almost reduced to blank spaces and the degenerating blood cells were seen distributed at random with a few patches of diffused material within.

3.3b. Histology of the kidney exposed to 0.03 ppm of cadmium:

The kidney of the fish exposed to this concentration appeared normal upto 4 days. However by 10th day of exposure the protoplasmic material of some cells of the proximal tubules as well as parenchymatous cells of hematopoietic tissue showed signs of precipitation (Plate XXVIII, Fig. 2). By 17th day the glomeruli were also affected and the cellular material of blood cells flowing through them started precipitating resulting in
Microphotographs of the kidney of fish exposed to
0.01 ppm (Fig. 1) and 0.03 ppm (Figs. 2-6) of cadmium
to show the damage caused to the:

Fig. 1 collecting tubule (960 X). EP— 51 days.
Fig. 2. proximal tubule (960 X). EP— 10 days.
Fig. 3. glomerulus (960 X). EP— 17 days.
Fig. 4. proximal tubules (960 X). EP— 17 days.
Fig. 5. collecting tubule (960 X). EP— 20 days.
Fig. 6. glomerulus (960 X). EP— 28 days.

EP— Exposure period.

Abbreviations are same as in Plate XXVI.
the formation of a few empty spaces (Plate XvIII, Fig. 3). There was a marked reduction of cytoplasmic material and shrinkage of nuclear material in proximal tubular cells (Plate XXVIII, Fig. 4). The cytoplasm of some of the parenchymatous cells was reduced and nuclear material was seen as a darkly stained mass in the centre.
Consequently some empty spaces were formed in them. By 20th day the damage spread to distal and collecting tubular cells also (Plate XXVIII, Fig. 5).

By 28th day even the cells of the glomerular capillaries showed degenerative changes and a large number of empty spaces were seen within the glomeruli (Plate XXVIII, Fig. 6). The proximal tubular cells developed some empty spaces due to destruction of the cellular material (Plate XXVIII, Fig. 1). Distinct patches of precipitated protoplasm and intermittent empty spaces were also seen in distal and collecting tubular cells. The nuclear material of these tubular cells was reduced (Plate XXIX, Fig. 2). The parenchymatous cells of hematopoietic tissue which were damaged could be differentiated into two types, one with empty spaces and the other with diffuse debris. The developing blood cells started losing their cellular structure (Plate XXII, Fig. 3).
PLATE XXIX

Microphotographs of the kidney of fishes exposed to 0.03 ppm of cadmium to show the damage caused to the:

Fig. 1. proximal tubule (960 X). EP. - 28 days.
Fig. 2. distal and collecting tubule (960 X). EP. - 28 days.
Fig. 3. hematopoietic tissue (960 X). EP. - 28 days.
Fig. 4. glomerulus (960 X). EP. - 40 days.
Fig. 5. proximal and collecting tubule (960 X).
EP. - 40 days.
Fig. 6. proximal tubule (960 X). EP. - 47 days.

EP. - Exposure period.

Abbreviations are same as in Plate XXVI.
By 40th day some of the glomeruli were practically disintegrated and others were in the process of destruction. The decomposed material of the blood cells and capillaries was deposited as patches with large empty spaces within them (Plate XXIX, Fig. 4). The cytoplasm was drastically reduced in the proximal tubular cells and nuclei which were shrunk became irregular in shape. The brush border was completely obliterated. Further both the distal as well as collecting tubular cells were severely damaged. Their cytoplasm and nuclear material was almost reduced and debris was deposited as clusters with intermittent empty spaces (Plate XXIX, Fig. 5). The parenchymatous cells of the hematopoietic tissue were severely damaged and the latter was represented by debris and large empty spaces. The developing blood cells lost their cytoplasm and were seen as dark specks of shrunken nuclear material.

By 47th day the injury became very acute. A complete destruction of blood and cell constituents of capillaries was noticed in almost all the glomeruli. By now the proximal tubular cells were completely necrotised. The lumen of some tubules appeared indistinct (Plate XXIX, Fig. 6). Due to further reduction of protoplasmic substance large empty spaces were formed in the distal as well as collecting tubular cells (Plate XXI, Fig. 1).
The hematopoietic tissue cells also appeared as large empty spaces and the developing blood cells were seen at various stages of destruction.

The glomerular structure became entirely indistinct by 51st day of exposure with remnants of diffused mass of damaged protoplasm and few disintegrating blood cells (Plate XXX, Fig. 2). Most of the proximal tubular cells lost their cell borders and were seen with some debris distributed at random. The nuclei were pressed towards the periphery and some of them were pushed outside the cells (Plate XXX, Fig. 3). By now the debris of distal as well as collecting tubular cells was drastically reduced resulting in the formation of large sized empty spaces. Their tubular structure was lost and the lumen became irregular. Hematopoietic tissue developed numerous empty spaces along with clusters of the cytoplasmic material, shrunken nuclei and few remains of developing blood cells (Plate XXX, Fig. 4).

3.30. Histology of the kidney exposed to 0.05 ppm of cadmium:

The degenerative changes induced by 0.05 ppm of cadmium were detectable within 10 days of exposure. Cytoplasmic and nuclear components of most of the proximal tubular cells were damaged and deposited in the precipitated form (Plate XXX, Fig. 5). Also affected
Microphotographs of the kidney of fishes exposed to 0.03 ppm (Figs. 1-4) and 0.05 ppm (Figs. 5-6) of cadmium to show the damage caused to the:

Fig. 1. distal and collecting tubule (960 X). 
EP.- 47 days.

Fig. 2. glomerulus (960 X). EP.- 51 days.

Fig. 3. proximal tubule (960 X). EP.- 51 days.

Fig. 4. hematopoietic tissue (960 X). EP.- 51 days.

Fig. 5. proximal tubule (960 X). EP.- 10 days.

Fig. 6. proximal and collecting tubule (960 X). 
EP.- 20 days.

EP.- Exposure period.

Abbreviations are same as in Plate XXVI.
were the parenchymatous cells of hematopoietic tissue. A reduction of cytoplasm and shrinkage of nuclei was marked in some of its cells.

By 20th day of exposure the damage became severe affecting all the tissues of kidney. The blood flowing through the glomeruli as well as the glomerular capillaries tissue started degenerating. The cytoplasm of the proximal tubular cells was reduced and the nuclei were shrunk. As a result empty spaces were formed in them. The protoplasm of both distal as well as collecting tubular cells was precipitated and deposited as clusters with empty spaces in between (Plate XXX, Fig. 6). Due to the disintegration of parenchymatous cells empty spaces were seen in the hematopoietic tissue. The damage spread to the developing blood cells also and there was a reduction in their cellular material (Plate XXXI, Fig. 1).

By 29th day of exposure the cellular material within the glomeruli was drastically reduced and as a result large number of empty spaces were developed (Plate XXXI, Fig. 2). The debris was deposited in the proximal tubular cells and the brush border became an irregular structure (Plate XXXI, Fig. 3). The distal as well as collecting tubular cells were represented by small patches of precipitated protoplasm and intermittent
PLATE XXXI

Microphotographs of the kidney of fishes exposed to 0.05 ppm of cadmium to show the damage caused to the:

Fig. 1. hematopoietic tissue (960 X). EP.- 20 days.
Fig. 2. glomerulus (960 X). EP.- 29 days.
Fig. 3. proximal tubule (960 X). EP.- 29 days.
Fig. 4. collecting tubule (960 X). EP.- 29 days.
Fig. 5. glomerulus (960 X). EP.- 40 days.
Fig. 6. proximal tubule (960 X). EP.- 40 days.

EP.- Exposure period.

Abbreviations are same as in Plate XXVI.
large empty spaces. The nuclei were observed at various stages of disintegration. The lumen of few tubules became indistinct (Plate XXI, Fig. 4). By now the hematopoietic tissue was full of diffused mass of decomposed material and remnants of degenerating developing blood cells along with vacuoles.

By 40th day the glomeruli lost their structural and functional integrity and were filled with diffused mass of debris, remains of blood cells and large empty spaces (Plate XXII, Fig. 5). The proximal tubular cells were severely affected and the tubular structure was lost. Clusters of damaged material were seen spread at random along with shrunken nuclei. Very little protoplasm was left in the distal and collecting tubular cells and large empty spaces were found in them. Hematopoietic tissue was more or less destroyed completely and was represented by little debris of parenchymatous cellular material, developing blood cells as dark specks and large vacuolar structures (Plate XXXI, Fig. 6).