6333

FINAL *REPORT ON*

CONTRACT NO DA -92-557-FEC-35699

INCLUSIVE DATES 15 November 1961 *TO* 14 November 1962

SUBJECT OF INVESTIGATION

STUDIES ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND IN HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY

RESPONSIBLE INVESTIGATOR

Dr. Fumikazu Takaki

Professor of Pathology

Jikei University School of Medicine
2-105, Atago-cho, Shiba, Minato-ku,
Tokyo, Japan.

U.S. Army Research & Development Group (9852) (Far East)

Office of the Chief of Research and Development
United States Army
APO 343

AD Div 16/10

Jikei University, Tokyo (Japan)
STUDIES ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND IN HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY by Fumikazu Takaki. Final report No. 2, 15 Nov 61 to 14 Nov 62. 16 p. illus. tables, 18 refs. (Contract DA92-557-FEC-35699)Unclassified report

Thioacetamide and yellow phosphorous were respectively administered into the stomach of Wister rats and periodical changes (15 min - 48 days) occurring in liver cells were observed with light and electron microscopes.

In the initial stage of acute thioacetamide poisoning, liver cell injury appears in the central zone and then spreads to the periphery. Electron microscopy reveals (a) transitional figures from REr to SEr, (b) single limiting membrane of mitochondria and increased electron density, (c) decreased glycogen content,

UNCLASSIFIED

1. Liver - Cells
2. Kidney - Cells
3. Heart - Cells

I. Title: None
II. Takaki, Fumikazu
III. U.S. Army Research and Development Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-35699

Armed Services Technical Information Agency

UNCLASSIFIED

AD Div 16/10

Jikei University, Tokyo (Japan)
STUDIES ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND IN HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY by Fumikazu Takaki. Final report No. 2, 15 Nov 61 to 14 Nov 62. 16 p. illus. tables, 18 refs. (Contract DA92-557-FEC-35699)Unclassified report

Thioacetamide and yellow phosphorous were respectively administered into the stomach of Wister rats and periodical changes (15 min - 48 days) occurring in liver cells were observed with light and electron microscopes.

In the initial stage of acute thioacetamide poisoning, liver cell injury appears in the central zone and then spreads to the periphery. Electron microscopy reveals (a) transitional figures from REr to SEr, (b) single limiting membrane of mitochondria and increased electron density, (c) decreased glycogen content,

UNCLASSIFIED

1. Liver - Cells
2. Kidney - Cells
3. Heart - Cells

I. Title: None
II. Takaki, Fumikazu
III. U.S. Army Research and Development Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-35699

Armed Services Technical Information Agency

UNCLASSIFIED

AD Div 16/10

Jikei University, Tokyo (Japan)
STUDIES ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND IN HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY by Fumikazu Takaki. Final report No. 2, 15 Nov 61 to 14 Nov 62. 16 p. illus. tables, 18 refs. (Contract DA92-557-FEC-35699)Unclassified report

Thioacetamide and yellow phosphorous were respectively administered into the stomach of Wister rats and periodical changes (15 min - 48 days) occurring in liver cells were observed with light and electron microscopes.

In the initial stage of acute thioacetamide poisoning, liver cell injury appears in the central zone and then spreads to the periphery. Electron microscopy reveals (a) transitional figures from REr to SEr, (b) single limiting membrane of mitochondria and increased electron density, (c) decreased glycogen content,

UNCLASSIFIED

1. Liver - Cells
2. Kidney - Cells
3. Heart - Cells

I. Title: None
II. Takaki, Fumikazu
III. U.S. Army Research and Development Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-35699

Armed Services Technical Information Agency

UNCLASSIFIED

AD Div 16/10

Jikei University, Tokyo (Japan)
STUDISS ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND IN HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY by Fumikazu Takaki. Final report No. 2, 15 Nov 61 to 14 Nov 62. 16 p. illus. tables, 18 refs. (Contract DA92-557-FEC-35699)Unclassified report

Thioacetamide and yellow phosphorous were respectively administered into the stomach of Wister rats and periodical changes (15 min - 48 days) occurring in liver cells were observed with light and electron microscopes.

In the initial stage of acute thioacetamide poisoning, liver cell injury appears in the central zone and then spreads to the periphery. Electron microscopy reveals (a) transitional figures from REr to SEr, (b) single limiting membrane of mitochondria and increased electron density, (c) decreased glycogen content,

UNCLASSIFIED

1. Liver - Cells
2. Kidney - Cells
3. Heart - Cells

I. Title: None
II. Takaki, Fumikazu
III. U.S. Army Research and Development Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-35699

Armed Services Technical Information Agency

UNCLASSIFIED

(d) enlargement of nucleoli, and (e) decrease or complete disappearance of Palade's granules, etc. These findings appear from about 30 minutes and become prominent with the lapse of time.

In the initial stage of acute yellow phosphorous poisoning, liver injury appears in the peripheral zone. Electron microscopically the main changes consist of (a) transitional figures between REr to SEr (b) swelling of mitochondria, (c) decrease of glycogen, (d) appearance of lipid droplets, (e) enlargement of nucleoli, (f) decrease and disappearance of Palade's granules, etc. These figures are first encountered at 15 minutes, and they become prominent with the lapse of time.

The electron microscopic results observed in present experiments were compared with those previously reported by others and those of the previous final report. Functionally, these findings suggest the disturbance of the enzymatic system and unclear biochemical activity, but further histochemical and biochemical studies are necessary in order to prove this assumption. (Author)

UNCLASSIFIED

DESCRIPTORS

Poisoning
Cells
Electron Microscopes
Liver
Kidneys

(d) enlargement of nucleoli, and (e) decrease or complete disappearance of Palade's granules, etc. These findings appear from about 30 minutes and become prominent with the lapse of time.

In the initial stage of acute yellow phosphorous poisoning, liver injury appears in the peripheral zone. Electron microscopically the main changes consist of (a) transitional figures between REr to SEr (b) swelling of mitochondria, (c) decrease of glycogen, (d) appearance of lipid droplets, (e) enlargement of nucleoli, (f) decrease and disappearance of Palade's granules, etc. These figures are first encountered at 15 minutes, and they become prominent with the lapse of time.

The electron microscopic results observed in present experiments were compared with those previously reported by others and those of the previous final report. Functionally, these findings suggest the disturbance of the enzymatic system and unclear biochemical activity, but further histochemical and biochemical studies are necessary in order to prove this assumption. (Author)

UNCLASSIFIED

UNCLASSIFIED

DESCRIPTORS

Poisoning
Cells
Electron Microscopes
Liver
Kidneys

(d) enlargement of nucleoli, and (e) decrease or complete disappearance of Palade's granules, etc. These findings appear from about 30 minutes and become prominent with the lapse of time.

In the initial stage of acute yellow phosphorous poisoning, liver injury appears in the peripheral zone. Electron microscopically the main changes consist of (a) transitional figures between REr to SEr (b) swelling of mitochondria, (c) decrease of glycogen, (d) appearance of lipid droplets, (e) enlargement of nucleoli, (f) decrease and disappearance of Palade's granules, etc. These figures are first encountered at 15 minutes, and they become prominent with the lapse of time.

The electron microscopic results observed in present experiments were compared with those previously reported by others and those of the previous final report. Functionally, these findings suggest the disturbance of the enzymatic system and unclear biochemical activity, but further histochemical and biochemical studies are necessary in order to prove this assumption. (Author)

UNCLASSIFIED

UNCLASSIFIED

DESCRIPTORS

Poisoning
Cells
Electron Microscopes
Liver
Kidneys

(d) enlargement of nucleoli, and (e) decrease or complete disappearance of Palade's granules, etc. These findings appear from about 30 minutes and become prominent with the lapse of time.

In the initial stage of acute yellow phosphorous poisoning, liver injury appears in the peripheral zone. Electron microscopically the main changes consist of (a) transitional figures between REr to SEr (b) swelling of mitochondria, (c) decrease of glycogen, (d) appearance of lipid droplets, (e) enlargement of nucleoli, (f) decrease and disappearance of Palade's granules, etc. These figures are first encountered at 15 minutes, and they become prominent with the lapse of time.

The electron microscopic results observed in present experiments were compared with those previously reported by others and those of the previous final report. Functionally, these findings suggest the disturbance of the enzymatic system and unclear biochemical activity, but further histochemical and biochemical studies are necessary in order to prove this assumption. (Author)

UNCLASSIFIED

D-I-S-T-R-I-B-U-T-I-O-N

The distribution of this report as made by USA R&D Gp (FT) is as follows:

Army Research Office, OCRD, Washington 25, D. C.	(4)
Army Attache, American Embassy, Tokyo, Japan	(1)
U.S. Army Medical R & D Command	(4)
ASTIA	(10)
~~Office of Primary Scientific Liaison~~	~~(1)~~
~~Office of Scientific Cognizance~~	~~( )~~

ABSTRACT

Thioacetamide and yellow phosphorus were respectively administered into the stomach of Wister rats and periodical changes (15 min.- 48 days) occurring in liver cells were observed with light and electron microscopes.

1. In the initial stage of acute thioacetamide poisoning, liver cell injury appeared in the central zone and then spreaded to the periphery. Electron microscopy revealed (a) transitional figures from REr to SEr, (b) single limiting membrane of mitochondria and increased electron density, (c) decreased glycogen content, (d) enlargement of nucleoli, and (e) decrease or complete disappearance of Palade's granules, etc.. These findings appeared from about 30 minutes and become prominent with the lapse of time.

2. In the initial stage of acute yellow phosphorus poisoning, liver injury appeared in the peripheral zone. Electron microscopically the main changes consisted of (a) transitional figures between REr to SEr, (b) swelling of mitochondria, (c) decrease of glycogen, (d) appearance of lipid droplets, (e) enlargement of nucleoli, (f) decrease and disappearance of Palade's granules, etc. These figures are first encountered at 15 minutes, and they become prominent with the lapse of time.

3. The electron microscopic results observed in the present experiments were compared with those previously reported by others and those of the former final report. Functionally, these findings suggest the disturbance of the enzymatic system and nuclear biochemical activity, but further histochemical and biochemical studies are necessary inorder to prove this assumption.

Final Report On

Contract No. DA-92-557-FEC-35699

Inclusive Dates: 15 November 1961 to 14 November 1962

SUBJECT OF INVESTIGATION

STUDIES ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY

RESPONSIBLE INVESTIGATOR

Dr. Fumikazu Takaki

Professor of Pathology

Jikei University School of Medicine,

2-105, Atago-cho, Minatoku, Tokyo, Japan.

1. Introduction:

Although there are various noxas (chemical, physical or deficiency state) which cause so-called regressive changes of the cell, it cannot be denied that there is a lack of pathochemical studies on the noxic process and intracellular damage. The mode and process of injury caused by them still remain an unexplored field to the scientists of today. The studies on cell injury in this sense are one of the most important basic problems in the field of pathology. The object of the present series of study which have been done in the first year of our project[1], and yet unfinished is to examine the degenerative changes of cells morphologically at the level of subcellular morphology, to detect the characteristic pathological processes in cells induced by various toxic agents, and to find some clues which will contribute to clarify the basic biological processes underlying such cell injuries.

From our experience with several series of experiments, it could be assumed that among various noxas, the pathological processes commencing in cells are different from one chemical to another. In this report, results obtained in thioacetamide, and yellow phosphorus poisoning will be described.

However, since we still feel it necessary to try other chemicals or noxic substances which are said to have different effects on the biochemical processes of cells inorder to obtain a more firm basis for biological analysis. Experiments using several other agents are now in progress.

2. Material and Method:

The experimental animals used in the present study consisted of 54 Wister albino rats (male, 100-210gm) bred for more than 10 days with standard diet*.

a. Eight groups of animals each consisting of 3 rats were given 60mg/kg of thioacetamide (CH_3-CSNH_2) through the stomach and sacrificed by decapitation 15min., 30min., 1, 3, 6, 12, 24 and 48hrs. later. Another group consisting of 3 rats was used as control.

b. Seven groups of animals each consisting of 3 rats were given 20mg/kg of yellow phosphorus as 0.2% olive oil solution through the stomach and sacrificed by decapitation 15min., 30min., 1, 3, 6, 12 and 24 hrs. later. Two other groups of animals each consisting of 3 rats were given 0.1cc/kg of olive oil through the stomach and sacrificed 15min. and 24 hrs. later. Immediately after the animals were sacrificed, small pieces of the liver were fixed in 1% OsO_4 in acetate veronal buffer(pH7.4) with sucrose added to make an isotonic solution for 2 hrs. at 0-4°C. Epon 812 and styrene-n-butylmethacrylate(mixed ratio 3:7) were used for embedding. Whenever necessary, electron staining was carried out with saturated uranium nitrate for 30-120min., or lead hydroxide for 5-20min. On the other hand, paraffin sections were prepared from the formalin fixed tissues and stained with H.E. and PAS.

*Standard diet
Wheat flour 78gm, milk casein 10gm, lard 3gm, cod-liver oil 1gm, McCollum salt 5gm, yeast 3gm: Total 100gm(350 plus alpha Cal.)

3. Observations:

a. Thioacetamide poisoning.

(1) Light Microscopy. The decrease in cytoplasmic basophilia in the centrolobular region starts to appear after 3 hours. Six hours after drug administration, decrease in PAS positive substance could be observed in the same area mentioned above. The nucleolus becomes conspicuous and increases in number (2 to several). After 12 hours, the changes described above become more severe and extensive. The cytoplasm contains fine vacuolar structures. Mitotic figures of liver cells are also observed in the peripheral zone. After 24 hours, the changes encountered are necrobiosis of liver cells in centrolobular region, decrease in PAS positive substance, decreased cytoplasmic basophilia throughout the lobules, pyknosis, thickening or hyperchromatosis of nuclear membrane, and prominent nucleoli stained red with eosin.

(2) Electron Microscopy.

(a) Nucleus. Within one hour, the nucleoli become slightly enlarged, followed by prominent nucleolonema formation after 3 hours (Fig. 1 and 2). However, after 24 to 48 hours, most of the nucleoli become a homogenous, highly electron dense mass. At the same time, the matrix of nucleus shows a mottled appearance and occasionally contains irregularly shaped, moderately electron dense, fine structures of unidentified nature. The nuclear membrane is intact in all cases examined showing a triple layered structure (Fig. 9 and 10).

(b) Mitochondria. After 30 minutes, some of the mitochondrias show slight swelling together with increase in number of microbody (Rouiller) (Fig. 1). Irregularity in shape and size of mitochondria and increase in mitochondrial granules are observed after 3 hours. After 48 hours, these changes become more severe and extensive, and there is an increase in number of microbody and dense body having similar structure as the granule assumed to be bilirubin granule. At this stage, all of the mitochondria lose their triple membrane. Cristae mitochondriales show irregular arrangement, cristolysis and cristorrhexis in many mitochondria (Fig. 9).

(c) Endoplasmic Reticulum. After 30 minutes, the endoplasmic reticulum (REr) begins to lose its grouped parallel arrangement and decreases in number. After one hour, there are disarrangement, shortening, decrease in number, dispersion, partial dilatation of lumina and partial lose of RNA granules of rough surfaced endoplasmic reticulum (REr). On the contrary, the smooth surfaced endoplasmic reticulum (SEr) appears

as if slightly increased after 30 minutes. In cases examined after 1 hour, there is grouped SEr forming a network (Fig. 2). Transitional figures of REr to SEr are encountered in many places (Fig. 3 and 4). These changes progress with the lapse of time and after 48 hours, the REr shows marked decrease in number. They are found surrounding the mitochondria in single or double layers. The RNA granules attached to the membrane and in cytoplasmic matrix decrease remarkably. The latter is often entirely absent in the cytoplasm. The SEr on the other hand, appears diffusely in the cytoplasmic stroma as a crowded small vesicular net-work (Fig. 2,8 and 9). Occasionally, myelin figures of SEr origin are encountered. After 6 hours or more, there are vacuolar structures containing amorphous or thread-like material of low electron density in the cytoplasm. They are limited by a single membrane and originate from SEr by marked distension of lumina (Fig. 6, 7 and 9).

(d) Golgi Complex. After 6 hours, the Golgi complex is hardly ever found in the peribiliary and perinuclear areas.

(e) Lysosome. It disappears almost completely after 24 to 48 hours.

(f) Bile Capillary. Narrowing of lumina and decrease in number of surface microvilli are the only findings.

(g) Glycogen. Glycogen is well preserved up till 15 minutes. It starts to decrease after one hour and disappears completely after 24 hours.

(h) Lipid. Although lipids increase after one hour, there is no marked increase throughout the whole series.

(i) Disse's Space. Disse's space is intact up till 12 hours. Narrowing of lumina, decrease in number of microvilli, and proliferation of connective tissue fibrils are observed after 48 hours.

b. Yellow phosphorus poisoning.

(1) Light Microscopy. At 30 minutes, there is enlargement of nuclei and nucleoli of the liver cells in both intermediate and peripheral zones. Basophilic body also becomes somewhat obscure. Lipid droplets appear after 6 hours or more from the peripheral zone and become prominent with the lapse of time. These consist of mainly small droplets. PAS reaction becomes weak after the 3rd hour and shows a negative reaction at 24 hours. No remarkable necrotic figures are encountered even after 24 hours.

(2) Electron Microscopy.

(a) Nucleus. No clear-cut changes can be observed between 15 and 30 minutes. Within one hour, the nucleoli become enlarged accompanied with prominent nucleolonema formation (Fig. 12 and 19). The nucleus is sometimes indented, and its membrane structure is well preserved. The nuclear substance is irregularly distributed, and small aggregates of fine granules similar to ribosomes are confirmed (Fig. 12). At 3 to 6 hours, these changes become more clear-cut with increased aggregates and appearance of large nucleoli (Fig. 16). The intermediate clear layer of the nuclear membrane (composed of 3 layers) frequently shows figures of mild dilatation and transitional figures to Er (Fig. 14). At 12 hours, most of the cells have large nucleoli, and at times 3 or more are found in one nucleus. The matrix of nucleus is irregularly distributed and consists of a clear and dark portion (Fig. 17, 19 and 20). Chromatin margination is also observed. At 24 hours, the above changes become more prominent, but the nucleolonema becomes obscure (Fig. 19).

(b) Mitochondrion. Although some cells tend to become somewhat swollen at 15 minutes, the majority is apparently normal appearing. At 30 minutes, however, swelling becomes more prominent, and figures of cristolysis and dilatation of intermediate clear layer are sometimes observed. The stroma of mitochondria is pale. The limiting membrane shows no changes and its triple layered structure is well preserved (Fig. 12, 13 and 14). After an hour up till 12 hours, the swelling becomes more prominent with the lapse of time. With the appearance of irregular shaped and atypical mitochondria, they tend to show small and large figures on one plane. At 24 hours, the swelling becomes extremely prominent with the stroma showing marked low density. The mitochondria is in most instances ovoid, but some are irregular shaped and elongated. The triple layered structure is still well preserved at this stage. Although not prominent, there are figures of cristolysis, cristorrhexis and dilatation of the intermediate clear layer. Mitochondrial granules are obscure in those showing remarkable swelling, but can be found in most of the cells (Fig. 19 and 20).

(c) Endoplasmic Reticulum. Transitional figures between rough surfaced endoplasmic reticulum (REr) and smooth surfaced endoplasmic reticulum (SEr) are looked upon as a noteworthy feature. This process consists of the following chain of events: Normal REr becomes dispersed, shortened, and the surfaced Palade's granules drop off. The lumina shows a localized, irregular dilatation, vesiculation and then transforms into SEr. At 15 minutes, these changes are seen in only a few of the cells (Fig. 11) with the majority

remaining apparently normal. They then become rather prominent in 30 minutes to an hour (Fig. 13, 14 and 15). Dense granules measuring 70 - 100 mu presumably originating from olive oil are frequently found in the lumina of SEr in all cases (Fig. 17, 18 and 20). The accumulation of these granules lead to the formation of large lipid droplets. At 3 - 6 hours, there is a prominent decrease of REr, while SEr indicates a remarkable increase (Fig. 16). However, there are a few cells remaining with typically arranged REr. At 12 - 24 hours, these cells with typically arranged REr become remarkably few in number, and in some instances only SEr can be noted (Fig. 18). SEr shows isolated, irregular shaped vesicular sacs or a net-work. Free Palade's granules disappear and the cell matrix becomes clear. Occasionally, myelin-like structures of SEr origin are observed, and they are sometimes found from the 1st hour. At 24 hours, one may frequently encounter disfigures REr.

(d) Golgi Complex. Developed profiles of Golgi complex are no longer seen with the lapse of time and progress of cell injury. At 12 - 24 hours, poorly developed Golgi complex is sometimes observed in the surrounding area of bile capillaries (Fig. 20).

(e) Microbodies, Secretion Granules, Lysosomes and Bilirubin Granules. Microbodies (Rouiller) are found not only in the surrounding of bile capillaries but also in other areas of the cytoplasm. With the lapse of time, there is an increase in number of microbodies, while lysosomes (Novikoff) or secretion granules (Takaki, Hagiwara) decrease in number. At 12 - 24 hours, numerous microbodies are observed, while there are hardly any lysosomes (Fig. 19). Large, dense, irregular shaped bodies considered to be bile pigments, are found between the nucleus and surrounding of bile capillaries (Fig. 20).

(f) Bile Capillary. With the atrophy of Golgi complex and decrease in number of secretion granules or lysosomes, there is also a decrease in number of microvilli protruding into a rather narrowed lumina (Fig. 20). Bizzozero nodules show no marked changes.

(g) Glycogen. There is a rapid decrease soon after the administration of yellow phosphorus, and a remarkable decrease can already be noted at 15 minutes (Fig. 11). Glycogen can hardly be detected in any of the liver cells at 24 hours (Fig. 19 and 20).

(h) Lipid. Lipid droplets measuring 70 - 100 mu appear in Disse's space from 15 minutes after administration of yellow phosphorus. They enter the endoplasmic reticulum (especially SEr) of liver

cells through pinocytosis (Fig. 18). They then accumulate, fuse and increase in size, and appear as large lipid droplets measuring 2 - 3 u. These figures are found in all cases up to 24 hours.

(i) Disse's Space, Endothelia or Kupffer Cell, Microvilli. In all cases after 30 minutes or longer, there are sometimes desquamation of endothelial cells, swelling of microvilli, and flow-in of destructed liver cell contents into the sinusoid.

4. Discussions:

a. Thioacetamide poisoning. There are many literatures on experimental injuries of the rat liver cells in thioacetamide intoxication. Most of them have described the development of liver cirrhosis or hepatic carcinoma, especially emphasizing nuclear changes after prolonged thioacetamide administration of small or subtoxic doses. On the other hand, there are only a relatively few reports on acute intoxication or early phase of poisoning of thioacetamide (Gallagher[5], Gupta[6], Salomon[10].). In the present experiment, the primary or the most important finding is the changes of Er. From about 30 minutes after the administration of thioacetamide, the REr begins to decrease in number, while the SEr increases in number and forms an irregularly connected vesicular structure. Connecting figures between these structures and REr with fall-off of surfaced Palade granules (Fig. 3). Similar changes are observed in rat liver during fasting (Takaki et al)[16], fluoroacetate poisoning (Hongo)[7], carbon tetrachloride poisoning (Aizawa)[2] and yellow phosphorus poisoning. Surfaced or free Palade granules consist of large molecules of ribonucleoprotein and are mainly responsible for the cytoplasmic basophilia seen under the light microscope. Decreased basophilia in centrolobular region observed under the light microscope in the present study coincides with the change of the aforementioned extreme decrease of REr and free Palade granules. On the other hand, the fact that glucose-6-phosphatase is located on the smooth surfaced endoplasmic reticulum (Chauveau)[4] also coincides with the decrease of glycogen which might be specifically interfering with carbohydrate metabolism of the cells. According to the recent study of Gupta[6] and Gallagher[5], rat liver cells in thioacetamide poisoning undergo a permeability change whereby calcium and sodium ions stream into the cells and "fur" the mitochondria, while magnesium and potassium ions are lost. Furthermore, they have shown by means of histochemical tests, that accumulation of calcium ions occurs at the surface of mitochondria. In this experiment, abnormal mitochondria having single layered limiting membrane with high electron density might indicate accumulation of calcium ions on the membrane (Cameron)[3]. To say the least of it, the above mentioned figures point to a permeability change and following dysfunction of enzymatic systems of mitochondria. In 48 hours, a homogenous, non-structural substance with low electron density appears after the disappearance of glycogen and free Palade granules (Fig. 9). This finding together with chromatin situated close to the border of the nucleus is similar to those pointed out by Takaki[14] in autolysis. Further studies are needed inorder to clarify the exact nature of the above substance. Cells having pyknotic nuclei, degenerated intranuclear organellae, and increased density of cell matrix appear in great numbers after 24 hours or more (Fig. 6). It is not clear whether these cells represent necrobiosis or not. Similar figures are also found at 24 hours in carbon tetrachloride poisoning (Aizawa)[2]. At 30 minutes after administration of thioacetamide, there are some cells showing gradual enlargement of nucleoli and marked formation of nucleolonema, but at 48 hours, the nucleoli become homogenous having a high electron density (Fig. 8). This is compatible to the eosin stained substance when observed under the light microscope. Histochemically and cyto-

chemically, the nucleus contains desoxyribonucleic acid, while the nucleoli are composed of ribonucleic acid. The aforementioned figures are presumably those of degenerated or vanished RNA particles.

b. Yellow phosphorus poisoning. Electron microscopic studies on the fine structures of the liver cells in acute yellow phosphorus poisoning have been carried out by Onoe[10] and Jézéquel[8]. Onoe using mice, injected 0.05ml of 0.25% phosphorus intraperitoneally. He observed swelling and drop in density of mitochondria, disappearance and vacuolization of crista, disappearance of endoplasmic reticulum, lipid granules, decrease and disappearance of Palade's granules, etc. On the other hand, Jézéquel described almost complete disappearance of ergastoplasma and swelling of mitochondria with only mild alteration of its internal structure. He emphasized that there are no direct morphologic connection between mitochondria and the formation of lipid droplets. In the present experiment, some of the mitochondria already show swelling at 15 minutes, and this change becomes prominent in 24 hours. This finding coincides with that reported by Onoe, but the alteration of internal structure of mitochondria with some showing cristolysis and cristorrhexis is much more remarkable than those described by Jézéquel. The triple layered structure of the limiting membrane remained well preserved in all cases. As has been previously mentioned, we are of the opinion that transitions from REr SEr play an important role in the disappearance of REr. An increase in number of microbodies (Rouiller)[11] is found in parallel with the swelling of mitochondria and transitions between REr and SEr, and their distribution is not limited to the surrounding area of bile capillaries. On the other hand, the number of lysosomes (Novikoff)[9] or secretions granules (Takaki)[15-17] and the development of Golgi complex and bile capillaries decrease or become poor in contrary to the increase of microbodies. These findings indicate that it is inadequate to include microbody (Rouiller) in lysosomes from just the existence of acid phosphatase containing granules in the surrounding of bile capillaries and from biochemical and electron microscopic findings of granules isolated by fraction centrifugation[9, 18]. The appearance of lipid droplets is one of the characteristic findings encountered in phosphorus poisoning. Since olive oil was used as a solvent in the present experiment, its intake from the sinusoid into the liver cells and formation of lipid droplets by its accumulation in endoplasmic reticulum was found in all of the cases (Suzuki)[13]. For this reason the morphologic figures indicating the mechanism of endogenous lipid formation in the liver cells could not be observed in this experiment. However, from the points that there are no figures suggesting lipid formation without the participation of SEr and that there are no transitional figures between mitochondria and lipid, it may be assumed that lipid formation in phosphorous poisoning takes place with the participation of SEr. Although the morphologic changes of liver cells in phosphorus poisonings[1,2,7], the following changes are considered to appear at an early stage and remarkably in phosphorus poisoning: (1) transitional figures between REr SEr, (2) swelling of mitochondria and increase in number of microbodies, (3) decreased glycogen, (4) appearance of lipid droplets, (5) enlargement of nucleoli, (6) decrease and disappearance of Palade's granules, etc. The changes of endoplasmic reticulum and swelling of mitochondria are similar to those observed in fluoroacetate poisoning, but the number of lipid droplets is much more numerous in

phosphorus poisoning. Although all these changes are found also in carbon tetrachloride poisoning, the vesicles formed by distension of Er lumina are smaller and much more numerous in phosphorus poisoning. In thioacetamide poisoning, there are characteristic figures of mitochondrial limiting membrane, lesser swollen mitochondria, and net-work arrangement of SEr, while in phosphorous poisoning the limiting membrane is well preserved and SEr shows a vesicular dilatation. The existence of a difference in mode of action in the various poisonings can be assumed from the difference in electron microscopic changes of the injured cells. The accumulation and comparative studies of various experimental poisonings should prove valuable in obtaining further information concerning the pathogenesis of cell injury.

5. Summary

a. Thioacetamide poisoning.

(1) Wister rats were orally administered with 60mg/kg of thioacetamide, and the changes in liver cells were observed under the light and electron microscopes at intervals of 15 min., 30 min., 1, 3, 6, 12, 24 and 48 hours, respectively.

(2) The changes in acute thioacetamide poisoning consisted of (a) transitional figures from REr to SEr, (b) single limiting membrane of mitochondria and increased electron density, (c) decreased glycogen, (d) enlargement of nucleoli, and (e) decreased or complete disappearance of Palade granules. These findings appear from about 30 minutes and become prominent with the lapse of time.

(3) The electron microscopic figures observed in the present experiment were compared with the hitherto reports and those of fluoroacetate, carbon tetrachloride and yellow phosphorus poisoning.

(4) The single limiting membrane of mitochondria and its increased electron density are characteristic figures found in acute thioacetamide poisoning. These findings suggest a change in permeability of mitochondrial membrane.

b. Yellow phosphorus poisoning.

(1) Wister rats were orally administered with 20mg/kg of yellow phosphorus dissolved in olive oil, and electron microscopic changes of liver cells were observed at intervals of 15 minutes, 30 minutes, 1, 3, 6, 12 and 24 hours, respectively.

(2) The main liver cell changes consisted of (a) transitional figures between REr SEr, (b) swelling of mitochondria, (c) decrease of glycogen, (d) appearance of lipid droplets, (e) enlargement of nucleoli, (f) decrease and disappearance of Palade's granules, etc. These figures were first encountered at 15 minutes, and they became prominent with the lapse of time.

(3) The results obtained in the present experiment were compared with those previously reported by others and those of fluoroacetate, carbon tetrachloride and thioacetamide poisoning.

(4) The difference in morphologic changes in acute yellow phosphorus poisoning and other acute poisonings has been confirmed and discussed.

6. References:

(1) Final Report on Contract No.DA-92-557-FEC-34390, 1 Nov. 1960 to 31 Oct. 1961. sponsor-ship of the U.S. Department of Army, through its Far East Research Office.

(2) Aizawa, S: Acta Path. Jap., 12:155, 1962.

(3) Cameron, G.R.: New Pathways in Cellular Pathology. Edward Arnold, (Publishers) Ltd, London, 1956.

(4) Chauveau, Y., Moulé, Y., Rouiller, C., and Schneebeli, J.: J. Cell Biology, 12:17 1962

(5) Gallagher, C.G., Gupta, D.N., Judah, D. & Rees, K.: J. Path. Bact., 72:193, 1956.

(6) Gupta, D.N.: J. Path. Bact., 72:183, 1956.

(7) Hongo, H.: Jikeikai Ikadaigaku Zassi, 77:250, 1961 (in Japanese).

(8) Jézéquel, A.M.: Ann. Anat. Path., 3:512, 1958.

(9) Novikoff, A.B. et al: J. Biophysic Biochem. Cytol., 2(suppl.), 179, 1956.

(10) Onoe, T. et al.: Electronmicroscopy, 6:111, 1956 (in Japanese).

(11) Rouiller, C. & Bernhard, W.: J. Biophysic. Biochem. Cytol., 2 (suppl.), 335, 1956.

(12) Salomon, J.C.: J. Ultrastructure Research, 7:292, 1962.

(13) Suzuki, Y.: J. Electronmicroscopy, 9:24, 1960.

(14) Takaki, F. et al.: Jikei Med. J., 2:27, 1955.

(15) Takaki, F. et al: Jikei Med. J., 8:51, 1961.

(16) Takaki, F. et al: Jikei Med. J., 8:85, 1961.

(17) Takaki, F. et al: Jikei Med. J., 8:68, 1961.

(18) Essner, E. and Novikoff, A.B.: J. Biophysic. and Biochem. Cytol., 9:773, 1961.

APPENDIX "A"

EXPLANATION OF FIGURES

Abbreviations of special terms used are as shown in the following paragraph.

B : Bile capillary

C : Collagen fibril

Cs : Cytoplasmic stroma

D : Dense body

F : Fat droplet

G : Golgi complex

Ga : Glycogen area

L : Lysosome

M : Mitochondria

Mb : Microbody (Rouiller & Bernhard)

N : Nucleus

Nl : Nucleolus

REr : Rough surfaced endoplasmic reticulum

S : Sinusoid

SEr : Smooth surfaced endoplasmic reticulum

V : Vacuole

Fig. 1. Thirty min. after administration of thioacetamide. There is a marked development of nucleolonema in the upper left. The glycogen area is not too well preserved and although most of the REr show typical parallel arrangement, some increase in number of SEr is observed in the right. There is slight increase of microbodies.

12,700X

Fig. 2. One hr. after administration of thioacetamide. Typical arrangement of ERr is no longer seen in this figure. Grouping of irregularly connected mesh structure of SEr. Glycogen storage is remarkably decreased. Golgi complex is normally developed.

8,100X

Fig. 3. Six hrs. after administration of thioacetamide. The glycogen area is almost obscure. Slight increase of fat droplets. Some of the mitochondria already demonstrate swelling and mitochondrial matrix with low electron density. Typical arrangement of REr is no longer seen and not a few of the REr show shortening, dispersion and enlargement of internal space with fall-off of surfaced granules. Increase of SEr with grouping of irregularly connected mesh structure of SEr. Transitional figures between REr and SEr (arrow).

18,400X

Fig. 4. Twelve hrs. after administration of thioacetamide. Similar figures to those observed in the above. Double membrane structure of the mitochondria is well preserved.

18,400X

Fig. 5. Twenty-four hrs. after administration of thioacetamide. Moderate swelling of mitochondria. Initial figures of fainting or disappearance of the outer limiting membrane of mitochondria.

23,000X

Fig. 6. Forty-eight hrs. after administration of thioacetamide. There are several atypical shaped or irregularly borderd mitochondriae and extremely decreased REr. Closly packed SEr is filling the cytoplasmic stroma and there is complete disappearance of glycogen area. There are some vesicles containing amorphous thread-like materials with low electron density. Moderate increase of collagen fibers in Disse's space. In the upper right of this figure, a dark appearing cell is observed.

3,700X

Fig. 7. Same case as the above. Similar figures to those observed in the above. Some nuclei with mottled appearance of chromatin and homogenous electron dense nucleoli.

3,700X

Fig. 8. Forty-eight hrs. after administration of thioacetamide. Two large large nucleoli with homogenous electron dense appearance are observed in the nucleus with mottled chromatin. Irregularly connected SEr in the cytoplasmic stroma. Numerous irregularly shaped mitochondriae with single layered limiting membrane. Moderate increase of microbodies are observed.

15,000X

Fig. 9. Same case as the above. A vesicular structure with single limiting membrane containing of amorphous or thread-like material is observed at the center. Mitochondriae show a single layered limiting membrane with rather high electron density. Cristolysis or cristorrhexis of cristae mitochondriales (arrow) is demonstrated. Completely homogenous, non-structural substance with low electron density filling the cytoplasmic stroma instead of disappearance of glycogen and free Palade granules. There are also marked increase of SEr in vesicular net-work.

24,300X

Fig. 10. Same case as the above. Swollen mitochondriae show a single layored limiting membrane with rather high electron density. Cristae mitochondriales are shortened and increased in number and reveal cristolysis or cristorrhexis in some portions. Markedly decreased REr with scarce number of surfaced Palade granules are surrounding the swollen mitochondria.

25,300X

Fig. 11. Fifteen min. after administration of yellow phosphorus. Early transformation from the rough surfaced endoplasmic reticulum (REr) to the smooth surfaced variety (SEr) is seen on the right lower field. Mitochondria appear almost normal.

3,300X

Fig. 12. Thirty min. after administration of yellow phosphorus. An enlarged nucleoli with nucleolonema formation, swollen mitochondria and increase of microbodies (Rouiller) can be noted.

4,600X

Fig. 13. Another liver cell of the same rat as shown in fig. 12. The initial change of transformation from REr to SEr as seen in a rather typically arranged REr.

29,700X

Fig. 14. A liver cell from the same case with that of Fig. 12. and 13. The transformation is a little more advanced. Mitochondria are slightly swollen and show mild cristolysis (arrow a). Three microbodies showing a figure suggesting the possible relationship to SEr in one of them, and extrusion of outer nuclear membrane (arrow b) are observed.

30,300X

Fig. 15. One hr. after administration of yellow phosphorus. Swollen mitochondria with disarrangement of their cristae and decreased profiles of REr are seen. Nucleus is found on the left and a fat drop on the right lower corner.

29,700X

Fig. 16. Three hrs. after administration of yellow phosphorus. Peribilecanalicular region between two liver cells. A liver cell occupying the right upper two third has an irregular shaped nucleus with small aggregates (arrow) of rather dense granular material as well as moderately dense fine granular nucleic substance. In these two cells increase of SEr is prominent.

26,400X

Fig. 17. Six hrs. after administration of yellow phosphorus. A ring-shaped transformation of REr (arrow a), small fat granules in SEr (arrow b), rather dense granular aggregates in the nucleus (arrow c) and two microbodies (Rouiller) showing connection to SEr (arrow d) are demonstrated.

25,900X

Fig. 18. Twelve hrs. after administration of yellow phosphorus. In this cell, most of the REr have disappeared and transformed into SEr. Fine fat granules (arrow) measuring about 70-100mu are being absorbed by pinocytotic mechanism from the cell surface facing the space of Disse (Ds) and accumulating within the internal space of SEr. Three large fat droplets are also seen.

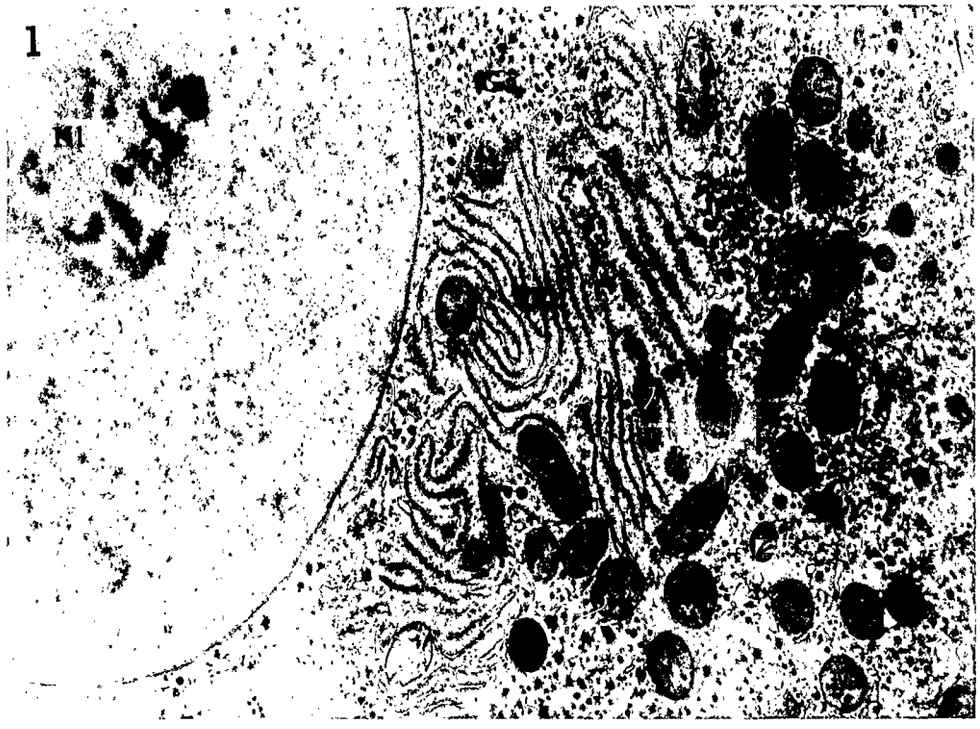
17,400X

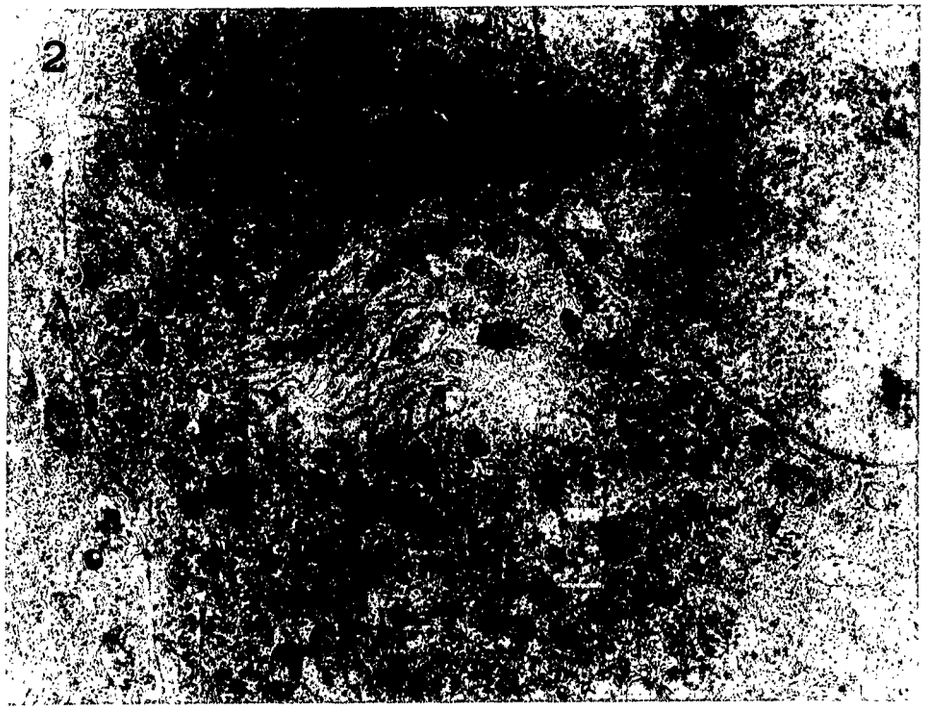
Fig. 19. Twenty four hrs. after administration of yellow phosphorus. Marked swelling of mitochondria, remarkable increase of microbodies, enlarged nucleoli and mottled appearance of the nucleus are seen.

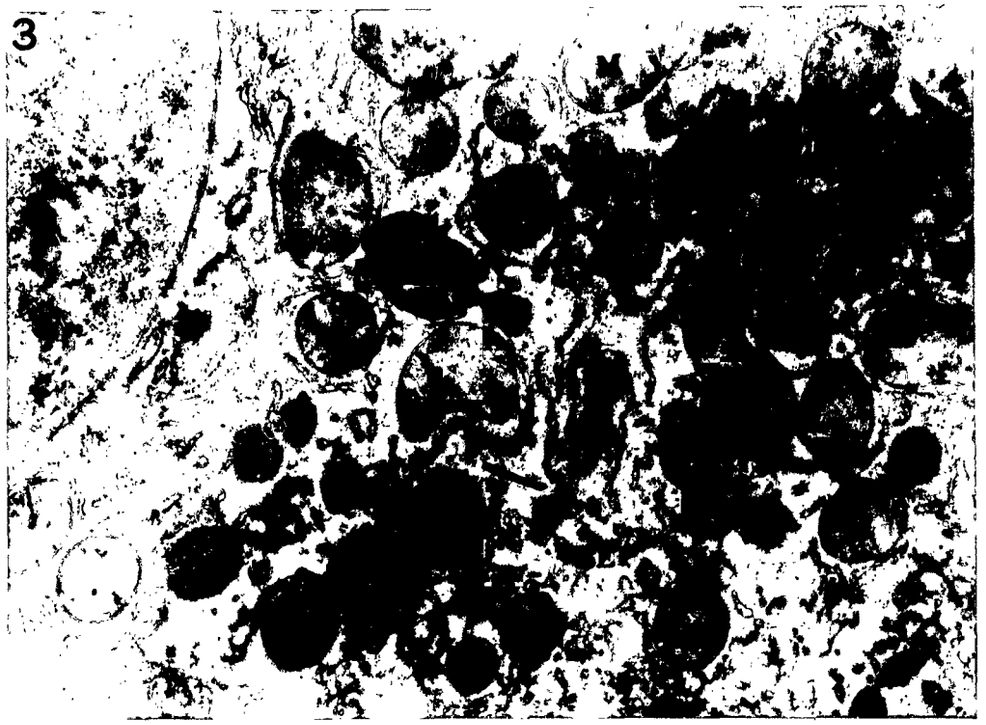
20,800X

Fig. 20. Same case as of Fig. 19. Marked swelling with highly decreased stromal density in mitochondria, an atrophic bile canalicule, many small cystic structures originated from endoplasmic reticulum(arrow) and a large dense body are observed.

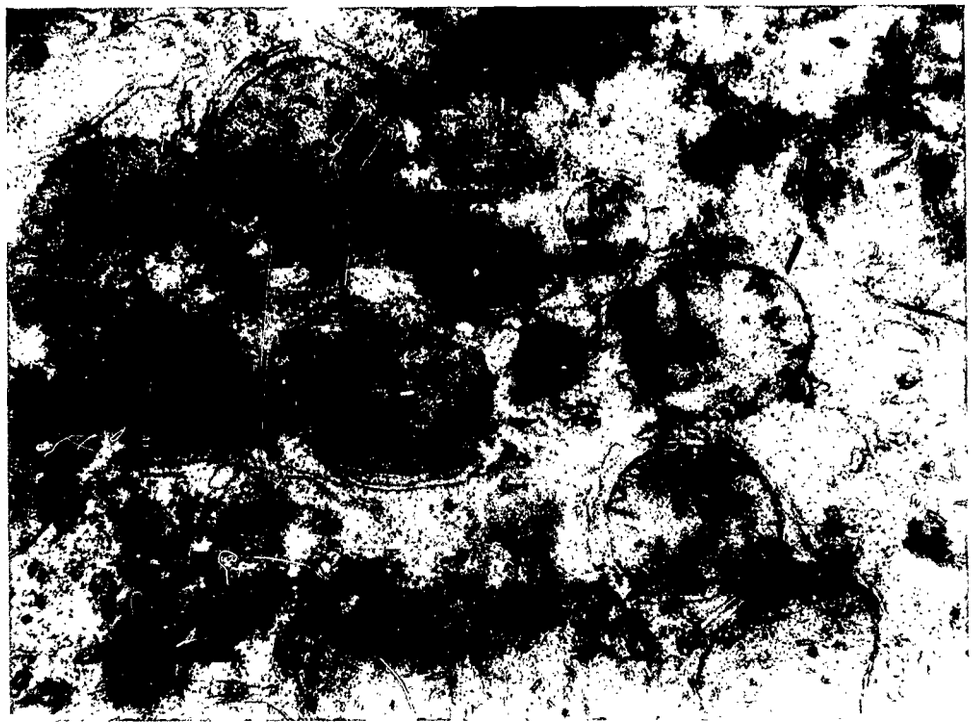
20,800X

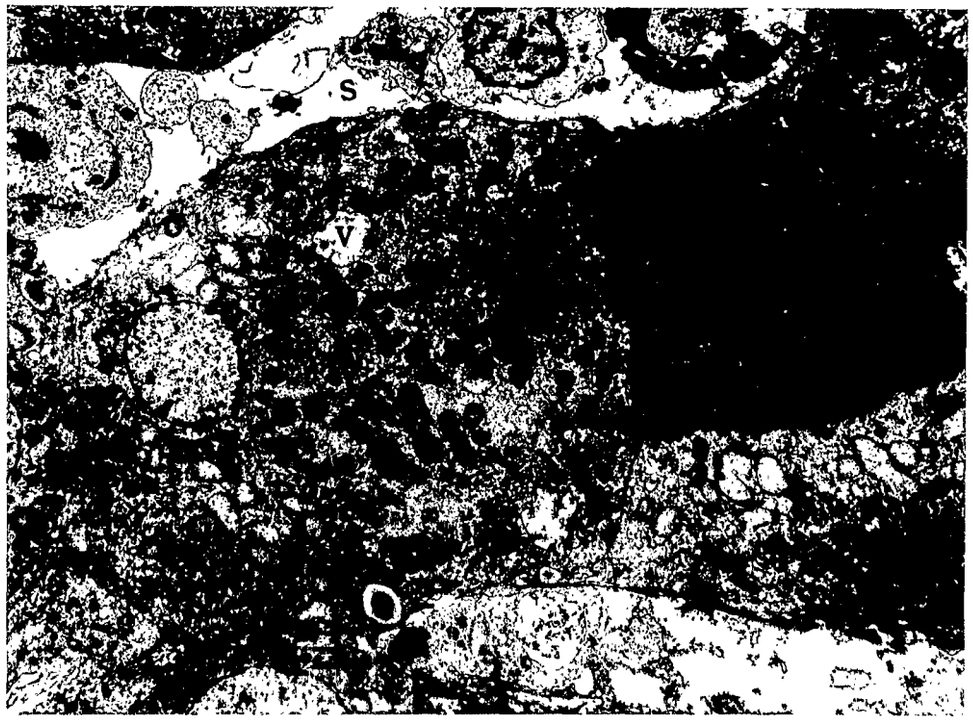

1

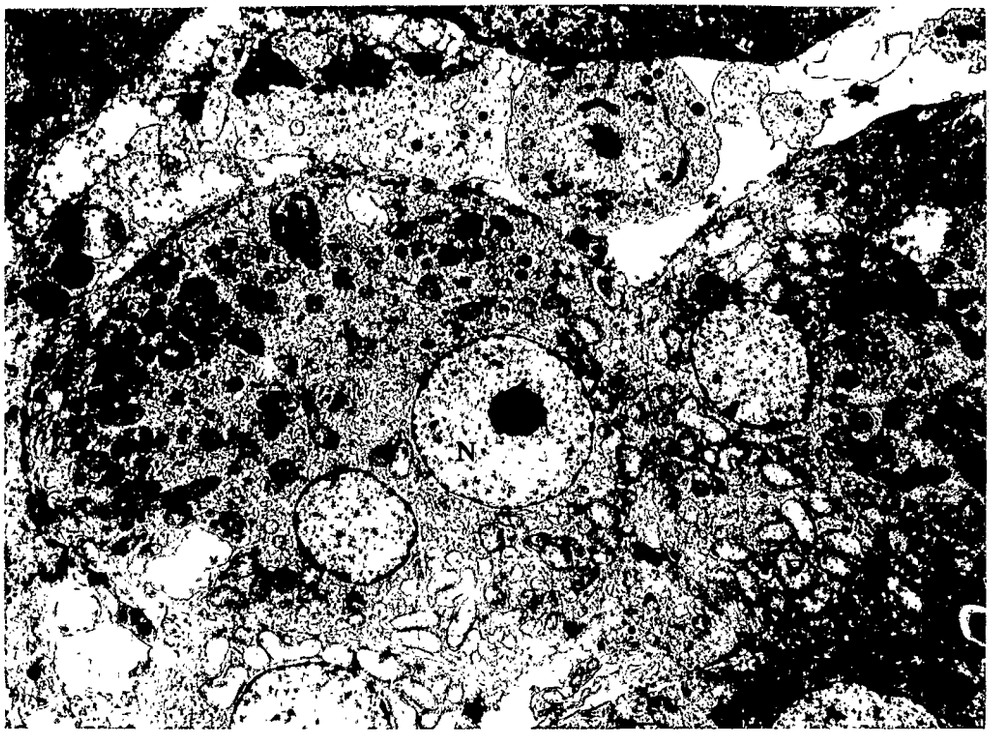

2

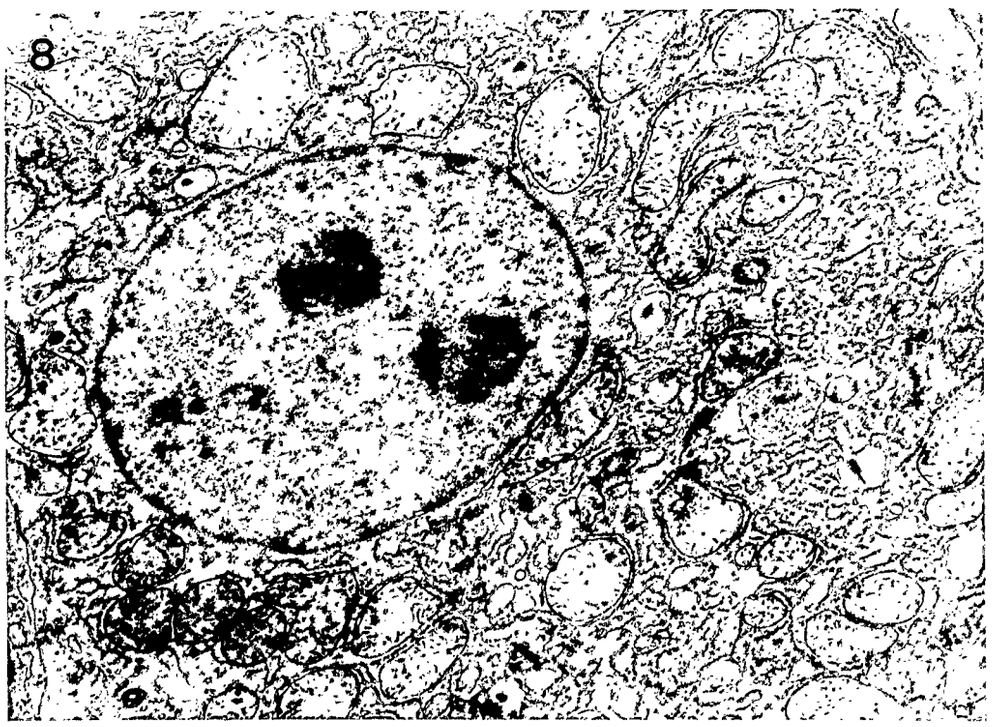

3

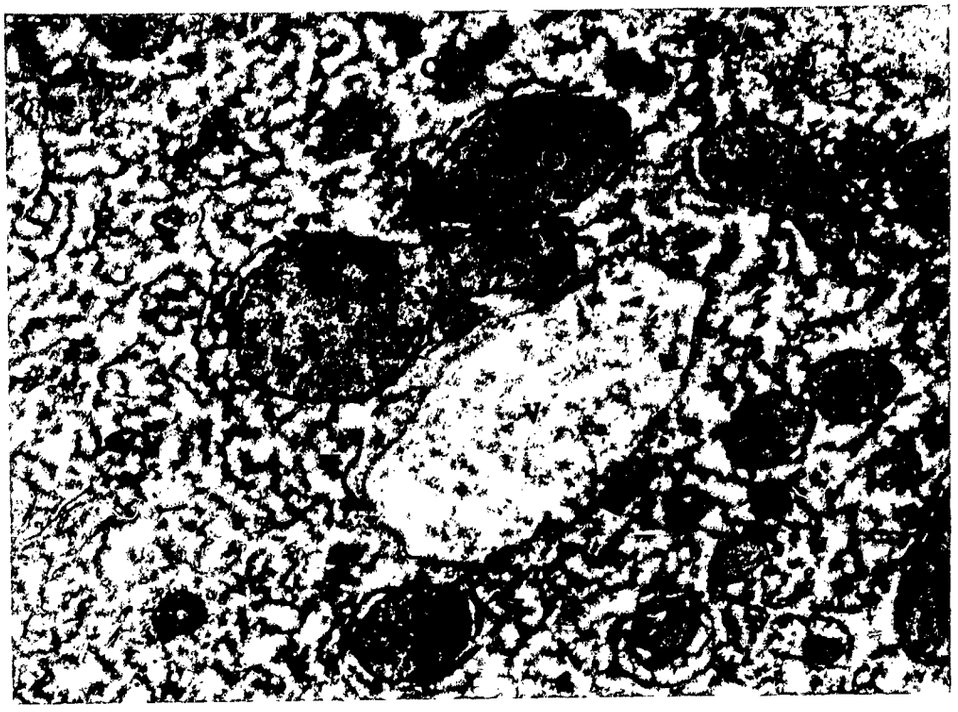

4

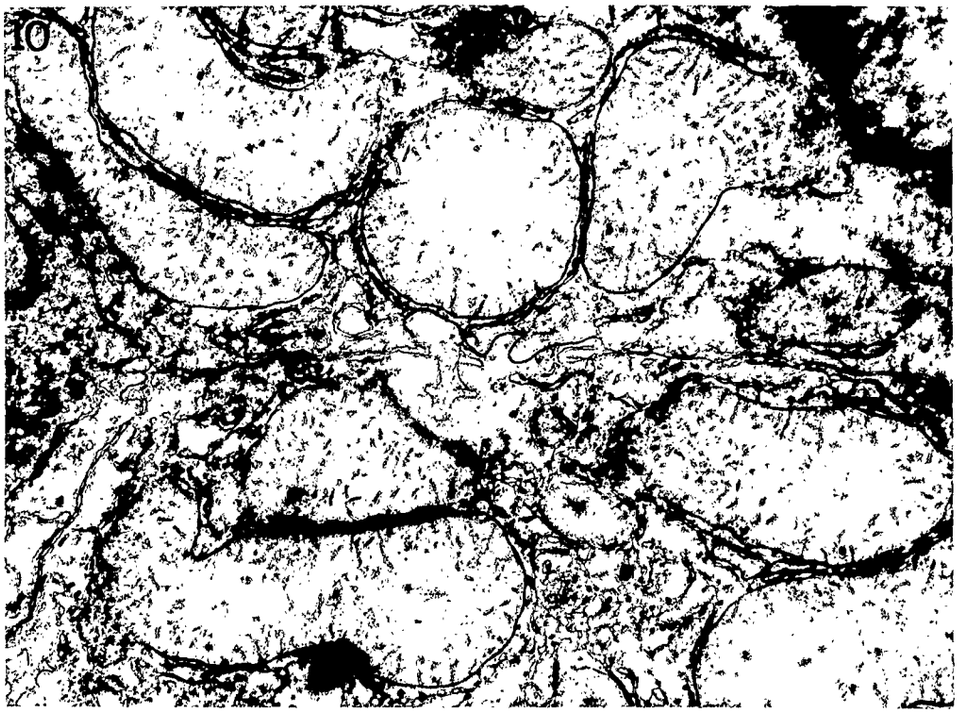


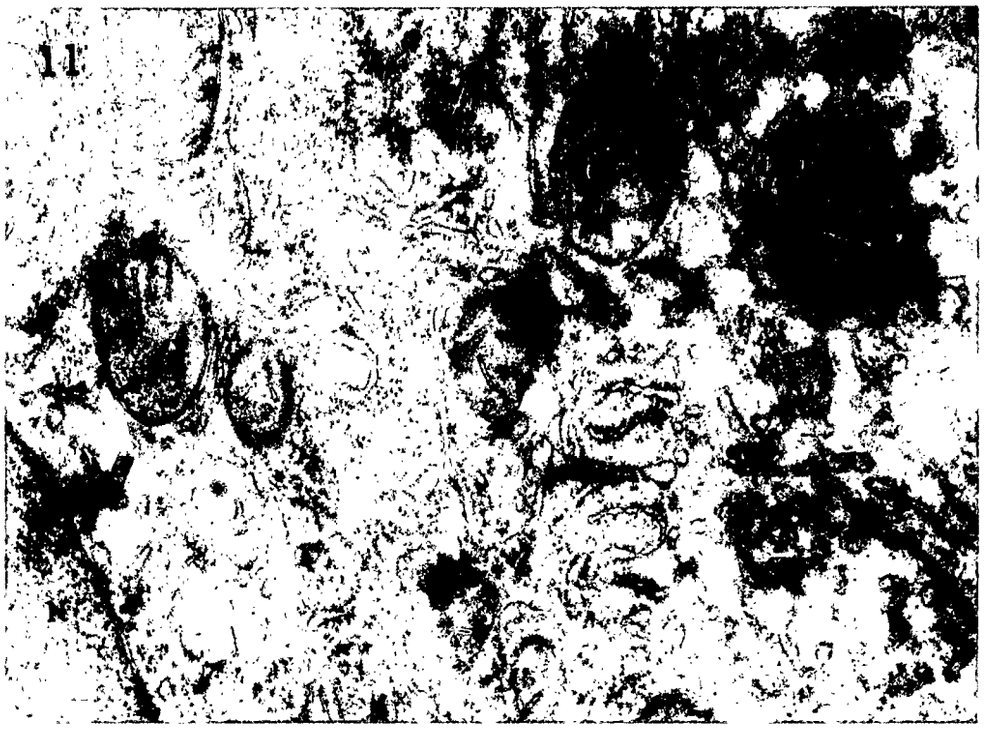

S
V

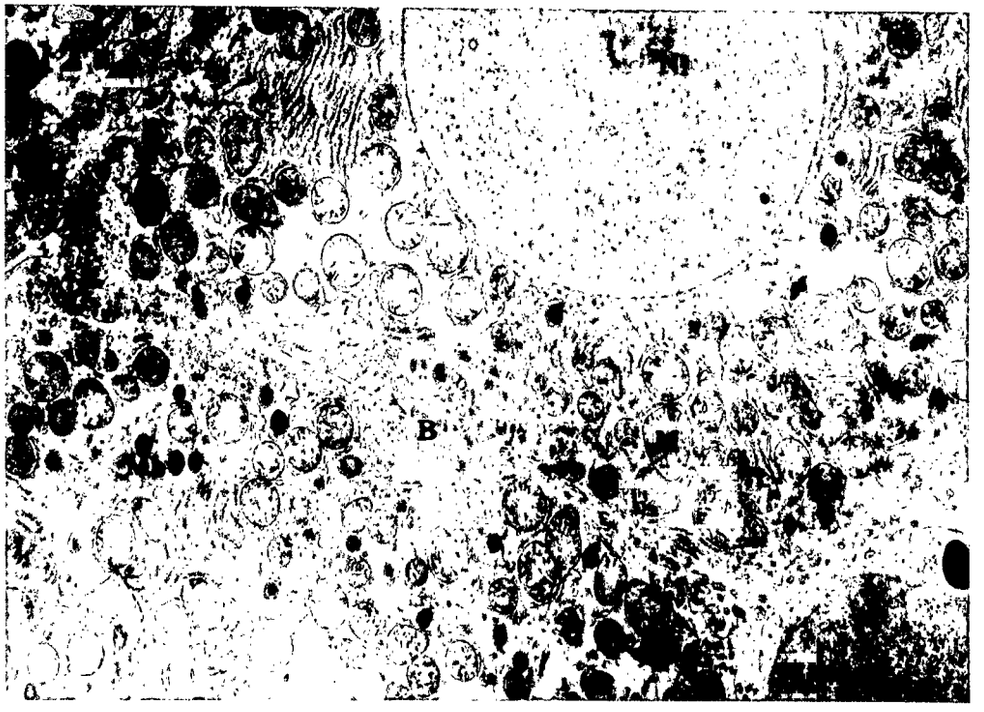

N

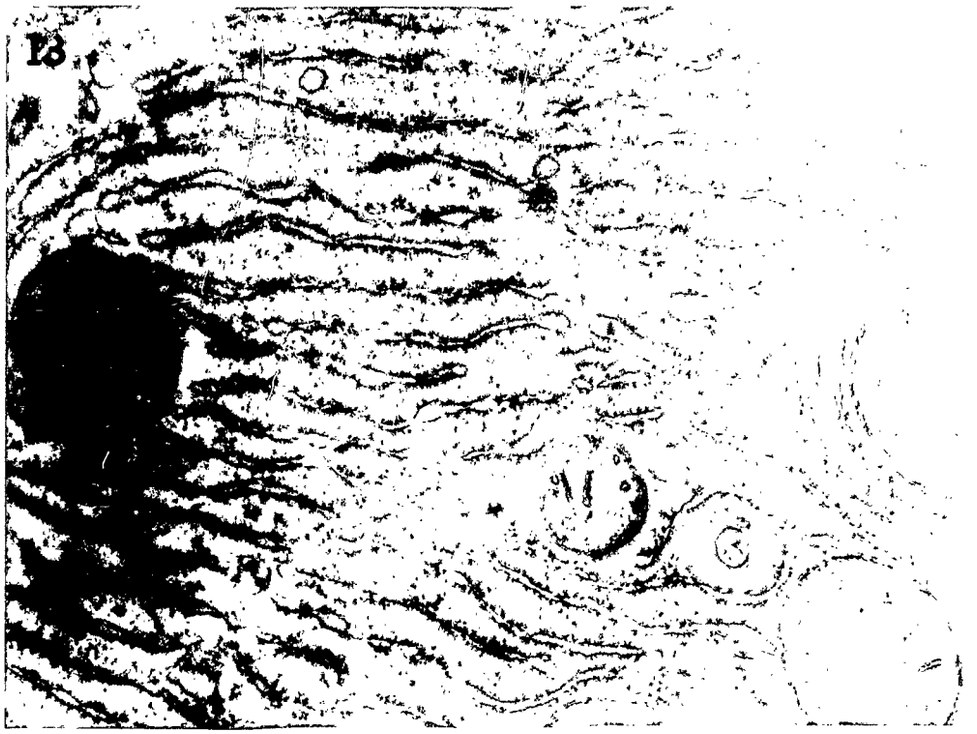

8



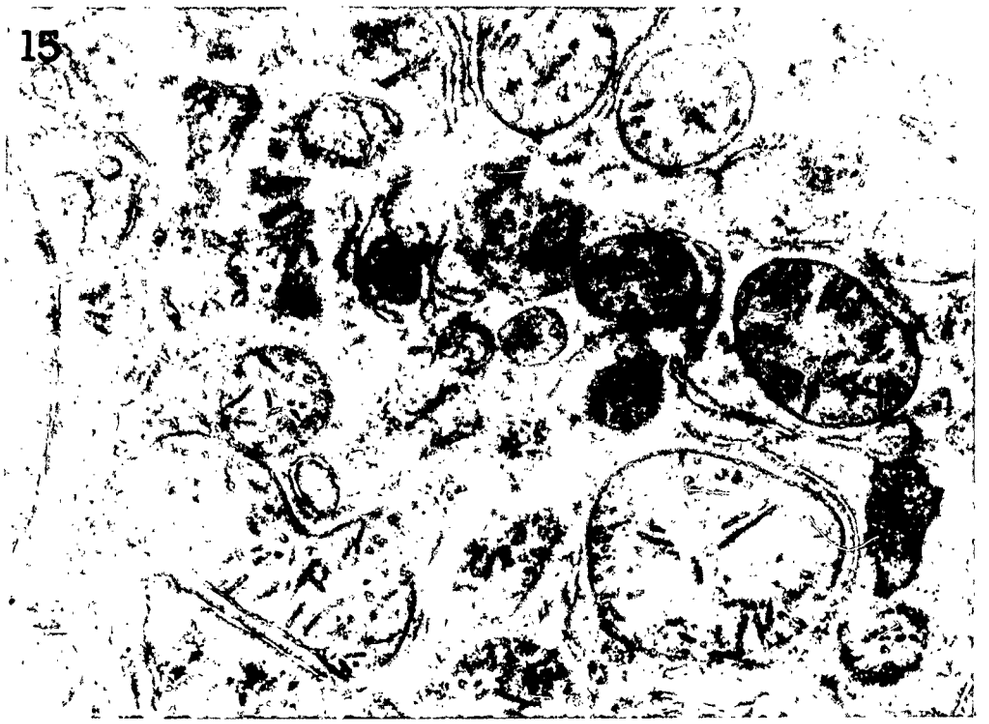


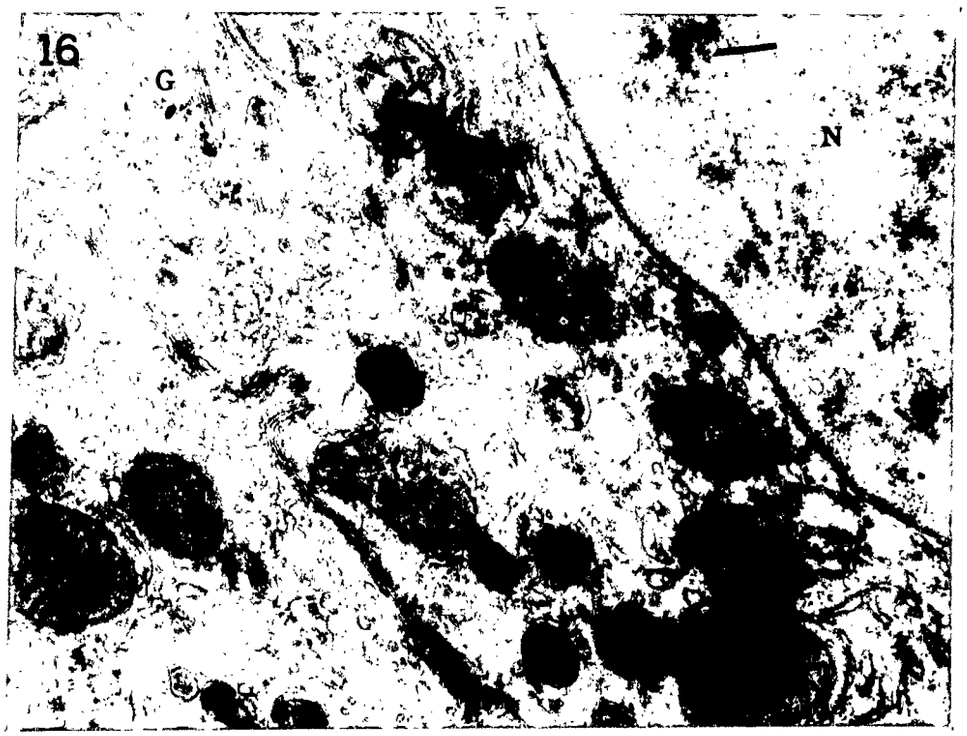




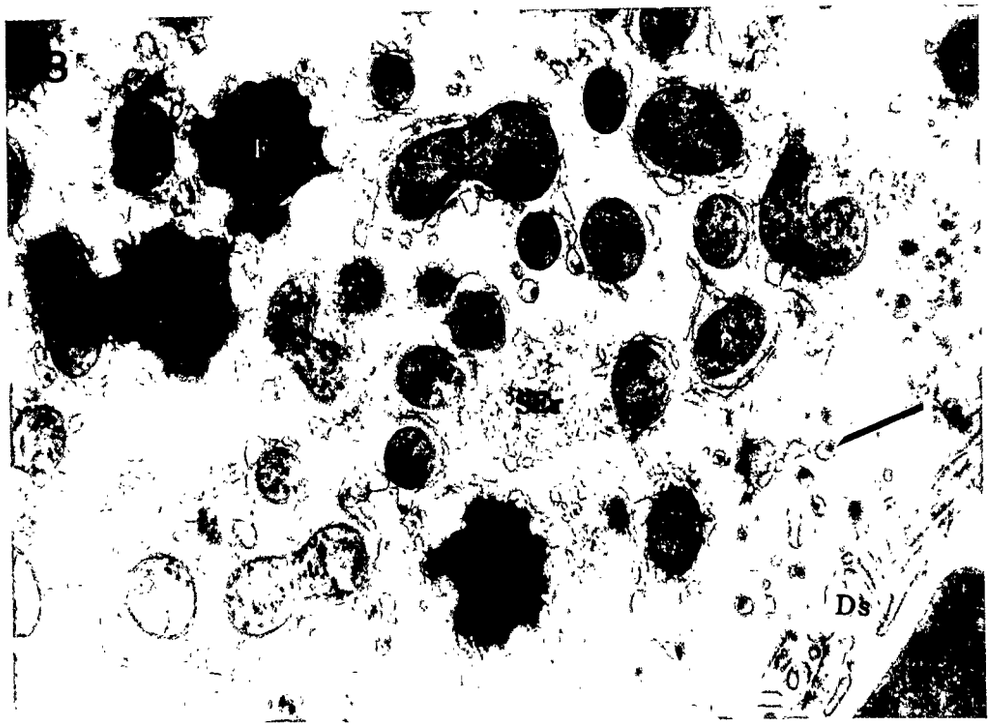

13


14
N

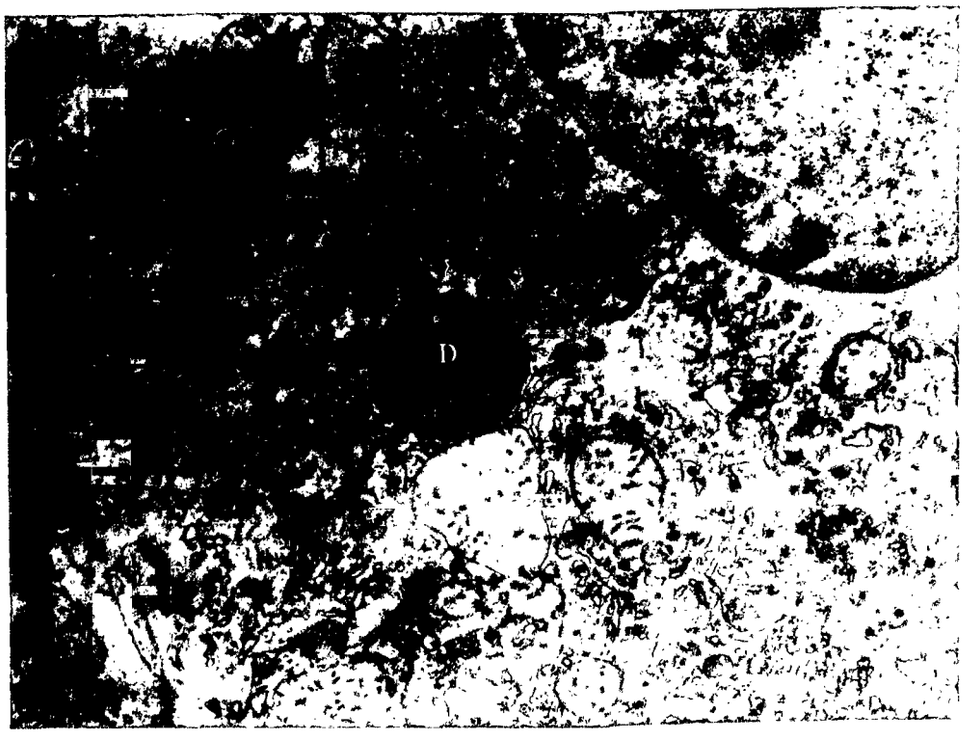

15


16
G
N


d
N
c


Ds


19


D