INTRACELLULAR CHANGES DUE TO NEUTRAL RED AS REVEALED IN THE PANCREAS AND KIDNEY OF THE MOUSE BY THE ELECTRON MICROSCOPE

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Plates 10 to 15

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Neutral red is taken up by living cells, isolated into cytoplasmic inclusions, and excreted after some time to leave the cell apparently as it was before exposure to the dye (25). Too much of it causes the death of cells or of animals. The mechanism by which tolerable amounts are segregated by the cell, and then excreted, has been the subject of controversy since the phenomenon was first observed. On the one hand, segregation might occur within preexisting structures; on the other hand, the presence of the dye might cause the development of new cell structures; or both mechanisms may be involved. Neutral red segregation by the pancreatic acinar cell is of special interest because neutral red inclusions have been extensively studied in this cell (2, 3, 13, 15, 20, 21, 25, 29, 30, 31). The present study represents an attempt to visualize neutral red inclusions with the electron microscope in order to study the relationships, if any, of these structures to well defined cytoplasmic organelles.

The animals used in the present experiments were Swiss albino mice. The pancreas was the principal organ studied; in addition, observations were made on the kidney. The experiment performed by Morgan (25) was repeated. In it, mice are injected subcutaneously with an "optimal" amount of an aqueous solution of neutral red, an amount which causes the appearance in the pancreatic acinar cell of neutral red inclusions which gradually increase in size and number, reach a peak, and then slowly disappear. In the present study, observations were made directly on fresh tissue, as well as on fixed tissue with the light and the electron microscope. It was found that neutral red, under the conditions of this experiment, is a cytoplasmic toxin, causing specific and reproducible pathology of the ergastoplasm, the zymogen granules, the mitochondria, and possibly the Golgi complex. Segregation occurs within pathologically modified mitochondria, in vacuoles containing the dye in relatively high concentration. The dye is finally excreted by the cell, and the cell returns to normal.

Materials and Methods

Neutral red chloride (C.F. 825) was obtained from the National Aniline Division of the Allied Chemical and Dye Corp. This sample was certified for use in vital staining by the

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Biological Stain Commission. The dye content of the sample was 69 per cent. The manufacturer states that the remaining 31 per cent is composed almost entirely of sodium chloride and water. Twelve normal male Swiss albino mice, weighing from 20 to 25 gm., were injected subcutaneously with 0.5 cc. of a 1 per cent solution of this dye. This "optimal dosage" according to Morgan (25) is sufficient to show neutral red inclusions clearly, but does "not cause gross pathological changes nor prevent the cell from returning in the course of time to a normal condition." At intervals from 1/2 hour to 16 hours after the time of injection animals were killed by decapitation, and small pieces of pancreas were obtained within 2 minutes of the time of death. Small pieces of kidney cortex were obtained from three animals killed about 2 hours after injection of the dye. Observations were made on spreads of fresh tissue with the light microscope, on osmic-fixed, silver-stained tissue with the light microscope, and on osmic-fixed, unstained tissue with the electron microscope.

For study of fresh, unfixed pancreas and kidney, a small fragment of tissue was placed in a drop of isotonic saline on a slide and covered with a No. 1 coverslip. For further study tissue was fixed for 4 hours in 1 per cent osmic acid in half-strength Ringer's solution buffered with acetate and veronal to pH 7.5 (27). It was then washed in distilled water and dehydrated by successive passage through 70, 95, and 100 per cent ethanol. The tissue was transferred from absolute ethanol to a half-and-half mixture of absolute ethanol and methacrylate. The methacrylate was composed of three parts n-butyl methacrylate to one part methyl methacrylate. After three changes of methacrylate, the tissue was embedded in methacrylate to which the catalyst, benzoyl peroxide, had been added. Polymerization was carried out at 47°C for 24 hours. Sections were cut with a Minot microtome, modified as described by Dempsey and Lansing (9) and equipped with glass knife. For light microscopy sections 1 to 2 μ in thickness were cut and floated onto a drop of water placed on a glass slide. The section was flattened and the water evaporated by gentle heating. To remove the plastic, the slides were passed through benzene and then xylene, after which they were dehydrated by passage through decreasing concentrations of ethanol and stained with Wilder's ammoniacal silver method for reticulum fibers (see Houck and Dempsey (18) for details of this technique). For electron microscopy sections approximately 300 A thick were used. The sections were examined without removing the plastic in an RCA model EMU electron microscope with a 40 μ objective aperture. Electron micrographs were taken at original magnifications of from 2,000 to 11,000 times and enlarged photographically as desired.

OBSERVATIONS

Within 5 minutes of the time of injection of neutral red, the mouse no longer manifests his usual alert and lively behavior, but becomes somewhat lethargic and unresponsive. Lethargy and unresponsiveness become more marked for the next 1/2 hour until by 20 minutes after injection the mouse sits with back hunched, fur standing erect, and eyes half-closed, rubbing his nose with his forepaws. This condition remains unchanged until about 3 hours after injection, when the mouse begins to show progressively greater responsiveness, until by 4 or 5 hours after injection his behavior is once again normal. No physiological observations were made on the animals except to note that their urine becomes pink within 15 minutes and remains red or pink for at least 16 hours.

Fresh Pancreas.—Under the light microscope, a squash preparation of living pancreas from an uninjected mouse is seen to be composed of many spherical acini embedded in a delicate network of connective tissue, each acinus composed of many conical cells attached at their bases to a basement membrane. The blunt apices of the acinar cells possess a free
border surrounding the lumen of the acinus. In their homogenous basal area the cells contain spherical, optically clear nuclei, and in their apices they contain many refractile zymogen granules. No other structures are seen with transmitted light under oil immersion.

15 minutes after injection with neutral red the entire pancreas has become stained a uniform dark pink. Upon microscopic examination, the acinar cells appear normal except for a diffuse light pink color which persists for approximately 2 hours. 45 minutes after injection neutral red vacuoles first appear. They are single, about 0.3 μ in diameter, few in number, and located in a zone basal to the zymogen granules and capping the nucleus apically and laterally. At 1 hour after injection more neutral red vacuoles have appeared. They are no larger in size, and occur in the same region of the cell. However, here and there, two or three of these tiny vacuoles may be grouped together. By 2 hours there is an increase in both number and clumping of the vacuoles and they are present in the basal part of the cell, as well as lateral and apical to the nucleus. The size of the clumps, the number of vacuoles in a clump, and the number of clumps in a cell all gradually increase, until, by 7 hours after injection, the cell is full of vacuoles 0.4 to 0.5 μ in diameter arranged in clumps of three, four, or more. A few clumps are scattered among zymogen granules, but most are located in a region lateral to the nucleus, and in the basal part of the cell. For the next 6 or 7 hours the number of clumps decreases, although the size of the separate vacuoles, and the number of vacuoles in a clump do not appear to change. By 12 hours after injection the clumps have disappeared from the cell; it once again appears completely normal. No other abnormalities in the structure of the acinus or of its constituent cells were observed. The zymogen granules and the nucleus appeared unchanged throughout the 16 hours after injection of neutral red.

Fixed Pancreas Stained with Silver.—Silver-stained sections of normal pancreas are toned a light yellow-brown. Certain elements stand out against this background. Most conspicuous is the dark black skeleton of reticulum fibers surrounding acini and blood vessels. Within the acinar cell the nuclear membranes and nuclear chromatin are sharply etched; the separate zymogen granules are delicately outlined by a thin brown line; and between the zymogen granules and nucleus a few small, irregular granules are stained light brown.

Following neutral red injection the appearance of the nucleus and zymogen granules remains unchanged; but around the nucleus new structures appear in the cytoplasm. The new structures are essentially similar to the normally present supranuclear granules, except that they are more intensely argyrophilic, larger, and more irregular in shape. They first appear about 45 minutes after injection, are spherical and about 0.5 μ in diameter (Fig. 15). A few appear lobulated. The number and size of these inclusions increase further with time until by 7 and 8 hours as many as ten or more of these black bodies are present in a section of a single pancreatic cell (Fig. 16). Almost all these structures have three or more spherical lobules; the entire inclusion may measure as much as 1.5 μ in diameter. After 8 hours the number of inclusions gradually decrease until, by 12 hours after injection, only a few can be seen within the cell, which once again appears normal. The variation in number, size, shape, and distribution of these argyrophilic inclusions with time is entirely similar to that described above for the neutral red vacuoles.

Electron Microscopy

The following points of normal anatomy appear pertinent:

The basal homogenous region of the cell contains ergastoplasm with interspersed mitochondria. The granular membrane of the ergastoplasmic sacs (32) can be resolved into a
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smooth membrane 60 Å thick, on the outer surface of which ergastoplasmic granules, about 160 Å in diameter, are fixed at more or less regular intervals (Figs. 1, 2, 8 to 11, 18). The mitochondria can be recognized by their double outer membrane and by internal folds which appear as double membranes (Figs. 4, 8 to 10). Sharply outlined zymogen granules which are uniformly electron-dense are packed together in the apical cytoplasm with a few mitochondria and ergastoplasmic sacs interspersed among them. Just apical to the nucleus, a system of membroaneous sacs, small granules, and vacuoles is present (Figs. 6, 7, 9, 10). The membranes are about 60 Å thick, smooth, and packed closely together; they appear in section to be closed loops up to 0.5 μ long, and represent the limiting membranes of closed sacs, as is true of the ergastoplasmic membrane. The small granules are approximately 400 Å in diameter, are bounded by a membrane which is smooth and 60 Å thick, and contain electron-lucent material. The vacuoles are irregularly spheroid, vary in greatest dimension up to 0.5 μ, contain electron-lucent material, and are bounded by a poorly defined cytoplasmic border which cannot be resolved into a membrane. This three-component system was first described by Dalton and Felix (8) in the epididymis, duodenum, pancreas, liver, and plasma cell, and has been seen by us in the duodenum (33), and by Dempsey and Peterson (10) in thyroid cells. Dalton and Felix have pointed out that whenever this system is found, it is located in the region of the Golgi apparatus, and is the only structure visualized in that region with the electron microscope. They have shown that, following buffered osmic fixation, the three components of the system can be impregnated with osmic acid. These elements, then, represent the components of the Golgi complex. These components will be referred to in this paper as Golgi sacs (bounded by Golgi membranes), Golgi vacuoles, and Golgi granules.

The first change observed after neutral red injection occurs within ¾ of an hour. In the base of the cell, regions of amorphous granular gray material appear, bordered on all sides by ergastoplasm (Figs. 1 to 3). The number of these regions is greatest at about an hour after injection, as many as three appearing in a section of a single cell. Such regions are present, though not as numerous, up to 15 hours after injection. The junction between recognizable ergastoplasm and these granular regions is fairly sharp. No membrane is present, however, the interface consisting on the outside of ergastoplasmic granules and apparently sheared-off ergastoplasmic sacs, and on the inside of granular material (Figs. 2 and 3). The granular regions are irregularly circular or oval in section, and measure as much as 3.5 μ in greatest dimension. Certain favorable sections appear to show stages of transition from normal ergastoplasm to granular material. In such regions the ergastoplasmic granules and membranes can still be distinguished, but are less electron-dense and no longer sharply defined (Figs. 1 and 2).

Soon after the appearance of these alterations involving the ergastoplasm, changes become apparent in the zymogen granules. 1½ hours after injection a few granules appear to have a poorly defined border and are slightly less dense than normal ones. Varying degrees of these alterations can be seen within a single cell (Figs. 4 to 6). By 2 hours after injections some cells contain no nor-
real zymogen granules, their apices being filled with an homogenous granular gray material (Fig. 6). Embedded in this granular gray material are normal ergastoplasm, Golgi complex, and mitochondria. The reaction at 2 hours is a maximal one. A few zymogen granules continue to show degenerative changes up to 8 hours after injection, but by 10 hours only normal ones are present.

An hour after neutral red administration, isolated, irregularly shaped osmiophilic inclusions appear in the supranuclear region of the cell (Figs. 7 to 10). These inclusions increase in number, size, and complexity during the interval up to 7 hours after neutral red administration. Thereafter, although they remain large and complex, they decrease in number until none remain after 15 hours. The size, shape, number, and distribution of the osmiophilic inclusions are consistent with the interpretation that they represent sections through the argyrophilic inclusions which have been described in the fixed, silver-stained sections seen in the light microscope.

Small spherical vacuoles appear within a few of the osmiophilic inclusions seen 1 hour after injection. As time passes, these vacuoles increase in size and more appear in each inclusion. At about 7 hours after injection, the size of the individual vacuoles and the number of vacuoles in a single osmiophilic inclusion reaches a maximum (Figs. 11 and 12). Thereafter they do not change except to decrease in total number as the number of separate osmiophilic inclusions decrease. In every animal killed, the number, size, and location of the individual vacuoles parallel the number, size, and location of the individual neutral red vacuoles in the separate neutral red clusters. Therefore we conclude that the vacuoles seen within osmiophilic inclusions are identical with the neutral red vacuoles observed in fresh tissue. It is not surprising that vacuoles are not seen with the light microscope, for the shell of argyrophilic material would mask the presence of a vacuole within. Only in thin sections observed in the electron microscope would the central vacuoles be unmasked.

The osmiophilic inclusion is a complex structure composed of three main components—a central body and two types of granules. The central body is light gray. It is usually spherical or ovoid, but may be irregular in form (Figs. 6, 8, 10 to 12, 17, 19). The structure is always bounded by a membrane about 150 A thick, which may appear as a single or a double line. Projecting inward from this bounding membrane, in certain cases, are short stubs of double membrane, about 150 A in total thickness, and of varying length (Figs. 8, 9, 11, 17). Such double membranes rarely transect the central body (Fig. 8). As many as three central bodies may be seen in a section of a single osmiophilic inclusion (Figs. 6 and 17). The vacuoles described above occur within the substance of the central body, single at first, and then multiple (Figs. 6, 11, 12, 17, 19). Adhered to a pole of the central body is a mantle of variable size composed of two
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types of granules. The larger of these is ovoid, and measures from 200 A to 2,000 A in greatest diameter (Figs. 6, 8, 10 to 12, 17 to 19). As many as thirty may be present in a single section (Fig. 18). They may be more or less osmiophilic, varying in density from a light gray, like that of the central body, to a dense black. The larger granules are bounded by a fairly well defined membrane about 150 A thick; they differ from the central bodies in their small size, their lack of internal structure, and their variability in electron density. The second type of granule is small, electron-dense, and 160 A in diameter.

It is probable that the central bodies represent mitochondria which pass through various phases of degeneration, first losing their internal folds, and later developing vacuoles. That this is the case is suggested by the presence of an outer limiting membrane which occasionally exhibits a double lined fold projecting into the substance of the body. Positive identification of the central bodies as degenerate mitochondria may be made more difficult in the pancreas, however, because pancreatic mitochondria normally do not possess many internal folds. Kidney tubule mitochondria, on the other hand, contain many closely packed internal folds. Neutral red vacuoles develop in the kidneys of injected animals, and when such kidneys are studied in the electron microscope, osmiophilic inclusions are found which contain vacuoles. Like the pancreatic osmiophilic inclusion, those found in the renal tubule consist of a central body and adsorbed granules (Fig. 14). The vacuolated central body of the renal inclusion, moreover, possesses many internal folds characteristic of a mitochondrion (Figs. 13 and 14). The central body of the osmiophilic inclusion of the renal tubule then, can be positively identified as derived from a mitochondrion. In the case of the pancreatic osmiophilic inclusion, all that can be said is that the central body frequently exhibits an internal structure consistent with its derivation from a mitochondrion.

The origin of the two sorts of granules of the osmiophilic inclusion is not clear. The larger granules are similar in appearance to immature zymogen granules, and may be derived from small ergastoplasmic sacs, as are immature zymogen granules (32). It is also possible that they may derive from the Golgi complex. Golgi granules are strikingly similar to newly formed buds from ergastoplasmic sacs (Fig. 10). In addition, the one change in the Golgi complex which is found in the neutral red-poisoned animals is an apparent increase in the number of Golgi granules, and their dispersion away from the region of the Golgi sacs (Fig. 6). Transitions either from Golgi granules or from small ergastoplasmic sacs to the large granules of the osmiophilic inclusions have not been seen, however. If the large granules do form by a process similar to the formation of zymogen granules, i.e. the accumulation of material within a small, membrane-bounded sphere, it would be difficult on the basis of the present evi-
The small granule in the osmiophilic inclusion is similar in size, electron density, and shape to the ergastoplasmic granules (Figs. 5, 12, 18). The possibility then exists that ergastoplasmic granules are involved in the formation of osmiophilic inclusions.

In summary, it appears that neutral red causes characteristic changes in ergastoplasm, zymogen granules, mitochondria, and possibly in the Golgi complex. No clear cut changes were found in the cell membrane or the nucleus.

DISCUSSION

Cytologists have, for many years, applied dyestuffs to living cells in an attempt to visualize cytoplasmic structures comparable to those demonstrable in fixed and stained tissues. The need of them was especially great for early investigators who did not have the dark field and phase contrast microscope, and who could see but little structure in the cytoplasm of living cells. Since late in the last century much work has been done on the effects of various dyes upon the living cell. The burden of the work on basic dyes indicates that these dyes readily diffuse into the cytoplasm of most cells, exert a more or less toxic effect, and are concentrated in certain cytoplasmic structures. One group of basic dyes, represented by Janus green (24), Janus blue, Janus gray, Janus black (22), and, under certain conditions, methylene blue (23), are known to be concentrated within mitochondria; the Janus dyes, indeed, have long been used in methods designed to specifically demonstrate mitochondria in living cells. A second group of basic dyes, the prototype for which is neutral red, stains certain cytoplasmic structures whose nature has been controversial. It has been variously held that these structures represent cytoplasmic granules or vacuoles of unspecified type (2, 6, 20, 21, 30), prozymogen granules (3, 29), elements of the Golgi system (1, 15, 28), mitochondria (12), and newly formed pathological inclusions (13, 21, 25).

It is not within the scope of this paper to review the extensive literature on the effect of neutral red and related basic dyes upon living cells. It would be valuable, however, to point out how the present results bear on some of the controversial points of this question.

The most extensive controversy has centered about the thesis that neutral red-stained inclusions represent an aspect of the "true" Golgi complex. To set this hypothesis in proper perspective it is necessary to outline briefly the different concepts of the morphology of the Golgi complex.

Soon after the turn of the century two main schools of thought regarding the structure of the Golgi complex had evolved, the first, represented by Golgi (14), Kopsch (19), and their followers, holding that the apparatus represented some variety of solid structure, membraneous, net-like, or otherwise, which could be impregnated
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with osmium or silver salts; and the second, originating with Holmgren (17), and supported by Cajal (5) and his followers, holding that the Golgi represented some sort of canalicular or vesicular intracellular aqueous phase bounded by a membrane which could be impregnated with osmium. Most subsequent workers have held to one or the other or a combination of these classical views. The notable exceptions have been influenced by observations on the effect of neutral red upon living cells. The French school, derived from Parat (28), has held that the Golgi consists of a system of neutral red-stainable vacuoles—the “vacuome”—the surfaces of which blacken after impregnation with osmium or silver to give rise to the classical network picture. The Oxford school, led by Baker (1), has held much the same view as the French. Baker looks on the Golgi as a collection of lipid droplets stainable with Sudan black, some of which droplets contain neutral red-stainable vacuoles; he considers that any metal-impregnated Golgi image not related to neutral red vacuoles is derived by myelin figure formation from the lipid in the coat of the neutral red vacuoles. Other workers who have been influenced by neutral red (for example, Hirsch (15)) have given different names to these vacuoles, have invested them with different materials, and have essentially concluded that any metal-impregnated organelle separate from the vacuoles is an artifact.

These non-classical concepts have been challenged effectively by many investigators (2, 7, 13, 16, 20, 26, 30) who maintain that neutral red inclusions and the Golgi complex are two separate classes of structure.

The clear cut demonstration by Dalton and Felix (8) of the structure of the Golgi complex as revealed by the electron microscope, and the present work showing that neutral red vacuoles are newly developing segregation vacuoles within mitochondria, together offer a new demonstration that neutral red vacuoles and Golgi complex are separate entities. A portion of the sudanophilic, argyrophilic, and osmiophilic material which adsorbs onto the surface of the abnormal, neutral red-segregating mitochondria may be derived from the Golgi complex, as Morgan’s and our works indicate. Our observation that the Golgi sacs, the Golgi vacuoles, and Golgi granules are present at all times after the injection of neutral red is, however, inconsistent with Morgan’s observation that the Golgi material disappears from the acinar cell as the neutral red inclusions develop.

Besides the question of neutral red’s relation to the Golgi complex, there have been other theories of what neutral red inclusions in living cells represent. Bensley (3) claimed that neutral red stained newly forming zymogen granules in pancreatic acinar cells. Recently Parks (29) has made observations consistent with this interpretation. It is impossible to judge the validity of these observations from the present work. They should be held open to question, however, until rapidly synthesizing acinar cells are exposed to neutral red in the low dose recommended by Parks, and then observed with the electron microscope.
Some investigators (6, 26, 30) have studied living cells in which vacuoles or granules observed before the exposure of the cell to neutral red, concentrate the dye when the cell is exposed to it. These vacuoles or granules may represent mitochondria, however, especially as it has been shown (34) that mitochondria may become vacuolated under certain circumstances without the use of vital dyes. On the other hand, it is possible that intracellular structures other than mitochondria may concentrate neutral red.

In summary, it appears that under certain conditions of administration of neutral red, it is segregated in vacuoles within mitochondria in the pancreatic acinar cell and in the kidney tubule cell. These intracellular vacuoles are distinct from the Golgi complex. Whether neutral red inclusions in other cells are different from mitochondrial segregation vacuoles remains to be seen.

In addition to the long recognized formation of segregation vacuoles, neutral red has other effects upon the pancreatic cell. It damages not only certain mitochondria, but also portions of the ergastoplasm and certain of the zymogen granules. The Golgi complex also appears to be altered in response to the presence of the dye.

That the concentration of neutral red by mitochondria represents a perversion of some normal physiological mechanism is suggested by the fact that these organelles also concentrate other cations. These include the Janus dyes, methylene blue, and probably those dyes which have been used to demonstrate the "vacuome," that is, toluidine blue, brilliant cresyl blue, bromocresyl purple, Nile blue sulfate, new methyl blue, bromphenol blue, tropeolin 00, methylene red, and Bismark brown (see Bowen's review (4)). In addition, Dempsey and Wislocki (11) have recently shown that mitochondria concentrate silver, and become abnormal in the process. These facts may be interpreted to signify that mitochondria are normally involved in the concentration of certain cations within the cell. The toxic effects of the vital basic dyes and of silver upon the mitochondria may be the result of some competitive interference with their normal cation-concentrating function.

SUMMARY

The effects of sublethal amounts of the cationic dye, neutral red, upon the structure of pancreatic exocrine cells, and upon the mitochondria of renal distal tubule cells, have been studied with the electron microscope. It was found that neutral red is a cytoplasmic toxin which causes reproducible and characteristic changes in the ergastoplasm, the zymogen granules, the mitochondria, and possibly in the Golgi complex. Ergastoplasmic membranes and granules and zymogen granules lose definition and become continuous with the cytoplasmic matrix. Mitochondria lose their internal folds, develop vacuoles which
contain a solution of neutral red in high concentration, and form the nidus for the development of sudanophilic, argyrophilic, osmiophilic inclusions which appear in the cytoplasm after neutral red administration. Golgi granules, one of the three elements of the Golgi complex, appear to increase in number and to be scattered more widely through the cytoplasm than is normal. No consistent changes were found in the cell membrane or nucleus.

The ability of the mitochondria to concentrate the cation, neutral red, taken with its well known ability to concentrate the cationic Janus dyes and methylene blue, and its probable role in concentrating those cationic dyes which have been used to demonstrate the "vacuome," is interpreted to signify that one of the functions of mitochondria may be to concentrate intracellular cations.

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BIBLIOGRAPHY

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EXPLANATION OF PLATES

PLATE 10

Fig. 1. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 12 hours after injection of neutral red. A portion of a zymogen granule (Z) is at the top of the micrograph. The rest of the field is filled with ergastoplasm. Many small ergastoplasmic sacs (ES) are present, represented by closed loops in section. The sacs are bounded by a 60 A thick ergastoplasmic membrane to which are adsorbed ergastoplasmic granules, 160 A in diameter. Some ergastoplasmic granules appear to be separate from membranes, although this appearance may result from a section tangential to the surface of a sac. In the center of the section is a region in which the ergastoplasmic granules have lost their sharpness and density, and appear to be melting into the cytoplasmic matrix. Also present in this region are small ergastoplasmic sacs (arrows), the membranes of which are fuzzy in outline, and less electron-dense than normal. This central region contains degenerating ergastoplasm and is surrounded by normal ergastoplasm. × 54,000.

Fig. 2. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 1 hour after injection of neutral red. Two regions of degenerated ergastoplasm are in the center of the field. Here the normal structure is completely disorganized. Transitions between normal and degenerated ergastoplasm can be seen surrounding the ovoid regions of degeneration. The normal ergastoplasmic sacs are bounded by a 60 A thick ergastoplasmic membrane to which are adsorbed ergastoplasmic granules approximately 160 A in diameter. Ergastoplasmic degeneration is more advanced here than in Fig. 1. × 41,000.

Fig. 3. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 1 hour after injection of neutral red. A region of degenerated ergastoplasm in the center of the field is surrounded by normal ergastoplasm. The normal sacs are bowed out of their straight course as they pass the region of abnormal ergastoplasm. × 24,000.
(Weiss: Intracellular changes caused by neutral red)
Fig. 4. Electron micrograph of a pancreatic cell from a mouse killed 2 hours after injection of neutral red. The plane of section is not through the nucleus. The base of the cell is at the left and below, the apex is at the right and above. Normal ergastoplasm and a few normal mitochondria are present in the base of the cell. The apex of the cell contains zymogen granules, both normal and abnormal, and normal mitochondria (M). The normal zymogen granules (Z) are oval, uniformly electron-dense, and sharply outlined. Markedly abnormal zymogen granules can be recognized only by their oval outline (arrows); they are large, electron-lucent, and tend to flow together. Transition forms (AZ) are larger, less electron-dense, and less clearly outlined than the normal granules. × 5,800.

Fig. 5. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 2 hours after injection of neutral red. The section is through the apical region of the cell, which contains abnormal zymogen granules interspersed with normal ergastoplasmic sacs. Beginning degeneration is marked only by a loss of the granule's sharp outline. With more advanced degeneration the granules become still less clearly outlined, less electron-dense, and larger. The most advanced degeneration is represented by a granular electron-lucent region were the liquified remnants of a number of zymogen granules have run together. This is seen at the very top of the figure. × 14,000.

Fig. 6. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 2 hours after the injection of neutral red. The section is through the apical region of the cell. Filling most of the section are degenerating zymogen granules, which are swollen, relatively electron-lucent, and poorly delimited from the cytoplasmic matrix. The markedly swollen zymogen granules (Z) tend to flow together. In the top half of the section Golgi complex (G) is present between the degenerating zymogen granules. The Golgi sacs are seen in section as elongated loops bounded by a 60 A thick Golgi membrane. The Golgi granules are about 400 A in diameter and are also bounded by a 60 A thick, smooth membrane. No Golgi vacuoles are present. The Golgi granules are scattered a greater distance from the Golgi sacs than is usually found in the normal pancreas. At the bottom center is an osmiophilic inclusion composed of three central bodies and a mantle of small and large granules. The upper and lower left central bodies contain vacuoles. The central bodies are bounded by a membrane, which is approximately 150 A thick. The large granules of the mantle are also bounded by a 150 A thick membrane. The small granules of the mantle (arrow) are approximately 160 A in diameter.

Several normal mitochondria (M) and a few normal ergastoplasmic sacs (ES) are scattered among the degenerating zymogen granules. × 30,000.
(Weiss: Intracellular changes caused by neutral red)
PLATE 12

Fig. 7. Electron micrograph of portions of two exocrine pancreatic cells from a mouse killed 1 hour after the injection of neutral red. The nuclei (N) are in the base of the cell, and are surrounded by ergastoplasm (E). Several normal mitochondria (M) are scattered through the cytoplasm. A few normal zymogen granules (Z) are in the apex of the cell. Golgi complex (G) is in the supranuclear regions of both cells. At this magnification only the Golgi vacuoles can be seen. Two osmiophilic inclusions are present in the supranuclear region of the upper cell, and one in the supranuclear region of the lower cell (arrows). These osmiophilic inclusions are separate from the Golgi complex. X 15,000.

Fig. 8. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 1 hour after the injection of neutral red. This figure is an enlargement of the region in the upper cell of Fig. 7 which contains the two osmiophilic inclusions. These inclusions can be resolved into complex structures composed of a central body bordered by a mantle of large and small granules. The central body in the upper left-hand osmiophilic inclusion is surrounded by a double membrane; a double membrane fold can be barely resolved transecting the waist of this body (arrow). The lower right osmiophilic inclusion has a central body which is bounded by a blurred single membrane. Normal mitochondria are present in the left-hand part of the field, and can be recognized by their outer double membrane, and their inner double membrane folds. In the bottom center is a mitochondrion the inner folds of which are not clear (M). Ergastoplasmic membranes and granules are also present. X 60,000.

Fig. 9. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 1 hour after the injection of neutral red. This figure is an enlargement of the region in Fig. 7 which contains the single lower osmiophilic inclusion. The fine structure of this inclusion is not as clearly outlined as that of the other two. Across the waist of the central body, however, a double membrane can be resolved (arrow). Portions of two normal mitochondria (M) are present, as is normal ergastoplasm. Golgi complex is present on the right. This is composed of Golgi vacuoles (V), Golgi sacs (S) bounded by a 60 A thick Golgi membrane, and Golgi granules (G) approximately 400 A in diameter and bounded by a 60 A thick membrane. This Golgi complex is normal in appearance. X 60,000.
(Weiss: Intracellular changes caused by neutral red)
Fig. 10. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 1 hour after the injection of neutral red. This section is through the supranuclear region of the cell. Occupying the center of the field is Golgi complex composed of Golgi vacuoles (V), Golgi sacs (S) bounded by 60 A thick Golgi membranes, and Golgi granules (G) approximately 400 A in diameter, and also bounded by a 60 A thick membrane. This Golgi complex is normal in appearance. At the upper right is an osmiophilic inclusion composed of two central bodies (C) and an adsorbed mantle of large and small granules. Bounding both the central bodies and the large granules are membranes approximately 100 A in thickness. Normal mitochondria (M) are also present, as is normal ergastoplasm. The ergastoplasmic sacs are bounded by a smooth membrane, 60 A thick, to which are adsorbed ergastoplasmic granules, approximately 160 A in diameter. Several buds (arrows) are pinching-off from the large ergastoplasmic sac to the left of the Golgi complex. Immediately to the right of these buds are small membrane-bounded spheres which may be either pinched-off ergastoplasm or Golgi granules. × 46,000.
(Weiss: Intracellular changes caused by neutral red)
PLATE 14

Fig. 11. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 7 hours after injection of neutral red. In the left lower corner is the midportion of a normal mitochondrion which contains internal folds each composed of a double membrane. Running up and down just to the right of this mitochondrion are a number of ergastoplasmic sacs, bounded by ergastoplasmic membranes with adsorbed ergastoplasmic granules. Occupying the center of the field is a large osmiophilic inclusion, which contains two central bodies and an adsorbed mantle of large and small granules. The lower central body contains four vacuoles, the upper one contains only one, in the plane of section. A double membraned internal fold is present in the substance of the lower central body, just below the left-hand vacuole (arrow). This double membrane appears to be attached to the outer limiting membrane of the central body. The membrane’s dimensions are the same as those of the double membraned folds of the normal mitochondrion in the lower left. × 46,000.

Fig. 12. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 7 hours after injection of neutral red. A large osmiophilic inclusion occupies most of the field; the left half of a second osmiophilic inclusion is present in the right-hand part of the field. In the large inclusion there are two central bodies, each vacuolated, one at the upper pole and one at the lower pole. Marked vacuolation makes sharp definition of the central bodies impossible. The larger granules in the mantle are clearly defined; they vary considerably in size, and in the electron density of their contents. The body at the upper right part of the inclusion (arrow) may be either a central body or one of the large adsorbed granules. Ergastoplasmic granules are present in the cytoplasm to the left of the field, and are the same size and electron density as those of the small granules in the osmiophilic inclusion (arrow). × 73,000.

Fig. 13. Electron micrograph of a small portion of a distal convoluted tubule cell from the kidney of a mouse killed 2 hours and 10 minutes after injection of neutral red. A mitochondrion occupies the center of the field, and can be recognized by the closely packed membranes of the internal folds. These membranes are 40 Å thick, and are separated by a 40 Å clear space. A large vacuole is present within the mitochondrion. × 100,000.

Fig. 14. Electron micrograph of a small portion of a distal convoluted tubule cell from the kidney of a mouse killed 2 hours and 10 minutes after injection of neutral red. A portion of the nucleus is present at the upper left, and an osmiophilic inclusion, consisting of a central body and an adsorbed mantle of granules, is present in the center of the field. The central body is recognizable as a mitochondrion, with internal membranes measuring about 40 Å in thickness, separated by clear spaces 40 Å wide. The section is in a plane tangential to the internal folds resulting in a target appearance of the membranes of these folds, which have probably been bent into a cup-like form. The granules of the mantle are not clearly defined. × 125,000.
(Weiss: Intracellular changes caused by neutral red)
PLATE 15

Fig. 15. Light micrograph of an acinus in the exocrine pancreas of an animal killed 1 hour after the injection of neutral red. The section is of osmic-fixed, plastic-embedded tissue which has been stained with Wilder's ammoniacal silver stain. The nuclear membrane and the chromatin within the nucleus (N) stand out with great contrast, as do the (small cytoplasmic) argyrophilic inclusions. In an animal which has not been given neutral red it is usual to find one or two such granules, about 0.5 μ in diameter, in the supranuclear cytoplasm. In this section, 1 hour after neutral red injection, the granules are no larger in size, but there are more in most cells, and a few are located in the basal part of the cell. × 1800.

Fig. 16. Light micrograph of an acinus in the exocrine pancreas of a mouse killed 2 hours after injection of neutral red. The section is prepared like that in Fig. 15. The argyrophilic inclusions in the cytoplasm are larger, measuring up to 1 μ in diameter, more irregular, and more numerous than those present at 1 hour. Several argyrophilic inclusions are in the basal region of the cell at the right in the acinus. × 1800.

Fig. 17. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 10 hours after injection of neutral red. Two vacuolated osmiophilic inclusions are in the center of the field. The central body of the upper one is bounded by a membrane, and contains a membrane structure within its substance (arrow). Several normal mitochondria (M) and portions of two normal zymogen granules (G) are present, as well as normal ergastoplasm. × 32,000.

Fig. 18. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 12 hours after the injection of neutral red. In the left part of the field is a section through the mantle of an osmiophilic inclusion. The membrane-bounded large granules are present in great number. There is a striking similarity between some of these granules and the ergastoplasmic sacs (E), present in the right-hand part of the field, some of which contain electron-dense material. The small granules in the mantle (arrow) are similar in size and electron density to ergastoplasmic granules. × 40,000.

Fig. 19. Electron micrograph of a portion of an exocrine pancreatic cell from a mouse killed 12 hours after the injection of neutral red. An osmiophilic inclusion is present, surrounded by ergastoplasm. Many vacuoles are present in this one section. A few large granules are adsorbed to the lower right surface of the inclusion. × 40,000.
(Weiss: Intracellular changes caused by neutral red)