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Abstract

1. Histochemical and cytochemical studies with respect to the sites of reaction were made on the succinic dehydrogenase system activity of human and animal tissues using ditetrazolium salts, namely, neotetrazolium chloride, nitro-neotetrazolium chloride, and nitra-blue tetrazolium chloride. 2. The advantages and disadvantages of each ditetrazolium salt for histochemical and cytochemical purposes and the reaction taking place in frozen tissue sections and that in fresh tissue blocks were compared, and the method of procedure suitable for each condition was established with some modification. 3. Selecting conditions suitable for cytochemical purpose, it was shown that the reaction took place at the sites coinciding with mitochondria, and the distribution of the enzyme reaction was also examined. In addition, several new findings in the brains and other tissues cytochemically made clear were pointed out.

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HISTOCHEMICAL AND CYTOCHEMICAL STUDIES ON THE SUCCINIC DEHYDROGENASE SYSTEM WITH THREE DITETRAZOLIUM SALTS, NT, NITRO-NT, AND NITRO-BT

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With the advent of various tetrazolium compounds a marked progress is being made in histochemical studies on the succinic dehydrogenase system as well as on various dehydrogenases[1–9], but there still remain many unsolved problems concernig the reduction mechanism of these compounds in living tissue, and also the question at what step of the oxidation-reduction system most of these reactions conjugate remains unanswered. Therefore, it is doubtful whether specific enzyme activity can be selectively verified by these reactions, and to date, the histochemical reactions have not received sufficient quantitative consideration. Moreover, it was difficult to obtain the reaction products of good pigment quality and to demonstrate the precise intracellular localization of the enzyme. Previously we pursued the reduction of ditetrazolium salts such as NT, Nitro-BT[10],and Nitor-NT[11, 12] by tissue cells and homogenates,and using various substrates and different inhibitory agensts we detected selectively the activity of individual enzymes or of the enzyme system of the terminal electron transport system involved in the energy production and described the method of the quantitative determination of the enzyme activities[13–16] and also it was proven microspectrophotometrically and electronmicroscopically that the cytochemical reaction of enzymes occurred at the sites of mitochondria[14, 16]. On the basis of these preliminary investigations an attempt was made to grasp histochemically and cytochemically the distribution of the enzyme activity in the principal organs of the body, mainly human body, under the normal condition. In this connection the method of procedure was studied from various angles and some modification was made, so that an improved method, when applied for pathological investigation, might be helpful. As for the reagents nitro-blue tetrazolium chloride (Nitro-BT), and nitro-neotetrazolium chlo-

ride (Nitro-NT), recently synthesized and generously supplied directly by SELIGMAN[10] and PEARSON[12], were used in additon to neotetrazolium chloride (NT), and the usefulness of each of these drugs for such a purpose was evaluated and merits of each were compared.

MATERIALS AND METHODS

The heart, liver, kidney, brains, spinal cord, intestines, pancreas, salivary gland, skeletal muscle, blood cells etc. of humans, cats and rabbits were used as the materials. Animals were sacrificed by decapitation, and human tissues were obtained a comparatively short time after death from autopsy materials having no pathological changes. Two procedures of histochemical reaction were tried; the one in which frozen tissue sections were put in the reagent, and the other in which a fresh tissue block was put into the reagent and then sections were prepared. For the substrate succinate was used and for ditetrazolium salts, three compounds mentioned above, but somewhat different method of procedure and the modified points will be discussed.

1) *In the case of NT (p-p'-diphenylene-bis-2 (3, 5-diphenyl) tetrazolium chloride).*

The reagent used was a mixed solution of 0. 2% NT aqueous solution, 0. 2M sodium succinate solution, and 0. 1 M phosphate buffer solution (pH 7. 6) each combined in an equal volume. When the reaction is made to take place using frozen sections, it is necessary to keep the proportion of the amount of tissue and the volume of reagent within a fixed limit, as the dilution of soluble factors involved in the reaction is apt to occur. For this reaction, the frozen section was pasted promptly onto slide glass as it is without drying and a fixed amount of reagent was dropped on it, or the whole frozen sections of a fixed amount of tissue were put into a fixed volume of reagent and had the reaction take place[12,14]. The reaction time was 30 minutes to one and half hours adjusted according to the strength of the enzyme activity in tissues. The specimen was fixed with a 10% formalin solution after the reaction and was embedded in glycerine. In the case of fresh tissue blocks, the reaction was made to take place similarly by soaking in a definite volume of reagent and fixed in formalin after reaction, and then frozen or carbowax embedded sections were made into pieces 2-4 μ thick.

2) *In the case of Nitro-BT (2, 2'-di-p-nitrophenyl-5, 5'-diphenyl-3, 3'-(3, 3'-dimethoxy-4, 4'-biphenylene) ditetrazolium chloride).*

As for the reagent the combination of equal volume of each of 0. 1%

Nitro-BT aqueous solution, 0. 2M sodium succinate solution, and 0. 1M phosphate buffer (pH 7.6) was used[10,14]. Nitro-BT readily permeates into tissue (about 2 mm) and its reaction is quite sensitive; and the reaction product dinitroformazan is histochemically excellent as it does not form needle-like crystals nor is it soluble in lipids nor in fat-solvent. Therefore, both in the case of frozen sections and fresh tissue sections 30-minute reaction time is practically sufficent, and it is possible to embed them in paraffin and counterstain with hematoxylin-eosin, and to embed in balsam.

3) *In the case of Nitro-NT (2, 2'-diphenyl-5, 5'(-m-nitrophenyl-)3, 3'-(-4, 4'-biphenylene)-ditetrazolium chloride).*

For the reagent the combination of equal volume of N, N-dimethyl formamide solution (10 mg./5 ml.), 0. 1M sodium succinate solution, and 0.1 M phosphate buffer (pH 7.6) was used[11,13]. Although Nitro-NT is somewhat difficult to dissolve in water, N, N-dimethyl formamide makes Nitro-NT water-soluble without affecting the enzyme reaction. Nitro-NT is like Nitro-BT sensitive to reaction. By lowering the reaction temperature down to 25°C, though the reaction becomes a little slower, reaction particles become finer and more distinct. As its permeability into tissue is weak, when the reaction is made to take place with fresh tissue blocks, it is demonstrated only on the surface layer. Diformazan is hardly soluble in alcohol, but parffin embedded sections can not be used as it is soluble in fat-solvents such as chloroform, acetone, and ether. Procedure for it is the same as for NT, but it dissolves a little in carbowax. It is also suitable for the reaction of liberated cells such as blood cells, and exudated cells or ascites cells. Moreover, in every case for the control succinate alone was excluded in order to investigate the endogenous dehydrogenase reaction, and on the other hand for the purpose of suppressing the endogenous reaction the tissue specimens were frozen.

RESULTS

Under an appropriate condition the reaction with any of these three salts shows distinct sphercial or rod-like particles in the cells of practically every tissue depending upon the enzyme activity. The nucleus is entirely negative to the reaction. The color of the reaction product is purple in the case with NT and Nitro-NT, while it is blue with Nitro-BT. On the whole the reaction particles appear more distinctly in the case where the reaction is made to take place first with fresh tissue block and then sectioned later, and these appear at the site more or less coinciding with mitochondria[14]. Therefore, for the cytochemical examination this method

is superior. The reaction with frozen sections is histochemically suitable for examining the strength and distribution of the activity in each tissue, but in this instance reaction particles tend to be rather irregularly large and scattered or crystallized. Especially in the case of frozen sections with NT such a tendency is great and the relationship of organellae in the cell becomes indistinct. The degree of reaction is in the descending order of Nitro-BT > Nitro-NT > NT; and the degree of permeability into the tissue is: Nitro-BT > NT > Nitro-NT; while the particles are finer and more minute in the case of Nitro-BT and Nitor-NT. Since diformazan of NT dissolves in lipid, sometimes lipids are stained at the same time. However, the lipid solubility of NT is weaker in the case of the reaction with fresh tissue block than in the case of the reaction with the frozen tissue sections[17]. Diformazan dissolved in lipids is readily distinguishable as it is light in color[11]. Both Nitro-BT and Nitro-NT do not dissolve in lipids and are not scattered. With any of these ditetrazolium salts in the case of fresh tissue there appears an endogenous reaction, though slight, especially with Nitro-BT, as the reaction is extremely sensitive, besides the particles coinciding with organellae, there is a tendency of diffuse light reaction appearing in the entire cytoplasm. On the other hand, in the frozen sections hardly any endogenous reaction can be recognized. Under the anaerobic condition the reaction of NT increases, but no change can be observed in the reaction of Nitro-BT and Nitro-NT.

Findings on Various Tissues

Cardiac muscle : Reaction particles appear in sarcoplasm between fibrils in line with longitudinal axis of the muscle fibers coinciding with the distribution of mitochondria as observed by the electron microscope. When NT and Nitro-NT are used, the particle picture is clear in the reaction of fresh tissue (Figs. 1, 5) but in frozen sections it is somewhat irregular, especially with NT such a unfavorable crystal formation is marked. In using Nitro-BT (Fig. 2), in the reaction of fresh tissue sections, besides the reaction particles as distinct as mentioned above, the reaction appears in the muscle fibrils, showing diffusely stained A band and leaving I band unstained thus clear-cut cross-striations are presented (Fig. 3). In frozen sections hardly any reaction can be observed in cardiac muscle fibrils themselves, obliterating cross-striations, while clear-cut particles appear coinciding with mitochondria (Fig. 4). Therefore, it is assumed that in the case of the reaction with fresh tissue block the reaction of cardiacmuscle fibrils themselves is caused by the endogenous reduction system (in all probability SH-group) other than the succinic dehydrogenase system.

Skeletal muscles and smooth muscles : The reaction in either one of these muscles is weaker than that of cardiac muscles, but it is fairly strong in skeletal muscles. Of them that of red muscle fibers is strong while it is weaker in white muscle fibers (Fig. 16).

Connective tissues : Connective tissue fibers react negatively but young connective tisssue cells are positive though weakly.

The liver: A strong reaction appears in the cytoplasm of liver cells of entire acini. The distribution of reaction particles is denser and also the reaction is stronger in the peripheral zone of acini than in the central zone (Figs. 7, 8). This is worthy of consideration in connection with the clarification of the histological structures and functions in the hepatic acini. Kupffer cells show an intermediate degree of activity, and the variation in the activity is demonstrated sharply in proportion to the acceleration or fall of the functions. The connective tissue fibers of Glisson's sheath are negative, and liver cell nuclei are, of course, negative. In the reaction of fresh tissue block with Nitro-BT, apart from the above-mentioned reaction particles, unspecific endogenous reaction appears diffusely in the cytoplasm of liver cells, lacking in the clarity of the particle picture. And with Nitro-BT, there is a strong tendency of the cell structures and boundary getting less distinct.

The pancreas : As a whole the activity in the pancreas is weak, but in the basal part of the gland cell the reaction appears in the form of long and short rods (Fig. 10). This agrees with the electronmicroscopic picture and the mitochondria-staining picture by acid fuchsin methyl green stain (Bensley). The activity of Langerhans island cells is extremely weak.

The salivary glands : The striated duct epithelium said to be involved in the excretion of calcium salts shows an extremely strong reaction, followed by the duct epithelium, and gland epithelial cells show a moderate reaction (Fig. 11).

The digestive canals : The stratified squamouse epithelium of the esophagus shows generally a weak reaction, especially the cells in the basal layer reveal the reaction to some extent while those in the upper layer are practically all negative. In the stomach the reaction of parietal cells is strongest. The epithelium of the intestinal mucous membrane shows a fairly strong reaction. In the columnar epithelial cells reaction particles are densely distributed at the basal part and in the surface part of the cytoplasm, but scanty in the central portion. Droplets of mucus of Goblet cells is entirely negative, and the nucleus is certainly negative (Fig. 12).

The kidney : Like the heart and liver the activity is strong in the kidney. In the uriniferous tubular epithelium, especially in the proximal covoluted tubules, reaction particles are densely distributed, and in Henle's duct the reaction is somewhat weaker. The glomerulus is nearly negative (Fig. 13), but with Nitro-BT, it reacts positively though weakly (Fig. 15).

Nerve tissue : Nerve cells show a markedly strong activity. Consequently the cortex generally shows a strong activity, while the white matter a weak one. When Nitro-BT is used, not only in the nerve cells but also in nerve fibers, expecially in the axon and dendrites reaction particles appear in conformity with their direction (Figs. 18, 22). And also glia cells show the reaction in the cytoplasm and in its numerous processes projecting like the branches of a tree (Fig. 19). Therefore, this histochemical reaction is an excellent method applicable to the study of the relationship between the structures and functions of neurons, glia cells and fibers on one hand their pathological changes on the other. In the cerebellum the molecular layer, Purkinje cells, and granular layer can be distinguished clearly (Fig. 20). The activity of Purkinje cells and cortical nerve fibers is strong, and in the granular layer there are two kinds of cells, namely, ones with a strong activity and the others with a weak activity. This distinction of the activity of cells in the granular layer was most marked in the cerebellum of the cat using NT (Fig. 20). In the case of Purkinje cells the activity is strongest in the part of cytoplasm surrounding the reaction negative nucleus situated in the center of the cell. Reaction particles in the nerve cell, differing from the distribution in Nissl bodies, appear coinciding with mitochondria distributed within the cell (Fig. 21). In the cortex particles are in a continuous line along with the direction of nerve fibers (Fig. 22).

The skin : The reaction is generally weak in the skin. The cells in the basal layer of the skin are positive; those in the upper layer weaker; and those in the stratum corneum are completely negative.

The mammary gland : The gland epithelium and duct epithelium are moderately positive, and the variation in the activity is pronounced according to the state of the function.

Blood cells and other free cells: For the reaction of free cells Nitro-NT is most suitable. According to the activity of the succinic dehydrogenase system of mitochondria in cytoplasm the reaction appears in the form of distinct granules. With exception of mature erythrocytes, blood cells are all positive. Rat ascitic tumor cells of the Yoshida sarcoma

show a fairly strong activity (Fig. 23). With Nitro-BT although there is a little tendency to destroy cellular structures, since the reaction is most sensitive, this method has an advantage of being able to induce reaction sufficiently even in the fresh smeared specimens without drying.

DISCUSSION

As the result of analyzing the conjugating step of the ditetrazolium reduction by tissue homogenate using various inhibitory agents with succinate as the substrate, in the case of Nitor-BT the reduction almost invariably takes place indirectly in step with that of succinic dehydrogenase; of Nitro-NT, about 50 per cent in step with succinic dehydrogenase and about other 50 per cent in step with Cyt. b or c_1; and of NT about 15 per cent in step with succinic dehydrogenase, about 35 per cent with Cyt. b or c_1 and about 50 per cent with Cyt. c oxidase[13,14,18]. In other words, it is construed that the Nitro-BT reaction is a reaction by succinic dehydrogenase; the Nitro-NT reaction is that by the succinic dehydrogenase system; and the NT reaction is by the succinoxidase system activity. The specificity of the reduction was discussed in the previous reports.[13,14,18].

Moreover, all these enzymes exist in mitochondria, and the reaction occurs at the sites coinciding with mitochondria according to the activity of enzymes[14]. However, the properties of reaction product differ according to the kind of ditetrzolium salts and conditions of tissues; for instance, when NT is made to react with frozen section, there is a strong tendency to form irregular, large crystals or to dissolve secondarly in lipids. This will rarely happen with fresh tissue block. As diformazan of NT and Nitro-NT is soluble in fat-solvent, the method with NT and Nitro-NT has an advantage in that at the time of histochemical observations the enzyme activity can be estimated by extracting the reaction product and by making colorimetric determination of the product[13,15]. Although Nitro-BT has many advantages histochemically, it has its disadvantages in that cellular structures are apt to be destroyed and the activity is difficult in determination. As for other advantages and disadvantages they were discussed in each section of results. Therefore, the kind of tetrazolium salt and method of procedure should be decided in such a manner as to fulfil best the purpose for which they are used.

An ideal tissue to be examined will be fresh one, but with this method even the autopsy material of human obtained a relatively short time after death can be sufficient for observations, and for the study of the mutual relationship between pathological changes and functions in tissues the

histochemical observation of this enzyme activity seems to have quite a significance.

SUMMARY

1. Histochemical and cytochemical studies with respect to the sites of reaction were made on the succinic dehydrogenase system activity of human and animal tissues using ditetrazolium salts, namely, neotetrazolium chloride, nitro-neotetrazolium chloride, and nitro-blue tetrazolium chloride.

2. The advantages and disadvantages of each ditetrazolium salt for histochemical and cytochemical purposes and the reaction taking place in frozen tissue sections and that in fresh tissue blocks were compared, and the method of procedure suitable for each condition was established with some modification.

3. Selecting conditions suitable for cytochemical purpose, it was shown that the reaction took place at the sites coinciding with mitochondria, and the distribution of the enzyme reaction was also examined. In addition, several new findings in the brains and other tissues cytochemically made clear were pointed out.

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LITERATURE

1. ANTOPOL. W., GLAUBACH, S., and GOLDMAN, L. : The use of neotetrazolium as a tool in the study of active cell processes. Trans. N. Y. Acad. Soi., Ser. **II**, **12**, 156—160, 1949—50.
2. RUTENBURG, A. M., GOFSTEIN, R. and SELIGMAN, A. M.: Preparation of a new tetrazolium salt which yields a blue pigment on reduction and its use in the demonstration of enzymes in normal and neoplastic tissues. Cancer Res. **10**, 113—121, 1950.
3. SELIGMAN, A. M. and RUTENBURG, A. M.: The histochemical demonstration of succinic dehydrogenase. Science **113**, 317—320, 1951.
4. RUTENBURG, A. M., WOLMAN, M. and SELIGMAN, A. M.: Comparative distribution of succinic dehydrogenase in six mammals and modification in histochemical technic. J. Histochem. and Cytochem. **1**, 66—81, 1953.
5. WACHSTEIN, M. : Histochemical demonstration of reducing activity in normal and leukemic blood and bone marrow cells. Proc. Soc. Exper. Biol. Med. **73**, 306—308, 1950.
6. ROSA, C. G. and VELARDO, J. T. : Histochemical demonstration of succinic dehydrogenase activity in tissue sections by a modified technique. J. Histchem. Cytochem. **2**, 110—114, 1954.
7. PEARSON, B. and DEFFENDI, V.: Histochemical demonstration of succinic dehydrogenase in thin tissue sections by means of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride under aerobic condition. J. Histochem. Cytochem. **2**, 248—257, 1954.

8. FARBER, E., et al.: Histochemical localization of specific oxidative enzymes. I—III. J. Histochem. Cytochem. 4, 254—294, IV —V. ibid. 347—362, 1956.
9. ITIKAWA, O.: Mechanism of dehydrogenating reaction in living cells (Japanese). Symposia Cell. Chem. 4, 113—146, 1956.
10. NACHLAS, M.M., TSOU, K.C., SOUZA, E.DE., CHENG, C.S. and SELIGMAN, A.M.: Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. J. Histochem. Cytochem. 5, 420—436, 1957.
11. PEARSON, B.: Use of nitro-neotetrazolium in enhancing the permanence and effecting finer histochemical localization of succinic dehydrogenase. Fed. Proc. 16, 368, 1957.
12. PEARSON, B.: Improvement in the histochemical localization of succinic dehydrogenase by the use of nitro-neotetrazolium chloride. J. Histochem. Cytochem. 6, 112—121, 1958.
13. ODA, T.: Cytochemical and biochemical studies on the terminal electron transport system. I. Analytical studies on the colorimetric estimations of the activities of the succinic dehydrogenase system and cytochrome oxidase (in Japanese with English abstract). Symposia Cell. Chem. 8, 157—172, 1958.
14. ODA, T.: Cytochemical and biochemical studies on the terminal electron transport system. II. Cytochemical demonstration of the succinic dehydrogenase system and cytochrome oxidase in mitochondria with the electron microscope (in Japanese with English abstract). Symposia Cell. Chem. 8, 173—190, 1958.
15. ODA, T., SEKI, S., SHIBATA, T., SAKAI, and OKAZAKI, H.: Analytical studies on the colorimetric estimation of the activity of the succinic dehydrogenase system with neotetrazolium chloride (in Japanese with English abstract). Okayama Igakkai Zasshi 70, 123—135, 1958.
16. ODA, T., YOSHIZAWA, K., SAKAI, A. and SENO, S.: Cytochemical observation on the succinic dehydrogenase activity of various blood cells including reticulocytes (Japanese abstract). Acta Haem. Jap. 19, 371—372, 1957.
17. ODA, T., OHTANI, K., AWAI, M. and SAKAI, A.: Histochemical studies on the nervous and humoral regulations of lipid and carbohydrate metabolisms. Acta Med. Okayama 11, 157—178, 1957.
18. ODA, T. and OKAZAKI, H.: An analytical study of the reduction of neotetrazolium chloride by the terminal electron transport system. Acta Med. Okayama, 12, 193—204, 1958.

EXPLANATIONS OF FIGURES

Fig. 1. Cardiac muscle (man): a fresh tissue block was incubated in the substrate solution containing NT., fixed with 10 per cent formalin solution, embedded in the carbowax, and cut into sections (2—4μ). Formazan is deposited in rows along the long axis of fibers. Nuclei and intercalated discs are negative. ($\times$500).

Fig. 2. Cardiac muscle (man): freezing sections (5—10μ) were incubated in the substrate solution containing NT. Some formazan shows crystal formation. ($\times$500).

Fig. 3. Cardiac muscle (man): reaction in fresh tissue block using Nitro-BT., paraffin sections (2—4μ). The fibers show a discontinuous manner to accentuate the cross striations which are inactive.

Fig. 4. Cardiac muscle (man): reaction in freezing sections (5—10μ) using Nitro-BT. Appearances of particles are clearer than in Fig. 3. ($\times$800).

Fig. 5. Cardiac muscle (man): reaction in fresh tissue block using Nitro-NT. ($\times$500).

Fig. 6. Liver (cat): stamped specimen of liver cells after the reaction was carried out in fresh tissue block using NT. (×1100).

Figs. 7. and 8. Liver (mouse): reactions in freezing sections (5—10 μ) using Nitro-BT. Succinic dehydrogenase activity in liver cells around the portal area (Fig. 7) is stronger than that around the central vein (Fig. 8). (×700).

Fig. 9. Liver (mouse): reaction in fresh tissue block using Nitro-NT. Particles are very distinct. (× 1500).

Fig. 10. Pancreas (cat): method is same as that in Fig. 6, using NT. Rod-like appearance of diformazan depositions at the basal part of the cell, which seems to correspond to mitochondria. At the another pole zymogen granules are accumulated, showing no activity (×2200).

Fig. 11. Salivary gland (submandibular gland of a rabbit): method is the same as that in Fig. 1, using NT. This picture shows slight activities of acinar cells and strong activities of striated convolution and excretory duct. (× 700).

Fig. 12. Small intestine (cat): method is the same as that in Fig. 1, using NT. The basal and surface parts of the columnar epithelial cells are strongly active. Mucinogenic substance in goblet cells is inactive. (× 700).

Fig. 13. Kidney (mouse): method is the same as that in Fig. 1, using NT. Glomerulus is almost negative. Proximal convoluted tubules show the strongest activities and the Henle's loop is less active than the former. (×600).

Fig. 14. Kidney (man): Method is the same as that in Fig. 1, using NT. Magnified picture of proximal convoluted tubules in Fig. 13, showing the details of the sites of succinoxidase system activity in the the cells. (×1300).

Fig. 15. Kidney (man): method is the same as that in Fig. 3, using Nitro-BT. This reaction is stronger than that of using NT. Glomerulus shows a slight activity. (×600).

Fig. 16. Skeletal muscle (mouse): reaction in freezing sections (10—15 μ), using NT. Red muscle fibers are very active, and white muscle fibers are less active.

Fig. 17. Cerebrum (man): method is same as Fig. 3, using Nitro-BT. Nerve cells and glia cells can be distinguished by the reactions in their cytoplasm and dendrites. (×600).

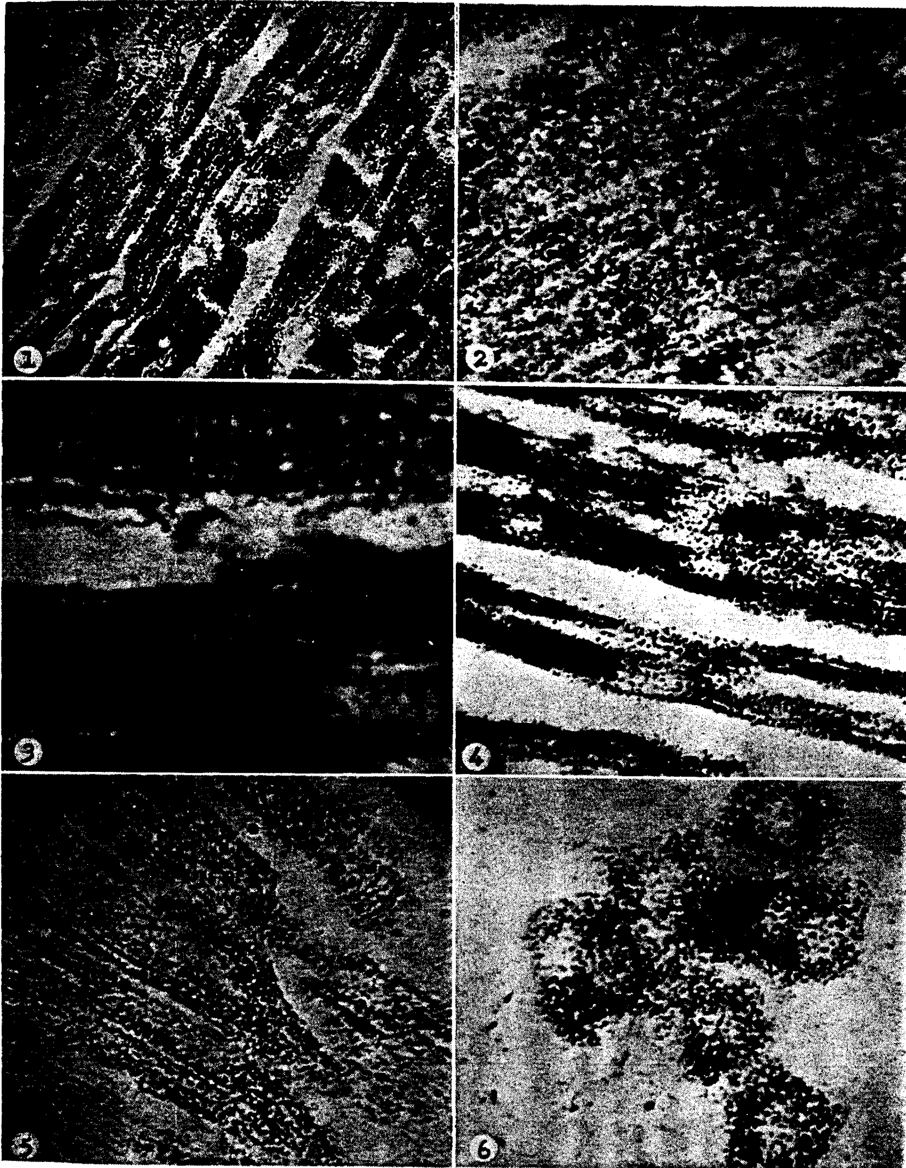
Figs. 18. and 19. Nerve cell and glia cell in the motor cortex of the cerebrum (man): These pictures are the magnifications of Fig. 17, showing many particles which are deposited in the cytoplasm, neurite and dendrites of the nerve cell. (Fig. 18.) (×200). Many fine processes of glia cell are also active. (Fig. 19) (×2400).

Fig. 20. Cerebellum (cat): method is the same as that in Fig. 1, using NT. Cortex, Purkinje cells and granular layer can be distinguished. Purkinje cells are very active. In the granular layer two kinds of cells, whose enzyme activies are different, are shown. (×600).

Fig. 21. A Purkinje cell of the cerebellum (man): method is the same as that in Fig. 3, using Nitro-BT. The nucleus is inactive. Intracytoplasmic particles follow into the neurite. Around the nucleus, activity is a little stronger than in the periphery. (×2200).

Fig. 22. Cortex of the cerebellum (man): This is the same specimen as Fig. 21. Nerve fibers can be seen as the continuation of dinitroformazan particles. (×2000).

Figs. 23. and 24. Blood corpuscles and tumor cells in ascites of a rat bearing the Yoshida-sarcoma: smeard specimens, being used Nitro-NT., demonstrate the formation of dinitroformazan particles coinciding with mitochondria in their cytoplasm. Five tumor cells show stronger activity than two leucocytes. (Fig. 23) (×2200). In Fig. 24, left one is a granulocyte and right one is a monocyte.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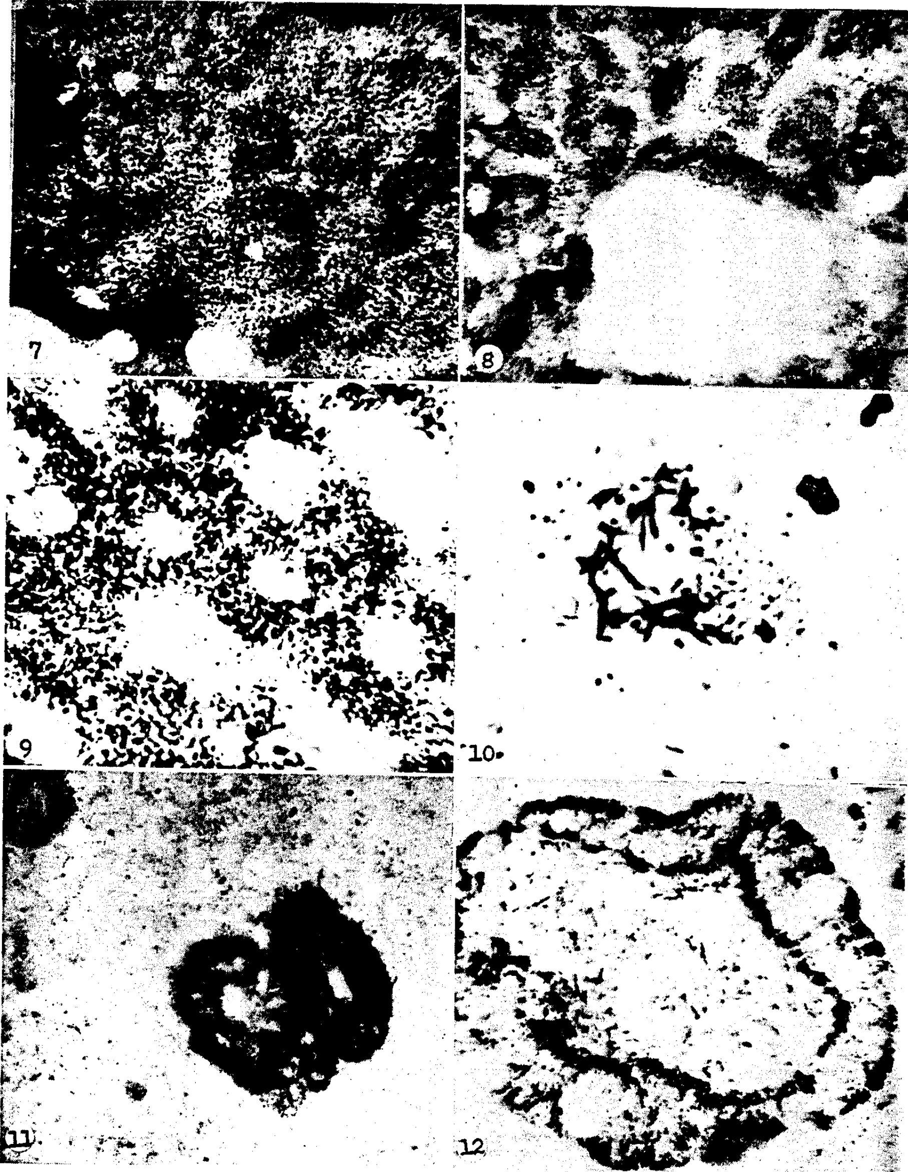
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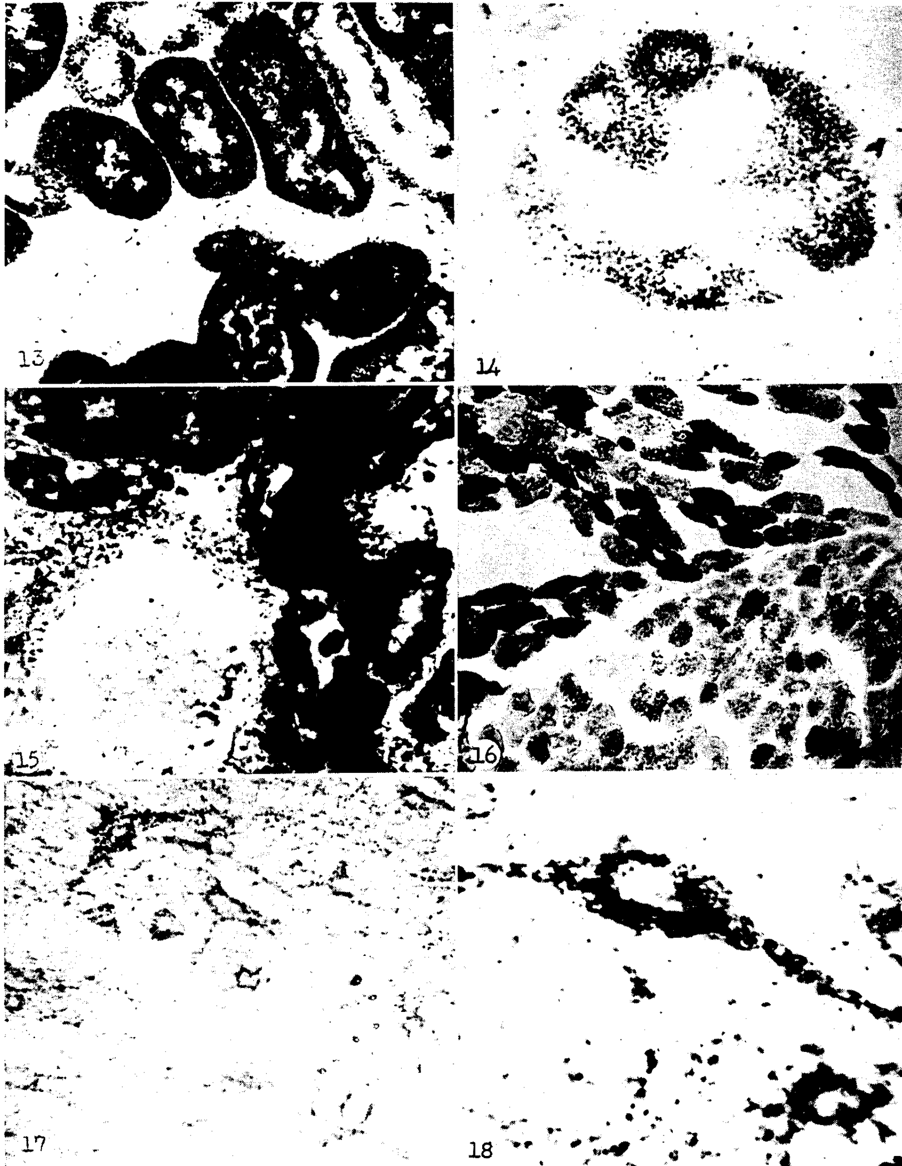
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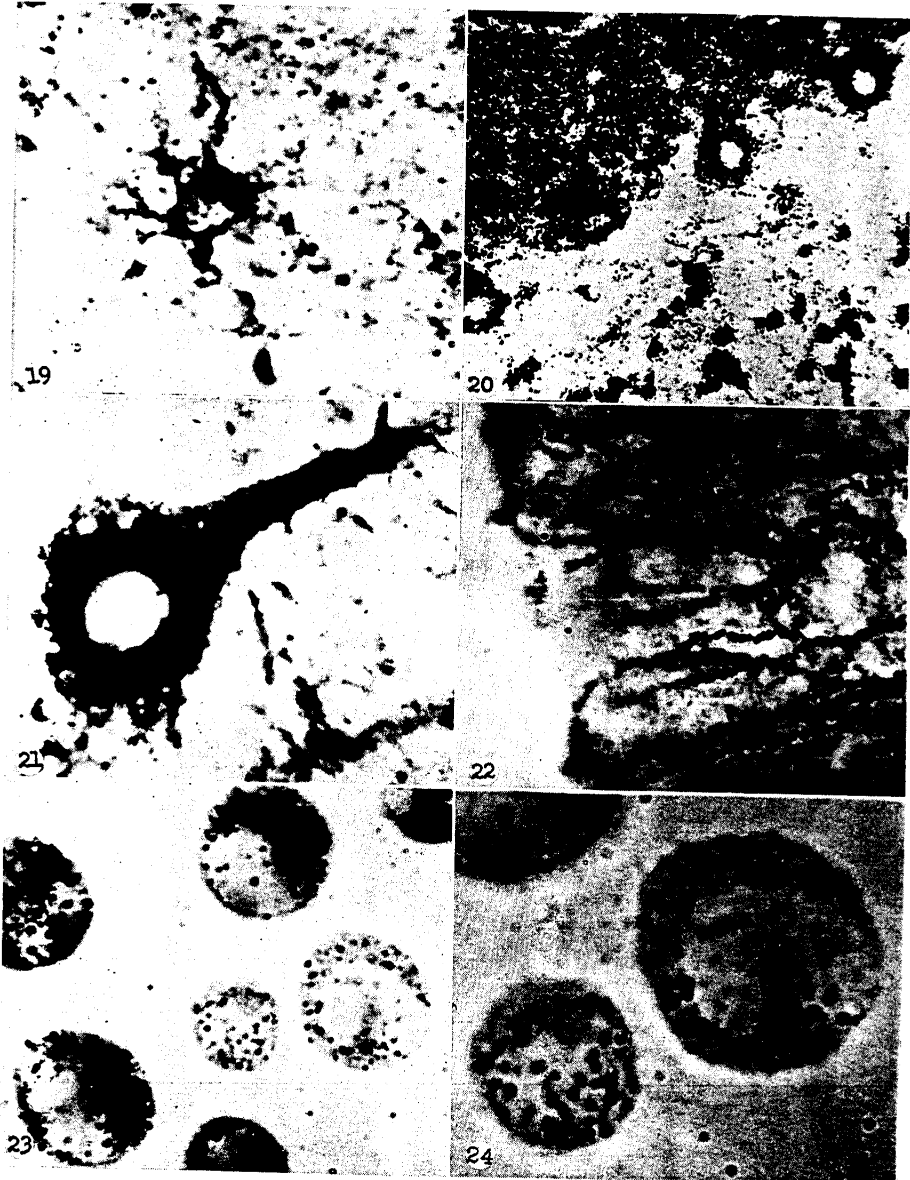
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