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Abstract

Carcinogenic effect of N, N'-dimethylnitrosourea (DMNU) on mice and hamsters was studied. Repeated subcutaneous injections of DMNU resulted in the induction of malignant lymphomas with an incidence of 100 per cent in adult C3HjBifBjKi mice and induced malignant tumors of forestomach, mammary gland and uterus with a high incidence in adult hamsters. Control animals showed no pathological changes. Electron microscopy revealed the presence of murine type C virus particles in some of the tiues examined. Many type C virus particles were found in a transplant of DMNU.induced malig. nant lymphoma. Some type C virus particles were shown in malignant lymphomas and lymph nodes of malignant lymphoma-bearing mice. A very small number of type C virus particles were observed in thymus of control mice and bone marrow of a malignant lymphoma.bearing mouse. A few particles, quite similar to murine type C virus particles, were detected in DMNU.induced mammary adenocarcinoma of hamster. No virus-like particles were seen in mammary glands of control hamsters. Whether these particles are merely paengers or are playing a significant role in the carcinogenesis of these tumors remains to be determined.

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N, N'-DIMETHYLNITROSOUREA-INDUCED TUMORS IN MICE AND SYRIAN HAMSTERS

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Abstract: Carcinogenic effect of N, N'-dimethylnitrosourea (DMNU) on mice and hamsters was studied. Repeated subcutaneous injections of DMNU resulted in the induction of malignant lymphomas with an incidence of 100 per cent in adult C3H/BifB/Ki mice and induced malignant tumors of forestomach, mammary gland and uterus with a high incidence in adult hamsters. Control animals showed no pathological changes. Electron microscopy revealed the presence of murine type C virus particles in some of the tissues examined. Many type C virus particles were found in a transplant of DMNU-induced malignant lymphoma. Some type C virus particles were shown in malignant lymphomas and lymph nodes of malignant lymphoma-bearing mice. A very small number of type C virus particles were observed in thymus of control mice and bone marrow of a malignant lymphoma-bearing mouse. A few particles, quite similar to murine type C virus particles, were detected in DMNU-induced mammary adenocarcinoma of hamster. No virus-like particles were seen in mammary glands of control hamsters. Whether these particles are merely passengers or are playing a significant role in the carcinogenesis of these tumors remains to be determined.

Since the discovery of the carcinogenic activity of dimethylnitrosamine by MAGEE and BARNES (1), numerous experimental studies have been reported on this and other nitrosamines. The extensive studies of DRUCKREY and his collaborators (2) on the relationship between chemical structure and carcinogenic activity of nitroso compounds have shown that a large number of organic nitroso compounds have powerful carcinogenic, mutagenic and teratogenic properties in a wide range of organs in rats. In 1967, SANDERS (3) has reported that secondary amines and amides can react with nitrite in human stomach juice to form carcinogenic nitrosamines and nitrosamides *in vitro*. Recently SANDERS (4) has successfully demonstrated the production of tumors of several organs including brain in rats by feeding inactive precursors of dimethylnitrosourea; namely, dimethylurea and sodium nitrite. It is well known that nitrites are food additives and also found in green vegetables and secondary amines are detected in certain samples of tea, tobacco, fish meat and grains. The presence of very small quantities of nitrosamine in a number

of commodities, *e. g.*, several types of cooked and uncooked meat, maize and beans has been reported (5). Thus the occurrence of nitroso compounds in the human environments has caused a great concern in recent years. Therefore, it is important to study the pathological effects of N, N'-dimethylnitrosourea (DMNU), one of nitroso compounds, in animals of various species. In 1967, the carcinogenic activity of DMNU has been demonstrated for the first time in rats by DRUCKREY *et al.* (2). HIRAKI (6, 7) has briefly reported the induction of tumors in adult mice and Syrian hamsters by DMNU. It is the purpose of this paper to describe the detailed microscopical and ultrastructural findings of tumors and other pathological lesions in mice and hamsters after weekly injections of DMNU.

MATERIALS AND METHODS

Experimental animals

Male and female C3H/BifB/Ki mice 8 to 10 weeks old, originally obtained from Baylor University, Houston, Texas, U. S. A. and bred in our laboratory, were used. Syrian hamsters 8 to 10 weeks old, of either sex, originally obtained commercially and bred in our laboratory, were also used. All the animals were separated by sex and housed in plastic cages. All of them were weighed and inspected every week until death.

N, N'-Dimethylnitrosourea

DMNU (synthesized by Nihon Kankohshikiso Laboratory, Okayama) dissolved in physiological saline solution was freshly prepared every week for subcutaneous injection.

Experimental procedures

All the animals were lightly anesthetized with ether before injection. The solution of DMNU was subcutaneously injected once a week at a dose of 80 mg/kg body weight into the interscapular region of 30 mice (17 males and 13 females). Thirty-four hamsters (20 males and 14 females) received weekly a dose of 40 mg DMNU/kg body weight by the same method as mice. The control 10 mice and 10 hamsters were injected subcutaneously with 0. 2 ml of physiological saline solution.

All the treated animals were allowed to die spontaneously or killed when in poor condition. Complete autopsy and histological study was performed on each of all the animals. Tissues were fixed in 10 per cent neutral formaldehyde and paraffin sections were routinely stained with hematoxylin and eosin. Periodic acid-Schiff, silver and Azan-Mallory stains were used when necessary. May-Giemsa stain was done on imprints of thymic malignant lymphomas of the treated mice. White blood cell counts were taken on 8 mice of the treated group at every 2 weeks and 5 mice of the control group at every 4 weeks.

For transplantation, several tumors were cut and made into about 20 per cent cell suspensions in physiological saline solution. One ml of these suspensions was inoculated subcutaneously or intraperitoneally into young adult mice

or hamsters, 3 weeks old, of the same strain.

For electron microscopy, the tissues were fixed in 2.5 per cent glutaraldehyde for 1.5 hr, postfixed in one per cent osmium tetroxide in Millonig's buffer for one hr, dehydrated in a series of graded ethanol solutions and embedded in epoxy resin. Thick sections were cut on a Porter-Blum MT-2 Sorvall ultramicrotome and following examination of 2 per cent toluidin blue-stained thick sections under the light microscope, adjacent thin sections were cut, double-stained with uranyl acetate and lead citrate, and examined in a Hitachi HU-11P or HS-8 electron microscope at magnifications varying from 2,000 to 15,000.

RESULTS

Effect of N, N'-dimethylnitrosourea in mice

1. *Induction of malignant lymphomas*: As summarized in Table 1, the repeated subcutaneous injections of DMNU showed a remarkable carcinogenic

TABLE 1 INCIDENCE OF MALIGNT LYMPHOMAS IN ADULT MICE GIVEN SUBCUTANEOUSLY ADMINISTRATION OF N, N'-DIMETHYLNITROSOUREA (DMNU)

Treatment	Number of mice	Sex	Single dose (mg/kg)	Average total dose (ma/kg)	Mice with malignant lymphoma (%)	Average latent period (days)
None	6	♂	—	—	0	—
None	4	♀	—	—	0	—
Total	10		—	—	0	—
DMNU	17	♂	80	1073	100	92
DMNU	13	♀	80	1015	100	86
Total	30		80	1047	100	89

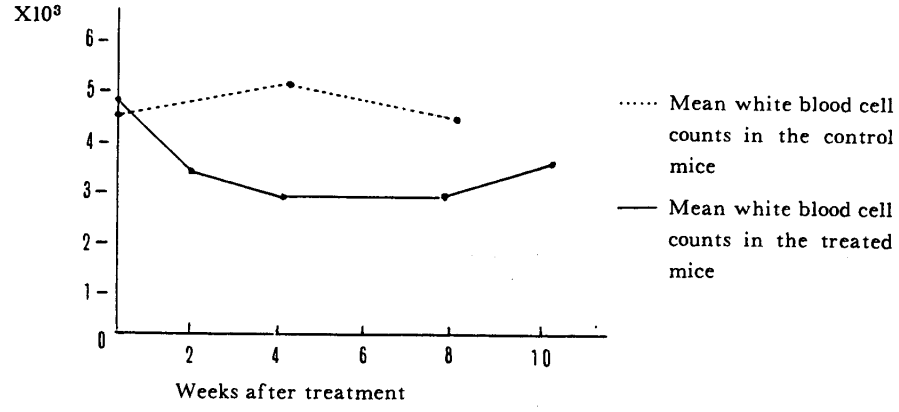
activity in C3H/BifB/Ki mice in which the development of spontaneous tumors is known to be very rare. All the DMNU-treated mice were found dead or killed in poor condition between 9 and 17 experimental weeks and had a malignant lymphoma originating in the thymus. The median latent period was 12 weeks. The total dose of DMNU was given per mouse varied from 720 to 1440 mg/kg with an average of 1047 mg/kg. No remarkable sex differences were noted with respect to the tumor incidence, latent period and total dose. No gross or histological evidence of other tumors was observed in any of the treated mice. All the control mice showed no pathological changes.

2. *Gross and histopathological appearance of malignant lymphomas*: An anterior mediastinal tumor mass consistent with an enlarged thymus was the most common gross finding in the majority of DMNU-treated mice (Fig. 1).

In most instances, this tumor infiltrated continuously into the bronchus, lungs and intercostal muscles. Extension beyound the chest cavity with involvement of the spleen, liver, kidney, bone marrow and lymph nodes occurred in 14 of 30 mice. In 7 instances, this was associated with hepatomegaly, splenomegaly, and enlargement of cervical, periaortic, mesenterial and thoratic lymph nodes. Meningeal involvement was found in four mice among them.

Histologically, all the anterior mediastinal tumors were composed of massive proliferation of lymphocytic or lymphoblastic cells associated with a scattering of so-called starry-sky cells; namely, non-neoplastic histiocytes containing nuclear and cellular debris (Figs. 2, 3). In the lungs the massive perivascular and peribronchial infiltration of neoplastic cells was most frequent. Metastatic foci of malignant lymphoma were usually pronounced in the perivascular region of the liver and kidney (Fig. 4). Other organs showed varying degrees of neoplastic infiltration when the disease was disseminated.

3. *Changes in peripheral blood*: As shown in Text-Fig. 1, white blood cells remarkably decreased following DMNU treatment, but slightly increased



Text-Fig. 1. Effect of N, N'-dimethylnitrosourea on white blood cell counts

to subnormal levels at the later period of life-span of the treated mice. No neoplastic cells appeared in the peripheral blood smears of any treated mice.

4. *Transplantation of malignant lymphoma*: All the ten young adult mice of the same strain transplanted with cell suspensions of the malignant lymphoma subcutaneously or intraperitoneally developed disseminated lymphomas that metastasized into the thymus, lymph nodes, liver, kidney, spleen and so on. The recipients died in four to five weeks after cell-graft.

5. *Fine strucuture of malignant lymphomas*: Electron microscopically, malignant lymphomas consisted of almost uniform lymphoblastic cells, 13 to 23 μ in diameter, with scanty fibrous stroma (Fig. 5). Their nucleus was

relatively large, 11 to 20 μ in diameter, and oval or horseshoe-shaped in profile. The chromatin was relatively evenly distributed in the nucleoplasm and showed some condensation on the periphery of the nucleus. Nucleoli were often seen. The narrow rim of the cytoplasm containing a few membranous organelles contained abundant ribosomes occurring singly or in clusters called polyribosomes. Rather big round or oval mitochondria, a few vacuoles and centrioles were present. The Golgi apparatus was seen but poorly developed.

The search for virus particles was attempted on the malignant lymphomas, spleen, liver, lymph nodes and bone marrow of two malignant lymphoma-bearing mice and the thymus, spleen and liver of two control mice. The transplant of the DMNU-induced malignant lymphoma was also examined. Extracellular particles with a diameter of about 110 nm were found in several samples. They were quite similar to type C virus particles of the known oncogenic murine leukemia viruses and seen in the process of the budding from the cell membrane (Fig. 6). The appearance of type C virus particles were summarized in Table 2. The transplanted tumor showed many type C virus

TABLE 2 APPEARANCE OF TYPE C VIRUS PARTICLES IN N, N'-DIMETHYLNITROSOUREA (DMNU)-TREATED AND CONTROL MICE AND TRANSPLANT OF DMNU-INDUCED MALIGNANT LYMPHOMA

Organs / Treatment	Type C virus particle						
	Lymphoma*	Thymus	Spleen	Lymph node	Liver	Bone Marrow	
DMNU	+		−	+	−	−	
DMNU	+		−	+	−	−	
None		+	−		−		
None		+	−		−		
Transplantation	+						

* originated in the thymus

particles (Fig. 7). Some type C virus particles were found in the malignant lymphomas (Fig. 6) and non-infiltrated lymph nodes of two malignant lymphoma-bearing mice. Only a very small number of type C virus particles were observed in the thymus of two control mice and the non-infiltrated bone marrow of the malignant lymphoma-bearing mouse. None of the other organs examined showed any virus particles.

Effect of N, N'-dimethylnitrosourea in hamster

1. *Survival time and total dosl*: All the DMNU-treated hamsters were found dead or killed between 17 and 31 experimental weeks and the average life-span was 24 weeks. The total dose of DMNU, the hamsters received, ranged between 680 and 1280 mg/kg body weight with an average of 957

mg/kg. The hair of all the treated hamsters began to fall out following ten injections of DMNU and the loss of hair increased progressively (Fig. 8). The control hamsters receiving physiological saline solution revealed no gross changes in the skin, subcutaneous tissue or other organs.

2. *Induction of malignant tumors*: All except three of the hamsters given weekly injections of DMNU developed malignant tumors, which occurred principally in forestomach, mammary gland and uterus. Seventeen hamsters had malignant tumors at more than one of these sites. Table 3 summarizes

TABLE 3 CARCINOGENIC EFFECT OF N, N'-DIMETHYLNITROSOUREA (DMNU) ADMINISTERED SUBCUTANEOUSLY TO SYRIAN HAMSTERS

Treatment	Hamsters		With fore-stomach cancer		With mammary cancer		With uterine cancer		Other malignant tumors
	Sex	No.	No.	(%)	No.	(%)	No.	(%)	
None	♂	5	0		0		0		None
None	♀	5	0		0		0		
DMNU	♂	20	13	65	0		0		8 cholangiocarcinomas, 6 pancreas cancers, 2 subcutaneous sarcomas
DMNU	♀	14	10	71	11	79	12	86	

the incidence and the site of malignant tumors observed in the DMNU-treated hamsters. The incidence of spontaneous tumors, especially in forestomach, and of uterine and mammary tumors in hamsters, is known to be extremely low (8, 9, 10). Mammary and uterine tumors were induced only in the treated females. However, there was no apparent difference in the incidence of other tumors due to sex. All the control hamsters showed no histological evidence of tumors or pathological lesions.

Forestomach tumors: In all the DMNU-treated hamsters, the mucous membrane of forestomach was thickened on the surface of which were scattered multiple nodules. These nodules were relatively small, up to 0.3 cm, firm and white. Microscopically the forestomach of all the treated hamsters showed more or less hyperplasia and hyperkeratosis of the epithelium. Small multiple nodules consisted of epithelium and keratin were squamous cell papillomas. In 23 instances (68 per cent), in addition to penetration of small tongues of epithelial tissue into the lammia propria, epithelial atypism characterized by variation in nuclear size and shape, hyperchromatism and increase of the nuclear-cytoplasmic ratio were observed (Fig. 9). Occasionally there was cellular invasion into the submucosa. These 23 instances were diagnosed as a well-differentiated squamous cell carcinoma, but showed no metastasis or infiltration into other organs.

Mammary tumors: After about 18 injections of DMNU, 11 (79 per cent) of all the treated females developed subcutaneous firm tumors derived from the mammary glands (Fig. 8). These tumors were usually small, measuring from 0.3 to 1.5 cm in their largest diameter. Often, as many as three or four mammary glands were involved and sometimes multiple tumor foci existed in the same gland. Some were ulcerated at the surface associated with necrosis, bleeding and inflammation. The histological pattern of the tumors indicated them to be either adenocarcinoma or adenoacanthoma (Fig. 10). No metastatic foci were found in lymph nodes or other organs. All the transplants of mammary tumors grew slowly in the subcutaneous tissue of male and female recipients of the same strain. Histologically the transplants were of completely anaplastic carcinoma.

Uterine tumors: Uterine tumors were recognized after about 19 injections of DMNU. Twelve (86 per cent) of all the DMNU-treated females had tumors of the uterine cervix. They were white, firm, round and relatively large, measuring from 1.0 to 2.0 cm in diameter. Microscopically the tumors were adenoacanthomas associated with remarkable necrosis and inflammation (Fig. 11). They invaded into the surrounding tissues, and in three instances metastasized to the inguinal lymph nodes.

Miscellaneous malignant tumors: Four males and two females (18 per cent) developed slightly firm, yellow-white and pea-sized tumors of the pancreas (Fig. 12). Microscopically they showed adenocarcinoma with periodic acid-Schiff positive material (Fig. 13). Besides cancer of forestomach, mammary gland, uterus and pancreas, cholangiocarcinoma of the liver was found in eight hamsters (24 per cent) (Fig. 14) and anaplastic sarcoma situated at the dorsal subcutis in two hamsters (6 per cent). No distant metastases were observed in the hamsters with these tumors.

3. *Induction of benign tumors and other lesions*: Twenty-five hamsters (74 per cent) had splenic nodules, often multiple, up to four in one animal. These nodules were soft, blackish red and fairly large, measuring from 0.3 to 1.5 cm in diameter (Fig. 12). They were cavernous hemangiomas with many thrombi in various stages of organization (Fig. 14). Six hamsters (18 per cent) had liver cirrhosis with proliferation of cholangioles.

4. *Fine structure of the DMNU-induced mammary tumors*: Ultrastructural observation was performed on the DMNU-induced adenocarcinoma and adenoacanthoma of the hamster mammary gland. In part of adenocarcinoma, neoplastic cells displayed a moderately electron-dense cytoplasm with fairly well developed organelles and irregular nuclei. Narrow and irregular lumens of ductules were frequently observed (Fig. 16). Neoplastic cells bordering the lumens of ductule showed numerous microvilli and often contained secre-

tory granules (Fig. 17). The cytoplasm formed conspicuous microvilli-like projections which interdigitated with those of neighboring cells. Other type of cell-to-cell attachments was the usual junctional complex (Fig. 18). In the cytoplasm, free ribosomes were relatively abundant and the Golgi apparatus was frequently well developed (Fig. 16). Some neoplastic cells displayed a cytoplasm rich in mitochondria and rough endoplasmic reticulum. Somewhat large nuclei were frequently indented and fairly large nucleoli were occasionally seen. Definite basement membranes appeared to be obscure.

A few virus-like particles with a diameter of about 125 nm were found in the extracellular spaces (Fig. 19). The particles originated from the cell membrane by a process of budding (Fig. 19). They were quite similar to type C virus particles of the known oncogenic murine leukemia viruses. No virus-like particles were detected in the mammary glands of control hamsters.

In the part of squamous cell carcinoma, irregularly shaped large neoplasmic cells had rather prominent nuclei. Nucleoli were usually single and large. Intranuclear filaments were found in some of them (Fig. 20). The most prominent features in the cypoplasm were large numbers of tonofilaments, which were organized in bundles or tonofibrils (Fig. 21). Several small cisternae of rough-surfaced endoplasmic reticulum as well as oval or round shaped mitochondria were seen in the cytoplasm. Free ribosomes were moderately abundant and the Golgi apparatus was poorly developed. Some of neoplastic cells contained lipid droplets or glycogen particles. The surfaces of the neoplastic cells were highly irregular with many ridges and cytoplasmic projections. These processes contacted with those of adjacent cells, and desmosomes were common in these regions (Figs. 20, 21).

DISCUSSION

The present study demonstrated that repeated subcutaneous injections of DMNU induced malignant lymphomas in 100 per cent of adult C3H/BifB/Ki mice and a high incidence of malignant tumors in the forestomach, mammary gland and uterus of adult Syrian hamsters. DRUCKREY *et al.* (2) reported the selective induction of neurogenic tumors such as glioma and neurinoma in rats by repeated intravenous or continuous oral administrations of the same nitrosourea. These results indicate that DMNU has a strong carcinogenic effect in many species of experimental animals, which differs remarkably between animal species. The carcinogenicity of a similar alkylnitrosourea, N-methyl-N-nitrosourea (MNU) also displays a marked species difference. DRUCKREY *et al.* (2, 11) produced tumors of the nervous system in 90 per cent of rats following repeated intravenous administrations of MNU. Brain tumors have also been produced in rabbits (12, 13) and dogs (14) by MNU. However, several

investigators (15, 16, 17, 18) reported the induction of malignant lymphomas by MNU in mice. HERROLD (19, 20) and GRAFFI *et al.* (21) demonstrated the development of malignant tumors in hamsters at the site of application, like the skin or bronchus, by either subcutaneous or intratracheal route. Thus species difference may have been due to several unrecognized factors.

Instability of DMNU in aqueous solutions and the induction of various tumors in experimental animals at the site remote from injection site, in contrast to the failure to induce tumors at the local, suggest that DMNU is rapidly absorbed from subcutaneous tissue into systemic blood circulation without loss of its carcinogenic activity, and its subsequent derivative, rather than DMNU itself, may induce the malignant tumors. Although little is known about the mode of action of alkylnitrosourea, it is considered in general that these substances methylate cellular macromolecles, in particular the guanine base of the DNA molecule (22, 23), as described by MAGEE and BARNES (24) for dimethylnitrosamine.

In the present study, electron microscopy revealed the presence of type C particles in DMNU-induced malignant lymphomas in mice. An important question that arises from this finding is whether these type C viruses have had any role in the cause of the malignant lymphoma, or whether they should be considered rather as accidental increase of a passenger virus. The leukemogenic potency of cell-free extracts and the presence of type C virus particles in the lymph nodes of 20-methylcholanthrene-induced leukemic mice have been reported by IRINO *et al.* (25). They concluded that the chemical carcinogen induces leukemia by activating a latent virus naturally resident in the mice. Subsequently the implication of leukemogenic virus, morphologically type C virus, in chemical leukemogenesis in mice has been demonstrated by several investigators (26, 27, 28, 29). Recently, IMAMURA (30) reported that N-nitrosobutylurea-, one of alkylnitrosourea, induced murine leukemia has been transmitted by the cell-free leukemic extracts to newborn mice or rats. The leukemogenicity of cell-free extracts prepared from DMNU-induced malignant lymphomas has been tested and leukemia developed one of 20 inoculated newborn mice with a latent period of 120 days (31). Because of its low incidence, leukemogenic potency of these cell-free extracts could not be determined. In the present study, after treatment with DMNU, white blood cell counts of the treated mice remarkably decreased. MOTOI *et al* (32) reported that a strong immunosuppression occurred in mice following DMNU-treatment. The immunosuppresive effect of DMNU in mice may be related to the increase of type C virus and the tumorigenesis of DMNU-induced malignant lymphoma.

Moreover, an interesting point to be mentioned here is that repeated

subcutaneous administrations of DMNU induced mammary carcinomas in 79 per cent of the treated female hamsters. Because of the extreme rarity of spontaneous and induced mammary tumors in hamsters, it seems to be relevant. Among the studies of chemical and viral carcinogenesis in hamster, only one (33) reported a high incidence of the mammary tumors following 3-methylcholanthrene feeding. In the present study, repeated administrations of DMNU to hamsters also resulted in the occurrence of adenoacanthomas of the uterine cervix. Although the mechanisms of action of DMNU in inducing mammary or uterine tumors in hamsters are obscure, it is thought that a hormonal mechanism may be involved. Furthermore, it is of interest that virus-like particles resembling known oncogenic murine leukemia and sarcoma viruses have been detected in the DMNU-induced hamster mammary carcinoma. To the best of our knowledge, no reference has been found reporting the presence of virus particles in spontaneous or experimentally induced hamster mammary tumors. It is now quite convincing that mouse mammary tumors are of viral etiology (34, 35). In recent years, particles resembling oncogenic murine RNA viruses have been reported in the human mammary cancers (36, 37, 38, 39, 40) as well as in a mammary tumor of rhesus monkey (41) and of collared lemming (42) and in the mammary tumors of rats (43, 44, 45) and cats (46). However, in contrast to the findings in mice, no unequivocal data have been presented on the biological activity or the active participation in tumorigenic process of these particles observed in the mammary tumors described above. The part played by virus-like particles found in the DMNU-induced hamster mammary tumor will have to be further investigated with respect to a possible etiologic significance.

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Explanation of Figures

Fig. 1-7. Gross, histological and electron microscopical findings of DMNU-induced malignant lymphomas in mice.

Fig. 1. A general view of DMNU-treated mouse. Note the anterior mediastinal tumor mass consisted of an enlarged thymus.

Fig. 2. Histology of the malignant lymphoma. H-E. ×100.

Fig. 3. Higher magnification of the malignant lymphoma. Note so-called starry-sky cells. H-E. ×400.

Fig. 4. Metastatic foci of the liver. Note the periportal infiltration of malignant lymphoma cells. H-E. ×100.

Fig. 5. Low magnification electron micrograph of a group of malignant lymphoma cells. ×5,000.

Fig. 6. A virus-like particle budding from the cell membrane (arrow) and two virus-like particles in the extracellular space of malignant lymphoma. ×20,000.

Fig. 7. Many virus-like particles in the extracellular space of transplant of malignant lymphoma. ×20,000.

Fig. 8-15. Gross and histological findings of DMNU-induced tumors in hamsters.

Fig. 8. A general view of DMNU-treated hamster. Note loss of hair and mammary tumors (arrows).

Fig. 9. Well-differentiated squamous cell carcinoma of the forestomach. H-E. ×100.

Fig. 10. Adenocarcinoma of the mammary gland. H-E. ×100.

Fig. 11. Adenoacanthoma of the uterine cervix. H-E. ×100.

Fig. 12. Gross finding of the miscellaneous tumors. Note the liver tumor (indicated by simbol a), pancreas tumor (b) and nodules of the spleen (c).

Fig. 13. Adenocarcinoma of the pancreas. H-E. ×250.

Fig. 14. Cholangiocarcinoma of the liver. H-E. ×100.

Fig. 15. Cavernous hemangioma of the spleen. H-E. ×100.

Fig. 16-21. Electron micrographs of DMNU-indced mammary carcinomas in hamsters.

Fig. 16. An electron micrograph of a group of adenocarcinoma cells. Note the narrow lumen of ductule and complex cell interdigitations. ×6,800.

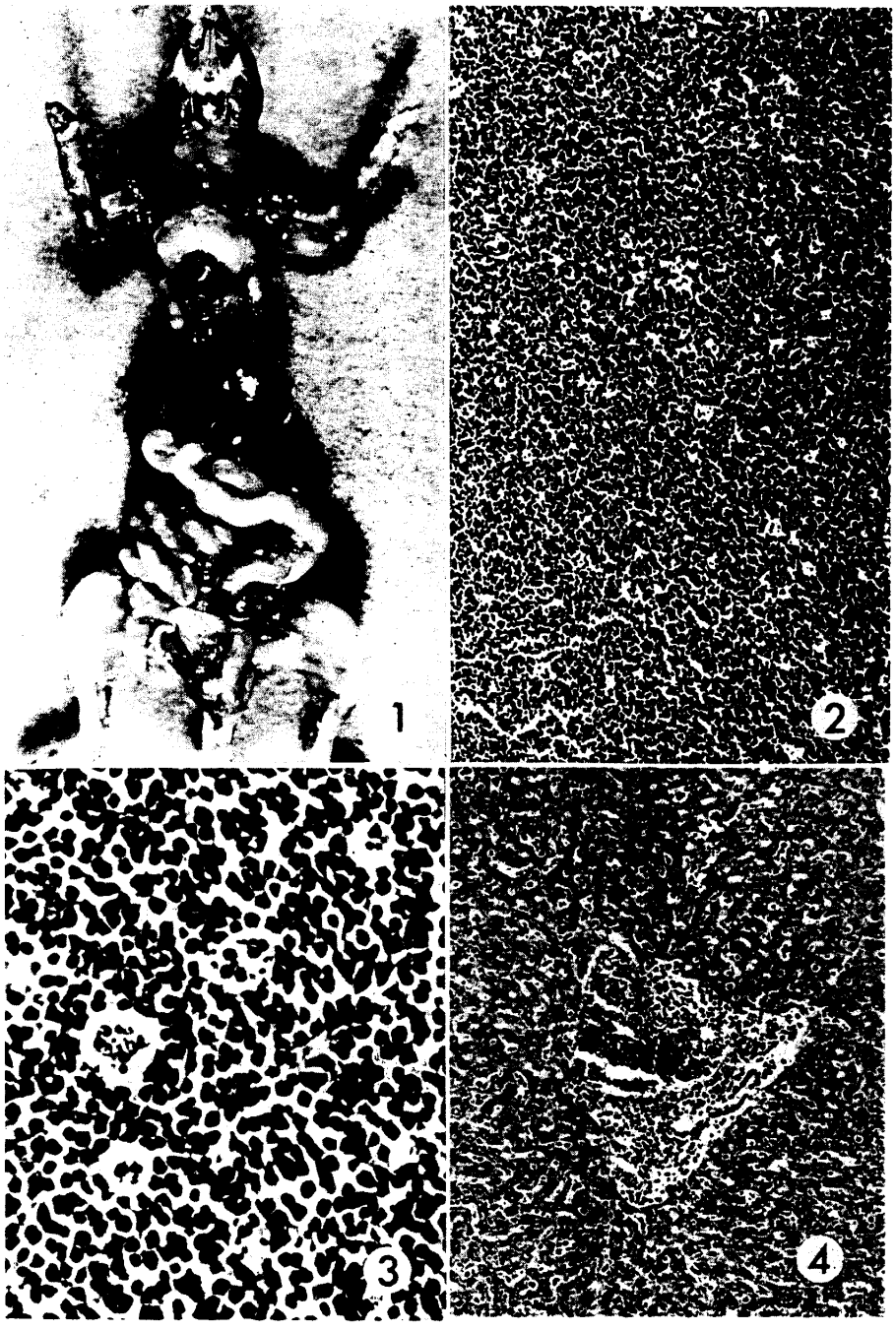
Fig. 17. Adenocarcinoma cells bordering the lumen of ductule. Note the numerous microvilli and secretory granules. ×10,500.

Fig. 18. Low magnification electron micrograph of a group of adenoacanthoma cells. Note the ductule, junctional complex and tonofilaments (arrow). ×3,650.

Fig. 19. Virus-like particles in the extracellular space of adenocarcinoma. Note a particle (arrow) budding from the cell membrane. ×20,000.

Fig. 20. Neoplastic cell in the part of squamous cell carcinoma. Note the intranuclear filaments (arrow), tonofilaments and desmosomes. ×5,100.

Fig. 21. Large numbers of tonofilaments and desmosomes in the part of squamous cell carcinoma. ×5,100.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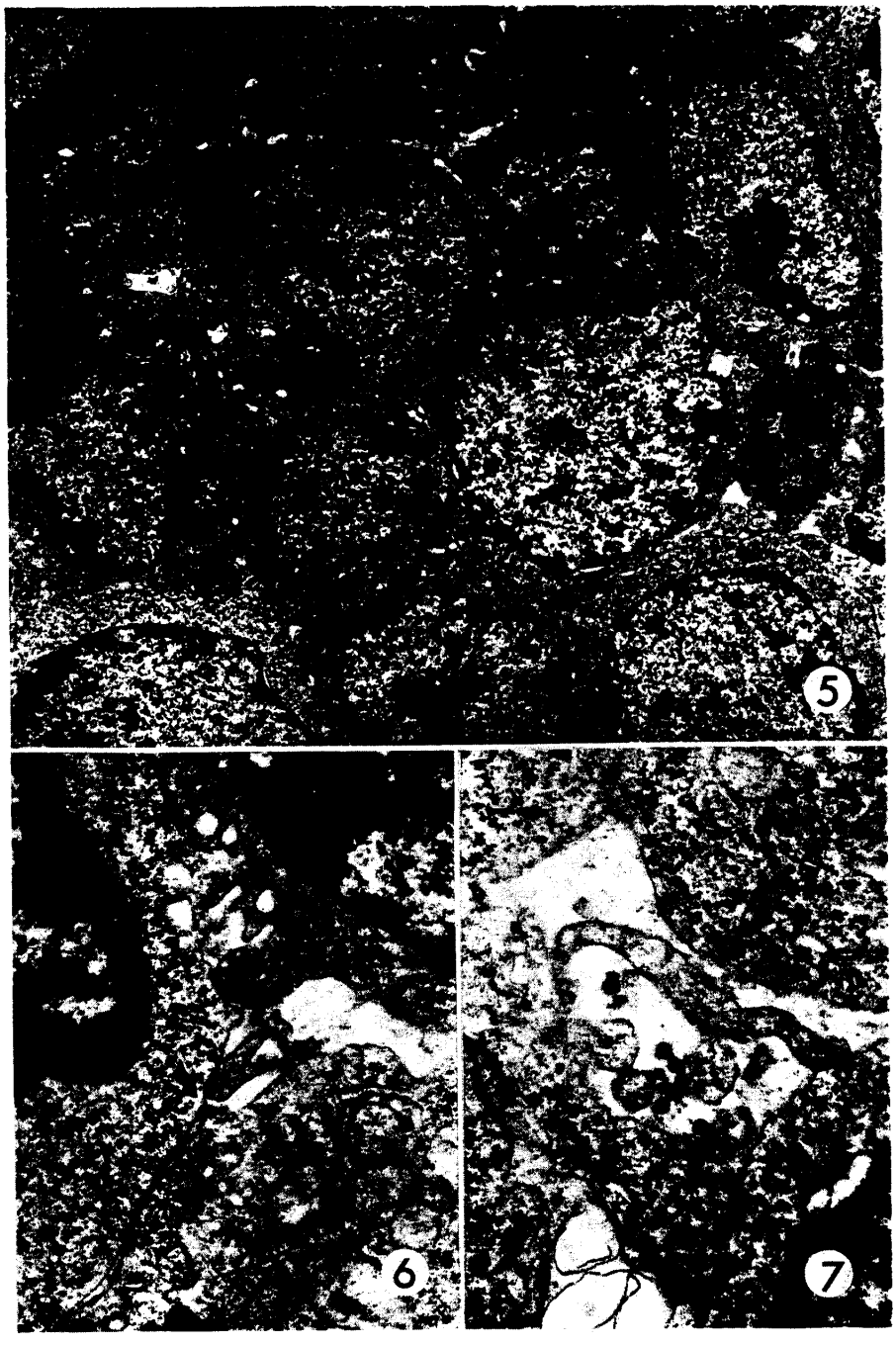
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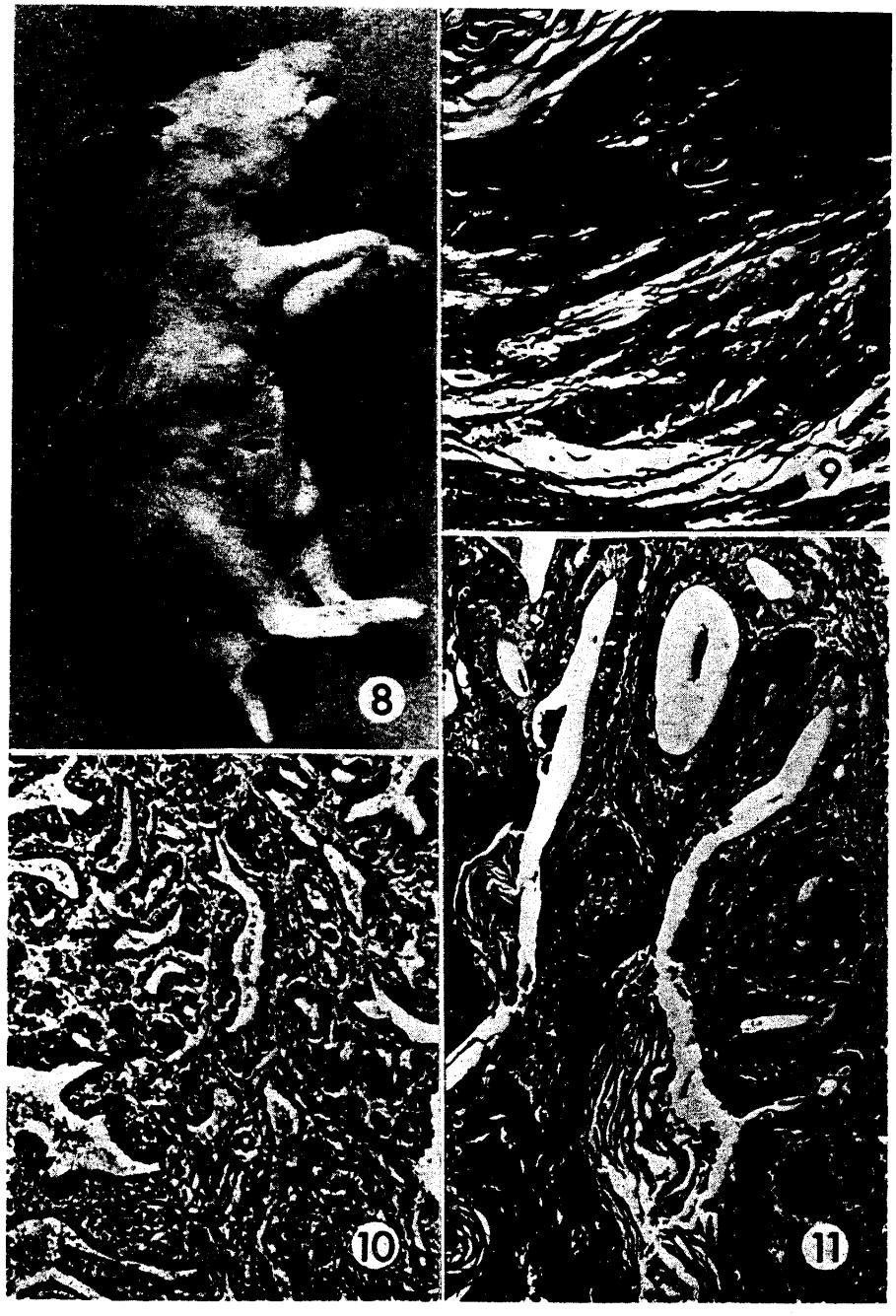
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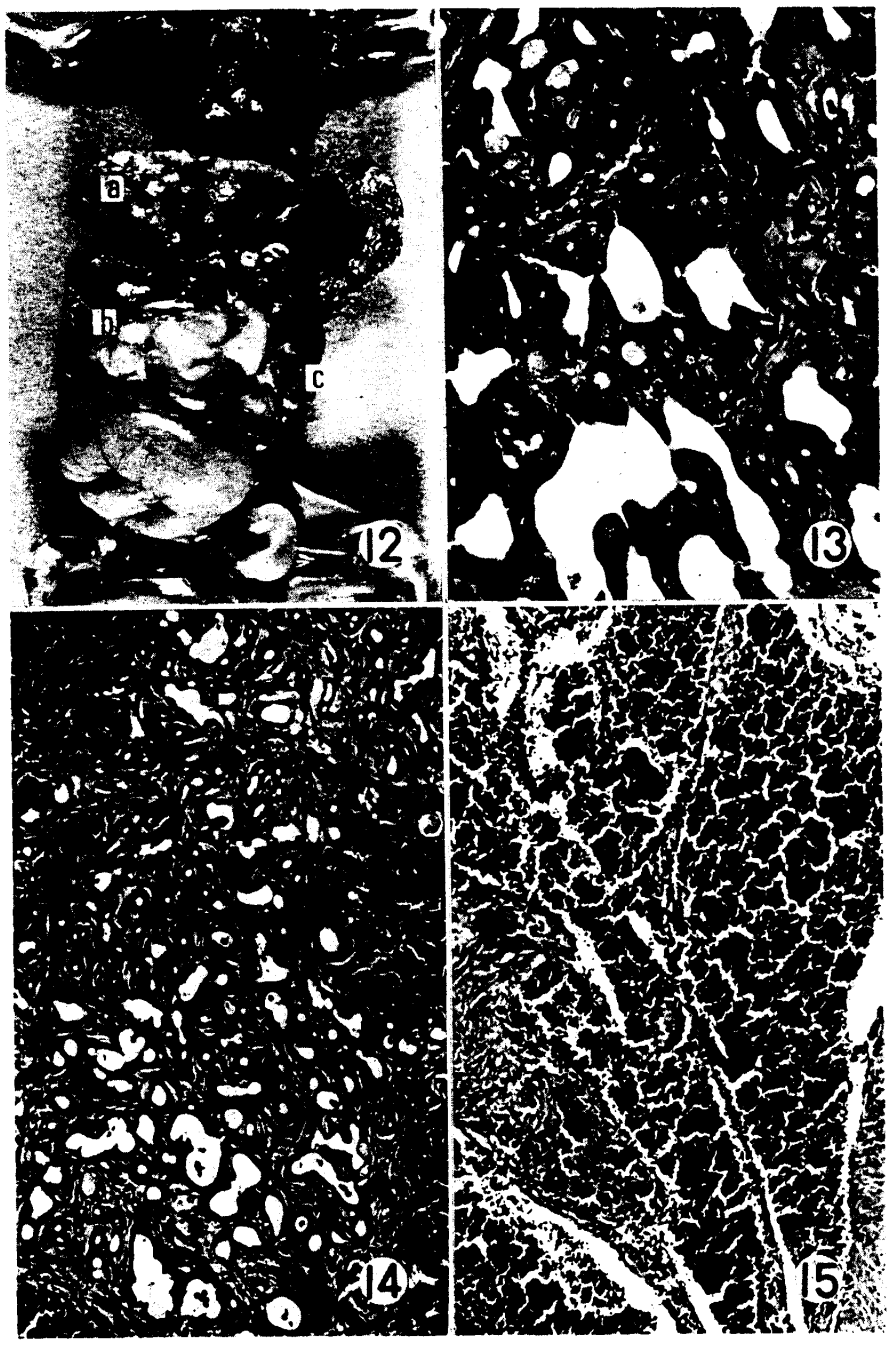
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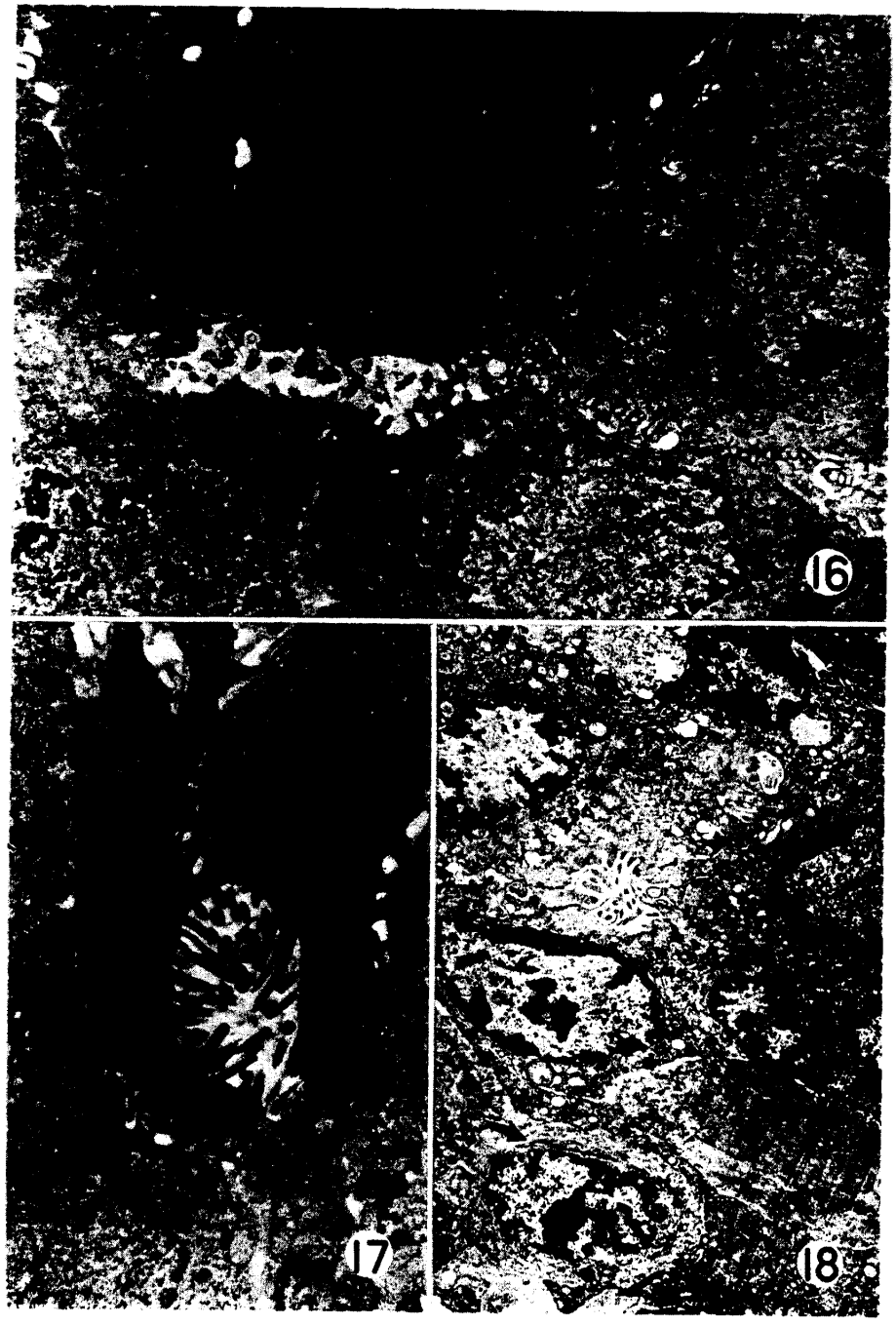
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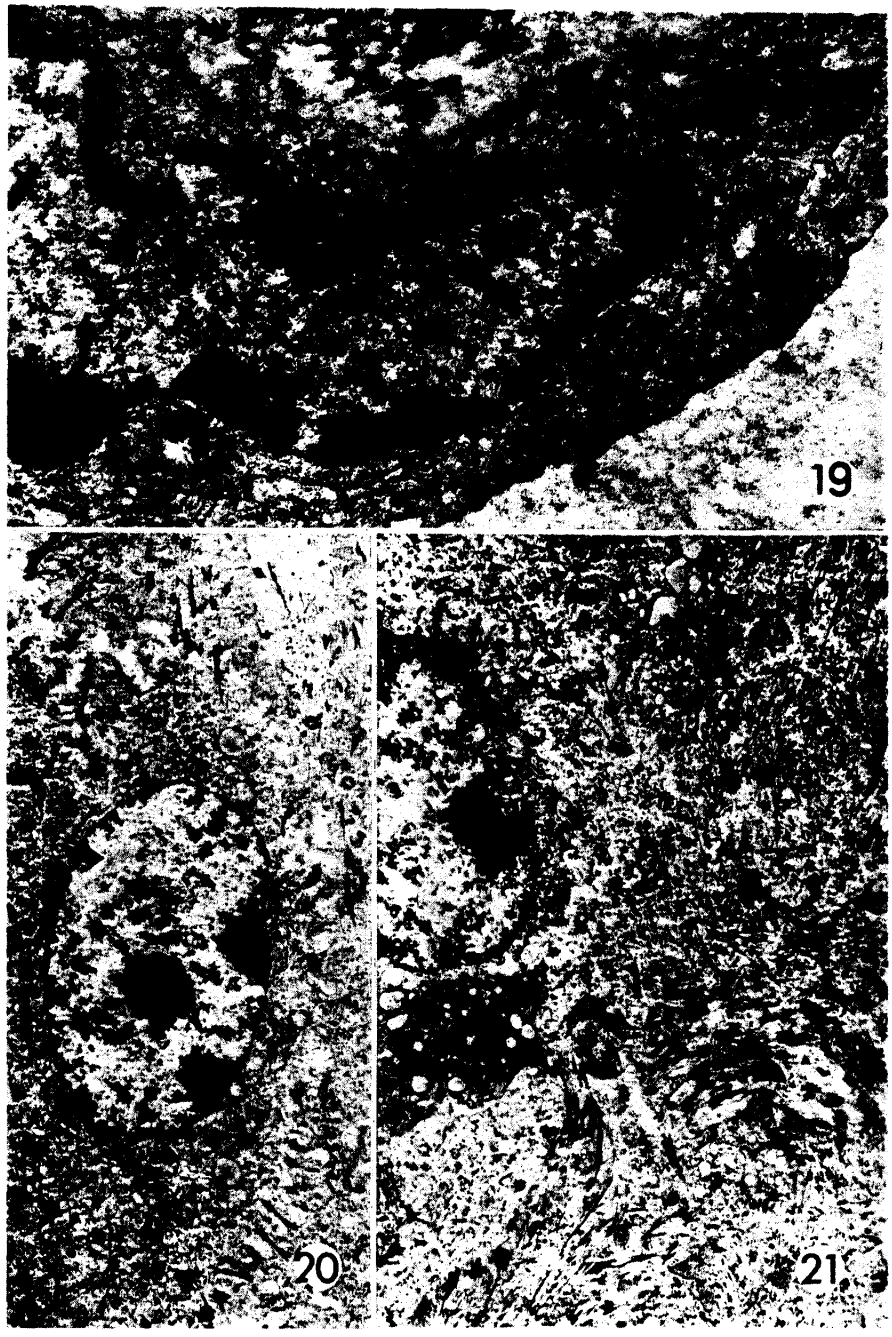
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