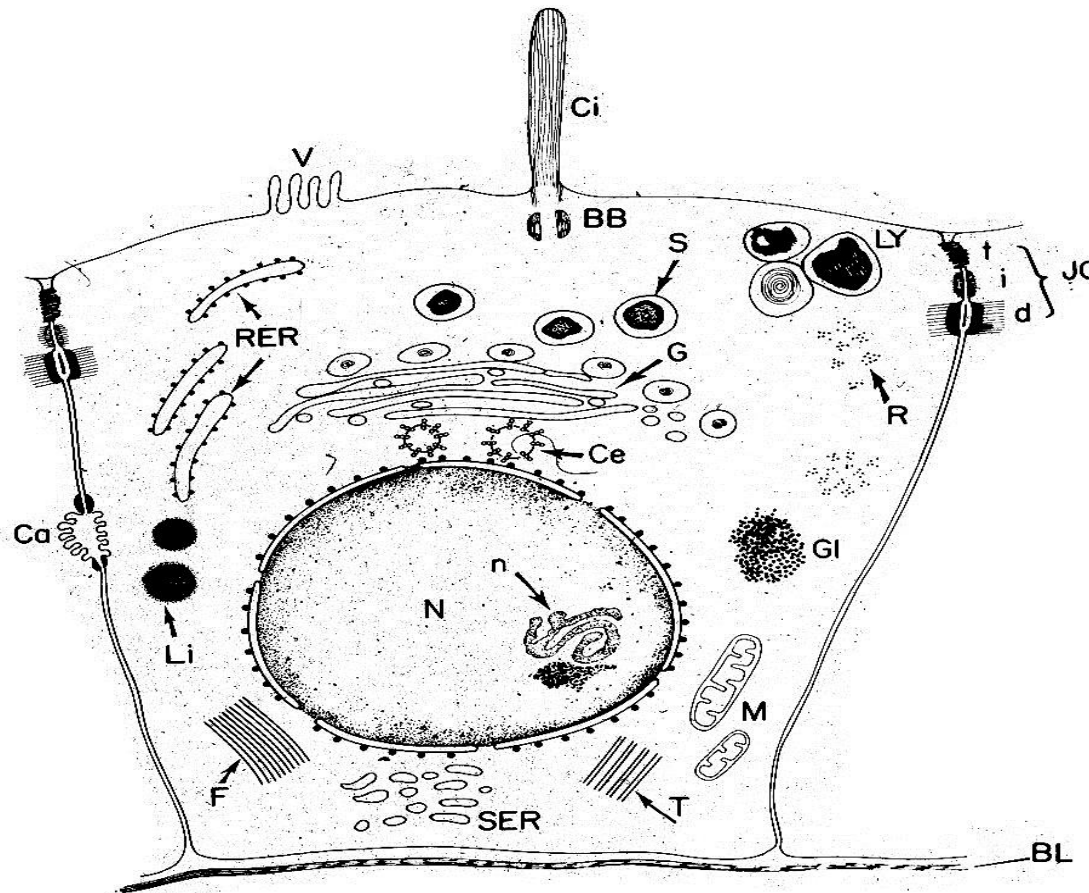


***ELECTRON MICROSCOPY
IN DIAGNOSTIC
PATHOLOGY***

Mark R. Wick, MD



- | | |
|---------------------------|------------------------------------|
| N = Nucleus | Ce = Centrioles |
| n = Nucleolus | G = Golgi apparatus |
| V = Villi | M = Mitochondria |
| Ci = Cilium | RER = Rough endoplasmic reticulum |
| BB = Basal body | R = Ribosomes |
| Ca = Canaliculus | SER = Smooth endoplasmic reticulum |
| BL = Basal lamina | LY = Lysosomes |
| JC = Junctional complex | S = Secretory granules |
| t = tight junction | GI = Glycogen granules |
| i = intermediate junction | Li = Lipid vacuoles |
| d = desmosome | F = Microfilaments |
| | T = Microtubules |

Basic Fine Structural Anatomy & Cellular Organelles

PROCESSING OF TISSUE FOR TRANSMISSION ELECTRON MICROSCOPY

- 1. Fix in 2 or 3% buffered glutaraldehyde immediately after receipt of the specimen, and after carefully mincing it into 1 mm cubes of tissue. The latter procedure is best done in a small pool of glutaraldehyde on a flat surface, after allowing a 4-5 mm cube of tissue to sit in it for roughly five minutes.**
- 2. Tissue is then embedded in epoxy resin and cut at 1 micron for staining with toluidine blue and selection of fields.**
- 3. Staining with osmium tetroxide and lead citrate is done next, followed by thin sectioning (0.3 to 0.4 microns) with a diamond knife, mounting on copper grids, and staining with uranyl acetate and lead citrate.**

PRECAUTIONS IN DIAGNOSTIC ELECTRON MICROSCOPY

- 1. Always correlate ultrastructural findings with histological observations and clinical data... REMEMBER-- "A fool with a fancy tool is still a fool!"**
- 2. Pay special attention to processing requirements for good preservation of tissue. Retrieval of samples from formalin or paraffin blocks yields vastly inferior results.**
- 3. Advise clinicians of time constraints that are inherent in electron microscopy. At least 3, and more likely 4 or 5 days are necessary to properly prepare and evaluate specimens submitted for ultrastructural analysis.**

Cytoplasmic Organelles That Strongly Suggest a Specific Diagnosis

--Neurosecretory Granules:

Neuroendocrine/neuroectodermal tumors

--Premelanosomes (types 1-3): Melanocytic tumors

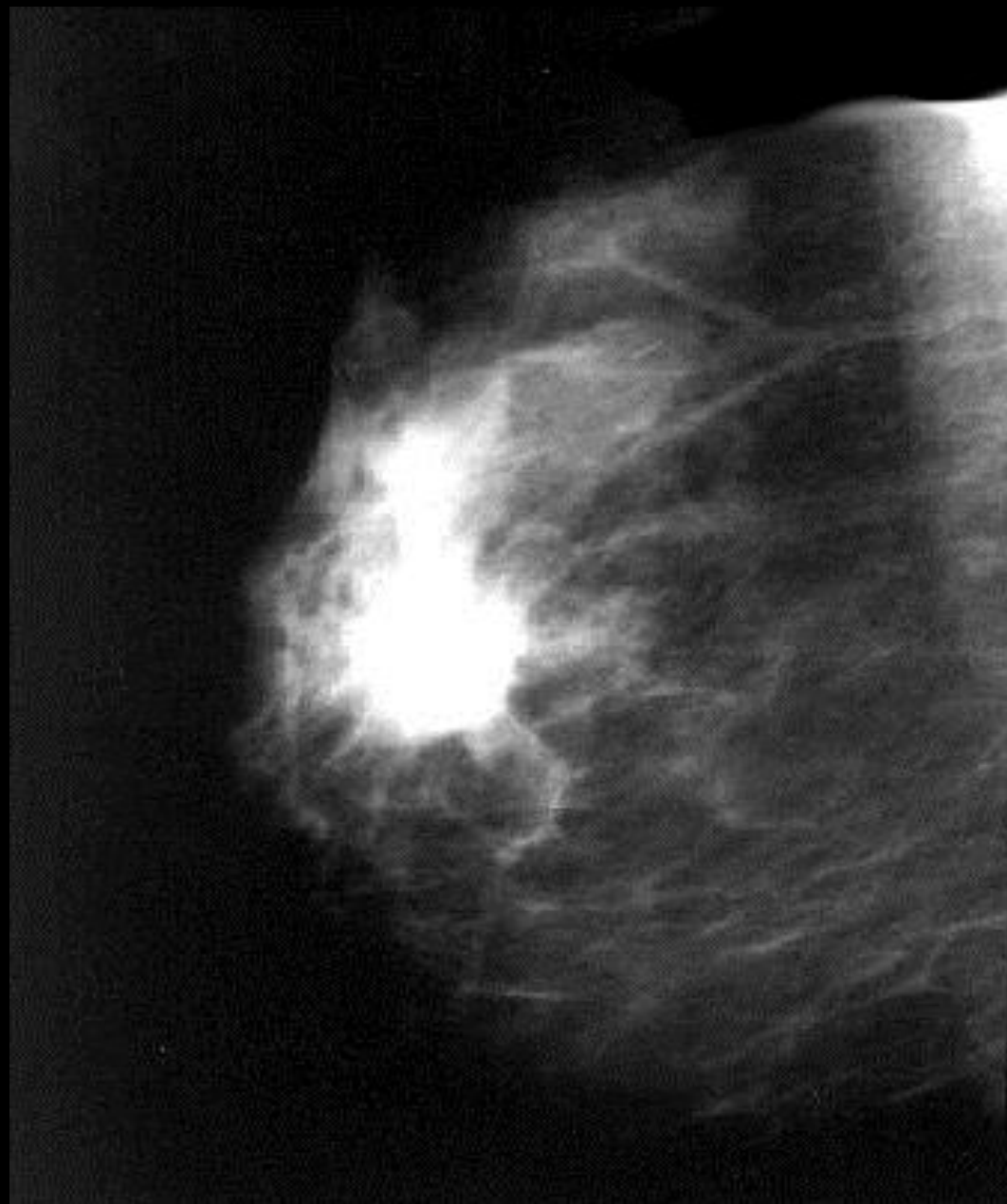
--Rhomboid crystalloids: Alveolar soft part sarcoma or Prostatic adenocarcinoma

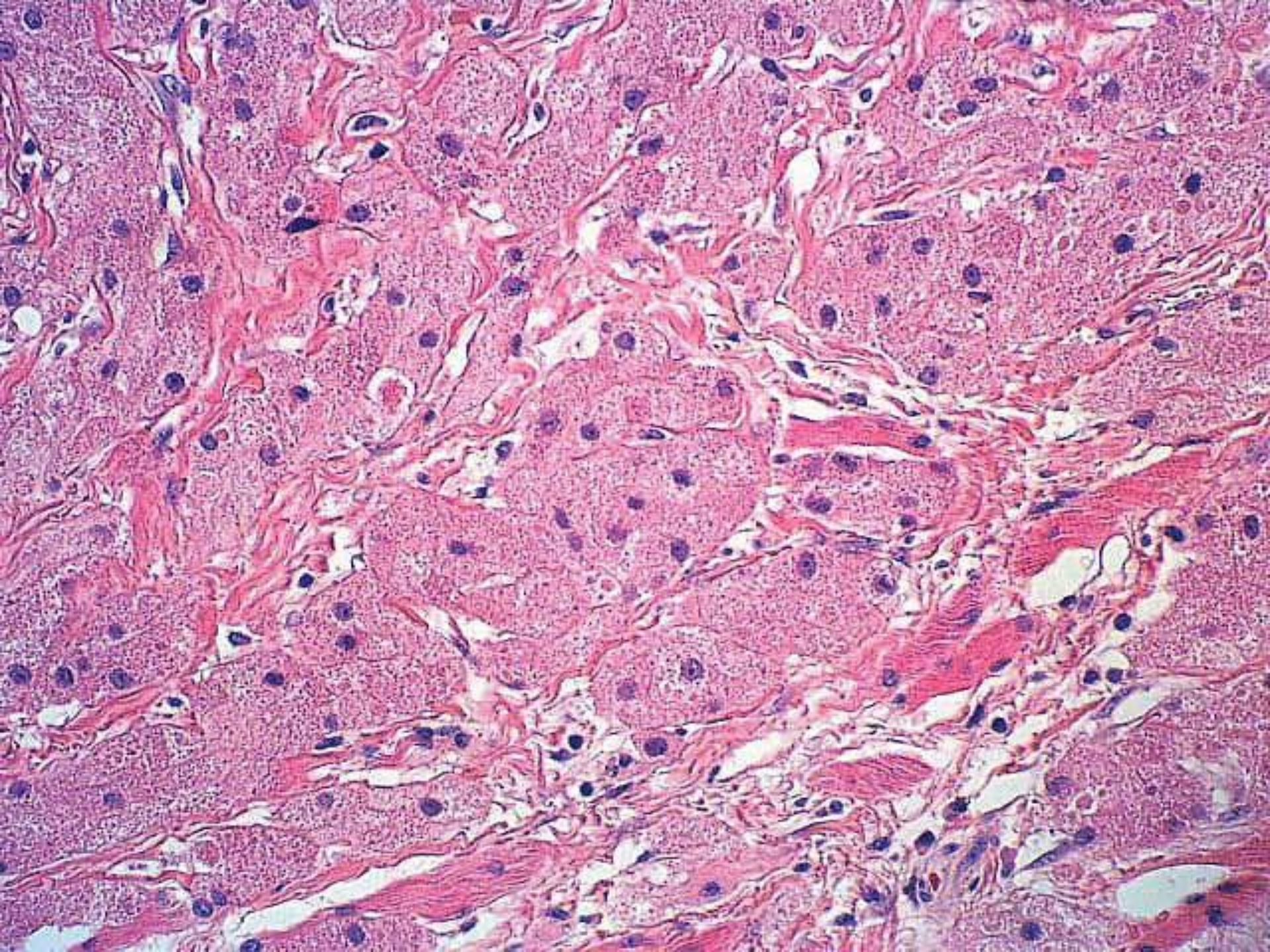
--Birbeck granules: Langerhans' cell proliferations

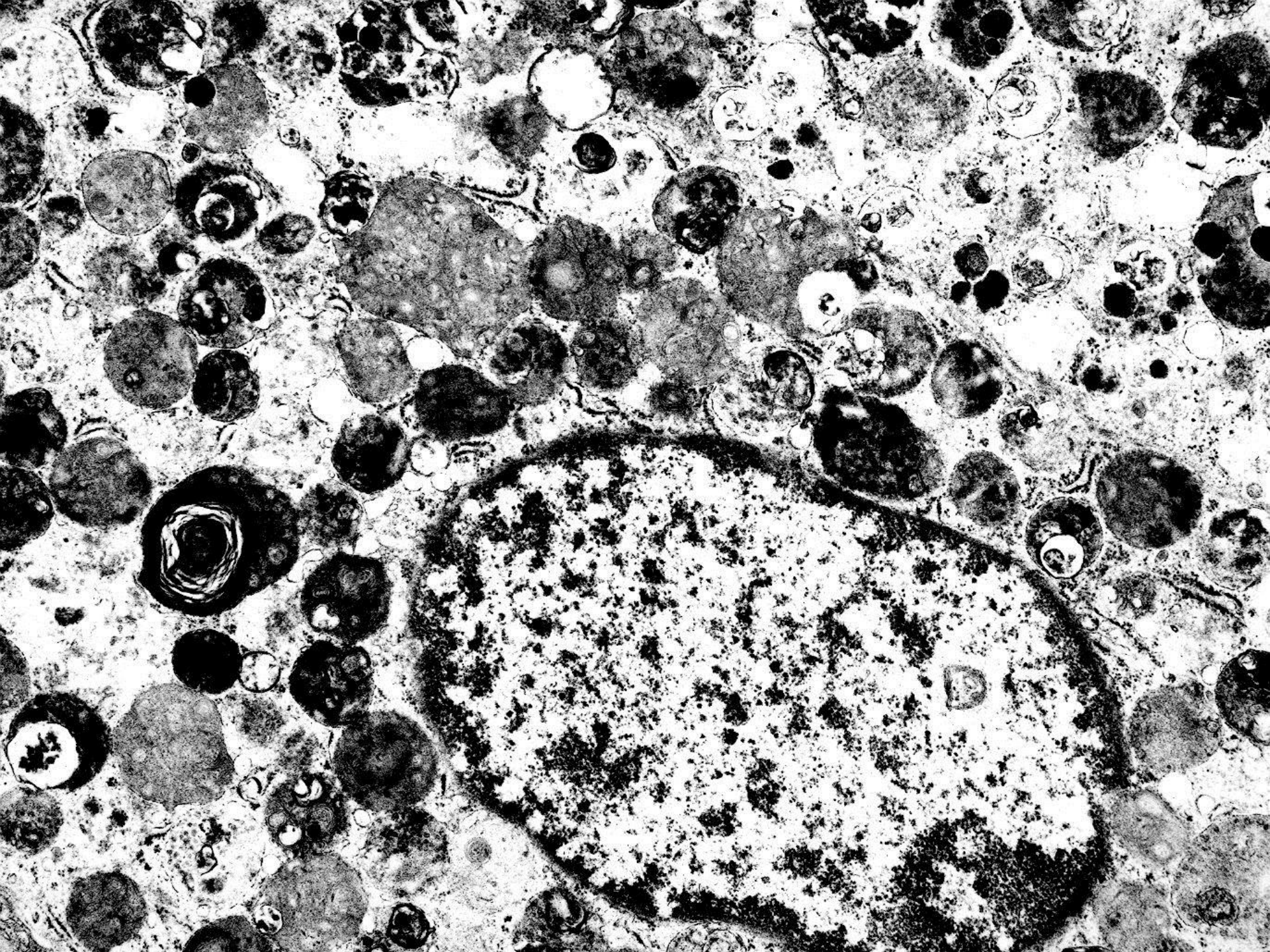
--Numerous phagolysosomes: Granular cell tumors

--Weibel-Palade bodies: Endothelial tumors

**38 year old woman with a left
breast mass**

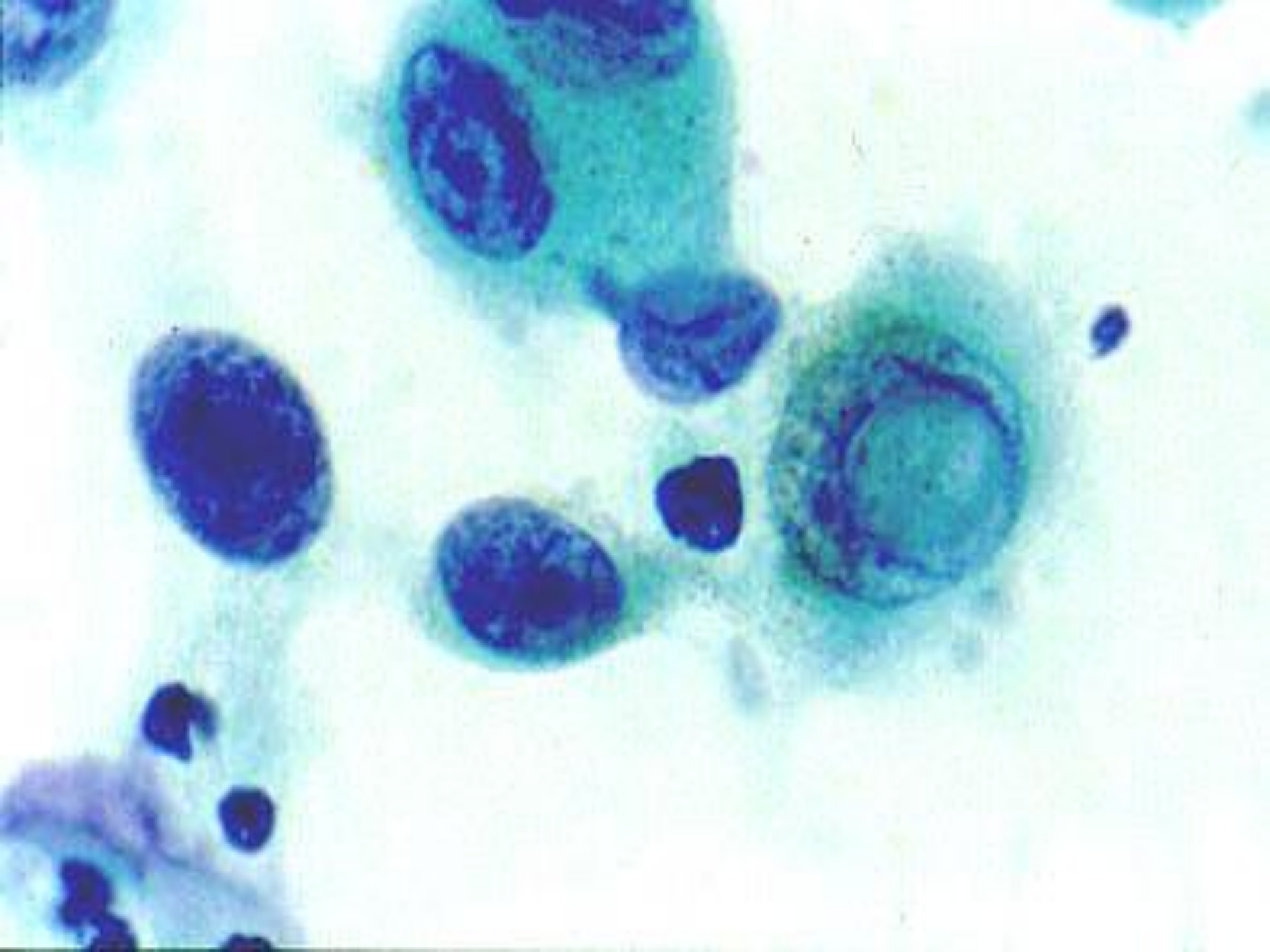


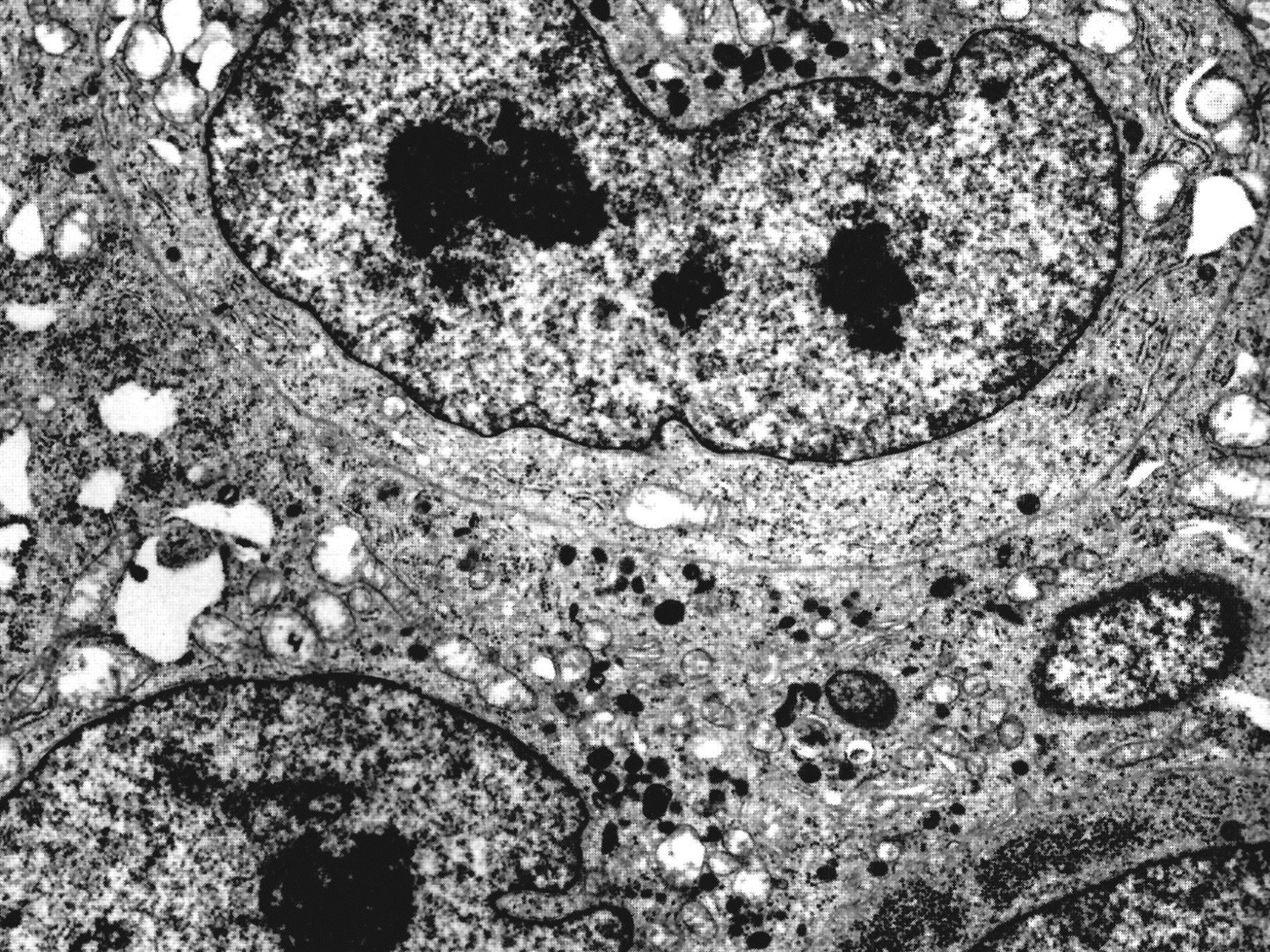


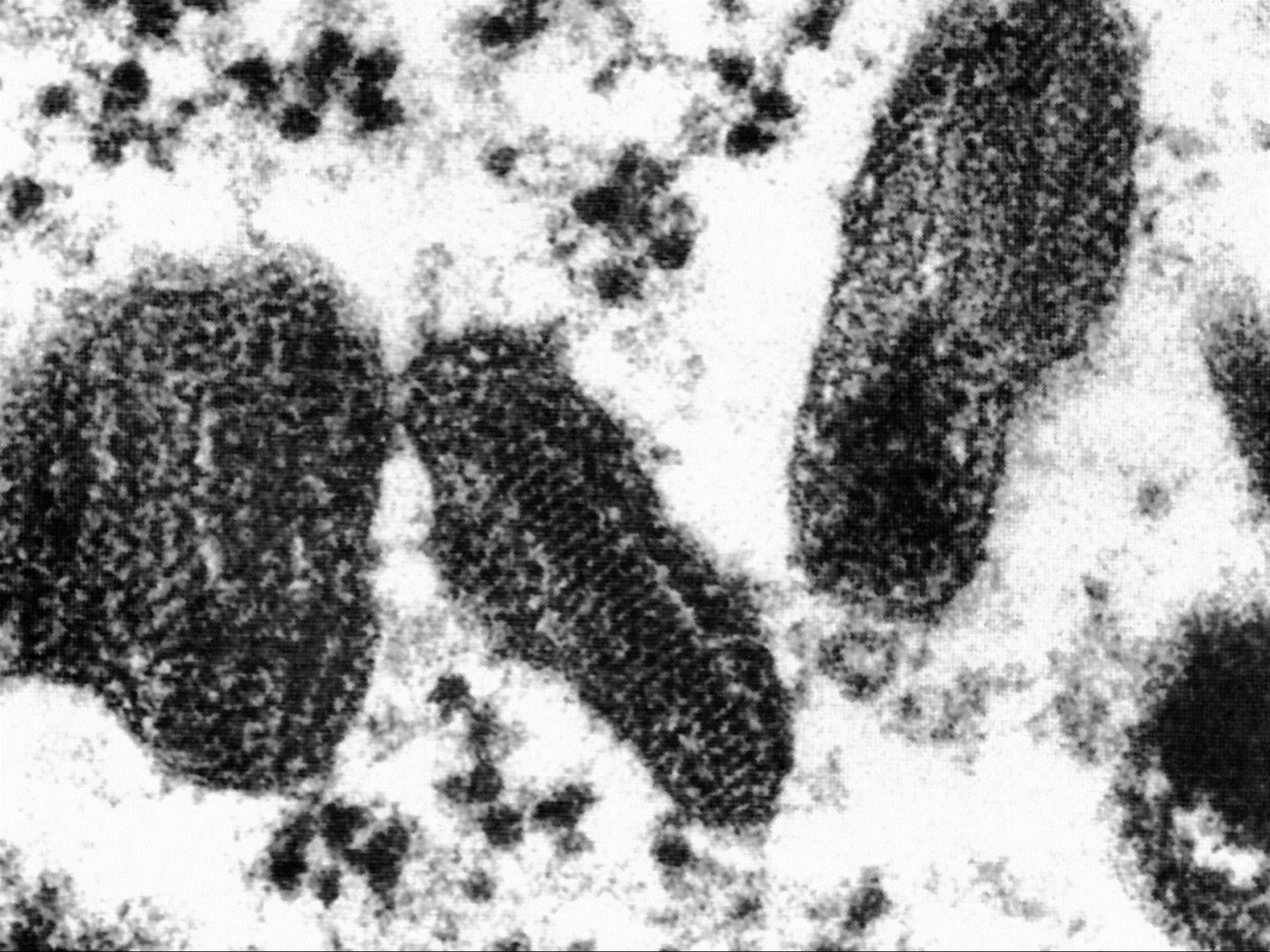


**61 year old man with elevated
serum alkaline phosphatase on
routine yearly health
assessment**

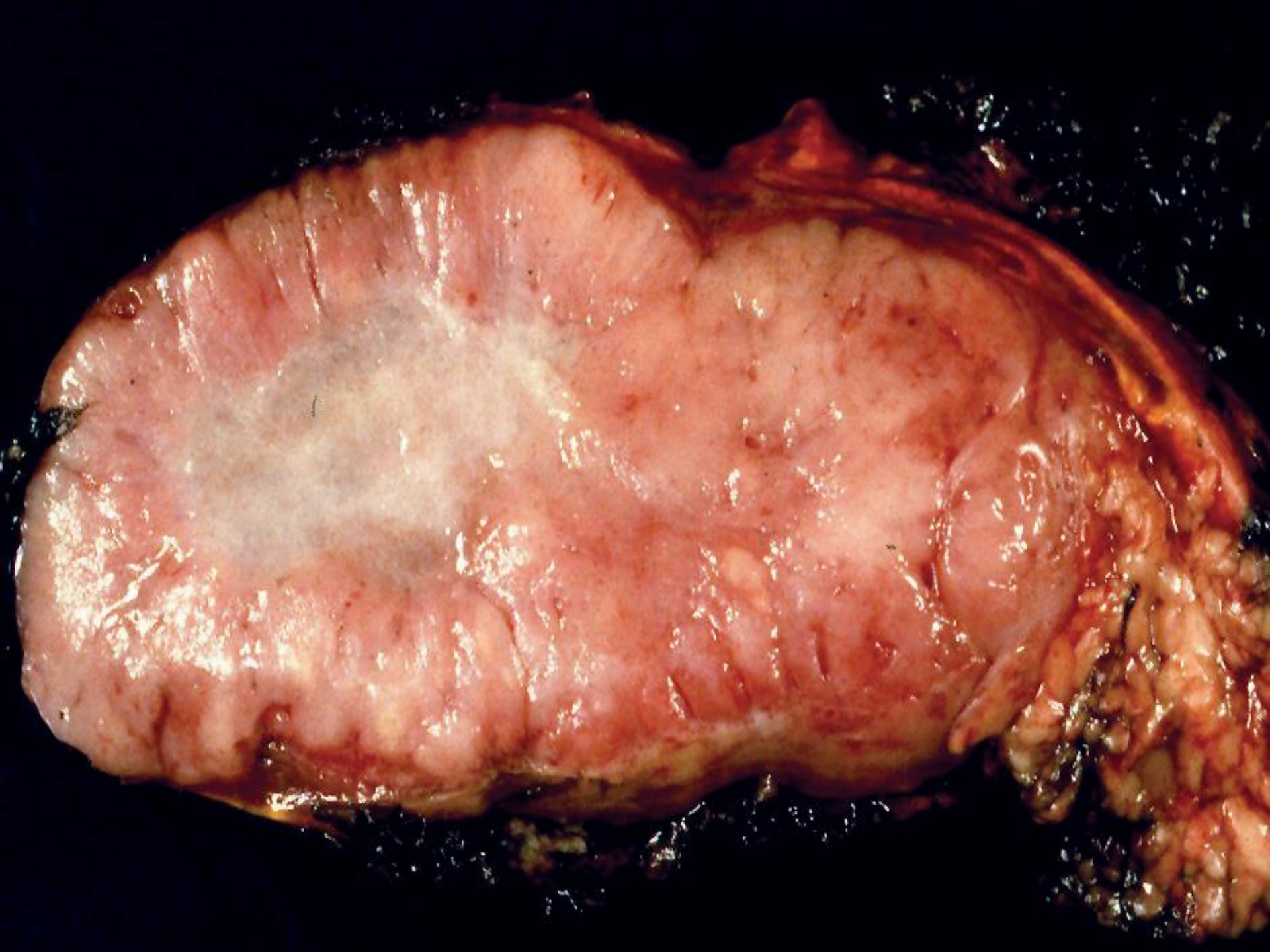


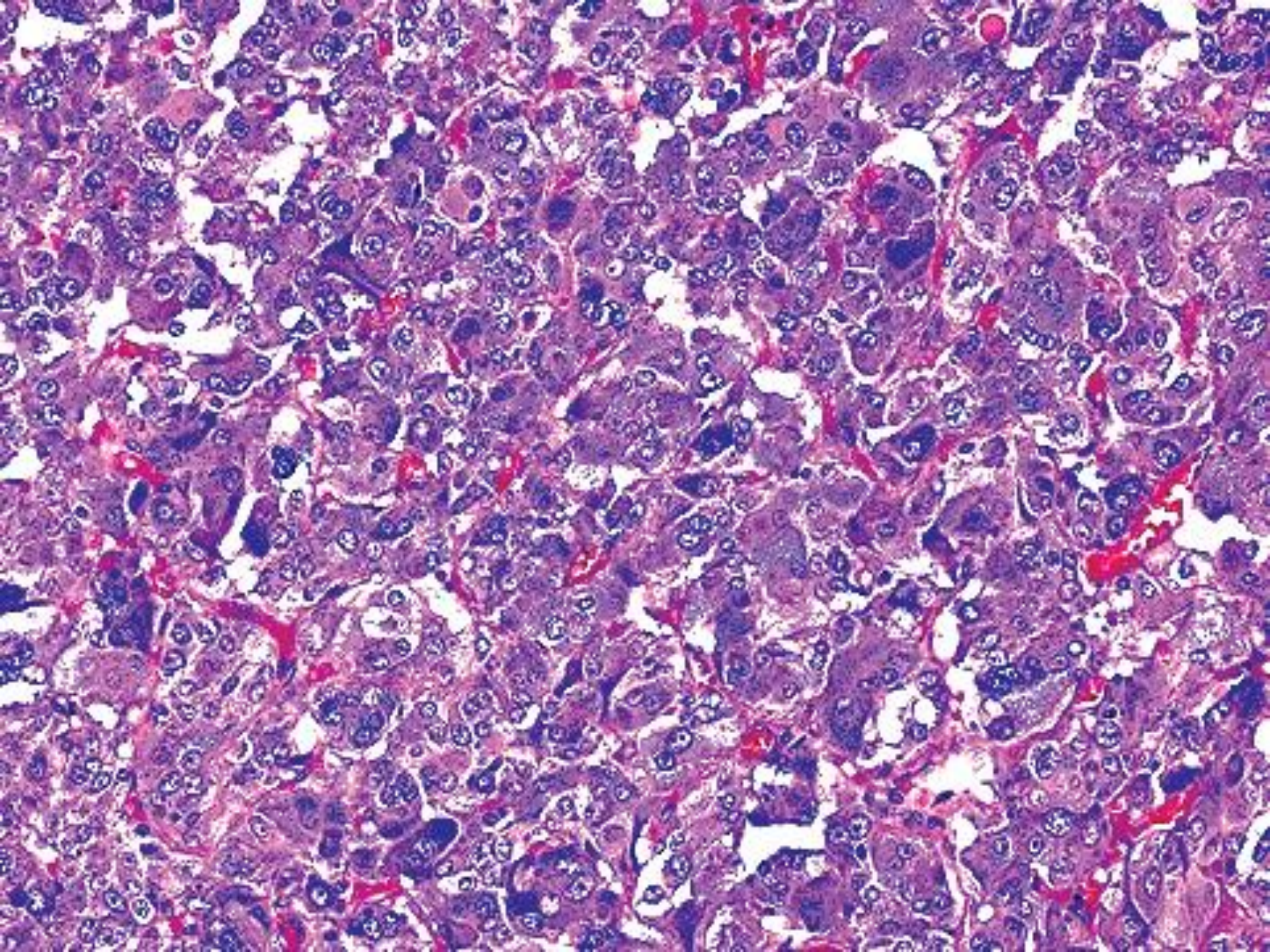


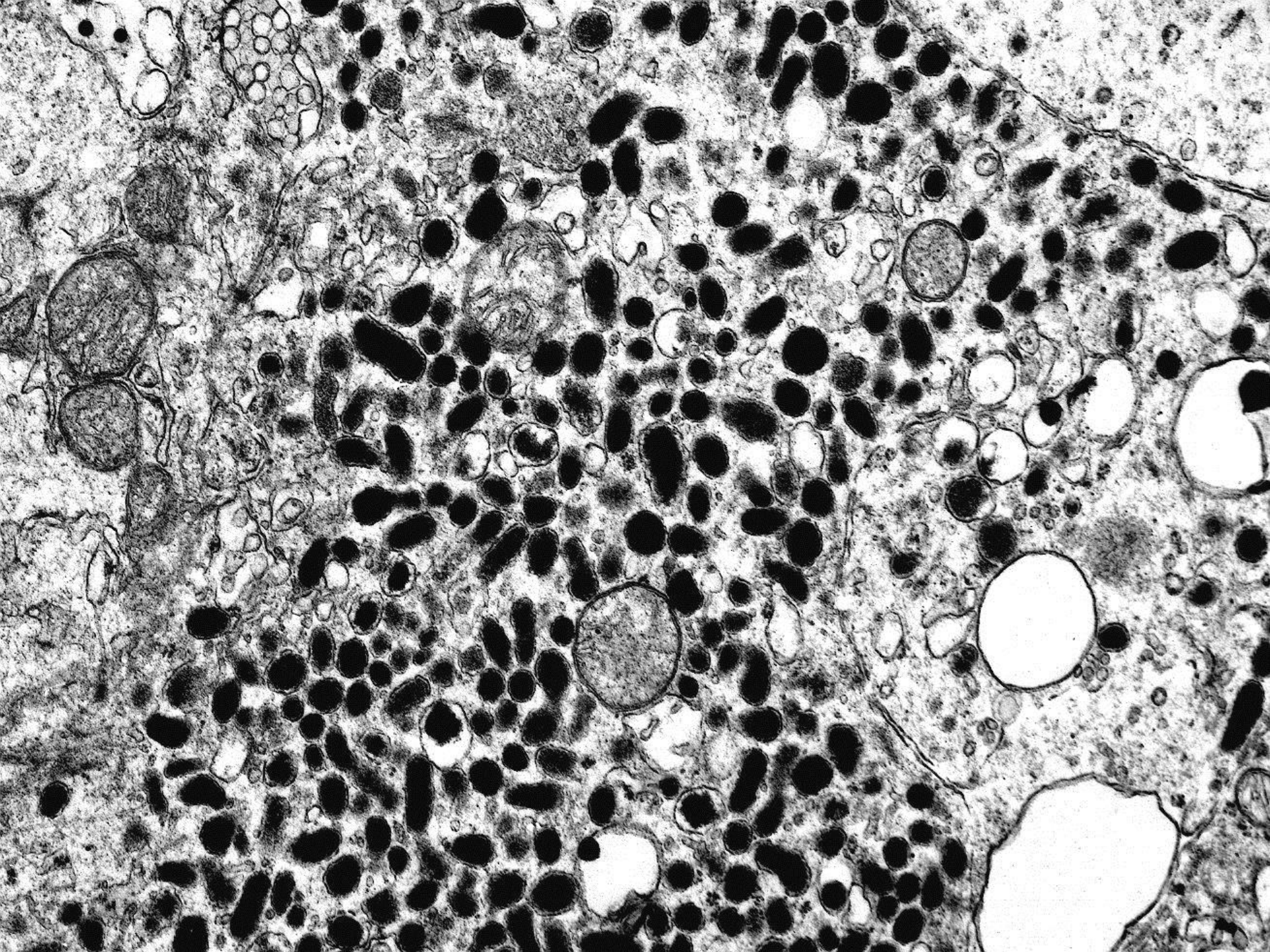




**37 year old woman with
“spells” of sweating,
palpitations, and dizziness; left
adrenal mass found on CT of
abdomen**



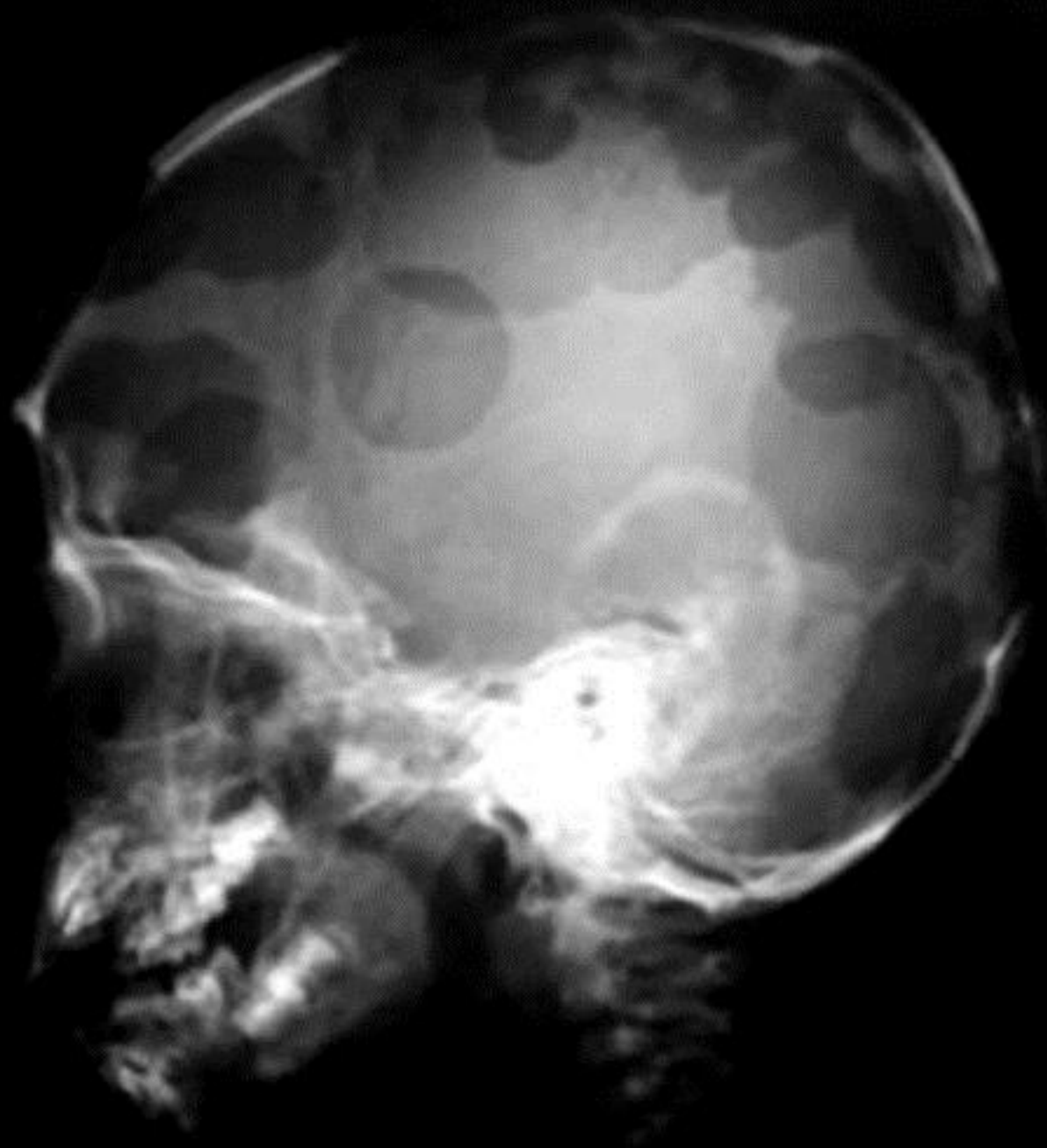


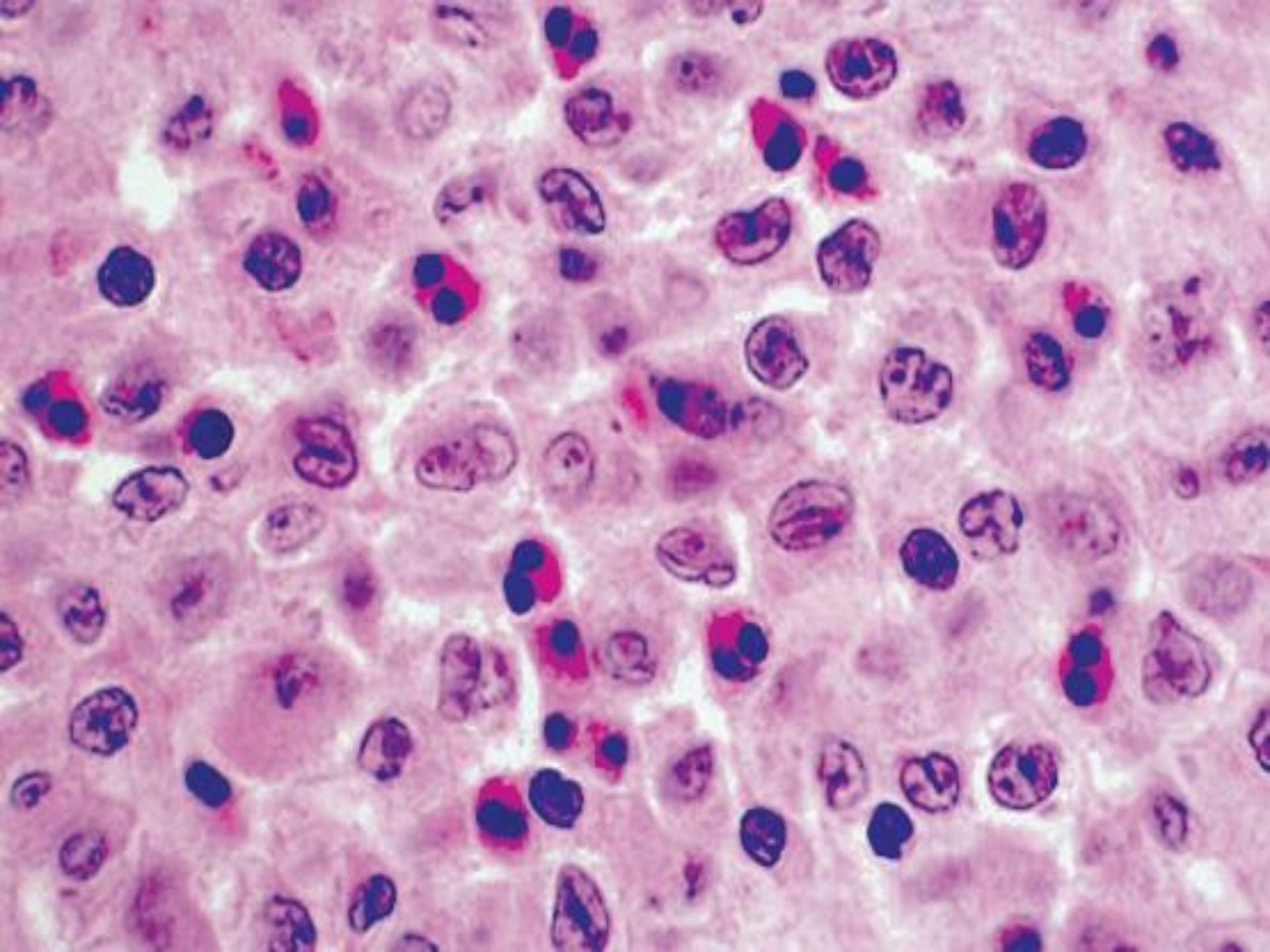


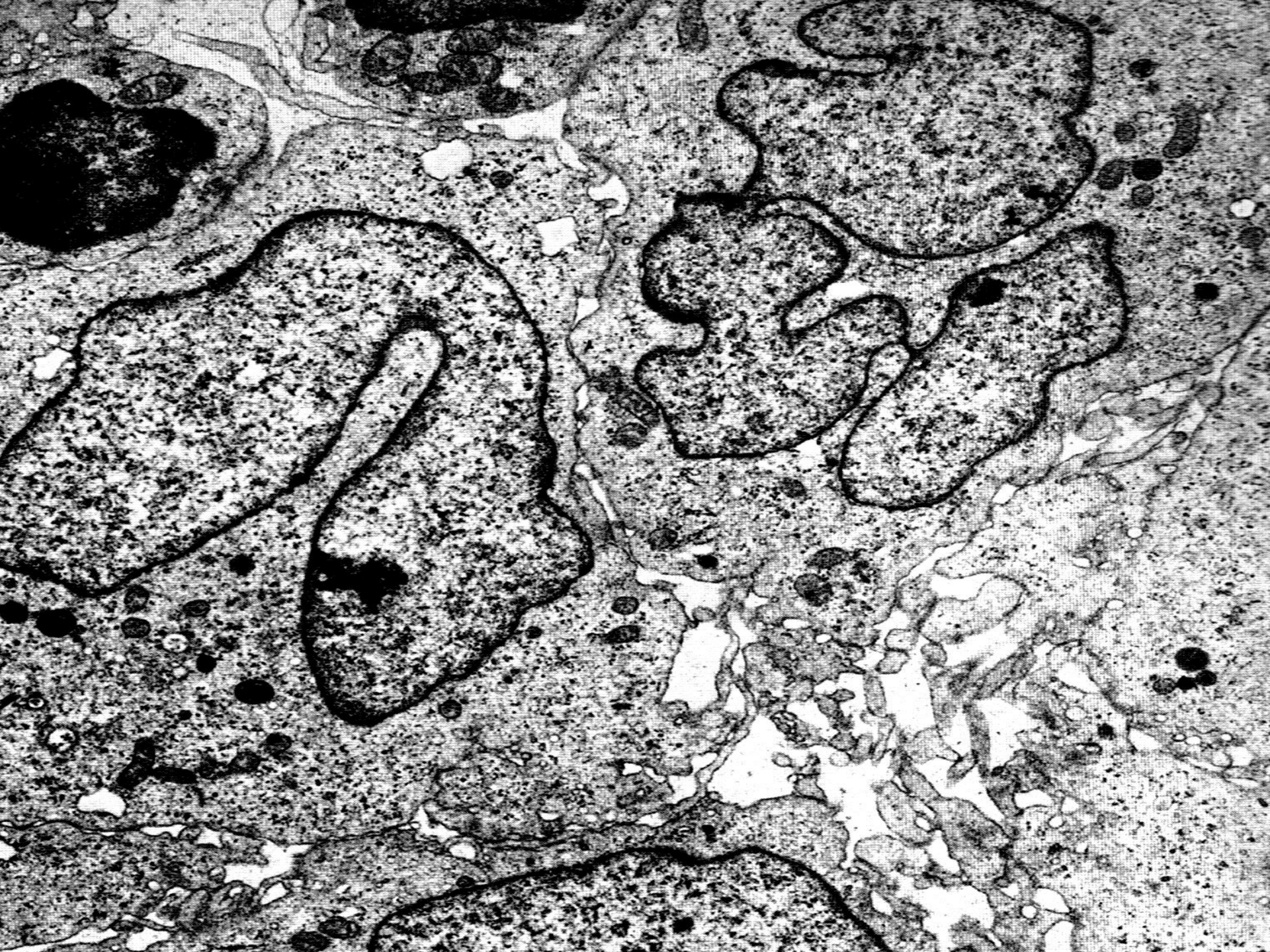
NEUROSECRETORY GRANULES VS. LYSOSOMES: THE URANAFFIN REACTION

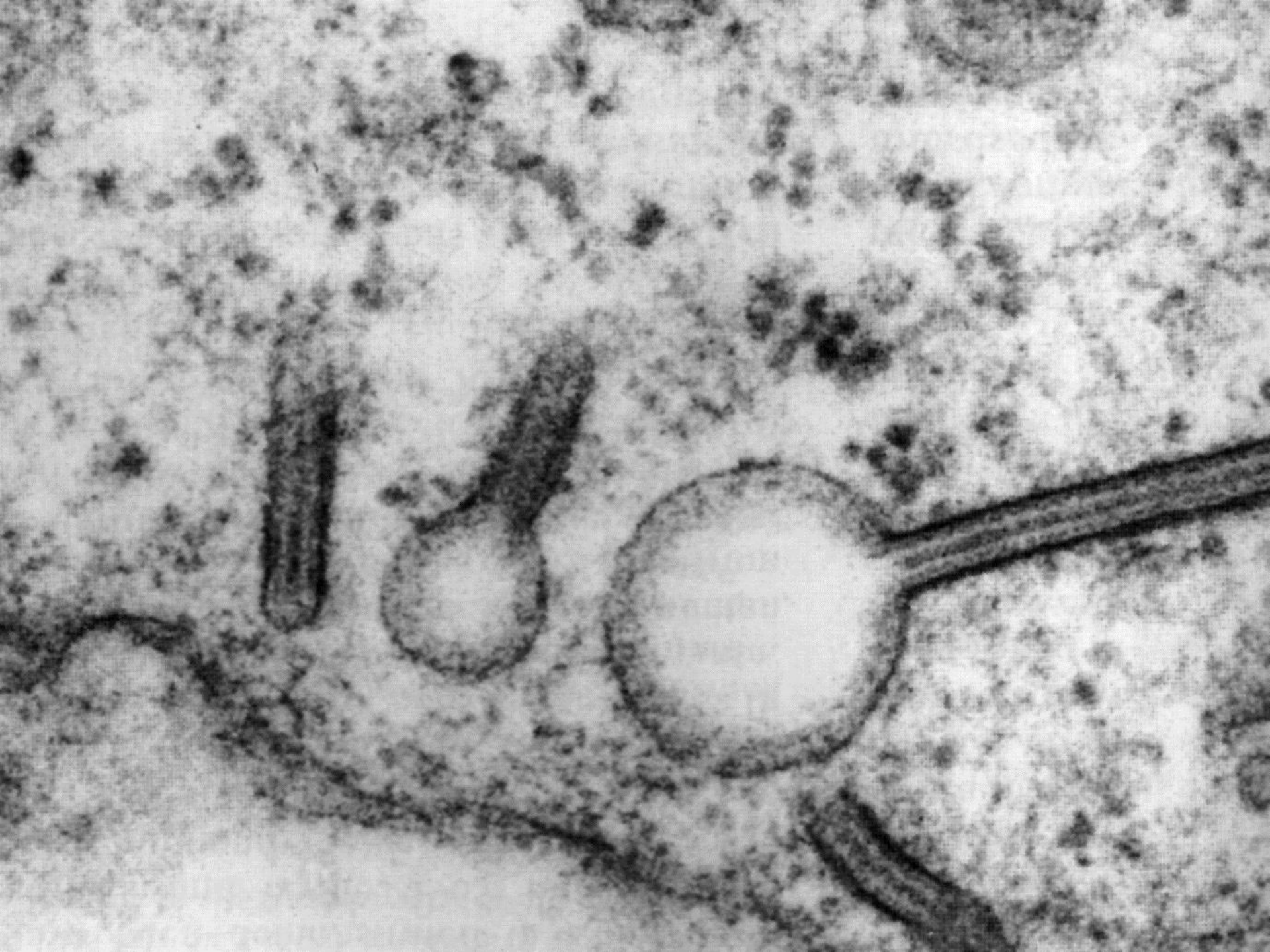
- Introduced by Payne et al. in 1985
- Ultrastructural-histochemical method for specific **deposition of silver salts on the matrices of neurosecretory granules**
- Consistently negative in lysosomes

**11 year old boy with headaches
and polydipsia**









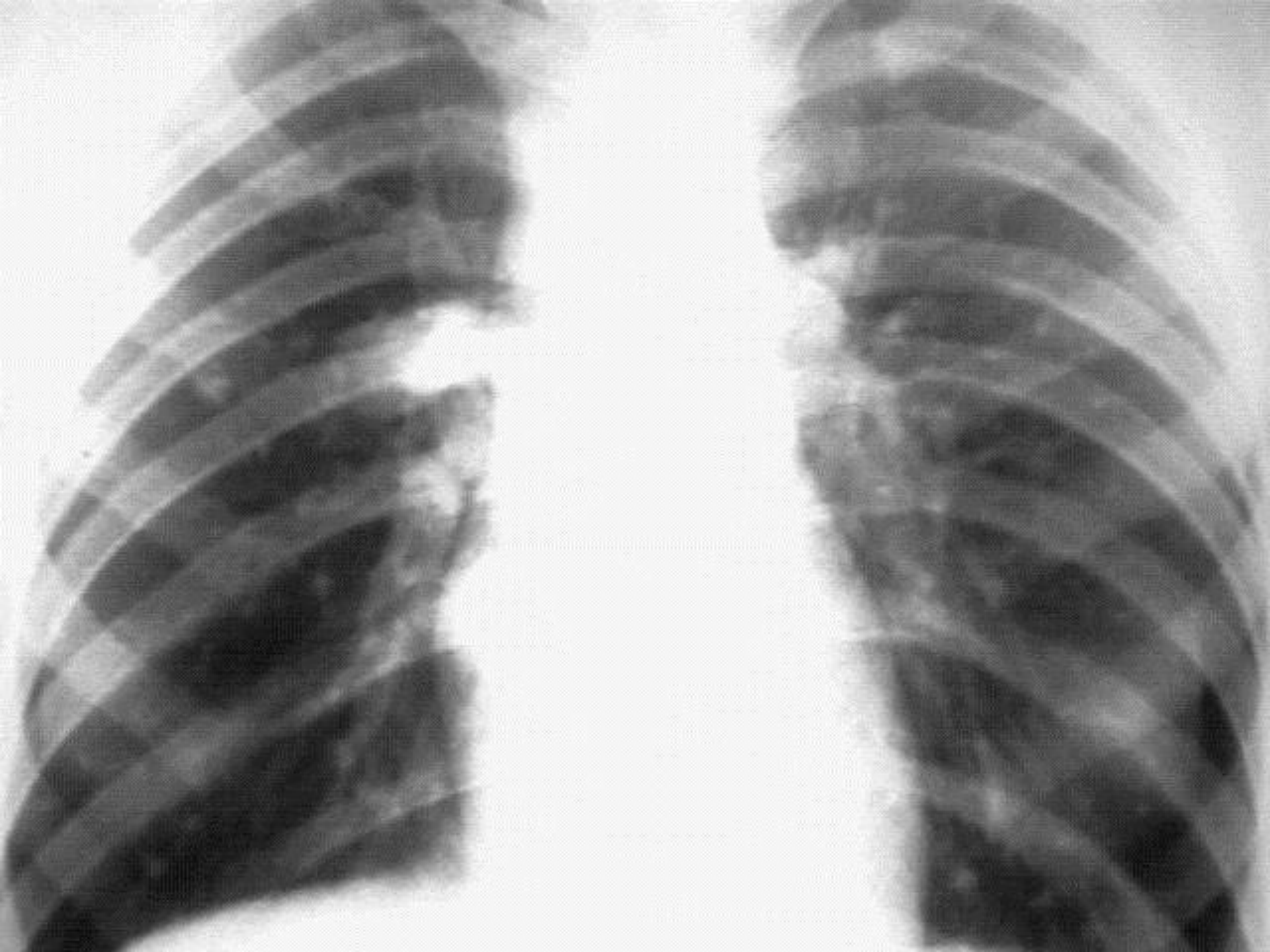
LANGERHANS' CELL PROLIFERATIONS: ULTRASTRUCTURAL FEATURES

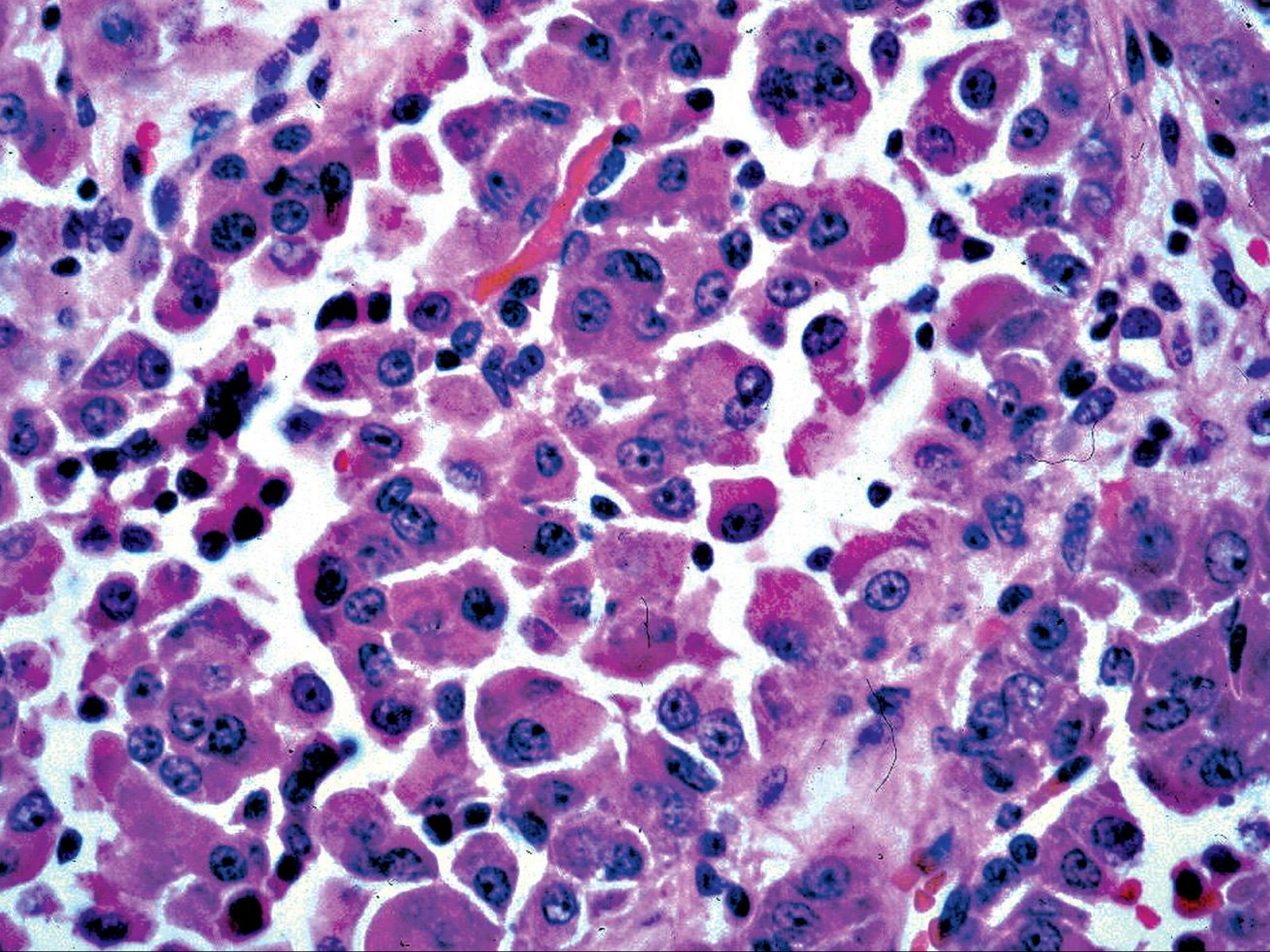
- Mononuclear cells with **"curved" nuclear contours**
- **Abundant cellular organelles**, (free ribosomes, rough endoplasmic reticulum, mitochondria, lysosomes)
- **Filopodial extensions** from cellular surfaces
- **Birbeck granules** (in 50 to 60% of cases only)
- In the skin, care must be taken to look for the above-cited features in dermal cells, to **avoid confusion with normal intraepidermal Langerhans' cells**

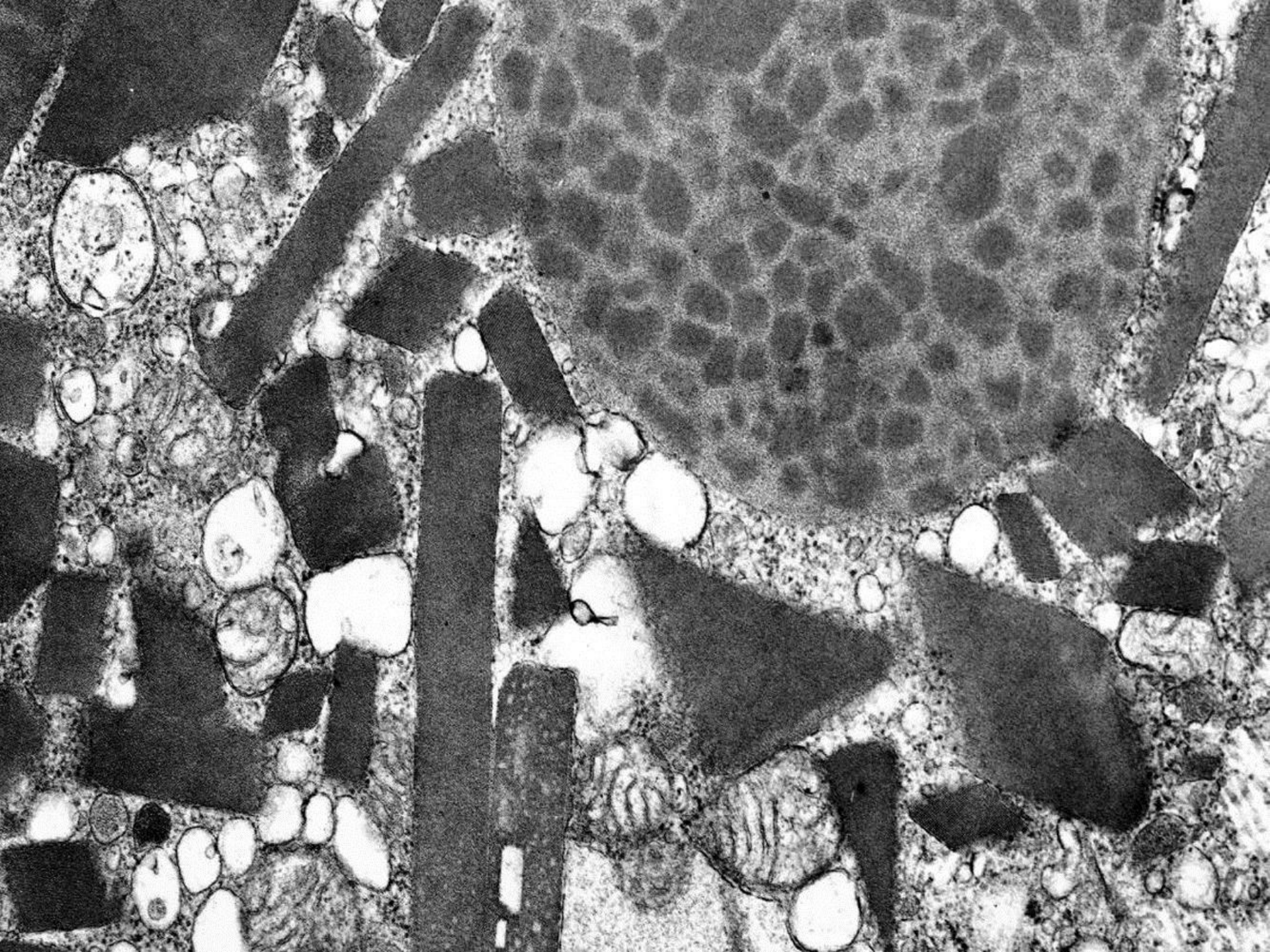
BIRBECK GRANULES IN LANGERHANS' CELLS: ULTRASTRUCTURAL CHARACTERISTICS

- Formed by invagination and detachment of plasmalemma into the cytoplasm**
- Tripartite (zipper-like) internal structure**
- May have rod-like, tennis racket-shaped, or angulated configurations in the same case or in different cases**

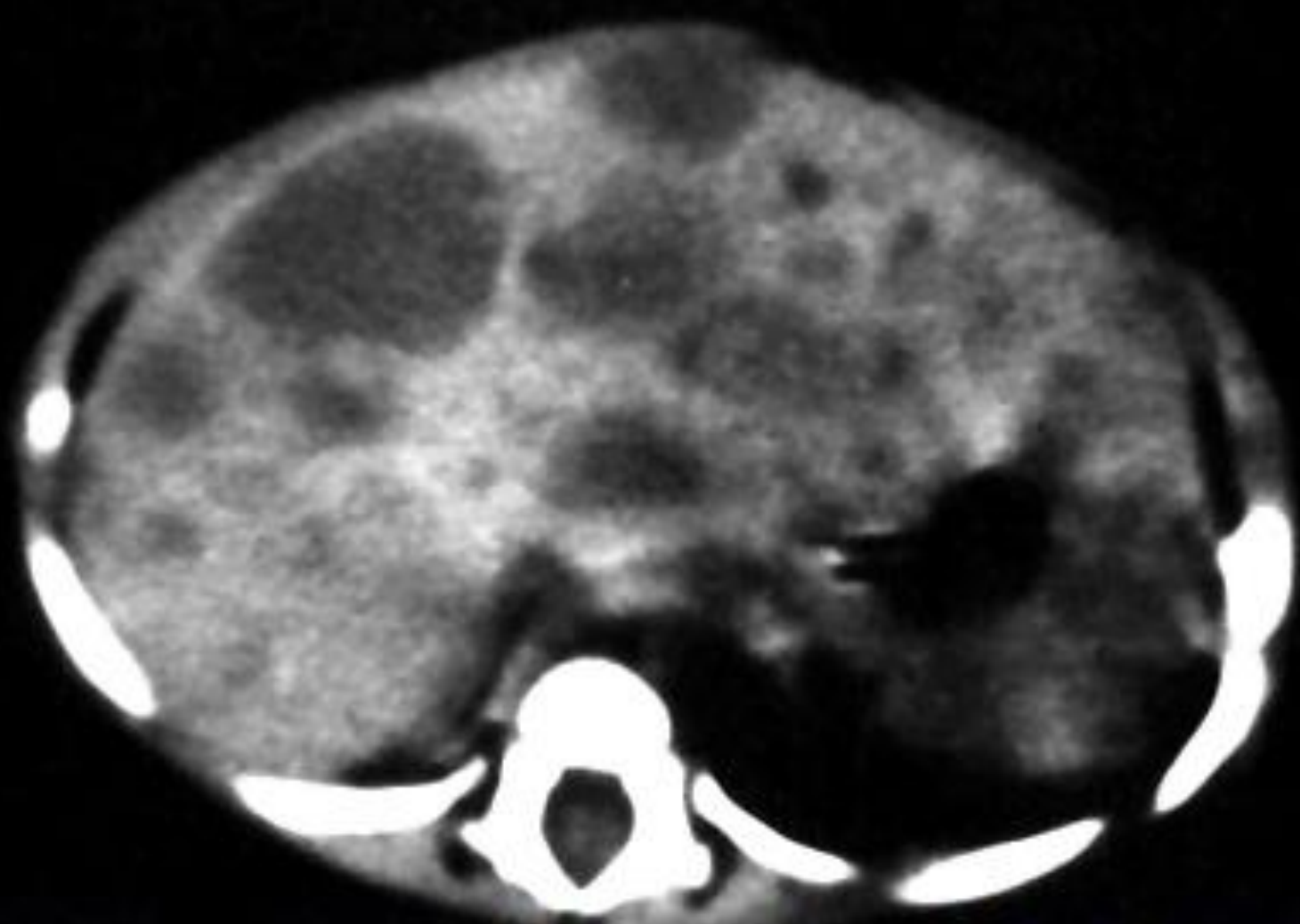
**16 year old boy with abnormal
chest radiograph on pre-
athletic school physical exam**

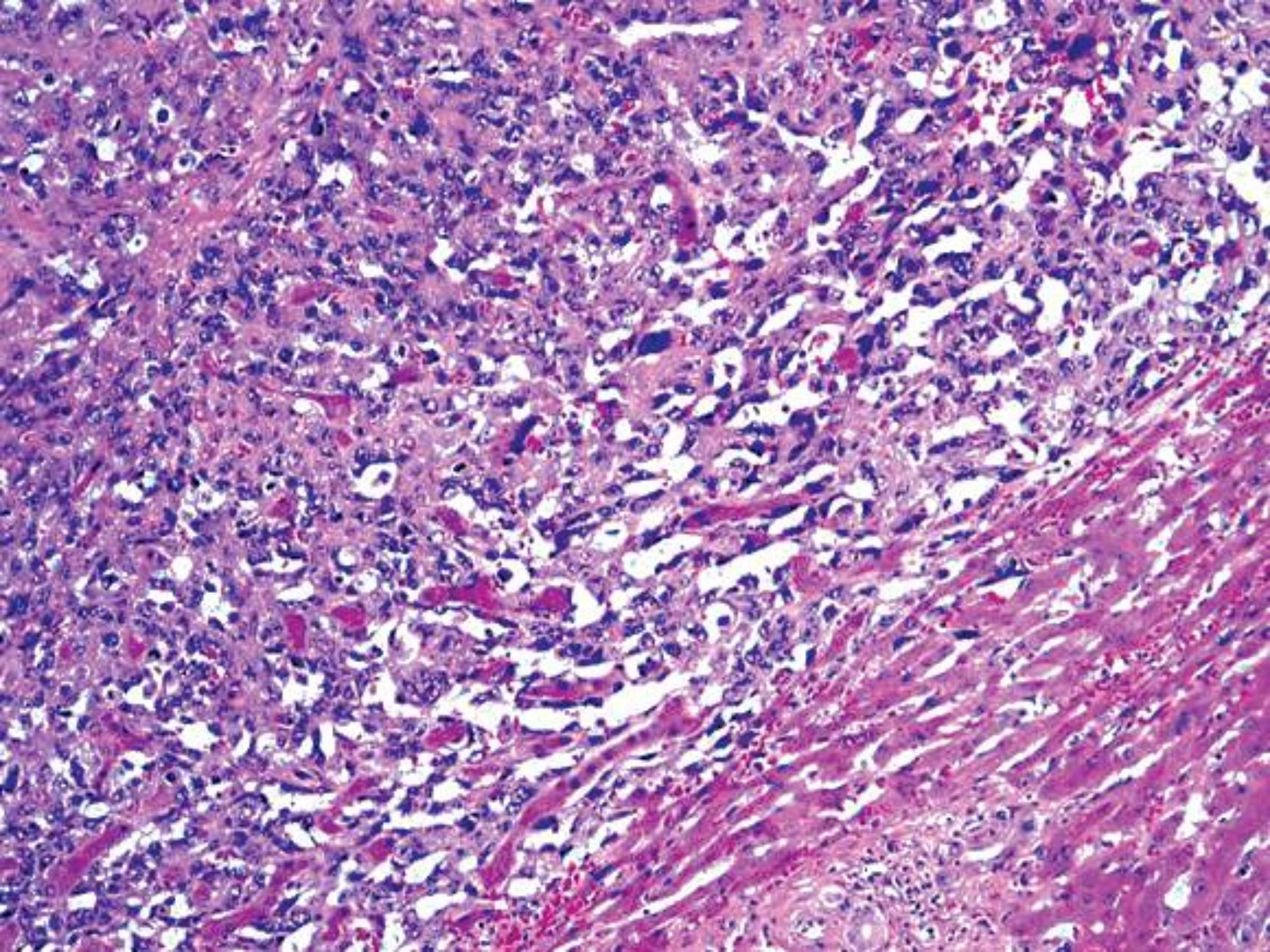


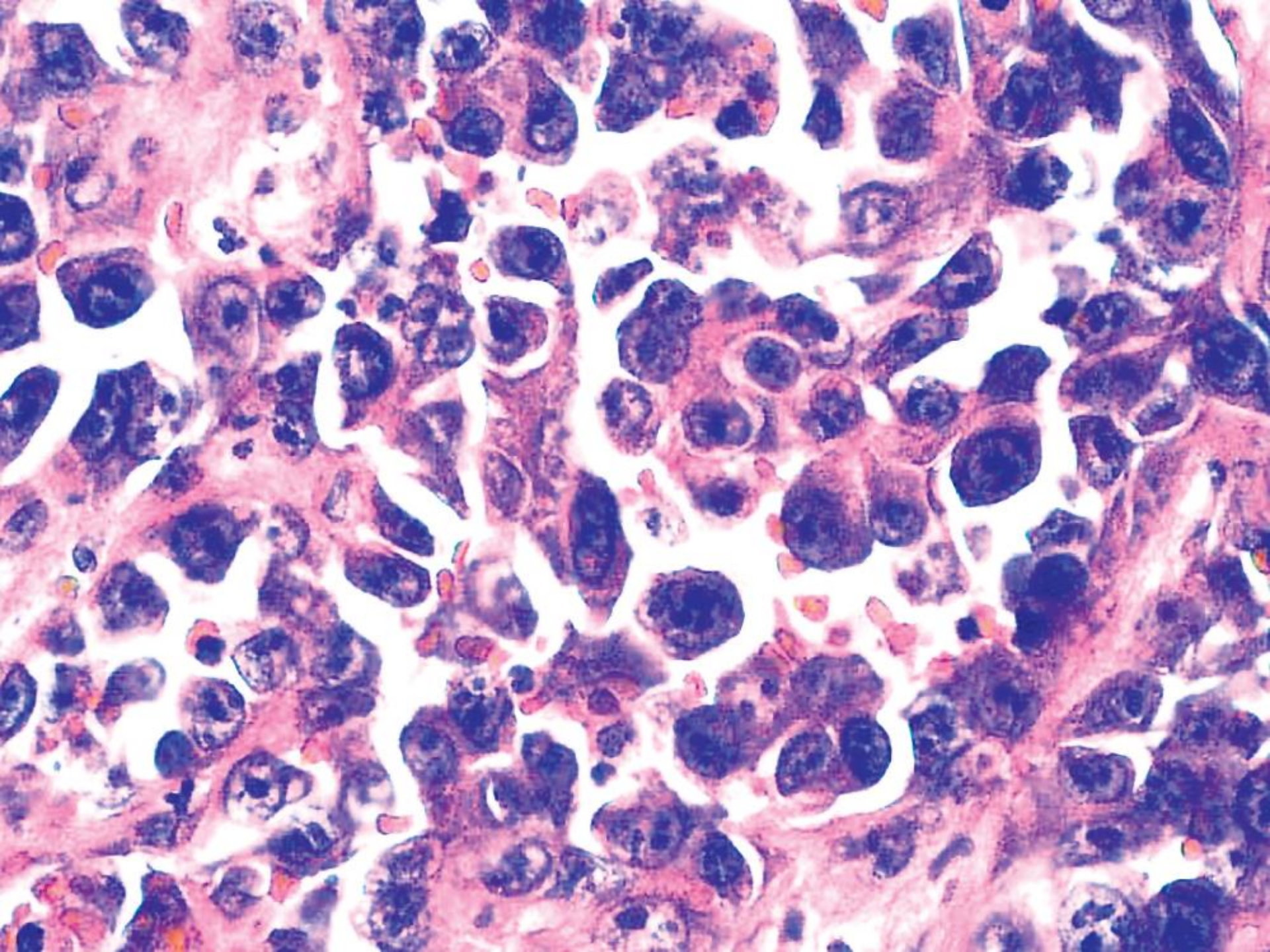


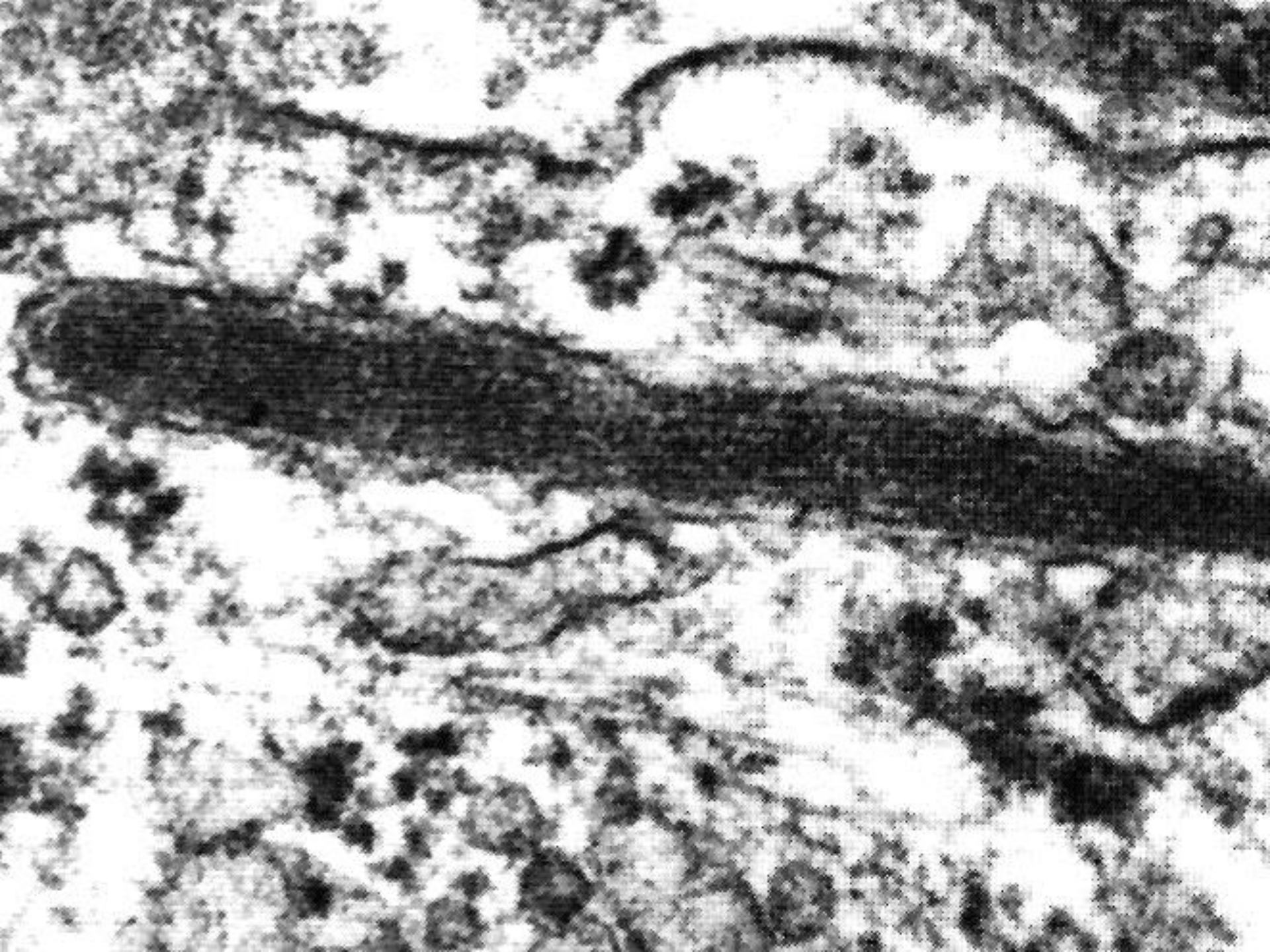


**78 year old man with
abdominal discomfort and liver
masses found on CT of
abdomen**







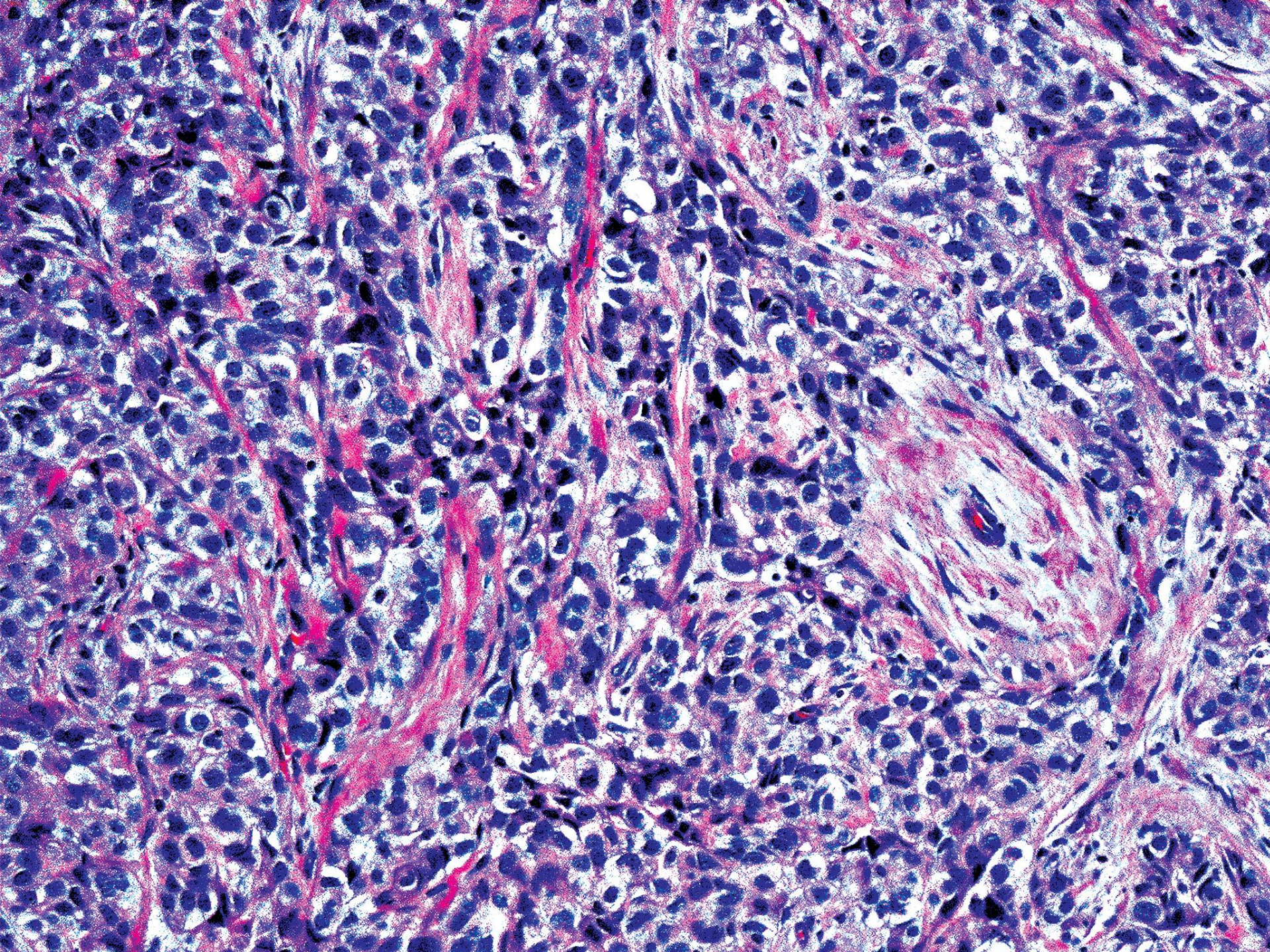


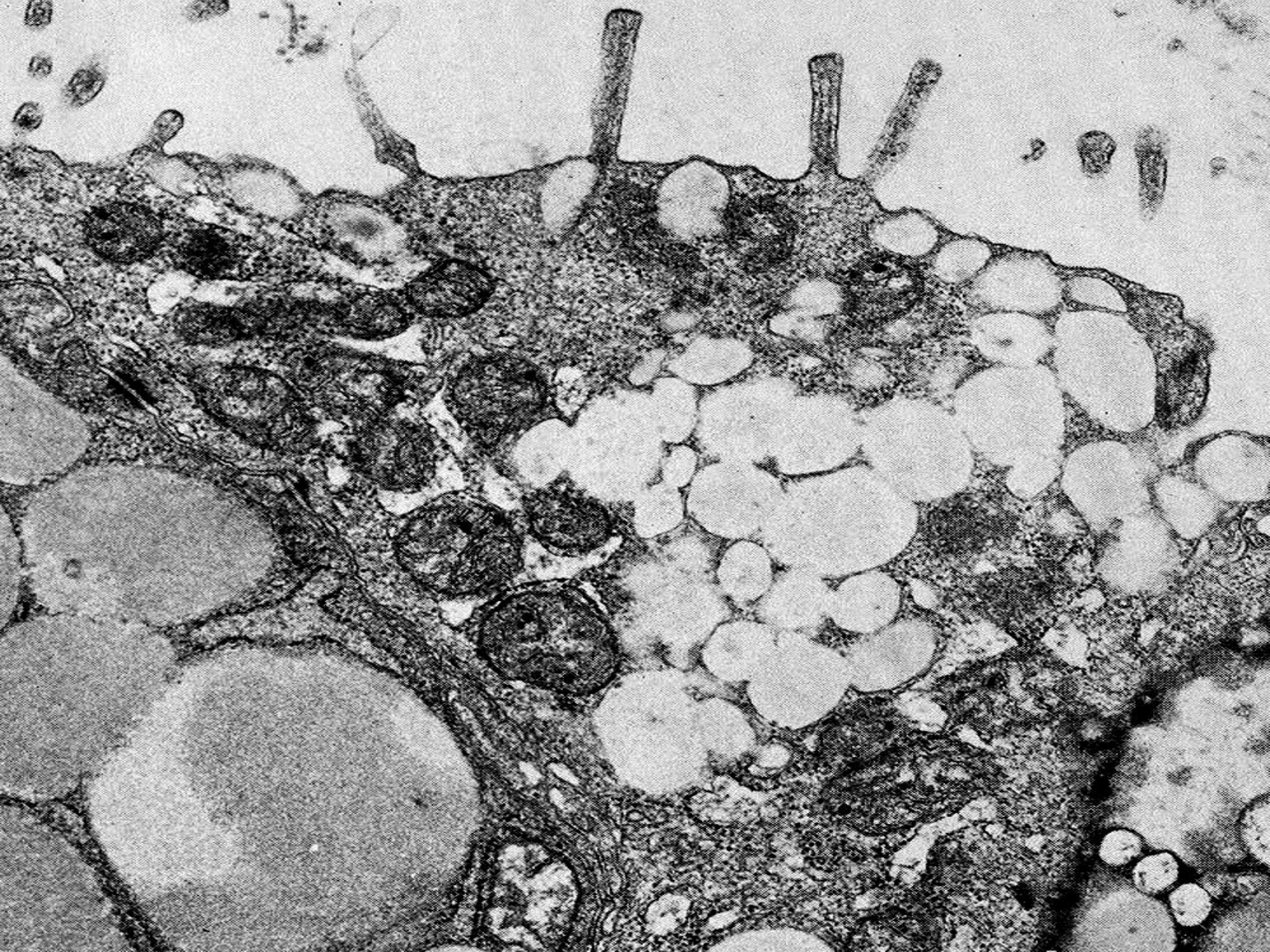
**OTHER SPECIFIC TUMOR
CATEGORIES AMENABLE
TO DIAGNOSIS BY
ELECTRON MICROSCOPY**

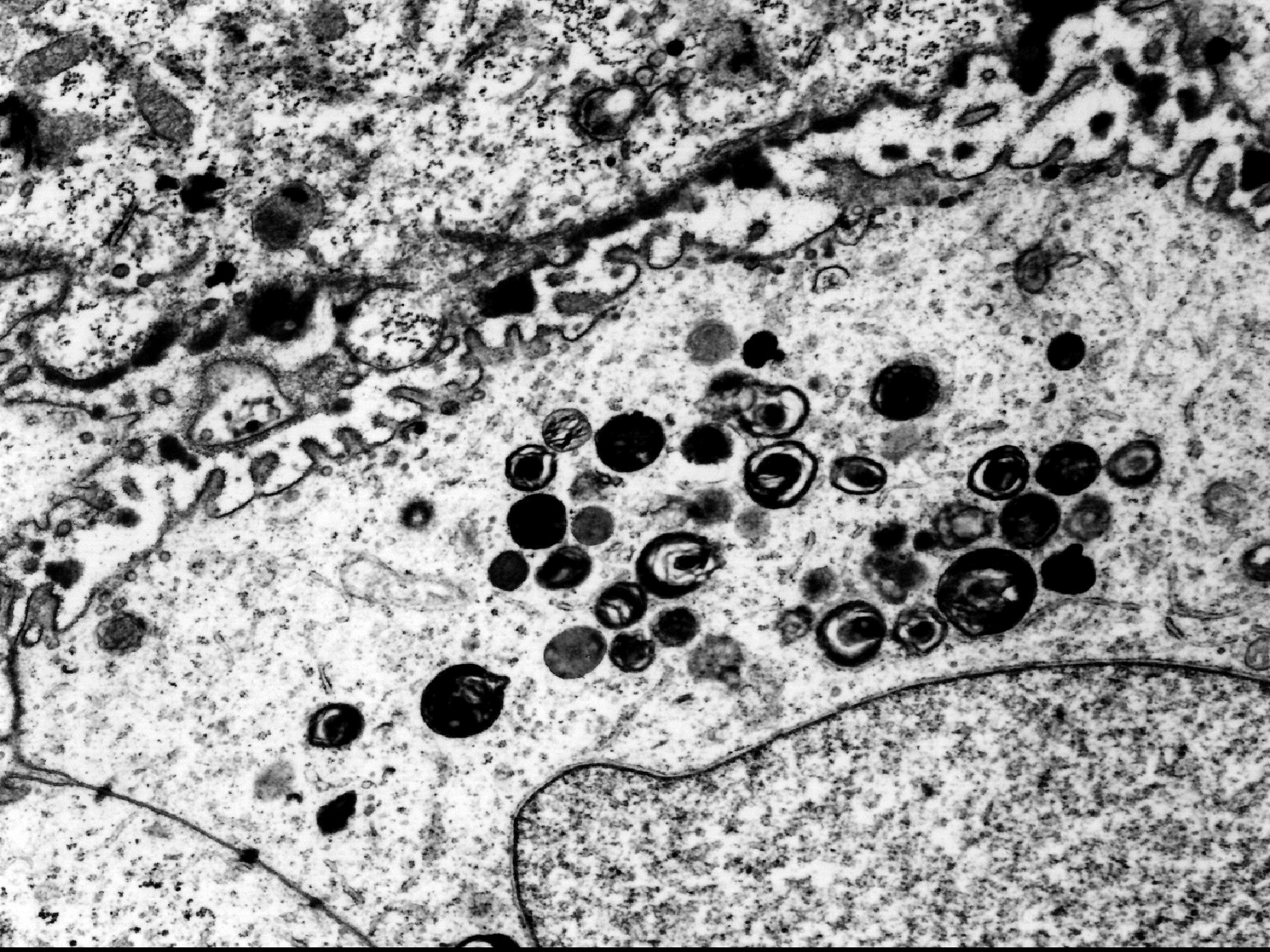
**MESOTHELIOMA VS.
METASTATIC
ADENOCARCINOMA**

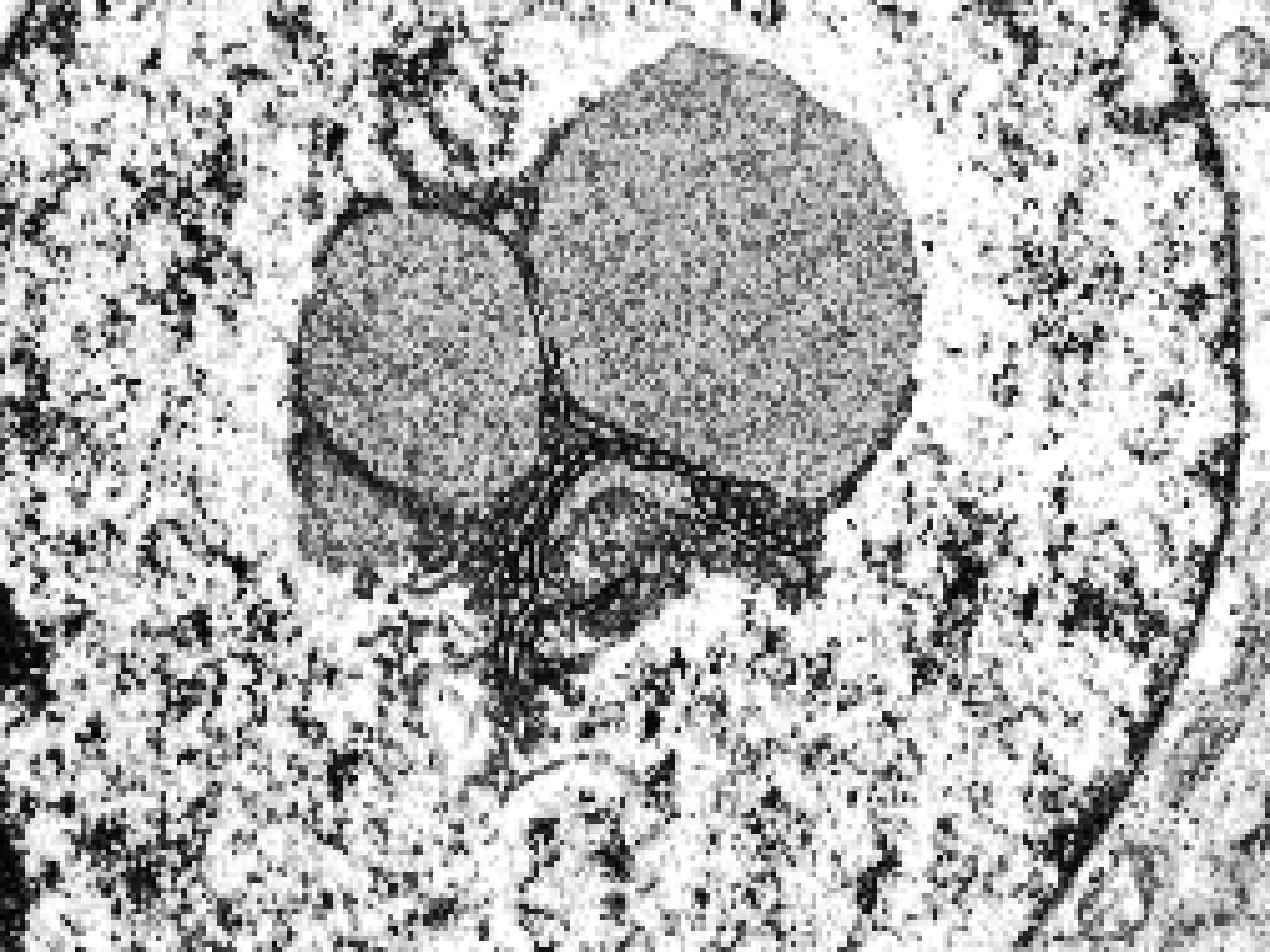
**59 year old man with
progressive shortness of
breath; chest radiographs show
large left pleural effusion and a
pleural-based mass. A pleural
biopsy is done.**









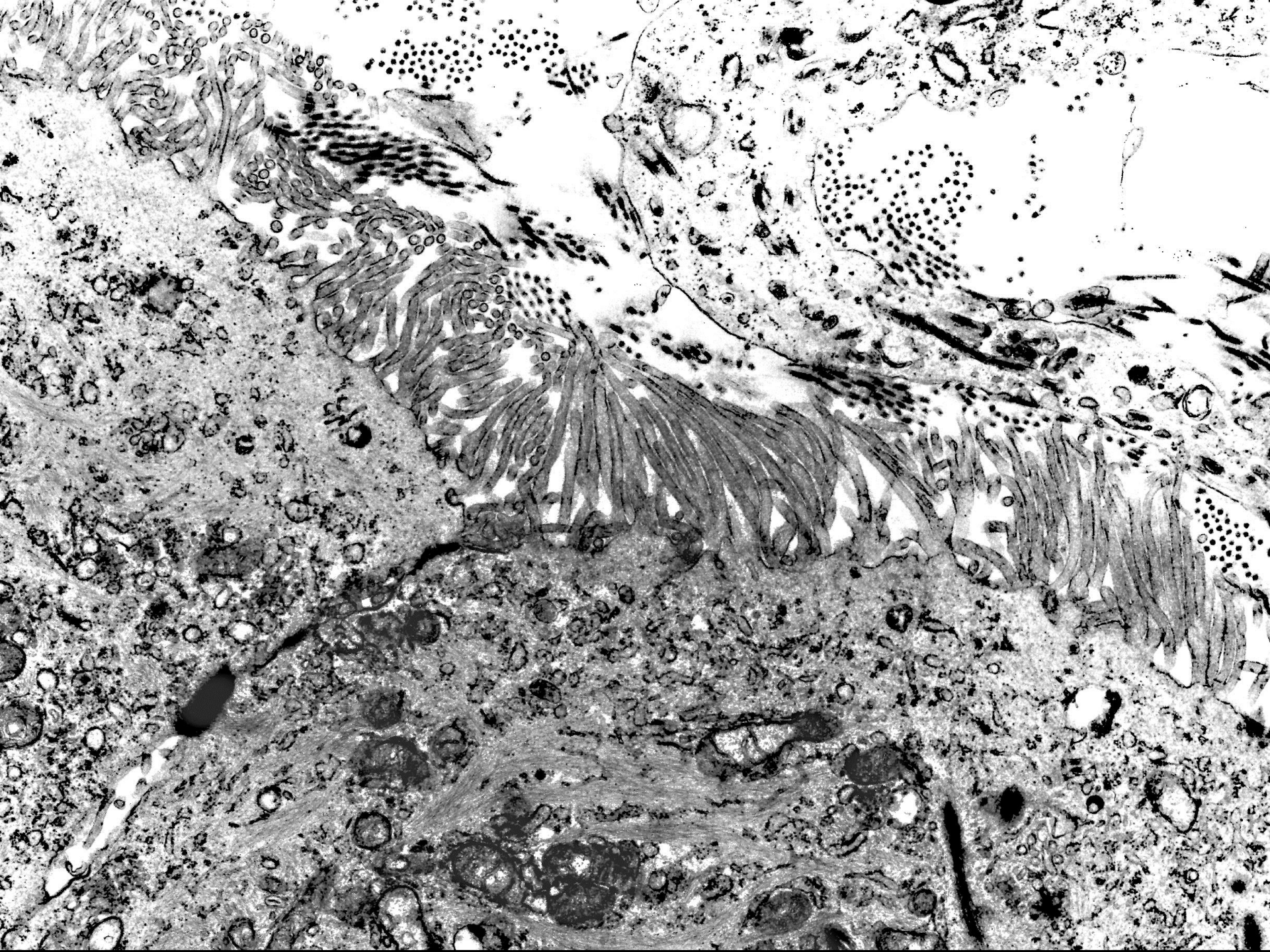


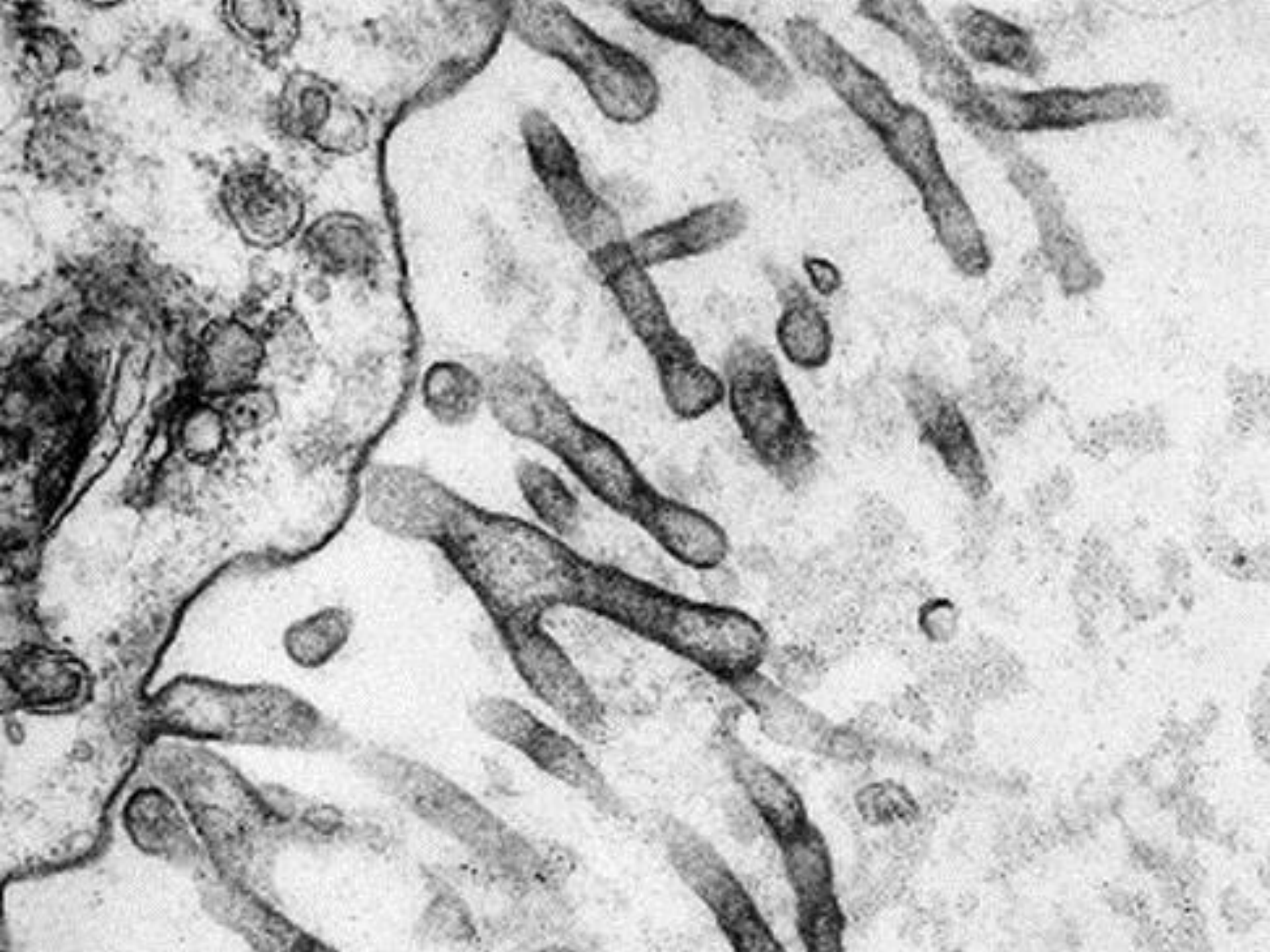
Diagnosis:

**Pseudomesotheliomatous
(pleurotropic) adenocarcinoma**

Mesothelioma: Characteristic Ultrastructural Findings

- Elongated branching microvilli with length-to-diameter ratios of at least 12:1
- Elongated complex intercellular junctions
- Prominent cytoplasmic filament skeins
- NO* cytoplasmic mucin granules





PEDIATRIC SMALL ROUND-CELL NEOPLASMS

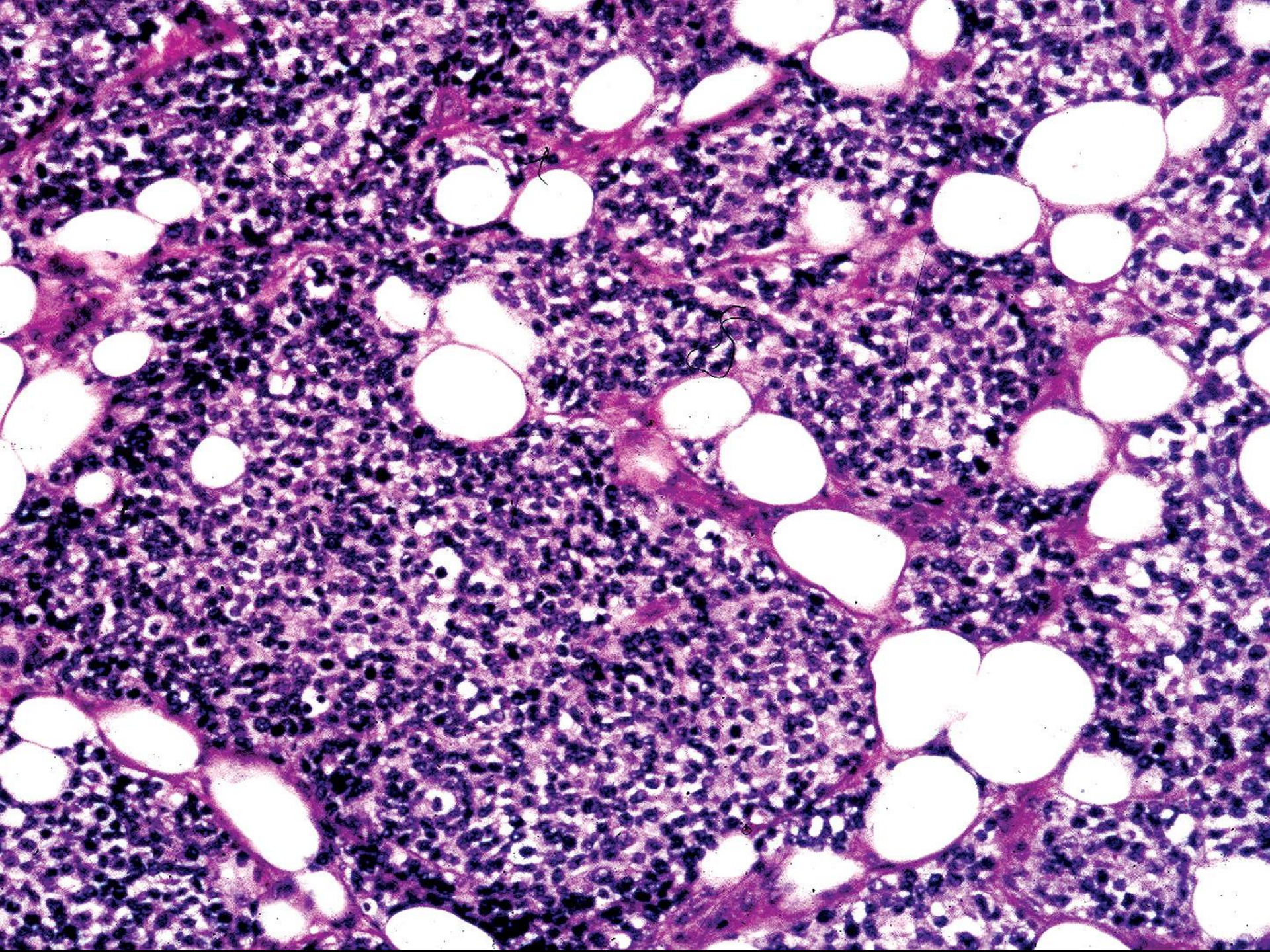
SMALL CELL TUMORS OF CHILDHOOD: ULTRASTRUCTURAL DIFFERENTIAL DIAGNOSIS

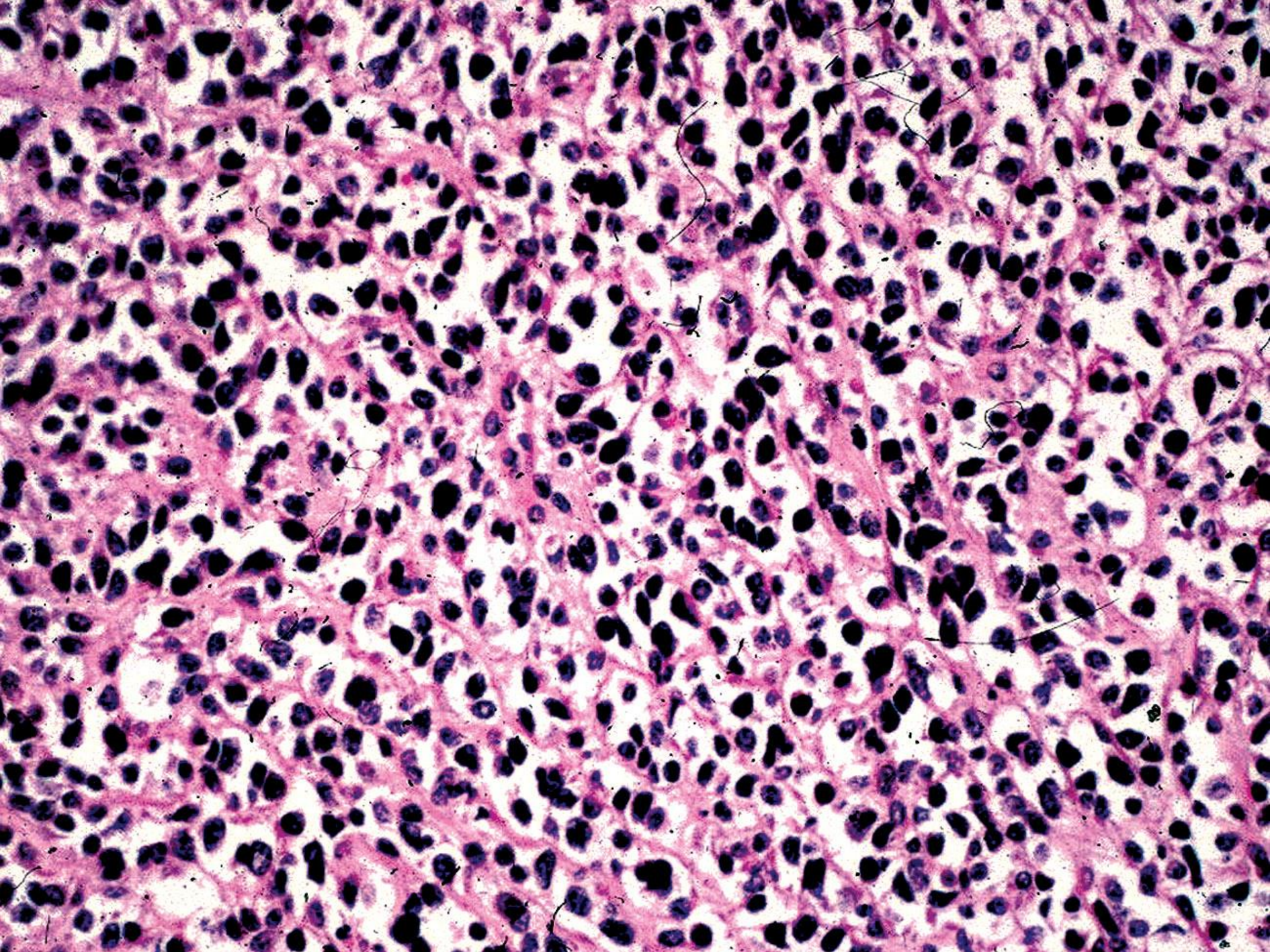
<u>TUMOR</u>	<u>JUNX</u>	<u>BL</u>	<u>NSG</u>	<u>FIL</u>	<u>GLY</u>	<u>CP</u>	<u>MT</u>
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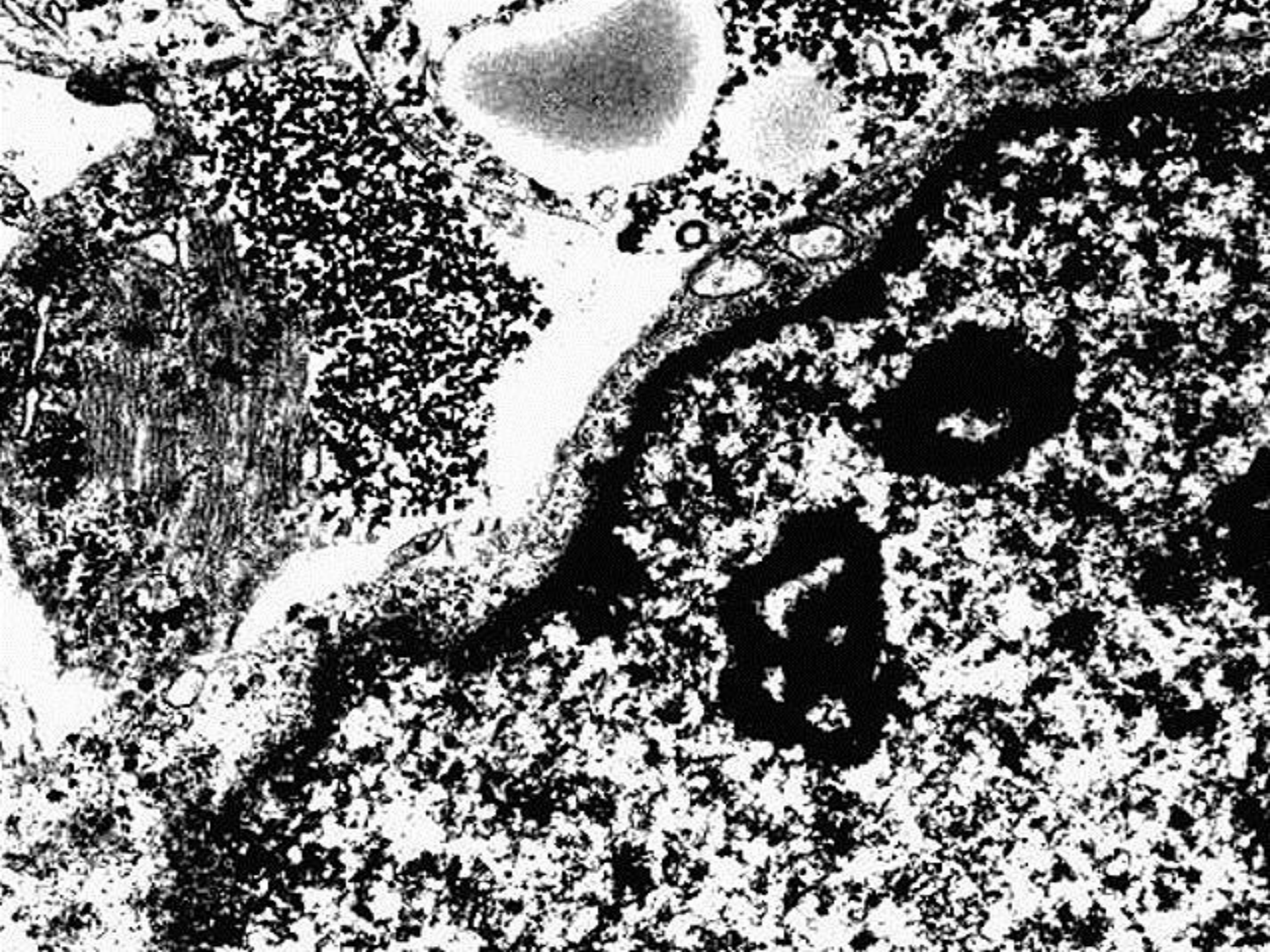
(JUNX = Intercellular junctions; BL = Basal lamina; NSG = Neurosecretory granules;
FIL = Cytoplasmic filaments; GLY = Glycogen pools; CP = Cytoplasmic processes;
MT = Cytoplasmic microtubules)

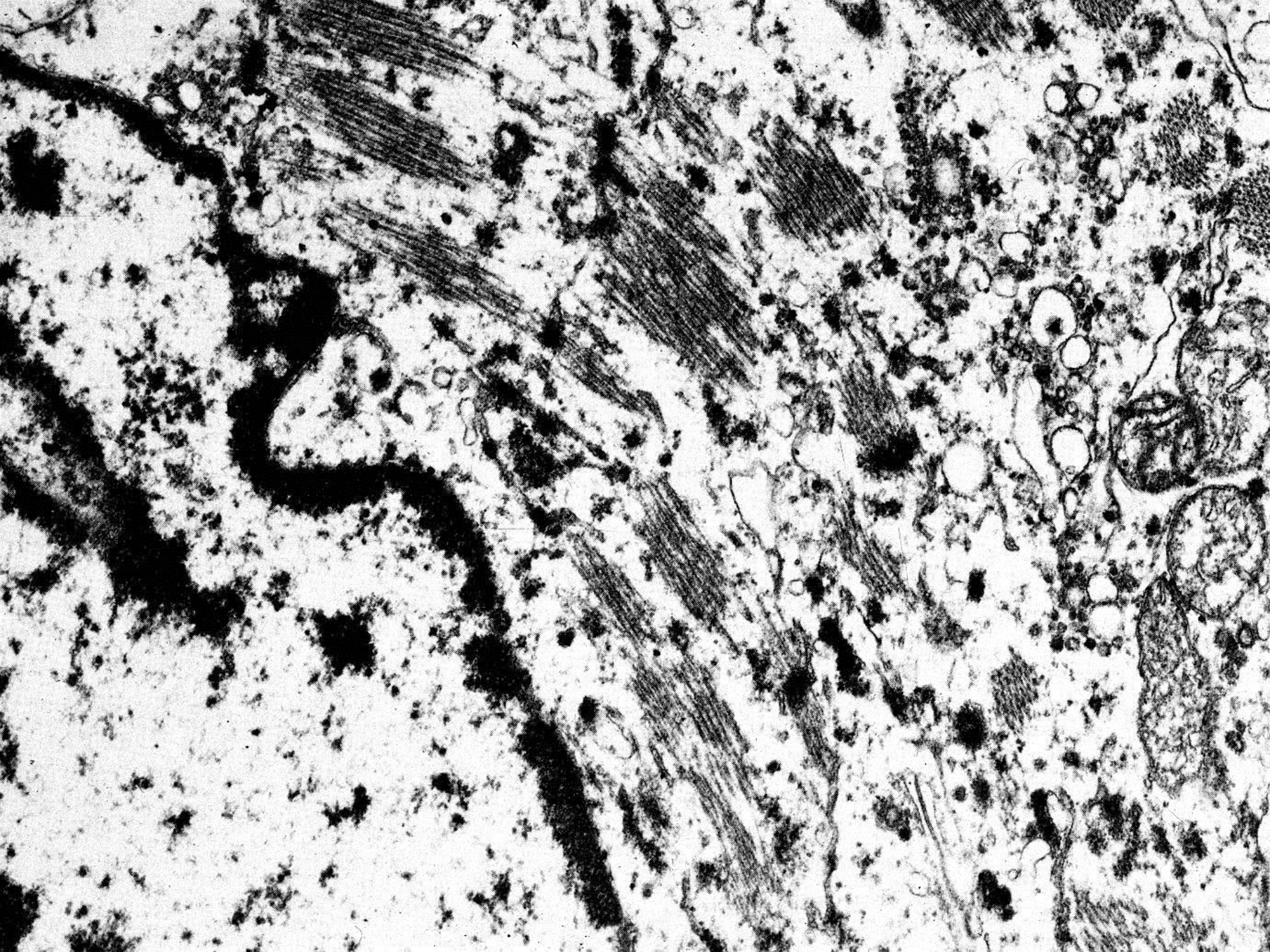
<i>PNET</i>	+/-	0	+/-	+/-	+/-	+	0
<i>NEUROBLASTOMA</i>	+/-	0	+	0	+/-	++	++
<i>LYMPHOMA</i>	0	0	0	0	0	0	0
<i>WILMS' TUMOR</i>	+	+	0	+/-	+/-	0	0
<i>RHABDOMYOSARC</i>	+/-	+	0	++	+	0	0

**Asymptomatic 13 year old boy
with a mass in the right
shoulder that he found while
bathing**





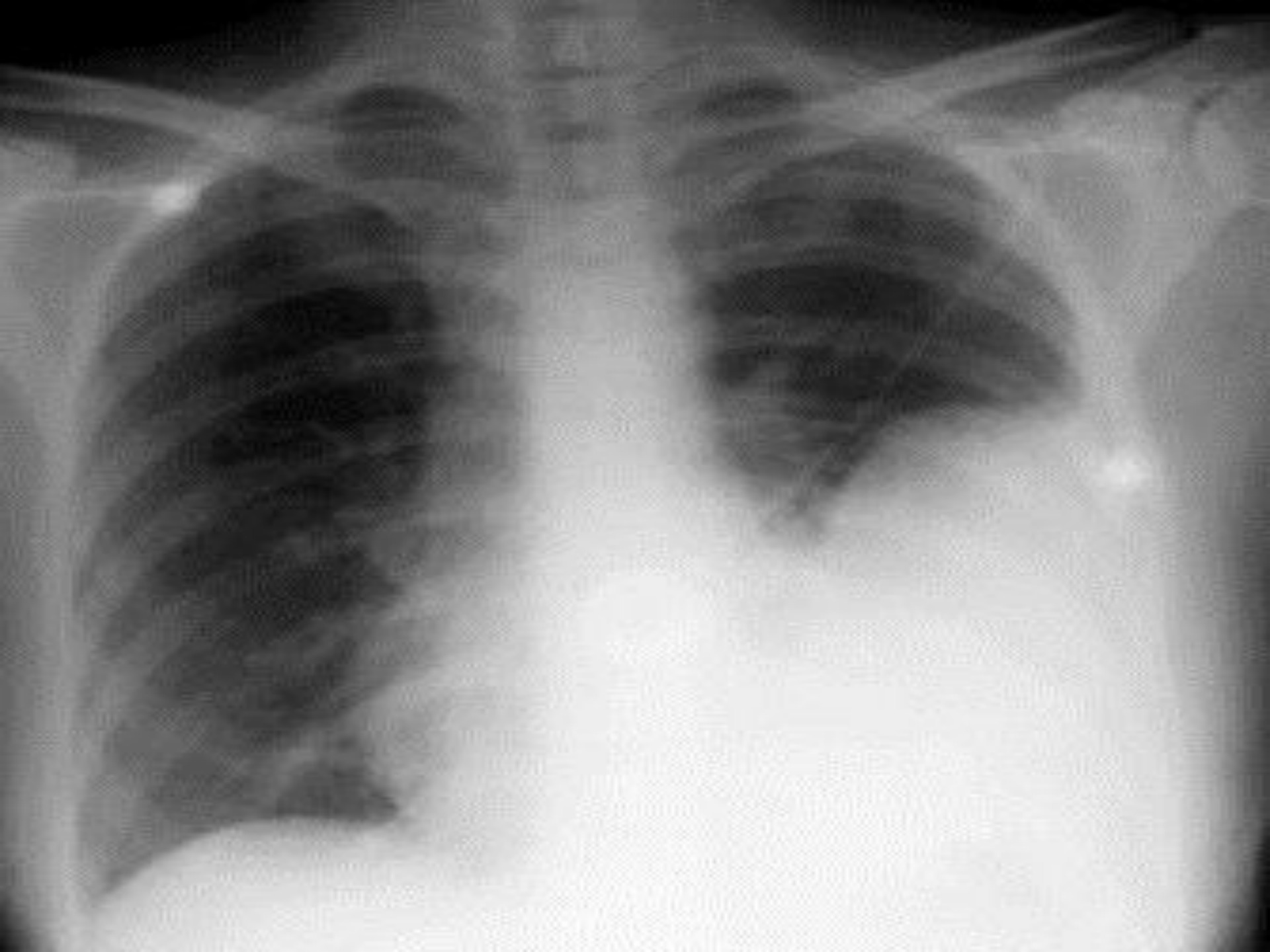


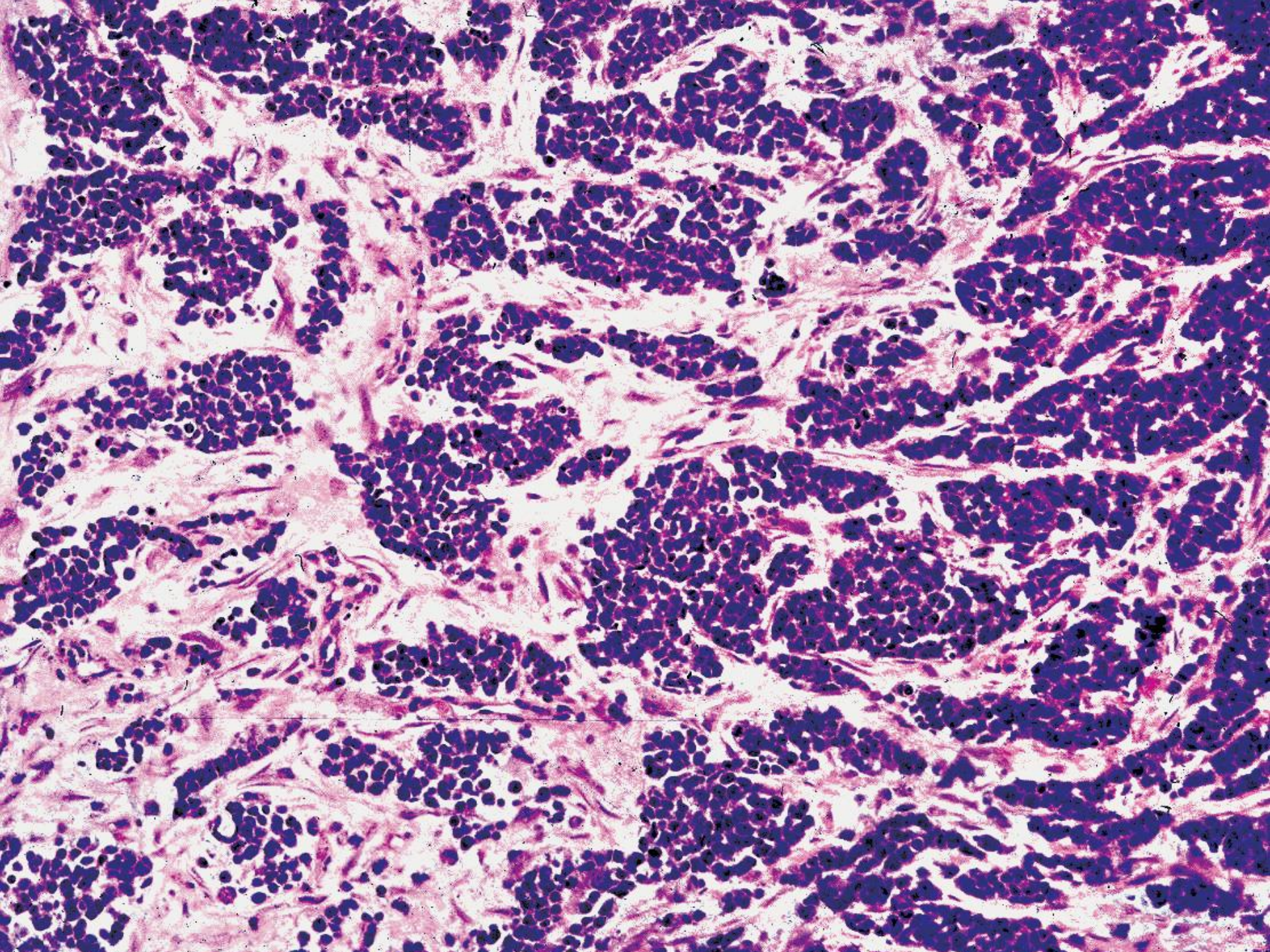


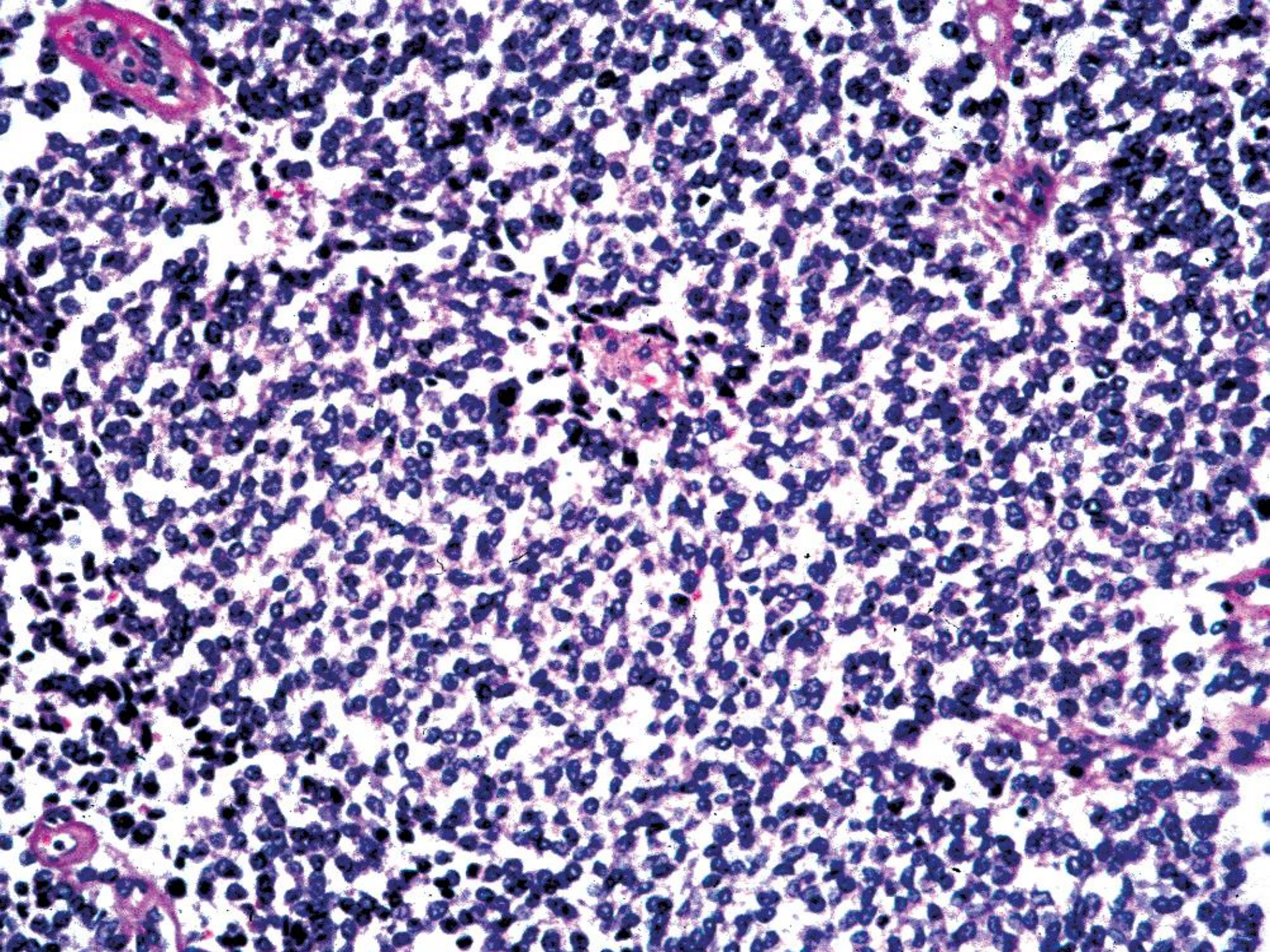
Diagnosis:

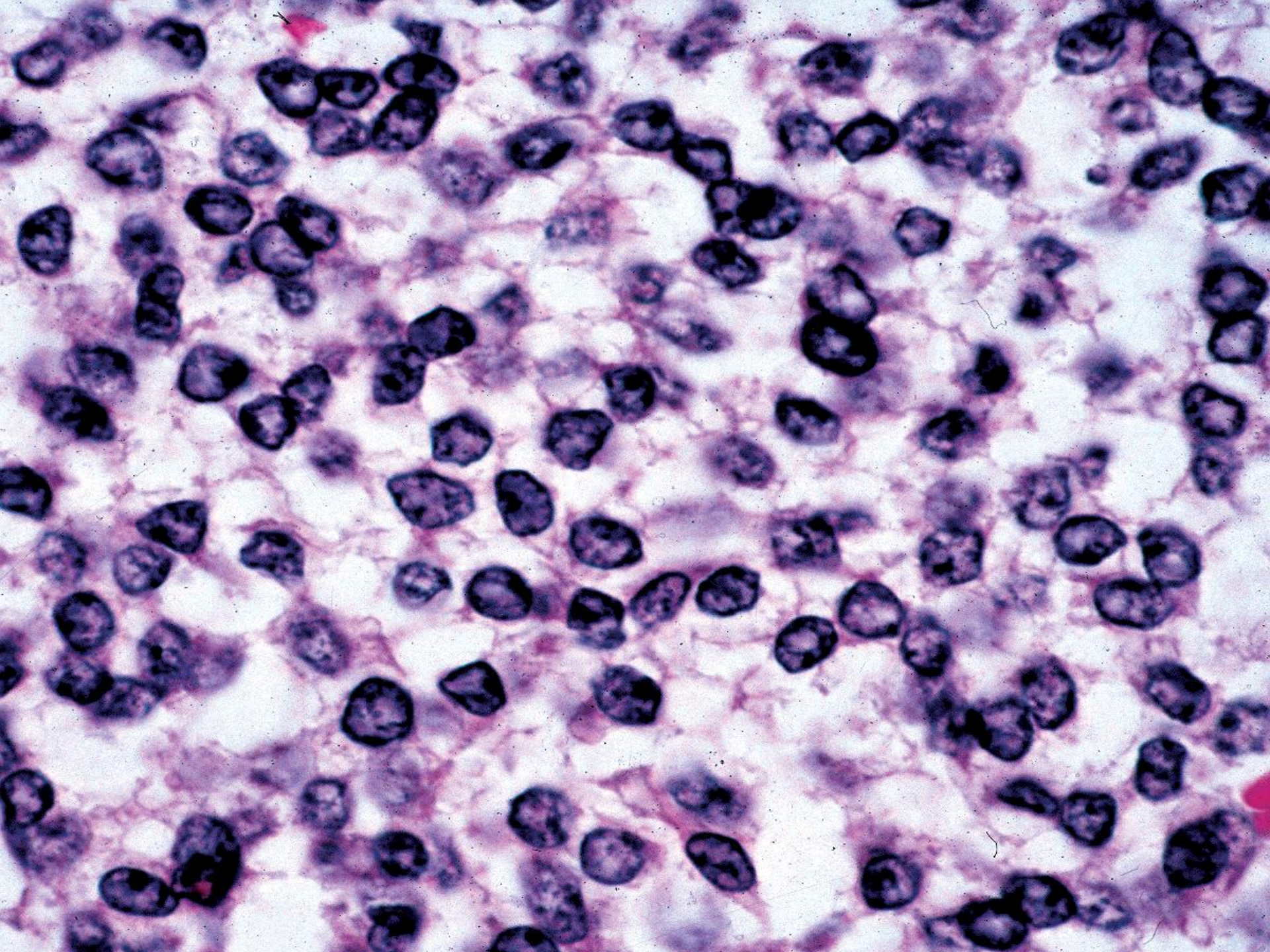
Alveolar rhabdomyosarcoma

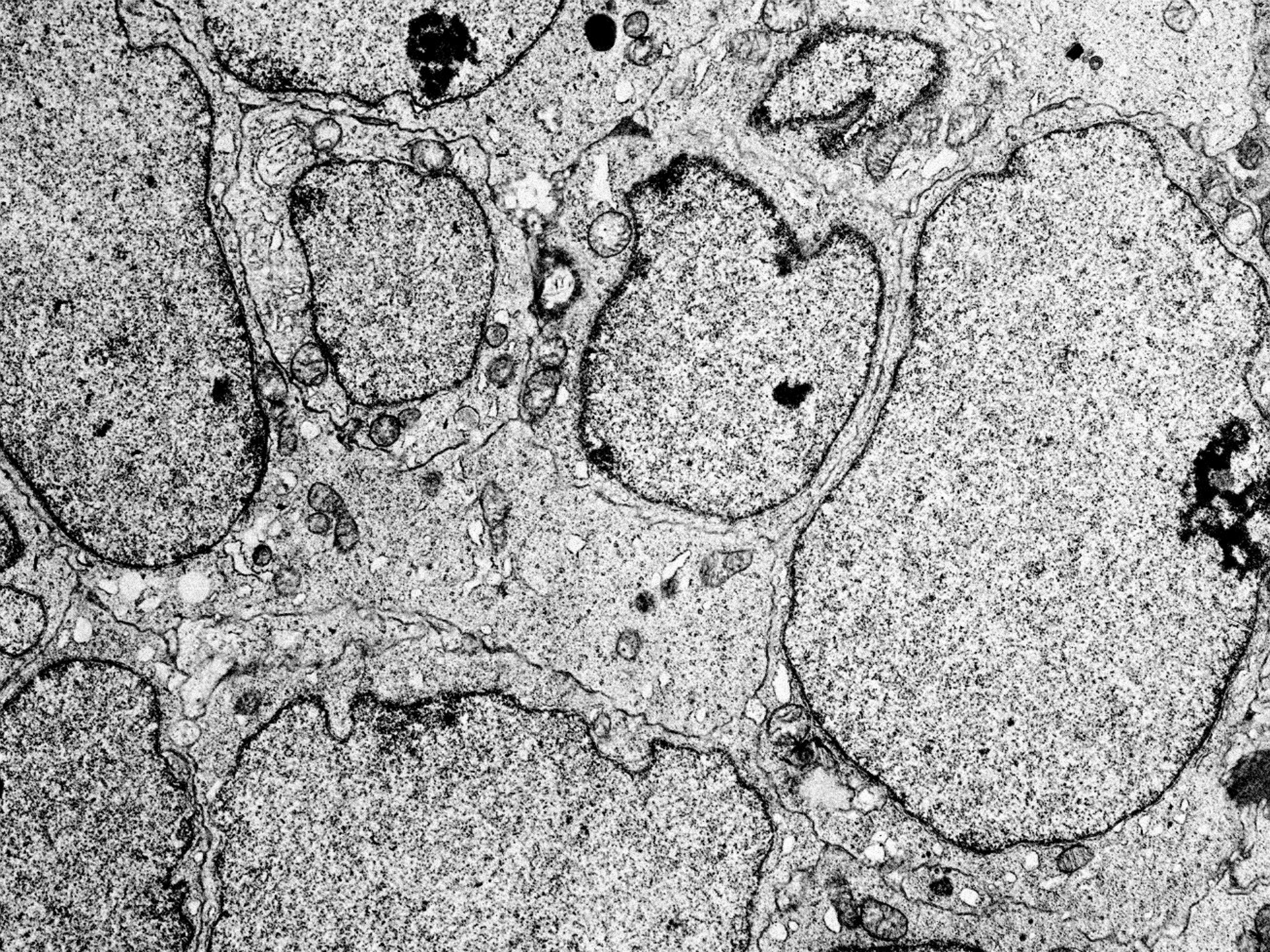
**19 year old boy with
progressive left-sided chest
pain and shortness of breath**







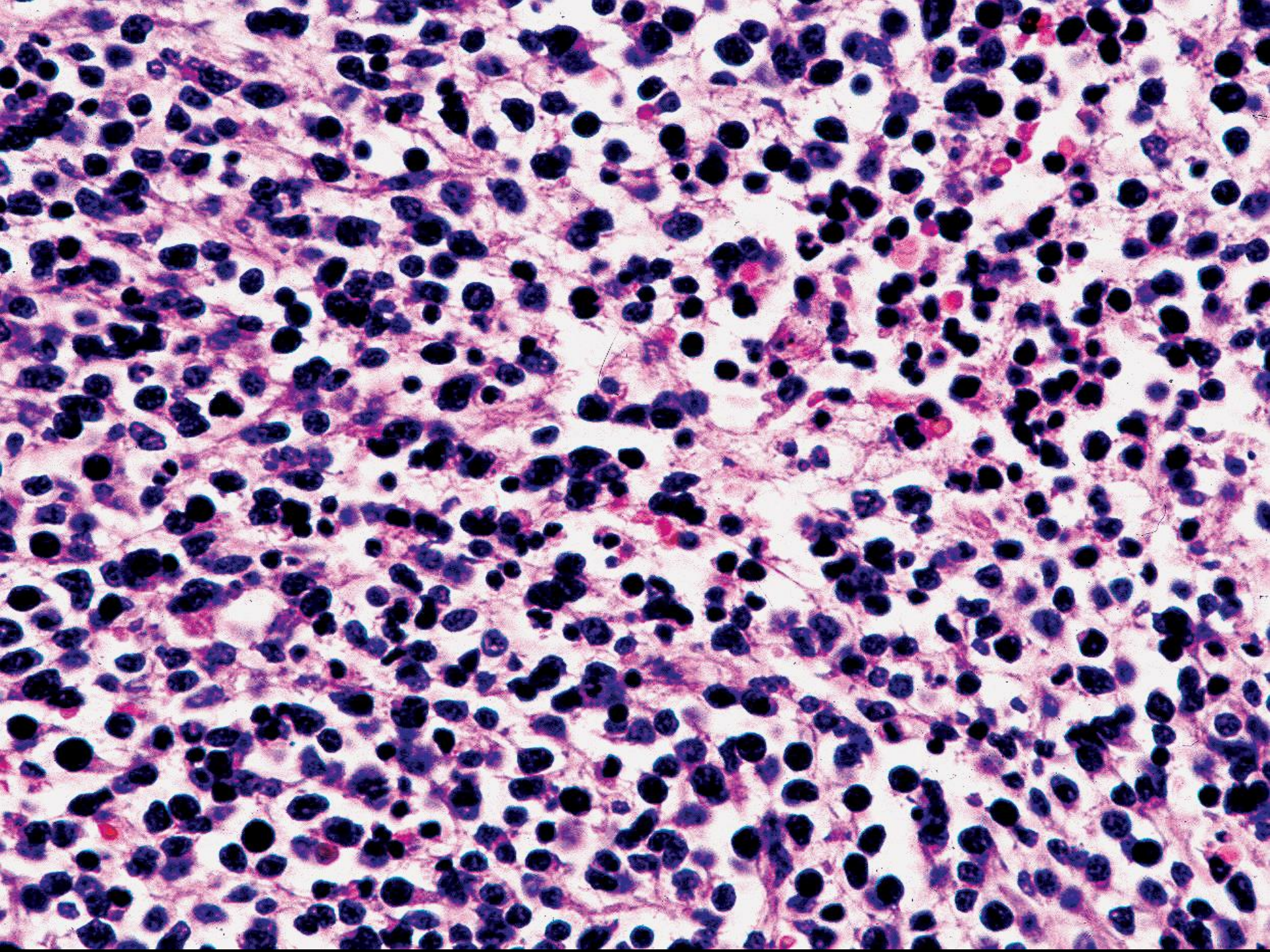




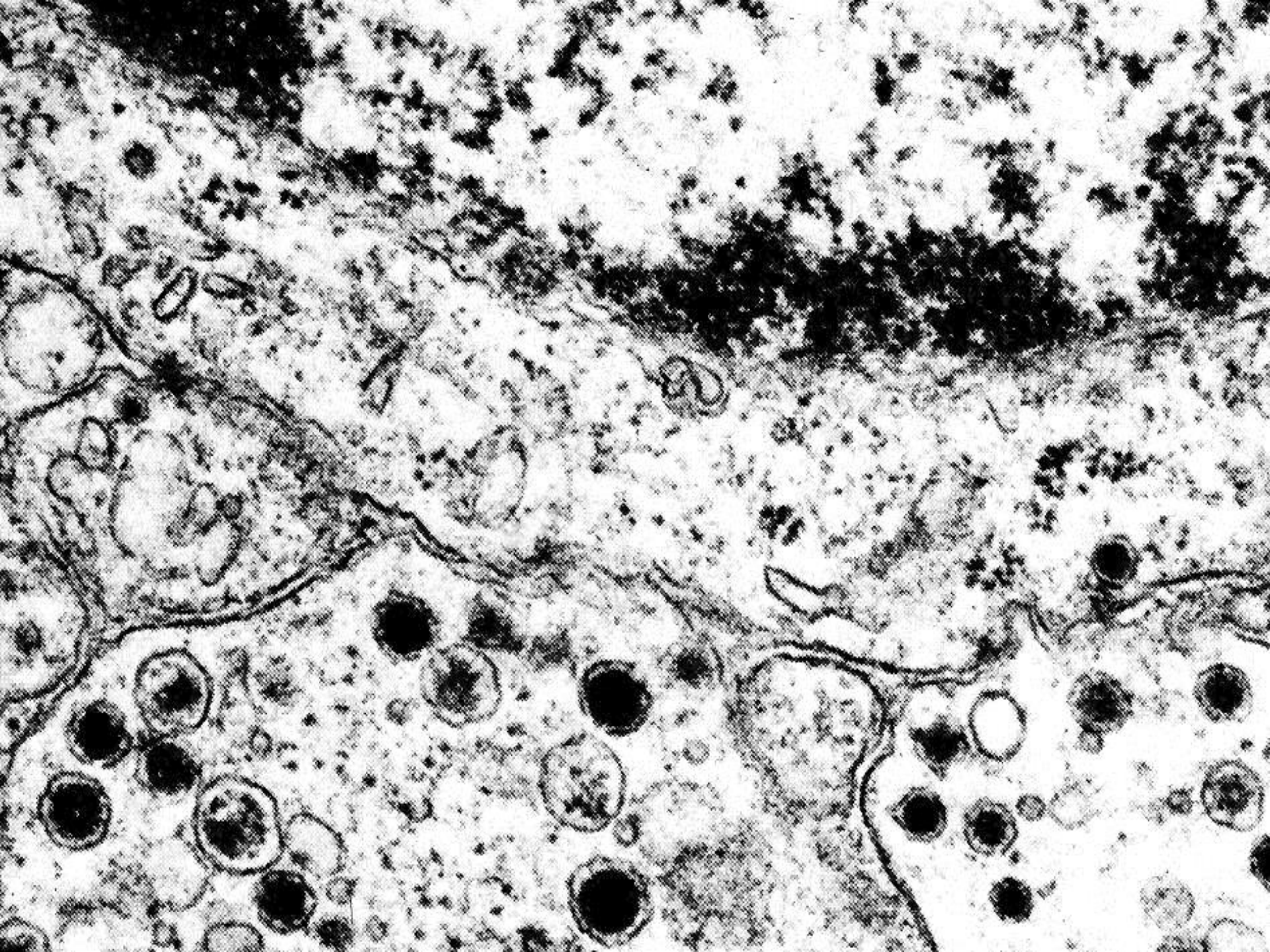
Diagnosis:

**Primitive neuroectodermal
tumor (Askin tumor)**

**2 year old boy with an
abdominal tumor found by his
mother; CT scan of the
abdomen demonstrated a
paravertebral retroperitoneal
mass**







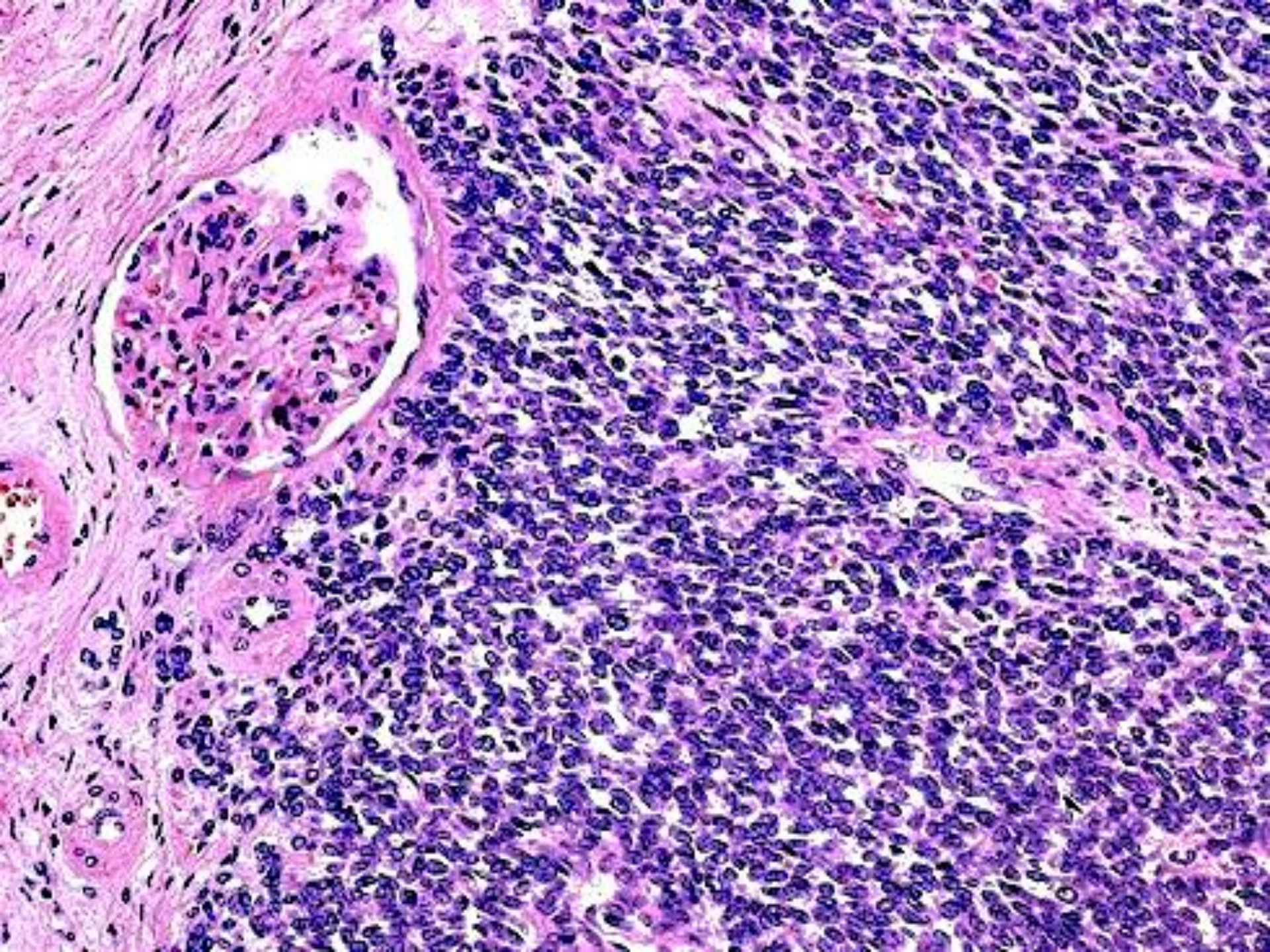
Diagnosis:
Neuroblastoma

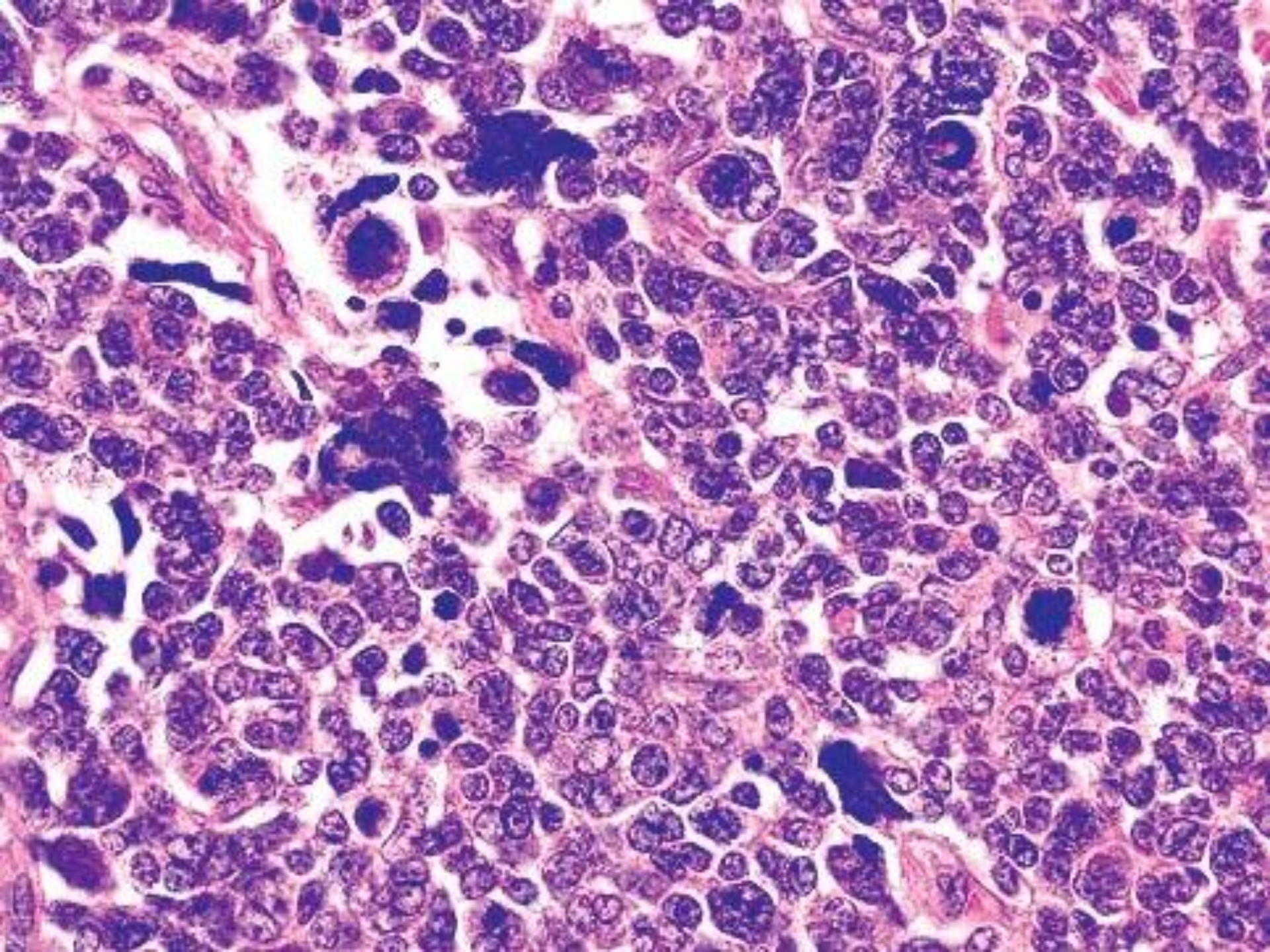
NEUROBLASTOMA VS. PNET: RELATED OR NOT?

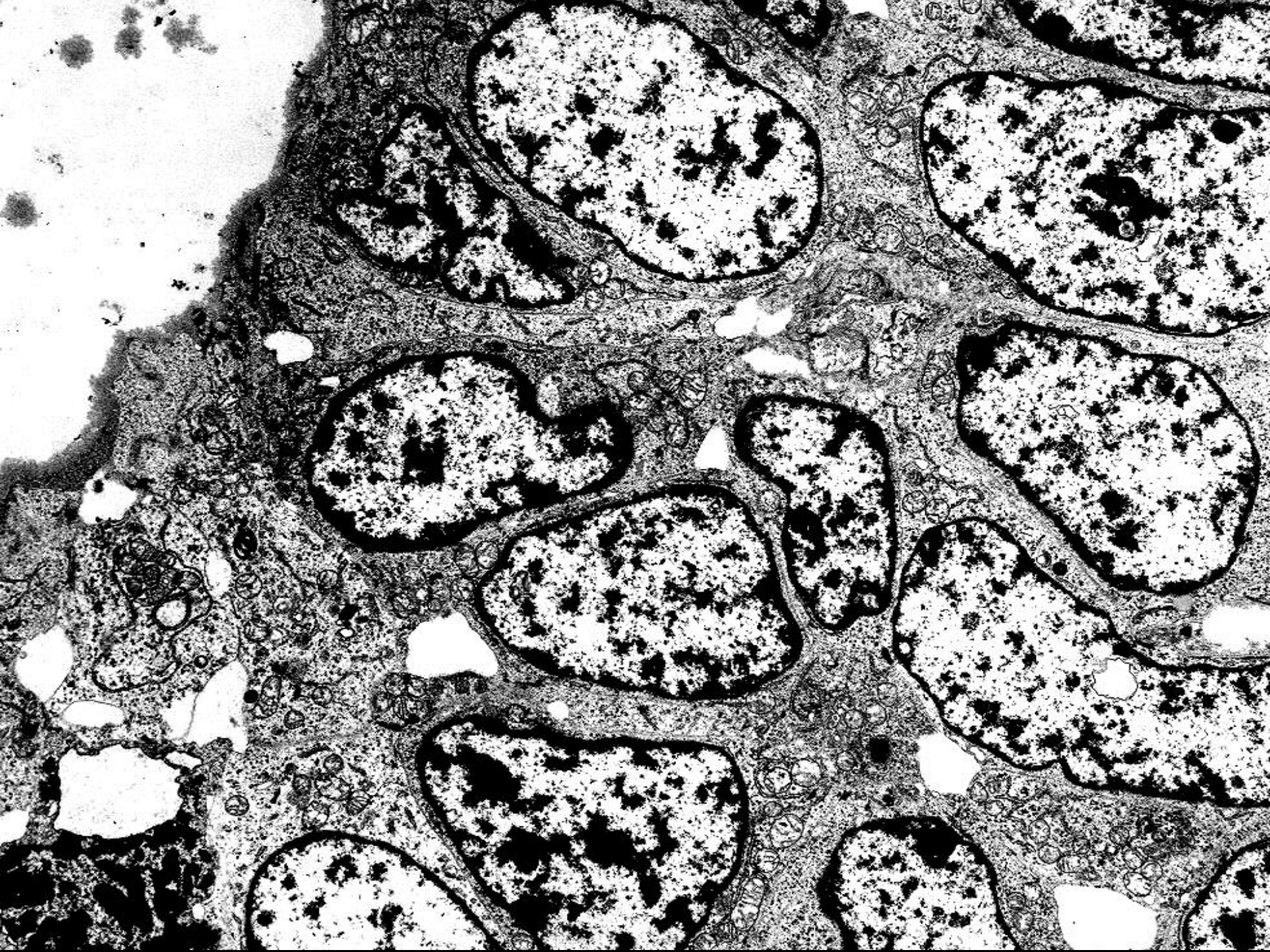
- **Similar but not identical ultrastructural features and immunophenotype**
(Neuroblastoma-- VIM \pm ; NF \pm ; SYN \pm ; NSE+; Leu 7+; DES--; CK--; CD99--
PNET-- VIM \pm ; NF \pm ; SYN \pm ; NSE+ ; Leu 7 \pm ; DES \pm ; CK \pm ; CD99+
- **Neuritic differentiation** (synaptic vesicles; microtubules) much more well-developed in NBL, as compared with PNET
- 11;22 chromosomal translocation of PNET not shared by neuroblastoma
- VMA/HVA synthesis of neuroblastoma is not shared by PNET

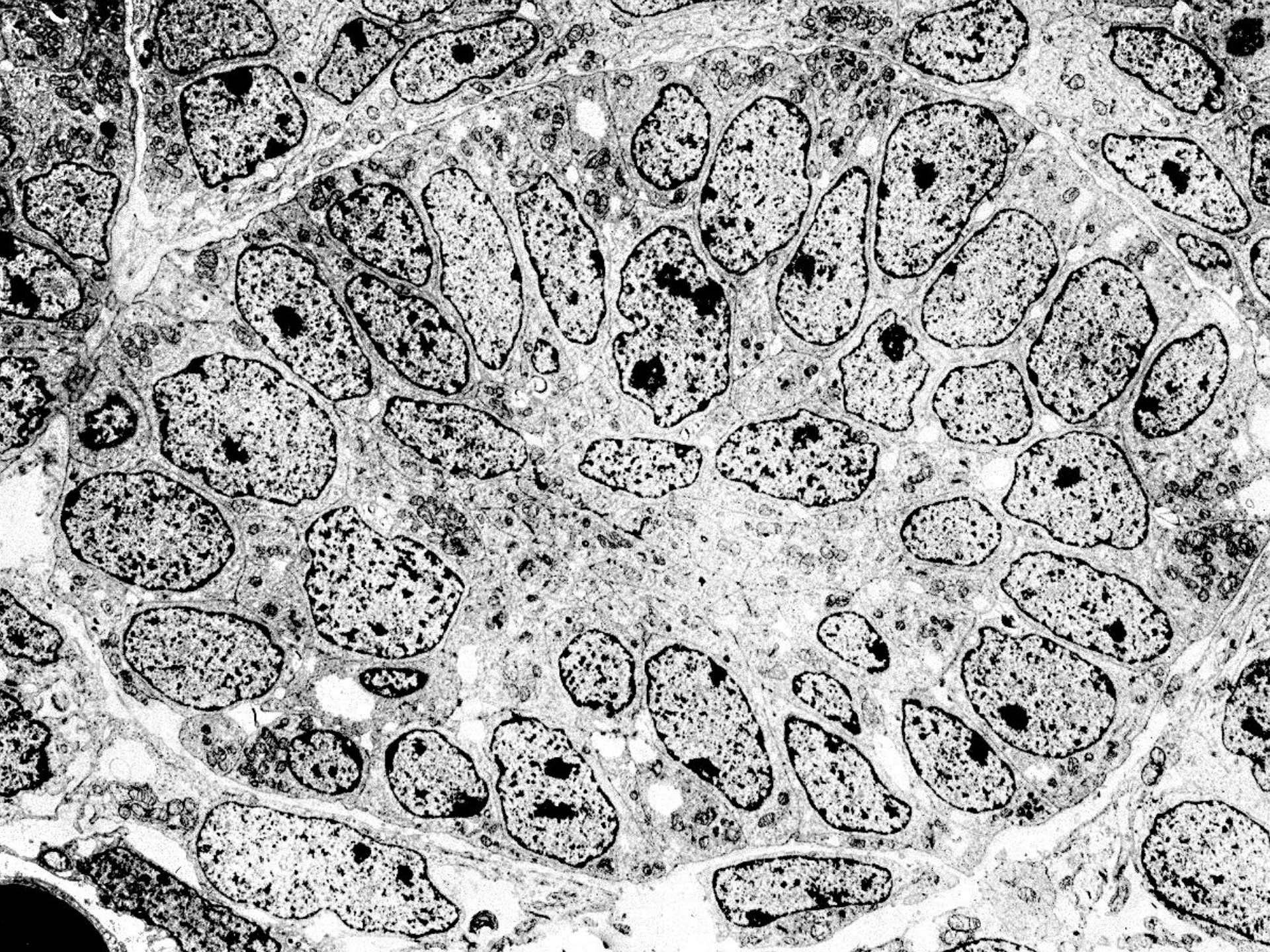
**2 year old girl with a left
abdominal mass, shown to be
renal in nature on CT scan**





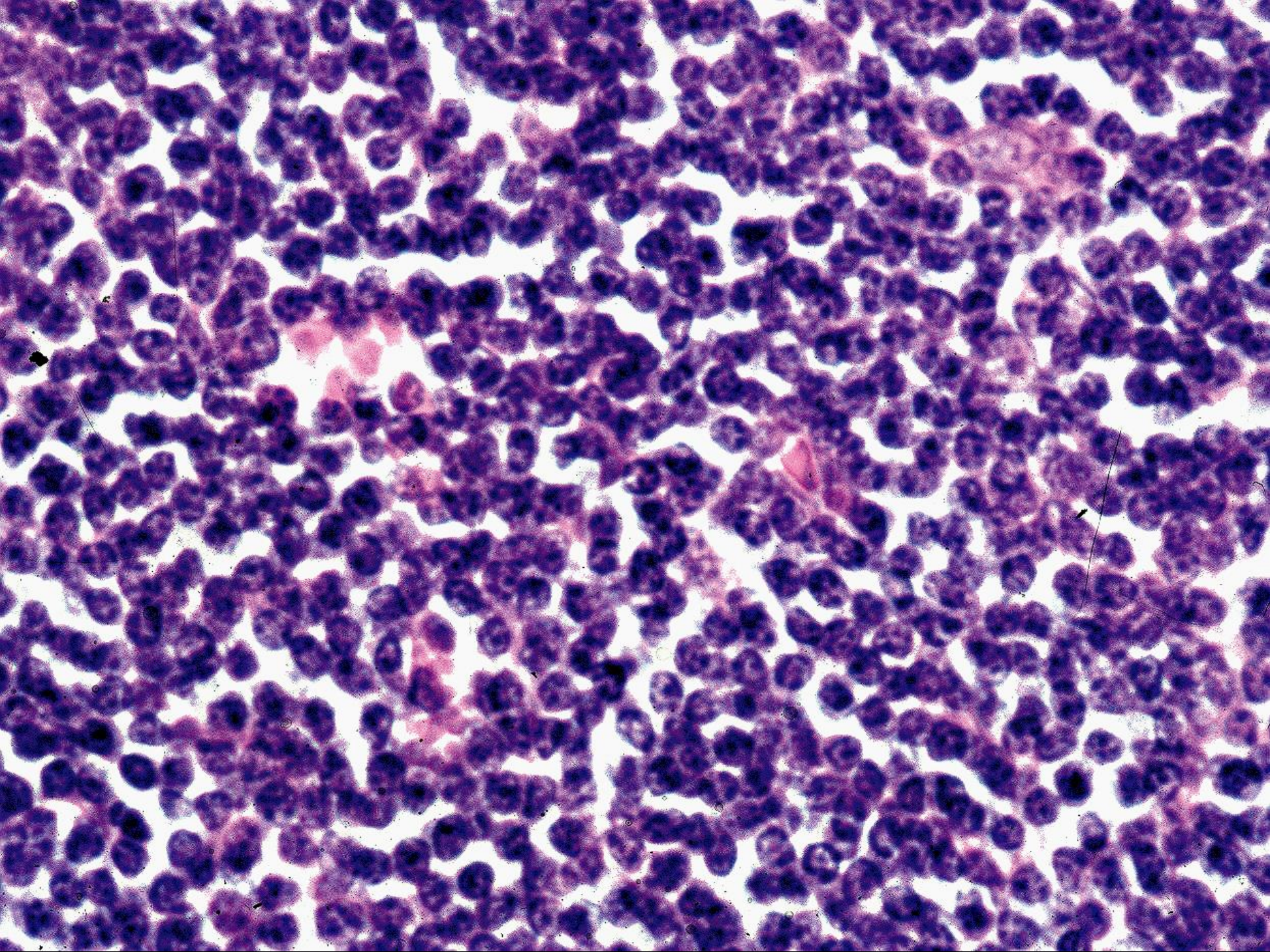






Diagnosis:

**Wilms' tumor (blastema-
predominant with anaplasia &
“occult” epithelial
differentiation)**



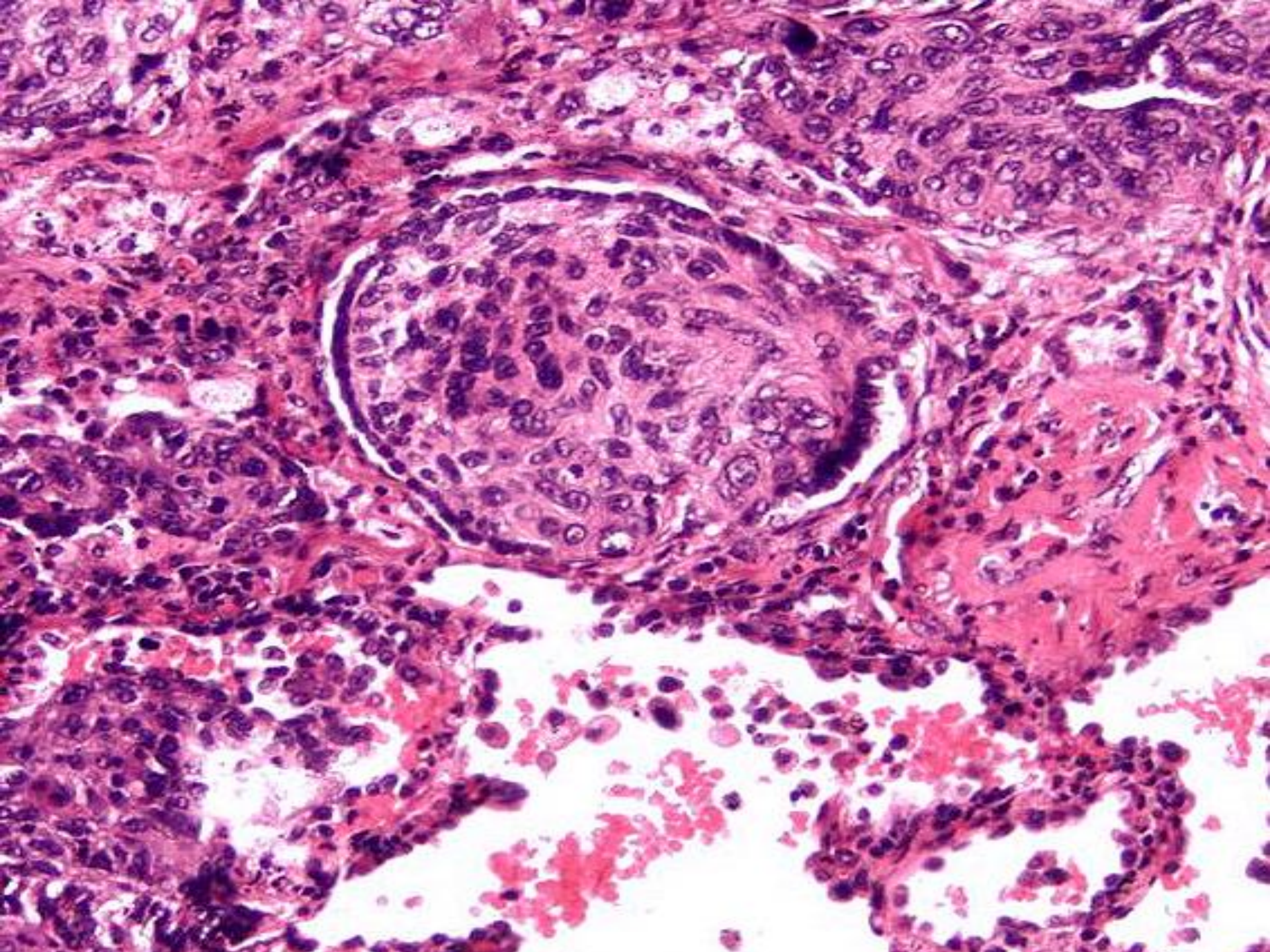
MALIGNANT LYMPHOMA: A “HAVE-NOT” SMALL ROUND- CELL TUMOR

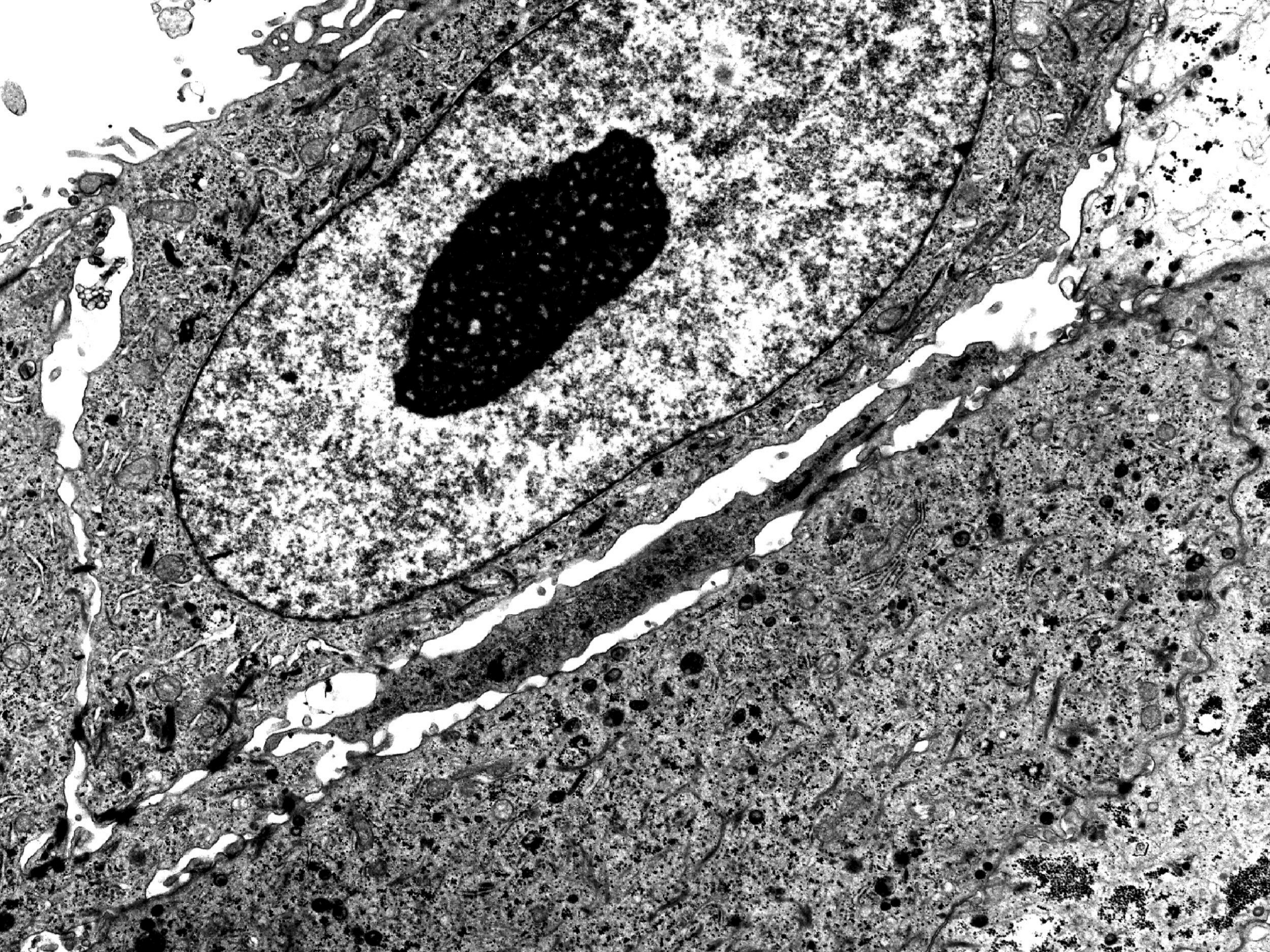
Lymphomas *lack*:

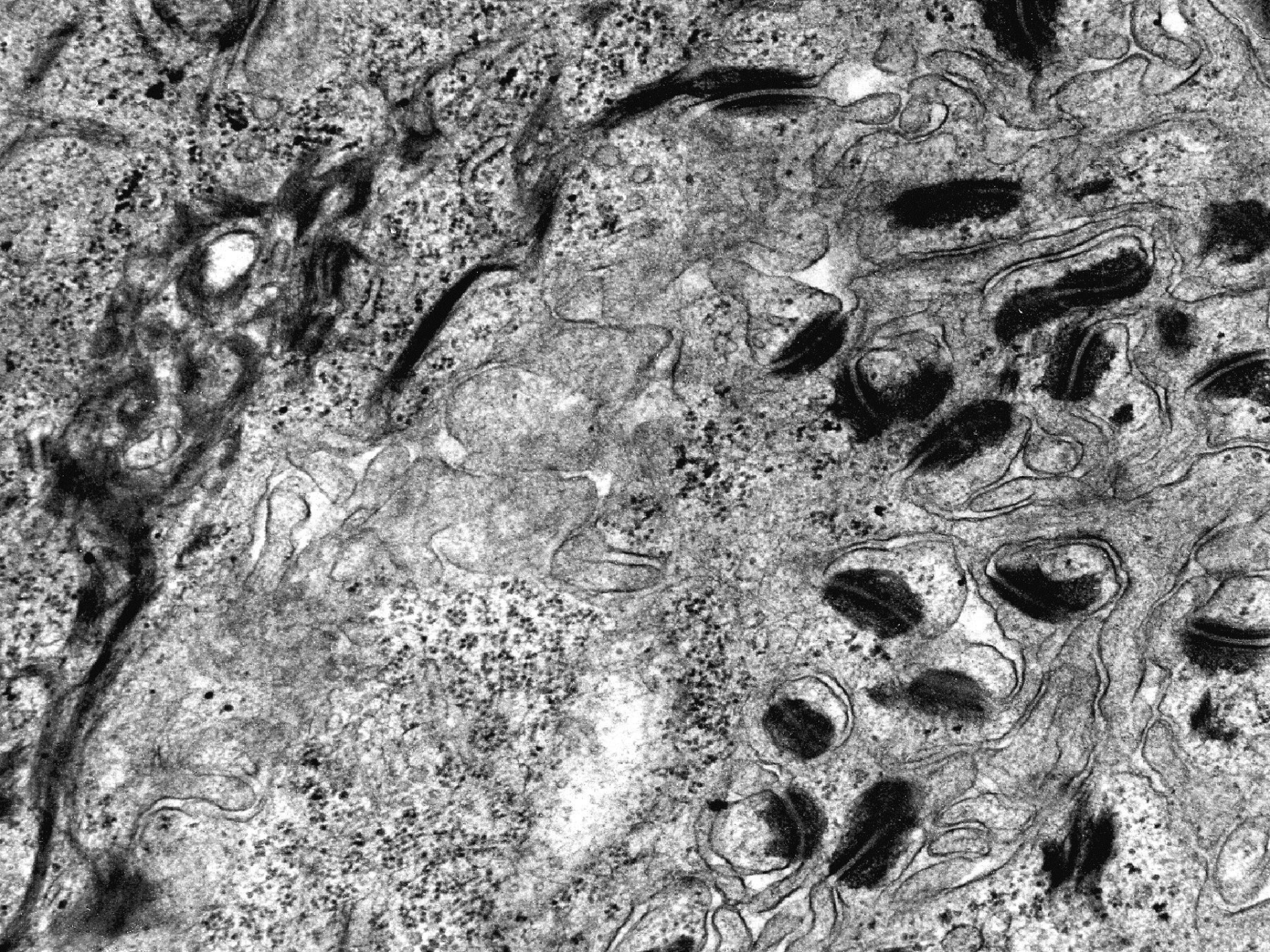
- Intercellular junctions
- Cytoplasmic filament skeins
- Microtubules
- Neurosecretory granules (*caution: lysosomes can resemble these*)
- Glycogen*
- Cell processes
- Basal lamina

POORLY-DIFFERENTIATED LUNG CARCINOMAS

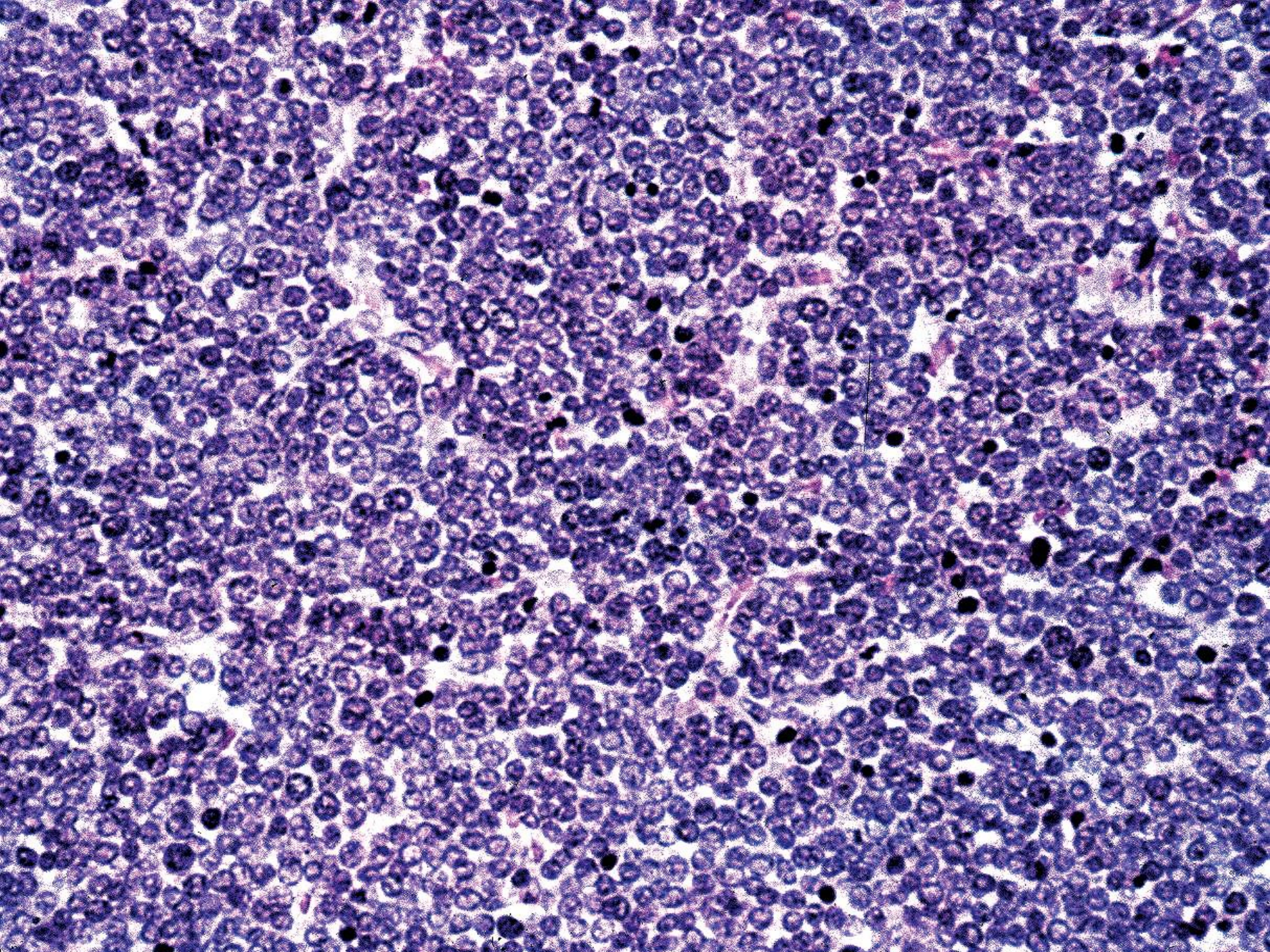
Squamous Cell Carcinoma

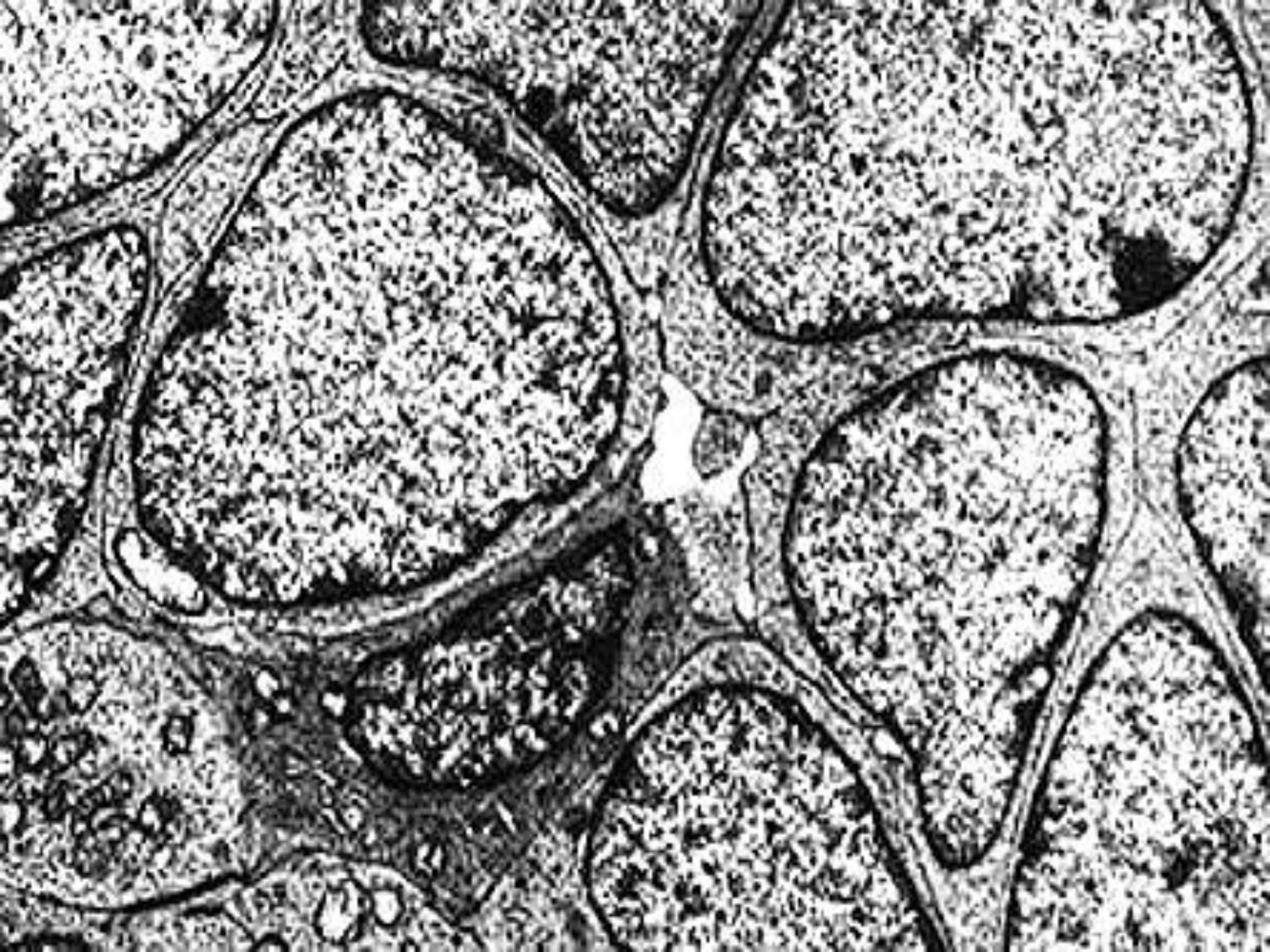


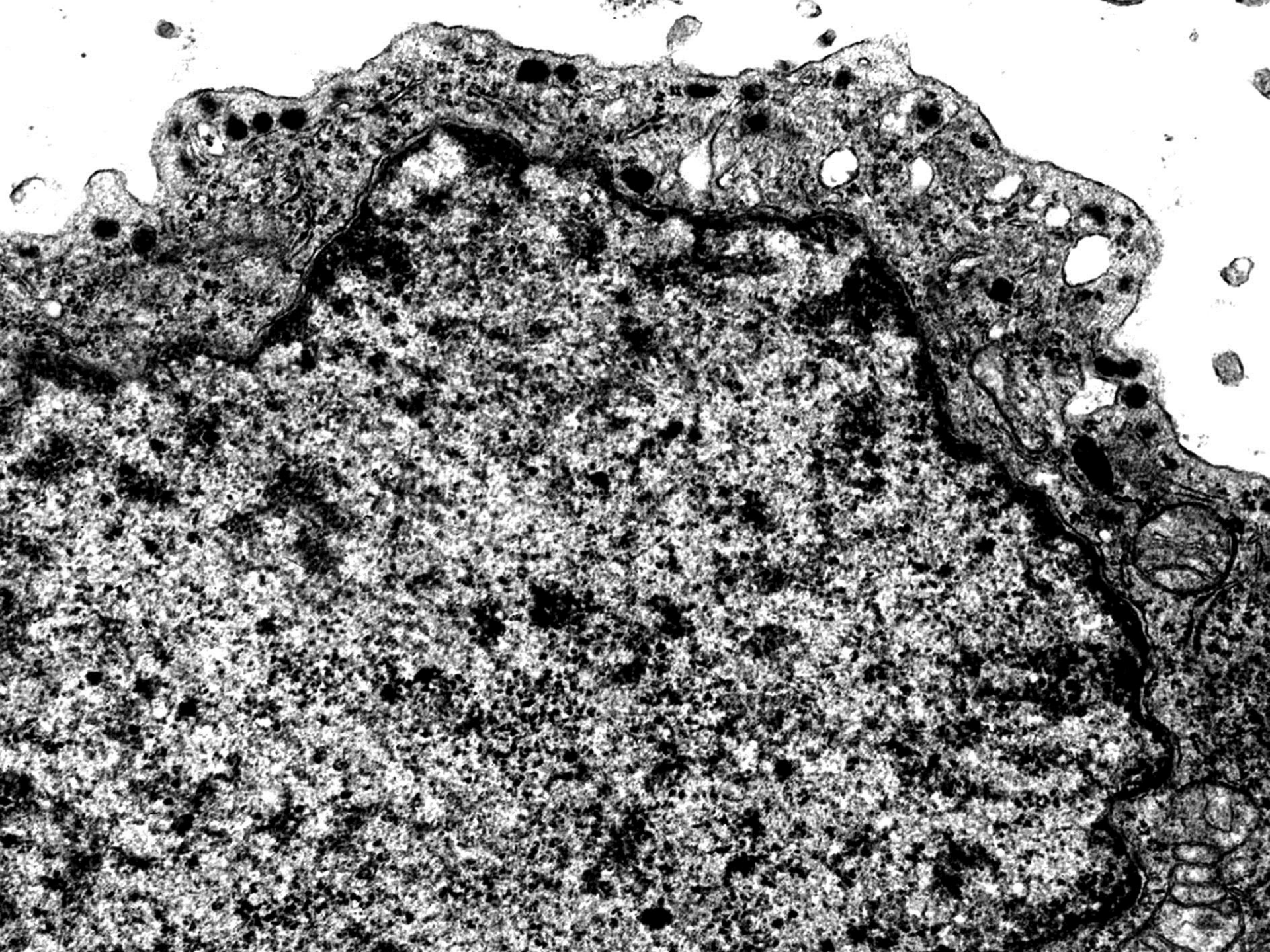




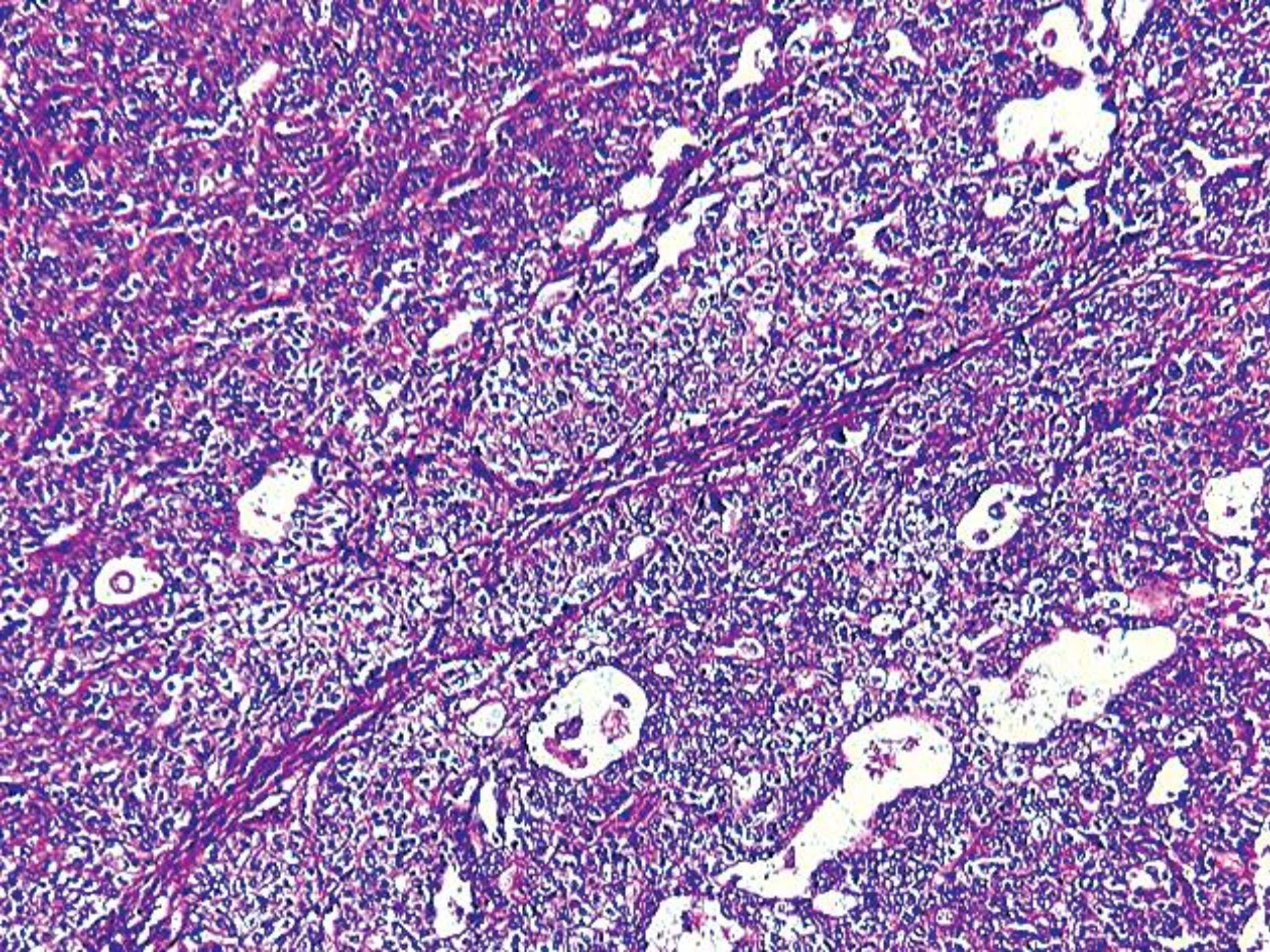
Small-cell Neuroendocrine Carcinoma

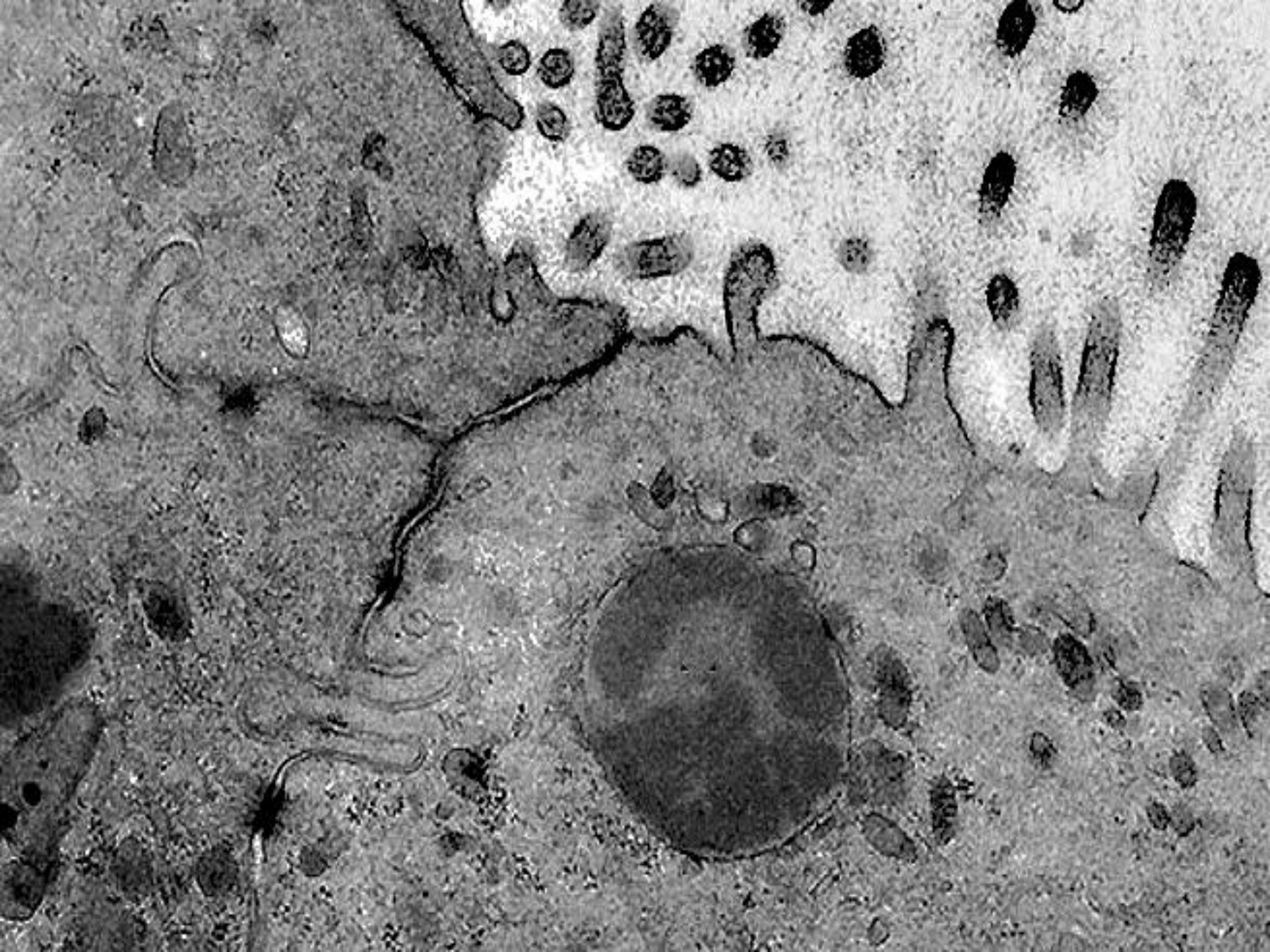






Poorly-differentiated Adenocarcinoma





METASTATIC ADENOCARCINOMAS: ULTRASTRUCTURAL CLUES TO SITE OF ORIGIN

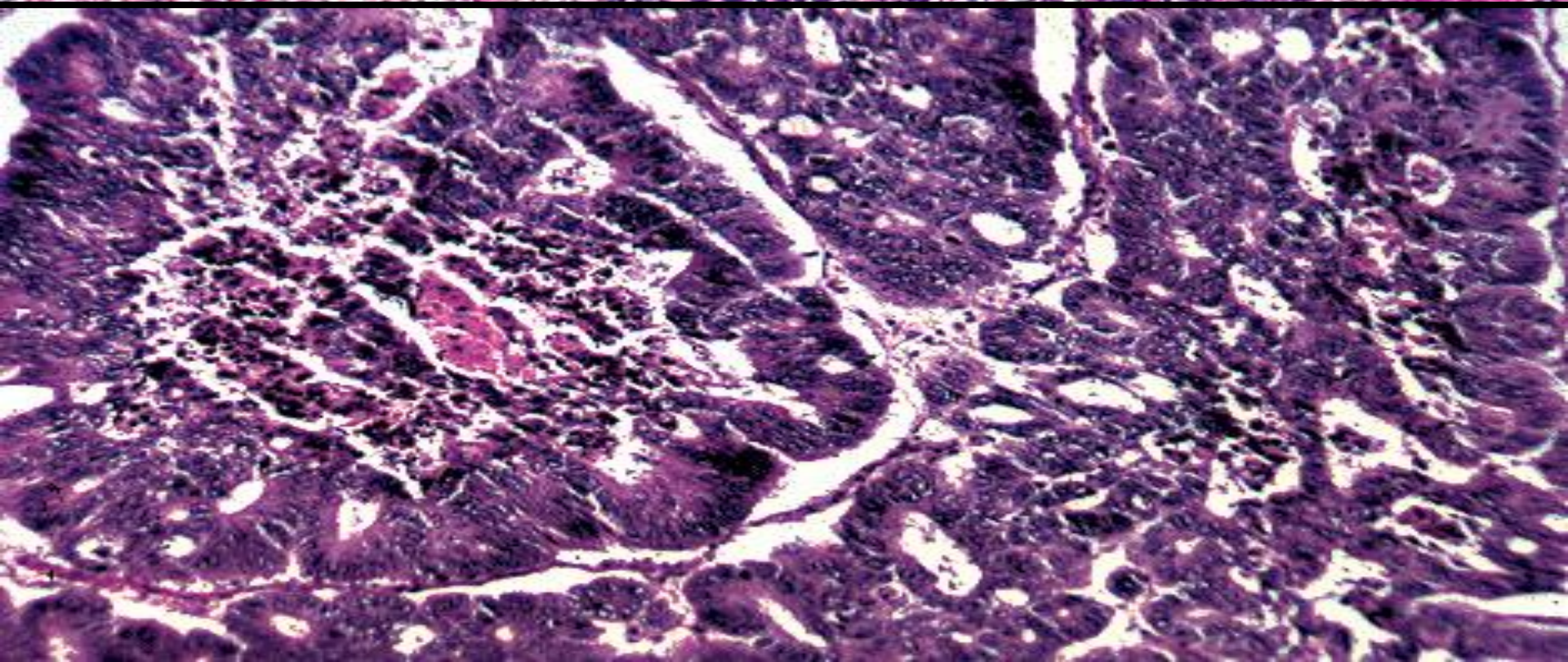
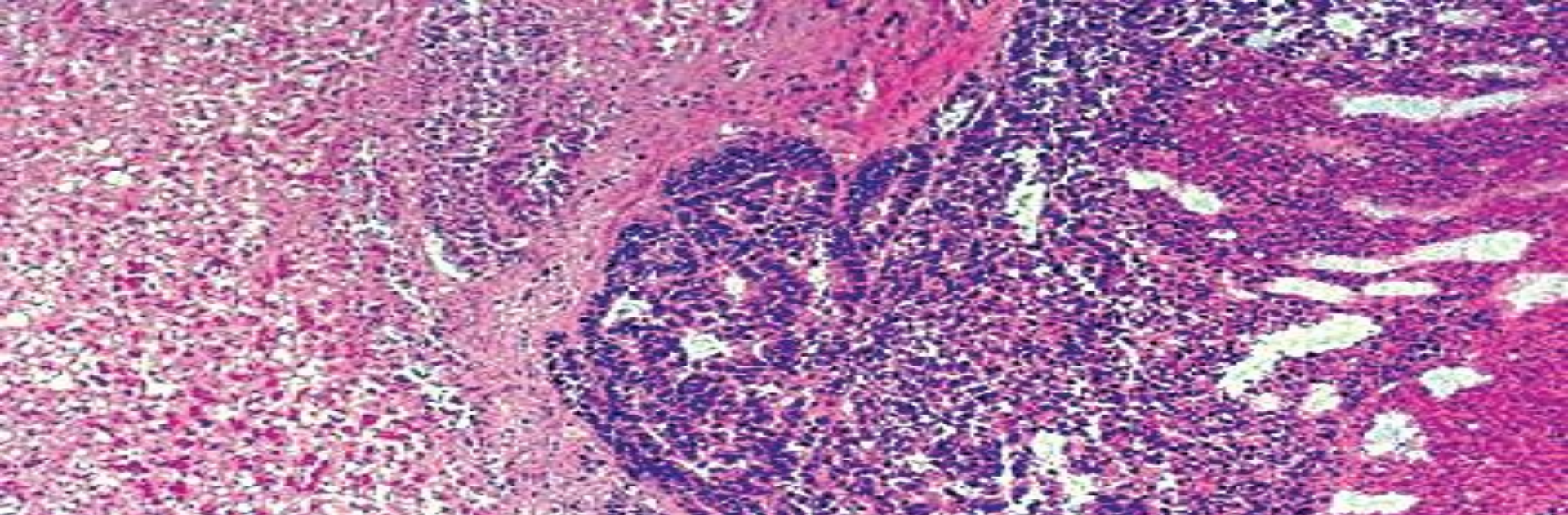
Gastrointestinal tumors: “Terminal web” of intermediate filaments and core-rootlets in tumor cells

Lung & Prostatic carcinomas:
Complex lysosomes or cytoplasmic crystalloids

Renal cell carcinomas: Concurrent cytoplasmic glycogen pools and lipid droplets

**71 year old woman with
abdominal discomfort and
elevated serum alkaline
phosphatase level**

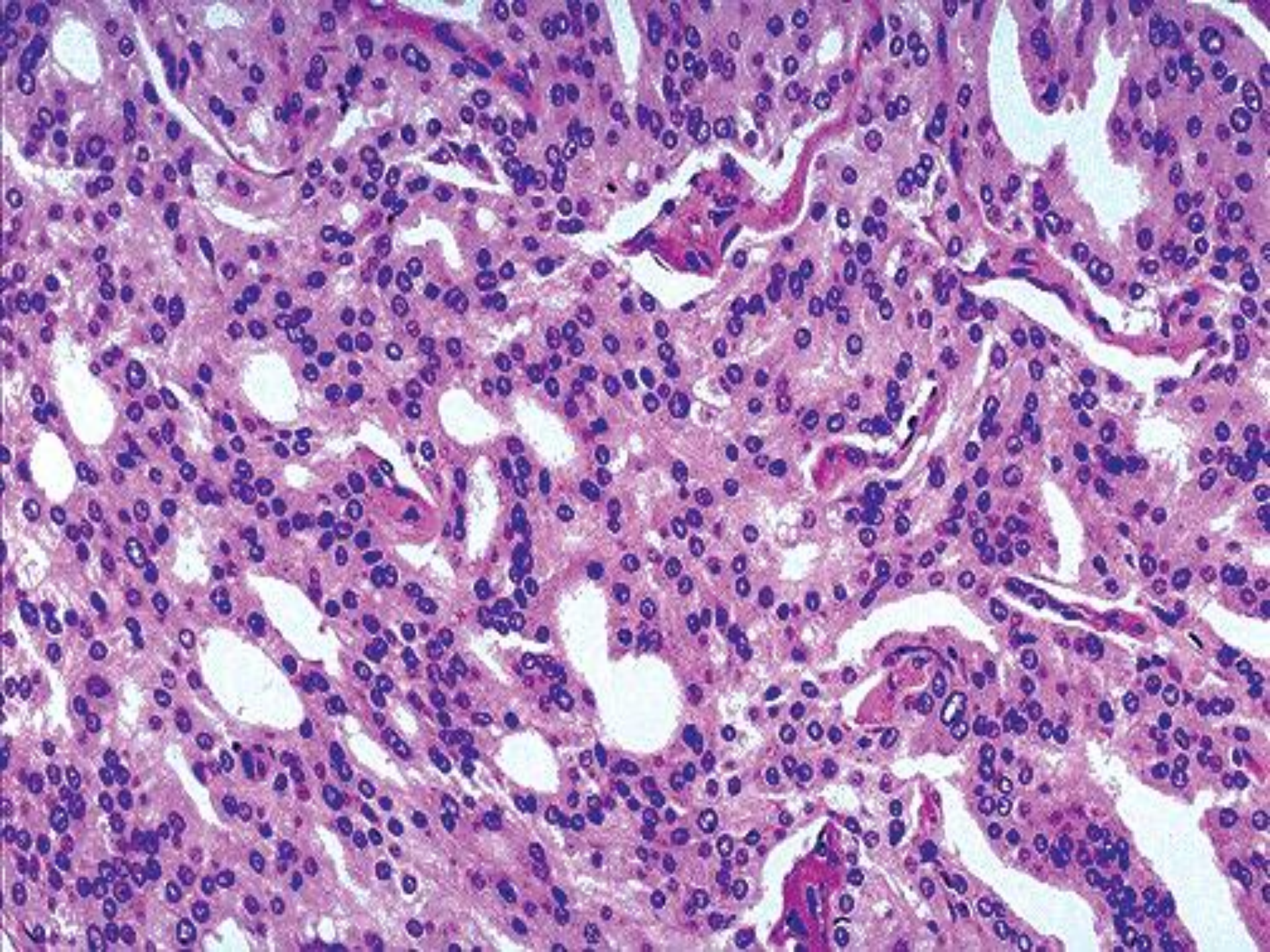


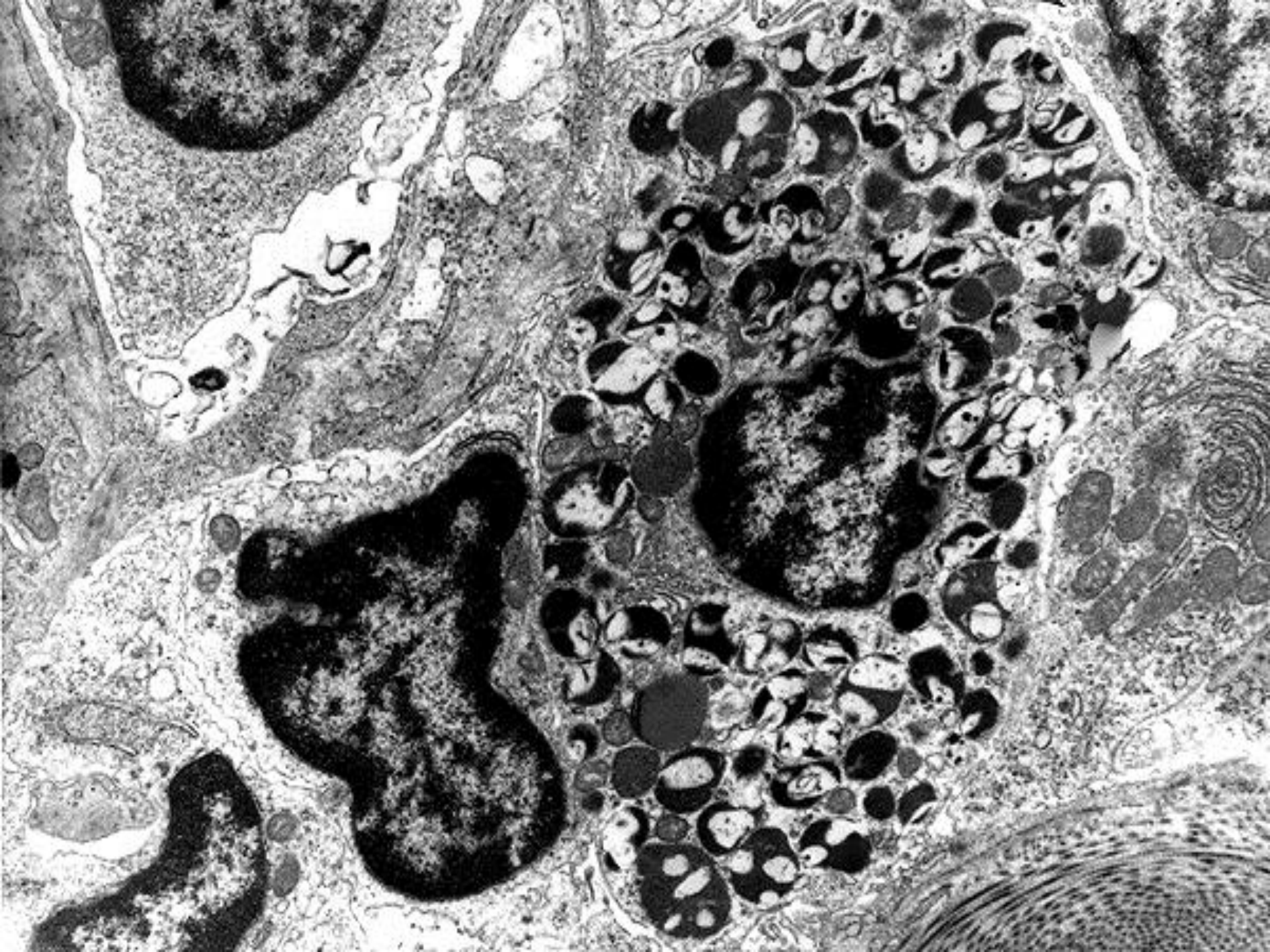




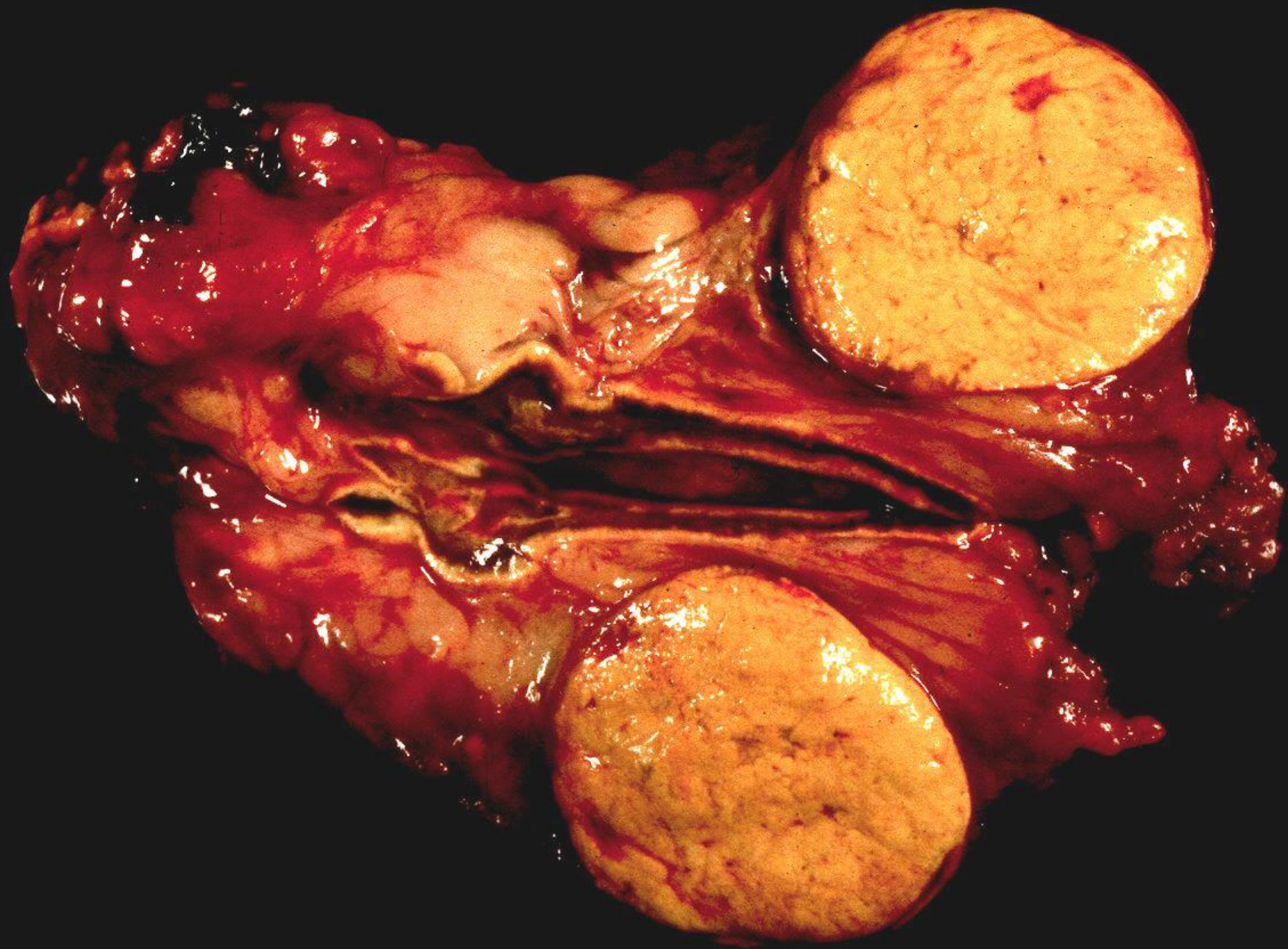
**67 year old man with back pain
and a serum PSA level of 42**

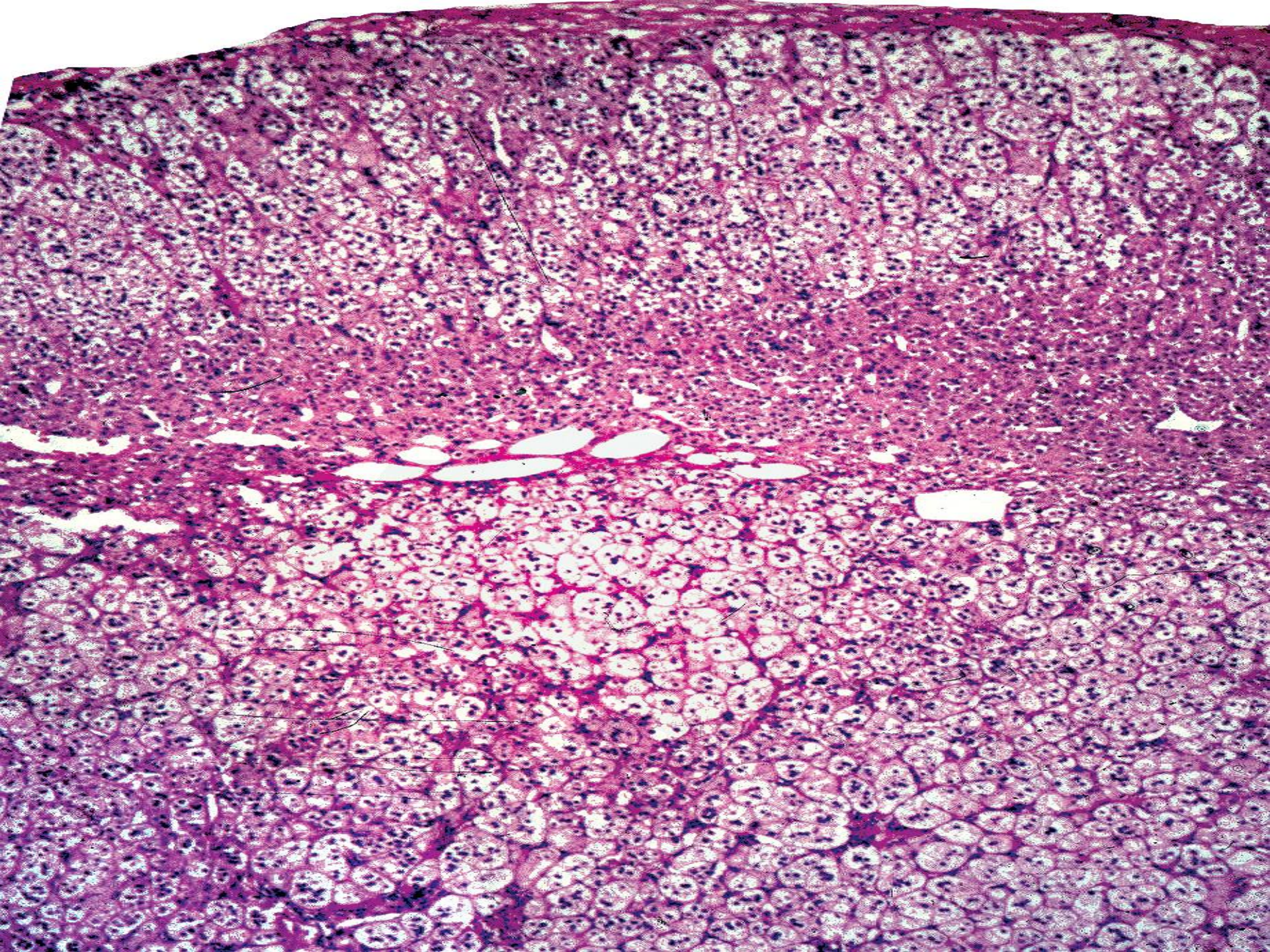


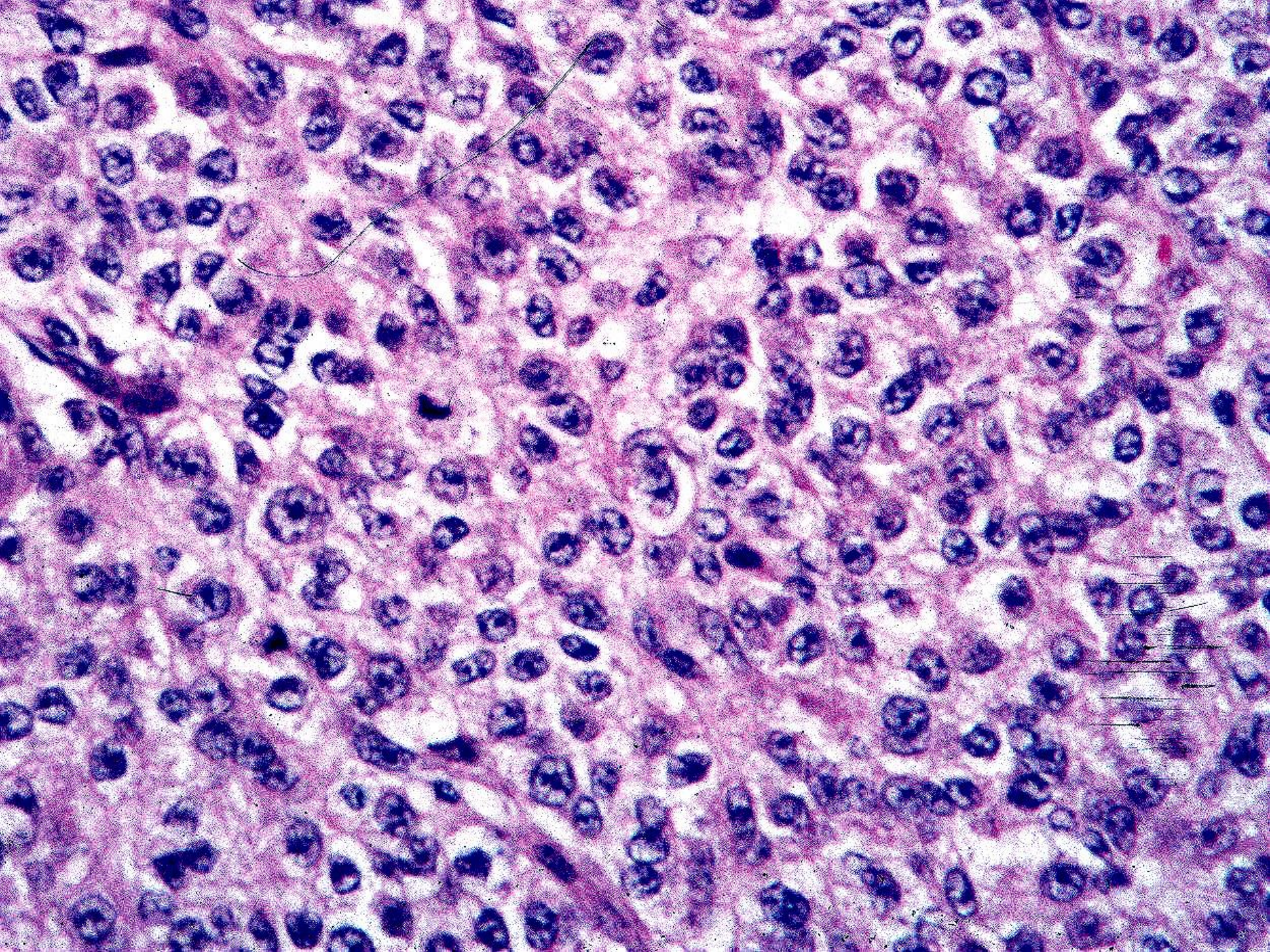


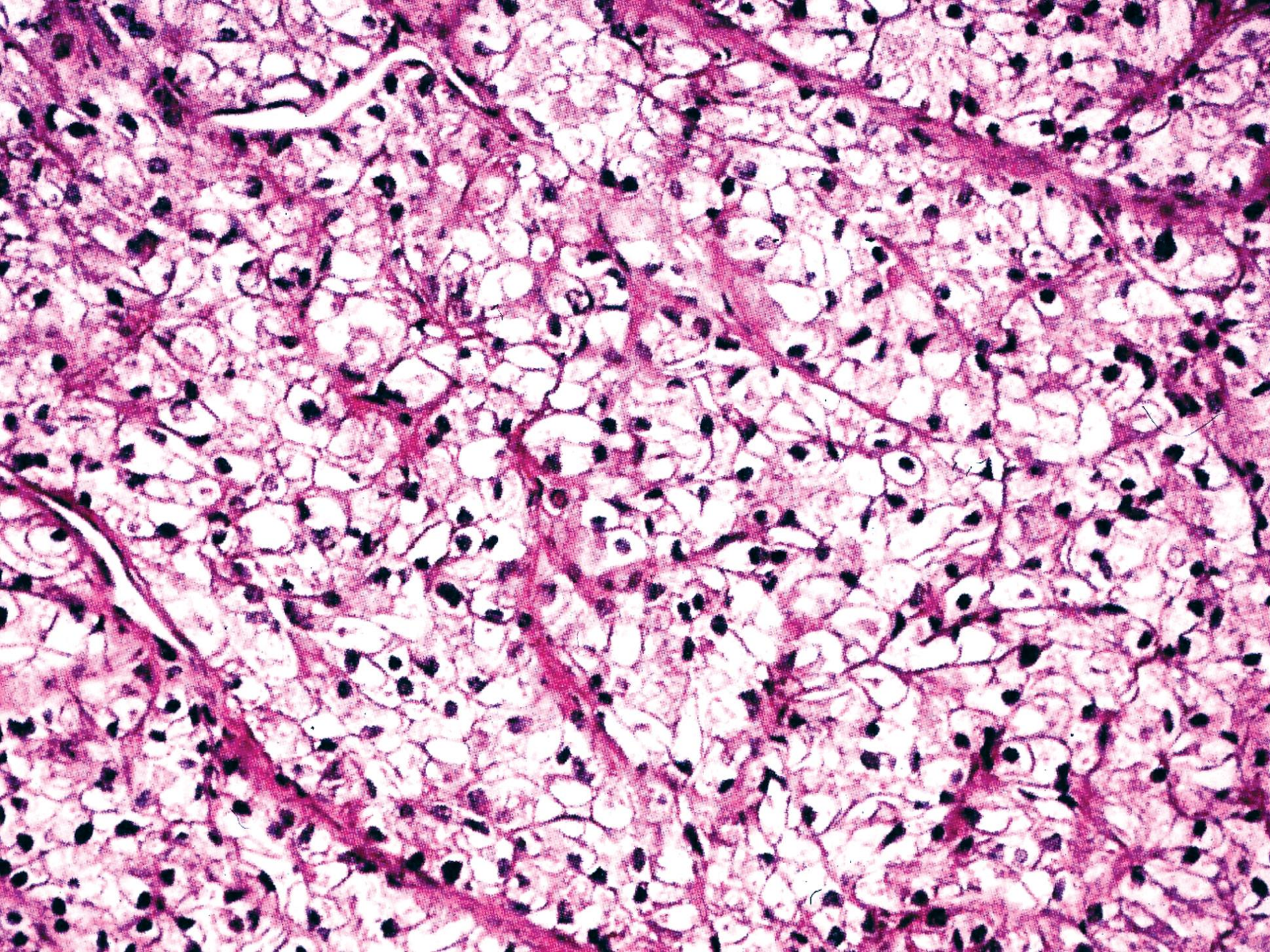


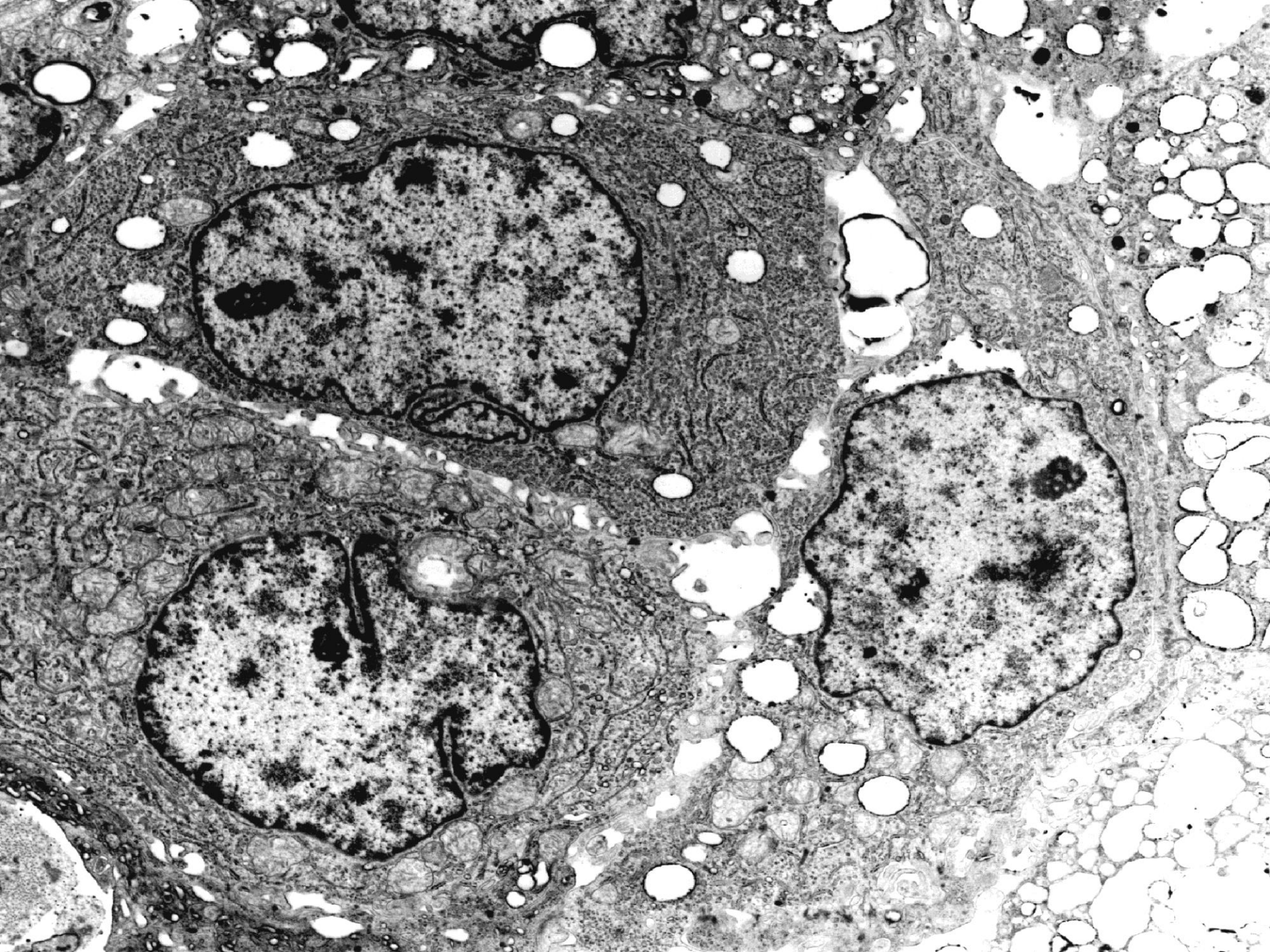
54 year old man with history of renal cell carcinoma 2 yrs ago. Now has a right adrenal mass on followup CT scan of the abdomen. An adrenalectomy is performed.

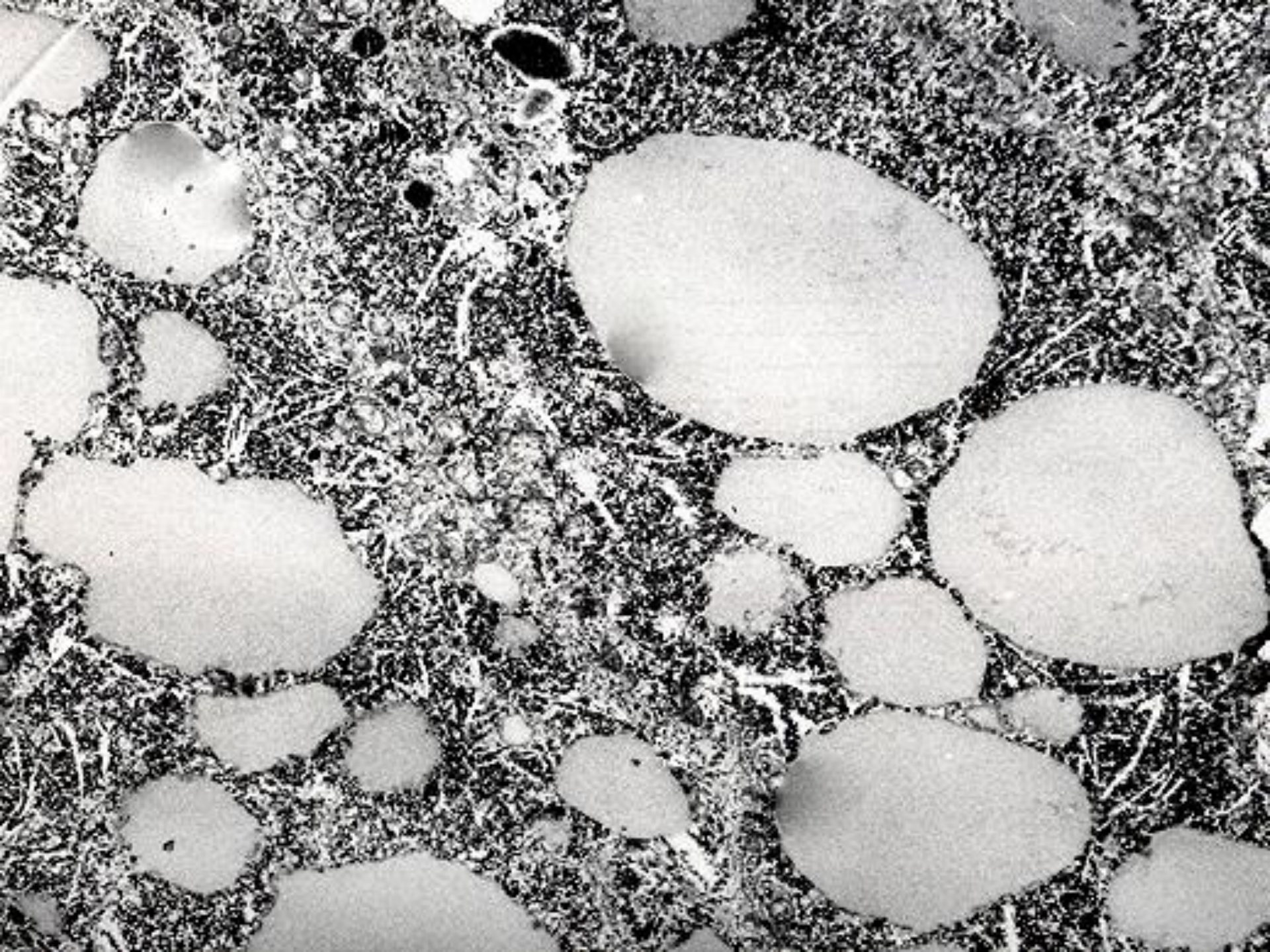


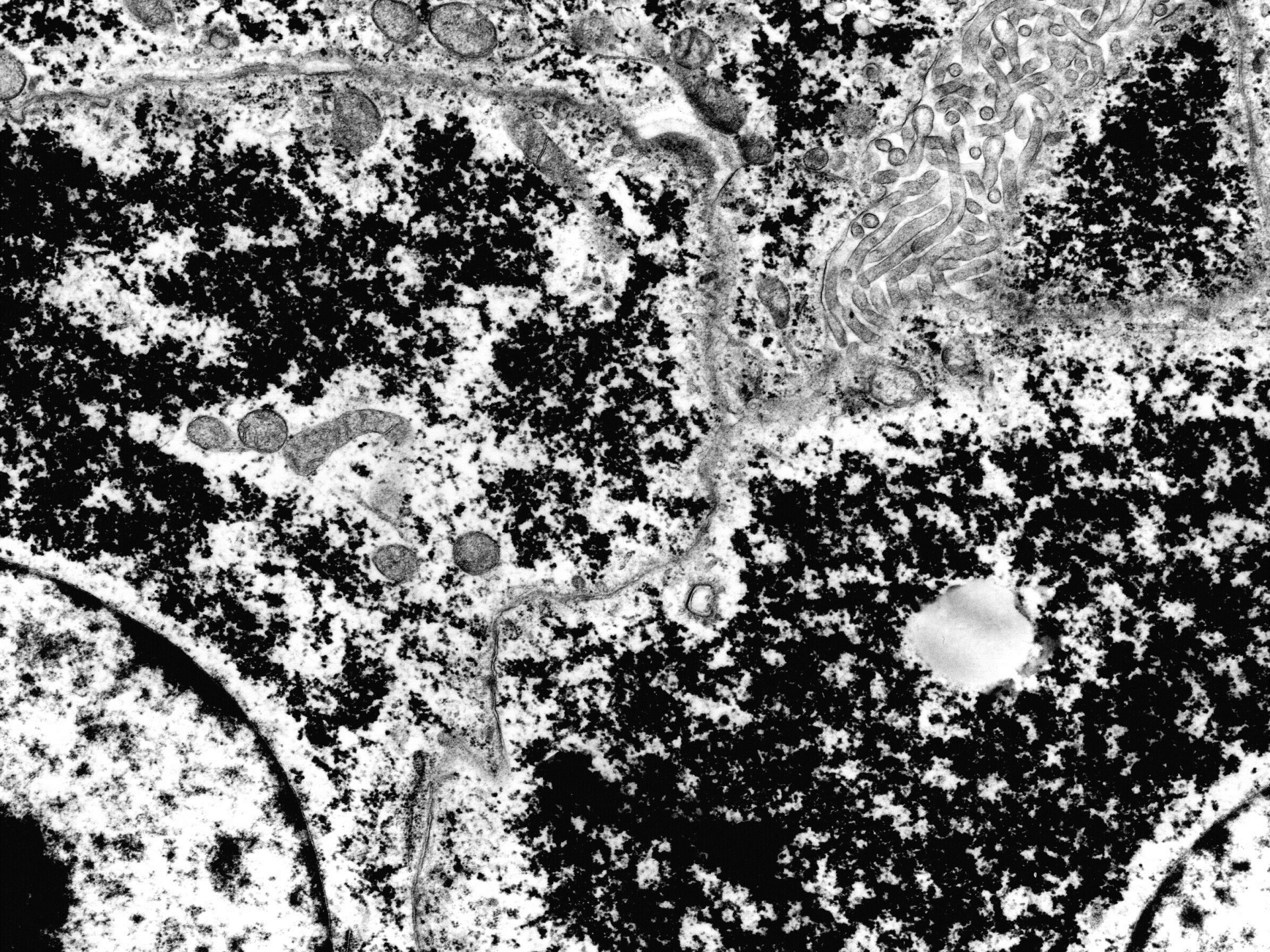










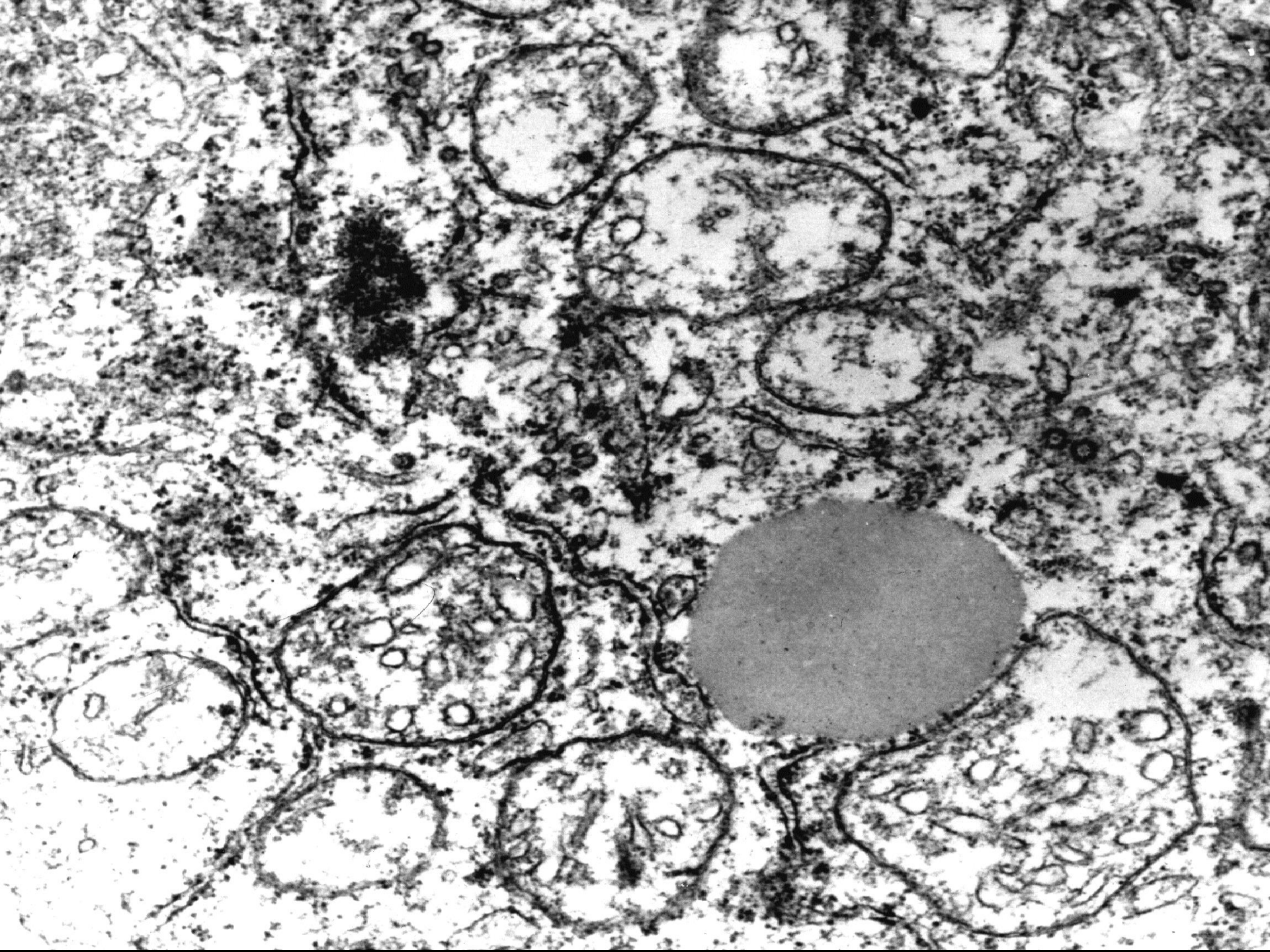


Diagnosis:

Metastatic renal cell carcinoma

Adrenocortical Neoplasms: Ultrastructural Features

- Few if any intercellular junctions
 - Little if any cytoplasmic glycogen
- No plasmalemmal microvilli
- Tubulovesicular mitochondrial cristae

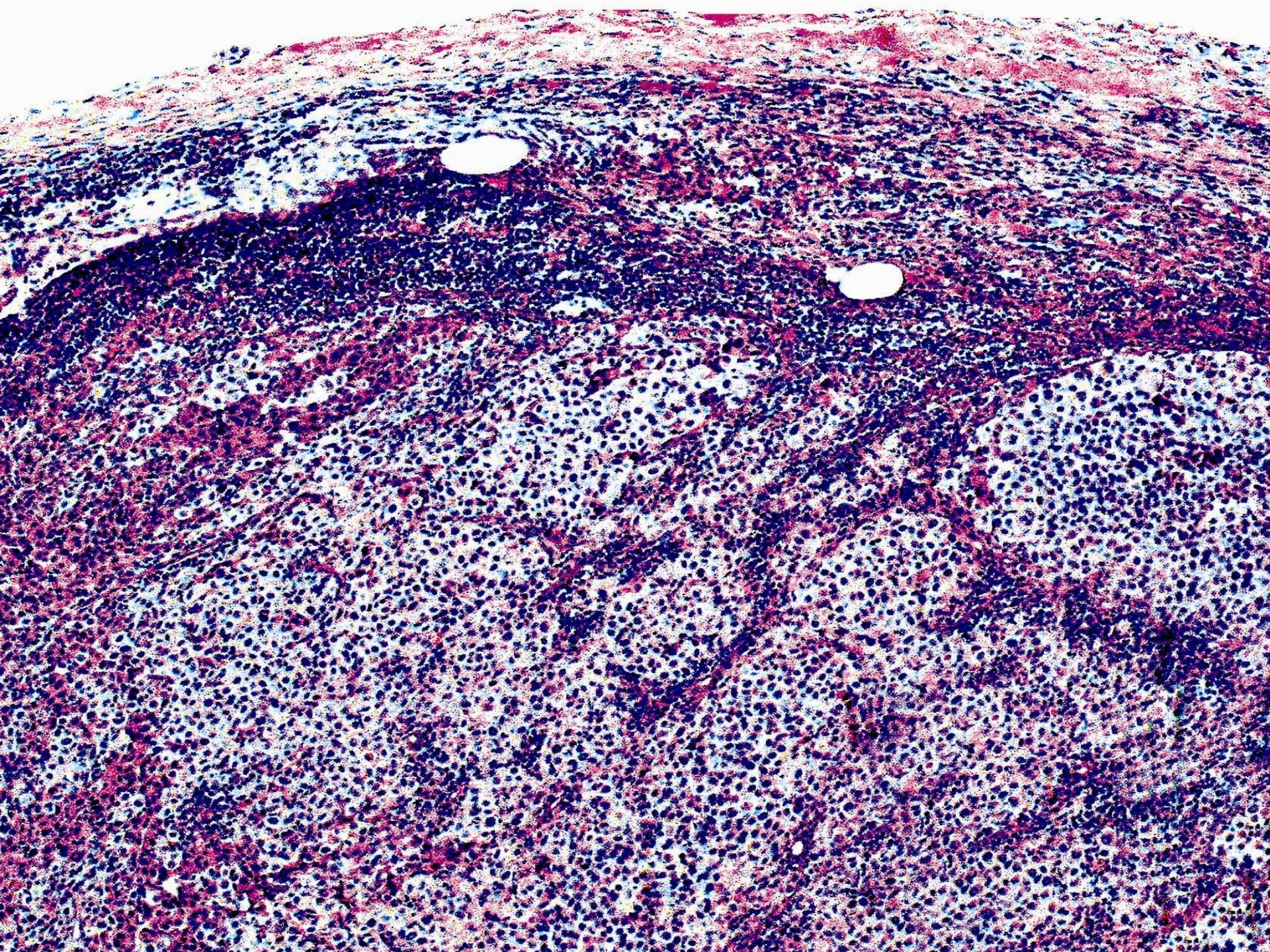


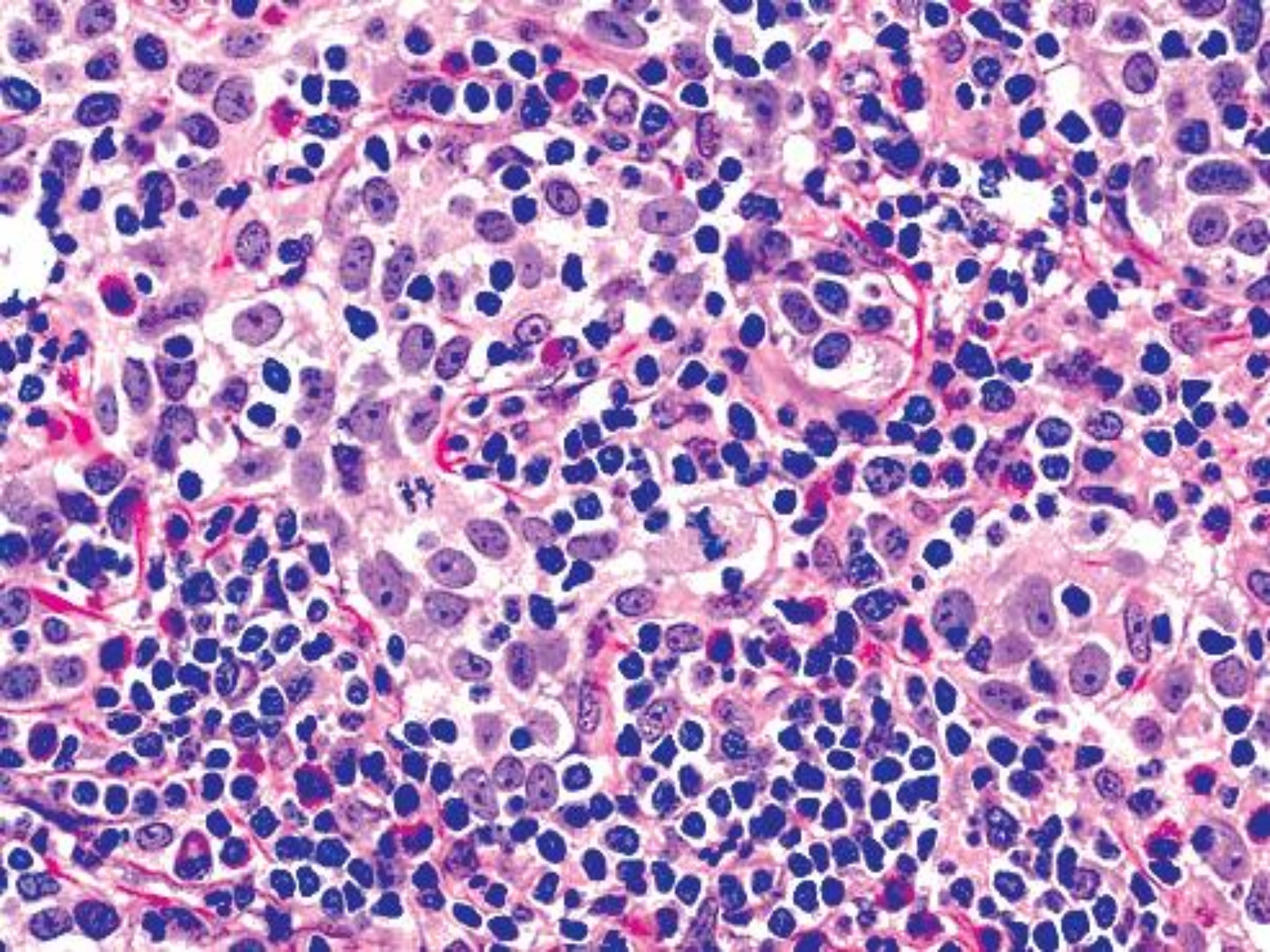
METASTATIC LYMPHOEPITHELIOMA- LIKE CARCINOMA: A FORM OF SQUAMOUS CARCINOMA

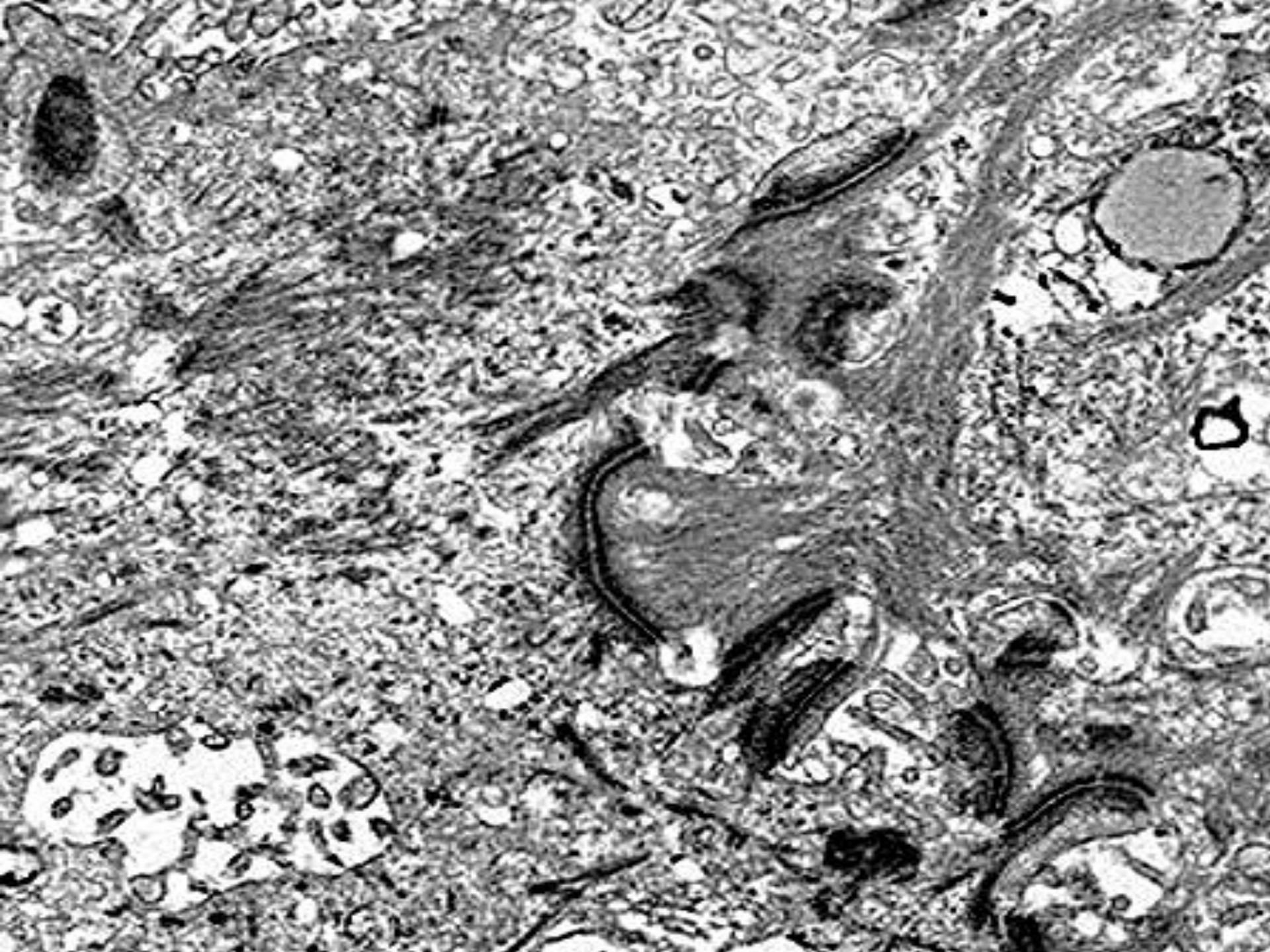
--Well-formed desmosomes

--Tonofibrils







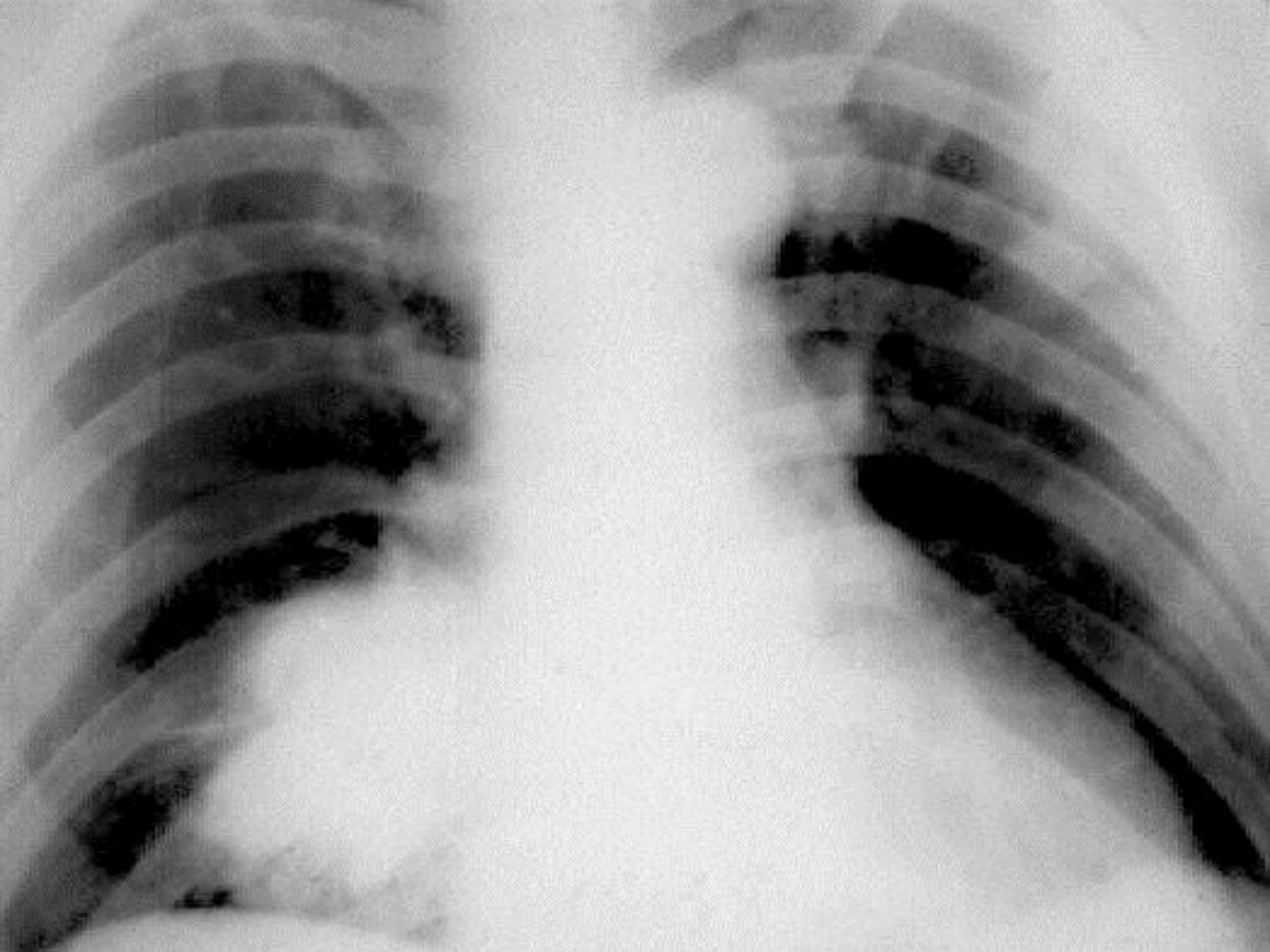


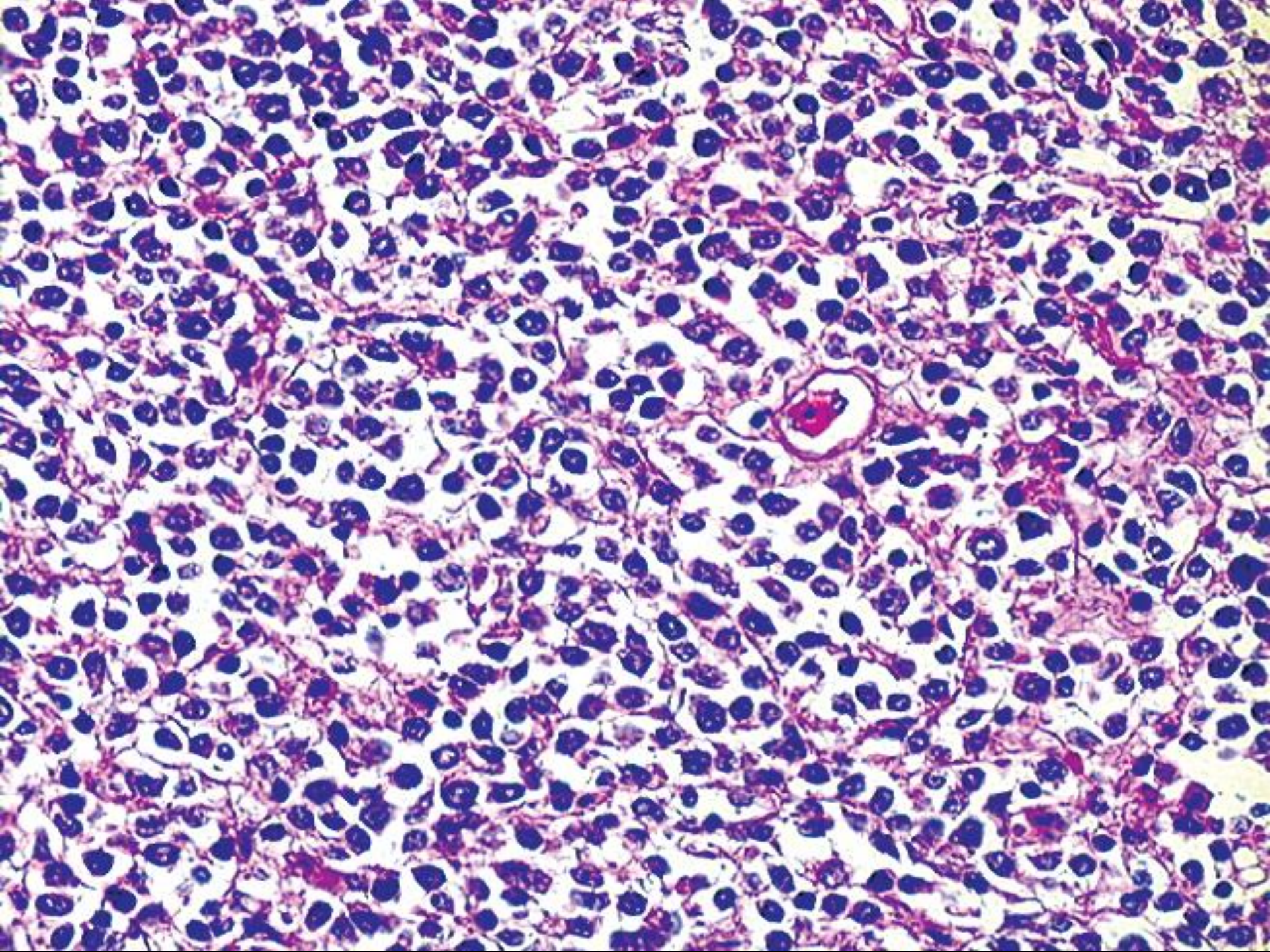
UNDIFFERENTIATED LARGE-CELL CARCINOMA VS. LARGE-CELL LYMPHOMA

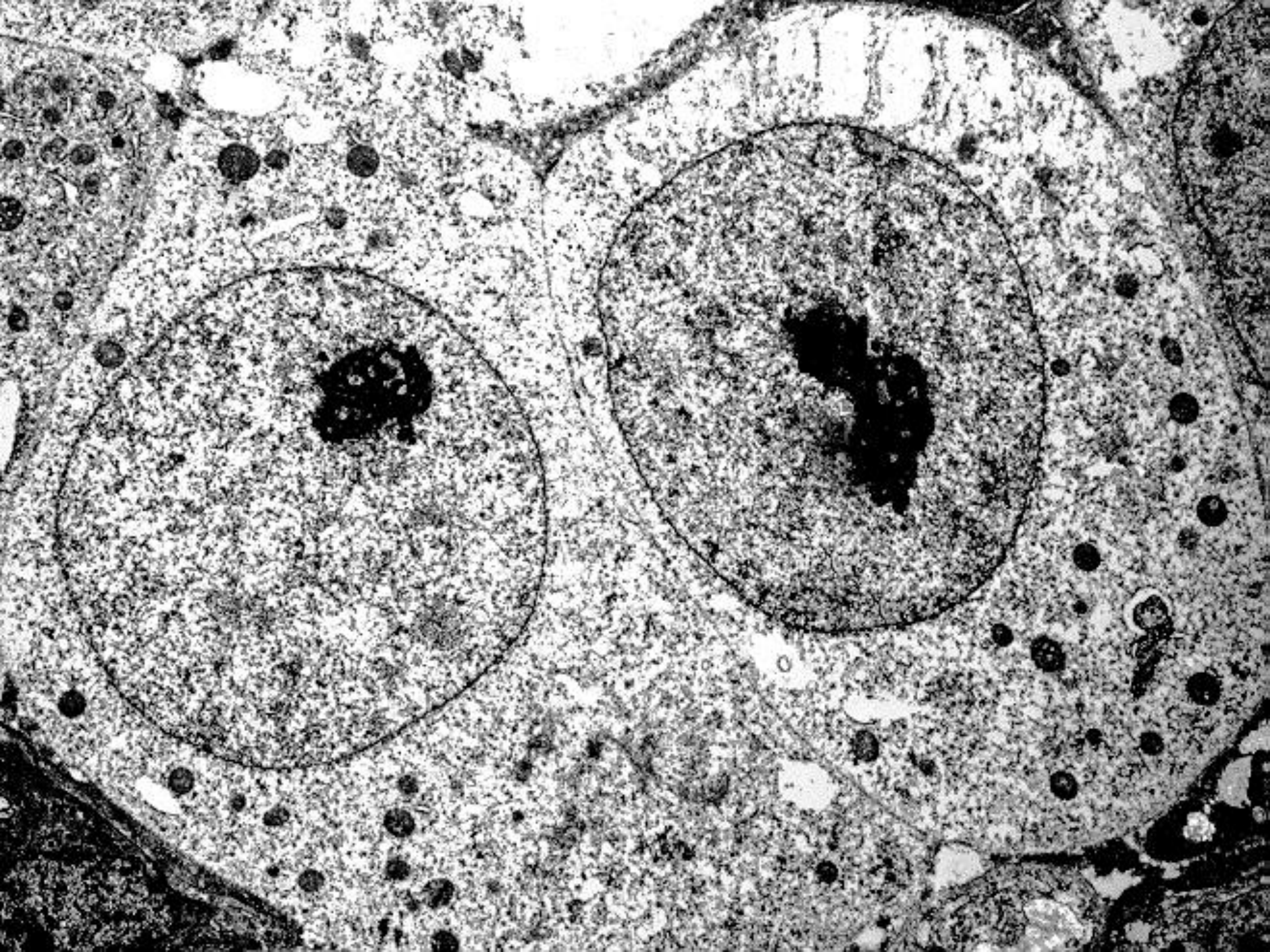
**-Carcinomas show discernible
intercellular junctions, with or
without pericellular basal
lamina**

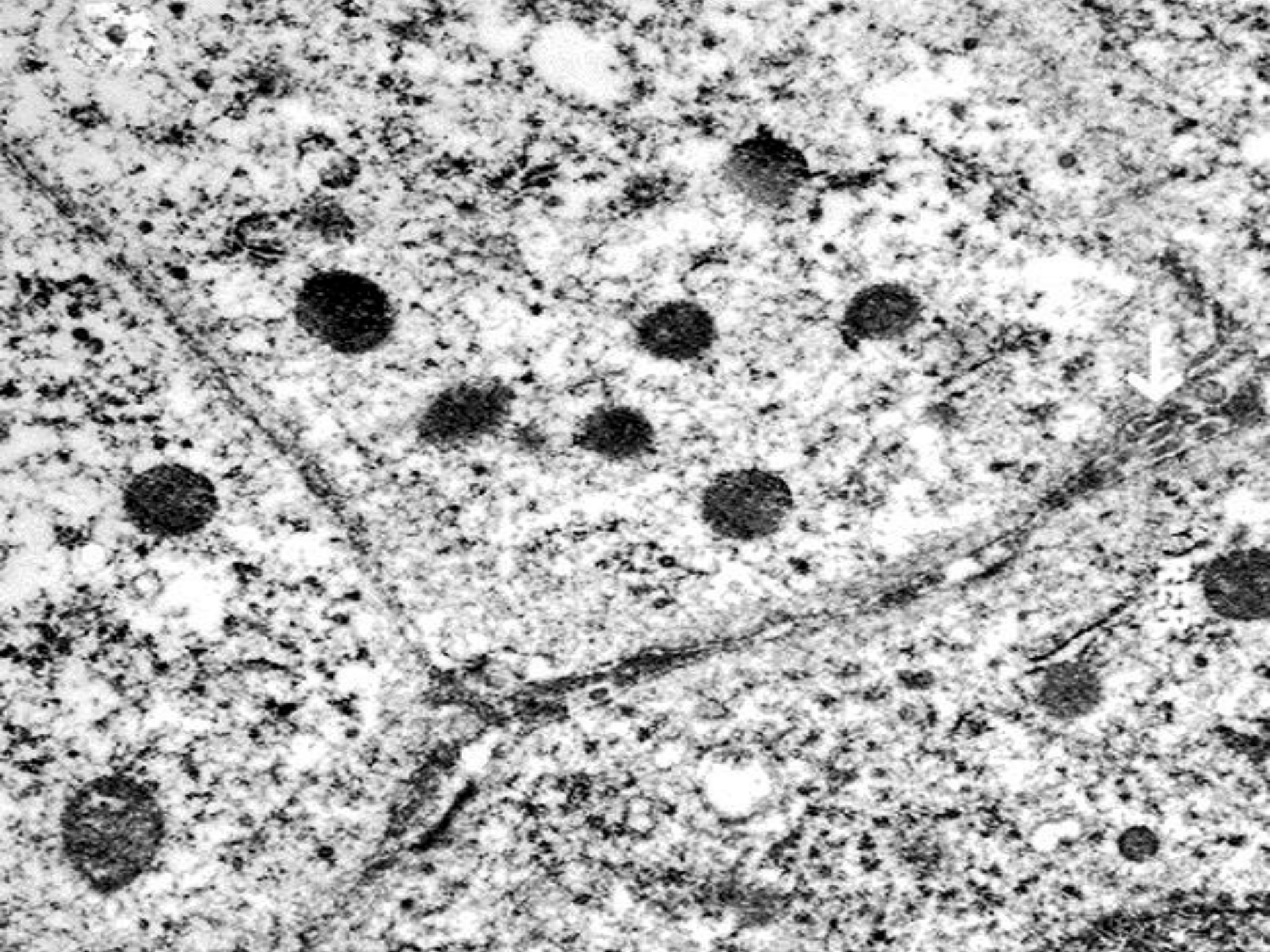
**-Like the case in small-cell
tumors, large-cell lymphomas
are “have-not” tumors**

**41 year old man with cough
and anterior chest pain**









Large-cell Lymphoma of Mediastinum

MALIGNANT GERM CELL TUMORS

MALIGNANT GERM CELL TUMORS: ULTRASTRUCTURAL FEATURES

Features Common to All Histotypes:

-Cytoplasmic glycogen, intercellular junctions, prominent nucleoli

Tumor Type-Specific Characteristics:

Seminoma: Glycogen lakes; nucleolonemata

Embryonal carcinoma: Primitive intercellular lumina; microvilli; basal lamina; long intercellular junctions

Yolk sac carcinoma: Basal lamina; intrareticular deposits of isodense material (alpha-fetoprotein) in rough endoplasmic reticulum

Choriocarcinoma: Microvilli; tonofibrils



