INCLUSIONS IN RENAL EPITHELIAL CELLS FOLLOWING THE USE OF CERTAIN BISMUTH PREPARATIONS *

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It is not the purpose of this paper to discuss the pathology of bismuth nephritis in general, but rather to call attention to peculiar epithelial inclusions, which seem to be definitely related to the administration of certain bismuth compounds. The fact that these bodies are found within the nucleus as well as in the cytoplasm lends them particular interest and justifies a detailed description. The bodies were first observed in Case 1. A second case, in which a different bismuth preparation was used, and which also showed similar inclusions in the renal epithelium, is also reported.

CASE REPORTS

CASE 1. P. H. History No. 357,602. The patient, E. R., a male, 46 years of age, was admitted to the Presbyterian Hospital on Oct. 28, 1932, and again on Dec. 9, 1932. The chief complaint was epigastric pain and loss of weight for 5 months. The family history was unimportant. The patient had been healthy and robust until the onset of the present symptoms. There was a history of gonorrhea 23, 20 and 9 years ago, but no syphilitic infection. The Wassermann reaction was reported negative in 1923, 9 years previous to the present illness.

Five months ago the patient began to have pain and a feeling of fullness in the epigastrium. The pains were gnawing in character, occurred 1 to 2 hours after meals and occasionally at night. The pain was relieved by vomiting. Over a period of 3 months there was a loss of 16 pounds in weight. He entered the hospital in October, remaining for 3 weeks. Physical examination at that time disclosed a palpable mass in the epigastrium. The Wassermann reaction was 4 plus, and because of the possibility that the mass was a gumma of the liver he was referred to the Dermatological Clinic for antiluetic treatment. On November 22nd, and again on the 25th, 29th and on December 2nd he received 2 cc. of bismocymol (0.1 gm. bismuth) intramuscularly. In addition, he was given three intravenous injections of Old Salvarsan, totalling 0.6 gm., and 1 gr. of mercuric salicylate intramuscularly.

There was no decrease in the size of the abdominal mass under treatment. The weakness and loss of weight progressed, and the patient was readmitted to the surgical service.

Physical Examination: The temperature was 98.4, pulse 80, respiration 20, blood pressure 115/90. The head and thorax were normal. In the epigastrium

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a large, hard, irregular mass could be felt with the lightest touch and its outlines could be seen through the abdominal wall. It extended two-thirds of the way to the umbilicus and was slightly tender. Cervical, axillary and right epitrochlear glands were definitely large but not tender. Examination otherwise negative.

Laboratory Findings: Hemoglobin 75 per cent, red blood cell count 4,150,000. Wassermann reaction 4 plus. Stool guaiac-negative. X-ray showed a broad crater shadow along the lesser curvature of the stomach. Urinalysis showed a trace of albumin, many hyaline and granular casts and occasional white blood cells.

An exploratory laparotomy was performed 3 days after admission and an extensive carcinomatous infiltration of the liver was found. This was confirmed by examination of an excised specimen. Following the operation a hematoma occurred in the lower third of the wound. The patient became increasingly weak and died on the 12th day after operation.

Clinical Diagnoses: Carcinoma of stomach with metastases to liver; syphilis.

Postmortem Examination

Autopsy No. 11,124, performed 16 hours after death.

Anatomical Diagnoses: Gastric ulcer and carcinoma, metastases in regional lymph nodes, liver and lung; syphilitic aortitis, syphilitic orchitis with gummas; atherosclerosis.

The stomach was the seat of a carcinomatous ulcer 2.5 by 3 cm., situated on the lesser curvature 4 cm. from the pylorus. Nodules of tumor tissue were seen in the base of the ulcer and in the lesser omentum. The liver weighed 5350 gm., was greatly enlarged and filled with carcinomatous nodules. Normal liver tissue was found only in one small area in the right lobe. The aorta presented gross and microscopic lesions of syphilitic aortitis. Both testes were fibrotic and contained gummatous areas of caseous necrosis.

Kidneys: No gross changes were visible. The right kidney weighed 140 gm., the capsule was easily removed, the surface smooth and brownish. The cortex was 7 mm. wide. The markings were regular and distinct. There was no gross vascular thickening. Pelves and ureters were normal. The left kidney was similar in appearance.

Microscopic Examination of Kidneys

Microscopically no significant changes are found in the glomeruli. The capillaries contain a moderate amount of blood, the basement membrane is not thickened and there is no increased cellular content. A small amount of granular coagulum is seen in some of the capsular spaces.

Interesting changes, however, are found in the epithelial cells of the convoluted tubules, and chiefly in the distal portions. The lining cells are atypical, often flattened or irregular in shape; the nuclei are variable in size and occasional mitotic figures are seen. Often the lumen is filled with exfoliated necrotic cells or cellular detritus which stains deeply with eosin. In some tubules one has the impression of a coagulative necrosis affecting the portion of the cell contiguous to the lumen, but leaving the basal portion of the cell still viable.

Peculiar bodies are found in many of these atypical or degenerating epithelial cells. They are both cytoplasmic and intranuclear. Their size varies from 2 to 5 microns, averaging perhaps half the size of a red blood corpuscle. Their shape is usually spherical, although elliptical and obovate forms are occasionally seen. They have, in unstained formalin-fixed frozen sections, a slightly brownish color, and are highly refractile with a dark, singly contoured membrane. In some tubules they are quite numerous; others contain only one or two.

Intranuclear bodies are usually surrounded by a pale halo (Fig. 1). The nucleus containing the inclusion is often slightly enlarged or hydropic and the nucleolus displaced to one side against the nuclear membrane. One nucleus is found in which the slightly elongated inclusion has apparently bulged out the nuclear membrane preparatory to escaping into the cytoplasm (Fig. 2).

The cytoplasmic bodies are usually located between the nucleus and free border, or at one side of the nucleus (Figs. 3 and 4). Frequently they are found within the necrotic desquamated cells within the lumen. In general, their presence is associated with, possibly leads to, necrosis of the containing cell. A characteristic inclusion, however, is found within a cell undergoing mitosis, and many of the cells with intranuclear bodies show little if any degenerative change. This suggests that the inclusions are first formed within the nucleus, to be later extruded as the cell degenerates.

CASE 2. P. H. History No. 405,156. The patient, a female, 60 years of age, was first admitted to the Neurological Institute on Dec. 11, 1933. For 3 years she had suffered from generalized headaches, attacks of flushing and dizziness. A year ago she had a slight stroke, with speech impairment and right hemiparesis. A second similar attack occurred in November, 1933.

On physical examination right hemiparesis, adiadokocinesis, rambling speech and exaggeration of reflexes on the right side were present.

Laboratory Findings: Red blood cells 4,300,000, white blood cells 7500, polymorphonuclears 70 per cent, hemoglobin 78 per cent. Blood Wassermann reaction 4 plus. Spinal fluid 0, gold curve 1111000000, Wassermann negative. Urine showed a faint trace of albumin and clumps of pus cells.

A diagnosis of meningovascular syphilis with thrombosis of left anterior and middle cerebral arteries was made. The patient was given a course of anti-syphilitic treatment. She received 10 intramuscular injections of potassium bismuth tartrate with butyn (Abbott Laboratories) over a period of 31 days, beginning December 19th. The total amount administered was the equivalent of 1 gm. of metallic bismuth.

On January 18th she developed what was thought to be pneumonia, and was transferred to the Presbyterian Hospital. The temperature was 103.2. The white blood count was 40,750, polymorphonuclears 89 per cent. The blood urea was 1.5 gm. per liter. The physical signs of pneumonia improved but several bed sores developed. A large calculus in the gall-bladder was demonstrated by X-ray. The urine contained considerable amounts of albumin and pus, and the left kidney was found enlarged. After 2 weeks Cheyne-Stokes respiration appeared and death occurred, apparently in uremia. Twenty-four hours before death the right leg became purplish, cold and edematous.

Postmortem Examination

Autopsy No. 11,469, performed 14 hours after death.

Anatomical Diagnoses: Generalized arteriosclerosis; thrombosis of arteries of right leg; gangrene of right leg; early confluent lobular pneumonia; acute tracheobronchitis; suppurative pyelonephritis and pyonephrosis, left; syphilitic aortitis; chronic cholecystitis and cholelithiasis; melanosis of colon; old salpingo-oophorectomy, left; pelvic adhesions.

Permission to examine the brain could not be obtained.

Kidneys: The left formed a sac-like mass 15 by 10 by 8 cm., weighing 110 gm. The capsule stripped easily and the surface was finely granular. The pelvis was distended with semipurulent fluid and there were several large abscesses in the substance of the kidney. The right kidney weighed 200 gm., was soft, flabby and the cortical markings were blurred, but there were no abscesses.

Microscopic Examination of Kidneys

Microscopically the left kidney shows a diffuse pyelonephritis, with the presence of numerous, short Gram-negative bacilli. The right kidney is almost free from acute suppurative changes. Autolysis is fairly marked but there appears to be little degeneration and no necrosis of the epithelial cells. Many of them, however, are

exfoliated and the lumens of the tubules are often filled with granular coagulum. There are no significant glomerular lesions and the arteriosclerotic lesions of the larger renal branches are not extreme.

The interesting feature of the kidney sections is the presence of numerous spherical globules, identical in location, size and appearance with those described in the previous case. They are found within the nuclei, but more abundantly in the cytoplasm of the epithelium of the convoluted tubules, or free in the lumen (Figs. 5 and 6).

Staining Reactions and Microchemical Tests of Refractile Bodies

1. With hematoxylin-eosin, after formalin or Zenker fixation, the refractile bodies are unstained but have a slight brownish tinge. They are, as has been stated, very refractile and sharply contoured.

2. With eosin-methylene blue the majority of the bodies, both intra- and extranuclear, stain intensely with the methylene blue. A few of the larger ones, however, retain their brownish tinge.

3. With Pappenheim's methyl green-pyronin they retain their brownish color, in contrast to the red-staining nucleoli, from which they can readily be distinguished.

4. They are unstained with scharlach R in frozen sections of formalin-fixed material. There are only occasional small fat droplets within the degenerating epithelial cells. The globules are not doubly refractive.

5. With Nile blue sulphate a faint greenish blue staining of the globules is obtained.

6. With 1 per cent osmic acid on frozen sections of formalin-fixed material, after washing in several changes of distilled water, a slight darkening of the globules is noted. They are not blackened, however.

7. With Spielmeyer's myelin stain intense blackening of both the intranuclear and cytoplasmic bodies is produced. The bodies are brilliantly and selectively brought out by this method (Fig. 7).

8. Ciaccio's method shows the presence of amorphous sudanophile masses in the cytoplasm of some of the epithelial cells. The globules are unstained.

9. After 10 days digestion of small slices of formalinized tissue with Merck's pancreatin the globules are unaffected, save that they show a tendency to stain with hematoxylin (Fig. 7).

10. The iron reaction with potassium ferrocyanide and hydrochloric acid is negative.

11. They are not dissolved by strong ammonia or by 20 per cent nitric acid.

12. Von Kossa's stain for calcium is negative.

The following microchemical tests for bismuth were applied.

1. With hydrosulphuric acid the bodies are slightly darker than in the unstained control sections.

2. Ammonium sulphide also produces darkening, but no diffuse black coloration.

3. With stannous chloride-sodium hydroxide the inclusions stain brownish black. Under the oil immersion minute black granules in active brownian motion are seen floating in a colorless menstruum.

4. With potassium iodide-sulphuric acid the bodies take a slightly more yellowish tinge than the unstained control.

5. Frozen sections treated according to the method of Komaya [1] with quinine sulphate and potassium iodide fail to give the characteristic bismuth reaction, although a deep brick red color was obtained with bismocymol on filter paper.

In addition to the refractive globules above described there is in Case 1 a considerable amount of yellowish brown pigment within the epithelial cells at the junction of pyramid and cortex. This is in the form of irregular, varying sized clumps. It does not blacken with hydrosulphuric acid or ammonium sulphide, nor does it give an iron reaction with potassium ferrocyanide and hydrochloric acid. It fails also to react with Komaya's reagent and the other microchemical tests for bismuth. It is not removed by the lipoid solvents used in dehydration and clearing, nor does it give a fat stain with scharlach R.

Chemical Determination of Bismuth in Kidneys

In view of the fact that the patient received intramuscular injections of bismuth it seemed of interest to determine the amount of bismuth still present in the renal tissues in each case.

A colorimetric determination was carried out on duplicate samples of formalin-fixed tissue, according to the method of Leonard.[2] The analysis of Case 1 gave the following results.

	Sample A	Sample B
Wet weight	8.2058 gm.	4.3730 gm.
Dry weight	1.9527 gm.	1.0368 gm.
Bismuth found	1.36 mg.	0.0727 mg.
Bismuth per 100 gm. wet tissue	16.57 mg.	16.62 mg.
Bismuth per 100 gm. dry tissue	69.7 mg.	69.8 mg.

Taking the combined weight of the two kidneys as 280 gm., the calculated total amount of bismuth retained by the renal tissue may be estimated as approximately 46.5 mg., or well over 10 per cent of the total amount injected. To what extent the extreme carcinomatous replacement of the liver tissue interfered with the normal storage of the bismuth in the reticuloendothelial cells (Komaya) of this organ, thus intensifying the toxic effect upon the kidney, must remain problematical. The finding of a considerable amount of bismuth in the kidney made it logical to ascribe the renal damage to this substance. Only a trace of mercury could be recovered.

An analysis of the kidneys of Case 2, carried out with the same technique, gave the following results.

Wet weight	4.6955 gm.
Dry weight	1.1016 gm.
Bismuth found	1.120 mg.
Bismuth per 100 gm. wet tissue	23.8 mg.
Bismuth per 100 gm. dry tissue	101.67 mg.
Total weight of both kidneys	310.0 gm.
Total bismuth content	73.8 mg.
Total amount injected	1.0 mg.

The kidney at the time of death therefore contained 7.38 per cent of the amount injected.

The nature of the refractile bodies within the epithelial cells still remained a problem. It seemed of interest to determine whether they could be reproduced experimentally or not.

Experimental Production of Similar Globules in Rats

A white rat, weighing 220 gm., was injected intramuscularly with four successive doses of bismocymol as follows.

On February 8, 1933, 0.1 cc. (equivalent to 261 mg. pro kg.) was injected, on February 9th 0.2 cc. was injected, on the 10th 0.45 cc., and on the 13th 0.4 cc. The animal showed no symptoms save a loss of weight of 30 gm. It was killed 3 days after the last injection. Bismuth was still present at the site of injection. About the material

was a grayish membrane, outside of which the muscle was edematous and hemorrhagic. The kidneys were dark red, the cortex showing grayish streaking. Other viscera were not abnormal.

Microscopically the kidney is the seat of an intense tubular necrosis. In many of the convoluted tubules the epithelial cells have lost their nuclei, stain intensely with eosin, are partially exfoliated and completely plug the tubules. There are no regenerative changes. Spherical inclusions, identical with those in the human tissue, are present both within the nuclei and in the cytoplasm (Figs. 8 and 9). They give similar microchemical reactions.

A section through the site of injection shows the bismocymol still present in the form of large granular deposits or agglomerations of spherical refractive masses having a greater variability in size than the inclusion bodies within the kidney. Treated with ammonium sulphite solution much of the deposit is immediately blackened. Many of the smaller globules take only a light brownish tint and thus closely resemble those found in the kidney. They are, however, entirely extracellular. With Komaya's reagent the injected material takes a brick red or orange color, rapidly decolorized by alkali but resistant to weak acid treatment. Some of the smaller globules react very feebly to the reagent. This suggests that the bismuth had been split off, leaving a non-reacting residue.

The injected material is surrounded by a broad zone of edematous granulation tissue. Many of the smaller globules and amorphous masses have been taken in by phagocytic cells, chiefly macrophages, but also polymorphonuclears. No globules, however, are found within the nuclei of the phagocytic cells or fibroblasts.

Chemical analysis of the rat kidney showed 0.042 mg. of bismuth in 0.1962 gm. of wet tissue, or 21.4 mg. of bismuth per 100 gm. of dry tissue.

The experiment was repeated on 7 other rats, as shown in Table I.

The inclusions were found in 5 out of 7 rats, that is, in all those surviving more than 4 days. There was no striking correspondence between the intensity of the tubular degeneration and the number of globules. Rats 11 and 15 showed only occasional tubules with necrotic epithelial cells, but intranuclear globules were numerous. The 2 rats with normal kidneys, killed on the 4th day, failed to show the globules.

The particular bismuth preparation used for treatment of the second case was not available for experimentation. Three rats were, however, given intramuscular injections of a similar preparation of sodium potassium bismuth tartrate with butyn suspended in peanut oil in doses equivalent to 153, 182 and 290 mg. per kg. respectively. None of the rats showed evidence of severe injury to the tubular

TABLE I

Summary of Injections

Rat No.	Total amount injected	Bismuth	Number injections	Days	Tubular degeneration	Globules
	cc.	*Mg/K*				
2	0.2	77.0	1	4	0	0
16	0.7	145.0	2	11	+++	+++
13	0.6	176.0	3	4	0	0
11	0.7	200.0	3	8	+	+++
1	1.15	287.5	4	8	+++	+++
15	0.82	297.0	2	5	0	++
12	1.78	393.0	4	15	+	+

epithelium, and in only 1 were there found characteristic inclusions within the nuclei. Further experiments extending over longer periods are planned. The preparation was either less toxic for the kidneys than the bismocymol or less bismuth was absorbed from the site of injection.

It seemed of interest to determine also whether similar inclusions appeared in the renal epithelial cells in response to injections of a water-soluble bismuth compound. A rat weighing 132 gm. was injected intramuscularly with 0.5 cc. of Loesser's solution of bismuth tartrate, equivalent to a dose of 107 mg. pro kg. The rat died on the 2nd day, showing extensive necrosis of many convoluted tubules but no refractile globules of the type described. Another rat, which received an injection of the same solution (0.178 mg. pro kg.) plus 0.5 cc. of a 10 per cent solution of camphorated oil into the muscles of the opposite leg, also showed severe tubular nephritis but no refractile inclusions.

Dr. G. H. Raizes of the Dermatological Research Laboratories of Philadelphia very kindly put at our disposal a sample of camphocarbonic acid, uncombined with bismuth. This was dissolved in olive oil by the aid of heat and injected intramuscularly into several

rats in amounts up to 150 mg. given in divided doses. No toxic effect upon the kidney was produced and no refractile globules were found within the epithelial cells.

Discussion

Following the intramuscular injection of 0.4 gm. of bismuth in the form of bismuth camphocarbonic acid in olive oil (bismocymol) into a syphilitic patient there occurred a tubular nephritis of moderate intensity, with regenerative change in progress at the time of the patient's death from a metastasizing gastric carcinoma. The unusual and, so far as we are aware, previously unobserved feature, was the presence of refractile spherical bodies within the nuclei and cytoplasm of the renal epithelial cells of the convoluted tubules. A second case, in which the patient had been given 1 gm. of potassium bismuth tartrate suspended in peanut oil, was found to have similar refractile globules in the epithelium of the renal tubules. The histochemical reactions in the two cases were identical.

One can say little that is definite as to the chemical nature of these bodies. Their failure to stain with sudan III or with Nile blue sulphate or osmic acid, and their resistance to lipoid solvents shows that they are not simple fats. The negative reaction to the Ciaccio method, and the fact that they are not anisotropic would indicate that they are not composed principally of lecithin-like substance or of cholesterol esters. On the other hand, the positive staining with Spielmeyer's method may suggest a chemical relation to the myelins. That they are not of a protein nature is shown by their prolonged resistance to tryptic digestion and their resistance to strong acid or alkali. The darkening with hydrosulphuric acid and ammonium sulphite suggests that they may contain traces of bismuth, but the more specific histochemical tests with Komaya's reagent and stannous chloride are negative, although the injected material at the site of injection gives clean-cut microchemical tests for bismuth.

Since similar intracellular bodies are not formed at the site of injection and cannot be detected within the glomeruli, one may assume that the material of which they are composed is eliminated from the glomerulus either in solution or in finely dispersed form, reabsorbed by the tubules, and segregated by the nucleus in the form of one or rarely two refractile globules. That the bodies are primarily formed

within the nucleus rather than the cytoplasm is indicated by the appearances in Rat 11 which received in divided doses 0.7 cc. of bismocymol, equivalent to 200 mg. pro kg. Killed on the 8th day after the first injection there was found only a slight tubular injury. Numerous globules were present in the epithelial cells, but almost exclusively within the nuclei. Often their presence was unaccompanied by any other evidence of cell injury.

In searching the literature for similar observations there was found a short paper by Kollert, Strasser and Rosner.[3] Following the administration of trepol (potassium sodium tartrobismuthate) there appeared in the urinary sediment small, polygonal, finely granular epithelial cells. The nucleus of these was usually not distinguishable, but a sharply contoured refractile body about the size of the nucleolus was often distinctly seen. No doubly refractile bodies were found with the polarizing microscope.

In the description of the tubular nephrosis produced in rabbits by toxic doses no further mention is made of these refractile bodies. Whether they were of the same nature as those described above or not cannot be decided. We have found no other reference to such structures in the rather extensive literature concerning the effects of bismuth upon the tissues.

Summary

Refractile globules were found within nuclei and cytoplasm of renal epithelial cells in 2 cases following intramuscular injection, in 1 instance of bismocymol (a bismuth derivative of camphocarbonic acid), and in the other of potassium bismuth tartrate with butyn. Similar globules were found in the renal epithelial cells of rats after the injection of appropriate doses of bismocymol. The chemical nature of these globules was not determined; they gave equivocal reactions for bismuth, were insoluble in lipoid solvents and in strong alkalis and acids, resisted tryptic digestion, did not react for iron or calcium but stained as myelin by the Spielmeyer method.

Note. We are indebted to Doctors Allen Whipple and Walter Palmer for permission to include the clinical records of these cases.

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DESCRIPTION OF PLATES

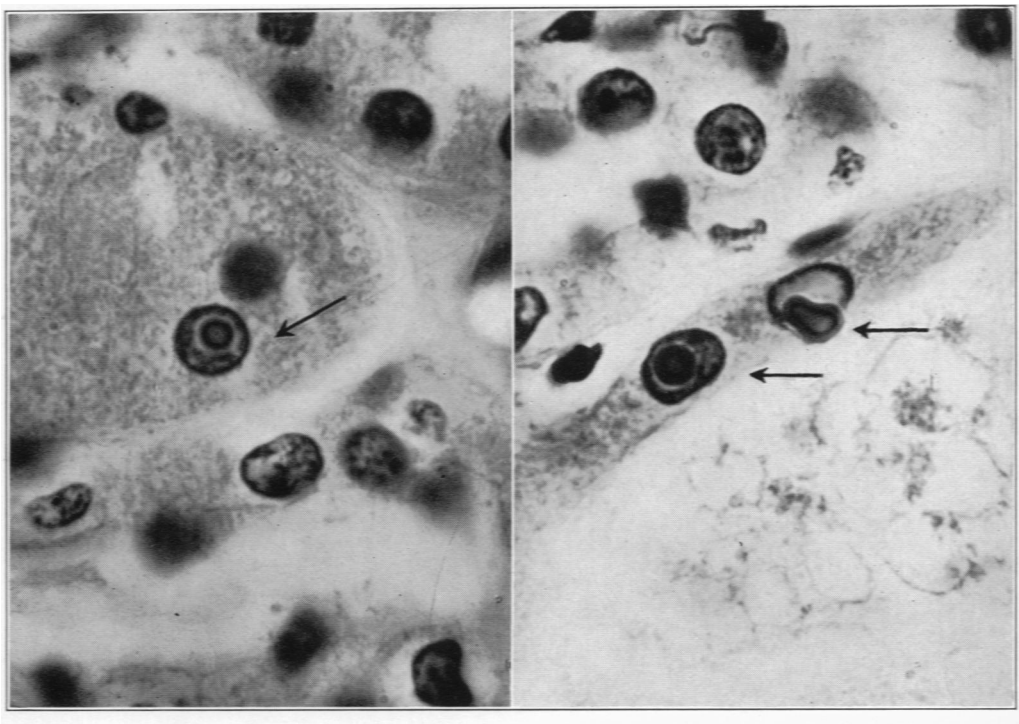
PLATE 138

FIG. 1. Case 1. Intranuclear body in renal epithelial cells. Hematoxylin-eosin stain. × 1680.

FIG. 2. Case 1. Two inclusion bodies are seen; one spherical in shape, completely within nucleus; another, pear-shaped, apparently escaping through a gap in the nuclear membrane. × 1680.

FIG. 3. Case 1. Refractile spherical inclusion in cytoplasm. The nucleus is pyknotic and irregular in shape. × 1680.

FIG. 4. Case 1. Two cytoplasmic inclusions are shown, one adjacent to a nucleus; another lies in a necrotic cell that has lost its nucleus and is desquamated into the lumen. × 1680.



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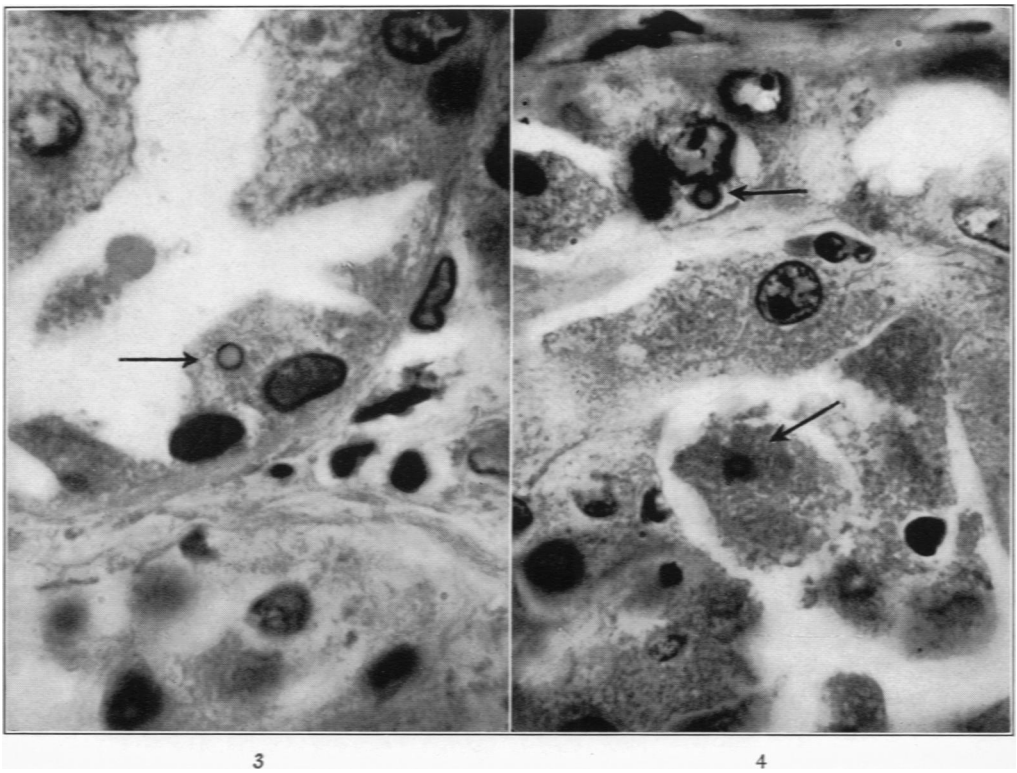


3 4

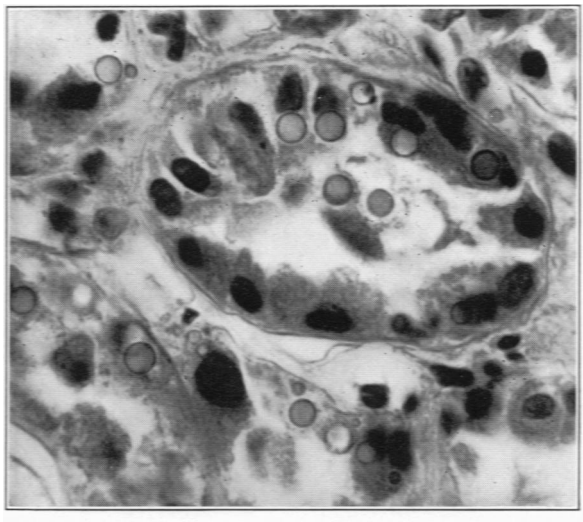
Pappenheimer and Maechling Inclusions in Renal Epithelial Cells

PLATE 139

FIG. 5. Case 2. Renal tubules containing numerous inclusions, chiefly cytoplasmic. Zenker fixation. Hematoxylin-eosin stain. × 720.

FIG. 6. Case 2. Frozen section of kidney, myelin stain, Spielmeyer's method. The globules are stained black. × 100.

FIG. 7. Case 2. Formalin-fixed tissue, washed, digested for 12 days with Merck's trypsin. The outlines of the tubules are still recognizable. The globules resist digestion and stain with hematoxylin. × 700.



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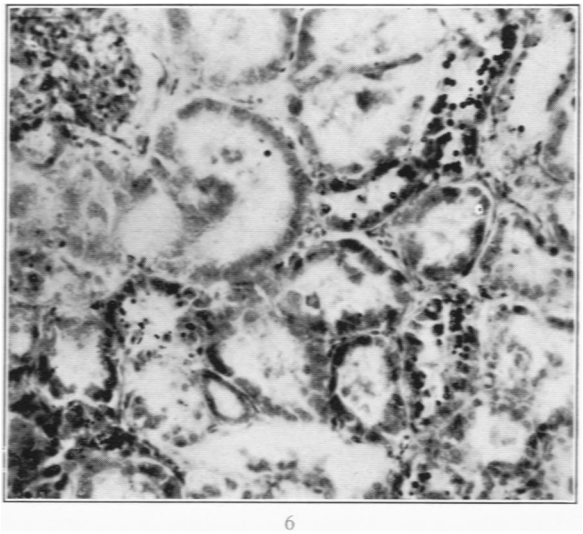

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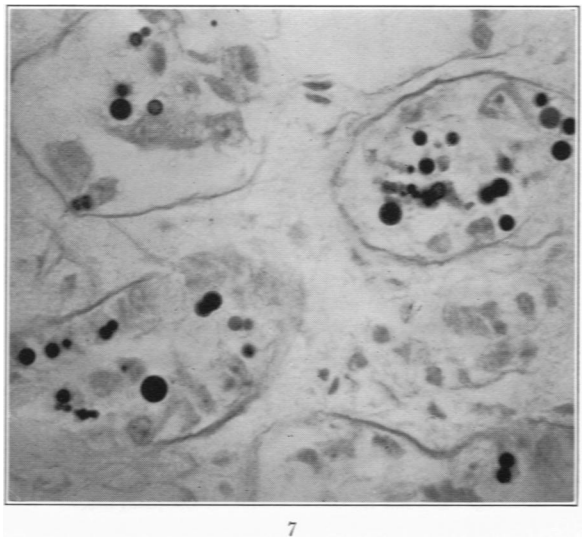

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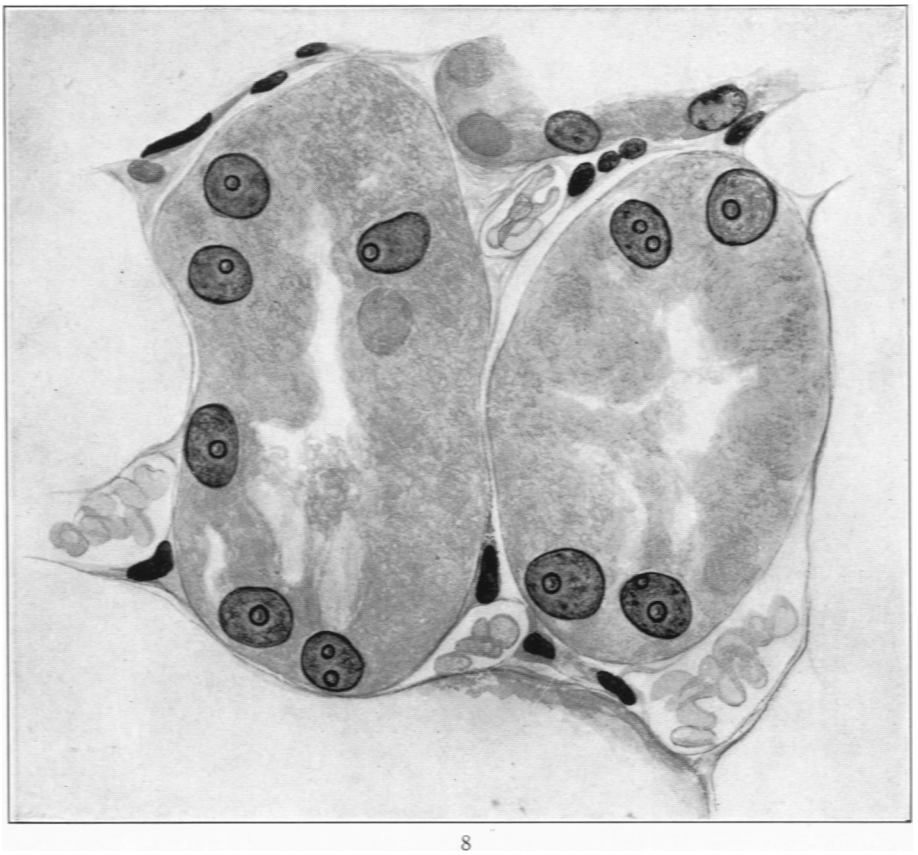
PLATE 140

FIG. 8. Rat 15. Numerous refractile globular inclusions within nuclei of renal epithelial cells, following injection of bismocymol. Zenker fixation, hematoxylin-eosin stain.

FIG. 9. Rat 15. Kidney with intranuclear inclusions. Unstained frozen section after formalin fixation. × 720.



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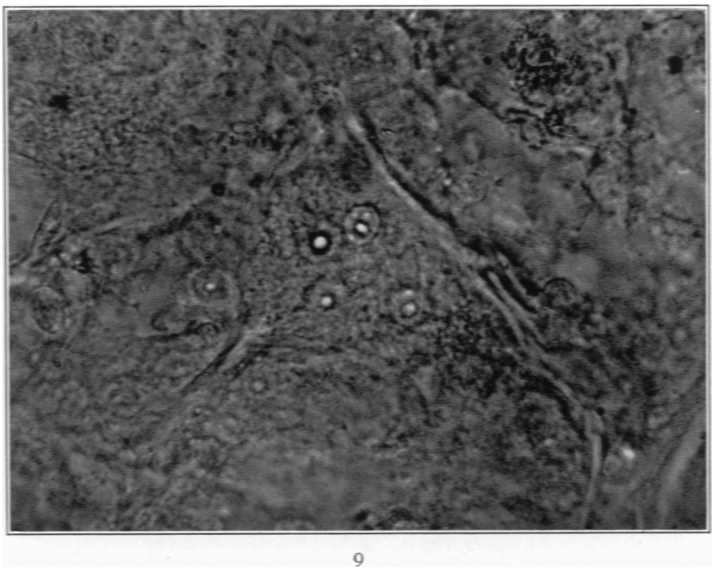

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