BISMUTH PIGMENTATION
Its Histochemical Identification *

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On occasion it is desirable to differentiate the pigmentation produced by compounds of bismuth from those due to other causes. Pigmentary deposits containing bismuth have been described as a diffuse, dark discoloration of the colon, beginning sharply at the ileocecal valve and involving the mucosa in varying degrees.[1-7] Bismuth is one of the several metals which may be deposited in the gingiva or other areas of the oral mucosa and induce brown, bluish, or black discoloration.[8,9] Deposition of black bismuth sulfide has also been described in the vagina [7,10,11] and in the bladder.[12,13] Deep pigmentation of the skin due to bismuth deposition may occur in exceptionally rare cases.[4,14,15]

In the present investigation, Castel's method [16] for the histochemical identification of bismuth was adapted for the demonstration of bismuth sulfide in tissue sections and in gross specimens. Thus, a simple histochemical procedure for the identification of bismuth, deposited as black sulfide as it may occur in surgical or autopsy material, is available. It was found that bismuth pigmentation of the large intestines occurs fairly frequently. Among the last 340 autopsies performed at the Mount Sinai Hospital, 4 examples were encountered.

Method

The method depends on the property of hydrogen peroxide of decolorizing bismuth sulfide instantaneously. Black bismuth sulfide is thus transformed into white bismuth sulfate. [17] By then treating the sections with a slightly modified Castel reagent, [16] containing brucine sulfate and potassium iodide, the bismuth sulfate is transformed into an orange-red deposit. This reaction, based on the work of Léger, [18] depends on the fact that numerous organic bases form insoluble double iodides with bismuth of the general formula BI_3. B. HI, in which B represents the base. [19]

In this study, frozen as well as paraffin sections were used. Paraffin sections were deparaffinized in the usual manner and were then treated with a few drops of superoxol (30 per cent hydrogen peroxide, Merck). This reagent is best kept in a dark bottle in the refrigerator. The black color of bismuth sulfide disappears in a few seconds. Sections

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are then washed thoroughly in tap water and placed in a Coplin jar, containing the modified Castel reagent.[16] The latter is made by dissolving 0.25 gm. of brucine sulfate (Merck or Eastman Kodak) in 100 cc. of distilled water containing 2 or 3 drops of concentrated sulfuric acid. After the brucine sulfate has dissolved, 2 gm. of potassium iodide are added. The reagent is kept in a brown bottle and filtered before use. After 1 hour, the sections are put into another jar containing the brucine reagent diluted with three parts of distilled water, and gently shaken in order to remove precipitates. Most of the remaining fluid is removed by gentle blotting and the slide covered with levulose solution (prepared by dissolving 30 gm. of levulose in 20 cc. of water by heating to 37° C. for 24 hours), to which a drop of the diluted Castel reagent is added with a glass rod or toothpick. If a counterstain is desired, the slide is stained for 4 minutes with a mixture containing 100 cc. of the nondiluted brucine sulfate reagent and 1 cc. of a 1 per cent aqueous solution of light green SF (Hartman-Leddon Co.), filtered before use. The stain will keep well although the orange color may darken to some degree.

This method can be applied also to gross specimens. Fixed material is preferable, but fresh tissues are also suitable. The concentrated hydrogen peroxide solution is added drop by drop to the area under investigation. Decolorization will take place immediately if bismuth sulfide is present. The specimen is then thoroughly washed in running water for several minutes to remove excess hydrogen peroxide. The modified Castel reagent is then applied to this surface. An intense orange precipitate is formed, giving the same color to the mucosa.

Cases with Bismuth Pigmentation

Case 1

A white male, 41 years old, was admitted to the hospital because of dyspnea, generalized edema, and severe anemia. Physical examination revealed marked enlargement of heart, liver, and spleen. Petechiae were found on the conjunctivae. A positive Wassermann reaction of the blood was present. For this reason the patient was given biweekly bismuth injections. He underwent a splenectomy for intractable anemia with good results, and was discharged after 4 months. Following this he continued to receive weekly bismuth injections. Five months later he was readmitted in cardiac failure, and died after 14 days in the hospital.

The pertinent post-mortem findings (autopsy No. 12768) were cerebral hemorrhage, healing stage of subacute bacterial endocarditis upon chronic rheumatic mitral and aortic valvular disease, and subacute glomerulonephritis. Patchy gray and black areas of discoloration were found in the cecum. In the tonsils similar pigmentation was visible in the crypts. Microscopic sections through the discolored areas

of the cecum showed black pigment in the walls of the most superficial capillaries. Characteristically, this pigment was found in the capillary endothelium, producing a singly or doubly contoured black line. Very little pigment was seen elsewhere. Sections through the tonsils showed lymphatic tissue covered by one layer of cylindrical epithelium frequently arranged in a papillary fashion. The subepithelial capillary walls showed marked impregnation by a dark pigment (Fig. 1). The intensity of this reaction varied in different vessels, so much so that in some the lumen was obscured by the very heavy deposit. Moreover, black pigment was seen also in some histiocytes as well as free in the submucosa, without definite relation to cellular structures.

Case 2

A white female, 30 years old, had a history of rheumatic fever and chorea in childhood. She had been suffering from mild dyspnea for the past 5 years. Two years before admission hypertension was noted. Wassermann reaction of the blood was 4 plus and therefore she was treated elsewhere with weekly bismuth injections for 1 year. Because of sudden severe dyspnea and cyanosis she was admitted to the Mount Sinai Hospital, where her blood pressure was found to be 250/140 mm. Hg. Urine examination revealed 3 plus albumin, hyaline and granular casts, and occasional red blood cells. She died 2 days later.

The pertinent post-mortem findings (autopsy No. 12933) were rheumatic heart disease and malignant nephrosclerosis. The cecum and ascending colon showed a very marked, diffuse, black discoloration. On microscopic study, the findings in regard to the location of pigment were exactly as in the previous case. Pigment-laden histiocytes and extracellular granules in the mucosa were, however, quite prominent. Again, no pigment was seen in the deeper layers (Fig. 2).

Case 3

A white male, 65 years old, gave a history of chancre 25 years before admission to the hospital. Eight months before, his blood and spinal fluid had given a positive Wassermann reaction and a paretic colloidal gold curve. He was then treated with fever therapy, arsphenamine, and two injections of 1 cc. of a bismuth preparation in oil. Five weeks before admission, a gastrostomy was performed elsewhere because of carcinoma of the esophagus. The patient entered Mount Sinai Hospital for resection, but died within a few days and before operation.

The most important post-mortem findings (autopsy No. 12952) were squamous cell carcinoma of the esophagus with metastases to the lungs and lymph nodes, anthracosilicosis, pulmonary tuberculosis with recent bronchogenic spread, and syphilitic mesaortitis. The mucosa of the cecum and ascending colon was diffusely brown. Microscopic sections through this area showed only occasional superficial capillaries impregnated with a dark pigment. Fine pigment granules were seen in the endothelial cells of less involved capillaries, and in an occasional histiocyte in the mucosa.

Case 4

A white male, 55 years old, had a history of chancre 34 years prior to admission and of treatment for 7 years with injections of bismuth and arsphenamine. Three years before admission examination of his blood revealed a 1 plus Wassermann reaction. He was treated for another year with bismuth and arsphenamine. Thereafter, his serologic tests became normal. A few months later he acquired a second syphilitic infection, proved by dark-field examination. Again he was treated with bismuth and arsphenamine parenterally. The patient was admitted to the hospital because of vomiting, severe headache, and mental confusion. His blood pressure was 250/110 mg. Hg and blood urea nitrogen, 150 mg. per cent. The patient died 3 days later.

The significant findings at the post-mortem examination (autopsy No. 13022) were malignant nephrosclerosis with marked cardiac hypertrophy and dilatation. The large intestine showed diffuse black pigmentation of the mucosa, especially severe in the cecum, starting sharply at the ileocecal valve and diminishing in intensity distally. A pigment line was visible on the upper gingival margins. Microscopic sections of the colon showed, as before, impregnation of many of the capillary walls by black pigment, and altogether a similar histologic picture. Sections through the gingiva revealed pigment deposited in superficial capillaries and histiocytes, as well as severe chronic and acute inflammation with superficial ulceration.

Case 5

A white male, 59 years old, had had a generalized rash, including palms and soles, at the age of 26. He was treated with mercury inunctions. Thirteen years later examination of his blood revealed a 4 plus Wassermann reaction. His spinal fluid was negative at that time. For the following 3 years he was treated continuously with bismuth and salvarsan injections. Examination of his blood 2 years prior to admission revealed a positive Wassermann reaction. He was given a course of eighteen bismuth and arsphenamine injections, following which the Wassermann reaction of the blood became negative. On one occasion, when the patient visited the Out Patient Department complaining of a swelling in his mouth, physical examination revealed a small growth extruding between the third and fourth left lower teeth. No mention was made of a black discoloration of the gingiva.

A specimen of this area, taken for biopsy (surgical specimen No. 81006), showed marked acute and chronic inflammation. Except for a greater intensity, the gingiva here revealed pigment deposition similar to that seen in the preceding case.

Comment

Microscopically, the outstanding feature of the deposition of bismuth is the impregnation of the capillary walls, predominantly in the superficial layers of the mucosa. When little is present, it is found only here; when much pigment is deposited, bismuth sulfide is seen also in histiocytes and free in tissue spaces. It is never encountered

in the deeper layers. This behavior is identical in the mucous membrane of the mouth and in that of the large intestine. In all cases in which bismuth sulfide was seen, it could be identified easily with the above-described method.

Among the pigments occurring in the oral cavity, melanin and iron are easily distinguished by their location. Iron, which may be present as the black sulfide, is oxidized by concentrated hydrogen peroxide and transformed into the usual golden brown hemosiderin. It does not react with Castel's reagent [16] but gives the typical iron reactions. Melanin is not bleached by a short treatment with hydrogen peroxide and does not give a reaction with the brucine reagent. Among the exogenous pigments, lead sulfide is the most important to exclude. Since it is deposited in vessel walls in the same manner as bismuth, it cannot be differentiated in routine sections. In order to test the bismuth reagent on lead sulfide in tissue sections, slides prepared according to Gomori's method [20] for the demonstration of acid phosphatase were used. By this technic, the phosphatase activity is demonstrated by the deposition of black lead sulfide. Preparations of this kind were oxidized with hydrogen peroxide. The lead sulfide is immediately discolored in the same fashion as bismuth sulfide, the sulfate being formed. However, treatment with Castel's reagent brings about only a slightly yellowish tinge of the lead deposits (PbI_2), in contrast to the brilliant orange-red formed with bismuth.

Copper, silver, and mercury may also be deposited in capillary walls. No tissue material was available for study. However, in the test tube, as already shown by Castel, [16] silver and mercury give yellow, and copper gives brown precipitates, thus differing from bismuth. All these metals react with the iodide but do not combine with the brucine to form double salts as bismuth does. [19]

In the large intestine, the most frequent cause of dark pigmentation is "melanosis." In this condition, a brown granular pigment is deposited in the histiocytes of the lamina propria and occasionally in the submucosa. This pigment has been grouped with the melanins.[21, 22] It is usually found in persons suffering from constipation. It has been shown that its occurrence is dependent upon the intake of the emodin-bearing group of cathartics (cascara, aloes, frangula, Rheum, and senna). [23, 24] On microscopic examination, a distinction between bismuth and this pigment is readily made, since the latter never impregnates the capillary walls. An immediate distinction can be made at autopsy by applying the previously described gross reaction to fresh tissue. This melanin is not discolored by the short application of hydrogen peroxide and does not give the reaction with Castel's reagent. [16]

A second specific test, also based on Léger's work, [18] is available for identification of bismuth in tissue sections. Quinine sulfate and potassium iodide give a yellow precipitate with bismuth. Komaya, [25] among others, [4] adapted this reaction for the use in tissue sections. While this method gives good results in frozen sections, we found Castel's reagent [16] much more reliable and simpler for use in paraffin-embedded material.

No attempt was made to apply the specific bismuth stain systematically to all organs. However, on applying the stain to the kidney sections of cases 2 and 4, as well as of another case not included in this series, it showed refractile globules in the epithelium of the proximal convoluted tubules, as described by Pappenheimer and Maechling. [26] These authors studied the staining properties of these inclusion bodies extensively. They found them to react regularly with the Weigert-Spielmeyer stain. They became dark on treatment with hydrogen or ammonium sulfide, but did not give the more specific histochemical reactions for bismuth with stannous chloride-sodium hydroxide and with Komaya's reagent. [25] Apparently, similar bodies were described also by Langhans [27] in tissue sections of experimental animals, and in epithelial cells of human urinary sediments by others. [28-32] In our material most of these inclusion bodies reacted with Castel's reagent. [16] The majority gave a distinct orange color, although some stained yellow and others remained unstained. Desquamated epithelial cells and casts gave an occasional positive reaction.

To our knowledge no statistics are available concerning the incidence of bismuth pigmentation in the colon. Wiener [11] and Heyman [7] stated that deposition of bismuth is of very rare occurrence. However, since special attention has been paid to proper identification of discolored areas in the mucosa of the large intestine, we have encountered this condition more frequently.

Case 3 is of special interest. Here bismuth pigmentation of the colon was found although only two injections of 1 cc. of a bismuth preparation were given. Grossly, "melanosis coli" was diagnosed. However, the pigment showed the typical microscopic appearance and histochemical behavior of bismuth sulfide. Because of this, inquiries were made which revealed that the patient had received this small amount of bismuth at another hospital. The amount of histologically demonstrable bismuth varied markedly in the other three patients, although all of them had received intensive treatment. This is in full agreement with the findings of Sollmann, Cole, and Henderson [33] who determined the bismuth content of various organs of patients who had received bismuth. In 23 cases the colon contained between 0.025 and

3.0 mg. of bismuth in 100 gm. of wet tissue. The average was 0.115 mg. per 100 gm. of wet tissue. They found that the colon ranked fifth in bismuth content, after kidney, liver, spleen, and bile. In one case, described previously by one of us,[6] in which bismuth therapy led to fatal intoxication, 5.8 mg. of bismuth per 100 gm. of wet tissue was found in the colon.

SUMMARY

Castel's method for the demonstration of bismuth in tissue preparations was adapted for the identification of bismuth sulfide in frozen sections, paraffin sections, and in gross specimens. The method permits histochemical identification of bismuth sulfide pigmentation in any tissue. Bismuth discoloration of the colon is apparently not infrequent. It was found in four of 340 consecutive autopsies. Even small amounts of injected bismuth may lead to the deposition of histochemically demonstrable bismuth sulfide in the large bowel. The inclusion bodies found in the renal epithelial cells following the use of bismuth preparations frequently give a positive reaction with Castel's reagent.

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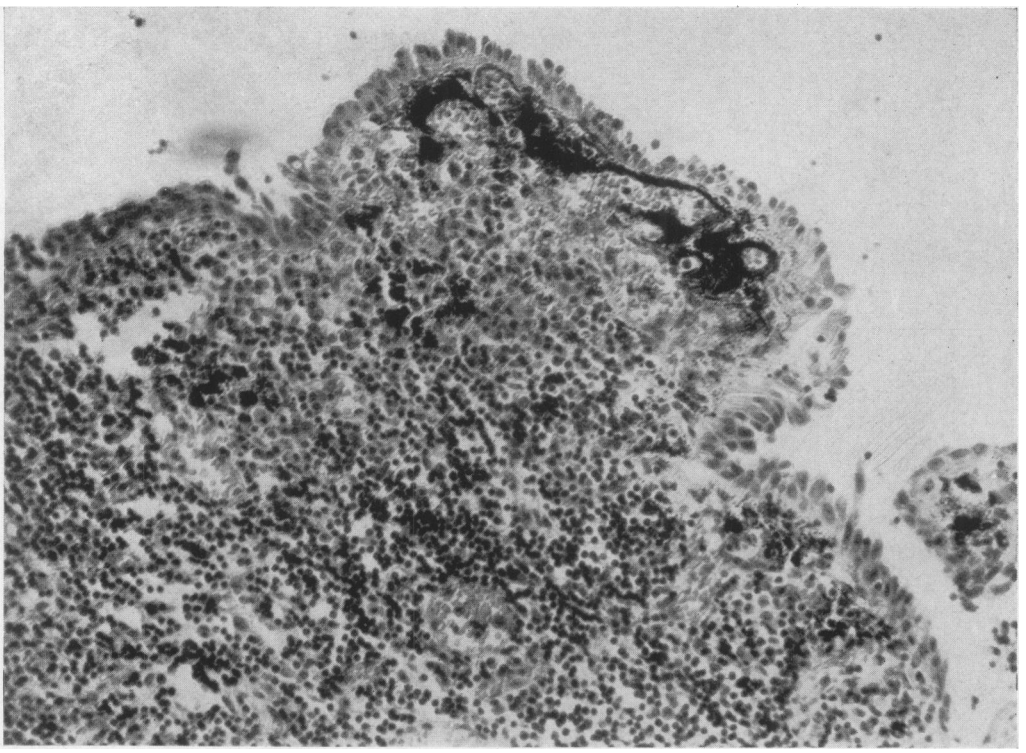
DESCRIPTION OF PLATE

PLATE 124

FIG. 1. Case 1. Section through tonsil, showing superficial capillaries impregnated by black bismuth sulfide. Occasionally pigment is seen in histiocytes. Hematoxylin and eosin stain. × 145.

FIG. 2. Case 2. Section through colon, showing extensive deposition of bismuth sulfide in mucosa. Hematoxylin and eosin stain. × 70.

1



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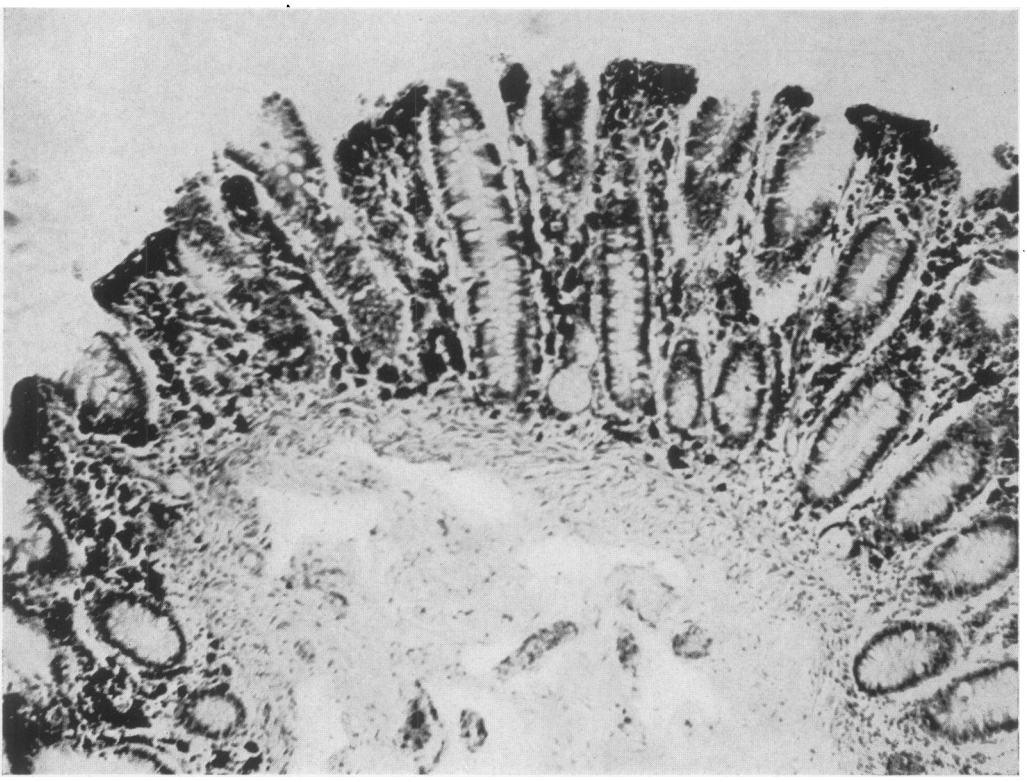

Wachstein and Zak

Histochemical Identification of Bismuth