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RELATIONS BETWEEN LEAD POISONING IN RABBIT AND MAN

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There is increasing concern over contamination of our highly populous areas with toxic substances. There is justifiable suspicion that the pathogenesis of some diseases, especially chronic diseases of insidious onset, may be related to prolonged exposure to these substances at concentrations insufficient to produce conspicuous manifestations. Among these substances lead has long been recognized as important but its relationship to many disturbances attributed to its toxicity has not been firmly established. Tanquerel was the first to bring the significance of lead poisoning as the cause of illness to the attention of physicians in Europe.[1] Dana translated his writings for the attention of physicians in this country.[2] Since then, an extensive literature on the subject has appeared and has been summarized.[3,4] As a result of the continued interest, the principal obvious sources of lead poisoning have been largely eliminated but new sources and less suspect old sources of intoxication are with us in ever increasing numbers.[5-7]

The overt manifestations of severe lead poisoning are well known. Anemia, neuropathy, nephropathy, gingival "lead line" and "gastric crises" have been emphasized in the adult.[3] In infancy and early childhood, anemia, nephropathy, osteopathy, encephalopathy and mental retardation seem most important.[8,9] The pathogenesis of these signs and symptoms is not understood nor is there agreement as to what the

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long-range effects of minor acute or chronic exposure to lead might be.[9-11] For instance, in Australia, chronic nephritis and arteriosclerosis with hypertension have been regarded as common delayed sequelae of chronic lead poisoning.[6] Also, mental retardation in children may at times be related to unrecognized or asymptomatic lead encephalopathy in infancy. Finally, there is the possibility that lead may act as a carcinogenic agent in man as it does in animals.[12-15]

The literature contains much speculation about lead poisoning and its relationship to many diseases. However, there is little experimental work designed to establish the suspected relationships beyond doubt or to define the means by which exposure to lead may produce such a variety of symptomatic and tissue structural disturbances.[16] With these matters in mind, we have undertaken an animal experimental study. The present report is concerned with a description of pathologic effects due to prolonged ingestion of lead by the rabbit and a comparison of these effects with those attributed to lead poisoning in man. Later reports will be concerned with the pathogenesis of these effects with particular attention to intercellular matrix formation, calcium metabolism and carcinogenesis.

Methods

Male New Zealand albino rabbits about 3 months old and weighing 5 to 6 pounds were used. Rabbits of German Checker and Belgian Hare strains were also used in studies which required pigmented retinal epithelium. All animals were housed individually and weighed each week. The basal diet was Purina rabbit chow. A record was kept of the amount of diet consumed.

Eighty-one animals were divided into 6 groups. Group I consisted of 16 rabbits fed the basal diet to which 500 mg per cent of lead subacetate (C.P.) was added. Group II consisted of 8 animals fed the basal diet to which was added 10 gm per cent of boiled commercial linseed oil containing the standard "lead drier." The concentration of components of the "lead drier" was given as follows: 0.20 per cent lead, 0.35 per cent manganese and 0.30 per cent cobalt naphthenates. Group III consisted of 15 animals fed the basal diet to which was added 500 mg per cent of lead subacetate (C.P.) and 10 gm per cent of boiled linseed oil containing the standard "drier." Group IV consisted of 8 animals fed the same diet as those in Group III with an additional supplement of 300 mg per cent of cholesterol. Group V consisted of 20 animals maintained on the basal diet. Group VI consisted of 14 animals given the basal diet supplemented with 300 mg per cent of cholesterol.

Animals in each group were killed at intervals of 4 to 8 weeks after 3 to 55 weeks on the different regimes. Complete necropsies were done. Tissues were placed in several different fixatives for microscopic study. A light microscopic study was made of sections of all organs fixed in formalin and stained with hematoxylin and eosin. A special study was made of the brain, spinal cord, nerve roots and peripheral nerves with myelin, axon and nerve cell stains. Particular attention was given to the retina, bone, muscle, kidney and the arterial system. Blocks were taken from the main pulmonary artery, aortic arch, thoracic aorta, abdominal aorta and the following arteries: iliac, femoral, carotid, brachial and renal. Abnormal pigment deposits and intracellular inclusions were investigated by use of Ziehl-Neelsen, iron, periodic acid-Schiff and Scharlach R stains.

Electron microscopic studies were made of the retina in several animals. The tissues were fixed promptly in osmic acid buffered according to the method of Palade. After fixation the tissues were embedded in Epon 812, cut with a diamond knife with an LKB microtome and studied with an RCA-EMU 3E electron microscope.

The following special laboratory procedures were done at 2 to 4 week intervals: serum cholesterol, blood hemoglobin and differential nucleated blood cell counts with stippled red cell counts of blood smears. X-ray studies of the long bones were made of 12 animals of Groups I, II and III over a period of 5 to 7 months.

Hematologic Studies

The most severe hematologic changes occurred during the first few months among animals on dietary regimes containing lead subacetate with or without supplemental linseed oil. Hemoglobin values declined from an average initial level of 12 to 14 gm per cent to a level of 8 to 10 gm per cent within the first 3 or 4 months and thereafter stabilized. The white cell count, as judged by a study of blood smears, did not change significantly except in the presence of infection. The usual infections were in the lungs though encephalitis and a generalized mild systemic inflammatory disease of unknown cause were rather common. These diseases occurred in control animals with about the same frequency. There was, however, late in chronic lead poisoning a relative lymphocytosis often as high as 80 to 90 per cent. Occasionally, there was an unexplained monocytosis as high as 20 per cent and basophils reached levels as high as 10 per cent in several animals. Platelets were consistently normal. Circulating erythroblasts increased in an irregular way in rough proportion to the increasing anemia. As a rule, they represented 1 to 6 per cent but occasionally as many as 20 per cent of nucleated cells. There was a reticulocytosis and an associated increase in stippled cells with counts as high as 4 to 6 per high power microscopic field. Anisocytosis, poikilocytosis and polychromatophilia were also often conspicuous. No atypical blasts were found except occasionally in the late stages of disease. As with hemoglobin values, there was no progressive depletion of red cells for they tended to stabilize at some abnormal state so long as the animal remained reasonably healthy on the lead regime.

Observations of blood smears were usually a suitable reflection of those made on the spleen and bone marrow. Hemosiderosis, excessive phagocytosis of red cells and increased erythropoietic activity in the spleen and bone marrow were conspicuous only after long periods on lead subacetate and lead subacetate-linseed oil-cholesterol regimes. The splenic hemosiderosis was at times complicated by a basophilic staining of the splenic reticulum (Fig. 12).

A diagnosis of lead poisoning in man is usually made by examination of the blood. As a rule, about 100 μg per cent of lead in the blood is

sufficient to indicate toxicity, and manifestations of toxicity usually increase with increasing blood levels.[17] Most of the lead is in the erythrocytes.[18] As exposure to lead is eliminated, the plasma is rapidly cleared, principally by the kidneys. Lead persists in the bones and erythrocytes for a much longer time. It is estimated that it would require 8×10^8 years to excrete 50 per cent of lead stored in bones which in adults with symptoms amounts to about 1.0 gm and in a child to about 100 to 1,000 mg.[17]

As lead accumulates in the blood of man, hematologic changes occur in about the following sequence: (1) increased blood protoporphyrin with increased urinary coproporphyrin, (2) basophilic stippling of red cells, (3) hypochromia of red cells and (4) reduced number of red cells. The principal disturbance, in common with that produced by intoxication with several other metals, is interference with porphyrin metabolism. This interference is generalized because the amount of coproporphyrin excreted is often far in excess of the amount expected from an involvement of the hematopoietic system alone.[17]

The principal chemical change in the erythrocyte is an increase in coproporphyrin and protoporphyrin with a decrease in the amount of heme. Rabbit and man are among the few species in which lead poisoning interferes with porphyrin metabolism. Furthermore, these two species, more so than others, readily develop basophilic stippling of erythrocytes. The degree of stippling in several animals of the present experiments was even greater than that encountered in man who seldom has more than 1 per cent stippled cells.[6] Stippling in both species is not restricted to lead poisoning. It is usually correlated, as in the present studies, with the common accompaniments of rapid erythropoiesis such as reticulocytosis, polychromatophilia and erythroblasts in the peripheral blood.[19] Soon after elimination of exposure to lead, the stippling, excessive coproporphyrinemia and associated hematologic findings disappear, though mild coproporphyrinuria may persist for months. Mitochondria of erythrocytes serve as the nidus for stippling and the materials involved, ribonucleic acid combined with protoporphyrin, are also characteristic of reticulocytes and red cells displaying polychromatophilia.[20] The stippled cells have an increased resistance to hypo-osmolar salt solutions, an effect which can be reproduced by treating normal erythrocytes with lead *in vitro*.[3] At the same time the cells display a reduced stickiness which interferes with the usual effect of isoagglutinins.[3] These altered properties of erythrocytes are accompanied by an increased mechanical fragility. This presumably contributes to the decrease in the number of red cells and occurrence of hemolytic anemia, common in human lead poisoning and in the present

studies. Splenic hemosiderosis and phagocytosis of red cells were conspicuous in our studies. It is implied that stippled cells are particularly susceptible to destruction by the spleen for following splenectomy in rats with lead poisoning, there is an increase in stippled cells accompanied by an alleviation of anemia.[21]

Many manifestations of patients with lead poisoning resemble those of patients with porphyria due to other causes.[17,22] This had led to the suggestion that lead principally exerts its toxic effect by disturbing porphyrin metabolism. In this connection it is of interest that hematoporphyrin may unite with proteins to form complexes. Upon exposure of these complexes to visible or ultraviolet light in the presence of oxygen, protein hydrolysis may occur.[23]

Most hematologic findings characteristic of lead poisoning in man were reproduced in these experiments. There was a reduction in the number of circulating erythrocytes accompanied by a greater relative reduction in hemoglobin. These changes which reached a plateau within a few weeks were accompanied by stippling, reticulocytosis, polychromatophilia and increased numbers of erythroblasts in the peripheral blood. Despite continued ingestion of lead for many months, these changes did not progressively increase in severity but fluctuated moderately as the period of lead ingestion was prolonged. A degree of tolerance seems to have been established. Blood platelets were unaffected. The differential counts, unlike those in man, tended to disclose a relative lymphocytosis, monocytosis and increased numbers of basophils.

The severity of anemia was reflected by increased erythropoietic activity in the bone marrow and occasionally in the spleen. Outstanding in the spleen, however, was the evidence of a hemolytic process characterized by conspicuous phagocytosis of fragmented red cells and accumulation of hemosiderin. At times, this was accompanied by incrustation of the reticulum and trabecular collagenous structure with basophilic materials resembling deposits of calcium or phosphate or both in combination with iron (Fig. 12). This resembled changes noted in some patients with chronic severe hemolytic anemias and secondary splenic hemosiderosis.

Nephropathy

There were three closely related cytopathologic changes in the kidneys. These occurred nearly simultaneously and increased in prominence in direct proportion to the duration and amount of lead ingestion. They were first noted in the straight narrow tubules near the cortico-medullary junction. They consisted of changes in the tubular lining cells characterized by pigmentation, cytomegaly and intranuclear inclusion bodies.

The first effect was the intracytoplasmic accumulation of light brown granules (Fig. 1). They had no consistent structural or staining properties. Occasionally, they stained for iron or were weakly positive with Scharlach R. As the granules of pigment increased in number the most severely affected tubular lining cells displayed degenerative changes. This led to the formation of desquamated pigmented cellular casts and the accumulation of pigment in extracellular locations. This degeneration was followed by a regenerative reaction, often characterized by appearance of large atypical tubular lining cells (Fig. 2). Each of these cells had a giant hyperchromatic nucleus and an abundant cytoplasm, usually containing granules of brown pigment. This type of cytomegaly has not been noted by us in regenerative reactions which follow customary endemic or experimental renal tubular degenerative diseases in the rabbit. During the period of initial accumulation of pigment and appearance of "anaplastic" giant tubular lining cells, intranuclear inclusion bodies made a somewhat delayed appearance (Fig. 1). They were most easily recognized in the giant cells but also occurred in adjacent tubular epithelium. Similar structures which were occasionally in the cytoplasm seemed to have formed originally in a nucleus. As the nephrotic disease progressed, the inclusion bodies became more numerous and often were found in cells lining convoluted tubules. The inclusions possessed properties fully described by others.[24-31]

The first signs of nephropathy, attributable to lead, appeared within about 8 to 12 weeks. Thereafter, the changes usually became increasingly conspicuous reaching near maximum severity after 28 to 36 weeks of maximum lead ingestion. At this time focal glomerulo-tubular replacement by subcapsular and radial cortical scars was a clear indication of progressive permanent renal damage. At the end of 55 weeks, there was evidence of further progression but not to a stage where the lesions might be held responsible for lethal renal insufficiency.

Lead nephropathy was impressive only in animals on the basal diet-lead subacetate regime. Addition of linseed oil to this regime did not influence the nephropathy. Addition of cholesterol to the diet, however, reduced the severity of the nephrosis and incidence of intranuclear inclusion bodies. This effect occurred at serum cholesterol levels between 180 and 350 mg per cent. These levels were much lower than those required to produce significant atheromatous arteriopathy or lipoid nephrosis in the rabbit.

No lead nephropathy occurred in animals maintained on the basal diet-linseed oil regime. This was unexpected because these animals developed a retinopathy comparable to that occurring in animals ingesting large amounts of lead subacetate. This apparent contradiction and the

antinephropathic effect of dietary cholesterol require further study. The latter observations would seem to be contrary to results of experiments in rats in which a high-fat diet containing lead induced necrosis and many intranuclear inclusion bodies in the liver.[30]

In human lead poisoning, the principal renal changes resemble those in the rabbit. The lining cells of Henle's loop and, to a lesser extent, those of proximal convoluted tubules are affected.[26] Cytoplasmic degenerative changes, giant tubular lining cells and intranuclear inclusion bodies are characteristic findings. Pigment accumulation seems insignificant. The associated functional disturbances in man, which have not been described in the rabbit, may resemble those of the Fanconi syndrome.[10]

Intranuclear inclusions in man, seldom found elsewhere except in the liver, have special properties by which they may be defined as specific for lead poisoning. They differ from viral-induced inclusion bodies, especially in their acid-fast staining property and their ultrastructure.[24-31] They are easily produced in the kidney of several experimental animals but are not found elsewhere except in the liver of rats, particularly when the diet is high in fat as well as lead.[30] Although other metallic compounds, such as aluminum oxide, may produce intranuclear inclusions in fibroblasts at sites of injection, the inclusions do not resemble those found in the kidney in lead nephrosis.[32] Also, attempts to produce renal inclusion bodies by injection or feeding toxic compounds of nickel, cobalt, mercury and uranium have failed.[29] The only other metal held responsible in man for the occurrence of renal intranuclear inclusions, also possessing the acid-fast property, is bismuth. The only bismuth compounds, among several tested, which produce renal intranuclear inclusions are bismuth camphocarbonic acid and bismuth subnitrate.[25,33,34] These inclusions differ from those of lead nephropathy in being more refractile and sharply spherical with staining properties similar to those of myelin. Other distinctive properties indicating the presence of a lipid component and absence of virus have been described in ultrastructural studies.[25]

The tubular degenerative changes in human lead nephropathy are not restricted to cells with intranuclear inclusions. Many changes are non-specific and resemble those produced by other toxic agents. There is no good evidence in this country that these changes lead progressively to chronic renal disease with hypertension. There is better evidence that renal disease with hypertension may have been a late complication of severe chronic poisoning often encountered years ago in Europe and Australia.[3,6]

There has been little comment about occurrence in the human kidney of intracytoplasmic pigment or atypical giant cells lining the tubules.[26]

Nor has anyone been impressed with the resemblance of the regenerating giant cells to some neoplastic cells. Nevertheless, the possible significance of pseudo-neoplastic cellular changes of this type should not be overlooked.

The location and sequence in development of renal pathologic changes in the rabbit resemble those in man more closely than those in the rat. The rat, however, has more often been used in the experimental study of lead nephropathy.[24,26-31] In the rabbit the proximal convoluted tubules are not involved until after conspicuous changes have occurred in the small straight tubules near the cortico-medullary junction. These tubules, analogous to Henle's loop, lie in the zone most susceptible to calcification in hypervitaminosis D and to interstitial xanthomatosis secondary to hyperlipemic hypercholesteremia.[35,36] In the rat the most conspicuous early changes and progressive disease with numerous intranuclear inclusions occur in the proximal convoluted tubules. In man, Henle's loop is first involved. In the rabbit, more so than in rat or man, an accumulation of intracytoplasmic pigment precedes the conspicuous appearance of giant cells and intranuclear inclusions. This pigment is refractory to study by special stains and is not clearly definable as hemosiderin or aposiderin as suggested by Tönz.[37] It may be a porphyrin-metal complex. This is in keeping with the traces of iron demonstrated by special stains in a small per cent of the pigment granules. Furthermore, large amounts of coproporphyrin, usually as a metallic compound, are excreted in the urine in severe lead poisoning.

The "anaplastic" type of cytomegaly, so characteristic of lead nephropathy, as distinguished from other renal diseases in the rabbit, occurs principally in cells containing brown granular pigment. These cells, as much as 3 to 5 times normal size, have correspondingly large hyperchromatic nuclei which seems peculiarly susceptible to the formation of more than one intranuclear inclusion and peculiarly resistant to mitotic division. These atypical cells acquire particular significance to us in view of the frequent occurrence of renal cortical carcinomas in rats fed diets similar to those used in these studies.[12-14] Similar tumors have also been found in wild rats exposed to lead fumes of burning refuse.[15]

The nephropathy in the present study occurred in animals on the different lead subacetate regimes. It was independent of the use of linseed oil alone or as a dietary supplement. It increased in severity with time to a near maximum at about 7 to 9 months. Thereafter, though it progressed slowly, it acquired characteristics of a chronic nephritis with loss of renal substance, scarring and irreparable damage. Late retardation in rate of progression of the disease indicated that adaptive mechanisms had developed to minimize the toxic effects of continuously ingested

lead. It is of interest that the addition of cholesterol to the lead diets reduced their nephrotoxic effects as indicated by a reduction in pigment granules, atypical giant cells and intranuclear inclusions. This effect was observed at serum cholesterol levels corresponding to those common in man.

The disturbances of renal function have been more fully studied in children than in adults with lead poisoning.[10,11] Hyperaminoaciduria often occurs in children with acute lead poisoning. It is a generalized aminoaciduria and the patterns represent an accentuation of normal excretory patterns. In some cases the hyperaminoaciduria, which correlates well with severity of acute plumbism, is associated with hypophosphatemia and the presence of reducing substances such as glucose and fructose in the urine.[10] This triad of the Fanconi syndrome is reversible during recovery from lead poisoning and is presumably due to an effect of lead on reabsorptive functions of proximal convoluted tubules. It does not correlate precisely with the amount of lead in the blood or the amount of lead or coproporphyrin in the urine. Inasmuch as impairment of phosphate reabsorption is important in the Fanconi syndrome, and x-ray changes are seldom present in the bones in acute plumbism, it is suggested that hypophosphatemia must persist for months to favor development of rachitic skeletal changes demonstrable by x-ray.[10] Apparently, the "lead line" in bones represents a stage of healing in lead osteopathy. Theoretically, hypophosphatemia should favor accumulation of lead in the soft tissues where it would exert toxic effects rather than in bone where it is sequestered and less available for production of symptoms.

Osteopathy

Examination of bones disclosed retardation of formation of new bone at epiphyseal lines without significant roentgenographic findings. This was accompanied by evidence of increased osteoclastic activity. These disturbances were manifest principally by delicacy of trabecular structure and excess lacunar resorption of trabeculae adjacent to provisional zones of ossification (Fig. 3). Osteoblasts in these regions produced loose fibrillary collagen and less than the normal amount of osteoid (Fig. 4). These changes were most conspicuous in young animals on the basal diet-lead subacetate regime for about 8 to 30 weeks and were not progressive beyond 30 weeks. Changes in bones were insignificant in animals given the basal diet supplemented with linseed oil alone. Nor did the addition of linseed oil to the basal diet-lead subacetate regime modify the effects described above. The addition of cholesterol to the diet containing lead subacetate, however, reduced the severity of the osteopathy.

These findings indicate that animals with the most severe lead nephropathy also had, as a rule, the best examples of osteopathy. In both instances, also, pathologic changes progressed to a near maximum in the same period and failed to progress very much despite prolongation of the dietary regime for several months. In general, there was no prominent difference in the overall growth patterns though there was a tendency for animals in all experimental groups to reach weight levels between 6 and 7 pounds while control animals often reached weight levels around 8 pounds. The observed changes were not those of classic rickets nor were they simply a reflection of retarded growth rates. They could be related to renal dysfunction; it is interesting that a form of the Fanconi syndrome has been described in children with acute plumbism. It would be still more interesting if the lead osteopathy were due to an interference in formation of osteoid matrices rather than an abnormality in mineralization of cartilaginous and osteoid matrices.

Following absorption of lead from the alimentary tract, much of it is removed from the portal circulation by the liver. It is then returned to the alimentary tract in the bile or otherwise recirculated and distributed to the various tissues. Though there is much less lead in blood plasma than erythrocytes, it is promptly either excreted from the plasma in the urine and feces or stored in the bones. In dogs the ratio of fecal to urinary excretion of Pb^{210} varies from 2.0 to 8.7 while for stable lead in man it is about 2.5 to 6.7.[38] In dogs about 90 percent of administered radio-lead, Pb^{210}, is stored in the bones. This is close to the value for storage of stable lead in man. The biologic half-time for loss of stored radio-lead from the canine skeleton is 346 days. This is to be compared to a period of 670 to 840 days in man.[7,38]

Lead in the plasma is presumably transported in "colloidal suspension" as the more soluble di-lead phosphate and the less soluble tri-lead phosphate. The latter is very stable at about pH 7.4 but with slight changes in acidity is converted to the former.[3] Conditions which favor precipitation of calcium phosphate in bone should also favor precipitation of lead as the highly insoluble phosphate. Actually, the form in which lead is transported, stored in bone or mobilized from bone is unknown. Crushed particulate bone adsorbs lead ions rapidly at pH 7.4 to 7.8 *in vitro* and liberates an equivalent amount of calcium.[3] If this is true *in vivo,* lead ions circulating under appropriate conditions might inhibit pathologic calcification or enhance resorption of such calcific deposits.

These concepts also bear upon the rationale for treatment of lead poisoning. It is generally accepted that calcium given in any other form than phosphate does not favor deposition of calcium in bones. From

this it may be assumed that sufficient phosphate must be available to favor deposition of lead in bones.[8] Thus, feeding calcium carbonate increases the toxicity of ingested lead while feeding sodium phosphate reduces its toxicity. Also, in rats the amount of lead in the blood increases as the calcium-phosphorus ratio increases.[18,39] Hence, as serum phosphorus increases, the lead content in the blood decreases and, under the influence of vitamin D, the amount of lead stored in bones is increased. When lead is in the diet, vitamin D leads to an increase of Ca × P and the Pb × P products in the plasma. From this, it is inferred that vitamin D should be used only in conjunction with disodium acid phosphate when it is desirable to divert lead from blood into bones. In the de-leading of patients, a diet low in calcium and high in phosphate without vitamin D seems indicated.[8]

Rickets was regularly demonstrated microscopically but not by x-ray in children with chronic lead poisoning.[40] This was attributed to formation of insoluble lead phosphate in the intestine with resultant interference in absorption of phosphate. Follis believed that the same mechanism was responsible for rachitic changes in rats fed several different heavy metals. The study made by Follis of osteopathy in children with lead poisoning is the best available but there seems to be no general agreement as to incidence or pathogenesis of osseous changes. In the adult, accumulation of lead in bones is not characterized by any conspicuous change in microscopic structure of bone. In infants, the principal effect prior to epiphyseal closure is either a delay in growth of bone or deficient mineralization of cartilage at epiphyseal lines. In the former instance, the changes resemble those accompanying retardation of growth such as often characterizes prolonged illness or a chronic nutritional disturbance. After exposure to lead ceases, growth is resumed and at this time the so-called "lead line" of increased density becomes demonstrable by x-ray.[10,41] In instances in which rachitic changes occur, it is assumed that the pathogenesis is the same as that obtaining in other forms of rickets.[40,41] In this connection attention has also been directed to lead nephropathy and the secondary Fanconi syndrome. There may be some relation between the hyperaminoaciduria of the syndrome and the osteopathy. It is more likely that hyperphosphaturia and hypophosphatemia contribute to the pathogenesis of the osteopathy. Infants with the syndrome, however, have acute plumbism and no rachitic changes demonstrable by x-ray.[10] This does not mean that rickets may not be demonstrable microscopically.[40]

Whatever the explanation may be—renal, serologic, nutritional or other—there were changes in bones in our experimental animals (Figs. 3 and 4). These increased in severity along with the lead nephropathy

and hematologic abnormalities for several months but tended to stabilize or even regress in later months without the formation of "lead lines" detectable by x-ray. The microscopic changes were not those of classic rickets in the sense of a continued irregular proliferation of cartilage and osteoid tissue without calcification at osteochondral junctions. On the contrary, the production of osteoid matrix and cartilage was reduced. The penetration of degenerate columns of cartilage cells by vascularized mesenchyme was regular. Osteoclastic resorption of delicate trabecular bone and inner cortical bone was conspicuous, resulting in a moth-eaten appearance (Fig. 3). These findings could not be explained by inanition because most animals either gained or maintained weight while control animals with inanition due to fortuitous illnesses failed to develop comparable lesions of bone. We regard the principal disturbance as due to an inhibition of osteoid matrix formation complicated by an enhanced rate of resorption of mineralized bone. This is in keeping with the opinion that the "lead line" in x-rays of bones represents a stage of healing characterized first by prompt deposition of osteoid tissue following elimination of exposure to lead.

Encephalopathy

One principal objective of this study was to inquire into the pathogenesis of neurologic manifestations in human lead poisoning. Symptoms in children ordinarily are attributed to an encephalopathy and those in adults to a neuropathy. In order to simulate conditions under which encephalopathy usually occurs in children, young animals were used, the peak period of chronic lead ingestion was established during the warm summer months and in several instances in another study excess vitamin D was added to the dietary regimes. Animals occasionally had convulsive seizures or became weak or paralyzed, especially in the hind legs. Similar symptoms were observed in several animals in control groups in these and other unrelated studies. As a rule, animals with convulsions were either terminally ill of an acute respiratory infection, an encephalitis or a meningo-encephalitis. Identical symptoms and diseases occurred with almost the same frequency in control animals. At times, weakness, progressing to paralysis of the legs, could be attributed to development of a severe myopathy. (Figs. 5, 6) In these instances, there was no microscopic evidence of a primary motor neuropathy. Similar lesions of muscle were found in occasional control animals but the conclusion was reached that, within the limits of detection by methods used, the nervous system was not involved in pathogenesis of symptoms or pathologic changes.

An acute encephalopathy is the most serious complication of human

lead poisoning.[9,10,26,42] This usually occurs in the summer months in children between 24 and 48 months of age. When it occurs in adults, the toxic exposure is either very great or involves unusually toxic metallo-organic compounds.[7,43] The onset of acute encephalopathy in the summer has been attributed to exposure to sunlight with generation of liberal amounts of vitamin D.[44] It has also been suggested that, inasmuch as many symptoms of lead poisoning resemble those occurring in patients with porphyria, the exposure of circulating porphyrins to excessive visible and ultraviolet light in the summer may be responsible.[22] In this connection it is of interest that porphyrins may acquire proteolytic properties when activated by light in the presence of molecular oxygen.[23]

Lead encephalopathy is not always acute. It may be subacute and recurrent. There is also good evidence that it may often be sub-clinical. When acute, it either soon subsides or, much too frequently, increases in severity and promptly terminates in death or, in event of survival, results in permanent impairment of cerebral function often characterized by mental retardation.[9,42] The acute encephalopathy is usually accompanied by signs and symptoms attributed to cerebral edema with increased intracranial pressure. The spinal fluid contains increased protein, increased numbers of lymphocytes and increased amounts of lead.[45,46] Pathologic changes in the brain are not specific.[26,47,48] Gross swelling and blotchy congestion of the brain are common. Microscopic studies usually disclose evidence of increased permeability of small blood vessels characterized principally by a perivascular serous exudate and a curious basophilic intramural degeneration. Less common findings are early patchy necrosis, Purkinje cell degeneration or gliosis accompanied at times with an accumulation of lymphocytes in the meninges and around intracerebral vessels.[47,48] There is no consistent correlation between microscopic structural changes and severity of cerebral manifestations.

The present experiments have not produced an encephalopathy attributable to lead. The amount of lead ingested by some young animals was very large and the period of ingestion at times exceeded one year. Some animals developed neurologic manifestations of diverse paralytic, convulsive and other types. Also, some animals had encephalitis or meningo-encephalitis of undetermined nature. However, similar symptoms and microscopic changes also occurred in a few control animals. Our conclusion, therefore, is that we have not found appropriate conditions for consistent production and recognition of lead encephalopathy in rabbits. Current experiments with young Wistar rats have led to the same conclusion even though other effects, such as those on the kidneys, are more severe than those in children with acute lead encephalopathy. Perhaps, recourse should be made to the use of younger animals or

primates. Alkylated compounds of lead might yield better results for it has been shown that triethyl tin causes cerebral edema and that tetraethyl lead is converted to triethyl lead in the body.[7,43,47] Metallo-organic compounds of this type are often highly toxic and may cause severe cerebral symptoms after moderate exposure.[7,43] The preceding remarks indicate that the pathogenesis of human lead encephalopathy is obscure. In view of the epidemiology of the disorder in infants and experimental data in animals, attention should be directed to the possibility that factors other than or in addition to ingestion of inorganic lead are responsible.

Myopathy and Neuropathy

After several months on the diets, some animals displayed increasing weakness. This was more conspicuous in groups ingesting the basal diet containing linseed oil with or without supplemental lead subacetate. Suspicion was directed, therefore, to the possible relation between linseed oil and muscular weakness rather than lead and muscular weakness. As a further complication, normal control animals also occasionally showed progressive weakness. Paralysis, usually of the hind legs, occurred in a few instances but these manifestations were not restricted to any particular group of animals. Microscopic studies disclosed no degeneration of peripheral nerves. In many animals, however, there was a peculiar degenerative myopathy (Figs. 5 and 6). This occurred in three forms. In the simplest form there was a proliferation of capillary endothelium and adjacent sarcolemmal cells between muscle fibers (Fig. 6). In the second form the principal deteriorative changes were in the skeletal muscle cells (Fig. 5). Within and around these, there was a mild infiltration of leukocytes. The muscle showed no regenerative activity. The third form of myopathy resembled the preceding type but was further characterized by accumulation of calcium in degenerated muscle cells. These studies did not define the distribution of the myopathies in detail. As a rule, certain paravertebral, diaphragmatic, foreleg and intercostal muscles were affected in the same animal. There was no consistent relation to disease in other organs.

The relation between diet and the different forms of myopathy has not been established because similar though less conspicuous changes were occasionally found in control animals. The type of myopathy most likely to be related to or enhanced by the ingestion of lead is the first form described above. This occurred in 5 of 16 animals on the basal diet-lead subacetate regime. The second type of myopathy occurred in 15 of 21 animals maintained on the basal diet-lead subacetate-linseed oil regime. The third type of myopathy was found in 4 of 8 rabbits fed the basal diet supplemented only with boiled linseed oil. It is premature to assign any specific relation between the incidence of these myopathies

and the ingestion of lead. It may be appropriate, however, to direct attention to the problem and, in view of the occasional presence of similar changes in control animals, to emphasize caution in drawing conclusions concerning the cause of myopathies in rabbits.

Weakness and paralysis in man with lead poisoning usually first involves extensor muscles of the arms.[3,4] These symptoms are ordinarily late manifestations of chronic plumbism and seldom occur in children. For many years they were attributed to a motor neuropathy. More recently, in the absence of microscopic evidence of sufficient peripheral neural degeneration to account for the symptoms, the primary lesion has been regarded as a neuronitis and attention directed to a study of motor nerve roots.[49] Others have taken the view that the weakness and paralysis are due to a toxic effect of lead on muscle, especially muscle which is fatigued.[50] This view is supported by two lines of evidence. First, there is no effect of lead on nerve conduction.[50] Second, in animals poisoned with lead, there is a reduction in phosphocreatine and an increase in inorganic phosphate in muscle.[51] This is attributed to a reduced ability of muscle to resynthesize phosphocreatine. The weakness and paralysis, therefore, are attributed to inhibition by the lead ion of enzyme systems responsible for synthesis of energy-yielding phosphorylated compounds.[4,52] If this is true in muscle, it might be equally true in some other tissues and especially so if the mode of action of lead is to interfere competitively with coenzyme functions of other cations such as Ca^{++} or Mg^{++}, both of which regulate numerous energy-yielding and energy-storing enzyme systems.

In the present experiments, animals with symptoms which could be attributed to a neuropathy had no consistent pathologic change in the nervous system. Changes which were found were also present in asymptomatic and control animals. Many animals, however, had one or more of three types of myopathy, seldom encountered in control animals. We have no explanation for the frequency of myopathy nor can we explain the lack of relations between changes in skeletal muscle, the duration of ingestion of lead or the severity of pathologic changes produced by lead in the blood, bone, kidney or retina. It is apparent that factors, other than ingestion of lead, contributed to the pathogenesis of structural changes in muscle. Such evidence as there is, however, directs attention to the probability that the muscle cell in lead poisoning is more vulnerable than the motor nerve, at least that portion proximal to the end-plate.

Retinopathy

The retinal epithelium of the New Zealand albino rabbit is throughout most of its extent a slightly elevated pavement epithelium without pigment granules or other conspicuous cytoplasmic particulates (Fig. 7).

Occasionally, however, retinal epithelial cells adjacent to the optic disc have brown intracytoplasmic granules. There are no pigmented melanocytes. The strains of German Checker and Belgian Hare rabbits, used occasionally, have a retinal epithelium containing numerous rods and granules of melanin, which are also abundant in melanocytes in the choroid, sclera and iris (Fig. 9).

A prominent change occurred in the retinal epithelium of all animals ingesting lead for about 6 to 8 weeks. This was a gradual accumulation of a light brown granular intracytoplasmic pigment. As the period of lead ingestion was prolonged, the pigment increased in amount and the cytoplasmic volume increased greatly until at the end of 24 to 36 weeks almost all retinal epithelial cells resembled inflated pigmented hemispheres elevating interdigitating processes of retinal photoreceptors (Fig. 8). Often, these changes occurred first in retinal epithelial cells spaced at fairly regular intervals so that it was possible to define progression of the disturbance until all intervening cells were equally affected. The melanin-rich retinal epithelium of the Belgian Hare and German Checker rabbits were similarly affected but the accumulation of atypical pigment among the normal melanin granules was less easily detected (Fig. 10). Therefore, this was an indolent, progressive cytopathologic change restricted to retinal epithelium and not found in any normal control animal.

The cytoplasmic granules varied in size, shape and other physical properties. After formalin fixation they displayed an affinity for hematoxylin. They were stained positively by PAS methods and were lightly acid-fast. They were refractory to stains for iron and had little or no affinity for Scharlach R. After Zenker fixation, they had a greater affinity for eosin than hematoxylin and showed other modifications in staining reactions.

Electron microscopic studies disclosed a variety of cytoplasmic structures. Most were spherical or nearly so. Some had no definite internal detail. Others were uniformly granular. Others contained angular structures resembling crystals. Many were in the form of myelin figures. Relations between various types of particles need not be described in detail here.

The present experiments were not designed to disclose the maximum sensitivity of retinal epithelium to ingested lead or to define the degree of specificity of the cytopathologic change. It was clear, however, that the retinal epithelium was quite sensitive to lead subacetate in a normal diet in concentrations no greater than 100 mg per cent. We are not prepared to assess the reason for the sensitivity of retinal epithelium to normal diets supplemented with nothing other than 10 gm per cent of

boiled commercial linseed oil. If the changes are due to the presence of "lead drier" in the oil, further studies are indicated to determine what the responsible component of the "drier" might be. Other evidence indicates that cholesterol in the diet does not minimize changes in the retina as it does in the kidneys and that there is no relationship between lead nephropathy and the pigmentary lead retinopathy.

Experiments now in progress indicate that the retinopathy is not necessarily specific for lead poisoning nor is it producible in all species. It is not produced in the Wistar rat maintained on diets with lead subacetate supplements as large as 1 gm per cent. It is found in mild degree in rabbits given diets containing large amounts of fluorenylacetamide. It is not produced, except occasionally in mild degree, in rabbits by chronic starvation, chronic chloroform intoxication, chronic carbon tetrachloride administration, chronic infection with debilitation or excessive doses of viosterol. As yet, no experimental situation has been found which will produce the retinopathy in a degree comparable to that found in rabbits ingesting lead for many months. Furthermore, there is as yet no evidence that the retinopathy disappears after restoration of the animal to a normal diet.

Many diseases of the eye have been attributed to lead poisoning.[53] In most instances, proof is lacking. In other instances, the diseases might be regarded as a complication of choking of the optic discs accompanying lead encephalopathy. We have found no description of an effect on human retinal epithelium resembling the change encountered in the present experiments.

The retinal epithelium is the outermost layer of the retina.[54,55] It lies on Bruch's membrane between the choroid and the photoreceptors. It is rich in vitamin A and contains the "pigment layer factor" of Bliss which promotes the reconstitution of rhodopsin.[56] The retinal epithelium of pigmented species contains melanin granules which in the presence of light elaborate free radicals thought to be semiquinones, a matter of interest because of the polyquinone structure of many melanins.[57]

In carnivora there is a layer of special choroidal cells, the *tapetum lucidum,* held responsible for high reflectivity of the fundus. This property is attributed to intracellular crystals composed of a cysteine complex of zinc.[58] An intravenous injection of dithizone, which also complexes zinc, induces retinal detachment and blindness in animals with this type of tapetum.[59] This effect also may be produced in rabbits, which, though they have no *tapetum lucidum,* do have a high concentration of zinc in the chorioretinal layers. From this it may be inferred that the retinal epithelium of the rabbit may contain zinc complexes similar to those in the tapetum of carnivora and presumably demonstrated by

electron microscopy.[60] Along with these constituents, ultrastructural studies of the retinal epithelium disclose many myeloid bodies, lipid granules and elements resembling lysosomes.[54,55]

In view of the essential role of the retinal epithelium in support of photoreceptor functions and its probable high content of zinc, the pathologic changes occurring in the rabbit during ingestion of lead and the refractoriness of the rat to these changes are of interest. It is of still greater interest that similar changes occurred in the rabbit ingesting commercial boiled linseed oil containing the usual commercial "drier." According to our best information, the "drier" in the oil is a mixture of lead, cobalt and nickel naphthenates. In each instance, the accumulation in the retinal epithelium of granules of brown pigment increased with prolongation of the dietary regime. This pigment had unusual staining properties which varied with the type of fixative, indicating a ready susceptibility to oxidation.

There is no proof that the lead ion is directly implicated in this conspicuous cytopathologic change despite the attractiveness of the idea that it may act by displacement of the zinc ion or participate in an abortive mechanism of melanogenesis. That lead alone is responsible seems unlikely because of the occurrence of retinal epithelial changes in rabbits fed boiled linseed oil which contained insufficient lead to produce renal lesions. Furthermore, similar changes, though much less impressive, were produced in rabbits on other "toxic" dietary regimes. It is doubtful that the changes, however, represent a non-specific cytoplasmic modification induceable by any noxious agent.[61] The retinal epithelium of the rat, for instance, is not affected by prolonged ingestion of lead subacetate even though it produces a lead nephropathy which is much more severe than that encountered in rabbits. It is perhaps justifiable to speculate that we may be dealing with zinc ion displacement, an interference in local metabolism of vitamin A, an unmasking of a melanogenetic potential, or, if the changes prove to be permanent, a means for converting nonpigmented retinal epithelium into a permanently pigmented structure. Along with these speculations, however, it must be kept in mind that the retinal epithelium with or without involvement of photoreceptors is peculiarly sensitive to x-irradiation, high concentrations of inspired oxygen and the intravenous ingestion of certain metabolic inhibitors such as sodium iodoacetate.[62,63]

Arteriopathy

Many references in the literature imply that there is a relation between prolonged exposure to lead and intensification of arteriosclerosis in man. The present studies disclosed no specific arterial lesions attributable to

lead or a combination of lead and cholesterol in the diet. With lead alone the serum cholesterol levels remained in the normal range between 50 and 100 mg per cent. When the lead regime was supplemented with cholesterol, the serum cholesterol levels increased to between 150 and 350 mg per cent. These are the levels which are expected when rabbits are given similar cholesterol-oil dietary supplements without the addition of lead.

However, the basal diet-lead subacetate mixture favored accumulation of calcium in areas of medial degeneration in the arch of the aorta (Fig. 11). This occurred in 8 of 16 animals on this regime and was encountered in only 1 of 8 animals on the basal diet-linseed oil regime. Among 21 animals on the lead subacetate-linseed oil regime, 8 had calcified plaques in the aortic arch. None of 8 animals given diets containing lead subacetate, linseed oil and cholesterol had aortic calcification. This is in keeping with other studies which have disclosed an antagonism between viosterol-induced calcification and hypercholesteremia.[86]

Atheromatous plaques were not conspicuous in any animal. Two of 8 animals on the cholesterol regime had small atheromas of the intima in the proximal aorta but no lesions elsewhere. The aortic lesions were different from those in control animals in other experiments in which comparable serum cholesterol levels were maintained.[86] The difference seemed related to excessive inhibition of fibrillogenesis in relation to intimal plaques occurring in animals on diets containing lead subacetate. At first, it was thought that this was simply the usual inhibition of collagen formation accompanying atheromatous deposits. However, intimal fibrillogenesis was also often completely inhibited over calcified plaques in the inner media. These unexpected observations and those in connection with inhibition of osteoid matrix formation direct attention to the possibility that some manifestations of lead poisoning may be due to interference in formation of intercellular materials, especially collagen.

The evidence in the literature which directs attention to the possible relation between chronic lead poisoning in man and the occurrence of chronic nephritis, hypertension and arteriosclerosis should not be ignored. On the contrary, studies should be undertaken to inquire more fully into the subject. The present experiments show that the incidence of medial degeneration with calcification in the arch of the aorta is increased by chronic ingestion of lead. It was also apparent that the same conditions favored the progression of a chronic nephropathy with irreversible changes in the kidneys. Accompanying these findings, there was often a disturbance in osteogenesis indicating the possibility of some interference in calcium or phosphate metabolism. Of still greater interest

was an associated inhibition or retardation of formation of certain intercellular matrices, especially those concerned in the repair of arterial lesions and the growth of bone. It seems probable that these effects are related through some fundamental displacement by the lead ion of one or more other ions which govern normal metabolism. The search for such a displacement, as elementary to an understanding of miscellaneous seemingly unrelated toxic effects of lead, might properly engage the attention of future investigators.

Other Pathologic Changes

A comparison of pathologic changes in the experimental groups with those in control groups and animals used in unrelated experiments justifies a few comments. The excessive splenic hemosiderosis accompanying the hemolytic anemia of chronic lead poisoning was occasionally complicated by accumulation of material which conferred on the stroma and reticulum a basophilic staining property (Fig. 12). This material was assumed to be either a ferric or calcium phosphate or a mixture of both.

Hyperplasia of the thymus and thyroid was unusually conspicuous in many animals ingesting lead subacetate for several months. The persistence and enlargement of the thymus was particularly impressive even in some animals which had lost weight. In 1 animal, after 50 weeks on the lead diet, a large thymoma developed. There was no tendency toward renal neoplastic changes such as those found in rats developing renal tumors while on diets containing lead.

Summary

Young rabbits were fed a basal diet variably supplemented with lead subacetate, cholesterol and commercial boiled linseed oil containing a "lead drier" for periods of 3 to 55 weeks. A study was made of the pathologic effects of the supplements and these effects were compared with those commonly attributed to lead poisoning in man.

The hematologic disturbances produced experimentally were similar to those found in human cases of lead poisoning. There was a self-limited progressive anemia, characterized by decreased hemoglobin, stippling of erythrocytes, poikilocytosis, polychromatophilia, erythroblastemia and evidence of hemolytic activity with accumulation of erythrocytes and hemosiderin in reticuloendothelial cells. As these changes increased in severity they were often accompanied by a relative lymphocytosis and monocytosis without undue immaturity of circulating leukocytes or evidence of interference in blood platelet production.

The experimental lead nephropathy was also similar to that found

in patients with lead poisoning. It was characterized principally by an accumulation, first in the straight tubules and later in the convoluted tubules, of an intracytoplasmic brown granular pigment followed by the appearance of giant "anaplastic" tubular lining cells and eosinophilic intranuclear inclusion bodies. As the nephropathy became more chronic, cortical subcapsular and radial scars developed in fairly direct proportion to the amount of lead ingested and in inverse proportion to the presence of cholesterol in the diet.

There was no resemblance between the osteopathy occurring in young rabbits and rachitic changes described in children with lead poisoning nor was there any "lead line" demonstrated microscopically or by x-ray in bones of the experimental animals. However, there was a retardation of formation of osteoid tissue and increased osteoclastic lacunar resorption of bone. These changes were most easily detected in young animals and they progressed over a period of about 6 months in proportion to increasing severity of lead nephropathy. The only recognized association between the nephropathy and osteopathy and the occurrence of an arteriopathy, so often emphasized as a late complication of chronic lead poisoning in man, was the frequency of medial calcification in the proximal aorta. In these instances, however, the formation of fibrous or fibroatheromatous intimal plaques was negligible.

A form of encephalopathy or motor neuropathy, attributed to lead poisoning in infants and adults respectively, was not produced experimentally. Weakness and paralytic manifestations were usually best explained by diseases also encountered with frequency in control animals or by the occurrence of myopathies which had no demonstrable relation to lesions of the nervous system. Inasmuch as similar myopathies also occasionally occurred in control animals, it was concluded that lead poisoning was responsible for nothing more than an increase in the severity and incidence of intercurrent myopathic disorders.

Many different disturbances in visual function and neuroretinal structure have been attributed to lead poisoning in man. None of the described pathologic changes resembles those which occurred in the retina of the rabbit. The retinopathy in the rabbit progressed to a maximum over a period of about 8 months. It was characterized by an accumulation of closely-packed brown granules in the greatly expanded cytoplasmic volume of retinal epithelial cells. The retinopathy occurred regularly at low levels of ingestion of lead and was not necessarily accompanied or preceded by any easily recognized cytopathologic change in other organs or tissues. Once developed, it was either permanent or only very slowly reversible upon restoration of the animal to a normal diet.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. An intranuclear inclusion body characteristic of lead poisoning, located in a giant nucleus of a cell lining a renal convoluted tubule. It shows a homogeneous central density with a diffuse margin which merges into an external less dense zone at the periphery of which there are radially arranged abnormal threads of chromatin. There are at least two less distinct inclusions in nuclei of neighboring cells. Numerous granules of pigment appear in the cytoplasm of most of the cells. × 600.

FIG. 2. A narrow straight tubule of the cortico-medullary zone in the kidney of an albino rabbit fed a basal diet supplemented with 500 mg per cent of lead subacetate for 55 weeks. The large tubular lining cells are characterized by giant hyperchromatic nuclei and intracytoplasmic pigment granules. These cells resemble neoplastic cells more closely than normal regenerating tubular epithelium. × 600.

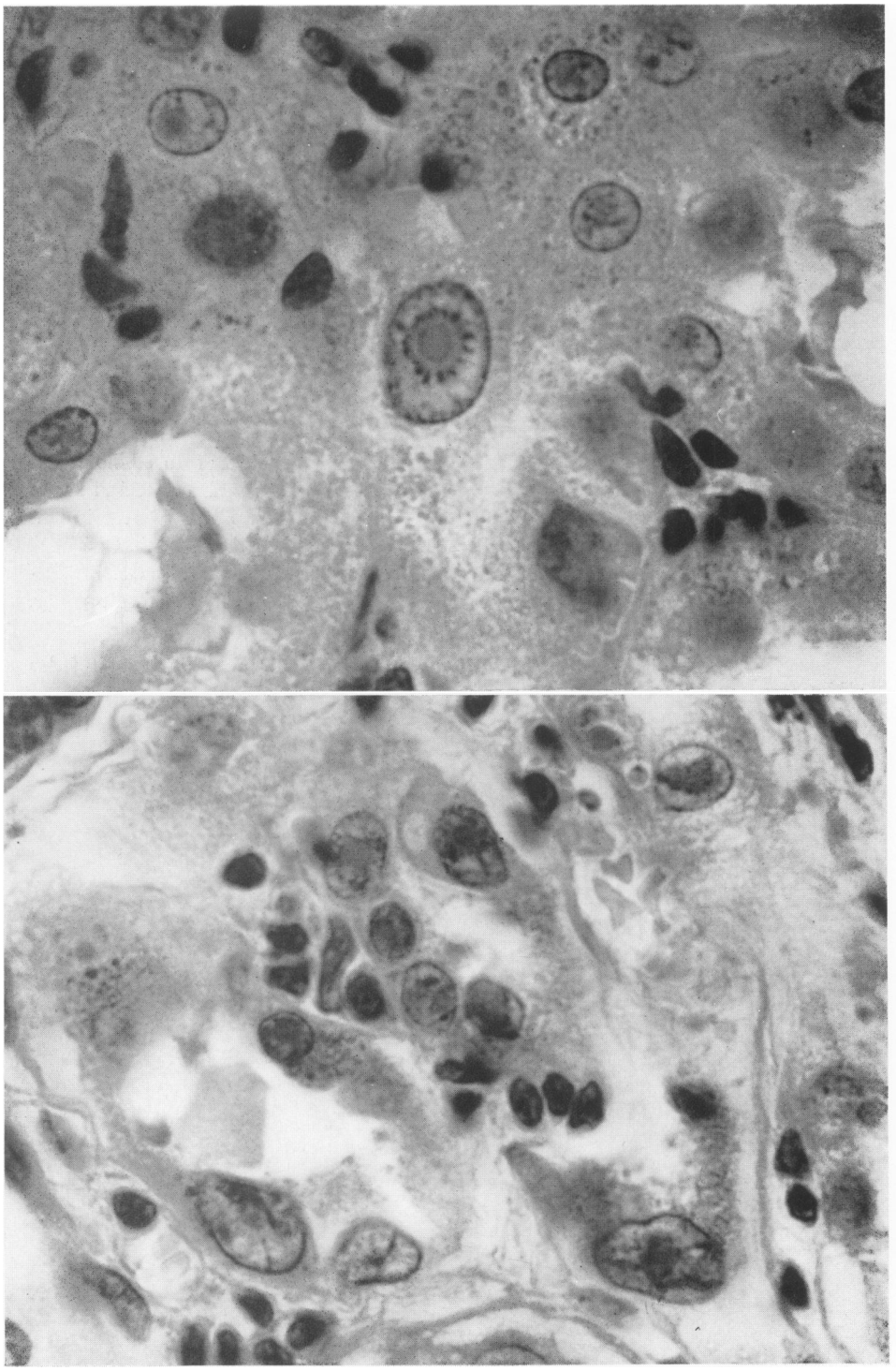

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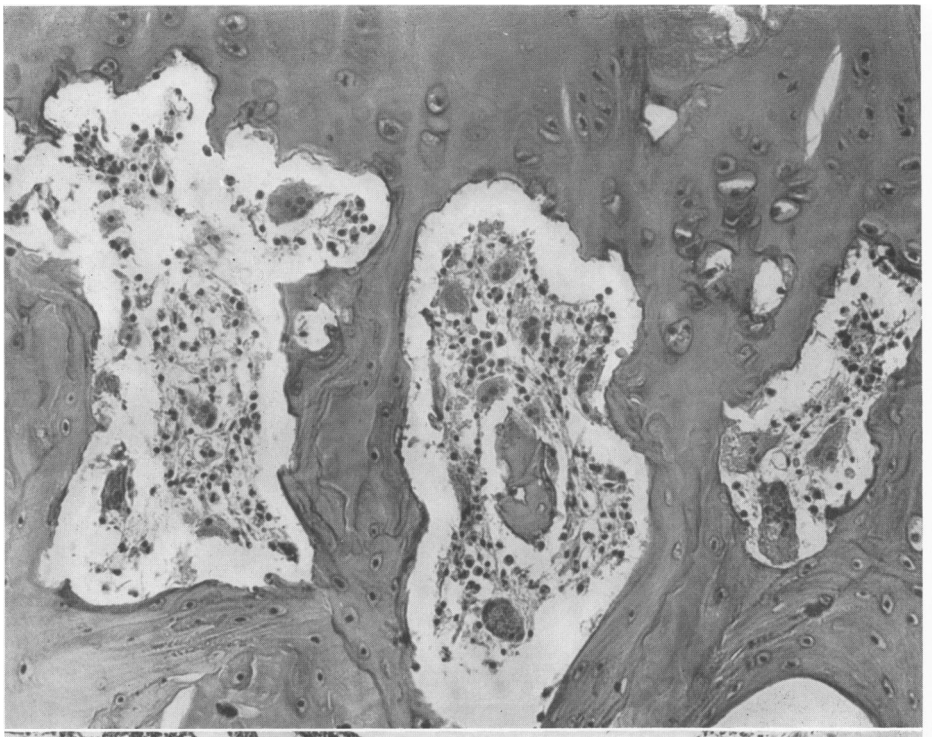
FIG. 3. The osteochondral junction in the body of a vertebra of an albino rabbit fed a basal diet supplemented with 500 mg per cent of lead subacetate and 10 gm per cent of commercial boiled linseed oil for 5 months. There is great irregularity at the junction; osteoclasts are numerous. Lacunar resorption of bone lends a moth-eaten appearance to the margins of the bony trabeculae. Production of osteoid tissue is minimal. This is the most severe form of lead osteopathy. × 150.

FIG. 4. The osteochondral junction of a vertebral body in an albino rabbit fed a basal diet supplemented with 500 mg per cent of lead subacetate for 3 months. There is a slight increase in osteoclastic activity. Osteoblasts in large numbers have formed a loose reticular collagenous tissue along the margins of the trabeculae. Osteoid tissue is minimal in amount and there is no recognizable defect in mineralization of cartilagenous matrix or its overproduction. × 75.



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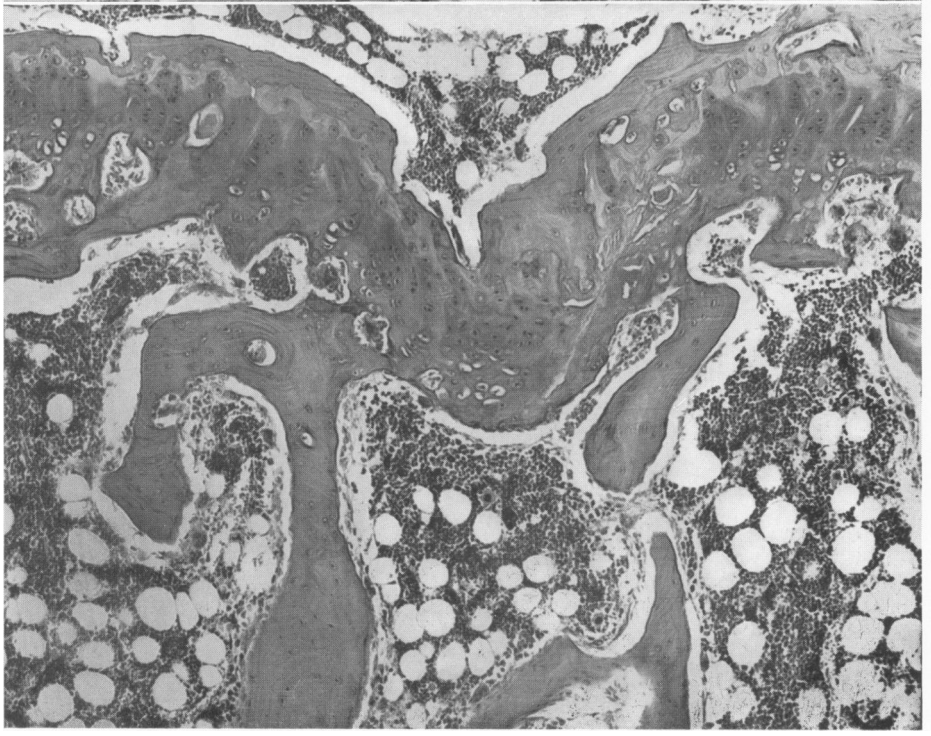

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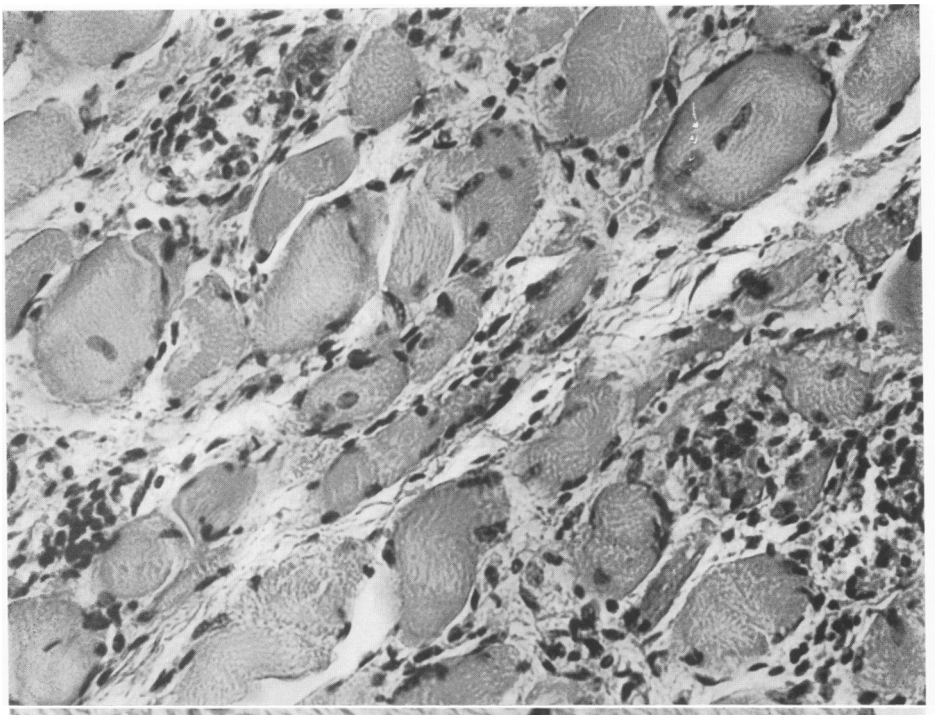
FIG. 5. Skeletal muscle from the foreleg of a rabbit given a diet supplemented with 500 mg of lead subacetate and 10 gm per cent of commercial boiled linseed oil for 52 weeks. There is chronic progressive necrosis of muscle cells characterized by an invasion of the degenerate cells by macrophages. Clusters of sarcolemmal cells and histiocytes form small aggregates in the interstitial tissues, usually marking the former location of a degenerate absorbed muscle cell. This is the most severe form of myopathy encountered in the experimental animals and is to be compared with the more frequent and less severe type of disease shown in Figure 6. In neither instance is there any evidence of a primary encephalomyelitis or motor neuropathy. × 200.

FIG. 6. A cross-section of skeletal muscle from the foreleg of a rabbit given a diet supplemented with 500 mg per cent of lead subacetate and 10 gm per cent of commercial boiled linseed oil for 17 weeks. The conspicuous feature of the myopathy is a local proliferation and accumulation of mesenchymal cells in and around capillaries between muscle cells. This occurs before the more advanced lesions illustrated in Figure 5 become conspicuous. × 600.



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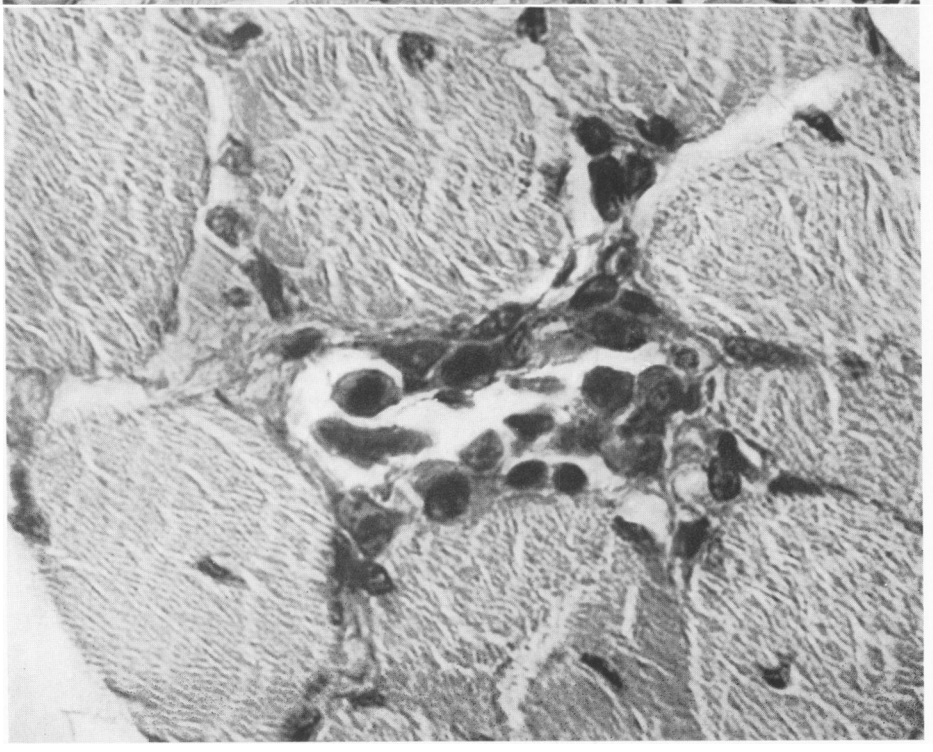

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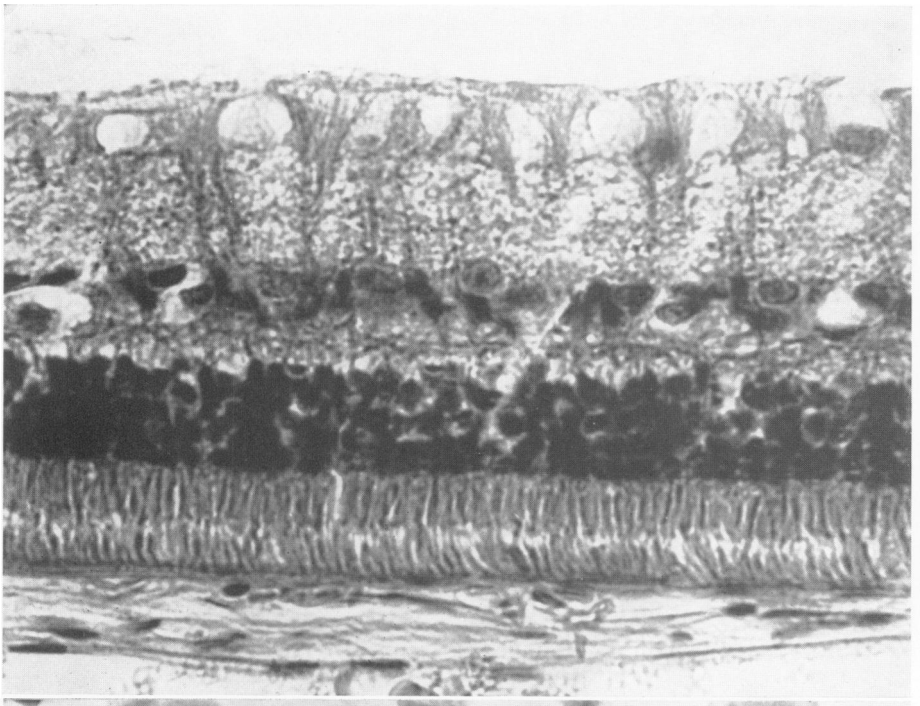
FIG. 7. Retina in an albino rabbit. The eye was removed surgically prior to feeding the animal a diet containing lead subacetate. A slightly elevated elongated chain of retinal epithelial cells lies between the photoreceptor rods and the choroid. As a rule the cells of normal retinal epithelium are even more like a pavement epithelium than this. After 9 months on the lead regime the opposite eye of this animal was studied. The retinal epithelium had the same appearance as that shown in Figure 8. × 400.

FIG. 8. Retina of an albino rabbit fed a basal diet supplemented with 500 mg per cent of lead subacetate and 10 gm per cent of commercial boiled linseed oil for 10 months. The animal had a severe lead nephropathy and osteopathy with a consistently high stippled red cell count and a low blood hemoglobin. The cytoplasm of all retinal epithelium is distended with closely-packed granules. The remainder of the retina and the adjacent choroid are normal. × 350.



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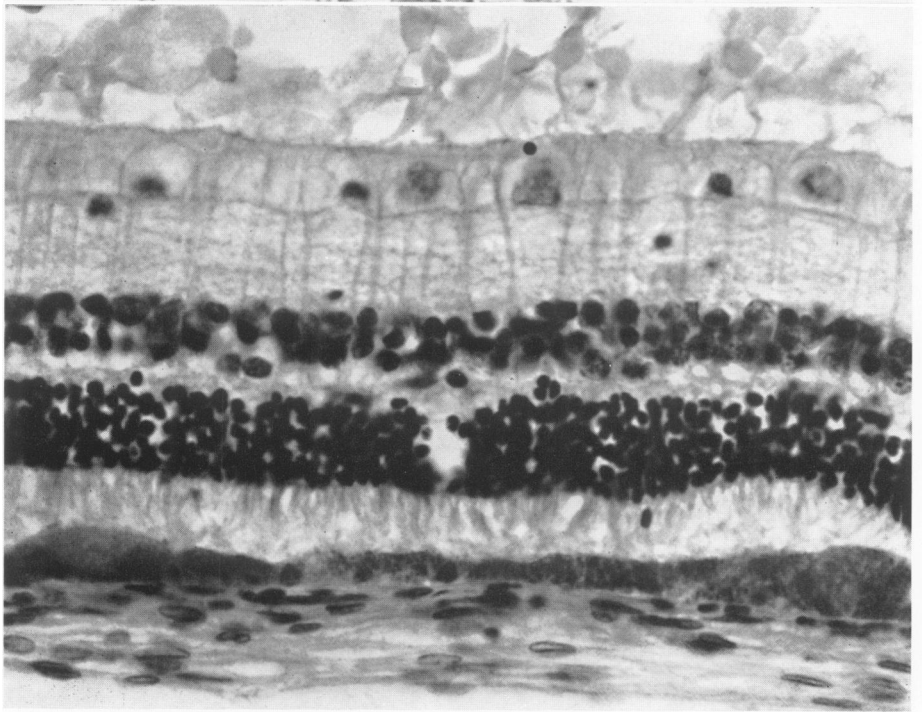

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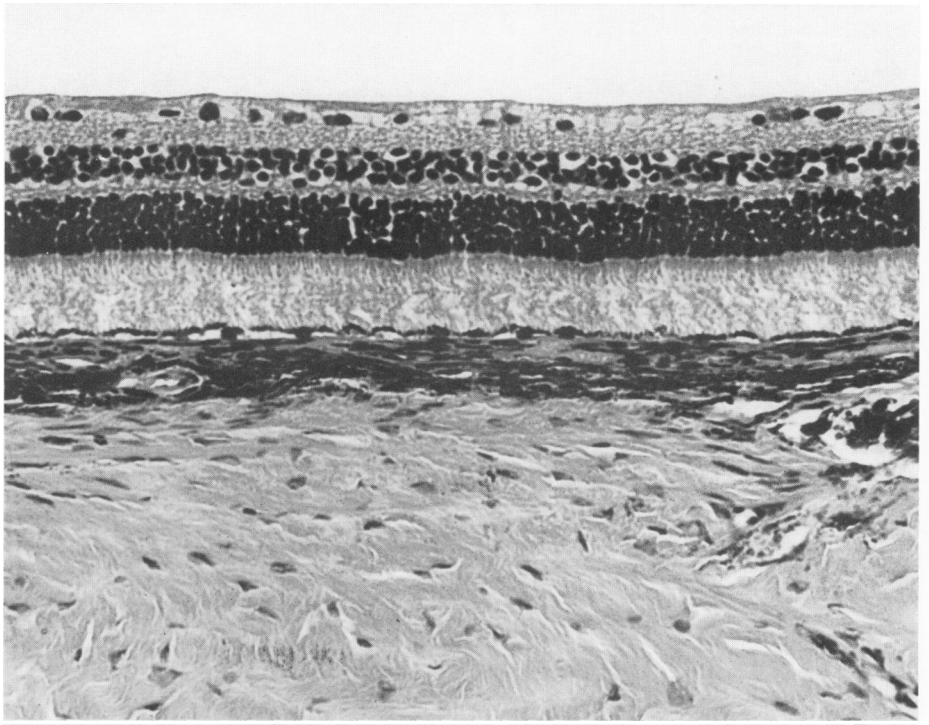
FIG. 9. Retina of a pigmented German Checker rabbit. Between the photoreceptor rods and the heavily pigmented choroid and sclera, there is a continuous layer of slightly elevated retinal epithelial cells. These are filled with melanin granules which are more numerous adjacent to the rods than to Bruch's membrane. This is the usual appearance of the retinal epithelium in animals wtih darkly pigmented eyes. × 125.

FIG. 10. Retina of a pigmented German Checker rabbit fed a basal diet supplemented with 500 mg per cent of lead subacetate for 3 months. Three retinal epithelial cells are enlarged due to an increase in cytoplasmic volume which accommodates an accumulation of abnormal pigment granules. These granules have disturbed the usual orientation and distribution of the rods and granules of melanin. The distinction between the melanin granules and the accumulated yellow granules of pigment often cannot be made. × 600.



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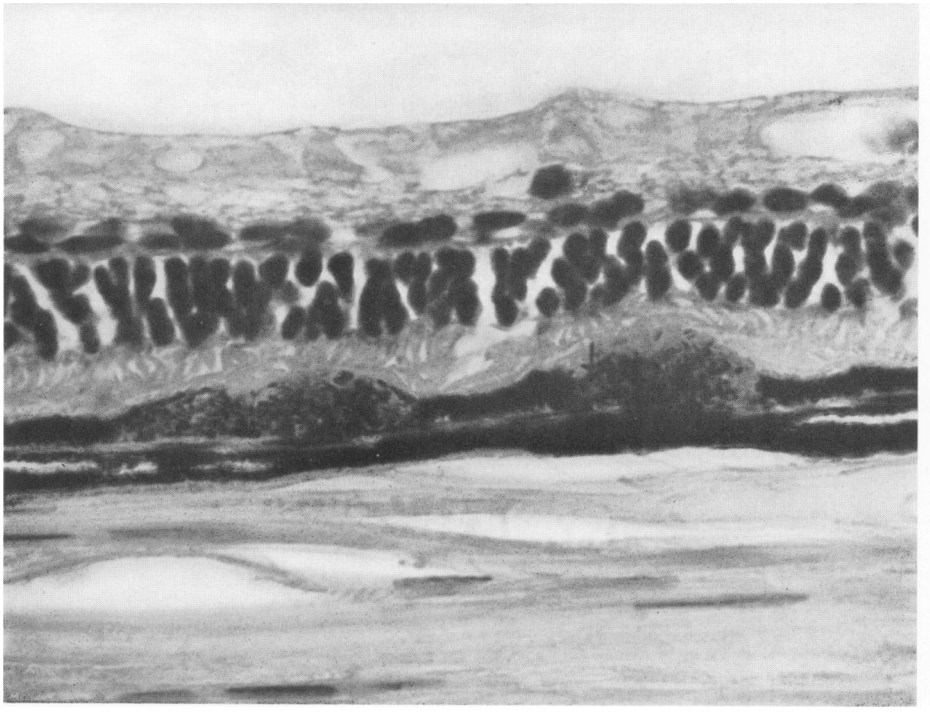

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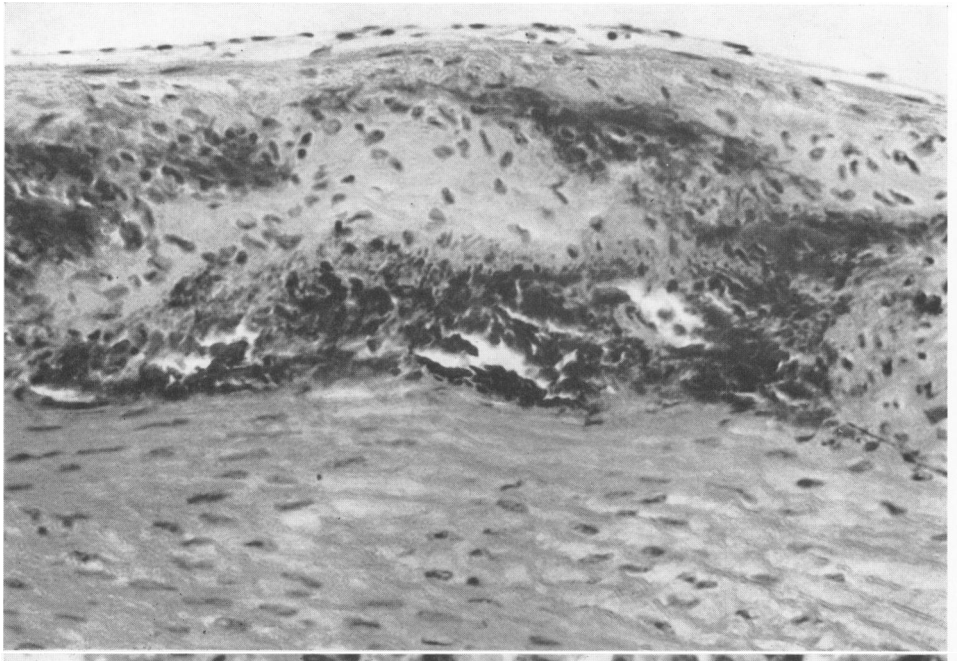
FIG. 11. A cross-section of the aortic arch in a rabbit fed a basal diet supplemented with lead subacetate for 34 weeks. The inner one-third of the media is degenerate, fibrous and partly calcified. There is no fibrous thickening of the overlying intima. Similar lesions are occasionally found in normal control rabbits but they are smaller and much less common than in rabbits given diets containing lead subacetate. × 250.

FIG. 12. Red pulp of the spleen of a rabbit given a diet containing 500 mg per cent of lead subacetate and 10 gm per cent of commercial boiled linseed oil for 50 weeks. The splenic reticulum around and between the larger vascular sinuses is deeply stained with hematoxylin. This property was demonstrable only when there was a large amount of hemosiderin and excessive phagocytosis of fragmented erythrocytes in the spleen. Administration of vitamin D accentuates development of this basophilic property by the reticulum. × 450.



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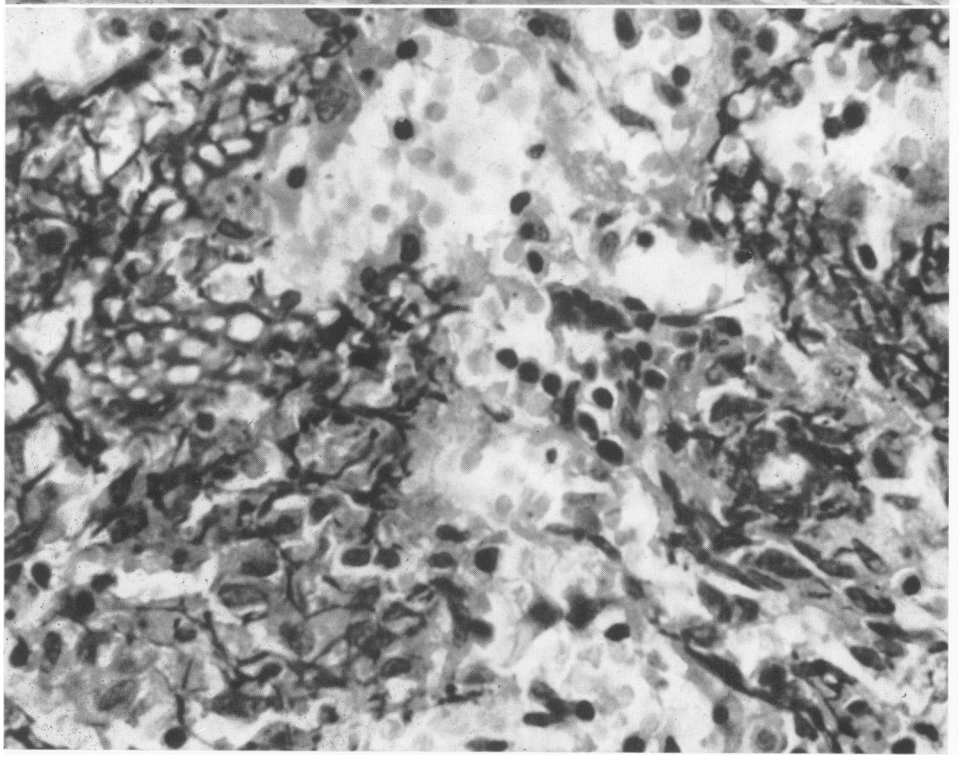

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