

HEREDITARY OSTEOPETROSIS OF THE RABBIT

IV. PATHOLOGIC OBSERVATIONS; GENERAL FEATURES

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PLATES 31 TO 35

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The signs and course of hereditary osteopetrosis of the rabbit have been reported (1, 2), while the pathologic condition of the skeleton has been described in the preceding paper (3). The disease which is present at birth is identified by peculiar abnormalities of the incisor teeth and by the dense homogeneous shadows of the skeleton in x-ray photographs. Growth is retarded, an anemia of increasing severity develops, deterioration with malnutrition and cachexia are evident within 2 to 3 weeks, and an early death, generally at 4 to 5 weeks of age, invariably occurs.

The present paper contains the results of postmortem examinations and of histologic study with the exception of those pertaining to the skeleton.

Materials and Methods

A postmortem examination was made on the majority of the 293 cases which constituted the basis of this work. Tissues for microscopic study were obtained from representative rabbits killed in most instances with chloroform anesthesia; certain very young animals were decapitated. Control material was similarly obtained from normal litter mates of the same age.

Tissues for routine study were fixed in Petrunkevitch's cupric-phenol solution and stained with hematoxylin and eosin or phloxine methylene blue. Blocks from the heart and aorta were fixed in Bouin's solution and stained with Weigert's elastic tissue stain. Tissues examined for fat were fixed in 10 per cent formol-saline and stained with Scharlach red. The pituitary gland was fixed in Susa's fixative, cut sagittally in series and stained with Heidenhain's azan stain. The adrenal glands were fixed in Orth's fluid and Weisel's stain for chromaffin was used. The thyroid gland and pancreas were fixed in Bouin's solution and stained with Heidenhain's azan stain. The anterior portion of the neck, including the upper part of the thymus, of representative cases was cut sagittally in series for a survey of parathyroid distribution; approximately every twelfth section was stained.

A limited histochemical study was made using Gomori's method for the demonstration of alkaline phosphatase, von Kossa's silver nitrate method for calcium, and Gomori's Prussian blue reaction for hemosiderin; the tissues were fixed in 80 per cent alcohol. Best's carmine stain for glycogen was used for tissues fixed in Gendre's alcoholic acetic picroformalin solution.

Organ weights of 40 osteopetrosis and 32 normal litter mates aged from 1 to 36 days were determined. The organs weighed were: brain, heart, lungs, liver, kidneys, spleen, thymus,

pituitary, adrenals, and gastrointestinal tract with contents. The results were analyzed on a relative or percentage basis using a net body weight value, that is, the value obtained by subtracting the weight of the gastrointestinal mass and contents from the gross body weight. Comparisons of mean group values at consecutive age levels using a 3 day interval, were made. In 8 age groups, there were from 2 to 8 osteopetrosis and from 2 to 6 normal rabbits while 4 groups contained only 1 diseased and 1 normal rabbit.

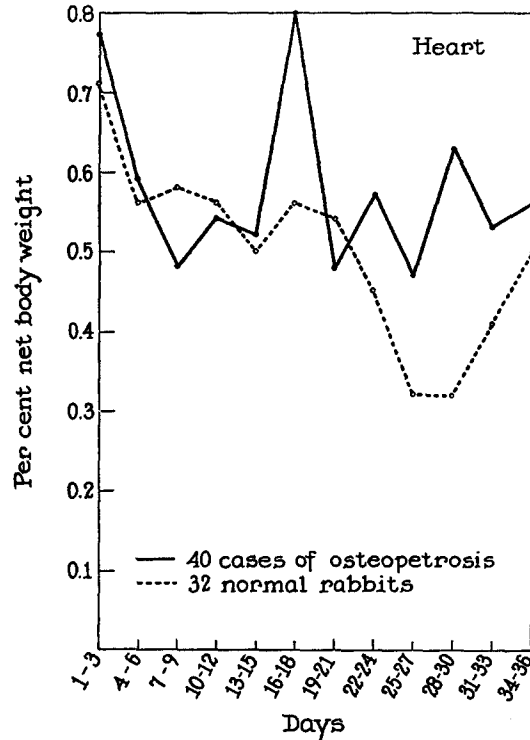


CHART 1. Mean relative weights of the heart of 40 osteopetrosis and 32 normal litter mate rabbits.

RESULTS

The results of postmortem examinations of early and advanced cases will first be presented and then the histologic observations will be described.

General Autopsy Observations

Early Cases.—In very young rabbits from birth to about 2 weeks of age, postmortem examination usually failed to reveal any striking abnormal features other than the characteristic skeletal and tooth changes and an occasional instance of internal hydrocephalus.

The skin and subcutaneous tissues were normal, the fat envelope was abundant, and the muscles generally were well developed. The diaphragm showed no visible change. The body cavities were free of fluid. The viscera appeared normal except for an occasional instance of

pallor of the heart, liver and kidneys, a finding more frequent in older animals. The thymus was abundant and the spleen rather large, red and firm. The popliteal lymph nodes tended to be small. No unusual features of the gastrointestinal tract were noted.

Beginning at the ages of 7 to 10 days, the mean relative weights of the heart, the lungs, the kidneys, the spleen, and the adrenal glands and the brain of osteopetrosis rabbits were greater than those of normal litter mates (Charts 1, 2, 3, 5, 7, and 8). In the case of the liver and the thymus, smaller values for the diseased as compared with those for normal rabbits were obtained (Charts 4 and 6).

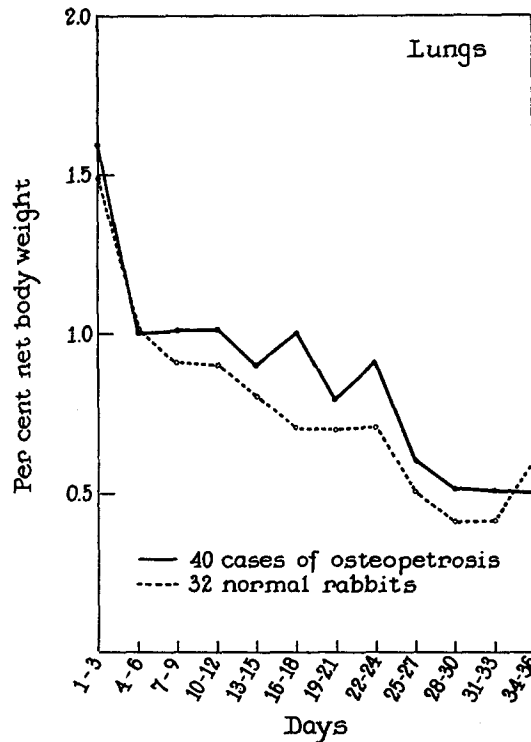


CHART 2. Mean relative weights of the lungs of 40 osteopetrosis and 32 normal litter mate rabbits.

The thyroid was usually quite pale and sometimes almost colorless and difficult to delimit. The appearance of the external parathyroids, the hypophysis, the pineal gland, and the adrenals was normal.

The brain and meninges were normal except for the occasional occurrence of internal hydrocephalus of variable degree. However, the mean relative weight values of the brain were consistently larger than those of normal litter mates (Chart 8). Hydrocephalus was obvious in some cases at or within a few days of birth, it usually increased steadily, death shortly ensued, and postmortem examination confirmed the diagnosis (1). In other rabbits, however, the condition did not increase but apparently completely subsided and the post-mortem appearance of the brain was normal. In contrast to these cases, there were certain rabbits in which hydrocephalus was not suspected during life but in which a typical lesion

was disclosed at autopsy. Examples of these findings are illustrated by the following observations on a litter containing 4 osteopetrosis cases.

The 4 rabbits, the photograph of whose brains is shown in Fig. 1, were killed at 7 days of age. Hydrocephalus was perfectly evident in the first case during life, but was thought not to be present in the 2nd rabbit and postmortem examination confirmed both opinions. In the 3rd animal a possible hydrocephalus was diagnosed and a comparatively minor lesion was found at autopsy. But in the 4th rabbit in which hydrocephalus was not suspected, post-

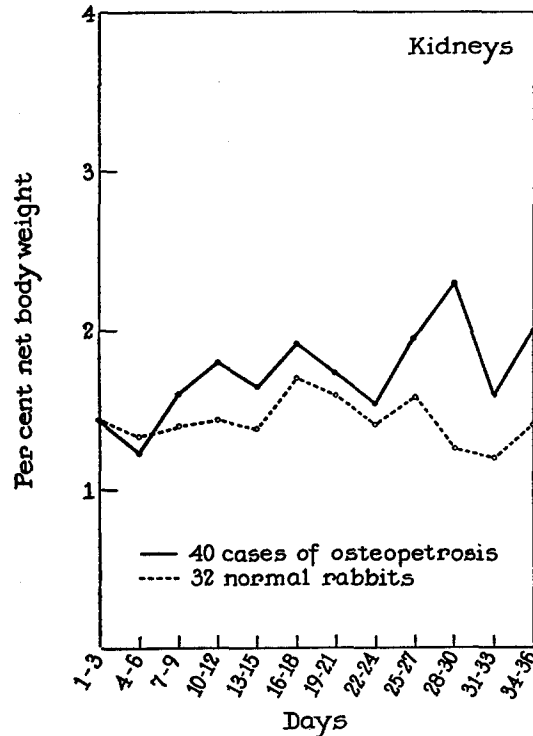


CHART 3. Mean relative weights of the kidneys of 40 osteopetrosis and 32 normal littermate rabbits.

mortem examination disclosed a typical lesion. It was not as extensive as in the first case but it was considerably greater than in the 3rd rabbit. With the exception of the first rabbit all were in good condition, growing and gaining weight.

Advanced Cases.—In older rabbits, there were some changes which appeared to be directly associated with the condition of progressive malnutrition and deterioration while others antedated this state as far as could be determined. It will be recalled that the age at which malnutrition was detected was variable but that frequently it was obvious at about 3 weeks of age.

The skin and subcutaneous tissues were not noteworthy except in animals which survived for longer periods and in such cases the tissues generally tended to be dry. The superficial fat envelope and the fat deposits of the omentum, mesentery, and perirenal areas eventually showed degrees of depletion, but in many cases there was a surprising amount of fat.

The muscles showed a progressive tendency toward pallor and softness. In a good many cases, there was apparently some degree of wasting, especially of the lumbar and thigh groups. Congestion, hemorrhage, or necrosis of the skeletal muscles was not observed but in the rapidly fatal or in the prolonged type of case, small pale opaque streaks suggesting degeneration and necrosis were sometimes seen in the diaphragm.

The body cavities did not contain free fluid. The lungs showed nothing of interest. The aorta and great vessels were normal.

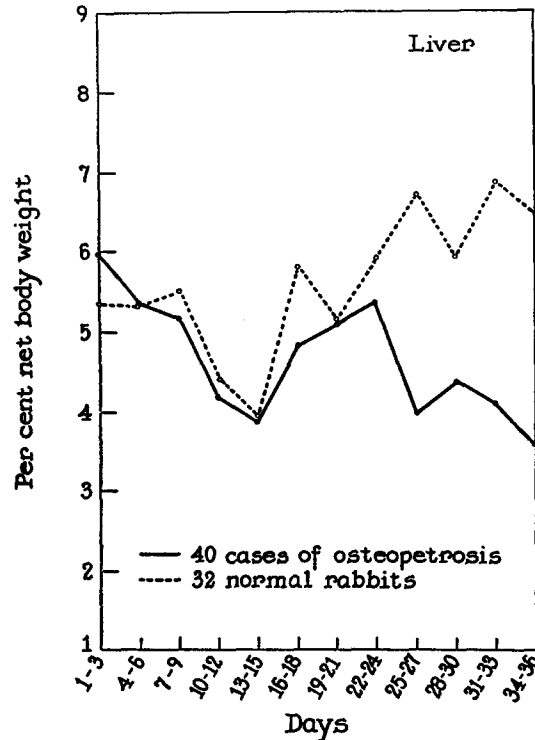


CHART 4. Mean relative weights of the liver of 40 osteopetrosis and 32 normal litter mate rabbits.

The heart, the liver, and the kidneys were often pale and whitish with a greyish yellowish or faint brownish tint resembling the color of ivory (Figs. 2 to 4). This characteristic "ivory" appearance was seen in rabbits which were in a good state of nutrition and still gaining weight, that is, before a cachectic state was obvious, but it was more pronounced in advanced cases with malnutrition and marked growth retardation. It was not observed in very young cases.

As a rule, the ivory appearance was less striking on the cut than on the external surface of the heart and liver, but in the case of the kidney, it was about the same for both surfaces. The characteristic pallor of the kidney as compared with the normal color is well shown in the photographs of the cut surfaces in Fig. 2; the respective rabbits were 40 days old. The change in the appearance of the external surface of the kidneys is seen in the photographs of the organs *in situ* of a 30 day old case and a normal litter mate (Figs. 4 and 5). In many

cases, all three organs had a similar pale appearance but in others, the marked pallor of the kidneys was conspicuous while the heart and liver showed a comparatively minor change.

The ivory appearance of the heart is indicated in the photograph in Fig. 2. As a rule, the change was not as conspicuous as that of the kidneys and liver but occasionally it was marked. An excess of pericardial fluid was only rarely observed. In 2 cases in which sudden death unexpectedly occurred, the cause was acute cardiac failure. The ventricles and right auricle were distended with soft red clots and the greater part of the outer wall of the left ventricle

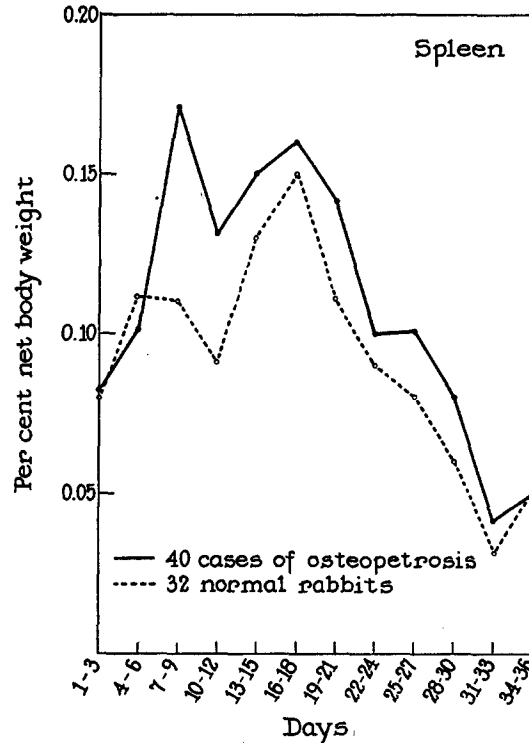


CHART 5. Mean relative weights of the spleen of 40 osteopetrosis and 32 normal litter mate rabbits.

was opaque, yellow, and apparently necrotic. The wall of the right ventricle was relatively pale and slightly striated. These cases were aged 25 and 35 days respectively.

The variable appearance of the liver in the advanced disease is illustrated by the photograph in Fig. 3 of specimens from 2 litter mate cases 40 days of age. The color of the liver in 1 case was essentially normal as shown by the lobe on the left, but the liver of the other case as is illustrated by the lobe on the right had a typical ivory appearance. Another but less striking example of a pale liver in a 30 day old case is shown in Fig. 4. The architecture of ivory livers and to a lesser degree, of ivory kidneys tended to be obscure or indefinite. In some instances the liver was mottled with small faint pinkish areas on the ivory background. In extremely advanced and practically moribund cases, the liver and kidneys were apt to have a pale bluish semitranslucent or "watery" appearance.

The relative size of the heart and the kidneys of advanced cases as indicated by mean

relative weight values, was larger than that of normal litter mates (Charts 1 and 3). A similar but less pronounced difference was found for the lungs (Chart 2). In the case of the liver, however, the mean values of the osteopetrosis rabbits were consistently smaller than those of the normal animals (Chart 4).

The gall bladder was filled with bile and in the older cases beginning at about 3 weeks of age, it was invariably distended and frequently to a marked degree (Figs. 3 and 4). The wall of the distended gall bladders was obviously much thinner than normal but no other changes

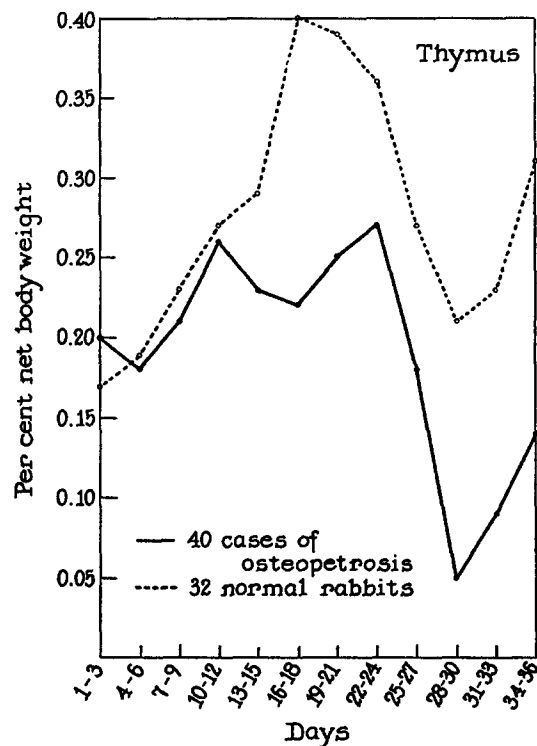


CHART 6. Mean relative weights of the thymus of 40 osteopetrosis and 32 normal litter mate rabbits.

were observed. In younger cases, the bile was usually a bright green color and of a thin, watery consistency. In the older cases, the color was a dark greenish black and the consistency somewhat viscous.

The gastrointestinal tract and contents showed no unusual features until the general state of the animal became affected. With a failure to gain weight and with the development of cachexia, a variable degree of distension of the lower ileum and caecum with gas and semi-fluid contents was found (Fig. 4); occasionally there were areas of congestion and petechial hemorrhages in the intestinal wall. The colon contained only comparatively small amounts of very pale greenish or yellowish mucus or a few small soft fecal masses which were yellowish or almost colorless. As the disease progressed, the extent and degree of intestinal distension increased, and the wall of the duodenum and upper jejunum were practically always clay

colored. In the terminal stages, the contents of the stomach and small intestine were scanty and the colon practically empty.

The pancreas, including the pancreatic tissue in the duodenal mesentery, showed no gross abnormalities.

The spleen in the majority of cases was somewhat enlarged. As indicated by the mean relative weight values in Chart 5, the spleens of osteopetrosis cases were larger than those of normal litter mates. The consistency was firm and the color normal or a slightly brighter

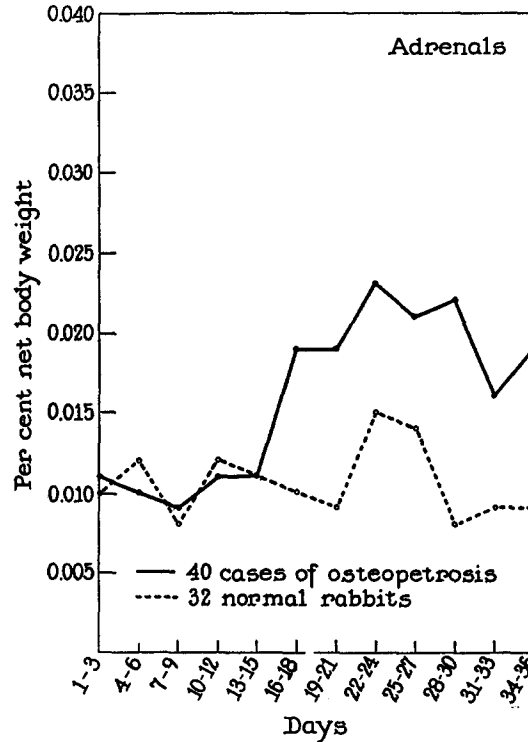


CHART 7. Mean relative weights of the adrenals of 40 osteopetrosis and 32 normal litter mate rabbits.

red than is generally found in normal rabbits of the same age. The cut surface was moist and the Malpighian bodies tended to be large. In the terminal stages of the disease, some spleens were moderately swollen, somewhat soft and paler than normal, but others from apparently similar cases were firm with a moist but not a drippy cut surface. As a rule, the splenic capsule of advanced cases showed some diffuse or patchy thickenings.

The thymus was firm, fleshy, and fairly abundant as may be seen in Fig. 4, but it was not as large as in the normal litter mates and the difference is indicated by the respective curves of mean relative values in Chart 6. In very advanced cases with a comparatively long survival period, the thymus was represented by a small stringy fibrous mass. No instance of hemorrhage in the thymus was observed.

The principal superficial lymph nodes and the popliteal nodes in particular were generally smaller than those of normal litter mates of the same age. The central mesenteric lymph

nodes were not remarkable, except that they too tended to be small and in very advanced cases were usually dry.

The thyroid gland was generally pale and more translucent than normal and in certain cases was almost colorless. Although its limits were not always easily determined, it appeared to be small rather than large.

The external parathyroids were usually identified without difficulty as pale straw-colored opaque bodies. The impression that they were enlarged was strengthened by the microscopic findings.

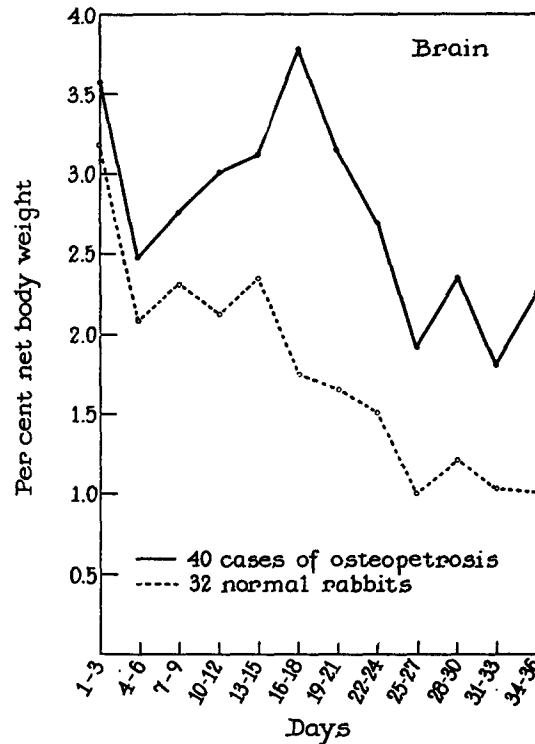


CHART 8. Mean relative weights of the brain of 40 osteopetrosis and 32 normal litter mate rabbits.

The adrenal glands were large in the majority of advanced cases but otherwise there were no unusual features externally or on section. The enlargement antedated the development of symptoms of malnutrition which it will be recalled were often present at 3 weeks of age. Beginning at about 2 weeks, as is shown in Chart 7, the mean relative weights of the adrenals of osteopetrosis cases consistently exceeded those of normal litter mates.

The pituitary, the pineal gland, and the gonads were not remarkable. In the younger cases up to about 2 weeks of age, and again in the oldest cases 4 and 5 weeks of age, the mean relative weights of the pituitary were smaller than those of normal litter mates while in the cases aged 2 and 3 weeks, the mean values for the osteopetrosis pituitaries were larger.

Histologic Observations

The abnormal conditions found on microscopic examination of the organ and tissues other than the bones will now be described.

Heart.—Except in advanced cases with symptoms of malnutrition in which there was a variable patchy distribution of cloudy swelling and of fatty degeneration, no unusual features were found.

Liver.—The most conspicuous feature in routine preparations was the large amount and persistence of hemopoietic tissue (Fig. 6). In normal rabbits as a rule these cells are not found in the liver after about 2 weeks of age but they were practically always present in advanced osteopetrosis cases, that is, up to 5 weeks of age (Fig. 7).

In cases with gall bladder distension, the bile ducts in the vicinity of the bladder were usually dilated, often considerably, and some of them showed slight branching. The connective tissue in these portal spaces was also moderately increased. There were no obvious vascular peculiarities except in areas showing dilated bile ducts and here the central veins and the sinusoids were usually dilated and congested.

The hepatic cells in sections stained in the routine way showed no particular changes until the disease was advanced. With the development of malnutrition, cloudy swelling was a constant finding and in some cases fatty degeneration was conspicuous as illustrated in the photograph of a specimen from a 31 day old case (Fig. 7). The distribution of the degenerative changes was chiefly in the central portion of the lobule.

The phosphatase content of the liver was considerably greater in osteopetrosis cases than in normal litter mates and notably so in young cases. The difference will be appreciated by comparing the photographs of preparations from an early and an advanced case with those of their normal litter mates; the respective ages were 8 days (Figs. 8 and 9) and 35 days (Figs. 10 and 11). In both normal specimens the peripheral lobular distribution is well shown, that of the younger animal (Fig. 9) being very much more abundant than that of the older (Fig. 11). In the younger osteopetrosis case (Fig. 8), the entire lobule was characteristically affected but in some areas a central and peripheral distribution was especially pronounced; in advanced cases with a less marked content the distribution was primarily peripheral (Fig. 10). The liver capsule of both early and advanced cases showed a much greater phosphatase content than was found in normal specimens. In the conspicuous dark rim of the capsule the cytoplasm in addition to the nuclei of the outer layers of cells was heavily involved.

Consistently larger amounts of calcium were demonstrated in the liver of osteopetrosis rabbits, and particularly in advanced cases, than in normal litter mates. In the normal liver there was comparatively little calcium but up to 4 times as much was estimated for the osteopetrosis specimens. The tendency toward a peripheral distribution is illustrated in the photograph of a preparation from a 35 day old case (Fig. 12).

Scattered hemosiderin granules in the liver were a characteristic feature of osteopetrosis cases in contrast to the absence of such deposits in normal livers. They were found in specimens of both young and advanced cases; the one pictured in Fig. 13 was obtained from a 27 day old case in excellent physical condition.

The glycogen content of the hepatic cells was smaller in advanced osteopetrosis cases than in normal sibs of the same age beginning at about 3 weeks of age and the difference was quite striking in individuals with a well marked cachexia.

Gall Bladder.—In the characteristically distended gall bladder of advanced cases, the wall was thinned and the lining mucosa flattened to a variable degree. A cross-section of a characteristic specimen from a 27 day old case is shown in Fig. 14, and a similar preparation from a normal litter mate of the same age is illustrated in Fig. 15. That these changes were initiated at an early period before they were evident at autopsy is shown in the photomicro-

graph from an 8 day old case (Fig. 8). Goblet cells in the mucosa and often in considerable numbers, were not infrequently found (Fig. 16).

The phosphatase content of the mucous cells was not as heavy as in normal specimens; in both cases the outer or brush border was particularly involved but in the submucosa there was much more phosphatase in osteopetrosis than in normal rabbits. No consistent difference in the degree of phosphatase content of the bile was noted.

The calcium content of the gall bladder was greater in the osteopetrosis cases. The cells of the mucosa and submucosa were thickly peppered with large coarse black granules in sharp contrast to the much less numerous distribution of fine granules in the normal specimens.

Kidney.—No abnormal features were observed in sections stained in the routine way other than variable degrees of cloudy swelling of epithelial cells in advanced cases. The convoluted tubules of the cortex were the structures chiefly affected. Calcification of the kidney was not seen. Only an occasional sample of urine obtained from the bladder was available for analysis. The phosphorus content of the urine of 3 osteopetrosis cases aged 18 to 22 days was 6.04, 6.22, and 7.24 mg. respectively per 100 cc. as compared with 4.54 and 4.82 mg. respectively for 2 normal litter mates. The calcium values were 12.0, 11.4, and 10.8 mg. for the osteopetrosis cases and 8.0 and 7.82 mg. for the normal rabbits.

The phosphatase staining of the kidney was more intense than in normal kidneys of both early and advanced cases (Figs. 17 and 18). The distribution was largely in the epithelial cells of the proximal convoluted tubules, but small amounts in other tubular epithelial cells including those of the pelvis were occasionally seen. There was a considerable amount of phosphatase in the kidneys of young normal rabbits 1 or 2 weeks of age, but much less in older normal rabbits 3 to 5 weeks of age.

The calcium content, in the form of fine granules, was also consistently greater than that of normal kidneys and its distribution was similar to that of phosphatase. The amount in all normal specimens was comparatively slight.

The glycogen content was apparently unaffected in young or moderately advanced cases in comparatively good condition. In cases with malnutrition there was less glycogen in the epithelium of both cortex and pelvis than in normal rabbits of the same age.

Urinary Bladder.—The phosphatase reaction in osteopetrosis cases was heavier than in normal sibs of the same age and the difference was particularly marked in advanced cases 3 to 5 weeks old. As shown in Fig. 19, the distribution was predominantly in the lamina propria.

Spleen.—In the young cases both the Malpighian bodies and the red pulp tended to be hypoplastic but beginning at about 10 days of age, the lymphoid areas became hyperplastic and considerably larger than those of normal litter mates (Figs. 20 and 21). This change continued to be a feature of advanced cases as may be seen by comparing the photomicrographs of specimens from an osteopetrosis rabbit and a normal litter mate aged 35 days (Figs. 22 and 23). As a rule, the pulp was not prominent and many of the sinuses were empty, or the red blood cell content comparatively scanty. Small collections of hemopoietic cells were scattered about in the pulp and as in the case of the liver, continued to be present in older cases. As the disease advanced, the splenic capsule became thickened both diffusely and in a patchy fashion and the trabeculae too were thickened. Occasionally in advanced cases, very fine hemosiderin granules in the red pulp cells and in the giant cells were observed.

The phosphatase staining of the osteopetrosis spleens was more intense than the small amount in normal spleens and the difference was most marked in advanced cases. The staining occurred in lymphoid cells and almost entirely in those of the Malpighian bodies. A similar but less marked difference in the small calcium content of osteopetrosis and normal spleens was observed.

Lymph Nodes.—The superficial lymph nodes were smaller than in normal rabbits of the same age judging from the popliteal and cervical glands. The considerable size difference is illustrated by the photographs at 5 days of age (Figs. 24 and 25) and similar findings were observed in advanced cases. In early cases, the nodules and medullary cords tended to be in an inactive or static condition (Fig. 24) as compared with an active or somewhat hyperplastic state in the normal node (Fig. 25), and the number of lymphatic cells in the spaces and sinuses was rather scanty. Beginning at about 2 weeks of age, however, the osteopetrosis nodes showed varying degrees of hyperplasia as is illustrated by the 13 and 27 day old examples in Figs. 26 and 27.

Multinucleated giant cells were fairly frequently found in the lymph nodes at all ages although the incidence was somewhat higher in advanced than in early cases (Fig. 28). In Heidenhain's azan stained preparations, the homogenous cytoplasm was a pale greyish blue color and the nucleus very vesicular. Fine hemosiderin granules were demonstrated in some of these giant cells. Small areas of hemopoiesis and myelopoiesis were occasionally observed; in the photograph of the 14 day old node shown in Fig. 28, there are 3 megakaryocytes.

The phosphatase, and particularly the calcium content, of the lymphoid cells in the lymph nodes of osteopetrosis cases was much greater than in those of normal litter mates. The difference was found in young as well as in older rabbits.

The deep lymph nodes as represented by the central mesenteric group presented generally similar but less clearly defined histologic changes to those in the cervical and popliteal nodes. Giant cells and hemopoietic tissue were very infrequently seen.

Thymus.—No unusual histologic features were found in sections stained in the routine way. The phosphatase staining in the few advanced cases studied was much more intense in all areas than in normal specimens, the greatest amount being in the medullary portion and the capsule. In the normal preparations, the heaviest staining was in the capsule and the lightest in the cortex. There was a considerable amount of calcium in both cortex and medulla in contrast to the very sparse distribution observed in the normal thymus.

Gastrointestinal Tract.—In older cases beginning at about 3 weeks of age the histologic changes observed comprised a flattening and thinning of the gastric and intestinal mucosa and to some extent of the submucous and muscle layers. There was also some indication of a decrease in the number of parietal cells of the gastric mucosa and of argentaffine cells in the gastric and duodenal mucosa. The lymphoid elements generally were less prominent than in normal rabbits of the same age and in cachectic individuals the lymphoid nodules tended to be comparatively small and hypoplastic.

Histochemical studies were limited to the duodenum. The phosphatase and calcium contents of the mucosa were greater in osteopetrosis cases of all ages as compared with their respective normal litter mates. The glycogen content was smaller as compared with similar preparations from normal litter mates of the same age.

Thyroid Gland.—In differentially stained sections, a large proportion of the colloid of osteopetrosis thyroids was acidophilic. Although this feature was found at all ages, it was especially prominent in young cases. In 25 cases aged 1 to 14 days, 16 of the thyroids showed more acidophilic than basophilic colloid and in 3 glands no difference was noted. In 23 cases aged 16 to 27 days, 12 of the thyroids showed more acidophilic than basophilic colloid and in 1 gland no difference was noted. In normal rabbits of this age range, that is, from 1 day to 6 weeks, it was usual to find a mixture of red and blue staining colloid, but with the blue basophilic reaction predominating. The size of the follicles and the amount of colloid together with the follicular epithelium showed nothing unusual. Small cysts lined with a low cuboidal epithelium and usually empty were occasionally observed, most frequently in advanced cases.

No consistent difference was found in the small phosphatase and calcium contents of the

thyroid epithelium of osteopetrosis cases and their normal litter mates. Neither phosphatase nor calcium was demonstrated in the colloid of either the abnormal or the normal rabbits.

Parathyroid Glands.—The most conspicuous feature disclosed by microscopic examination was the large amount of parathyroid tissue. This was a constant finding. The 4 external glands, and particularly the upper pair, were generally enlarged (Fig. 29) while nodules of varying size were practically always found in the thymus and frequently also along the trachea, often in association with long, thin strands of thymic tissue (Figs. 30 and 31). In normal litter mates, a small nodule or two was not infrequently seen in the thymus but in no instance did the total amount of parathyroid tissue approach that of the osteopetrosis cases. To obtain an idea of the amount of parathyroid tissue in an osteopetrosis animal as compared with that of a normal litter mate of the same age, counts were made of the parathyroid nodules in the sections from a series of representative cases and their controls at ages from 1 to 35 days, including a group of serially cut sagittal sections of the neck. The consistent results obtained are illustrated by the following example from rabbits 24 days old: There were 131 nodules of parathyroid tissue in 18 sections from the osteopetrosis case and 22 nodules in 28 sections from the larger normal sib, that is 7.3 and 0.8 nodules per section for the osteopetrosis rabbit and its normal litter mate respectively.

Microscopically the parathyroid glands showed nothing unusual except that small cysts containing granular debris were relatively frequent (Figs. 29 and 31), in contrast to their great rarity in normal parathyroids. Increased mitotic frequency was not observed.

Phosphatase staining of parathyroid cells was observed in advanced cases, the distribution and intensity, however, being quite variable. None was found in the normal specimens. Calcium was similarly demonstrated in the parathyroid cells of advanced cases as compared with little or none in the normal glands.

Adrenal Glands.—There was apparently an increase in the relative amount of the medulla of the adrenal glands, particularly in advanced cases, as compared with that of the adrenals of normal litter mates of the same age. The photomicrograph of an adrenal from a 21 day old case (Fig. 32) together with one from a normal litter mate (Fig. 33) gives an idea of the difference in the extent or size of the respective medullas. Increased mean relative weights of the adrenal glands of advanced cases have already been pointed out (Chart 7). In routinely stained preparations, the appearance of cortical and medullary cells showed nothing unusual. Occasionally a small area of hemopoietic tissue in the cortex was observed.

The fat content of the cortex was somewhat greater in osteopetrosis rabbits than in normal litter mates (Figs. 32 and 33). The zona glomerulosa especially stained more deeply while the zona reticularis in its outer portion and the zona fasciculata showed a similar but less pronounced difference.

The phosphatase staining of the cortex was more intense in the osteopetrosis than in the normal adrenal. In the normal gland, the zona reticularis showed a moderately heavy and the inner portion of the zona glomerulosa a somewhat less pronounced reaction. In the osteopetrosis adrenal marked staining was found in all 3 zones. Phosphatase was not demonstrated in the medulla of either normal or osteopetrosis rabbits.

The calcium content of both cortex and medulla was greater in osteopetrosis than in normal adrenals. There was more in the cortex than in the medulla, the greatest amount being in the zona reticularis.

Pituitary Gland.—In the first day or two of life, there was less differentiation of both basophilic and acidophilic cells of the osteopetrosis than of the normal pituitary. The differentiation of basophil cells occurred somewhat earlier in osteopetrosis cases than in their normal litter mates. At 5 and 6 days of age, the chromophobes, acidophils, and basophils of a normal pituitary were generally distributed in approximately equal numbers and only a few dark basophils were observed. In osteopetrosis rabbits of this age, more than a third of the

cells were basophils, and dark ones were not infrequent while the chromophobes represented somewhat less than a third of the cells. There were usually more deep staining acidophils in the normal than in the osteopetrosis gland.

These differences in the osteopetrosis glands continued into the 2nd and 3rd weeks with a gradually increasing proportion of acidophils. In a typical 16 day old osteopetrosis case, for example, the estimated distribution was: acidophils 50 per cent; basophils 30 per cent; and chromophobes 20 per cent. In the normal litter mate, the estimated values were 60, 20, and 20 per cent respectively. Orange staining acidophils were first seen in osteopetrosis specimens in the 3rd week of life, and antedated by a few days their appearance in normal glands. In older advanced cases 4 and 5 weeks of age, an obvious increase in the proportion of basophils was occasionally but not consistently found.

A chance observation should be mentioned because of its indication of pituitary disturbance although its precise significance is not known. In 2 cases aged 14 and 22 days, sections of the pituitary glands stained with toluidine blue after alcohol fixation showed a number of unusual cells not found in corresponding sections of normal glands. These cells were large, one-third again the size of other anterior lobe cells and occasional ones were twice as large. The cytoplasm as well as the nucleus stained a deep blue and the cytoplasm was coarsely granular. They tended to occur in clumps in the posterior half of the anterior lobe and principally along its lateral aspects. These conspicuous cells represented about 5 per cent of the total number. In all the other cells only the nucleus and cell membrane showed a comparable staining; the cytoplasm of many of them stained a faint bluish tint and was finely granular while the cytoplasm of the remainder was perfectly colorless.

Phosphatase was demonstrated in anterior lobe cells of an advanced 22 day old case and probably in greater intensity than in a normal litter mate specimen. There was little or no phosphatase in the preparations of a 14 day old case and its normal sib. In the posterior lobe of the advanced case, there was considerable phosphatase staining of both nuclei and fibers while in the normal, practically only the nuclei were involved. In the younger case and its normal sib, only a few nuclei of the posterior lobe were stained.

Calcium was found in greater amounts in the anterior lobe cells and in the nuclei of the posterior lobe of the 22 day old case than in the normal litter mate specimen. However, this distribution was reversed in the 14 day old animals, the normal gland showing larger amounts, particularly in the anterior lobe, than the osteopetrosis preparation.

Nervous Tissue.—Routine histologic preparations of representative central and peripheral nervous tissue revealed nothing of interest. The limited histochemical study was confined to portions of the brain, spinal cord, and optic nerve of a few advanced osteopetrosis cases together with the peripheral nerves and ganglia included in other sections.

The phosphatase staining of the osteopetrosis optic nerve was pronounced as shown in the photograph of a specimen from a 27 day old case reproduced in Fig. 34. A narrow outer portion of the nerve was less heavily stained. In the normal optic nerve phosphatase was demonstrated only in the blood vessels and meninges (Fig. 35). Longitudinal sections showed a fairly uniform phosphatase distribution. In some preparations, the phosphatase content of the meninges appeared to be less heavy than in normal specimens. The calcium content of optic nerve sections was quite heavy in contrast to an absence of calcium in the normal specimens.

In both the grey and the white matter of the spinal cord of osteopetrosis cases, the phosphatase staining was intense (Fig. 36) while in sections of normal cords, only the grey matter, and principally the anterior horn area was stained (Fig. 37). In neither specimen was there staining of the cytoplasm of the nerve cells. Calcium was demonstrated in comparatively large amounts in the grey matter of osteopetrosis spinal cords, and conspicuously so in the large anterior horn cells; elsewhere in the grey matter and to a somewhat less degree in the white

matter, there was a diffuse sprinkling of fine black granules. In preparations of normal cords, calcium was found to be practically limited to small amounts in the anterior horn cells.

The phosphatase staining of the cerebral cortex and the outer molecular layer of the cerebellum was much more intense in the osteopetrosis than in the normal rabbit. A similar difference obtained with respect to calcium. The deposits were especially heavy in the nuclei and cytoplasm of the large nerve cells and the neuroglia nuclei of the cerebral cortex, the Purkinje cells of the cerebellum, and the epithelial cells of the pia mater.

In sympathetic ganglia, the satellite cells and the connective tissue separating the ganglion cells showed a generally heavier phosphatase reaction in the osteopetrosis than in the normal specimens. Calcium too was easily demonstrated in the ganglion and satellite cells whereas it was scanty or absent in normal specimens. In peripheral nerves, both the phosphatase staining reaction and the distribution of calcium deposits were greater in osteopetrosis than in normal preparations.

Blood and Bone Marrow.—The phosphatase reaction of the blood cells in smears obtained from 5 osteopetrosis rabbits 6 to 30 days of age was much more intense in the osteopetrosis cases than in their corresponding normal litter mates. The nuclei and cytoplasm of the neutrophils and the nuclei of the normoblasts were heavily stained. There was also some staining of lymphocyte nuclei but little or none in normal lymphocytes.

In bone marrow smears from osteopetrosis cases aged 6, 15, and 23 days, the phosphatase reaction was comparatively intense in the nuclei of normoblasts, and in the nuclei and cytoplasm of premyelocytes, myelocytes, and neutrophils, and of megakaryocytes. In the corresponding normal preparations, these cells showed only a slight and variable reaction. Calcium was not found in the bone marrow cells of either the osteopetrosis case or its normal litter mate aged 23 days.

DISCUSSION

Other than the characteristic bone changes found in all cases of osteopetrosis (3), postmortem examination of early cases up to about 2 weeks of age revealed nothing of special note except pallor of the thyroid gland, possible enlargement of the external parathyroids, the small size of the popliteal lymph nodes, and occasional instances of internal hydrocephalus of varying grades. In advanced cases and particularly in those with well marked growth retardation and eventually malnutrition and cachexia, the autopsy findings included depletion of fat, atrophy of the thymus, pallor of the muscles, a pale or ivory appearance of the heart, liver, and kidneys, marked distension of the gall bladder with a very dark bile, gaseous distension of the small intestine and caecum, and marked reduction or practically an absence of gastrointestinal contents. Enlargement of the external parathyroids seemed certain, the thyroid continued to be very pale, the adrenal glands were generally large, and the spleen was often somewhat pale and swollen and usually somewhat enlarged.

The hydrocephalus reported in some of the severe infantile and juvenile cases of human osteopetrosis has been explained on the basis of interference with vascular communications resulting from the osteosclerotic changes of the cranial bones. Similarly, the symptoms of optic atrophy which are comparatively common and the somewhat less frequent disturbances of hearing have been attributed to a narrowing of the optic foramen and the internal acoustic

meatus. In osteopetrosis of the rabbit, exophthalmos of varying degrees was not infrequent, nystagmus was occasionally observed, and visual disturbances were sometimes suspected (1) and it was thought that in some at least of these cases the optic foramen was smaller than it should be. In any event the development of any of these various conditions could well be related to the pathologic changes of the bones of the base of the skull and of the vault (3). Considering the constancy and the degree of these changes, however, it is surprising that hydrocephalus was not more frequent.

The hematologic disturbance, as previously pointed out (2), was remarkably similar to the condition observed in the severe form of human osteopetrosis. The progressive anemia was severe and thrombocytopenia and a moderate myeloid leucocytosis regularly developed. The anemic condition, it was thought, was responsible for the pale appearance at autopsy of the heart, liver, kidneys, and muscles. At any rate, there was nothing in the gross or histologic findings of these organs which suggested another explanation.

Extramedullary hemopoietic foci in the liver and spleen and occasionally in such sites as the adrenal cortex and the lymph nodes was a noteworthy feature. The cells of these foci comprised normoblasts, metamyelocytes, myelocytes and young neutrophilic leucocytes in variable proportions, an occasional myeloblast, and comparatively numerous megakaryocytes. The presence of hemosiderin granules in the liver and less frequently in the spleen and occasionally in the lymph nodes was an additional indication of the severe hemopoietic disturbance.

Hyperplasia of the follicles of the lymph nodes and of the Malpighian bodies of the spleen was regularly observed in all but the youngest cases a few days old. Multinucleated giant cells were quite frequently seen in sections of the lymph nodes and occasionally in the spleen and some of them contained fine hemosiderin granules. The capsule and trabeculae of the spleen of advanced cases showed fibrous thickenings.

These pathologic features are the counterpart of those described in severe human osteopetrosis. Extramedullary erythropoiesis and myelopoiesis in the liver and spleen and sometimes the kidney and lymph nodes are noteworthy features. In addition, enlargement of the lymph nodes and hyperplasia of the Malpighian bodies of the spleen together with enlargement and fibrosis are characteristically observed. In a case aged 3 years 9 months Gerstel (4), draws special attention to the presence of large multinucleated giant cells containing hemosiderin granules in the spleen.

The initiation and development of a general deterioration with cachexia which was a typical feature of rabbit osteopetrosis (1) was thought to be directly related to the steadily progressive anemia. Malnutrition and weakness usually increased rather rapidly and eventually food was refused. In advanced cachectic cases, there was regularly observed little or no gastrointestinal food contents,

a marked gaseous distension of the intestine, and accumulations of mucus in the lower colon and rectum. Depletion of the thymus gland, as in other examples of malnutrition from various causes in the rabbit, was a characteristic feature in advanced cases.

In the advanced disease, marked distension of the gall bladder was regularly observed and microscopically, the wall was thinned, the folds flattened, and in the lining mucosa clear goblet cells were found fairly frequently. Distended bile ducts in the neighborhood of the affected gall bladder were often seen. No instance of jaundice was observed but the small intestine had a dull clay or colorless appearance. The liver sinusoids and central veins particularly in the gall bladder lobe, were slightly or moderately dilated. The hepatic cells showed a variable incidence and degree of parenchymatous and fatty degeneration distributed predominantly in the central portion of the lobule.

Similar changes in the liver cells and vessels are reported as characteristic findings in severe cases of human osteopetrosis, but distension of the gall bladder is apparently not a typical feature. Its occurrence in the rabbit is probably to be explained on the basis of the rapidity and degree of development of malnutrition and cachexia for in other conditions of the rabbit in which rapid emaciation occurs, gall bladder distension is rather frequently found.

Limited histochemical studies showed, as was to be expected, a very marked disturbance of phosphatase and calcium distribution. As compared with corresponding preparations from normal litter mates of the same age, a much more intense phosphatase staining was found in the following organs and tissues: the hepatic cells and the liver capsule, the submucous layer of the gall bladder, the epithelial cells of the renal convoluted tubules, the lamina propria of the urinary bladder, the duodenal mucosa, the lymph nodes, the thymus, the Malpighian bodies of the spleen, the nuclei of normoblasts, the bone-marrow cells, the white blood cells, the parathyroid cells, the cortex of the adrenal gland, the pituitary in the advanced disease, the optic nerve, the cerebral cortex, the outer molecular layer of the cerebellum, and the spinal cord. The phosphatase staining of the gall bladder mucosa was not as marked as in normal specimens. No consistent difference was found in the thyroid gland.

An increased calcium content with a distribution generally similar to that observed with phosphatase was also observed. An exception to this was the comparatively large amount in the gall bladder mucosa of osteopetrosis cases.

Certain tissues of advanced cases were found to have less glycogen than those in corresponding normal preparations as determined by histochemical procedure. This result was observed in the following sites: hepatic cells, renal epithelium of cortex and pelvis, duodenal mucosa, bronchial mucosa, and muscles (skeletal, diaphragm, tongue, and possibly the myocardium). It is not known at what age this change took place but presumably well before 2 weeks at which time growth retardation was obvious. In this connection, it should be

recalled that, as compared with the results for normal litter mates, smaller mean values for liver glycogen were found in all age groups and smaller mean values for muscle glycogen in the 2 weeks and older groups of osteopetrosis cases (2).

From a general biological standpoint, it is of interest that the so called essential or vital organs were comparatively so well preserved during the last weeks of the disease and were not involved in growth retardation and loss of weight to the same degree as the body mass. This feature is shown by the respective relative weight values. The heart, the lungs, the kidneys, the adrenals, and the brain all showed higher mean relative weight values than those of normal litter mates (Charts 1, 2, 3, 7 and 8). The values for the liver, however, were lower (Chart 4), a result which might be interpreted on the basis of a large factor of safety in terms of size for this organ.

Certain characteristic changes were found in the parathyroid, thyroid, and adrenal glands and possibly also in the pituitary gland of osteopetrosis cases.

The amount of parathyroid tissue identified microscopically greatly exceeded that of normal rabbits. Not only were the glands enlarged but accessory nodules were frequent. This condition of hyperplasia was found in both early and advanced cases. The parathyroid cells themselves showed no abnormal features and there was no increased mitosis. Small cysts containing granular debris were not infrequently found in contrast to their rare occurrence in the parathyroid glands of normal rabbits of this age range, that is, from birth to 6 weeks.

The thyroid gland in both early and advanced cases was pale or almost colorless and apparently was not enlarged. The outstanding histologic feature was the predominately acidophilic reaction of the colloid in contrast to the mixed but usually predominately basophilic colloid of the normal gland. It was most prominently displayed in young cases.

The pituitary gland generally appeared to be small, although in older cases, the mean relative values were usually somewhat larger than the corresponding normal figures. As compared with corresponding normal sections, cell differentiation was somewhat delayed in cases a few days old, while in those 1 and 2 weeks of age, the proportion of basophils as a rule was somewhat increased and that of the acidophils and to some extent the chromophobes reduced. Although this pattern of cell distribution was noticeable in certain older cases, it was not consistently found.

The adrenal glands showed no unusual gross features except an enlargement, most marked in advanced cases with well established growth retardation and malnutrition but also noted in younger cases beginning at about 2 weeks of age (Chart 7). Microscopically, there appeared to be a relative increase in the size of the medulla, most definite in advanced cases and in these cases also the stained fat content of the cells of the cortex, particularly of the zona glomerulosa, was greater than that observed in corresponding preparations from normal litter mates of the same age.

In this hereditary disease of the rabbit, the profound skeletal abnormality, which was present at birth, was obviously the most conspicuous feature (2, 3). Normal osteogenesis did not occur. The evidence from the clinical, x-ray, and pathologic studies suggested that the essential or basic condition concerned the mesenchymal cell and in general this is the conclusion reached in human cases from both postmortem observations and clinical studies. In a few human cases, a parathyroid disturbance has also been invoked but in the great majority of postmortem reports, the endocrine glands are described as normal.

In osteopetrosis of the rabbit, the possibility of a hypoparathyroid state is suggested by the characteristic manifestations of decreased bone resorption and progressive osteosclerosis together with increasing serum phosphorus levels and a progressively low serum calcium (2). In Albright and Reifenstein's (5) classification of metabolic bone diseases in adults, osteopetrosis is placed in the division of "Too-Much-Calcified-Bone" and in the sub-division of "Bone-Resorption-Too-Little." The other condition in this category is hypoparathyroidism. According to these authors' interpretation, the resorption of calcium salts (dahllite) from the matrix is decreased because of the increased phosphate level in the body fluids which is not quite offset by the lowered calcium level.

On the other hand, the large amount of parathyroid tissue in the disease of the rabbit suggested a hyperparathyroid state. There were no deviations from the normal histologic picture, it should be pointed out, except in respect to the increased total amount of glandular tissue.

Twenty years ago Péhu, Policard, and Dufourt (6) and Dupont (7) suggested on the basis of a parathyroid adenoma in a juvenile case of marble bone disease observed for several years, that a primitive hyperactive condition of the parathyroid glands might be causally implicated. Parenti (8) described the parathyroid glands of his case as hypertrophic, stating that the augmentation of the number of chromophil cells gives the impression of a hyperactivity of the glands. In a recent review on marble bone and other bone diseases, however, Péhu, Guichard, and Jeune (9) abandoned the hyperparathyroid theory largely on the ground of the great rarity of parathyroid abnormalities in autopsy reports. In addition, they pointed out that the almost constant absence of an osteoclastic reaction speaks against hyperparathyroidism and they remark that, although a parathyroid insufficiency would theoretically better explain deficient bone resorption, such a state cannot be reconciled with the clinical symptoms, the serum chemical findings, and the anatomic features of the disease.

The question of hyperparathyroidism in human osteopetrosis was recently reopened by Pincus, Gittleman, and Kramer (10) on the basis of extensive metabolic studies in 2 cases of juvenile osteopetrosis. Their most important finding was the high level of urinary phosphorus excretion; hypophosphatemia was persistent but hypercalcemia was absent. These authors concluded that, while in osteopetrosis there exists a congenital malformation of the primordial

mesenchymal cell, there is also an associated metabolic disturbance which in its manifestations resembles a chronic state of hyperparathyroidism. They expressed no opinion on whether this disturbance is of maternal or fetal origin. In the pathologic report on 1 of these 2 cases, Pines and Lederer (11) state that the thyroid and parathyroid glands appeared to be normal and the adrenal glands showed a moderate degree of hypoplasia.

The experimental results of Selye and of Pugsley are of considerable interest in the present connection. These authors (12-16) produced in rats a state of increased bone density which was considered comparable to marble bone disease of man. This was accomplished by the administration of parathyroid extract after the animals had become tolerant to the normal action of the hormone. The osteoclastic reaction (osteitis fibrosa) changed over to an osteoblastic one (marble bone formation) at the same time as the increased calcium excretion returned to normal levels. With this change serum calcium levels became decreased and serum phosphorus levels increased. Furthermore, minute doses of the hormone caused an excessive proliferation of active osteoblasts with the deposit of excessive amounts of bone and there was no initial phase of osteoclastic bone absorption (13). Recently Selye (17) expressed the opinion that it is highly problematic whether marble bone disease should be regarded as primarily of parathyroid origin.

As far as the available observations on osteopetrosis of the rabbit are concerned, the data with respect to the role of the parathyroid hormone are confusing. On the basis of the actual amount of gland tissue observed, a state of hyperparathyroidism might be assumed. On the other hand hypoparathyroidism was suggested by some of the blood chemical observations (2). In the majority of cases, the initially very low serum phosphorus levels became progressively higher and the low serum calcium levels became progressively lower. The serum phosphatase levels were high. Unfortunately, only a few specimens of urine were obtained, but analysis of these showed higher levels of both phosphorus and calcium than were found in normal specimens. It should be recalled in this connection that calcification in such sites as the kidney, which might be expected under conditions of hyperparathyroidism, was never observed. However, the amount of calcium demonstrated by histochemical methods in many tissues including the kidney considerably exceeded that in corresponding normal tissues.

With respect to the thyroid, the marked tendency toward acidophilic staining of the colloid was noteworthy. It was observed in all stages of the disease but most frequently in the younger cases, before growth retardation was obvious and before the development of definite malnutrition. The size of the follicles and the appearance of the columnar epithelium, however, were normal. If the acidophilic condition of the colloid be interpreted as evidence of thyroid hypofunction, it seems reasonable to assume some degree of lowered oxidative

balance. From the standpoint of endocrine relationships such a condition would not be out of harmony with a hypoparathyroid state.

It is also of interest that the younger age range in which acidophilic colloid of the thyroid was most prominent was the period in which there was a definite tendency toward an increased proportion of basophil cells in the pituitary. In the more advanced stages of the disease, the proportion of acidophils generally exceeded that of the basophils, but there was no instance of a marked reduction of the percentage of basophils. In some of the older cases, on the other hand, the proportion of basophils was high.

The adrenal glands were enlarged as is indicated by relative weight values of cases 2 weeks of age and older. In addition, a probable relative increase in the size of the medulla was noted. Under the critical conditions of a progressive anemia and a rapidly developing malnutrition and cachexia, a hypertrophic reaction of such vital tissues as the cortex and especially the medulla of the adrenal would be expected.

Considering the entire picture of this hereditary disease with its marked skeletal manifestations present at birth through its rapidly progressive course to an invariably fatal termination at 4 or 5 weeks of age, it seems reasonable to conclude that the skeletal abnormality represents the basic or primary condition and is the expression of a genetic mutation.

The disease manifestations, however, also included parathyroid gland hyperplasia and, from what is known of the action of the parathyroid hormone, two obvious questions immediately arise. Does the large amount of parathyroid tissue represent a reaction to, or a result of a primary condition of abnormal bone development? Or on the other hand does the parathyroid condition represent the basic or primary hereditary character which wholly or in part determines or is responsible for the skeletal abnormality? The thyroid, the adrenal, and the pituitary glands also showed certain deviations from the normal pattern but, whether any or all of the changes were the result of or were independent of the parathyroid hyperplasia, is unknown.

These several questions which have a direct bearing on the essential nature of hereditary osteopetrosis of the rabbit cannot be answered until additional information is available. From the observations reported in this and previous papers (1-3), it would seem probable that embryological investigations with this material would clarify many of the presently obscure aspects of the disease.

CONCLUSIONS

The results of postmortem examination of cases of hereditary osteopetrosis of the rabbit together with histologic observations on organs and tissues other than the skeleton have been described.

The principal findings were, first, those associated with the characteristic progressive anemia of the disease, such as extramedullary foci of hemopoietic

tissue, lymphoid hyperplasia, and the occurrence of hemosiderin in the liver, spleen, and lymph nodes.

There was a widespread tissue distribution of intense phosphatase staining and of fine calcium deposition as would be expected in the circumstances of the profound skeletal abnormality (3). In advanced cases with established growth retardation, malnutrition, and deterioration, the tissues generally showed a decreased glycogen content.

The large amount of parathyroid tissue found in both early and late cases suggested a state of hyperparathyroidism. Low serum calcium, high serum phosphorus and phosphatase levels (2), and a predominately osteoblastic reaction (3) were suggestive of hypoparathyroidism. The possibility that an involvement of the parathyroid glands was a basic or primary condition of the disease is discussed.

Evidence of a disturbance of other endocrine glands was shown by the predominately acidophilic staining reaction of the colloid of the thyroid, an enlargement of the adrenals in which both cortex and medulla participated, and the tendency toward a basophilia of the anterior lobe of the pituitary.

It was pointed out that before an explanation of the part played by the parathyroid glands in this disease could be made, other data, including particularly embryological studies, must be available. Similarly, an interpretation of other endocrine gland changes must await additional information.

BIBLIOGRAPHY

1. Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1948, **88**, 579.
2. Pearce, L., *J. Exp. Med.*, 1948, **88**, 597.
3. Pearce, L., *J. Exp. Med.*, 1950, **92**, 591.
4. Gerstel, G., *Frankf. Z. Path.*, 1937, **51**, 23.
5. Albright, F., and Reifenstein, Jr., E. C., *The Parathyroid Glands and Metabolic Bone Disease*, Baltimore, The Williams & Wilkins Company, 1948.
6. Péhu, M., Policard, A., and Dufourt, A., *Presse méd.*, 1931, **39**, 999.
7. Dupont, J., *L'Osteopetrosis*, la Thèse de Lyon, 1930.
8. Parenti, G. C., *J. Chir.*, 1936, **48**, 213. Abstr. of article in *Chir. Org. Movim.*, 1935, **21**, 200.
9. Péhu, M. (*in memoriam*), Guichard, A., and Jeune, M., *J. méd. Lyon*, 1946, **27**, 173.
10. Pincus, J. B., Gittleman, I. F., and Kramer, B., *Am. J. Dis. Child.*, 1947, **73**, 458.
11. Pines, B., and Lederer, M., *Am. J. Path.*, 1947, **23**, 755.
12. Selye, H., *J. Am. Med. Assn.*, 1932, **99**, 108.
13. Selye, H., *Endocrinology*, 1932, **16**, 547.
14. Pugsley, L. I., *J. Physiol.*, 1932, **76**, 315.
15. Selye, H., *Arch. Path.*, 1932, **14**, 60.
16. Pugsley, L. I., and Selye, H., *J. Physiol.*, 1933, **79**, 113.
17. Selye, H., *Textbook of Endocrinology*, Montreal, Acta Endocrinologica, 1947.

EXPLANATION OF PLATES

All material used for illustrations was obtained from rabbits killed by chloroform anesthesia. The histologic preparations were stained with hemotoxylin and eosin unless otherwise stated. All the preparations in which Gomori's phosphatase method was used were counterstained with methyl green and phloxine.

The photographs were made by Mr. J. A. Carlile.

PLATE 31

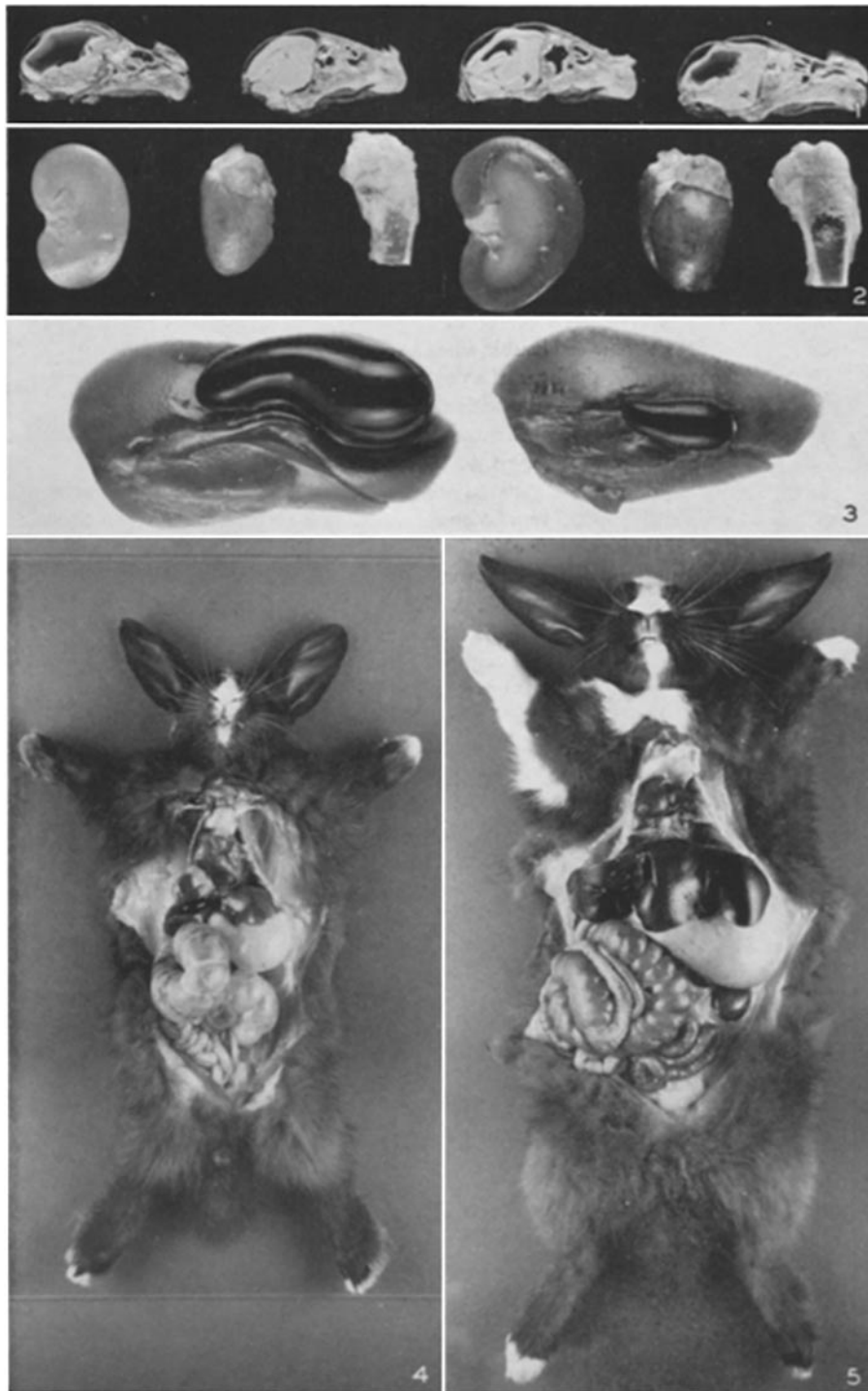
FIG. 1. Photograph of the brains of 4 osteopetrosis litter mate cases aged 7 days. A sagittal section was made close to the midline after fixation in 10 per cent formol-saline and hardening in Müller's fluid. The second brain from the left showed no gross changes. Note the marked hydrocephalus in the first and fourth brains and the moderate hydrocephalus in the third brain. During life the condition was evident in the first animal and was suspected in the third but was not thought to be present in the fourth case. $\times 0.7$.

FIG. 2. Photograph of a sectioned kidney, heart, and the proximal portion of a longitudinally sectioned femur from an osteopetrosis rabbit aged 40 days. On the right are shown similar specimens from a normal litter mate of the same age. Note the pale ivory appearance of the kidney and heart of the osteopetrosis rabbit and the extensive amount of spongy bone and dense fibrous tissue in the femur. $\times 0.9$.

FIG. 3. Photograph of the distended gall bladder and adjacent liver lobe from 2 litter mate cases of osteopetrosis aged 40 days. The ivory appearance of one liver is shown by the lobe on the right while the color of the other liver was normal. $\times 1.2$.

FIG. 4. Photograph of a postmortem preparation with the organs *in situ* of a case of osteopetrosis aged 30 days. The rabbit was in fairly good physical condition and weighed 280 gm. Compare with the corresponding photograph of a normal litter mate reproduced in Fig. 5. Note especially the marked distension of the large intestine, the distended gall bladder, the pallor or ivory appearance of the left kidney, the moderate pallor of the heart, and the slight pallor of the liver. The thymus was still fairly well preserved. The perirenal and pelvic fat masses were depleted. The incisor teeth were missing. $\times 0.4$.

FIG. 5. Photograph of a postmortem preparation with the organs *in situ* of a normal rabbit aged 30 days, a litter mate of the osteopetrosis case shown in Fig. 4. The rabbit was in excellent physical condition and weighed 716 gm. $\times 0.4$.



(Pearce: Hereditary osteopetrosis of rabbit. IV)

PLATE 32

FIG. 6. Photograph of the liver of an osteopetrosis rabbit 22 days old in fairly good condition. Note the considerable amount of hemopoietic tissue. $\times 99$.

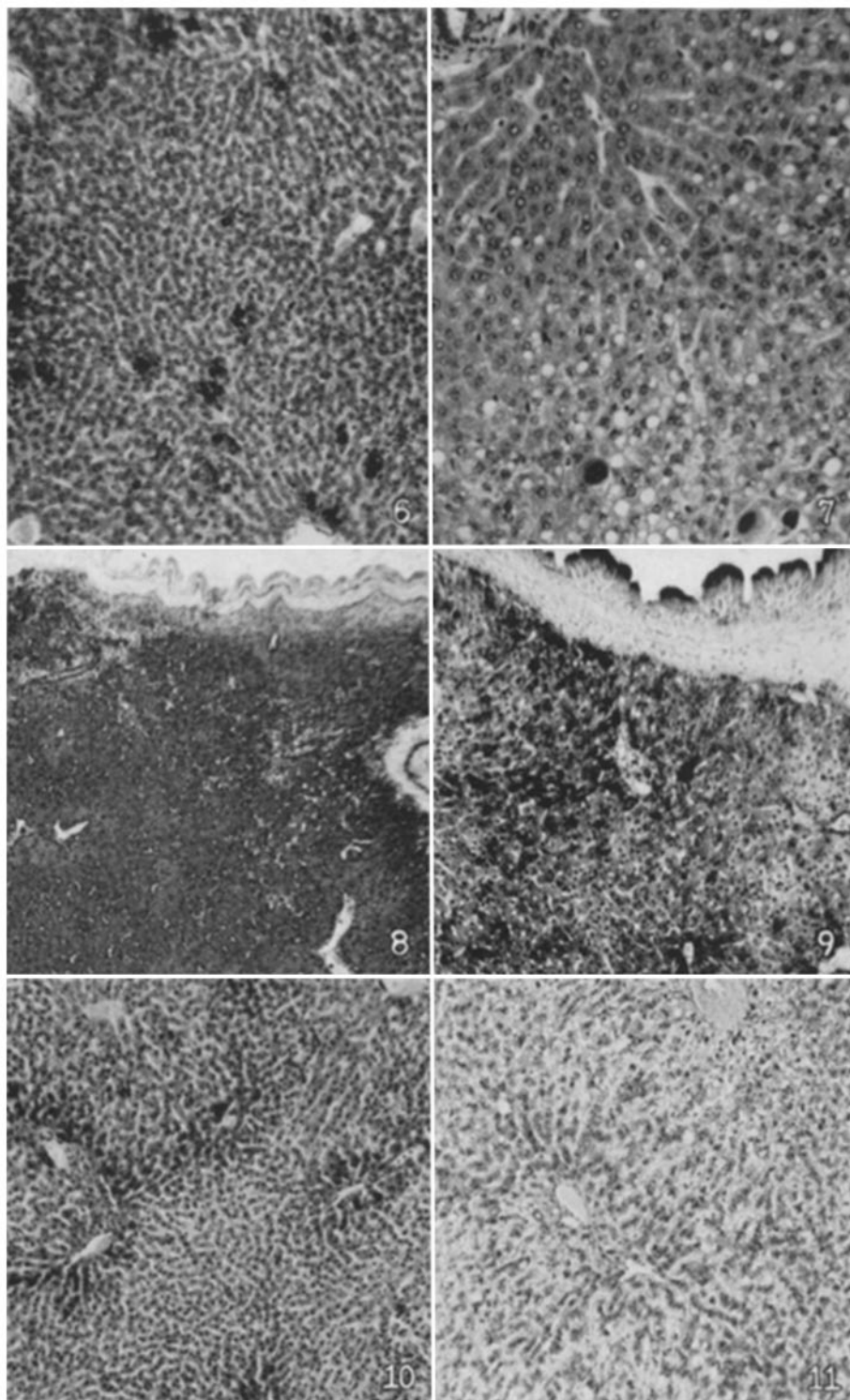
FIG. 7. Photograph of the liver of an osteopetrosis rabbit 31 days old in poor condition with marked anemia, malnutrition, and cachexia; no body weight gain for 6 days. Pronounced fatty degeneration of hepatic cells chiefly in central half of the lobules; 3 megakaryocytes are shown. $\times 188$.

FIG. 8. Photograph of the liver and wall of the gall bladder of an osteopetrosis rabbit aged 8 days. Note the very intense phosphatase staining of the hepatic cells and the negligible staining of the epithelial cells of the gall bladder as compared with the findings in the corresponding preparation from a normal rabbit shown in Fig. 9. Gomori's phosphatase method. $\times 89$. A photograph of a phosphatase kidney preparation from this rabbit appears in Fig. 17.

FIG. 9. Photograph of the liver and wall of the gall bladder of a normal rabbit aged 8 days. Gomori's phosphatase method. $\times 89$. A photograph of a phosphatase kidney preparation from this rabbit appears in Fig. 18.

FIG. 10. Photograph of the liver of an osteopetrosis rabbit aged 35 days in fair general condition. The phosphatase reaction was considerably greater than the very slight one in the liver of a normal litter mate of the same age (Fig. 11). Gomori's phosphatase method. $\times 74$. Other photomicrographs of the liver, urinary bladder, and spleen of this rabbit are illustrated in Figs. 12, 19, and 22.

FIG. 11. Photograph of the liver of a normal rabbit aged 35 days, a litter mate of the rabbit represented by the liver photograph in Fig. 10. Gomori's phosphatase method. $\times 74$. A photomicrograph of the spleen of this rabbit appears in Fig. 23.



(Pearce: Hereditary osteopetrosis of rabbit. IV)

PLATE 33

FIG. 12. Photograph of the liver of an osteopetrosis rabbit aged 35 days, the same animal represented in the photographs of Figs. 10, 19, and 22. Note the considerable calcium content, which is in sharp contrast to the very slight amounts present in the livers of normal rabbits aged 1 to 40 days. Von Kóssa's calcium method. $\times 73$.

FIG. 13. Photograph of the liver of an osteopetrosis rabbit aged 27 days in very good general condition. Note the considerable amount of hemosiderin; none was found in the livers of normal rabbits aged 1 to 40 days. Gomori's Prussian blue reaction. $\times 253$. Photomicrographs of the gall bladder and a popliteal lymph node of this rabbit appear in Figs. 14 and 27.

FIG. 14. Photograph of the distended gall bladder of the osteopetrosis rabbit of Fig. 13. The degree of distension together with the generally thinned condition of the wall and flattening of the epithelial lining are well shown. Compare with a similar photograph from a normal litter mate of the same age in Fig. 15. Heidenhain's azan stain. $\times 19.8$. The popliteal lymph node illustrated in Fig. 27 was obtained from this rabbit.

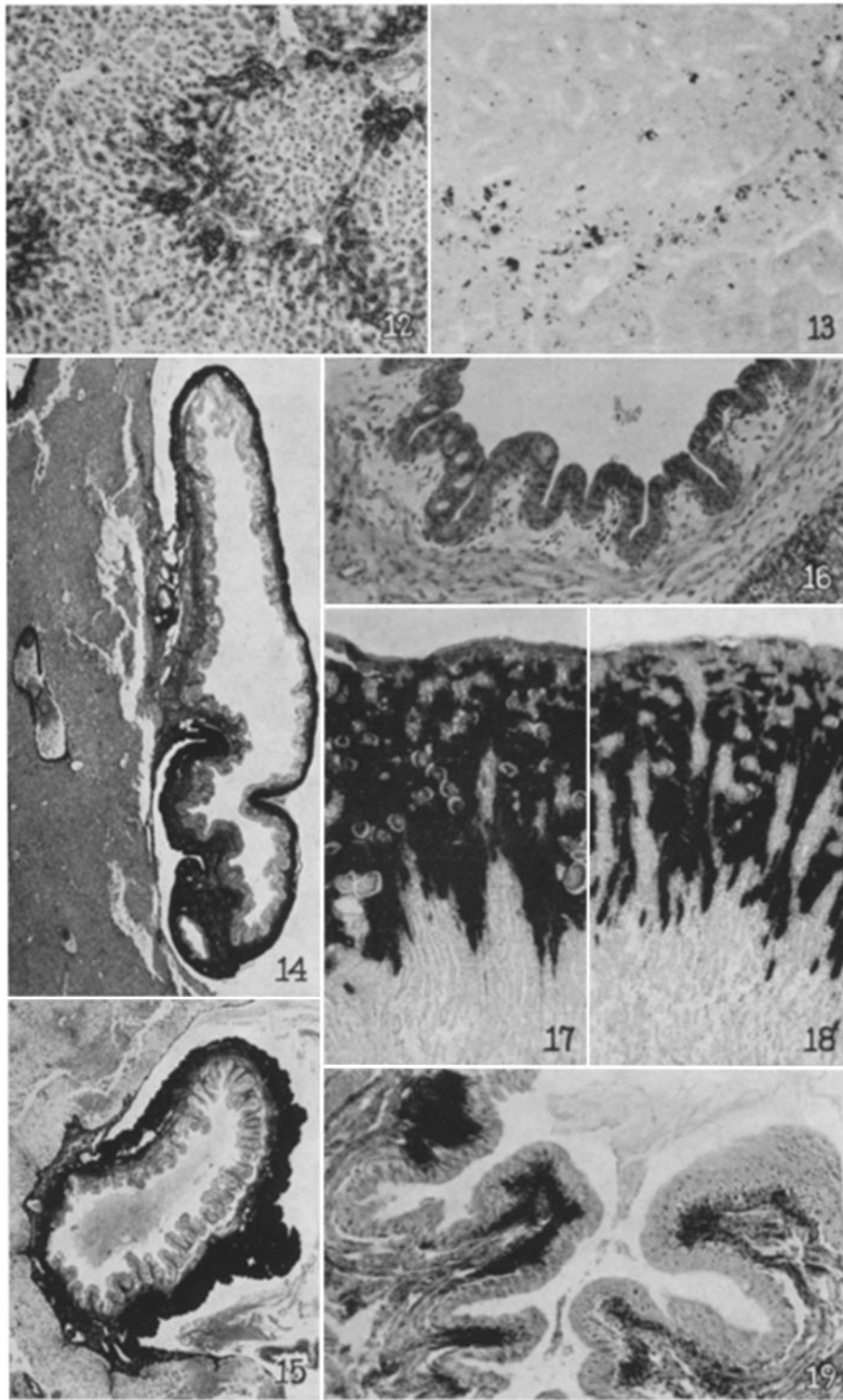
FIG. 15. Photograph of the gall bladder of a normal rabbit aged 27 days, a litter mate of the osteopetrosis rabbit represented in Fig. 14. Heidenhain's azan stain. $\times 19.8$.

FIG. 16. Photograph of the gall bladder wall of an 18 day old osteopetrosis rabbit in fairly good condition to show the goblet cells in the mucosa. $\times 115.6$.

FIG. 17. Photograph of the kidney of an 8 day old osteopetrosis rabbit—the same as represented in Fig. 8—to show the somewhat greater phosphatase reaction than that of the normal kidney illustrated in Fig. 18. Gomori's phosphatase method. $\times 38$.

FIG. 18. Photograph of the kidney of an 8 day old normal rabbit, the same represented in Fig. 9. Gomori's phosphatase stain. $\times 38$.

FIG. 19. Photograph of the urinary bladder of an osteopetrosis rabbit aged 35 days, to show the intense phosphatase staining of the lamina propria. In a corresponding specimen from a normal litter mate of the same age, the staining was comparatively slight. Gomori's phosphatase method. $\times 73$. Other photomicrographs of preparations from this rabbit appear in Figs. 10, 12, and 22.



(Pearce: Hereditary osteopetrosis of rabbit. IV)

PLATE 34

FIG. 20. Photograph of the spleen of an osteopetrosis rabbit aged 14 days in good condition. Note the hyperplastic Malpighian corpuscle and the hemopoietic tissue in the pulp. Compare with a corresponding section from a normal litter mate of the same age depicted in Fig. 21. $\times 96$. A photomicrograph of a cervical lymph node of this rabbit appears in Fig. 28.

FIG. 21. Photograph of the spleen of a normal rabbit aged 14 days, a litter mate of the osteopetrosis rabbit of Fig. 20. $\times 96$.

FIG. 22. Photograph of the spleen of an osteopetrosis rabbit aged 35 days, the same rabbit whose liver and urinary bladder sections are illustrated in Figs. 10, 12, and 19. The Malpighian bodies are markedly hyperplastic. Compare with the section from a normal litter mate of the same age shown in Fig. 23. $\times 96$.

FIG. 23. Photograph of the spleen of a normal rabbit aged 35 days, a litter mate of the osteopetrosis rabbit represented by Fig. 22. $\times 96$. A photomicrograph of the liver of this rabbit appears in Fig. 11.

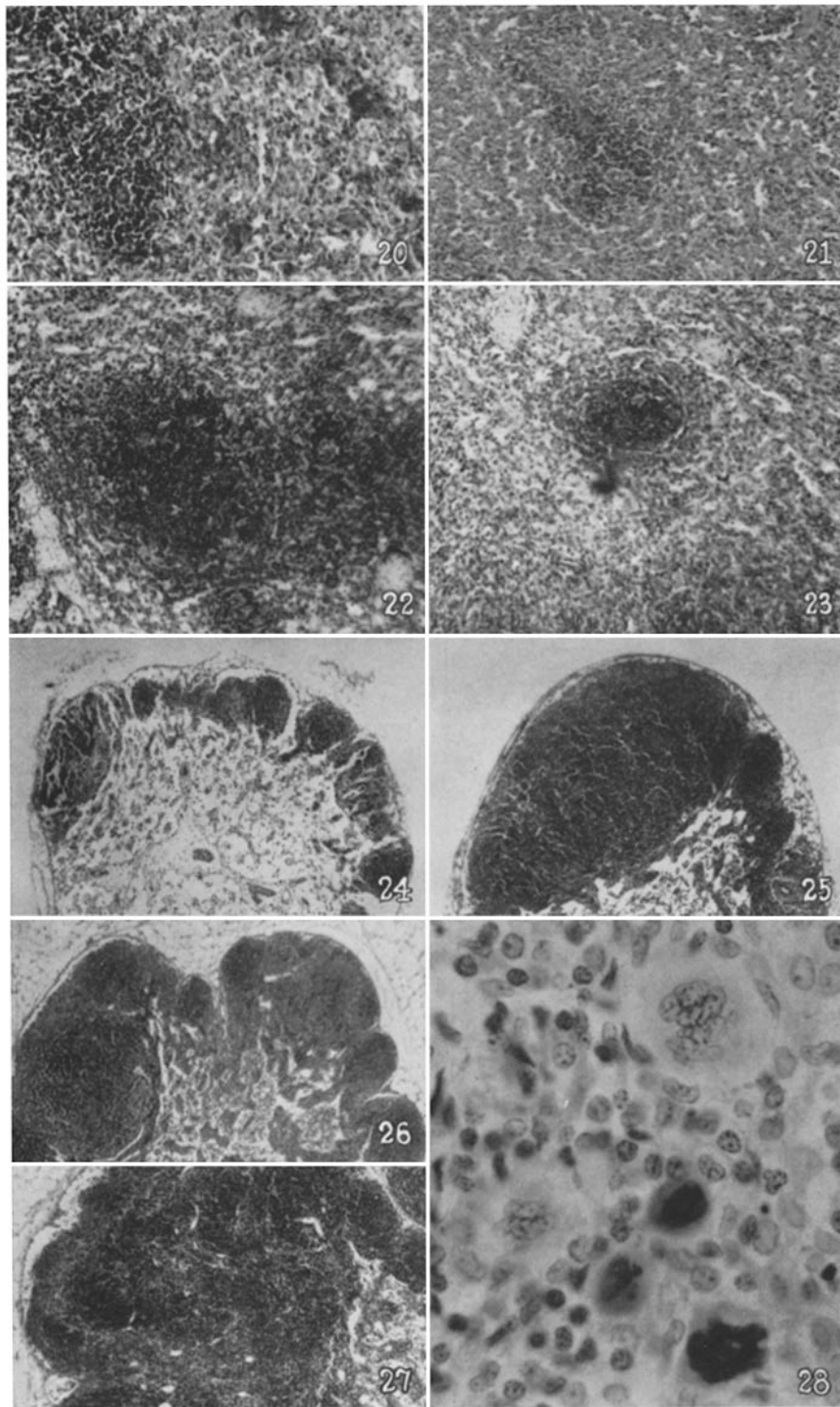
FIG. 24. Photograph of approximately one-half of a popliteal lymph node of an osteopetrosis rabbit aged 5 days and weighing 100 gm. Note the small size of the node and the inactive state of the nodules. Compare with the photomicrograph of a normal node shown in Fig. 25. $\times 37$.

FIG. 25. Photograph of less than one-half of a popliteal lymph node of a normal rabbit aged 5 days and weighing 128 gm., a litter mate of the osteopetrosis rabbit represented in Fig. 24. $\times 37$.

FIG. 26. Photograph of a portion of the popliteal lymph node of an osteopetrosis rabbit 13 days old. The nodules and medullary cords show a moderate hyperplasia. $\times 37$.

FIG. 27. Photograph of a portion of the popliteal lymph node of an osteopetrosis rabbit aged 27 days, the same rabbit represented in Figs. 13 and 14. Note the marked hyperplasia of the nodules and to a somewhat less degree, of the medullary cords. $\times 37$.

FIG. 28. Photograph of a superficial cervical lymph node of an osteopetrosis rabbit aged 14 days to show 2 multinucleated giant cells and 3 megakaryocytes. Heidenhain's azan stain. $\times 558$. A photomicrograph of the spleen of this rabbit appears in Fig. 20.



(Pearce: Hereditary osteopetrosis of rabbit. IV)

PLATE 35

FIG. 29. Photograph of a large external parathyroid gland of an osteopetrosis rabbit aged 7 days. The size of the parathyroid was about one-third that of the adjacent thyroid lobe. An oval cyst of the parathyroid is shown in the lower part of the photograph. Heidenhain's azan stain. $\times 86.8$. Practically all the colloid of the thyroid was acidophilic as determined by the azocarmine aniline blue stain.

FIG. 30. Photograph to show 5 nodules of parathyroid tissue situated along the border of the thymus and 2 other nodules in the connective tissue between the thymus and trachea adjacent to an artery. From an osteopetrosis rabbit aged 24 days. Heidenhain's azan stain. $\times 21.2$.

FIG. 31. Photograph of the lower end of a long mass of parathyroid tissue situated in the upper third of the thymus of an osteopetrosis rabbit aged 22 days. Note the cyst in the distal end of the parathyroid nodule. $\times 76.7$.

FIG. 32. Photograph of the central portion of the adrenal gland of an osteopetrosis rabbit aged 21 days in very good condition and weighing 200 gm. To show the greater fat content of the cortex especially of the zona glomerulosa, and the large size of the medulla in comparison with the findings in an adrenal from a normal litter mate of the same age, illustrated in Fig. 33. Scharlach red stain. $\times 27.9$.

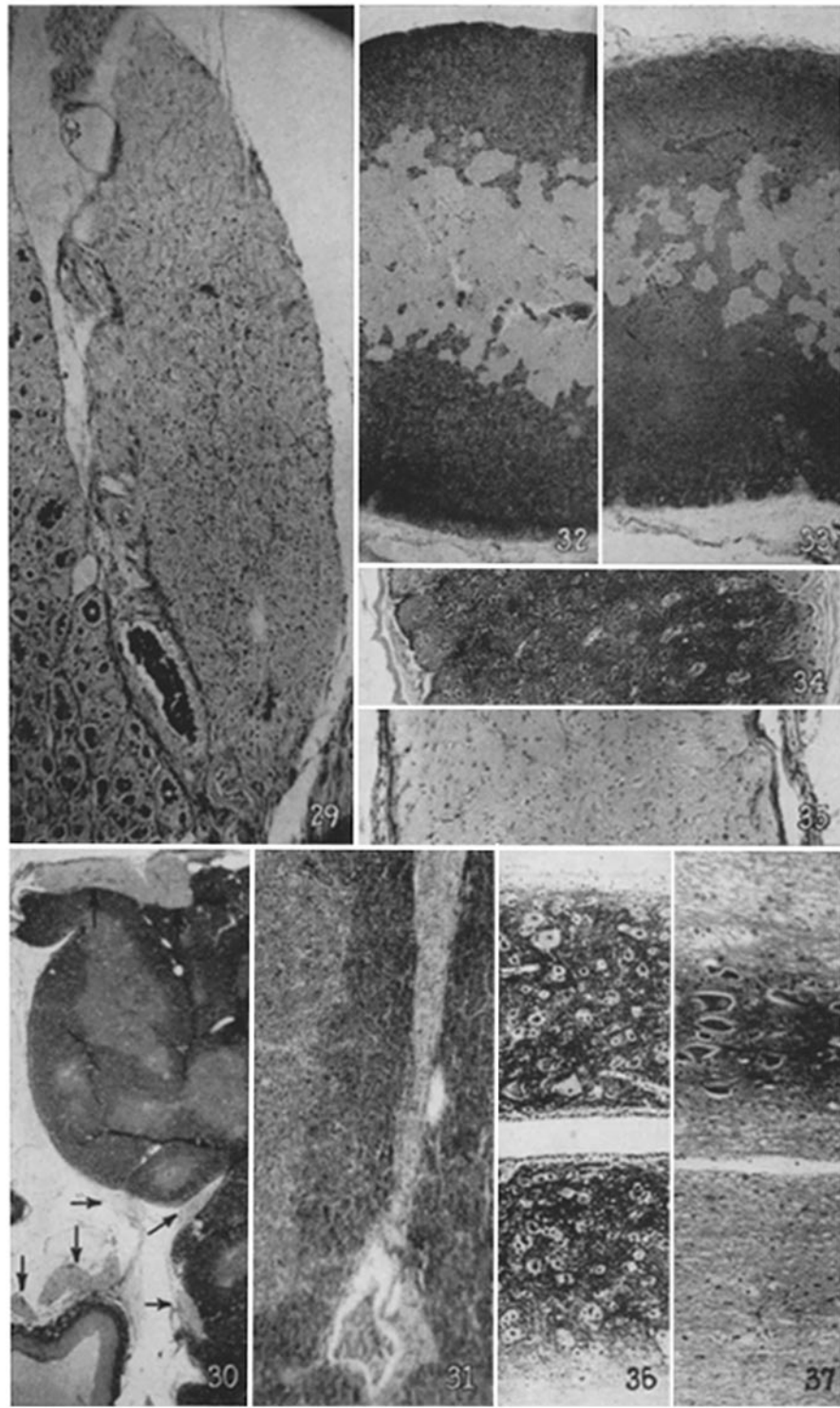
FIG. 33. Photograph of the central portion of the adrenal gland of a normal rabbit aged 21 days weighing 480 gm., a litter mate of the osteopetrosis rabbit represented in Fig. 32. Scharlach red stain. $\times 27.9$.

FIG. 34. Photograph of a portion of the cross-section of an optic nerve of an osteopetrosis rabbit aged 27 days to show the intense phosphatase reaction. Compare with a corresponding section from a normal litter mate of the same age illustrated in Fig. 35. Gomori's phosphatase method. $\times 79.8$.

FIG. 35. Photograph of a portion of the cross-section of an optic nerve of a normal 27 day old rabbit, a litter mate of the osteopetrosis rabbit represented in Fig. 34. Phosphatase is present in practically only the meninges. Gomori's phosphatase method. $\times 79.8$.

FIG. 36. Photograph of a longitudinal section of the spinal cord of the osteopetrosis rabbit whose optic nerve is depicted in Fig. 34. Note the intense phosphatase staining of both the grey and white matter. Compare with the much lighter reaction of a normal cord as shown in Fig. 37. Gomori's phosphatase method. $\times 79.8$.

FIG. 37. Photograph of a longitudinal section of the spinal cord of a normal rabbit whose optic nerve is depicted in Fig. 35. The phosphatase reaction is quite well marked in the anterior horn area but is only slight in the posterior horn area and practically absent in the white matter. Gomori's phosphatase method. $\times 79.8$.



(Pearce: Hereditary osteopetrosis of rabbit. IV)