HEREDITARY OSTEOPETROSIS OF THE RABBIT

I. General Features and Course of Disease; Genetic Aspects

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From our increased knowledge of the natural history and biology of the rabbit largely attained in recent years, it appears that this species is subject to a much greater number of inherited morbid conditions than had heretofore been generally thought. The conditions vary widely, ranging from those which are comparatively simple and apparently non-injurious to those with more or less severe manifestations and even lethal effects. In the first category are found such anatomical and physiological variations as the Rex type of coat, yellow fat, and brachydactylia and allied abnormalities (1). In extreme contrast to the essentially harmless conditions which are in nowise incompatible with growth and development are those associated with non-viability at or within a few hours of birth. Some of these so called lethals are ill defined and little of a precise nature is known about them but in the case of achondroplasia, for example, many features have been fully described (2–4).

Hereditary osteopetrosis, which is reported in this and succeeding papers (5, 6), is likewise present at birth but the uniformly lethal effect is delayed, usually to the 4th or 5th week. It is one of a number of disease complexes which have been encountered in a colony of rabbits maintained in this laboratory for experimental investigation on constitutional factors associated with susceptibility and resistance to disease.

Marble bone disease of man was first described in 1904 by Albers-Schönberg (8). The name osteopetrosis, which was introduced by Karshner (9) in 1926, is now commonly employed although other descriptive names such as osteosclerosis are sometimes used. The disease was long considered a rare affection, but with the great increase of x-ray examination, its incidence now appears to be not infrequent. Its outstanding feature is the remarkable appearance and structure of the bones, the chief characteristics of which are an extremely thick cortex and a narrowed marrow cavity which is filled to a greater or less extent with abnormal medullary bone. In severe cases, there is an accompanying anemia which is often pronounced, and various symptoms such as physical underdevelopment, tooth defects, spontaneous fractures, hydrocephalus, and optic atrophy are described.

* Dr. Brown died on August 4, 1942. The disease here described occurred in stock with which he was working and the early phases of this study were carried out by him.—L.P.

The disease is most frequently seen in infants and young children and it now seems clear that its onset takes place at some phase of intrauterine development (10). Its etiology is unknown. A significant feature is its strong familial tendency, and its hereditary character is generally accepted. In the opinion of most observers, the mode of inheritance is on the basis of an autosomal recessive factor, but the occurrence of certain parent-offspring cases raises the question of an irregular dominant factor (11–13).

The hereditary disease of the rabbit here described for the first time closely resembles in many respects osteopetrosis of man. Investigations on the human disease have been greatly limited by the relatively small number of cases on which comprehensive studies were possible. The present comparable condition of the rabbit should be of value, particularly from the standpoint of an experimental approach to the human problem. Furthermore, since embryological material is now available, it seems not unlikely that investigations in this field might have important applications in the basic problems of osteogenesis and hematogenesis.

The observations on hereditary osteopetrosis of the rabbit have been divided into three categories for presentation. The present paper is concerned with the manifestations and course of the disease and the results of genetic studies. The following paper (5) contains a description of x-ray examinations of the skeleton and the results of hematologic observations and of chemical determinations. In a third paper (6) the results of postmortem examinations and histological studies will be described. A summary of these studies has recently been reported (7).

Materials and Methods

The foundation of the osteopetrosis stock was a small group of pure bred Dutch rabbits purchased from a dealer, which included in particular a male rabbit some of whose offspring from backcross matings were the first observed examples of the condition. As soon as the hereditary nature of the condition appeared probable, extensive interbreeding of a large number of more or less closely related animals was undertaken for the purpose of obtaining additional transmitters. The identification of transmitters was made on the basis of affected progeny and several were discovered in these early experiments. Enlargement of the stock was accomplished by using identified transmitters for further inbreeding with related animals and also for outcrosses with various unrelated stocks. Progeny derived from such outcross matings constituted an F_1 hybrid generation which was used as the basis for genetic studies.

The present report is based upon 293 rabbits which at birth or within a few days showed the characteristic physical signs of the disease complex. These rabbits were contained in 169 litters. The control material comprised the 722 normal litter mates and other normal rabbits of comparable ages derived from both related and unrelated stock.

A detailed description of affected individuals was included in the notes dictated at the time the litter was first examined a few hours after birth. The animals were weighed on an automatic balance calibrated in gram intervals and their sex recorded. Subsequent examinations were made 3 times a week or oftener, particular attention being paid to the growth and nutri-

tional state of the abnormal as compared with the normal members of the litter. Body weight determinations were usually a reliable index of the general condition of the animal. In the great majority of cases, the diagnosis was confirmed by x-ray photographs and post-mortem examination of the skeleton.

All adult rabbits in the colony are housed in large individual cages, and the diet comprises a constant water supply, timothy and alfalfa hay, and a standard pellet preparation of the best quality obtainable. Supplementary feeding of fresh cabbage and carrots is carried out during the winter months. An excellent condition of health is generally maintained. In breeding experiments, the duration of pregnancy can be calculated to within a few hours. The time of most matings is exactly known and in those cases in which the doe is left with the male, it is known to within 18 hours. Pregnancy checks are made 10 days after mating and pregnant females are examined at regular intervals throughout gestation. The detection of abnormal or unusual features in this period is thus reasonably assured.

RESULTS

Osteopetrosis of the rabbit has as characteristic features abnormal bone and tooth development, retardation of growth, anemia, progressive cachexia, and an invariably fatal effect. The dense bone shadows with reduced marrow spaces, particularly of the long bones, are striking features of x-ray photographs (5).

Of 293 animals showing the disease, 275 were born alive while 18 were found dead when the litter was first examined a few hours after birth. Almost all litters were born at term and there was only an occasional instance of prematurity or of slightly prolonged gestation. No abnormal or unusual conditions were observed during pregnancy which could be associated with the disease. The affected animals were the only members of the litter which at birth or within the first few days of life showed abnormalities that could be attributed to the disease. Transmitters could be identified only by breeding tests.

Many of the cases were among the smaller individuals of the litter at birth. The great majority were well nourished and continued to grow for some days. The fur and nails were well developed and the nares were patent. The characteristic tooth abnormality observed during life comprised the subnormal development or the absence of one or more of the incisor teeth. The disease is uniformly fatal; the survival period of most cases was 4 to 5 weeks.

There were 17, or 5.8 per cent, of the rabbits classified as tarda cases because of the delayed development of incisor tooth abnormalities. The teeth appeared to be normal at birth but within 1 to 10 days characteristic changes occurred. The animals were generally large individuals and several lived somewhat longer than the usual case.

The signs and course of the disease will now be described, after which the results of genetic studies are presented. It will be helpful in reading this description to refer to the x-ray photographs in an accompanying paper (5); these illustrations have been arranged in order of disease progression.

General Description

Teeth.—The first clue to the diagnosis of osteopetrosis is an abnormal condition of the incisor teeth which is usually present at birth. The molar teeth are similarly affected as postmortem examination has regularly revealed; but the posterior portions of the buccal cavity are not easily examined during life.

In the normal rabbit born at term, the four incisors are fully erupted and the teeth of each pair are evenly placed in line with and in close approximation to each other. The enamel is smooth, glistening, and white, usually with a faintly bluish tint and slightly translucent.

The chief attributes of the incisor tooth abnormality were an initial subnormal development and subsequent retarded and abnormal growth. The most frequently observed condition at birth was the absence of, or the rudimentary state of one or more of the teeth. In a good many cases, the point of an unerupted tooth was palpable through the gum but in others nothing of this sort could be felt. The absence of one or both uppers occurred more frequently than a similar condition of the lower incisors while the absence of all four teeth was occasionally observed. In some cases, only one incisor was seen. The appearance of the incisor teeth on the 2nd day of life of a normal rabbit and a litter mate with osteopetrosis is illustrated in Fig. 1; in this case the upper incisors were not erupted and the lowers were slanting and convergent.

There were also at birth less obvious tooth abnormalities comprising some peculiarity of size, shape, color, or position. In many of these instances, the tooth was somewhat small, and was apt to be out of line with the other member of the pair and more or less separated from it, the cutting edge was frequently curved or slanting or somewhat pointed instead of straight, and the enamel had an opaque slightly yellowish color. Various combinations of incisor abnormalities were seen, as for example, an absent pair and one abnormal and one normal appearing tooth of the other pair. Occasionally only one of the four teeth was suspiciously small.

Within a few days, the condition noted at birth became accentuated and other abnormalities were apt to occur as well, as can be seen in the photographs of several cases from 6 to 35 days of age (Figs. 2 to 7).

The abnormalities most frequently observed as the disease progressed were a retardation or suppression of growth of the teeth and shedding of them.

Small stunted teeth were apt to be deformed and abnormally placed and often the pair was convergent (Fig. 2) or more or less widely separated (Fig. 5). The enamel of these abnormal teeth was often an opaque dirty grey color or a dull yellowish white. In some cases the incisor teeth retained their infantile proportions with no apparent growth whatever. Such teeth were usually widely separated and rotated or twisted in position. The photograph in Fig. 6 illustrates this condition of tiny peg-like teeth in a 21 day old case. On the other hand, there were a few instances in which an originally small tooth became a thin spindly fragile structure which might be longer than normal (Fig. 7).

The shedding of an abnormal tooth or even of an apparently normal one was often observed (Figs. 2, 3, 4, and 7). It usually occurred in the first few days of life but sometimes not until the animal was a week or 10 days old. The absence of all four incisors in a 9 day old rabbit is shown in Fig. 3. In this case at birth the left upper incisor was missing, the right upper was a small short tooth, and the lowers appeared to be normal; the three teeth were shed between the 6th and 9th days. An x-ray photograph of this rabbit is reproduced in the following paper (5, Fig. 5).

In another case of a 13 day old rabbit shown in Fig. 4, a small right lower incisor was shed between the 4th and 6th days. Both upper incisors were missing from birth. X-ray photographs of the jaws and other bones of this animal also appear in the following paper (5, Fig. 9).

The eruption of a second tooth in the gum area of the shed incisor was a fairly common occurrence; usually the new tooth was rudimentary and grew but little (Fig. 5). There also were instances in which an incisor erupted, usually within the first 2 or 3 days of life, in an area in which at birth no tooth was seen or felt. Such teeth were practically always stunted and deformed and remained in this condition.

As has already been mentioned, certain animals have been classified in a group designated as osteopetrosis tarda on the basis of a delayed occurrence of incisor teeth abnormalities. At birth the teeth appeared to be normal but typical changes soon developed and in the majority of animals at 3 to 4 days of age. The subsequent general course of events was comparable to that of the usual case.

Shedding of incisor teeth was relatively frequent in tarda cases and was often followed by the abnormal growth of one or more of the remaining teeth as is illustrated by the photograph of a rabbit 35 days of age (Fig. 7). At this time the right upper incisor was a short stumpy tooth which slanted toward the midline and was widely separated from the smaller somewhat pointed left upper incisor. Both lower incisors were abnormally long and widely separated. The enamel of all four teeth was dull, opaque, and white. At birth, the teeth appeared normal but at 3 days of age little or no development of the uppers was apparent and by the 10th day the subnormal condition was marked. By the 16th day, the left upper was missing and the lowers were longer than normal. On the 25th day, there were two very short stumpy upper incisors, a second left upper meanwhile having erupted. In the next 10 days, there was slight growth of the uppers but a considerable overgrowth of the lowers. During this period the general condition of the animal underwent a rapidly progressive deterioration.

It has been mentioned that abnormalities of the molar teeth similar to those of the incisors were observed at postmortem examination. Many of these teeth were not erupted while others were small and irregularly placed. It was evident that the underlying condition responsible for the abnormal condition of the incisor teeth was not confined to the anterior regions of the jaws but that it extended throughout both bones. Cleft palate was not seen.

Size.—At birth, rabbits with osteopetrosis tended to be slightly smaller than their normal litter mates. On the basis of mean body weights the difference was comparatively minor. In a group of 161 cases and 353 normal sibs, for example, the mean birth weights were 44.5 and 46.2 gm. respectively. The frequency distribution curves of birth weight values, however, give a better idea of the size variations encountered.

The curves in Chart 1 were drawn at 5 gm. intervals. The one representing the birth weights of normal rabbits approaches a normal frequency curve and its peak practically coincides with the mean value for the group. The curve representing the diseased rabbits is more irregular and does not reach as high a level. Furthermore, the position of its two highest

points is to the left and below the peak of the normal curve. One of these two points representing the 35–39.9 gm. class is only slightly lower than that of the next highest class in which the mean value of 44.5 gm. falls. These classes comprise 60 animals or 37.3 per cent of the group; with the inclusion of the next highest class, 45–49.9 gm., a total of 86 cases, or 53.4 per cent of the group, is represented. A similar comparison with the normal sibs shows that in the 45–49.9 gm. class which contains the mean value, there were 79 or 22.4 per cent of the total group represented. With the inclusion of the two adjacent classes of 40–44.9 and 50–54.9 gm., the number of animals represented is 183 or 51.8 per cent of the group. Slightly more than half the rabbits of each group fall in the class containing the mean weight value and

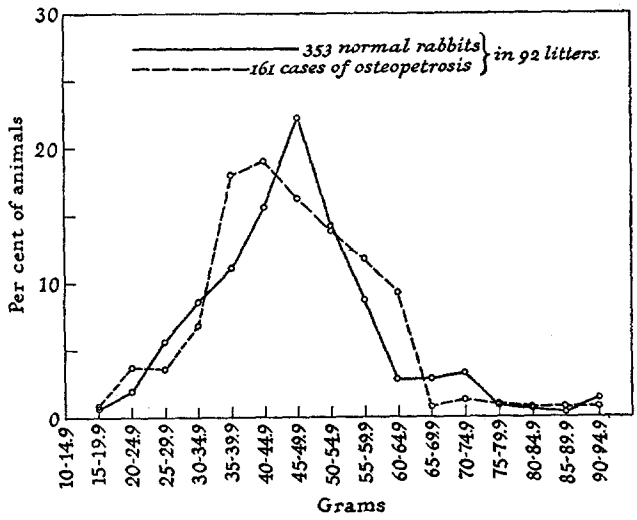

CHART 1. Distribution frequencies of birth weight values.

the two adjacent weight classes, but the position of these classes for the diseased rabbits is to the left and slightly below that for the normal litter mates.

A comparison has also been made of the relationship of the weight of each individual in a litter expressed in terms of a percentage of the weight of the heaviest litter mate (14). This procedure eliminates the variations due to differences in the absolute weights of animals of different litters since all observations are given a relative value. The percentage values were grouped in classes in descending order of magnitude while the frequencies in each class were expressed in relative or percentage values of the total number of rabbits. The results of this analysis are shown in Chart 2. The curve representing the 161 cases of osteopetrosis generally resembles that for 353 normal litter mates but its comparative height and position indicate the tendency of these cases toward small rather than large size.

Course of Disease and Length of Survival.—As has already been pointed out, there is nothing unusual or peculiar in the general appearance of osteopetrosis animals at birth or for the first few days of life except the abnormal condition

of the teeth. The animals are well developed and well nourished. Nursing usually proceeds normally, there are no obvious gastrointestinal disturbances,

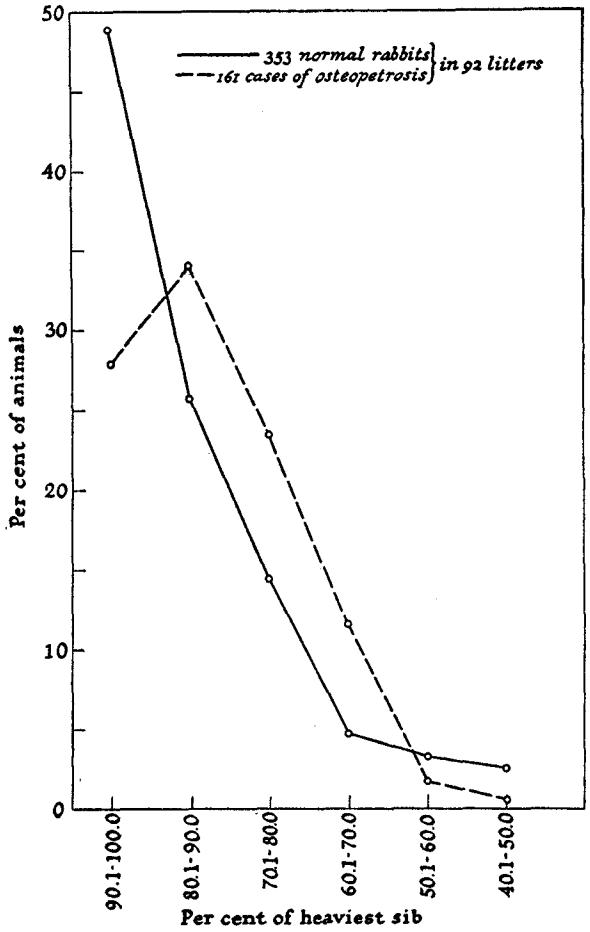

CHART 2. Distribution frequencies of birth weight values in terms of the per cent of the heaviest sib. The number of animals in each weight class is expressed as a per cent of the total number of animals.

the body weight increases, and growth is evident. Within a short time, however, often by the 14th day or even earlier, signs occur, the most striking being a retardation of growth followed by the development of an outspoken cachexia. The rate of body weight gain decreases and ultimately there is actual weight loss. These several features are illustrated by the weight curves in Charts 3 and 4.

It will be noted from the curves in Chart 3 representing 3 cases of oste-

opetrosis, that all body weight values were smaller than the mean values of 5 normal sibs.

All the diseased rabbits, A, B, and C, continued to gain weight for a fortnight at approximately the same rate as the normal animals. From this time on there was a progressive weight loss with the exception of one temporary reversal in the case of rabbit C from the 19th

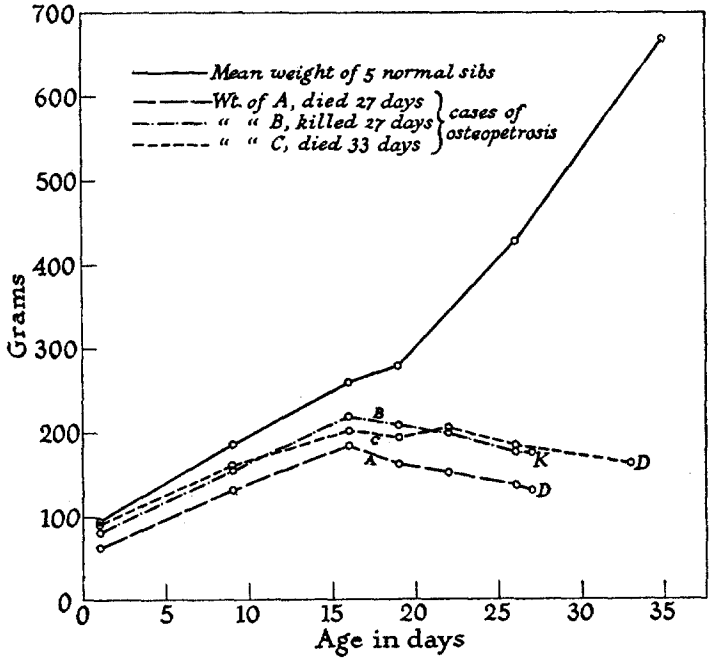

CHART 3. Body weight values of 3 rabbits with osteopetrosis and 5 normal litter mate rabbits from birth to 33 days of age. For the normal sibs, mean values are used.

to the 23rd day. The first death, that of rabbit A, occurred on the 27th day; the body weight had decreased to the level reached on the 9th day. Rabbit B, killed on the 27th day, was in quite good physical condition with a weight only slightly lower than that on the 16th day. Rabbit C survived to the 33rd day; the body weight was then at a somewhat lower level than the peak value on the 16th day. Meanwhile, all 5 normal sibs continued to grow rapidly, particularly after the first fortnight. At the time rabbit C died the mean weight value of the normals was approximately 4 times the weight of C.

Chart 4 contains the curves representing body weight values of two normal and two osteopetrosis litter mates.

At birth and at 3 days of age, the weights of all the animals were practically identical but from then on, both normal animals, A and B, gained weight much more rapidly than C and D, the rabbits with osteopetrosis. In the case of rabbit D there was a stabilization of weight on the 9th and 11th days and a slight loss on the 13th day when the animal was killed. In the

case of rabbit C, a similar weight stabilization occurred but on the 13th day a slight gain was found and further gains took place until the 23rd day; thereafter there was a loss of weight and the animal was killed on the 27th day. At this time the weight of one normal was 2½ and that of the other normal was 3 times the weight of rabbit C.

There was a considerable variation in the course of the disease particularly with respect to the time of development of the cachexia and its rate of progress. The opening of the eyelids usually took place at the normal age of 10 to 11 days

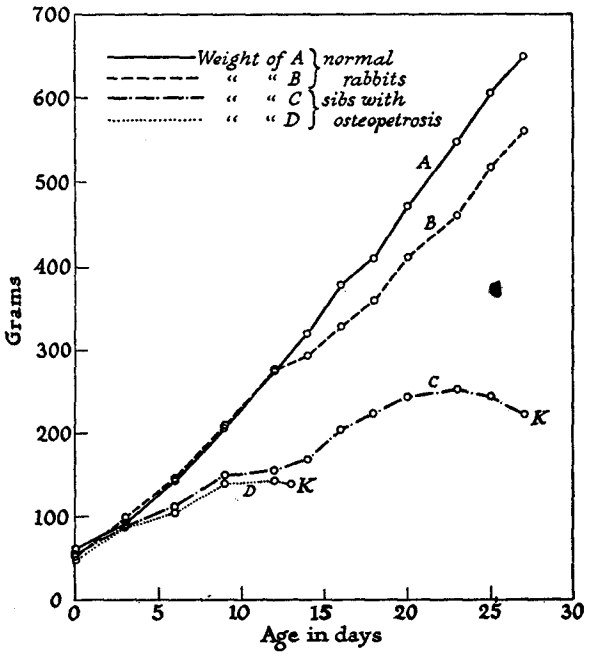

CHART 4. Body weight values of 2 rabbits with osteopetrosis and 2 normal litter mate rabbits from birth to 27 days of age.

but a delay of 2 or 3 days was not infrequent and in these cases the gain in weight was often comparatively slight and death was apt to occur early, that is, in the 2nd and 3rd weeks. In the majority of cases, however, the course of events was more prolonged, the animals continued to nurse, and many exhibited a surprising activity in spite of evident malnutrition and progressive weight loss. The small size of affected rabbits particularly after the 2nd week was very striking.

An idea of the manifestations and course of the disease can be obtained by comparing the photographs of three cases with those of three normal litter mates. The appearance of the first case and a normal sib on the 7th, 15th, and 27th days is shown in Figs. 8, 9, and 10, respectively. The photographs of

another pair on the 16th and 24th days appear in Figs. 11 and 12, while a third example on the 34th day is shown in Fig. 13.

The most obvious feature of these photographs is a difference in size, the retardation of growth becoming more striking with increasing age and disease progression. It was already apparent at 7 days of age in the case pictured in Fig. 8 and it was marked at the older ages illustrated in Figs. 9 to 13. As the disease progressed other signs developed. The fur became dull and somewhat rough or irregular and fluffy or nappy (Figs. 10 and 12). Eventually, a spotty thinning of the coat might occur and sometimes there were small areas of alopecia particularly in the cervical region. An ophthalmia was frequent. The eyelids were apt to droop, the corneas appeared dull, lacrimation was likely to be excessive, and often tiny crusts or scales accumulated on the lids (Figs. 10 to 12). Not infrequently the secretion was thick and gummy and occasionally the lids were sealed.

By the 4th week or even sooner, emaciation and evident weakness were usually present. In these cases the skin lost its normal tautness and the musculature felt soft and flabby. As the disease progressed, malnutrition and weakness became increasingly pronounced with eventual prostration and death. A few days before death, a thin mucous or watery diarrhea often developed. In some cases, the abdomen became prominent and bloated. The rabbit whose photograph on the 16th and 24th days is reproduced in Figs. 11 and 12 showed a steadily deteriorating condition and was killed, together with the normal litter mate, on the 27th day. The x-ray photographs of the skeletons appear in the following paper (5, Figs. 17 and 19).

The great majority of deaths occurred in the 4th and 5th weeks as is shown by the frequency distribution curve of the length of survival of 243 cases (Chart 5). It should be mentioned that 34 of these animals, or 14 per cent, were killed for blood chemistry determinations and other studies. Their distribution is indicated on the chart and it will be noted that in most age groups, their number in proportion to the total number of animals in the group is small. There were two exceptions. In the 13–15 and the 19–21 day groups, the proportion of killed animals was 53.3 and 38.9 per cent respectively. From what is known of the disease, it is probable that the older cases at least would not have survived much longer. However, it should be pointed out that contrary to the usual experience with diseased rabbit nurslings, predictions of probable length of life, especially of cases surviving more than a fortnight, were not too reliable. Some rabbits whose general condition did not seem immediately critical promptly died while others continued to live for several days in spite of marked emaciation.

As has already been pointed out, certain of the longer lived cases belonged in the tarda class of the disease. There were 17 such animals in the total group of 243 cases represented in Chart 5; their distribution is shown by vertical bars at the base of the chart. It will be noted

that 13 lived longer than 2 weeks and that 8 of these died in the 5th, 1 in the 6th, and 1 in the 10th week of life respectively.

Hydrocephalus.—Other signs of the disease which have been seen more or less frequently will now be described beginning with hydrocephalus. At first this condition was thought to be fairly common and together with the manifestation of prominent eyeballs, was responsible for the laboratory nickname of "hydro-bugeye." Although some instances of hydrocephalus were found at birth most of them developed within the 1st week of life. Further experience, however,

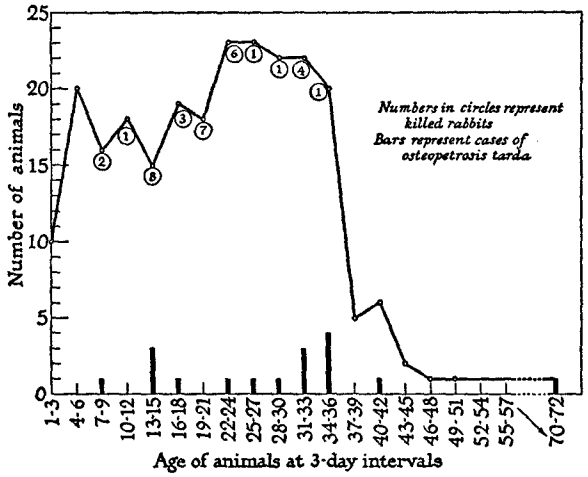

CHART 5. Distribution frequencies of length of survival of 243 rabbits with osteopetrosis, including 34 that were killed.

did not support the early impression of the comparative frequency of hydrocephalus and, actually, its incidence rate is not known. Postmortem examination of many cases in which a slight or a fair degree of hydrocephalus was thought to be present failed to reveal any evidence of it. The prominent calvarium and apparently increased fluctuation over the posterior fontanelle were deceptive. The history of similar cases together with the character of the skull bones at different ages suggests that the probable explanation lies in the deficient calcification of the skull and delayed closure of the sutures which give the impression of floating bones. The pressure of the growing brain on pathological bones may also be a contributing factor.

On the other hand there were definite instances of hydrocephalus in which the diagnosis made during life was confirmed at autopsy. In most of these unmistakable cases, a progressive dome-like expansion of the posterior calvarium occurred over a period of a few days. Some of these cases were held for observation and in a few of them gradual regression of the condition took

place and only a minor degree of hydrocephalus was found at postmortem examination. Finally there were still other cases in which hydrocephalus was not suspected during life but which showed this condition at autopsy (6).

Exophthalmos.—As has just been mentioned, prominence of the eyeballs was observed and in a few cases extreme grades amounting to well marked protuberance developed. Certain conspicuous examples are known to have occurred in hydrocephalic cases, but in others scarcely less pronounced, no evidence of hydrocephalus was found at autopsy. Although the condition was not marked in the majority of cases, the incidence of some slight or minor degree of eyeball prominence was fairly frequent. It was noted at birth or within a day or so and usually it had subsided after about a week or 10 days. It appears likely that the circumstances just suggested as being responsible for the erroneous diagnosis of hydrocephalus were also directly related to the manifestation of prominent eyeballs.

Neurologic Signs.—There were a few cases in which uncoordinated and athetoid movements of the head and body were seen. These symptoms usually developed within the first 3 or 4 days but sometimes they were not noted until the 2nd week. Tremors of the head and occasionally of the entire body have also been observed. In certain animals there was a lateral spasmodic shaking of the head toward one side only or sometimes toward one side and then toward the other. Other animals displayed a peculiar weaving motion of the head. There were a few instances of opisthotonos and in one case, it was marked. Jerking movements of the eyeballs, a nystagmus, have occurred. In some rabbits, the movements suggested an interference with vision and in addition, the face had a strange blank expression. In these cases the pupillary light reflex was thought to be sluggish or absent.

The majority of the cases were examples of an early fatal outcome but a few survived for upwards of 3 weeks. In the latter individuals particularly it was noted that there was a considerable variation with respect to the constancy of the peculiar movements. At one examination, for example, the condition was striking, but on the next it might be comparatively minor or practically absent. It should also be mentioned that the development of the neurologic signs was not necessarily associated with malnutrition or advanced cachexia.

With the development of unusual disturbances of movement and possibly of vision, the presence of hydrocephalus was suspected and searched for. Again, it was demonstrated in some animals but not in others.

Genetic Data

As has already been stated, the individuals known to represent cases of osteopetrosis were the only members of their litters which showed any abnormalities at birth or subsequently which could be directly attributed to the disease. X-ray photographs of a large number of these apparently normal rabbits were taken not only for control purposes in connection with their osteopetrosis litter mates but also with the possibility in mind that abnormal bone conditions might be disclosed in some animals. None was found.

Since the disease was invariably lethal within a few weeks and since transmitters could be identified only by breeding tests, genetic studies were necessarily carried out with such animals. A number of identified transmitters were mated with unrelated rabbits, including examples of pure breeds, to form an F_1 hybrid generation, and these animals in turn were tested for transmission of the condition. Those found to be transmitters were then interbred to form an F_2 generation.

The 293 cases of osteopetrosis here considered occurred among 1015 total births contained in 169 litters. The incidence of the condition is 28.87 per cent. On the basis of a simple recessive factor, one-fourth, or 254 animals, would be expected to show the condition. There is no significant deviation between the observed distribution of rabbits with osteopetrosis and normal litter mates and the expected distribution on the basis of genetic principles as shown by the chi-square test of homogeneity ($N = 1$, $P = 0.05+$). Of the total number of progeny, 314 rabbits contained in 51 litters were derived from F_2 matings, their F_1 parents being outcrossed hybrids. The number of cases of osteopetrosis in the groups was 79, an incidence of 25.16 per cent.

The disease is not sex-linked. Of a total of 212 cases whose sex was determined, 109 were males and 103 were females. There was likewise no preponderance of either sex in the normal progeny of the osteopetrosis stock nor in the pure bred Dutch stock in which the condition was first seen.

There was no unusual relationship between the frequency distribution of osteopetrosis cases and the size of the litters. The 169 litters contained from 2 to 11 animals, the average number being 6. A curve drawn to illustrate the frequency distribution of the litters in relation to litter size approaches a normal frequency curve. The same is true of the distribution curve representing the number of cases in the several litter classes or sizes.

In analyzing the proportion of the total number of osteopetrosis rabbits to the total number of births in each litter class it was found that in the smaller litters of 2, 3, and 4 animals, the proportion of diseased rabbits ranged from 50.0 to 39.3 per cent. In the litter classes of 5 and 6 animals, the ratios were 34.4 and 30.3 per cent respectively. In the remaining classes of 7 to 11 animals, the ratios varied from 25.8 to 18.1 per cent. The largest number of total births in any litter class, 203, occurred in the class of 7 animals; of these births, 49, or 24.1 per cent, were cases of osteopetrosis.

DISCUSSION

The hereditary morbid condition of the rabbit here designated as osteopetrosis is considered to be a disease entity on the basis of, first, a well defined complex of manifestations and a distinctive course which have been described in the present paper and, second, characteristic bone changes observed in x-ray photographs and in postmortem material. These latter are discussed in later reports (5, 6). The several manifestations are peculiar to this condition and have not otherwise been observed in a large rabbit stock maintained as a colony in this laboratory nor have they been reported elsewhere so far as is known.

The disease is notable in several respects. In the first place, it is present at birth and is invariably fatal; the majority of cases survive to the 4th or 5th week of age. The diagnosis which is made at or within a few days of birth is based upon the peculiar appearance of the incisor teeth. The underlying condition is in reality one of subnormal and disturbed development of the entire skeleton. In anticipation of the observations described in the following paper (5), it may be stated here that the characteristic appearance of the bones in x-ray photographs of the younger cases is one of practically uniform density or opacity with little or no suggestion of differentiation. In older cases, some degree of differentiation is seen.

Retarded growth is constantly observed. At birth, the size of the animal tends to be somewhat small in comparison with normal litter mates and the difference is indicated by body weight values. With increasing age, growth becomes increasingly and markedly retarded and eventually there is actual weight loss. The smaller size of the bones as compared with those of normal litter mates of the same age is very striking (5). Malnutrition, weakness, gastrointestinal disturbances, and prostration are characteristic manifestations. Hydrocephalus has occurred and variable degrees of prominence of the eyeballs are frequently observed. Peculiar muscular movements have occasionally been seen.

The disease is hereditary and the mode of inheritance is on the basis of a simple recessive unit factor. The appearance of unaffected litter mates is entirely normal and transmitters of the disease (heterozygotes) can be identified only by breeding tests. The condition was first observed in the backcross progeny of a pure bred Dutch rabbit.

The condition bears a striking resemblance to the disease of man first described as marble bones and more recently as osteopetrosis. In the severe form of infants and young children, physical underdevelopment is usually observed and the characteristic dense appearance of the skeleton in x-ray photographs is pathognomonic. Other manifestations such as tooth defects, hydrocephalus, and optic atrophy are often present. An anemia is generally found and this is also a prominent feature of the disease of the rabbit (5). Again in anticipation of a later report (6), the resemblance of the bone lesions to those of human osteopetrosis is striking. In both conditions there is a failure or a deficiency in bone absorption. Finally, the human disease shows a strong familial tendency and many authors have stated that it is hereditary, an autosomal recessive factor being concerned. However, certain parent-offspring cases have occurred. Nussey (13) refers to the records of 7 families with 26 cases in whom the disease was observed in more than 1 generation; in 6 families, 2 generations and in 1 family, 3 generations were affected. These observations raise the question of an irregular dominant factor. This difference in inheritance of the human and the rabbit disease, if it exists, is commonly observed in a comparison of the inheritance of the same abnormality in man and in lower animals.

Human cases in all age periods have been observed but the majority are in infants and children (15). The occurrence of cases in young infants together with the general character of the symptoms, and in particular retardation of growth and dentition, has led to the opinion that the onset of the disease is in fetal life. That this actually occurs has been shown by the demonstration of typical findings in a 6 months fetus (10). In a recent monograph, Dierickx (16) states that the disease is characterized as a recessive hereditary morbid condition, the onset coinciding with the first appearance of ossification, about the 2nd fetal month. It has been suggested by several authors that probably many cases die *in utero* and others soon after birth.

The disease is much more severe in younger than in older patients and a comparatively rapid fatal outcome is frequent. In adult cases, on the other hand, the disease is characteristically benign and the presence of typical bone changes is generally discovered only by chance, as from a dental x-ray examination. It is not known when the bone abnormality of these cases was initiated although the presumption is that it began in early or even in fetal life. As the matter now stands, it appears that the survival of the adult case depends largely on the absence of what have been called secondary characteristics of the disease, whereas the early development of these manifestations causes death in a relatively short time (9). Such symptoms as physical underdevelopment, imperfect dentition, hydrocephalus, and in particular anemia, are in all probability incompatible with life.

No explanation for the development of severe manifestations in one individual and not in another is available. The suggestion has been made that actually there are two, or less probably three, distinct conditions having the feature of characteristic skeletal change in common but with other dissimilar symptoms and in addition, different genetic relationships (13, 17, 18). In the case of the severe infantile disease, the operation of a recessive factor has been invoked and for the benign adult condition, a dominant factor. Up to the present, however, the number of cases adequately investigated from an hereditary standpoint is too small for a final opinion on the subject.

The condition of hereditary osteopetrosis in the rabbit is considered to be the counterpart of the severe infantile form of the human disease and the question of a benign adult form with characteristic bone changes as the chief or only abnormality, has not arisen. At least, in an extensive survey of adult rabbits of the stock, including a large number of x-ray examinations, no example of adult marble bones has been found.

Finally, mention should be made of a skeletal condition resembling osteopetrosis which occurs in two other species, the mouse and the Florida manatee, and also in the dugong, which is closely related to the manatee. It is of interest that these species represent such distinctive orders as the Rodentia and Sirenia among the mammals.

In the grey-lethal mouse described by Grüneberg (19–22) the formation of all the yellow pigment of the coat is suppressed, secondary bone absorption is completely absent, the teeth fail to erupt because of the resistance of the bone, growth is retarded, and death occurs between the 22nd and the 30th day of age. Anemia, however, does not develop as is the case in the rabbit (5). An autosomal recessive gene is the basis for the condition. The gene arose presumably by a spontaneous mutation in a laboratory stock of the house mouse.

The order Sirenia includes 2 living families of aquatic placental mammals, the Manatidae or manatees and the Halicoridae or dugongs. The members of both families are remarkable for their exceedingly dense and massive bones. The medullary cavity is entirely lacking in the long bones and ribs.

Histologic studies on fetal and adult specimens of the Florida manatee made by Fawcett (23) showed that there was a suppression of bone absorption. Fawcett

considered that osteogenesis in Sirenia, in athyreotic states of man and experimental animals, and in marble bone disease of man, has certain features in common: delayed growth in length of bones with relatively normal growth in diameter; incomplete differentiation of periosteal bone into Haversian systems; retardation of endochrondral ossification, with disorderly arrangement at the diaphyseoepiphyseal junction; a diminution in number and activity of osteoclasts; unusual persistence of unabsorbed calcified cartilage and primary non-lamellar bone; and a reduction in the amount of functional bone marrow. There also was evidence of an inherent hypofunctioning of the thyroid gland. Because of the similarity of the microscopic structure of the bone in marble bone disease of man, to those in sirenian bone and in skeletons of athyreotic animals, Fawcett suggested a possible endocrine basis for all three conditions.

The descriptions of the skeletal abnormalities in the grey-lethal mouse suggest many points of resemblance to osteopetrosis of the rabbit. Furthermore, both conditions have a lethal effect and both are inherited on the basis of a single recessive gene which in both cases arose as a presumably spontaneous mutation. The analogy between osteopetrosis of the rabbit and the severe infant and juvenile form of marble bone disease in man has already been drawn and it seems not unlikely that the grey-lethal mouse may belong in the same category.

In the case of Sirenia, however, the skeletal peculiarity is a characteristic feature of the species and must be considered a normal, not an abnormal, condition. How close a comparison may be drawn with the benign adult form of human osteopetrosis is not clear but except for certain skeletal changes, there is actually little resemblance to hereditary lethal osteopetrosis of the rabbit or to the severe osteopetrosis disease of infants and young children.

SUMMARY

The manifestations and course of an hereditary disease of the rabbit are reported. The condition is present at birth and is invariably fatal, generally in the 4th and 5th weeks of age. Retardation and eventual cessation of growth with marked reduction in size are conspicuous characteristic symptoms.

The condition, which first occurred in the backcross progeny of a pure bred Dutch male rabbit, is inherited. It is determined by the expression of a simple recessive unit factor, affected individuals being homozygous for the factor. Rabbits heterozygous for the factor are identified only by appropriate breeding tests. The condition is not sex-linked.

The disease has a remarkable resemblance to osteopetrosis or marble bone disease of infants and children with respect to signs and general course and also, as may be stated in anticipation of later discussions (5, 6), to the characteristic abnormal condition of the skeleton.

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EXPLANATION OF PLATES

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PLATE 33

FIG. 1. The incisor teeth of a normal rabbit and a litter mate with osteopetrosis 2 days old. Each weighed 65 gm. and was in excellent condition. In the diseased rabbit on the right no upper incisors were visible and only tiny points were palpable through the gum. The slightly separated lower incisors were convergent. The right lower was approximately normal; the left lower was a small very slanting tooth placed slightly posteriorly. × 1.84.

FIG. 2. The incisor teeth of a normal rabbit and an osteopetrosis litter mate 6 days old. Both were in excellent condition; the normal weighed 132 gm. and the diseased animal 112 gm. In the osteopetrosis rabbit on the right the right upper was a small slanting tooth with an opaque slightly bluish white color. At birth, both uppers were erupted; they were small teeth and the shorter left tooth was missing on the 4th day. The lower incisors were also erupted at birth but they were slightly smaller than normal. The difference in size persisted and the teeth became convergent. Their distal half was an opaque yellowish white while the proximal portion was a semiopaque grey. × 1.04.

FIG. 3. The incisor area of a normal rabbit and of an osteopetrosis sib 9 days old. Both were in good condition; the normal weighed 200 gm. and the diseased animal 154 gm. In the osteopetrosis rabbit on the right all 4 incisor teeth were missing. At birth, the left upper was missing and the right was a very small short tooth but the lowers appeared to be normal. These 3 teeth were shed between the 6th and 9th days. × 1.04. X-ray photographs of the jaws and other bones of this animal are shown in the following paper (5, Fig. 5).

FIG. 4. The incisor teeth of an osteopetrosis rabbit aged 13 days. The body weight was 140 gm. and the general condition was fairly good. Both upper incisors were missing from birth. There was also no right lower incisor and the left was small and poorly developed. At birth, both lowers were short and small and the shorter right tooth was shed between the 4th and 6th days. × 0.49. Skeletal x-rays of this rabbit appear in the following paper (5, Figs. 9 and 10).

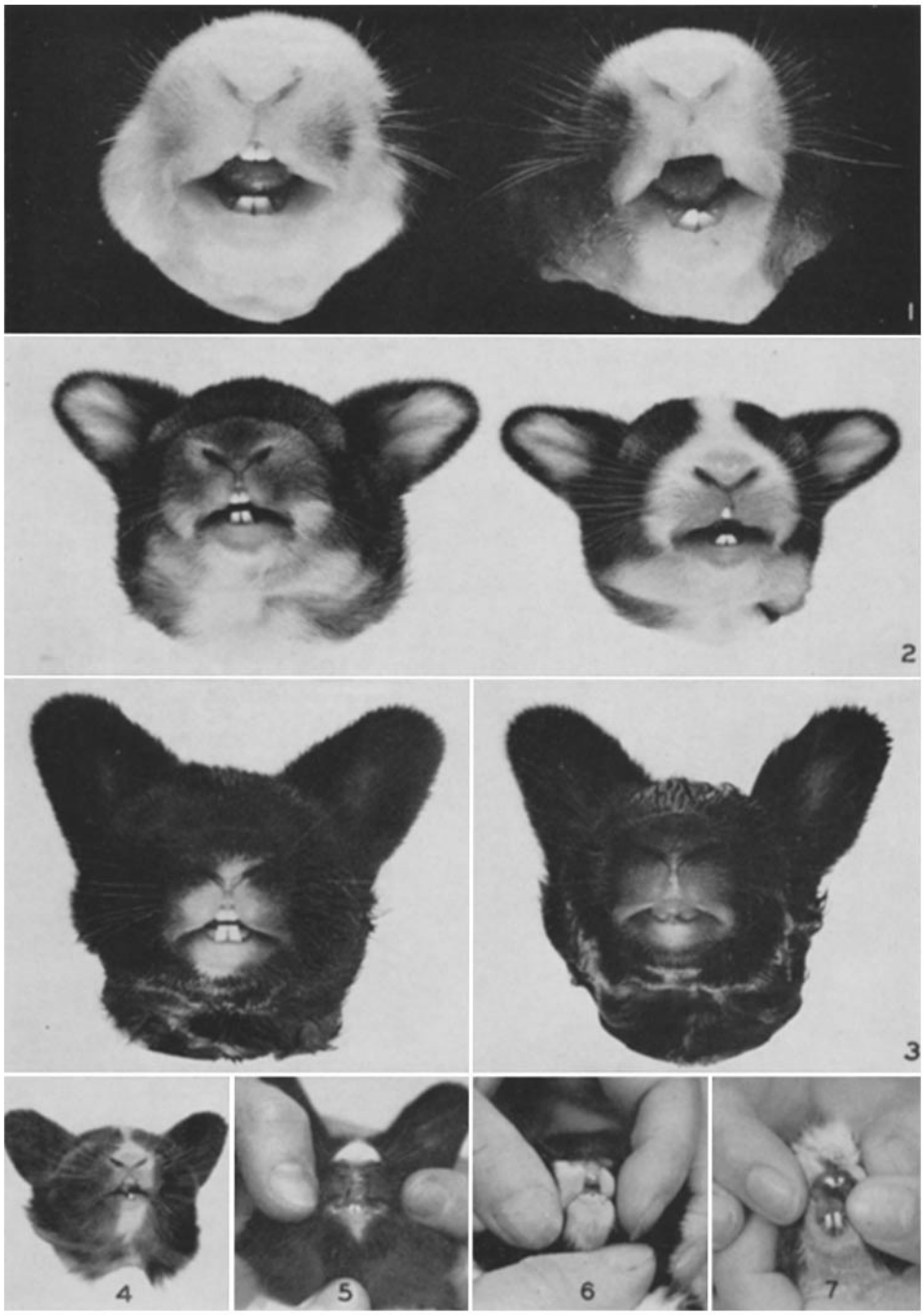
FIG. 5. The incisor teeth of an osteopetrosis rabbit aged 16 days, weighing 294 gm. and in good condition. The point of the right upper could just be felt deep in the gum; the left upper was a tiny thin sliver-like tooth. The lower incisors were very small frail short teeth, quite widely separated and slightly convergent. × 0.50.

At birth, there was a small right upper incisor but the left upper was not seen or felt; the lower incisors had a normal appearance. By the 7th day, the right upper was shed and the lowers were developing very slowly. Toward the end of the 2nd week, the point of the left upper appeared but there was comparatively little growth of the lower teeth. The appearance of this animal on the 16th and 24th days is shown in the photographs in Figs. 11 and 12. It was killed with chloroform on the 27th day and an x-ray photograph of the anterior skeleton is shown in the following paper (5, Fig. 17).

FIG. 6. The incisor teeth of an osteopetrosis male rabbit, aged 21 days, weighing 280 gm., well nourished, and growing. The upper incisors were still of infantile proportions; they were practically perfect in form but their position was twisted, the right being rotated upward and the left downward. The lower incisors were minute peg-like, separated teeth. There had been practically no change in the appearance of all 4 teeth since birth except as regards changes in position relative to one another. The animal died 15 days after this photograph was taken. × 0.50.

FIG. 7. The incisor teeth of a rabbit with osteopetrosis tarda aged 35 days. The slanting right upper was a short stumpy tooth widely separated from its smaller somewhat pointed mate. The separated lowers were abnormally long. The enamel of all 4 teeth was dull, opaque, and very white.

At birth, the incisors appeared normal but at 3 days of age, the uppers were small and separated and at 10 days were tiny fragile teeth. By the 16th day, the left upper was missing and the lowers were thin, long, slightly curved, and separated. At 30 days, both sets were growing and continued to do so, although the general physical condition rapidly deteriorated. Death occurred a few hours after this photograph was taken. × 0.63.



(Pearce and Brown: Hereditary osteopetrosis of rabbit. I)

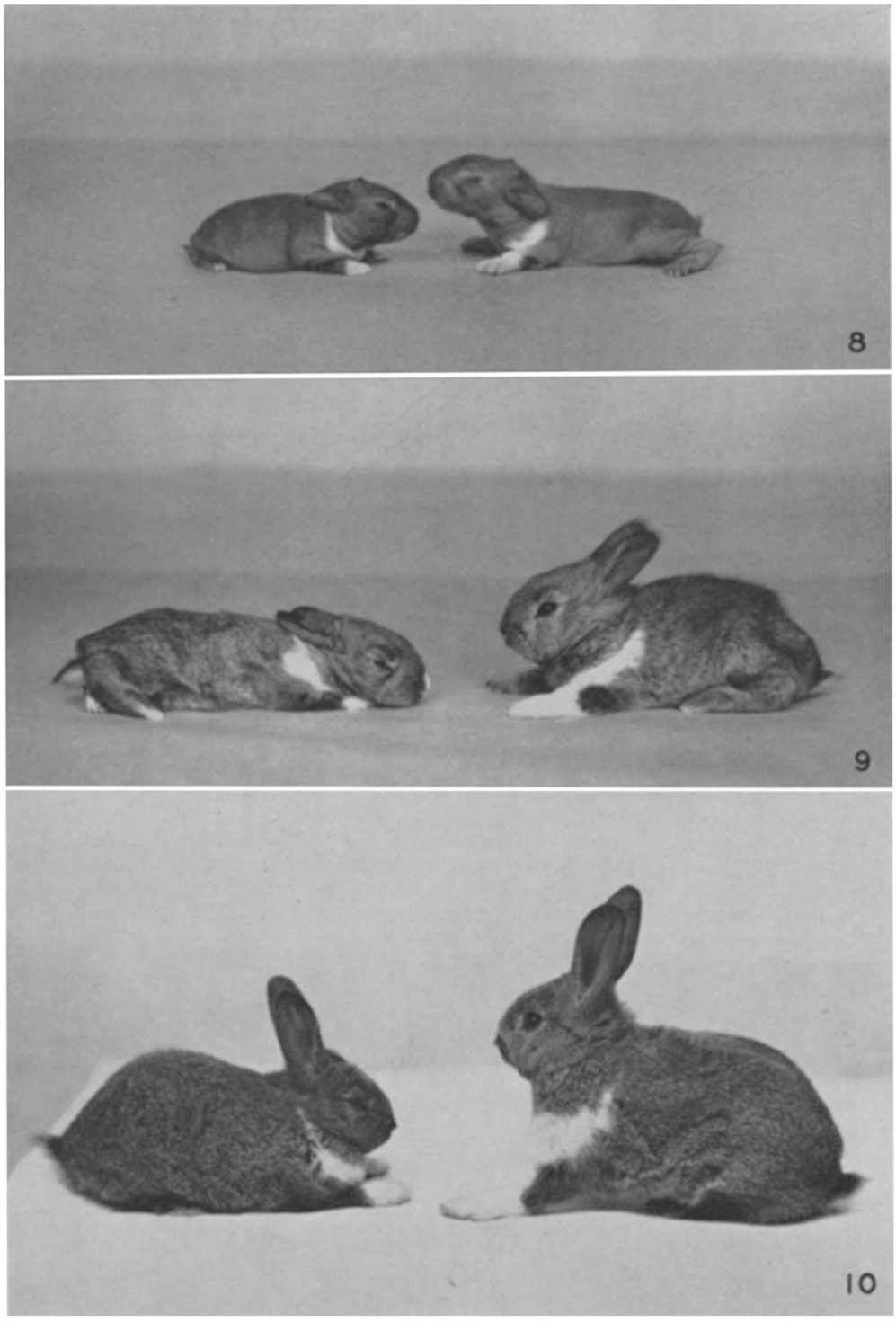
PLATE 34

FIG. 8. Photograph of a rabbit with osteopetrosis on the left and a normal litter mate on the right aged 7 days. The respective body weights were 148 and 190 gm.; at birth the affected rabbit was slightly the heavier, the weights being 57 and 50 gm. respectively. Other than the small size and the condition of the teeth there was nothing unusual in the general appearance of this animal. The state of nutrition was excellent and the coat was thick, uniform, and sleek. The upper incisor teeth were missing while the right lower was slightly shorter than the left lower. × 0.27.

FIG. 9. The same rabbits shown in Fig. 8 at 15 days of age; the diseased rabbit weighed 224 gm. and the normal litter mater 286 gm. The difference in size had now become well marked but the nutritional state of the osteopetrosis rabbit was still good and growth was continuing. The condition of the coat was also good. The eyelids happened to be closed in the photograph. The upper incisor teeth could still not be seen nor felt but development of the lower incisors was progressing well. × 0.27.

FIG. 10. The same rabbits shown in Figs. 8 and 9 at 27 days of age. The size and weight differences were now pronounced; the osteopetrosis animal weighed 320 gm. and the normal litter mate 530 gm. The diseased animal was still lively and active and the nutritional state was still comparatively good but the coat had become somewhat fluffy and dull and the lacrimal secretion was slightly excessive and somewhat thickened. × 0.27.

The points of the upper incisors were first seen 5 days before this photograph was taken but in the interval, very little growth had occurred. The lower incisors were rather long narrow slightly separated teeth, of an opaque dull yellowish white color. For about 10 days after this photograph was taken, the general condition continued to be fair and the body weight increased to 374 gm. But on the 39th day the weight had decreased to 356 gm. and death occurred 2 days later.



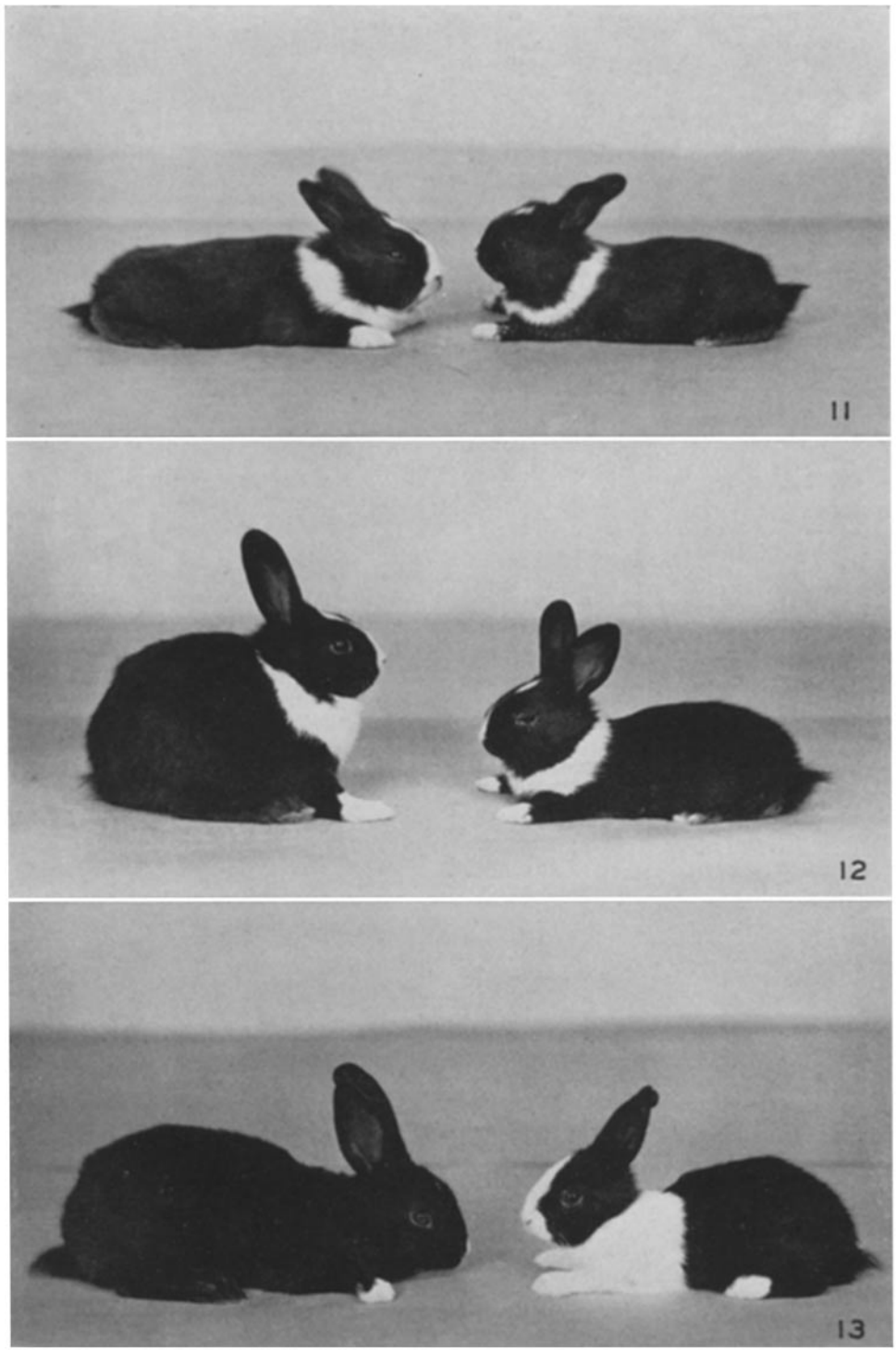
(Pearce and Brown: Hereditary osteopetrosis of rabbit. I)

PLATE 35

FIG. 11. Photograph of a rabbit with osteopetrosis on the right and a normal litter mate on the left aged 16 days. The body weights were 294 and 420 gm. respectively; at birth the diseased rabbit weighed 65 gm. and the normal sib 74 gm. The diseased animal was lively and still in a good state of nutrition and growing. The coat was normal. The lacrimal secretion had increased in amount and was more viscous than normal and there were small gummy crusts on the eyelids. The lids were sealed until just prior to the time the photograph was taken. The condition of the incisor teeth on this day is shown in Fig. 5. × 0.24.

FIG. 12. Photograph of the same rabbits shown in Fig. 11 at 24 days of age. The normal litter mate on the left weighed 592 gm. The diseased rabbit on the right weighed 318 gm.; the general physical condition was still fairly good but an indication of the progress of the disease is indicated by body weight values. The peak weight of 336 gm. was reached on the 20th day and was maintained for 2 days; thereafter there was a progressive loss of weight amounting to 18 gm. on the 24th day, when this photograph was taken, and a further 10 gm. during the next 3 days when the animal was killed with chloroform. The photograph shows the somewhat droopy eyelids, dull cornea, and the accumulation of tiny crusts on the lids. The coat was beginning to be rough, irregular, and dull and there was some fecal staining about the anus and external genitalia. × 0.24. The appearance of the incisor teeth was essentially unchanged from that shown in the photograph of Fig. 5 taken at 16 days of age. X-ray films of the anterior skeletons at 27 days appear in the following paper (5, Figs. 17 and 18).

FIG. 13. Photograph of a rabbit with osteopetrosis on the right and a normal litter mate on the left aged 34 days. The normal sib weighed 640 gm. and was growing well. The much smaller osteopetrosis animal was in the terminal stage of cachexia; the body weight was 222 gm. The highest recorded weight of this animal was 271 gm. on the 25th day; thereafter there was a steady loss of weight. During the week prior to the day this photograph was taken, the loss had amounted to 35 gm. while the normal litter mate had gained 198 gm. There were marked weakness and apathy, and an excessive lacrimal gummy secretion, first noted at 2 weeks of age, had become pronounced. An edematous swelling of the external genitalia developed at 3 weeks of age, persisted for several days, and subsided. The coat changes in this animal were comparatively minor. None of the incisor or the molar teeth was erupted and none could be felt through the gum. × 0.24.



(Pearce and Brown: Hereditary osteopetrosis of rabbit. I)