Chicks infected as 12-day-old embryos with an end-point-purified derivative of avian myeloblastosis virus developed a rapidly progressive osteopetrosis that manifested within 1 week of hatching. A detailed comparison of osteopetrotic chicks and normal hatchmates revealed the following. (i) Osteopetrotic chicks exhibited a stunting syndrome, growing at a mean rate that was 26% of the control rate. (ii) At autopsy, the mass of the lymphoid organs was reduced, whereas the mass of the heart, pancreas, kidneys, lungs, brain, liver, and bones of osteopetrotic chicks was increased. Edema was likely responsible for most of the increase in organ weight. (iii) Infected chicks exhibited a normochromic, normocytic anemia that was virus dose dependent and was not required for the development of osteopetrosis. (iv) Bone collagen content was normal. (v) Osteopetrotic bone was initially hypomineralized, but later became more fully mineralized. (vi) The concentrations of alpha, beta, and gamma globulins in the plasma were elevated in osteopetrotic chicks, whereas albumin concentration was decreased. (vii) The level of plasma alkaline phosphatase was elevated in osteopetrotic chicks, yet the level of acid phosphatase was unchanged. (viii) Body and bone temperatures were unchanged.

Osteopetrosis is a metabolic bone disease that occurs in several forms in different species. Affected bones are generally enlarged, demonstrating growth in trabecular bone at the expense of the medullary cavity (19). Osteopetrosis has been considered to result from a failure of bone maintenance, specifically a lack of bone resorption (19).

The etiology of mammalian osteopetrosis seems to be genetic (21), and it is usually associated with an autosomal gene. For example, the malignant form of human osteopetrosis is associated with a recessive gene (13, 16), whereas the benign form is associated with a dominant gene (13, 28).

Osteopetrosis in chickens is caused by viruses of the avian leukemia complex (10). A number of detailed pathological descriptions of osteopetrosis have been presented (3, 11, 14, 26). However, most of these studies were complicated by the following factors. First, field strains of virus were used, which induced osteopetrosis as only one of a number of other neoplasms, including erythroblastosis, nephroblastoma, and visceral lymphomatosis. The simultaneous occurrence of osteopetrosis and other tumors, particularly visceral lymphomatosis, undoubtedly complicated the interpretation of pathological changes observed. Second, animals examined for osteopetrosis were relatively old. For example, birds characterized by Blitz and Pelligrino (3) were 20 to 28 weeks old. Further, the earliest lesions observed by Sanger et al. (26) were detected microscopically at 4 weeks. Third, osteopetrosis was usually induced in a relatively low proportion of infected animals. For example, transmission experiments described by Holmes (12) showed that 67% of infected birds failed to develop osteopetrosis.

Only two viruses have been described that overcome the disadvantages mentioned above. The first to be described was the ARC isolate (Dougherty et al., Fed. Proc. 27:681, 1968), which induced a high incidence and rapid onset of osteopetrosis. However, a full description of the disease induced by the ARC virus has not been published. A second virus, derived from avian myeloblastosis virus, induced an incidence of osteopetrosis approaching 100%, and palpable lesions were present in chicks within 7 to 10 days after hatching (29, 30). The high incidence and reproducibility of disease induction, combined with early onset, permitted examination of homogeneous groups of animals at the same stage of osteopetrosis. This communication presents a biological and chemical characterization of age-matched virus-infected and control chickens.

MATERIALS AND METHODS

Virus. An end-point-purified derivative of avian myeloblastosis virus was used in these experiments (29, 30). This virus was designated MAV-2(O), de-
noting that it is a myeloblastosis-associated virus of subgroup B inducing osteopetrosis. Virus was propagated by serial passage in chickens. Sera were collected from birds showing heavy osteopetrosis, pooled, and stored at −70°C in 0.1-ml portions. Virus was diluted 1:20 (10⁴ plaque-forming units per 0.1 ml) prior to use.

Experimental animals. Fertile chicken eggs certified free of avian leukosis group-specific antigen and chick helper factor were obtained from SPAFAS, Inc., Storrs, Conn. MAV-2(0) (10⁴ plaque-forming units per 0.1 ml in phosphate-buffered saline, pH 7.4, containing 10% calf serum) was administered to 12-day-old embryos by injection of a chorioallantoic membrane vein (1). Eggs were maintained in a humidified incubator until 1 day prior to hatching, at which time infected and control eggs were transferred to separate incubators. Birds were maintained in an animal isolation facility designed to prevent spread of viruses among groups of infected animals and were fed Purina Grower Chow and water ad libitum (chow contained 8.4 IU of vitamin D per g, 0.92% Ca, and 0.73% P). Osteopetrosis in diseased animals was essentially as described by Sanger et al. (26). In most cases, osteopetrosis was observed within 2 weeks, and birds were incapacitated by heavy bone growth within 4 weeks.

Animal and organ mass determination. Birds were weighed daily on a Harvard laboratory balance. At intervals of 2 and 4 weeks, birds were exsanguinated; then organs were excised and weighed, using a Mettler analytical balance. The mean and standard error values for body and organ masses were calculated for each group.

Packed cell volume. Blood samples were collected on alternate days in heparinized 15 μl-microhematocrit tubes (Clay-Adams, Parsippany, N.J.). Packed cell volume was determined for each sample by comparison to a standard scale. Mean and standard error values were calculated for each determination.

Bone parameters. (i) Caliper measurements. Bone length and width were determined with a metric micrometer caliper. Tibiotarsi and femurs were stripped of soft tissue and disarticulated. Determinations were made on paired bones from each animal. Mean and standard error values were calculated for each group at 2 and 4 weeks after hatching.

(ii) Water content. Epiphyses and marrow were removed from bones previously stripped of soft tissue. Wet weight measurements of clean bone diaphyses were made on a Mettler analytical balance. Diaphyses were frozen, lyophilized, and weighed. The percentage of water content in bones was calculated as 100 − [(dry weight/wet weight) × 100].

(iii) Collagen content. Hydroxyproline content was determined by the method of Prockop and Udenfriend (22). The amount of collagen per bone was calculated as mass hydroxyproline/0.12.

(iv) Ash content. Clean bone diaphyses from control and experimental bones were frozen in liquid nitrogen and reduced to 200- to 400-mesh powder with a Spex Freezer Mill apparatus (Spex, Inc.). Bone powder samples (100-mg portions) were weighed in tared porcelain crucibles and reduced to ash by heating at 600°C for 24 h. The inorganic residue was weighed and the percentage of ash content was determined [(mass after 600°C for 24 h mass lyophilized bone powder) × 100]. Hydroxyapatite crystals were analyzed by examining the diffraction patterns obtained by the use of a 114.59-mm Picker camera (Picker X-ray Corp., Cleveland, Ohio) and a copper K-alpha X-ray source operating at 38 keV and 10 mA (15).

Plasma protein electrophoresis. Plasma protein electrophoretic analyses were performed with a Beckman Microzone electrophoresis system. Plasma samples (0.25 μl) were applied to cellulose-acetate membranes and subjected to 4.5 mA at 250 V for 20 min. Membranes were stained in Ponceau red, cleared, scanned with a Quick-Scan Jr. (Helena Laboratories, Inc.), and analyzed with the aid of a Digital PDP-1 computer. Areas under the peaks were integrated and expressed as a percentage of the total area scanned. Plasma protein concentrations were determined by the method of Lowry et al. (18). All determinations were performed in triplicate. Plasma proteins were expressed as grams per milliliter (percentage of plasma protein × grams of protein per 100 ml of plasma).

Enzyme determinations. Acid and alkaline phosphatase activities were determined by a modification of the method of Messer et al. (20). Alkaline phosphatase activity in 10 μl of plasma was measured at 26°C in 0.1 M tris(hydroxymethyl)aminomethane buffer, pH 8.0, containing 10 mM Mg²⁺ and 5 mM p-nitrophenyl phosphate. Release of p-nitrophenol was measured spectrophotometrically at 410 nm, and enzyme activity was expressed as moles of p-nitrophenol released per milligram of protein per minute (international units). The extinction coefficient for p-nitrophenol at 410 nm was assumed to be the same as that at 405 nm.

Acid phosphatase activity in 20 μl of plasma was measured at 25°C in 50 mM citrate buffer, pH 4.8, containing 5 mM p-nitrophenyl phosphate. Enzyme activity was expressed in international units above.

Temperature measurements. Temperature measurements were performed by an electric thermistor (Frigitronics, Inc., model TTI). Rectal temperatures were measured and expressed as body temperature. Temperatures of the humerus and femur were measured by piercing the skin and muscle over the bone and positioning the thermistor probe (which had dimensions of a 22-gauge needle) against the bone.

Statistical analysis. Levels of significance (P) were calculated by Student's two-tailed t test. In all cases, P values refer to the difference between normal and osteopetrotic samples of the same age.

RESULTS

Body mass. Osteopetrotic chicks demonstrated a stunting syndrome, growing at a rate that was 26% of the normal value (Fig. 1). Normal chicks grew at a mean rate of 7.0 g/day, whereas osteopetrotic chicks grew at a mean rate of 1.83 g/day. Although both groups of birds had similar initial body mass, osteopetrotic birds were distinguishable from their
normal counterparts by 10 to 14 days after hatching.

**Organ mass.** A comparison of the mass of individual organs of normal and osteopetrotic birds at 2 and 4 weeks after hatching is shown in Table 1. The following observations were obtained.

(i) Heart, pancreas, kidneys, and lungs from osteopetrotic birds had a 1.3-fold increase in mass compared with normal counterparts, whereas brain and liver had a 1.6- to 1.7-fold mass increase at 2 weeks and a 1.8- to 1.9-fold mass increase at 4 weeks after hatching. The livers of osteopetrotic birds were green (presumably from the presence of excessive bile), and the gallbladder was distended with bile fluid. Histological sections of the osteopetrotic liver showed distension of the bile ducts, with no evidence of lymphoid infiltration.

(ii) The crop, gizzard, and intestines were similar in mass in both groups during the experimental period. In addition, an examination of the intestinal contents of osteopetrotic birds indicated that they were consuming the chow, and thus the stunting syndrome documented in the previous section was not a consequence of starvation due to incapacitation.

(iii) The lymphoid organs of osteopetrotic birds greatly decreased in mass; the spleen

---

**Fig. 1.** Body mass of normal and osteopetrotic chicks. Eight normal chicks and 13 osteopetrotic chicks were weighed daily on a Harvard laboratory balance over a 28-day period after hatching. Data points shown are the error bars for each determination. Differences in body mass became statistically significant ($P < 0.01$) on day 7. Heavy bars represent osteopetrotic chicks; light bars represent normal chicks.

---

**Table 1.** Organs of normal and osteopetrotic chicks expressed as fraction of body mass

<table>
<thead>
<tr>
<th>Vtr. Age (wk)</th>
<th>Brain</th>
<th>Heart</th>
<th>Kidney</th>
<th>Lung</th>
<th>Pancreas</th>
<th>Liver</th>
<th>CG + N</th>
<th>Pancreas</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>1.85</td>
<td>1.17</td>
<td>1.78</td>
<td>1.32</td>
<td>1.66</td>
<td>0.99</td>
<td>0.87</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2.17</td>
<td>1.58</td>
<td>1.76</td>
<td>1.42</td>
<td>1.74</td>
<td>1.05</td>
<td>0.94</td>
<td>1.02</td>
</tr>
</tbody>
</table>

*NS = No significant difference.

---

After animals were sacrificed by exsanguination, organs were removed and weighed on a Mettler analytical balance.

---

*Combined mass of heart, pancreas, and intestine.

---

*Standard error.

---

*NS = No significant difference.
mass was unchanged at 2 weeks, but was 26% of the control mass at 4 weeks, whereas the bursa was 33 and 11% of the control values at 2 and 4 weeks, respectively. The mass of the thymus in osteopetrotic birds was also decreased compared with control levels (data not shown).

Anemia. MAV-2(0)-infected birds displayed a normochromic, normocytic anemia. The extent of the anemia was documented by determining the packed cell volume of normal and osteopetrotic chicken blood for a 37-day period (Fig. 2). Two animals in the infected group had packed cell volumes of 5 and 3% before dying 35 days after hatching. The anemia was detectable before bone changes (nodule formation and thickening of the diaphyses) had occurred, and the anemia persisted in infected birds. However, some infected birds simultaneously demonstrated bone changes, stunting, and anemia, whereas others displayed anemia and stunting in the absence of palpable osteopetrosis.

Bone parameters. (i) Bone growth. Measurements of length and width of normal and osteopetrotic bones at 2- and 4-week intervals after hatching revealed that bones from MAV-2(0)-infected birds were shorter and thicker than were the normal counterparts (Fig. 3). In addition, the periosteum of osteopetrotic bones was as great as 2 mm in diameter. The lengths of both tibiotarsi and femurs in osteopetrotic birds were approximately 82% of control values at 2 weeks and 67% of control values at 4 weeks after hatching. It is likely that the stunting observed in osteopetrotic animals (Fig. 1) contributed to the decreased bone length observed (Fig. 3). The widths of both tibiotarsi and femurs in osteopetrotic birds were elevated 1.3- to 1.5-fold at 2 weeks and 2.2-fold at 4 weeks after hatching. In older osteopetrotic birds (2 to 4 months), the elevation in bone width was as high as sixfold greater than normal. Total bone mass (for a given long bone—tibiotarsus, femur, or humerus) was consistently two- to sixfold greater in osteopetrotic birds than in bones of normal birds, regardless of the stunting syndrome accompanying osteopetrosis.

(ii) Water content. Bones from osteopetrotic birds contained approximately 2.8 times as much water as did normal counterparts (Table 2). Tibiotarsus, femur, and humerus of normal 2- or 4-week-old birds contained a mean value of 24% water, whereas osteopetrotic bones at the same time intervals contained a mean value of 67% water.

(iii) Collagen content. The collagen content of both osteopetrotic and normal bones (tibiotarsus, femur, humerus) was approximately 22 to 26% (Table 2). Bones of older osteopetrotic birds had a decreased collagen content. Specifi-
that they tibiotarsi petrotic metatarsals lyophilization.

birds revealed had ens tibiotarsi larly, was trotic animals chickens month-old hatching, the osteopetrotic bones of determined. 33.63%, whereas birds revealed X-ray crystallographic analysis mineral (Table of proportions and elevated in concentration of bone.

9.2-fold, a2-globulins respectively, globulins (Table 4). In the plasma of osteopetrotic birds, albumin content was decreased to 55 and 70% of control levels at 2 and 4 weeks, respectively, after hatching. The concentration of each of the globulin classes was elevated in plasmas of osteopetrotic birds. At 2- and 4-week intervals after hatching, the concentration of α1-globulins was elevated 3.4- and 9.2-fold, α2-globulins 3.5- and 6.5-fold, and β-

globulins 1.5- and 1.6-fold, respectively. Gamma globulins were elevated 1.7- and 1.3-fold at 2 and 4 weeks, respectively. The albumin-total globulin ratio in normal birds was 1.60 and 1.57 for the 2- and 4-week intervals, respectively, whereas the ratio for osteopetrotic birds was 0.51 and 0.74 for the same time intervals.

Alkaline and acid phosphatase levels. Plasma alkaline phosphatase levels were elevated in osteopetrotic birds (Table 5). Enzyme levels were increased 2.4- and 4.6-fold at 2- and 4-week intervals, respectively. Alkaline phos-

<table>
<thead>
<tr>
<th>Virus</th>
<th>Age (wk)</th>
<th>No. examined*</th>
<th>Bone examined*</th>
<th>Water (%)</th>
<th>Collagen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>2</td>
<td>5</td>
<td>T</td>
<td>23.2 ± 2.3</td>
<td>29.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>24.1 ± 2.2</td>
<td>21.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>23.8 ± 2.1</td>
<td>26.6 ± 1.2</td>
</tr>
<tr>
<td>+</td>
<td>2</td>
<td>6</td>
<td>T</td>
<td>64.8 ± 2.3e</td>
<td>23.1 ± 0.2e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>66.65 ± 0.45e</td>
<td>18.9 ± 2.1'</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>67.45 ± 3.05e</td>
<td>22.6 ± 0.3e</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>3</td>
<td>T</td>
<td>23.3 ± 2.1</td>
<td>30.1 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>23.8 ± 2.4</td>
<td>22.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>24.0 ± 2.1</td>
<td>27.1 ± 1.8</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>6</td>
<td>T</td>
<td>65.25 ± 2.35e</td>
<td>24.95 ± 1.65'</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>66.1 ± 1.0'</td>
<td>20.5 ± 0.7'</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>69.95 ± 1.35e</td>
<td>26.25 ± 1.05'</td>
</tr>
</tbody>
</table>

* Number of animals examined. Water and collagen content of two bones from each animal were determined. Collagen determinations were made in duplicate.

† T, Tibiotarsus; F, femur; H, humerus.

§ Water content was determined by weighing bone samples immediately after autopsy and then weighing after lyophilization. Numbers represent mean ± standard error.

¶ Percentage of collagen established by determining the hydroxyproline composition of hydrolyzed decalcified bone. Numbers represent mean ± standard error.

* P < 0.01.

† No significant difference.

(iv) Ash content. Analysis of the ash content of 3- and 8-week-old normal and osteopetrotic birds revealed that, at 3 weeks after hatching, osteopetrotic bones were hypomineralized in that they contained 80% of the control levels of mineral (Table 3). However, at 8 weeks after hatching, the mineral content of osteopetrotic bones was 95% of the control level. In addition, X-ray crystallographic analysis of the hydroxyapatite in osteopetrotic bone revealed a diffraction pattern identical to that of normal bone.

Plasma protein composition. Electrophoresis of plasma proteins from normal and osteopetrotic birds revealed striking differences in the proportions of albumin and alpha, beta, and gamma globulins (Table 4). In the plasma of osteopetrotic birds, albumin content was decreased to 55 and 70% of control levels at 2 and 4 weeks, respectively, after hatching. The concentration of each of the globulin classes was elevated in plasmas of osteopetrotic birds. At 2- and 4-week intervals after hatching, the concentration of α1-globulins was elevated 3.4- and 9.2-fold, α2-globulins 3.5- and 6.5-fold, and β-

<table>
<thead>
<tr>
<th>Virus</th>
<th>Age (wk)</th>
<th>No. examined*</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>3</td>
<td>6</td>
<td>63.12 ± 0.41</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>6</td>
<td>50.07 ± 0.79c</td>
</tr>
<tr>
<td>-</td>
<td>8</td>
<td>6</td>
<td>61.45 ± 0.23d</td>
</tr>
<tr>
<td>+</td>
<td>8</td>
<td>6</td>
<td>59.36 ± 0.71d</td>
</tr>
</tbody>
</table>

* Two bones were examined from each of six animals.

† Percentage of ash was determined by establishing a dry weight for the bone samples, then ashing the bone at 600°C for 24 h. Numbers represent mean ± standard error.

‡ P < 0.01.

§ P < 0.02.
AVIAN OSTEOPETROSIS CHARACTERIZATION

Table 5. Alkaline and acid phosphatase activities in plasma from normal and osteopetrotic chickens

<table>
<thead>
<tr>
<th>Virus</th>
<th>Age (wk)</th>
<th>No. birds examined</th>
<th>Alkaline phosphatase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>± standard error</td>
<td>± standard error</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>9</td>
<td>0.032 ± 0.002</td>
<td>0.00335 ± 0.001</td>
</tr>
<tr>
<td>+</td>
<td>2</td>
<td>6</td>
<td>0.077 ± 0.012c</td>
<td>0.00414 ± 0.001d</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>3</td>
<td>0.021 ± 0.003</td>
<td>0.00689 ± 0.003</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>6</td>
<td>0.097 ± 0.020*</td>
<td>0.00858 ± 0.002*</td>
</tr>
</tbody>
</table>

* Data refer to the number of animals in each group. Each determination was performed in duplicate.

Enzyme values are expressed in international units, defined as the activity liberating 1 μmol of p-nitrophenol per min at 25°C. Numbers refer to mean ± standard error.

P < 0.01.

No significant difference.

P < 0.05.

Phosphatase activity was destroyed by heating the plasma at 56°C for 15 min, indicating that the enzyme was derived from bone, since liver alkaline phosphatase is heat stable. Acid phosphatase levels were unchanged.

Body and bone temperature. Body and bone (humerus and femur) temperatures were similar in both 2- and 4-week-old normal and osteopetrotic birds (Table 6). In fact, the temperature of osteopetrotic animals (rectal and bone) was consistently lower than that of normal animals, a finding which may reflect generalized debilitation.

DISCUSSION

Avian osteopetrosis is a hyperplastic condition resulting in excessive bone deposition, with no evidence of metastasis to other organs (12, 26). Previous studies have failed to implicate an imbalance in the hormonal control of calcium metabolism in the causation of osteopetrosis. The levels of calcitonin and parathyroid hormone in the osteopetrotic chicken have been evaluated, but the small hormone changes observed are secondary to the hyperplastic changes in the bone (7). Serum calcium levels have been reported as normal (2, 7, 33) or elevated (12), whereas serum phosphorus concentration was either normal (33) or lowered (7, 12). The level of serum alkaline phosphatase has been reported to be elevated (2, 25). Maintenance of osteopetrotic birds on calcium-free diets does not lead to an arrest of the disease (7). Although evidence of parathyroid gland and ultimobranchial body hyperactivity has been noted (7, 33, 34), administration of either parathyroid hormone or calcitonin to normal birds does not lead to osteopetrosis (4) or to a reversal of osteopetrosis in diseased birds (7).
TABLE 6. Temperature measurements of normal and osteopetrotic chicks

<table>
<thead>
<tr>
<th>Virus</th>
<th>Age (wk)</th>
<th>Rectal</th>
<th>Humerus</th>
<th>Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>2</td>
<td>41.5 ± 0.2</td>
<td>38.2 ± 0.2</td>
<td>39.75 ± 0.3</td>
</tr>
<tr>
<td>+</td>
<td>2</td>
<td>40.5 ± 0.2a</td>
<td>37.2 ± 0.4a</td>
<td>36.8 ± 0.4a</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>42.0 ± 0.4</td>
<td>38.5 ± 0.1</td>
<td>37.0 ± 0.5</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>40.15 ± 0.6c</td>
<td>35.85 ± 0.5d</td>
<td>35.3 ± 0.5e</td>
</tr>
</tbody>
</table>

a P < 0.01.
b P < 0.05.
c No significant difference.
d P < 0.02.
e P < 0.1.

The purpose of the experiments presented in this communication was to document some of the in vivo manifestations of a virus that induced a high incidence of osteopetrosis in susceptible chickens (29, 30). The virus was chosen for study because it induced a rapid onset of osteopetrosis. Furthermore, the virus differed from field strains of avian leukosis virus, both in oncogenic spectrum and in the number of genome copies present in affected tissue (29).

The severity of the body-mass depression, anemia, and the rapid onset of bone growth observed in MAV-2(O)-infected animals may be due to two factors: (i) MAV-2(O) was administered in a relatively high dose, and (ii) the onset of osteopetrosis was uncomplicated by other lymphoid leukosis manifestations, such as erythroblastosis or visceral lymphomatosis. Nevertheless, it should be noted that nephroblastoma was sometimes observed in MAV-2(O)-infected chickens (29, 30), especially when chickens survived to the age of 8 to 10 weeks.

A stunting syndrome was the most striking characteristic of avian osteopetrosis discernible prior to bone enlargement. At times when growth rates were linear, normal birds grew at a rate 3.8 times that of MAV-2(O)-infected birds (Fig. 1). Chicks were fed Purina Growena chow containing all the essential and nonessential components for normal growth and development (Purina Technical Bulletin no. 5065), including five times the normal daily requirement of vitamin D, a component vital for normal bone development.

Autopsy data indicated that several organs in osteopetrotic birds increased in mass during the experimental period (Table 1, Fig. 3). The increase in mass of soft tissues was probably due to edema. The elevation in osteopetrotic bone mass was likewise caused in part by edema (osteopetrotic bone contained 67% water, whereas normal bone contained 24% water; Table 2). However, total bone mass measured on a per bone basis indicated that osteopetrotic bone was heavier than normal bone.

Bone size was substantially increased in MAV-2(O)-infected chickens compared with bones of normal counterparts (Fig. 3). Edema was responsible for a large part of elevated bone volume. However, osteopetrotic bones contained more material on a dry-weight basis than did normal bones.

Osteopetrotic bone contained less mineral per unit mass at 3 weeks but approximately the same amount of mineral per unit mass at 8 weeks after hatching (Table 3). X-ray crystallographic analysis of hydroxyapatite crystals indicated that there was no difference in the diffraction patterns obtained with normal and osteopetrotic bone, a finding consistent with that reported by Biltz and Pellegrino (3). Preliminary experiments indicated that the amount of hot perchloric acid-extractable deoxyribonucleic acid per gram (dry weight) of bone was the same in osteopetrotic and normal bones. Therefore, the increased volume of osteopetrotic bone was principally caused by cellular hyperplasia, rather than by excessive accumulation of mineral or organic substances. Our results are consistent with the previous conclusions that avian osteopetrosis is largely the result of cellular hyperplasia (26).

Osteopetrotic bone contained approximately the same amount of collagen as normal bones (Table 2). However, a chemical comparison of normal and osteopetrotic collagen indicated that a significant difference existed in the cross-linking of the collagen solubilized from the bones (A.J. Banes, R. Smith, P. Bernstein, and G. Mechanic, manuscript in preparation).

Analysis of plasmas from osteopetrotic and normal birds indicated that osteopetrotic birds had 55 and 70% of the control levels of albumin at 2 and 4 weeks, respectively, after hatching (Table 4). Therefore, edema in osteopetrotic birds may result from osmotic imbalances in blood caused by decreased albumin concentration (24).

The decrease in the mass of lymphoid organs (bursa, spleen, and thymus) may have been
caused by virus infection of lymphocytes and subsequent reduction in cell number. An involvement of the avian lymphoid system in osteopetrosis is particularly attractive, since results in the mouse show that lymphoid cells play a role in osteopetrosis. For example, parabiosis of normal and osteopetrotic mice resulted in remission of the disease (31), and it was later shown that infusion of lymphocytes from bone marrow or spleen of normal mice reversed osteopetrosis in diseased mice (32). However, similar lymphocyte-transfer experiments in chickens have not yet effected a cure for the avian disease (unpublished data). Although the lymphoid organs are involved in osteopetrotic birds, the plasma gamma globulin concentration was normal or slightly elevated compared with control levels (Table 4). This result suggests that the humoral immune response in osteopetrotic birds may not be diminished, but quantitative responses to specific antigens have not been measured.

An involution of the lymphoid system in avian osteopetrosis is likely to have been overlooked by previous workers, because the viruses used induced visceral lymphomatosis, which results in transformation of bursa cells (6), and a proliferative response that increases the weight of both the spleen and bursa.

The anemia accompanying MAV-2(O)-induced osteopetrosis is extremely interesting. Loss of marrow space probably contributes to a decrease in the number of circulating erythrocytes; however, examination of marrow smears of osteopetrotic animals indicated that an aplastic anemia was present in the small amount of marrow space remaining (R. Scott, Medical College of Virginia, Richmond, personal communication). It is interesting to note that equine infectious anemia virus has recently been shown to have reverse transcriptase and 70S ribonucleic acid (5). One might speculate that a portion of the osteopetrosis virus genome may code for anemia, or a second virus may be present that induces anemia. Although the presence of a second virus is unlikely because animals were isolated and a cloned virus was used, such a possibility cannot be excluded. Examination of several organs by electron microscopy failed to show morphological evidence for any viruses other than C-type particles. Therefore, the probability is minimal that a lytic virus unrelated to ribonucleic acid tumor viruses caused the anemia. However, it is possible that a situation exists similar to that recently demonstrated for avian erythroleukemia virus, in which a defective transforming virus is responsible for erythroid malignancy, and a nondefective associated virus is responsible for anemia (9).

The plasma levels of alkaline phosphatase, which is the principal enzyme associated with synthesis of bone, were elevated in osteopetrotic birds, whereas the concentration of acid phosphatase was unchanged (Table 5).

The level of α₂-macroglobulin was elevated in plasmas of osteopetrotic birds. α₂-Macroglobulins complex with enzymes, such as trypsin and collagenase, and inhibit substrate degradation in some cases (8, 23). The physiological role of α₂-macroglobulins and other plasma proteins in the pathogenesis of avian osteopetrosis remains unknown. However, the levels of plasma proteins are clearly abnormal in osteopetrotic birds (Table 4), a finding documented in other leukemia virus systems (17).

Our investigation of the bone and body temperature of normal and osteopetrotic chicks showed that there was no difference in temperature (Table 6). Our results differ from those previously reported by Sanger et al. (27), who found that osteopetrotic bone was warmer than normal bone. The discrepancy likely stems from three sources. First, the osteopetrotic animals examined by Sanger et al. (27) were considerably older (9 to 19 weeks) than those observed in the present study (2 to 4 weeks). A difference in metatarsal surface temperature in older chickens might result from a high degree of bone vascularization in osteopetrotic bones compared with less vascularization of normal metatarsal bones. Second, the measurements by Sanger et al. (27) were made on the surface of the metatarsal, whereas the probe in the current study was inserted into the leg and placed in direct contact with the bone. Third, different bones were measured in the two studies. When osteopetrosis was induced by MAV-2(O) in very young chicks, the femurs and tibiotarsi were more involved than were the metatarsals; consequently, they were measured for temperature. Involvement of the femurs and tibiotarsi seemed to be dose dependent in that high doses of virus resulted in rapid bone growth in the deeper long bones, whereas low doses of virus resulted in excessive growth of the metatarsals after a long latent period.

The results presented in this communication indicate that MAV-2(O) infection causes major changes in the metabolism of the infected animal. The role of MAV-2(O) in the induction of the pathological changes that accompany osteopetrosis requires further investigation.

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LITERATURE CITED


