THE USE OF RADIOACTIVE PHOSPHORUS IN TOOTH METABOLISM STUDIES OF CARIES RESISTANT AND CARIES SUSCEPTIBLE STRAINS OF ALBINO RATS

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Dedicated
to my wife

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INTRODUCTION

In 1931-32 Hoppert et al. (1, 2) of the Department of Chemistry, Michigan State College, published data demonstrating that dental caries could be developed in rats by feeding diets containing coarsely ground corn or rice. Subsequently, a genetic study was undertaken with Hunt, of the Department of Zoology, with Albino rats (rattus norvegicus) in which two distinct strains have been developed that vary markedly in their susceptibility to dental caries (3, 4, 5, 6).

The susceptible and resistant strains have been inbred through eighteen and fourteen generations, respectively. In the case of the susceptible strain studies have been made to determine the prophylactic value of various types of treatment both topical and dietary. These have included the topical application of fluorides (7), addition of fluoride to the drinking water (8), the use of diets varying in their content of carbohydrate and fat (9) and the introduction into the diet of urea plus urease, and diammonium phosphate (8).

However, until the present study was undertaken, little work had been done on the chemical constituents of the teeth. In this investigation a comparison of the whole teeth of the resistant and susceptible strains and stock animals has been made with regard to (1) weight of the teeth, (2) per cent ash in the teeth, (3) per cent phosphorus in the teeth calculated on the (a) dry weight basis, and (b) in the ash, (4) the rate of deposition and removal of radioactive phosphorus in the teeth of rats injected intraperitoneally with the isotope, (5) the occurrence of labelled phosphorus in the teeth of offspring whose mothers were injected intraperitoneally with the isotope during pregnancy, and (6) the adsorption of radioactive phosphorus in vitro by the teeth of the different strains.

The use of radioactive phosphorus in tooth metabolism studies is founded on the assumption that stable and radioactive isotopes are chemically alike and behave in the same manner in the animal organism except in cases where the radiations are so intense as to produce pathological tissue changes.

The advantages of using radioactive phosphorus in tooth metabolism studies are (1) the radioactivity makes it possible to trace the element easily, (2) purification processes associated with direct chemical approach methods are not necessary to determine the concentration of the isotope, (3) the element upon administration may be distinguished from that already present, thereby making it possible to determine the rate of uptake by the teeth of a given dose as well as the subsequent rate of removal of the element from the tissue. Limitations in the use of any radioactive isotope are chiefly the half-life of the isotope, and the energy of the radiation. Fortunately, of the radioactive elements none are freer from these limitations than phosphorus-32. Phosphorus is particularly well adapted to the study of dental tissues, since it comprises about 17 and 12.5 per cent of the dry weight of the enamel and dentin, respectively (10).

FACTS PERTINENT TO RADIOACTIVE ISOTOPES AND THE USE OF RADIOACTIVE PHOSPHORUS-32 AS A "TRACER"

A. Definition of radioisotope (11): A radioactive isotope is an isotope of an element that has the property of emitting alpha or beta particles, or gamma rays. When an element emits such particles it undergoes a change into another element. For example, radioactive phosphorus thirty-two (P^{32}) gradually decomposes to stable sulfur as:

$$P^{32}\ (15p,\ 17n) \longrightarrow \text{Beta particle}\ (1.69\ \text{mev}) + S^{32}(16p,\ 16n).$$

B. Types of emissions (11, 12): Emissions from radioactive isotopes may be alpha or beta particles or gamma rays. The alpha particles have a positive electrical charge of two units. They have two protons (from which they derive their positive electrical charge) and two neutrons. The alpha particles are nuclei of helium atoms which contain two protons and two neutrons. These particles have very great ionizing power but very small penetrating power. They may be stopped by a fraction of a millimeter of lead.

Gamma rays are uncharged and because of this they can not be deflected from their path by either electric or magnetic fields as can both the alpha or beta particles. The action of gamma rays is quite similar to that of x-rays although the gamma rays have a shorter wave length and a higher frequency than the x-rays which gives them great penetrating power.

Beta particles are the same as electrons and therefore have a negative charge of one unit. The penetrating power of these particles is about 100 times that of alpha particles. Radioactive phosphorus-32 which was used in this investigation emits only beta particles.

C. Half-life (11, 12): The length of time required for a radioisotope to decay to half its original activity is known as its half-life. The half-lives of different isotopes vary greatly. For example, one of the isotopes of carbon, C-11, has a half-life of 21 minutes whereas C-14 has a half-life of about 5000 years. Radioactive phosphorus-32 has a half-life of 14.3 days. The decay rate of any isotope can be conveniently shown by graphically plotting time against the activity remaining. Figure 1 shows a decay curve obtained with a standard solution of radioactive phosphorus in this investigation. As indicated in the graph the determined decay rate paralleled very closely the theoretical decay rate.

D. Types of measuring equipment (11, 12, 13, 14, 15): Whereas measurements of stable isotope concentration are normally based on the difference in physical constants of aggregates containing the tracer, or in spectrometric analysis of mass ratios, the concentration of a radioactive tracer is usually far too small to be measured by such means. Use is made instead of its emission properties due to its disintegration into other atoms with accompanying emission of alpha or beta particles or gamma rays.

All radioactivity measuring instruments make use of the secondary effects of the radiation. One method of studying the distribution of radioactive materials, known as the radioautographic method, is the direct exposure of a photographic plate to a section of the subject in question.

Much more sensitive measurements may be made with electroscopes or electrometers. These instruments determine the amounts of ionization in a gas produced by radiations from the active materials by measuring the conductivity of the ionized gas. In the simple electroscope this is accomplished by charging two relatively displacable parts alike. Their rate of approach to their uncharged positions is dependent on the discharge rate which measures the amount of ionization. Electrometers use more elaborate measuring methods, gaining in sensitivity, range, accuracy, or convenience.

Where samples are of weak radioactivity or where the greatest possible accuracy is desired a Geiger-Mueller counter is generally used. This instrument is sensitive to individual ionizing particles. In conjunction with its accompanying circuits the Geiger counter actually counts and records the number of emitted particles passing into its tube. An ionizing emission in the specially gas filled counter tube will produce a number of free electrons which will be accelerated toward the positive electrode. Collisions between these and other gas particles will free more electrons in "avalanche" fashion with the result that a measureable electric pulse is produced for each emission that enters the Geiger-Mueller tube. Then, either because the tube is "self-quenching" by virtue of the composition of the gas it contains or by a special quenching circuit, the pulse is quickly extinguished and the system is ready to record the next particle. In this investigation a Bale-Haven dipping-type Geiger-Mueller tube (16) was used. This tube is of the non-self-quenching type and a modified Neher-Harper circuit was attached to the counter by the Tracerlab, Inc., Boston, for quenching purposes.

E. Equipment required for radioactive phosphorus assay (13): The necessary equipment for the routine evaluation of radiotracer phosphorus includes the Geiger-Mueller tube (in this investigation a dipping-type tube (16) supplied by the Distillation Products Corp., 755 Ridge Road, Rochester, N. Y. was used), and a unit for supplying the operating voltage for the counter tube, counting the impulses from the tube, and a timer to measure the time required for the reception of a given number of these impulses (a compact unit is marketed by Tracerlab, Inc., Boston, which has these three capacities and was used in this investigation). In addition lead shields may be necessary to protect personnel for certain operations with concentrated samples. Tongs help to avoid too close contact with the active materials. The use of area monitoring equipment is mandatory, a Tracerlab Monitor Su-3 having been employed in this investigation.

F. Sample preparation and measurement (15): For tracer work it is not necessary to know the absolute value of the activity of a sample but only the activity relative to a standard solution of the isotope. Standard positions are necessary since the measured activity is partly dependent on the absorption of the rays in the sample itself. The same mounting should be used to eliminate deviations due to the difference in secondary radiations and back-scattering. The dipping-type tube such as was used in this investigation modified many of the geometry problems existing for "dry" tubes.

In this investigation the teeth were ashed in a muffle furnace. The ash was dissolved in 10 ml. of 2.5 N hydrochloric acid and made to volume in a suitable volumetric flask. A 5 ml. aliquot of the solution was delivered to a cylinder-like cup having a flanged top and the dipping-type Geiger-Mueller tube was then inserted into the cup in a reproducible manner and the activity of the solution determined.

G. Counter measurement standardization (15): A counter-circuit system when operating properly should give a voltage-counting rate curve like that shown in Figure 2. The important range, called the "plateau", is that portion between A and B in which the counter registers close to 100 per cent of the ionizing particles introduced. The operating voltage should normally lie on the flattest portion of the curve as voltage fluctions will then be reflected least in the counting rate. To increase the lifetime of the counter the operating voltage should be at the low voltage end of the flattest portion. When an extinguishing circuit is employed which is required for a non-self-quenching type of Geiger-Mueller tube, inflections are sometimes introduced into the plateau curve. A non-self-quenching tube was used in this investigation and inflections were common on the plateau of the voltage-activity curve. However, a satisfactory operating voltage was obtained without such inflections on the low voltage side of the plateau. In practice the plateau region was found not to be very flat and the higher the activity of the sample the greater was the slope with the dipping-type tube used in this investigation. In order to have comparable results it was found to be of the greatest importance to maintain the voltage constant for all counting operations.

Preliminary to ordering the radioactive phosphorus from Oak Ridge the Geiger-Mueller counter assay equipment was tested for reproducibility and precision by determining the counts per minute of 0.4, 0.02, and 0.004 N solutions of thorium nitrate. Essentially a straight line was obtained upon plotting activity versus concentration of thorium nitrate.

Five ml. of 0.4 N solution had an activity of approximately 1500 counts per minute. The counter was also demonstrated to give the same total counts per minute for a bottle of uranium acetate and a bottle of thorium nitrate as the sum of their individual counts at a given distance from the Geiger-Mueller tube, thereby obtaining a value for the resolving time of the tube.

A. Corrections. Measurements, as previously mentioned, were taken with the source of radiation at a standard position relative to the counter and were corrected for systematic variations before any attempt was made at interpretation. The necessary corrections were:

1. Resolving time correction.
2. Background correction.
3. Efficiency changes with time.
4. Absorption and geometry corrections.

1. Resolving time correction: The Geiger-Mueller counter registers the passage of individual particles through the tube, but there is a short interval following each registered count (resolving interval) during which it is not able to register such emissions. In this investigation the counter was demonstrated to count linearly with increasing concentration up to 5000 counts per minute. Also, to minimize resolving time error standard sample concentrations were chosen that approximated the concentrations of the experimental samples.

2. Background correction: The background count which is due to cosmic rays and stray radiations in the laboratory was measured under the exact conditions of the other measurements, except with the absence

of the radioactive sample. This value was subtracted from the counting rate of the sample. Throughout this investigation the background count was always in the range of 12 to 20 counts per minute.

3. Efficiency changes with time: Such changes may be corrected for by counting a standard long-lived source regularly. After sample counts and standard counts are corrected for resolving time and background, their ratio is a measure of the strength or efficiency of the calibrated system. Uranium or radium sources are frequently used for such standards. In this investigation solutions of radioactive phosphorus which were equivalent to the doses of isotope injected into the animals were analyzed with the Geiger-Mueller counter at approximately the same time as the samples of teeth containing the labelled phosphorus thereby eliminating the necessity for decay and efficiency corrections.

4. Geometry and absorption corrections: For comparative studies as in tracer investigations where absolute values are not sought geometry and absorption errors should be cancelled by treating the samples and standards alike during the assay procedure. This principle was followed in the investigation.

H. Precision of measurements (15): The precision of all measurements is limited by the statistical accuracy of counting. The reproducibility of measurements of samples that were rechecked in the course of this investigation is shown in Table 1. Satisfactory reproducibility was obtained on samples that were analyzed as long as 25 days apart.

J. Radioactive principles pertinent to radioanalysis (17, 18): The equation expressing the basic law of radioactive decay is,

$$-\frac{dN_t}{dt} = \frac{0.693}{T_{\frac{1}{2}}} N_t,$$ where $\frac{dN_t}{dt}$ is the absolute disintegration rate at the time, t; N_t is the number of atoms potentially radioactive which are present at the time t; and $T\frac{1}{2}$, the half life, is that interval of time required to reduce N_t two-fold. This equation may be used to become oriented as to the amounts of radioactive material normally used in "tracer" investigations. By substituting Avogadro's number (6.02×10^{23} molecules per mole) and the millicurie count per second 37×10^6) into equation (1), equation (2) is obtained as:

(2) γ = K (mc) ($T\frac{1}{2}$) (A). γ is the weight in micrograms of radioactive substance present; (mc) is the millicurie strength of the sample; ($T\frac{1}{2}$) is the half-life; and (A) is the atomic or molecular weight of the radioactive substance in question. K is a constant whose value depends upon the time units of the half-life. For convenience, these numerical values are tabulated below:

	Half-life, $T\frac{1}{2}$, expressed in				
Numerical value	seconds	min.	hrs.	days	years
K	8.8×10^{-11}	5.5×10^{-9}	3.2×10^{-7}	7.7×10^{-6}	2.8×10^{-3}

The following four examples illustrate the use of equation (2):

1. What weight of P-32 (half-life = 14.3 days) is equivalent to 1 mc.?

$\gamma = 7.7 \times 10^{-6} \times 1 \times 14.3 \times 32 = 0.0035$ micrograms.

2. A patient with hyperthyroidism is treated with 10 mc. of 8 day I-131. What weight of iodine was given?

$\gamma = 7.7 \times 10^{-6} \times 10 \times 8 \times 131 = 0.08$ micrograms of I-131.

3. A $BaCO_3$ (Mol. Wt. = 197) precipitate containing C-14 (half-life =

5000 yrs.) and weighing 10 mg. gives a radioassay of 37 counts per second which is estimated to be equivalent to about 3700 absolute disintegrations per second (10^{4} mc.). What fraction of the sample is barium radiocarbonate?

$$\gamma = 2.8 \times 10^{-3} \times 10^{-4} \times 5000 \times 197 = 0.28 \text{ micrograms.}$$

Thus, the fraction of $BaCO_4$ containing C-14 is 2.8×10^{-5} or approximately 4 molecules in 10,000 are potentially radioactive.

4. If a 10 mg. precipitate of $BaSO_4$ (mol. wt. = 233) contained 10^{-4} mc. of 87 day S-35, what fraction of the precipitate contains S-35?

$$\gamma = 7.67 \times 10^{-6} \times 10^{-4} \times 87 \times 233 = 1.56 \times 10^{-5} \text{ micrograms.}$$

Thus this fraction is 1.56×10^{-9} or approximately 6 barium sulfate molecules in 10^{8} contain S-35.

LITERATURE REVIEW OF THE APPLICATION OF RADIOACTIVE PHOSPHORUS TO RESEARCH ON THE METABOLISM OF TEETH

One of the first investigations of the metabolism of teeth in which a radioactive element was used was that of J. Aub (19), who demonstrated that a naturally occuring radioisotope of lead could be deposited in living dental tissues, and that, with the passage of time, such deposits apparently disappeared.

In 1935 Chievitz and Hevesy (20) employed radioactive phosphorus in the first study of teeth utilizing an artificial radioactive element. In 1937 Chievitz and Hevesy (21) added radioactive phosphorus in the form of disodium hydrogen phosphate to the diets of rats and reported that the phosphorus quickly found its way into the teeth. Krogh (22) reported in a review article that Hevesy had found that a week after an adult rat had been given radioactive phosphorus 29 per cent of the dose was found in the skeleton, 3.3 per cent in the incisors, and 0.2 per cent in the molars. The ratio of radioactive phosphorus to total phosphorus per mg. of ash of the incisors was found by Chievitz and Hevesy (21) to be 2.5 times that of the molars. These investigators also reported that when the rats were allowed to live longer the isotope decreased in the various tissues due to its replacement by phosphorus from the diet. Manly and Bale (23) gave radioactive phosphorus by stomach tube to 26 rats weighing 110 to 150 grams and reported that the isotope in the growing incisors showed increasing amounts from the tips through the middle and root portions. These investigators found that the incisors contained from 2.0 to 3.5 per cent of marked phosphorus 4 hours after receiving the dose, 4 to 7 per cent two days later, and increased slowly up to 8 per cent in 20 days, the results being calculated on the basis of per cent dose found per gram of ash in the teeth. In contrast the molars contained only 1 per cent of the dose in 4 hours after receiving the isotope and remained fairly constant from 10 hours up to 8 days after which time the percentage of labelled phosphorus decreased slowly. At the end of 20 days approximately two-thirds of the maximum amount of labelled phosphorus was still present in the molars. The rate of deposition of the radioactive phosphorus during the first day was about 0.07 per cent of the total phosphorus present, or about one-third that deposited in the incisors in the same period.

Hevesy (24) demonstrated that labelled phosphorus is removed rapidly from the blood of rats. Only two per cent of the dose remained in the plasma two hours after injection of the isotope, while 92 per cent was recovered in the calcified tissues. Hodge et al. (25, 26), suggested that the greater part of the radioactive phosphorus going into the bones and teeth was probably due to the natural affinity of the apatite structure for phosphate. In equilibrium the phosphate content of bone is 1000 times that of the plasma (27).

Manly and Bale (23) determined the retention of labelled phosphorus in the molars and incisors of 28 rats whose teeth were extracted with an ethylene glycol solution of 3 per cent potassium hydroxide. The results agreed well with previous studies made by Hevesy et al. (28) on unextracted teeth, thereby indicating that the exchange phenomenon took place in the inorganic constituents of the teeth.

After consideration of the available evidence from histological, chemical, and physical studies Chase (29) in 1931 concluded that enamel must be "a lifeless, inert, mostly inorganic substance". However, since then radioactive phosphorus has made possible a new approach in the study of this dental tissue. Hevesy and Armstrong (30) reported that, in vitro, the exchange of labelled phosphorus in the enamel was less than 10 per cent that in the dentin. Volker and Sognnaes (31) reported that some of the inorganic phosphorus in the enamel apparently could be derived from the saliva. These workers (32) demonstrated that the surface enamel of cats retained on the average, three times as much marked phosphorus as the remaining enamel when the isotope was administered through a stomach tube. The distribution of the isotope in the various density fractions of the whole crowns of the teeth of a dog showed a direct relationship between the density and the concentration of isotope in the enamel, but an inverse relationship in the dentin. The surface enamel of the teeth of a dog also contained the greatest deposit of labelled phosphorus. When the enamel was protected from the saliva by a plaster covering a reverse in the density-phosphorus-32 relationship was obtained. It thus appeared that the surface of the enamel adsorbed appreciable amounts of inorganic phosphorus from the saliva.

Barnum and Armstrong (33) covered various parts of human teeth with collodion and exposed them in vitro to a solution of radioactive phosphorus and found that the phosphorus reached the enamel through the dentin and also reached the dentin through the enamel. Inasmuch as the quantity passing through the enamel was relatively small compared to that which passed through the dentin they postulated, that, in vivo, more phosphate reached the enamel through the blood circulation and the pulp than was absorbed from the saliva. They found that the ratio of the activity of 1 gram of enamel to that of 1 gram of dentin was 0.07. On the basis of the specific activities of the dental tissues, the rate of phosphorus exchange in the enamel was approximately 5 per cent of that in the dentin. Armstrong and Barnum (34) gave a male rat, weighing 238 grams, by stomach tube 8 ml. of a solution containing about 0.6×10^6 counts per minute of calcium-45 isotope and 9×10^6 counts per minute of phosphorus-32 isotope and determined the concurrent uptake and retention of both elements by the teeth. They found that the uptake and retention of both the radiocalcium and radiophosphorus stood in the following decreasing order: incisor dentin, incisor enamel, molar dentin, and molar enamel.

Pederson and Schmidt-Nielson (35) were unable to demonstrate any appreciable concentration of radioactive phosphorus in cement-capped enamel surfaces of several teeth from two young girls who had been given a dose of the isotope several days previous to the extraction of the teeth.

Manly and Levy (36) fed labelled phosphorus in the form of disodium hydrogen phosphate to 31 experimental rats. One group was mated 2 days after administration of the dose, and the other group 7 days afterwards. The data obtained indicated that when the mothers were on an adequate diet no significant exchange of inorganic phosphorus took place between her skeleton and the offspring.

EXPERIMENTAL AND RESULTS

General procedure: In this investigation a comparison was made of the whole teeth of resistant and susceptible strains and stock Albino rats with regard to (1) weight, (2) ash content, (3) phosphorus content expressed as per cent of (a) dry weight of the teeth and (b) of the ash, (4) rates of deposition and removal of radioactive phosphorus-32 in the teeth after intraperitoneal injection of the isotope, (5) occurrence of radioactive phosphorus in the teeth of offspring whose mothers were injected intraperitoneally with the isotope during pregnancy, and (6) the adsorption of radioactive phosphorus in vitro by the teeth of the different strains.

Three rations were used in this investigation. They were designated as fine rice, stock, and pmas, respectively, and consisted of the following ingredients:

Fine rice	Stock	Pmas
rice* - 66%	corn meal - 35%	powder. milk - 79%
milk powder - 30%	ground wheat - 25%	alfalfa meal - 20%
alfalfa meal - 3%	w. milk powd. - 20%	salt - 1%
salt - 1%	linseed oil meal - 10%	
	alfalfa - 6%	
	brewers yeast - 3%	
	salt - 1%	

*The rice was ground so that 2 per cent was held on a 20 mesh screen and 40 per cent on a 40 mesh screen.

The above rations gave the following results when analyzed for per cent moisture, ash, and phosphorus according to the recommendations of the A.O.A.C. Methods of Analysis:

Constituent	Fine rice %	Stock %	Pmas %
moisture	9.80	9.56	5.50
ash	4.42	4.97	9.10
phosphorus (dry basis)	0.37	0.47	0.66

Approximately 224 rats of the resistant and susceptible strains and stock colony were compared in the age range of 6 to 60 days in regard to weight of teeth, per cent ash in the teeth, per cent phosphorus in the teeth calculated on the basis of the dry weight and in the ash. An individual member of each litter used in the investigation was isolated, fed the fine rice ration, and observed for development of caries in order to check if the various strains were responding as expected. The stock colony rats appeared to be more like the susceptible than the resistant strain in susceptibility to dental caries. Rats of the susceptible strain would almost invariably have small cavities developing at 60 days of age and for this reason older rats were not used in this investigation. Unpublished data obtained by Hoppert et al. show that the stock ration is less cariogenic than the fine rice ration, and that the pmas ration is essentially non-cariogenic. Both of the developed strains and the stock rats were compared on the fine rice ration in the age range of 6 to 60 days. The susceptible strain and stock rats were also compared on the stock ration in the age range of 6 to 21 days. A few rats of the susceptible strain were fed the pmas ration. The resistant strain was maintained entirely on the fine rice ration. The fine rice diet was also used in all of the tracer studies.

To obtain the teeth for analysis the rats were killed with ethyl ether, and the teeth removed from the jaws and cleaned of all tissue with a scalpel and cheesecloth. The pulp was removed from all incisors, but in the case of the molars the pulp was removed only in rats 20 days of age or younger. Although the teeth do not erupt in young rats until about the 14th to 16th day, eight molars and the four incisors were removed from rats ranging from 6 to 14 days. In the case of older rats the four back molars were also included.

The various phases of this investigation listed above will be presented in turn.

Weight of teeth: The molars and incisors were dried at least 2 hours (to constant weight) in a hot air oven at 103° C. The data obtained with the susceptible and resistant strains and stock rats on the fine rice ration are presented in Table 2. Figures 3 and 4 present in graphical form the data obtained with the molars and incisors, respectively. The molars increased in weight linearly up to 20 days of age after which time the rate of growth gradually decreased, whereas the incisors grew linearly throughout the investigational period i.e. 6 to 60 days. The weight of both the molars and incisors of the resistant strain tended to be slightly lighter in weight after 30 days of age than those of the susceptible strain and stock colony rats.

Thirty rats of the susceptible strain and 19 rats of the stock colony whose mothers were maintained on the stock ration were investigated in the age range of 6 to 21 days for rate of tooth growth and were found to give comparable results to rats on the fine rice diet.

Per cent ash in the teeth: (1) The per cent ash was obtained by heating the dried teeth in a muffle furnace overnight at 600° C. The data obtained with the susceptible and resistant strains and stock rats on the fine rice ration at 6 to 60 days of age are shown in Table 2. Figures 5 and 6 present in graphical form the data obtained on the molars and incisors, respectively. The per cent ash in the molars increased rapidly from approximately 40 per cent at 6 days of age to approximately 77 to 80 per cent at 20 days of age after which time the values tended to be constant throughout the period of observation. The per cent ash in the incisors increased from approximately 60 per cent at 6 days of age to approximately 75 per cent at 20 days after which time it remained quite constant. Table 3 shows that the average concentration of ash in the molars from 20 to 60 days of age was 76.7 $\pm$ 0.9, 78.8 $\pm$ 0.9, and 77.6 $\pm$ 1.0 per cent for the susceptible, resistant, and stock colony groups, respectively. The incisors of the susceptible strain contained approximately 0.6 per cent less ash than those of the resistant and stock rats.

(2) The per cent ash in the teeth was also obtained by extracting the organic matter with an ethylene glycol solution of 3 per cent potassium hydroxide at the boiling temperature according to the procedure of Crowell et al. (37). The data obtained by this technique is shown in Table 4. The per cent ash was found by this extraction procedure to be approximately 5 to 8 per cent higher than by muffle furnace ashing at 600° C. However, the resistant and susceptible rats of the same age gave similar values by the extraction method in both molars and incisors.

Per cent phosphorus in the teeth: The phosphorus in the teeth was determined by the volumetric phosphomolybdate method (38). The ash residues of the teeth were used for the phosphorus analysis and the results were calculated on the dry weight basis of the teeth and also on the basis of the phosphorus in the ash. The susceptible and resistant strains and stock animals were compared on the fine rice ration from 6 to 60 days of age and the data obtained is shown in Table 2. The data are presented graphically for the molars and incisors in Figures 7 and 8, respectively, in which the per cent phosphorus was calculated on the basis of the dry weight of the teeth, and in Figures 9 and 10, respectively, in which the per cent phosphorus was calculated on the basis of the ash content of the teeth. Figure 7 shows that the per cent phosphorus in the molars on the dry weight basis increased from 8 to 10 per cent at 6 days of age to approximately 14 to 15 per cent at 20 days of age at which time the values became constant. Figure 8 indicates that the per cent phosphorus in the incisors on the dry weight basis followed closely the pattern of the molars. Figures 9 and 10 show that when phosphorus was expressed in terms of the per cent of the ash, age appeared to have little influence. The somewhat greater variations found in the case of the very young animals may have been due to the inherent difficulties in the preparation of comparable samples of dental tissue. The aggregate weight of the eight molars of 6 day old rats was as little as 5 mg. in some cases.

As shown in Table 3 in which average phosphorus values are given for the age range of 20 to 60 days, the molars of the resistant strain contained 15.5 ± 0.4 per cent calculated on the dry weight basis as compared to 15.0 ± 0.2, and 15.1 ± 0.4 per cent for the susceptible and stock colony rats, respectively. However, no significant difference was demonstrated between the 3 groups for phosphorus in the incisors on this basis, nor in the phosphorus content of the ash of either the molars or incisors. Thirty-three rats of the susceptible strain and 19 rats of the stock colony maintained on the stock ration were sacrificed in the age range of 6 to 21 days, and 14 rats of the susceptible strain on the pmas ration that were sacrificed in the age range of 40 to 100 days, gave phosphorus values comparable to those found above in the case of rats fed the fine rice ration. Also, a 167 day old resistant rat kept on the fine rice ration gave similar phosphorus values. The data obtained indicate that the phosphorus content of the ash of the teeth is relatively independent of age, sex, heredity, and diet.

Rate of deposition in and removal of radioactive phosphorus from the teeth: This phase of the investigation of the role of phosphorus in the teeth of the specialized strains and stock rats involved two shipments of radioactive phosphorus from Oak Ridge, Tennessee, after allocation from the Atomic Energy Commission. The amount received each time was 1 ml. of an aqueous solution of phosphoric acid containing 3 millicuries of the radioactive isotope. The 1 ml. of solution was diluted before use to approximately 150 ml. with distilled water, sufficient sodium chloride added to give a 0.9 per cent solution, and the pH adjusted to approximately 7.5 with sodium hydroxide. On the basis of the Oak Ridge assay report 1 ml. of the diluted solution, hereafter designated as "stock solution", should have contained approximately 15 to 20 microcuries of the isotope and was found to have an activity of approximately 1,600,000 counts per minute on the dipping-type Geiger-Mueller tube used in this investigation. The activity determination was made by diluting 1 ml. of the stock solution plus a few drops of concentrated nitric acid to 2000 ml. with distilled water and using a 5 ml. aliquot for analysis.

One ml. of the stock solution was injected into the peritoneal cavity of resistant, susceptible, and stock rats at different ages i.e. at 20, 24, 29, 32, and 35 days of age. At intervals of a few hours up to 40 days after injection of the isotope the animals were sacrificed and the teeth removed. The molars and incisors were separated, dried at 100° C., and ashed in a muffle furnace at 600° C. The ash was then dissolved in 10 ml. of approximately 2.5 N hydrochloric acid and transferred to a suitable volumetric flask from which aliquots were taken for activity measurements and analysis for total phosphorus.

The data for the weight of teeth, per cent ash, and per cent phosphorus is shown in Table 3 (see column headed "age P^{32} injected" for guide to rats that were injected with labelled phosphorus). The data obtained for the deposition and removal of the injected radioactive phosphorus is presented in Table 4. The results are expressed in terms of per cent of injected dose found, per 100 mg. of ash in the teeth, at the time of sacrifice of the animals. Figures 11 and 12 show graphically the results obtained for molars and incisors, respectively, on the resistant and susceptible strains and stock rats that were injected with the isotope at 20 days of age. Figures 13 and 14 show graphically the results for molars and incisors, respectively, on rats that were injected at 32 to

35 days of age. Rats of both specialized strains and stock rats rapidly deposited the isotope in the teeth, approaching a maximum percentage of the injected dose at 24 to 48 hours after injection.

As shown in Figure 11, the molars of the 20 day old rats deposited approximately 4 to 7 per cent of the injected isotope at 24 to 48 hours after receiving the dose. The per cent deposition increased slightly up to 15 days, after which time the concentration began to decrease. Figure 12 shows the per cent of phosphorus-32 found in the incisors of the rats injected with the isotope at 20 days of age. These animals had a deposition of approximately 5 to 9 per cent of the injected isotope in the incisors within 24 to 48 hours after receiving the dose and tended to increase slightly up to 5 to 10 days after which time the concentration decreased gradually to 3 to 4 per cent after 30 to 40 days.

Figure 13 shows graphically the data obtained on the molars of rats that were injected with the isotope at 32 to 35 days of age. Both the specialized strains and stock rats had approximately one per cent of the dose deposited in the molars by 24 to 48 hours following the injection. The per cent deposition was relatively constant at 1 to 1.5 per cent throughout the range of 5 to 15 days after the isotope was administered, after which time the concentration decreased. Figure 14 presents graphically the data obtained on the incisors of rats that were injected with the isotope at 32 to 35 days of age. The incisors of the resistant strain contained slightly more of the isotope than those of the stock and susceptible strains. The incisors in general deposited 2 to 3 times more radioactive phosphorus than the corresponding molars. The 35 day old rats deposited only a third to one-half as much of the isotope in their molars and incisors as the 20 day old rats.

Radioactive phosphorus found in the teeth of offspring of mothers that were administered the isotope during pregnancy: In this experiment pregnant females of the resistant and susceptible strains and stock rats were injected intraperitoneally during pregnancy with 5 ml. of the stock isotope solution which contained approximately 90 to 100 microcuries of radioactive phosphorus. The offspring were then sacrificed at 6, 9, 14 and 20 days of age and the activity in the molars and incisors determined after drying at 100° C. in a hot air oven, ashing at 600° C. in a muffle furnace overnight, and dissolving in dilute hydrochloric acid. Two females of each of the specialized strains and stock rats were injected with the isotope. However, one resistant strain mother and one stock colony mother destroyed all of their offspring, whereas the other stock rat mother destroyed all but one and the second susceptible strain mother destroyed all but three of their offspring. The data obtained are shown in Table 5. These data indicate that the offspring were normal in regard to weight of teeth, per cent ash, and per cent phosphorus in the teeth at the age that they were sacrificed. An appreciable amount of activity was found in the teeth of all offspring. However the longer the gestation period after the injection of the isotope the lower the percentage found in the teeth of the offspring.

Fortunately, a comparison of the susceptible and resistant strains was made possible by the birth of a litter of each type two days after the females were administered the isotope. The data obtained with these two litters are shown graphically in Figures 15 and 16, for molars and incisors, respectively. The per cent of injected isotope found per 100 mg. of ash in the molars of both strains decreased from approximately 5 per cent at 6 days of age to approximately 2.1 per cent at twenty days of age. In the case of the incisors the percentage of dose found decreased from approximately 6 to 7 per cent at 6 days of age to 2.4 per cent at 20 days of age. A representative line drawn through the points on these graphs would be essentially identical for both strains.

In vitro adsorption of radioactive phosphorus by the teeth: In this experiment the teeth of the susceptible and resistant strains and stock colony rats that had been on the fine rice ration were investigated with regard to in vitro adsorption of radioactive phosphorus. Rats at 20, 30, 40, and 60 days of age were used for the study. The procedure used was similar to that used by Hodge et al. (25). The teeth were extracted with an ethylene glycol solution of 3 per cent potassium hydroxide to remove the organic matter. After filtering the extracting solution the teeth were ground to medium fineness, and agitated for 8 hours in 25 ml. of a neutral aqueous solution of radioactive sodium hydrogen phosphate at 40° C. The radioactive solution had an activity of approximately 2000 counts per minute per 5 ml. as determined on the dipping-type Geiger-Mueller tube used in this investigation. After exposure the teeth were washed by decantation and transfer to a Selas crucible with 5 washings of 25 ml. of distilled water at 40° C. The ground dental tissue was then dissolved in approximately 2.5 N hydrochloric acid, diluted to volume in a volumetric flask, and an aliquot taken for measurement of the activity adsorbed from the radioactive phosphorus solution. In the case of the 40 day old rats the molars were equally divided and half were ashed in the muffle furnace at 600° C. and the capacity of the ash to adsorb radioactive phosphorus was determined as above. The data obtained in these in vitro studies are presented in Table 6. The teeth of the 60 day old rats adsorbed appreciably less phosphorus than those of the younger rats i.e. both the molars and incisors adsorbed radioactive phosphorus equivalent to 20 to 30 counts per minute per mg. of ash, whereas the teeth of the 20, 30 and 40 day old rats adsorbed sufficient isotope to give 50 to 60 counts per minute in the case of the molars, and 70 to 80 counts per minute in the case of the incisors. The teeth of the 40 day old rats that were ashed in the muffle furnace adsorbed only enough isotope to give approximately 15 counts per minute per mg. of ash, in the case of both the susceptible and resistant strains.

DISCUSSION

Very little chemical work has been done on the teeth of the caries resistant and caries susceptible strains of Albino rats previous to this investigation. Whole teeth only were used in this study in order to determine whether gross chemical differences existed between the strains. Circumstances did not permit a separation of the enamel and dentine for individual study altho it is recognized that more detailed and valuable information would have been obtained by so doing.

A few ash determinations made by extracting the organic matter from the teeth with 3 per cent potassium hydroxide in ethylene glycol gave 5 to 8 per cent higher values than those obtained on corresponding teeth ashed at 600° C. Bowes and Murray (39) found that ashing at high temperatures decomposed all the combined carbon dioxide in the dentine and about 75 per cent of that in the enamel. They reported that dry dentine and dry enamel contained 3.18 and 1.95 per cent of combined carbon dioxide, respectively. These investigators also reported that extraction methods remove approximately 0.4 per cent of the phosphorus as organically combined phosphorus. Further work is obviously necessary to determine what part of the molars of the specialized strains is responsible for the small differences in ash and phosphorus encountered in this investigation. The data obtained in this study indicated that the phosphorus content of the ash in the whole teeth of the specialized strains and stock rats is independent of age, sex, and heredity.

The variation in the capacity of members of the same strain to deposit and remove radioactive phosphorus from the teeth indicates that small differences between the strains cannot be determined easily. The apparent biological variation encountered in this study is of the same order as that obtained by Manly and Bale (23) when they gave radioactive phosphorus by stomach tube to Albino rats and determined the rate of turn-over of the isotope in the teeth. In studies of the absorption, distribution, and excretion of labelled phosphorus in the rat Cohn and Greenberg (40) administered the isotope by stomach tube and by intraperitoneal injection. They found both techniques to give appreciable variations in the isotope content of the various tissues of the body.

The experiment, in which the two specialized strains were compared in regard to the occurrence of labelled phosphorus in the teeth of the offspring of mothers that had been administered the isotope during pregnancy, indicates that the early phosphorus metabolism of the teeth of the two strains is very similar. Sognnaes and Volker (32) demonstrated that the enamel and dentine of the unerupted teeth of a monkey adsorbed the same amount of radioactive phosphorus. Since the teeth were removed from the young rats in the present experiment in the pre-erupted stage or soon afterwards, it might be assumed that the enamel and dentine of the two strains are equivalent in their capacity to assimilate phosphorus at least in the early period of their tooth development.

Hodge et al. (25) found that Albino rat teeth adsorbed radioactive phosphorus in vitro from an aqueous solution in accordance with the Freundlich adsorption isotherm. Subsequently, Hodge et al. (41) showed that when enamel and dentine were exposed to radioactive phosphorus in vitro fairly rapid adsorption of the isotope occurred in each substance over a period of at least 8 hours. For this reason, the teeth were exposed to the labelled phosphorus in aqueous solution for 8 hours in the in vitro adsorption tests made in the present investigation.

CONCLUSIONS

The data obtained in this investigation of the whole teeth of two strains of Albino rats that are markedly different in their susceptibility to dental caries indicate that they vary slightly in the following respects:

1. Weight of teeth.
2. Per cent ash in the molars.
3. Per cent phosphorus in the molars.

They were found to be essentially similar in the following respects:

1. Per cent ash in the incisors.
2. Per cent phosphorus in the incisors on the dry weight basis, and per cent phosphorus in the ash of the molars and incisors.
3. Rate of deposition and removal of radioactive phosphorus in the teeth when the isotope was administered intraperitoneally.
4. Occurrence of radioactive phosphorus in the teeth of offspring of females that were injected intraperitoneally with the isotope during pregnancy.
5. In vitro adsorption of the isotope by the teeth from aqueous solution.

Table 1. Data showing reproducibility of assay technique for rechecking per cent radioactive phosphorus in acid solutions of rat teeth ash.

designation of teeth	1st analysis date	1st analysis % P^{32}	2nd analysis date	2nd analysis % P^{32}	difference days	difference % P^{32}
M1	7/11	0.88	8/5	0.89	25	-0.01
2	7/12	0.66	8/5	0.70	24	-0.04
3	7/24	1.16	8/5	1.09	12	-0.07
4	7/27	0.94	8/9	1.00	13	-0.06
5	7/29	0.76	8/9	0.85	11	-0.09
12	7/23	0.94	8/5	0.87	13	-0.07
13	8/9	1.02	8/12	0.86	3	-0.16
16	7/11	0.95	8/5	0.94	25	-0.01
17	7/12	0.96	8/5	1.08	24	-0.12
18	7/27	1.81	8/9	1.90	13	-0.09
19	7/30	2.42	8/9	2.37	10	-0.05
28	7/23	2.01	8/12	2.15	20	-0.14
29	7/28	2.77	8/12	2.85	15	-0.08
39	7/25	2.00	8/6	2.18	12	-0.18
41	7/25	2.05	8/12	2.03	18	-0.02
42	7/31	4.34	8/12	4.37	13	-0.03
43	8/4	4.44	8/12	4.59	8	-0.15
44	8/6	1.75	8/12	1.74	6	-0.01
47	8/2	1.70	8/7	1.83	5	-0.13
48	8/7	1.84	8/12	2.03	5	-0.19
51	8/11	2.79	8/12	2.72	1	-0.07

Note: The above values for % P^{32} were calculated on the basis of per cent injected dose found in the teeth.

Table 2. Weight of teeth, per cent ash, per cent phosphorus in the teeth, and per cent phosphorus in the ash of the teeth of the resistant and susceptible strains and stock rats on the fine feed ration in the age range of 6 to 60 days.

strain	age P^{32} inject. (days)	age rat sacrific. (days)	wt. of rat (gms)	sex	wt. of teeth (mgs) mol.	wt. of teeth (mgs) inc.	% ash mol.	% ash inc.	% phosphorus dry basis mol.	% phosphorus dry basis inc.	% phosphorus in ash mol.	% phosphorus in ash inc.
susc.	-	6	16	M	9.5	6.5	48.4	61.5	10.1	13.3	20.9	21.6
"	-	6	15	F	6.9	4.9	43.5	55.1	-	-	-	-
"	-	6	12	M	5.6	4.6	41.1	54.4	-	-	-	-
"	-	6	15	F	6.9	5.2	55.0	46.2	10.5	14.1	19.1	30.6
"	-	9	17	F	14.7	9.9	57.2	68.7	12.1	15.4	21.1	22.4
"	-	9	25	M	17.7	9.4	56.5	58.5	-	-	-	-
"	-	9	15	M	15.2	9.0	54.6	66.7	10.9	14.6	20.0	21.9
"	-	9	13	M	11.8	7.7	50.9	62.3	10.8	13.5	21.2	21.7
"	-	9	13	M	12.1	7.6	52.1	64.5	10.9	14.1	21.0	21.9
"	-	10	-	-	15.4	9.0	67.5	83.3	12.0	15.3	17.7	18.4
"	-	10	-	-	15.9	9.4	67.3	85.1	12.2	15.7	18.1	18.5
"	-	10	11	M	11.5	7.6	50.4	63.2	10.8	13.7	21.4	21.7
"	-	14	26	F	33.5	22.4	74.6	77.7	14.4	15.4	19.3	19.8
"	-	14	35	M	34.5	21.1	72.5	73.5	14.7	17.9	20.3	24.7
"	-	14	22	M	27.5	18.9	69.9	69.5	-	-	-	-
"	-	19	39	F	54.8	35.4	77.4	73.7	15.1	14.8	19.5	21.1
"	-	20	35	M	48.2	29.6	78.2	72.6	15.2	15.0	19.5	20.6
"	-	20	32	F	60.7	38.3	76.9	73.9	15.1	15.0	19.6	20.3
"	-	20	48	M	66.5	44.9	77.9	74.8	15.1	15.1	19.4	20.1
"	-	20	30	F	35.4	25.6	78.3	75.2	16.0	16.1	20.5	21.4
"	-	20	37	F	61.2	42.5	77.3	74.4	15.0	15.1	19.4	20.3
"	20	21	37	F	64.3	45.4	78.4	74.2	-	-	-	-
"	20	22	39	F	70.8	51.6	77.1	75.7	-	-	-	-
"	20	25	55	M	77.4	61.1	77.1	74.8	-	-	-	-
"	20	25	50	M	84.3	61.6	76.3	75.3	-	-	-	-
"	20	30	70	F	92.8	70.6	-	-	-	-	-	-
"	20	30	75	M	91.1	75.7	76.3	73.4	15.0	15.4	19.7	21.1
"	-	30	77	F	83.2	61.1	79.0	77.9	15.4	15.7	19.5	20.2
"	-	30	82	F	84.5	76.0	76.9	77.6	15.1	15.9	19.6	20.5
"	-	30	66	F	89.3	71.9	77.6	76.6	15.3	15.8	19.7	20.6
"	-	30	70	F	85.6	67.8	76.4	74.9	15.1	15.6	19.8	20.8
"	-	32	83	F	80.4	62.7	76.9	74.5	15.1	15.6	19.6	20.7
"	29	34	91	M	78.2	63.5	77.8	75.8	-	-	-	-
"	20	35	98	M	95.2	82.2	77.2	76.5	-	-	-	-
"	20	35	70	M	100.0	85.0	76.1	75.9	14.4	14.8	18.9	19.5
"	35	37	97	M	104.2	102.0	77.3	77.4	-	-	-	-
"	33	38	98	F	103.9	95.4	76.3	75.9	15.4	16.1	20.2	21.3
"	-	39	100	F	95.9	90.6	75.8	74.4	15.2	15.5	20.0	20.8
"	29	39	110	M	102.0	94.3	77.5	76.4	15.2	15.8	19.6	20.4
"	-	40	127	-	100.1	96.9	77.7	77.6	15.2	15.8	19.5	20.3
"	-	40	103	F	102.1	95.9	75.7	74.9	14.9	15.4	19.7	20.6
"	-	40	79	M	90.5	88.3	75.8	75.3	15.0	15.3	18.6	20.3
"	-	40	119	M	-	108.4	-	75.8	-	15.9	-	21.0
"	20	40	70	F	95.8	97.0	75.7	77.3	14.3	15.0	18.9	19.4

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(Table 2 continued)

strain	age P^{32} inject. (days)	age rat sacrific. (days)	wt. of rat (gms)	sex	wt. of teeth (mgs) mol.	inc.	% ash mol.	inc.	% phosphorus dry basis mol.	inc.	in ash mol.	inc.
susc.	33	43	134	F	108.4	123.3	-	-	14.7	15.6	-	-
"	-	49	145	M	90.1	106.9	75.4	76.2	15.1	15.9	20.0	20.8
"	-	49	135	F	117.4	112.8	76.4	75.3	15.0	15.3	19.7	20.3
"	-	50	133	F	112.6	135.5	76.7	78.7	15.0	16.0	19.5	20.3
"	-	50	136	M	108.8	120.6	76.3	76.2	14.8	15.4	19.3	20.2
"	35	50	132	F	112.0	136.7	77.2	76.5	14.9	15.2	19.3	19.9
"	35	50	140	F	97.7	139.0	-	78.5	-	16.1	19.9	20.5
"	-	55	132	F	113.7	153.3	74.7	75.7	15.1	15.8	20.2	20.8
"	20	55	70	M	91.8	105.1	75.1	76.3	14.3	15.1	19.1	19.8
"	-	57	163	F	130.5	168.9	76.1	75.3	15.1	15.6	18.7	20.8
"	-	60	146	F	-	156.4	-	76.7	-	15.7	-	20.5
"	-	60	155	F	125.6	167.4	75.3	75.6	15.0	15.7	20.0	20.7
"	-	60	147	F	121.7	172.3	75.5	75.8	14.8	15.7	19.5	20.7
"	20	60	85	M	105.7	124.3	75.1	76.4	14.3	15.1	19.0	19.8
resist.	-	5	7	-	2.5	2.1	40.0	57.1	14.4	13.3	36.0	23.3
"	-	5	7	-	2.1	2.3	23.8	52.2	8.2	11.0	34.4	21.0
"	-	5	7	-	2.4	2.2	29.2	45.5	8.8	12.0	30.3	26.4
"	-	6	8	F	5.7	3.3	43.9	63.6	8.5	14.3	19.4	22.5
"	-	6	10	M	5.2	4.6	42.4	58.7	-	-	-	-
"	-	6	10	M	5.0	3.3	36.0	42.5	-	-	-	-
"	-	9	7	F	8.0	5.0	48.8	66.0	9.6	12.6	19.7	19.0
"	-	9	12	M	11.5	-	52.2	-	10.4	-	20.0	-
"	-	9	6	M	6.7	3.2	47.8	63.6	10.6	16.5	22.3	25.9
"	-	9	18	F	15.9	10.5	58.0	71.5	11.9	14.5	20.6	20.3
"	-	10	17	F	16.8	10.2	54.8	68.6	10.5	13.7	19.2	20.0
"	-	10	12	M	14.7	8.1	53.7	66.7	10.5	14.1	19.4	21.2
"	-	10	16	M	14.5	8.8	52.4	64.8	11.0	13.9	21.0	21.4
"	-	10	17	F	15.1	6.6	53.0	62.1	10.9	13.8	20.7	22.2
"	-	13	20	M	26.9	16.7	70.2	73.4	14.2	15.4	20.2	21.0
"	-	14	20	M	29.4	19.3	72.5	74.6	14.5	15.4	20.1	20.6
"	-	14	22	F	25.4	17.4	72.2	70.7	-	-	-	-
"	-	19	31	F	49.1	33.8	78.8	73.7	15.4	15.1	19.4	20.5
"	19	19	25	M	43.2	27.3	78.3	72.0	-	-	-	-
"	19	19	22	M	41.3	25.1	78.7	73.7	-	-	-	-
"	-	20	35	F	46.6	34.4	79.6	76.5	15.8	16.0	18.9	18.8
"	20	20	31	M	56.5	39.4	80.3	77.2	15.3	14.2	18.7	18.4
"	20	21	28	F	57.8	39.2	79.3	77.1	15.0	14.5	18.9	18.8
"	20	21	27	F	55.4	39.8	80.0	77.9	15.0	14.5	18.7	18.4
"	20	21	30	M	67.4	44.2	78.5	76.0	-	-	-	-
"	20	22	-	F	62.4	45.7	79.5	76.4	-	-	-	-
"	20	25	-	F	68.3	51.9	78.5	75.5	-	-	-	-
"	20	25	-	M	51.0	38.8	77.8	73.7	-	-	-	-
"	25	26	-	F	76.3	61.1	79.6	76.7	-	-	-	-

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(Table 2 continued)

strain	age P^{32} inject. (days)	age rat sacrific. (days)	wt. of rat (gms)	sex	wt. of teeth (mgs) mol.	wt. of teeth (mgs) inc.	% ash mol.	% ash inc.	% phosphorus dry basis mol.	% phosphorus dry basis inc.	% phosphorus in ash mol.	% phosphorus in ash inc.
resist.	25	27	-	M	76.0	64.7	79.2	76.0	-	-	-	-
"	20	30	-	F	83.4	66.0	80.6	78.3	16.1	16.5	20.0	21.0
"	20	30	55	F	72.9	66.5	79.0	75.3	15.7	13.6	19.9	18.0
"	20	30	-	M	71.6	65.6	-	-	15.2	15.7	-	-
"	25	30	-	F	62.1	58.3	79.0	75.8	15.6	16.1	19.7	21.
"	20	20	28	F	53.8	36.7	79.6	76.5	16.2	16.1	20.3	21.1
"	20	20	33	M	54.4	39.0	79.6	75.8	16.3	15.8	20.4	20.9
"	20	22	-	M	57.8	42.8	79.6	76.0	16.1	16.1	20.2	21.2
"	20	35	53	F	81.1	79.9	78.8	75.3	15.6	15.6	19.8	20.8
"	20	35	-	M	87.7	79.0	80.7	76.8	15.1	15.2	18.8	19.7
"	29	31	62	F	63.6	69.0	76.9	75.2	15.2	15.5	19.8	20.7
"	35	36	-	F	79.3	75.7	78.8	76.2	-	-	-	-
"	35	37	-	F	83.7	87.5	79.2	76.2	-	-	-	-
"	29	39	84	F	75.8	94.3	77.0	76.2	15.3	15.6	19.8	20.5
"	-	40	93	M	-	100.5	-	76.7	-	16.1	-	21.0
"	35	40	-	M	83.1	84.2	78.2	76.2	16.1	16.1	20.5	21.2
"	25	40	-	F	78.5	77.3	75.7	74.3	15.4	15.6	20.4	21.0
"	35	50	-	M	91.7	116.1	78.3	75.4	15.8	15.8	20.3	21.0
"	20	50	77	M	96.5	105.1	78.5	77.9	14.8	15.3	18.8	19.6
"	20	55	68	F	98.1	116.5	78.2	77.9	14.8	15.4	18.9	19.8
"	-	60	134	F	113.6	154.3	78.1	78.3	15.6	16.3	20.0	20.8
"	-	60	122	F	-	146.4	-	77.5	-	16.1	-	20.7
"	20	60	98	M	104.2	134.7	78.3	77.0	15.0	15.3	19.1	19.9
"	-	167	-	F	119.3	228.6	78.6	77.0	15.3	15.3	19.4	19.9
stock	-	6	-	-	6.7	4.8	46.3	60.4	9.6	12.5	20.7	20.7
"	-	6	17	-	8.3	5.9	45.8	54.2	9.5	12.3	20.8	23.6
"	-	6	13	-	4.3	3.6	72.1	91.7	11.6	14.4	16.1	14.8
"	-	6	10	F	3.0	3.2	46.7	68.8	10.8	13.4	22.9	19.5
"	-	6	10	-	5.3	4.4	54.7	59.2	-	-	-	-
"	-	9	14	M	12.9	8.5	51.9	65.9	10.5	12.2	20.1	18.6
"	-	9	12	F	12.9	7.5	54.3	66.7	10.9	14.4	30.1	21.6
"	-	9	21	M	14.9	8.9	61.1	78.7	15.4	14.7	18.9	18.6
"	-	9	23	F	18.2	11.3	61.5	82.3	11.1	14.9	18.0	18.1
"	-	9	17	F	12.1	8.0	50.4	62.5	10.6	13.5	21.0	21.6
"	-	11	19	M	22.2	14.0	61.7	71.4	12.4	14.8	20.1	20.7
"	-	14	-	F	31.9	22.0	68.7	71.4	13.5	14.8	19.7	20.8
"	-	14	-	M	34.6	21.2	74.0	78.3	14.0	15.5	18.9	19.8
"	-	14	33	F	35.1	22.1	71.2	71.0	14.2	14.4	19.9	20.3
"	-	18	37	F	56.2	35.0	76.3	72.0	15.5	15.0	20.3	20.8
"	-	19	31	M	52.8	35.7	77.5	74.2	15.3	15.1	19.7	20.4
"	-	19	29	M	53.4	35.7	77.7	72.8	14.4	14.9	18.5	20.4
"	-	19	31	F	55.6	36.4	77.7	75.0	15.7	16.5	20.2	20.7
"	-	20	36	F	56.8	36.9	78.0	73.7	15.3	15.2	19.6	20.6

Continued next page

(Table 2 continued)

strain	age P^{32} inject. (days)	age rat sacrific. (days)	wt. of rat (gms)	sex	wt. of teeth (mgs) mol.	inc.	% ash mol.	inc.	% phosphorus dry basis mol.	inc.	in ash mol.	inc.
stock	-	20	32	M	53.0	36.3	80.8	78.5	15.6	15.7	19.3	19.9
"	20	21	-	F	52.4	39.6	80.0	76.8	-	-	-	-
"	20	22	-	F	64.8	46.9	-	-	15.0	14.9	-	-
"	24	29	-	M	72.8	60.7	76.9	74.5	-	-	-	-
"	-	30	72	F	95.8	73.3	78.6	77.5	15.3	15.9	19.4	20.5
"	-	30	60	F	79.0	64.1	79.0	77.4	15.6	16.0	19.7	20.7
"	-	30	54	F	75.8	60.8	78.7	76.8	15.6	16.1	19.8	20.9
"	20	30	-	M	82.9	67.0	77.9	76.8	15.5	16.1	20.0	21.0
"	24	34	-	M	84.1	80.6	-	-	15.0	15.9	-	-
"	20	35	-	M	93.6	83.6	77.5	76.9	15.1	15.6	19.6	20.4
"	29	34	77	M	83.6	77.7	76.7	74.4	15.0	15.4	19.6	20.7
"	32	35	-	F	87.6	84.3	78.3	75.2	-	-	-	-
"	24	39	-	F	84.5	76.0	77.5	75.5	-	-	-	-
"	29	39	96	M	95.2	85.1	76.5	75.9	14.2	14.9	18.5	19.5
"	-	40	87	F	90.4	99.5	77.4	77.0	15.2	15.8	19.6	20.5
"	35	40	120	M	94.4	111.9	76.0	76.6	14.3	14.7	18.9	19.5
"	32	42	-	F	106.2	111.8	-	-	15.0	15.7	-	-
"	-	46	132	F	118.7	144.0	76.0	76.6	15.3	15.8	20.1	20.9
"	-	46	131	F	124.1	138.8	76.6	76.9	15.3	16.0	20.0	20.8
"	32	47	-	M	103.7	124.2	77.5	77.5	-	-	-	-
"	35	52	134	F	108.5	135.8	75.3	76.3	14.3	14.9	19.0	19.6
"	20	55	-	F	109.0	136.0	78.0	77.9	15.6	16.0	20.0	20.5
"	35	55	159	M	101.2	142.0	77.2	77.5	14.5	15.1	18.7	19.4

Table 3. Ash and phosphorus values obtained in the age range of 20 to 60 days.

Strain	No. Rats	% Ash		% Phosphorus			
				dry basis		in ash	
		Mol.	Incis.	Mol.	Incis.	Mol.	Incis.
Susceptible	37	76.7±0.9	75.8±1.1	15.0±0.2	15.5±0.3	19.6±0.3	20.5±0.4
Resistant	25	78.8±0.9	76.4±0.8	15.5±0.4	15.5±1.0	19.6±0.5	20.3±0.8
Stock	18	77.6±1.0	76.4±1.0	15.1±0.4	15.6±0.4	19.5±0.4	20.3±0.5

Table 4. Rates of deposition in and removal of radioactive phosphorus from the teeth of the resistant and susceptible strains and stock rats that were injected intraperitoneally with the isotope.

strain	at time of injection age (da.)	wt. (gm.)	sex	time after injection (hrs.)	% inject. dose found/100 mg. ash molars	incisors
stock	20	27	F	24	5.30	3.34
"	"	33	F	48	6.67	8.78
"	"	32	F	72	7.05	10.1
"	"	31	M	240	7.49	9.98
"	"	32	F	360	5.85	8.32
"	"	33	M	480	6.97	8.62
"	"	30	F	840	5.17	3.34
susc.	20	38	F	24	4.03	8.75
"	"	40	F	48	3.99	6.98
"	"	41	M	120	5.04	10.1
"	"	39	M	240	6.31	12.2
"	"	48	F	240	4.11	6.99
"	"	40	M	360	6.24	9.17
"	"	38	F	480	3.91	5.41
"	"	28	M	840	2.82	3.14
"	"	34	M	960	3.12	3.68
resist.	19	22	M	2	6.28	7.99
"	"	25	M	9	8.25	10.6
"	20	28	F	1	1.47	1.99
"	"	33	M	4	3.39	3.49
"	"	28	F	18	3.34	4.97
"	"	30	F	24	3.44	8.30
"	"	34	F	48	4.50	8.25
"	"	32	M	48	3.70	4.52
"	"	37	F	120	5.34	9.26
"	"	28	M	120	5.59	10.9
"	"	37	F	240	4.04	7.58
"	"	32	M	240	5.32	8.05
"	"	35	M	360	7.64	8.58
"	"	25	F	360	4.85	7.38
"	"	29	M	480	4.79	6.03
"	"	28	M	720	3.60	3.52
"	"	29	F	840	3.84	3.47
"	"	28	M	960	3.46	2.99

Continued next page

(Table 4 continued)

strain	at time of injection age (da.)	wt. (gm.)	sex	time after injection (hrs)	% inject.dose found/100 mg. ash molars	incisors
stock	24	33	M	24	2.50	4.75
"	"	31	M	48	3.72	8.79
"	"	30	F	72	4.27	8.45
"	"	33	M	120	4.00	8.98
"	"	35	M	240	3.76	8.08
"	"	31	F	360	4.02	9.08
resist.	25	55	F	24	1.55	3.41
"	"	54	M	48	1.79	3.41
"	"	44	F	120	4.13	8.10
"	"	50	F	240	2.77	5.52
"	"	35	F	360	3.99	7.81
stock	29	52	M	120	1.72	4.12
"	"	55	F	240	1.69	4.25
susc.	29	58	M	120	3.00	6.11
"	"	74	M	240	2.20	5.84
resist.	29	53	F	41	1.47	3.55
"	"	50	F	240	1.54	4.42
stock	32	73	M	24	0.97	2.41
"	"	66	M	48	1.07	2.27
"	"	61	F	72	1.50	3.90
"	"	59	F	120	1.31	3.75
"	"	65	F	240	1.55	4.42
"	"	58	M	360	1.52	5.06
"	35	74	M	480	0.82	2.27
resist.	35	42	F	24	1.41	5.01
"	"	64	F	48	1.06	3.04
"	"	39	M	120	1.68	6.23
"	"	55	F	240	1.46	4.98
"	"	63	M	360	1.19	4.70
susc.	33	85	F	120	1.10	3.44
"	"	90	F	240	1.05	3.12
"	35	110	M	48	0.92	2.90
"	"	83	F	360	1.39	3.83
"	"	90	F	480	1.08	3.77

Table 5. Radioactive phosphorus found in the teeth of offspring of females of the resistant and susceptible strains and stock rats that were injected with the isotope during pregnancy.

strain	gestat.* time bet. ing. and birth(da.)	age** offspr. sacrif. (da.)	wt. of offspr. (gms)	sex	wt. of teeth		% ash		% P in ash		%inj.dose found per 100 mg.ash	
					mol.	inc.	mol.	inc.	mol.	inc.	mol.	inc.
susc.	2	6	15	F	6.9	4.9	43.5	55.1	-	-	4.66	6.30
"	"	"	12	M	5.6	4.6	41.1	54.4	-	-	5.65	6.40
"	"	9	17	F	14.7	9.9	57.2	68.7	21.1	22.4	2.73	5.29
"	"	14	22	M	27.5	18.9	69.9	69.5	-	-	2.60	2.90
"	"	20	30	F	35.4	25.6	78.3	75.2	20.5	21.4	2.09	2.40
resist.	2	6	10	M	5.2	4.6	42.4	58.7	-	-	5.45	7.04
"	"	6	10	M	5.0	3.3	36.0	42.5	-	-	7.22	-
"	"	9	18	F	15.9	10.5	58.0	71.5	20.6	20.3	3.59	3.47
"	"	14	22	F	25.4	17.4	72.2	70.7	-	-	2.24	2.60
"	"	20	35	F	46.6	34.4	79.6	76.5	19.6	20.9	2.08	2.36
stock	5	6	10	F	5.3	4.4	54.7	59.2	-	-	0.20	0.24
susc.	15	6	15	F	6.9	5.2	55.0	46.2	19.1	30.6	0.11	0.19
"	"	9	25	M	17.7	9.4	56.5	58.5	-	-	0.96	1.06
"	"	14	35	M	34.5	21.1	72.5	73.5	20.3	24.7	0.77	0.82

* Abbreviated reading should read "gestation time between injection and birth (days)."

** Abbreviated reading should read "age offspring sacrificed (days)."

Table 6. In vitro adsorption of radioactive phosphorus by the teeth of the resistant and susceptible strains and stock rats.

strain	age (da.)	wt. of rat (gms.)	sex	wt. of teeth (mg.) mol.	wt. of teeth (mg.) inc.	% ash eth.gly.ex. mol.	% ash eth.gly.ex. inc.	% ash 600°C. mol.	pH	adsorp. of P^{32} (c/m/mg.ash) eth.gly.ex.ash mol.	adsorp. of P^{32} (c/m/mg.ash) eth.gly.ex.ash inc.	adsorp. of P^{32} (c/m/mg.ash) 600°C.ash mol.
susc.	20	40	M	61.8	43.6	85.1	78.4	-	7	46.8	78.6	-
stock	"	37	M	58.1	40.5	83.8	79.3	-	"	54.9	73.9	-
"	"	30	F	53.8	38.1	82.9	82.2	-	"	56.3	70.3	-
susc.	30	75	M	81.6	60.0	85.2	86.5	-	3	54.6	83.0	-
resist.	"	49	F	73.4	59.4	86.2	87.2	-	"	57.9	77.8	-
"	"	58	F	72.1	61.6	86.7	86.2	-	"	63.7	81.7	-
susc.	40	119	M	114.4*	-	88.3	-	76.5	"	57.2	-	14.6
resist.	"	93	M	93.2*	-	84.7	-	76.8	"	54.8	-	16.6
susc.	60	176	F	120.3	159.8	82.6	84.7	-	7	29.3	20.3	-
resist.	"	131	M	105.8	119.7	84.4	86.5	-	"	27.2	24.2	-

* Intact teeth were put into isotope solution during adsorption period of 8 hours. All other samples of teeth were ground with a mortar and pestle to fine crystals before tests were made.

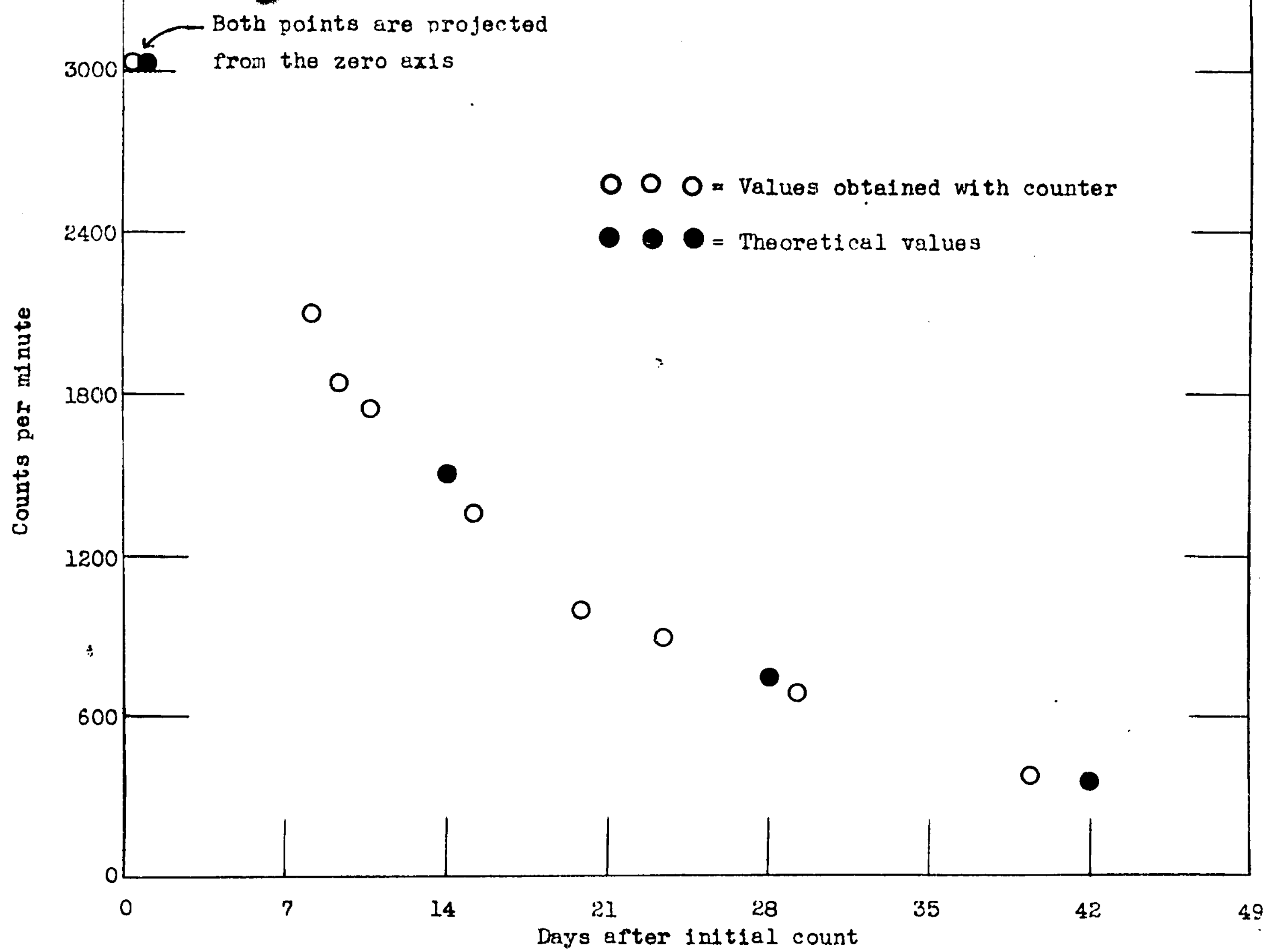

Figure 1. Comparison of the decay rate obtained on a standard solution of radioactive phosphorus and the theoretical (14.3 day half-life) decay rate.

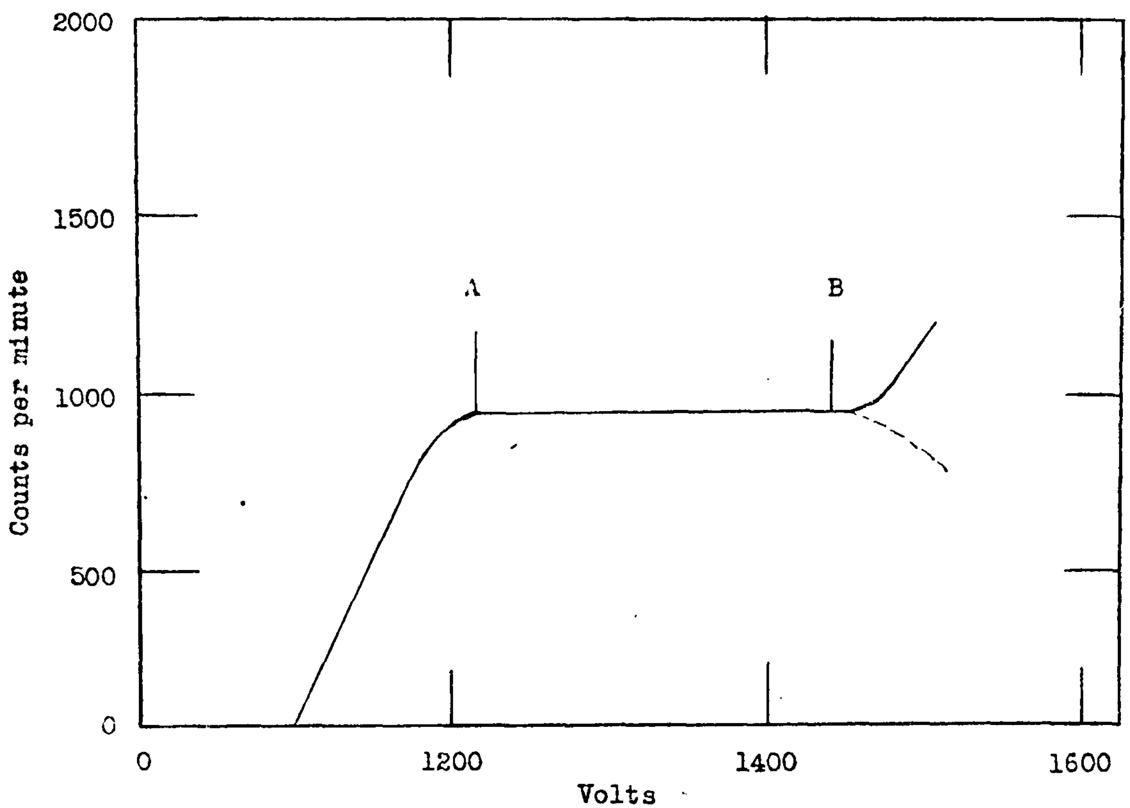

Figure 2. A characteristic voltage-counting rate curve .

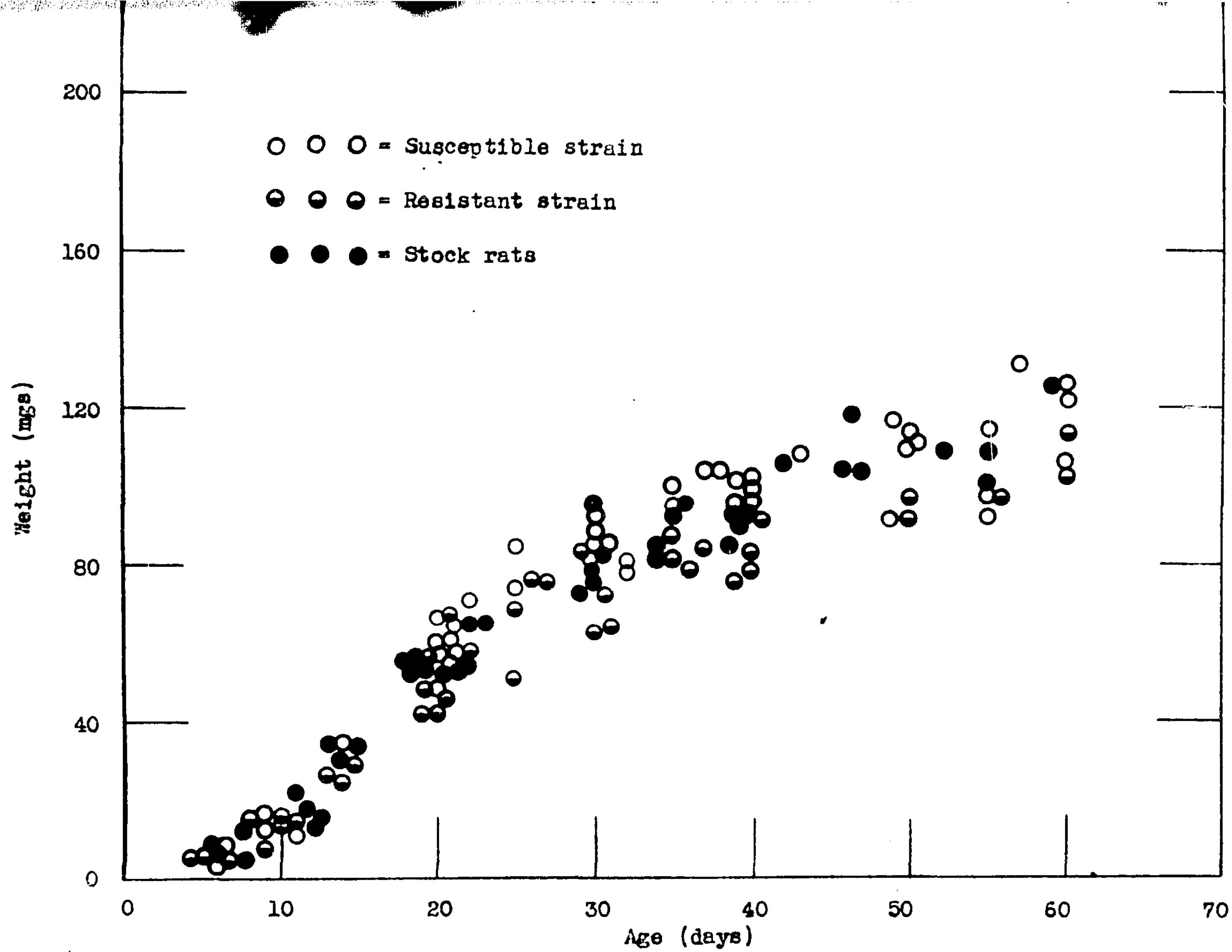

Figure 3. Weight of the molars of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

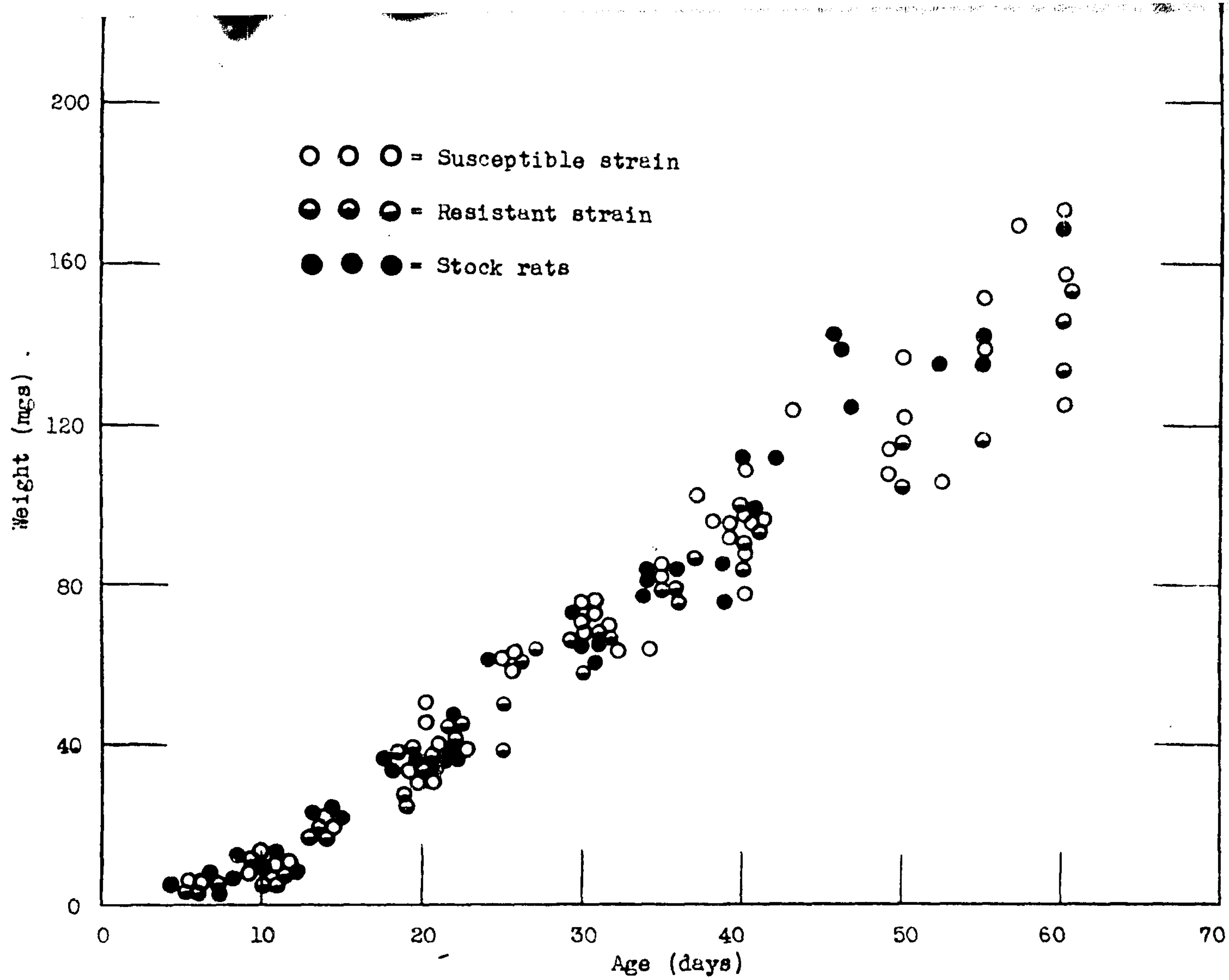

Figure 4. Weight of the incisors of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

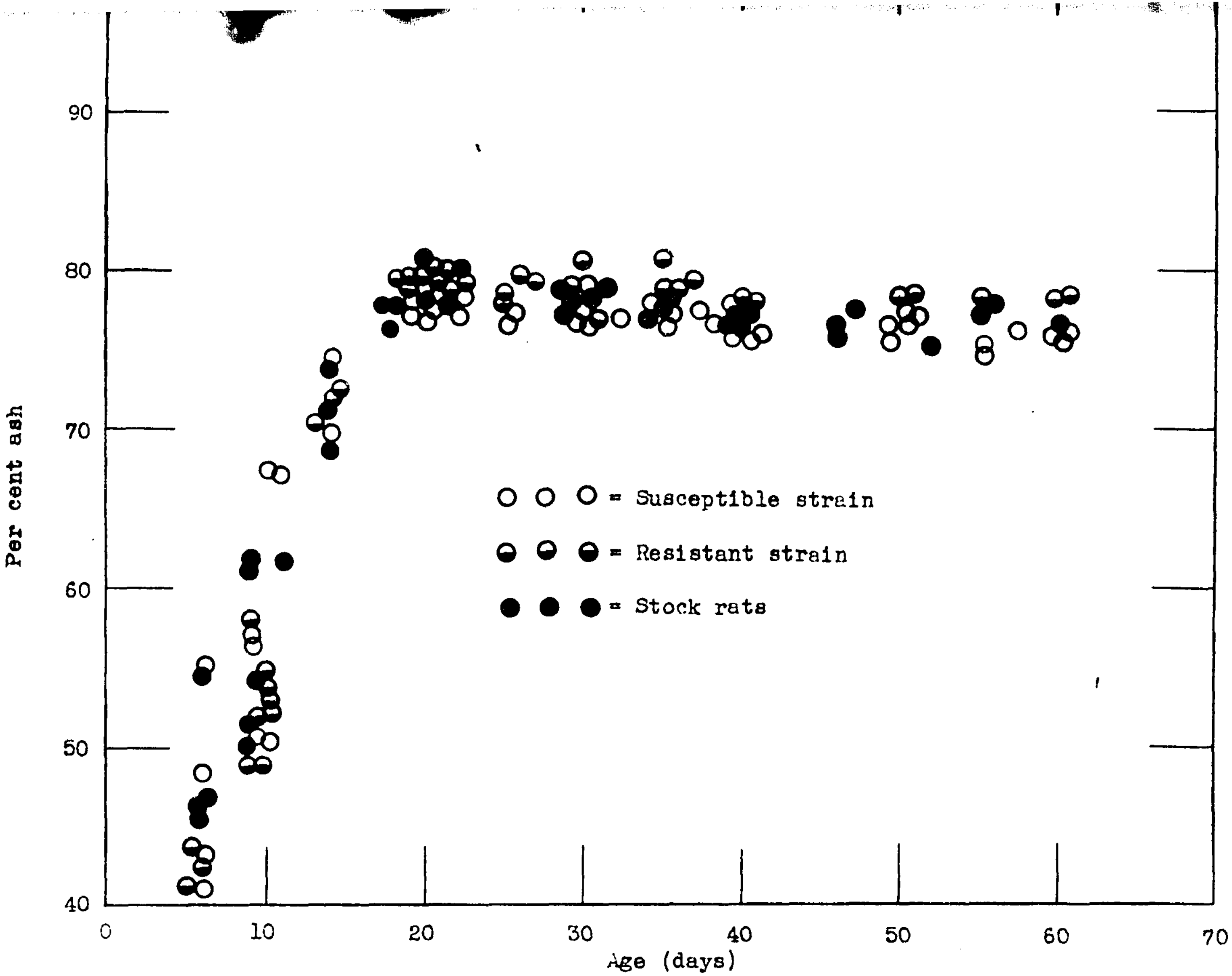

Figure 5. Per cent ash found in the molars of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

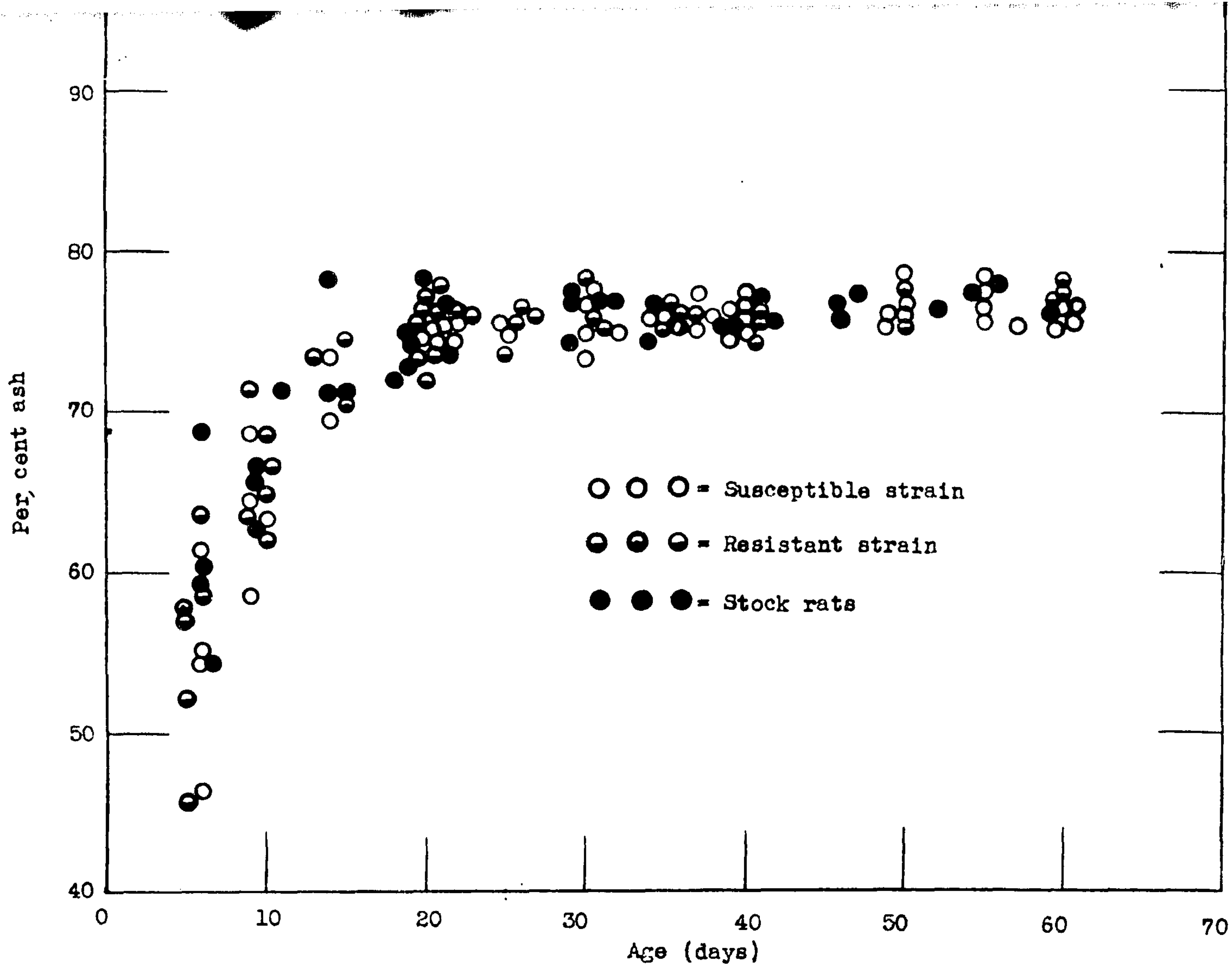

Figure 6. Per cent ash found in the incisors of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

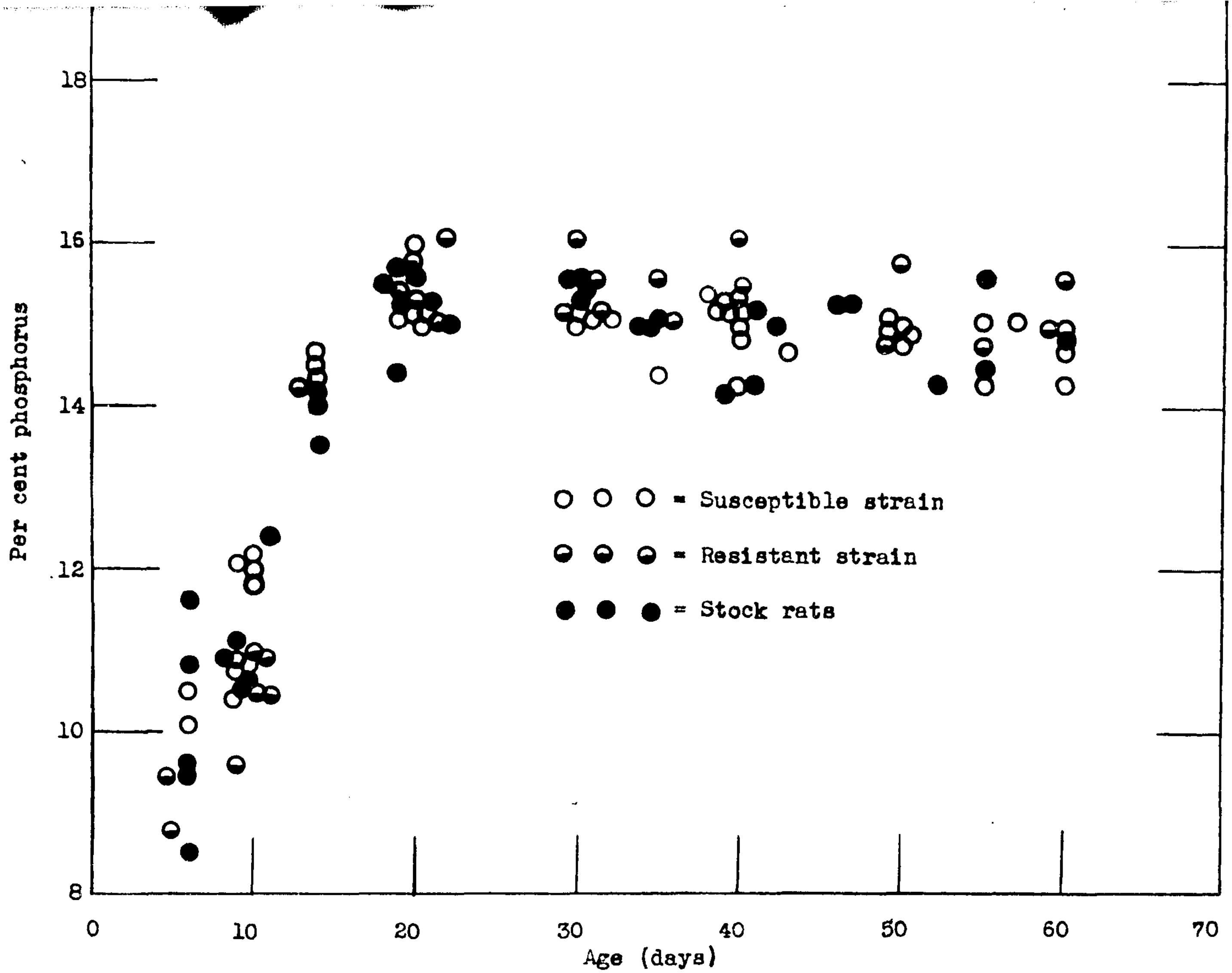

Figure 7. Per cent phosphorus found in the molars (dry basis) of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

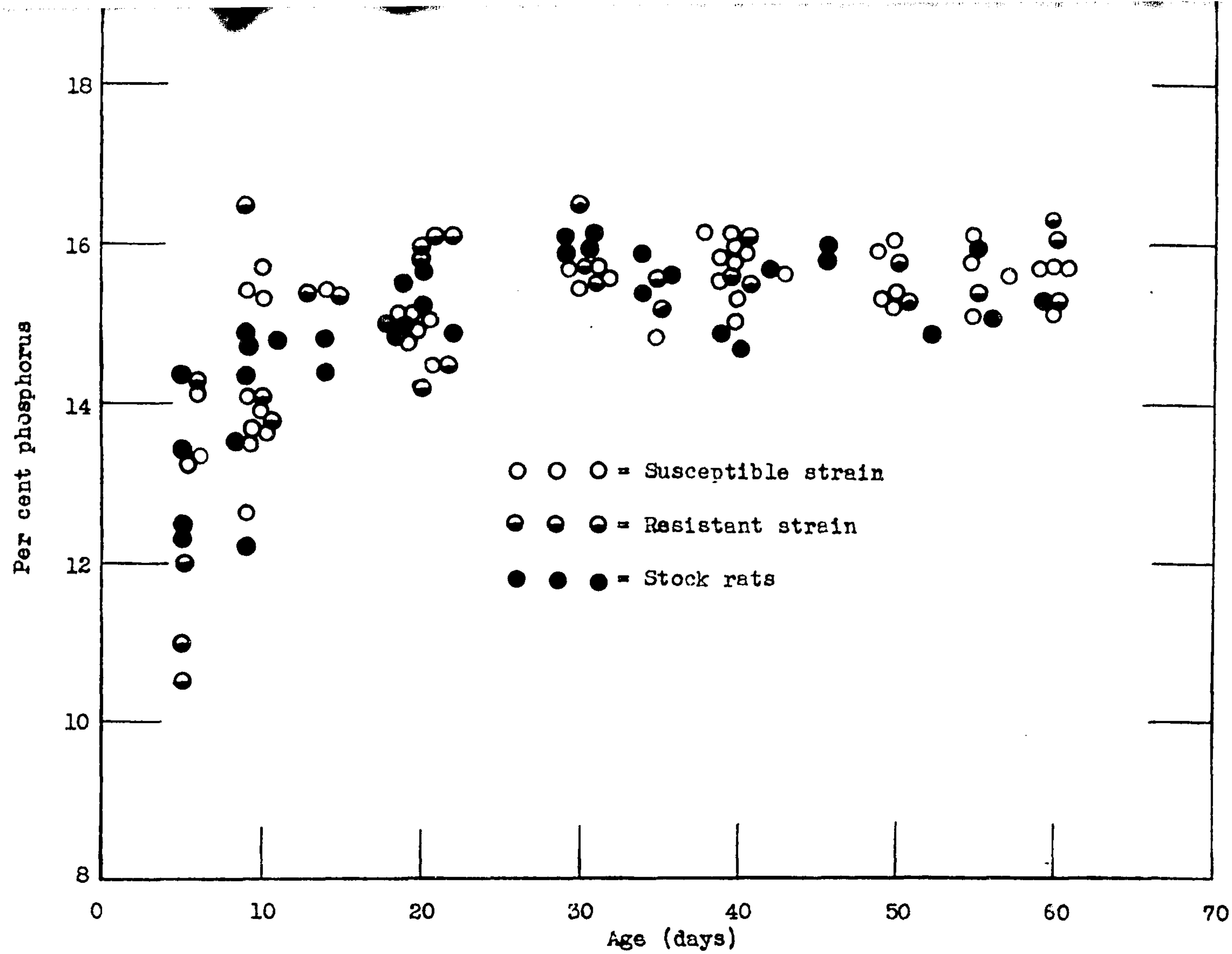

Figure 8. Per cent phosphorus found in the incisors (dry basis) of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

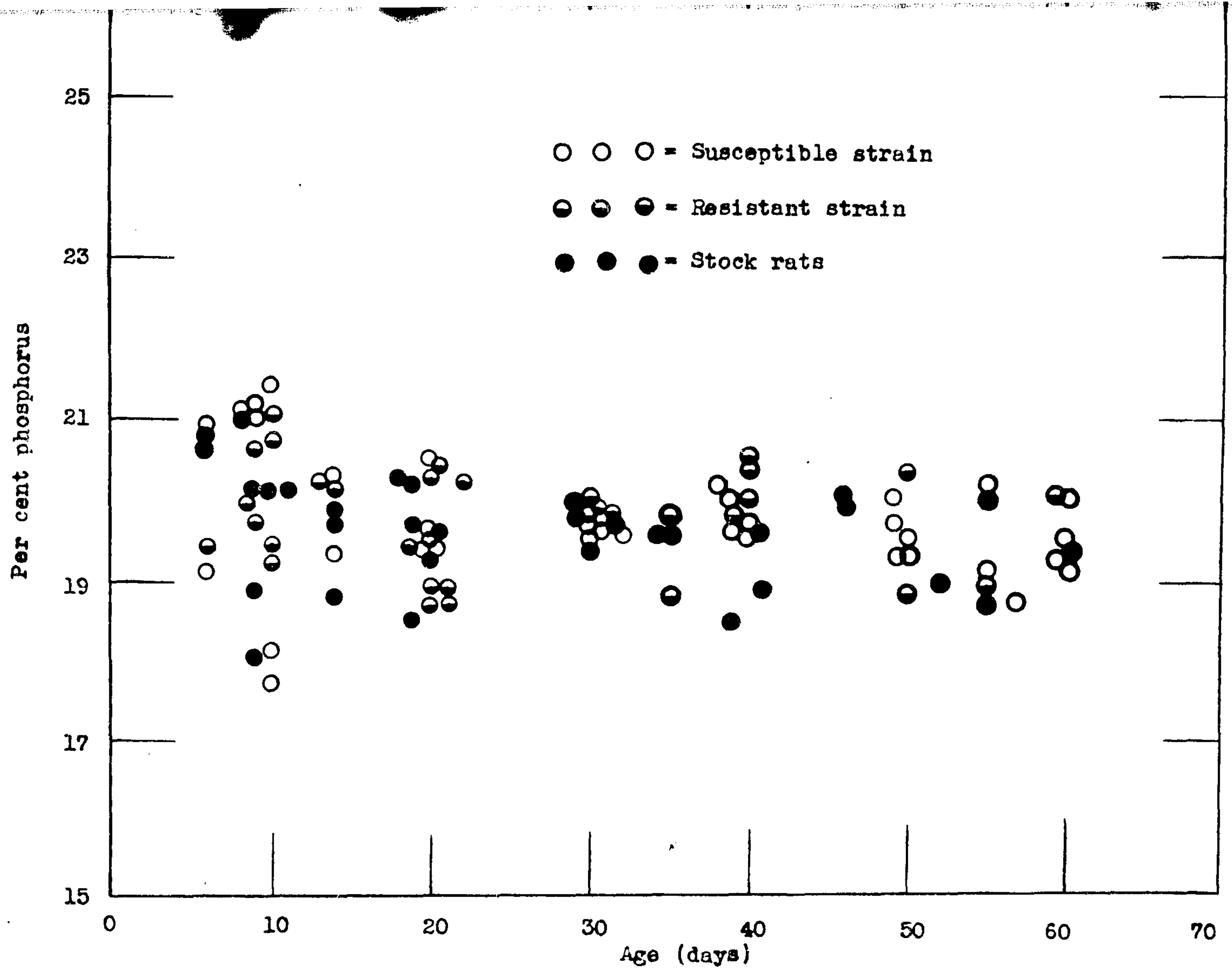

Figure 9. Per cent phosphorus in the ash of the molars of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

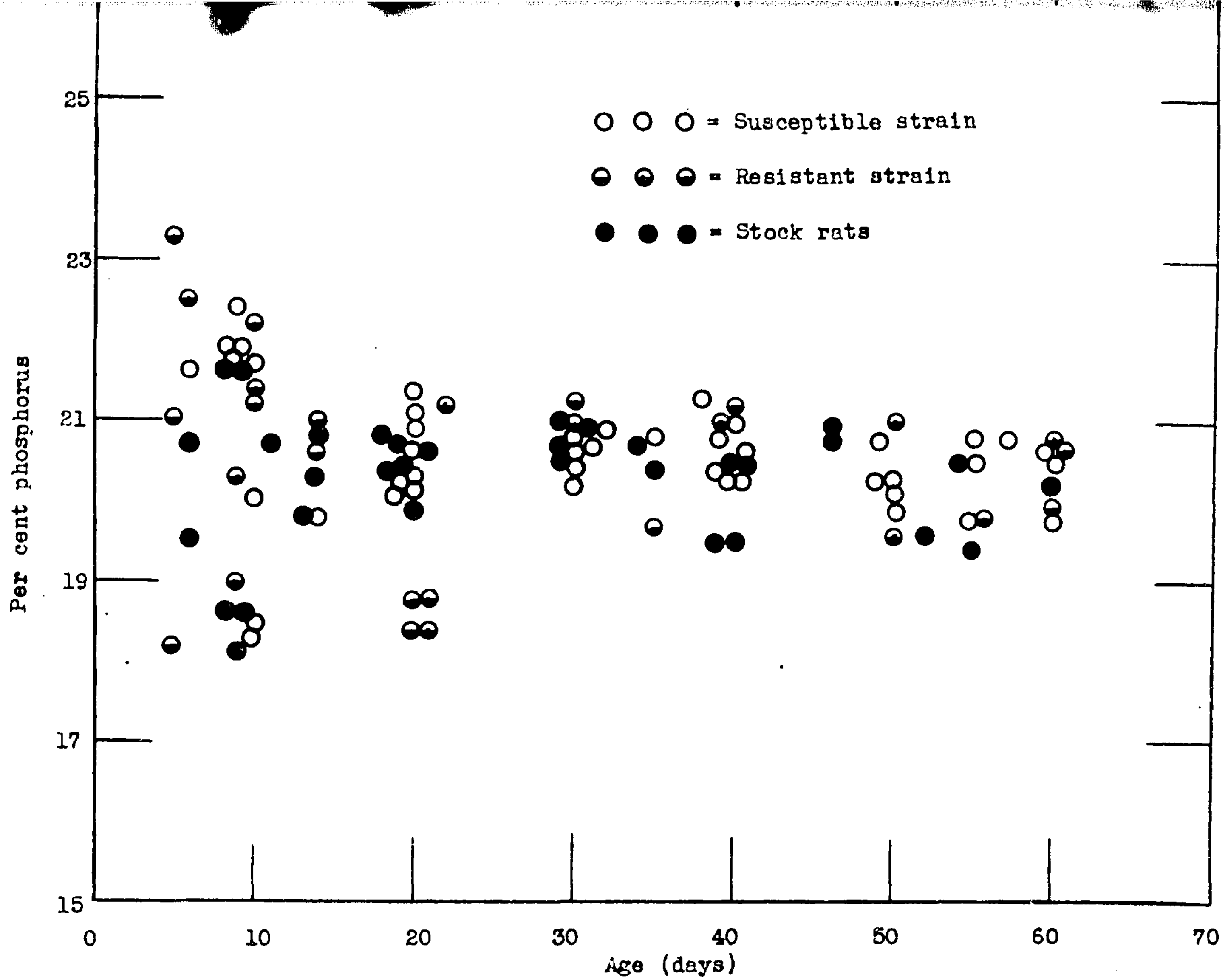

Figure 10. Per cent phosphorus found in the ash of the incisors of the susceptible and resistant strains and stock rats in the age range of 6 to 60 days.

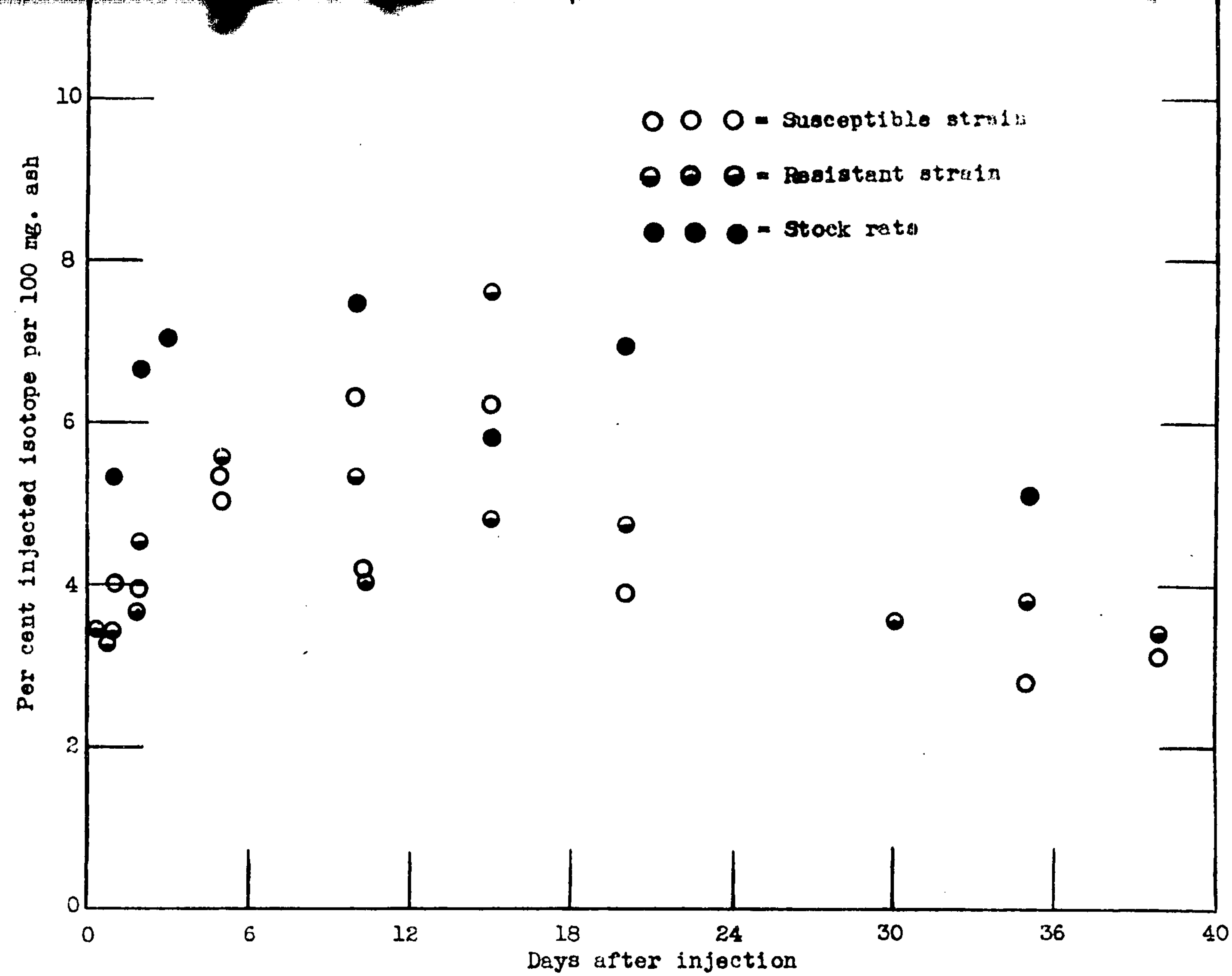

Figure 11. Rates of deposition and removal of labelled phosphorus in the molars of the susceptible and resistant strains and stock rats that were injected intraperitoneally with the isotope at 20 days of age.

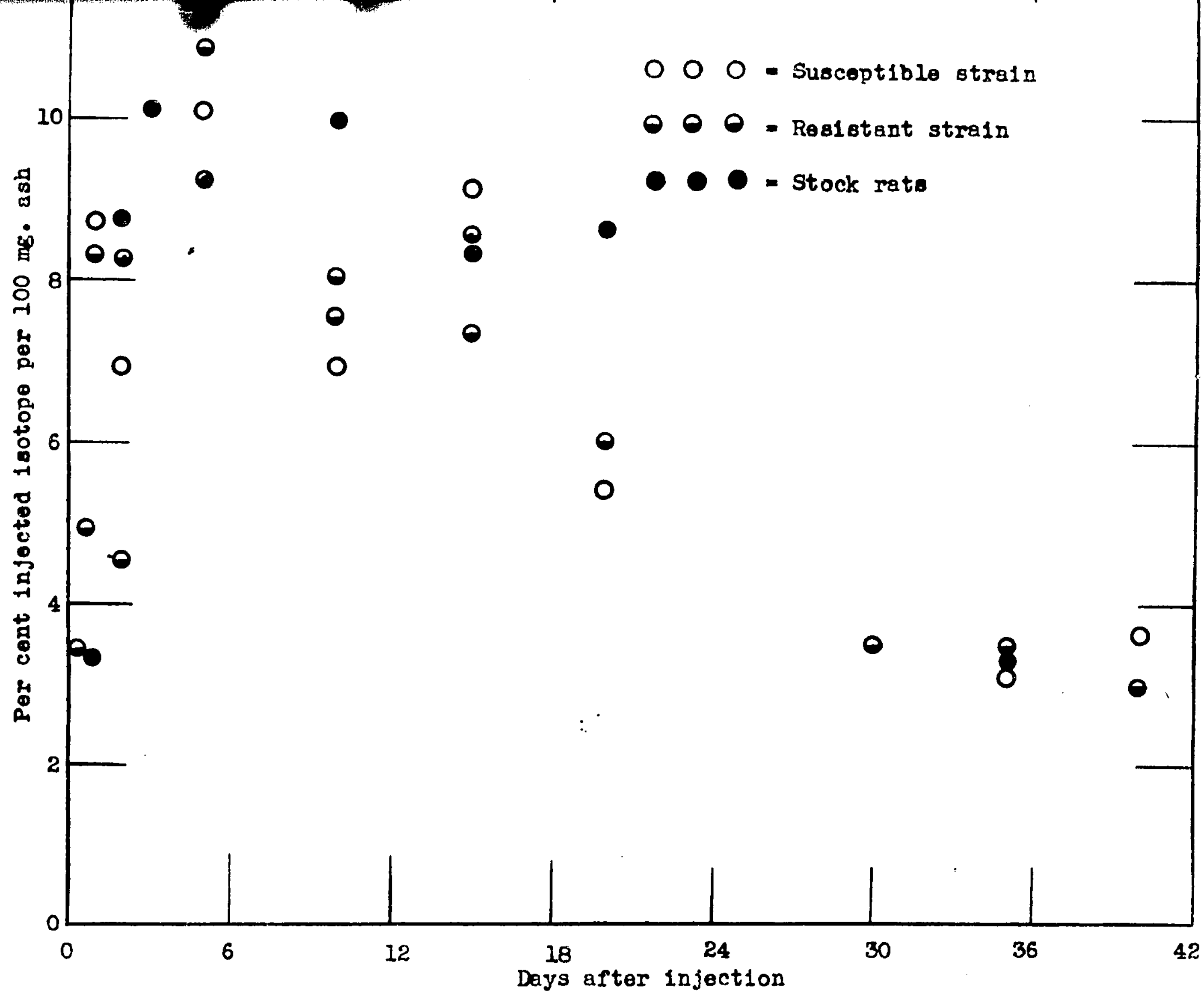

Figure 12. Rates of deposition and removal of labelled phosphorus in the incisors of the resistant and susceptible strains and stock rats that were injected intraperitoneally with the isotope at 20 days of age.

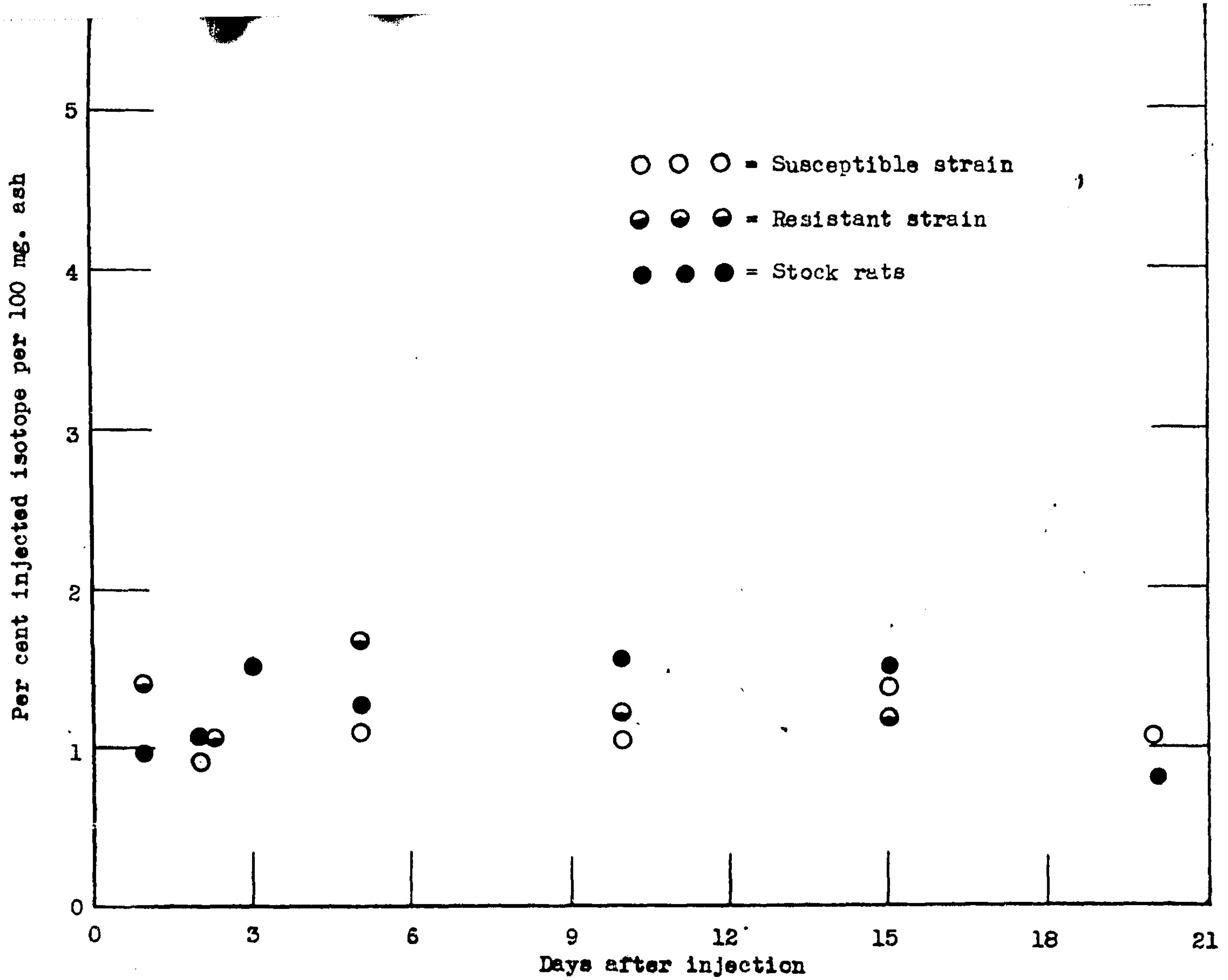

Figure 13. Rates of deposition and removal of labelled phosphorus in the molars of the susceptible and resistant strains and stock rats that were injected intraperitoneally with the isotope at 32 to 35 days of age.

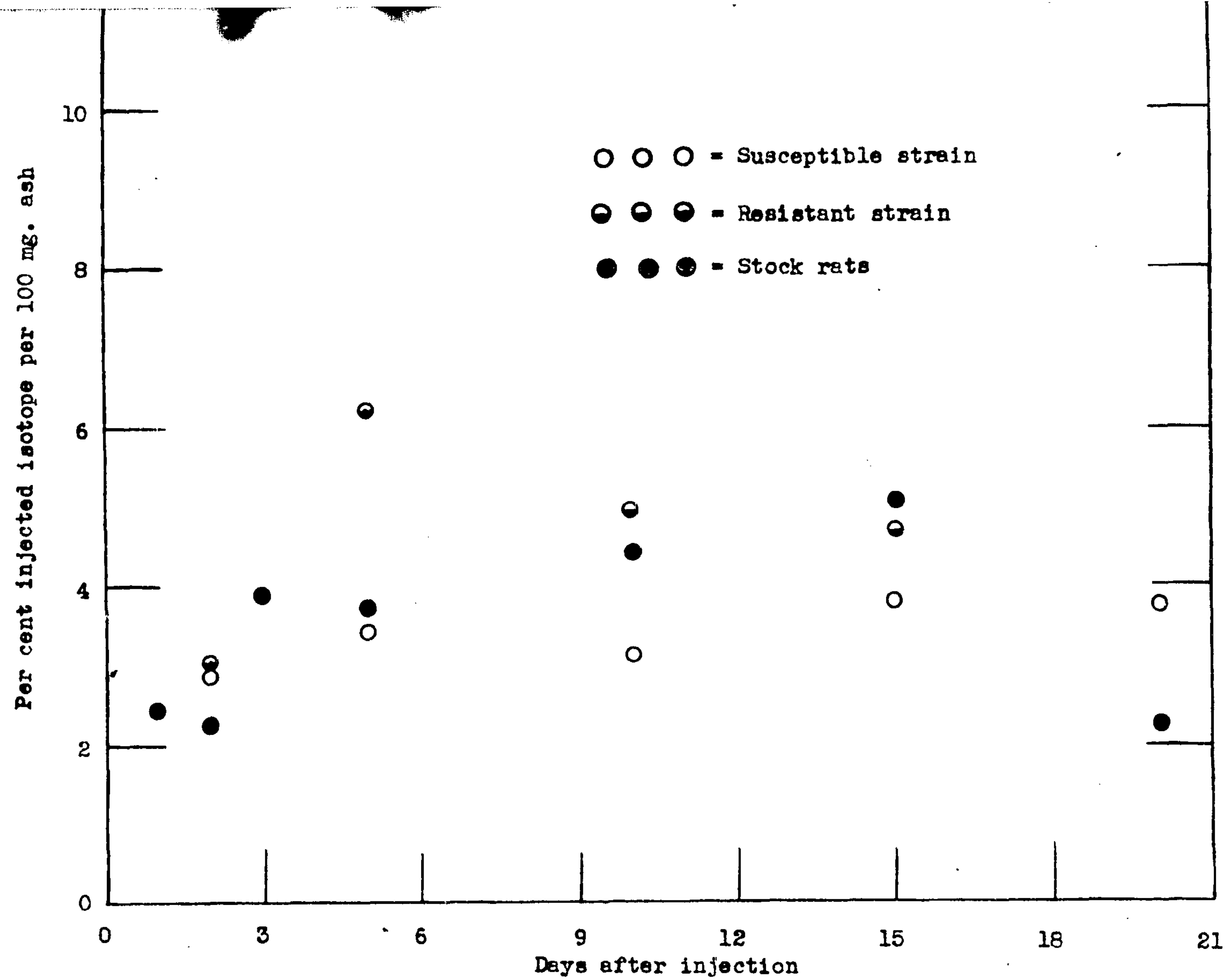

Figure 14. Rates of deposition and removal of labelled phosphorus in the incisors of the susceptible and resistant strains and stock rats that were injected intraperitoneally with the isotope at 32 to 35 days of age.

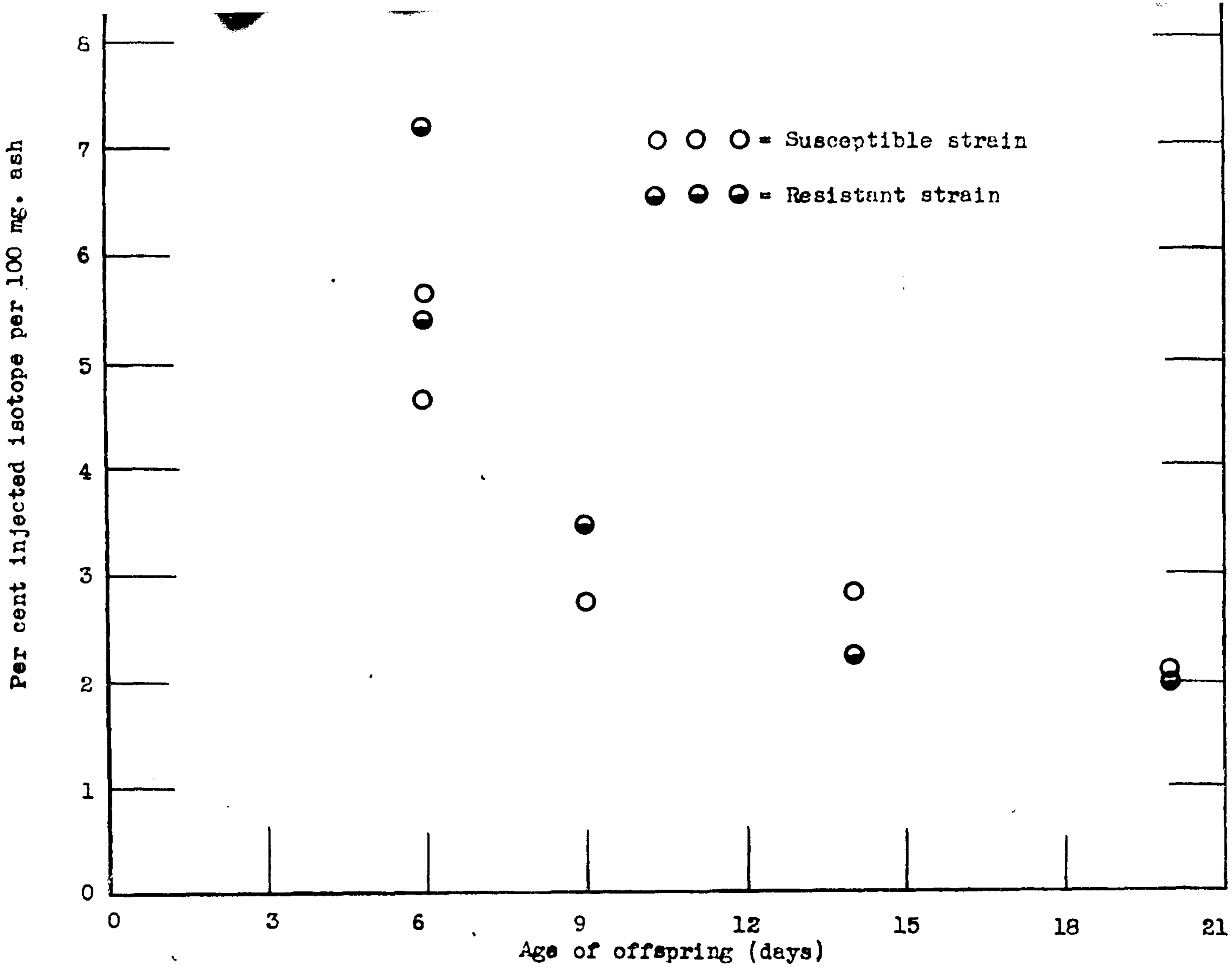

Figure 15. Per cent labelled phosphorus found in the molars of young rats of the susceptible and resistant strains whose mothers had been injected intraperitoneally with the isotope during pregnancy.

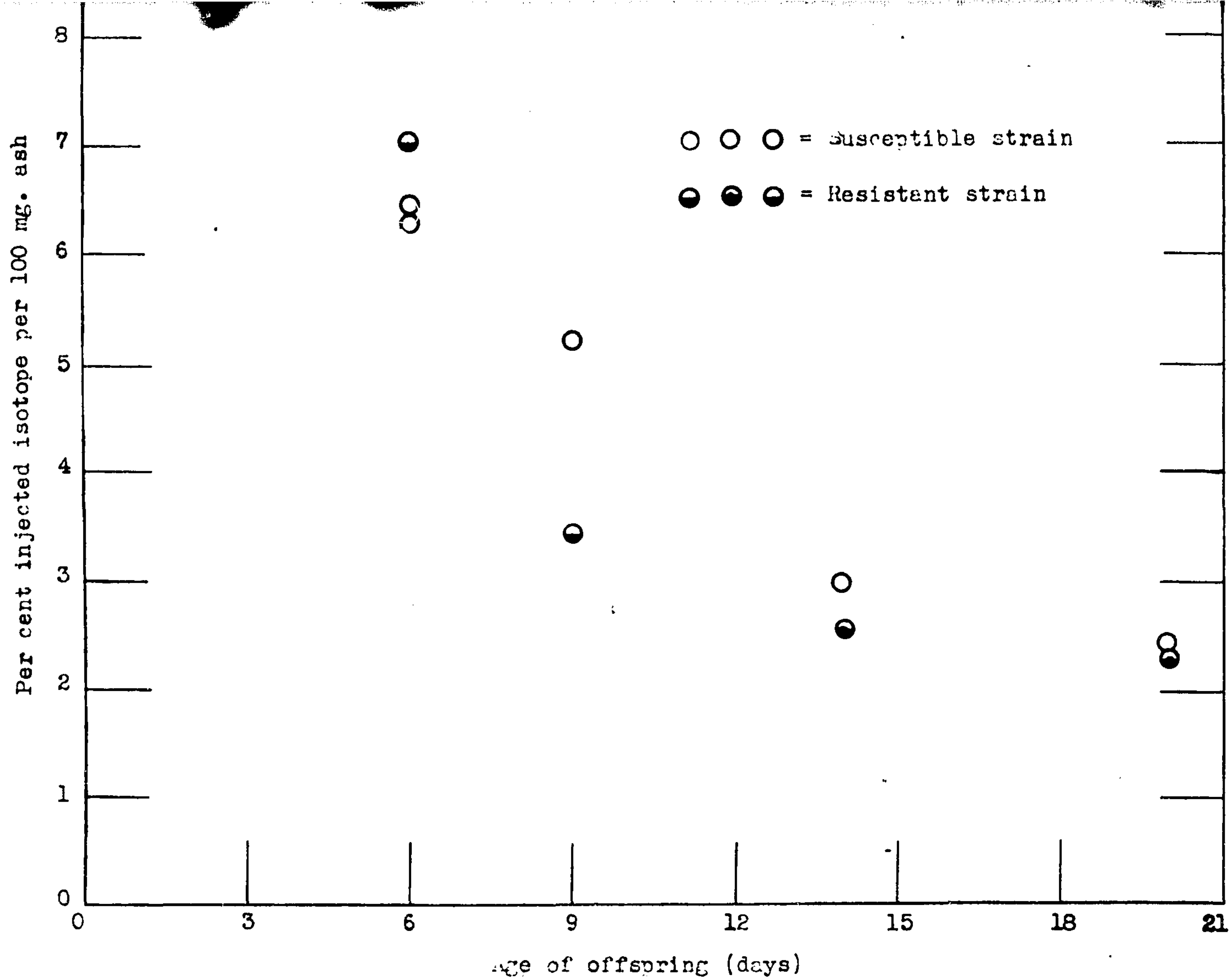

Figure 16. Per cent labelled phosphorus found in the incisors of young rats of the susceptible and resistant strains whose mothers had been injected intraperitoneally with the isotope during pregnancy.

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