

Fig. 1. Average body potassium concentrations of males and females as a function of chronologic age (grams per kilogram of gross body weight).



Fig. 2. Change in male and female potassium concentrations in relation to growth (as indicated by weight gain).

tween the ages of about 9 and 14, potassium concentration continues to drop rapidly; this suggests that relatively less muscle mass is added with increasing age and weight. Accelerated muscular growth occurs in the male at puberty, giving rise to a second maximum in potassium concentration at about age 16. The absolute rate of muscular development, however, is even more pronounced than is indicated by the potassium values (since this is also a period of continuing rapid skeletal growth which would tend to lower the potassium concentration). The pronounced decline in potassium concentration in males between the ages of 16 and 20 was rather surprising, since this is a period during which the rate of weight gain is decreasing also. Evidently gain in weight after age 16 is a result of a "filling out" process involving the addition of fat, connective tissue, and bone, with relatively little addition of muscle tissue. The foregoing interpretation of changes in potassium concentration in males and females with age is quite in accord with observations of age and sex variations in total body water reported by Edelman et al. (8).

The steady decrease in potassium concentration beyond age 20, with similar slopes for males and females (Fig. 1), is very interesting and worthy of further study. Multiplication of the potassium concentration by the average weight for each adult age group gives a measure of the amount of lean, oxidizing, protoplasmic mass and an indication of its variation with age and sex. Mean weights for the adult male age groups measured in our study showed essentially no increase in weight with age; therefore, the observed decrease in potassium concentration must represent a net loss in lean, protoplasmic mass. Between ages 20 and 60, this net loss in males amounted to about 18 percent. After correction for increase in gross body weight with increasing age, the net decrease in lean protoplasmic mass in females between ages 20 and 60 was only about 6 percent. This interpretation is in general agreement with observations of decrease in basal metabolic rate with increasing age (9) and supports von Döbelin's suggestion (10) that standard metabolic rate be expressed in relation to fat-free body mass.

Measurement of potassium-40 may offer an effective, statistical means of studying some aspects of the physiology of aging, exercise, starvation, and such wasting diseases as muscular dystrophy. The ease with which the potassium-40 measurements can be made [over 4000 people were measured, to a precision of 10 percent, during the recent 15-day Geneva Conference on Peaceful Uses of Atomic Energy, with an improved  $2\pi$ counter (11) and counting time of 40 seconds] enhances the attractiveness of this method for the study of large population groups (12).

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12. We are grateful to Mrs. B. E. Clinton for making the measurements on which this report is based. The cooperation of the many volunteer subjects is acknowledged, especially that of the Cub Scouts and Brownies who supplied data in the interesting region from ages 8 to 10. H. I. Israel was most helpful in programing for electronic data processing. This work was performed under the auspices of the U.S. Atomic Energy Commission.

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# Changes in the Pattern of Nitrogen Excretion during the Life Cycle of the Newt

Abstract. In the course of its life cycle the eastern newt, Triturus (Diemyctylus) viridescens, undergoes two metamorphoses, the first from the aquatic larva to the terrestrial red eft; the second, 2 to 3 years later, from the eft to the permanently aquatic and sexually mature adult newt. The pattern of nitrogen exretion changes during both transformations. Older larvae excrete about 75 percent of the nitrogen as ammonia, 25 percent as urea; during the first metamorphosis the ratio of ammonia to urea is about 57:43; completely transformed efts excrete 87 percent of the nitrogen as urea. Adult aquatic newts show a partial return to the larval pattern, with an increase in the proportion of ammonia from the 13 percent typical for the eft to 26 percent, a highly significant difference.

The studies reported in this paper were inspired by G. Wald's article "The significance of vertebrate metamorphosis," which appeared in the 12 Dec. 1958 issue of Science (1). Wald emphasized the basic biochemical changes that occur during metamorphosis from aquatic to terrestrial existence, and that are correlated with morphological and ecological changes. He also stressed the occurrence of a "second metamorphosis" at the time of reproduction, and the return of many sexually mature vertebrates to their natal environment, which they had left during the first metamorphosis.

No other species of amphibia exhibits these two metamorphoses as strikingly as the eastern newt, Triturus (Diemyctylus) viridescens. During the first transformation, the aquatic larva changes into the terrestrial red eft, which, during its 2 or 3 years of life on land, shows many morphological adjustments to the new environment. The epidermis is roughened and covered by a thicker cuticle; numerous multicellular glands appear in the skin; the lateral-line organs sink below the surface and become nonfunctional; nasal glands of various types are formed; eyelids and lacrymal glands protect the eye against drying; the tongue becomes muscular, protrusible, and well equipped with glands whose secretion aids in the capture of prey.

At the end of its terrestrial growth

phase the eft has reached sexual maturity and migrates back to a pond, impelled by an increased output of one of the pituitary hormones, probably the lactogenic factor (2). In the laboratory, the metamorphosed animal (eft) can be sent back to the water at any time, beginning with the day when it first leaves the water, by implantation of one-half or one-quarter of an anterior lobe of an adult pituitary, or by injection of minimal amounts of a prolactin preparation. In contrast to other amphibia, which leave the water at the end of the breeding season, the adult newt remains in its pond permanently.

The morphological manifestations of the second metamorphosis are also numerous: the epidermis becomes smooth and covered with a thin cuticle; the lateral-line organs move to the surface and become functional once more; the tail acquires a broad keel; the tongue is reduced in size and no longer protrusible; the lingual glands are less prominent.

At the biochemical level, Wald found that the photosensitive pigment of the

90 10-80 20 NITROGEN CUREA + AMMONIA) EXCRETED 70-30-60-50-50 40--60-TOTAL 30-70-ОF PERCENTAGE 20 80 10 90red efts young larvae adults mature metamorphosis newly larvae metamorphosed STAGE OF DEVELOPMENT

Fig. 1. Percentage of total nitrogen (urea plus ammonia) excreted as ammonia and urea in various stages of the life cycle of the newt.

eye, which in the eft is predominantly rhodopsin, as in other terrestrial vertebrates, is replaced by porphyropsin, which presumably was the pigment of the larval retina, as he demonstrated in tadpoles of the bullfrog *Rana catesbiana*.

A second, and functionally even more important, biochemical change takes place in all amphibia during the first metamorphosis from aquatic to terrestrial or amphibious life: a shift in the form in which nitrogen is excreted. Like other aquatic vertebrates, amphibian larvae excrete primarily ammonia. A terrestrial vertebrate must conserve water and cannot afford to use large amounts to eliminate the highly toxic ammonia. Nitrogen must be excreted in a more harmless form, which demands less water for its removal. Adult amphibia, like mammals, transform the ammonia into urea by the ornithine cycle, the essential step of which is the splitting of the arginine molecule into urea and ornithine by the enzyme arginase (3). The detailed studies of Brown and Cohen (4) on Rana catesbiana showed

that the change to ureotelism occurs simultaneously with a rise in activity of several enzymes of the ornithine cycle.

Munro (5) demonstrated that a similar change from ammoniotelism to ureotelism at metamorphosis occurs in the toad (*Bufo bufo*) and in two species of European newts (*Triturus vulgaris* and *T. cristatus*) and also in the Mexican axolotl when metamorphosis is induced in the latter by injection of thyroxin.

The close relationship between the biochemical metamorphosis and the change in habitat is strikingly shown by the excretory events that take place in the South African clawed frog, Xenopus laevis (5, 6). In the metamorphosing animal the excretory pattern changes: instead of excreting approximately 80 percent ammonia and 20 percent urea (typical for the tadpole), the animal excretes 50 percent ammonia and 50 percent urea. However, the young frog never leaves the water, and its nitrogen excretion returns to the earlier pattern (74 percent ammonia and 26 percent urea).

The existence of two clear-cut metamorphoses in *Triturus viridescens*, the first leading from water to land, the second back to water, suggested that a study be made of the pattern of nitrogen excretion in all three phases of its life cycle (7).

Larvae at various stages of development were starved for from 1 to 3 days and transferred in small groups to a tightly stoppered collection flask containing 6 ml of tap water, where they were kept for 48 hours. Samples of the water were then analyzed for ammonia and urea. Urine from red efts was obtained directly by gentle pressure applied to the region of the bladder. Adult newts were starved for 3 days, placed in separate flasks containing 7 ml of tap water each, and left for 10 hours. The percentage of total nitrogen (ammonia plus urea) excreted as ammonia and urea was determined by the Conway microdiffusion method, which had been used by Munro (5) and by Underhay and Baldwin (6).

The results are summarized in Fig. 1. The transition from a predominantly ammoniotelic excretion to a predominantly ureotelic pattern is gradual and is not completed until several weeks after metamorphosis. Most important, the adult aquatic newt shows a partial return to the larval pattern; the percentage of nitrogen excreted as ammonia rises from 13 percent in the eft to 26 percent in the adult. This difference is statistically highly significant and would be expected to occur by chance in less than 1 case in 10,000.

Thus, the biochemical events that occur during the second metamorphosis of the newt, when it returns from a terrestrial to an aquatic life, include not only the return to the larval form of retinal pigment but also a change in the mode of nitrogen excretion, in the direction of the larval pattern.

In Triturus viridescens, both metamorphoses can be modified experimentally with great ease. The first metamorphosis can be accelerated with thyroxin or suppressed by thryoidectomy in the embryo or by raising the larvae in solutions of thiouracil. The second metamorphosis can be precipitated, and practically fused with the first, by treatment with pituitary or prolactin. The patterns of nitrogen excretion under these experimental conditions will be investigated.

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## Mummified Seal Carcasses in the McMurdo Sound Region, Antarctica

Abstract. Information was collected on 90 mummified carcasses of the "crabeater" seal in the ice-free areas of the McMurdo Sound region, Antarctica. The carcasses range from relatively well-preserved bodies to merely old, twisted, winddissected fragments of tissue. They are hard and dry and lie on the surface of the ground, mostly in valley bottoms. The arid, cold climate is ideal for retarding organic decay. One carcass was dated by radiocarbon analysis and found to be between 1600 and 2600 years old.

Mummified carcasses of the "crabeater" seal (Lobodon carcinophagus) lie scattered over the land surface 1 to 30 miles from the sea and up to 3000 feet above sea level in the ice-free areas of the McMurdo Sound region, Antarctica. A few such carcasses were noted on land many miles from the sea in this area almost 60 years ago by scientists of the early British antarctic expeditions (1). We noted 90 mummified seal carcasses during the 1957-58 field season (2). No doubt many others exist in the McMurdo

All except one of the identifiable carcasses are of the crabeater seal. One is a Leopard seal  $(Hydruga \ leptonyx)$  (4). A fairly well-preserved carcass of an Adelie penguin was found lying on the ground 15 miles from the sea near seal carcasses on the west side of the sound.

The leathery dry carcasses are in various states of preservation; some are relatively well-preserved, and others are merely old, twisted, wind-dissected fragments of tissue. The well-preserved ones range in length from  $3\frac{1}{2}$  to 7 feet and in diameter from 1 to  $1\frac{1}{2}$  feet. They are dry and hard, and they have hair only on the side in contact with the ground; this side is generally flat and has a strong smell.

We found seal carcasses in every icefree area we visited in the McMurdo Sound region except Black Island and Ross Island. Twenty-five percent of the remains were found within a mile of the sea, but scattered groups of 2 to 19 specimens were found as much as 17 miles inland. The carcasses in each group were spaced 10 to 100 feet apart.

Most of the carcasses were found in the valley bottoms, many along courses of ephemeral streams. Most of the streams do not drain into the sea but into small, ice-covered lakes in valleys blocked from the sea by a moraine or a glacier. Several seal carcasses were found along the edges of these lakes. Many were found at the heads of ephemeral streams where the streams issue from glaciers, or at the heads of stream valleys.

All the carcasses noted were on land except one which was found half embedded in the ice cover of Lake Bonney at the upper end of Taylor Dry Valley. All of those on the land lie on top of the ground and most have 2 to 4 inches of coarse, windblown sand banked against their windward sides.

The age of the mummified seal carcasses in the ice-free land of the Mc-Murdo Sound region is intriguing. The remains have been thought to be perhaps 100 years or so old, because the arid and cold climate of the area is ideal for retarding organic decay. Radiocarbon analysis of one carcass showed that it is between 1600 and 2600 years old; another is being analyzed.

The material, which was dated at the Lamont Geological Observatory, Columbia University (sample L-462B), was from a brown, weathered fragment 1 foot wide and 4 feet long. It was found at an elevation of 1640 feet above sea level on glacial drift overlying a bedrock bench on the north side of Mount Nussbaum in Taylor Dry Valley. E. A. Olson and W. S. Broecker of Lamont Geological Observatory report as follows (5):

"Since the radiocarbon age of any organic sample requires a knowledge of initial radiocarbon concentration, it is customary to assume this to be the same as in a similar contemporary sample. In the case of antarctic seals, no presentday material was available, so that we have had to assume two extreme values and thus to quote an age interval rather than a discrete age. A lower age limit involves the assumption that the seal's diet consisted entirely of marine organisms deriving their carbon from surface water adjacent to Antarctica. Based on measurements of the dissolved carbonate in antarctic water which show it to be relatively depleted in radiocarbon, an age of 1700 ( $\mp$  100 years is obtained. An upper limit of 2500 ( $\mp$  100) years is obtained if the Lamont contemporary wood standard is used in the age calculation. Hence, the seal age almost certainly lies within the interval 1600-2600 years."

We believe that the antarctic seals, which occasionally wander inland, find no food in the fresh or alkaline lakes and therefore die. The cold, arid climate preserves their carcasses an incredible length of time, and the remains of seals and other animals that have wandered inland during the last 2000 years probably still exist to attest the animals' last journey (6).

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- The work was done under the auspices of the National Academy of Sciences, U.S. National Committee for the International Geophysical Year.
- An earlier report was made by the senior au-3. All carlier report was made by the scholar du-thor at the Symposium on Antarctic Research, Wellington, New Zealand, 18–22 Feb. 1958. See *Polar Times* (June 1958), p. 11, col. 4. Identified by David H. Johnson, curator, Di-vision of Mammals, Smithsonian Institution, Washington, D.C., from a lower jaw of a
- 5. Written communication to the senior author. 16 Apr. 1959. Publication of this article was authorized by
- 6. the director of the U.S. Geological Survey.

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