CESIUM-137 METABOLISM BY *BLATTA ORIENTALIS* IN VARIOUS THERMAL ENVIRONMENTS

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ABSTRACT

The effect of temperature on cesium-137 metabolism in the oriental cockroach, *Blatta orientalis*, was studied. Adult and nymphal animals were fed cesium-137 to investigate the biological half-time, excretion rate, retention curve and distribution of the isotope among the body tissues.

Metabolic rate studies at different temperatures showed an exponential relationship between oxygen consumption and temperature. The Q_{10} was 2.56 for adults and 2.20 for nymyhs in the 10°C-35°C range.

Cesium-137 elimination rate increased as environmental temperature increased. The Q_{10} for cesium-137 elimination was constant (1.2) over the 15°-35°C range and greatly increased over the 10°C-15°C range (2.05 and 16.4). The gut had a high concentration of cesium-137. Concentrations in the cuticle, as well as in other tissues or organs examined were very similar.

INTRODUCTION

Radioisotopes as tracers are clearly one of the most powerful tools in biological science research. Not only can radioisotope-labeled compounds be readily traced, but the ease and sensitivity of a radioactivity assay makes possible the detection of extremely minute amounts. For example, radiotracer techniques are used to measure of materials and energy along food chains (Crossley, *et al.*, 1962; Crossley, 1963b; Crossley and Howden, 1961; Reichle and Crossley, 1965; Auerbach, 1963), and to estimate feeding rates, metabolic rates, and productivity for the heterotrophic parts of food chains (Crossley, Corley and Tietjen, 1963; Odum and Golley, 1963; Crossley, 1963a; Crossley, 1963c; Davis and Foster, 1958). Radioisotopes are also used to study the dispersal, migration, life history, and behavior of insects in order to furnish information leading to their control, as well as to study the role of predators of disease vectors (Rings and Layme, 1953; Jenkins, 1963; Schmidt and Smith, 1963; Tomes and Brian, 1946). In addition to their ecological uses, tracers are also employed in the study of transfer rates, turnover rates, and metabolism of substances in living organisms.

Considerable attention has been given to the metabolism of fission products by health physicists, radioecologists, physiologists, and other radiobiologists. As aspect of radiobiology that has interested physiologists has been the relationship between electrolyte metabolism and temperature. An attempt to evaluate the effects of temperature on the metabolism of cesium-137 in the oriental cockroach, *Blatta orientalis*, is the subject of the study reported herein.

MATERIALS AND APPARATUS

Cesium–137, an alkali metal, is a member of the homologous series Na-K-Rb-Cs, members of which are group I elements of the periodic table. These elements have many similar properties. Cesium is widely distributed in nature and is usually associated with the other alkalis in small amounts. Cesium–137 is one of the most important contaminating radionuclides because of its high fission yield, chemical nature, long radiological half-time, and relatively high energies of emitted radiation.

Isotopes, to be useful in physiochemical studies, must have suitable radiation spectra, reasonably long physical half-times, and must be economically produced at high specific activities. Cesium–137 was chosen for this study for the following reasons; First, cesium–137 is an important radioisotope in radio-biology and has a long physical half-time (26.6 years) (Finston and Kinsley, 1961; Wiles and Tomlinson, 1955). In addition, cesium–137 has physical and chemical characteristics similar to potassium. Their physiological processes are also grossly analogous (Ringer, 1882; Kornberg, 1961; Relman, 1956). Cesium–137 emits beta particles with engergies of 1.7 Mev. and 0.52 Mev., and gamma rays with an energy of 0.66 Mev. Cesium–137 transforms by beta decay into barium–137 (Finston and Kinsley, 1961).

The oriental cockroach, *Blatta orientalis* Linnaeus, was used in the studies described. The nymphal cockroaches had an average weight of 0.427 g (range of 0.250 g–0.600 g). The male adult animals had an average weight of 0.338 g (range of 0.247 g–0.495 g). Adult females were excluded as experimental animals.

Stock animals were kept in an opaque aquarium-type container with a sandy floor and a wire screen top. Room temperature (22±2°C) prevailed. The cockroaches were fed Purina Lab Chow and water. After the initial whole body count was taken (see under Procedures), the cockroaches were deposited individually into clean cardboard cartons, each being 9 cm in diameter and 9 cm in height, and each having a 4 cm×4 cm transparent window top. Roaches were kept in the cartons, which were placed in temperature controlled incubators, for the duration of the cesium retention experiments to be described. Purina Lab Chow and a cotton ball saturated with distilled water were furnished.

Metabolic rates were measured with Gilson Medical Electronics' Gilson differential respirometer, Model G 20. The advantages of this unit are described by Gilson (1963). All radiation measurements were made with a Parkard Instrument Co.. Model 410A auto-gamma single-channel spectrometer in conjunction with a Model 440 Armar scintillation detector. This type of detector is ideally for studying the retention and excretion of various gamma-emitting radioisotopes in experimental animals due to its high counting efficiency and sensitivity. At least one millimicrocurie (1×10^{-9} curie) of cesium–137 can be detected with an accuracy of ±3% in 4 minutes counting time. Counting efficiency for cesium–137 is approximately 30%. Whole body counts were measured while animals were individually contained in a plastic counting cyclinder, 20.3 cm long, which insured centering the specimen in the

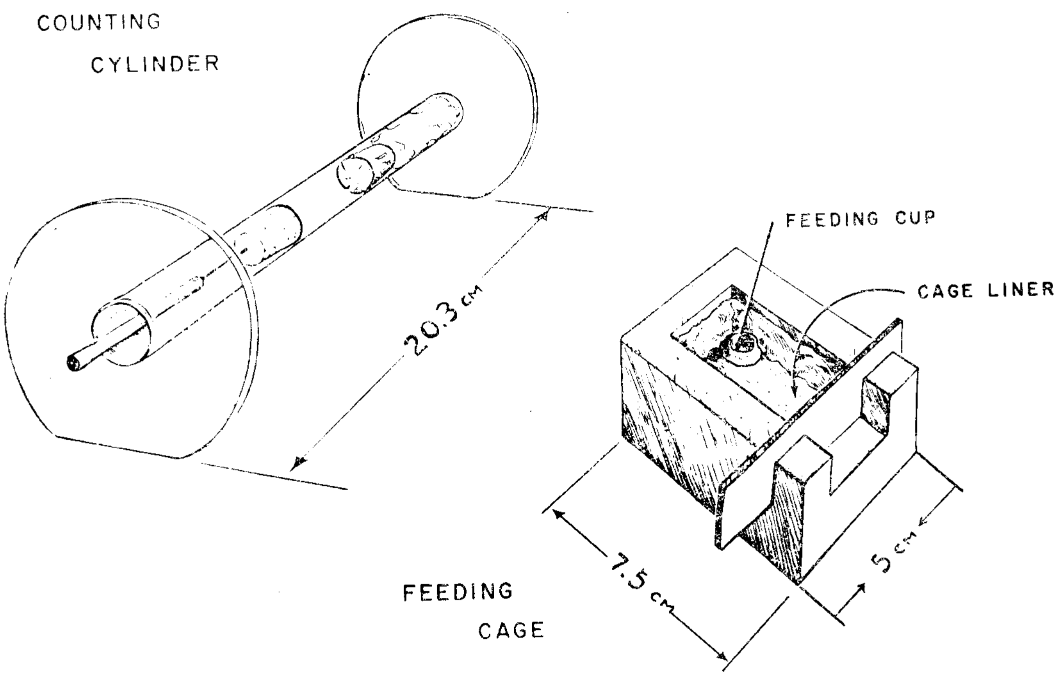

Fig. 1. Counting Cylinder and Feeding Cage

counting chamber (Figure 1). The chamber is a cyclinder 10.8 cm in diameter and 23 cm in length.

PROCEDURES

Metabolic Rate Measurement

A water bath provided a constant temperature during data collecting. Carbon dioxide produced by the animal was absorbed by 0.2 ml of 10% potassium hydroxide solution placed in the center jar of the reaction vessel. Precautions were taken to avoid dropping the cockroach into the potassium hydroxide solution. Prior to data collecting a minimum of 20 minutes shaking was required for equilibration. Six ten-minute readings were taken for each animal. The frequency of vessel shaking, as counted by the experimenter, was 72 per minute.

The micromenter readings were given digitally in microliters (μl). To convert to standard conditions, microliters of dry gas at 760 millimeters Hg and 0°C temperature, the microliter readings were multiplied by the following:

$$\text{Multiplying factor } (M_f) = \frac{(273)\ (P_b - 3 - P_w)}{(t+273)(760)}$$

where,

t = water bath temperature, °C.

P_b = operating pressure (usually the same as barometric pressure)

P_w = pressure of water vapor

3 was substracted to compensate for the specific gravity of mercury at room temperature.

The rate of oxygen consumption by individual animals was expressed as microliters (μl) of oxygen consumed per gram of fresh weight of animal per hour (μl/g/hr).

All metabolic rate measurements were made between 3:00 PM and 4:30 PM to eliminate biological rhythms as a variable.

Cesium-137 Administration and Measurement

Pilot studies included attempts to administer the cesium-137 by intra-thoracic punctures with small gauge needles, by feeding from a dropper, and by feeding from a cup.[1] The latter method, as indicated above, was eventually utilized in all cases.

The complete set of procedures employed for administering the isotope and for counting are given as follows: (1) the animals were starved, at room temperature, for six days (2) they were individually anesthetized with carbon dioxide, (3) weighed, and (4) placed in carboard cartons. After resumming activity each roach was induced to the feeding cage and finally reached the aluminum foil drinking cup which contained the isotope solution. Approximately one hour after feeding was initiated. each cockroach was allowed to crawl into a rectangular wire screen cage and was washed in running distilled water to eliminate the isotope from its mouth parts. The roaches were dried with paper tissue, their initial whole body count was measured with the auto-gamma spectrometer.

After establishing back ground radiation by making ten one-minute counts, three one-minute counts were averaged for all subsequent radioactivity measurements at each session of counting. The whole body counts were measured daily for the first eight days and thereafter at four day intervals.

The biological half-time is defined as the time required for the amount of a particular radionuclide in the body to decrease to one-half of its initial value due to elimination by natural biological process. For most radioisotopes the biological half-time should be corrected by following formula (Broda, 1960):

$$T_e = \frac{T_b T_p}{T_b + T_p}$$

where T_e is the effective half-time, or the observed half-time without correction for radioactive decay; T_b is the biological half-time; and T_p is the physical radioactive half-time. The data presented in this study, however, were not corrected for radioactive decay because the physical half-time of cesium-137 (26.6 years) is long in comparison with the observed half-time and radioactive decay can be ignored. The elimination rate may, therefore, be characterized in the term of biological half-time. Biological half-time may be determned by of several methods. Two methods

1) The solution in each cup was 0.1 μc of cesium-137 in 125 λ of distilled water. Cesium-137 had previously been obtained in a more concentrated form of cesium chloride from the Oak Ridge National Laboratory.

have been utilized in this study. (1) The method described by Crossley and Pryor (1960) appears to be the preferred method and is determined by extension of the straight-line portion of the log per cent retention versus time plot. The straight-line portion of the curve was obtained during the third to eighth day period, whereas Crossley and Pryor used the second to ninth day period for grasshoppers (1960). (2) An alternative method for determining biological half-time involves visual inspection of the log per cent retention versus time plot and estimating of the point at which the plot crosses the 50% retention line. The first method has advantage of reducing investigator prejudices as the 50% retention is calculated by the method of least squares. The second method has advantages regarding (1) ease of visual inspection, (2) the straight-line portion of the curve may not exist at the same time for different animals because of (a) regurgitation, (b) differences among individuals, (c) differences among species, and (d) some external factors, for instance, temperature.

Experimental Design

Eleven series of cockroaches were administered cesium-137 as described above. Each series was subjected to photoperiods of twelve hours dark and twelve hours light with a 60 watt bulb approximately 3 ft above the cartons containing the test animals. Four series (series I, II, III, and IV) consisted of adult male cockroaches exposed to 31±2°C, 15±2°C, and 10±2°C respectively, while four nymphal animal series (series V, VI, VII, and VIII) were placed in 35±2°C, 22±2°C, 15±2°C, and 10±2°C respectively. Additional nymphal roaches exposed to 35±2°C, 22±2°C, and 10±2°C were anesthetized, weighed and decapitated at 54 hours after initial feeding. Organs or tissues were removed and weighed immediately after sacrifice. Three one-minute counts were then made for each tissue, and the tissue retention indices (TRI) were calculated.

Biological half-times and retention curves were determined by the method of least squares. The small sample statistical technique was employed for analysis of data (Schueler, 1953). The 0.05 level of confidence was accepted as being significant.

RESULTS

Metabolic Rate

Metabolic rate, expressed as microliters of oxygen consumption per gram of live animal weight per hour ($\mu l/g/hr$), was plotted against temperature on semilogarithmic paper. Straight lines were obtained for both adult and nymphal cockroaches (Table 1 and Figure 2). The exponential relationship between temperature and metabolic rate may be expressed as

$$M = {}_{a\,b}T$$

or

$$\log M = \log a + (\log b)T$$

or

$$Y = a_0 + a_1 T$$

Table 1. Metabolic rate of *Blatta orientalis*

	Temp. °C	Number of animals	O_2 consumption μl/g/hr.	SD
Adult	25		450*	
	22	19	314.8	39.5
	20		277*	
	15	20	172.3	27.7
Q_{10} for adults=2.56				
Nymph	35	17	960.0	169.4
	22	20	374.5	80.6
	15	16	196.3	38.6
	10	33	129.9	22.9
Q_{10} for nymphs=2.20				

* values taken from Altman and Dittmer, 1964

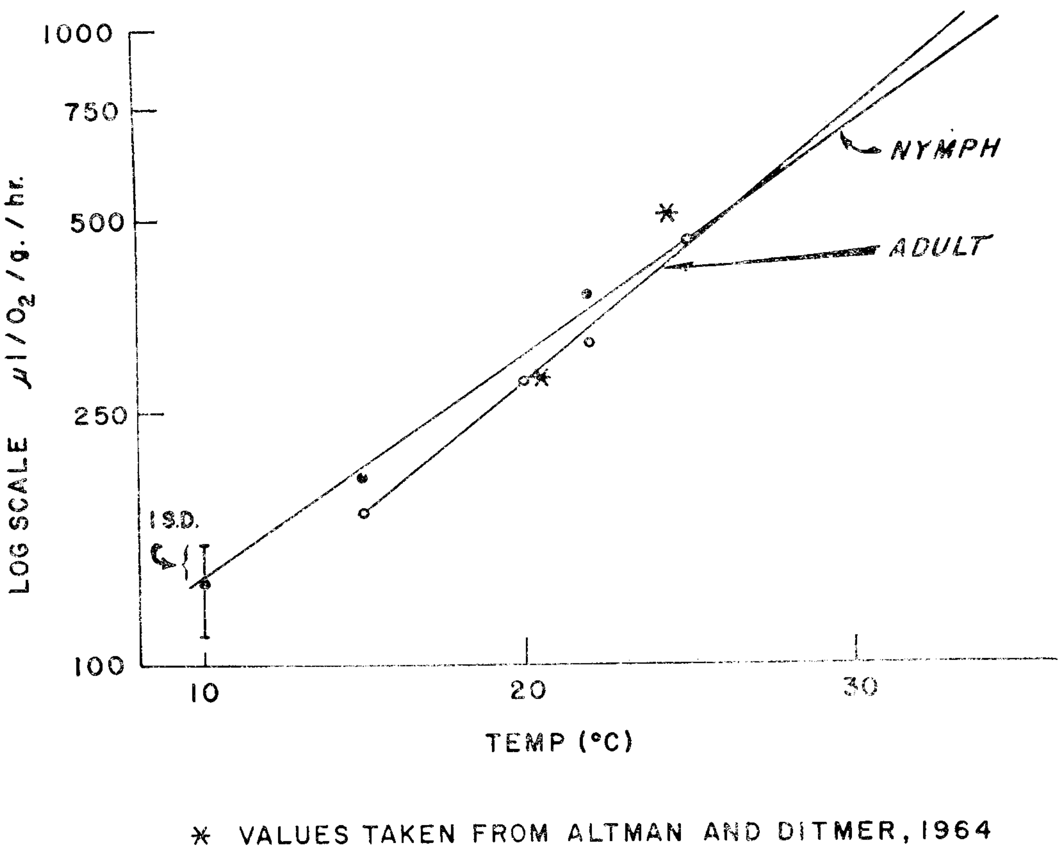

Fig. 2. Metabolic Rate of *Blatta orientalis*

where M = total metabolism or oxygen consumed per gram of animal per hour; T = temperature in degree Centigrade. The constant a_1 is the slope of the straight line. The constant a_0 is the Y intercept which is the value of Y (or log M), when T = 0. The equation calculated from data for nymphal cockroaches was:

$$Y(\text{or } \log M) = 1.777 + 0.03488\ T$$

and that for adults was:

$$Y(\text{or } \log M) = 1.6187 + 0.0409\ T$$

The slope for the adult animals was found to be steeper than that of the nymphs (0.0409 and 0.03488 respectively). The Q_{10} of nymphs was 2.20 and adults 2.56 within the experimental temperature range (10°C—35°C for nymphs; 15°C—25°C for adults) (Table II and Figure 2).

Cesium-137 Retention Curves and Biological Half-time

Data for cesium-137 retentions by nymphal and adult animals were plotted on semilogarithmic paper as per cent retention versus time in days. The retention curves exhibited an initial rapid drop, then a straightline portion, and after eight days a slower loss of cesium-137 (Figure 3 and Figure 4). The high rate of excretion shortly after administration of cesium-137 is similar to the pattern of cesum-137 excretion observed in mammals (Richmind, 1958). However, a slightly different curve was obtained for nymphal cockroaches at 10°C environmental temperature (Figure 4). The relationship between metabolic rate and cesium excretion rate can be examined by consideration of the Q_{10} values for these processes. Whereas the Q_{10} values for metabolic rate are nearly constant for the full range of experimental temperature (Table II and Figure 2), the Q_{10} values for biological half-time at low and high temperature are different (Table III and Figure 5).

Table II. Biological half-time of cesium-137 in *Blatta orientalis*

	Temp. °C	Number of animals	Tb½* d	Tb½** d	25% Ret.** d
Adult	31	10	5.2	1.7	4.3
	22	10	6.4	2.5	7.8
	15	13	7.4	5.5	18.8
	10	9	30	56	
Nymph	35	14	6.7	1.8	6.8
	22	11	8.4	2.6	12.2
	15	5	9.5	10.0	26(approx.)
	10	11	13.6	22(approx.)	

* Biological half-time, determined by extrapolation of the straight-line portion from the third to the eighth day of log per cent retention vs. time.

** Biological half-time and 25% retention, determined by visual inspection of log per cent retention vs. time.

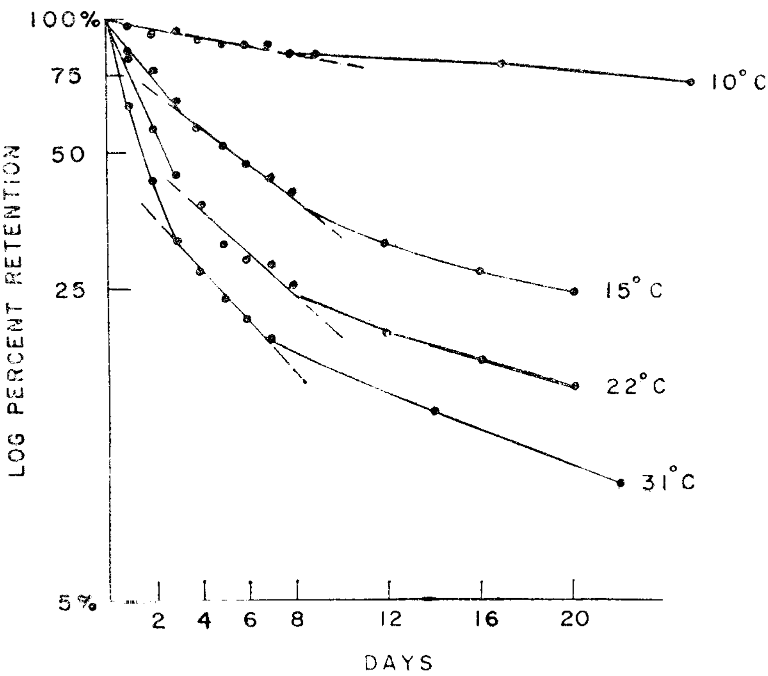

Fig. 3. Cesium-137 Retention by Adult *Blatta orientalis* in Various Thermal Environments

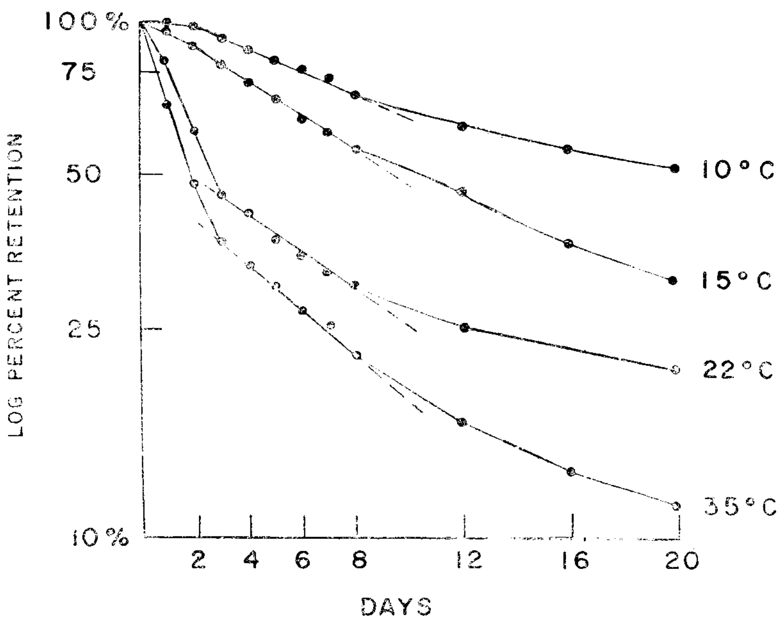

Fig. 4. Cesium-137 Retention by Nymphal *Blatta orientalis* in Various Thermal Environments

Table III. Q_{10} values for biological half-time

	Temp. range °C	Q_{10}**	Q_{10}**
Adult	31–22	1.26	1.54
	22–15	1.23	3.80
	15–10	16.40	10.40
Nymph	35–22	1.20	1.33
	22–15	1.19	6.85
	15–10	2.05	4.82

* Determined from the biological half-times which were determined by extrapolation of the straight-line portion from the third to the eighth day of log per cent retention vs. time.

** Determined from the biological half-times which were determined by visual inspection of log per cent retention vs. time.

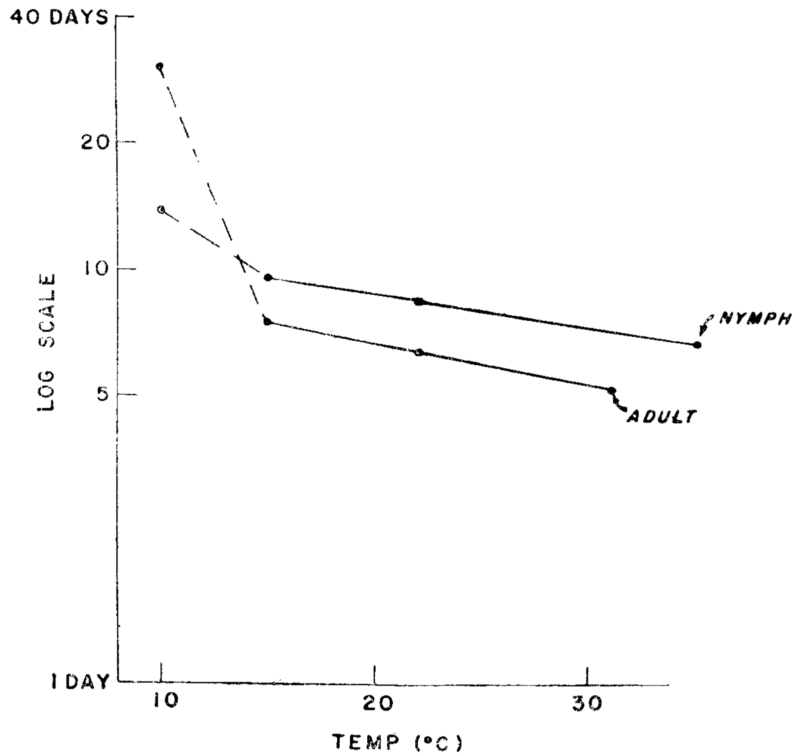

Fig. 5. Biological Half-times and Environmental Temperatures

Cesium-137 Distribution in Tissues

Absorptions and retentions of cesium-137 in tissues and organs were expressed as tissue retention indices (TRI). The tissue retention index is the ratio of radioactivity of the tissue to that of the whole body in units of weight, or

$$\mathrm{TRI}=\frac{\dfrac{\text{Counts per minute of tissue}}{\text{g. of tissue weight}}}{\dfrac{\text{Counts per minute of whole body}}{\text{g. of whole body}}}$$

Therefore, an index of more than 1.00 at any time indicates that cesium-137 concentration in the given tissue is greater than the average concentration of cesium-137 in the whole body (Lin, 1964).

Additional data indicated cesium-137 was unevenly distributed during the first few days and then became more evenly distributed (Chuang, 1966). The TRI of the gut exceeded 1.00 at all times but generally dropped rapidly from a relatively high figures after the first few days. The higher the temperature, the more rapid was the movement of cesium-137 from blood to the extravascular pool. In contrast, the TRI of other tissues or organs increased with time, although the total amount of cesium-137 in these tissues was decreasing. After 486 hours slightly more isotope was found in the leg (a very muscular structure) at 35°C and 22°C than in tissues other than the gut. The cesium-137 content of cuticle was similar to that of other tissues at all temperatures. A comparison of TRI from the three thermal environments indicates that animals exposed to the higher temperatures had the highest rates of absorption from the gut as well as highest rate of uptake of cesium by the tissues (Figure 6).

Table IV. Excretion rate* of cesium-137 at various thermal environments

	Temperature °C	Excretion rate
Adult	31	12.5
	22	10.48
	15	8.81
	10	2.06
Nymph	35	9.55
	22	7.86
	15	7.20
	10	4.93

* Excretion rate=average per cent excreted per day during the third to the eighth day period.

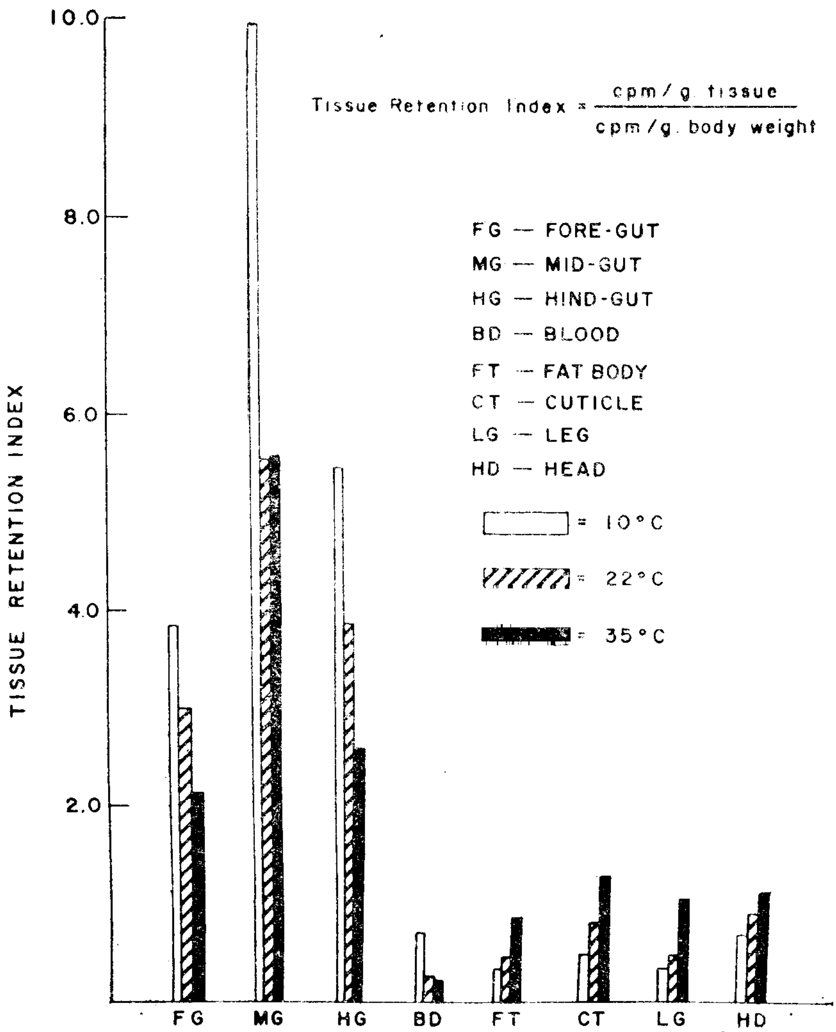

Fig. 6. Tissue Retention Index 54hr. After Administration of Cesium-137

DISCUSSION

Metabolic Rate

Metabolic rate, or oxygen consumption rate of animals vary betweem species and fluctuate according to activity, sex, temperature, nutrition, body size, stage in life cycle, season, time of day, precious oxygen experience and genetic background (Prosser, 1962).

In the present study the metabolic rate of *Blatta orientalis* correlated well with temperature changes in the 10°C to 35°C range and the 15°C to 25°C range for nymphs and adults respectively (Figure 2). This is similar to the results reported for many insects, but is different from the data regarding temperature regulating homeotherms, which have a fairly constant metabolic rate over a relatively wide temperature range.

The data suggest a Q_{10} of 2.20 for nymphs and 2.56 for adults. Adult animals had a faster increase in oxygen consumption with elevated temperature than did nymphs. At the lower temperature range (lower than 25°C) the oxygen consumption of adults seemed slightly lower than that of nymph. An explanation for this might be that nymphal animal are in the process of growth and utilize more energy for growth in a given time and processes of growth are less sensitive to temperature.

Cesium-137 Retention Curves and Biological Half-time

Crossley and Pryor (1960) presented a typical curve for the retention of cesium-137 by the grasshopper, *Romalea*. This curve is similar to the results in this study, an initial drop in radioactivity, then a long straight-line portion, and at the end of that portion there are a few points which suggest a further decrease in elimination rate. The value for the biological half-time of cesium-137 in *Romales* was described as being four to five days. The biological half-time of grasshoppers may be expected to be longer than that of roaches because larger animals usually have longer biological half-times than small animals (Bacq and Alexander, 1961). The mean biological half-time of cesium-137 in adult and nymphal roaches at 22°C ranged from 6.4 to 8.4 days (Table II). The differences between biological half-times of cesium-137 in grasshopper and roaches may be due to starvation prior to administration, diet as well as other factors. The high potassium content of plant food may be expected to facilitate cesium excretion.

The cesium-137 retention curve for grasshoppers and cockroaches were similar. The characteristics of the cesium-137 retention by various physiological compartments may be quite different among insects. However, additional information is needed to describe the cesium-137 metabolism in various physiological systems which make up overall retention curve.

In addition to methods involving retention, the excretion rate of the isotope, expressed as the average per cent excreted per day from the third to the eighth day, may also be useful in evaluating the relationship between metabolism of the isotope and temperature (Table IV). It is interesting to note that although animals at 15°C−35°C contained less cesium-137 during the third to eighth day period they excreted a larger percentage of the cesium than did animals at 10°C.

Effect of Temperature on Cesium-137 Retention

Metabolic rate of poikilotherms increases with temperature. This is principally due to the increased rates of chemical processes in the body as temperatures rise. Increased metabolic rate may result in a rapid elimination of cesium. However, the data from this study suggest that metabolic rate alone may not be entirely responsible for cesium-137 elimination. This conclusion is obtained when one compares Figure 3 with Figure 6. These illustrate, respectively, that while Q_{10} for metabolic rate is more than two, Q_{10} calculated from biological half-time of cesium-137 is less than two at higher temperatures, out greater than two between 10°C and 15°C.

Crossley (1963a) states that biological half-times of cesium-137 for immature stages of two grasshoppers and a chrysomelid beetle are shorter than those for the adults (his adult animals were larger than immatures). The half-times were not as short as were predicted from his calculated weight-biological half-time relationships. In other words, for animals of similar weight the nymphs had a longer biological half-time than adults. In the present study the average weight of the male adult cockroaches was less thant hat of the nymphs (0.338 g and 0.426 g respectively). The result of shorter half-times for the adult cockroaches at temperatures above 15°C parallels Crossley's results when weight is considered.

Extensive data, not included in this report, indicate that without doubt the route of cesium-137 elimination in *Blatta* is primarily via the feces. A relatively high tissue index for cuticle suggested molting may be an important route of elimination in roaches. Relatively low radioactivity was obtained from oöthecae, so it seems unlikely that any significant amount of cesium is lost by oviposition in these animals.

Cesium-137 Distribution in Tissues

As the fore-gut is considered, in part, to be a storage organ, it is reasonable to assume that cesium-137 may be temporarily stored there after feeding and prior to absorption via mid-gut and hind-gut. After being assimilated into the circulating blood, cesium-137 reaches the extravascular pool, including all the tissues that nutrient-carrying blood can reach. That most of the ingested cesium-137 concentrates in the soft tissues of vertebrates has been reported (Hood and Comar, 1953; Yamagata, 1962), and the same is true for the grasshopper, *Romalea* (Crossley and pryor, 1960). In this study, however, a considerable amount of cesium was also found in the cuticle (Figure 6). The tissue retention indices of cesium-137 for cuticle at 486 hours after ingestion were close to the average amount of cesium-137 in the whole body at the same time (Chuang 1966). Therefore, as indicated above, a considerable amount of cesium-137 may be lost by molting. The slightly higher TRI of the leg than of other tissues at 22°C and 35°C may indicate that more cesium is contained in muscle tissue, as was described in grasshoppers (Crossley and Pryor, 1960).

ACKNOWLEDGMENTS

The author expresses his gratitude to Drs. M. L. Riedesel, C. S. Crawford for their generous and thoughtful guidance through this study and in the prepartion of this report. Mr. W. R. Joyce's assistance during the experiments was very helpful and is appreciated.

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