AN ABSTRACT OF THE THESIS OF

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Title: METABOLIC ADJUSTMENT TO TEMPERATURE IN STRONGYLOCENTROTUS PURPURATUS (STIMPSON)

Abstract approved: Redacted for Privacy

Austin W. Pritchard

The metabolism of Strongylocentrotus purpuratus (Stimpson) was investigated at several levels of urchin organization. Some experiments were performed with tissues from recently collected (acclimatized) urchins. Other experiments were performed with tissues from urchins held at one of two temperatures (acclimated) for 30 days.

Body-component oxygen consumption was measured for body-wall, gut, testis, and ovary slices from seasonally acclimatized and temperature-acclimated urchins. Metabolic rates of testis and ovary underwent seasonal shifts appearing to compensate for changes in habitat temperature -- i.e., higher and lower rates during cold and warm periods, respectively. Rates of body wall and gut underwent seasonal shifts in a direction opposite those of testis and ovary -- i.e., inverse compensation. This gonadal-nonreproductive difference was

observed less conclusively with temperature-acclimated urchins. Compensatory shifts occurred with testis. Equivocal shifts occurred with body wall, gut, and ovary. Inverse compensation of metabolic rate resulted when oxygen-consumption measurements were repeated with gut slices and homogenates from temperature-acclimated urchins.

Radiotracers -- glucose-1-^{14}C, glucose-6-^{14}C, and acetate-1-^{14}C -- were incubated with gut, testis, and ovary homogenates from temperature-acclimated urchins. Cold acclimation caused higher CO_2 and lower lipid activities in the testis. Conversely, warm acclimation caused higher CO_2 and lower lipid activities in the gut. Conversion of glucose to CO_2 was greater in gut than in testis. Conversion of acetate to lipid was generally greater in testis than in gut. Phosphogluconate oxidation and lipid synthesis relative to glycolysis and oxidation, respectively, increased in the testis following warm acclimation. Conversely, glycolysis and oxidation relative to phosphogluconate oxidation and lipid synthesis, respectively, increased in the gut following warm acclimation. Cold acclimation resulted in higher levels of glycogenesis for testis and ovary but in equivocal differences for gut.

Cytochrome c oxidase activity was determined in gut, testis, and ovary extracts from seasonally acclimatized and temperature-acclimated urchins. Changes in enzyme activity paralleled those in the oxygen-consumption and radiotracer results. Gut activity was

highest in September, whereas testis and ovary activities were highest in December and March, respectively. Gut activity was higher following warm acclimation, but testis and ovary activities were higher following cold acclimation.

Glucose 6-phosphate dehydrogenase (G6PDH) activity was determined in gut, testis, and ovary extracts from temperature-acclimated urchins. Changes in G6PDH activity were the reverse of those in the oxygen-consumption, radiotracer, and cytochrome c oxidase results, suggesting that changes in the effectiveness with which glycolysis and phosphogluconate oxidation compete for the common substrate, glucose 6-phosphate (G6P), may compensate for each other. Warm acclimation generally increased gonadal G6PDH activity but failed to alter gut activity. Gonadal G6PDH activity was generally much higher than was that of the gut. The apparent G6PDH-G6P affinity for gut, testis, and ovary was increased following cold acclimation.

Body-component weights were determined from the urchins used in the metabolic rate-season studies. Gonadal ash-free dry weights expressed as a percentage of the total were maximal in the fall and minimal in the spring, whereas those of the body wall were maximal in the spring and minimal in the fall. Seasonal changes in the gut were negligible by comparison.

The above temperature-induced changes in urchin metabolism are discussed relative to temperature-induced changes in whole-urchin

metabolism and to situations confronting intertidal populations of urchins. Gonadal catabolism appears maximal in the winter or spring and minimal in the fall, whereas gonadal anabolism appears maximal in the fall and minimal in the spring. Nonreproductive catabolism appears maximal in the fall and minimal in the winter, whereas nonreproductive anabolism appears maximal in the spring and minimal in the fall.

Metabolic Adjustment to Temperature in *Strongylocentrotus purpuratus* (Stimpson)

by

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INTRODUCTION

Populations of *Strongylocentrotus purpuratus* (Stimpson), a common intertidal urchin, are exposed to great variation in temperature. If the metabolic rate of this poikilotherm were to change with temperature, optimal levels of activity for numerous metabolic functions (e. g. , the movement of spines, tube feet, and cilia) might be difficult to maintain. Metabolic regulation in the face of changing temperature would be a means to maintain relatively uniform levels of activity.

The objective of the research reported herein is to ascertain the influence of temperature on metabolism at various levels of urchin (*S. purpuratus*) organization. The existence of apparently adaptive shifts in metabolism may help to explain how this poikilotherm can function properly during temperature changes of 15°C or more within several hr. Urchins were partitioned into subdivisions, and each was studied at several levels -- oxygen consumption of tissue slices, incubation of tissue homogenates with radiotracers, and assays of single enzymes in crude extracts.

Gonadal tissues generally changed in a direction opposite that of the nonreproductive tissues. For example, anabolism was enhanced in gonadal tissues following warm acclimation but was enhanced in

nonreproductive tissues following cold acclimation. Conversely, increases in catabolism were observed in gonadal tissues following cold acclimation but were observed in nonreproductive tissues following warm acclimation.

Appropriate reviews, particularly those concerning temperature effects in poikilotherms, include those of Bullock (1955), Prosser (1955, 1958, 1964, 1967, 1969), Fry (1958), Precht (1958, 1968), and Newell (1969, 1973). Physiological variation may be a survival response to an environmental extreme or an alteration following exposure to "normal" environmental values. Prosser (1967, 1969) called these "resistance adaptation" and "capacity adaptation," respectively.

Some confusion has resulted from the terms acclimatization and acclimation. Prosser and Brown (1961) and Prosser (1969) referred to the former as an adjustment to multiple environmental factors which vary simultaneously. The latter is a response to a carefully controlled situation in which only a single factor is varied. This distinction will follow throughout the thesis.

Studies of respiratory function in marine invertebrates are numerous. Those restricted to echinoids are few by comparison. However, this area has been expanded considerably during the past decade. A variety of factors has been shown to influence sea-urchin (echinoid) respiration -- body size (McPherson, 1968), nutritional

state (Farmanfarmaian, 1966), reproductive state (Giese et al., 1966), oxygen tension (Johansen and Vadas, 1967), salinity (Giese and Farmanfarmaian, 1963), and substrate habit (Lewis, 1968).

The effect of temperature on urchin metabolism has been studied as well. Ulbricht (1970) and Ulbricht and Pritchard (1972) reported capacity adaptation in the oxygen-consumption rate-temperature (R-T) relationship of summer-acclimatized urchins from the central Oregon coast. Oxygen consumption of intertidal purple urchins (S. purpuratus) was essentially independent of temperature between 12° and 21°C, whereas that of the predominantly subtidal Strongylocentrotus franciscanus (A. Agassiz) and benthic Allocentrotus fragilis (Jackson) showed more temperature dependence throughout most of the test range. Metabolic rate-temperature independence is presumably adaptive for poikilotherms, such as purple urchins, which are exposed to considerable temperature variation.

Some studies report the effect of temperature acclimation on poikilotherm R-T relationships. Higher rates for cold-acclimated forms at a given temperature are the usual response and are presumably adaptive, although other responses are also described (Bullock, 1955; Prosser, 1955, 1964; Precht, 1958, 1968; Prosser and Brown, 1961). Farmanfarmaian and Giese (1963) reported higher oxygen-consumption rates for S. purpuratus following cold acclimation (type 3 response, see Precht, 1958, 1968). On the other hand,

Boolootian and Cantor (1965) reported no compensation (Precht type 4) or even inverse compensation (Precht type 5) in the oxygen consumption of temperature-acclimated Arbacia punctulata (Lamarck). No compensation was observed in the R-T curves of temperature-acclimated S. purpuratus and A. fragilis collected from Oregon waters (Ulbricht, 1970, 1973a, b). In fact, inverse compensation was suggested for temperature-acclimated S. purpuratus at test temperatures of 12° and 15°C and for temperature-acclimated S. franciscanus at all test temperatures.

Inverse compensation of oxygen consumption following temperature acclimation appears paradoxical and is difficult to explain. Inverse compensation following acclimatization may result from environmental factors other than temperature (Prosser, 1958, 1964; Webb and Brown, 1961; Pickens, 1965). Presumably, such factors were not operative during the temperature acclimation discussed by Ulbricht (1970, 1973a, b).

A second major point emerged from the R-T curves of temperature-acclimated S. purpuratus, S. franciscanus, and A. fragilis (Ulbricht, 1970, 1973a, b). Temperature coefficients (Q_{10} values) generally were lower for cold-acclimated urchins than warm-acclimated forms at lower test temperatures but were lower for warm-acclimated urchins than cold-acclimated forms at higher temperatures. Apparently, temperature acclimation resulted in greater R-T

independence at test temperatures close to those of acclimation. Ulbricht (1973a) suggested that following temperature acclimation of urchins, rotational (slope) changes in R-T curves may be more important than translational (ordinate) changes (see Prosser, 1958, 1969; Prosser and Brown, 1961, for appropriate definitions).

Oxygen consumption by body components of S. purpuratus was discussed by Giese et al. (1966). They suggested that the oxygen available to internal tissues is limited by exchange surfaces and translocation. They further suggested that the resulting attenuation leads to wet weight-organic weight as well as intact urchin-body component differences in the oxygen-consumption rate. The effect of temperature as well as recent thermal history on the oxygen consumption of urchin body components has not been reported.

The relative activity of a metabolic pathway may be altered following temperature change or temperature acclimation (Ekberg, 1958; Kanungo and Prosser, 1959; Hochachka and Hayes, 1962; Hochachka, 1968; Dean, 1969). The activity of glycolysis relative to that of phosphogluconate oxidation may be inferred from incubation studies utilizing variously labelled substrates (e.g., glucose-1-^{14}C and glucose-6-^{14}C). Activation of glycolysis along with considerable shunt activation was observed in selected tissues of several fish species following cold acclimation (Ekberg, 1958; Kanungo and Prosser, 1959; Hochachka and Hayes, 1962).

Although no regulatory function has been ascribed to cytochrome c oxidase, changes in the activity of this enzyme may reflect metabolic alterations observed at higher levels of organization. Freed (1965) reported increasing cytochrome c oxidase activity in goldfish trunk muscle following cold acclimation. Higher cytochrome c oxidase activity was observed in brain, gill, "red" muscle, and to a lesser extent liver homogenates of cold-acclimated goldfish (Caldwell, 1969).

The importance of conducting enzyme-regulation studies at physiological rather than saturating substrate levels was stressed by Atkinson (1966). Enzyme activators are generally thought to increase enzyme-substrate affinity. Consequently, changes in the apparent Michaelis constant (K_m) -- inversely related to enzyme-substrate affinity -- may provide information helpful toward understanding metabolic regulation. Henceforth, K_m refers to the substrate concentration at which the reaction velocity is half of the maximum. The effect of temperature on various enzyme systems is viewed as analogous to that of enzyme modulators (Hochachka and Somero, 1968, 1973; Somero, 1969b; Somero and Hochachka, 1971). Temperature-dependent K_m values have been observed for a variety of poikilotherm enzymes (Hochachka and Somero, 1968; Somero and Hochachka, 1968, 1969, 1971; Behrisch, 1969, 1971, 1972; Behrisch and Hochachka, 1969; Somero, 1969a, b; Baldwin and Hochachka, 1970; Hochachka and Lewis, 1970, 1971; Moon, 1970; Baldwin, 1971; Moon and Hochachka,

1971; Boernke, 1973; Hoskins and Aleksiuk, 1973; Meşter, Iordăchescu, and Niculescu, 1973; Wernick and Künnemann, 1973). Minimal K_m values may be observed at or close to habitat and acclimation temperatures. These correlations suggest mechanisms which may operate at physiological concentrations.

The purpose of the present study, as described above, is to ascertain the influence of temperature on metabolism at various levels of organization. For example, what mechanism(s) might contribute to the varying degrees of R-T independence found in summer-acclimatized and temperature-acclimated urchins? Secondly, what mechanism(s) might contribute to the inverse compensation of urchin R-T curves following temperature acclimation? Interpretations involving mechanisms for phenomena at any given level of organization may be apparent from studies at the adjacent but lower level. For example, the oxygen consumption of tissue slices may improve the understanding of whole-urchin metabolic responses. Similarly, multienzyme studies using radiotracers may improve the understanding of tissue-slice results, and assays of single enzymes may improve the understanding of multienzyme results. A temperature-induced shift in metabolism (e.g., an oxidative increase following cold acclimation) may occur from one level of organization to another -- whole urchins to tissue slices to multienzymes (metabolic pathways) to single enzymes. Patterns persisting from one level to another can emphasize the

validity of the observations at any one level. However, the lack of recurring patterns should not by itself diminish the validity of the observations at any one level.

MATERIALS AND METHODS

Collection and Maintenance of Urchins

Strongylocentrotus purpuratus was collected from two locations along the central Oregon coast. Urchins used in the cytochrome c oxidase studies were collected from Whale Cove. Urchins used for all other studies were collected from Yaquina Head.

Urchins were held for short periods of time in aerated, running sea water at the Marine Science Center, Newport, Oregon. Those used for acclimatization studies were sacrificed on the days of collection. Those used for acclimation studies were held at 9^{o} or 18^{o} for 30 days. These latter urchins were fed macroscopic algae to satiety during the initial two weeks of acclimation but were not fed anything thereafter. These urchins were subjected to a 12-hr light -- 12-hr dark photoperiod throughout the acclimation period.

Body-component Oxygen Consumption

Urchins were partitioned into subdivisions (body components) -- body wall, lantern, perivisceral fluid, gonad, and the remaining internal, nongerminal tissues (hereafter called "gut"). Studies were conducted to ascertain the influence of the type of preparation on oxygen consumption. Homogenates of components were compared with sections (1-g slices) from intact "organs." Two suspending

media -- filtered perivisceral fluid and filtered sea water -- were compared.

Perivisceral fluid was prepared by first removing large masses of coagulated coelomocytes by filtration. Smaller particulate matter was removed upon centrifugation. Gut homogenates were obtained by removing entire digestive tracts and the adherent mesenteries. These were washed several times in filtered sea water in order to remove all debris. Pieces were homogenized in either the supernatant of centrifuged perivisceral fluid or filtered sea water (1 g component: 10 ml chilled medium). Sections of components were prepared by adding 1-g slices to 4-5 ml of chilled medium (perivisceral fluid or sea water).

Metabolic rates of components were determined from oxygen-consumption measurements using Gilson differential respirometry. A 1-hr equilibration period at the temperature of measurement (12° for all preliminary studies) preceded the oxygen-consumption measurements.

Additional preliminary studies were performed to compare the metabolic rates of the body components and to determine preparation viability.

Sections of body wall, gut, testis, and ovary were used in the R-T experiments. Each reaction flask contained a standardized respiring mixture of 1 g of body-component section added to 4 ml of

filtered sea water. Urchins were either field acclimatized (collected seasonally from October 1970 through August 1972) or temperature acclimated (acclimation temperatures of 9° and 18°). Test temperatures were 9°, 12°, 15° (acclimation study only), and 18°. Three rate determinations were made for each section at each test temperature (10-20 min per determination).

Total dry and ash weights were determined for most component sections. Sections were burned to ash in a muffle furnace (500°C for 24 hr). Ash-free dry weights were assumed to be the difference.

Metabolic rates derived from body-component oxygen consumption are expressed as $\mu l\ O_2/g$ wet per hr (some preliminary studies) or $\mu l\ O_2/g$ dry per hr (all remaining studies). The latter rates are ash-free dry weights and are corrected to standard conditions.

Multienzyme Systems

Body-component homogenates were incubated with radiotracers in order to ascertain the relative activities of alternate metabolic pathways. Components were taken from urchins acclimated at 9° and 18° as previously described.

Body components -- gut, testis, and ovary -- were quickly removed from urchins and placed in chilled "Instant Ocean Sea Salts" (Aquarium Systems, Inc.). Debris was removed as previously discussed. In every instance, components from several urchins were pooled.

Homogenates were made up in "Instant Ocean Sea Salts" (1 g tissue:10 ml homogenate). Six ml of homogenate were placed in each reaction flask and incubated with approximately 0.5-1.0 μc of glucose-1-^{14}C, glucose-6-^{14}C, or acetate-1-^{14}C (New England Nuclear Company). Incubation temperatures were 9°, 13.5°, and 18°. Incubation was terminated with the addition of 5 ml of 10% trichloroacetic acid (TCA).

Following several preliminary studies to establish the viability of the homogenate system at various incubation intervals, a 3-hr incubation period was decided upon for the radiotracer studies.

Respired $^{14}CO_2$ was trapped in a center well containing 0.4 ml of 10% KOH. Strips of laboratory filter paper were transferred from the center wells to 15 ml of scintillation fluid (4 g PPO[1] and 100 mg POPOP[2] in 667 ml toluene and 333 ml triton X-100) for counting.

The incubation mixtures were then centrifuged at 10,000 X g for 30 min. Lipids were extracted from the TCA-insoluble material with three volumes of chloroform-methanol (2:1) for 3 hr (Folch, Lees, and Sloane Stanley, 1957). Extracts were washed twice with two volumes of water. One-tenth ml of the lipid extract was added to 15 ml of scintillation fluid for counting.

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[1] 2,5-diphenyloxazole

[2] 1,4-bis-2-(5-phenyloxazolyl)-benzene

Glycogen was extracted as described by Clegg (1964). Eight ml of 100% ethanol were added to 2 ml of the supernatant fluid. The mixture was allowed to stand for 15 min and then centrifuged for 15 min. Two ml of 30% KOH were added to the precipitate, and the mixture was well shaken. The mixture was heated in boiling water for 10 min and then cooled in ice. This was mixed with 3 ml of 95% ethanol and well shaken. After overnight refrigeration, the resulting mixture was centrifuged for 10 min. The precipitate was washed twice with 6 ml of 95% ethanol and then dissolved in 2 ml of water. One-tenth ml of the extract was added to 15 ml of scintillation fluid for counting.

Two instruments were used for determining radioactivity, either a Nuclear-Chicago liquid scintillation system (model 6819) or a Packard Tri-Carb liquid scintillation spectrometer (model 3310). In either case, standard methods were used to correct for background and quenching. Protein was measured as described by Lowry et al. (1951).

Cytochrome c Oxidase Activity

Cytochrome c oxidase activity was determined in tissues obtained from both seasonally acclimatized and temperature-acclimated urchins.

Body components -- gut, testis, and ovary -- were quickly removed from urchins and transferred to chilled, filtered sea water. These were pooled from several urchins and then frozen in liquid nitrogen. The tissues were held at -84° until assayed for enzyme activity.

Homogenates were made up in a chilled solution consisting of 250 mM sucrose, 10 mM Tris-HCl (pH, 7.5), and 0.1 mM EDTA (1 g tissue:10 ml homogenate). Homogenates were centrifuged at 3000 X g for 10 min. All assays were promptly performed on the supernatant fluid. Preliminary studies were conducted to ascertain the effects of freezing, pH, homogenate medium, and cytochrome c dilution on cytochrome c oxidase activity. The influence of possible interfering enzymes -- cytochrome c peroxidase or reductase activity -- was determined with extracts from gut and ovary. Preliminary studies were generally conducted at 25°.

Cytochrome c oxidase activity was determined as described by Smith (1955) and as modified by Caldwell (1969). Oxidation of reduced cytochrome c was determined from decline in the optical density at 550 nm. Reaction-mixture components were 2.5 ml of 0.1 M potassium phosphate (pH, 7.0), 0.4 ml of 225 μM reduced cytochrome c dissolved in 10 mM potassium phosphate (pH, 7.0), and 0.1 ml of the extract. Final concentrations were 84.7 mM potassium phosphate, 30 μM reduced cytochrome c, 8.33 mM sucrose, 0.33 mM Tris-HCl, and

3.3 μM EDTA. Cytochrome c (Type III, horse heart, from Sigma Chemical Company) was reduced with the addition of sodium hydrosulfite (1 mg/ml cytochrome c solution).

Following temperature equilibration, extracts were added to the cytochrome c-buffer mixture, and changes in absorbance were followed for approximately 5 min. Oxidation was generally quite linear. Each reaction was terminated upon the addition of 5 μl of saturated $K_3Fe(CN)_6$. The assays were conducted at 13.5°. All assays were performed using a Beckman Instruments, Inc., spectrophotometer (model DU) in conjunction with a Gilford Instrument Laboratories, Inc., absorbance indicator and recorder (model 2000). A water jacket surrounding the cells permitted precise temperature control.

Activity for cytochrome c oxidase is expressed as μmoles of cytochrome c oxidized per mg of protein per min. This expression is the product of the first-order velocity constant and the initial reduced cytochrome c concentration. The first-order velocity constant (K') may be calculated from the following expression:

$$K' = \frac{[\log(OD_1 - OD_\infty) - \log(OD_2 - OD_\infty)] \cdot 2.3026}{t_2 - t_1}$$

where: t_1 is time 1;
t_2 is time 2;
OD_1 is the optical density at time 1;
OD_2 is the optical density at time 2; and
OD_∞ is the optical density of totally oxidized cytochrome c. Totally oxidized cytochrome c is obtained following the addition of saturated $K_3Fe(CN)_6$.

> The cytochrome c concentration is determined from the fully reduced molar absorbance index of 2.77×10^4 cm^{-1} M^{-1} (Margoliash, 1954).

Protein was measured as described by Lowry et al. (1951).

Glucose 6-phosphate Dehydrogenase Kinetics

Glucose 6-phosphate dehydrogenase (G6PDH)[3] activity was determined from tissues of temperature-acclimated urchins. Body components -- gut, testis, and ovary -- were quickly removed from urchins and transferred to chilled "Instant Ocean Sea Salts." In each case, tissues were pooled from several urchins.

Homogenates were made up in chilled "Instant Ocean Sea Salts" (1 g tissue:10 ml homogenate). Homogenates were centrifuged at 15,000 X g for 30 min. All assays were then performed on the supernatant fluid. Preliminary studies were conducted to ascertain the effects of pH, buffer systems, Mg^{2+} (salt and dilution), and dilution of enzyme and coenzyme on G6PDH activity.

Glucose 6-phosphate dehydrogenase activity was determined as described by Kuby and Noltmann (1966). Formation of NADPH was followed by the increase in optical density at 340 nm. No effort was

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[3]Other abbreviations used: ATP: adenosine 5'-triphosphate; G6P: glucose 6-phosphate; NADH: reduced nicotinamide adenine dinucleotide; NADP: oxidized nicotinamide adenine dinucleotide phosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate; 6PG: 6-phosphogluconate; 6PGDH: 6-phosphogluconate dehydrogenase.

made to isolate the G6PDH from interfering 6PGDH, and presumably some of the NADPH which formed resulted from the oxidation of 6PG. Glock and McLean (1953) discussed a suitable method for determining G6PDH activity from crude extracts. Extracts were assayed using a substrate mixture of G6P and 6PG. Assays were repeated with 6PG alone. The resulting difference corresponded quite closely to activity levels obtained with purified G6PDH (Glock and McLean, 1953). This difference method was used in the present study to determine G6PDH activity. Reaction-mixture components were 2.5 ml of 0.1 M glycylglycine (pH, 8.1), 0.1 ml of 0.03 M (or some dilution thereof) G6P and 6PG (or 6PG alone), 0.1 ml of 0.01 M NADP, 0.2 ml of 0.15 M $MgCl_2$, and 0.1 ml of properly diluted extract. Glucose 6-phosphate and 6PG concentrations were 3×10^{-6}, 3×10^{-5}, 3×10^{-4}, 3×10^{-3}, or 3×10^{-2} M. Glucose 6-phosphate dehydrogenase activity was determined at 6°, 10°, 14°, 18°, 22°, and 26°.

Following temperature equilibration, extracts were added to the remaining reaction components, and changes in absorbance were followed for approximately 7 min. Glucose 6-phosphate oxidation was quite linear. All determinations were made using the previously mentioned Beckman spectrophotometer-Gilford recorder combination.

Activity for G6PDH is expressed as μmoles of NADPH formed per mg of protein per min. This expression is the product of the number 0.0161 and the number of arbitrary enzyme units per mg of

protein (Kuby and Noltmann, 1966). One arbitrary unit of G6PDH is defined as the amount of enzyme in 1 ml of reaction mixture which oxidizes G6P at 0.1 absorbance unit per min at 30°. The number 0.0161 follows from the NADPH molecular extinction coefficient -- 6.22×10^6 cm^2/mole (Horecker and Kornberg, 1948). Protein was measured as described by Lowry et al. (1951).

Statistical Methods

Data dispersion about mean values is expressed in standard ways (e.g., one standard error of the mean, two standard errors of the mean, and 95% confidence intervals). Confidence intervals for ratios were determined as discussed by Bliss (1967).

RESULTS

Body-component Oxygen Consumption

Preliminary Studies

Oxygen consumption of intact sections and homogenates of Strongylocentrotus purpuratus gut tissues are compared in Figure 1. The metabolic rates of component sections generally appeared unaffected by time, whereas those of homogenates declined. The effect of suspending medium on gut-section metabolic rate is seen in Figure 2. Somewhat greater variation appeared to result with perivisceral fluid, and for this reason filtered sea water was used in the remaining experiments. The points in Figures 1 and 2 represent single rate determinations but are representative of the overall trends observed.

Figure 3 depicts the metabolic rates of intact sections from various body components. Sections of gut and testis maintained the highest rates, and those of body wall, lantern, and perivisceral fluid had successively lower rates.

Figures 4-6 illustrate the metabolic rates of body-wall, gut, and testis sections, respectively, for a period in excess of the 4 hr needed to conduct the rate-temperature studies. Levels of variation were observed, but there was little indication of any loss of viability for any of the component sections during this period.

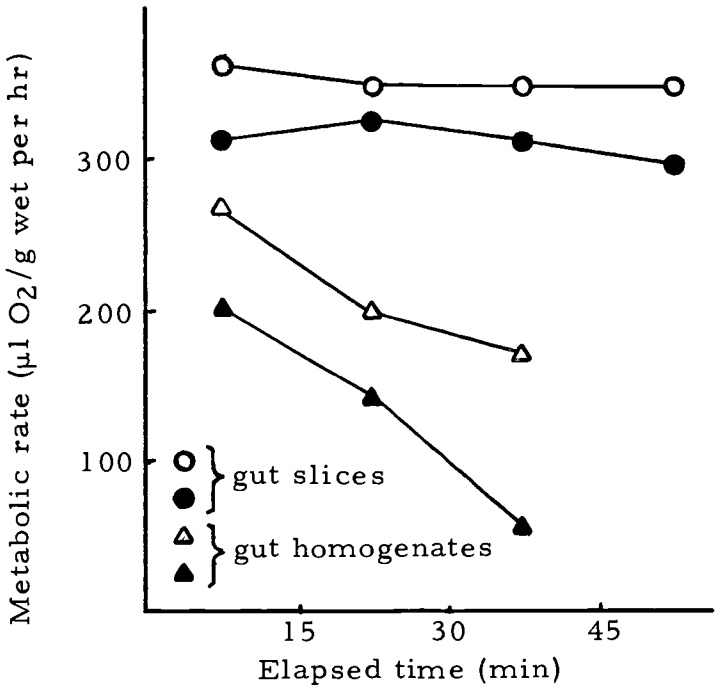

Figure 1. Effect of preparation on the gut metabolic rate-time relationship.

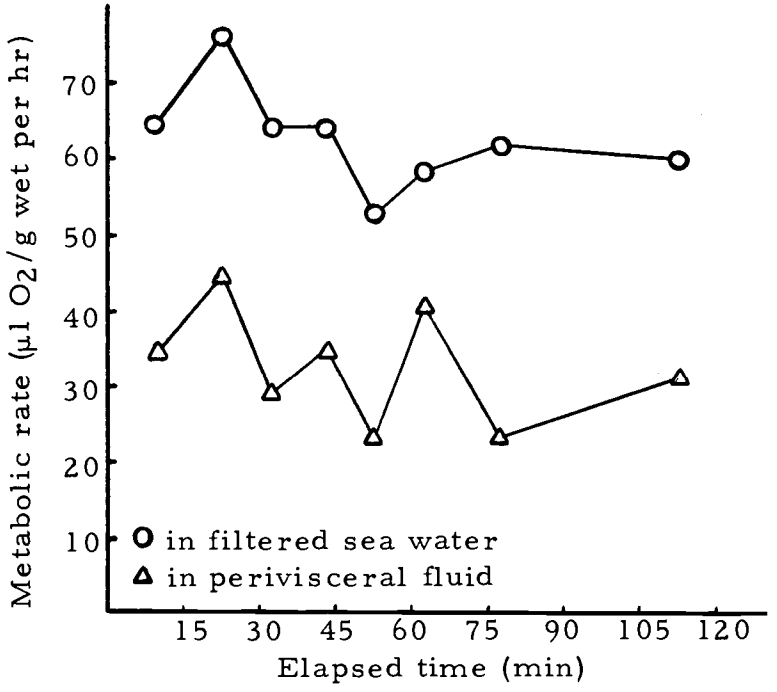

Figure 2. Effect of suspending medium on the gut metabolic rate-time relationship.

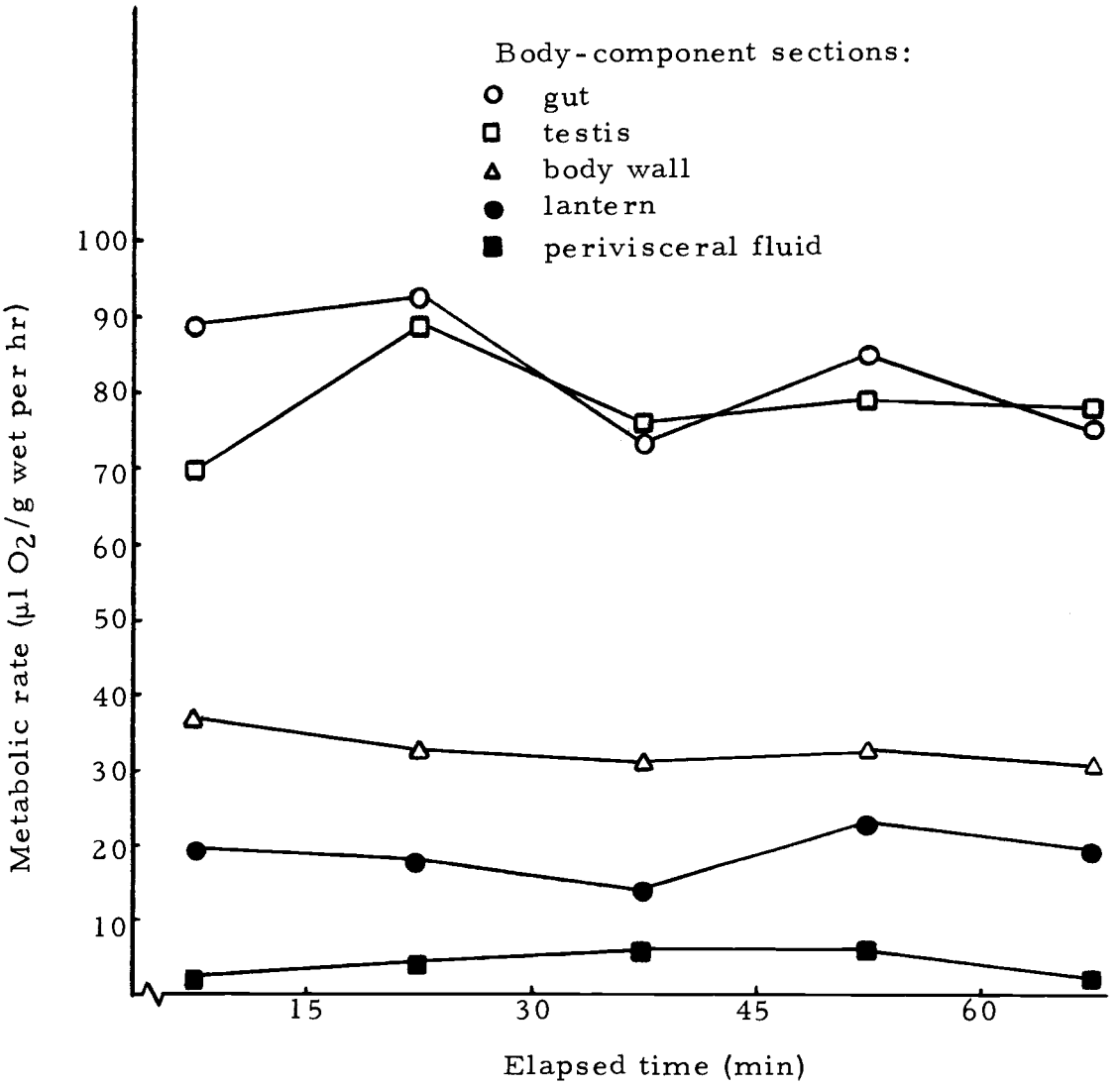

Figure 3. Body-component metabolic rate-time relationships. Rates are averages of components from 2 urchins.

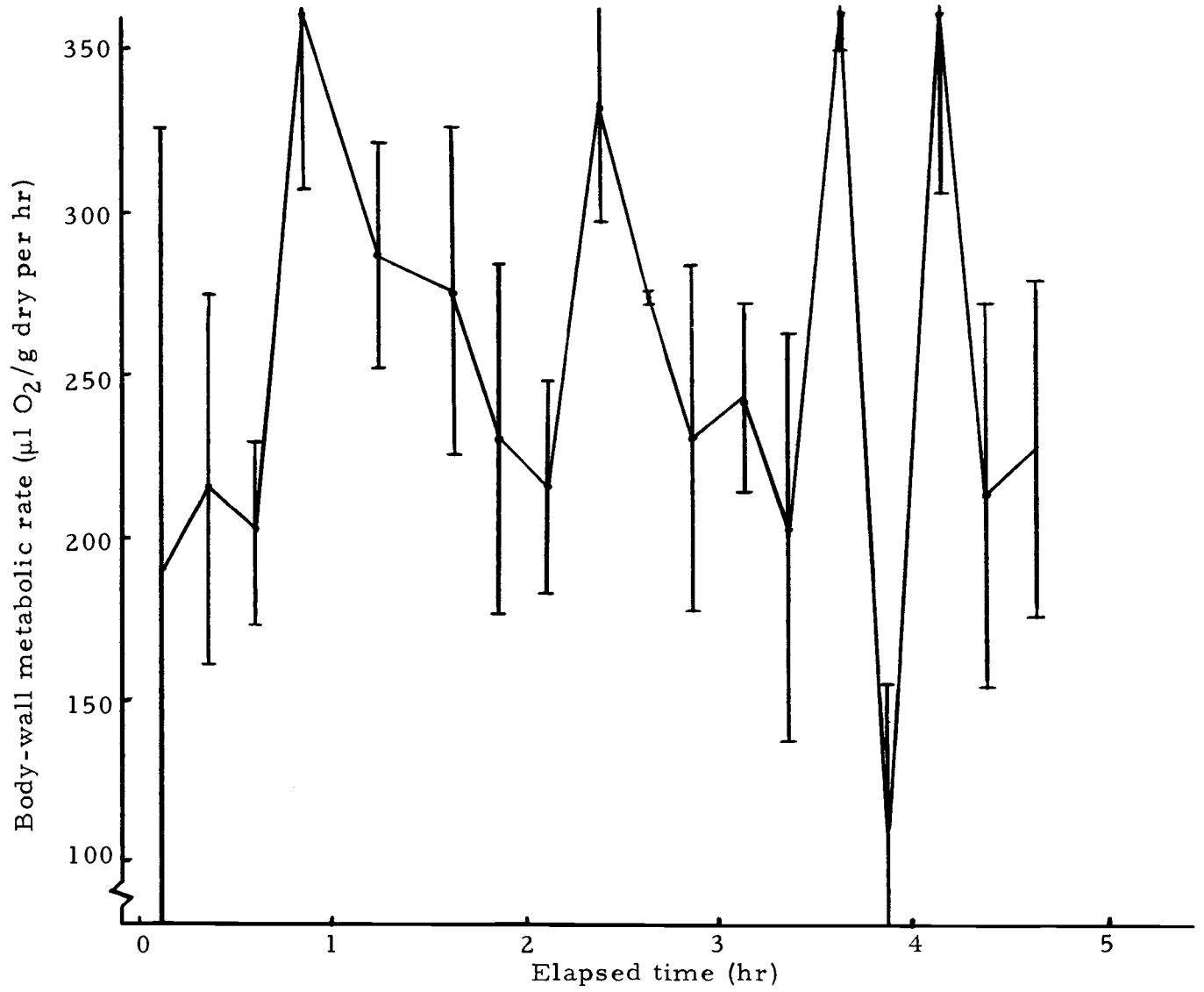

Figure 4. Body-wall metabolic rate-time relationship. Vertical bars indicate ±2 SEM (N = 3).

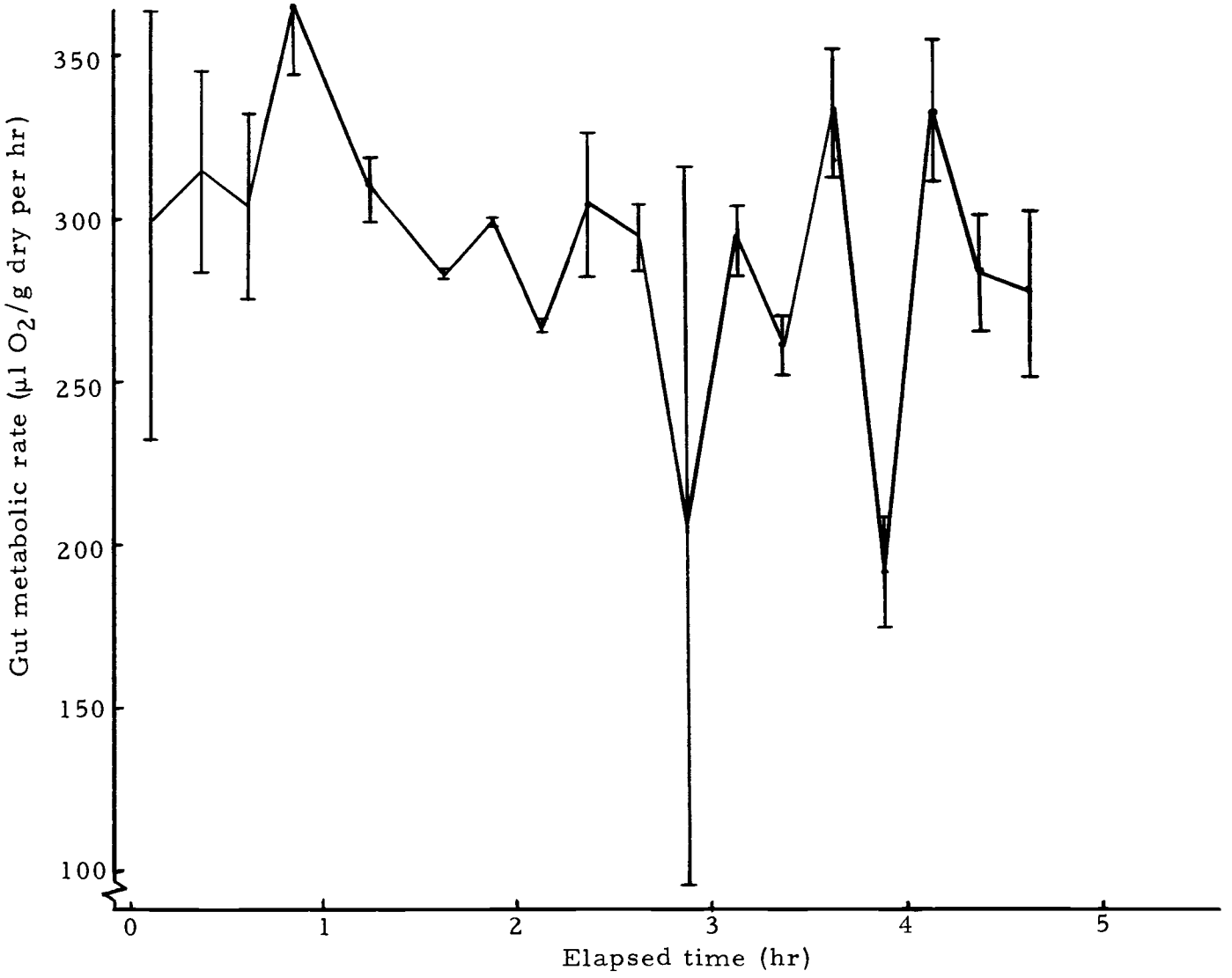

Figure 5. Gut metabolic rate-time relationship. Vertical bars indicate ± 2 SEM (N = 3).

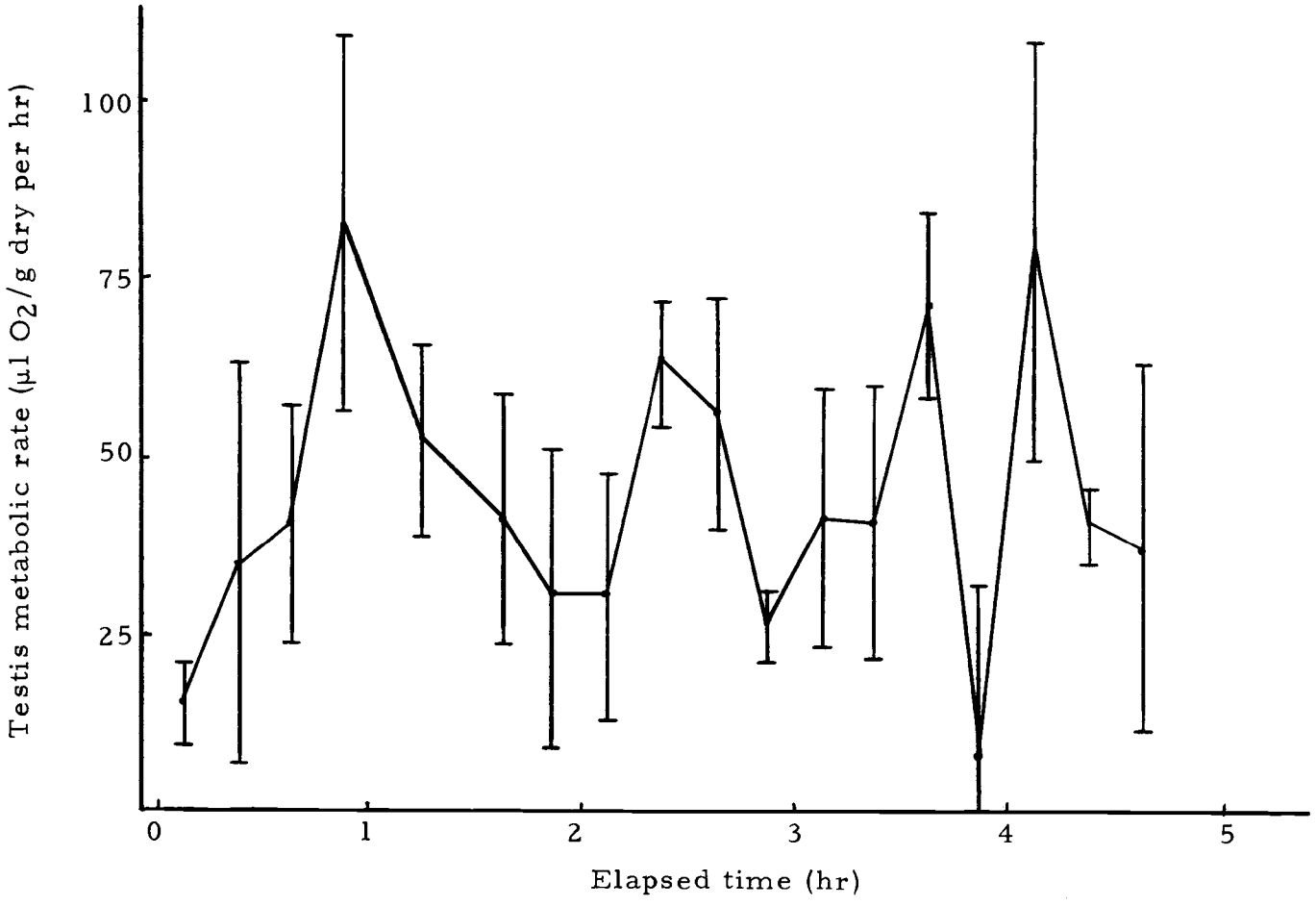

Figure 6. Testis metabolic rate-time relationship. Vertical bars indicate ± 2 SEM (N = 3).

Rate-temperature Studies

Seasonal changes in the metabolic rates of body-component sections from field-acclimatized urchins are shown in Figures 7-10. Changes for body wall and gut were generally similar but contrasted with those of testis and ovary. Rates of the former two were at high levels during the fall. These rates decreased drastically by midwinter. The rates then generally increased for each subsequent season through the next fall. Essentially the reverse was observed for testis and ovary. Lowest rates occurred in the fall. By midwinter relatively high or the highest rates were observed. These rates underwent a general decline seasonally through the following fall.

Metabolic rate-temperature curves of body-component sections from temperature-acclimated urchins are presented in Figures 11-15. Differences between rates at any given test temperature were not significant for body wall, gut, and ovary (Figures 11, 12, and 14, respectively). However, significant differences between acclimation groups were observed for testis (Figure 13). Testis R-T curves illustrate overcompensation (type 1, Precht, 1958, 1968). Figure 15 depicts the R-T curves of all four components plotted on the same pair of coordinates.

Multienzyme Systems

Preliminary studies were performed to determine the relationship between $^{14}CO_2$ evolution and elapsed time. Radioactivity from

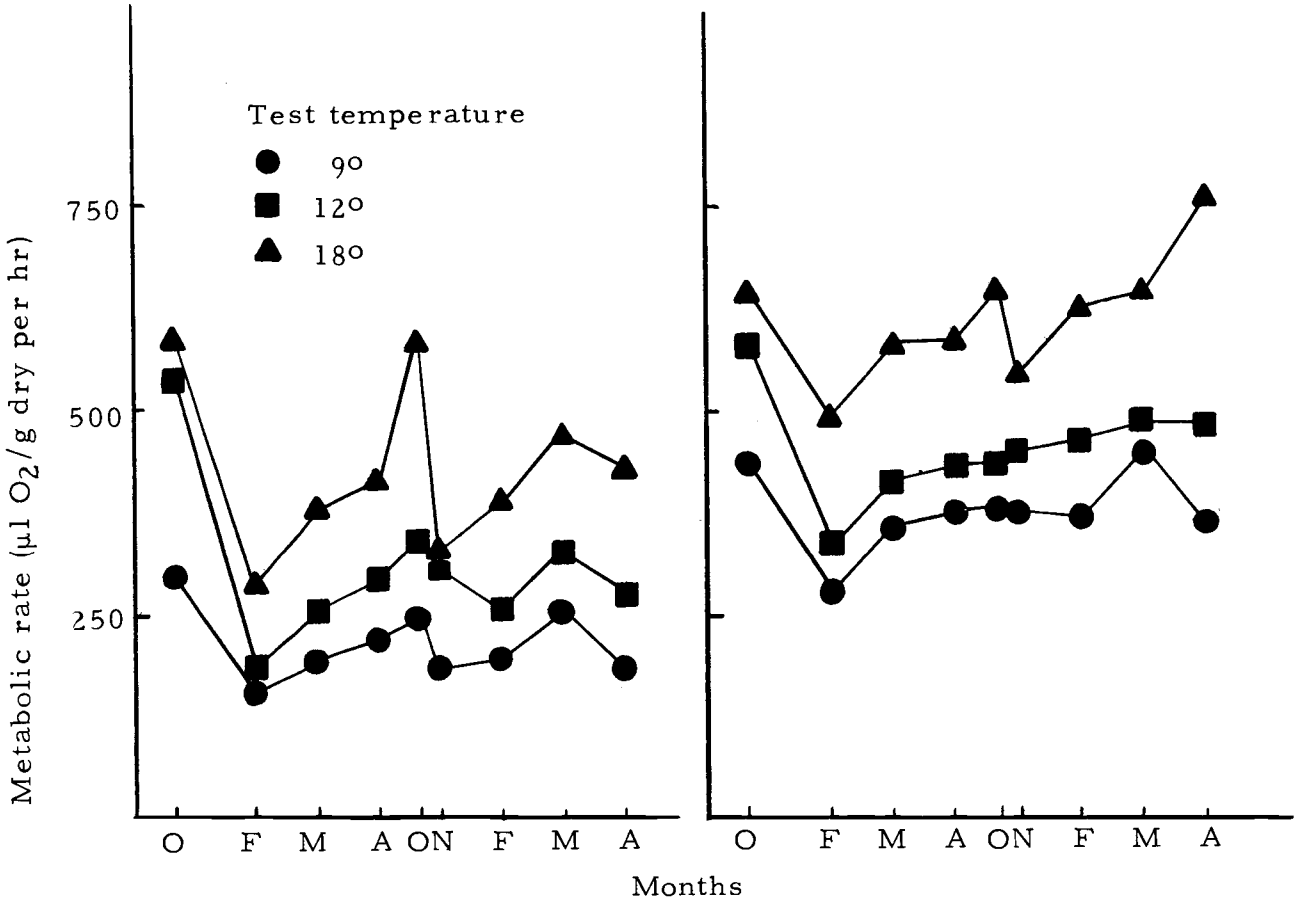

Figure 7. Body-wall metabolic rate-season relationship. Triplicate rates were determined for 1-4 body-wall sections from each of 2-4 urchins.

Figure 8. Gut metabolic rate-season relationship. Triplicate rates were determined for 2-3 gut sections from each of 2-4 urchins.

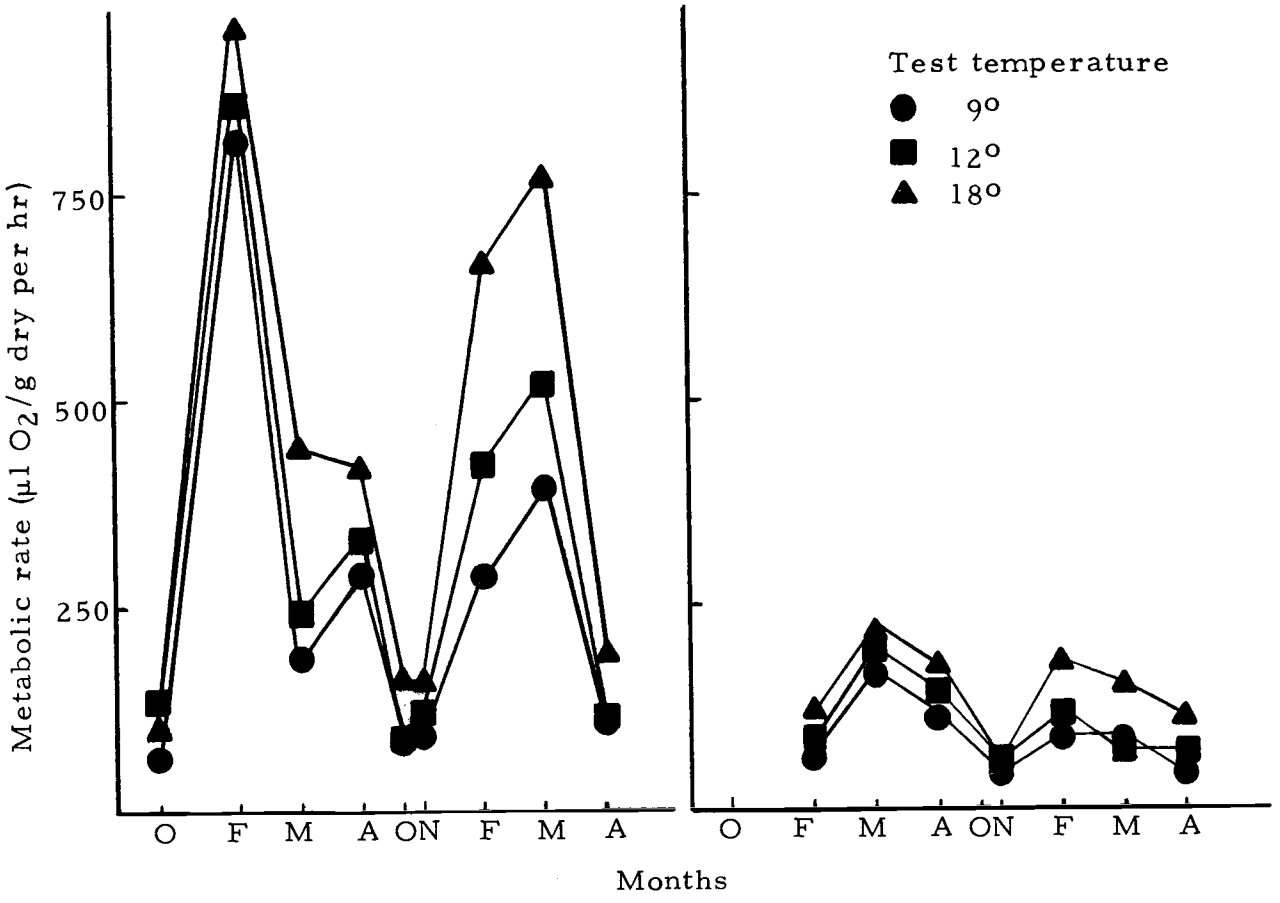

Figure 9. Testis metabolic rate-season relationship. Triplicate rates were determined for 1-4 testis sections from each of 2-3 urchins.

Figure 10. Ovary metabolic rate-season relationship. Triplicate rates were determined for 1-2 ovary sections from each of 1-2 urchins.

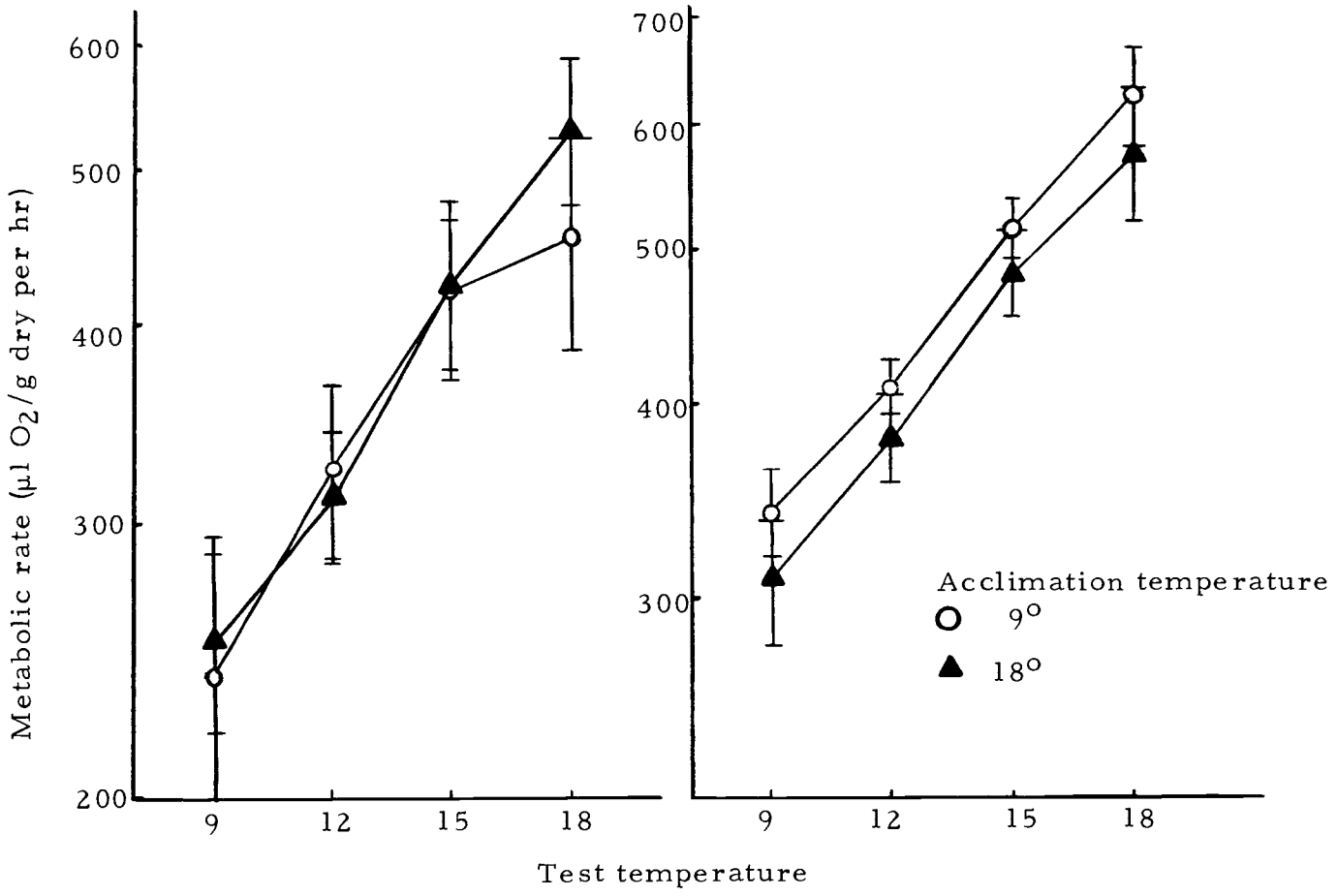

Figure 11. Body-wall metabolic rate-temperature relationship following temperature acclimation. Triplicate rates were determined for 1-2 body-wall sections from each of 4 urchins. Vertical bars indicate 95% confidence limits.

Figure 12. Gut metabolic rate-temperature relationship following temperature acclimation. Triplicate rates were determined for 2 gut sections from each of 4 urchins. Vertical bars indicate 95% confidence limits.

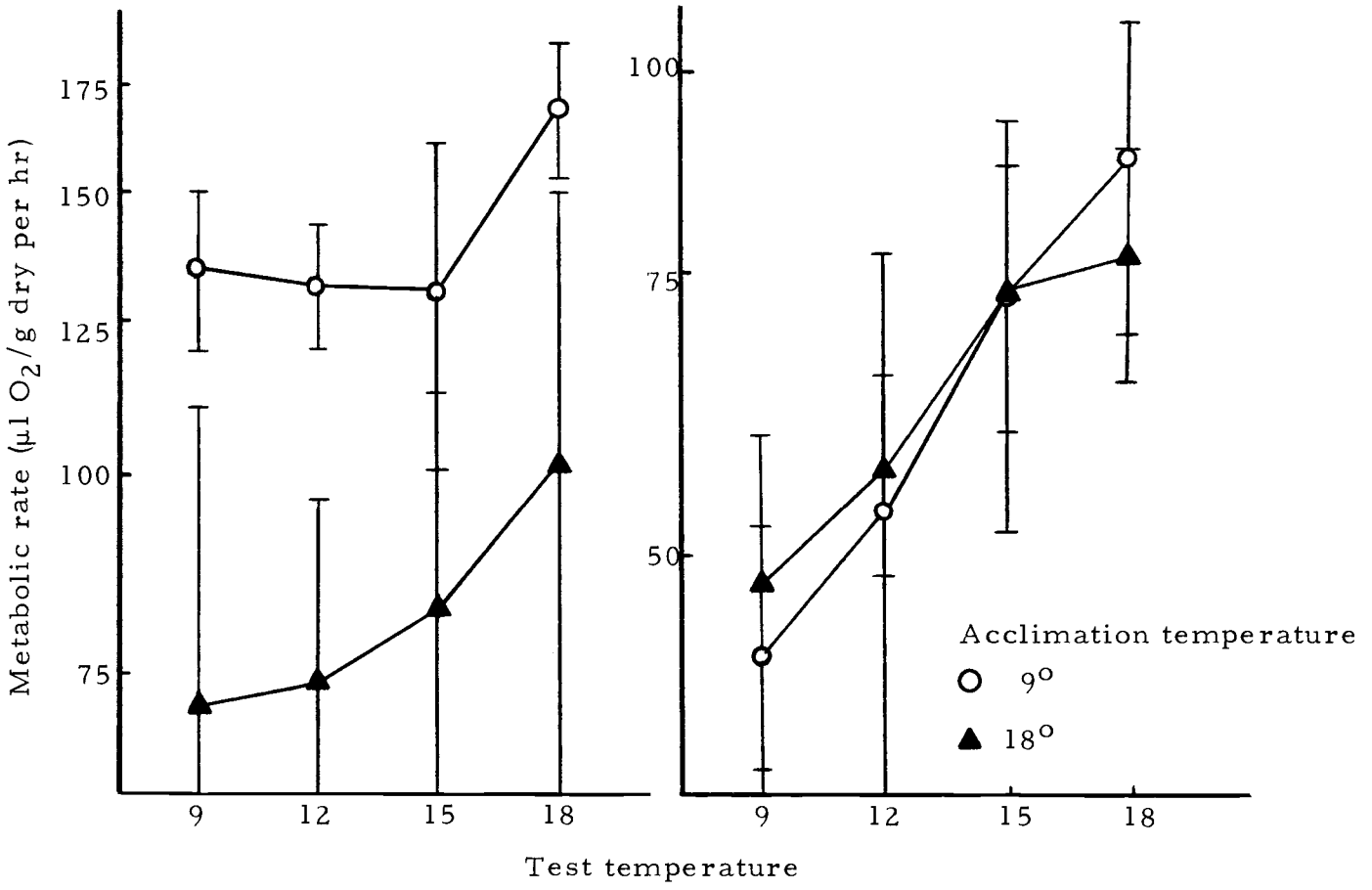

Figure 13. Testis metabolic rate-temperature relationship following temperature acclimation. Triplicate rates were determined for 2 testis sections from each of 1-2 urchins. Vertical bars indicate 95% confidence limits.

Figure 14. Ovary metabolic rate-temperature relationship following temperature acclimation. Triplicate rates were determined for 2 ovary sections from each of 2-3 urchins. Vertical bars indicate 95% confidence limits.

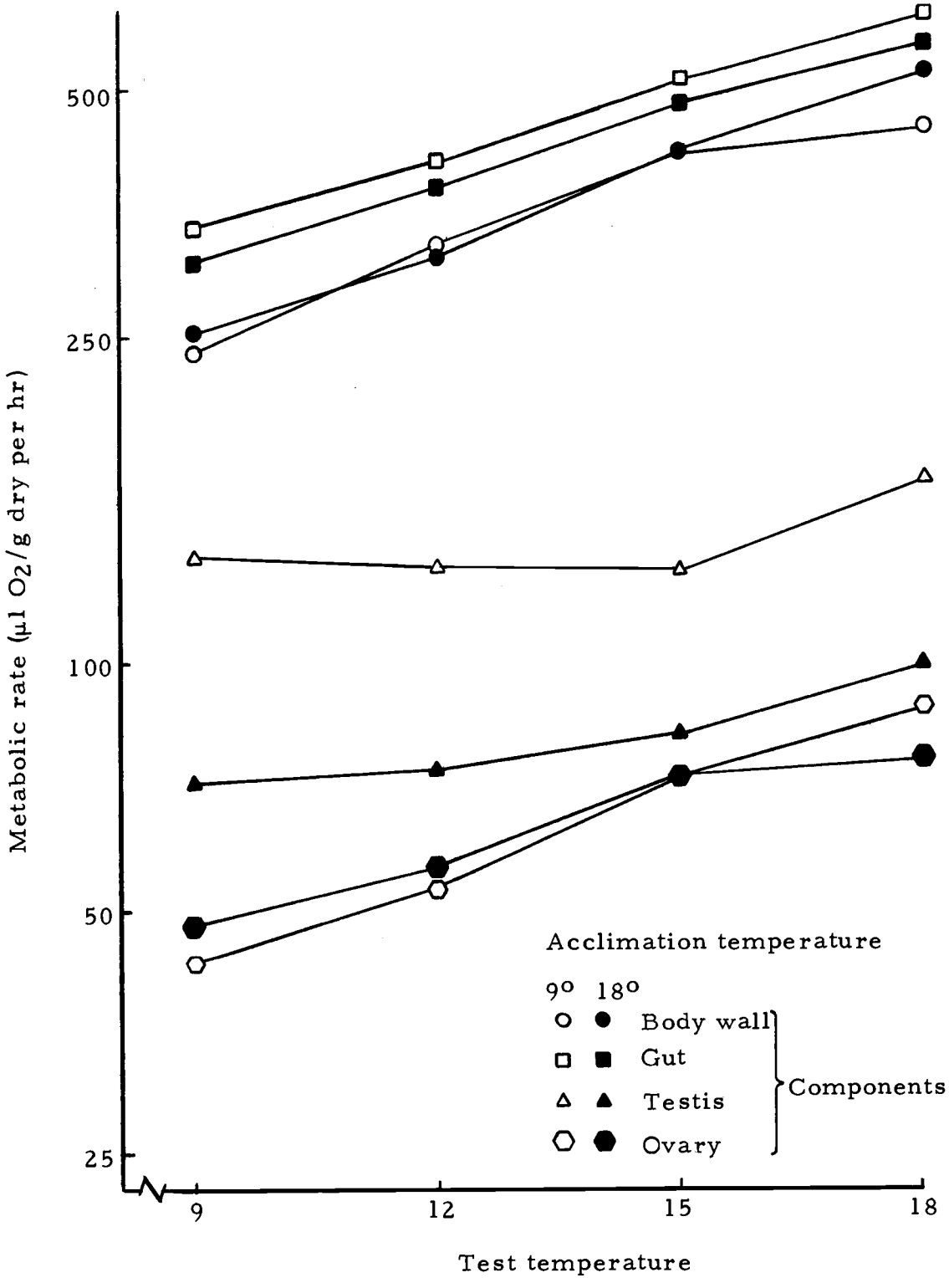

Figure 15. Body-component metabolic rate-temperature relationships following temperature acclimation.

$^{14}CO_2$ began to decline significantly with time after 4 hr of incubation. If there were no change in metabolism with time, a CO_2-saturation problem might have occurred with the trapping system. Increases in radioactivity from extracted lipids and glycogen were linear with time. On the basis of these tests a 3-hr maximal incubation period was decided upon for all radiotracer experiments.

Gut and testis homogenates from temperature-acclimated urchins were incubated with several radiotracers -- glucose-1-^{14}C, glucose-6-^{14}C, and acetate-1-^{14}C. Incubation periods were 3 hr for all of these studies.

Gut and testis homogenates were incubated with glucose-1-^{14}C and glucose-6-^{14}C. The appearance of ^{14}C in the trapped CO_2 is depicted in Figure 16. Regardless of the label or incubation temperature, $^{14}CO_2$ activity was higher for all "warm-acclimated" gut homogenates. Conversely, $^{14}CO_2$ activity was higher for all "cold-acclimated" testis homogenates. When incubation was at 18°, all differences between the means were significant (95% level). Gut homogenates appeared to metabolize more of the labelled glucose to CO_2 than did those of the testis. Greater amounts of $^{14}CO_2$ appeared from glucose-1-^{14}C than from glucose-6-^{14}C in both homogenate systems.

Gut and testis homogenates were incubated with acetate-1-^{14}C. The appearance of ^{14}C in the extracted lipids as well as in the trapped

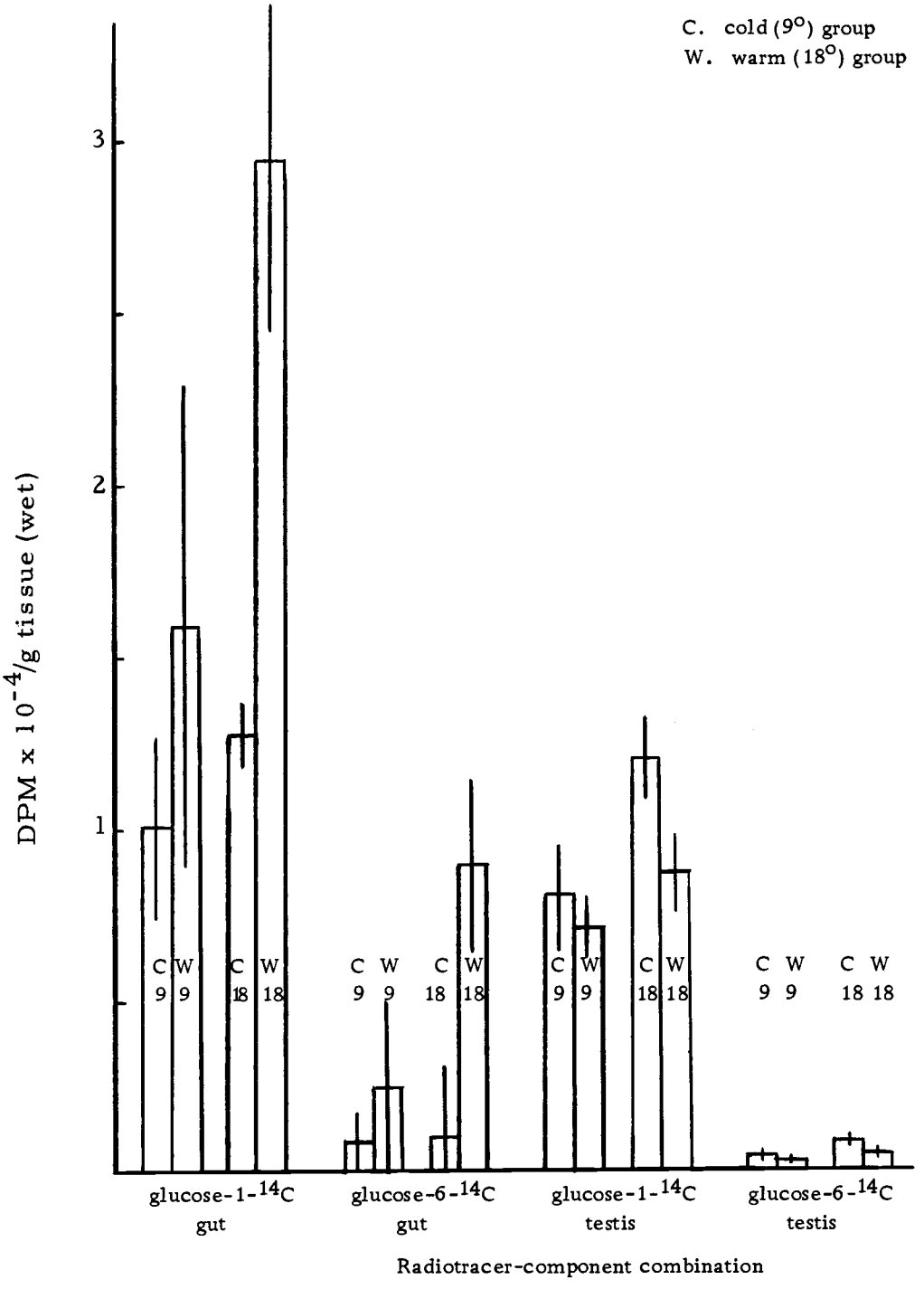

Figure 16. Evolution of $^{14}CO_2$ in homogenates from temperature-acclimated urchins incubated with ^{14}C-glucose. Tissues for each homogenate were obtained from 6 or more urchins. Determinations were made in triplicate (vertical bars indicate 95% confidence limits). Acclimation temperatures were 9° (C) and 18° (W). Incubation temperatures were 9° (9) and 18° (18).

CO_2 is depicted in Figure 17. Regardless of the incubation temperature, ^{14}C activity in the trapped CO_2 was higher for "warm-acclimated" gut homogenates. However, no significant difference was observed between the means at either test temperature. On the other hand, $^{14}CO_2$ activity was higher for "cold-acclimated" testis homogenates. The difference between the means was significant (95% level) at either test temperature.

Regardless of the incubation temperature, ^{14}C activity in the extracted lipids was higher for "cold-acclimated" gut homogenates. Conversely, ^{14}C-lipid activity was higher for "warm-acclimated" testis homogenates. The difference between the means was significant (95% level) for the testis at both incubation temperatures. A significant difference was obtained for the gut only at 18°.

Ratios of ^{14}C activity are presented in Table 1. When accompanied by appropriate assumptions, changes in these ratios permit limited inferences about the relative activities of alternate metabolic pathways. One important ratio compares the $^{14}CO_2$ resulting from incubation with glucose-1-^{14}C (G1^{14}C) and glucose-6-^{14}C (G6^{14}C). This ratio -- G1^{14}C:G6^{14}C -- decreased with gut homogenates from warm-acclimated urchins. However, this ratio increased with testis homogenates from warm-acclimated forms. The differences were significant (95% level) for both gut and testis when incubation occurred at 18°.

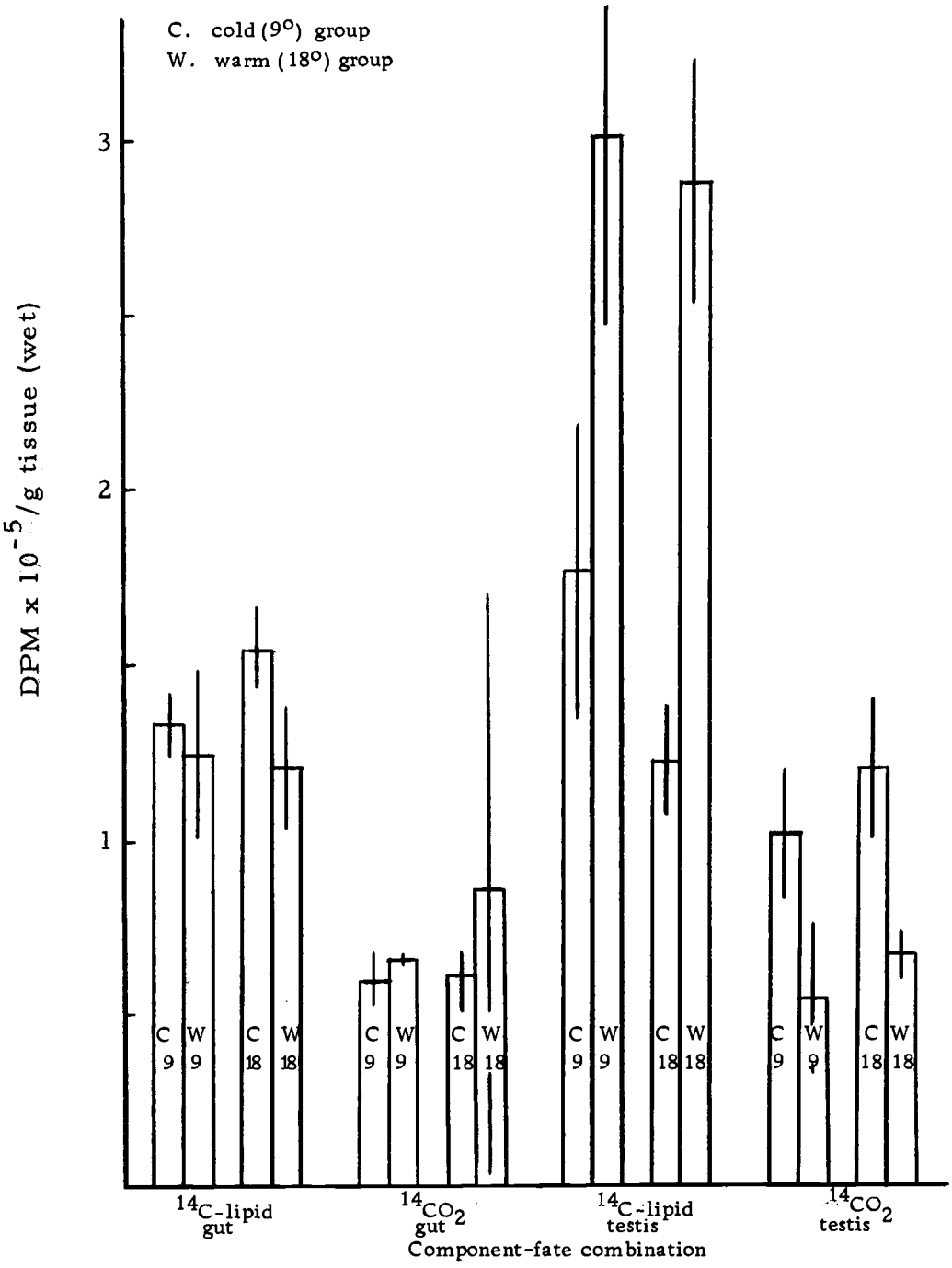

Figure 17. Evolution of ^{14}C-lipid and $^{14}CO_2$ in homogenates from temperature-acclimated urchins incubated with acetate-1-^{14}C. Tissues for each homogenate were obtained from 6 or more urchins. Determinations were made 9 and 3 times for ^{14}C-lipid and $^{14}CO_2$, respectively (vertical bars indicate 95% confidence limits). Acclimation temperatures were 9° (C) and 18° (W). Incubation temperatures were 9° (9) and 18° (18).

Table 1. Radioactivity ratios in body-component homogenates from temperature-acclimated Strongylocentrotus purpuratus.

Incubation Temp.	Acclimation Temp.	$G1^{14}C$ as $^{14}CO_2$:$G6^{14}C$ as $^{14}CO_2$ Gut	$G1^{14}C$ as $^{14}CO_2$:$G6^{14}C$ as $^{14}CO_2$ Testis	$A1^{14}C$ as ^{14}C-lipid:$A1^{14}C$ as $^{14}CO_2$ Gut	$A1^{14}C$ as ^{14}C-lipid:$A1^{14}C$ as $^{14}CO_2$ Testis
9°	9°	11.651	19.112	2.214	1.731
		NS	NS	NS	*
	18°	6.356	21.910	1.877	5.484
18°	9°	12.897	12.775	2.526	1.016
		*	*	*	*
	18°	3.266	17.260	1.397	4.275

$G1^{14}C$ glucose-1-^{14}C
$G6^{14}C$ glucose-6-^{14}C
$A1^{14}C$ acetate-1-^{14}C
NS not significant
* significant difference (95% level)

Tissues for each homogenate were obtained from a minimum of six (6) urchins.

A second important ratio compares the ^{14}C-lipid and $^{14}CO_2$ resulting from incubation with acetate-1-^{14}C. This ratio -- ^{14}C-lipid: $^{14}CO_2$ -- decreased with gut homogenates from warm-acclimated urchins. However, this ratio increased with testis homogenates from warm-acclimated forms. The differences were significant (95% level) for testis at both incubation temperatures but significant for gut only at 18°.

The final radiotracer experiments were a series of time-course studies conducted at 13.5°. Uptake of ^{14}C by ovary homogenates was studied as well as that by gut and testis. The results were generally equivocal throughout the first hour of incubation, but increasing differences were observed after 3 hr. These differences were consistent with previously discussed trends. For example, gut homogenates from warm-acclimated urchins metabolized more glucose-1-^{14}C to $^{14}CO_2$ than those from cold-acclimated forms (Figure 18). The reverse held for testis and ovary homogenates (Figures 19 and 20).

Apparently significant differences in the G1^{14}C:G6^{14}C and ^{14}C-lipid:$^{14}CO_2$ ratios emerged and continued throughout 3 hr of incubation with "temperature-acclimated" gut homogenates (Figures 21a and 22a). Differences in the testis and ovary ratios were much more equivocal (Figures 21b, c, 22b, and c).

Gut, testis, and ovary homogenates were incubated with glucose-1-^{14}C and glucose-6-^{14}C, and the evolution of ^{14}C-glycogen was

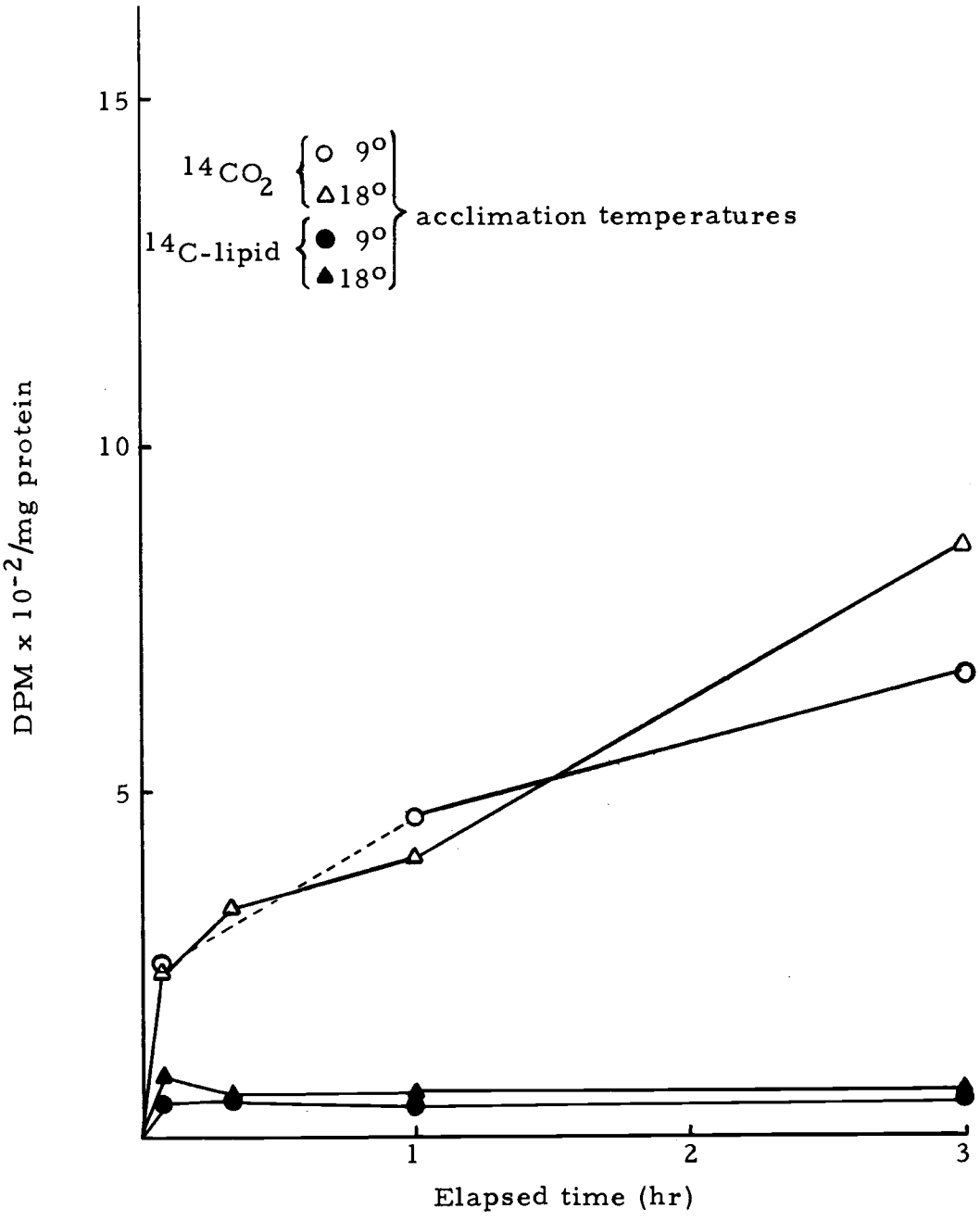

Figure 18. Evolution of ^{14}C-lipid and $^{14}CO_2$ in gut homogenates from temperature-acclimated urchins incubated with glucose-1-^{14}C. Tissues for the "cold" and "warm" homogenates were obtained from 9 and 10 urchins, respectively.

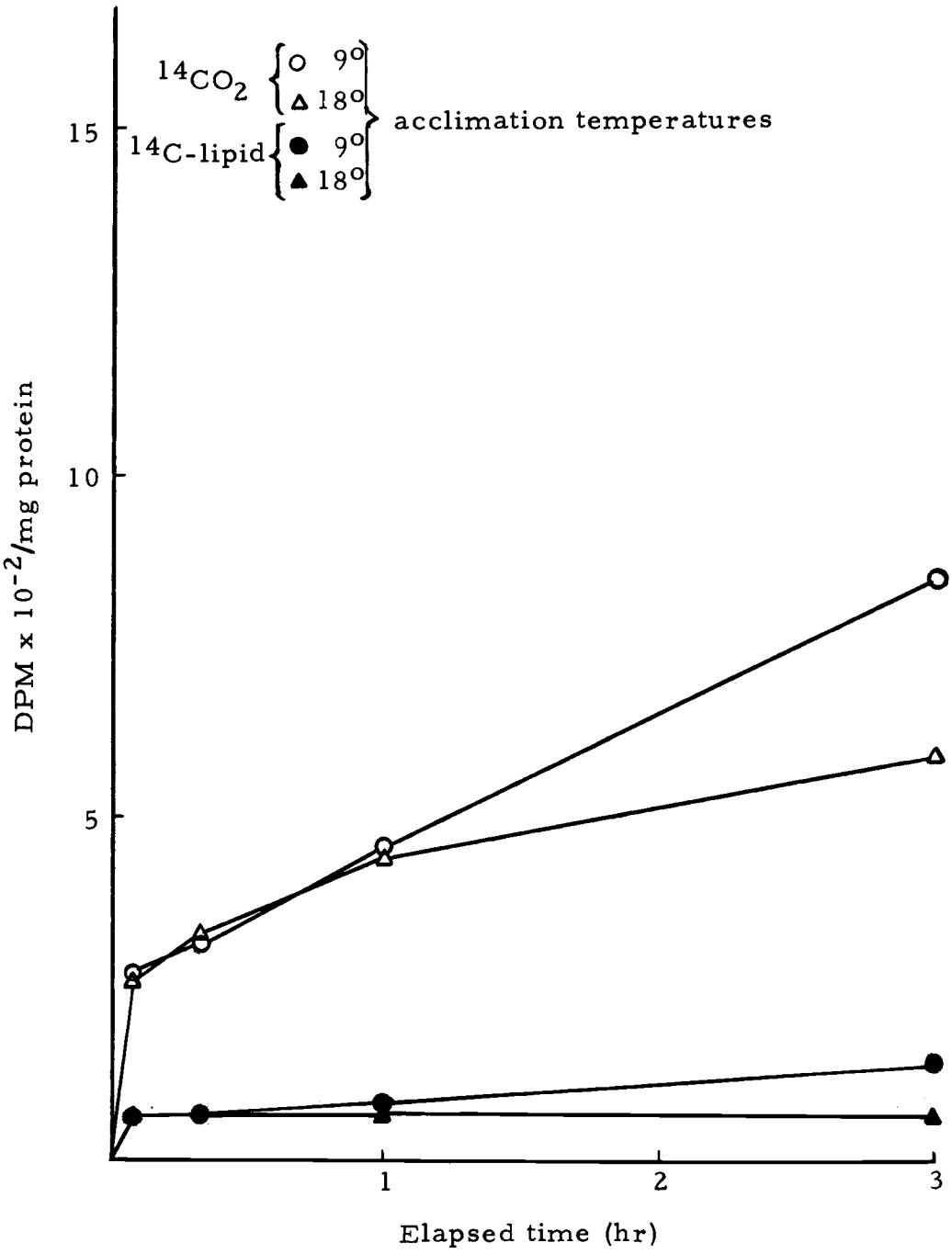

Figure 19. Evolution of ^{14}C-lipid and $^{14}CO_2$ in testis homogenates from temperature-acclimated urchins incubated with glucose-1-^{14}C. Tissues for each homogenate were obtained from 5 urchins.

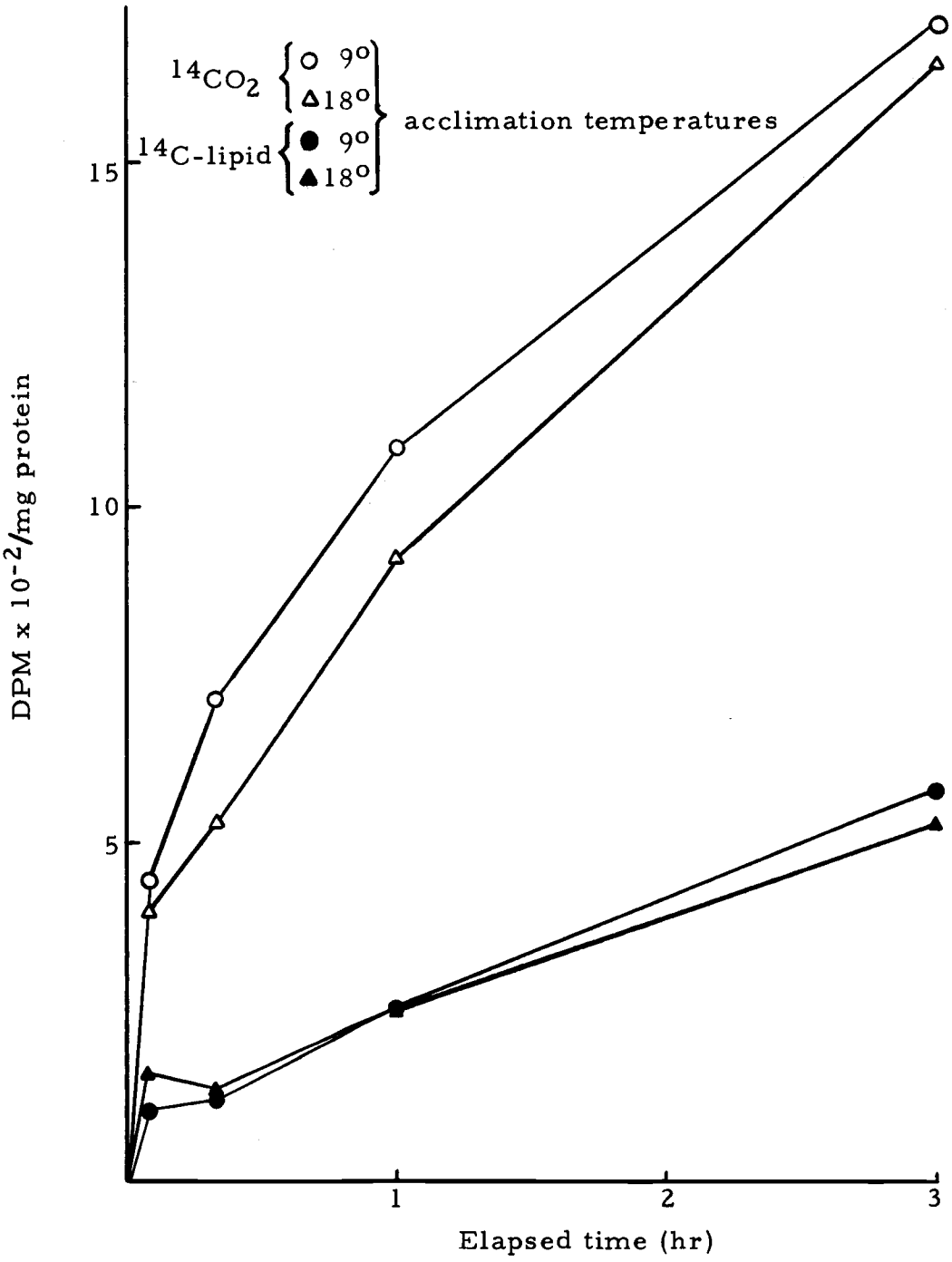

Figure 20. Evolution of ^{14}C-lipid and $^{14}CO_2$ in ovary homogenates from temperature-acclimated urchins incubated with glucose-1-^{14}C. Tissues for the "cold" and "warm" homogenates were obtained from 4 and 5 urchins, respectively.

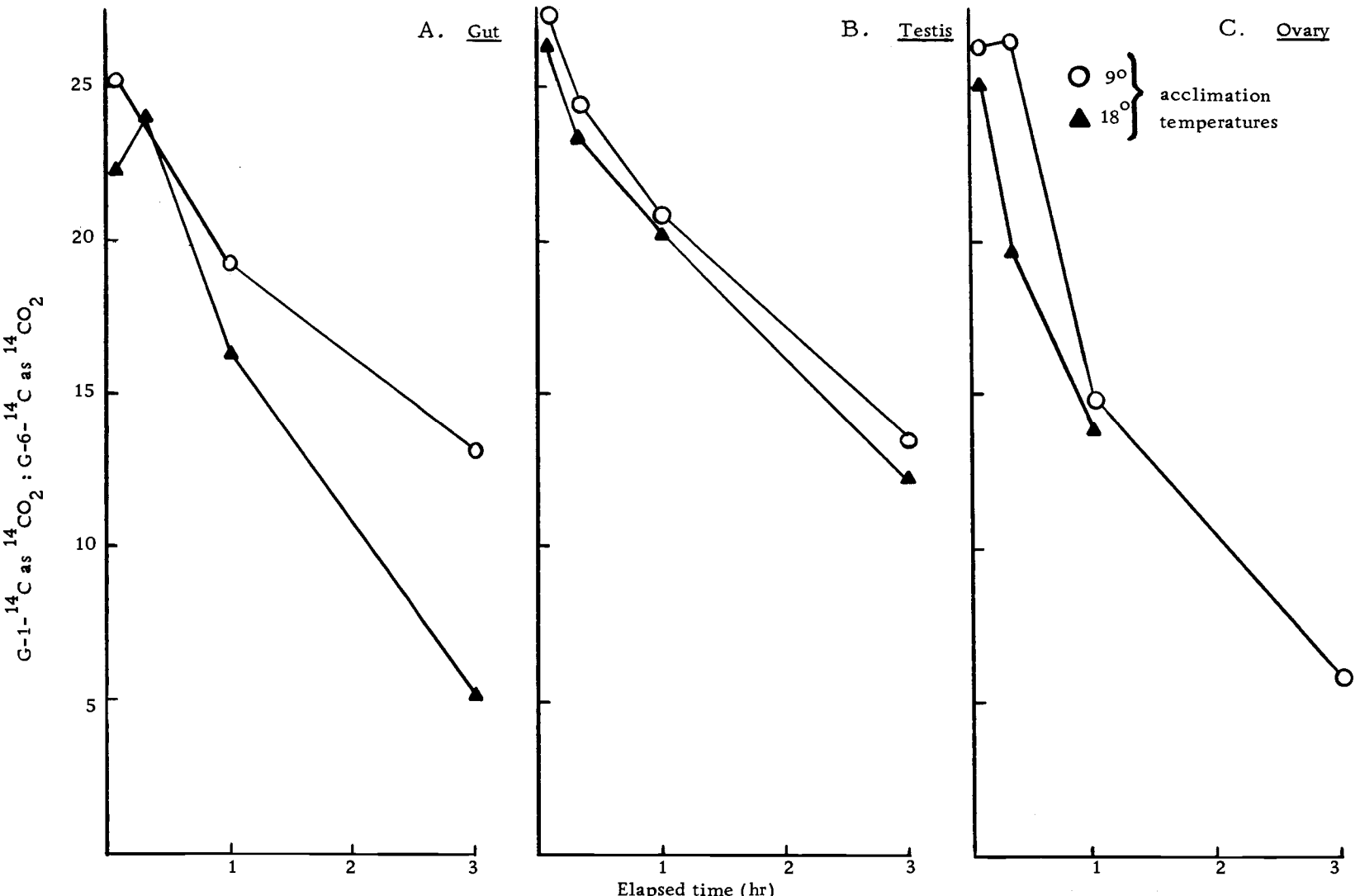

Figure 21. Ratio of $^{14}CO_2$ from glucose-1-^{14}C to $^{14}CO_2$ from glucose-6-^{14}C in homogenates from temperature-acclimated urchins as a function of time. Tissues for gut, testis, and ovary homogenates were obtained from the urchin numbers specified in Figures 18-20, respectively.

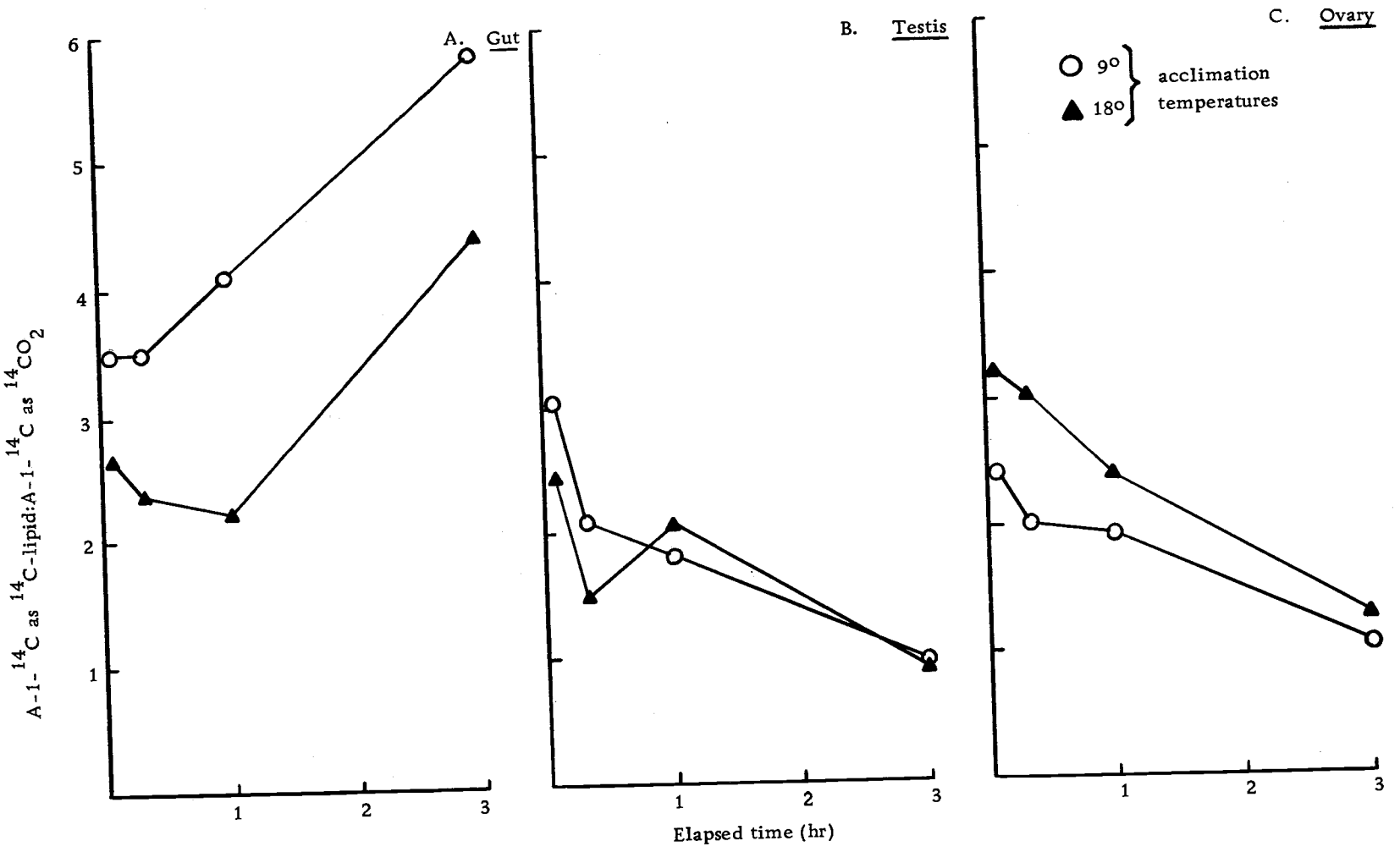

Figure 22. Ratio of ^{14}C-lipid from acetate-1-^{14}C to $^{14}CO_2$ from acetate-1-^{14}C in homogenates from temperature-acclimated urchins as a function of time. Tissues for gut, testis, and ovary homogenates were obtained from the urchin numbers specified in Figures 18-20, respectively.

followed with time. After 3 hr of incubation approximately twice as much glycogenesis occurred with testis and ovary homogenates from cold-acclimated urchins as from warm-acclimated forms, regardless of the label (Figure 23). Lesser and equivocal differences occurred with the gut.

Cytochrome c Oxidase Activity

Cytochrome c oxidase activity was not adversely affected by freezing ovary sections in liquid nitrogen (Table 2). Limited time-course studies indicated little or no loss of cytochrome c oxidase activity with longer time. For example, gut-extract activity went from 6.491 $\pm$ 4.397 to 7.276 $\pm$ 0.6453 μmoles of cytochrome c oxidized per mg of protein per min after 19 days of freezing.[4]

Table 2. Cytochrome c oxidase activity in an ovary extract (Strongylocentrotus purpuratus) following freezing (μmoles cytochrome c oxidized/g wet per min).

Treatment	N	$\bar{X}$	SEM
Fresh	4	24.78	1.952
Frozen for several min	2	23.05	3.000
Frozen for 3 hr	2	24.87	3.680
Fresh after 3 hr	2	9,610	1.140

The extract was obtained from a single urchin.

[4]Data dispersion is expressed as standard error of the mean.

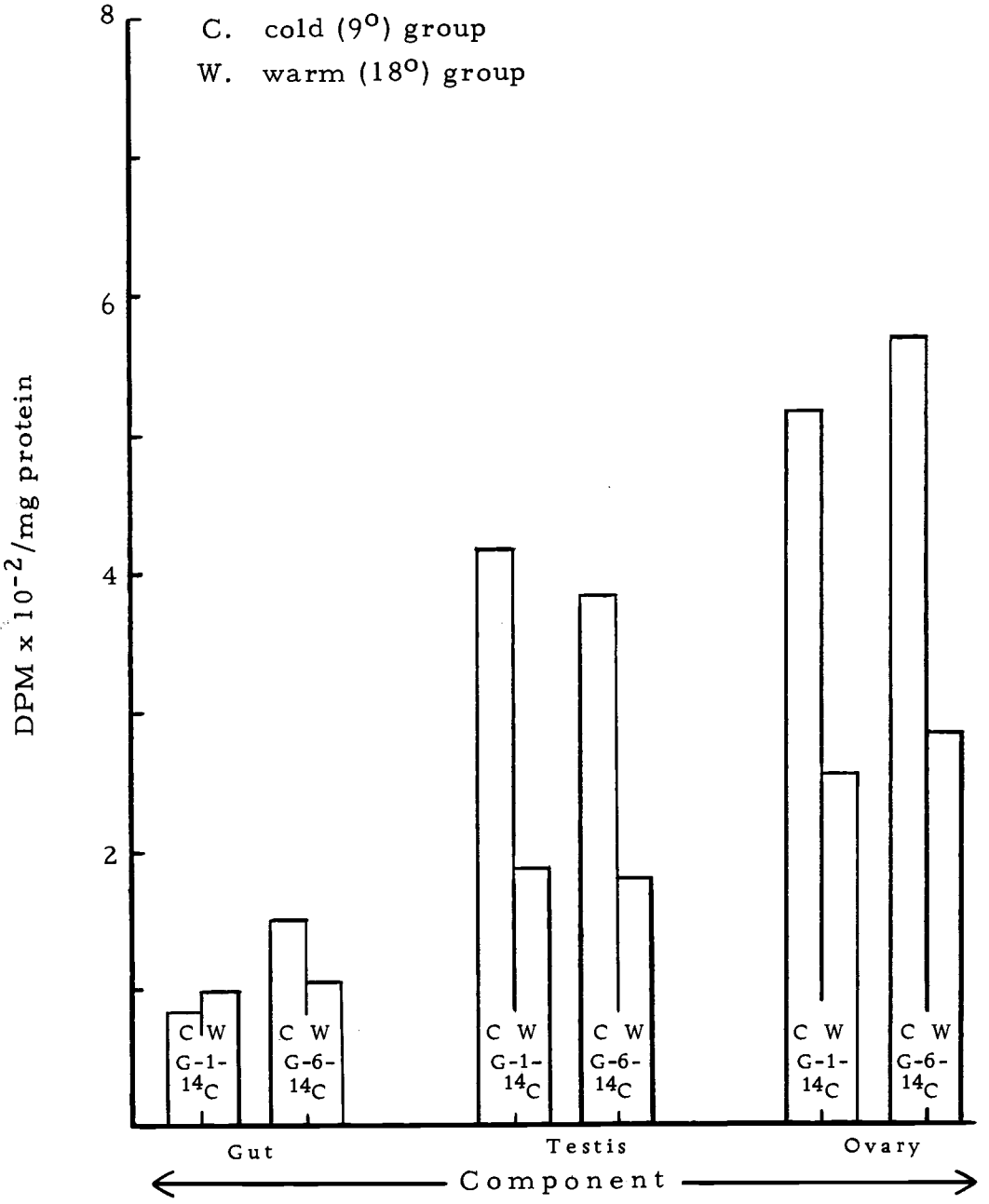

Figure 23. Evolution of ^{14}C-glycogen in homogenates from temperature-acclimated urchins incubated with ^{14}C-glucose. Tissues for gut, testis, and ovary homogenates were obtained from the urchin numbers specified in Figures 18-20, respectively. Acclimation temperatures were 9°(C) and 18°(W). Radiotracers were glucose-1-^{14}C (G1^{14}C) and glucose-6-^{14}C (G6^{14}C).

The influence of pH on cytochrome c oxidase activity is depicted in Figure 24. For the pH values studied, 7.0 was optimal for enzyme activity in gut and ovary extracts. All remaining assays were conducted at a pH of 7.0.

Possible interfering enzymes were considered. Reductase activity should appear following the addition of 5×10^{-3} M NaCN to the reaction mixture (Smith, 1955). No evidence for reductase activity was observed in gut and ovary preparations. Peroxidase activity should appear after passing carbon monoxide through the cytochrome c solution for 90 sec (Smith, 1954). Peroxidase activity accounted for approximately 3.1% and 13.9% of the cytochrome c oxidation in gut and ovary extracts, respectively.

Cytochrome c oxidase activity in gut, testis, and ovary extracts is depicted in Figures 25a, b, and c, respectively. Gut activity was highest in September (Figure 25a), and rather low activity levels were observed in December, March, and June. Slight seasonal changes in activity appeared in testis extracts (Figure 25b). Activity was highest in December but lowest in March. Ovary activity was highest in March (Figure 25c). Little difference was observed among the relatively low rates of September, December, and June.

Large differences in cytochrome c oxidase activity are seen between temperature-acclimated groups in Figures 25a, b, and c. Reciprocal patterns of response were again observed in gut and

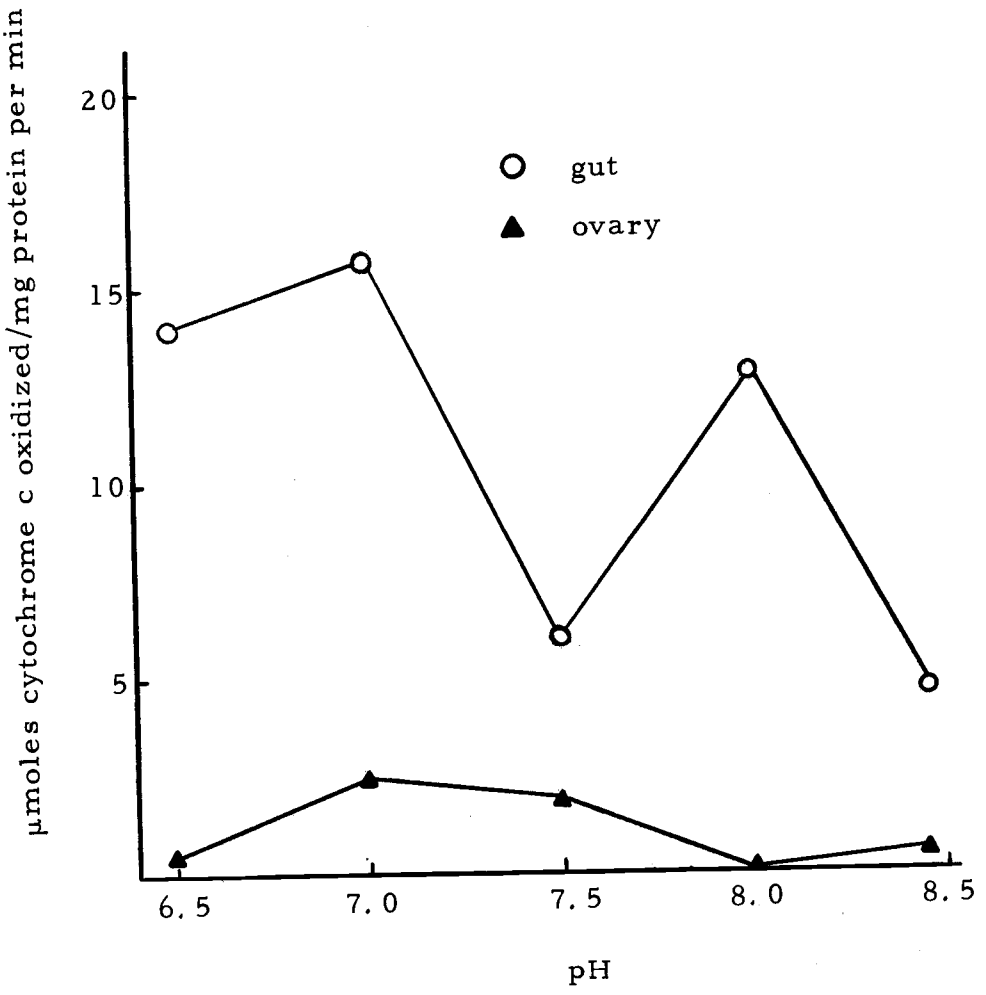

Figure 24. Effect of pH on the reaction velocity of gut and ovary cytochrome c oxidase.

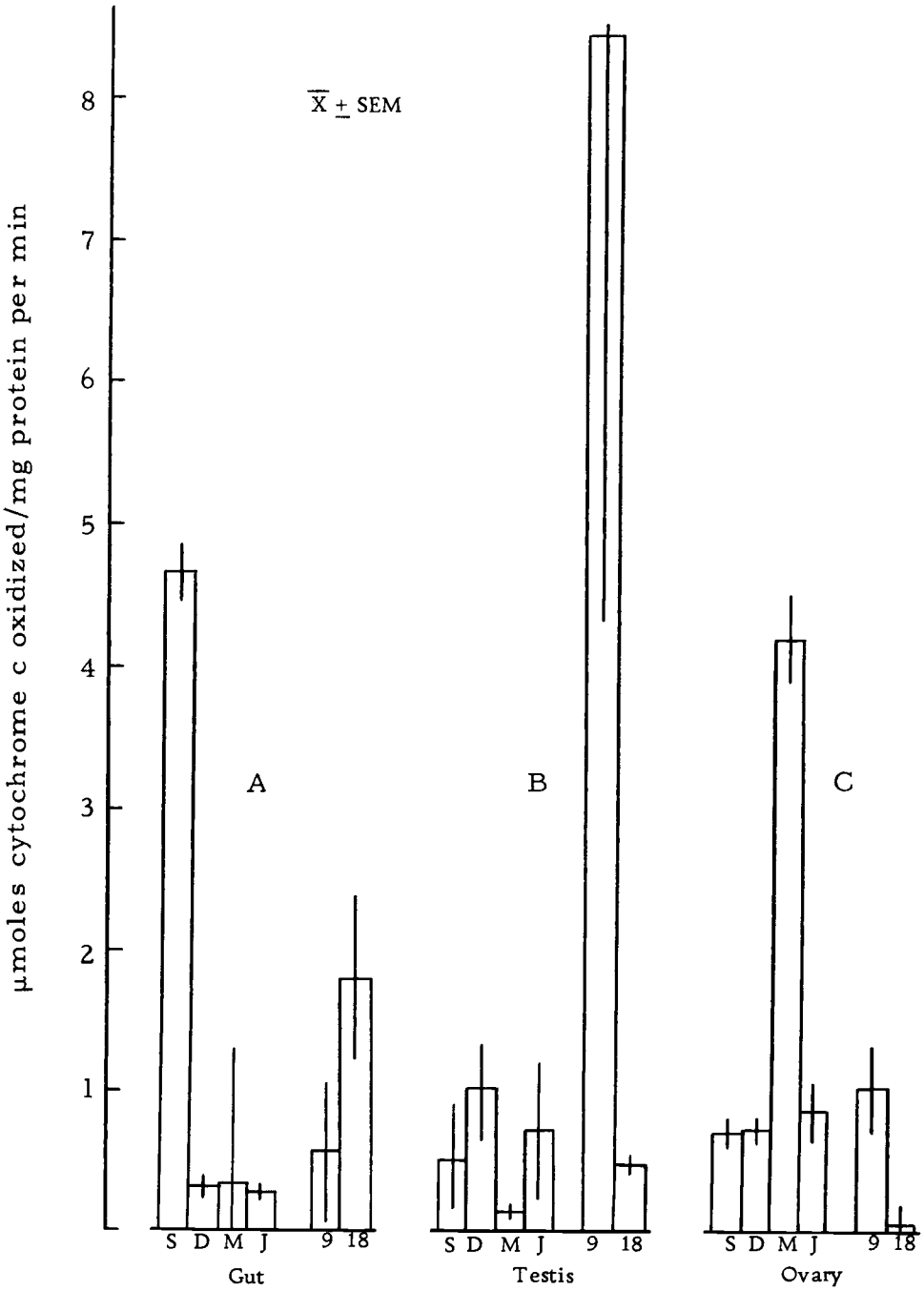

Figure 25. Effects of season and acclimation temperature on the reaction velocity of gut, testis, and ovary cytochrome c oxidase. Tissues for September gut, testis, and ovary extracts were obtained from 8, 2, and 6 urchins, respectively. Tissues for December gut, testis, and ovary extracts were obtained from 12, 7, and 5 urchins, respectively. Tissues for March gut, testis, and ovary extracts were obtained from 13, 8, and 5 urchins, respectively. Tissues for June gut, testis, and ovary extracts were obtained from 14, 6, and 8 urchins, respectively. Tissues for "cold" gut, testis, and ovary extracts were obtained from 11, 6, and 6 urchins, respectively. Tissues for "warm" gut, testis, and ovary extracts were obtained from 11, 1, and 10 urchins, respectively.

gonadal tissues. Higher activity was observed in gut extracts from warm-acclimated urchins than from cold-acclimated forms (Figure 25a). Conversely, both testis and ovary extracts from cold-acclimated urchins had higher activities (Figures 25b and c).

Statistical dispersion about several means in Figures 25a and b is rather large and results, in part, from small sample sizes (a minimal N of 3).

Glucose 6-phosphate Dehydrogenase Kinetics

The effect of pH on G6PDH activity is depicted in Figures 26a and b. Maximal activity resulted at pH values of 8.0 and 8.1. All subsequent G6PDH assays were performed at a pH of 8.1.

Similar G6PDH activities resulted with glycylglycine and Tris-HCl buffer systems. A glycylglycine system was favored due to the tendency of the Tris-HCl pH to vary with temperature. Maximal activity resulted with final concentrations of 333 μM NADP and 10 mM $MgCl_2$.

Reciprocal plots of velocity and substrate concentration (Lineweaver-Burk method) yield straight lines with slopes of K_m/V_m--V_m being the maximal velocity. Linear regression was used to determine the K_m and V_m values. Williams (1969) observed that Lineweaver-Burk plots are particularly suitable for this type of treatment due to the separation of dependent and independent variables.

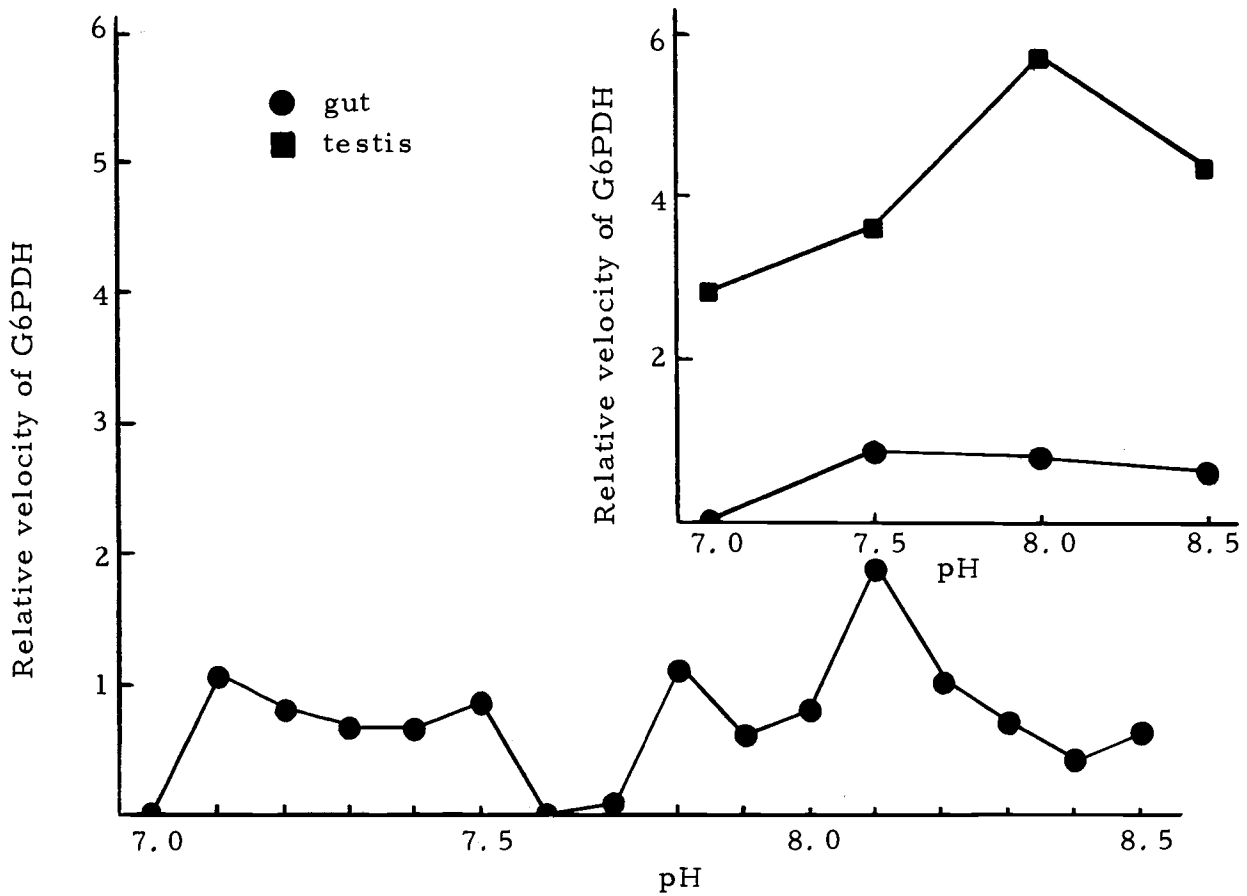

Figure 26. Effect of pH on the relative velocity of gut and testis glucose 6-phosphate dehydrogenase.

Apparent Michaelis constant-temperature relationships of extracts from temperature-acclimated urchins are depicted in Figures 27-29. Low $K_{m(G6P)}$ values -- reflecting high enzyme-substrate affinity -- were obtained with gut extracts at intermediate test temperatures of 14^{o} and 18^{o} (Figure 27). Higher values were obtained at 6^{o}, 10^{o}, and 22^{o}. The influence of temperature acclimation becomes apparent at these temperature extremes.

Different $K_{m(G6P)}$-temperature patterns were obtained with testis extracts (Figure 28). Enzyme-substrate affinity was much greater with extracts from cold-acclimated urchins than those from warm-acclimated forms at 6^{o}, 10^{o}, 14^{o}, and 18^{o}. This difference was reversed or at least equivocal at temperatures of 22^{o} and 26^{o}. In contrast to both gut and testis extracts, those of the ovary exhibited greatest enzyme-substrate (G6P) affinity at 6^{o}, the lowest test temperature (Figure 29). A gradual decrease in enzyme-substrate affinity occurred with ovary extracts between 6^{o} and 18^{o} with higher $K_{m(G6P)}$ values obtained over this range with the "warm-acclimated" extract. Ovary $K_{m(G6P)}$ values showed changes similar to those of the testis at the higher test temperatures.

Maximal-velocity values are the reciprocals of Y-axis intercepts of Lineweaver-Burk plots. These values for G6PDH are plotted against test temperature in Figures 30a, b, and c. Very low V_m values were obtained for gut extracts from temperature-acclimated

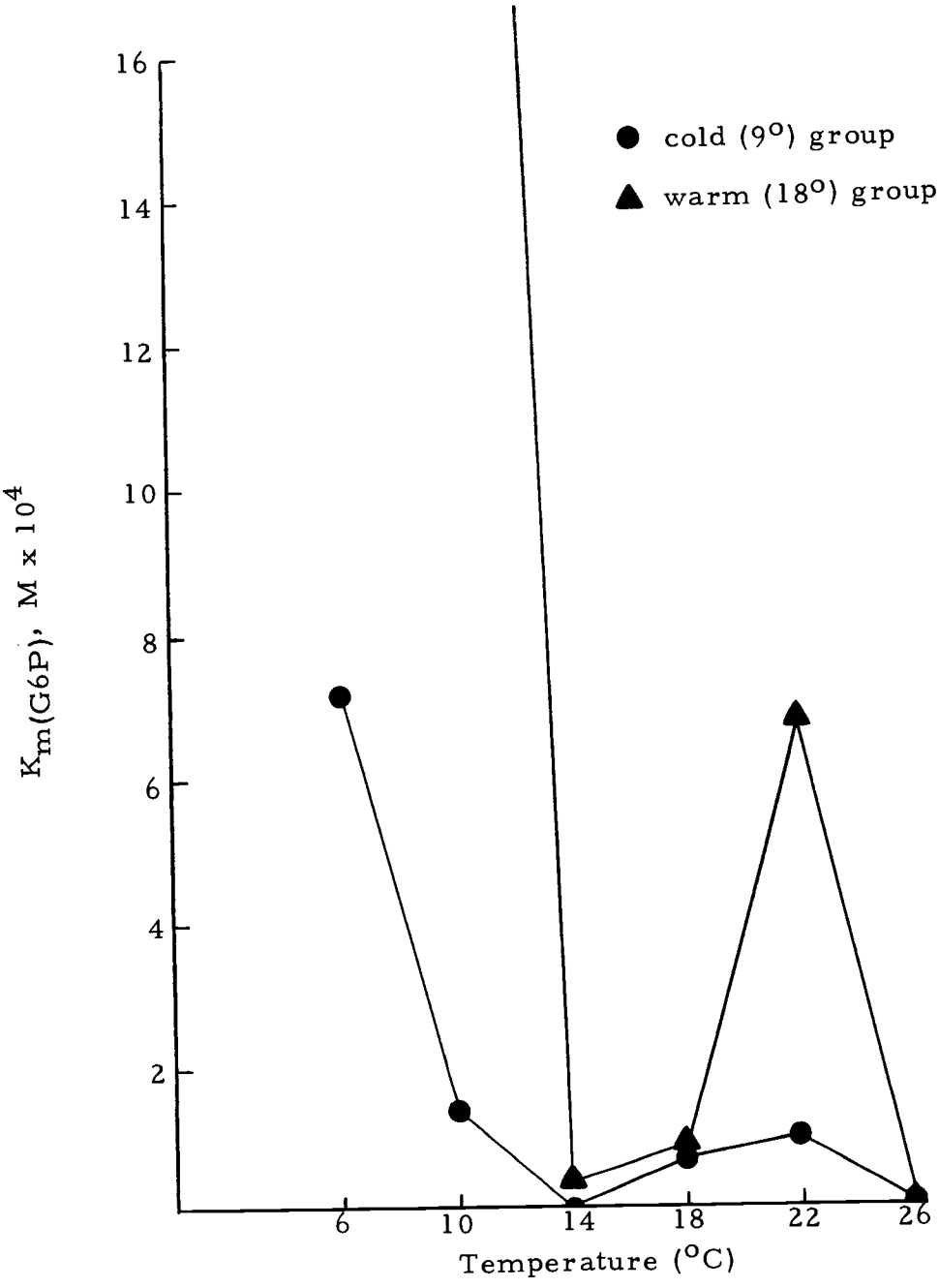

Figure 27. Glucose 6-phosphate dehydrogenase K_m-temperature relationship for gut extracts from temperature-acclimated urchins. Tissues for the "cold" and "warm" extracts were obtained from 9 and 8 urchins, respectively.

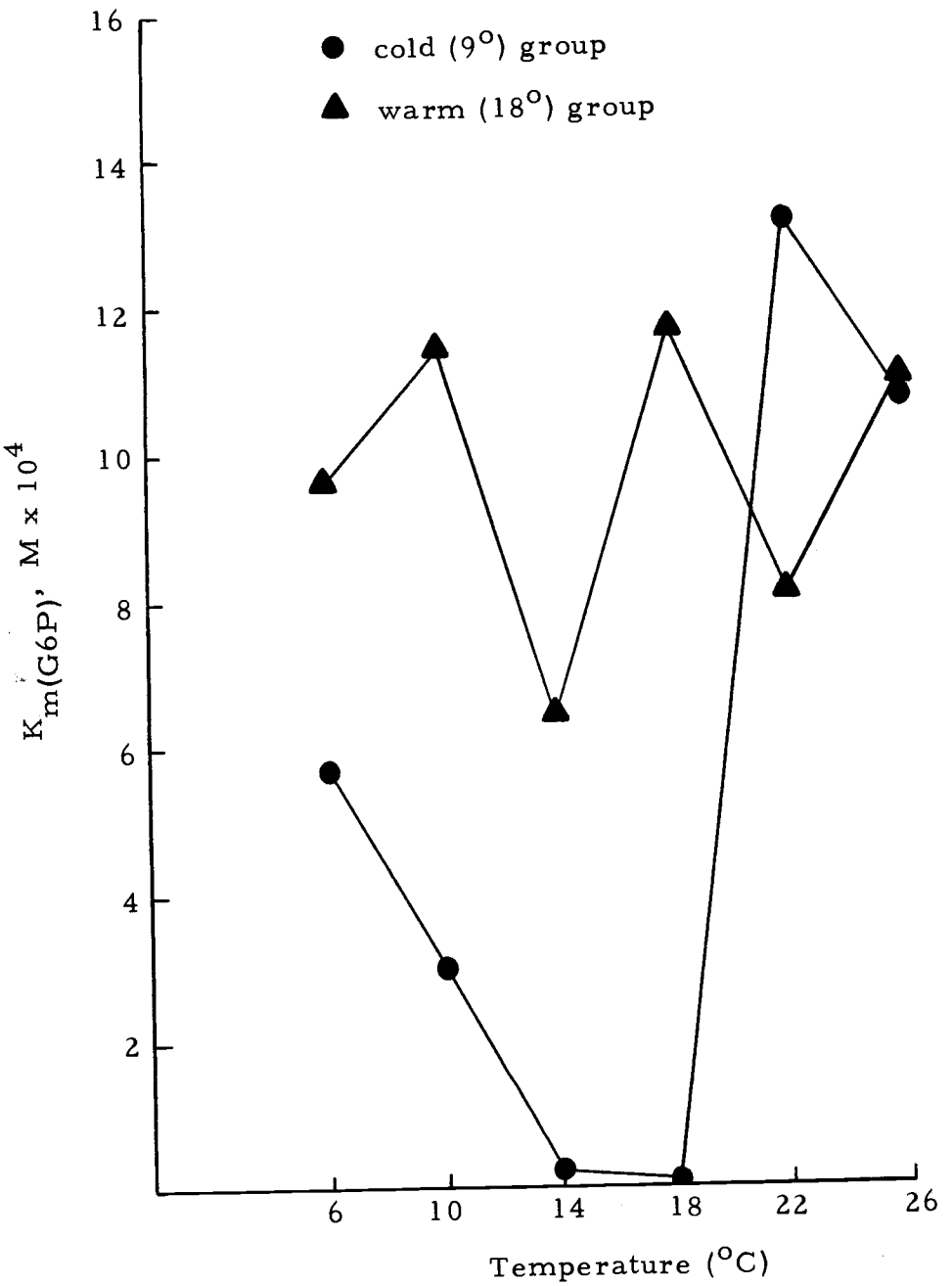

Figure 28. Glucose 6-phosphate dehydrogenase K_m-temperature relationship for testis extracts from temperature-acclimated urchins. Tissues for each extract were obtained from 2 urchins.

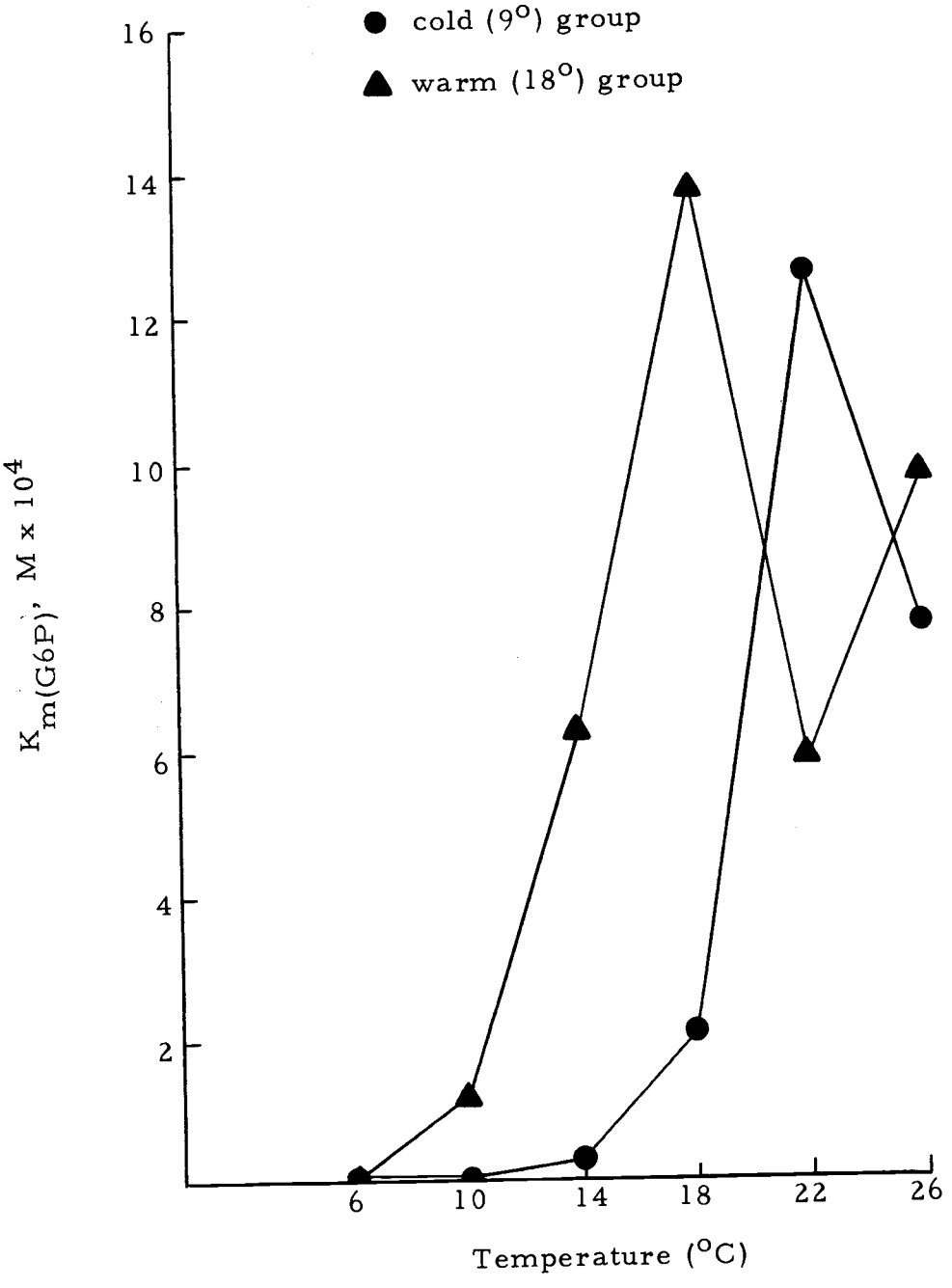

Figure 29. Glucose 6-phosphate dehydrogenase K_m-temperature relationship for ovary extracts from temperature-acclimated urchins. Tissues for the "cold" and "warm" extracts were obtained from 7 and 6 urchins, respectively.

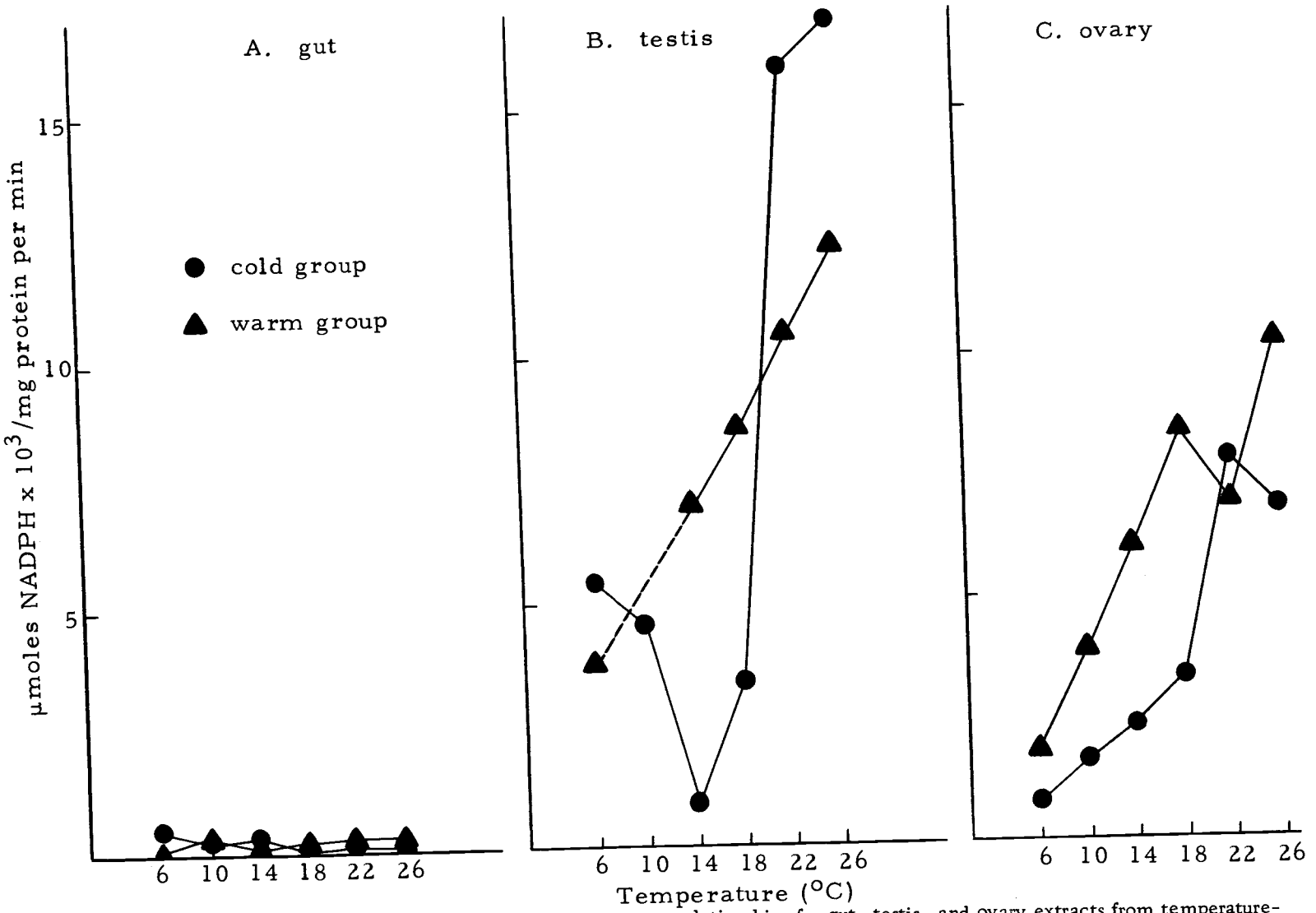

Figure 30. Glucose 6-phosphate dehydrogenase maximal velocity-temperature relationships for gut, testis, and ovary extracts from temperature-acclimated urchins. Extracts were obtained from the urchin numbers specified in Figures 27-29.

urchins (Figure 30a). Gonadal extracts showed different V_m-temperature patterns (Figures 30b and c). Testis activity from warm-acclimated urchins was higher than that from cold-acclimated forms at 10°, 14°, and 18°. Similarly, ovary activity from warm-acclimated urchins was higher than that from cold-acclimated forms at 6°, 10°, 14°, and 18°. Gut G6PDH-V_m values appeared independent of temperature, whereas those of either type of gonad appeared temperature dependent. Gonadal activity was markedly higher than that of the gut.

Body-component Weights

In an ancillary study, the urchins used in the oxygen-consumption experiments were completely partitioned into body wall (including the lantern apparatus), gut, gonad, and perivisceral-fluid components. Wet and ash-free dry weight determinations were made for each of these partitions. These are plotted in Figures 31 and 32 as a percentage of the total weight in relation to season. Seasonal changes in percent gonadal wet weight correspond well with those suggested by Lawrence, Lawrence, and Holland (1965) and Gonor (1973a). Compensatory seasonal changes in percent perivisceral-fluid wet weight corroborate the observation of Giese et al. (1966), Fuji (1967), and Giese (1967) that changes in gonadal index lead to reciprocal changes in perivisceral fluid. Seasonal differences in gonadal

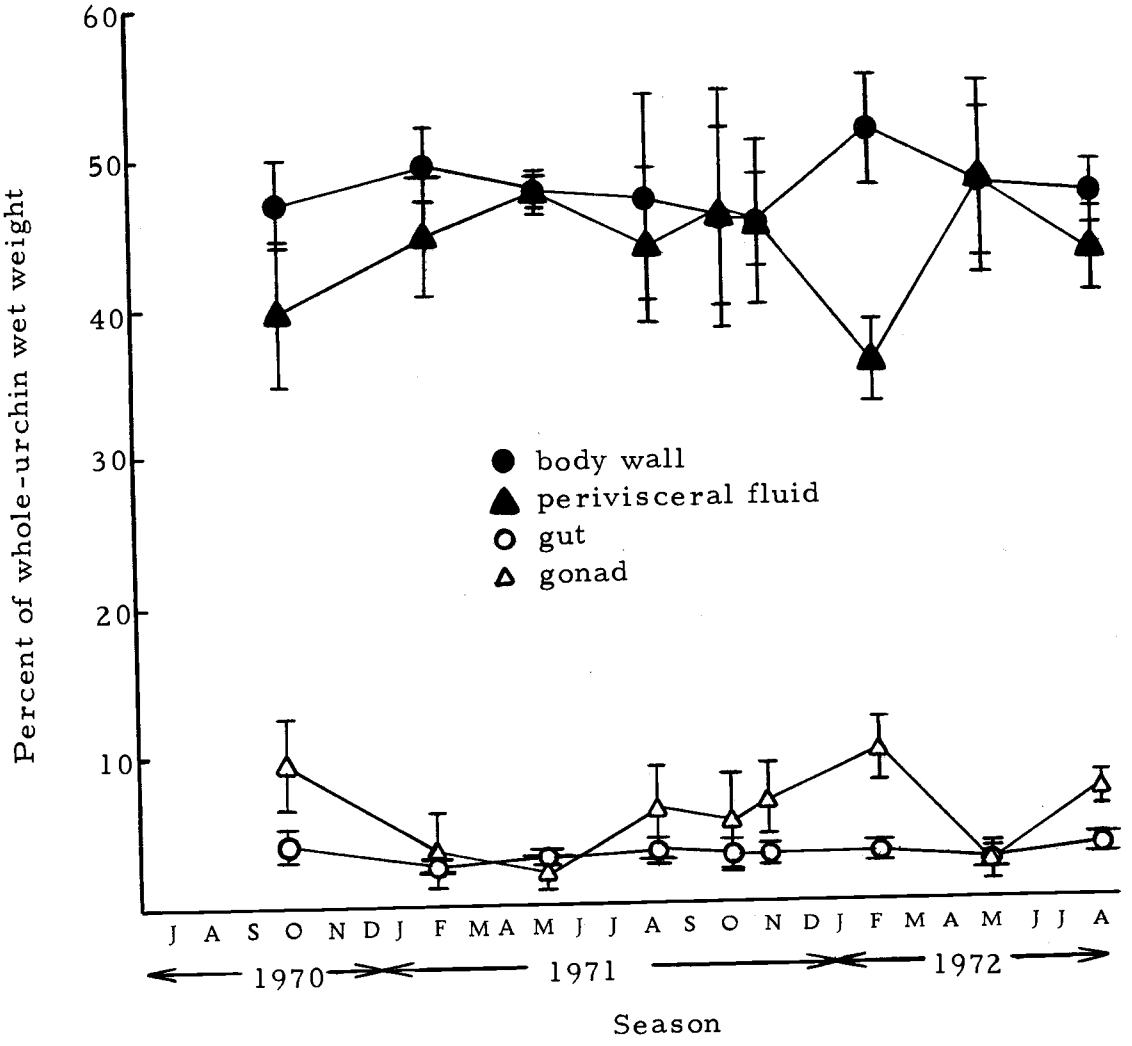

Figure 31. Body-component percent wet weight-season relationships. Vertical bars indicate 95% confidence limits (4-6 urchins per sample).

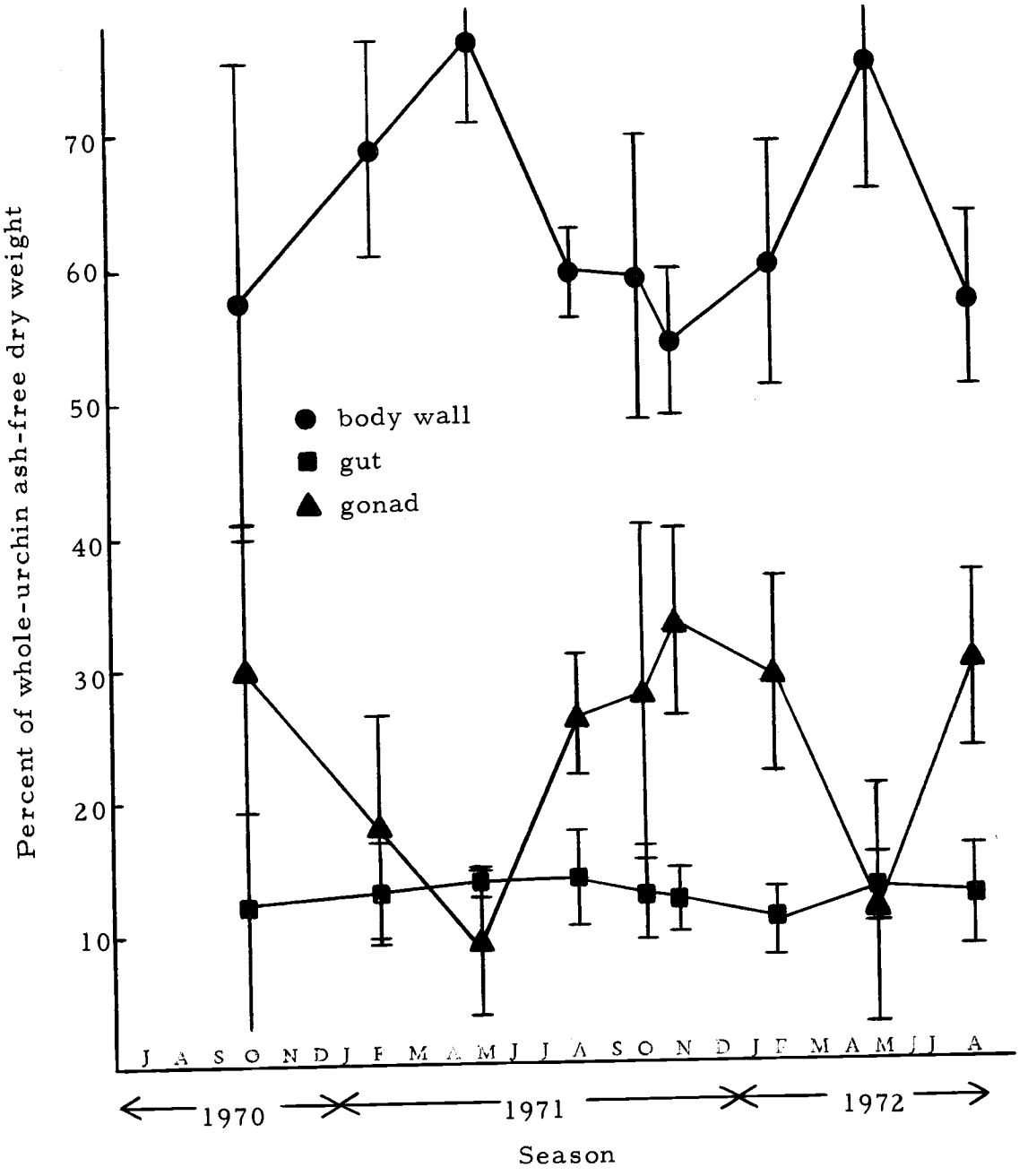

Figure 32. Body-component percent ash-free dry weight-season relationships. Vertical bars indicate 95% confidence limits (4-6 urchins per sample).

percentage when expressed as ash-free dry weight were similar to those expressed as wet weight, but this method of expressing relative weights brought out a reciprocal relationship with the body wall not evident with the latter expression. Changes in gut percentage -- whether expressed as wet weight or ash-free dry weight -- were negligible by comparison.

DISCUSSION

Body-component Oxygen Consumption

Giese et al. (1966) and Giese (1967) suggested that the internal oxygen consumption of intact Strongylocentrotus purpuratus may be low due to internal oxygen tensions which are far below those at air saturation. Direct measurements of internal oxygen tensions have supported this idea. Webster (1972) mentioned that gonadal oxygen tensions are low regardless of season and generally range from 0.0 to 10.0 mm Hg. These findings might appear to detract from the results of the in vitro body-component oxygen-consumption experiments. Oxygen tensions in perivisceral fluid, however, are much higher than those in the gonads. Webster (1972) indicated with representative data that although gonadal tension is about 10 mm Hg, that of perivisceral fluid is approximately 40 to 60 mm Hg. Johansen and Vadas (1967) reported rather constant levels of oxygen consumption for intact S. purpuratus at ambient oxygen tensions above 60 to 70 mm Hg. They further indicated that decreases in perivisceral-fluid oxygen tension and overall oxygen consumption are observed below this range.

Webster (1972) indicated that perivisceral-fluid oxygen tension increases with size. Larger urchins had relatively high perivisceral-fluid tensions (74 and 80 mm Hg for 103.1 and 75.3 g wet,

respectively). The temperature-acclimated S. purpuratus discussed by Ulbricht (1970, 1973a, b) averaged 96.6 g wet -- approximately the size of the larger urchins studied by Webster (1972). Webster (1972) did not present any gut oxygen-tension data. Sea-urchin gut tissues resemble convoluted envelopes. Diffusion problems would appear to be minimized on organ systems similar to the gut -- those with extensive surface areas. Therefore, oxygen appears to be more available to the tissues of this organ system than to those of the gonads. Greater oxygen availability for the internal tissues would appear to characterize the urchins discussed by Ulbricht (1970, 1973a, b) and Ulbricht and Pritchard (1972).

Immersing sections of the body wall in sea water may be justified for studies involving oxygen consumption, but some question exists for the internal components -- i.e., gut, testis, and ovary. The choice of sea water as the ambient medium for the internal components does appear justified because of the relatively uniform rates obtained (Figure 2).

Giese et al. (1966) conducted Warburg-Barcroft respirometry studies on component sections of S. purpuratus and obtained results consistent with those presented in Figure 3. They reported metabolic rates of gut sections which are somewhat higher than those reported herein.

As a result of the preliminary tests, sections of body wall, gut, and gonad were selected for the metabolic rate-temperature experiments. The lantern was excluded from further study owing to both its comparatively small contribution to whole-urchin metabolism (small mass of metabolizing tissue and relatively low metabolic rate) and the variation in rate which resulted from random partitioning. As shown in Figures 4-6, viability of the preparations throughout the experimental period does not appear to be a problem.

Respiration of gametes -- particularly the spermatozoa -- probably accounted for part of the oxygen consumed by gonadal sections. An apparently continuous release of gametes often nullified efforts to wash the loosely attached ones from the gonads. However, released gametes appeared most numerous during October and November, months when gonadal metabolism was minimal. On the other hand, relatively high oxygen consumption was observed for gonads during May, at which time there was no macroscopic evidence for the release of gametes. Also, gametic viability probably declined during the equilibration periods (preceding the rate determinations at any given temperature). This would further reduce the possibility of errors from gametic respiration. Seasonal changes in "gonadal metabolism" are thus thought to reflect metabolic changes in the gonads themselves. Oxygen consumption of the gametes presumably was superimposed upon the gonadal changes in metabolism.

Seasonal shifts in the metabolic rates of the various components fell into one of two categories. Highest rates for body wall and gut generally occurred in the fall (Figures 7 and 8), but highest rates for testis and ovary occurred in the winter or spring (Figures 9 and 10). Hedgpeth and Gonor (1969) noted that the surface temperature of inshore waters off Oregon falls abruptly in early winter. Presumably, air temperatures would emphasize this difference for exposed urchins. Both air and sea temperatures are lowest and have narrow ranges during the winter (Hedgpeth and Gonor, 1969). Therefore, compensatory shifts with respect to temperature were observed seasonally in the metabolic rates of testis and ovary, and apparently paradoxical shifts were observed in the rates of body wall and gut. The possible role of temperature in producing these seasonal effects is further analyzed in the following discussion of temperature acclimation.

A compensatory shift in testis metabolic rate was observed following temperature acclimation (Figure 13), and, in fact, overcompensation (Precht type 1) was observed for this component. Temperature-acclimation shifts for testis sections agree with those following seasonal acclimatization. This was not the case with the remaining components -- body wall, gut, and ovary. Significant rate differences were not observed with any of these components (Figures 11, 12, and 14).

Ulbricht (1970, 1973a, b) reported that inverse compensation of whole-urchin metabolic rate was maximal at test temperatures of 12° and 15°. Therefore, a separate experiment was performed in order to measure the oxygen consumption of both gut sections and homogenates from temperature-acclimated urchins at an intermediate temperature of 13.5°. Temperature acclimation was conducted as previously described. As shown in Table 3, inverse compensation of metabolic rate resulted with both gut sections and homogenates. Although the higher rate for gut sections from warm-acclimated urchins was not significantly different from that of cold-acclimated forms, the difference in rates between the acclimation groups for gut homogenates was highly significant (99% level). Therefore, comparison of the field-acclimatized and temperature-acclimated urchins suggests that temperature is an important but certainly not the only factor effecting seasonal changes in urchin metabolic rate.

Table 3. Oxygen consumption of gut preparations from temperature-acclimated Strongylocentrotus purpuratus (μl O_2/g ash-free dry per hr).

Preparation	Acclimation Temperature	N	$\overline{X}$	SEM	P
sections (1-g slices)	9°	12	506.9	26.5	0.2<P<0.3
	18°	12	542.3	11.1	
10% homogenates	9°	9	409.1	24.0	P<0.01
	18°	9	634.7	72.9	

Tissues for each acclimation group were obtained from two (2) urchins.

Multienzyme Systems

As previously stated, one of the objectives of the present study is to explain the enigmatic inverse compensation observed in whole-urchin metabolic rate following temperature acclimation. Inverse compensation was observed for the oxygen-consumption rates of body-wall and gut components but was not observed with testis and ovary components. Results of the radiotracer studies may support those obtained from higher levels of organization. For example, higher CO_2 production in the radiotracer studies should reflect higher metabolic rates -- assuming that all other factors remain unchanged. This is in fact what occurred in the radiotracer experiments. Gut homogenates from warm-acclimated urchins had higher CO_2 production than those from cold-acclimated forms. On the other hand, testis homogenates from cold-acclimated urchins had higher CO_2 production than those from warm-acclimated forms. These differences occurred regardless of the incubation temperature -- 9° and 18° -- and resulted following incubation with three different radiotracers -- glucose-1-^{14}C, glucose-6-^{14}C, and acetate-1-^{14}C (Figures 16 and 17).

Gonadal metabolism is apparently influenced in a manner opposite to that of the nonreproductive organs. For example, partial compensation and even overcompensation of testis and ovary metabolic rate were observed after temperature acclimation. Inverse

compensation was observed for the body wall and gut. Testis-homogenate CO_2 production from cold-acclimated urchins was higher than that from warm-acclimated forms, but gut-homogenate CO_2 production from warm-acclimated urchins was higher than that from cold-acclimated forms.

A further gonadal-nonreproductive difference is found in the ^{14}C activity determined from lipid extracts. Lipid synthesis was higher in gut homogenates from cold-acclimated urchins than those from warm-acclimated forms (Figure 17). Lipogenesis was higher in testis homogenates from warm-acclimated urchins than those from cold-acclimated forms (Figure 17).

Changes in the relative activities of alternate metabolic pathways under different temperature regimes may be inferred from radioactivity ratios. For example, the relative effectiveness with which the glycolytic and phosphogluconate-oxidative pathways compete for a common substrate -- G6P -- may follow from incubation studies with labelled glucose. Changes in the ratio of $^{14}CO_2$ activity from glucose-1-^{14}C to that from glucose-6-^{14}C -- $G1^{14}C$:$G6^{14}C$ -- imply changes in the relative effectiveness with which these two pathways compete for G6P (Hochachka, 1968). If all of the G6P were metabolized through glycolysis, carbon from both the 1 and 6 positions of glucose would appear as CO_2 with equal probability, and the resulting $G1^{14}C$:$G6^{14}C$ ratio would approach unity. An increase in this ratio

would be anticipated as phosphogluconate oxidation begins to compete for the G6P (Hochachka, 1968). The CO_2 produced during phosphogluconate oxidation comes exclusively from the 1 position of glucose (Larner, 1971). Katz and Wood (1960) cautioned that this approach fails to consider the recycling of hexosemonophosphate through the phosphogluconate-oxidative pathway. Therefore, the precise contribution of G6P to each pathway is not understood without additional information (Katz and Wood, 1960). However, this approach does appear fruitful if interest lies in obtaining information about the relative activities of these two pathways.

Additional gonadal-nonreproductive differences are evident in the $G1^{14}C:G6^{14}C$ and ^{14}C-lipid:$^{14}CO_2$ ratios (Table 1). Increases in the $G1^{14}C:G6^{14}C$ ratio suggest increasing effectiveness for phosphogluconate oxidation relative to that of glycolysis in competing for G6P. In comparing the ratio from warm-acclimated urchins with that of cold-acclimated forms, there was a decrease for gut homogenates and an increase for testis homogenates. Warm acclimation apparently increased the relative activity of glycolysis (compared with that of phosphogluconate oxidation) for gut tissues. Conversely, warm acclimation increased the relative activity of phosphogluconate oxidation (compared with that of glycolysis) for testis tissues.

Increases in the ^{14}C-lipid:$^{14}CO_2$ ratio suggest increasing effectiveness for lipid synthesis relative to that of oxidation in

competing for the common substrate of citric acid. In comparing the ratio from warm-acclimated urchins with that of cold-acclimated forms, there was a decrease for gut homogenates and an increase for testis homogenates. Warm acclimation apparently increased the relative activity of oxidation (compared with that of lipid synthesis) for gut tissues. Conversely, warm acclimation increased the relative activity of lipid synthesis (compared with that of oxidation) for testis tissues.

Both the $G1^{14}C$:$G6^{14}C$ and ^{14}C-lipid:$^{14}CO_2$ ratios support the observations of metabolic differences between gonadal and nonreproductive tissues. Larner (1971) observed that the NADPH and H^+ produced during phosphogluconate oxidation constitute a reducing-power potential for fatty acid synthesis. Whenever the relative activity of lipid synthesis changes, a corresponding change in that of phosphogluconate oxidation could provide the reducing equivalents necessary for this change in biosynthesis. This appears to have occurred. Following warm acclimation, the relative activities of lipid synthesis and phosphogluconate oxidation (compared to those of oxidation and glycolysis, respectively) increased with testis tissues. However, under the same conditions, the relative activities of oxidation and glycolysis (compared to those of lipid synthesis and phosphogluconate oxidation, respectively) increased with gut tissues.

Time-course experiments were conducted over a 3-hr period in order to compare ^{14}C evolution from "cold- and warm-acclimated" homogenates. Exogenous glucose or acetate was added to each reaction flask with incubation occurring at 13.5°. Steady-state levels were apparently achieved at times following 5 min of incubation. The results for gut and testis homogenates after 3 hr of incubation corroborate the gonadal-nonreproductive differences of the above experiments (Figures 16 and 17) in which incubation was terminated after this length of time. For example, CO_2 production was higher in gut homogenates from warm-acclimated urchins than those from cold-acclimated forms (Figure 18). Conversely, CO_2 production was higher for both types of gonad in homogenates from cold-acclimated urchins than those from warm-acclimated forms (Figures 19 and 20). Equivocal results were generally obtained throughout the first hour of incubation.

Radioactivity for the time-course studies is expressed as disintegrations per min (dpm) per mg of protein, contrasting with the dpm per g of tissue of the earlier experiments. Changes in the protein levels of gut and testis following temperature acclimation appeared to be proportional to changes in the wet weights of these component sections. This is shown by the protein concentrations of gut and testis homogenates from warm-acclimated urchins expressed as a percentage of those from cold-acclimated forms (98.2% and 100.4%, respectively).

However, a disproportionate change in ovary protein relative to the wet-weight change was observed following temperature acclimation -- i.e., the concentration of ovary protein from warm-acclimated urchins was 113.9% of that from cold-acclimated forms.

Lipid levels in the ovary may be disproportionate relative to the tissue weight and, in effect, may balance the disproportionate levels of protein. For example, a decrease in the relative amount of lipid may accompany the relative increase in protein following warm acclimation. Lawrence, Lawrence, and Giese (1966) noted increases in gonadal protein (percent dry weight) and decreases in gonadal lipid (percent dry weight) during a post-spawning period (from early April to June) for S. purpuratus. Gonor (1973a) indicated a general increase in sea-surface temperature along the central Oregon coast during this period. Also, the air temperature undergoes a general increase during this period. The urchins studied by Lawrence et al. (1966) were field acclimatized and not temperature acclimated. Nevertheless, the parallels between the protein and lipid changes and the temperature conditioning of this study (Lawrence et al., 1966) and the present study are intriguing.

Temperature acclimation of S. purpuratus (from the time-course studies) caused apparent differences in the G1^{14}C:G6^{14}C and ^{14}C-lipid: $^{14}CO_2$ ratios of gut homogenates (Figures 21a and 22a). These results of the time-course study thus seem to corroborate those of the earlier

experiments, but confidence levels cannot be assigned to any of the points in Figures 21 and 22 as each represents a single observation.

Negligible differences in the G1^{14}C:G6^{14}C and ^{14}C-lipid:$^{14}CO_2$ ratios of testis and ovary homogenates were observed following temperature acclimation. The incubation temperature for these studies was 13.5^{o} as it was for the gut extracts. These results do not corroborate the earlier experiments in which a reciprocal relationship was shown between the gonadal and nonreproductive tissues. Part of the explanation may lie in the fact that urchins used in these studies were collected in midwinter, and incubation occurred in late winter. Urchins used in the earlier radiotracer experiments were collected in midsummer, and incubation occurred in late summer. Seasonal variation in urchin gonads is well documented (Stott, 1931; Bennett and Giese, 1955; Giese et al., 1959; Giese, 1961, 1966; Holland and Giese, 1965; Fuji, 1967; Chatlynne, 1969, 1972; Gonor, 1973a, b). Seasonal changes in gonadal growth, gametogenesis, levels of organic constituents, or other factors might have led to the differences observed for testis homogenates from summer and winter urchins. On the other hand, these differences may reflect an acute response to temperature change in testis metabolism irrespective of season.

The ^{14}C-ratio differences between the time-course and the previously discussed radiotracer studies may be a function of incubation temperature. This interesting possibility may serve as an explanation

for the inverse compensation observed at higher levels of urchin organization. The ^{14}C ratios for all radiotracer experiments are presented in Table 4. Gut ratios present a consistent trend regardless of the incubation temperature -- warm acclimation increased glycolysis and oxidation relative to phosphogluconate oxidation and lipid synthesis, respectively. The opposite occurred for testis homogenates when incubated at the acclimation temperatures -- 9^{o} and 18^{o}. However, differences between the acclimation groups were negligible at the intermediate incubation temperature of 13.5^{o}.

An interesting comparison involves the radiotracer results (the present study) and the metabolic rates of whole urchins at 12^{o} and 15^{o} (Ulbricht, 1970, 1973a, b) following temperature acclimation. Giese et al. (1966), following in vitro studies, inferred that oxygen consumption by the gonads is from 1.6% to 57.0% of the theoretical total, dependent upon the gonadal index and oxygen-consumption rate. However, due to the low metabolic rates for gonadal tissues (Figure 15), the gonadal contribution to whole-urchin metabolism is less than or no more than that of the nonreproductive tissues. If gonadal and nonreproductive levels of metabolism are comparable, negligible differences in whole-urchin metabolic rate appear likely between the acclimation groups when tested at 9^{o} and 18^{o}. This in fact is what was observed by Ulbricht (1970, 1973a, b). The inverse compensation observed in whole-urchin rates at 12^{o} and 15^{o} might, partially at

Table 4. Radioactivity ratios in body-component homogenates from temperature-acclimated Strongylocentrotus purpuratus.

Incubation Temp.	Acclimation Temp.	$G1^{14}C$ as $^{14}CO_2$:$G6^{14}C$ as $^{14}CO_2$ Gut	Testis	Ovary	$A1^{14}C$ as ^{14}C-lipid:$A1^{14}C$ as $^{14}CO_2$ Gut	Testis	Ovary
9°	9°	11.651	19.112	---	2.214	1.731	---
	18°	6.356	21.910	---	1.877	5.484	---
*13.5°	9°	13.157	13.476	14.849	5.821	0.955	1.005
	18°	5.264	12.313	13.851	4.377	0.906	1.273
18°	9°	12.897	12.775	---	2.526	1.016	---
	18°	3.266	17.260	---	1.397	4.275	---

$G1^{14}C$ glucose-1-^{14}C
$G6^{14}C$ glucose-6-^{14}C
$A1^{14}C$ acetate-1-^{14}C

* conducted in a season different from that of 9° and 18°.

Tissues for each homogenate were obtained from a minimum of four (4) urchins.

least, have resulted from the inverse compensation of the nonreproductive-tissue rates and the negligible differences between the gonadal-tissue rates. A correct assessment of the role of acute metabolic responses to temperature, seasonal changes in the gonads, or any other factor in effecting the inverse compensation of whole-urchin metabolic rate is not possible without additional information.

Radioactivity in the trapped CO_2 was greater when homogenates were incubated with G1^{14}C than with G6^{14}C. Consequently, all of the G1^{14}C:G6^{14}C ratios (Tables 1 and 4) are greater than unity. An estimate of phosphogluconate-oxidative participation follows from the difference between $^{14}CO_2$ from G1^{14}C and $^{14}CO_2$ from G6^{14}C. An estimate of glycolytic participation follows from the remaining ^{14}C activity trapped as CO_2. These estimates gain precision as participation of the nontriose-phosphate pathways -- conversion of G6P to glycogen, lactose, glucosamine, uronic acids, pentose, etc. -- declines (Katz and Wood, 1960).

Levels of oxidation may be inferred from the trapped $^{14}CO_2$. Undoubtedly, a part of the oxidation implicit in Figures 16-20 was complete -- that is electrons from the oxidation of metabolites were channelled through the electron transport system. If this relative amount were not to change appreciably, the trapped $^{14}CO_2$ would be an index of oxidative phosphorylation.

The metabolism of acetate, as acetyl-S-CoA, may not be restricted to lipid synthesis and oxidation. Gluconeogenesis is one possible alternative. No measurable ^{14}C-glycogen (with one marginal exception) was extracted from homogenates incubated with acetate-1-^{14}C for up to 3 hr. However, significant levels of glycogenesis were detected in replicate homogenates incubated with Gl^{14}C and G6^{14}C (Figure 23). Apparently, most of the $^{14}CO_2$ trapped following incubation with acetate-1-^{14}C resulted from complete oxidation. This reasoning must not necessarily be applied to the homogenates incubated with Gl^{14}C and G6^{14}C. Nevertheless, changes in the trapped $^{14}CO_2$ following temperature acclimation were the same for any given homogenate, regardless of the radiotracer -- glucose-1-^{14}C, glucose-6-^{14}C, or acetate-1-^{14}C.

Voogt (1972) observed that squalene and 3β-sterols are synthesized from acetate injected into three urchin species. Fagerlund (1969) noted that cholesterol is frequently a major component of urchin sterols. The biosynthesis of cholesterol originates with acetyl-S-CoA and continues with successive additions to an isoprenoid precursor. Temperature acclimation may influence cholesterol synthesis relative to both fatty acid synthesis and complete oxidation. Cholesterol synthesis utilizes oxygen and involves several decarboxylations (Larner, 1971). The changes in $^{14}CO_2$ from acetate-1-^{14}C following temperature acclimation are supported by the oxygen-consumption

measurements of component sections and homogenates -- oxygen consumption and CO_2 production when higher for gut were lower for testis tissues (Figures 13 and 17 and Table 3). However, the differences in lipid-extract ^{14}C activity were quite large (Figure 17). Therefore, even if the activity of fatty acid synthesis relative to that of cholesterol synthesis is temperature sensitive, some other phenomenon -- probably the activity of lipid synthesis relative to that of complete oxidation -- appears to exert a greater influence on urchin metabolism at the higher levels of organization.

The evidence previously cited suggests that complete oxidation -- oxidative phosphorylation -- is sufficiently temperature sensitive so that oxidation relative to lipid synthesis is altered following temperature acclimation. The radiotracer results correlate quite well with the body-component oxygen-consumption results. For example, gut tissues from warm-acclimated urchins had higher metabolic rates (Table 3) along with increasing glycolysis and oxidation relative to phosphogluconate oxidation and lipid synthesis, respectively, (Figures 16-18) than those from cold-acclimated forms. Conversely, testis tissues from warm-acclimated urchins had lower metabolic rates (Figure 13) along with increasing phosphogluconate oxidation and lipid synthesis relative to glycolysis and oxidation, respectively, (Figures 16, 17, and 19) than those from cold-acclimated forms. The gonadal-nonreproductive differences are obvious.

An additional gonadal-nonreproductive difference is apparent in Figure 23. Temperature acclimation had equivocal and apparently negligible effects on glycogenesis in gut tissues. However, temperature acclimation led to consistent differences in glycogenesis in testis and ovary tissues. Gonadal tissues from cold-acclimated urchins converted approximately twice as much glucose to glycogen as those from warm-acclimated forms. These gonadal differences in glycogenesis appear to be supported by the observations of Giese et al. (1959). Glycogen levels in either testis or ovary increased proportionally more than the bulk of the gonad throughout its growth phase. Although Gonor (1973a) noted that the correlation between gonadal growth and sea temperature is not good for populations of S. purpuratus along the central Oregon coast, he suggested that the gonadal growth of this species occurs from July through October or November. Gonor (1973a) indicated that sea-surface temperature undergoes a general decline during this period.

Cytochrome c Oxidase Activity

The suggestion that changes in urchin cytochrome c oxidase activity may reflect changes occurring at higher levels of organization follows from studies of fish metabolism. Cytochrome c oxidase activity in extracts of several tissues from cold-acclimated goldfish, Carassius auratus (Linnaeus), was higher than that from

warm-acclimated forms (Freed, 1965; Caldwell, 1969). Freed (1965) and Caldwell (1969) suggested that quantitative changes in the enzyme and changes in the environment of the enzyme, respectively, may have led to this effect. Caldwell (1969) observed that cytochrome c oxidase and reductase activities in gill mitochondrial extracts were increased following cold acclimation and suggested that the entire electron transport system in these tissues is activated when exposed to cold.

Changes in cytochrome c oxidase activity in tissue extracts from temperature-acclimated S. purpuratus correlate well with results from the body-component oxygen-consumption studies. For example, gut metabolic rate was generally highest in the fall as was cytochrome c oxidase activity (Figures 8 and 25). Testis and ovary metabolic rates were highest in the winter or spring as were the respective enzyme activities (Figures 9, 10, and 25). Gut cytochrome c oxidase activity from warm-acclimated urchins was higher than that from cold-acclimated forms -- as with metabolic rate (Figure 25 and Table 3). Conversely, gonadal enzyme activities from cold-acclimated urchins were higher than those from warm-acclimated forms -- as with metabolic rate (Figures 13, 14, and 25). Therefore, gonadal-nonreproductive differences in enzyme activity occurred following seasonal acclimatization and temperature acclimation.

The results in Figure 25 were obtained with extracts from quick-frozen tissues. A separate experiment was conducted in order to

verify the preliminary control experiment. Cytochrome c oxidase activity was determined with freshly prepared gut extracts from temperature-acclimated S. purpuratus. All assays were conducted at 13.5°. The results are summarized in Table 5. These results -- higher enzyme activity in extracts from warm-acclimated urchins than those from cold-acclimated forms -- corroborate the observations obtained with frozen tissues. The difference between the means is highly significant (99% level).

Table 5. Cytochrome c oxidase activity in gut extracts (Strongylocentrotus purpuratus) following temperature acclimation (μmoles cytochrome c oxidized/mg protein per min).

Treatment	N	$\overline{X}$	S^2	SEM	P
Cold group	6	0.1876	0.0005	0.0087	P<0.001
Warm group	3	0.4178	0.0020	0.0257	

Extracts for each acclimation group were obtained from two (2) urchins.

Comparison of the cytochrome c oxidase results from the quick-frozen and freshly prepared gut extracts (using urchins collected in early September and late May, respectively) discloses what appears to be a persisting seasonal influence. Enzyme activity in both studies (Figure 25 and Table 5) was higher in gut extracts from warm-acclimated urchins than those from cold-acclimated forms, but activity in the early fall was several times higher than that in the late spring. This seasonal difference is precisely what was observed following

seasonal acclimatization (Figure 25).

Glucose 6-phosphate Dehydrogenase Kinetics

Gonadal-nonreproductive differences, apparent in most of the results, are not evident in the $K_{m(G6P)}$-temperature relationships following temperature acclimation of S. purpuratus (Figures 27-29). Greater similarity was frequently observed for gut-testis or gut-ovary comparisons than was observed between the gonads themselves. For example, $K_{m(G6P)}$ values for gut and testis extracts increased at the lower extreme of the test range (6^{o} and 10^{o}), whereas ovary $K_{m(G6P)}$ values were lowest at these temperatures. Secondly, negligible differences occurred between minimal $K_{m(G6P)}$ values of the acclimation groups for gut and ovary extracts. This difference was much greater for testis extracts.

Changes in enzymatic activity occurring at temperatures below the biological temperature range resemble those resulting from negative modulators (Somero and Hochachka, 1971; Hochachka and Somero, 1973). These decreases in activity, accompanied by enormous Q_{10} values, presumably result from increasing K_m values as well as decreases in kinetic energy.

Low $K_{m(G6P)}$ values for ovary extracts at 6^{o} and 10^{o} (Figure 29) suggest that G6P oxidation may readily occur at these temperatures, regardless of the acclimation temperature. The $K_{m(G6P)}$ values for

testis extracts at 6° and 10° (Figure 28) were high by comparison. If G6PDH is a control point for phosphogluconate oxidation (Kather, Rivera, and Brand, 1972) and phosphogluconate oxidation parallels lipogenesis (Ball, 1966), gonadal $K_{m(G6P)}$ values at 6° and 10° appear to correspond to lipid changes in the gonads. Giese et al. (1959) noted that during the gonadal growth of S. purpuratus ovary lipid increases proportionally more than the gonad itself but that testis lipid increases proportionally less. Gonor (1973a) indicated that S. purpuratus gonads increase in size from July through October or November and that during this period sea-surface temperature undergoes a general decline to 10° or lower. Higher $K_{m(G6P)}$ values for ovary extracts might occur with further decreases in temperature -- below 6°.

Minimal K_m values for seasonal groups are generally similar (Somero and Hochachka, 1971; Hochachka and Somero, 1973). This apparently occurred with G6PDH in gut and ovary extracts following temperature acclimation (Figures 27 and 29, respectively). However, minimal $K_{m(G6P)}$ values were quite different in the two acclimation groups for testis extracts (Figure 28). Enzyme-substrate affinity was apparently much higher at 6°, 10°, 14°, and 18° for testis extracts from cold-acclimated urchins than for those from warm-acclimated forms.

Several metabolic pathways -- glycolysis, phosphogluconate oxidation, and glycogenesis -- compete for G6P. If one of these pathways becomes increasingly effective in competing for G6P and the G6P level does not change, compensatory decreasing effectiveness for one or more alternate pathways appears likely. For example, a reciprocal relationship may exist between glycolysis and phosphogluconate oxidation regarding the relative effectiveness in utilizing G6P. This appears to have occurred in the multienzyme studies (Figure 16 and Table 1).

The $K_{m(G6P)}$-temperature curves for G6PDH do not correlate well with the body-component oxygen-consumption, multienzyme, and cytochrome c oxidase results. These latter results in association with each other suggest that following warm acclimation gonadal tissues favor phosphogluconate oxidation relative to glycolysis. They also suggest that nonreproductive tissues favor glycolysis relative to phosphogluconate oxidation. Cold acclimation increased gut G6PDH-G6P affinity at 6^{o}, 10^{o}, and 22^{o}, but negligible $K_{m(G6P)}$ differences occurred at the intermediate temperatures (Figure 27). Increased enzyme-substrate affinity was expected and was generally observed following cold acclimation. On the other hand, decreased G6PDH-G6P affinity was expected for testis and ovary following cold acclimation. The opposite -- increased affinity -- was generally observed (Figures 28 and 29).

Maximal-velocity (V_m) differences for G6PDH were observed in gonadal extracts following temperature acclimation (Figures 30b and c). These differences -- generally higher enzyme activity following warm acclimation -- correlate well with earlier results (body-component oxygen-consumption, multienzyme, and cytochrome c oxidase studies). All of these results when combined suggest that warm acclimation favors phosphogluconate oxidation relative to glycolysis. Test temperatures of 10^{o} and 18^{o} are of particular interest. Enzyme (G6PDH) activity was higher in gonadal extracts from warm-acclimated urchins than those from cold-acclimated forms. This correlates well with the multienzyme results at 9^{o} and 18^{o} (Figure 16 and Table 1).

Temperature acclimation produced negligible V_m differences in gut extracts (Figure 30a). Gut V_m values were negligible compared with those of either gonad. Doezema (1967, 1969) indicated that G6PDH activity of S. purpuratus gut extracts ranges from 0.013 to 0.023 μmoles of NADPH per mg of protein per min, much higher than that of the present study. Although Doezema (1967, 1969) used crude extracts, he did not mention any procedure correcting for contaminating 6PGDH activity. Also, differences in the reaction-mixture components are sufficient to at least partially explain the lower G6PDH activity reported herein.

Atkinson (1966) indicated that most positive modulators of enzymes function by increasing enzyme-substrate affinity (lowering the substrate K_m) and that decreasing affinity usually results from negative modulators. Hochachka and Somero (1973) observed that enzymatic assays ought to be performed at physiological, non-saturating substrate concentrations if the results are to be biologically meaningful. Some question may exist regarding the significance of the G6PDH-V_m results (Atkinson, 1966), inasmuch as physiological levels of substrate (approximately 1 mM or less, Somero, 1969b) are normally far less than those used in V_m assays. However, the relative velocities of the G6PDH reaction at $K_{m(G6P)}$ levels of substrate -- physiological concentrations -- should not change from those in Figure 30 as the velocities at the $K_{m(G6P)}$ levels equal $V_m/2$.

The G6PDH results substantiate and contradict the hypothesis that glycolysis and phosphogluconate oxidation, in competing for G6P, undergo changes which compensate for each other. The V_m and $V_m/2$ interpretations appear consistent with the earlier results. However, the $K_{m(G6P)}$ interpretations fail to support the other results. This disparity -- between the $K_{m(G6P)}$ and the other results -- might have occurred because of the possibility that G6PDH is not a control point for phosphogluconate oxidation. For example, 6PGDH may be more important than G6PDH in regulating

phosphogluconate oxidation in the gills of the Crucian carp, Carassius vulgaris Nilsson, and in the hepatopancreas of the blue crab, Callinectes sapidus Rathbun (see Ekberg, 1962, and Robert and Gray, 1972, respectively). On the other hand, Kather, Rivera, and Brand (1972) concluded that the control of phosphogluconate oxidation in adipose tissue isolated from rats lies with G6PDH but depends upon the amount of NADPH needed for lipid synthesis. Temperature acclimation of urchins may affect the G6PDH-NADP affinity in a manner consistent with the other results of the present study. Some other aspect, singly or together with the above, may help to explain the apparent discrepancies between the $K_{m(G6P)}$ and the other results.

Additional Interpretations

The present study was conducted to ascertain the influence of temperature on urchin metabolism at various levels of urchin organization. This effort has disclosed metabolic differences between gonadal and nonreproductive tissues at several levels of organization. One additional difference between gonadal and nonreproductive tissues is depicted in Figure 32. Ash-free dry weights of gonadal tissues fluctuated seasonally, and seasonal shifts in nonreproductive-tissue weight -- body wall in this case -- almost perfectly compensated for these changes.

The seasonal reciprocity between gonadal and body-wall weights (Figure 32) may be indicative of nutrient storage by the body wall. Gonadal tissues (Moore, 1937) as well as gut tissues (Fuji, 1961; Giese, 1961; Farmanfarmaian and Phillips, 1962; Lawrence et al., 1965; Lawrence et al., 1966) have been implicated as nutrient-storage sites for echinoids.

Lawrence et al. (1965) noted that gut indices (percent body wet weight) of S. purpuratus undergo seasonal variation and that variation for the gonads lags one to three months behind that of the gut. In the present study, seasonal variation for the gut appears negligible when expressed as percent wet weight (Figure 31) but becomes apparent when expressed as percent ash-free dry weight (Figure 32). Also, gonadal fluctuations in the present study follow those of the gut by approximately three months. The gut may store nutrients for gametogenesis as speculated by Lawrence et al. (1965). However, the more perfect reciprocal relationship (season as well as magnitude) between the organic weights of the body wall and gonads prompts the suggestion that the body wall may store nutrients for gametogenesis.

A significant difference (95% level) was observed between gut ash-free dry weights of S. purpuratus following temperature acclimation (Ulbricht, 1973b). Guts from warm-acclimated urchins were significantly smaller than those from cold-acclimated forms. The urchins were unfed during the final 16 days of acclimation. Greater

levels of tissue resorption apparently occurred with the warm group than with the cold group due to the increased metabolic demands for maintenance-level activity at the higher temperature. Lawrence et al. (1966) reported a decrease in the relative gut size of S. purpuratus following starvation and attributed the decline to decreasing levels of neutral lipid and -- to a lesser extent -- carbohydrate. Ulbricht (1973b) noted that following temperature acclimation this nutritional aspect along with the appropriate metabolic-rate expression produces results which may be interpreted as inverse compensation. This aspect can be at least a partial explanation for inverse compensation as it can effect translational shifts (see Prosser, 1958, 1969; Prosser and Brown, 1961), but it does not explain the rotational (slope) shifts observed.

Further explanations for the inverse compensation of S. purpuratus metabolic rate following temperature acclimation are suggested in the present study. In general, gonadal tissues from cold-acclimated urchins had higher oxygen-consumption rates, CO_2 production, levels of glycogenesis, and cytochrome c oxidase activity but lower levels of lipid synthesis and G6PDH activity than those from warm-acclimated forms. Conversely, nonreproductive tissues from cold-acclimated urchins generally had higher levels of lipid synthesis but lower oxygen-consumption rates, CO_2 production, and cytochrome c oxidase activity than those from warm-acclimated forms. Differences

between the levels of glycogenesis and G6PDH activity for nonreproductive (gut) tissues were equivocal. Glycolysis and oxidation relative to phosphogluconate oxidation and lipid synthesis, respectively, increased with gonadal tissues following cold acclimation, but the opposite occurred with nonreproductive tissues. Numerous gonadal-nonreproductive differences are apparent.

Gonor (1973a) observed that gonadal growth results from the accumulation of nutritive materials and not gametes. Gonor (1973a) suggested that food availability is one important environmental factor influencing the size of gonads. If gonadal size is a function of the food available, one possible adaptation would be to minimize catabolic activity during the period of continuous growth -- July through October or November (Gonor, 1973a). This appears to have occurred (see gonadal metabolic rate-season relationships, Figures 9 and 10).

During the early fall -- the conclusion of gonadal growth for S. purpuratus -- gonadal size (ash-free dry weight) may be more than twice that of the gut (Figure 32). However, gut metabolic rate ranges from approximately 2 to almost 10 times that of the gonads during the same period. Therefore, changes in gut catabolism following temperature acclimation at this time of year would appear to outweigh those of the gonads, in spite of the former component probably having less organic mass than the latter.

If gut metabolism (nonreproductive-tissue metabolism in general) exerts a greater influence on whole-urchin metabolism relative to that of the gonads, inverse compensation of whole-urchin metabolic rate ought to follow temperature acclimation. This is what occurred when the oxygen consumption of intact urchins was measured from August through October (Ulbricht, 1970, 1973a, b).

Oxygen availability is probably not as much a problem for gut tissues as it is for gonadal tissues. Webster (1972) reported gonadal oxygen tensions ranging from 0.0 to 10.0 mm Hg. Oxygen levels of 60 to 70 mm Hg (Johansen and Vadas, 1967) and 40 to 60 mm Hg (Webster, 1972) were observed for perivisceral fluid. Apparently, higher levels of oxygen tension are available to gut rather than gonadal tissues due to the reduced distance for oxygen to diffuse from the perivisceral fluid. This apparently is an additional factor favoring the expression of the gut relative to that of the gonads in the aerobic metabolism of whole urchins.

Varying degrees of temperature independence were observed for intact summer and temperature-acclimated urchins (Ulbricht, 1970, 1973a, b; Ulbricht and Pritchard, 1972). Evidence explaining this relative R-T independence is not too apparent. One explanation, which must be regarded as extremely tentative for the present, lies with the multienzyme results. Phosphogluconate oxidation and lipid synthesis relative to glycolysis and oxidation, respectively, did not appear to

change with incubation temperature or season for gut tissues (Table 4). Cold acclimation apparently increased the relative activities of phosphogluconate oxidation and lipid synthesis. Conversely, warm acclimation increased the relative activities of phosphogluconate oxidation and lipid synthesis in gonadal tissues incubated at 9° and 18°. However, these trends for gonadal tissues were reversed or at least equivocal when incubation occurred at 13.5°. If all of the gonadal and nonreproductive tissues in whole urchins respond as these examples, equivocal differences in metabolic rate appear likely at 9° and 18° following temperature acclimation. However, the whole-urchin metabolic rate from warm-acclimated urchins would be higher than that from cold-acclimated forms at 13.5° -- inverse compensation. This interpretation corroborates the whole-urchin metabolic rates determined by Ulbricht (1970, 1973a, b) and suggests how R-T slope changes may be effected.

Seasonal changes in the gonads (Stott, 1931; Bennett and Giese, 1955; Giese et al., 1959; Giese, 1961, 1966; Holland and Giese, 1965; Fuji, 1967; Chatlynne, 1969, 1972; Gonor, 1973a, b) might have led to the slope changes in whole-urchin R-T curves. The radiotracer incubation at 13.5° (time-course studies) was performed with tissues from urchins collected in February, whereas that at 9° and 18° (3-hr incubation period) used urchins collected in August. Any interpretation suggesting that the whole-urchin R-T slope changes were acute

responses to temperature must be tentative.

Increased oxygen consumption has been associated with the activation of phosphogluconate oxidation. For example, Kanungo and Prosser (1959) reported that the oxygen consumption of cold-acclimated goldfish increases because of activated phosphogluconate oxidation. Studies which indicate how changes in phosphogluconate oxidation effect changes in respiration are not common. In a study of this kind, Flatt and Ball (1964) suggested that phosphogluconate oxidation does not supply all of the NADPH necessary for lipogenesis in rat adipose tissue. The remaining reduced coenzymes, presumably NADH, are apparently produced during the conversion of glucose to acetyl-S-CoA. Approximately half of these reduced coenzymes are oxidized by oxygen. In short, increases in oxygen consumption appear to parallel increases in lipid synthesis.

The multienzyme (radiotracer) results of the present study -- increasing phosphogluconate oxidation and lipid synthesis relative to glycolysis and oxidation, respectively, or the opposite (Figures 16 and 17 and Table 1) -- run contrary to the interpretation of Flatt and Ball (1964). Increases in the relative activity of phosphogluconate oxidation for urchin tissues were accompanied by decreases in the relative activity of oxidation. Apparently, most of the oxidation of acetate-1-^{14}C was oxidative phosphorylation, because negligible levels of gluconeogenesis were detected when homogenates were

incubated with this label. Metabolic-rate differences for urchin components generally corroborate the radiotracer results (Figure 13 and Table 3).

Kornacker and Ball (1965) proposed a scheme in which pyruvate is converted to extramitochondrial acetyl-S-CoA. Pyruvate enters the mitochondria where it is converted to citrate. After citrate leaves the mitochondria, it is cleaved into acetyl-S-CoA and oxaloacetate. The latter is reduced to malate which in turn is converted to pyruvate and CO_2, completing one cycle. Reducing equivalents are transferred from NADH to NADPH -- transhydrogenation -- during the last two reactions. Two high-energy phosphates are required for each turn of the cycle. The pyruvate carboxylase and citrate cleavage enzyme reactions require ATP. Presumably, the increased level of oxygen consumption reflects the ATP necessary to drive the cycle.

The results of the present study run contrary to those suggested by Kornacker and Ball (1965). Decarboxylation occurs in the NADP-malate dehydrogenase and pyruvate dehydrogenase reactions. The pyruvate carboxylase reaction requires CO_2. If extramitochondrial acetyl-S-CoA is produced as suggested by Kornacker and Ball (1965), the pyruvate dehydrogenase reaction presumably operates, producing mitochondrial acetyl-S-CoA. Therefore, after an arbitrary number of turns of the cycle, CO_2 production ought to exceed CO_2 consumption. In other words, CO_2 production would increase with lipid

synthesis. Results with urchin tissues repeatedly oppose what is anticipated from the scheme of Kornacker and Ball (1965). Decreases in CO_2 production accompany increases in lipogenesis in urchin tissues (Figures 16 and 17). Fatty acid synthesis involves additional CO_2 production (Larner, 1971). These additional decarboxylations further emphasize the disparity between the present results and the scheme of Kornacker and Ball (1965).

Lipogenesis in urchin tissues did not occur precisely as suggested by Flatt and Ball (1964) and Kornacker and Ball (1965) who studied lipogenesis in rat adipose tissue. The information at hand does not permit alternate suggestions for urchin metabolic pathways.

Giese (1966) observed that the body wall of urchins may store nutritive materials. Results of the present study (Figure 32) suggest that the body wall may be a nutrient reservoir for gonadal growth. A precedent for this type of gonadal-nonreproductive reciprocity was reported by Fuji (1967) who noted that the test growth (mean diameter and height) for large specimens of Strongylocentrotus intermedius (A. Agassiz) varies inversely with gonadal growth.

Gonadal-nonreproductive differences in metabolism at several levels of urchin organization appear to be closely tied to the gonadal-nonreproductive weight differences. Gonadal catabolism was maximal in the winter or spring and minimal in the fall. Gonadal anabolism was maximal in the fall and minimal in the spring. Catabolism of

nonreproductive tissues appeared maximal in the fall and minimal in the winter. Anabolism of nonreproductive tissues appeared maximal in the spring and minimal in the fall. Ebert (1968) reported that the feeding rates for populations of S. purpuratus at Sunset Bay, Oregon, are maximal in September and October and minimal in March. This seasonal cycle correlates nicely with those for food availability and gonadal growth (see Ebert, 1968; Gonor, 1973a). Nutritive materials from nonreproductive tissues presumably augment those from the food assimilated during gonadal growth.

Temperature acclimation alters urchin metabolism at several levels of organization (Figures 13, 16-23, 25, and 30 and Tables 1, 3, and 5). Field acclimatization also alters urchin metabolism (Figures 7-10 and 25), and frequently the seasonal changes appear to be the result of temperature differences. However, the similarities between the results from field-acclimatized and temperature-acclimated urchins are not perfect. Also, the metabolism of field-acclimatized urchins does not always change as would be predicted if temperature alone were influencing it (see Figure 33 for seasonal changes in habitat temperature). Apparently, temperature is an important factor influencing urchin metabolism, but it does not function alone in this regard. Presumably, other environmental factors -- body size, nutritional state, reproductive state, oxygen tension, salinity, and substrate

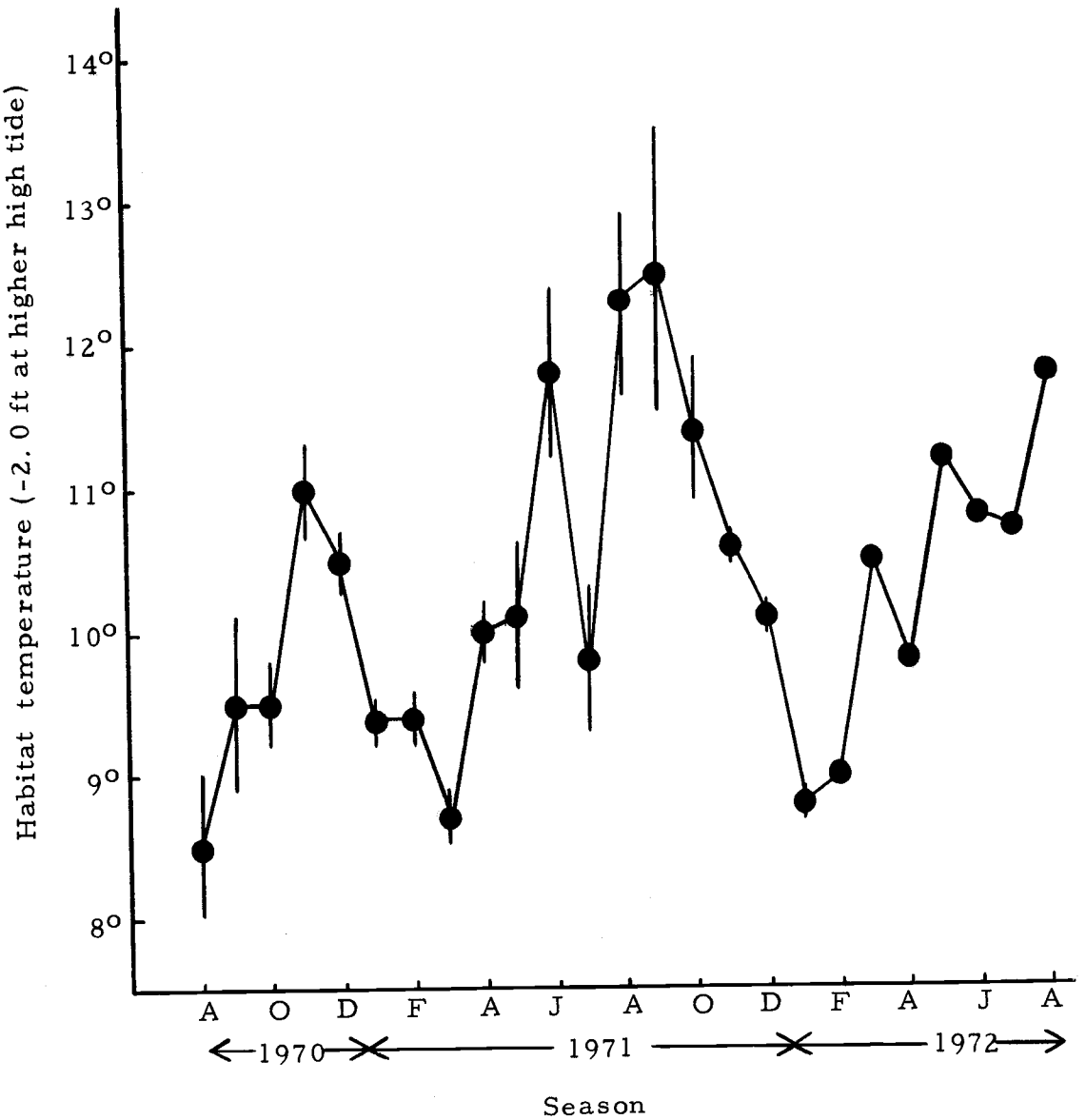

Figure 33. Intertidal (-2.0 at HH) temperature-season relationship (D. W. Elvin, Oregon State University, personal communication). Temperatures from August 1970 through January 1972 were measured at Whale Cove (vertical bars indicate ± 2 SEM). Temperatures from February 1972 through August 1972 were measured at Yaquina Head (mean values with N ranging from 2 to 4). Intertidal temperatures at Yaquina Head correlate well with those at Whale Cove (D.W. Elvin, Oregon State University, personal communication). An equation describing this relationship throughout one year is as follows:

$$Y = 0.0316 + 0.9866X$$

where

Y = Yaquina Head values,
X = Whale Cove values,
N = 16 pairs, and
$r^2 = 0.8348$

habit -- influence urchin metabolism, and the resulting effects when superimposed upon those from temperature alone produce the seasonal changes observed.

SUMMARY

The metabolism of Strongylocentrotus purpuratus was investigated at several levels of urchin organization.

Oxygen-consumption rates for testis and ovary underwent seasonal shifts which appeared to compensate for changes in habitat temperature -- i.e., high and low rates during cold and warm periods, respectively. Rates for body wall and gut underwent seasonal shifts in a direction opposite that of testis and ovary -- i.e., inverse compensation. This gonadal-nonreproductive difference was observed less conclusively with temperature-acclimated urchins. Compensatory shifts occurred with testis sections (slices). Equivocal shifts occurred with body-wall, gut, and ovary sections. Inverse compensation of metabolic rate resulted when oxygen-consumption measurements were repeated with gut sections and homogenates from temperature-acclimated forms.

Radiotracer -- glucose-1-^{14}C, glucose-6-^{14}C, and acetate-1-^{14}C -- incubation with gut, testis, and ovary homogenates from temperature-acclimated urchins disclosed additional gonadal-nonreproductive differences. Higher CO_2 and lower lipid activities were observed for testis homogenates following cold acclimation regardless of the test temperature or radiotracer. Conversely, higher CO_2 and lower lipid activities were observed for gut homogenates following warm

acclimation. Conversion of glucose to CO_2 was greater in gut than in testis homogenates regardless of the test temperature or radiotracer. Conversion of acetate to lipid was generally greater in testis than in gut homogenates. Phosphogluconate oxidation and lipid synthesis relative to glycolysis and oxidation, respectively, increased in testis homogenates following warm acclimation. Conversely, glycolysis and oxidation relative to phosphogluconate oxidation and lipid synthesis, respectively, increased in gut homogenates following warm acclimation. Cold acclimation resulted in higher levels of glycogenesis for testis and ovary tissues but in equivocal differences for gut tissues.

Changes in cytochrome c oxidase activity in extracts from field-acclimatized and temperature-acclimated urchins paralleled the oxygen-consumption and radiotracer results. Additional gonadal-nonreproductive differences were disclosed. Enzyme activity was highest in gut tissues during September, whereas it was highest for testis and ovary tissues in December and March, respectively. Enzyme activity was increased in gut tissues following warm acclimation, but it was increased for testis and ovary tissues following cold acclimation.

Changes in G6PDH activity in extracts from temperature-acclimated urchins were consistent with the hypothesis that changes in the effectiveness with which glycolysis competes for G6P are accompanied by compensatory changes in phosphogluconate oxidation, providing that

the competition from other pathways does not change. Another gonadal-nonreproductive difference was observed. Warm acclimation generally increased the G6PDH activity for testis and ovary tissues but failed to produce any significant change in gut activity. Gonadal activity was generally much higher than that of the gut. Cold acclimation led to increased G6PDH-G6P affinity in gut, testis, and ovary extracts.

The changes in urchin metabolism which resulted from season and temperature suggest explanations, at least in part, for phenomena characteristic of whole-urchin metabolic rate. In general, oxidation in gonadal tissues appears to compensate for temperature (i.e., higher rates at any given temperature following cold acclimation), whereas that in nonreproductive tissues appears to undergo inverse compensation. If the combination of metabolic rate and tissue mass for nonreproductive tissues exceeds that for gonadal tissues, inverse compensation of whole-urchin metabolic rate appears likely. Phosphogluconate oxidation and lipid synthesis increase relative to glycolysis and oxidation, respectively, for testis tissues at 9^{o} and 18^{o} following warm acclimation. Negligible differences are observed at 13.5^{o}. Glycolysis and oxidation increase relative to phosphogluconate oxidation and lipid synthesis, respectively, for gut tissues at all temperatures following warm acclimation. If no factor other than temperature brings about these differences, slope changes in the metabolic

rate-temperature relationship of intact urchins appear likely.

Ash-free dry weight for gonadal tissues expressed as a percentage of that for the whole urchin was maximal in the fall and minimal in the spring, while that for body-wall tissues was maximal in the spring and minimal in the fall. Seasonal changes in the gut were negligible by comparison.

The metabolic differences between gonadal and nonreproductive tissues correlate well with the body-component weight changes. Gonadal catabolism appears maximal in the winter or spring and minimal in the fall, whereas gonadal anabolism appears maximal in the fall and minimal in the spring. Nonreproductive catabolism appears maximal in the fall and minimal in the winter, whereas nonreproductive anabolism appears maximal in the spring and minimal in the fall. In short, seasonal shifts in the levels of biosynthesis and oxidation of gonadal tissues are opposite those of nonreproductive tissues. These differences may reflect a metabolic economy. Food consumption may not be sufficient for concomitant increases in the biosynthesis and oxidation of both gonadal and nonreproductive tissues. The body wall may store nutritive materials which can be used to augment assimilated food during gonadal growth.

Temperature evidently influences urchin metabolism at several levels of urchin organization: body-component level, multienzyme systems, and single enzymes. Apparently, the effects of other

factors, operative in the field, are superimposed upon the effects due to temperature alone.

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