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CARBOHYDRATE LEVELS IN *PATELLA*

By R. J. C. BARRY
Department of Physiology, University of Sheffield
and K. A. MUNDAY
Department of Physiology and Biochemistry, University of Southampton

(With Text-figs. 1–9)

The limited observations available on blood sugar levels of Mollusca suggest that they are very low. Values of 2–14 mg % glucose have been reported for *Aplysia* (see Kisch, 1929; Berthoumeyroux, 1935), and even the higher levels recorded for *Octopus* and *Sepia* species by Bierry & Giaja (1909), Berthoumeyroux (1935) and Derrien (1938) range only from 20 to 32 mg % glucose. The majority of these blood sugar analyses were based on the Hagedorn & Jensen (1923) technique. Landgrebe & Munday (1954) have shown that this technique may have an inherent blank error of 5 mg % glucose equivalent, and consequently it is not a suitable method for critical analyses of these low molluscan blood sugar levels.

Seasonal analyses of molluscan blood sugar levels have been restricted to the terrestrial *Helix pomatia* L. From monthly analyses, Schwarz (1935) could not detect any definite seasonal variation, beyond a possible increase before and at the end of hibernation. Wolf-Heidegger (1935) obtained higher mean blood sugar values (22 mg %) for summer as compared with hibernating *Helix* (11 mg %), but this seasonal difference was not confirmed by the investigations of Lustig, Ernst & Reuss (1937) and Holtz & von Brand (1940). It was desirable to extend these seasonal investigations to other Mollusca, using a method particularly suitable for small blood samples and low concentrations of glucose. The marine gastropod *Patella* was selected for these studies.

Rather more investigations have been carried out on polysaccharide reserves in Mollusca. Seasonal variation in the glycogen reserves of *Ostrea* species has been demonstrated by Mitchell (1915–16), Russell (1923) and Okazaki & Kobayashi (1929). From analyses of the individual tissues of *Gryphaea angulata* Lamk., Couteaux-Bargeton (1947) concluded that glycogen is used both during the overwintering period and in the formation of sexual products. The dual role of polysaccharides has been most clearly demonstrated in *Helix pomatia* (see von Brand, 1931; May, 1934), in which the two polysaccharides, glycogen and galactogen, have concentration maxima at different periods of the year. Glycogen is apparently used in the maintenance of metabolism

during hibernation, with a maximum concentration at the beginning of this period. Galactogen, which is restricted to the albumen gland, forms the main polysaccharide reserve of the eggs, and has a maximum concentration at the onset of egg-laying.

The work reported in this paper records the seasonal levels of blood glucose in *Patella* paralleled by tissue glycogen concentrations. In addition, starvation effects on these carbohydrate levels have been established under laboratory conditions, and the effects of glucose administration on blood glucose concentration studied.

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MATERIALS AND METHODS

Patella, used in these studies, occurred as a sheltered population between two natural rock barriers, which project southwards into the sea at Pevril Point, Swanage. For laboratory investigation they were transported packed in sea-weed and then maintained under starvation conditions in sea-water aquaria. Under these conditions, *Patella* readily re-attached themselves to a rocky substrate and could be maintained in a healthy condition for several months; the maximum period of survival recorded being 11 months.

In blood sampling, the head was pushed gently to the back of the head cavity, which was dried out with cotton-wool. Uncontaminated blood was withdrawn into a hypodermic syringe from the large vessel, which runs from the pallial vessel dorsally into the heart (Fig. 1). In the field, sampling was completed within 2 min of prising the limpet from its substrate. The blood was immediately mixed with tungstic acid protein precipitant and the deproteinised supernatant analysed on return to the laboratory.

The blood total reducing value, taken as an estimate of the blood glucose concentration, was determined by Landgrebe & Munday's (1954) modification of the Folin & Malmros (1929) technique. Duplicate analyses of 0·5 ml. *Patella* blood in 5 ml. tungstic acid gave values agreeing within 0·4 mg % glucose equivalent, confirming the suitability of the technique at these low levels.

Tissue glycogen concentrations were estimated by the Good, Kramer & Somogyi (1933) technique, the glycogen hydrolysate having been chromatographically identified as glucose. Tissues dissected from the living animal were weighed and immersed in hot KOH solution within 10 min of removing the animal from its shell. A homogeneous solution was obtained within 45 min of heating in a boiling water-bath, using a maximum of 200 mg wet weight of tissue per ml. 30 % KOH. The alcohol-precipitated glycogen was redissolved

in 2 ml. 15% KOH and reprecipitated by adding 2 ml. absolute alcohol. After acid hydrolysis, the total reducing value was determined by the Landgrebe & Munday (1954) technique. Glycogen concentrations were expressed as % wet weight; that is, the weight of reducing substances, as g glucose, resulting from hydrolysis of the glycogen present in 100 g wet weight of tissue (glycogen as g glucose/100 g tissue).

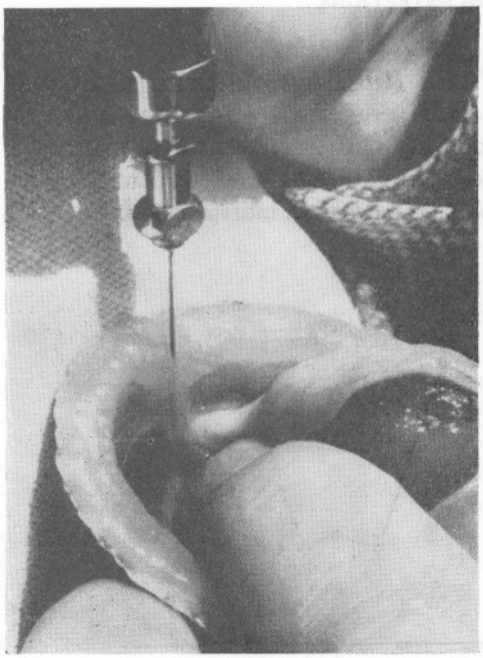

Fig. 1

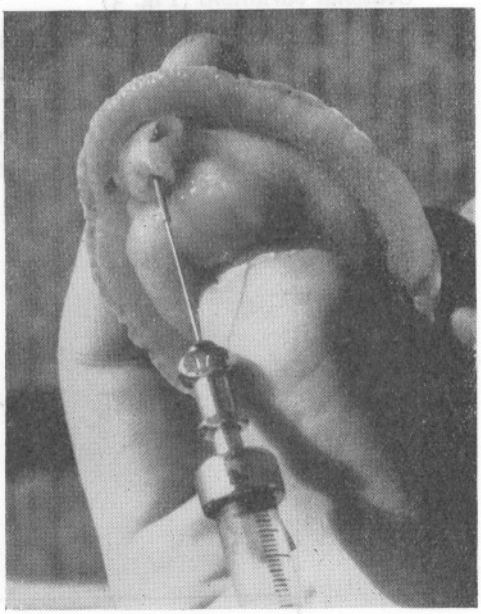

Fig. 2

Fig. 1. Methods of taking blood samples from *Patella*, showing the hypodermic needle in the vessel which runs dorsally from the pallial vessel into the heart.

Fig. 2. Method of oral administration of glucose solutions, after the tube has been passed down the gut into the stomach of *Patella*.

Glucose was administered intravascularly and orally. Glucose solutions were injected into the visceral sinus through the foot medianly from the ventral side. Oral glucose was administered by plastic tube (external diameter 1 mm and length 3 cm) passed over the buccal mass down the gut into the stomach (Fig. 2).

The three species of *Patella* present on the Swanage coast are not readily identified from their external characteristics, because of the considerable intergrading between species (Evans, 1953). Examination of the pleuricuspid teeth of a sample of *Patella* confirmed Evans's observation that *P. vulgata* L.

was the commonest species in sheltered habitats, such as exist at Pevril Point. All *Patella* used were of the same external appearance. Although most specimens were probably *P. vulgata*, this investigation has been assigned to the genus *Patella*, rather than to any one species.

RESULTS

BLOOD GLUCOSE CONCENTRATIONS

Seasonal variations (Fig. 3)

The blood glucose concentrations of field *Patella* were determined by analyses of blood samples taken in the field between February 1954 and July 1955. Limpets were taken from the full range of their vertical distribution and from different vertical faces of exposed rocks. No correlation was detected between the blood glucose level and the ecological position of individual *Patella*.

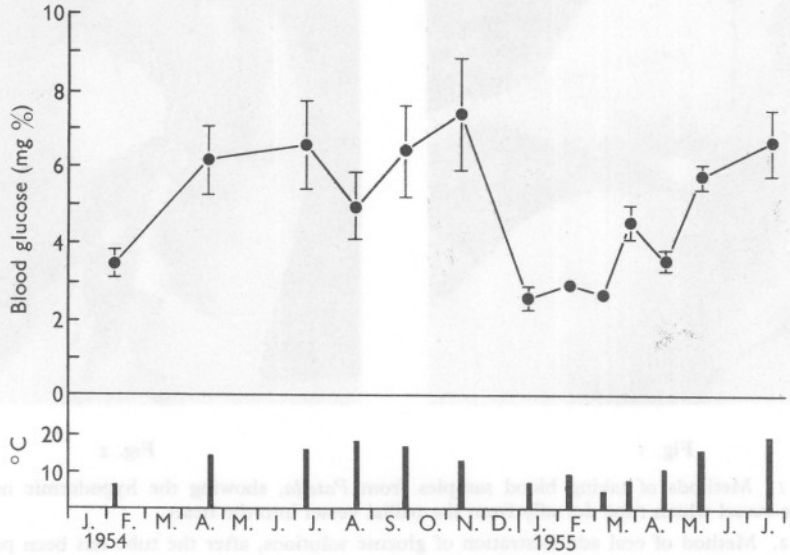

Fig. 3. Seasonal variation in blood glucose concentration of field *Patella*, showing the mean ± standard error of each series of analyses (10 individuals) and the sea temperature (shown below) at the time of blood sampling.

Between May and November, called the summer period, the mean glucose concentrations approximated to 6 mg % glucose. The means, with their standard errors, ranged from $4{\cdot}9 \pm 0{\cdot}85$ mg % to $7{\cdot}3 \pm 1{\cdot}45$ mg %, but this variation between means was not significant, because of the wide range of individual values in each series of analyses. A single very high value often accentuated the wide range in any particular series, the highest value recorded being 18·0 mg %. Between November and January, the mean blood glucose

concentration decreased to the relatively low level of $2{\cdot}5 \pm 0{\cdot}29$ mg %. This low level persisted from January to the early part of March, with means ranging from $2{\cdot}5 \pm 0{\cdot}29$ mg % to $2{\cdot}9 \pm 0{\cdot}12$ mg %. This winter period was also characterized by the narrow range of individual values in each series of samples. The late-March and April series in 1955 gave values intermediate between the low winter level and the higher summer level, the latter having been re-attained in the May limpets (mean $5{\cdot}7 \pm 0{\cdot}30$ mg %).

The results indicate a definite seasonal variation of the blood glucose concentrations of *Patella* in their natural environment, the very variable summer values contrasting with the low relatively constant winter values.

Effect of starvation (Fig. 4)

Patella were maintained under starvation conditions in laboratory sea-water aquaria at a temperature similar to that occurring in the natural environment. In July 1954 the individual *Patella* sampled in the field (sea temperature 17° C) were brought into the laboratory and starved in sea-water aquaria at 17° C. After 4 days starvation, a second series of blood samples from these individual *Patella* was analysed. The mean blood glucose level of eight field *Patella* was $6{\cdot}5 \pm 0{\cdot}84$ mg %, with a range of 3·1–13·8 mg %. After 4 days starvation the mean blood glucose level had decreased to $4{\cdot}9 \pm 0{\cdot}14$ mg % and the range narrowed to 4·2–5·6 mg %. This latter level approximated to the lower values recorded in the July field samples. After this initial starvation effect, the blood glucose concentration decreased more gradually up to 40 days starvation (Fig. 4). Similar results were obtained with *Patella* collected in November.

The lower and more constant blood glucose concentrations of starved *Patella* suggest that the relatively high concentrations of some individual field *Patella* result from an alimentary hyperglycemia. On this hypothesis, the wide range of individual field values results from differences in the degree of this hyperglycemia and the lower values of starved *Patella* represent a post-absorptive blood glucose level.

During the winter period, the low and relatively constant glucose concentrations of field *Patella* suggest that they do not feed during this period. This was supported by starvation experiments, in which January *Patella* were maintained in sea-water aquaria at the field sea temperature of 6° C. After 11 days starvation, the mean blood glucose concentration was $2{\cdot}9 \pm 0{\cdot}24$ mg %, as compared with $2{\cdot}5 \pm 0{\cdot}29$ mg % for the field analyses. In contrast to the summer *Patella*, the blood glucose had not decreased on starvation, indicating that it was already at a basal level in field *Patella* during the winter period. That the blood glucose concentration of January field *Patella* collected at 6° C could decrease was shown by raising the aquarium temperature to 17° C. From a mean field value of $2{\cdot}5 \pm 0{\cdot}29$ at 6° C, the mean blood glucose concentration after 5 days starvation at 17° C was $1{\cdot}9 \pm 0{\cdot}36$ mg %, and after

17 days starvation at 17° C 1·6 ± 0·13 mg %. The higher aquarium temperature at which these January field *Patella* were maintained may have caused an increased utilization of available carbohydrate. The results suggest that the disappearance of glucose from January starved *Patella* is temperature dependent.

Yeast fermentation experiments suggested that the true blood glucose concentrations of starved *Patella* were even lower than the total reducing values determined by the Landgrebe & Munday technique. In July *Patella* starved

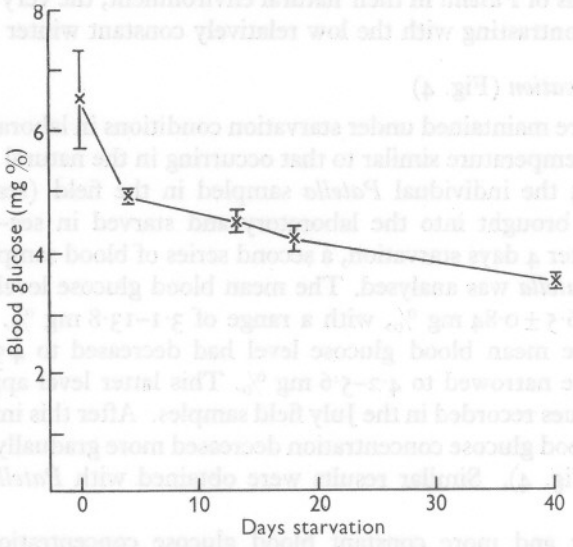

Fig. 4. Effect of starvation on the blood glucose concentration of *Patella*, showing the mean ± standard error of each series of analyses (8 individuals). July *Patella* collected at sea temperature of 17° C and maintained at that temperature in the aquaria.

for 4 days in the laboratory, the true blood glucose concentrations approximated to 40 % of the total reducing values. The decrease in the total reducing value of winter *Patella* starved at 17° C appeared to result from a decrease in the true blood glucose concentration. The marked individual variation of total reducing value of summer *Patella* also apparently represents a real variation in true blood sugar level. However, the yeast fermentation technique at these low glucose concentrations was liable to considerable error, and consequently this technique was not applied as a routine procedure to all blood samples.

Glucose administration experiments (Fig. 5)

The disappearance of glucose from the blood was investigated by injection experiments. 2 mg glucose in 0·2 ml. sea water was injected into the visceral sinus of starved summer *Patella* maintained at 17° C, and a hyperglycemia

of approximately 30 mg % was recorded 10 min after injection (Fig. 5). Within 4 h the blood glucose concentration had returned to the pre-injection level of 2·5 mg %. Injection of the same volume of sea water alone had no hyperglycemic effect. The sea water surrounding the limpet was analysed for glucose, in order to establish whether any glucose was being excreted during the experiment. Even after injection of up to 15 mg glucose, which induced a hyperglycemia of over 200 mg %, less than 2 % of the total glucose injected could be recovered from the sea water. Thus, there was no appreciable escape of glucose through the body surface of the limpet, nor was the injected glucose excreted by the kidney during the period of the experiment.

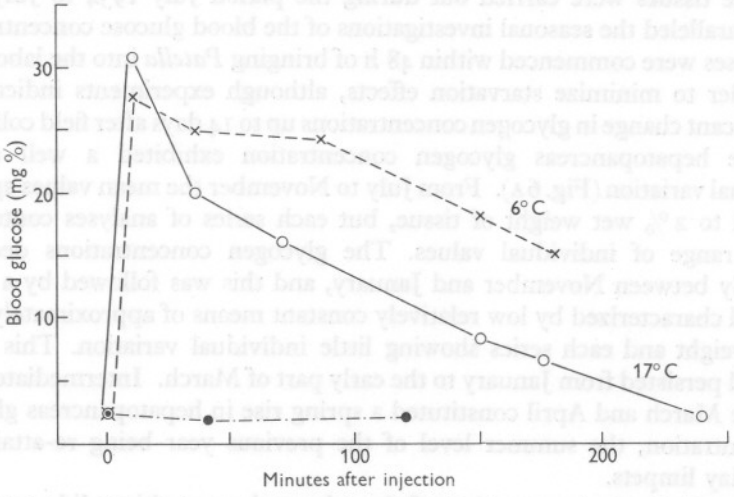

Fig. 5. Variation of blood glucose concentration of *Patella*: (○——○), after the intravascular injection of glucose solution at 17° C, mean 8 individuals; (× - - - - - ×), glucose solution at 6° C, mean 6 individuals; and (●–·–·–●), of sea water, mean 8 individuals, showing mean blood glucose values with increasing periods of time.

This rapid removal of glucose from the blood probably resulted from its metabolism to a non-reducing product. Using starved winter *Patella* maintained at 6° C, the rate of disappearance of injected glucose from the blood was considerably slower than in the summer *Patella* at 17° C. This also suggested that some active metabolic process was involved in the removal of glucose.

Similar results were obtained from April and May *Patella*, to which glucose was administered orally. Oral administration of small volumes of sea water had no effect on the blood glucose concentration, but oral administration of 0·3 ml. 1 % glucose/sea-water solution induced a hyperglycemia of approximately 30 mg % within 15 min. This suggested a rapid transfer of glucose from the alimentary canal to the blood stream. In animals at 17° C, the initial glucose levels were re-attained within 4 h, but animals maintained at 6° C still possessed elevated blood glucose levels 5 h after oral administration.

TISSUE GLYCOGEN CONCENTRATIONS

Seasonal variations (Fig. 6)

The hepatopancreas (including the intestine), the odontophore cushion and the foot were shown to be the main glycogen storage tissues of *Patella*, each normally having a glycogen concentration greater than 1 % wet weight during the summer months. In contrast, the glycogen concentrations of the gonad, the mantle skirt and the remainder of the head region never exceeded 1 % wet weight.

Seasonal determinations of the glycogen concentrations of the three main storage tissues were carried out during the period July 1954 to July 1955, and paralleled the seasonal investigations of the blood glucose concentrations. Analyses were commenced within 48 h of bringing *Patella* into the laboratory, in order to minimize starvation effects, although experiments indicated no significant change in glycogen concentrations up to 14 days after field collection.

The hepatopancreas glycogen concentration exhibited a well defined seasonal variation (Fig. 6A). From July to November the mean values approximated to 2 % wet weight of tissue, but each series of analyses contained a wide range of individual values. The glycogen concentrations decreased sharply between November and January, and this was followed by a winter period characterized by low relatively constant means of approximately 0·3 % wet weight and each series showing little individual variation. This winter period persisted from January to the early part of March. Intermediate values in late March and April constituted a spring rise in hepatopancreas glycogen concentration, the summer level of the previous year being re-attained in the May limpets.

The glycogen concentration of the odontophore cushion did not exhibit a marked seasonal pattern (Fig. 6B). Very variable values were obtained throughout the year. Following a sharp decrease in the mean value between November and January, low mean glycogen concentrations did not persist in the February and March series, although the majority of the individual values of < 1 % wet weight occurred during this period.

Foot glycogen concentrations were determined by analyses of pieces of tissue from the mid-foot region (Fig. 6C). The general pattern of the seasonal variation resembled that of the hepatopancreas. During July to November, the foot glycogen concentrations of individual *Patella* exhibited considerable variations, and the mean level tended to decrease in the October and November series. In January, the glycogen concentration had fallen to a low winter level of approximately 0·8 % wet weight, a somewhat higher level than that recorded in the hepatopancreas (0·3 % wet weight). The hepatopancreas also differed from the mid-foot, in that the spring rise in its glycogen concentration preceded that of the mid-foot. The low winter level of mid-foot glycogen persisted until April and the spring rise was not evident before the May

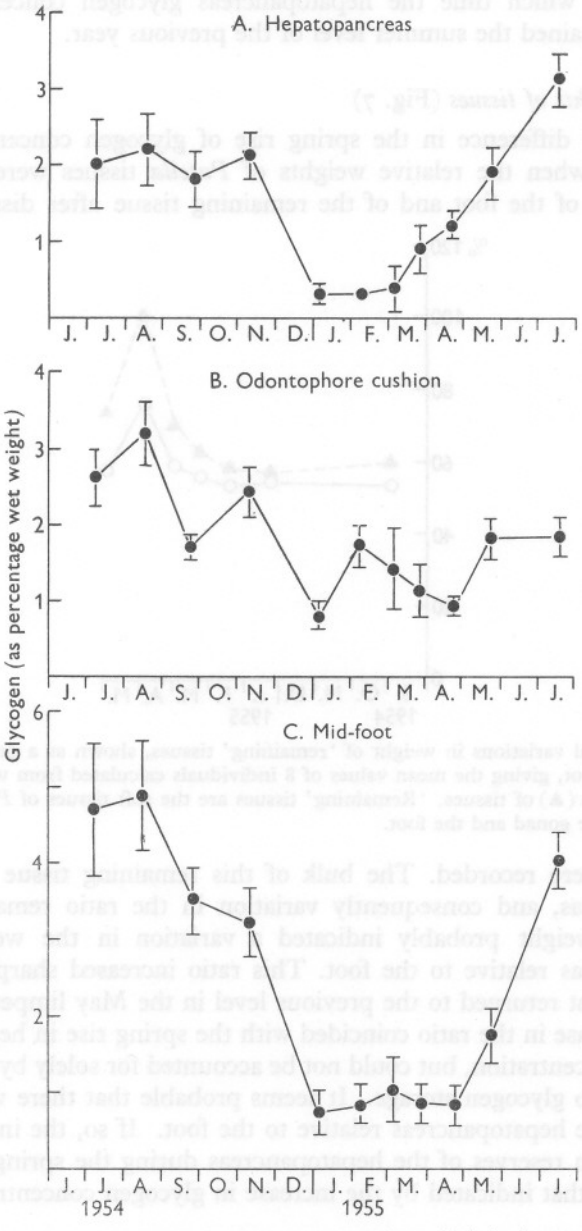

Fig. 6. Seasonal variations in tissue glycogen concentrations of (A) the hepatopancreas, (B) the odontophore cushion, and (C) the mid-foot of *Patella*, showing the mean ± standard error of each series of analyses (7 individuals).

analyses, by which time the hepatopancreas glycogen concentration had already re-attained the summer level of the previous year.

Relative weights of tissues (Fig. 7)

The tissue difference in the spring rise of glycogen concentrations was accentuated when the relative weights of *Patella* tissues were considered. The weights of the foot and of the remaining tissue after dissecting away

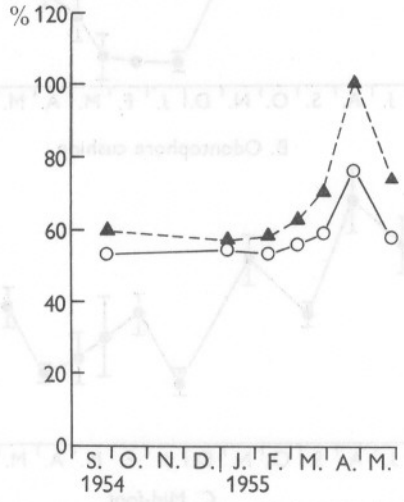

Fig. 7. Seasonal variations in weight of 'remaining' tissues, shown as a percentage of the weight of the foot, giving the mean values of 8 individuals calculated from wet weights (○) and dry weights (▲) of tissues. 'Remaining' tissues are the soft tissues of *Patella* after dissecting away the gonad and the foot.

the gonad were recorded. The bulk of this remaining tissue consisted of hepatopancreas, and consequently variation in the ratio remaining-tissue-weight/foot-weight probably indicated a variation in the weight of the hepatopancreas relative to the foot. This ratio increased sharply in March and April, but returned to the previous level in the May limpets (Fig. 7).

This increase in the ratio coincided with the spring rise in hepatopancreas glycogen concentration, but could not be accounted for solely by an increased weight due to glycogen storage. It seems probable that there was an actual growth of the hepatopancreas relative to the foot. If so, the increase in the total glycogen reserves of the hepatopancreas during the spring rise is even greater than that indicated by the increase in glycogen concentration.

Effect of starvation (Fig. 8)

Glycogen concentrations of the main storage tissues were determined with increasing periods of starvation. *Patella*, collected in July, were maintained

in sea-water aquaria at the July sea temperature of 17° C. During the first month's starvation there was no marked decrease in the tissue glycogen concentrations. After 60 days starvation, the concentration of the hepatopancreas (0·4 % wet weight) had decreased to the winter level. The concentrations of the odontophore cushion and mid-foot had also fallen, but they still contained

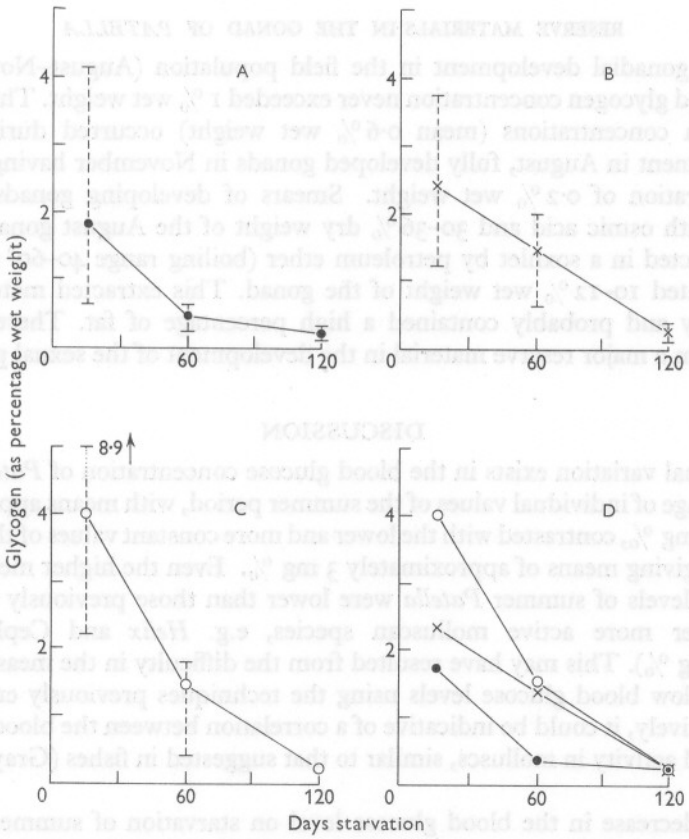

Fig. 8. Variations in tissue glycogen concentrations of *Patella* collected in July and maintained in sea-water aquaria at 17° C. Graphs A, B and C show the means and range of individual values (12 for 5 days, 4 for 60 and 4 for 120 days) with increasing periods of starvation for the hepatopancreas (●), odontophore cushion (×) and the mid-foot (○) respectively. Graph D shows the mean values for the three tissues superimposed.

appreciable glycogen concentrations with mean values 1·4 and 1·5 % wet weight respectively. On prolonged starvation of 120 days, the glycogen concentrations of all tissues were extremely low, no individual value exceeding 0·4 % wet weight.

Well-developed gonads were present in *Patella* starved for 60 and 120 days. This gonadial development paralleled that occurring in the natural population

over the same period (August–November). The decrease in the tissue glycogen reserves of starved *Patella* may have resulted from glycogen utilization both in maintaining metabolism and in the development of the gonads. However, in starved *Patella* the glycogen concentrations of the gonads themselves did not exceed 0·1 % wet weight.

RESERVE MATERIALS IN THE GONAD OF *PATELLA*

During gonadial development in the field population (August–November), the gonad glycogen concentration never exceeded 1 % wet weight. The highest glycogen concentrations (mean 0·6 % wet weight) occurred during early development in August, fully developed gonads in November having a mean concentration of 0·2 % wet weight. Smears of developing gonads stained black with osmic acid and 30–36 % dry weight of the August gonads could be extracted in a soxhlet by petroleum ether (boiling range 40–60° C). This represented 10–12 % wet weight of the gonad. This extracted material was very oily and probably contained a high percentage of fat. Therefore, fat may form a major reserve material in the development of the sexual products.

DISCUSSION

A seasonal variation exists in the blood glucose concentration of *Patella*. The wide range of individual values of the summer period, with means approximating to 6 mg %, contrasted with the lower and more constant values of the winter period, giving means of approximately 3 mg %. Even the higher mean blood glucose levels of summer *Patella* were lower than those previously reported for other more active molluscan species, e.g. *Helix* and Cephalopoda (8–33 mg %). This may have resulted from the difficulty in the measurement of very low blood glucose levels using the techniques previously employed. Alternatively, it could be indicative of a correlation between the blood glucose level and activity in molluscs, similar to that suggested in fishes (Gray & Hall, 1930).

The decrease in the blood glucose level on starvation of summer *Patella* suggests that the higher concentrations in individual field *Patella* result from an alimentary hyperglycemia. The similarity between the low concentrations of both field and starved *Patella* during the winter months suggests that *Patella* do not feed during this winter period, and that they may pass into an inactive state comparable to the hibernation of terrestrial molluscs.

A close correlation exists between the seasonal variations of the blood glucose and hepatopancreas glycogen concentrations (Fig. 9). Between November and January, these concentrations decreased to a relatively low and constant winter level, which persisted into the early part of March. The spring rise in the concentration of the hepatopancreas glycogen also paralleled that of the blood glucose, but preceded the increase in foot glycogen concentration.

The total increase in the glycogen reserves of the hepatopancreas was probably even greater than indicated by the glycogen concentration alone, because of an actual growth of the hepatopancreas relative to the foot (March–April). This close parallel between the spring rise in blood glucose and hepatopancreas glycogen concentration may be associated with the function of the hepatopancreas as a digestive and absorptive organ and the recommencement of feeding. It is not suggested, however, that there is any functional analogy with the vertebrate liver as the main glycogen storage organ.

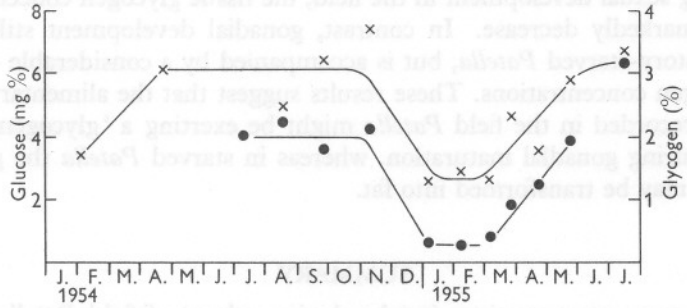

Fig. 9. Patterns of seasonal variations in blood glucose concentrations (upper line, ×) and hepatopancreas glycogen concentrations (lower line, ●) of field *Patella*.

The decrease in glycogen concentrations of all tissues of *Patella* between November and January coincided with the shedding of the genital products. The resultant low levels of hepatopancreas and foot glycogen remained constant throughout the winter period, no further decrease being detected before the spring rise commenced at the end of March. In the decrease of glycogen reserves with the shedding of the genital products, the seasonal pattern of tissue polysaccharide reserves in *Patella* resembled that reported for *Helix* (von Brand, 1931; May, 1934) and oysters (Couteaux-Bargeton, 1947). But in these latter species, this decrease was followed by a period of accumulation before the onset of the winter period, during which there was a second decrease in the polysaccharide reserves. It has been suggested that in *Helix* and oysters glycogen is utilized in maintaining the metabolism during the winter period. In *Patella* the absence of changes in the low levels of glycogen in the hepatopancreas and foot between January and March offer no evidence of a similar glycogen utilization.

In field *Patella* gonadial development occurred between August and November. At no period of the year did the gonad contain appreciable glycogen reserves, not even preceding sexual maturity. This contrasts with the large reserves of galactogen laid down in the albumen gland of *Helix* (May, 1934) and of glycogen in the gonad of oysters (Couteaux-Bargeton, 1947) prior to sexual activity. However, petroleum ether extracts of developing gonads of *Patella* suggest that fat rather than polysaccharide forms a

major reserve material in the sexual products, in a manner somewhat similar to the storage of fat rather than glycogen in the digestive gland of *Pila* (George & Desai, 1954). Nevertheless, the rapid decrease in glycogen reserves between November and January is unlikely to result solely from the maintenance of metabolism after the cessation of feeding, since short-term starvation (1 month) at a much higher temperature (+10° C) does not result in a rapid utilization of glycogen. Glycogen may be used up rapidly in the final stages of gonadial maturation.

During sexual development in the field, the tissue glycogen concentrations do not markedly decrease. In contrast, gonadial development still occurs in laboratory-starved *Patella*, but is accompanied by a considerable decrease in glycogen concentrations. These results suggest that the alimentary carbohydrate recorded in the field *Patella* might be exerting a 'glycogen-sparing effect' during gonadial maturation, whereas in starved *Patella* the glycogen reserves may be transformed into fat.

SUMMARY

Blood glucose concentrations (total reducing values) of field *Patella* showed a seasonal variation. The variable summer values, whose means approximated to 6 mg % glucose (May–November), contrasted with the relatively low and constant winter values, whose means approximated to 3 mg% glucose (January–March). On starvation of summer *Patella*, the higher field values rapidly decreased to a constant basal level, more comparable to the low winter field values. A hyperglycemia of 30 mg % induced by intravascular glucose injection was followed by a rapid adjustment of the blood glucose to the pre-injection level within 4 h.

Seasonal variations in the glycogen concentrations occurred in the hepatopancreas and foot, but were not evident in the odontophore cushion, the other main site of glycogen storage. The patterns of the seasonal glycogen variations were similar to those of blood glucose, the parallelism being closer in the hepatopancreas than in the foot glycogen changes. As with the blood glucose, the spring rise in hepatopancreas glycogen concentration occurred during March and April, and was accompanied by a growth of the hepatopancreas. The spring rise in the foot glycogen concentration did not commence until May.

The developing gonads of both field and starved *Patella* (August–November) contained no appreciable glycogen reserves, but petroleum ether extracts indicated considerable fat storage. The shedding of the genital products coincided with the sharp decrease in blood glucose and tissue glycogen concentrations (November–January). Unlike oysters, the glycogen levels remained low throughout the winter period.

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