SEASONAL CHANGES IN BLOOD SUGAR, FAT BODY, LIVER GLYCOGEN, AND GONADS IN THE COMMON FROG, *RANA TEMPORARIA*

By C. L. SMITH
From the Zoology Department, University of Liverpool

(*Received* 18 *May* 1949)

(With Ten Text-figures)

INTRODUCTION

In recent years a large volume of research, mainly on the Mammalia, has shown the important role played by the endocrine glands in the general control of carbohydrate metabolism and in the regulation of the blood-sugar level. The work in this field has been comprehensively reviewed by Cori (1931), Soskin (1941), and Soskin & Levine (1946). The extent to which a similar endocrine control might be operative in lower vertebrates, such as the Amphibia, has not been so extensively investigated. The isolated frog's liver has been used by several workers to test the action of various hormones on liver glycogenolysis (Lesser, 1920; Issekutz, 1924; Fluch, Greiner & Loewi, 1935; Kepinov, 1937). Houssay and his co-workers (Houssay & Biassotti, 1931, 1933; Houssay, Biassotti & Rietti, 1934) have shown that the anterior pituitary in a toad, *Bufo arenarum*, exerts a 'diabetogenic' action, and the severity of the diabetes resulting after pancreatectomy is greatly ameliorated if the anterior lobe of the pituitary is removed. Slome (1936) also found that the blood sugar of *Xenopus laevis* was reduced after anterior lobe hypophysectomy. No reference has been found in the literature to any physiological work on seasonal changes in the activity of the endocrine glands of the frog, though Sklower (1925) has described seasonal differences in the histological appearance of various glands. Aron & Schwartz (1925) and Aron (1926) demonstrated the occurrence of seasonal histological changes in the Islets of Langerhans in the pancreas and in the interstitial tissue of the testis. Smith (1938) was able to correlate the latter with the increasing intensity of the induced clasping action of the male during the autumn and winter.

In contrast with the rather incomplete picture of the role of the endocrine glands in amphibian metabolism, there is a good deal of information available regarding the nature and extent of certain seasonal changes which occur in the frog (Holmes, 1927; Holzapfel, 1937). Among the organs showing well-defined changes are the liver, fat bodies, and the gonads. Athanasiu (1899) and Pflüger (1907) first reported the occurrence of marked seasonal changes in the glycogen content of frogs, and their work has subsequently been confirmed by Kato (1910), Bleibtreu (1911), and Goldfederowa (1926). All these authors are agreed that the liver glycogen and total body glycogen of the frog attain a high maximum value in the autumn, and fall to

a minimum in the spring. It was considered that the physiological mechanism controlling this seasonal change in glycogen content might prove to be of general interest if it could be elucidated. By analogy with the conditions found in the higher vertebrates it would seem probable that the endocrine system would be involved in such metabolic changes, and that there would be a reasonable expectation of associated changes in the post-absorptive blood-sugar level. The literature provides several references to estimations of blood sugar in the frog, but some of these relate to one particular season only (Bang, 1913; Lesser, 1913; Slome, 1936), while in those cases where the observations extend over a few months the duration and conditions of captivity are not always well defined (Besson, 1945; Scott & Kleitman, 1921). As a first approach to the problem it was therefore decided to carry out a seasonal survey to investigate the possible occurrence of correlations between the changes in the post-absorptive blood-sugar level, the development of the fat bodies, the glycogen content of the liver, and the development of the gonads.

MATERIAL AND METHODS

As the primary object of the present work was the investigation of the occurrence of seasonal variations in the post-absorptive blood sugar, it was necessary to standardize the sampling procedure so that all the samples should be comparable. To ensure this, interference by alimentary factors also had to be avoided as far as possible. From preliminary observations it was found that frogs examined after being kept in captivity for 48 hr. without access to food, in many cases still had partly digested food in the stomach. On examination after 72 hr., however, the stomach was usually found to be empty, and in the few exceptional cases digestion was far advanced. This agrees with the work of Frost (1932), who found that food required 48 hr. to pass through the gut of *Rana pipiens*. Hill (1911) has shown that the heat production of frogs falls considerably in the first 15 days of captivity without food. This decline in metabolic rate due to captivity and inanition raised the question of whether the blood-sugar level might also be affected. It was for this reason considered advisable to deal with the samples after the minimum time in captivity necessary to avoid errors due to alimentary hyperglycaemia. The third day after capture was finally selected as the time for taking all blood samples, and unless otherwise stated all blood-sugar values in this paper relate to frogs which have been in captivity without feeding for approximately 72 hr.

Most of the frogs used in this work were obtained from a collector in Shropshire. They were received in the laboratory 24 hr. after capture, and at once put in a glass-sided aquarium tank which was slightly tilted and contained enough water to form a pool at one end, while leaving the other end dry. The slate bottom of the tank provided a dark background, and all the frogs used for the routine observations were therefore dark-ground adapted. Slome (1936) has reported a difference in blood sugar between dark- and light-ground adapted *Xenopus laevis*. While no indication of such a difference was found in preliminary observations made by the author (*vide infra*), it was nevertheless thought advisable to standardize the background conditions. The room in which the frogs were kept was unheated and well ventilated,

the temperature being approximately atmospheric. At times during the summer samples of frogs were obtained locally, these being used as parallel samples for comparison with those from the usual source. In no case were any significant differences found and the results have not been shown separately.

A standard procedure was adopted for dealing with each frog in the samples. The frog was removed from the tank with the minimum of disturbance, care being taken to prevent the frog's struggling, and immediately pithed. The heart was quickly exposed and the blood sample withdrawn from the ventricle. The liver was then excised, blotted between filter-paper to remove the excess moisture, rapidly weighed and transferred to a centrifuge tube containing hot 40% caustic potash. The frog itself was then weighed, after which the fat bodies and, in some samples, the gonads were removed, blotted and weighed. The blood samples were obtained from the ventricle by means of a hypodermic syringe which had previously been rinsed out with a dilute solution of potassium oxalate and air dried. The volume of blood obtainable by this method varied considerably, but usually from 0·3 to 0·4 ml. could be drawn off quite rapidly. The blood was transferred to an oxalated crucible from which a 0·1 ml. sample was pipetted into the zinc hydroxide protein coagulant. The sugar content of these samples was estimated by the method of Hagedorn & Jensen (1923). Duplicate analyses were made on numerous occasions, and the final burette readings never differed by more than 0·01 ml. Liver glycogen was estimated by Sahyun's (1931) method, the glucose content of the digest after hydrolysis being determined on an aliquot sample by reduction of potassium ferricyanide as in the blood-sugar estimations. The glycogen found was expressed as a percentage of the wet weight of the liver. The fat body, liver and gonad weights were expressed as percentages of the total body weight.

Samples of frogs were obtained every month (with the exception of February) from January 1948 to January 1949. The number in each sample varied from 12 to 26, the average sample number for the whole series being 16. The weight of the frogs used usually lay between 20 and 25 g., the extreme range being from 12 to 50 g. (usually gravid females). The lower size limit was set by the difficulty of obtaining blood samples from small frogs. At the lowest size used all the frogs had attained sexual maturity.

The seasonal changes in fat bodies, gonads, liver weight, and liver glycogen content

(*a*) *Fat bodies*

The cycle of fat-body development found in 1948 is shown in Fig. 1. If a true comparison between the sexes is to be made it is necessary to make allowance for the differential development of the reproductive organs, for, as March (1937) has pointed out, the great development of the ovaries and oviducts causes a disproportionate increase in the body weight of the female. Therefore the weight of the reproductive organs was deducted from the total body weight of the females in the July to January samples, inclusive, before calculating the percentage weight of the fat body. As the testes represent a significant proportion of the body weight in

August, their weight was similarly deducted from the total male body weight in the July, August and September samples to achieve uniformity of treatment. At other times of the year the correction for testis weight is insignificant. Only in January 1949 was a significant sex difference found, and this is the only month when the fat-body weights have been shown by sexes in Fig. 1.

Previous workers have found a very similar cycle for the changes in the fat bodies of frogs (Gaule, 1901; Victoroff, 1908; Kennel, 1912). The fat body attains its

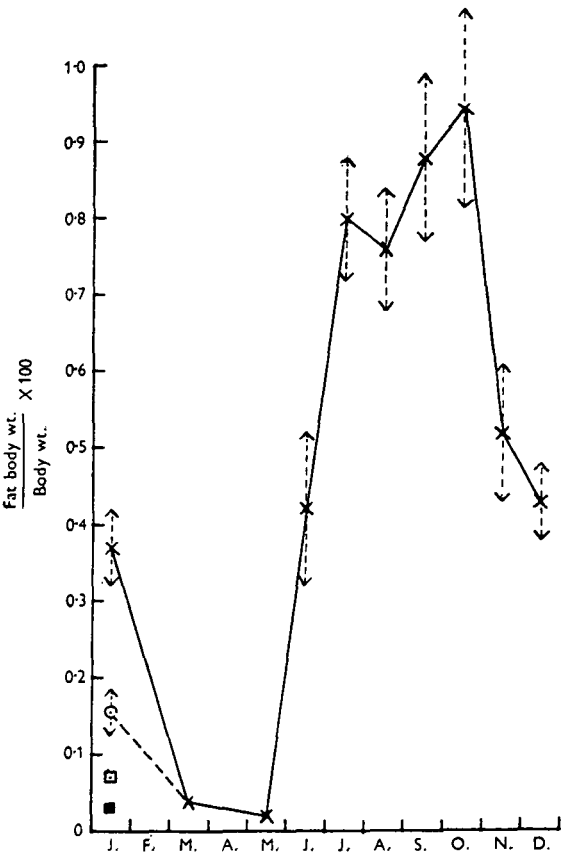

Fig. 1. Seasonal variation in weight of fat body of *R. temporaria* in 1948. ⊙, females Jan. 1949; ⊡, sexually active males Jan. 1948; ■, sexually active females Jan. 1948. Vertical broken lines show the standard errors of the sample means.

maximum development in October, this being followed by a decrease through the winter culminating in a rapid fall to a minimum at the spawning season. The effect of sexual activity on the fat body is shown by the difference between the two January samples. All the frogs received in January 1948 were in amplexus, while those in January 1949 were not sexually active, and it can be seen that the fat body had not been so extensively depleted in the latter group. There is some conflict in the literature as to the existence of a sex difference in the fat-body development in the autumn. Kennel (1912) found the female fat body in *Rana temporaria* to be con-

siderably larger than that of the male from June to March. Gaule (1901), however, found it to be better developed in the male *R. esculenta* from September to June. In the present work, where a correction has been made for the weight of the reproductive organs, a significant sex difference was found only in January ($P < 0{\cdot}01$), when the male fat body was the larger. This is perhaps an indication that the female draws more extensively on the stored reserves of nutriment during the winter than the male. It may be noted that there was always a wide range of individual variation in fat-body weight, as can be seen from the standard errors of the means plotted in Fig. 1, and much larger sample numbers would have to be used before a reliable test of possible sexual differences could be made.

The most striking feature of the present results is the occurrence of a very rapid regeneration of the fat body in June and July. Eleven frogs caught on 31 May showed no difference in fat-body condition from the post-spawning samples, but examination of thirteen frogs caught on 15 June revealed that fat-body regeneration was well under way. The average fat-body weight for the last group was 0·42% of the body weight compared with 0·02% for those caught at the end of May. This rapid increase was continued in the July sample, but a definite check was seen in August. A second small increase was found in September and in October when the maximum for the year was attained. Gaule (1901) found a similar rapid increase in fat-body weight for *R. esculenta* during July. In the early part of the month the fat body represented 0·03% of the body weight, compared with 0·88% at the end of the month.

(*b*) *Gonads*

The seasonal changes in weight of the gonads are shown in Fig. 2. The data on which these curves are based were not obtained from the samples examined in 1948 as the gonads were not weighed in all cases, but were obtained by the author in 1934 (previously unpublished). When average gonad weights were determined in 1948 they showed no significant departures from the corresponding 1934 records. The general cycle of gonad development agrees closely with that found by other workers (Holmes, 1927). The most obvious change in the testis weight was the sharply defined maximum in August accompanying spermatogenesis. In late July the testis weight was 3·2%, in August it reached 6·0%, and by September it had fallen again to 3·1% of the body weight. During the winter there is a slight decrease in weight, with a more obvious fall at the spawning period due probably to the transfer of spermatozoa to the vesicula seminales. The regeneration of the ovaries is a more extended process. They show a gradual increase in weight during July and August, followed by a more rapid rise to November when the maximum weight is nearly attained. The ovarian weight shows a steep fall at the spawning season when ovulation takes place.

(*c*) *Liver weight*

The variation in liver weight during the year is shown in Fig. 3. As in the case of the fat body adjustment has been made where necessary for the disproportionate

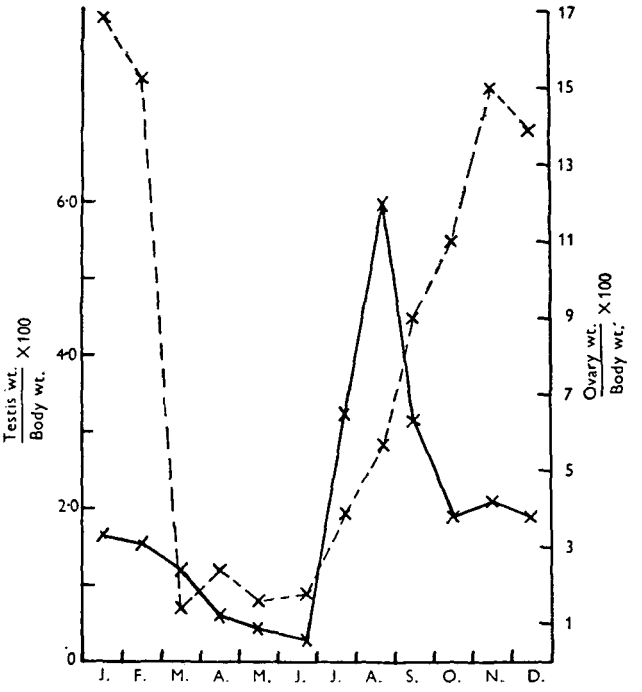

Fig. 2. Seasonal variation in weight of the gonads of *R. temporaria* in 1934. ×———×, testis; ×– – – –×, ovary.

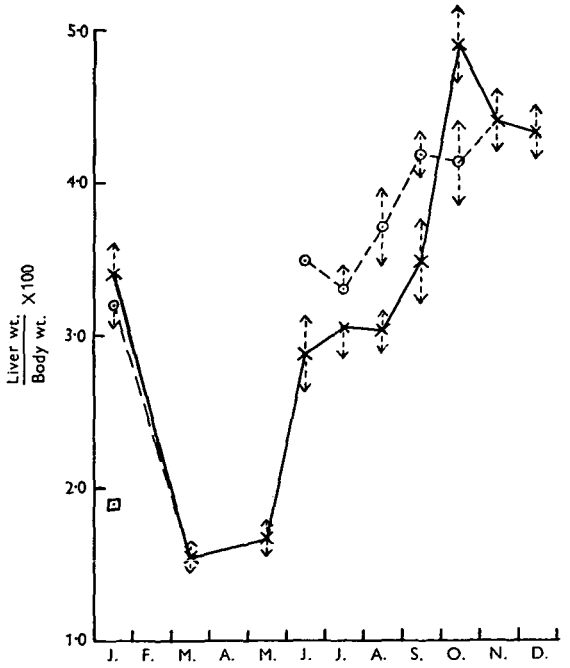

Fig. 3. Seasonal variation in weight of the liver of *R. temporaria* in 1948. ×———×, males; ⊙– – – –⊙, females; ⊡, sexually active males Jan. 1948. Vertical broken lines show the standard errors of the sample means.

increase in the reproductive organs in the two sexes. The weight of the ovaries and oviducts or of the testes has been deducted from the total body weight in the same way and for the same months as detailed in the fat-body section. March (1937) found that the female liver weight always exceeded that of the male when adjustment was made for the weight of the reproductive organs. The present results confirm this with the exception of those for October and January when the male liver was the heavier. The standard errors of the means are, however, too large for any statistical significance to be attached to these differences. The influence of the onset of sexual activity on liver weight is shown by the mean value given by the January 1948 frogs, which were all in amplexus. The mean liver weight of the males in this sample was 1·9% of the body weight compared with 3·4% in January 1949 when the frogs were not sexually active. Apart from the sex difference there was a regular cycle of liver-weight change. The minimum occurred during and after the breeding season in March, April and May. The first obvious post-spawning increase took place in June to be followed by a further rise in the autumn, the maximum for the year being reached in October.

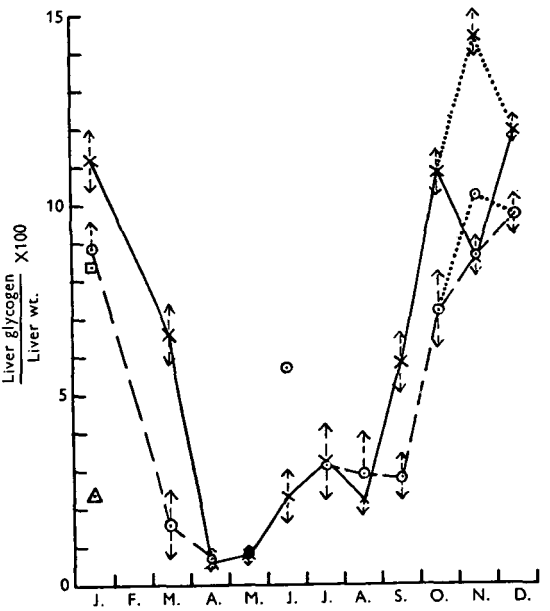

Fig. 4. Seasonal variation in liver glycogen, calculated as a percentage of the wet weight of the liver, of *R. temporaria* in 1948. ×———×, males; ⊙- - - -⊙, females; ⊡, sexually active males Jan. 1948; △, sexually active females Jan. 1948. Dotted lines in November show the liver glycogen content found after 4 days in captivity. Vertical broken lines show the standard errors of the sample means.

(*d*) *Liver glycogen*

The monthly means of the estimations of the glycogen content of the liver are shown in Fig. 4 as percentages of the wet weight of the liver. In view of the fact, however, that the relative weight of the liver itself has been shown to fluctuate

seasonally, it was considered that these results would more accurately depict the seasonal changes in glycogen storage if they were expressed as liver glycogen contained in 100 g. of total body weight of frog. The data when recalculated in this way are shown in Fig. 5, where, as in the case of the fat-body and liver weights, the weight of the reproductive organs has been deducted from the total body weight for the same months previously detailed.

In the main the changes in liver glycogen follow a similar cycle to that just described for the liver weight. There is a post-spawning minimum in April and May, followed by a slight increase in June. The June sample contained only three female frogs, which showed liver glycogen contents of 7·6, 7·2 and 2·3% of the wet weight of the liver (mean 5·7 ± 1·7%). In view of the small sample number and large

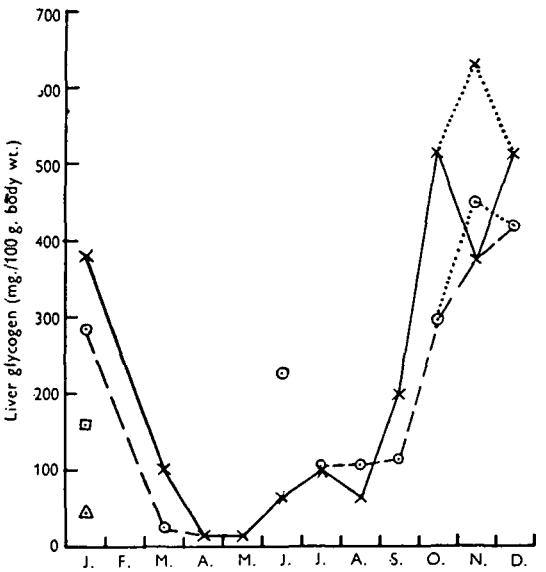

Fig. 5. Seasonal variation in liver glycogen, calculated as mg. of liver glycogen per 100 g. of frog. For explanation of symbols, see legend to Fig. 4.

variation this mean value for female liver glycogen has been shown as an isolated point in Figs. 4 and 5. It is considered that this transient rise in female liver glycogen shown by this sample is probably due to sampling errors. In July and August there is little further change, but in the male there is a rapid increase in September, while in the female this autumn increase is delayed until October and is not as great as that of the male. The points shown joined by dotted lines in Figs. 4 and 5 relate to glycogen values found after frogs from the same sample had been in captivity for 4 days. They show that a significant increase in glycogen content had taken place, especially in the male, in the 24 hr. since the normal 72 hr. observations were made. This phenomenon is referred to again below (p. 428). There is a gradual fall in liver-glycogen content during the winter, largely owing to the decrease in liver weight. A sharper fall occurs at the spawning season, the female liver glycogen being depleted more rapidly than that of the male. The influence of sexual activity

is again well shown by the sexually active sample received in January 1948. This annual cycle is similar to that described by previous authors (Athanasiu, 1899; Pflüger, 1907; Kato, 1910; Bleibtreu, 1911; Goldfederowa, 1926).

Seasonal changes in blood sugar

The annual cycle of variation of the sugar content of the blood of frogs kept in captivity for 72 hr. is shown in Fig. 6. It can be seen that during the greater part of the year the blood sugar did not depart very far from a value of 40 mg./100 ml., with

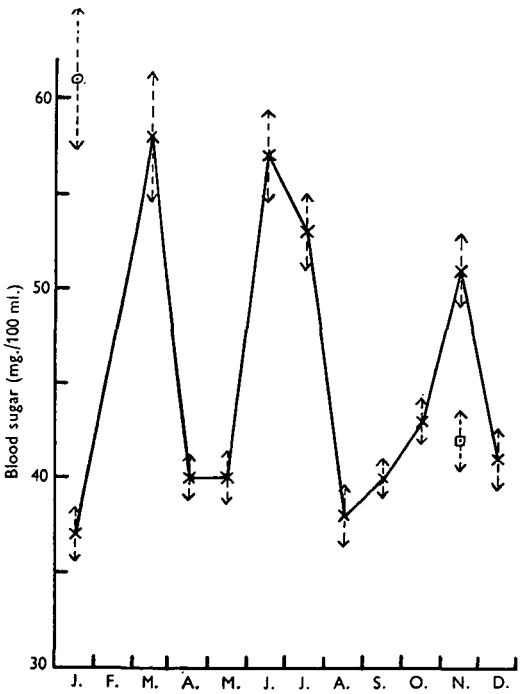

Fig. 6. Seasonal change in blood sugar of *R. temporaria* during 1948, after 3 days in captivity without food. ⊙, sexually active sample Jan. 1948; ⊡, after 4 days in captivity in Nov. 1948. Vertical broken lines show the standard errors of the sample means.

the exception of three periods when a significant increase was found. The first of these occurs at the spawning period, the mean blood sugar in March being $58 \pm 3{\cdot}5$ mg./100 ml. The correlation between this high blood sugar and sexual activity is indicated by the high mean of $61 \pm 3{\cdot}7$ mg./100 ml. given by the sexually active sample in January 1948, while the quiescent sample received in January 1949 gave a mean value of only $37 \pm 1{\cdot}4$ mg./100 ml. After the spawning season the blood sugar fell to a steady level of $40 \pm 1{\cdot}4$ mg./100 ml. in April and May. In June, however, there was a return of the hyperglycaemic condition, the mean value being $57 \pm 2{\cdot}4$ mg./100 ml. There was a slight, but not statistically significant, fall in July, followed by a significant decrease to $38 \pm 1{\cdot}6$ mg./100 ml. in August. From August onwards there was a steady rise, the mean reaching $51 \pm 1{\cdot}9$ mg./100 ml. in November.

From November until the onset of sexual activity the blood sugar remained in the neighbourhood of 40 mg./100 ml. In Fig. 6 the standard error of the mean of each sample has been plotted, and it can be seen that the increases in blood sugar in the hyperglycaemic periods are statistically highly significant ($P < 0.001$). The standard errors for the higher (± 2.0 to 3·5) were usually greater than those for the lower means (± 1.0 to 1·5 mg./100 ml.).

A search of the literature has revealed a few estimates of blood sugar in the frog which may be compared with those reported here. Loewit (1909) found 540 mg./100 ml. in winter frogs, while spring frogs contained 810 mg./100 ml. It is generally agreed by other authors (Bang, 1913; Lesser, 1913; Scott & Kleitman, 1921) that these values are much too high, but it is interesting to note that Loewit found a higher blood sugar in spring than in winter frogs. Bang (1913) estimated the blood sugar of freshly caught frogs (*R. temporaria*) and found it to be 40 mg./100 ml. in May, while it varied between 40 and 50 mg./100 ml. in August, values very similar to those obtained in the present investigation for the corresponding months. Lesser (1913) used composite samples of frog blood in July which on analysis by Bang's method showed 35, 40 and 36 mg. of sugar per 100 ml. of blood. Brinkmann & Van Dam (1919) found blood sugars ranging from 40 to 65 mg./100 ml. Scott & Kleitman (1921) determined the blood sugar of *R. pipiens* in February, March and April and obtained an average for their whole series of 37 mg./100 ml., there being no apparent increase during the spring, but the frogs were kept in a vivarium at 18° C., and the duration of captivity is not stated. Slome (1936) determined the blood sugar of *Xenopus laevis* when dark- and light-background adapted, and found it was significantly higher in the dark than in the light specimens (35·0 and 25·6 mg./100 ml. respectively). Similarly, after removal of the anterior lobe of the pituitary the blood sugar was reduced to 22 mg./100 ml. Zwarenstein & Bosman (1932), however, also investigated the influence of various agencies which affect the state of the melanophores on the fasting blood-sugar level of *X. laevis*. They found that exposure to white or dark backgrounds, total darkness, or removal of the eyes led to no alteration of the blood sugar. They also found that hypophysectomy, either complete or anterior lobe only, had no effect on the blood sugar, but did lead to a considerable increase in glucose tolerance. Besson (1945) carried out a similar investigation on *Rana temporaria* during the months of February, March and April. He found that the blood sugar was higher for the frogs kept on a light ground, but the difference decreased throughout his series and in April there was no background effect. Besson's mean values on a light background were as follows: February 79 mg.%, March 59 mg.% and April 36 mg.%, while on a dark background he obtained: February 48 mg.%, March 43 mg.% and April 36 mg.%. The experimental details of this work are not very clear, but it appears that the frogs were in captivity without food during the whole 3-month period of the experiment. As far as the blood sugars of the light-background adapted frogs are concerned they follow rather closely the cycle shown in Fig. 6, although Besson does not mention the occurrence of any sexual activity. The difference in blood sugar of the light- and dark-background adapted animals is

directly opposed to that found by Slome (1936) for *Xenopus laevis*. The present author made a short series of observations on the relation between blood sugar and background in March 1948. Twenty-seven frogs which had been in captivity and unfed for 2 weeks were divided into two groups, one of which was placed on a black and the other on a white background. At the time of killing all the frogs showed good adaptation to their particular background. The mean blood sugar of the frogs on the light ground was $49 \pm 3{\cdot}5$ mg.%, compared with $45 \pm 3{\cdot}6$ mg.% for those on the dark ground, the difference being without significance. It would appear, therefore, that the existence of a relation between blood sugar and background is not certainly established at present for either *X. laevis* or *Rana temporaria*.

While the previous work on the blood sugar of the frog provides little information which can be regarded as confirming the seasonal variation found in the present investigation, the extreme range of the mean values (37–62 mg.%) observed lies within that found by other workers, namely, 34–79 mg.% if the very high figures reported by Loewit are neglected.

Scott & Kleitman (1921) found that the blood sugar of the female *R. pipiens* was slightly higher than that of the male. To ascertain whether the present data provide any indication of a sex difference the monthly means for the males and females have been computed separately and are shown in Fig. 7. The splitting of the monthly samples into sex groups has, of course, reduced the sample number and there is a general tendency for the standard errors of the means given in Fig. 7 to be increased by the reduction in the number of observations in each group. In some of the samples the proportion of the sexes was unequal; for instance in June and July the samples contained only three and four females respectively, while the males in the same samples numbered eight and ten. In general, the annual cycle seen in Fig. 7 follows that already described for the undivided samples, but the detailed relationship between the sexes is of considerable interest. From October to May the female blood sugar lies slightly below that of the male in every sample, the maximum difference being 7 mg.% in November and the minimum only 1 mg.% in April and May. With the onset of the hyperglycaemia in June, however, the female blood sugar rises above that of the male and remains slightly higher until September. Again the difference is not very great, the maximum being 6 mg.% in June and falling to 2 mg.% in September. It is apparent from Fig. 7 that the differences between the sexes are not statistically significant. This is because the number of observations in each group was too small. The June, July and August observations on the one hand, and those for October, November and December on the other, may be pooled. If a statistical analysis is now made for the sex variable only, it can be shown that, in both groups, the sex difference in blood sugar is statistically significant, P being $< 0{\cdot}02$ in June, July and August, and $< 0{\cdot}001$ in October, November and December. It therefore seems probable that there was a sex difference in the 72 hr. blood-sugar level, and that there was a reversal of the relation between the sexes taking place near the end of September. The sex difference found by Scott & Kleitman (1921) for *R. pipiens* was only 7 mg.% for their whole series, and is only statistically significant if one very hypoglycaemic female (11 mg.%)

is not included. Bosman & Zwarenstein (1930) found no sex difference in the fasting blood sugar of *Xenopus laevis*. It will be advantageous to defer further discussion of the sex difference found in this work to a later section of the paper.

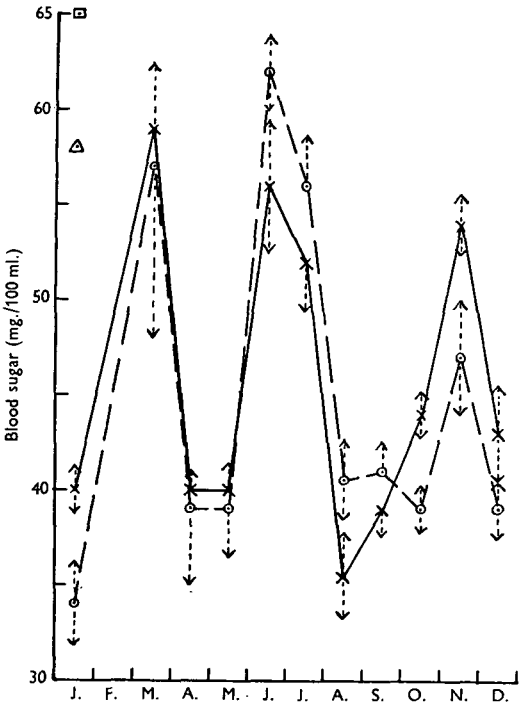

Fig. 7. Seasonal change in blood sugar of male and female *R. temporaria* during 1948, after 3 days in captivity without food. ×———×, males; ⊙- - - -⊙, females; ⊡, sexually active males Jan. 1948; △, sexually active females Jan. 1948. Vertical broken lines show the standard errors of the sample means.

Comparison of the various seasonal cycles

During the early part of the year, up to and including the spawning period, comparison of Figs. 1, 3, 5 and 6 shows that the fat body, liver weight, and liver glycogen were all decreasing. This decrease was most rapid during the breeding season itself when the blood sugar was high. It seems, then, that the hyperglycaemia accompanying sexual activity was correlated with a rapid consumption of the stored food reserves. This is not unexpected in view of the fact that not only is the metabolism of the frogs high at this time (Krogh, 1904; Dolk & Postma, 1927), but also they probably are not feeding to any appreciable extent. In connexion with this high blood-sugar level and the mobilization of liver glycogen during the breeding season it is interesting to compare the rate of sugar production by the isolated liver. Lesser (1921) perfused isolated livers of *R. esculenta* with isotonic Ringer solution. The total glucose formed during 4 hr. perfusion between August and February varied from 300 to 500 mg., but in March, April and June it increased to approximately 1080 mg., in a 4 hr. period. Fluch *et al.* (1935) also perfused isolated livers of

R. esculenta, and found this marked increase in sugar production in February only. These workers were also able to show that liver glycogenolysis was considerably reduced by previous hypophysectomy, but that such treatment did not abolish the increased sugar production observed in February. They therefore concluded that the increased glycogenolysis at this time was independent of the influence of the anterior pituitary, but might have had a connexion with spawning. The spawning season is later in the year for *R. esculenta* than for *R. temporaria*, but in view of the results of perfusion experiments given above it would seem probable that the high blood-sugar level associated with sexual activity was due to a potentiation of glycogenolysis in the liver.

In the post-spawning months of April and May the frogs were feeding actively but there was no regeneration of the fat body, or deposition of liver glycogen, while the blood sugar remained steady at 40 mg.%. This phase was succeeded by a marked change in June. As has already been shown, there was a rise in blood sugar, a simultaneous slight increase in liver weight and liver glycogen, and the fat-body weight increased rapidly. These changes in June are shown compared in Figs. 8 and 9, and it can be seen that the main feature accompanying the raised blood sugar of this period was the rapid regeneration of the fat body.

During the remainder of the year the sequence of events was different for the two sexes, and it is simpler to consider them separately. In Fig. 8 the changes in blood sugar, testis weight, fat-body weight, and liver-glycogen content are shown for the male. It can be seen that the fall in blood sugar found in August coincided very closely with the peak of the wave of spermatogenesis in the testis, as indicated by the increase in testis weight. It may also be noted that the male blood sugar attained its lowest value for the year at this time. As the wave of spermatogenesis subsided, then the blood sugar slowly rose again. It is also apparent that during spermatogenesis there was no further deposition of liver glycogen or regeneration of the fat body. When spermatogenesis was subsiding, however, there was a slight increase in the fat-body weight to its October maximum, but the main event was a large increase in liver glycogen which rose to 518 mg./100 g. of frog in October. The comparable cycle in the female for the same period is shown in Fig. 9. In this case also the blood sugar fell in August, though not as far as that of the male, and this was also accompanied by an increase in the weight of the gonads. Unlike spermatogenesis in the testis, the regeneration of the ovary continued until November, and this process was accompanied, at least until October, by a low blood-sugar level. The liver glycogen in the female did not begin to increase until October, that is, a month later than in the male, and the maximum attained was also lower in the female.

Fig. 10 shows the relationship between the blood-sugar level and gonad regeneration for the two sexes. Here the course of regeneration in the male is again indicated by the changing weight of the testis, but instead of using ovary weight in the female the ovary glycogen per 100 g. of frog has been plotted. The data for this part of the figure have been taken from the work of Bleibtreu (1911) on *R. temporaria*. The sequence of events in the male is, of course, as described above. In the female, however, the use of ovary-glycogen content instead of ovary weight shows a clearer

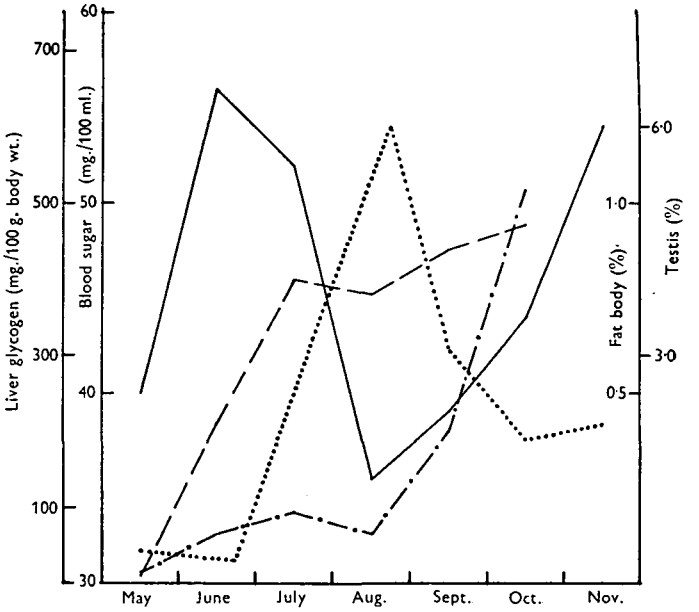

Fig. 8. Comparison of seasonal changes in blood sugar, fat-body weight, gonad weight, and liver glycogen of male *R. temporaria*. ————, blood sugar; — — —, fat body; ....., gonad; —·—·—·, liver glycogen.

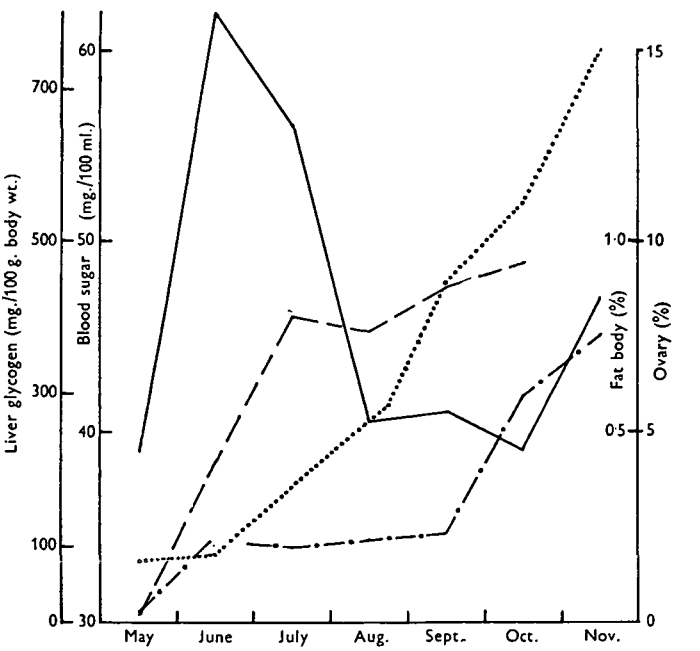

Fig. 9. Comparison of seasonal changes in blood sugar, fat-body weight, gonad weight, and liver glycogen of female *R. temporaria*. For explanation of symbols, see legend to Fig. 8.

relation between the period of low blood sugar and gonad regeneration. Thus the fall in blood sugar in August coincided with an increase in ovary glycogen, which continued through September and October. The blood sugar remained low during the whole of this period, but in November, when the blood sugar showed a slight rise, the increase in ovary glycogen was considerably reduced. A slight increase was found by Bleibtreu to continue until December.

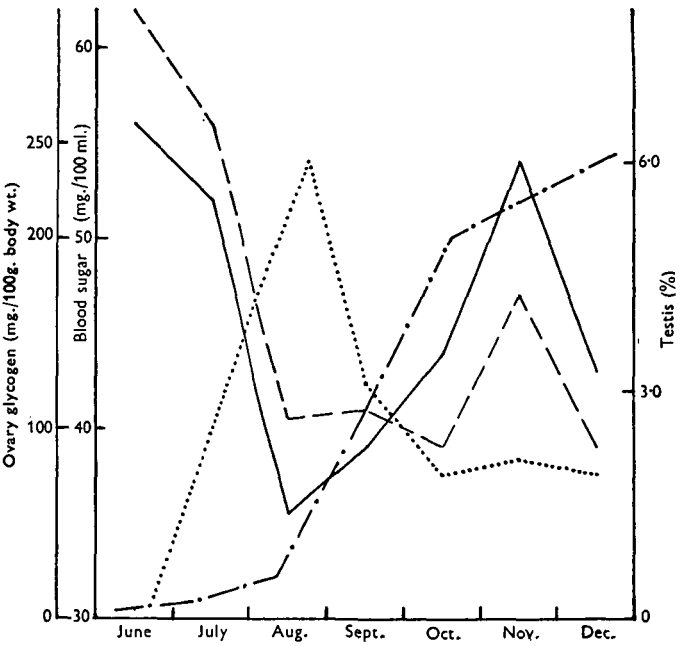

Fig. 10. Comparison of seasonal changes in blood sugar and gonad regeneration in male and female *R. temporaria*. ————, male blood sugar; — — —, female blood sugar; . . . . . , testis weight; — · — · —, glycogen content of the ovary (from Bleibtreu, 1911).

DISCUSSION

A question which arises from the results described in the preceding sections is the extent to which the observed changes may be attributed to the feeding habits of the frog. A quantitative survey of the food intake at different times of the year has not been attempted, but a note was made of the nature and amount of the stomach contents of all freshly caught frogs examined during the year. The stomach contents of the December and January samples indicated a reduction in food intake, but even in these winter months several aquatic insect larvae were found in their stomachs. These samples had been taken under water in a shallow ditch, and it must be noted that the weather of the winter 1948–9 was very mild, so that it is quite possible that in more inclement weather the frogs would have retreated into the mud at the bottom of the ditch and have ceased to feed. The fat-body cycle (Fig. 1) would suggest that even in November the food intake was inadequate for the frogs' metabolic needs, as the fat body showed a marked decline from the October level. Presumably the frog

does not feed during the spawning season, for all stomachs examined at this time were found to be empty. The liver glycogen and fat-body cycles show that the frog is compelled to draw on its stored reserves from November onwards, as both decrease steadily during the winter. This mobilization is accelerated greatly by the enhanced metabolism of the breeding season, and results in the almost complete utilization of the fat body and a marked depletion of the liver glycogen. It would seem then that the changes in the fat body and liver glycogen during the winter months may be attributed to the diminished food intake being inadequate to supply even the reduced energy requirements of the winter frog.

No change in the intensity of feeding was indicated by the stomachs examined between April and November. During this period all freshly caught frogs had stomachs well filled with a variety of insects, earthworms, slugs and caterpillars. In the first part of this period there was no increase in either the fat body or the liver glycogen, and the blood sugar remained low. This survey does not provide any indication of the fate of any food ingested in excess of that needed to supply the animal's immediate energy requirements at this time. It is possible, however, that this is a period of tissue repair after the heavy demands of the spawning season and perhaps also of growth. Examination of stomach contents has, therefore, provided no evidence to indicate that an alimentary factor might be responsible for the metabolic changes observed in summer and early autumn. Such independence of food supply is also indicated by the fact that Pflüger (1907) found little difference in glycogen content in October between frogs which had been kept without access to food since the end of August and frogs which were freshly caught.

The data provided by the present survey are not sufficient to show the physiological significance of the changes in blood sugar observed. For instance, during the period of active feeding there are two phases characterized by a low blood sugar, namely, April and May, and August and September. The first of these was accompanied by no obvious change in any of the organs examined, while the second synchronized with the regeneration of the gonads, and in the female the accumulation of glycogen in the ovary. It is suggested, therefore, that the low level of the blood sugar at these times is not due to the same endocrine balance in each case, despite the similarity of the blood-sugar values. The incidence of hyperglycaemia in June could be due to an increase in hepatic gluconeogenesis from either exogenous or endogenous material. This is indicated by the concomitant rapid storage of fat, and increase in relative liver weight and liver glycogen. When the regeneration of the gonads begins it seems that another factor enters, and the glucose formed in the liver may be utilized to provide the energy needed for gametogenesis and for storage of food reserves in the ovary. When maturation of the sex products is nearing completion the glucose formed in excess of the animal's energy requirements is apparently stored as glycogen rather than as fat, as it was in June and July. It is considered that the dependence of the incidence of glycogen storage in the liver on the completion of gonad regeneration is strongly indicated by the reversal of the sex difference in blood sugar found in September, and the earlier appearance and greater extent of the liver glycogen increase in the male. Another factor contributing

to glycogen storage in the autumn may be the decreasing mean air temperature, which will reduce the basal metabolism of a poikilothermous animal.

During the year a few estimations of blood sugar have been made at intervals other than 72 hr. after capture. These have not been either regular or very numerous, but they have indicated that the blood sugar of frogs in the field is fairly high (about 70 mg. %) from April to November. It is, therefore, possible that the seasonal changes in the 72 hr. observations may be due to different rates of fall of the blood sugar from such an initial value to a steady level of about 40 mg. %, which always seems to be attained when the frogs have been kept in captivity for longer periods (over 2 weeks). The observed variations might then be reflexions of alterations in the glucose tolerance of the frog. In this connexion reference may be made to the values for liver-glycogen content found after 4 days in captivity in November (shown by dotted lines in Figs. 4 and 5). This increase in glycogen during the additional 24 hr. of captivity was accompanied by a fall in blood sugar in both sexes. Such an observation has several interesting theoretical implications, but as it is still subject to confirmation with larger sample numbers, discussion of these will not be pursued here. In the present year the intention is to follow the changes in blood sugar and liver glycogen due to captivity and inanition continuously from the time of captivity to the attainment of a stable condition. It is hoped to obtain such data for all the periods where changes in metabolism have been indicated in this preliminary survey. If possible, glucose-tolerance estimations will also be made at the same periods. When information on points such as these is available, discussion of the physiology of seasonal changes in frog metabolism should be facilitated.

SUMMARY

1. Seasonal changes in the weights of fat body, gonads and liver, liver-glycogen content, and blood sugar have been followed by observations on monthly samples of *Rana temporaria* which had been kept in captivity for 3 days without food.

2. Three periods during which the blood sugar was high (from 54 to 62 mg./100 ml. of blood) were observed during the year: at the spawning season in March, in June and July, and in November.

3. The hyperglycaemia of June and July was accompanied by the rapid development of the fat body. This phase was succeeded by that of gonad regeneration, during which there was no further storage of fat or liver glycogen, and the blood sugar was low (about 40 mg./100 ml. of blood).

4. When gonad regeneration was nearing completion glycogen storage in the liver became the dominant feature, and the blood sugar tended to rise again.

5. A sex difference in blood sugar was observed at certain periods. From June to September the female blood sugar was higher than that of the male, but from September to January the relationship was reversed. This may be correlated with the differing rates of gonad regeneration in the two sexes.

6. During the winter months, when the food intake was reduced, the frogs utilized some of their stored fat and glycogen and the blood sugar was low (about

40 mg./100 ml.). The hyperglycaemia found at the spawning season was accompanied by the rapid depletion of the fat and glycogen stores.

7. In April and May, after the breeding season, when the frogs were feeding actively, the blood sugar was low and apparently neither fat nor glycogen was being accumulated. It is suggested that this was a period of tissue repair and possibly of growth.

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