THE DIURNAL VARIATIONS IN THE TISSUE GLYCOGEN CONTENT AND THEIR RELATION TO MITOTIC ACTIVITY IN THE ADULT MALE MOUSE

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(With Two Text-figures)

INTRODUCTION

In a recent series of papers on the epidermis of the adult mouse (Bullough, 1949a, b, 1950a, b), it has been repeatedly stressed that the concentration of glucose or glycogen is the most important single factor affecting mitotic activity. In normal circumstances a high mitosis rate is seen only during rest or sleep, and it was suggested by Bullough (1949a) that this may be related to the deposition of glucose from the blood at this time. While it is well known that most of the deposited glucose is stored in the liver in the form of glycogen, it also appeared possible that some may be stored on other tissues including the epidermis.

Opportunity has now been found to check this hypothesis and to discover whether the diurnal rhythm in the glycogen content of the skin shows any relation to the diurnal rhythm in epidermal mitotic activity.

MATERIAL AND METHODS

(1) The mice. The animals used were males of the Kreyberg’s white label and Strong’s CBA strains, and were between the ages of 3 and 9 months. All were healthy and in breeding condition. They had been kept from birth at a controlled temperature of 20° C., and were fed on an abundance of commercial rat cake with added cod-liver oil, flaked maize, and dog biscuit. Invariably they received their food between 09.00 and 10.00 hr. so that they were accustomed to being awake at that time. This habit determined the form of their diurnal cycle of bodily activity, which in turn determined that of their diurnal cycle of mitotic activity (Bullough, 1948).

(2) Estimation of glycogen. Groups of animals were killed with chloroform at various times between 08.00 and 22.00 hr., and samples of skin and liver were immediately removed for glycogen determination. The method used was the Good, Kramer and Somogyi (1933) modification of Pflüger’s technique. For hydrolysis, 5 or 10 ml. samples of glycogen solution, to which 5 or 10 drops of concentrated HCl were added, were maintained for 2½ hr. in a bath of boiling water. That hydrolysis is complete in these conditions was shown by duplicate determinations on

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samples treated for longer periods, and on solutions of glycogen of known concentration.

The glucose content of the neutralized hydrolysate was determined by the Somogyi-Shaffer-Hartmann method (Somogyi, 1930), that is by the iodometric titration of reduced copper. The results were also checked by the method of Folin and Wu (Folin & Wu, 1920; Folin, 1929), which is a colorimetric technique.

(3) Histological demonstration of glycogen. In order to observe the distribution of glycogen in the dermis and epidermis, pieces of ear were fixed in Bouin’s alcoholic fluid, embedded in ester wax, and cut into sections 7 μ thick. The sections were stained either in Best’s carmine or, after treatment in periodic acid, in Schiff’s reagent (McManus, 1946).

(4) Estimation of mitotic activity. The epidermal mitosis rate was determined in small pieces of ear removed at the time of death. All stages of mitosis were included in the counts which were made on unit lengths (1 cm.) of sections of epidermis cut 7 μ thick. From each piece of ear ten such counts were made and the average taken. Each experimental group consisted of six mice, and therefore in each case six figures were available from which a grand mean and standard error were calculated according to the method recommended by Simpson & Roe (1939) for small samples.

OBSERVATIONS

(1) Diurnal glycogen cycles. Judging from previous results on the diurnal variations in mitotic activity and in the blood sugar concentration (Bullough, 1949a), it appeared that sufficient information could be obtained from those fluctuations occurring between 08.00 and 22.00 hr. This interval includes a period of waking and feeding at about 10.00 hr., a period of sleep centred round 14.00 hr., and a period of evening activity beginning at about 17.00 hr. (Bullough, 1948).

Groups of six mice each were killed at 08.00 hr. and at 2 hr. intervals until 22.00 hr. Males were used in order to avoid both the complications of the oestrous cycle (Bullough, 1946) and the possible differences in glycogen concentration due to sex (Denel, Butts, Hallman, Murray & Blunden, 1937, 1938; Sjögren, Nordenskjöld, Holmgren & Møllerström, 1938). From each animal two samples of liver and two samples of skin were taken for glycogen determination, the skin samples including dermis, epidermis and hair. The results were expressed in mg. of glucose (formed by hydrolysis) per 100 g. of fresh tissue, averages were taken, and the results are shown in Table 1.

These results are also expressed as a graph in Fig. 1, and it is at once obvious that the glycogen content of both liver and skin reached a minimum at about 10.00 and 20.00 hr. and a maximum at 14.00 hr.

As already mentioned, the figures for the skin refer to the total glycogen content of dermis and epidermis, since it was not found practical to separate these two layers and to estimate the glycogen content of the epidermis alone. Consequently, it was necessary to check that a proportion of the glycogen measured was indeed present in the epidermis. Sections of ears taken from mice killed at 10.00 hr. and at 14.00 hr. were prepared, and were stained for glycogen. During staining slides were placed
Table 1. The average quantities of glycogen present in the liver and skin and the average numbers of mitoses present per unit length (1 cm.) of sections (7 μ thick) of ear epidermis at various hours of the day in groups each of six adult male mice

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Glycogen concentration expressed as mg. glucose per 100 g. of fresh tissue</th>
<th>No. of mitoses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In liver</td>
<td>In skin</td>
</tr>
<tr>
<td>08.00</td>
<td>932 ± 60</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>10.00</td>
<td>820 ± 84</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>12.00</td>
<td>1068 ± 132</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>14.00</td>
<td>1211 ± 87</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>16.00</td>
<td>968 ± 66</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>18.00</td>
<td>879 ± 118</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>20.00</td>
<td>792 ± 80</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>22.00</td>
<td>788 ± 14</td>
<td>65 ± 2</td>
</tr>
</tbody>
</table>

Fig. 1. Diurnal variations in the glycogen content of the liver and skin of the adult male mouse compared with diurnal variations in the epidermal mitosis rate.
back to back in pairs, one member of each pair holding sections taken from a mouse killed at 10.00 hr. and the other from a mouse killed at 14.00 hr. Comparisons between the depths of staining in such pairs confirmed that much more glycogen was present at 14.00 hr. than at 10.00 hr., and showed that at both times the greatest concentration of glycogen lay inside the epidermal cells.

(2) **Diurnal cycle in epidermal mitotic activity.** When each animal was killed a piece of ear was also removed, and from it the epidermal mitosis rate was determined. The results (see Table 1) show clearly that the diurnal variations in epidermal mitotic activity and in skin glycogen concentration ran parallel to each other. The times of high glycogen concentration were the times of high mitotic activity, and the times of low glycogen concentration were the times of low mitotic activity.

(3) **Experimental modifications of glycogen concentration.** Since glycogen concentration and epidermal mitotic activity seem normally to be linked together, it was decided to test whether this same connexion is evident in experimental conditions. To do this injections of starch or of insulin were given in the manner described by Bullough (1949a).

The first experiment involved the subcutaneous injection of 20 mg. starch in 0.4 ml. water into each animal, two groups each of six male mice being used. The injections were given at 08.30 hr., and one group was killed at 10.00 hr. and the other at 12.00 hr. The results are given in Table 2.

Table 2. *The effects of an injection of 20 mg. starch at 08.30 hr. on the skin glycogen concentration and the mitotic activity of the ear epidermis in groups each of six adult male mice*

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Skin glycogen concentration in mg. glucose per 100 g. fresh tissue</th>
<th>Numbers of mitoses per cm. length of sections cut 7 μ thick</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninjected</td>
<td>Injected starch</td>
</tr>
<tr>
<td>10.00</td>
<td>52 ± 4</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>12.00</td>
<td>68 ± 4</td>
<td>94 ± 4</td>
</tr>
</tbody>
</table>

Evidently the injection of starch induced an abnormal rise in the skin glycogen concentration, the final figure of 94 mg./100 g. of fresh tissue being considerably higher than anything seen during the normal diurnal cycle. This steep rise in the glycogen concentration was accompanied by an unusual increase in epidermal mitotic activity, although by 12.00 hr. this had not yet reached abnormal heights. A similar increase in glycogen concentration was also found in the liver.

The converse experiment was performed by means of two subcutaneous injections each of 1/6 unit insulin. The first injection was given at 12.30 hr., when the mice were beginning their afternoon rest, and the second at 13.30 hr. The animals were killed at 14.00 hr., and the results are shown in Table 3.

The treatment induced a sharp fall in the skin glycogen concentration, and this was accompanied by a deep mitosis depression.
Table 3. The effects of two injections, each of $\frac{1}{4}$ unit insulin, at 12.30 and 13.30 hr. on the skin glycogen concentration and the mitotic activity of the ear epidermis in groups each of six adult male mice

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Skin glycogen concentration in mg. glucose per 100 g. fresh tissue</th>
<th>Numbers of mitoses per cm. length of sections cut 7 $\mu$ thick</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninjected</td>
<td>Injected insulin</td>
</tr>
<tr>
<td>14.00</td>
<td>80 ±3</td>
<td>54 ±6</td>
</tr>
</tbody>
</table>

Thus in experimental, as in normal, conditions the skin glycogen content and the epidermal mitotic activity fluctuated in unison.

DISCUSSION

There have been many previous studies of diurnal variations in glycogen concentration, but these seem to have referred mainly to the liver. Several species have been studied and the results obtained have been widely divergent. In 1931, Ågren, Wilander & Jorpes described cyclic variations in the liver glycogen content of rabbits, rats and mice, and found that they were to a considerable extent independent of the intake of food. Their results showed that glycogen is deposited into the liver during the night and withdrawn in the morning. As regards the rabbit these results were confirmed by Förgren (1928, 1929), who studied the deposition of glycogen in relation to the secretion of bile. He found the greatest liver glycogen concentration during the night, and the highest rate of bile secretion during the day.

As regards the guinea-pig, rhythmic fluctuations in the liver glycogen content were described by Petrén (1939), there being a minimum concentration at 09.00 hr. and a maximum at 15.00 hr. This result was challenged by Marble, Grafflin & Rachel (1940) and by Grafflin (1940a, b), who concluded that there are, in fact, no significant differences in liver glycogen content at these times.

In mice, Higgins, Berkson & Flock (1932, 1933) concluded that the diurnal cycle of liver glycogen is probably influenced by the times of feeding and of digestion, and Deane (1942a, b, 1944) confirmed this after extensive experiments with both controlled and uncontrolled feeding. A diurnal cycle was also described by Ekman & Holmgren (1949), who found maximum values during the night and minimum values during the day.

It has now been shown that the skin glycogen concentration varies with that of the liver, and from the various data available some understanding of the glycogen rhythm may be obtained. In the first place it is evident that the deposition of glycogen into the tissues occurs after feeding, as it also does after a subcutaneous injection of starch. In the second, a comparison of the present results with those described earlier by Bullough (1948) indicates that glycogen deposition occurs at the onset of a period of rest or sleep. Studies of the daily cycles of bodily activity in mice accustomed to being fed regularly between 09.00 and 10.00 hr. indicate that they have two periods of wakefulness, in the morning and evening, and two periods of rest or sleep, in the afternoon and early morning. Later results (Bullough, 1949a) showed
that during the periods of wakefulness the blood sugar level is high, while during rest or sleep it falls. Co-ordinating these various results it can now be concluded that at the onset of sleep glucose is deposited from the blood to form glycogen, while on waking the reverse process occurs. These relations are illustrated in Fig. 2.

![Fig. 2. The diurnal variations in the bodily activity (Bullough, 1948) and in the blood sugar concentration (Bullough, 1949a) compared with those in the skin glycogen concentration and in the epidermal mitosis rate.](image)

However, it is important to stress that the timing of cycles illustrated in Fig. 2 refers only to adult male mice of between 3 and 12 months of age, which are accustomed to being fed between 09.00 and 10.00 hr. (Bullough, 1949b). With other age groups, with the other sex, with other species, and with other times of feeding, different forms of graphs may be expected. It is most probably the lack of standardization in these respects that is responsible for the divergent results previously reported.

The conclusions expressed graphically in Fig. 2 also lend support to the suggestion that the glucose deposited from the blood during sleep may be the critical factor causing increased mitotic activity at this time. In both normal and abnormal conditions a high concentration of glycogen in the skin coincides with a high rate of epidermal mitotic activity, while a low glycogen concentration coincides with low mitotic activity. Histologically it has also been shown that a mitotically active epidermis contains within its cells relatively large quantities of glycogen, while a mitotically inert epidermis does not. It is interesting to add, however, that it is now known that any quick depletion of tissue glycogen does not stop, or even delay, the completion of those mitoses which are at that time in progress (Bullough, 1950a). Apparently it is only the transition from the interphase to the prophase that is sensitive to the presence or absence of glucose or glycogen, and once a division is under way it becomes entirely insensitive.
Diurnal variations in tissue glycogen content in male mouse

SUMMARY

1. A description is given of the hour-to-hour variation in the liver glycogen content in adult male mice, and it is shown that the concentration is highest while the animals are asleep and lowest while they are awake.

2. A similar cycle is also described in the glycogen content of the skin. Histologically it is shown that a high proportion of the skin glycogen lies in the cytoplasm of the epidermal cells, and that during sleep both the epidermal glycogen content and the epidermal mitotic rate increase considerably. The skin glycogen content and the epidermal mitotic activity also show a marked increase after a subcutaneous injection of 20 mg. starch, while they are both abnormally depressed after two injections of \( \frac{1}{10} \) unit insulin.

3. These results, together with others previously reported, are in agreement with the theory that at the onset of sleep glucose is deposited from the blood into the tissues where it appears in the form of glycogen. Since it is known that glucose, or glycogen, is a critical substance affecting mitotic activity in the adult mouse, it is logical to find that an increase in the epidermal glycogen content is accompanied by a greatly increased mitosis rate. On waking, the reverse process takes place, glycogen being withdrawn as glucose into the blood and mitotic activity falling to a low level.

REFERENCES