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# THE EFFECT OF DIET ON GLYCOGEN FORMATION IN RAT LIVER

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There is some evidence (e.g. Long, 1953) that the composition of an animal's diet may affect the ability of its tissues to metabolize various substrates. We have carried out experiments to test the effect of varying amounts of dietary carbohydrate, fat and protein on some aspects of carbohydrate metabolism. In this paper we report differences in glycogen formation and utilization in rat liver, both *in vivo* and *in vitro*, and in the following communication some effects on glucose and acetate metabolism in isolated rat diaphragm.

## METHODS

### *Diet and animal regime*

Rats, mostly albino but including some black and white, bred in this Department, were weaned at approximately 4 weeks (a few litters at 3 or 5 weeks) and fed immediately on one of the experimental diets, rats from each litter being divided as evenly as possible among the different diets. Consumption of the experimental diets was started at weaning to allow full opportunity for any changes in tissue composition to develop.

In the first series of experiments the diets were based on those used by Lawrie & Yudkin (1949), and were intended to include 60–70 % of carbohydrate, fat or protein respectively, without entirely excluding fat or carbohydrate from any diet. In contrast to the findings of Lawrie & Yudkin the rats on the high fat and high protein diets did not grow as well as did those on the high carbohydrate diet, so after 10 days the amounts of fat and of casein in the high fat and high protein diets respectively were reduced from 60 to 50 g per 100 g diet. The rats on the high fat diet still grew less well than those on the other two diets, and the fat content of this diet was again reduced by 10 g per 100 g of diet. This meant that the carbohydrate content of the high fat and high protein diets were not identical, so in one series of experiments a group of rats was fed a medium protein diet (Q) of carbohydrate content equal to that of the high fat diet (F). Table 1 shows the composition of the diets finally used. The composition of the high carbohydrate diet (C) was close to that of the stock pellet diet used in this Laboratory; in some later experiments rats were fed the stock diet from weaning until transfer to individual cages with a restricted feeding period (see below) when diet C was substituted for the pellet diet.

Animals in subsequent series of experiments were fed diets of the composition shown in Table 1 from weaning; those on diets C and P grew at approximately the same rate (Fig. 1), but those on diet F (high fat), especially the male rats, grew more slowly. This was partly due to a poor growth rate during the first few weeks after weaning (even when fed the diet with the reduced fat content); later, when the rats had become accustomed to the diet, their growth, as well as their general con-

TABLE 1. Composition of diets (g/100 g)

| | Stock rat cake | High carbohydrate (C) | High fat (F) | Medium carbohydrate and protein (Q) | High protein (P) |
|---|---|---|---|---|---|
| Sucrose | — | 65 | 30 | 30 | 20 |
| Casein* | — | 15 | 15 | 50 | 60 |
| Animal fat† | — | 5 | 40 | 5 | 5 |
| Yeast‡ | — | 10 | 10 | 10 | 10 |
| Salt mixture§ | — | 5 | 5 | 5 | 5 |
| | Analyses of typical batches of diet | | | | |
| Carbohydrate‖ | 64·7 | 73·2 | 38·6 | 34·6 | 28·8 |
| Protein | 15·7 | 14·5 | 13·4 | 48·9 | 54·0 |
| Fat | 4·6 | 5·2 | 40·0 | 5·2 | 5·3 |
| Ash | 2·5 | 3·9 | 4·0 | 3·8 | 4·5 |
| Moisture | 12·5 | 3·2 | 4·0 | 7·5 | 7·4 |

In addition each rat received weekly 1–2 drops Radiostoleum (B.D.H.) diluted 10 times with olive oil.

* Glaxo, lactic casein, unextracted. N = 13·5 %.

† Carcass fat rendered down from condemned beef.

‡ Dried yeast, Pharmacochemical Co. Ltd.

§ B.D.H. salt mixture no. 2 (U.S.P. xiv and xii).

‖ Calculated by difference.

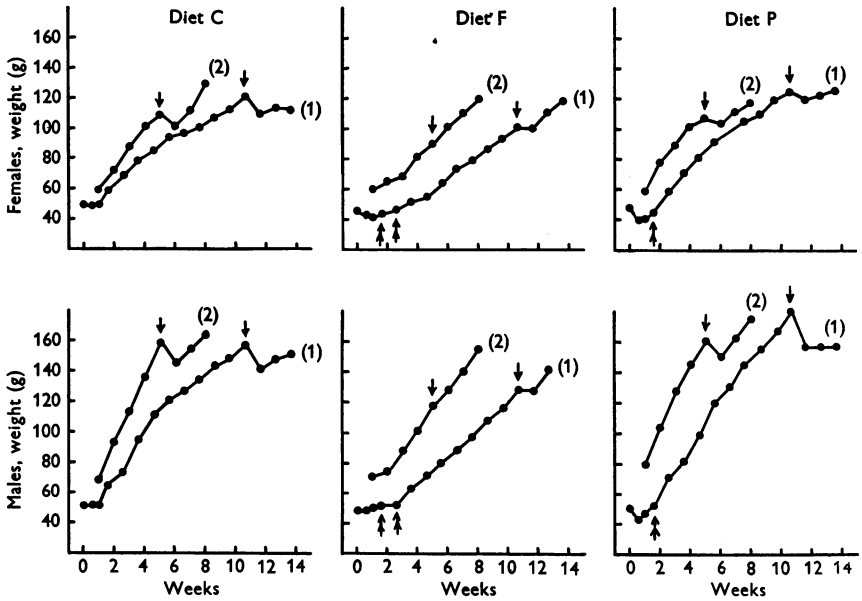


Fig. 1. Growth of rats on diets C, F and P (see Table 1); series 1, litters A–D, series 2 litters F–I. At ↟ the amount of fat or of protein in the diet was reduced (see text). At ↓ restricted feeding period started (series 1, lower curves, 2 hr; series 2, upper curves, 4 hr).

dition, improved. After these experiments had been completed Long (1953) reported the results of experiments in which he had used Lawrie & Yudkin's diets and he also observed that rats on the high fat diet did not grow as well as did those on the high protein or high carbohydrate diets. It is possible that rats on this high caloric diet did not eat a sufficient quantity to obtain an adequate amount of protein.

For 6–10 weeks after weaning small groups of rats (4–6) were kept together in a cage with food always present. Then the rats were moved to individual cages and an excess of food was presented for a limited period only each day, so that the rats could be killed at a known time after consumption of food. In the first series of experiments food was left in the cages for 2 hr only each morning. The rats lost weight for a few days on being transferred to this regime, and although later they maintained their weight, they did not regain their former rate of growth, except for those rats (both sexes) on diet F (Fig. 1, series 1). The feeding period for all animals was therefore increased to 4 hr each day; on this regime the rats on diets C and P still lost weight at first but soon resumed growth at approximately the original rate. Rats on diet F (both sexes) continued to grow without interruption (Fig. 1, series 2). It was not appreciated until after these experiments had been completed that such a restricted feeding regime would influence the manner in which the animals disposed of ingested food (Tepperman, Brobeck & Long, 1943). Therefore the findings reported here, whilst valid for the conditions of these experiments, do not necessarily apply to animals fed similar diets but with food available continuously.

In the majority of experiments the rats were fed at 9.30 each morning and killed the same afternoon for investigation of 'fed' animals and the next or the following morning when 24 or 48 hr fasted rats were required. (The time after removal of food of the '24 hr fasted' rats varied from 22 to 29 hr, but this difference did not account for the variations observed in the liver glycogen content in these groups.) In one series of experiments, the rats were fed at 6 a.m. and killed later the same or the following morning; but this change in feeding time did not affect the results.

### *Preparation of liver slices*

Rat livers, partially drained of blood by bleeding from the aorta, were chilled in ice-cold saline (0·9% NaCl). (When the diaphragm was also used it was dissected out before removal of the liver.) A number of slices weighing about 70 mg were cut (with the aid of a Stadie & Riggs (1944) slicer) from one or more lobes of the liver and placed on a chilled plate in a moist chamber. The slices were weighed and then either dropped directly into centrifuge tubes containing 0·3 ml. of 30% (w/v) KOH for estimation of the glycogen content or placed in Warburg flasks containing 1 ml. of the appropriate incubation medium (see below) and standing in a bath of crushed ice.

### *Incubation media*

The incubation medium used when glucose or pyruvate was to be the substrate for glycogen synthesis was that described by Teng, Simex & Hastings (1950). In most experiments glucose was added to give a final concentration of 1% (w/v; approximately 0·05 M), but in a few the concentration was increased to 3% (w/v). Commercial 'pure' pyruvic acid, containing approximately 75% pyruvic acid, was used without further purification and was added in place of HCl, as recommended by Buchanan, Hastings & Nesbett (1949), to give a final concentration of approximately 0·05 M. A considerable synthesis of glycogen in liver slices from glucose-1-phosphate can be observed if fluoride is present in the incubation medium to inhibit the activity of phosphoglucomutase. Preliminary experiments showed that synthesis of glycogen from this substrate is not so dependent on the ionic composition of the medium as is that from glucose. The final composition of the medium used was 0·1 M-NaF, 0·1 M-NaCl, 0·02 M-$KHCO_3$, and 2% (w/v) potassium glucose-1-phosphate (approximately 0·05 M), prepared by a modification* of Hanes's (1940) method. Warburg flasks containing these media were gassed with oxygen containing 5% $CO_2$ for 5 min at room temperature. For measurement of glycogen breakdown in liver slices from fed rats the

* Private communication to Dr E. M. Crook.

incubation medium was made up of one part of 0·1 M phosphate buffer, pH 7·4, diluted with 9 parts of 0·9 % (w/v) NaCl. The flasks in these experiments were not gassed, but were shaken in air. In all experiments the flasks were shaken in a bath at 37° C for the required time; the slices were then dropped into centrifuge tubes containing 0·3 ml. of 30 % (w/v) KOH for determination of glycogen, and when necessary the glucose content of the medium was determined on an aliquot after deproteinization with barium hydroxide and zinc sulphate.

*Estimation of glycogen*

Glycogen was estimated by the Good, Kramer & Somogyi (1933) method, except that it was not found necessary to dissolve and re-precipitate the glycogen. Glucose was determined after acid hydrolysis by Somogyi's modification (1945) of Nelson's (1944) method; values are reported as glucose found without conversion to glycogen. Cori & Cori (1940) point out that glucose-1-phosphate is not destroyed by alkaline hydrolysis and that the potassium salt is sufficiently insoluble for it to be precipitated along with the glycogen when alcohol is added. They recommend the use of NaOH for hydrolysis of tissue for estimation of glycogen when glucose-1-phosphate may be present. We did not find this procedure satisfactory; although the sodium salt of glucose-1-phosphate is more soluble than the potassium salt, sufficient is still precipated by alcohol to cause interference in glycogen estimation if much glucose-1-phosphate is present; also we found that glycogen precipitates do not adhere as well to the centrifuge tube when precipitated from sodium instead of from potassium hydroxide. Experience showed that in the experiments described here, in which glycogen was estimated only in slices, interference from glucose-1-phosphate was negligible and it has been disregarded.

Sometimes glycogen was estimated in slices from more than one lobe of a rat's liver. Whilst close agreement was observed in determinations on slices from the same lobe, considerable variation was found in the glycogen content of different lobes of liver from both fed and fasted rats, whatever their previous diet; the mean inter-lobe difference was 17 %, but differences as high as 90 % were recorded. Comparison of glycogen content before and after incubation was always made on slices (often in duplicate) cut from the same lobe; when more than one lobe was used each set of slices was regarded as a separate experiment. When *in vivo* differences in liver glycogen values were to be compared, if estimations had been made on more than one lobe the mean value was recorded.

In a number of experiments liver glycogen was also determined by a modification of the trichloroacetic acid extraction method described by Bloom, Lewis, Schumpert & Shen (1951). A piece of liver weighing from 0·5 to 1·0 g was cut from the same lobe as the slices to be used for estimation of glycogen by the alkali extraction method. It was frozen on solid $CO_2$, weighed and ground in a chilled mortar with sand and 10 ml. of cold 10 % trichloroacetic acid, the extract being separated by centrifugation. 1·0 ml. aliquots were treated with 1·2 ml. ethanol and allowed to stand overnight in the refrigerator. Glycogen was separated by centrifugation, hydrolysed in 1·0 N-HCl and the glucose estimated as before by Somogyi's (1945) method.

## RESULTS

### *Glycogen content of rat liver* in vivo

*Effect of diet, sex and time after feeding.* Wide variations in glycogen content were observed in all the groups examined (Table 2*a*). The values in most groups when plotted in a dot diagram showed a skew distribution, making comparison difficult. However, when logarithms of the observed values were plotted the distribution was more symmetrical; therefore, for statistical analysis of these results, $\log_{10}$ of ten times the observed values have been used for comparison (multiplication by 10 avoided negative logarithms). An analysis

TABLE 2*a*. The effect of time of fast, diet and sex on the glycogen content of rat liver (mean ±S.D. about the mean).

| Sex | Diet C | | Diet F | | Diet P | | Diet Q | |
|---|---|---|---|---|---|---|---|---|
| | No. of rats | Glycogen (mg/g wet wt.) | No. of rats | Glycogen (mg/g wet wt.) | No. of rats | Glycogen (mg/g wet wt.) | No. of rats | Glycogen (mg/g wet wt.) |
| | | | | Time of fast 0 hr | | | | |
| Male | 8 | 57·2±18·6 | 10 | 35·3±9·7 | 8 | 29·3±15·3 | 4 | 63·9±7·1 |
| Female | 10 | 52·3±18·3 | 9 | 33·9±9·2 | 7 | 22·0±10·8 | 4 | 37·7±2·7 |
| | | | | Time of fast 22–29 hr | | | | |
| Male | 47 | 2·80±1·63 | 10 | 8·13±5·70 | 11 | 8·87±11·1 | — | — |
| Female | 9 | 1·23±1·42 | 6 | 2·97±2·97 | 7 | 11·27±12·5 | — | — |
| | | | | Time of fast 36 hr | | | | |
| Not recorded | — | — | 6 | 2·79±1·61 | 6 | 6·73±2·80 | — | — |
| | | | | Time of fast 48 hr | | | | |
| Male | 3 | 3·66±1·01 | 4 | 0·70±0·25 | 4 | 1·81±0·40 | — | — |
| Female | 3 | 2·87±2·01 | 3 | 1·85±1·01 | 3 | 0·83±0·22 | — | — |

TABLE 2*b*. Analysis of variance of data from Table 2*a* (omitting rats fed diet Q or fasted 36 hr).

*Note.* $1+\log_{10}$ observed values used for analysis (see text). Significant values marked $*P<0{\cdot}05$, $**P<0{\cdot}01$.

| Source | Sum of squares | Degrees of freedom | Mean square |
|---|---|---|---|
| | 1st analysis | | |
| Between diets (*D*) | 0·0468 | 2 | 0·0234 |
| Between times (*T*) | 6·4354 | 2 | 3·2177** |
| Between sexes (*S*) | 0·0977 | 1 | 0·0977* |
| $D \times T$ | 0·6507 | 4 | 0·1627** |
| $T \times S$ | 0·0508 | 2 | 0·0254 |
| $D \times S$ | 0·0097 | 2 | 0·0048 |
| $D \times T \times S$ | 0·3283 | 4 | 0·0821* |
| | 7·6194 | 17 | — |
| Corrected residual | | 148 | 0·0250 |
| | 2nd analysis | | |
| Between diets at | | | |
| $T=0$ hr | 0·1293 | 2 | 0·0647 |
| $T=24$ hr | 0·3409 | 2 | 0·1704** |
| $T=48$ hr | 0·2271 | 2 | 0·1136* |
| Between diets within times $=D \times D \times T$ | 0·6973 | 6 | — |

of variance (omitting results for rats fed diet Q or fasted 36 hr) is shown in Table 2*b*. This analysis can only be approximate owing to the variation in the number of rats in each group, but a further calculation using a small random sample of the large number of 24 hr fasted male rats gave similar results and the original analysis can be considered valid.

The significant difference ($P<0{\cdot}01$) in liver glycogen content at different times after feeding is well known. A number of previous workers (e.g. Deuel, Butts, Hallman, Murray & Blunden, 1937) have observed an effect of sex and our results, taken altogether, show a significant difference ($P<0{\cdot}05$), the males usually, but not always, having a larger amount of glycogen in their livers.

The effect of diet over all times is not significant, but in fasted rats there is a difference in liver glycogen content between livers from rats fed different diets. In 24 hr fasted rats the level was significantly higher ($P < 0{\cdot}01$) in livers of rats fed diets F and P than in those fed diet C. In contrast, 48 hr after feeding, the liver glycogen level in rats fed diet C had risen above that for 24 hr fasted rats and was now higher than that of rats fed either of the other diets ($P < 0{\cdot}05$) whose liver glycogen had continued to fall during the second day of fasting.

In spite of the large differences obvious in Table 2, the mean values for liver glycogen content in fed rats on diets C, F and P are not significantly different. That this is due to some unidentified factor or factors which mask the effect of diet can be shown by expressing the results in a different manner. Since it was recognized that unsuspected factors might interfere with effects due to differences of diet, as far as possible one rat from each dietary group was killed each day. If the liver glycogen content of fed rats receiving diets F and P was compared with that of the rat fed diet C killed on the same day, it was found that those rats fed diet C always had a larger amount of glycogen in their livers than did those fed diet P and usually a larger amount than those fed diet F. If C, F and P represent the liver glycogen content of rats (both male and female) fed the three diets, then $\log_{10} C/P = 0{\cdot}446 \pm 0{\cdot}177$, $\log_{10} C/F = 0{\cdot}181 \pm 0{\cdot}113$ and $\log_{10} F/P = 0{\cdot}264 \pm 0{\cdot}316$, all of which are significantly greater than 0 ($P < 0{\cdot}01$), so that on any one day rats fed diet C had more glycogen in their livers than those fed diet F, which in turn had more than those fed diet P. The nature of the factors which caused such a large daily variation in the liver glycogen content are unknown. Season may have had a small effect (Fig. 2), and it may be noted that the temperature of the room in which the rats were housed was poorly controlled. Records of daily food intake were kept for a number of animals; these showed considerable variation from day to day and this may have been the direct cause of variation in liver glycogen content, but the cause of this daily variation in food intake must still be sought.

The values for the small number of animals fed a medium-high protein diet (Q) suggest that a fall in carbohydrate content affects the female rats before the males and, comparing male rats on diets Q and F (which had the same carbohydrate content), that addition of fat has an effect in addition to that of lowering of the carbohydrate content of the diet.

*Proportion of liver glycogen extracted by trichloroacetic acid.* The proportion of the total glycogen (i.e. that extracted by hot alkali) which could be extracted by 10% trichloroacetic acid varied significantly ($P < 0{\cdot}05$) in livers of fed rats receiving different diets (Table 3), being highest in livers of rats fed diet F and lowest in those of rats fed diet P. During a 24 hr fast, whatever the rats' previous diet, the glycogen which could be extracted by trichloroacetic acid

disappeared from the livers more rapidly than the non-extractable fraction (Fig. 3), but this difference was not apparent until the total glycogen content of the livers had fallen to a low level (less than 8·0 mg/g). This observation, that rat liver contains a small amount of glycogen which cannot be extracted

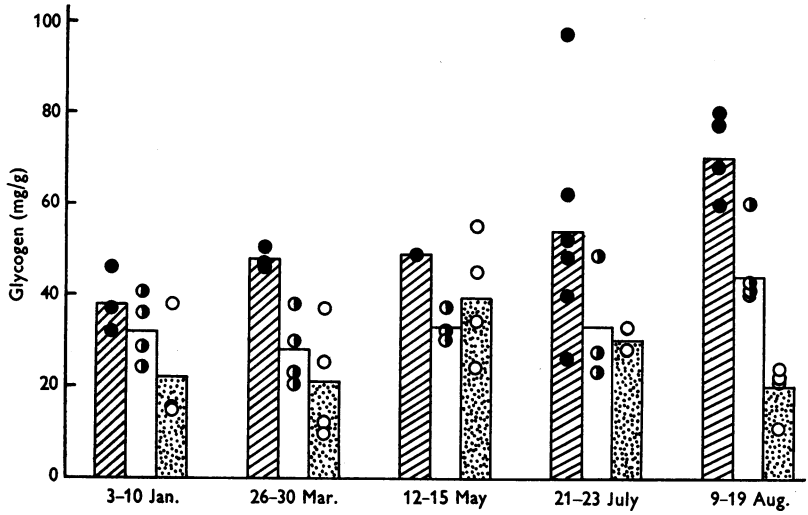


Fig. 2. Effect of season on liver glycogen (mg/g) in rats fed diets C (●), F (◑) and P (○).

TABLE 3. The effect of diet on liver glycogen in fed rats.

Comparison of the amount of glycogen extracted by (*a*) hot alkali (=total glycogen) and (*b*) 10% cold trichloroacetic acid (mean values ± standard deviation about the mean).

| Diet ... | C | F | P |
|---|---|---|---|
| No. of rats ... | 7 | 8 | 7 |
| Glycogen mg/g wet wt. | | | |
| (*a*) | 56·2±19·3 | 38·3±9·0 | 20·7±9·0 |
| (*b*) | 50·5±17·2 | 36·9±7·9 | 15·8±7·8 |
| * Percentage of total glycogen extracted by trichloroacetic acid | 91·2±1·05 | 97·7±1·05 | 74·0±1·25 |

* Calculated from mean $\log_{10} 100\ b/a$.

by trichloroacetic acid, agrees with the findings of Bloom *et al.* (1951). On the other hand, Kleij (1951) found no difference in the amount of glycogen extracted from liver by trichloroacetic acid or by hot alkali, in either fed or fasted rats.

*Changes in glycogen content of liver slices during incubation*

We found that changes in glycogen content of liver slices during incubation *in vitro* depended on the glycogen content of the slices at the beginning of the incubation period, so that it was essential to take this value into account when comparing the effects of different conditions, e.g. the previous diet of the rat,

or the substrate present during the incubation period. The same considerations which led to the use of logarithms of ten times the observed values when comparing *in vivo* liver glycogen levels (which were the initial values for a period of incubation) made it reasonable to compare logarithms of glycogen values

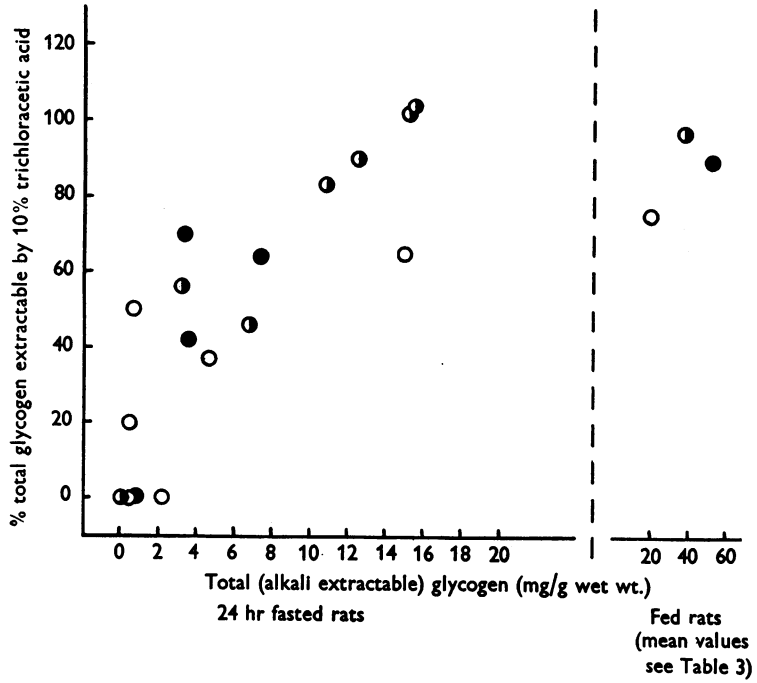


Fig. 3. Effect of diet on proportion of total liver glycogen extractable by 10% trichloroacetic acid in rats fed diets C (●), F (◑) and P (○). Comparison of results for fed and for 24 hr fasted rats.

at the beginning ($I$) and at the end ($E$) of the incubation period. In the following sections the values for $\log_{10} E/I$ (in Fig. 9, $\log_{10} 10E$) have been plotted against the values for $\log_{10} 10I$).

*Synthesis of glycogen from glucose-1-phosphate.* As fluoride does not prevent the synthesis of glycogen from this substrate it could be used here to inhibit glycogen breakdown, although even a high concentration (0·1 M) did not completely stop glycogenolysis. A considerable increase was observed in the glycogen content of slices (up to 4·0 mg/g in 60 min incubation) with a low initial glycogen level, but when the initial level was high (above 10 mg/g) there was little increase, and in some cases glycogenolysis exceeded synthesis of glycogen in spite of the presence of fluoride. The results for a number of experiments are shown in Fig. 4. The slopes of the lines relating $\log_{10} 10I$ and $\log_{10} E/I$ for slices from rats fed different diets and fasted 24 or 48 hr are not

significantly different from one another, but the overall slope of the line calculated for all slices is significantly different from zero. For comparison with the results for synthesis of glycogen from glucose shown in Fig. 6, where only values for slices from 24 hr fasted rats have been included, the line drawn in Fig. 4 is calculated for slices from 24 hr fasted rats only. It will be seen that the values for slices from rats fed diet C tend to be somewhat higher than those from rats fed diet F.

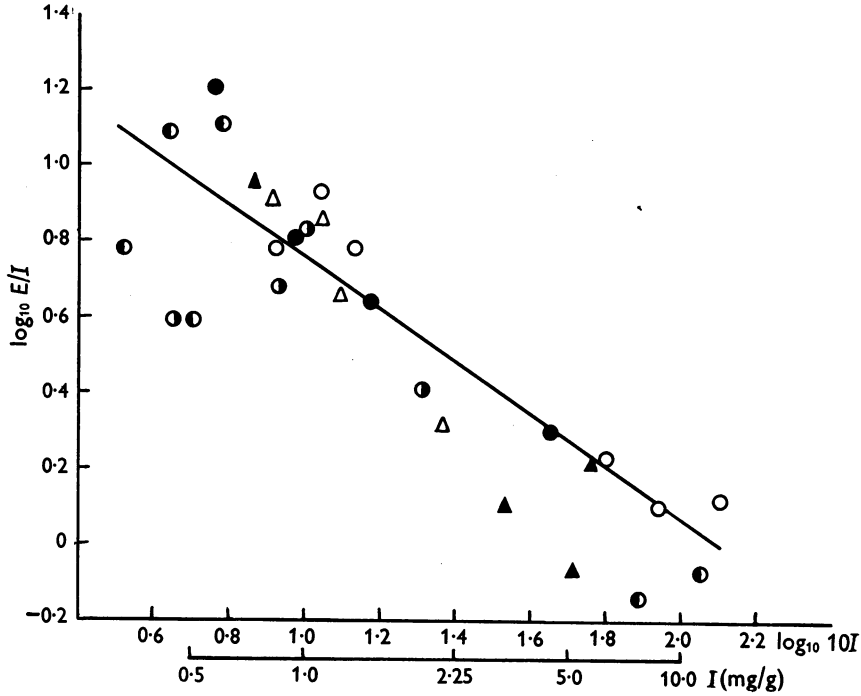


Fig. 4. The effect of diet, time of fasting and initial glycogen content ($I$) on the change in glycogen content of rat liver slices incubated 60 min at 37° C in a medium containing 2% potassium glucose-1-phosphate. ($E$ = glycogen content after incubation.) 24 hr fasted rats, diet C, ●, diet F, ◐, diet P, ○: 48 hr fasted rats, diet C, ▲, diet F, ◑, diet P, △. Calculated equation, for slices from 24 hr fasted rats only (all diets): $\log_{10} E/I = 1{\cdot}46 - 0{\cdot}70\ (\log_{10} 10I)$.

*Synthesis of glycogen from glucose.* Preliminary experiments confirmed the observation of Buchanan *et al.* (1949) that glycogen synthesis from glucose is favoured by a high concentration of potassium in the incubation medium. In the first series of experiments after incubation for 1 hr an increase in the glycogen content was observed only in slices with a low initial glycogen level (1–2 mg/g wet weight). Few of the slices from rats fed diets F or P (fasted for 24 or 48 hr), or from 48 hr fasted carbohydrate-fed rats, had a sufficiently low initial glycogen level to allow an increase in glycogen content during incubation, and no conclusion could be drawn as to a possible effect of diet.

It was thought possible that any such effect might be better observed by following the change in glycogen content of the liver slices at various times during the incubation period. An experiment was carried out with twelve rats fasted 24 hr, four from each of the groups fed diets C, F or P. Five slices were cut from each of two lobes of the livers and glycogen determined in one slice from each set after 0, 30, 60, 90 and 120 min incubation. (The glucose content

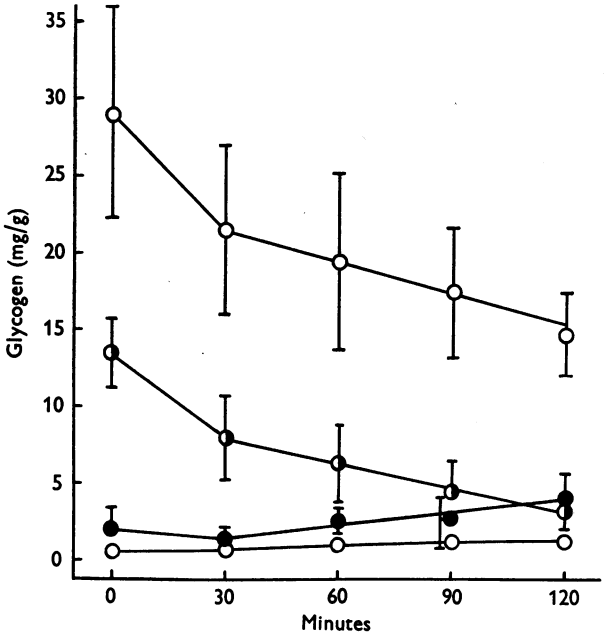


Fig. 5. Effect of previous diet on the change in glycogen content of liver slices from 24 hr fasted rats during incubation in a medium containing 3% glucose. Mean values and standard deviation: diet C, ● (7 expts.), diet F, ◑ (8 expts.) diet P, ○ (8 expts., 4 in each group).

of the incubation medium was increased to 3% for this experiment.) The results are shown in Fig. 5. In half the slices from the protein-fed rats the initial glycogen level was very low and there was a small increase during the first hour of incubation, the level thereafter remaining nearly constant. In two of the slices from carbohydrate-fed rats there was an increase in glycogen content during the first 30 min of incubation, but in all other slices breakdown of glycogen exceeded synthesis during this time. All but one of the slices from carbohydrate-fed rats showed an increase in glycogen content during the rest of the incubation period. In all the slices from the fat-fed rats, and in the remainder of those from the protein-fed rats, the glycogen content fell throughout incubation, the rate being fastest during the first 30 min period and then remaining nearly constant.

This difference in behaviour of the liver slices might have been due only to the difference in the initial glycogen content; therefore a further comparison of the effect of diet on the synthesis of glycogen from glucose was made by plotting the changes in glycogen content of all liver slices from 24 hr fasted rats which had been incubated in a glucose-containing medium for 60 min

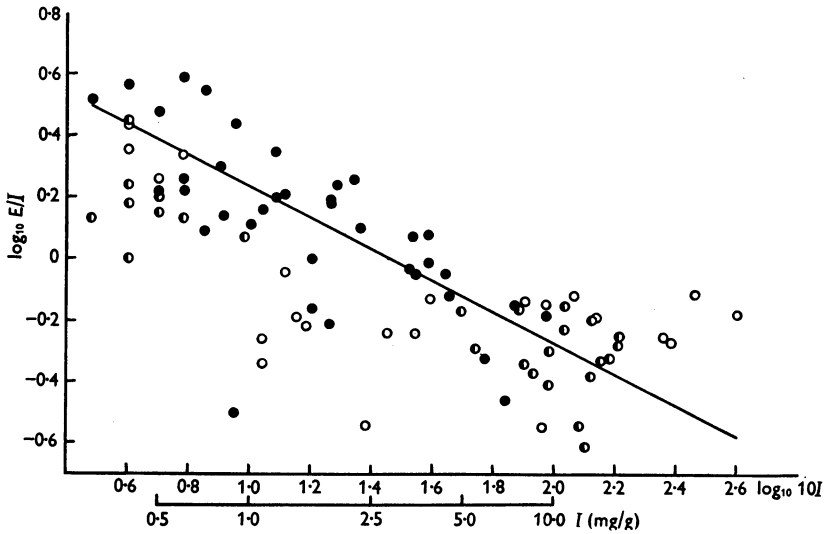


Fig. 6. Effect of diet and initial glycogen content ($I$) on the glycogen content of liver slices from 24 hr fasted rats incubated 60 min at 37° C in a glucose-containing medium (1% or 3%). ($E$ = glycogen content after incubation.) Diet C, ●, diet F, ◐, diet P, ○. Calculated equation for slices from rats fed diet C: $\log_{10} E/I = 0{\cdot}71 - 0{\cdot}51\ (\log_{10} 10I)$.

(both those from experiments already described and from a number of others, Fig. 6). Calculation showed that the points representing slices from rats fed diet C clustered about a line such that $\log_{10} E/I = 0{\cdot}714 - 0{\cdot}515\ (\log_{10} 10I)$ and that at an initial glycogen content of 2·8 mg/g no change in glycogen content would be expected. The slices from rats fed diet F had either a high or very low initial glycogen content and there was insufficient evidence to determine whether or not the points lay on a straight line, and so to calculate the glycogen level at which no change would have been expected during incubation, but in those slices in which an increase in glycogen content was observed, the increase was less than that to be expected in slices from carbohydrate-fed rats with the same initial glycogen content. In slices from rats fed diet P changes in glycogen content during incubation were irregular and did not appear to be related to the initial glycogen content in the same manner as in slices from carbohydrate-fed rats. An increase in glycogen content was observed in only five slices (diet P), in all of which the initial level was less than 1·0 mg/g; again the increases were less than would have been expected from slices with a similar

glycogen content from rats fed diet C. The results from rats fasted 48 hr were not included in the calculations but they showed the same general effect; possibly somewhat less glycogen was synthesized than would have been expected from slices from 24 hr fasted rats with a similar initial glycogen content.

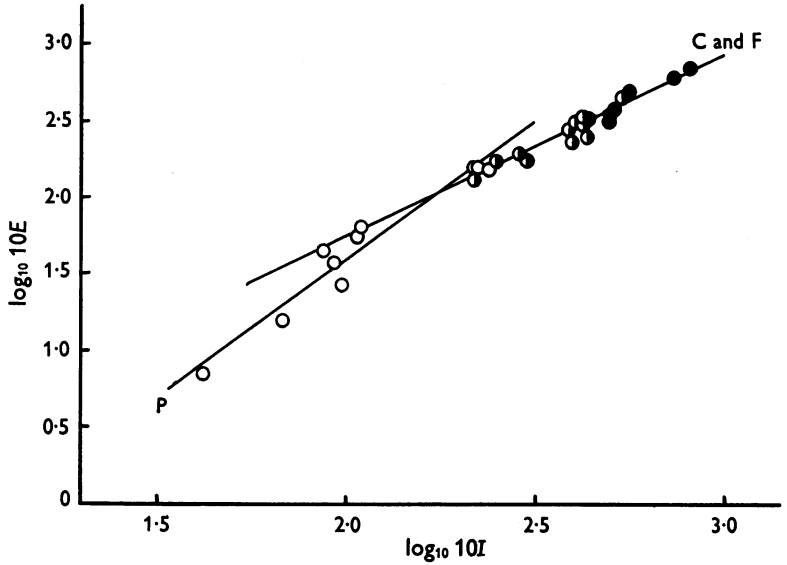


Fig. 7. Effect of diet and of initial glycogen content ($I$) on final glycogen content ($E$) of liver slices from fed rats incubated 30 min at 37° C in a phosphate buffer. Diet C, ●, diet F, ◑ and diet P, ○. Calculated equation for slices from rats fed diets C and F: $\log_{10} 10E = 1{\cdot}20\ (\log_{10} 10I) - 0{\cdot}66$; for slices from rats fed diet P, $\log_{10} 10E = 1{\cdot}82\ (\log_{10} 10I) - 2{\cdot}04$.

*Synthesis of glycogen from pyruvate.* A similar relationship was observed between the initial and final glycogen content of liver slices incubated in a pyruvate-containing medium. The initial level below which an increase in glycogen content was observed (about 0·8 mg/g) was less than that for slices from rats fed diet C incubated in a glucose-containing medium, and even in those slices in which an increase in glycogen content was observed the changes were very small. There was no indication of an effect of diet or of time of fasting.

*Breakdown of glycogen in slices from fed rats.* There was no difference between the results obtained after an incubation period of 30 min for slices from rats fed diet C and those from rats fed diet F (Fig. 7). In slices from protein-fed rats comparatively more glycogen was broken down during incubation. This may have been due to the low initial glycogen content of the slices or directly to the high protein content of the diet.

The amount of glucose found after 30 min incubation was approximately

constant and therefore was not directly related to the amount of glycogen broken down, which depended on the initial content. In slices from rats fed diets C and F, 8·6 ± 0·80 mg/g glucose was found after incubation; this was less than the glycogen broken down when the initial level was high. In contrast, with slices from rats fed diet P, although less glucose was found, 6·4 ± 0·7 mg/g in 30 min, it was greater in amount than the glycogen broken down. Again, it cannot be decided whether this difference in behaviour was due directly to the high protein diet or to the small amount of glycogen present at the beginning of incubation. The failure of glucose formation to increase when the amount of glycogen present is large would be expected if the maximum rate of glucose formation were less than that for glycogen breakdown, but this explanation does not fit in with Sutherland & Cori's (1951) observation that during incubation of liver slices, phosphorolysis is the rate-limiting step in conversion of glycogen to glucose.

## DISCUSSION

A large number of factors is known to affect the level of glycogen in liver (see Swensson, 1945, for a full discussion) and the variability which we, like other workers (e.g. Mirsky, Rosenbaum, Stein & Wertheimer, 1938, but contrast Cori & Cori, 1929), have observed in rats treated so far as possible in the same manner, is a reflexion of our inability to control some at least of these unknown factors, and this complicates the interpretation of effects due to diet.

Previous workers have shown that a high proportion of protein (Mirsky *et al.* 1938) or of fat (Stein, Tuerkischer & Wertheimer, 1939) in the diet depresses the level of glycogen in the livers of fed rats and prevents glycogen breakdown during fasting. Greisheimer & Johnson (1930) have also reported similar findings in fed rats, but MacKay & Bergman (1933) who used rats fasted for 24 hr found the lowest level of glycogen in the livers of rats previously fed protein. Our results for fed rats and for those fasted 24 hr are in general agreement with those of Mirsky *et al.* (1938) and Stein *et al.* (1939) (Table 4), but we did not find that the stabilizing effect on liver glycogen of diets with a high protein or fat content lasted for as long as 48 hr.

Mirsky *et al.* (1938) suggested that this high fasting level was due to gluconeogenesis rather than to lack of utilization of liver glycogen and the maintenance of this high level in their rats (particularly in those on the high protein diet) during a 48 hr fast lent support to this view. In our experiments conditions were different, as the rats were accustomed to long periods (20 hr) without food. Some calculations of the amount of glycogen which our rats stored in their livers and utilized during their 20 hr fasting period (Table 5) lead to the following general considerations (which the very approximate nature of the data does not invalidate). First, 2–3 hr after feeding, liver glycogen accounted for very little of the dietary carbohydrate whatever diet the rats were

TABLE 4. Effect of diet on the glycogen content (mg/g wet wt.) of rat liver (males): Comparison of mean values, (*a*) this paper, Table 2, (*b*) Mirsky *et al.* (1938) and (*c*) Stein *et al.* (1939).

| | High carbohydrate (65–70 %) | High fat (40 % fat or 50 % butter + margarine) | High fat (68 % butter + margarine) | High protein (60–70 % casein) |
|---|---|---|---|---|
| | | Fed rats | | |
| *a* | 57·2 | 35·3 | — | 29·3 |
| *b* | 41·0 | — | — | 13·8 |
| *c* | — | 19·8 | 10·8 | — |
| | | Fasted 24 hr | | |
| *a* | 2·8 | 8·1 | — | 8·9 |
| *b* | 0·7 | — | — | 14·2 |
| *c* | — | 1·4 | 3·6 | — |
| | | Fasted 48 hr | | |
| *a* | 3·7 | 0·7 | — | 1·8 |
| *b* | 3·8 | — | — | 14·2 |
| *c* | — | 2·7 | 4·5 | — |

TABLE 5. Calculation of food intake and carbohydrate storage of male rats. Food intake, mean for five rats on each diet over a 10-day period. Remaining data calculated from values shown in Tables 1 and 2, assuming livers weighed 7 g (mean for seven fed rats).

| | Diet C | Diet F | Diet P |
|---|---|---|---|
| Food intake (g) | 9 | 8 | 9 |
| Carbohydrate intake (g) | 6·5 | 3 | 3 |
| Glycogen content of liver | | | |
| (i) 2–3 hr after removal of food: | | | |
| (*a*) g | 0·40 | 0·25 | 0·20 |
| (*b*) as percentage of carbohydrate intake | 6·0 | 8·0 | 7·0 |
| (ii) 24 hr after removal of food (g) | 0·02 | 0·08 | 0·06 |
| Liver glycogen used in 21 hr | | | |
| (*a*) g | 0·38 | 0·17 | 0·14 |
| (*b*) as percentage of carbohydrate intake | 5·7 | 5·7 | 4·8 |

receiving. As the rats were not fed again for 20 hr the carbohydrate they had eaten (less that metabolized during the feeding period and the subsequent 2–3 hr before death) must have remained in the body in some other form, probably as fat, since the high respiratory quotients observed by Tepperman *et al.* (1943) in rats fed only one meal each day suggest that in these animals ingested carbohydrate is converted to fat. Second, the glycogen removed from the livers during this 20 hr fasting period represents much the same fraction of the dietary carbohydrate for rats fed any one of the three diets. We think this supports the view that direct utilization of dietary carbohydrate was less in our rats fed diets F and P than in those fed diet C. On the other hand, it is clear that glucose formation from sources other than liver glycogen must have taken place in all our rats, although the data do not provide evidence for the source of this new-formed carbohydrate. When fasting was continued it is probable that in the rats fed diet F at about 24 hr after feeding the remaining glycogen in the liver was rapidly exhausted, as these 24 hr fasted rats fell into two distinct groups, those with a moderate

amount of glycogen in their livers and those with only a very small amount (Fig. 6).

The results of the experiments in which liver slices were incubated in a glucose-containing medium also support the suggestion that tissue from the rats fed a high carbohydrate diet utilized more carbohydrate. In slices with a similar initial glycogen content, there was a larger increase in glycogen in those from rats fed diet C than in those from rats fed diets F or P. It is likely that the difference lay in the ability to utilize glucose rather than in the ability to synthesize glycogen, since the increase in glycogen content was not significantly affected by diet when the substrate was glucose-1-phosphate.

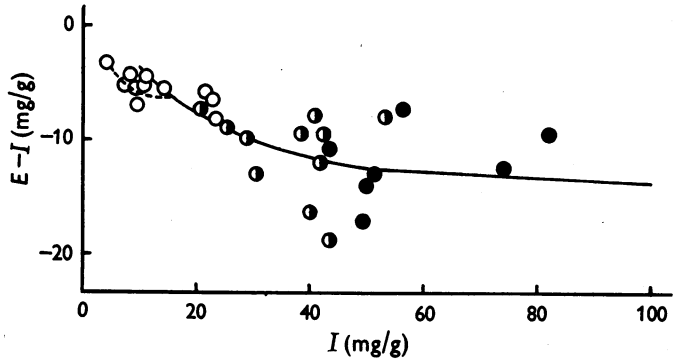


Fig. 8. Effect of diet and initial glycogen content (*I*) on the change in glycogen content (final glycogen content, *E*, less *I*) of liver slices from fed rats incubated 30 min at 37° C in a phosphate buffer. Comparison of observed values (diet C, ●, diet F, ◑, diet P, ○) with values calculated from equations shown in Fig. 7 (diets C and F solid line, diet P broken line).

No simple relationship is to be expected between the initial and final glycogen content of incubated slices. The rate of change is not uniform throughout the incubation period (Fig. 5) and any glycogen which is formed is deposited at the periphery of the slices. This has been demonstrated for slices incubated in a glucose-containing medium by Deane, Nesbett, Buchanan & Hastings (1947) and Mr Galton, working in this laboratory, has made similar observations for slices incubated in a medium containing glucose-1-phosphate. In slices from fed rats synthesis is likely to be negligible in comparison with the breakdown processes, and since the initial glycogen content still influences the amount broken down it may be inferred that a high concentration of glycogen is necessary to saturate phosphorylase. An estimate of the minimum concentration may be made by comparing the observed changes in glycogen content during incubation with the curve calculated from the equation relating the logarithms of initial and final glycogen values (Fig. 8). The maximum rate of glycogen breakdown (12–15 mg/g in 30 min) is not reached until the slices contain 40–50 mg/g glycogen.

Calculations have also been made (from the equations shown in Figs. 4 and 6) for changes in glycogen content of slices from 24 hr fasted rats incubated in media containing glucose or glucose-1-phosphate. The observed values are scattered so widely round the calculated curves (Fig. 9) that these can only be regarded as a very approximate expression of the actual relationship. It is of interest that both curves are of the same general shape and that for initial glycogen values above about 4 mg/g the curves are approximately parallel,

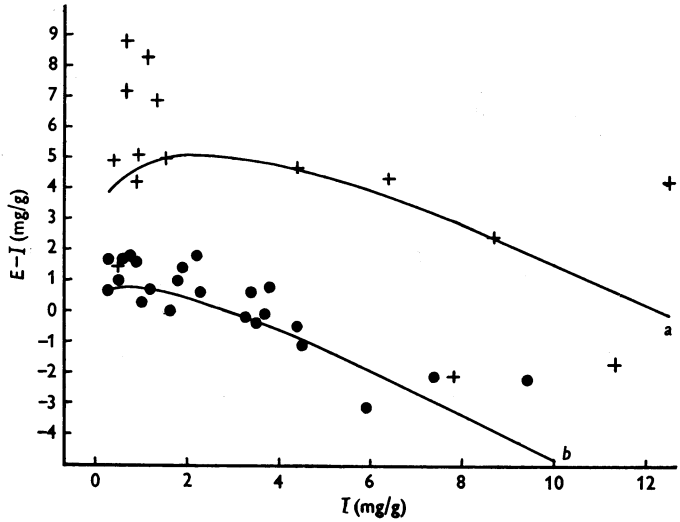


Fig. 9. Effect of substrate and of initial glycogen level (*I*) on the change in glycogen content (final glycogen content, *E*, less *I*) of liver slices from 24 hr fasted rats incubated 60 min at 37° C. Comparison of observed values (glucose-1-phosphate, all diets, +; glucose, diet C only, ●) with values calculated (*a*) for glucose-1-phosphate from the equation shown in Fig. 4, and (*b*) for glucose from that shown in Fig. 7.

indicating that in both media, in spite of the presence of $F^-$ in the medium containing glucose-1-phosphate, breakdown of glycogen was the same when the initial content was the same. This somewhat surprising conclusion may be related to the fact noted above, that glycogen formation takes place only at the periphery of the slices. In a few experiments in which slices were incubated in a medium containing glucose-1-phosphate, but no fluoride, changes in glycogen content were similar to those found when a glucose-containing medium was used.

The curves shown in Fig. 9 indicate that in slices with a very low initial glycogen content, synthesis of glycogen actually increases with an increase in the amount of glycogen in the slice, i.e. that synthesis proceeds faster when some glycogen is already present. If this could be substantiated, it would seem that, in these conditions, branching enzyme (Hehne, 1951) is the limiting factor in glycogen synthesis.

SUMMARY

1. The glycogen content of livers of rats fed diets rich in carbohydrate, fat or protein has been determined at various times after feeding.

2. The sex of the rats and the composition of the diet were found to affect the amount of glycogen in the livers of both fed and fasted rats.

3. A small fraction of liver glycogen (as estimated by the method of Good *et al.* 1933) could not be extracted by 10% trichloroacetic acid.

4. During incubation, changes in glycogen content of liver slices from both fed and fasted rats depended on the initial glycogen content.

5. Slices from fasted rats previously fed the high carbohydrate diet synthesized rather more glycogen during incubation in a glucose-containing medium than did those from rats previously fed the high fat or protein diets. There was no significant effect of previous diet on the amount synthesized when glucose-1-phosphate or pyruvate were used as substrates.

6. On incubation of slices from fed rats, the amount of glucose formed exceeded the amount of glycogen broken down in slices from protein-fed rats which had a comparatively low initial glycogen content, but was less than the glycogen broken down in slices from rats fed the high carbohydrate or fat containing diets which had a much higher glycogen level at the beginning of the incubation period.

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