liver to be considerably less than previously reported values for DPN–cytochrome *c* reductase.

The total TPN contents of the tissues investigated show some rough positive correlation with levels of activity of glucose 6-phosphate and 6-phosphogluconate dehydrogenases (see Glock & McLean, 1954) and with glutathione reductase (see Rall & Lehninger, 1952).

SUMMARY

1. The DPN^+, DPNH, TPN^+ and TPNH contents of a variety of animal tissues have been determined.

2. In all the tissues investigated DPN is present mainly in the oxidized form, whereas TPN is present chiefly and sometimes exclusively in the reduced form.

3. High total concentrations of both DPN and TPN are found in liver, adrenals, kidney and lactating mammary glands. The high level of TPN in ovary and the low levels in brain and voluntary muscle are also of interest.

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A Preliminary Investigation of the Hormonal Control of the Hexose Monophosphate Oxidative Pathway

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An earlier investigation (Glock & McLean, 1954) of levels of activity of enzymes of the hexose monophosphate oxidative pathway in mammalian tissues and tumours suggested that this pathway is probably under hormonal control. In addition, recent preliminary experiments (Glock & McLean, 1955) have shown that the levels of activity of glucose 6-phosphate and 6-phosphogluconate dehydrogenases are significantly reduced in the livers of rats in alloxan diabetes. These experiments on diabetes have now been extended and the effects of starvation, variations in food intake and administration of growth hormone, thyroxine and thiouracil have also been investigated. A later paper will deal with the effects of sex hormones, hypophysectomy and adrenalectomy.

EXPERIMENTAL

Materials

D-*Glucose* 6-*phosphate* (*G* 6-*P*) *and* 6-*phosphogluconate* (6-*PG*). These were preparations of the barium salts used previously (Glock & McLean, 1953).

Triphosphopyridine nucleotide (*TPN*). This was made from horse liver by the method of Kornberg & Horecker (1953). It contained 75% TPN (analysed with G 6-P dehydrogenase according to Kornberg, 1950) and no diphosphopyridine nucleotide (DPN) (by the alcohol-dehydrogenase method of Racker, 1950).

Methods

Estimation of G 6-*P and* 6-*PG dehydrogenase activities.* Liver and voluntary-muscle dehydrogenase activities were determined spectrophotometrically by following the rate of reduction of TPN at 340 mμ. in 1 cm. cells in a Hilger

Uvispek spectrophotometer as described previously (Glock & McLean, 1953). In the assay of G 6-P dehydrogenase activity, the reaction mixture consisted of 0·5 ml. 0·25M glycylglycine, pH 7·6, 0·5 ml. 0·1M-$MgCl_2$, 0·1 ml. 6-PG dehydrogenase [dialysed $(NH_4)_2SO_4$ liver fraction], 0·1 ml. liver supernatant or 0·5 ml. muscle supernatant and 0·2 mg. TPN in a total volume of 2·4 ml. The reaction was started by the addition of 0·1 ml. 0·05M G 6-P to both cells, the blank being devoid of TPN. In the assay of 6-PG dehydrogenase activity, the $(NH_4)_2SO_4$ liver fraction was omitted, G 6-P was replaced by 0·1 ml. 0·05M 6-PG and the reaction was carried out in glycylglycine buffer at both pH 7·6 and pH 9·0. A unit of enzyme activity is defined as the quantity of enzyme which reduces 0·01 μ-mole TPN/min. at 20°.

Determination of nucleic acids. In some of the experiments both the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contents of the livers were determined. The extraction procedure adopted was that of Schneider (1946). DNA was estimated by a modification of the diphenylamine method of Dische (1930) as described by Racker (1952), except that the readings were taken at 600 mμ. RNA was determined by the orcinol method of Mejbaum (1939), the time of heating being increased to 45 min. The standard RNA was a commercial yeast preparation purified according to Fletcher, Gulland, Jordan & Dibben (1944). The standard DNA was a preparation of the sodium salt prepared by the late Professor J. Gulland and his colleagues and was kindly given by Dr J. M. Creeth. According to the notation suggested by Chargaff & Zamenhoff (1948), the atomic extinction coefficients, $\epsilon(P)$, of samples treated with hot trichloroacetic acid as in the extraction procedure were 10 910 for RNA and 8350 for DNA. These values are in fairly good agreement with those quoted by Ogur & Rosen (1950) and Robinson (1952).

Nitrogen contents of tissues. These were determined by a micro-Kjeldahl procedure.

Dietary procedure. In general, albino rats between 160 and 180 g. in weight were used and, with the exception of the growth hormone experiments, were all males. In order to accustom the animals to the diet (that of Greenbaum & Young, 1953), they were fed this for 7 days before the experiment was started. In all the experiments, the control and treated group of animals were pair-fed.

Effect of variations in food intake and of starvation. In this experiment, the control group was fed *ad lib.* (20–21 g./rat/day), three groups received respectively 5, 10 and 15 g. of food daily for 8 days, and one group was starved for 48 hr. before being killed.

Treatment with growth hormone. Two samples of crystalline growth hormone were used, both being prepared by the method of Wilhelmi, Fishman & Russell (1948). One (GH 1) was prepared and kindly provided by Dr A. L. Greenbaum and the other (GH 2) was a commercial preparation presented by Dr Steelman of Armour and Co. Ltd. Adult female rats were subjected to both short-term and long-term treatment with growth hormone. In the short-term experiments, 2 mg. GH 1 were injected intraperitoneally into albino rats and the animals killed after 5 hr. In the long-term experiments, 400 μg. GH 1 or 500 μg. GH 2 were injected daily subcutaneously into hooded Norway or albino rats for 8 or 10 days respectively before being killed.

Treatment with thyroxine. These animals received a daily subcutaneous injection of 0·5 mg. DL-thyroxine (as the Na salt) for 8 days.

Treatment with thiouracil. This group of animals was fed on the diet supplemented with 0·1 % thiouracil for 3 weeks.

Production of alloxan diabetes. After preliminary starvation for 48 hr. the rats were made diabetic by the subcutaneous injection of alloxan according to Kass & Waisbren (1945) and killed 10 days after the injection. The urinary excretion of reducing sugar was determined daily.

In vitro *effects of hormones.* The *in vitro* effects of growth hormone (GH 1), crystalline insulin (Boots Pure Drug Co. Ltd.) and DL-thyroxine (Roche) were tested on the G 6-P and 6-PG dehydrogenase activities of dialysed liver supernatants. Dehydrogenase activities were determined as described above, except that the reactions were allowed to proceed at room temp. for 15 min. and the $(NH_4)_2SO_4$ liver fraction containing 6-PG dehydrogenase was omitted from the G 6-P dehydrogenase assay system. The final concentrations of hormones employed were 0·001M thyroxine, 200 μg. growth hormone/ml. or 0·4 or 1·0 unit of insulin/ml.

RESULTS

Starvation and variations in food intake

The results for the ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP) contents and for the G 6-P and 6-PG dehydrogenase activities of liver are shown in Table 1. The DNAP/g. liver increased progressively with decreasing food intake and was markedly increased after 48 hr. starvation, but the nitrogen and RNAP contents/g. liver were relatively constant. On the other hand, the total DNAP contents in mg./liver were relatively constant in the different groups, whereas the total RNAP in mg./liver showed a steady decline with decreasing food intake (see Fig. 1). Similar effects on the total RNAP and DNAP contents of rat liver after 72 hr. starvation have been reported by Thomson, Heagy, Hutchinson & Davidson (1953). The G 6-P and 6-PG dehydrogenase activities expressed in units/g. liver were scarcely affected by restricted food intake, whereas the total units of both dehydrogenases in the whole liver showed a steady fall (see Fig. 1). A similar pattern of change of G 6-P and 6-PG dehydrogenase activities is obtained if the dehydrogenase activities are expressed in units/mg. DNAP. It is of interest that the rate of decrease of total liver RNAP parallels the decline in total dehydrogenase activities. After 48 hr. starvation, the total dehydrogenase activities were close to those of the group fed 5 g. food/day, namely, approximately 50 % of the control values.

Alloxan diabetes

The RNAP and DNAP contents and the levels of activity of G 6-P and 6-PG dehydrogenases in the livers of alloxan-diabetic and pair-fed control rats are shown in Table 2. There is a very significant decrease in the levels of activity of both dehydrogenases in the livers of the diabetic rats which is accentuated if the results are expressed as total

Table 1. *Effect of starvation and variations in food intake on levels of glucose 6-phosphate and 6-phosphogluconate dehydrogenases in rat liver*

Results expressed as means ± S.E.M. For details, see Methods section.

Daily food intake	Unlimited (Average 20·5 g.)	15 g.	10 g.	5 g.	Starved for 48 hr.
No. of animals	12	6	6	6	6
Initial body wt. (g.)	164±1	163±2	162±1	161±2	155±3
Final body wt. (g.)	187±3	166±3	147±4	119±3	141±3
Liver wt. (g.)	7·99±0·25	6·86±0·25	5·46±0·20	4·30±0·14	5·39±0·14
Liver nitrogen (mg./g.)	35·2±0·3	36·0±0·5	38·2±0·2	40·8±0·3	38·9±0·4
DNAP (μg./g. liver)	259±10	269±14	313±13	383±12	321±16
RNAP (μg./g. liver)	606±13	542±30	574±12	543±21	632±11
Units of enzyme/g. liver					
G 6-P dehydrogenase, pH 7·6	61±5	54±2	52±4	50±3	39±3
6-PG dehydrogenase, pH 9·0	163±7	137±7	158±19	159±9	148±8
6-PG dehydrogenase, pH 7·6	80±3	60±3	57±7	57±3	76±4
Units of enzyme/mg. DNAP:					
G 6-P dehydrogenase, pH 7·6	238±19	200±11	166±9	133±9	120±8
6-PG dehydrogenase, pH 9·0	637±37	515±35	510±70	415±14	462±17
6-PG dehydrogenase, pH 7·6	307±18	222±16	182±25	148±5	238±9

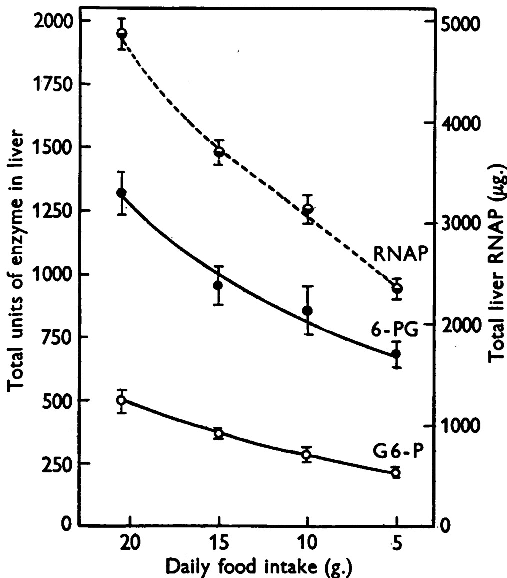

Fig. 1. Effect of variations in food intake on the levels of activity of G 6-P and 6-PG dehydrogenases and on the ribonucleic acid content of rat liver. ○, G 6-P dehydrogenase, pH 7·6; ●, 6-PG dehydrogenase, pH 9·0; ⊖, RNAP. The vertical lines represent twice the S.E.M.

units of enzyme in the whole liver. The total RNAP (μg./whole liver) is also significantly lower in the diabetic animals. This latter finding is not in agreement with Thomson *et al.* (1953), who found no significant alterations in either total DNAP or RNAP contents of liver in alloxan diabetes. The dehydrogenase activities have not been expressed on a DNA basis, since Diermeier & Di Stefano (1954) have shown that the DNA content/liver cell nucleus is increased in alloxan-diabetic rats.

Similar changes in dehydrogenase levels to those shown in Table 2 were obtained in a preliminary experiment in which the animals were less diabetic, excreting on an average only 2 g. reducing sugar/day.

Treatment with growth hormone

The effects of short- and long-term treatments with growth hormone on the levels of activity of G 6-P and 6-PG dehydrogenases of rat liver are shown in Tables 3 and 4. Short-term treatment, in which the animals were killed 5 hr. after the injection of 2 mg. of growth hormone, produced no significant changes in the levels of either dehydrogenase (see Table 3). After long-term treatment with two different crystalline growth-hormone preparations (see Table 4), 6-PG dehydrogenase activities expressed in units/g. liver were significantly reduced in both experimental groups. There was also a marked increase in the average G 6-P dehydrogenase activity in the first group. When expressed as total units/liver or on a DNA basis, however, none of these differences was statistically significant. This can be attributed to wide variations in the individual dehydrogenase activities, which are encountered only in groups of female rats.

Thyroxine treatment

The levels of activity of G 6-P and 6-PG dehydrogenases in the liver and voluntary muscles of control and pair-fed thyroxine-treated rats are given in Table 5. There is a very significant increase in the levels of activity of both dehydrogenases in the livers of the thyroxine-treated animals. Although it was previously shown (Glock & McLean, 1954) that muscle G 6-P and 6-PG dehydrogenase levels are very low, they were, nevertheless, determined in this experiment on account of the peripheral action of thyroxine. No significant differences were found.

Table 2. *Levels of glucose* 6-*phosphate and* 6-*phosphogluconate dehydrogenases in the livers of control and diabetic rats*

Results expressed as ±S.E.M. For details, see Methods section.

	Control group	Alloxan-treated group	*P*
No. of animals	6	6	—
Initial body wt. (g)	170±2	170±1	—
Final body wt. (g.)	202±4	146±8	—
Liver wt. (g.)	8·6±0·5	6·6±0·5	—
Average daily sugar excretion (g.)	—	6±1	—
Liver nitrogen (mg./g.)	34·6±0·9	38·3±0·8	0·013
DNAP (μg./whole liver)	1738±73	1651±103	0·502
RNAP (μg./whole liver)	4628±245	3561±216	0·010
Units of enzyme/g. liver:			
G 6-P dehydrogenase, pH 7·6	149±9	58±6	<0·001
6-PG dehydrogenase, pH 9·0	187±13	82±4	<0·001
6-PG dehydrogenase, pH 7·6	111±4	40±4	<0·001
Total units of enzyme in liver:			
G 6-P dehydrogenase, pH 7·6	1296±144	377±41	<0·001
6-PG dehydrogenase, pH 9·0	1612±175	544±68	<0·001
6-PG dehydrogenase, pH 7·6	952±69	261±25	<0·001

Table 3. *Effect of short-term treatment with growth hormone on the levels of glucose* 6-*phosphate and* 6-*phosphogluconate dehydrogenases in rat liver*

Female albino rats injected intraperitoneally with 2 mg. growth-hormone preparation 1 (see Methods section) and killed 5 hr. later. Results expressed as means±S.E.M.

	Control group	Growth-hormone-treated group	*P*
No. of animals	5	5	—
Body wt. (g.)	160±1	161±2	—
Liver wt. (g.)	6·11±0·16	6·39±0·21	—
Units of enzyme/g. liver:			
G 6-P dehydrogenase, pH 7·6	106±18	82±6	0·230
6-PG dehydrogenase, pH 9·0	309±32	338±23	0·480
Total units of enzyme in liver:			
G 6-P dehydrogenase, pH 7·6	656±129	525±34	0·345
6-PG dehydrogenase, pH 9·0	1914±227	2147±119	0·351

Table 4. *Levels of glucose* 6-*phosphate and* 6-*phosphogluconate dehydrogenases in the livers of control and growth-hormone-treated rats*

For description of growth-hormone preparations see under experimental section. Results expressed as means±S.E.M.

	Control group	Growth-hormone-treated group 1*	*P*	Control group	Growth-hormone-treated group 2†	*P*
No. of animals	5	5	—	11	11	—
Initial body wt. (g.)	158±4	157±4	—	182±6	184±5	—
Final body wt. (g.)	164±7	180±4	—	192±5	205±4	—
Liver wt. (g.)	5·95±0·51	7·50±0·81	—	6·53±0·27	7·16±0·26	—
Total DNAP (μg./liver)	1104±142	1403±124	0·148	—	—	—
Total RNAP (μg./liver)	2756±266	2994±261	0·541	—	—	—
Units of enzyme/g. liver:						
G 6-P dehydrogenase, pH 7·6	120±27	177±39	0·269	139±14	130±8	0·596
6-PG dehydrogenase, pH 9·0	366±27	261±33	0·040	305±16	249±7	0·006
6-PG dehydrogenase, pH 7·6	—	—	—	142±6	110±3	<0·001
Total units of enzyme in liver:						
G 6-P dehydrogenase, pH 7·6	762±237	1345±331	0·191	907±101	935±66	0·820
6-PG dehydrogenase, pH 9·0	2187±233	1974±363	0·631	1906±104	1727±92	0·212
6-PG dehydrogenase, pH 7·6	—	—	—	931±67	793±45	0·101
Units of enzyme/mg. DNAP:						
G 6-P dehydrogenase, pH 7·6	651±114	954±223	0·264	—	—	—
6-PG dehydrogenase, pH 9·0	2089±329	1406±208	0·119	—	—	—

* Female hooded Norway rats injected subcutaneously with 400 μg. growth-hormone preparation 1 for 8 days.
† Female albino rats injected subcutaneously with 500 μg. growth-hormone preparation 2 for 10 days.

Thiouracil treatment

The dehydrogenase activities of the liver and voluntary muscle of thiouracil-treated and pair-fed control rats are given in Table 6. Although there is a significant decrease in the levels of activity of both dehydrogenases when expressed in units/g. liver, there is no significant change if the results are expressed in units/whole liver, on account of the significant increase in liver weight in the thiouracil-treated group of animals. The muscle-dehydrogenase levels are likewise unaffected.

Seasonal variations in dehydrogenase activities

Tables 1–6 show that although the dehydrogenase activities of the livers of the control rats show only small variations in any one experiment, the average dehydrogenase activities of the control groups of animals vary considerably in different experiments. This cannot be attributed to the paired-feeding technique and indicates seasonal variations in enzyme activities, as is shown in Fig. 2. The levels of activity of both dehydrogenases are considerably higher in summer than in winter.

Table 5. *Levels of glucose* 6-*phosphate and* 6-*phosphogluconate dehydrogenases in the livers of control and thyroxine-treated rats*

Results expressed as means ± S.E.M. For details, see Methods section.

	Control group	Thyroxine-treated group	*P*
No. of animals	6	6	—
Initial body wt. (g.)	175 ± 1	172 ± 1	—
Final body wt. (g.)	192 ± 3	167 ± 3	—
Liver wt. (g.)	7·52 ± 0·37	6·90 ± 0·24	—
Liver nitrogen (mg./g.)	35·8 ± 1·2	37·4 ± 0·4	0·258
Muscle nitrogen (mg./g)	35·2 ± 0·3	36·4 ± 0·3	0·019
Units of enzyme/g. liver:			
G 6-P dehydrogenase, pH 7·6	76 ± 10	151 ± 14	0·002
6-PG dehydrogenase, pH 9·0	264 ± 8	634 ± 32	<0·001
6-PG dehydrogenase, pH 7·6	119 ± 8	312 ± 18	<0·001
Total units of enzyme in liver:			
G 6-P dehydrogenase, pH 7·6	557 ± 61	1056 ± 128	0·006
6-PG dehydrogenase, pH 9·0	1985 ± 129	4394 ± 320	<0·001
6-PG dehydrogenase, pH 7·6	895 ± 66	2165 ± 172	<0·001
Units of enzyme/g. muscle*:			
G 6-P dehydrogenase, pH 7·6	7·5 ± 0·4	8·0 ± 0·4	0·431
6-PG dehydrogenase, pH 9·0	16·9 ± 0·5	16·6 ± 2·5	0·883

* Five rats in this group.

Table 6. *Levels of glucose* 6-*phosphate and* 6-*phosphogluconate dehydrogenases in the livers of control and thiouracil-treated rats*

Results expressed as means ± S.E.M. For details, see Methods section.

	Control group	Thiouracil-treated group	*P*
No. of animals	6	6	—
Initial body wt. (g.)	170 ± 1	169 ± 1	—
Final body wt. (g.)	204 ± 3	201 ± 5	—
Liver wt. (g.)	6·77 ± 0·23	8·09 ± 0·42	0·014
Thyroid wt. (mg.)	18 ± 1	53 ± 5	<0·001
Liver nitrogen (mg./g.)	37·4 ± 0·4	35·3 ± 0·9	0·055
Muscle nitrogen (mg./g.)	37·0 ± 1·4	34·2 ± 0·4	0·064
Units of enzyme/g. liver*:			
G 6-P dehydrogenase, pH 7·6	73 ± 11	44 ± 5	0·041
6-PG dehydrogenase, pH 9·0	196 ± 13	148 ± 7	0·011
Total units of enzyme in liver:			
G 6-P dehydrogenase, pH 7·6	479 ± 67	367 ± 61	0·251
6-PG dehydrogenase, pH 9·0	1293 ± 67	1210 ± 126	0·604
Units of enzyme/g. muscle:			
G 6-P dehydrogenase, pH 7·6	5·2 ± 0·4	4·3 ± 0·4	0·114
6-PG dehydrogenase, pH 9·0	11·1 ± 0·5	10·2 ± 0·6	0·272

* Five rats only in this group.

In vitro experiments

Neither G 6-P nor 6-PG dehydrogenase activities of rat-liver supernatants were affected by the addition of growth hormone, thyroxine or insulin in the concentrations employed and under the particular conditions of assay described in the experimental section.

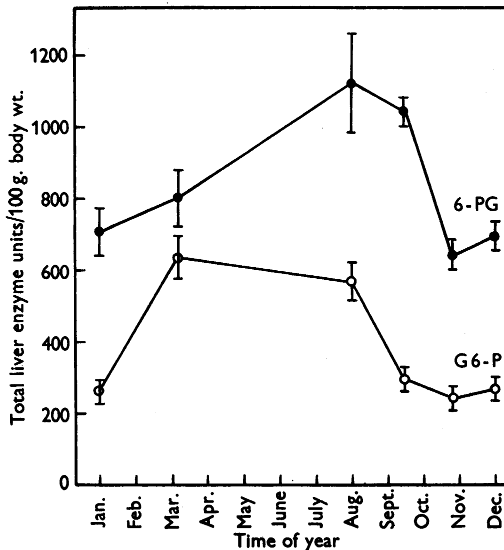

Fig. 2. Seasonal variations in the levels of activity of G 6-P and 6-PG dehydrogenases in rat liver. Adult male rats used. ○, G 6-P dehydrogenase, pH 7·6; ●, 6-PG dehydrogenase, pH 9·0. The vertical lines represent twice the S.E.M.

DISCUSSION

This investigation has shown that levels of activity of G 6-P and 6-PG dehydrogenases are significantly reduced in the livers of rats maintained on a restricted food intake for 8 days and also after 48 hr. starvation. The levels of both dehydrogenases are in addition markedly decreased in alloxan diabetes and increased as a result of thyroxine treatment. Although G 6-P dehydrogenase activity shows a tendency to increase as a result of long-term treatment with growth hormone, this is not statistically significant when expressed on a whole-liver basis, on account of the wide variations in the individual dehydrogenase activities in groups of female rats. The paired-feeding technique was employed in all these hormonal experiments in order to eliminate nutritional variations in dehydrogenase levels.

Alterations in enzyme contents of liver as a result of restricted food intake, low-protein diets and starvation have been reported frequently. In general, loss of enzyme activity has been shown to parallel a decrease in total liver protein (Potter & Klug, 1947; Seifter, Harkness, Rubin & Muntwyler, 1948; Miller, 1948, 1953), suggesting that liver-enzyme proteins enter into protein metabolism in a dynamic but more or less non-specific fashion. The alterations in levels of G 6-P and 6-PG dehydrogenases in whole liver as a result of restricted food intake and starvation appear to be no exception to this general rule.

The ubiquitous distribution of enzymes of the hexose monophosphate (HMP) oxidative pathway in plant and animal tissues and the high levels of activity of G 6-P and 6-PG dehydrogenases in certain mammalian tissues (Glock & McLean, 1954) suggest that there is active competition between the glycolytic and HMP oxidative pathways for available G 6-P. Since in the steady state of intracellular metabolism the concentration of G 6-P is very low, the relative affinities of G 6-P for competing enzymes of the two alternative pathways must play a decisive part in determining its ultimate fate under physiological conditions. If the assumption is made that hexose phosphate isomerase is never a limiting factor, published values for the Michaelis constants (K_m) of 6-phosphofructokinase and G 6-P dehydrogenase, the first enzyme steps in the glycolytic and HMP oxidative pathways respectively, indicate that the latter pathway can indeed actively compete for G 6-P. Thus the K_m (F 6-P) of rabbit-muscle phosphofructokinase is 1×10^{-4} M (see Stumpf, 1954) and the K_m (G 6-P) for G 6-P dehydrogenase of rat liver is $1{\cdot}3 \times 10^{-5}$ M (Glock & McLean, 1953). Since G 6-P is the common substrate for both metabolic pathways, hormonal effects at the hexokinase level would be expected to affect both pathways in a similar fashion. Indirect evidence, particularly from experiments on the utilization of [^{14}C]glucose and [^{14}C]fructose by rat-liver slices, indicates that there is in fact a metabolic block at the glucokinase stage both in experimental diabetes and as a result of starvation for 48 hr. or longer (see Chernick & Chaikoff, 1951; Chernick, Chaikoff & Abraham, 1951; Wyshak & Chaikoff, 1953; Renold, Teng, Nesbett & Hastings, 1953; Renold, Hastings & Nesbett, 1954). The existence of such a block could account for the lowered levels of activity of G 6-P and 6-PG dehydrogenases observed under similar conditions in the present investigation. No data are available on the effects of growth hormone and thyroxine on liver hexokinase, although thyroxine treatment has been shown to increase muscle-hexokinase activity (Smith & Williams-Ashman, 1951).

The most important contributions to an evaluation of alternative pathways have come from isotope studies with tissue slices in which the

yields of $^{14}CO_2$ from (6-^{14}C)glucose and (1-^{14}C)-glucose are compared. Preferential conversion of C_1 of glucose into CO_2 indicates the occurrence of a non-glycolytic pathway. Results obtained by Agranoff, Brady & Colodzin (1954) are in support of active competition between the glycolytic and non-glycolytic pathways for G 6-P, since, in liver slices from rats fed on a high-glucose diet, there was an approximately threefold increase in $^{14}CO_2$ from both (1-^{14}C)glucose and (6-^{14}C)glucose without an increase in the quotient (yield of $^{14}CO_2$ from C_6)/(yield of $^{14}CO_2$ from C_1), this quotient remaining approximately constant at 0·33–0·29. This work also corroborates our findings about the reduced activity of the HMP oxidative pathway after 48 hr. starvation. The non-glycolytic pathway was still further reduced after 72 hr. starvation, the C_6/C_1 quotient rising to 0·7. Although Katz, Abraham, Hill & Chaikoff (1954) consider that not more than 10% of the glucose utilization by liver slices can proceed by non-glycolytic pathways, the work of Agranoff *et al.* (1954) and of Bloom & Stetten (1955) indicates a much more active participation of non-glycolytic routes. In diaphragm, however, glucose metabolism proceeds probably exclusively via glycolysis (Bloom & Stetten, 1953; Agranoff *et al.* 1954), which is in accord with the very low levels of activity of enzymes of the HMP oxidative pathway in voluntary muscle (Glock & McLean, 1954).

It is of some interest that the steady decline in total dehydrogenase activities in the livers of rats on a restricted food intake parallels an accompanying decrease in total liver RNA levels. Lowered dehydrogenase levels in alloxan diabetes are accompanied by a similar reduction in the total RNA content (cf. Thomson *et al.* 1953). Although these changes in dehydrogenase and RNA levels are probably non-specific manifestations of alterations in protein synthesis in general, it is attractive to conjecture that RNA levels parallel dehydrogenase activities, since the HMP oxidative pathway may serve as a source of ribose for RNA synthesis.

SUMMARY

1. Total levels of activity of both glucose 6-phosphate (G 6-P) and 6-phosphogluconate (6-PG) dehydrogenases are significantly reduced in the livers of rats maintained on restricted food intakes for 8 days and also after 48 hr. starvation.

2. Concentrations of both dehydrogenases are markedly reduced in alloxan diabetes, total liver activities being only approximately one-third of the control values.

3. Thyroxine treatment results in an approximately twofold increase in the levels of activity of both dehydrogenases, whereas thiouracil treatment, although decreasing enzyme activities/g. liver, does not significantly affect total liver values.

4. Although long-term treatment with crystalline growth hormone causes some increase in G 6-P dehydrogenase activity/g. liver, total liver-enzyme activities are not significantly affected.

5. Liver-dehydrogenase concentrations show seasonal variations and are significantly higher in summer than in winter.

6. Crystalline insulin, crystalline growth hormone and DL-thyroxine have no *in vitro* effect on either G 6-P or 6-PG dehydrogenase activity under the particular conditions employed.

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Effects of Hormones on Levels of Oxidized and Reduced Diphosphopyridine Nucleotide and Triphosphopyridine Nucleotide in Liver and Diaphragm

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Variations in tissue levels of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), as well as the relative proportions of oxidized and reduced coenzymes, presumably play an important part in the regulation of alternative pathways of carbohydrate metabolism. For this reason, the methods described by Glock & McLean (1955*a*) for the separate determination of oxidized and reduced DPN and TPN in animal tissues have been applied to the estimation of DPN^+, DPNH, TPN^+ and TPNH in the livers and voluntary muscles of rats subjected to various dietary and hormonal procedures. The present paper deals with the effects of starvation, alloxan diabetes and treatment with growth hormone, thyroxine and thiouracil. Changes in the total DPN content of liver, as well as in the proportion of oxidized to reduced coenzyme, have recently been reported by Helmreich, Holzer, Lamprecht & Goldschmidt (1954) in alloxan diabetes and in starvation.

METHODS

Treatment of animals. Young adult albino rats were used. The dietary procedure, production of alloxan diabetes, conditions for studying the effects of starvation and treatment with growth hormone, thyroxine and thiouracil were the same as described in the preceding paper (Glock & McLean, 1955*b*).

Estimation of coenzymes. The preparation of substrates and enzymes was carried out as described previously (Glock & McLean, 1955*a*), except for cytochrome *c*, which was a commercial preparation (Sigma Chemical Co.). Oxidized and reduced coenzymes were determined, respectively, in neutralized acid and alkaline tissue extracts (Glock & McLean, 1955*a*).

RESULTS

Alloxan diabetes and starvation

The contents of oxidized and reduced DPN and TPN in the liver and diaphragm of alloxan-diabetic and pair-fed control rats are given in Table 1. Liver values after 24 and 48 hr. starvation are shown in Table 2. In the case of the alloxan-diabetic rats, there was a significant fall in the concentration of oxidized DPN/g. liver with a corresponding decrease in the DPN^+/DPNH quotient. Although the total DPN (DPN^+ + DPNH) content was unaltered on a g. liver basis there was a significant decrease on a whole-liver basis. There were much less striking changes in the TPN contents. The total TPN (principally TPNH)/g. liver was unaffected, but again total TPN/whole liver was significantly reduced. In diaphragm there was some rise in the concentration of oxidized DPN/g. muscle, with a small but significant increase in the DPN^+/DPNH quotient, the total DPN concentration being unaffected. When expressed on a whole-diaphragm basis, there was a small but significant decrease in both DPNH and total DPN (DPN^+ + DPNH) contents. The total TPN content of diaphragm is too small to warrant further investigation.

The liver values of rats starved for 24 and 48 hr. (see Table 2) indicate that, as in alloxan diabetes, there is a marked fall in the DPN^+/DPNH quotient when compared with the average quotient of control rats (Table 1). In addition, there was a marked reduction in the concentration of both oxidized and reduced DPN when the starvation period was extended to 48 hr.