Holzer, H., Goldschmidt, S., Lamprecht, W. & Helmreich, E. (1954). *Hoppe-Seyl. Z.* **297**, 1.
Kaplan, N. O., Colowick, S. P. & Neufeld, E. F. (1953). *J. biol. Chem.* **205**, 1.
Keilin, D. & Hartree, E. F. (1952). *Biochem. Prep.* **2**, 1.
Kornberg, A. (1950). *J. biol. Chem.* **182**, 805.
Kornberg, A. & Horecker, B. L. (1953). *Biochem. Prep.* **3**, 24.
Kornberg, A. & Pricer, W. E. jun. (1953). *Biochem. Prep.* **3**, 20.
Mahler, H. R., Sarkar, N. K., Vernon, L. P. & Alberty, R. A. (1952). *J. biol. Chem.* **199**, 585.
Margoliash, E. (1954). *Biochem. J.* **56**, 529.
Negelein, E. & Wulff, H. J. (1937). *Biochem. Z.* **293**, 351.
Racker, E. (1950). *J. biol. Chem.* **184**, 313.
Schlenk, F. (1951). In *The Enzymes*, vol. 2, part 1, p. 250. New York: Academic Press Inc.
Singer, T. P. & Kearney, E. B. (1950). *J. biol. Chem.* **183**, 409.
Singer, T. P. & Kearney, E. B. (1953). *Fed. Proc.* **12**, 269.
Singer, T. P. & Kearney, E. B. (1954*a*). *Advanc. Enzymol.* **15**, 79.
Singer, T. P. & Kearney, E. B. (1954*b*). In *The Proteins*, vol. 2, part A, p. 123. New York: Academic Press Inc.
Spirtes, M. A. & Eichel, H. J. (1954). *Arch. Biochem. Biophys.* **53**, 308.
Vernon, L. P., Mahler, H. R. & Sarkar, N. K. (1952). *J. biol. Chem.* **199**, 599.
Warburg, O., Christian, W. & Griese, A. (1935). *Biochem. Z.* **282**, 157.

Levels of Oxidized and Reduced Diphosphopyridine Nucleotide and Triphosphopyridine Nucleotide in Animal Tissues

By GERTRUDE E. GLOCK and PATRICIA McLEAN
Courtauld Institute of Biochemistry, Middlesex Hospital, London, W. 1

(*Received* 10 *March* 1955)

The preceding paper (Glock & McLean, 1955) describes sensitive methods for the separate determination of oxidized and reduced diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) in animal tissues. Reduced coenzymes are coupled with their respective cytochrome *c* reductases and the rate of reduction of cytochrome *c* is followed spectrophotometrically. These methods have now been applied to the estimation of DPN^+, DPNH, TPN^+ and TPNH in a variety of animal tissues.

EXPERIMENTAL

The preparation of substrates and enzymes and the estimation of coenzymes were carried out as described previously (Glock & McLean, 1955). Oxidized and reduced coenzymes were determined, respectively, in neutralized acid and alkaline tissue extracts.

RESULTS

Our results for the levels of oxidized and reduced DPN and TPN in a variety of animal tissues are incorporated in Table 1. The rate of reduction of cytochrome *c* was in all cases directly proportional to the volume of tissue extract. The presence of relatively high concentrations of ascorbic acid in the neutralized acid extracts of adrenals does not invalidate the assay procedures, since, although ascorbic acid reduces cytochrome *c* directly, the rate of reduction is so rapid that it is virtually complete before the first spectrophotometric reading is taken.

In all the tissues investigated, the total DPN concentrations ($DPN^+ + DPNH$) are considerably higher than the total TPN concentrations ($TPN^+ +$ TPNH). Moreover, whereas DPN is present chiefly in the oxidized form, TPN on the contrary is present mainly in the reduced form. Thus the $DPN^+/DPNH$ quotients are in all cases greater than 1 and the $TPN^+/TPNH$ quotients always considerably less than 1. In many tissues the levels of oxidized TPN are negligible. The highest total concentration of TPN is found in liver but fairly high concentrations also occur in adrenals, lactating mammary glands, ovary and kidney. Most of the other tissues examined contain very little TPN either in the oxidized or reduced form. The low values in brain and voluntary muscle (diaphragm), amounting to less than 5% of the total DPN concentration, are particularly interesting. The most variable results were obtained with the adrenals both for the total concentrations of DPN and TPN and for the proportions of oxidized to reduced coenzymes. This may prove to be related to the physiological state of activity of the gland, particularly since both DPN and TPN have been shown to be necessary for various steroid interconversions in adrenal homogenates (Hayano, 1954; Hayano & Dorfman, 1953; Plager & Samuels, 1953). The high TPN levels in ovary might also be associated with steroid metabolism.

Table 1. *Levels of oxidized and reduced coenzymes in animal tissues*

Figures in parentheses represent number of animals.

Tissues	Coenzyme content (μg./g. tissue)					
	DPN^+	DPNH	$DPN^+ + DPNH$	TPN^+	TPNH	$TPN^+ + TPNH$
Rat liver (6)	370±13	204±9	574±17	6±1	205±6	211±6
Rat adrenal (5)	315±136	154±45	469±134	17±9	116±24	133±24
Rabbit adrenal (1)	295	117	412	<2	62	62
Rabbit adrenal cortex (1)	356	133	489	14	68	82
Rat diaphragm (6)	289±7	138±9	427±14	<2	13±1	13±1
Rat cardiac muscle (3)	299±15	184±38	483±23	4±1	33±5	36±2
Rat kidney (3)	223±12	212±54	435±60	3±1	54±1	57±2
Rat mammary gland (18 days' lactation) (3)	227±9	83±3	310±10	<2	51±13	51±13
Guinea-pig ovary (1)	214	38	252	6	116	122
Rabbit ovary (1)	181	34	215	<2	42	42
Rat brain (3)	133±6	88±36	221±42	<2	8±3	8±3
Guinea-pig brain cortex (1)	155	67	222	8	16	24
Rat spleen (3)	135±12	61±15	196±18	<2	12±3	12±3
Rat thymus (3)	116±17	35±13	151±20	<2	12±2	12±2
Rat lung (2)	108	52	160	9	18	27
Rat pancreas (2)	115	78	193	<2	12	12
Guinea-pig thyroid (1)	126	30	156	<2	<2	<2
Rat seminal vesicles (1)	128	11	139	<2	12	12
Rat ventral prostate (2)	80	17	97	<2	11	11
Rat testis (1)	80	71	151	<2	6	6
Rat placenta (20-day pregnancy) (2)	90	11	101	<2	3	3
Rat blood* (3)	55±3	36±10	91±8	5±2	3±2	8±3
Rabbit blood* (2)	33	4	37	3	<2	3

* Coenzymes expressed in μg./ml. whole blood.

Our figures for the total DPN content of lactating mammary glands are in good agreement with those obtained by Ringler, Becker & Nelson (1954) in the guinea pig, using alcohol dehydrogenase, and our combined values for the $DPN^+ + DPNH + TPN^+ + TPNH$ contents of muscle, heart, kidney, spleen, lung and pancreas agree moderately well with the figures for total pyridine nucleotide contents of these tissues determined polarographically by Carruthers & Suntzeff (1953).

DISCUSSION

The values reported in this paper for the DPN^+, DPNH, TPN^+ and TPNH contents of animal tissues are, with a few exceptions, the first to be published. Apart from recent figures for the DPN^+ and DPNH contents of liver, which are discussed in the previous paper (Glock & McLean, 1955), very few reliable figures are available (see Schlenk, 1951) and there are none for TPNH.

It is of interest that whereas in all the tissues studied DPN is present mainly in the oxidized form, the reverse is true of TPN, which is present predominantly and sometimes exclusively in the reduced form. The steady-state ratios of oxidized to reduced coenzymes are presumably controlled both by concentrations of substrates and of competing dehydrogenases. Chance (1954) has found large variations in the steady-state level of DPN^+/DPNH in respiring yeast cells, and has concluded that this ratio is governed chiefly by competing dehydrogenases, in this case chiefly by triosephosphate dehydrogenase and α-glycerophosphate dehydrogenase. The steady-state quotient of DPN^+/DPNH is also presumably controlled by oxidative phosphorylation. Thus Lehninger (1951) and Vishniac & Ochoa (1952) have shown that oxidative phosphorylation of ADP to ATP can be coupled with the oxidation of DPNH. This would explain the oxidation of reduced pyridine nucleotides recently observed by Connelly & Chance (1954) in the contracting sartorius muscle of the frog, and the oxidation of reduced pyridine nucleotides on addition of low concentrations of ADP to rat-liver mitochondrial preparations respiring in the presence of β-hydroxybutyrate (Chance & Williams, 1954). It seems probable, although not yet proved, that oxidative phosphorylation can also be coupled with the oxidation of TPNH. The very low TPN^+/TPNH quotients in all the tissues examined presumably indicates that the TPN-dependent dehydrogenase systems are relatively irreversible and that the steady-state levels of TPN^+/TPNH are controlled more by these dehydrogenases than by TPN–cytochrome *c* reductase and glutathione reductase, which cause reoxidation of TPNH. It is of interest in this connexion that Hogeboom & Schneider (1950) found the TPN–cytochrome *c* reductase activity of mouse

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.

We are indebted to Professor F. Dickens, F.R.S., for interest, and to Miss Audrey Bowles for valuable technical assistance. This work was carried out during the tenure of the Hugh Percy Noble Scholarship by G.E.G. and has been in part financed by a grant to the Medical School from the British Empire Cancer Campaign.

REFERENCES

Carruthers, C. & Suntzeff, V. (1953). *Arch. Biochem. Biophys.* **45**, 140.
Chance, B. (1954). In *The Mechanism of Enzyme Action*, p. 399. Baltimore: Johns Hopkins Press.
Chance, B. & Williams, G. R. (1954). *Fed. Proc.* **13**, 190.
Connelly, C. M. & Chance, B. (1954). *Fed. Proc.* **13**, 29.
Glock, G. E. & McLean, P. (1954). *Biochem. J.* **56**, 171.
Glock, G. E. & McLean, P. (1955). *Biochem. J.* **61**, 381.
Hayano, M. (1954). *Fed. Proc.* **13**, 226.
Hayano, M. & Dorfman, R. I. (1953). *J. biol. Chem.* **200**, 175.
Hogeboom, G. H. & Schneider, W. C. (1950). *J. biol. Chem.* **186**, 417.
Lehninger, A. L. (1951). *J. biol. Chem.* **190**, 345.
Plager, J. E. & Samuels, L. T. (1953). *Arch. Biochem. Biophys.* **42**, 477.
Rall, T. W. & Lehninger, A. L. (1952). *J. biol. Chem.* **194**, 119.
Ringler, I., Becker, N. & Nelson, W. L. (1954). *Arch. Biochem. Biophys.* **52**, 348.
Schlenk, F. (1951). In *The Enzymes*, vol. 2, part 1, p. 250. New York: Academic Press Inc.
Vishniac, W. & Ochoa, S. (1952). *J. biol. Chem.* **198**, 501.

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

By GERTRUDE E. GLOCK and PATRICIA McLEAN
Courtauld Institute of Biochemistry, Middlesex Hospital, London, W. 1

(*Received* 5 *April* 1955)

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