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Levels of Oxidized and Reduced Diphosphopyridine Nucleotide and Triphosphopyridine Nucleotide in Tumours

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It is probable that the availability of pyridine nucleotides as well as the relative proportions of the oxidized and reduced forms of these nucleotides are among the factors controlling the metabolic behaviour of cells. For this reason, previously published work (Glock & McLean, 1955*a*) on the distribution of oxidized and reduced diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) in animal tissues has now been extended to a variety of tumours. This supplements the recent investigation of Jedeikin & Weinhouse (1955), who determined the contents of oxidized and reduced diphosphopyridine nucleotide (DPN^+ and DPNH respectively) of several tumours by a spectrophotometric method employing alcohol dehydrogenase and appropriate substrates. Besides supplying additional data on the DPN^+ and DPNH contents of various tumours, the present investigation provides the first reliable figures for TPN contents. Oxidized and reduced pyridine nucleotides have been determined by extremely sensitive methods (Glock & McLean, 1955*b*) which involve coupling enzymically reduced pyridine nucleotides with their respective cytochrome *c* reductases and then following the rate of reduction of cytochrome *c* spectrophotometrically.

EXPERIMENTAL

Tumour material. Most of the animals bearing transplantable tumours were very kindly supplied by Professor A. Haddow of the Chester Beatty Research Institute, London. In addition, we are indebted to Dr R. Stein of the Marie Curie Hospital, London, for rats bearing Jensen sarcomas. Animals with liver tumours induced by *p*-dimethylaminoazobenzene were provided by this Institute. (For details of dietary procedure, see Campbell, 1955.)

Preparation of substrates and enzymes. These were prepared as described in a previous publication (Glock & McLean, 1955*b*) except that cytochrome *c* and D-glucose 6-phosphate, the latter in the form of the heptahydrate of the barium salt, were purchased from Sigma Chemical Co., St Louis. TPN–cytochrome *c* reductase was prepared from Löwenbraü (Munich) bottom-ale yeast.

Estimation of oxidized and reduced pyridine nucleotides. The oxidized and reduced pyridine nucleotides were determined respectively in neutralized acid and alkaline tissue extracts (Glock & McLean, 1955*b*). The reaction mixture for the TPN assay consisted of 0·5 ml. of 0·25 M glycylglycine, pH 8·7, 0·1 ml. of 0·05 M glucose 6-phosphate (sodium salt), 0·1 ml. (1 mg.) of glucose 6-phosphate dehydrogenase, 0·1–0·5 ml. of neutralized tissue extract and 0·1 ml. (0·25 mg.) of TPN–cytochrome *c* reductase in a total vol. of 2·4 ml. The reaction was started by the addition of 0·1 ml. of 0·001 M cytochrome *c* and the rate of reduction of cytochrome *c* was followed spectrophotometrically at 550 mμ. in 1 cm. cells against a blank containing only buffer, water and cytochrome *c*. DPN was estimated by a comparable procedure in which glucose 6-phosphate, glucose 6-phosphate dehydrogenase and TPN–cytochrome *c* reductase were replaced by 0·1 ml. of ethanol, 0·1 ml. (0·1 mg.) of alcohol dehydrogenase and 0·1 ml. of DPN–cytochrome *c* reductase. These assay procedures were standardized daily by using four levels of each nucleotide (0·1–0·8 μg. of DPN and 0·05–0·4 μg. of TPN). The DPN^+, DPNH, oxidized and reduced triphosphopyridine nucleotide (TPN^+ and TPNH respectively) contents of the tissue extracts were calculated from the increase in optical density at 550 mμ. between 1 and 6 min., after correction for enzyme and extract blanks.

RESULTS

Results for the DPN^+, DPNH, TPN^+ and TPNH contents of a variety of tumours and of a few normal tissues are given in Tables 1 and 2. Table 1 includes the values for the pyridine-nucleotide contents of normal rat liver and also of liver tumours and liver from *p*-dimethylaminoazobenzene-treated animals. The liver tumours were in most cases mixed hepatomas and cholangiomas, but one pure hepatoma gave values that were not markedly different from the average for the mixed tumours. Although the total DPN ($DPN^+ + DPNH$) content of liver from tumour-bearing rats is not significantly different from that of normal liver, the average value for liver tumours is only about one-half of this. The total TPN (chiefly TPNH) contents of the same pieces of tissue show much more striking differences. Even in the liver from tumour-bearing rats the total TPN content is less than one-half that of normal liver, whereas the average value for the liver tumours is as low as 14 % of the value for normal liver.

Table 1. *Pyridine-nucleotide contents of normal livers and of liver tumours from* p-*dimethylaminoazobenzene-treated rats*

The assay conditions are given in the Experimental section. Figures in parentheses represent no. of animals. Results are expressed as means ± S.E.M.

	Pyridine-nucleotide contents (μg./g. of tissue)					
Tissue	DPN^+	DPNH	$DPN^+ + DPNH$	TPN^+	TPNH	$TPN^+ + TPNH$
Normal rat liver (23)	398±10	182±8	580±16	7±1	217±5	224±5
Liver tumours:						
Mixed cholangiomas and hepatomas (7)	226±15	83±12	309±19	3±2	27±9	30±9
Hepatomas (1)	255	25	280	<3	28	28
Liver from tumour-bearing rats (5)	441±75	151±35	592±32	4±1	92±21	96±23

Table 2. *Pyridine-nucleotide contents of transplantable rat and mouse tumours*

The assay conditions are given in the Experimental section. Figures in parentheses represent no. of animals. Results are expressed as means ± S.E.M.

	Pyridine-nucleotide contents (μg./g. of tissue)					
Tissue	DPN^+	DPNH	$DPN^+ + DPNH$	TPN^+	TPNH	$TPN^+ + TPNH$
Rat skeletal muscle (5)	278±16	27±5	305±19	8±1	9±2	17±2
Mouse skeletal muscle (4)	314±18	22±4	336±20	<3	<3	<3
Mouse thymus (4)	165±30	65±6	230±27	<3	9±1	9±1
Jensen rat sarcoma (5)	118±12	41±9	159±21	<3	4±1	4±1
Crocker mouse sarcoma (5)	112±22	61±6	174±27	5±2	9±3	14±4
Sarcoma 37 (5)	111±17	22±3	133±17	<3	<3	<3
Carr lymphosarcoma (4)	134±7	37±7	171±9	<3	<3	<3
EL4 mouse leukaemia (5)	101±7	33±2	134±7	<3	<3	<3
Walker rat carcinoma (5)	94±9	50±13	145±24	<3	<3	<3
MC/63 mouse carcinoma (5)	131±31	40±3	172±30	<3	<3	<3
Krebs ascites tumour (4)	132±13	36±0·3	168±13	<3	<3	<3

Results for the pyridine-nucleotide content of various transplantable solid tumours and for skeletal muscle and lymphatic tissue (thymus) are given in Table 2. The total TPN contents of all the tumours are extremely low or negligible. The total DPN contents of these transplantable tumours are, in general, lower than those found in most normal tissues (Glock & McLean, 1955*a*), although they do not differ markedly from such tissues as thymus, lung, testis and thyroid. In contrast with the wide range in total DPN contents of normal tissues (100–600 μg./g. of tissue), these solid tumours show a much narrower range (134–174 μg. of total DPN/g. of tissue).

The relative proportions of the oxidized and reduced forms of both pyridine nucleotides are not significantly different in normal and neoplastic tissues. In both types of tissue DPN is present preponderantly in the oxidized form and TPN largely and often almost exclusively in the reduced form.

DISCUSSION

In a recent valuable review by Weinhouse (1955) certain abnormalities in the oxidative reactions of tumour homogenates and mitochondria are discussed in terms of availability of pyridine nucleotides. Tumour homogenates and mitochondria have been shown to be much more dependent on an additional supply of DPN than are similar preparations from normal tissues in order to oxidize pyruvate and components of the citric acid cycle (Wenner, Spirtes & Wienhouse, 1951; Wenner & Weinhouse, 1953; Williams-Ashman & Lehninger, 1951; Kennedy & Williams-Ashman, 1952; Williams-Ashman & Kennedy, 1952). This pronounced DPN requirement of tumour mitochondria has not yet been satisfactorily explained. It could be due to a low DPN content of intact tumour cells, to a loose conjugation of the nucleotide with mitochondrial apoenzymes together with increased mitochondrial permeability, to a high diphosphopyridine nucleotidase activity, or to an impaired ability of tumour cells to synthesize pyridine nucleotides.

The results of Jedeikin & Weinhouse (1955) on the DPN^+ and DPNH contents of a number of normal and neoplastic mouse and rat tissues indicate a generally much lower content of total pyridine nucleotide in neoplastic tissues. In both types of tissue, however, the same pattern of oxidized and reduced forms was observed, the oxidized form being always preponderant. A low DPN content of tumours had also been observed by earlier workers (Bernheim & Felsovanyi, 1940; Kensler, Sugiura & Rhoads, 1940; Fisher & Schlenk, 1948; Carruthers & Suntzeff, 1953), but most of these results are open to criticism on account of the non-specificity of the methods used; in some cases only total pyridine nucleotide was determined. This low total DPN content of tumours cannot be due to increased destruction of this nucleotide since the work of Quastel & Zatman (1953) shows that the diphosphopyridine nucleotidase activity of a variety of tumours is within the range of normal tissue activities. Recent work of Branster & Morton (1956) indicates that nuclear DPN pyrophosphorylase activity is significantly reduced in mammary-gland carcinomata from C_3H mice. Whether this reduced capacity for DPN synthesis is a general property of all neoplastic tissue is at present unknown, although Waravdekar, Powers & Leiter (1956) have published a preliminary note to the effect that DPN synthesis in liver homogenates of CAF mice bearing sarcoma 37 is considerably reduced.

In the present investigation the TPN^+ and TPNH contents of tumours have been determined in addition to DPN^+ and DPNH. These figures for TPN appear to be the first reliable ones to be published. Our values for DPN^+ and DPNH as well as for the relative proportions of the oxidized and reduced forms are in very good agreement with the recent results of Jedeikin & Weinhouse (1955) and support the general view that the total DPN content of tumours is relatively low. Of great interest is the finding that the total TPN (chiefly TPNH) contents of all the tumours investigated are extremely low or even negligible. The gradual reduction in the total TPN content of rat liver undergoing carcinogenesis by *p*-dimethylaminoazobenzene is most striking. The average values for normal rat liver and liver and liver tumours from tumour-bearing rats are respectively 224, 96 and 22 μg. of total TPN/g. of tissue. Much less dramatic changes were found in the total DPN contents of the same pieces of tissue, in contrast to earlier findings of Kensler *et al.* (1940). These results suggest, in addition, that the synthesis of TPN from DPN is severely curtailed in these tumours. The relative proportions of oxidized and reduced forms of both pyridine nucleotides are not significantly different from those of normal tissues (Glock & McLean, 1955*a*). In both neoplastic and normal tissues DPN is present preponderantly in the oxidized form and TPN largely and often almost exclusively in the reduced form. The finding of Euler, Schlenk, Heiwinkel & Högberg (1938) that Jensen sarcomas contain much more DPNH than DPN^+ has not been confirmed.

Weinhouse (1955) has re-emphasized the very marked dependence of glycolysis on the availability of various cofactors, and has suggested that the characteristically high rate of glycolysis of tumours might conceivably be due to an abnormal intracellular distribution of necessary cofactors,

including pyridine nucleotides. Carruthers & Suntzeff (1954) and Carruthers, Suntzeff & Harris (1954) have studied the distribution of total pyridine nucleotide in cellular fractions of normal tissues, various transplantable tumours and *p*-dimethylaminoazobenzene-induced cholangiomas. Although the total pyridine nucleotide content of many of these tumours was low, the pattern of distribution among the various cellular fractions was not markedly different from that of normal tissues, although very little, if any, pyridine nucleotide could be detected in the microsomal fraction. We have determined the intracellular distribution of DPN^+, DPNH, TPN^+ and TPNH in normal rat liver (Glock & McLean, 1956) and a few preliminary experiments have indicated that both total DPN and particularly total TPN contents of mitochondria are very low in *p*-dimethylaminoazobenzene-induced liver tumours. This work is being continued.

Previous work on the pyridine-nucleotide content of normal tissues (Glock & McLean, 1955*a*) indicated that the total TPN contents showed a rough positive correlation with levels of activity of the TPN-dependent 6-phosphogluconate oxidative pathway. This correlation does not seem to apply to tumours, however, since glucose 6-phosphate and 6-phosphogluconate dehydrogenase activities of a variety of tumours were found to fall within the range of normal tissues (Glock & McLean, 1954), although all the tumours used in the present investigation had very low TPN contents. Additional evidence indicating an active 6-phosphogluconate pathway in neoplastic tissues is provided by experiments using [1-^{14}C]glucose and [6-^{14}C]glucose (Agranoff, Brady & Colodzin, 1954; Abraham, Hill & Chaikoff, 1955; Emmelot, Bosch & van Vals, 1955; Kit, 1956).

SUMMARY

1. The oxidized and reduced forms of di- and triphosphopyridine nucleotide have been determined in a number of transplantable solid tumours and also in *p*-dimethylaminoazobenzene-induced liver tumours.

2. Although liver from tumour-bearing rats has a normal diphosphopyridine nucleotide (DPN) content, the total triphosphopyridine nucleotide (TPN) content is less than one-half that of normal liver. In liver tumours, however, the concentration of both pyridine nucleotides is considerably reduced, total TPN being affected much more than total DPN.

3. The total DPN contents of the solid transplantable tumours investigated are, in general, lower than those of most normal tissues. The total TPN contents are in all cases very low or negligible.

4. The relative proportions of the oxidized and reduced forms of both pyridine nucleotides are not significantly different in normal and neoplastic tissues.

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