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Apparent increases in tumour NAD^+ levels induced by treatment with vitamin K_1 or its synthetic substitutes

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IT HAS been shown that treatment with Synkavit (tetrasodium salt of 2 methyl 1,4-naphthohydroquinone diphosphate), a synthetic substitute for the natural vitamin K_1, causes a statistically significant rise in the phosphate levels of tumours.[1] Further work has now shown that both vitamin K_1 and Menadione (2 methyl 1,4-naphthoquinone bisulphite) will also cause temporary increases in the phosphate levels of a variety of transplanted tumours. Similar peaks in phosphate levels can be induced by agents, such as nicotinamide or 3 acetyl pyridine, which cause a new synthesis of NAD^+ (nicotinamide adenine dinucleotide, diphosphopyridine nucleotide or coenzyme I).[2] Since new NAD^+ synthesis and increased phosphate levels have been associated with increased radio-responsiveness[2] we have been prompted to examine the effects of vitamin K_1, Synkavit and Menadione on tumour NAD^+ levels as all three compounds are claimed radiosensitizing agents.

All experiments have been carried out with Balb/c or CBA mice aged 12–14 weeks. Animals of either sex were used according to availability. Solid tumours were transplanted in the flanks of animals and only used when they had grown to a volume of 1 cm^3. The ascites tumour was used 5 days after the intraperitoneal transplant of 1×10^6 cells.

Details of the tumours used are given in Table 1.

TABLE 1. TUMOURS USED

Mouse strain	Tumour	Tumour type
Balb/c	NK/Ly/R	Lymphoma (ascitic) Resistant form of Lymphoma NK/Ly
Balb/c	ADJ/PC5	Plasma cell tumour
Balb/c	PL 64	Carcinoma (skin)
Balb/c	H.P.	Harding Passey melanoma
Balb/c	S.180	Crocker sarcoma
Balb/c	Bp 64/12	Sarcoma
CBA	Bp 65/2	Spindle celled sarcoma

Chemicals and dosage (by intraperitoneal injection) were: Vitamin K_1 as Konakion (Roche, Welwyn Garden City, England), 5 mg/mouse; Synkavit (Roche), 2·5 mg/mouse; Menadione (Sigma, London), 2·5 mg/mouse in distilled water at 10 mg/ml.

Measurement of NAD^+ was by the enzymatic method of Klingenberg.[3]

Experimental results with the ascites tumour (Nk/Ly/R) are shown in Fig. 1 and some results obtained with solid tumours in Fig. 2. Each experimental point represents the mean of three separate measurements. Untreated controls have been measured at the same times as the experimental animals

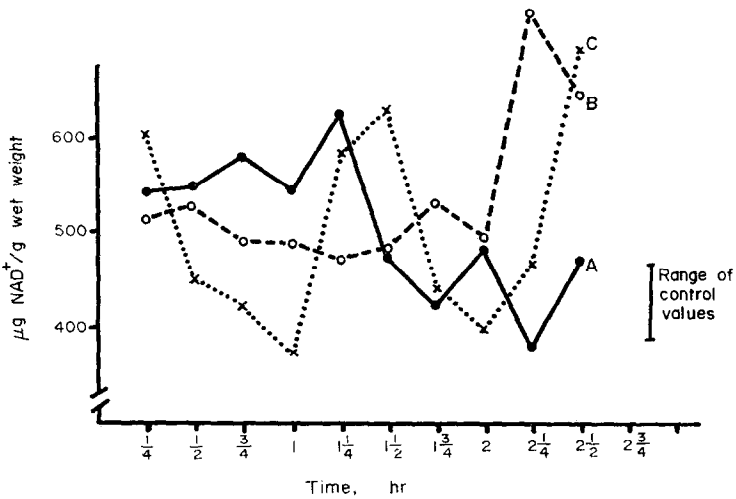

FIG. 1. The response of tumour NK/Ly/R to: Vitamin K_1—curve A. Synkavit—curve B. Menadione—curve C.

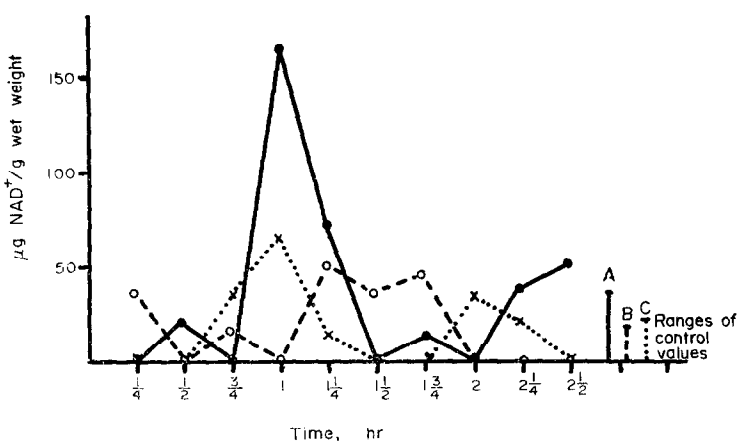

FIG. 2. Response of some solid tumours. Curve A—S.180 and vitamin K. Curve B—PL.64 and Synkavit. Curve C—H.P. and Menadione.

and since one of us (S.M.T.) has shown the occurrence of circadian rhythms in NAD^+ levels the range over which the controls fluctuated during the experiment is shown instead of a mean control value. To illustrate the variation in timing with which the response occurs all results have been tabulated in Table 2.

TABLE 2. THE RESPONSE OF TUMOURS TO VITAMIN K_1 OR ITS SUBSTITUTES

Tumour	Time of maximal response		
	K_1	Synkavit	Menadione
NK/Ly/R	1·25	2·25	1·5
ADJ/PC5		1	
PL.64		1·25	
H.P.			1
S.180	1	2	1
Bp 64/12		1	
Bp 65/2		1	1

It is evident that the response is a major one and not any casual response to the injection of foreign material. Control experiments in which distilled water or normal saline were injected showed no alterations in NAD^+ levels. The time at which the response occurs varies according to the tumour and the eliciting agent used. Repeat experiments have shown that for any one tumour and any one agent the time of response is constant. Again the timing found in these experiments is completely different to that found in experiments with the recognized inducers of new NAD^+ synthesis such as nicotinamide or 3 acetyl pyridine.

At the moment it can only be assumed that it is NAD^+ that has been measured, this being based on the believed specificity of the enzymatic technique used. The origin of this apparent new NAD^+ is unknown. The variable time delays in the response and the suddenness of the response when it does occur would appear to be arguments against any suggestion of the inhibition of normal metabolism of preexisting NAD^+. The formation of a compound based on a naphthoquinone structure also seems very unlikely.

In the light of these findings and the fact that new NAD^+ synthesis is associated with an increase in radioresponsiveness[2] the role of vitamin K_1 or its substitutes as radiosensitizers needs further examination.

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