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SHORT COMMUNICATIONS

The effects of vitamin K_1 and synthetic substitutes on tumour cell sulphydryl levels

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THE USE of the synthetic substitute—Synkavit—(tetrasodium salt of 2 methyl 1, 4 naphtho-hydroquinone diphosphate) of the natural vitamin K as a radiosensitising agent has been considered in detail by Mitchell and Marrian.[1] The activity is envisaged as a reversal of radioprotection by thiol groups, it being proposed that after dephosphorylation the naphthohydroquinone is oxidised to the full quinone (Menadione) which in turn reacts with and removes tumour sulphydryl groups. This was supported by Gronow's[2] finding of considerable falls in non protein sulphydryl (-SH) levels in tumour cells 30 min after treatment with Synkavit.

In the experience of Mitchell[1] and his associates neither Vitamin K_1 nor Menadione (2 methyl 1, 4 naphthoquionone) has proved an adequate radiosensitiser, but Adams[3] has found Menadione to act as a radiosensitiser under anoxic conditions.

TABLE 1. DETAILS OF EXPERIMENTAL TUMOURS

Mouse strain	Tumour	Tumour type
Balb/C	NK/Ly	Lymphoma (Ascites)
Balb/C	ADJ/PC5	Plasma cell tumour
Balb/C	PL/64	Carcinoma (skin)
Balb/C	S.180	Sarcoma
Balb/C	37S	Sarcoma
CBA	Bp 65/2	Sarcoma
CBA	Bp 65/3	Sarcoma
CBA	Bp 65/4	Sarcoma

A more detailed investigation of the effects of Vitamin K_1, Synkavit and Menadione (as the water soluble bisulphite) is now reported. Particulars of the tumours used are given in Table 1. All transplants were in mice of 25–28 g and the tumours were only used when at least 0·5 g of non-necrotic tissue was available.

All three agents were given by i.p. injection, dosages being: Synkavit (Roche, Welwyn Garden City, England) 2·5 mg/mouse; Vitamin K_1 (as Konakion from Roche) 5 mg per mouse and Menadione bisulphite (Sigma, London) 2·5 mg/mouse in distilled water at 10 mg/ml.

-SH measurements were by methods described by Calcutt and Doxey[4] and Calcutt, Doxey and Coates.[5] Since Calcutt[6] showed that diurnal variations exist in tumour -SH levels all experiments were commenced at the same time of day. Further, to minimise any short term variations separate groups of control animals were killed at the same time as every experimental group. All experiments and appropriate controls were in duplicate, the results being averaged.

Measurements were made at 15-min intervals up to 2 hr after treatment and the results are shown in Figs. 1–4. Findings with the ascites tumour have been shown separately (Fig. 1) since in this case the agents were applied directly to the tumour cells and not after transport by the blood stream. For the sake of clarity total -SH figures have been omitted from the diagrams, these data being held in this laboratory. The results are shown as percentage changes against the controls, separate experiments having shown that control injections had no effect on -SH levels.

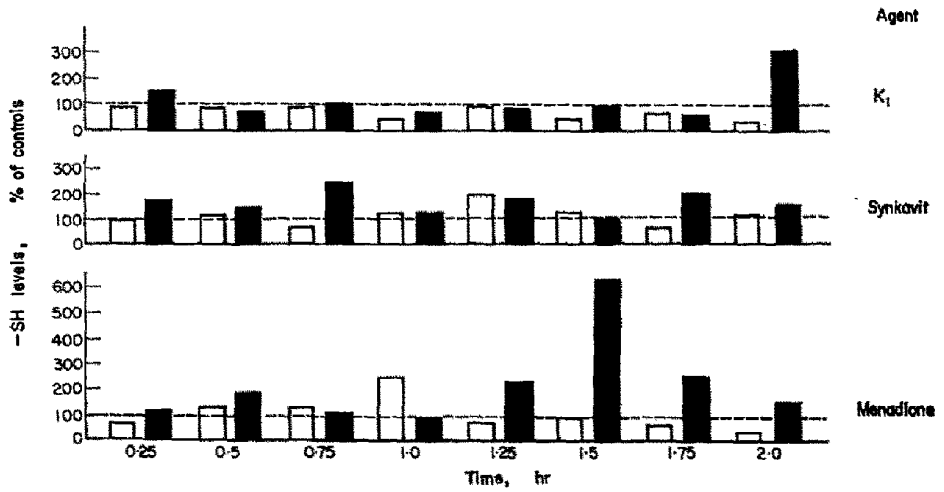

FIG. 1. Changes in -SH levels in the ascites tumour—NK/Ly—after treatment with Vitamin K_1, Synkavit or Menadione bisulphite.
In this and subsequent figures -SH levels are shown as a percentage of the control level at that time.
Open blocks—acid soluble -SH; Solid blocks—protein-bound -SH.

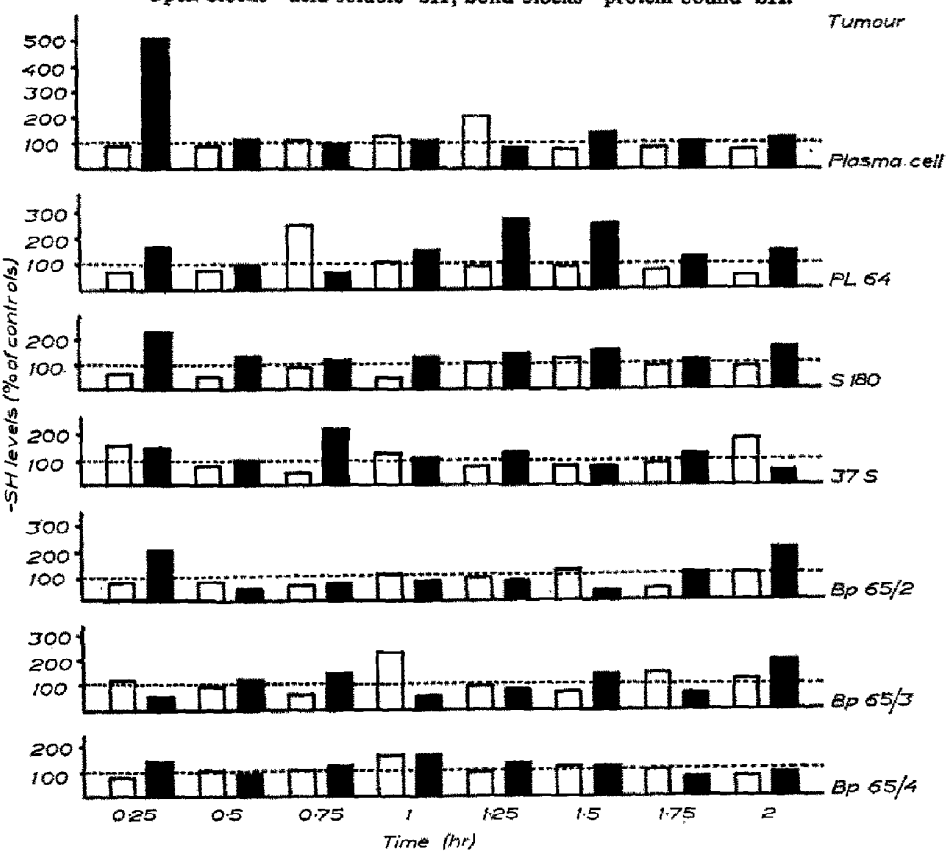

FIG. 2. Changes in -SH levels in solid tumours after treatment with Vitamin K_1.

Inspection of the figures shows considerable changes in both acid soluble and protein bound -SH levels after treatment with all three agents, but there is no evident distinction between the results with the ascites and the solid tumours. Nothing in these results supports the view that Synkavit selectively reduces acid soluble -SH levels ½ hr after injection and thereby increases radiosensitivity. There appears to be little distinction between the results with the three different agents used.

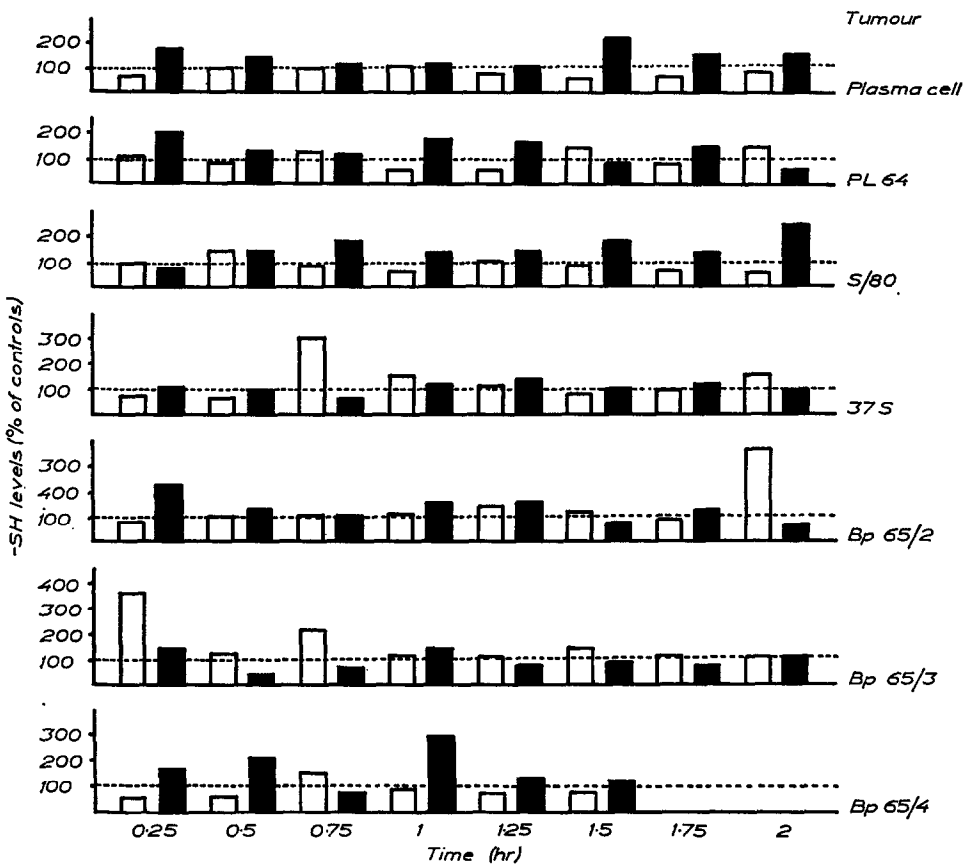

FIG. 3. Changes in -SH levels in solid tumours after treatment with Synkavit.

Careful examination of the Figures shows that a distinct pattern of rise and fall occurs in both protein bound and acid soluble -SH measurements. This occurs with a time interval of 1–1¼ hr between peak and peak or trough and trough. These patterns show no fixed relationship to one another nor to the time base. The smoothness and regularity with which these changes have occurred must be regarded as evidence that they are specific in nature and not the result of random variations in the experimental material. Since separate controls were taken at each time interval these rhythmic changes would appear to be the result of the treatments applied and not the expression of something normally occurring in the tumour.

Concluding, it may be said that Vitamin K_1, Synkavit and Menadione all readily induce changes in the sulphydryl levels of tumours, these changes falling into rhythmic patterns. Nothing in these

changes supports the view that the specific radiosensitising action of Synkavit is mediated via changes in the level of tumour sulphydryl groups.

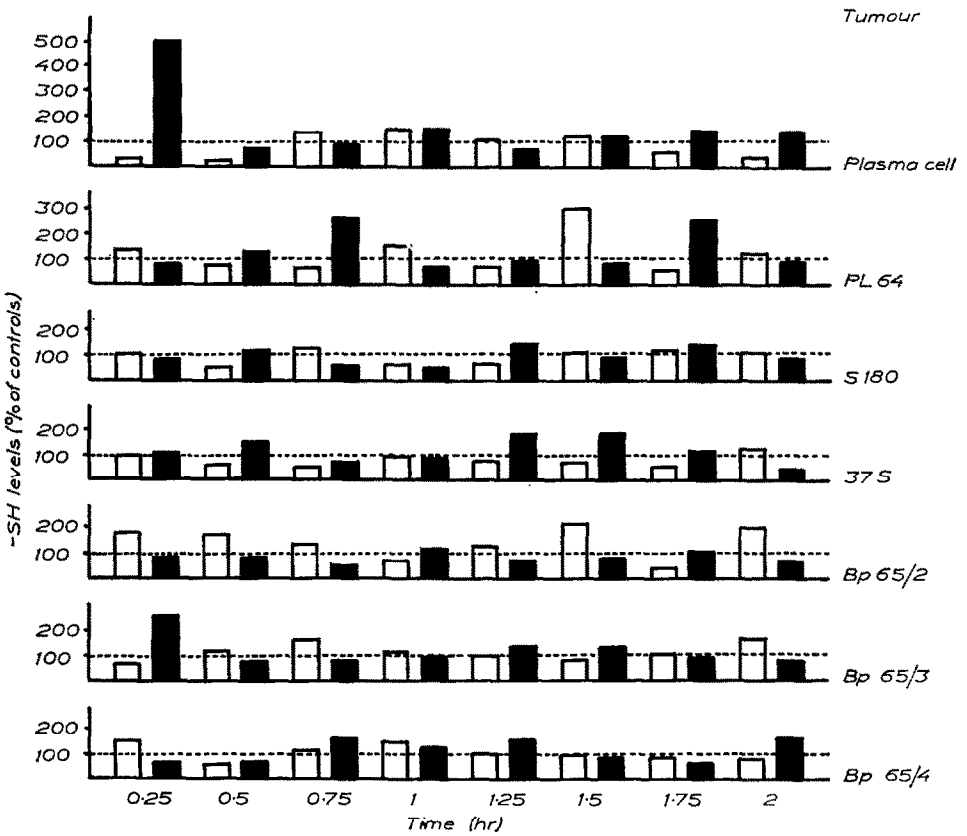

FIG. 4. Changes in -SH levels in solid tumours after treatment with Menadione bisulphite.

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