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THE EFFECT OF CO, KCN AND NaN_3 ON THE NUCLEIC ACID CONTENT OF EHRLICH ASCITES TUMOR CELLS

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Abstract—Ehrlich ascites cells were treated *in vivo* with a 2% aqueous solution of carbon monoxide or KCN or NaN_3. On the ninth day after transplantation the quantity of ascitic fluid, the number and average size of cells and the total packed cell volume (TPCV) were determined. Nucleic acid fractions of the cells were isolated and measured on the basis of their phosphorous content. Smears of the cells were stained with methyl-green pyronin and by the Feulgen method. It was found that after treatment with carbon monoxide the nucleic acid content of the cells increased similarly to that already shown for yeast cells. After treatment with KCN or NaN_3 the nucleic acid content of the cells decreased.

IN STUDIES on the mechanism of chronic carbon monoxide intoxication, we have previously investigated the effect of carbon monoxide, KCN and NaN_3 on the nucleic acid content of cell of *Saccharomyces Italicus Castelli*[1] and on the ability of these cells to decompose glucose.[2] In this paper we have investigated the effects of these three agents on Ehrlich ascites tumour cells with the aim of further elucidating the mechanism of carbon monoxide intoxication.

MATERIALS AND METHODS

Sandy mice 20–24 g/wt. were randomly divided into groups of ten. They were inoculated with 11×10^6 tumour cells taken from animals with eight-day-old tumours. All solutions were freshly prepared. Carbon monoxide was used as a 2% aqueous solution in phosphate buffer pH 6. It was obtained from the reaction with concentrated formic acid and concentrated sulphuric acid washed into sodium hydroxide as previously described.[1] M/1000 NaN_3 and KCN were dissolved in phosphate buffer pH 6. Two dosage schedules were used. Animals were either injected with 2·2 ml of the above solutions i.p. three times during one week or daily for one week. In the second experiments some animals receiving carbon monoxide also inhaled the gas in a chamber for 1 hr daily at a pressure of 1,850 mg/m^3 CO. On the ninth day the animals were decapitated and the ascitic fluids collected. The total cells were counted and the packed cells washed and suspended in physiological saline. The results were expressed in terms of dry weight. Fractionation of the cells was by the method of Schmidt–Tannhauser–Schneider as previously described[1] and the phosphorous content of each fraction was measured by the method of Robison–Martland.

The following fractions were isolated for measurement (1) The total cell suspension, (2) The total nucleic acid fraction, (3) The DNA fraction, (4) The RNA fraction and (5) The intermediate phosphorous fraction (IMP).

The ascitocrit indices and total packed cell volume of each group was determined according to Sassenrath *et al.*[3] The average diameter in microns of the cells was determined and cell smears were stained with methyl-green-pyronin or by the Feulgen procedure.

RESULTS

The phosphorous contents of the various fractions after three treatments are shown in Table 1, where the treated groups are expressed as a percentage of the control group.

TABLE 1

	Control	NaN_3	KCN	CO
I. Total P	100	63·2	49·3	142·7
II. Total nucleic acid NA-P	100	63·9	47·3	114·5
III. DNA-P	100	71·2	43·1	155·9
IV. RNA-P	100	75·7	59·4	101·9
V. IMP	100	57·1	48·4	168·9

These same values expressed on the basis of mg P/g/dry wt. are shown in Fig. 1. The phosphoprotein and the remaining phosphorous after extraction were also measured but as these values proved to be very small they were neglected.

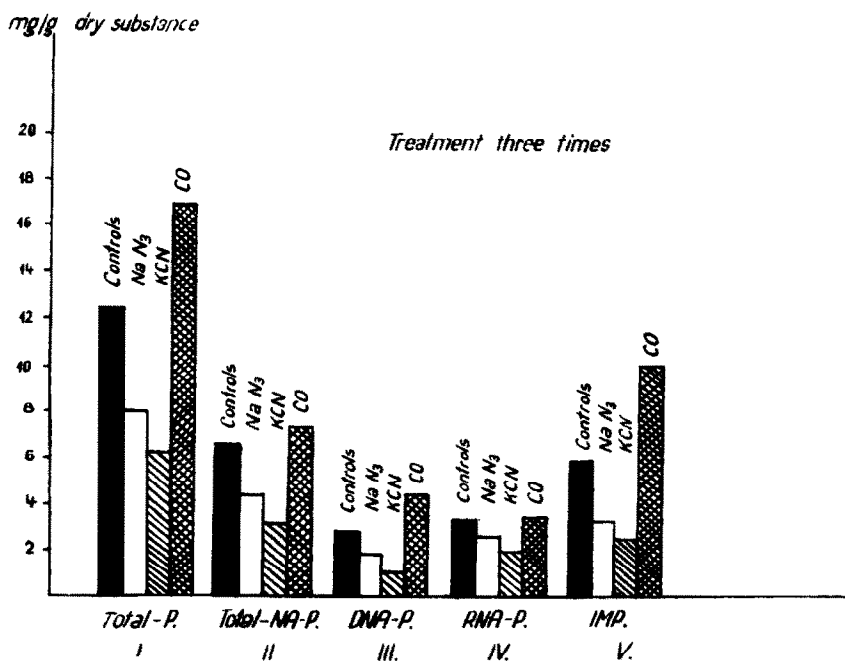

FIG. 1

Fig. 2 shows the values obtained for the various fractions after seven daily treatments. It is evident from this data (Figs. 1, 2) that only carbon monoxide increased the total nucleic acid content. In the experiment where three doses were given, only

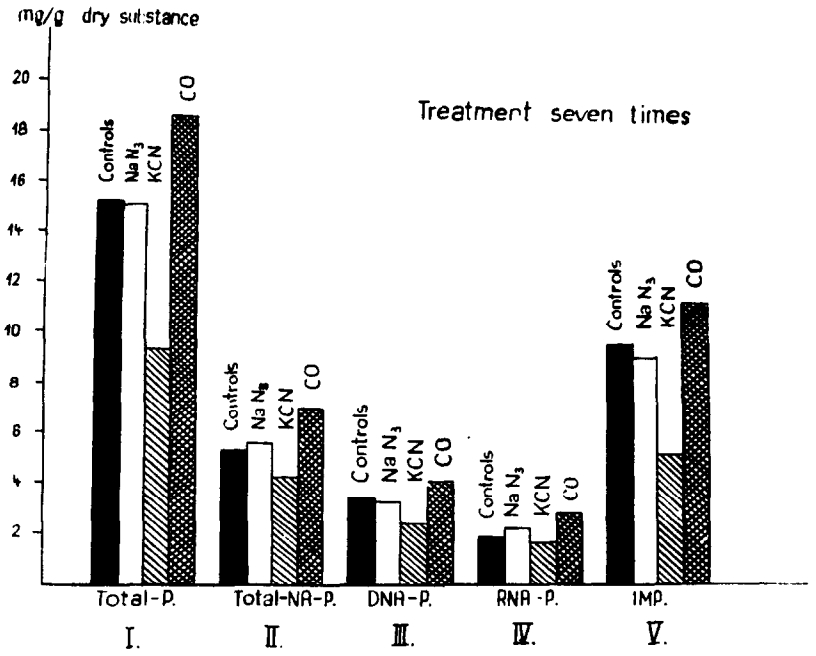

FIG. 2

DNA was seen to increase but in the experiment where seven daily doses were given the RNA increased also. The lowest nucleic acid values were found in animals treated with KCN. After NaN_3 treatment three times the nucleic acid content also fell but after seven daily doses there was little effect.

The changes in the behaviour of the ascites cells found on the ninth day after the various treatments are listed in Table 2. Although the carbon monoxide treated group

TABLE 2. BEHAVIOUR OF THE ASCITES CELLS AFTER DIFFERENT TREATMENTS DURING NINE DAYS

Mode of treatment	Number of ascites cells (mill/ml)	Average diameter of the ascites cells (μ)	Total ascitic volume (TV) (ml)	Ascitocrit	Total packed cell volume (TPCV) (ml)
KCN	63	14·63	12·90	6·2/1·1	2·30
NaN_3	54	14·77	8·80	7·6/1·6	1·85
CO	71		5·50	6·5/1·8	1·52
	81	11·64			1·58
CO injected a. inhalated	91		4·50	7·1/2·6	1·65
Control	68	14·77	7·20	5·8/1·7	2·11

had the lowest packed cell volume they nevertheless had a relatively large total cell number because the cells were smaller in diameter than the other groups despite the presence of 10–12 per cent inflammatory cells. In fact on the second day after treatment with carbon monoxide smaller cells could already be observed while in the NaN_3 treated animals the cells appeared to be relatively larger. Cytotoxicity was marked after treatment with KCN. After NaN_3 treatment the cells were of uniform medium

size with sharp nucleus borders while in the carbon monoxide treated group there were numerous tiny tumour cells with the sharply defined nucleus occupying almost the whole of the cell. Smears stained with methyl-green-pyronin and by Feulgen indicated that the increase of the nucleic acid content of the cells especially the DNA is most pronounced after carbon monoxide treatment and is in agreement with the biochemical measurements.

DISCUSSION

We can conclude that when carbon monoxide is given three times the DNA content increased about 50 per cent ($P = 0{\cdot}05$). After NaN_3 treatment there was little effect or at the most a slight decrease in DNA content while after KCN there was a 20–40 per cent decrease. In the seven daily treatment series the carbon monoxide group showed a greater nucleic acid increase than in the first experiment. One might speculate that since it has been shown that tumour cells contain a ribonuclease inhibitor[4] the carbon monoxide may influence this inhibitor and so lead to an accumulation of RNA.

'Active formate' is N^{10} formyltetrahydrofolic acid and leucovorin, N^5 formyltetrahydrofolic acid, can be converted by ATP to the N^{10} formyl derivative. The one carbon fragment is derived from the metabolism of serine and glycine although histidine and glyoxylic acid can also serve. Active formate is responsible for the formation of thymidine-5'-phosphate from deoxyuridine-5-phosphate. This in fact is the basis of the method of the measurement of DNA synthesis by measuring the incorporation of ^{14}C formate into DNA.[5] It is then a possibility that carbon monoxide can be regarded as a precursor of active formate and so stimulate DNA synthesis. In this connection Swallow[6] has studied the products formed by the irradiation of dilute aqueous solutions of carbon monoxide with CO^{60} γ rays. In the presence of 0·1 N NaOH, formic acid is formed. There is then a possibility that *in vivo* formic acid is formed from the administered carbon monoxide. This is in fact the first time that dilute aqueous solutions of carbon monoxide have been used in animal experiments of this type. In previous experiments such as those described by Warburg the carbon monoxide was administered as a gas.

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