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The analysis of changes in the activation energy of succinate dehydrogenase as influenced by some antitumour agents*

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CHANGES in the activation energy of enzymes may bring about marked changes in the metabolism of cells. Many experiments have been carried out dealing with the inhibitory action of alkylating and related agents on enzyme activity[1] but no data are available concerning closer details of enzymic function under the action of such agents, though studies of such type might considerably contribute to the completeness of our concept of cytostatic action which is still unknown at the molecular level as pointed out by Wheeler.[1] Succinate dehydrogenase was chosen for such studies because it had been thoroughly investigated in cancer research and because its activity can be readily determined.[2]

EXPERIMENTAL AND RESULTS

The activity was determined in triplicate using liver homogenates of normal untreated mice[2] at 5 different temperatures, 42, 37, 32, 27 and 22°. Three experiments were made with each substance on 3 different days using the pooled livers of 2 male 'Swiss' mice fasted overnight. 1,000 μg test substance were used per 10 mg wet tissue per ml.

Donor mice were anaesthesized by ether, the chest opened so as to avoid dissection of the internal mammary vessels, right auricle incised and the left ventricle cannulated. The cannula was attached by rubber tubing to a Mariotte flask which was filled with warm physiologic saline and mounted so as to be able to perfuse with an approximate hydrostatic pressure of 120 cm water. After a 3-min perfusion the livers grew maximally pale.

The energy of activation was calculated from 2 velocity constants, obtained at 22 and 42°, from the Arrhenius plot, using the following formula:

$$E = \frac{2{\cdot}303\ (\log k_2 - \log k_1)R}{1/T_1 - 1/T_2}$$

where E is the energy of activation per g mole, k is the specific reaction rate, R is the gas constant per g mole and T the absolute temperature. For details of the calculation see West.[3]

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The activity of the following substances was investigated: Sodium arsenite* (British Drug Houses Ltd.), cyclophosphamide (Endoxan, Asta Werke GmbH), Nitrogen mustard (Merck, Sharp and Doehme Research Laboratories) and mannitol mustard (Degranol, Chinoin Budapest).

The results of each experiment are represented in Table 1. Enzyme activity was given as logarithm of the reciprocal of the decolorization time of methylene blue expressed in seconds. T/C indexes represented in Fig. 1. are products of the enzyme activity obtained in the presence of the test substance (T) and of enzyme activity in the presence of a control solution (C). Latter is to correct for ionic strength deviation due to the neutralization of the test substance.

It can be seen from Table 1 that sodium arsenite causes a slight decrease of activation energy in each case. This decrease means a 14 per cent change. Nitrogen mustard caused a more marked decrease while cyclophosphamide and amnnitol mustard resulted in no change of the activation energy.

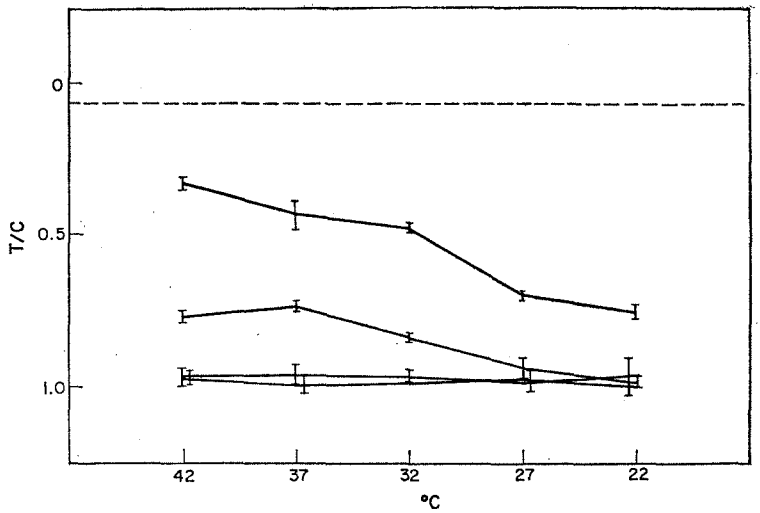

FIG. 1. Effect of various cytostatics on the activity of liver succinate dehydrogenase; 1000 μg drug and 10 mg wet liver tissue per final ml.

T/C indexes are products of the enzyme activity obtained in the presence of the test substance (T) and in the presence of a control solution. Control solution serves to give compensation for ionic strength deviation due to the neutralization procedure in the tests. Enzyme activities are expressed as log of the reciprocal of the decolorization time of methylene blue in secs.

$\frac{T}{\perp}$ are standard deviations $\left(\text{S.D.} = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}\right)$

T/C indexes of the enzyme activities clearly demonstrated that inhibitory substances did not modify this enzyme out of the physiologic or nearly physiologic temperature range. The inhibitory action of these drugs approaches zero at lower temperatures (see Fig.1).

DISCUSSION

In quest for newer and more exact screening methods, formerly we devised a simple test which permitted the investigation of potential anti-tumour agents by a biochemically defined approach. It was demonstrated that succinate dehydrogenase was influenced differently by substances belonging to groups of various types of activity, i.e. one could find inhibitory as well as non-inhibitory substances among alkylating agents, while inhibition could also be found by general cytotoxic substances and antimetabolites.[2]

* Instead of potassium arsenite.

Many experiments were carried out concerning inactivating effects of various alkylating agents on various enzymes but the effect of such drugs on activation energy was not subject of any detailed investigations yet, though if they occurred, such changes might be responsible for alterations in the biochemistry of cells.

TABLE 1. CHANGES IN THE ACTIVATION ENERGY OF LIVER SUCCINATE DEHYDROGENASE AS INFLUENCED BY CERTAIN ANTITUMOUR AGENTS

Compounds	Ass. No.	Activation energy in g cal/mol				% change		
		Controls		Tests				
Sodium arsenite	I	17,040	16,160* ± 930	14,060	13,900* ± 835	−17	−13·7 ± 1·7	$t = 8{\cdot}059$ $0{\cdot}01 < P < 0{\cdot}02$
	II	17,140		15,260		−11		
	III	14,300		12,380		−13		
Nitrogen mustard	I	14,690	15,730 ± 570	5,800	7,330 ± 869	−60	−53·3 ± 3·7	$t = 14{\cdot}405$ $P < 0{\cdot}01$
	II	16,660		8,810		−47		
	III	15,830		7,390		−53		
Cyclophosphamide	I	13,370	12,860 ± 404	12,470	12,570 ± 369	−7	− 2·3 ± 2·0	$t = 1{\cdot}150$ $P > 0{\cdot}05$
	II	12,060		11,980		−1		
	III	13,150		13,250		+1		
Mannitol mustard	I	11,100	13,620 ± 1266	10,970	13,280 ± 1170	−1	− 2·0 ± 0·6	$t = 3{\cdot}333$ $P > 0{\cdot}05$
	II	14,660		14,130		−3		
	III	15,100		14,750		−2		

* Resulting each from 9 determinations per each degree of temperature in 3 assays on 3 different days. All values are means ± standard error of the mean (S.E.) P and t values concern significance of the difference from 0%.

The present experiments showed that some antitumour agents cause a decrease in activation energy while others are without effect. The most pronounced decrease was with nitrogen mustard while sodium arsenite caused only a small decrease in activation energy. Conversely, mannitol mustard, known to be approximately 50-times less toxic,[4] but not less effective than the parent drug nitrogen mustard, did not influence activation energy. Similarly, no change was produced by cyclophosphamide another alkylating agent, though it must be added that this latter agent possesses no cytostatic activity unless first degraded to the active compound by enzymic attack *in vivo*.[5, 6] It cannot be definitely stated whether this difference between active and transport forms might be responsible for the difference of action, since this transformation takes place in the liver microsomes.[7] It can be concluded for the substances investigated that a cytostatic effect does not necessarily lead to changes in the activation energy of this enzyme, but if an inhibition of the enzyme activity was encountered, it was always parallelled by a similar decrease in activation energy.

Departments of Physiology and Biochemistry,
Medical University of Szeged,
Hungary

G. A. DOMBRADI
IRENE CSÁNYI
GY. DOMJÁN

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