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THE ACTION OF SOME CYTOSTATICS ON THE HEXOSE MONOPHOSPHATE SHUNT AND GLYCOLYSIS IN EXPERIMENTAL RAT TUMOURS

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Abstract—The effect of the currently used cytostatics on the glycolytic pathway EMKP and the hexose monophosphate shunt (HMP) using ^{14}C-labelled glucose was investigated. Typical alkylating agents inhibited both these metabolic pathways. Mitomycin C and Trenimon—both quinone derivatives—stimulated HMP, while simultaneously depressing EMKP. Antimetabolites are inactive on both metabolic pathways of glucose. Mitomycin C and Trenimon were influenced in their action on HMP by some enzyme inhibitors in a very similar way as are known electron acceptors. The results point to the possibility that the mechanism of action of Trenimon and Mitomycin C on HMP might be due to the electron-acceptance capacity of these drugs.

IT IS well established that the metabolic pathways of tumour tissues differ from those of normal tissues by increased aerobic glycolysis (Warburg, 1926). It has been shown that several cancerostatically active alkylating agents, apart from the inhibition of biosynthesis of nucleic acids and proteins and the blockade of reactive groups of important enzymes, for example, -SH groups, have an inhibitory effect upon the glycolysis of tumour tissue.[5–10]

There are at least two main pathways for glucose metabolism in tumour tissue, *viz.* the Embden–Meyerhof–Krebs glycolytic pathway (EMKP) and the hexose monophosphate shunt (HMP).[1, 2] The HMP is energetically less important but by this pathway it proceeds the *de novo* synthesis of ribose, indispensable to the formation of nucleosides and nucleic acids, the biosynthesis of $TPNH^-$, necessary to the reduction synthesis of fatty acids, proteins as well as some of the hormones of the steroid group.[1, 3, 4]

Recently investigation of the action of cytostatic drugs upon the carbohydrate metabolism in tumour tissue has been in the foreground. Most of the papers dealt with the action of cytostatic agents on the glycolysis. In this study we are investigating the effect of some clinically useful cytostatic drugs on the two main metabolic pathways of glucose, i.e. on EMKP and HMP.

MATERIALS AND METHOD

The cytostatics used in the present work, Endoxan, Mitomen, TS-160, Thio-tepa, Mytomycin C, †Trenimon, 6-Mercaptopurine, 6-Azauracil riboside, are currently

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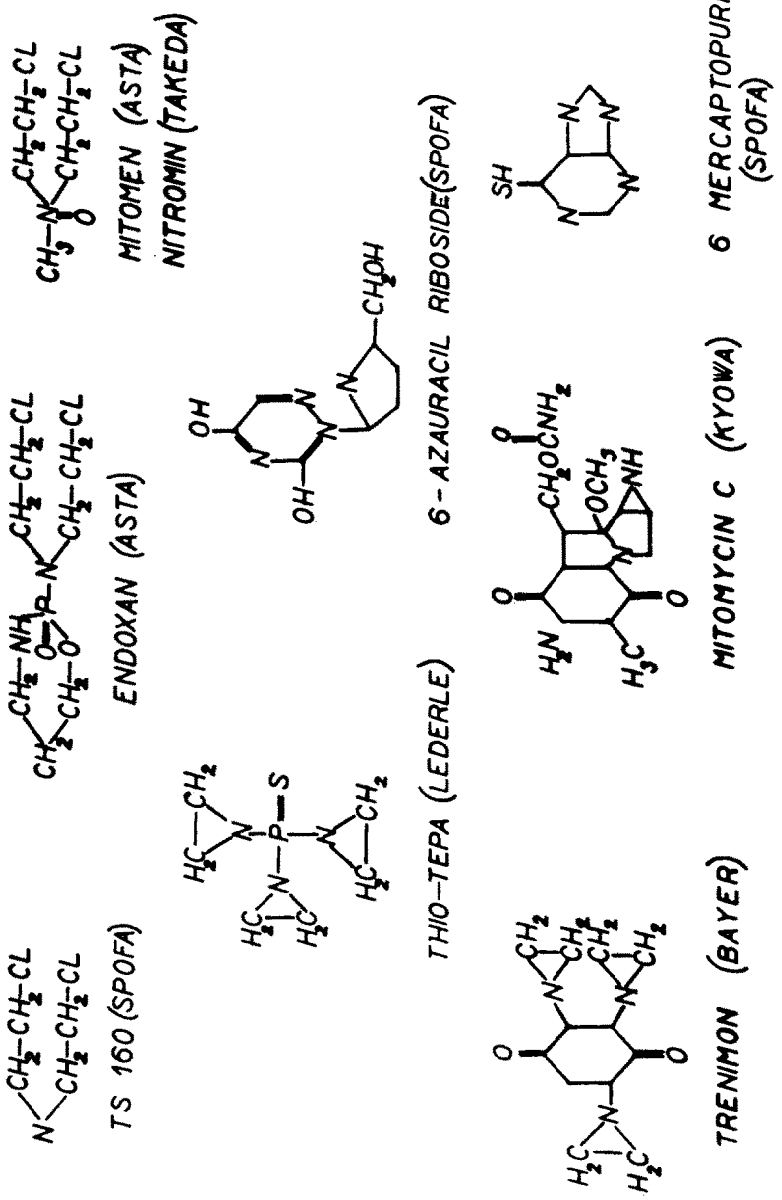

FIG. 1. The chemical structure of cytostatics used in present work.

used in therapy at the clinical departments of our Institute. The chemical structure of the cytostatics used is illustrated in Fig. 1.

In the experiments two types of rat sarcomas were used: *viz.* the BS tumour (21) and a tumour induced by Ferridextran in this Institute. Both the tumours grow subcutaneously in Wistar rats and are maintained by transplantation. The effect of cytostatic agents upon the HMP and glycolysis was studied in slices of viable parts of the tumours by *in vitro* incubation.

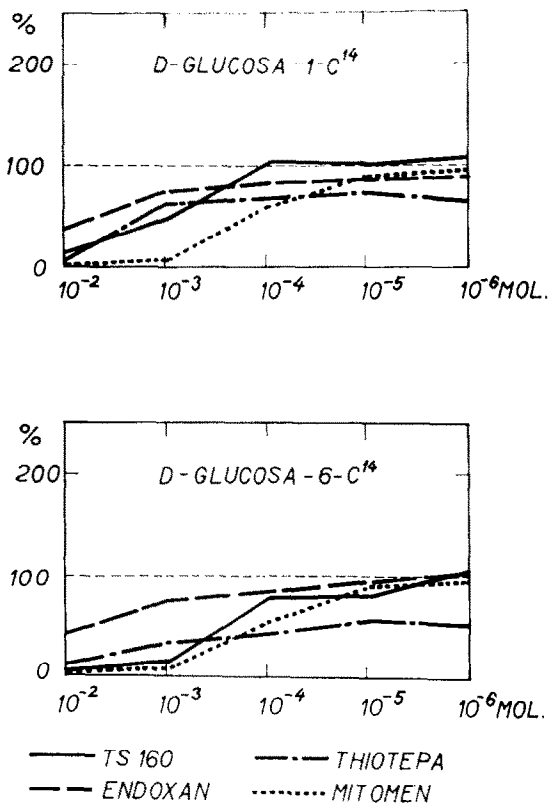

FIG. 2. The action of typical alkylating agents on glucose carbon 1 and glucose carbon 6 oxidation by tumour slices. The effect of cytostatics is expressed in per cent of the values obtained for the control. The parallel decrease of radioactivity released from glucose-1-^{14}C and -6-^{14}C proves the depression of EMKP.

Incubation was carried out in 25 ml Erlenmeyer flasks with a central well. The basic incubation medium contained 2,6 ml Krebs–Ringer–Phosphate buffer of pH 7,5; 3,2 mg bovine albumin (Armour Ltd.), 3,6 mg non-radioactive glucose, and radioactive glucose labelled with the radioisotope ^{14}C in the 1st or 6th positions (Radiochemical Centre, Amersham) with an activity of 0,8 μC per l. incubation flask. The basic incubation medium, as described above, was added with cytostatic after tissue slices of about 50 mg wet weight had been placed in it. The added volume of each of the cytostatics and their concentration was adjusted so that the resulting concentration in the flasks was 10^{-2}–10^{-6} M. The flasks were incubated for two hours in a

Dubnoff metabolic shaker under oxygen atmosphere at 37°C. The method of determining the radioactive yield of $^{14}C\ O_2$ and the evaluation of the results have already been published.[19, 22] The final values (mean of 8–10 close measurements) we expressed in the percentage of control values, obtained without addition of cytostatics, and these were considered as being 100 per cent. Both pathways of glucose metabolism were evaluated on the basis of the generally admitted presumption that the $^{14}C\ O_2$ radioactivity released from glucose-6-^{14}C is a measure of glycorespiration while the radioactivity released from glucose-1-^{14}C comprises both metabolic pathways. Thus, the metabolic efficacy of HMP can be inferred from the difference between these two pathways.

RESULTS

By the effect on EMKP and HMP the cytostatics investigated can be classified into three groups: Figure 2 illustrates the effect on the oxidation of the first and the sixth carbon of glucose of the typical alkylating agents–Endoxan, Mitomen, TS-160, and Thiotepa, which are added in concentrations of 10^{-2}–10^{-6} M. From these results it is clear that this group of alkylating cytostatics inhibits the glucose metabolism in both the pathways. The finding of depressive effect on glycolysis or glycorespiration is in keeping with literary data.

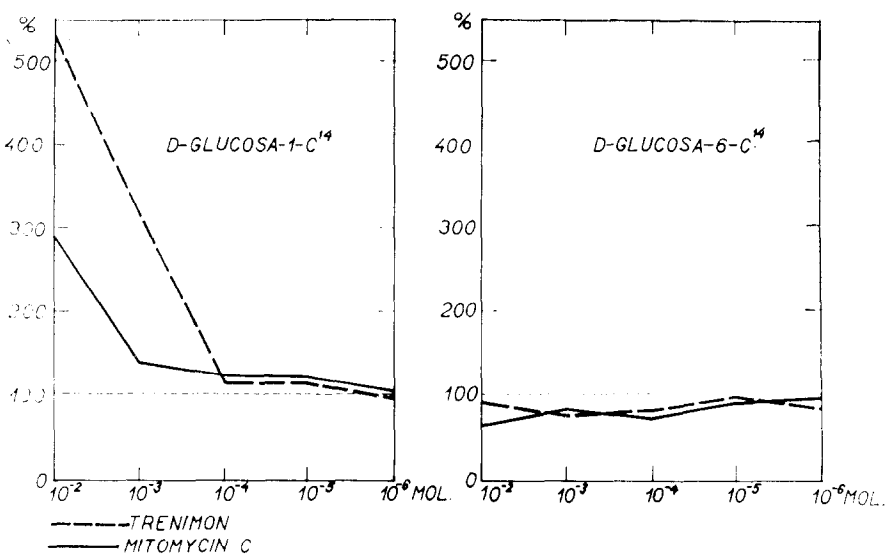

FIG. 3. The action of Mitomycin C and Trenimon on glucose 1 carbon and glucose 6 carbon oxidation by tumour slices. The effect of the cytostatics is expressed in the same way as in the above chart. The increase of activity released by oxidation of glucose-1-^{14}C proves the selective stimulation of HMP.

However, the effect of Mitomycin C and Trenimon is interesting. These substances are both quinone derivatives, Trenimon being an ethylene imino derivative, while Mitomycin C is an antibiotic (from *Streptomyces verticillatus*). As can be seen in Fig. 3, the oxidation of the first carbon of glucose is potently enhanced and at the same time the oxidation of the sixth carbon of glucose is depressed by these

substances; this points to a stimulation of HMP. Figure 4 shows the failure of the cytostatics of the antimetabolite series on the metabolic pathways of glucose. The cytostatic effect of these substances consists in a competitive-inhibitory interference in nucleic acid synthesis. The partial depression of glucose metabolism at high concentrations of antimetabolites is ascribed to the toxic action of these drugs.

It is known that HMP can be increased also by other compounds whose mechanism of action consists of acceptance of electrons and thus forming TPN^+. In order to

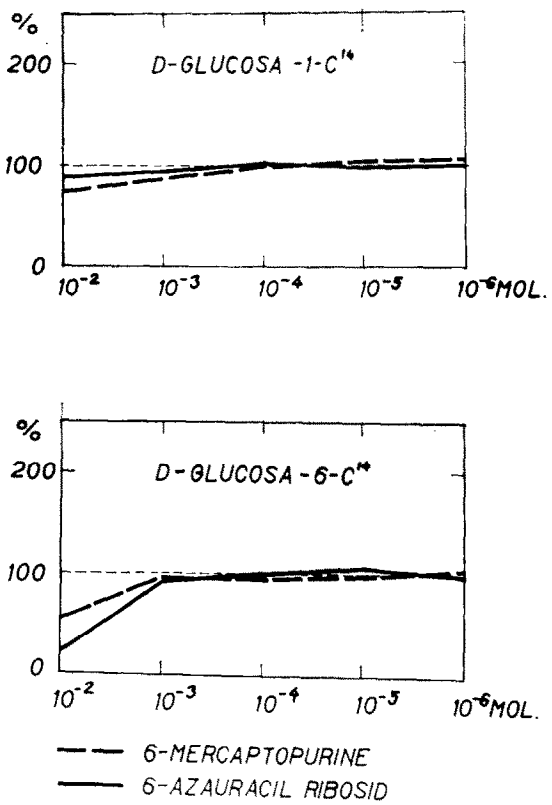

FIG. 4. The action of 6-Azauracil riboside and 6-Mercaptopurine on glucose carbon 1 and glucose carbon 6 oxidation. Results are expressed in per cent of the values obtained for the control flasks. These drugs are ineffective, the depressive activity is seen at high concentrations only.

ascertain whether Mitomycin C and Trenimon had a similar effect we also tested the effect of some known artificial electron acceptors on HMP. The compounds used were: methylene blue, Synkavit, pyruvate and l-epinephrine. As can be seen from Table 1, all these compounds increase HMP. As a check we used the inhibitory effect of known enzyme inhibitors (iodoacetate, 2,5-dinitrophenol, KCN) on the stimulation of HMP by Mitomycin and Trenimon as well as by the electron acceptors mentioned above. By all these inhibitors the stimulatory effect of cytostatics and electron acceptors was affected in the same way.

TABLE 1. COMPARISON OF THE STIMULATING EFFECT OF MITOMYCIN C AND TRENIMON ON THE OXIDATION OF CARBON 1 AND 6 OF GLUCOSE WITH THE EFFECT OF SOME KNOWN ELECTRON ACCEPTORS. THE ACTION OF IODO-ACETATE, 2,5-DINITROPHENOL AND KCN ON THIS EFFECT.

	Control	Iodoacetate 10^{-3} M	KCN 10^{-4} M	2-5 Dinitrophenol 10^{-5} M
Control	100	17	44	90
Mitomycin C 10^{-4} M	135	28	67	108
Trenimon 10^{-4} M	153	27	72	112
Pyruvat 10^{-4} M	195	23	68	164
Methyl blue 10^{-4} M	374	31	64	332
Synkavit 10^{-5} M	187	23	87	155
L-Adrenaline 10^{-3} M	168	23		

DISCUSSION

The relation between the concentration of cytostatics and the amount of effect was studied after 2 hr incubation. A time course of this effect was not studied after incubations at shorter periods than 2 hr, therefore the possibility of non-linearity of this effect during incubation with different agents must be taken into consideration.

The inhibitory effect of the alkylating cytostatics investigated by us on the EMKP of glucose metabolism is described in literature and is interpreted by the blocking of hexokinase,[6, 7, 9, 17] phosphoglyceraldehyde dehydrogenase,[9, 15] succinic dehydrogenase,[6] and DPN^+ synthesis,[5, 15–17] More recently, it has been supported that $DPNH^-$ is blocked by a mechanism similar to the Crabtree effect. In this the utilisation of inorganic phosphate and the phosphate acceptor is blocked, and this leads to the inhibition of the electron transfer from $DPNH^-$ to the cytochrome b.[11, 12] Our results, too, argue in favour of an inhibitory effect of alkylating cytostatics on hexokinase, as they show that these substances brought a simultaneous depression of glucose-1-^{14}C and -6-^{14}C oxidation. The data found in literature seem to indicate that in reality this effect is a complex one.

Two cytostatics—Mitomycin C and Trenimon, both quinone derivatives—have proved to stimulate HMP intensively. However, the activation of this metabolic pathway of glucose metabolism by these cytostatics is a new hitherto undescribed effect, and the question of its mechanism arises.

Recent investigations have proved that HMP is responsible for the formation of reduced $TPNH^-$; important for reduction syntheses. The oxidation of the first carbon of glucose via HMP depends on the oxidated TPN^+ supply, which is the rate-limiting reaction of this metabolic pathway. $TPNH^-$ dehydrogenation proceeds through reduction syntheses, or directly, using some electron acceptors, such as pyruvate. Beside this natural electron acceptor several artificial electron acceptors have been described, such as methylene blue, epinephrine, and some quinone derivatives, such as Synkavit (vitamin K), Menadione, Plumbagin etc.[1, 13, 19] Electron acceptance is a common feature of quinones and, therefore, we considered this mechanism of action on glucose-1-^{14}C oxidation as most probable also in the case of Trenimon and Mitomycin C. This effect was observed before the chemical structure of Mytomycin C was published.[18] The structural formulae of both the compounds show (Fig. 1), that both these substances are in agreement with the presumption of Hoskin[13] which regards

as the condition of the electron-acceptance capacity the presence of the methyl group, or of some other short alkylating group, in the position 2 (or 3) and also the presence of a methyl group or a side ring in the position 5 and 6 of the basic quinone nucleus. The mechanism of electron-acceptance is supported also by our experiments. They confirmed that enzyme inhibitors inhibit the stimulating effect of the above mentioned cytostatics on HMP just as they inhibit some known electron-acceptors of quinone character. (Table 1).

There seems to exist one more possibility of increasing the TPN^+ supply. No attempts have been made to verify this possibility in the present work. Dallner and Ernster[12] have shown, that vitamin K_3 can establish an electron shunt between $DPNH^-$ and cytochrome b. This shunt was shown to involve the pyridine nucleotide non-specific flavoenzyme, DT diaphorase, which can act as $DPNH^-$ and $TPNH^-$ dehydrogenase. Even though the shunt effect of vitamin K_3 is specific—in view of the non-specificity of DT diaphorase—one could presume the existence of a shunt between $TPNH^-$ and the cytochrome system in the presence of a suitable substance. Mitomycin C and Trenimon could participate in such a shunt.

With the tumours used, the effect of Mitomycin C and Trenimon on HMP is marked in the concentration range of 10^{-5}–10^{-4} M, and has been confirmed also in ascitic cells and in other experimental rat tumours. With regard to these relatively higher concentrations, further investigations will be necessary to verify the possibility that this effect takes place also *in vivo*. This could represent a new, hitherto unknown factor of the cytostatically active substances.

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