A Note on the Action of Tannin upon Tumour Glycolysis

By A. LASNITZKI, *Department of Pathology (Cancer Research Laboratory),
Medical School, University of Birmingham*

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Considerations dealing with the relation between cell proliferation and carbohydrate metabolism (particularly that of glucose), as well as with certain effects of variation in the water content of growing tissue, have suggested that the intensity of carbohydrate breakdown in proliferating cells is largely dependent upon the degree of hydration of corresponding enzyme proteins, in such a manner that, within limits, an increase in hydration stimulates and a decrease inhibits enzymatic activity (Lasnitzki, 1928, 1945). This assumption is supported by the results of investigations on the influence of hydrating and dehydrating ions upon glycolysis and related processes (see Lasnitzki, 1945).

Some further evidence in favour of this assumption, obtained in a different way, is recorded in this note. It was felt desirable to carry out similar investigations with substances whose mode of action differed from that of the electrolytes, although under such conditions research might be confined mainly to the study of the effects of dehydration. The most suitable agents appeared to be represented by the group of vegetable tannins, having regard to the available evidence of their dehydrating power, in low concentrations, upon proteins and other hydrophilic colloids (Kruyt, 1922; Bungenberg de Jong, 1923, 1924), irrespective of the nature of the underlying chemical changes. Experiments on the action of a tannin upon the anaerobic glycolysis of tumour tissue were thus undertaken, and ordinary tannic acid was chosen as the agent to be tested. A transplanted rat tumour, Jensen sarcoma, served as material.

EXPERIMENTAL

Methods

The preparation of the tumour tissue and measurement of glycolysis were carried out according to Warburg (1930). The tissue slices, obtained from the peripheral (non-necrotic) zone of the tumour, were placed in Ringer's solution which contained, apart from NaCl and KCl in the usual concentrations, 0.025 M-bicarbonate and 0.2% glucose, but was free of Ca in order to avoid formation of sparingly soluble calcium tannate. A similar Ringer's solution (1.0 ml.), but of double concentration, was then pipetted into each of two metabolism vessels. To the fluid in vessel 1 was added 1.0 ml. of distilled water to make up 2.0 ml. of control Ringer's solution; while the fluid in vessel 2 was similarly mixed with 1.0 ml. of a dilute, freshly made solu-

tion of tannic acid in distilled water, a commercial tannic acid preparation of high purity being employed. Within a few seconds the tissue slices were immersed in the media thus prepared and the latter were immediately saturated with about 5% CO₂ in N₂ (pH ~7.4). This procedure prevented the partial oxidation of tannic acid in the Ringer's solution during the preparatory period of the experiment. The measurements were performed at 37.5°.

Results

The results of four experiments, in which the effect of 0.1% tannic acid was tested, are given in Table 1. It will be seen that the rate of glycolysis

Table 1. *Effect of 0.1% tannic acid on the glycolysis of Jensen rat sarcoma*

Exp. no.	Glycolysis, Q _O ^N					
	Ringer's solution (control)		Ringer's solution + 0.1% tannic acid		Inhibition by tannin (%)	
	First 30 min.	Second 30 min.	First 30 min.	Second 30 min.	First 30 min.	Second 30 min.
1	26.3	—	16.2	—	38	—
2	30.8	28.0	22.8	13.6	26	51
3	26.8	22.4	17.8	12.9	34	42
4	27.2	22.8	17.1	13.0	37	43

Under conditions similar to the above, tannin-containing Ringer's solution, without tissue, showed a minute but continuous increase in manometric pressure, probably due to CO₂ liberation indicating a slight hydrolysis of tannic acid. In calculating the rates of glycolysis, this extra CO₂, amounting to 2-3 μl./ml. of solution/hr., was allowed for in nos. 2-4, but not in no. 1. In nos. 3 and 4 the bicarbonate content of the Ringer's solution to which the tannic acid had been added was increased to 0.028 M, in order to compensate for the loss of bicarbonate caused by that addition (amounting to about 10%).

in the tannin-containing medium was clearly decreased as compared with that in the control medium. In Exps. 3 and 4 the percentage inhibition was little different during the first and second 30 min. of observation, but in Exp. 2 the inhibition during the latter interval was about twice as great as during the former. The average inhibition of glycolysis was approximately 40%. Exps. 3 and 4, which were made on slices from the same tumour, showed a fair agreement in corresponding values; the average course of lactic acid formation in these

two experiments, for the control and the tannin-containing medium, is illustrated in Fig. 1. In a further experiment, in which the tannic acid content of the medium was raised to 0.2 %, the inhibition of glycolysis was about 60 %. It is evident, therefore, that tannic acid, in low concentration, has a marked inhibitory effect upon the glycolytic activity of tumour tissue. It is to be noted that the rate of glycolysis in the control medium (see

Fig. 1. Glycolysis of Jensen rat sarcoma in Ringer's solution, with and without addition of tannin (0.1 % tannic acid).

specially Exps. 1, 3 and 4) was here somewhat lower than that normally observed, owing to the use of calcium-free Ringer's solution, the lack of calcium causing an outward diffusion of potassium from the tumour cell, which in turn decreases glycolysis to some extent (Lasnitzki, 1938).

At the end of the experiments the tissue slices removed from the tannin-containing medium were somewhat turbid in appearance as compared with the fairly transparent slices removed from the control medium.

DISCUSSION

The process of 'protein tanning' very likely consists in a chemical combination of tannin with the protein concerned, the nature of this combination being not yet fully understood. The protein molecule thus changed shows physical properties essentially different from those of the normal protein molecule, and the most important of these alterations appears to be its partial or total conversion into a hydrophobic colloid. It is an obvious assumption that the series of enzyme proteins involved in the anaerobic breakdown of glucose by the tumour cell will, under the influence of tannic acid, be altered in a similar manner, and there is a probability that not the chemical change *per se*, but the associated dehydration of the enzyme protein molecule is the factor responsible for the inhibitory effect of tannic acid upon that process. This view agrees with the supposed connexion between enzymatic carbohydrate breakdown in proliferating cells and the degree of hydration of corresponding enzyme proteins, as is substantiated by the investigations already mentioned on the action of ions.

The experiments described, which were carried out some time ago, have a bearing on more recent investigations by Powell (1944), who found that the growth of a transmissible mouse carcinoma could be strongly inhibited by tannic acid, among other substances, when a low concentration (especially as sodium salt) was administered continuously by intraperitoneal injection. These findings are in agreement with the result that tannic acid, in low concentration, is likewise able to cause a definite inhibition of tumour glycolysis.

SUMMARY

The anaerobic glycolysis of tumour tissue (Jensen rat sarcoma) can be markedly inhibited by tannic acid in low concentration.

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