EFFECT OF TRANSPLANTED TUMOUR ON THE PYRIDINE NUCLEOTIDE SYNTHESIS IN THE MOUSE LIVER

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It is well known that the level of pyridine nucleotide (PN) is generally very low in actively growing tissues such as tumour or foetal tissues (1–8). It has been suggested that the decrease of PN level in these tissues may be caused by a diminished rate of the PN synthesis rather than by an increase of its destruction (8). On the other hand, a marked increase of the PN content has been observed in the mouse liver following the injection of nicotinamide which is acting thereby as a precursor substance of PN (9, 10). In a previous paper, the author has reported that the intrasplenic implantation of a nicotinamide pellet is very effective in elevating the PN level of normal and regenerating rat liver and that the numbers of mitoses are thereby greatly reduced (11). It has also been reported that the decrease of the PN level is a general occurrence in livers of the tumour-bearing animals, indicating that there may be a sort of hormonal intervention (12). It is possible that the adrenal, especially adrenocortical hormones are involved in such hormonal intervention, since the presence of a tumour may act as a stressor. In the present study, the capability of PN synthesis in livers of tumour-bearing mice was tested by injecting nicotinamide, and furthermore the effect of cortisone acetate on the PN level in these mice was examined.

MATERIALS AND METHODS

Male albino mice weighing from 15 to 20 g were used as material throughout the experiments, and Ehrlich ascites tumour was exclusively used, being intraperitoneally transplanted. One or two weeks after the transplantation of the tumour, animals were sacrificed and examined for the PN level, since tumour cells undergo a rapid growth during this period.

Nicotinamide (1,000 mg/kg of body weight) was administered intraperitoneally or subcutaneously 10 hours before the sacrifice. In experiments in which cortisone acetate was used, 100 micrograms of it were injected subcutaneously for two days, the last injection being performed 15 hours before the sacrifice.

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The PN content of the liver and tumour cell was determined by the method of Feigelson, Williams and Elvehjem (13) with a slight modification. Animals were decapitated and the livers were quickly removed and about 300 mg of the tissues were weighed on a torsion balance before the determination of PN. The details of the procedure have already been described in the previous report (11), but in the present experiment, 100 mg of acid-washed and dried Norit SX 30 were used to adsorb PN.

Another piece of the liver weighing about 200 mg was used for the determination of protein N, this being done by the micro-Kjeldahl method, and the values obtained served as the basis of calculation of PN level. The PN was calculated as diphosphoryridine nucleotide (DPN).

For the determination of PN in tumour cells, the ascites was quickly pipetted out before the removal of the liver, and transferred into a centrifuge tube which had been placed in an ice-bath. Then, about 2 volumes of ice-cold isotonic NaCl solution were added and centrifuged at 500 r.p.m. for 5 minutes to separate the red blood cells. To the precipitated tumour cells were added about 3 volumes of ice-cold isotonic NaCl solution and centrifuged at 3,500 r.p.m. for 2 minutes to remove the ascites. About 2 ml of washed tumour cells were suspended in an ice-cold isotonic NaCl solution, and the volume was adjusted to 10 ml. Each 2 ml of the suspension were used for the PN and protein N determination respectively. For the extraction of PN, 2 ml of the suspension were decanted into a glass homogenizer tube containing 2 ml of ice-cold 10 per cent trichloroacetic acid and 0.5 ml of 30 per cent hydrogen peroxide, then homogenized for a minute. After the addition of 6 ml of deionized water and 1 ml of 2 per cent trichloroacetic acid, it was again homogenized for a minute. The other procedure was the same as in case of the liver.

**RESULTS**

The changes in the PN content of livers of normal and ascites-tumour-bearing mice following the administration of nicotinamide are represented in Table 1. It will be seen that a definite increase of the PN level is brought about in the livers of both normal and tumour-bearing animals by the administration of nicotinamide, although the increase is not very marked in the case of tumour-bearing mice as compared with the normal. In livers of normal mice which received no injection, 1.97 mg of PN per 100 mg of protein N was found on an average and 10 hours after the subcutaneous injection of nicotinamide, the PN was found to rise to 14.0 mg per 100 mg of protein N. It is to be noted that during a week or two after the transplantation of Ehrlich ascites tumour, the average PN value for the liver decreased to 1.35 mg per 100 mg of protein N from the value of 1.97 mg, indicating that the PN level of the liver of the tumour-bearing animal is about 70 per cent of that of
However, 10 hours after the intraperitoneal or subcutaneous injection of nicotinamide, the PN content of the liver of tumour-bearing animals was found to increase to 7.05 or 7.40 mg per 100 mg of protein N.

Table 1. The changes of the PN content in livers of normal and tumour-bearing mice 10 hours after the injection of nicotinamide (1,000 mg/kg of body weight.) The values represent mg of PN/100 mg of protein N. Changes in the PN content of the tumour cells under the same conditions are also given.

<table>
<thead>
<tr>
<th>Animal</th>
<th>No Treatment</th>
<th>Intraperitoneal Injection of Nicotinamide</th>
<th>Subcutaneous Injection of Nicotinamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Tumour</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.2</td>
</tr>
<tr>
<td>Normal Mouse</td>
<td>1.21</td>
<td>1.41</td>
<td>2.23</td>
</tr>
<tr>
<td>(Average)</td>
<td>2.29</td>
<td>2.33</td>
<td>13.3</td>
</tr>
<tr>
<td>Tumour-bearing</td>
<td>1.22</td>
<td>9.61</td>
<td>2.75</td>
</tr>
<tr>
<td>Mouse</td>
<td>1.79</td>
<td>5.84</td>
<td>2.81</td>
</tr>
<tr>
<td>(Average)</td>
<td>1.03</td>
<td>5.13</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>1.13</td>
<td>7.62</td>
<td>2.13</td>
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<tr>
<td></td>
<td>1.60</td>
<td>2.52</td>
<td>7.59</td>
</tr>
<tr>
<td></td>
<td>1.35</td>
<td>1.97</td>
<td>2.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.29</td>
</tr>
</tbody>
</table>

The changes in the PN content of tumour cells as influenced by nicotinamide injection are also shown in Table 1. From this Table it is obvious that the PN content of the tumour cells differs little from that of livers of intact normal mouse, and the PN level of the tumour itself increases to 2.84 and 3.29 mg per 100 mg of protein N after the intraperitoneal and subcutaneous injection of nicotinamide, respectively. From these results, it is also clear that there is no difference in the effect produced on the PN content of the liver or tumour cells between the intraperitoneal and subcutaneous injection of nicotinamide. Therefore, only the subcutaneous injection was employed in the next experiments.

After having confirmed that nicotinamide is very effective in accelerating the synthesis of PN whether in livers of normal and tumour-bearing animals or tumour itself, the following experiments were performed in order to decide whether the hormones of the adrenal have some effect on the PN level of these tissues.

Cortisone acetate was subcutaneously injected into mice and the PN levels of livers and tumour cells were examined. The results are shown in Table 2. It was found
that in livers of normal mice, the PN content was lowered to 1.34 mg per 100 mg of protein N after the administration of cortisone acetate, and this level is the same as that in the liver of tumour-bearing animal. When cortisone acetate was administered to normal mouse prior to the injection of nicotinamide, the PN content of the liver increased to 8.56 mg per 100 mg of protein N. This increase was about half that caused by nicotinamide alone and almost identical to that observed in the liver of tumour-bearing mouse after the administration of nicotinamide. These results may be taken to indicate that the depression of the PN synthesis in livers of tumour-bearing mice is brought about by the intervention of some such adrenal hormone which is liberated in excess in these animals. However, it is to be noted that the livers of tumour-bearing mice and ascites tumour cells were not affected so strongly by cortisone acetate as in the case of normal liver. Especially, in the case of tumour cells, the injection of cortisone together with nicotinamide caused an increase of the PN level nearly to that found after the injection of nicotinamide alone. In the case of the liver of tumour-bearing mouse, only a slight reduction of PN synthesis was observed when cortisone acetate was injected combined with nicotinamide.

It may be concluded therefore that cortisone acetate exerts a definite influence on the synthesis of the PN in the liver of normal animal, depressing the PN level to

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Tumour</th>
<th>Liver</th>
<th>Tumour</th>
<th>Liver</th>
<th>Tumour</th>
<th>Liver</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nicotinamide Alone</td>
<td>1.44</td>
<td>2.13</td>
<td>1.23</td>
<td>2.18</td>
<td>1.97</td>
<td>1.34</td>
<td>3.63</td>
<td>3.11</td>
</tr>
<tr>
<td>Cortisone Acetate Alone</td>
<td>1.22</td>
<td>1.95</td>
<td>1.47</td>
<td>2.45</td>
<td>1.35</td>
<td>1.42</td>
<td>2.08</td>
<td>2.84</td>
</tr>
<tr>
<td>Cortisone Acetate &amp; Nicotinamide</td>
<td>9.89</td>
<td>7.37</td>
<td>6.92</td>
<td>5.94</td>
<td>1.51</td>
<td>2.26</td>
<td>5.64</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Table 2. The effect of cortisone acetate on the changes in the PN content of livers of normal and tumour-bearing mice following the subcutaneous injection of nicotinamide. The values represent mg of PN/100 mg of protein N. The effect on the PN content of tumour cells is also given.
that of the liver of the tumour-bearing animal and that the decreased PN level of the liver of the tumour-bearing animal or tumour itself is no longer influenced by the cortisone administration.

**Discussion**

In was previously reported by Waravdekar (12) that the livers of animals bearing various forms of tumour showed a decreased capacity for DPN synthesis, and that following the surgical removal of the tumour the DPN synthetic activity returned to normal. He also suggested that some hormonal intervention is likely in the reduction of DPN synthesis in the livers of tumour-bearing animals.

On the other hand, it is well known that the liver catalase activity of tumour-bearing animals is generally depressed, and Nakahara and his colleagues (14) have reported that a fraction extracted from the tumour tissues and named Toxohormone, invariably causes a marked decrease in the catalase activity in the mouse liver in vivo. Furthermore, it was reported by Ono and Tomaru (15) that the DPN synthesis in mouse liver was also depressed by this Toxohormone both in vivo and in vitro, although it was less effective in vitro than in vivo. In this connection, the report of Utsugi (16) is very interesting. He has shown that the characteristic depression of catalase activity no longer occurs in tumour-bearing rats and mice, when they are hypophysectomized and kept alive, and concluded that a certain pituitary hormone, presumably the growth hormone, plays a definite role in the depression of liver catalase activity which takes place in livers of these tumour-bearing animals. From these findings it is likely that the adrenal plays some part in the decrease of PN content in the liver of tumour-bearing animal through the intervention of the pituitary.

In the present study, a marked reduction of the capability of PN synthesis in the liver of tumour-bearing and cortisone-treated mouse has been demonstrated. The increase of PN which is brought about by the administration of nicotinamide in the liver of tumour-bearing mouse is only half that observed in livers of normals. If cortisone acetate is administered prior to the injection of nicotinamide, there occurs a marked reduction of PN synthesis in the case of normal liver, the PN level becoming very similar to that in the liver of tumour-bearing animal. However, the administration of cortisone acetate produces no longer any marked effect upon the already reduced synthesis of PN in the liver of tumour-bearing animal. These results seem to indicate that there is the participation of the adrenal hormone in the reduction of PN synthesis in the liver of tumour-bearing animal, although it is not certain whether the adrenal hormone is acting directly or through the pituitary.

In the case of the Ehrlich ascites tumour cells, the level of PN was relatively high. However, their activity of PN synthesis as influenced by the administration of nicotin-
amide was very low. Even if the intraperitoneal injection of nicotinamide was employed, the PN level of tumour cells was no more than 150 per cent of that observed in the animals received no injection. Also PN synthesis in tumour cells was not affected by cortisone acetate.

Lastly, it may be mentioned that there is some evidence that the lowered PN content of the tumour or other growing tissues is associated with the rate of cell proliferation (11, 17, 18). Morton (17) and Sahsrabudhe (18) are of the opinion that the lowered PN level causes the cell to divide by some ‘feed-back’ mechanism in connection with energy metabolism. In these discussions, it has been assumed that the respiratory activity is low and glycolytic activity high in tumour tissues. However, Weinhouse (19) has shown that the low respiratory activity is only found in some specific types of tumour such as Ehrlich ascites and it is not always found in all tumours. From these facts, it is suggested that the energy metabolism in the tumour tissues is sufficiently maintained by such a decreased activity of PN synthesis as found in these tissues, whereas the relatively high level of PN and the high activity of its synthesis as observed in normal liver cell must be utilized for the functional activity such as secretion or detoxication and so on. Therefore, it will be interesting to examine closely the functional activity of the liver of tumour-bearing animals, in which the PN level has been greatly lowered.

**Summary**

1. The capability of PN synthesis as influenced by nicotinamide administration is very low in the Ehrlich ascites tumour cell and the liver of its host mouse, as compared with that of normal mouse liver. In the case of the liver of tumour-bearing mouse, the increase of PN after the administration of nicotinamide is half that observed in normal mouse liver.

2. After the administration of cortisone acetate, the activity of PN synthesis in normal mouse liver always decreases to the level of tumour-bearing liver, suggesting some such hormone may be acting in the tumour-bearing animal.

3. However, the already reduced activity of PN synthesis in the liver of mouse bearing ascites tumour and tumour cell itself is hardly affected by the administration of cortisone acetate.

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**References**