The Effects of Uncoupling Agents on the Uptake and Incorporation of Glycine by Transplantable Tumors

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SUMMARY

The effects of dinitrophenol (DNP) on the uptake and incorporation of glycine by tumors have been studied. DNP (0.05 mM) inhibited glycine incorporation into the protein of the solid and ascitic forms of the Ehrlich carcinoma and Sarcoma 87 by approximately 60 per cent. The uptake of glycine by the ascitic forms was inhibited to the extent of 25 per cent, but no effect on the uptake of glycine by slices of the solid form of the tumors was observed. In ascites cells, glucose reversed all the inhibitory effects of DNP if the medium was adequately buffered. Bicarbonate proved far more effective as a buffer than phosphate, and it was only in the presence of this buffer that any reversal of the inhibitory effects of DNP on slices could be obtained.

Decanoate and dicoumarol acted as uncoupling agents and gave rise to effects which were similar to those produced by DNP. In addition, decanoate stimulated uptake and incorporation of glycine in some media, an effect which has been interpreted as being due to an interaction of the fatty acids with cellular and subcellular lipide membranes.

Low concentrations of dinitrophenol (DNP) are able to bring about a stimulation of respiration (see, for example, the account by Ronzoni and Ehrenfest [24]) but, at the same time, cause an inhibition of certain biosynthetic events, such as cell division (5) and assimilation of acetate and butyrate by microorganisms (3). It was also demonstrated (24) that the presence of DNP caused a depletion of the creatine phosphate and adenosine triphosphate (ATP) of frog muscle. Subsequently, Loomis and Lipmann (19) showed that DNP prevents the aerobic synthesis of ATP by liver mitochondria without inhibiting respiration, a process now known as the uncoupling of oxidation from phosphorylation.

The synthesis of ATP during glycolysis is resistant to DNP (18). It should therefore be possible to overcome some of the inhibitory effects of DNP on tumors by adding glucose, because of the high aerobic glycolysis of which tumors are capable (31). This type of phenomenon has actually been demonstrated by Rabinovitz, Olsen, and Greenberg (23), who showed that the inhibition by DNP of amino acid uptake in Ehrlich ascites cells is reversed on addition of glucose. A similar reversal of the action of uncoupling agents on an energy-requiring process was obtained in the experiments of Quastel and Bickis (21). These workers showed that the presence of glucose led to a reversal of the inhibitory effects of DNP and bilirubin on glycine incorporation into the proteins of Ehrlich ascites cells. Later results (7) have confirmed that the inhibitory effect of 0.05 mM DNP on P32 turnover in the ATP of Ehrlich ascites cells is largely reversed by the presence of glucose.

The high aerobic glycolysis of Ehrlich ascites cells is increased approximately to the level of anaerobic glycolysis (reversal of the Pasteur effect) by uncoupling agents, such as dinitrocresol (4), DNP (29), and fatty acids (26). At the same time, the presence of DNP leads initially to a reversal of the suppression of respiration by glucose—a reversal of the Crabtree effect (6,8,16). Subsequently, the respiratory activity may fall quite suddenly to very low levels (17,38), an effect which has been shown (26) to be due to an inadequate capacity to buffer the increased lactic acid produced. This conclusion has been confirmed by Emmelot and Bos (9).

It was of interest to us to determine how far
such factors may also be involved in the metabolism of tumor slices and to what extent they influence the uptake of radioactive glycine into the alcohol-soluble fraction (free glycine pool) and its incorporation into protein in both tumor slices and ascites cells.

MATERIALS AND METHODS

Animals.—All tumors were grown in CF1 Swiss white mice weighing 15−20 gm.

Tumors.—The Ehrlich carcinoma and S-37 sarcoma were grown as solid and ascitic tumors. The solid tumors were obtained by subcutaneous injection of the ascitic form and were generally ready for use about 12−14 days later. The ascitic (liquid) form of the sarcoma was generally maintained by intraperitoneal transplantation of S-37 ascites cells. Originally the Ehrlich ascites were maintained by a similar procedure, but it was found that, after a variable number of transfers, the yield decreased greatly (see Bennette [1]). Subsequently, and for all the present work, the Ehrlich ascites cells were continually re-isolated from the solid form, and only the cells obtained from the first liquid to liquid transfer were used. In this way, the yields were consistently maintained at a high level, and none of the metabolic activities measured changed appreciably over the course of 18 months.

Tissue preparations.—Slices were cut with the aid of a Stadie-Riggs tissue slicer, only the peripheral non-necrotic tissue being used.

Ascites cells were freed from blood elements, washed in isotonic saline, and packed as previously described (15).

Incubation technic.—Slices were incubated in Warburg vessels at 37.6°C. in oxygen (10 mM phosphate, pH 7.4 as buffer), or in 98 per cent oxygen: 7 per cent CO2 (25 mM bicarbonate as buffer to yield a final pH of 7.4). The incubation media were always essentially a calcium-free Krebs-Ringer medium containing 145 mM NaCl; 5.8 mM KCl; 1.5 mM KH2PO4, and 1.5 mM MgSO4 (“salts solution”), the final volume in the vessel being 3 ml.

Ascites cells were either incubated in air or in Warburg vessels when measurements of respiratory activity or an atmosphere other than air was required.

The duration of the incubation was 45 minutes for ascites cells or 60 minutes for tumor slices unless otherwise stated. The reaction was begun by adding the suspension of ascites cells to the Earlmeneyflasks or by tipping radioactive glycine (0.5 μc/3 ml) and inhibitor from the side-arms of Warburg vessels.

Assay of radioactivity.—The “alcohol-soluble” glycine and “alcohol-insoluble” glycine of ascites cells were estimated as previously described (15). The method consisted, essentially, of washing with ice-cold salts solution and extraction with 95 per cent ethanol. An aliquot of the supernatant was plated and counted. The residue was washed with 95 per cent ethanol and with acetone, then plated as a suspension in acetone and counted.

When tumor slices were used, the Warburg vessels were first removed from the incubation bath and placed on cracked ice. The slices and medium were then mixed with 5 ml. ice-cold salts solution and centrifuged. The residue was washed with a further 8 ml. ice-cold salts solution and then homogenized in 3 ml. 95 per cent ethanol using a Teflon pestle homogenizer. An aliquot (300 μl) of the supernatant was plated and counted. The protein of the residue was isolated, as described by Rabinovitz, Olsen, and Greenberg (22), and its radioactivity assayed.

All samples were plated on aluminum discs (5 sq. cm.) and counted with the use of the thin end-window tube and Baird Atomic 123 scaler, corrections being applied for self-absorption where necessary. Glycine-1-C14 was obtained from Merck and Co., Limited, Montreal.

RESULTS

The Effects of DNP on Glycine-1-C14 Uptake and Incorporation by Tumor Slices

The effects of DNP and glucose on the respiratory activity, on glycine uptake into the alcohol-soluble fraction, i.e., on the free glycine pool, and glycine incorporation into the proteins of slices of the Ehrlich carcinoma and Sarcoma 37 were first investigated. The results obtained are presented in Table 1. Similar experiments were also performed with Ehrlich ascites cells, and the results obtained in these experiments are included in this table for purposes of comparison.

Respiration.—In all cases, addition of 0.05 mM DNP alone stimulated respiration by about 15 per cent. Addition of 0.05 mM DNP in presence of glucose more than reversed the Crabtree effect in ascites cells (16) but gave only partial reversal in the tumor slices.

Glycine uptake.—Uptake of glycine into the alcohol-soluble fraction was usually stimulated by about 10 per cent on addition of 10 mM glucose to ascites cells but was consistently inhibited to the same extent on the addition of glucose to tumor slices. The presence of 0.05 mM DNP caused a decrease of 25 per cent in the uptake of glycine-1-C14 by the ascites cells but had no effect on the
uptake by tumor slices. Concentrations of DNP greater than 0.08 mM, which inhibit the respiration of slices (compare Shacter [30]), did produce an inhibition of glycine uptake. An even more striking difference between the two forms of the tumors became apparent when DNP was added to preparations incubated with 10 mM glucose. The inhibitory effects of DNP on uptake in ascites cells were completely reversed, in agreement with previous observations (23). However, the uptakes of glycine-1-C\textsuperscript{14} by the tumor slices in the presence of DNP and glucose were 20--25 per cent less than the control values.

Glycine incorporation.—Addition of 0.05 mM DNP produced an inhibition of glycine-1-C\textsuperscript{14} incorporation into protein amounting to approximately 60 per cent in all cases. This inhibition was largely reversed by addition of glucose to the ascites cells (21,23) but remained unaltered or slightly increased in the case of the tumor slices.

### Table 1

**Effects of 10 mM Glucose and 0.05 mM DNP on the Uptake and Incorporation of Glycine-1-C\textsuperscript{14} by Solid and Ascitic Tumors**

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>DNP (mM)</th>
<th>Sarcoma 37</th>
<th>Incorporation*</th>
<th>Ehrlich carcinoma</th>
<th>Incorporation*</th>
<th>Ehrlich ascites</th>
<th>Incorporation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.5</td>
<td>4.23</td>
<td>3.70</td>
<td>5.8</td>
<td>5.02</td>
<td>3.73</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3.8</td>
<td>3.81</td>
<td>3.49</td>
<td>4.1</td>
<td>4.00</td>
<td>3.41</td>
</tr>
<tr>
<td>0</td>
<td>0.05</td>
<td>6.4</td>
<td>4.38</td>
<td>1.36</td>
<td>6.7</td>
<td>5.11</td>
<td>1.42</td>
</tr>
<tr>
<td>10</td>
<td>0.05</td>
<td>5.8</td>
<td>3.29</td>
<td>1.21</td>
<td>6.0</td>
<td>4.08</td>
<td>1.34</td>
</tr>
</tbody>
</table>

\* Uptake = \(\text{gmoles glycine-1-C}^{14}\) uptake per gm. wet weight of tumor.

\(\text{Incorporation} = \text{gmoles glycine-1-C}^{14}\text{ incorporated per mg. protein.}\)

\(\text{Incorporation} = \frac{\text{gmoles glycine-1-C}^{14}\text{ incorporated per ml. packed cells.}}{\text{mg packed cells}}\)

The results represent mean values obtained from at least four experiments for each tumor. The ascites cells were incubated at 37.6°C for 45 minutes and the tumor slices for 60 minutes. All incubations were carried out in the standard medium containing 10 mM phosphate buffer, pH 7.4, and 6 \(\mu\)moles glycine-1-C\textsuperscript{14} (0.5 gc.) in 3 ml. of medium.

The Effects of Altered Buffering Capacity

Previous experiments (36) had shown that the sudden decrease in respiratory activity in the presence of both DNP and glucose could be correlated with an increase in the rate of intracellular lactic acid production, i.e., a decrease in the intracellular pH. The same effect might account for the further depression in slices of the glucose-inhibited levels of glycine uptake and incorporation on addition of DNP. Studies in which the buffering power of the medium was varied confirmed this suggestion. Slices of the Ehrlich carcinoma and of glycine uptake were similar but not so marked.

When the tumor slices were incubated in a medium buffered by CO\textsubscript{2}-bicarbonate to pH 7.4 (7 per cent CO\textsubscript{2}; 25 mM bicarbonate) the values for the uptake and the incorporation of glycine increased by 15--20 per cent. The further addition of 10 mM glucose caused a 40 per cent increase in the rate of glycine incorporation into the proteins of both tumors and approximately 5 per cent increase in the amount of amino acid taken up. The inhibition by DNP of glycine incorporation was almost the same in bicarbonate buffer as it was in 10 mM phosphate, and again DNP had no effect on glycine uptake. However, the addition of glucose to slices of the Ehrlich carcinoma or Sarcoma 37 incubated in a bicarbonate medium largely reversed the inhibition by DNP of the glycine incorporation into protein.
### TABLE 2

**EFFECT OF VARIOUS BUFFERS ON THE UPTAKE AND INCORPORATION OF GLYCINE-1-C\(^{14}\) BY TUMOR SLICES**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Additions</th>
<th>(\mu)MOLES GLYCINE-1-C(^{14}) UPTAKE/GM WET WEIGHT OF TUMOR</th>
<th>(\mu)MOLES GLYCINE-1-C(^{14}) INCORPORATED/MG PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(O_2)</td>
<td>(O_2/CO_2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phosphate (10 mM)</td>
<td>Phosphate (25 mM)</td>
</tr>
<tr>
<td>Ehrlich carcino-ma</td>
<td>-</td>
<td>4.97</td>
<td>5.02</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3.64</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>4.95</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>3.05</td>
<td>4.08</td>
</tr>
<tr>
<td>Sarcoma 37</td>
<td>-</td>
<td>4.16</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3.12</td>
<td>3.81</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>4.21</td>
<td>4.38</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.38</td>
<td>3.29</td>
</tr>
</tbody>
</table>

The experimental conditions were as in Table 1. The values in parentheses refer, in each case, to percentages of the control values obtained in the absence of added glucose or DNP. All results are the mean of six to eight determinations in four experiments with each type of tumor.

### TABLE 3

**INCORPORATION OF GLYCINE-1-C\(^{14}\) INTO ASCITES CELLS IN THE PRESENCE OF VARIOUS INHIBITORS IN VARIOUS MEDIA**

<table>
<thead>
<tr>
<th>Additions</th>
<th>(\mu)MOLES GLYCINE-1-C(^{14}) INCORPORATED/ML PACKED CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate (10 mM)</td>
</tr>
<tr>
<td>Nil</td>
<td>310</td>
</tr>
<tr>
<td>0.4 mM decanoate</td>
<td>139</td>
</tr>
<tr>
<td>0.05 mM DNP</td>
<td>125</td>
</tr>
<tr>
<td>0.01 mM dicoumarol</td>
<td>78</td>
</tr>
</tbody>
</table>

The results presented are the averages of at least three experiments with each inhibitor. The incubation conditions were as described in Table 1. When 25 mM bicarbonate was present phosphate buffer was omitted, and the atmosphere was 93% \(O_2\): 7% \(CO_2\).

### TABLE 4

**EFFECT OF 0.6 mM DECANOATE AND 0.01 mM DICOUMAROL ON GLYCINE-1-C\(^{14}\) UPTAKE AND INCORPORATION BY EHRLICH CARCINOMA SLICES**

<table>
<thead>
<tr>
<th>Additions</th>
<th>10 mM PHOSPHATE</th>
<th>(\pm 5) mM BICARBONATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (10 mM)</td>
<td>Decanoate (0.6 mM)</td>
<td>Dicoumarol (0.01 mM)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* \(\mu\)moles glycine-1-C\(^{14}\)/gm tumor tissue.
† \(\mu\)moles glycine-1-C\(^{14}\) incorporated/mg tumor protein.

The incubation conditions were as described in Tables 1 and 3.
The Effects of DNP, Decanoate, and Dicoumarol

The general pattern of the inhibitory effects of DNP has been ascribed to its capacity to uncouple oxidation from phosphorylation. The effects of DNP on glycine incorporation into protein have now been compared with those obtained on addition of decanoate and dicoumarol, which are known to produce similar uncoupling effects (20, 25, 26). The results obtained are presented in Table 3, from which it may be seen that these three compounds inhibit glycine incorporation into protein, their effects are partially reversed in a phosphate-buffered medium by the addition of glucose.

The Effects of Various Concentrations of Decanoate in a Phosphate-Buffered Medium

The results presented in Table 3 show that there is an unexpected stimulation of glycine incorporation into the protein of Ehrlich ascites cells on addition of decanoate to a medium containing glucose and buffered by bicarbonate. The effect is not seen when slices of the Ehrlich carcinoma are studied (Table 4). The effects of decanoate in ascites cells were further investigated, and the results obtained are presented in Table 5. They indicate that there is also a small but consistent stimulation of the uptake of glycine into the alco-

TABLE 5

Effect of Concentration Series of Decanoate on Glycine-1-C14 Uptake and Incorporation in Ehrlich Ascites Cells

<table>
<thead>
<tr>
<th>Decanoate concentration (mM)</th>
<th>μmoles glycine-1-C14 uptake/mL packed cells</th>
<th>μmoles glycine-1-C14 incorporated/mL packed cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No glucose present</td>
<td>10 mM glucose present</td>
</tr>
<tr>
<td>0.0</td>
<td>11.0</td>
<td>11.3</td>
</tr>
<tr>
<td>0.2</td>
<td>12.0</td>
<td>11.8</td>
</tr>
<tr>
<td>0.4</td>
<td>12.1</td>
<td>11.0</td>
</tr>
<tr>
<td>0.6</td>
<td>11.0</td>
<td>10.9</td>
</tr>
<tr>
<td>0.8*</td>
<td>7.2</td>
<td>10.4</td>
</tr>
<tr>
<td>1.0</td>
<td>3.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>

The experimental conditions were as in Table 1.

* At concentrations of decanoate greater than 0.8 mM respiration was markedly inhibited.

their effects are somewhat less in a bicarbonate-buffered medium, and completely reversed in the latter medium on addition of glucose. It should be noted that, in a bicarbonate-buffered medium containing glucose, decanoate led to a consistent increase in the rate of glycine incorporation.

Similar experiments were performed to compare the roles of 10 mM phosphate and 25 mM bicarbonate in controlling the inhibitory effects of 0.6 mM decanoate and 0.01 mM dicoumarol on glycine uptake and incorporation in Ehrlich carcinoma slices. The results, which are presented in Table 4, confirm the superiority of the bicarbonate buffer under these circumstances. It is important to note, however, that, in contrast to the results obtained with ascites cells, glucose did not completely reverse the effect of decanoate or dicoumarol.

The Duration of Incubation

When ascites cells are incubated in 10 mM phosphate buffer, pH 7.4, with decanoate and glucose, there is a cessation of respiration (26) and of fatty acid oxidation (27) after 30-60 minutes' incubation. The partial reversal of the inhibitory effects of decanoate and DNP on glycine incorporation into proteins of ascites cells might therefore be a combination of an initial complete reversal of the inhibition followed by a phase during which no glycine incorporation occurred. Incubations, simi-
lar to those described in Tables 3 and 4, were therefore carried out for various times, and the results obtained are presented in Chart 1. In the first 15 minutes of incubation, 0.05 mM DNP inhibited glycine incorporation by 65 per cent, and the extent of this inhibition remained constant during the 60-minute incubation period (Chart 1). When 10 mM glucose and 0.05 mM DNP were both present the inhibitory effect of the DNP was initially completely reversed. Subsequently, the rate of glycine incorporation fell rapidly. During the period from 40 to 60 minutes an inhibition by glucose alone became apparent (compare the inhibitory effects of glucose in slices seen in Tables 1 and 2). Results obtained when the incubations with DNP and glucose were carried out in bicarbonate buffer are also shown in Chart 1. After 60 minutes' incubation no inhibitory effects had become apparent. Similar results were obtained when 0.4 mM decanoate was used in place of 0.05 mM DNP.

DISCUSSION

The behavior of slices of the Ehrlich carcinoma and of Sarcoma 37 in the above experiments was strikingly similar. In studies of $Q_{10}$ values, of rates of glycine incorporation into protein, extent of glycine uptake, sensitivities to glucose, DNP, decanoate and dicoumarol, and in the reactions of their ascitic forms, these two tumors have yielded results which did not differ from one another by more than 10 per cent. On the other hand, the two ascitic forms differed considerably in their reactions from the two corresponding solid forms of these tumors. For example, the uptake of glycine by Ehrlich ascites cells was greatly decreased by 0.05 mM DNP, but the uptake of glycine by slices of the Ehrlich carcinoma was not influenced by DNP at this concentration. It was shown by Christensen and Riggs (2) and by Heinz and Mariani (12) that DNP greatly depresses the uptake of glycine by ascites cells, but both these groups of workers used DNP at concentrations at which it is known to cause inhibitions of respiratory activity. In the present work care has been taken to employ a concentration (0.05 mM) of DNP which stimulates rather than inhibits respiration of Ehrlich ascites cells. When DNP was added to slices at concentrations at which it inhibits their respiration, the uptake of glycine was correspondingly decreased. This raises the question of the direct participation of ATP in the process of concentration by the cell of amino acids, such as glycine. The supply of ATP is definitely depleted in tumor slices on addition of 0.05 mM DNP (unpublished observations), and the inhibition of glycine incorporation by 0.05 mM DNP to the extent of 60 per cent in the carcinoma slices (Table 1) supports this conclusion. It had previously been noted, however, that the incorporation of glycine into ascites cells ceased immediately upon addition of DNP; but there was an appreciable delay before loss of glycine, previously concentrated by the cells, began to occur (15). The suggestion previously made is that the role of ATP in amino acid transport is the rather indirect one of maintaining the carrier in an active form. This would be in agreement with the scheme outlined by Heinz and Walsh (13). If the carrier system present in slices is more stable than it is in the ascites cells, then it would be less dependent on a supply of ATP and therefore less sensitive to the inhibitory effects of DNP. The incorporation of glycine into protein requires the direct participation of ATP, and the rate of this reaction may well be limited by the amount of available ATP. The depletion of the ATP of slices and of ascites cells to an equal extent by DNP would then lead to the equal inhibitions of the rates of glycine incorporation noted in Table 1.

Slices and ascites cells also differed in the effects on their metabolic pathways resulting from the simultaneous addition to them of 10 mM glucose and 0.05 mM DNP. The glucose initially reversed the effects of DNP on glycine uptake and incor-
poration in ascites cells. The duration of this reversal depends upon the buffering power present in the medium. It should be emphasized that a change in the pH of the medium is only a reflection of the change in pH of the cells present in the medium. Previous results (26) have shown that Ehrlich ascites cells respire well in a phosphate-buffered medium of pH 5.7, but when the pH of a phosphate-buffered medium was caused to fall from 7.4 to 5.7 by the addition of glucose and DNP, the respiratory activity was almost abolished. This experiment has now been repeated and confirmed by Emmelot and Bos (9), who have drawn the same conclusion that the rate of acid production inside may be so rapid that the buffering capacity of the environment may not be sufficient to maintain the cell in its normal pH range. When 25 mm bicarbonate buffer was used instead of 10 mm phosphate, such pH effects were no longer apparent, suggesting that bicarbonate is more able to equalize the intracellular and the extracellular pH's.

It is of interest to note that Farber, Kit, and Greenberg (10) observed a similar effect of 25 mm bicarbonate on glycine incorporation. A suspension of cells of the Gardner 6C3HED lymphosarcoma was used, and incubation was carried out in a medium containing glucose but not DNP.

It has been reported by Wrba, Seidler, and Allmann (32) that the rate of phosphate entry into ascites cells is rapid, a steady state being attained within 5 minutes; and the results of Ibsen, Coe, and McKee (14) are in agreement with this report. Such results, however, can yield no information concerning the pH of the phosphate entering or leaving the cell. These may be the same, and hence phosphate may buffer inside the cell but have little capacity to remove excess hydrogen ions from the cell to the medium. Bicarbonate could accomplish this by leaving the cell as carbon dioxide (11).

Addition of glucose to tumor slices incubated in a phosphate-buffered medium, in the presence of DNP, did not reverse the inhibitory effect of DNP. In fact, when the medium contained only 3.3 mm phosphate buffer, the inhibition was much greater. This effect was due to a decreased pH, since it did not occur when slices were incubated in bicarbonate buffer. The use of the latter buffer did permit a partial reversal by glucose of the effects of DNP on slices, but the reversal was never complete.

Caution must, therefore, be exercised when interpreting the results of experiments in which glucose is employed, particularly when the buffering capacity of the medium is low and when agents which increase aerobic glycolysis are present. It should also be emphasized that such results, which are consequent upon the decrease of pH, are more apparent in slices than in the corresponding ascitic forms. This is presumably owing to the inability of tumor slices to neutralize the excessive amounts of lactic acid formed or to the slow passage of lactic acid from the cells of such slices into the surrounding medium.

Mention should also be made of the effects of the two other uncoupling agents, dicoumarol and decanoate. The former behaved in a manner completely analogous to that of DNP and was about 5 times as effective an inhibitor as DNP. The effects produced by decanoate as a result of its uncoupling action were also analogous to those produced by DNP, but approximately 8 times as much decanoate was required to duplicate the effects of DNP. In addition, decanoate appeared to possess properties not connected with its capacity to uncouple oxidation from phosphorylation. It caused about 10 per cent increase in the uptake of glycine by ascites cells and up to 30 per cent increase in the rate of glycine incorporation into the protein of ascites cells in a bicarbonate medium in the presence of glucose. These stimulations may both be the result of effects of decanoate on the lipide membranes of the ascites cells and their subcellular constituents.

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