Action of Dinitrophenol and Lanatoside C on the Canine Heart-Lung Preparation

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Dinitrophenol (DNP) induces failure of the canine heart-lung-preparation and increases specifically its $\text{O}_2$-consumption. Lanatoside C compensates this failure as long as the degree of dinitrophenol poisoning is moderate and the $\text{O}_2$-supply sufficiently ample. This cardiac glycoside does not influence the metabolic action of dinitrophenol, i.e. the increased $\text{O}_2$-consumption, nor does it counteract the failure due to a large degree of dinitrophenol poisoning. From these findings the mechanism of action of Lanatoside C is discussed.

DINITROPHENOL (DNP) has been shown to exert a marked effect in accelerating the metabolism of animals and increasing the $\text{O}_2$-consumption of various tissues in vitro.\textsuperscript{1-3} Despite this stimulating action of dinitrophenol on cell metabolism, the function of various organs is impaired: cell division of the sea urchin, viral growth, cardiac contractility, etc., are inhibited.\textsuperscript{3-4} This effect appears to be the result of an interference of dinitrophenol with the synthesis of high energy phosphate bonds, i.e. the dissociation between oxidation and phosphorylation; this property was designated as an uncoupling effect by Loomis and Lipman\textsuperscript{7} and by Cross et al.\textsuperscript{8} While oxygen uptake is increased, the function of the cell is impaired due to a lack of an adequate supply of energy rich phosphate bonds. Actually it was found by Wollenberger and Karsh\textsuperscript{8} that dinitrophenol concentration inducing cardiac failure lowered the phosphocreatine content of the myocardium.

It is of particular interest to study the action of cardiac glycosides on dinitrophenol induced cardiac failure, as such findings could contribute to the problem of the mechanism of action of these drugs. Wollenberger and Karsh\textsuperscript{8} found that ouabain had no or only a weak cardiotonic action on the dinitrophenol poisoned and depressed Langendorff heart. Ellis\textsuperscript{9} confirmed these results using the isolated frog heart. This resistance to the action of cardiac glycosides would postulate some analogy between dinitrophenol and anoxia induced failure. There are, however, important objections to such an argument, because $\text{O}_2$-supply and work performance of the Langendorff and isolated frog hearts are insufficient and unphysiological. It appeared therefore desirable to study the action of cardiac glycosides on the dinitrophenol-poisoned heart-lung preparation working under more physiological conditions, i.e. with greater load and larger $\text{O}_2$-supply. This approach has been tried in the present study.

METHOD

Experiments were performed on denervated heart-lung preparations (HLP) of 30 dogs weighing between 7 and 14 kg, and anaesthetized with morphine and chloralose. The pressures in the right and left auricle, pulmonary artery and aorta were recorded simultaneously with the cardiac output; in some experiments changes of heart volume were registered using the pericardium as oncometer. Heparinized blood from donor dogs under light thiopental anaesthesia was used to obtain a total blood volume in the system of about 900 cc. Blood temperature was kept constant either at 39°C or at 30°C. The distance between the heart and the venous reservoir remained fixed. A variable orifice in the venous inflow tube was used to regulate the backflow to the auricle thus imposing a certain minute volume to the heart. Pressure values were recorded at different levels of cardiac output; cardiac pressure-volume work/100 gm. heart weight was calculated from these data and related to the right auricular pressure. The curves thus obtained were used as a competence test of the heart-lung preparation (HLP) (compare fig. 1). Changes in cardiac competence, evaluated from these curves, were expressed as changes in work performance at a constant right.
RESULTS

a) Blood temperature at 39 C. After determination of initial cardiac competence, dinitrophenol was added to the venous reservoir at concentrations of 0.75-1.35 × 10^-4 M. A negative inotropic action of dinitrophenol becomes evident as right and left auricular pressure are increasing while cardiac output decreases (fig. 2). Despite a progressively diminishing cardiac work performance, O2 uptake rose steadily. After 15 to 30 minutes O2 uptake and cardiac competence reached a relatively steady level. Any increase of cardiac output rose further the O2 consumption. Lanatoside C exerted its cardiotonic action by lowering the filling pressure and increasing the cardiac output. Effective doses of lanatoside C correspond to those necessary to obtain a positive inotropic action in spontaneous or Nembutal induced failure of the HLP. Equally, no difference in the incidence of abnormal cardiac rhythm following the administration of the cardiac glycosides has been noted. Cardiac oxygen consumption remained increased during the cardiotonic action of lanatoside C.

Fig. 3 summarizes the results obtained on 7 heart-lung preparations: 1.1 × 10^-4 M. Dinitrophenol decreased the work performance of the HLP from 0.88 kgm/100 gm h.w. to 0.32 kgm/100 gm h.w. or to 36% of the initial value. Lanatoside C (0.25-0.42 mgm/100 gm h.w.) restored initial work performance. Myocardial oxygen consumption (at constant work) rose from 6.6 cc./min./100 gm h.w. to 12.5 cc. or to 190 per cent after the dinitrophenol, whereas no further change in O2 uptake was noted after the administration of the cardiac glycoside. These results demonstrate that the cardiac glycoside, although counteracting the insufficiency, does not interfere with the oxidative metabolism during dinitrophenol induced failure.
The QR of the heart dropped during the dinitrophenol induced increase in O$_2$-consumption from an average of 0.85 to 0.69 and began then to rise very slowly. A clear effect of the cardiac glycoside on the RQ has not been observed. Dinitrophenol had no influence on the heart rate, whereas the cardiac glycoside decreased the heart rate slightly in three out of seven experiments.

These findings contrast the results obtained by Wollenberger and Karsh$^4$ on the Langendorff preparation, where ouabain was found to be ineffective against dinitrophenol induced failure. The concentrations of dinitrophenol used in this study corresponded to those used by Wollenberger in the Langendorff heart. A possible explanation for this difference could be that the isolated heart is working under a low O$_2$-reserve, and that the increased demand of O$_2$ after dinitrophenol is leading, therefore, to a relative anoxia. Indeed, we found that dinitrophenol induced a failure in the heart-lung preparation, perfused with anoxic blood, similarly as in the previous experiments, but that lanatoside C was completely ineffective under these circumstances; rather there seemed to be an early appearance of extrasystoles. These findings suggest that an ample supply of O$_2$ is indispensable for the cardiotonic action of lanatoside C on dinitrophenol induced failure. On the other hand, it could be assumed that the Langendorff or frog heart is more sensitive to dinitrophenol poisoning, and the degree of metabolic blockade is such that it resembles anoxic failure which is resistant to the cardiotonic action of the glycosides. The effect of lanatoside C on the heart poisoned with larger doses of dinitrophenol should, therefore, be studied. Preliminary trials with high concentrations of dinitrophenol showed such a deteriorating effect on the heart-lung preparation that the cardiotonic action of lanatoside C could not be demonstrated. To circumvent this difficulty of a too rapidly progressing failure under the influence of high dinitrophenol-concentrations, this part of the experiments was repeated at a lower temperature, since it is known that hypothermia increases the tolerance for dinitrophenol and decreases its toxicity$^{11}$. 

**Fig. 2.** Record showing the effect of DXP (1.2 x $10^{-4}M$) on the canine HLP. BP = blood pressure, RA = right auricular pressure, PA = pulmonary artery pressure, LA = left auricular pressure; minute volume and time: 6 sec. Lanatoside C compensates the DXP induced failure. O$_2$-consumption: before DNP 7.9 cc./min., after DNP 11.5 cc./min., after Lanatoside C 12.2 cc./min.
b) Blood temperature at 30°C. This temperature did not impair cardiac contractility and seemed most suitable; however, the myocardial O₂-uptake was reduced and cardiac efficiency therefore increased (see table 1). The decrease of heart rate could at least partially account for this effect. The cardiotonic action of lanatoside C during spontaneous and Nembutal failure at 30°C was fully preserved. Oxidation stimulation induced by moderate concentrations of dinitrophenol (1.8 × 10⁻⁴M) at 30°C was not essentially different from that at 39°C (table 2). High doses of dinitrophenol (2.8 × 10⁻⁴M) further increased the O₂-consumption. These results seem to indicate that the metabolic action of dinitrophenol is not essentially different at 30 and 39°C, and that high concentrations of dinitrophenol produce a larger degree of metabolic blockade at 30°C as well as at 39°C.

The effect of dinitrophenol on the heart-lung preparation during hypothermia is illustrated in fig. 4. A concentration of dinitrophenol decreasing the work capacity of the heart-lung preparation at 39°C exerted no negative inotropic action at 30°C. Dinitrophenol even decreased the left auricular pressure, whereas cardiac output and right auricular pressure were not affected. The latter finding is surprising and unusual, as it could point rather to a cardiotonic action of dinitrophenol. However, analysis of the heart volume changes revealed a pronounced cardiac dilatation following dinitrophenol administration. On the other hand, the cardiac competence test did not point to a decreased work performance, as the auricular pressures and the heart volume rose only slightly during the period of increased cardiac load. This change of heart volume opposite to that of the left auricular pressure could, therefore, hardly be interpreted as a change in myocardial contractile force, but pointed rather to a decrease in cardiac tonus. At 30°C myocardial competence was consequently more resistant to dinitrophenol poisoning, whereas on the other hand the cardiac O₂-uptake increased after the administration of dinitrophenol to a similar extent as at 39°C (see table 2). Equally, the RQ of the heart fell slightly.

High concentrations of dinitrophenol were required to induce cardiac failure during hypothermia; concomitantly, a further increase of the O₂-consumption was noted (compare table 2). The heart rate dropped moderately after poisoning with large doses of dinitrophenol. Lanatoside C, even in high concentrations, had no cardiotonic effect and was without influence on the increased O₂-consumption. In some experiments, an early appearance of an abnormal

Table 1.—Effect of Hypothermia on Cardiac O₂-uptake

<table>
<thead>
<tr>
<th>Temp.</th>
<th>No. of exper.</th>
<th>Average work (kgm/100 gm h.w.)</th>
<th>Right auric. press. (cm HO)</th>
<th>O₂-consumption (cc/min./100 gm h.w.)</th>
<th>Heart rate/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>39°C</td>
<td>7</td>
<td>0.52</td>
<td>4.8</td>
<td>5.6 ± 0.18 S.E.</td>
<td>146 ± 11.8 S.E.</td>
</tr>
<tr>
<td>30°C</td>
<td>7</td>
<td>0.58</td>
<td>5.3</td>
<td>5.3 ± 0.27 S.E.</td>
<td>115 ± 12.0 S.E.</td>
</tr>
<tr>
<td>Difference in p-value...</td>
<td>-</td>
<td>-</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>
ACTION OF DINITROPHENOL AND LANATOSIDE C


dog f 10 kg

Fig. 4. Effect of DNP on cardiac dynamics during hypothermia (30 °C). At 1, lowering of the left auricular pressure with concomitant cardiac dilatation suggests decrease in cardiac tonus after DNP. Between 2 and 3 the minute volume is increased; loading of the heart increases slightly the filling pressure—good cardiac competence. Further poisoning with DNP (at 4) puts the heart into failure. Pulmonary artery pressure at 1: 16.4 cm H₂O, before 2: 15.8, before 4: 16.4, after 4: 20.0 cm H₂O.

Table 2.—Effect of DNP and Lanatoside C on the HLP at 30 °C and 39 °C.
(Average of 7 experiments for each dose)

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>After 1.8 × 10⁻⁴ M. DNP</th>
<th>After 1.8 × 10⁻⁵ M. DNP</th>
<th>After 0.3–2.0 mg/100 mg h.w. Lanatoside C</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 °C</td>
<td>work kgm/100 gm h.w.</td>
<td>0.85 = 100%</td>
<td>100%</td>
<td>39%</td>
</tr>
<tr>
<td></td>
<td>O₂-consumption cc./min./100 gm h.w.</td>
<td>5.3 = 100%</td>
<td>207%</td>
<td>270%</td>
</tr>
<tr>
<td>39 °C</td>
<td>O₂-consumption cc./min./100 gm h.w.</td>
<td>6.6 = 100%</td>
<td>190%</td>
<td>—</td>
</tr>
</tbody>
</table>

Cardiac rhythm was noted after the administration of the cardiac glycoside. These results indicate that high concentrations of dinitrophenol block the cardiotonic action of lanatoside C at 30 °C, as well as at 39 °C, independent of the degree of cardiac failure induced by this metabolic blocking agent. Gruhzit and Farah described also ineffectiveness of ouabain during severe dinitrophenol poisoning.

Discussion
The present experiments demonstrate that dinitrophenol increases the O₂-consumption of the heart-lung preparation while impairing its function. Consequently, there is a dissociation between the energy uptake of the myocardium and its energy output, namely its work performance. This finding is expressed in the pronounced decrease of cardiac efficiency after
dinitrophenol poisoning. A rise of cardiac $O_2$-consumption during failure has repeatedly been described and it is assumed that any increase of the diastolic volume stimulates the oxidative metabolism. However, it appears from the data in the literature that this increase of myocardial respiration is considerably smaller than that observed after dinitrophenol. Using the same procedure of evaluation we found that the cardiac $O_2$-uptake during spontaneous or Nembutal failure increases only 5-10%, a negligible effect compared to the 90 per cent rise after dinitrophenol (unpublished data). These data indicate that the increase in myocardial ($O_2$-consumption after dinitrophenol is not or only to a small extent due to the cardiac failure, but it is rather the effect of a direct influence of dinitrophenol on the myocardial metabolism. The concentrations of dinitrophenol administered in the present study correspond to those used by Wollenberger and Karsh and which were found to depress the phosphocreatine content of the heart. Similar concentrations inhibit phosphorylation while increasing the $O_2$-consumption in various isolated tissues. It is, therefore, probable that the cardiac failure of the heart-lung preparation develops as a consequence of a lowered supply of energy rich phosphate bonds.

Lanatoside C exerts its typical cardiotonic action in dinitrophenol failure only under two conditions: a sufficient supply of $O_2$ has to be present and the degree of dinitrophenol poisoning should be moderate. It has been found also by Gruhzit and Farah that ouabain restores only the failure due to moderate dinitrophenol poisoning. In contrast to the present findings, ouabain does not compensate dinitrophenol failure of the Langendorff and frog heart. It has been suggested above that the low $O_2$-reserve of the Langendorff or frog heart and/or a larger degree of dinitrophenol poisoning can explain the different results obtained by Wollenberger and Karsh and Ellis. In the heart-lung preparation, the cardiac glycoside can completely compensate the failure due to moderate degrees of dinitrophenol poisoning. This effect, however, is achieved without changing the marked increase of $O_2$-consumption of the DNP poisoned heart and is, therefore, not related to the metabolic action of dinitrophenol.

**Conclusions and Summary**

Dinitrophenol (DNP) induces failure of the canine heart-lung-preparation (HLP) at 39 C. blood temperature and increases cardiac $O_2$-consumption specifically. During the hypothermia (30 C.), the metabolic action of dinitrophenol on the $O_2$-uptake is similar to that at 39 C.; the cardiac competence, however, is no longer depressed by moderate doses of dinitrophenol.

Analysing the action of lanatoside C on dinitrophenol failure, we observed two findings which are of special interest: a) Lanatoside C exerts its cardiotonic effect during moderate dinitrophenol poisoning without inhibiting the metabolic stimulation of dinitrophenol (i.e. the increased $O_2$-consumption). b) Cardiac failure cannot be influenced by lanatoside C during a large degree of metabolic blockade obtained either by low concentrations of dinitrophenol and relative anoxia or by high doses of dinitrophenol.

These findings point to the tentative conclusion that the cardiac glycoside does not act as an anti-metabolite at the level where dinitrophenol, as an uncoupling drug, inhibits phosphorylation. Rather it appears that the cardiotonic action of lanatoside C is performed despite a persisting metabolic blocking activity of dinitrophenol as long as a minimal adequate supply of energy is present. Ellis draws a similar conclusion from his experiments on isolated frog heart. It can, therefore, be assumed that the cardiac glycoside interferes with energy utilisation rather than with energy production.

**References**

Effect of Barbiturate and Chloralose Anesthetics on the Circulation

It is considered as fairly well established among experimentalists that barbiturate anesthesia reduces cardiac output and that arterial pressure is maintained through increased peripheral resistance. Recent studies at the Wm. G. Kerckhoff Institute in Bad Nauheim indicated that chloralose acts in an identical manner; the only difference found was that barbiturates, which increase heart rate, reduce mean stroke volumes more than chloralose.

However, the authors' studies also demonstrated that when cardiac output is determined over many months on the same trained unanesthetized dogs, the so-called "resting output" varies extremely from day to day, presumably because the "minimal resting output" is raised occasionally by unknown mechanisms. Their studies revealed that the cardiac output during barbiturate or chloralose anesthesia never fell below the minimal values obtained on resting unanesthetized trained dogs. They conclude that the decrease in cardiac output by these anesthetics should be regarded as a reduction to a minimal resting output.

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