COMBINATION OF EPINEPHRINE AND 2,4-DINITROPHENOL WITH MUSCLE OF THE NORMAL RAT*

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The synthesis of glycogen from glucose by the isolated rat diaphragm in vitro is altered by several compounds. Insulin is the only substance known at present which increases glycogen synthesis, while several substances, epinephrine, most steroid hormones, and 2,4-dinitrophenol, are known to decrease it. The mechanisms by which these effects are brought about are obscure. We have found (1, 2) that a rat hemidiaphragm placed in an insulin solution for 1 minute or less, then washed, will synthesize more glycogen than its paired untreated hemidiaphragm when incubated for 90 minutes in a glucose medium. We interpreted this to mean that insulin combines rapidly with some structural element of the muscle and exerts its effects in combined form. Presumably such combination is a prerequisite for the effect of insulin upon metabolic processes.

The present paper reports experiments designed to determine whether or not such combinations can be demonstrated by the same method in the case of epinephrine, 2,4-dinitrophenol, and two adrenal steroids, all of which diminish glycogen formation from glucose when they are present during the entire period of synthesis.

Methods

The techniques of preequilibration of the diaphragms with the test substance, the washing procedure, the character of the medium during the 90 minute synthetic period, the method for the determination of glycogen synthesis, and the measurement of the oxygen uptake are identical to those employed in previous experiments (1).

Results

Epinephrine—Table I contains the data of experiments in which rat hemidiaphragms were immersed for 1 minute in solutions of epinephrine

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of different concentrations, washed, and equilibrated in the medium with glucose. There was a significant decrease in the glycogen synthesis of the experimental hemidiaphragm compared to its control in experiments in which the concentration of epinephrine was 4 or 10 \( \gamma \) per ml. during the initial 1 minute preequilibration period.

2,4-Dinitrophenol—This compound causes an increase in the oxygen uptake and a decrease in the glycogen synthesis from glucose in the isolated rat diaphragm. Table II shows that preequilibration with 2,4-dinitrophenol for 1 minute at a concentration of 10 \( \gamma \) per ml. is sufficient to cause, subsequently, a marked increase in \( O_2 \) uptake and a decrease in glycogen synthesis of the diaphragm. In all experiments, the oxygen uptake was linear during the entire 90 minute equilibration period.

**Adrenal Cortical Steroids**—Verzar and Wenner (3) demonstrated that glycogenolysis occurs when diaphragms are equilibrated in media con-

### Table I

*Combination of Epinephrine with Isolated Rat Diaphragm*

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Concentration of epinephrine (( \gamma ) per ml.)</th>
<th>Decrease of glycogen synthesis (( \mu M ) per gm.) (glucose equivalents)</th>
<th>Per cent of control</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>4</td>
<td>2.2 ( \pm ) 0.83</td>
<td>22 ( \pm ) 8.5</td>
<td>( &lt;0.05 )</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>2.9 ( \pm ) 0.87</td>
<td>30 ( \pm ) 8.9</td>
<td>( &lt;0.01 )</td>
</tr>
</tbody>
</table>

* Probability that the difference is due to chance.

### Table II

*Combination of 2,4-Dinitrophenol (DNP) with Isolated Rat Diaphragm*

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Oxygen uptake, ( \mu M ) per gm. per hr. ( \pm ) s.e.m.</th>
<th>Decrease of glycogen synthesis with DNP (( \mu M ) per gm.) (glucose equivalents)</th>
<th>Per cent of control series</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>48</td>
<td>22 ( \pm ) 5.4*</td>
<td>3.7 ( \pm ) 1.0*</td>
</tr>
</tbody>
</table>

* Probability that the difference is due to chance, \( <0.01 \).
taining various steroid hormones. The experiments reported in Table III are in agreement with these findings. Two representative steroids, 17-hydroxycorticosterone and 11-desoxycorticosterone, when present during

Table III
Effect of Adrenal Cortical Hormones on Glycogen Synthesis by Isolated Rat Diaphragm

Diaphragm equilibrated for 90 minutes (38°) in phosphate-saline medium (0.4 per cent glucose). Adrenal cortical steroid as indicated. Solvent, propylene glycol or alcohol (1.5 per cent), also added to control paired hemidiaphragm.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Adrenal steroid</th>
<th>Concentration</th>
<th>Glucose in medium</th>
<th>Decrease in glycogen compared to control, μg (glucose equivalents) per gm. ± s.e.m.</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg. per ml.</td>
<td>per cent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17-Hydroxycorticosterone</td>
<td>0.05</td>
<td>0</td>
<td>4.7 ± 1.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>11-Desoxycorticosterone</td>
<td>0.05</td>
<td>0.2</td>
<td>6.6 ± 0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>0.05</td>
<td>0.4</td>
<td>4.8 ± 0.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>0.5</td>
<td>0.4</td>
<td>22.4</td>
<td></td>
</tr>
</tbody>
</table>

* Probability that the difference is due to chance.

Table IV
Failure to Demonstrate Combination of Adrenal Hormones with Isolated Rat Diaphragm

Diaphragms equilibrated (25°) for the times indicated in media containing cortical steroids. Washed twice (30 seconds) in 25 ml. of medium. Assay period, 90 minutes (38°) in phosphate-saline with 0.4 per cent glucose.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Adrenal steroid</th>
<th>Concentration</th>
<th>Time of fixation</th>
<th>Mean effect on glycogen synthesis compared to control, μg (glucose equivalents) per gm. ± s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg. per ml.</td>
<td>min.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Desoxycorticosterone</td>
<td>0.1</td>
<td>2</td>
<td>−1.3 ± 1.19</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>0.2</td>
<td>5</td>
<td>+0.6 ± 0.87</td>
</tr>
<tr>
<td>4</td>
<td>17-Hydroxycorticosterone</td>
<td>0.1</td>
<td>1</td>
<td>+2.1 ± 1.41</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>0.1</td>
<td>5</td>
<td>+0.9 ± 0.98</td>
</tr>
</tbody>
</table>

the 90 minute equilibration of the diaphragm with glucose, caused a marked decrease in glycogen synthesis.

However, when we attempted to demonstrate a combination of these substances with muscle tissue by the method which was successful in the case of insulin, epinephrine, and 2,4-dinitrophenol, we failed. Diaphragms were treated with solutions of desoxycorticosterone and 17-hydrox-
ycorticosterone for 1 to 6 minutes, washed, and then equilibrated for 90 minutes in a glucose medium. There was no difference (Table IV) in the ability of these diaphragms compared to controls to synthesize glycogen.

**DISCUSSION**

The phenomenon discussed in this paper may be stated in general terms as follows: Certain substances which are metabolically active combine with considerable rapidity with elements of tissue structure with sufficient firmness to resist the dissociating action of washing and, in combined form, exert characteristic effects on tissue metabolism. We demonstrated (1, 2) this phenomenon in the case of rat muscle and insulin as follows: A rat hemidiaphragm was equilibrated for a brief period (10 seconds or more) in a medium containing insulin and then thoroughly washed. When equilibrated in a glucose medium for 90 minutes the pretreated hemidiaphragm utilized more glucose and synthesized more glycogen than its paired control. We also showed in the following way that an anterior pituitary protein combines with rat diaphragm: A rat diaphragm was equilibrated for a brief period in a solution containing pituitary protein and then washed. When we attempted to combine insulin with this diaphragm by the method described above, we failed. We interpreted this to mean that pituitary protein had combined first and made the diaphragm refractory to insulin. The experiments reported in this paper further demonstrate the combination with muscle of epinephrine and 2,4-dinitrophenol which, in bound form, bring about a diminution of glycogen synthesis from glucose. In the case of the latter substance we also observed the characteristic effect of a marked increase in oxygen uptake.

The literature discussing this phenomenon is quite fragmentary. In the field of immunochemistry there are references to combinations of biologically active compounds, but we have been unable to find discussions of combinations of substances with mammalian tissue resulting in alterations of metabolic activity. Rothstein and coworkers (4, 5) reported interesting experiments which demonstrate the formation of complexes of uranium and proteins of the cell surface layer of yeast. They proposed a mass action interrelation between the concentrations of uranium and yeast which they tested by measuring free and combined uranium and the percental inhibition of anaerobic glucose utilization.

Most pertinent to the present study are the important observations made by Boehm (6) in 1910. He studied the effect of curarine on the excitability of frog muscle to electrical stimulation and found that the maximal effect of the drug was obtained after dipping the muscle for about 1 minute into a solution containing curarine. The drug was not removed
by extensive washing. The author drew the conclusion that curarine combined chemically or by adsorption with structural elements of the muscle and that this firm combination was an important part of the physiological action of this compound.

It is remarkable how similar this phenomenon is to the chemical combination of insulin with the rat diaphragm described by us.

The ability of certain hormones such as insulin, pituitary hormone, and epinephrine to combine chemically with muscle tissue is obviously of considerable physiological significance. For example, only small amounts of hormones need be secreted since, owing to combination, blood levels could be essentially zero. Again competitive interaction between hormones of opposed metabolic effects for sites of chemical combination on tissue offer a simple explanation for regulatory mechanisms. Since both proteins and simple organic compounds have been shown to combine with tissue, it is possible that key groups in the molecule are responsible for the binding. In the case of hormones, it is conceivable that certain groups are concerned with combination and others with specific metabolic effects. Such a dual mechanism would lead to interesting possibilities in specificities of action on metabolic processes.

Attempts to demonstrate the combination of representative adrenal steroid hormones were unsuccessful. Since these compounds are quite active in causing glycogenolysis when present in the medium during the entire equilibration period, they are apparently easily removed from the tissue during the washing period. The explanation of this difference in behavior is speculative. Perhaps metabolically active substances range in character from that of insulin, which appears to bind very firmly so that prolonged washing does not remove it from tissue, to steroid hormones which do not bind at all, or so loosely that a minimal amount of washing removes them from combination. Uranium may be in between in that complex formation with yeast protein is easily demonstrable, but dissociation occurs when the cells are subject to a moderate amount of washing (4). In certain respects the coenzymes are analogous: they are metabolically active; they combine with protein very firmly, as in the case of the flavins, and relatively loosely, as in the case of the pyridine nucleotides.

SUMMARY

1. The phenomenon of the chemical combination of metabolically active substances with structural elements of tissues was further studied. Such combination, originally observed to occur with insulin and pituitary hormone, was also demonstrated in the case of epinephrine and 2,4-dinitrophenol.
2. The implications of the combination of metabolically active substances with tissue are discussed.

BIBLIOGRAPHY
COMBINATION OF EPINEPHRINE AND 2,4-DINITROPHENOL WITH MUSCLE OF THE NORMAL RAT
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