THE POTENTIAL AND RESPIRATION OF FROG SKIN

I. THE EFFECT OF THE HOMOLOGOUS CARBAMATES.  II. THE EFFECT OF CERTAIN LYSINS

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INTRODUCTION

The subject of the relation between metabolism and the spontaneously occurring potential differences observed in certain tissues has been extensively investigated by means of experiments which fall into two general categories. The first type of experiment consists in showing that regions which have a higher metabolic rate, either because they are actively growing, or because their O₂ consumption or CO₂ output is greater, or because they reduce methylene blue more quickly, are positive to regions where the metabolism is lower. This has been done for stems of Obelia and for several varieties of growing root tip (Lund (1926), Lund and Kenyon (1927)), and Lund interprets the potential difference in general as due to “the flux equilibrium of the oxidation-reduction systems” in the cells; the experiments, however, are inconclusive in that differences in metabolism or in oxidation-reduction potential are not the only factors which can give rise to the potential differences. The second type of experiment seeks to show that the O₂ consumption of a tissue in which spontaneously occurring potential differences can be measured is depressed by O₂ lack or by the addition of various substances in the same way as are the observed potential differences. More specifically, Lund (1928 a) has shown that KCN depresses the O₂ consumption and the potential difference to the same extent in the case of frog skin, and Francis (1934) agrees with the conclusion in a general sort of way. The results of experiments on the effect of varying the O₂ tension to which the skin is exposed are very conflicting, probably because of the different methods used. Lund (1928 b) finds both O₂ consumption and potential to be reduced when
the O₂ tension is diminished, although not to the same extent; indeed, he says that the same increase in O₂ tension may stimulate respiration as much as 1600 per cent and yet increase potential by 100 per cent only. His method for measuring O₂ concentration and consumption (the Winkler method for dissolved O₂) is open to criticism, however, and both Adolph (1929) and Francis (1934) find a different relation between O₂ tension and O₂ consumption from that described by Lund. Francis (1934) concludes that the potential and respiration depend in the same way on the O₂ concentration, but Taylor (1935) says that the lowering of potential is usually about 20 per cent greater than the lowering in O₂ consumption when the skin is exposed to N₂/O₂ or CO/O₂ mixtures; finally, there is disagreement as to the effect of high concentrations of O₂ on the respiration and potential (Lund (1928 b), Francis (1934), Taylor (1935)).

No quantitative work has yet been done on the effect of narcotics on potential and O₂ consumption when these are measured simultaneously, although Boell and Taylor (1933a) have investigated the effect of the homologous carbamates on frog skin potential and shown that they depress it; the purpose of the first part of this paper is accordingly to show that the effect of the carbamates on frog skin potential is not closely related to the effect on O₂ consumption. In the second part of the paper it will be shown that several lysins, which have certain properties in common with the carbamates, abolish the skin potential without reducing the respiration.

I. The Effect of the Homologous Carbamates

Materials and Methods

1. Frog Skin.—The frogs (Rana pipiens) were used during the winter months (December–March), kept in the cold, and acclimatized at 20°C. for 72 hours before the skin was removed. For the measurement of respiration, pieces of skin about 200 mg. in weight were taken from the ventral surface, the dorsal surface, and from the upper part of the legs, but for the potential measurements only skin from the ventral surface and from the legs was used, as dorsal skin usually gives small potentials. In all cases the measurements of O₂ consumption and of potential were begun within a few minutes after the skin was removed.

2. Respirometry.—The measurements of O₂ consumption were made at 25°C. in Fenn respirometers with side cups from which the carbamates, etc., could be added by tipping the instrument. One piece of skin was used in each respirometer cup, which also contained 0.5 cc. of frog Ringer (without carbonate), and
1 cc. of the carbamate solution to be added was placed in the corresponding side cup. After 30 minutes for equilibration, the normal O₂ consumption was measured over a period of 30 minutes; this normal O₂ consumption remains remarkably constant for a period of at least 2 hours.

It is convenient here to give the values for O₂ consumption which we have obtained. These can be given either in terms of the wet weight of the skin (mm.³/gm./hr.), or in terms of dry weight (Qₒ₂),¹ and vary with the part of the body from which the skin is taken. The results of some 400 determinations are shown in Table I.

These values are about the same as those obtained by Francis (1934), who finds a Qₒ₂ of −1.08, but lower than those of Taylor (1935), who gives 330 mm.³/gm./hr. for wet skins, and higher than those of Adolph (1929) and of Lund and Moorman (1931), whose values for wet skins are 125 mm.³/gm./hr. and 139 mm.³/gm./hr. respectively. The only investigators who seem to have noticed that the O₂ consumption depends on the region from which the skin is taken are Williams and Sheard (1932).

<table>
<thead>
<tr>
<th>Location</th>
<th>Wet skin, mm.³/gm./hr.</th>
<th>Dry skin, Qₒ₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>185</td>
<td>−0.87</td>
</tr>
<tr>
<td>Ventral</td>
<td>236</td>
<td>−0.97</td>
</tr>
<tr>
<td>Leg</td>
<td>265</td>
<td>−1.15</td>
</tr>
</tbody>
</table>

After the normal O₂ consumption has been observed for 30 minutes, the apparatus is tilted, and the narcotic thus added to the skin in the respirometer vessel. Readings are made each 10 minutes for another 30 minutes, at the end of which time a new and steady rate of O₂ consumption is always attained, the effect of the narcotic on the respiration being complete within 20 minutes, irrespective of the narcotic concentration. The rate of O₂ consumption at the end of this time is then expressed as a percentage of the normal rate, and, to allow for small variations in the effect, the results of eight experiments are averaged for each concentration of narcotic. The extent to which any given concentration of carbamate depresses the skin respiration may vary by ±10 per cent, and the average depression of respiration observed in eight experiments with one narcotic concentration varies by ±3 per cent at most.

3. Potential Measurements.—These were made by the use of very simple apparatus. The skin is stretched over the end of a small glass tube, and secured in

¹ Qₒ₂ is defined as mm.³ O₂ consumed per hr. per mg. of dry tissue. The O₂ consumption in mm.³/gm./hr. for wet skin, when divided by 213, gives the Qₒ₂, with great exactness.
position with rubber bands; the tube is then inverted, so that the skin dips below
the surface of Ringer's solution contained in a small flat receptacle. A small
amount of Ringer is placed in the tube, so as to bathe the inner surface of the
skin and equalize the hydrostatic pressure, and calomel half-cells, prepared with
0.6 per cent NaCl, dip into the fluid in the tube and in the receptacle respectively.
We have found agar-silver-silver chloride electrodes very unsatisfactory. The
potentials are measured in the usual way, with a high resistance galvanometer
as a null instrument, and at a temperature of 25 ±1°C.

The skin potentials are observed at 15 minute intervals for periods from 1 to 3
hours, and only if a steady potential is finally attained is the experiment con-
tinued. The final potential attained is very variable from frog to frog, and,
as Williams and Sheard (1932), Boell and Taylor (1933a), and Francis and
Pumphrey (1933) have observed, the ventral skin gives consistently higher
potentials than the dorsal skin. The skin of the leg usually gives a potential
between the two. In view of this variability, we have made it a rule to use
only skins which give a potential of between 25 and 40 mv. at equilibrium.
There seems to be no relation between the O₂ consumption of the skin and its
potential at equilibrium, as follows from the fact that the former is very con-
stant, whereas the latter is exceedingly variable.

When a steady potential is attained, the Ringer's solution on both sides of
the skin is removed and replaced by a solution of the narcotic in Ringer. The
change in potential is followed, and at the end of 30 minutes a new and steady,
or sometimes slowly falling, potential is reached. In order that the determina-
tion may be comparable to the determination of the effect of the narcotic on the
O₂ consumption, the potential at the end of 30 minutes is taken as the final
one, and is expressed as a percentage of the equilibrium potential before the
addition of the narcotic. The variability in the effect of the carbamates on
skin potential is much greater than the variability in their effect on respiration,
and so the results have to be based on the average of a number of experiments,
and a standard error attached. The number of individual experiments required
for the determination of the effect of a given concentration of carbamate depends,
in fact, on the magnitude of the effect itself; thus, it is easy to show that there
is no diminution in potential or that the potential is completely abolished, but

2 Boell and Taylor (1933a) appear to be satisfied that the effect of a carbamate
on frog skin potential is independent of the original potential difference observed
across the skin, provided the effect is expressed as a percentage of the original
equilibrium potential, even if this varies from 5 to 75 mv. This has not been
our experience, for skins with a low initial potential are frequently affected by
the addition of a carbamate to a greater extent than are skins with high initial
potentials. The percentage effect of a carbamate, however, seems to be rela-
tively independent of the initial potential when the latter is between 25 and 40 mv.
The same kind of difficulty does not arise with respect to the O₂ consumption,
for this is subject to much less variation.
a large number of individual experiments are required when the reduction in potential is only partial.

RESULTS

The results obtained with four carbamates, ethyl, isopropyl, n-butyl, and isoamyl, are shown in Table II.\(^3\) The first column gives the molar concentration of the narcotic, the second the \(O_2\) consumption 30 minutes after the application of the carbamate, expressed as a percentage of the normal \(O_2\) consumption, and the third the potential attained 30 minutes after the addition of the carbamate, also expressed as a percentage of the normal equilibrium potential of the skin. In all cases the concentration of narcotic is that actually present in the fluid bathing the skin in the respirometer vessel, for the 1 cc. of narcotic

\(^3\) All the carbamates used were obtained from the Eastman Kodak Co. In some of the earlier experiments we found that ethyl carbamate obtained from another source had less narcotic effect. Boell and Taylor use the words "carbamate" and "urethane" synonymously.
added from the side cup is diluted with the 0.5 cc. of Ringer in the vessel containing the skin.

These results are shown graphically in Figs. 1–4, the \( O_2 \) consumption being shown by the line passing through crosses and marked A, and the potentials by the dotted line marked P. In the case of the curve for the potentials, the line is drawn through the means for a variable number of experiments with each narcotic concentration, and the vertical lines show the size of the standard errors.

**Fig. 1.** Ethyl carbamate. Curve P, effect on potential, curve A, effect on \( O_2 \) consumption. (Curve B shows results obtained in the experiments on adsorption of the carbamate; see text for explanation.) Ordinate, potential or \( O_2 \) consumption as a percentage of initial potential or \( O_2 \) consumption; abscissa, concentration of carbamate in the system.

**Fig. 2.** Propyl carbamate. Ordinate and abscissa, etc., as in Fig. 1.
Speaking generally, the curve for the reduction of potential by increasing quantities of narcotic pursues a course different from that of the curve for the reduction of O₂ consumption. In the case of propyl, butyl, and amyl carbamates, the respiration is first reduced in a concentration which does not affect the potential, and the potential is abolished in a concentration which reduces the O₂ consumption to about 20 per cent, a point beyond which it does not seem possible to reduce it further. The curve for the reduction of the potential thus

4 In the case of all the carbamates, the curve relating residual respiration to concentration shows a tendency to rise when the carbamate concentration is very great. Thus the O₂ consumption is reduced to 18 per cent by the addition of 1.5 M ethyl carbamate, but only to 26 per cent by the addition of 2.25 M. This point is not shown in the figures.
crosses the curve for the reduction of the O₂ consumption. The difference between the two curves is very marked in the case of ethyl carbamate, where the potential is reduced to zero in a concentration which reduces the respiration to 50 per cent only.⁸

Traube's Rule and the Effective Concentration of the Carbamates

The data obtained in the foregoing experiments can be used to test the validity of Traube's rule for members of a homologous series, this rule stating, in its simplest form, that the ratio between the isoactive concentrations of any two adjacent members of a homologous series is 3:1. Boell and Taylor (1933a) conclude that the carbamates conform to Traube's rule in depressing the potential of frog skin, and obtain ratios between the isoactive concentrations of adjacent members of from 2.16 to 3.66, with an average value of about 3.0.

Our results certainly do not support Traube's rule, either as regards the effect of the carbamates in reducing skin potential or in reducing O₂ consumption. Table III shows the concentrations effective in producing a 50 per cent reduction in respiration and potential respectively, together with the ratio of the activity of each homologue to that of the one before it, and almost identical results are obtained if we consider an 80 per cent reduction in potential and in respiration.

In systems containing surface-active substances such as the carbamates, however, the effects produced are more likely to be related to

⁸ In the case of ethyl carbamate only, and in the higher concentrations, there is a sudden burst of O₂ consumption immediately after the narcotic is added. The burst is over within 30 seconds, but is nevertheless quite definite.
the concentration of narcotic actually present in the regions of the cells affected than to the concentration added to the system as a whole. The quantity of narcotic "adsorbed" by the skin at the end of any given time from a solution of any given concentration of carbamate can be found in the following way.

A piece of frog skin of the size ordinarily used in obtaining the curves marked A in Figs. 1-4 (about 200 mg. in weight) is placed in 1.5 cc. of a selected concentration $c_1$ of carbamate contained in a respirometer vessel, which is rocked to and fro for 30 minutes at 25°C. The respiration is not recorded, the only purpose of the respirometer being to duplicate the conditions under which the standard curves marked A were obtained. At the end of 30 minutes 1 cc. of the fluid surrounding the skin is transferred to the side cup of another respirometer containing a fresh piece of skin in 0.5 cc. of Ringer; the normal respiration of this piece of skin is recorded for 30 minutes, and then the fluid from the side cup is tipped into the respirometer vessel. The residual $O_2$ consumption at the end of another 30 minutes is found and expressed as a percentage of the normal $O_2$ consumption of the skin, already measured. Since a relation exists between the residual respiration at the end of 30 minutes and the concentration of a carbamate, this relation being shown in the curves marked A, the concentration $c_2$ of the carbamate added from the side cup can be read off from the standard curves. The difference $\Delta = (c_1 - c_2)$ obviously gives the quantity of carbamate adsorbed by the first piece of skin at the end of 30 minutes.

The results obtained for various concentrations of different carbamates are shown in the curves marked B in Figs. 1-4, these showing the relation between the residual respiration of the second piece of skin and $c_2$, the concentration of carbamate added to the first piece of skin. Each point is an average of six experiments, and the values found show a variation not exceeding ±5 per cent. Table IV shows the relation between $\Delta$, the quantity of carbamate adsorbed by the first piece of skin, and $c_2$, the quantity left free, both $\Delta$ and $c_2$ being given in molar concentrations. To convert them to milligrams of carbamate adsorbed or left free in the experimental systems, it is only necessary to multiply the figures in the table by 1.5 times the molecular weight of the carbamate.

When log $\Delta$ is plotted against log $c_2$, a good straight line results for each carbamate; the quantity of carbamate taken up by the skin is accordingly related to the amount left free by the expression

$$\Delta = A c_2^{4.6}$$
which is the same as that for the adsorption isotherm. The slope of the lines, which determines the constant \( n \), is substantially the same for each of the carbamates, but the numerical value of \( n \) is exceptionally small (0.2 approximately); this makes one reluctant to accept the linear relation of \( \log \Delta \) and \( \log c_2 \) as evidence of the carbamates being concentrated in the neighborhood of the skin by an adsorption process alone.\(^6\)

\(^6\) In interpreting the results of these experiments on adsorption, we are assuming that the difference between the quantity of carbamate added to the system and the quantity found in the fluid after 30 minutes represents a quantity of narcotic existing as such, and concentrated at the skin surfaces or distributed throughout its thickness. It is conceivable that the narcotic which disappears is metabolized or otherwise combined, so that it does not exist as a "narcotic concentration" in the sense in which one uses the term when speaking of adsorption or of Traube's rule. The fact that a few washings in Ringer's solution is sufficient to restore the \( O_2 \) consumption of a deeply narcotized skin to about 75 per cent of the normal \( O_2 \) consumption (and most of the 25 per cent reduction can be accounted for by the handling of the tissue) indicates that the narcosis is not the result of some irreversible process such as the oxidation of the carbamate to urea, and that the carbamates exist as a narcotic concentration in the sense in which we have been using the term. The local concentration, of course, need not be uniform, and may be brought about by adsorption at surfaces, solution in lipoids, or in other ways.

### TABLE IV

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>( c_1 )</th>
<th>( \Delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl</td>
<td>0.27</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.36</td>
<td>0.14</td>
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<tr>
<td></td>
<td>0.46</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>0.52</td>
<td>0.58</td>
</tr>
<tr>
<td>Propyl</td>
<td>0.056</td>
<td>0.0065</td>
</tr>
<tr>
<td></td>
<td>0.080</td>
<td>0.0080</td>
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<td>0.115</td>
<td>0.0350</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>0.10000</td>
</tr>
<tr>
<td>Butyl</td>
<td>0.024</td>
<td>0.0060</td>
</tr>
<tr>
<td></td>
<td>0.035</td>
<td>0.0100</td>
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<td>0.042</td>
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</tr>
<tr>
<td></td>
<td>0.050</td>
<td>0.0400</td>
</tr>
<tr>
<td>Amyl</td>
<td>0.0060</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td>0.0078</td>
<td>0.0032</td>
</tr>
<tr>
<td></td>
<td>0.0090</td>
<td>0.0060</td>
</tr>
<tr>
<td></td>
<td>0.0105</td>
<td>0.0120</td>
</tr>
</tbody>
</table>
The quantities of the carbamates taken up by the skin when the O₂ consumption is reduced to 50 per cent, or to 20 per cent, do not conform to Traube's rule any better than do the quantities which are present in the system as a whole. The quantities of adsorbed carbamates which correspond to a 50 per cent reduction in O₂ consumption,

![Figure 5: Adsorption of the carbamates. Curve E, ethyl, P, propyl, B, butyl, and A, amyl. Log Δ plotted against log c₂.](image)

for example, are in the ratios 1:8.7:11.6:58 for the successive homologues, instead of in the ratios 1:3:9:27, as Traube's rule requires.

**II. The Effect of Certain Lysins**

The effect of the carbamates on the potential and O₂ consumption of frog skin shows that these two properties are not affected identically,
but does not supply evidence that they can be affected independently. We have therefore studied the effect of another class of surface active substances, viz., the simple lysins, particularly as Boell and Taylor's work (1933 a, 1933 b) suggests a relation between the effect of the carbamates on skin potential and their surface activity. The lysins which we have used are saponin, sodium taurocholate, and sodium glycocholate, and the methods employed are identical with those described above.

Quite unlike the carbamates, these simple lysins in proper concentration completely abolish the skin potential, but leave the O2 consumption either unaltered or slightly increased. The first column of Table V gives the dilution of the lysin, the second the O2 consumption 30 minutes after the addition of the substance, and the third the potential after the same interval; as in the case of Table II, the O2 consumption and the potential observed are expressed as percentages of the normal O2 consumption and the normal potential respectively.

The increases in O2 consumption which Table V shows are probably not real, for the addition of the more concentrated lysins to the system results in CO2 production, and the absorption of the liberated CO2 by the KOH in the respirometer vessel results in what appears to be an increased O2 consumption. Observations of the O2 consumption over the first 30 minutes are accordingly apt to give a spuriously high value.7 The essential point is that the O2 consumption ultimately

7 This liberation of CO2 is probably due to the added lysins being acid (pH 4.7 for a 1 in 50 saponin, 5.7 for a 1 in 100 glycocholate), but it is very difficult to say whether its absorption by the KOH in the respirometer accounts for all
returns to its normal rate and continues at this rate for hours, whereas the skin potential is entirely abolished.

DISCUSSION

In interpreting these results, it must be borne in mind that frog skin is a heterogeneous tissue, and that the potential differences may depend on properties which are spatially defined; for example, they may arise in the neighborhood of the skin surfaces only. When one measures the $O_2$ consumption of the whole skin, one is not necessarily measuring the $O_2$ consumption in the regions which determine the potential difference, and so it is not remarkable that substances such as the carbamates or the simple lysins should affect the $O_2$ consumption and the potential differently, particularly as the substances themselves are in all probability heterogeneously distributed. To take an extreme case in the lysins which abolish potential but leave respiration unchanged, these substances may be concentrated in the neighborhood of the skin surfaces, and while these may be regions responsible for the maintenance of potential, their contribution to the total $O_2$ consumption of the skin may be very small. Even if one were to admit that the potential difference observed across a tissue had its origin in metabolic processes of which $O_2$ consumption was a measure, it would be impossible, strictly speaking, to determine the nature of the relation except in a tissue in which there was no spatial separation of the seat of the potential and of the respiration respectively.

Bearing this in mind, the results can be considered in relation to two theories regarding the origin of the potential difference across frog skin. The first is the general theory most clearly expressed by Francis (1934), which requires two things only: (a) a respiratory process affording a constant source of diffusible ions, and (b) a partial separation of oppositely charged ions, which may result from there being a concentration gradient, a distribution of dissolved salts between two

the apparent increase in the $O_2$ consumption. There are two reasons why we think that it may not. The first is that there is a very poor relation between the pH of the added lysin and the apparent increase in $O_2$ consumption, and the second is that the apparent increase sometimes lasts as long as 2 hours. There may thus be a real stimulating effect on the $O_2$ consumption, such as undoubtedly occurs in the case of ethyl carbamate.
immiscible solvents, or the presence of a semipermeable membrane, the integrity of which may also depend on metabolic processes. There are a sufficient number of possible ways in which a narcotic may act in such a system to account for all the experimental results; the effect of a carbamate, for instance, need not be such as to depress potential and respiration equally, and in an extreme case a lysin might destroy the integrity of a semipermeable membrane or other barrier to equal ionic diffusion without affecting the metabolic process which gives rise to the diffusible ions, and which is measured in terms of the O₂ consumption.

The second theory is that of Lund (evidence for which is well summarized in Lund (1930)), which accounts for the potential difference in terms of the “flux equilibrium potential of oxidation-reduction systems” in the cells. The supporters of this theory would have little difficulty in accounting for the results contained in this paper in so far as they concern the unequal effect on O₂ consumption and potential difference produced by the carbamates, for they have used the conception of “flux equilibrium” to account for the similar disparity in effect on O₂ consumption and potential observed with reduced tensions of O₂ (see Lund (1928 b)). The abolition of the potential by the lysins, without there being any reduction in O₂ consumption, cannot be explained in this way, but even this result does not disprove Lund’s hypothesis if the frog skin is recognized as a heterogeneous tissue, for it can be argued that the potential differences are due to oxidation-reduction processes in spatially delimited regions of the skin, these being affected by the lysins, but not contributing appreciably to the total O₂ consumption of the bulk of the skin. The idea of heterogeneity, however, is essential, and it ought to be remarked that a theory such as Lund’s, which postulates a specific relation between O₂ consumption and potential difference and yet is sufficiently elastic not to be disproved by a type of experiment which shows that the variables can be affected independently (the foregoing), cannot derive substantial support from similar experiments (those involving variations in O₂ tension) which show that, under other circumstances, the variables are affected in a like manner.
SUMMARY

Measurements of the \( O_2 \) consumption and of the potential of frog skin, made under comparable conditions, show that the homologous carbamates (ethyl, propyl, butyl, and amyl) reduce both the \( O_2 \) consumption and the potential, but not in a similar manner. In this respect, the effect of the carbamates is like the effect of reduction in \( O_2 \) tension. The simple lysins (saponin and the bile salts), on the other hand, abolish the potential without reducing the \( O_2 \) consumption at all.

Irrespective of whether one considers the concentration of carbamate in the entire system or the amount of carbamate adsorbed by the frog skin, Traube's rule relating the effect of a carbamate to its position in the homologous series does not seem to apply.

REFERENCES

Protoplasma, 1930, 13, 236.