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# The Oxygen Uptake, Glucose Utilization and Lactic Acid Production of Guinea-pig Skin in Relation to Oxygen Tension

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In spite of the many disorders of unknown aetiology to which the skin is subject, and which may be possibly caused by metabolic abnormalities, accounts of the metabolic activity of skin *in vitro* are relatively scanty as compared with those of internal organs or tumours. Among these reports are the investigations of Peters & Wakelin (1946) and Peters, Sinclair & Thompson (1946) leading to the development of 2:3-dimercaptopropanol (BAL), the work of Dixon & Needham (1946) providing evidence in favour of the enzyme theory of vesication, that of Barron, Meyer & Miller (1948) and of Thompson (1946) on the metabolism of skin and the effect of vesicant agents, the work of Berenblum, Chain & Heatley (1940) on the metabolism of skin and neoplastic tissue, that of Bullough & Johnson (1951) on respiration and mitotic activity, and that of Griesemer & Gould (1954) on the citric acid cycle in epidermal tissues.

Medawar (1948) first demonstrated that skin slices could be maintained *in vitro* for several days, and in a previous paper (Cruickshank, 1954) a differential micro-respirometer was described for making continuous observations of the oxygen uptake of skin slices under similar conditions of cultivation. Some of the factors affecting the respiration of skin slices were discussed in that study. The present work is concerned with the metabolism of glucose by skin under conditions of tissue culture and thus differs from previous reports in that serum was present in the medium and that observations were made over periods of 24 hr. as well as for the usual short initial periods of up to 5 hr. In earlier experiments it had been shown that the oxygen tension affected the respiratory rate, and it seemed desirable to establish the role of oxygen tension in the survival of skin *in vitro*, and to determine its effect upon glucose consumption and upon lactic acid production.

## MATERIALS AND METHODS

The respirometer consists essentially of two chambers in a nickel-plated brass block which are connected by a graduated capillary containing an indicator droplet. A special tap permits the addition of any desired gas mixture to the chambers by means of metal tubes projecting above the level of the water bath. This tap was slightly modified for the present experiments by increasing the size of the Perspex base-plate which was cut in the form of a circle. This was done to permit observations at gas pressures greater than atmospheric without leakage.

The skin slices float on the surface of a culture medium in a glass cup in one of the chambers, a cup containing only medium being placed in the control chamber. $CO_2$ is absorbed by filter paper soaked with 2% KOH in the floor of each chamber.

The medium consisted of serum 5 parts, Krebs–Ringer phosphate 3 parts, 5% glucose 1 part and streptomycin 500 $\mu$g./ml. 1 part. Amounts of 0·5–0·7 ml. were accurately measured into each cup and the utilization of glucose and the production of lactic acid were measured by comparing the final concentrations of these substances in the 'culture' and 'control' cups.

Glucose was estimated by the method of King, Haslewood & Delory (1937*a*). Portions of 0·2 ml. of medium were diluted to volume with isotonic sodium sulphate and deproteinized by precipitation with copper tungstate (King *et al.* 1937*b*).

Lactic acid was estimated by the method of Barker & Summerson (1941) as adapted for blood analysis (Hawk, Oser & Summerson, 1947).

Skin was obtained from the shaved guinea-pig ear. This was prepared the previous day and anointed with a fungicide, 'Mycostatin', to prevent the occasional contamination of 24 hr. cultures with a mould frequently found on guinea-

pig skin. The skin was cut free-hand with a stropped scalpel into thin slices consisting mainly of epidermis with only a fine supporting framework of dermis. Adult guinea pigs of about 800 g. were used. Each guinea pig provided enough skin for six respirometers—about 10 mg. being put in each. The skin from young guinea pigs showed respiration rates which were up to 20% higher than those of adults. All observations were expressed per mg. initial wet weight of skin per hour.

## RESULTS

### *Effects of oxygen tension on oxygen uptake*

The oxygen uptake of skin in relation to the oxygen tensions at which observations are made is shown in Table 1. These values represent mean rates based on readings at 15 min. intervals during (*a*) the first 5 hr. and (*b*) between 21 and 24 hr. It will be seen that in 0·05 atm. oxygen the rate is low, while maximum respiration during the first 5 hr. occurs in 0·75 atm. Increase of oxygen tension up to 1·5 atm. produces no further increase in respiratory rate. The mean rate in air (0·2 atm.) is 1·05 μl./mg./hr. and in oxygen 1·27 μl./mg./hr.—a difference of about 20%.

The respiratory rates obtained after 21–24 hr. exposure to these oxygen tensions show a somewhat different relationship. The low rates at oxygen tensions less than that of air are still observed, and maximal respiration is obtained at 0·5–0·75 atm. oxygen. The rate in pure oxygen is lower than that in 0·75 atm. by 0·15 μl./mg./hr. A comparison of the mean rates in oxygen and the rate obtained by combining observations at 0·5 and 0·75 atm. shows this difference to be statistically significant ($P > 0·01$). At 1·5 atm. oxygen a striking reduction occurs after 24 hr., the respiration being 0·38 μl./mg./hr. as compared with 1·00 μl./mg./hr. in oxygen at atmospheric pressure and with 1·32 μl./mg./hr. after only 5 hr. exposure to 1·5 atm. oxygen. After exposure for 24 hr. to 1·5 atm. oxygen the skin slices were histologically abnormal, many cells being necrotic. Exposure to air at 1·5 atm. pressure caused no harmful effects. It will be noted that the respiration rates after 24 hr. at 0·5 and 0·75 atm. oxygen are only some 10% less than the initial rates. This small reduction can be avoided by using less skin per unit volume of medium; but the amounts of tissue used in this study were rather large in order to increase the accuracy of the chemical estimations.

All of the skin cultures maintained at 0·05 atm. oxygen, and most of those in 0·1 atm. oxygen showed histological abnormalities which may be reasonably attributed to anoxia. The greatly reduced respiration obtained at these tensions suggests inadequate penetration of oxygen to the skin cells. These observations indicate that for the slice thickness used, the minimal oxygen tension is between 0·1 and 0·2 atm. Measurement of several slices from several experiments by means of a recording micrometer showed that the slices were between 0·1 and 0·2 mm. thick. The theoretical limiting thickness of tissue slices is usually based upon Warburg's formula (Warburg, 1923):

$$d = \sqrt{(8CO \times D/A)},$$

where $d$ = limiting thickness (mm.), $CO$ = oxygen tension in atm.; $D$ = diffusion constant ($1·98 \times 10^{-5}$, Carlson, 1911); $A$ = rate of oxygen consumption (ml./min./ml. of tissue). But, since oxygen diffusion through the keratin surface of skin is probably negligible, the effective surface area is reduced approximately by half and consequently the thickness must be similarly reduced. Table 2 shows the

Table 1. *Effect of oxygen tension on the respiration of skin slices*

Results are based on 12–20 experiments at each oxygen tension.

| Oxygen tension (atm.) | 0–5 hr. Mean respiration (μl./mg./hr.) | 0–5 hr. S.E. of mean | 0–5 hr. Successive difference | 21–24 hr. Mean respiration (μl./mg./hr.) | 21–24 hr. S.E. of mean | 21–24 hr. Successive difference |
|---|---|---|---|---|---|---|
| 0·00 | 0·00 | — | — | 0·00 | — | — |
| 0·05 | 0·37 | ±0·02 | +0·47 | 0·38 | ±0·06 | +0·41 |
| 0·10 | 0·84 | ±0·09 | +0·21 | 0·79 | ±0·08 | +0·11 |
| 0·20 | 1·05 | ±0·04 | +0·17· | 0·90 | ±0·03 | +0·26 |
| 0·50 | 1·22 | ±0·04 | +0·10 | 1·16 | ±0·03 | −0·01* |
| 0·75 | 1·33 | ±0·03 | −0·05* | 1·15 | ±0·07 | −0·15* |
| 1·00 | 1·27 | ±0·07 | +0·05* | 1·00 | ±0·05 | −0·62 |
| 1·50 | 1·32 | ±0·05 | | 0·38 | ±0·10 | |

* These differences fail to reach conventional levels of significance ($P < 0·05$). All other differences listed are 'significant'.

Table 2. *Theoretical limiting slice thickness*

For method of calculation, see text.

| Oxygen tension (atm.) ... | 0 | 0·05 | 0·1 | 0·2 | 0·5 | 0·75 | 1·0 | 1·5 |
|---|---|---|---|---|---|---|---|---|
| Limiting skin thickness (mm.) | — | 0·10 | 0·14 | 0·19 | 0·30 | 0·37 | 0·43 | 0·52 |

theoretical limiting thickness for skin slices based upon these considerations. The respiratory rate in 0·75 atm. oxygen was taken as being the highest obtainable. On this basis the limiting thickness in air is 0·19 mm. and in 0·1 atm. oxygen 0·14 mm. This corresponds with the experimental findings.

*Effects of oxygen tension on glucose utilization and lactic acid production*

As observations of glucose utilization were made by measuring the total consumption and dividing by the number of hours, figures are available for the periods of 0–5 hr. and for the period of 0–24 hr. The rates of glucose utilization (0–5 hr.) at the various oxygen tensions are shown in Table 3*a*. It will be seen that there is a decline in glucose utilization from 10·0 μg./mg./hr. at 0·05 atm. to 6·3 μg./mg./hr. at 1·5 atm., and also that the rate of utilization in nitrogen is slightly, but insignificantly, lower than that at 0·05 atm. oxygen. It is likely that this is due to diminishing viability of the tissues, since a few determinations of the glucose utilization of skin in nitrogen over 3 hr. gave values of 12–14 μg./mg./hr. The rates of glucose utilization measured over 24 hr. are much lower (Table 3*b*), being approximately one-half of those measured over 5 hr. The decline of glucose utilization with increasing oxygen tension is still present.

The rates of lactic acid production at the various oxygen tensions are also shown in Table 3. In both 5 and 24 hr. experiments the lactic acid production decreases as the oxygen tension of the gas phase is increased. As with glucose utilization and, presumably for a similar reason, the lactic acid production in nitrogen is slightly lower than in 0·05 atm. of oxygen, and the rates as measured in 24 hr. experiments only about half of those in the 5 hr. experiments.

As the observations on oxygen uptake, glucose utilization and lactic acid production were made on the same portions of skin, it is possible to compare their inter-relationship quantitatively. Such a comparison involves consideration of the respiratory quotient. The design of the apparatus precluded the addition of acid without opening the chambers to the atmosphere. It was thus necessary to measure the oxygen uptake in the manner previously described, and the carbon dioxide output indirectly by omitting the potassium hydroxide from the chambers and making observations in an atmosphere containing 5 % $CO_2$. Since serum was included in the medium, it was found necessary to allow it to equilibrate with 5 % $CO_2$ in oxygen overnight. The respiratory quotient was found to be 0·97 ± 0·03 and, consequently, in the following calculations it was assumed that oxygen was consumed entirely in the oxidation of glucose to carbon dioxide. Since 1 μl. of oxygen is used in the complete oxidation of 1·34 μg. of glucose, the 'theoretical' rate of lactic acid production can be calculated from the difference between the glucose actually used and that corresponding to the oxygen used. For measurements over 24 hr. the mean of the respiratory rates obtained at 0–5 hr. and 0–24 hr. were used in order to estimate the amount of glucose fully oxidized. This is valid, where the difference between the two rates is slight. Where a large drop occurred, as in 1·5 atm. oxygen, the comparison was not made.

The calculated amount of glucose oxidized increases with increasing oxygen tension (Table 3); this follows from the previous determination of the

Table 3. *Effect of oxygen tension on glucose metabolism*

| Oxygen tension (atm.) | Glucose utilized (μg./mg./hr.) | Glucose oxidized (μg./mg./hr.) | % Glucose oxidized | Lactic acid theoretical (μg./mg./hr.) | Lactic acid found (μg./mg./hr.) | Glucose unaccounted for (μg./mg./hr.) | No. of expts. |
|---|---|---|---|---|---|---|---|
| | | | (*a*) 0–5 hr. experiments | | | | |
| 0 | 9·8 | 0 | 0 | 9·8 | 6·5 | 3·3 | 6 |
| 0·05 | 10·0 | 0·8 | 8 | 9·2 | 7·4 | 1·8 | 6 |
| 0·10 | 9·8 | 1·1 | 11 | 8·7 | 7·0 | 1·7 | 6 |
| 0·20 | 9·4 | 1·5 | 16 | 7·9 | 6·2 | 1·7 | 6 |
| 0·50 | 8·4 | 1·8 | 22 | 6·6 | 3·1 | 3·5 | 6 |
| 0·75 | 8·6 | 1·9 | 22 | 6·7 | 2·9 | 3·8 | 6 |
| 1·00 | 6·4 | 1·8 | 28 | 4·5 | 3·0 | 1·5 | 6 |
| 1·50 | 6·3 | 1·7 | 27 | 4·6 | 3·5 | 1·1 | 6 |
| | | | (*b*) 0–24 hr. experiments | | | | |
| 0 | 4·3 | 0 | 0 | 4·3 | 3·3 | 1·0 | 13* |
| 0·05 | 4·6 | 0·6 | 13 | 4·0 | 3·0 | 1·0 | 13* |
| 0·10 | 4·5 | 1·1 | 23 | 3·4 | 2·6 | 0·8 | 16* |
| 0·20 | 4·3 | 1·3 | 30 | 3·0 | 2·3 | 0·7 | 20† |
| 0·50 | 4·0 | 1·6 | 40 | 2·4 | 1·8 | 0·6 | 19† |
| 0·75 | 4·0 | 1·7 | 43 | 2·3 | 1·8 | 0·5 | 16* |
| 1·00 | 3·7 | 1·5 | 41 | 2·2 | 2·0 | 0·2 | 25† |
| 1·50 | 3·2 | 1·1 | 40 | — | — | — | 8 |

* Estimations of lactic acid on six experiments only. † Estimations of lactic acid on nine experiments only.

relations between oxygen tension and respiration. The percentage of glucose oxidized also increases. Comparison of the observed lactic acid production with the amounts theoretically expected shows a discrepancy at all oxygen tensions during the first 5 hr. This difference, amounting approximately to 10–30 %, does not appear to be affected by oxygen tension.

In the 24 hr. experiments there is also a discrepancy between the 'observed' and 'theoretical' lactic acid production. But in this series of experiments, the amount of glucose unaccounted for becomes smaller as the oxygen tension is increased (Table 3).

## DISCUSSION

The values which we have observed for the $Q_{O_2}$ of guinea-pig-ear skin are considerably higher than those reported for other mammals. Since the average wet/dry weight ratio of the skin is 3·5/1 our figure of 1·27 $\mu$l./mg. (wet)/hr. in oxygen represents a value of about 4·5 $\mu$l./mg. (dry)/hr. No information concerning guinea-pig skin has been encountered in the literature, but the values for adult human skin have been given as 1·5–2 $\mu$l./mg. (dry)/hr. (Barron *et al.* 1948; Wohlgemuth & Klopstock, 1926). Rat skin is stated to respire at about 1 $\mu$l./mg. (dry)/hr. (Barron *et al.* 1948) and mouse at between 1 and 2 $\mu$l./mg. (dry)/hr. (Bullough & Johnson, 1951). Our values are thus some three to four times greater than those reported. The inclusion of serum in the medium does not account for this discrepancy. Observations in glucose–saline give figures for the $Q_{O_2}$ only slightly lower—at least in the initial 5 hr. period. It is likely that the explanation lies in the nature of the skin slices. Guinea-pig ear has a thick cellular epidermis which may be obtained by free-hand slicing relatively free from dermis. Current studies on the skin and separated epidermis of guinea pig and other mammals confirm this view.

As well as being composed mainly of epidermis, the skin slices are also exceedingly thin and, because of this, adequate penetration of oxygen to the cell is ensured. Calculations based on Warburg's formula for limiting slice thickness indicate that, assuming that no oxygen diffusion occurs through the keratin layer, the upper limit of thickness to ensure maximal respiration in pure oxygen is 0·4 mm. and in air 0·2 mm. This postulate is in agreement with the experimental results in that signs of anoxia are present when oxygen tension is reduced below that of air with skin slices of 0·1–0·2 mm. thickness, and at these oxygen tensions respiratory rates are very low. Barron *et al.* (1948) used slices of 0·7 mm. thickness in oxygen.

The finding that oxygen tension affects the respiratory rate (Bullough & Johnson, 1951) has been confirmed. Bullough, using mouse-ear shavings, showed a fairly steady increase, as oxygen tension was raised from that of air to pure oxygen, the total increase over the range being 55 %. With guinea-pig-ear slices the increase over the same range was 20 %. Maximal respiration occurred in 75 % oxygen and the even higher oxygen tensions of pure oxygen and 1·5 atm. oxygen caused no marked variation in short-term experiments. It is possible that the slight differences in the observations were due to the thickness of the slice used. The difficulty of distinguishing any true stimulating effects from those due to increased availability of oxygen is clear.

Our experiments have demonstrated the susceptibility of the skin to oxygen poisoning. Dickens (1946) has observed oxygen poisoning *in vitro* in brain and liver slices, and to a lesser extent in lung and muscle. As our methods differed considerably from those used by Dickens, it is not at present possible to compare the relative susceptibility of skin to that of the other tissues in which this phenomenon has previously been shown to occur. Nevertheless, a pronounced fall in respiration occurred after exposure for 24 hr. to 1·5 atm. oxygen and after this time histological examination showed great abnormality. There is also some suggestion from our results that even 1 atm. oxygen may be harmful to skin cultures over a period of 24 hr.; at this time there is undoubtedly some depression of respiration, but histologically the skin appears healthy, and consequently any effect must be slight.

From observations based on nucleic acid phosphorus instead of wet or dry weights, Berenblum *et al.* (1940) state that skin is a tissue with a high rate of glycolysis and with a low R.Q. While agreeing with the former observation, we have found a value for the R.Q. of $0·97 \pm 0·03$. This value, however, may be slightly high, since no account was taken of the possible liberation of carbon dioxide from serum bicarbonate due to the production of lactic acid, but, as no pH change was observed in the short period of the experiment, the error should be small.

Even under conditions of high oxygen tension skin maintains a high rate of glycolysis. In 5 hr. experiments we have found the total glucose utilization in oxygen to be 6·4 $\mu$g./mg./hr., of which 3 $\mu$g. are converted into lactic acid. Under relatively anaerobic conditions the glucose utilization was considerably higher (10 $\mu$g./mg./hr.), and this was accompanied by a corresponding increase in glycolysis as measured in terms of lactic acid production (7 $\mu$g./mg./hr.). The suppression of glycolysis by oxygen is an example of the Pasteur effect, a phenomenon discussed by Dixon (1937) and described more recently in kidney slices by

Stevenson & Smith (1948) and in bone marrow by Warren (1942). The Pasteur effect was demonstrable also in our 24 hr. experiments. The rates of glucose utilization and lactic acid production were, however, much lower when measured over 24 hr. as compared with 5 hr. This implies that glycolysis falls off rapidly with time, approximately half the total glucose utilization and lactic acid production occurring in the first 5 hr., and the remainder over the next 19 hr. The reduction of the respiration rate over the same period is only about 10%. Several possible explanations of this may be offered, but at this stage speculation seems hardly justified.

When the amount of lactic acid produced and the amount of glucose oxidized (as calculated from the $Q_{O_2}$) are deducted from the amount of glucose used there remains a gap of 'glucose unaccounted'. This may be due in part to synthetic processes. Wohnlich (1949) showed that glycogen could be formed by skin in the presence of lactic acid. The discrepancy might also be due to the relative diffusion rates of glucose into, and lactic acid from, the cell. If the former exceeded the latter, analysis of the medium would show a relative deficiency of lactic acid. The greater discrepancy in the short-term experiments adds some weight to this possibility. However, the fact that in the 24 hr. experiments there is a significant reduction of the amount of 'glucose unaccounted', as oxygen tension rises, suggests a third possibility—that metabolites of glucose other than lactic acid may be produced especially at lower oxygen tensions.

## SUMMARY

1. The oxygen uptake, total glucose utilization and lactic acid production of guinea-pig-ear skin have been determined at oxygen tensions varying from 0 to 1·5 atm. Observations were made over 5 hr. and over 24 hr.

2. The oxygen uptake has been shown to be greater at the higher oxygen tensions. Oxygen poisoning occurred when skin was exposed to 1·5 atm. pressure of oxygen for 24 hr. Exposure to pure oxygen for this period caused a greater fall in respiration than exposure to 0·5 or 0·75 atm. oxygen.

3. The thickness of skin slices in relation to oxygen tension has been investigated. It is suggested that the limiting thickness for skin slices in air is 0·2 mm. and in oxygen 0·4 mm.

4. Glucose utilization and lactic acid production are reduced as oxygen tension is increased, but even under high oxygen tensions glycolysis persists.

5. Glucose utilization and lactic acid production are high when measured over 5 hr. but low when measured over 24 hr.

6. Comparison of the lactic acid production and the amounts of glucose necessary to account for this and for the uptake of the observed amounts of oxygen indicate that a proportion of glucose is unaccounted for. In 24 hr. experiments this discrepancy decreases as oxygen tension increases.

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