TRANSCUTANEOUS FLUX OF
NITROGEN AND HELIUM

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Purpose:

To measure the flux of nitrogen through the skin.

Apparatus:

A stainless steel cup, inverted and sealed to the abdomen of a pig, was used to collect the nitrogen diffusing through the skin. The cup was sealed to the surface of the skin with cyanoacrylate cement and supported with a burette clamp. It was equipped with a water jacket of copper coils used to maintain the skin temperature at a desired level throughout an experiment. Four o-ring seal penetrations served as sampling port, thermistor port, in-flushing port, and out-flushing port which doubled as a chamber for $O_2$ replacement after each sample was withdrawn (See Fig. 1). The sampling line was hooked directly into the sampling loop of a gas chromatograph, all of which was evacuated prior to each sample by a vacuum pump distal to the sampling loop.

Procedure:

White Yorkshire pigs of female sex weighing 8-9 kg were used for this experiment. The animals were induced with an intraperitoneal injection of pentobarbital at a dose of 40 mg/kg. The animals were then maintained with the same drug at an intravenous dose of 17 mg/kg/hr. Atropine was also given I.M. at a dose of 25 mg/kg/experiment. An
Fig. 1

Top view showing the 4 penetrations

Flushing port equilibration chamber

Sampling line

Thermistor

Equilibration chamber

Sampling valve

Copper coil water jacket

Stainless steel cup

Cement seal

Fig. 1

Close up of typical penetration

Port

O-ring seal

Cup wall

Nuts
intravenous line was introduced into the external mammary vein for drug administration and a tracheal tube was inserted for breathing gas administration. A volume respirator was used for breathing the animal. The respirator was set to a tidal volume of 175 to 200 cc depending on the size of the animal and the frequency was set to maintain an alveolar CO$_2$ of 4.5 to 5%. The CO$_2$ was measured intermittently with a Beckman infrared CO$_2$ analyzer. Core temperature was also measured and maintained at 37°C with the aid of a heating blanket.

The animal was prebreathed with the breathing mix for at least 45 min prior to the start of an experiment so that equilibration of the skin to the new gas tensions could take place. The thermistor was then fixed to the skin with cyanoacrylate cement and the cup was then sealed around the thermistor with the same cement. After allowing about 20 min for drying of the cement, the cup was flushed with pure oxygen at the rate of 2-3 L/min for about 30 min. At the end of this time a sample of the cup gas was taken to insure a 100% pure oxygen environment. This purity signaled the start of the experiment. All valves on the cup were secured and a 1 L/min flush of the equilibration chamber was begun with the oxygen and continued throughout the experiment. The purpose of the
equilibration chamber was to replace the sampled gas (1 cc/sample) with oxygen thus maintaining an isobaric system. After each sample the cup was opened to the equilibration chamber for 5 sec. Samples of the cup gas were taken every 20-30 min and the percentage of \( O_2 \), \( N_2 \) and He was determined by gas chromatography (hydrogen carrier gas). Skin temperature under the cup was also maintained at a given level by heating or cooling the gas in the cup by pumping water through the copper coils around the cup. Breathing gas for the experiment was 36% He, 33% \( N_2 \), 31% \( O_2 \).

Data:

The percentage of helium and nitrogen in the cup was plotted against time and the slope determined (Figs. 2-10). The measured flux was directly proportional to the slope of the line:

\[
J(\text{cc/hr/M}^2) = \text{slope} \left( \% \text{ cup} \right) \cdot \left( \frac{1.00}{100\%} \right) \cdot \frac{150 \text{ cm}^3}{45 \text{ cm}^2}, \text{ cup volume} \cdot \frac{10^4 \text{ cm}^2}{\text{M}^2} \cdot \frac{60 \text{ min}}{\text{hr}} = 2.00 \times 10^4 \times \text{slope}
\]
Specific flux rates are obtained by normalizing the measured fluxes by the driving forces used:

\[ N(\text{cc/hr}/M^2/\text{ATA}) = J/\text{fraction inspired gas} \]

Lag times were calculated:

\[ t\text{-lag} = - (y\text{ intercept})/(\text{slope}) \]

(see Table 1)

Experiments were deleted if the scatter of the points was found to be too great or if the data revealed an obvious contamination problem.

Results:

The helium flux was found to be consistent over the 1.5°C temperature range of the experiments possibly because the flux does not vary greatly in this small range and there were too few experiments to see this small variation. The lag times were negative or very small as expected since the skin was equilibrated with the new gases prior to each experiment. The nitrogen fluxes, on the other hand, show a nice proportion variation with temperature and here the lag times are also low as expected. In experiment 4 the lag times for both helium and nitrogen are higher than expected, possibly due to insufficient equilibration time.
<table>
<thead>
<tr>
<th>EXPT #</th>
<th>SPECIFIC FLUX&lt;sub&gt;He&lt;/sub&gt; (cc/hr/M²/ATA)</th>
<th>t-LAG&lt;sub&gt;He&lt;/sub&gt; (MIN)</th>
<th>R</th>
<th>SPECIFIC FLUX&lt;sub&gt;N₂&lt;/sub&gt; (cc/hr/M²/ATA)</th>
<th>t-LAG&lt;sub&gt;N₂&lt;/sub&gt; (MIN)</th>
<th>R</th>
<th>SKIN X (°C)</th>
<th>TEMP. SD (°C)</th>
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<td>23.5</td>
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mean 45.86  mean 28.63  34.88
Fig. 10

- Expt #
- 4 - 35.6°C
- 6 - 34.8°C
- 8 - 33.9°C
In experiments 6 and 9, at the conclusion of the experiments, the animal was breathed with pure oxygen for 1 hour and the fluxes were measured during this time. In both cases the helium flux became negative indicating the reverse flux of the helium into the skin, and the nitrogen flux dropped to 30% of its experimental value. Since the washout time for N₂ is greater than that of He, it would take longer for the N₂ flux to become negative so that the reason the N₂ flux was not negative was simply a matter of insufficient experimental time.

Experiments 8 and 9 were performed on the same animal in the same 12-hour period.

**In vitro Experiments:**

These experiments consisted of two types. The first type had the purpose of testing the integrity of the cup and the second type tested the accuracy of this system for measuring gas fluxes and also indicated if lag times, such as those in experiment 4, could be due to a stratification of gases in the cup.

In all cases the cup was cemented onto a latex film (0.02 cm thick) which was in turn cemented to a beaker with cyanoacrylate cement. The beaker was then continuously flushed with the same breathing mix used for the animals and the cup was flushed with oxygen until it was pure.
During the integrity test samples were analyzed every 30 minutes for 4 hours with the exact sampling technique used for the animal experiments including the equilibration chamber. After 4 hours the sampled gas was found to contain 0% N₂, 0% He, 100% O₂.

In the second type of experiment samples were taken at 15 min intervals for 2 hours and the flux of He and N₂ determined. In half of these experiments the gases in the cup were mixed prior to each sample with the aid of glass syringe attached to the flushing port. In these latter experiments only He flux was determined as contamination was introduced with the syringe, thus preventing nitrogen determinations. It was found that mixing had no effect on the lag times in these experiments. The typical lag time was 10 min for He and 20 min for N₂. The specific flux for He was 355 (cc/hr/M²/ATA) and 103 (cc/hr/M²/ATA) for N₂. Science and Technology of Polymer Films by Sweeting and Orville quote the flux through "natural rubber" as 421/cc/hr/M²/ATA) and 105 (cc/hr/M²/ATA) in helium and nitrogen, respectively.
Summary

The techniques used in these experiments provide an easy and reliable method for the determination of nitrogen fluxes through the skin. The results from the experiments are consistent with predictions of fluxes based on permeability of the gases and previous experiments. From this experiment at 35°C:

\[
\text{Flux}_{\text{He}} = 46.9 \quad \text{SD} = 4.7 \ (\text{cc/hr/M}^2/\text{ATA})
\]

\[
\text{Flux}_{\text{N}_2} = 28.5 \ (\text{cc/hr/M}^2/\text{ATA})
\]