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Influence of Temperature on the Absorption of Inhaled Bovine Serum Albumin through Isolated Rabbit Lungs (41189)

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Abstract. We examined the influence of temperature on the absorption of inhaled ^{125}I-bovine serum albumin (BSA) and $^{125}I^-$ into the pulmonary circulation in isolated rabbit lungs. The lungs were insufflated for 15 min with an aerosol containing either ^{125}I-BSA or Na ^{125}I. The inhaled dose was the amount deposited in the lungs distal to the main bronchi. Blood samples were obtained from the perfusion circuit for 4.5 hr following insufflation. When ^{125}I-BSA was inhaled, the ^{125}I appeared in the blood in trichloroacetic acid (TCA)-soluble and TCA-precipitable fractions. At 38°, the TCA-precipitable and TCA-soluble fractions appeared in the blood at rates of about 1.4 and 1.9% of the inhaled dose per hour, respectively. Cooling to 12° reduced these rates to 0.25 and 0.12% per hour, respectively. The initial rate of $^{125}I^-$ absorption was much more rapid (4%/min) and no temperature dependence was detected. These results indicate that the rates of absorption and breakdown of inhaled albumin were markedly temperature dependent, while the absorption of the low molecular weight ^{125}I was not.

Considerable information is available about the mechanisms of mucociliary clearance of nongaseous material deposited proximal to the terminal bronchioles (1). However, relatively little is known about the mechanisms of clearance from the nonciliated surface of the lower respiratory tract. Particulate material reaching the alveolar lining may be largely cleared by alveolar macrophages (2). On the other hand, soluble material can also penetrate the epithelial surface and enter the pulmonary capillaries (3–11) or the lymphatic drainage of the interstitium (11). Although it may be of considerable importance with regard to the immunologic responses to inhaled antigens, the major transport mechanism for absorption of inhaled soluble macromolecules into the pulmonary capillary blood is not known. Microvesicular transport has been suggested by cytochemical studies (6). However, the quantitative importance of such a mechanism is not known. Temperature dependence is one criterion which has been used to evaluate the contribution of vesicular transport to the transcapillary passage of plasma proteins (12). The rationale has been that vesicular transport mechanisms are highly temperature dependent (13). Thus, if the reduction in protein transport produced by tissue cooling is no greater than that which would be expected for diffusion or bulk filtration, vesicular transport must be of little importance. In fact, Rippe *et al.* (12) ruled out a significant role for transendothelial vesicular transport of radiolabeled serum albumin in the rat hindquarters on this basis. Little information is available about the influence of temperature on protein transport across the pulmonary alveolocapillary membrane. However, Chinard and DeFouw (14) observed that the increase in pulmonary endothelial and epithelial vesiculation, which occurs when lungs become edematous at 37°, was eliminated by cooling to 15° and yet edema formation was not prevented by cooling. On this basis, they concluded that the vesicles were not primarily responsible for the fluid and solute transport within the alveolar membrane during the formation of edema. The purpose of the present study was to determine how tissue cooling would influence the rate at which inhaled ^{125}I-labeled bovine serum albumin (^{125}I-BSA) enters the pulmonary circulation in isolated rabbit lungs.

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Materials and Methods. *Isolated lung perfusion system.* The experiments were carried out using an isolated perfused rabbit lung preparation which we have previously described in detail (4). New Zealand white rabbits (2.2 ± 0.3 SD kg) were given 15 mg of chlorpromazine hydrochloride per rabbit (im) followed by 15–20 mg/kg sodium pentobarbital (iv). They were heparinized (1200 units/kg) through a carotid artery catheter and exsanguinated. The chest was opened and the pulmonary artery, left atrium, and trachea were cannulated. The lungs were then removed and placed in a perfusion chamber, and the arterial and venous cannulas were connected to the perfusion system which had been primed with 120 ml of the autochthonous blood. The blood was pumped (Masterflex roller pump) at a constant rate of 160 ml/min into the pulmonary artery from a reservoir open to atmospheric pressure. It then flowed out of the left atrium and back into the reservoir. The perfusion system included a heat exchanger which was used to maintain the temperature at 38 ± 0.5 or 12 ± 0.5°. Left atrial pressure was set at atmospheric pressure and the pulmonary arterial pressure (relative to left atrial pressure) was monitored throughout the perfusion period. Since in the isolated rabbit lungs there is a tendency for the perfusion pressure to increase with time (4), we added papaverine hydrochloride to the perfusion system (50 μg/ml of blood) (15). This minimized the rise in pressure, and preliminary experiments demonstrated that the drug had no effect on the rate of radioiodinated albumin absorption.

The lungs were ventilated by negative pressure cycling within the chamber at a frequency of 10/min and an end-inspiratory and expiratory chamber (pleural) pressure of approximately −8 and −2 cm H_2O, respectively. Blood gas composition was maintained by attaching a 40-liter meterological balloon filled with approximately 15% O_2 and 5.6% CO_2 in N_2 to the tracheal cannula.

After a 30-min stabilization period, the tracheal cannula was attached to a DeVilbiss Model 65 ultrasonic nebulizer filled with 5 ml of 0.9% saline solution containing ^{125}I-BSA (30–100 μCi with specific activity of 0.6–10 μCi/μg protein) plus 1 mg of unlabeled BSA or with 5 ml of saline containing Na^{125}I (1–6 μCi). The ^{125}I-BSA was prepared using the iodine monochloride method as previously described (4), or using the lactoperoxidase method (New England Nuclear, NEZ-151). Unbound iodine was removed by dialysis and the ^{125}I-BSA solution placed in the nebulizer was always ≥98.5% trichloroacetic acid (TCA) precipitable. The lungs rebreathed the aerosol for 15 min, resulting in approximately 8% of the radioactivity placed in the nebulizer being deposited in the lung distal to the main bronchi. This fraction did not vary significantly between the groups studied.

Groups Studied. *Group I* ($n = 7$). The lungs in Group I were maintained at 38° during the entire perfusion period.

Group II ($n = 3$). The lungs in Group II were maintained at 12° during the entire perfusion period.

Group III ($n = 6$). The lungs in Group III were maintained at 38° until 2 hr after the beginning of the aerosol insufflation period. They were then rapidly cooled to 12° and maintained at 12° for the remainder of the 4.5-hr perfusion period.

Group IV ($n = 5$). The lungs in Group IV were maintained at 12° until 2 hr after the beginning of the 15-min aerosol insufflation period. They were then rapidly warmed to 38° and maintained at 38° for the remainder of the 4.5-hr perfusion period.

The lungs in Groups I–IV were insufflated with ^{125}I-BSA.

Groups V ($n = 3$) *and VI* ($n = 3$). Groups V and VI were similar to Groups I and II, respectively, except that the aerosol contained Na^{125}I instead of ^{125}I-BSA.

Analysis of Samples. In all groups, blood samples (1 ml) were obtained at various intervals following initiation of the aerosol administration. At the end of 4.5 hr, the trachea and extraparenchymal bronchi were removed. The lungs were weighed, and then homogenized (Polytron) in a final volume of 50 ml H_2O. Total lung radioactivity was calculated from the radioactivity in samples of the homogenate. The total radioactivity entering the blood prior to

each sample time was determined by multiplying the radioactivity in each sample by the volume of perfusing blood at that time and adding the radioactivity in each preceding sample. The total blood ^{125}I after 4.5 hr was added to the total activity in the lungs to determine the total dose of ^{125}I deposited in the lungs during the aerosol exposure. The radioactivity in residual blood was subtracted from the total lung radioactivity, assuming that the lungs contained 0.28 ml of residual blood per gram wet weight. The blood radioactivity was divided into that in plasma and that associated with the blood cells by counting the radioactivity in an aliquot of plasma and then using the hematocrit to calculate the total plasma radioactivity in each whole blood sample. In the ^{125}I-BSA groups (I–IV), the cell-associated ^{125}I averaged about 13% of the total ^{125}I in the blood, with no significant difference between the groups studied. The plasma samples were precipitated in a final concentration of 12.5% plasma and 5% TCA. In several experiments from each group, the cell-containing fraction obtained after removal of most of the plasma from the 4.5-hr samples was washed three times in 5 vol of 0.9% saline solution. TCA precipitation was performed on the pooled supernatant from these washings after the addition of normal rabbit plasma to provide a final concentration of 12.5% plasma and 5% TCA. The fraction of total blood radioactivity which remained with the cells after the wash was less than 2%. In the ^{125}I-BSA insufflation groups, the cell-associated ^{125}I was approximately 91% TCA soluble. Therefore, 91% of the cell-associated radioactivity was added to the plasma TCA-soluble fraction and 9% to the TCA-precipitable fraction to determine the concentrations of TCA-soluble and TCA-precipitable ^{125}I in the whole blood. When $Na^{125}I$ was used (Groups V and VI), the ^{125}I in plasma and cell washings was virtually 100% TCA soluble. Thus, total blood radioactivity was equal to total blood TCA-soluble ^{125}I.

Results. Figures 1 and 2 show the accumulation of the ^{125}I in the blood in TCA-precipitable and TCA-soluble forms, respectively, when the lungs were at 38 or 12° throughout the perfusion period. The appearance of both the TCA-precipitable and TCA-soluble forms of ^{125}I was markedly reduced by cooling. The results were similar when the temperature change was made after the aerosol exposure (Figs. 3 and 4).

The results of the $Na^{125}I$ insufflation experiments are shown in Fig. 5. The rate of $^{125}I^-$ absorption was very rapid compared to that of ^{125}I-BSA. Equilibrium was nearly complete within approximately 2 hr at which time the blood contained about 90% of the inhaled dose of ^{125}I. This is in contrast to ^{125}I-BSA insufflation in which only about 15% of the total ^{125}I inhaled was in the blood at 4.5 hr. The ^{125}I in the blood following $Na\ ^{125}I$ insufflation was entirely TCA soluble. In order to determine the initial rate of $^{125}I^-$ absorption, the data were analyzed assuming that the absorption can be described by:

$$D(t) = D(\infty)\,[1 - e^{-kt}] \qquad (1)$$

where $D(t)$ is the percentage of the inhaled dose in the blood at time $= t$, $D(\infty)$ is the percentage of inhaled dose in the blood at equilibrium, and k is the absorption rate constant determined from the slope of the graph of $\ln\,(1 - D(t)/D(\infty))$. The initial rate of absorption ($t = 0$) was calculated from: $k \times D(\infty)$. The smooth line in Fig. 5 was obtained using the average k and $D(\infty)$ for the 38° experiments. The line for the 12° experiments is virtually congruent to that at 38°. For comparison with the $^{125}I^-$ absorp-

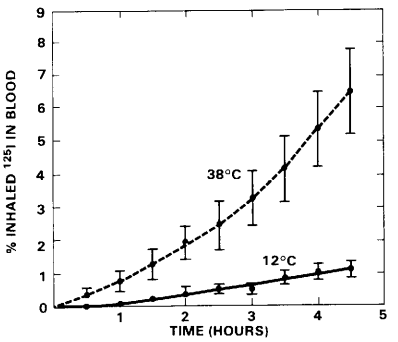

FIG. 1. Percentage of inhaled ^{125}I which appeared in the blood in TCA-precipitable form following insufflation with ^{125}I-BSA at 38 or 12° (Groups I and II). Vertical bars are SE.

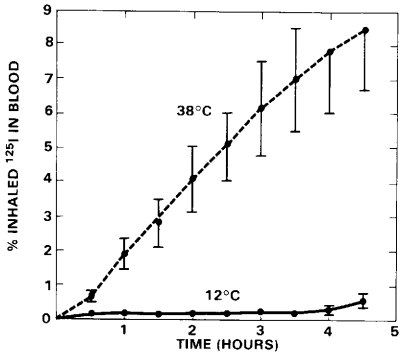

FIG. 2. Percentage of inhaled ^{125}I which appeared in the blood in TCA-soluble form following insufflation with ^{125}I-BSA at 38 or 12° (Groups I and II).

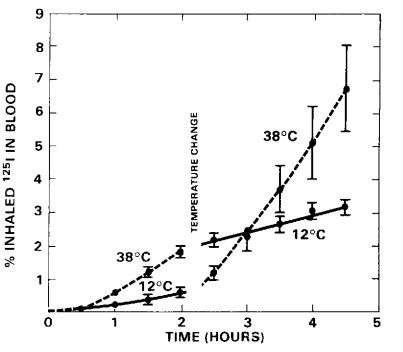

FIG. 3. Percentage of inhaled ^{125}I which appeared in the blood in TCA-precipitable form following insufflation with ^{125}I-BSA at 38 or 12°. The temperature was then either decreased or increased to 12 or 38°, respectively, during the time designated by the stippled bar (Groups III and IV).

tion (Table I), the initial rates of ^{125}I absorption were taken to be the average rates over the 4.5-hr period in the ^{125}I-BSA insufflation Groups I and II. At 38°, the initial rate of $^{125}I^-$ absorption was at least two orders of magnitude faster than that of the TCA-precipitable ^{125}I inhaled as ^{125}I-BSA. The rate of appearance of the ^{125}I-BSA was markedly dependent on temperature in both TCA-precipitable and TCA-soluble forms. The temperature coefficients (these are apparent Q_{10} values over the temperature range studied since they are based on measurements at only the two temperatures) were 2.0 for the TCA-precipitable ^{125}I and 2.9 for the TCA-soluble ^{125}I. On the other hand, for the ^{125}I which was inhaled as $^{125}I^-$, there was no detectable effect of temperature on the rate of appearance in the blood or on the percentage of inhaled dose in the blood at equilibrium.

Some aspects of the general condition of the lungs during the perfusion period may also be relevant in the interpretation of these data. The lung wet weight:body ratios averaged 4.4 ± 0.2 SE g/kg body wt in the 38° lungs (Group I). This is compared to a ratio of 4.0 g/kg for lungs of freshly killed rabbits in the same body weight range. This ratio averaged 5.3 ± 0.2 ($P < 0.01$ when compared to Group I) for the lungs from groups in which the lungs had been cooled

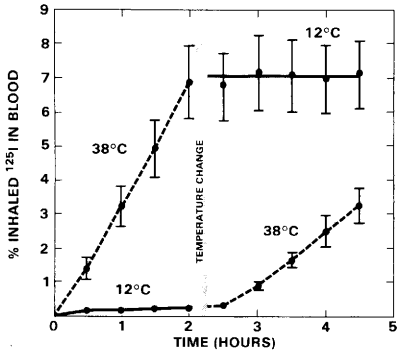

FIG. 4. Percentage of inhaled ^{125}I which appeared in the blood in TCA-soluble form following insufflation with ^{125}I-BSA at 38 or 12°, respectively. The temperature was changed during the time designated by the stippled bar (Groups III and IV).

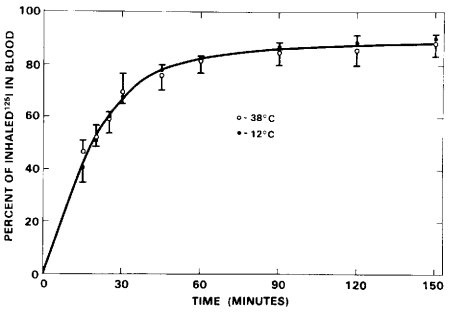

FIG. 5. Percentage of inhaled dose of ^{125}I which appeared in the blood following insufflation with Na ^{125}I at 38 or 12° (Groups V and VI). All of the ^{125}I was TCA-soluble.

TABLE I. EFFECT OF TEMPERATURE ON RATE OF ^{125}I APPEARANCE IN BLOOD

Temperature (°)	^{125}I-BSA inhaled		Na^{125}I inhaled	
	TCA-precipitable (%/hr)	TCA-soluble (%/hr)	TCA-soluble (%/min)	At equilibrium (%)
38	1.44 ± 0.31	1.88 ± 0.41	4.0 ± 0.6	88.3 ± 4.5
12	0.25 ± 0.05	0.12 ± 0.05	4.0 ± 0.1	90.1 ± 0.7

Note. Values are mean ± SE. % = percentage of inhaled dose of ^{125}I in the blood.

during all or part of the perfusion period. This suggests that cooling increased the rate at which these isolated lungs accumulated extravascular fluid. The pulmonary arterial pressures ± SE at the beginning and end of the 4.5-hr perfusion period were 6.9 ± 0.4 and 7.7 ± 0.7 Torr, respectively, in the 38° lungs (Group I). This pressure averaged about 13 Torr when the blood was cold (Groups II–IV), and returned to 7.7 Torr when the cold lungs were rewarmed (Group IV). This reversible temperature-dependent change in perfusion pressure is probably, at least in part, a consequence of the increase in blood viscosity with cooling.

Discussion. The results of this study indicate that the absorption of ^{125}I inhaled as ^{125}I-BSA was markedly reduced by tissue cooling. The ^{125}I appeared in the blood in both TCA-precipitable and TCA-soluble forms. On the basis of previous studies in which the absorption of radiolabeled albumin was examined in a similar preparation, it appears that the TCA-precipitable ^{125}I represents ^{125}I attached to intact BSA, while the TCA-soluble ^{125}I represents protein breakdown products, including ^{125}I$^-$ and ^{125}I bound to peptides and amino acids (4). Cooling reduced the absorption of the ^{125}I in both TCA-precipitable and TCA-soluble forms, suggesting that cooling inhibited the breakdown as well as the absorption of the iodinated protein. The influence of temperature was observable when the temperature was changed after aerosol administration during the course of the 4.5-hr study period, indicating that the major effect of cooling was not due to differences in aerosol deposition pattern, nor to some irreversible change in the tissue.

If diffusion or bulk filtration through aqueous pores were the only transport processes involved for the ^{125}I-BSA, cooling from 38 to 12° would be expected to reduce the rate of absorption by about 50%, primarily as the result of the increase in the viscosity of water (16). In fact, the influence of cooling was considerably greater than predicted on that basis, suggesting that the influence of cooling on ^{125}I-BSA transport is more complex. The concept that a vesicular transport mechanism is involved is compatible with these results. Thus, contrary to the case in rat hindquarters (12), a vesicular transport mechanism cannot be ruled out for the transport of inhaled albumin across the alveolocapillary barrier.

Whether microvesicular transport is in fact quantitatively important cannot be determined from the temperature effect alone and the entire alveolocapillary barrier is more complex than the endothelial barrier for which the influence of temperature on albumin transport has been previously described (12, 17). Calculated from the data of Rippe *et al.* (12), the Q_{10} for the transendothelial transport of ^{125}I-labeled serum albumin in the rat hindquarters was approximately 1.25 over a temperature range of 14 to 36°. They concluded that the temperature effect was too small to be compatible with significant vesicular transport. Siflinger *et al.* (17) obtained a considerably higher value of about 1.9 for the uptake of ^{125}I-labeled serum albumin by the endothelial surface of the dog carotid artery over a temperature range of 18 to 37°. They concluded that pinocytosis was responsible for the transendothelial transport, but they did not consider the temperature effect to be conclusive evidence. In the lung, the endothelium is only one of the transport barriers. The epithelium may provide the rate-limiting barrier (18), and at 12° changes

in the physical properties of the lipid lining of the alveoli may also be important. It is interesting that for $^{125}I^-$, which may be presumed to cross the barrier primarily by diffusion, we were unable to detect a decrease at 12°. Thus, whatever cold-induced change in the physical or physiological properties of the barrier occurred, they contributed to a decrease in the transport of only the large molecule.

It may be somewhat surprising that no detectable decrease in the $^{125}I^-$ absorption occurred. One explanation for this might be that tissue cooling had an additional effect on the barrier which tended to increase permeability. This is suggested by the observation that the lung weight:body weight ratios were higher in lungs which had been exposed to the cold, and the observation by Chinard and DeFouw (14) that isolated lungs maintained at 15° were more prone to edema than at 37°. Another contributing factor might be the increased surface area resulting from the higher vascular pressures due to the increased blood viscosity in the cold.

An interesting aspect of the $Na^{125}I$ insufflation experiments was that approximately 90% of the inhaled $^{125}I^-$ was in the blood at equilibrium. Thus, most of the aerosol deposited in the lungs distal to the main bronchi was deposited close enough to the pulmonary blood vessels that the $^{125}I^-$ could readily diffuse into the blood. Assuming that the aerosol deposition pattern was independent of whether the aerosol contained $Na^{125}I$ or ^{125}I-BSA, this result indicates that most of the ^{125}I-BSA which was deposited in the lungs was in close proximity to the pulmonary circulation.

Although direct quantitative comparisons between isolated preparations and the intact animal must be made with reservation, the rates of ^{125}I-BSA and $^{125}I^-$ absorption through the isolated rabbit lungs were not very different from those observed for similar substances in human lungs. In the isolated rabbit lungs in the present study, the half-time for $^{125}I^-$ clearance from the lungs was about 15 min. Yeates *et al.* (9) exposed human subjects to an aerosol (generated in a similar fashion) which contained the pertechnetate ion (comparable in size to $^{125}I^-$ with a molecular weight of 163 daltons). They concluded that clearance from the lungs occurred via diffusion into the pulmonary circulation with an average half-time of approximately 10 min. Using a similar approach, Rinderknecht *et al.* (8) drew similar conclusions and found that the clearance half-time for the pertechnetate ion in normal human subjects was about 13.5 min. Sanchis *et al.* (10) exposed human subjects to an aerosol containing ^{131}I-labeled human serum albumin and found that the half-time for clearance of ^{131}I from the peripheral zone by nonciliary mechanisms and, apparently, transport directly into the pulmonary circulation was about 23 hr. In the 38° rabbit lungs, a rough estimate of the half-time for clearance of ^{125}I-BSA is possible. If one assumes that the average rate of ^{125}I clearance over the 4.5-hr period following ^{125}I-BSA insufflation was the initial clearance rate, the clearance half-time calculated using Eq. [1] would be about 19 hr. Thus, it would appear that in the rabbit lungs the clearance of ^{125}I, inhaled as either $^{125}I^-$ or ^{125}I-BSA, followed a time course which was on the same order as those found in *in vivo* human studies for a similar substance, suggesting that the same basic mechanisms were involved.

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