Table 1. Inhibition in vitro of PRPP amidotransferase by adenine, guanine, AMP, and GMP. The incubation mixture contained 0.10 ml of crude enzyme extract, 20 μmole of glutamine, 0.35 μmole of $MgCl_2$, 0.28 μmole of ATP, and 30 μmole of tris buffer (*p*H 8.9). The incubations were carried out for 5 minutes at 37°C, and the reaction was stopped with 15 μmole of ethylenediaminetetraacetate. Residual activity expressed as percentage of control without inhibitor.

Substance	Concentration (mmole/liter)	Residual activity (%)
None	0.0	100
Adenine	0.5	70
Adenine	1.0	30
Adenine	2.0	20
Adenine	4.0	0
Guanine	0.5	70
Guanine	1.0	63
Guanine	4.0	66
AMP	1.0	50
AMP	4.0	30
AMP	8.0	15
GMP	1.0	90
GMP	4.0	60
GMP	8.0	60

de novo takes place in the cells that populate this organ during the disease. However, the reason for decrease in enzyme activity during the last phase of the disease is not entirely clear, since terminally the splenic architecture is almost completely disrupted by neoplastic cells. Several of the other enzymes studied (thymidylate synthetase, dihydrofolic reductase, and the formate-activating enzymes) have also shown a similar pattern of activity after viral infection (*2*).

GABRIELLE H. REEM
Department of Pharmacology, New York University Medical Center, New York

CHARLOTTE FRIEND
Department of Experimental Cellular Biology, Mount Sinai School of Medicine, New York

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1. Abbreviations: FLV, Friend leukemia virus; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GTP, guanosine triphosphate; and GMP, guanosine monophosphate.
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Absorption of Intact Protein Molecules across the Pulmonary Air-Tissue Barrier

Abstract. *The majority of heterologous serum albumin and globulin molecules introduced into the pulmonary alveoli of dogs are absorbed into the circulatory system antigenically intact. This function of the alveoli has both physiologic and pathologic importance.*

The aim of the experiments reported here was to determine to what extent protein molecules introduced into the alveoli can be absorbed intact. Heterologous serum protein was labeled with iodine-131, and the quantity in the blood was measured after precipitation with specific antibodies. Complete balance studies, however, proved impractical.

The experiments were carried out on 15 mongrel dogs of either sex weighing between 9 and 26 kg. Four of these animals were used to determine the specificity of the antiserums as well as the zone of equivalence. Data obtained from these animals are not included in this report. In ten dogs, 5 ml of human serum containing a tracer quantity of I^{131}-labeled human serum albumin or human γ-globulin (*1*) was instilled into the lungs (*2*). Blood samples were obtained at regular intervals from 15 minutes to 7 days after instillation or when the animal was killed. At death, the amount of the instilled material remaining in the lungs was also determined. A small quantity (2 to 4 ml) of each blood sample was heparinized and set aside for determination of the total radioactivity in the blood. The remainder was allowed to clot, and the serum was collected for the antibody studies.

Quantitative immune precipitation of the labeled protein in these serums was carried out with specific antiserums, usually in duplicate, after determination of the zone of equivalence. Goat antiserum to human serum albumin, as well as specific antiserums against human serum γ-globulin, was used (*3*). The formation of the antigen-antibody complexes was permitted to proceed at 4°C over a period of 4 days. After centrifugation of the samples at 10,000*g* for 30 minutes, the radioactivity of the supernatant and precipitate was measured with a Picker crystal-scintillation counter (counting efficiency approximately 45 percent).

One animal (K) was injected intravenously with 5 ml of human serum containing I^{131}-labeled albumin, and percentages of antibody-precipitate radioactivity were determined at intervals thereafter.

The stock solutions of the I^{131}-labeled albumin and γ-globulin, as well as the material instilled (mixture of serum with isotope-labeled albumin or globulin) into the lung, were subjected to precipitation with antibody. Over 96 percent of the radioactivity of these stock solutions and of the instilled material precipitated with the specific antiserums. Specificity of the antiserums was demonstrated by the absence of precipitins when serums containing I^{131}-labeled albumin were mixed under standard conditions with antiserum to human globulin, and vice versa.

To study absorption of human serum proteins from the canine lung, human serum containing I^{131}-labeled albumin was instilled into the lungs of six dogs. Three of these (A to C) were killed at 24, 48, and 72 hours, respectively. The percentages of radioactivity in the blood precipitable at these times with antibody to human serum albumin are recorded in Table 1 on the basis of triplicate determinations. Blood samples were obtained at regular intervals from the three other dogs (animals H to J), and the radioactivity precipitable with antibody was determined (Table 2). As much as 97 percent of the isotope in the blood remained attached to a protein molecule that could be precipitated with antibody to human serum albumin. Variation in the percentage of precipitable isotope was not great. A slight drop from the average of more than 90 percent was found 12 to 48 hours after the experiment was begun. Over 80 percent of the isotope which was not precipitated by the specific antibody was precipitable with 3.5 percent trichloroacetic acid. There was no radioactivity in the erythrocytes. Over 98 percent of the isotope remained in solution when serums obtained at various intervals after instillation were mixed with antiserums to human γ-globulin.

The results of the experiments with absorbed I^{131}-labeled γ-globulin (animals D to G) were more difficult to interpret in that there was a consistent

rapid decline in the percentage of the isotope that could be precipitated with antibody. This decrease was proportional to the interval between instillation and taking of the sample. The largest percentage of antigenically intact γ-globulin was present in the blood 24 hours after intrapulmonary instillation, and as much as 61 percent was present after 4 days (Table 1). In comparison with albumin, the difference could possibly be explained by a more rapid removal of heterologous serum globulin, or it could be due to incomplete precipitation of the γ-globulin by the antibodies. Gyenes and Sehon (*4*) observed that about 25 percent of human γ-globulin remained in solution in the initial part of the zone of equivalence in precipitin reactions with rabbit antiserum.

In the experiment in which 5 ml of human serum and I^{131}-labeled albumin were injected intravenously, more than 99 percent of the isotope in the blood could be precipitated by antiserum from 15 minutes to 6 hours after injection; the percentage then began to drop slowly to 95 percent at day 4; it was 99 percent at day 7 (Table 2). Again, 2 percent or less of the isotope in the blood samples was precipitable with antibody to γ-globulin.

Table 1. Percentage of radioactivity precipitable by antibodies in blood after intrapulmonary instillation of I^{131}-labeled albumin and I^{131}-labeled globulin. The radioactive proteins were precipitated with specific antibody.

Time (hr)	Radioactivity in blood (%)	
	I^{131}-albumin*	I^{131}-globulin†
24	91	86
48	88	68
72	96	62
96		57

* The mean of triplicate determinations with antiserum to albumin. † The mean of duplicate determinations with antiserum to globulin.

Table 2. Percentage of radioactivity precipitable by antibodies in the blood after intravenous injection or alveolar instillation of I^{131}-labeled albumin. Data were obtained by specific immune precipitation with antiserum to human serum albumin.

Time (hr)	Percentage of radioactivity in blood after	
	Injection*	Instillation†
0.16 to 6	99	93
12 to 24	97	88
48 to 96	95	88
120 to 168	96	93

* The mean of duplicate determinations at 12 intervals; one animal. † The mean of duplicate determinations obtained from each of three dogs at eight intervals.

Exact measurement of the proportion of the instilled protein absorbed intact by the lung is complicated by numerous factors, only some of which are known: (i) loss of instilled material by the ciliary activity of the bronchial mucosa and by cough, this loss representing approximately 20 percent of the instilled protein (*2*); (ii) continuing absorption as the time passes, shown previously not to be a first-order reaction (*2*); (iii) individual variation in the turnover in vivo of the absorbed heterologous serum; and (iv) difficulty of achieving maximum precipitation of the absorbed intact protein with the precipitation technique (for example, with a slight excess of antibody or antigen near the zone of equivalence). The percentages of protein absorbed intact in relation to the total amount absorbed by the lung have not been corrected for the factors cited, except that 20 percent has been subtracted from the volume of the instilled material to adjust for loss by the airways. The values for absorption are therefore minimum. They were obtained on the animals killed immediately after blood samples were taken (animals A to G). The labeled protein remaining in the lung was determined after homogenization of the entire lung; subtraction of this amount from the amount instilled (corrected for loss by the airways—less 20 percent) gave a quantity of protein assumed to have been absorbed by the lung and transferred into the blood compartment. The calculations necessary for the determination of the percentage of intact albumin in blood, relative to the total of the albumin absorbed within the various time intervals studied, are presented in Table 3 for animal A as an example. Identical calculations (Table 4) were carried out with the data obtained from the study with γ-globulin (animals D to G). Also determined were the percentages of intact albumin relative to the total albumin absorbed in experiments in which serial blood samples were obtained after intrapulmonary instillation of serum containing I^{131}-labeled albumin (animals H to J). In these animals the residual albumin in the lungs was estimated on the basis of data obtained in a previous study (*2*). Blood data for these animals are listed in Table 2. The largest percentage of intact albumin was present in blood 24 hours after instillation into the lung; it amounted to 67 percent of the absorbed albumin. As much as 54 percent of the γ-globulin absorbed by the lung can be demonstrated antigenically unchanged in the blood 24 hours after instillation.

Thus the lung can absorb large quantities of intact long-chained protein molecules. Drinker and Hardenbergh (*5*) demonstrated by immunologic methods that after intratracheal instillation small protein molecules were present in the pulmonary lymph; they concluded that only exceedingly small amounts of protein are absorbed unchanged and that removal of protein from the lumina of the alveoli occurs after degradation of the molecules. Courtice and Simmonds (*6*) confirmed these conclusions with respect to the slow absorption of protein, but found no necessity to postulate that breakdown of proteins is a prerequisite for their removal. The conclusions drawn by Courtice and Simmonds were based mainly on studies of serums labeled with Evans Blue (T 1824) and on precipitin titers; removal from the lung was thought to take place by way of lymphatics. Schultz *et al.* (*7*), in experiments with isolated lungs, found an appreciable quantity of instilled albumin in the perfusate; column chromatography was used for the identification of the albumin.

Table 3. Calculation of the percentage of intact albumin in blood relative to the total amount of the albumin absorbed within 24 hours (animal A). Results are given in 10^6 count/min, except when stated otherwise. R, radioactivity.

Item	Result
Albumin instilled	5.25
Loss by the airways (20%)	1.05
R in lungs after 24 hours	1.82
R absorbed	2.38
R detectable in blood	1.73
R precipitable with specific antibody*	1.60
Absorbed albumin accounted for in blood	67%

* Data for calculation: Animal weight, 18.6 kg; blood volume, 1440 ml; radioactivity of blood, 1205 count/min per milliliter; radioactivity of blood precipitable with specific antibody in optimal zone, 92.4 percent.

Table 4. Percentages of protein accountable for antigenically intact in the blood. Animals A to J, after pulmonary instillation; K, after intravenous injection; animals D and E received I^{131}-labeled γ-globulin; others, I^{131}-labeled albumin.

Time (hr)	Animal							
	A	B	D	E	H	I	J	K
24	67		54		66	51	45	57
48		53		36	37	34	23	39

The absorptive properties of the lung, which have not been adequately realized, are physiologically important because they conserve solutes that have escaped into the air spaces. On the other hand, these absorptive properties permit the introduction of antigenic substances into the general circulation, and thus may set the stage for allergic reactions not only in the lung but in the body as a whole.

KLAUS G. BENSCH
EDUARDO DOMINGUEZ
AVERILL A. LIEBOW
Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510

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Barometric Pressure Fluctuations: Effects on the Activity of Laboratory Mice

Abstract. *Fluctuations in naturally occurring levels of barometric pressure appear to be an important determinant of activity in laboratory mice. In three experiments, activity was higher after increases in barometric pressure than it was after decreases. When the barometric pressure remained relatively stable, intermediate levels of activity were observed.*

The role of ambient barometric pressure as a determinant of physical and metabolic activity in several plants and in marine invertebrates is a well-documented phenomenon (*1*). A correlation between "lunar related barometric pressure cycles" and activity of mice and rats has also been reported (*1*).

These reports indicate that barometric pressure may be an important determinant of the activity of mice in a variety of situations commonly used in studies of behavior. In order to investigate this possibility, two ongoing experiments were analyzed for an effect by barometric pressure, and a third experiment was set up to investigate the phenomenon directly. In all three experiments the mice were housed in a colony room (21.5 m^2) with a 12-hour light cycle (14 fluorescent lights, 40 watts each, on from 6 a.m. to 6 p.m.) and constant temperature (22°C maintained by steam heat in winter, air-conditioning in summer). Humidity and partial pressure of oxygen (pO_2) levels were not measured or controlled. A Taylor recording barometer was used to provide a constant monitor of barometric pressure.

In experiment 1 operant behavior scores of four male mice, strain C57BL/6J, were analyzed for an effect by barometric pressure. These mice had been run for 14 months in an operant conditioning chamber built for mice. Each mouse spent 1½ hours per day in the chamber, where it licked a small tube to receive a reward of sweetened condensed milk. Light [two 7-watt fluorescent lamps illuminating a soundproof box (0.61 by 1.22 m) in which the chambers were housed], temperature (24°C), and time of day were held constant. In analyzing the data, daily sessions were divided into three groups on the basis of a comparison of the level of barometric pressure during each session with the level during the session which preceded it by 24 hours. Results of this analysis are shown in part 1 of Fig. 1. The group means were compared by using two-tailed Student's *t*-tests. The mean number of responses per minute during sessions after rising barometric pressure [+0.1 inch (0.02 mm) of Hg or more during the 24 hours immediately preceding a session] differed significantly from the mean number of responses per minute during sessions after falling (− 0.1 inch of Hg or more) barometric pressure ($P < 0.05$). No significant correlations between activity scores and absolute levels of barometric pressure were observed.

In experiment 2, scores for wheel-running activity were analyzed in three groups of male mice (RX-GE, DX-GE, and DX-ML) (*2*). Data were analyzed in the same way as they were in experiment 1 in order to discover whether the same relation between changes in barometric pressure and activity obtained in this situation. Data from the three groups were combined, which resulted in a subject pool of 137 animals. The mice in this experiment were maintained, for 2 weeks before the start of the experiment, in the same colony room and under the same conditions as mice in experiment 1 were. Five mice were placed in individual activity wheels at 1 p.m. each day. They remained in the wheels for 23½ hours under conditions of constant illumination [two 7-watt fluorescent lamps illuminating a soundproof chamber (0.61 by 1.22 m) in which the wheels were housed] and constant temperature (24°C). Each mouse was run

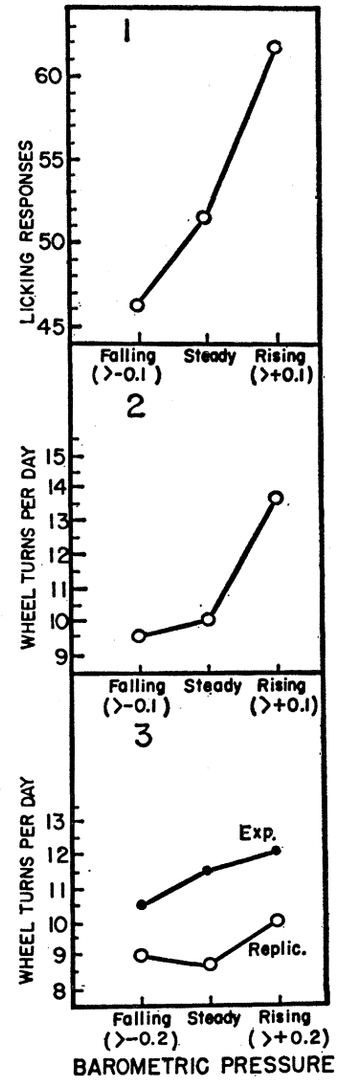

Fig. 1. Mean activity scores of laboratory mice as a function of changes in ambient barometric pressure in three experiments that correspond to parts 1, 2, and 3. Changes in barometric pressure are expressed in inches of Hg. For parts 2 and 3, the numbers on the ordinate must be multiplied by 10^3. (See text for rates of changes in barometric pressure.)