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Distribution of ^{35}S in the Blood and its Excretion in Urine of Dogs Exposed to $^{35}SO_2$

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Blood ^{35}S levels in anesthetized dogs rose progressively when the isolated upper airways were exposed to $^{35}SO_2$ for 30 to 60 minutes, and decreased slightly in the hours following exposure. The plasma contained more ^{35}S than the red blood cells (RBC); more than half of the plasma-^{35}S was dialyzable, the greatest radioactivity among the nondialyzable fraction of the plasma being associated with α-globulin proteins. Two thirds of the RBC-^{35}S appeared to be intracellular. RBC-^{35}S/Plasma-^{35}S was higher following in vivo exposure of animals than when plasma-^{35}S was mixed with nonradioactive RBC. Most of the urinary ^{35}S was in the form of inorganic sulfate.

EXPOSURE to sulfur dioxide (SO_2)may be associated with changes in the concentration of plasma proteins,[1,2] hemoglobin concentration,[3,4] and red blood cells (RBC).[5,6] The mechanism for these changes is not certain, nor is it clear whether impairment of nutrition, water or electrolyte balance, or both were in part responsible for their occurrence. The ^{35}S is known to enter the blood within minutes of the onset of exposure to both $^{35}SO_2$[7,8] and sulfuric acid mist.[9] Little is known of its subsequent distribution and exchange among the components of the blood, knowledge that might clarify the hematologic changes attending exposure to the gas. The primary purpose of our study was to extend preliminary observations that had been made on the interactions of ^{35}S and blood[10] as well as to identify the forms in which ^{35}S is excreted in the urine. Our results are based on the exposure of both the isolated upper airways of dogs and in vitro samples of blood to $^{35}SO_2$.

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Methods

In Vivo Experiments.—The surgically isolated upper airways of nine mongrel dogs were exposed to $^{35}SO_2$. In five animals the concentration of $^{35}SO_2$ was 22 ± 2 ppm[10] the level of radioactivity being 3.7×10^4 picocuries/ml for the first experiment (dog 1). The other four animals were exposed to 50 ppm of $^{35}SO_2$[11] the level of radioactivity was 2.1×10^4 picocuries/ml for the first animal (dog 7) in this

group. The details of the surgical preparation have been described separately.[10] The animals were anesthetized with intravenously administered pentobarbital sodium (initial dose, 30 mg/kg; maintenance dose, 30 to 60 mg, as needed), paralyzed with succinylcholine chloride (initial dose, 3 mg/kg; maintenance dose, 0.02% solution in 5% dextrose in saline infused continuously at the approximate rate of 1 ml/min), and ventilated on ambient air by a displacement pump. Arterial blood and urine samples were collected during and following the exposure; the postexposure period lasted up to three hours. The blood was sampled through indwelling polyethylene tubing that had been passed through one of the femoral arteries into the abdominal aorta. The sampling syringes were moistened with heparin sodium. Samples were drawn at 5 to 10 minute intervals during the exposure, less often following completion of the exposure. The blood, within minutes of collection, was separated into its plasma and cellular components by centrifugation. The urine was collected through a catheter that was implanted in the bladder through an incision in the lower anterior abdominal wall. At least 1.5 ml of urine were obtained with each sample. Urinary sampling was less frequent and less regular than that of blood, the interval depending on the rate of urinary flow. All samples were refrigerated at about 5 C until analyzed. One-milliliter samples of the plasma and cellular components of the blood and urine were counted with a thin window flow-type counter (Geiger-Müller). We calculated the whole blood activity usually by summing the radioactivity of the plasma and cellular components and correcting for the hematocrit value. To correct radioactivity of the RBC, we assumed that trapped plasma represented 5% of the sample volume.[12] All values for radioactivity were corrected for self-absorption.[10]

In Vitro Experiments.—The arterial blood of unexposed dogs and the venous blood of healthy volunteers were exposed to $^{35}SO_2$ in a slowly rotated tonometer at room temperature.

Distribution of ^{35}S in Plasma.—We determined the fraction of the plasma radioactivity that was bound to protein by the method of dialysis[13]: The cellophane tubing (Visking 9/16 inches inflated diameter) containing the radioactive plasma was placed in a 25% solution of polyvinylpyrrolidone (PVP) and refrigerated at about 5 C. The PVP was stirred continuously. Counts were made on 1-ml samples of the plasma before dialysis, and on the PVP after dialysis. All data reported for this technique were obtained after at least 24 hours of dialysis at which time the transfer was virtually complete. The percentage of dialyzable ^{35}S was calculated as follows:

$$\% \text{ dialyzable } ^{35}S = \frac{\text{PVP activity after}}{\text{Plasma activity before}} \frac{\text{dialysis x PVP vol}}{\text{dialysis x Plasma vol}} \times 100.$$

The nondialyzable radioactive plasma was subjected overnight to electrophoresis on 36- × 15-cm strips of filter paper in a buffer (Veronal) of pH 7.3. Following electrophoresis, the strips were dried at 100 C and then were developed in bromphenol blue solution. The dried and stained strips were cut into six segments: albumin, α-, β-, and γ-globulins, and two unstained portions of the paper beyond albumin and globulin. The segments were taped to planchets and were counted (Geiger-Müller). Because of the low ^{35}S radioactivity present on each segment of paper, we counted both the background and sample for more than half an hour.

Distribution of ^{35}S in RBC.—We determined the relative distribution of ^{35}S between the surface and the interior of the RBC by comparing the radioactivity of the cells before and after they were washed. To wash the cells, three parts of normal saline (NaCl) or isotonic phosphate buffer were added to one part of the radioactive cells, the mixture was rotated gently and centrifuged for 15 minutes; the supernatant fluid was then discarded. The procedure was repeated three times.

To test if any ^{35}S was fixed to the cell membrane, we counted the radioactivity of RBC ghosts that were prepared by the method of Dodge et al[14]: The ghost sample was prepared from 1 ml of the washed cells suspended in an equal volume of isotonic phosphate buffer, and then resuspended in 1 ml of 0.1N potassium hydroxide.

In Vitro Exchange of ^{35}S Between Plasma and RBC.—We combined equal volumes of radioactive plasma and nonradioactive RBC in a syringe and rotated the contents in a water bath at 37 C for at least 15 minutes. (In initial tests, we found no difference in the magnitude of the transfer of ^{35}S to RBC for periods ranging from 15 to 60 minutes). The mixture was then separated into plasma and RBC components for counting. We measured the concentrations of ^{35}S in the plasma before and after mixing with the RBC, and in the RBC component following the mixing. The radioactivity of the RBC was corrected for trapped plasma.

Urinary Sulfate.—We used the method of Folin[15] to analyze the urine for ^{35}S. Inorganic sulfate was precipitated by the addition of 5%

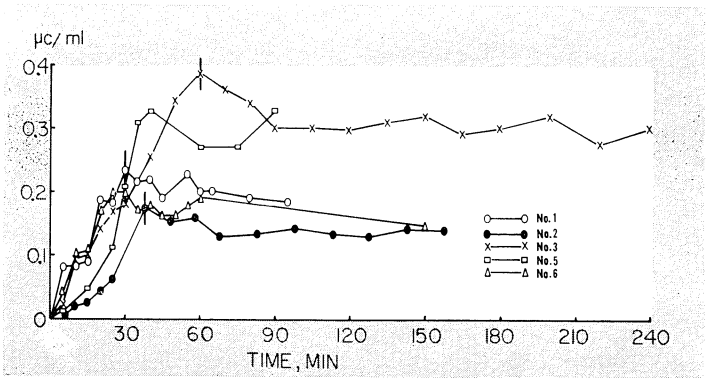
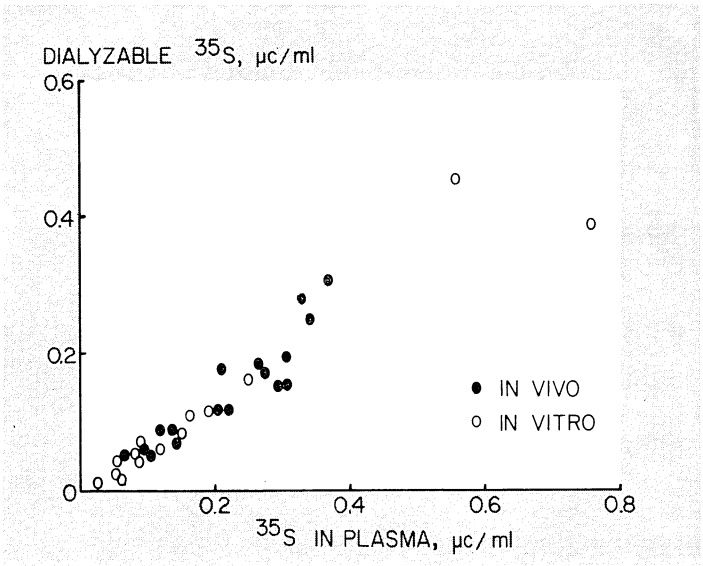


Fig 1.—Changes in level of ^{35}S in whole blood of five dogs. The $^{35}SO_2 = 22 \pm 2$ ppm. Decay constant of ^{35}S was used to correct all levels of radioactivity to the day at which the first dog was exposed. Vertical bars indicate end of exposure.

Fig 2.—Relation between total plasma ^{35}S and dialyzable ^{35}S following dialysis with 25% PVP for 24 hours.



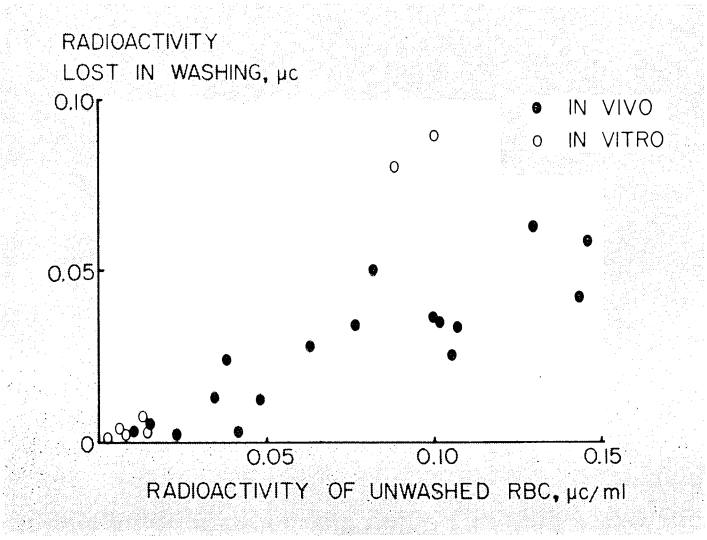


Fig 3.—Relation between the ^{35}S activity of unwashed RBC and activity lost from these cells by washing with normal saline or isotonic phosphate buffer.

Table 1.—*Relative Counts per Minute of Paper Segments Following Electrophoresis of Non-dialyzable Portions of Radioactive Plasma*

Dog No.	Blank	Albumin	α-Globulin	β-Globulin	γ-Globulin	Blank
9	6.6 (13)*	9.6 (18)	21.1 (41)	4.0 (8)	6.6 (13)	3.5 (7)
15	2.3 (10)	4.6 (20)	8.8 (38)	2.9 (12)	2.8 (11)	2.0 (9)

* Numbers in parentheses are equal to % of total count.

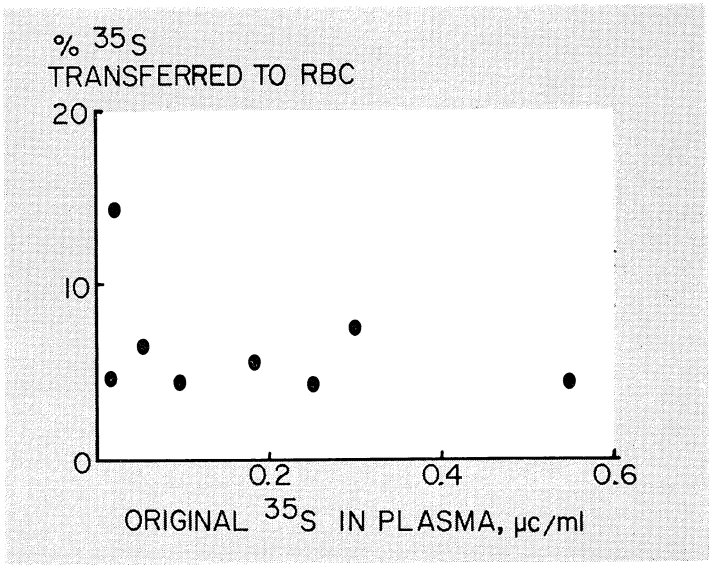


Fig 4.—Relation between plasma ^{35}S and percentage of ^{35}S transferred to RBC following mixing of plasma and RBC in a syringe at 37C for 15 minutes.

barium chloride to the diluted, acidified urine. To precipitate total sulfate, we heated the urine slowly to boiling before adding the barium chloride. The mixture was allowed to stand two to three hours, filtered through a funnel (Buchner), and the precipitate was washed with cold water. The radioactivity of the filtrate, including the washings, was determined with a counter (Geiger-Müller). The radioactivity of the sulfate was calculated from the difference in radioactivity between the urine and the filtrate.

Table 2.—*Relative Urinary Excretion of ^{35}S as Inorganic and Total Sulfate*

Sample Number	% ^{35}S as Inorganic Sulfate	% ^{35}S as Total Sulfate
1 (Dog 7)	88.2	91.5
2 (Dog 8)	82.9	99.1
3 (Dog 8)	81.2	90.1
4 (Dog 9)	86.1	...
5 (Dog 9)	87.3	91.3
6 (Dog 9)	83.2	90.2
Mean	84.8	92.4

Results

The ^{35}S in Whole Blood, Plasma, and RBC.—The absolute levels of radioactivity in whole blood, corrected to the day of the first experiment by application of the decay constant for ^{35}S,[16] are shown in Fig 1. In general, the whole blood levels rose steadily throughout the exposure (30 to 60 minutes). The lowest levels were found in dog 2 which was found to have pulmonary edema at autopsy. The edema is thought to be related to an excessive infusion that was given inadvertently. Four of the five animals showed a fall in ^{35}S blood levels once exposure was ended, though the fall was slight and was not necessarily progressive with time. The maximal postexposure period lasted three hours. The time course of the changes in

plasma and RBC levels of ^{35}S have been reported.[10] They may be summarized as follows: The ^{35}S rose progressively in both compartments during the exposure. In the postexposure period the plasma levels tended to fall whereas the RBC levels, following an initial fall, gradually rose. The ^{35}S concentrations were always higher in the plasma than in the RBC, although the difference between concentrations was variable.

The ^{35}S Distribution Within Plasma.—The sum of the ^{35}S in the dialyzable and nondialyzable fractions did not differ statistically from the ^{35}S measured independently in aliquots of the same whole plasma. The percentage of dialyzable ^{35}S was essentially constant over the entire range of radioactivity (Fig 2): 64.4% (SE ± 2.3%) in 31 measurements. The partitioning was similar for the in vivo and in vitro samples.

To test the results of dialysis, the radioactivity of eight samples of plasma was measured before and after precipitation of the proteins with trichloroacetic acid. The average percentage of ^{35}S remaining in the plasma filtrate was 74.7% (SE ± 8.8%), a value not significantly different from the percentage that was dialyzed.

Protein Fractions.—Only two samples had sufficient radioactivity for electrophoretic analysis. Since we did not correct for self-absorption or for differences in the paper size among the segments, the results are useful for describing only the relative distribution of ^{35}S. Table 1 lists the count number per minute for each segment of the paper. The highest count was found on the segment containing the α-globulin fraction, the next highest on the segment containing albumin.

RBC Radioactivity.—*Surface Versus Interior.*—Figure 3 shows the relation between the radioactivity lost from the cells through washing and the radioactivity of the unwashed cells for samples analyzed within one or two days of their exposure. The mean percentage of radioactivity lost from the RBC through washing was 35.1% (SE ± 3.6%) for the in vivo samples, and 63.8% (SE ± 10.3%) for the in vitro samples. The difference between the two groups was significant ($P < 0.01$).

Cell Membrane Radioactivity.—We found no radioactivity in the ghosts of six samples of RBC.

In Vitro Exchange of ^{35}S Between Plasma and RBC.—An average of only 8.0% (SE ± 1.5%) of the plasma ^{35}S underwent transfer to the RBC following the mixing of equal volumes of the two constituents (Fig 4). The ratio of RBC radioactivity per plasma radioactivity was significantly lower following this in vitro form of transfer than following exposure of the upper airway to $^{35}SO_2$ (in vivo).

Urinary Excretion of ^{35}S.—The time course of urinary excretion of ^{35}S and the relative concentrations of urinary-^{35}S and plasma-^{35}S were reported previously.[10]

An average of 84.4% of the urinary-^{35}S was in the form of inorganic sulfate; 92.4% was present as total sulfate (Table 2).

Comment

The level of ^{35}S in the blood should reflect the balance between the rate at which ^{35}S enters the circulation, principally from the mucosa of the upper airways, and the rates at which it is excreted from the body and deposited in peripheral tissues. There is no direct information about the magnitude of these functions, nor how they may change with the concentration of ^{35}S in different tissues and with time. In general, we found that the radioactivity of whole blood decreased relatively little during postexposure periods lasting up to three hours despite the continuous renal excretion of ^{35}S. The observation confirms Bystrova's experience with white rats.[7] It would appear that the rate of transfer of the bulk of the ^{35}S from the site of absorption to the circulation, though delayed, is sufficient to sustain whole blood levels for many hours after the exposure is ended. The time required to mobilize all ^{35}S from the adsorptive site is not known. Balchum et al[17] were able to detect ^{35}S in the airway tissues of a dog one week after the animal had been exposed to $^{35}SO_2$ through a tracheal cannula.

We found that ^{35}S was always more concentrated in the plasma than in the RBC. About one third of the radioactivity in plasma appeared to be in association with proteins, especially the α-globulins. The nature of the ^{35}S-protein bond was not determined. The α-globulins are considered to be hetero-

genous mixtures of proteins that are bound to carbohydrates, lipids, and other substances. They include most of the glycoprotein found in human plasma.[18] Smith et al[19] showed by means of paper electrophoresis that following the intravenous injection of $Na_2{}^{35}SO_4$ into rats, the greatest accumulation of radioactivity occurred on the strips containing the α_1-globulin. The authors assumed that the ^{35}S was principally contained in sulfonated carbohydrate-globulin complexes. In the light of this binding of ^{35}S to the α-globulins, it is interesting to note that both Erban[1] and Symon[2] reported elevated serum α-globulin in experimental rabbits that were exposed to an average concentration of 36 mg/cu m (12.6 ppm) of SO_2 for 80 days. Erban suggested that the change represented a nonspecific response to chronic stress rather than a consequence of the direct action of the gas; our finding suggests that a more specific mechanism may also operate. Alternatively, Navrotsky[20] reported no effects on the serum protein spectrum of rabbits after long-term exposure to SO_2.

In our experiments about two thirds of the ^{35}S associated with the RBC appeared to be intracellular. The remainder is assumed to have been adsorbed on the surface of the cell. We could not demonstrate any ^{35}S that was bound to the cell membrane. During the postexposure period the amount of ^{35}S in association with the RBC increased, while the plasma concentration of ^{35}S fell. The net gain in the RBC concentration of ^{35}S is difficult to explain, particularly if the radioactivity within the cell is assumed to reflect principally the sulfate ion. The latter is thought to be passively transported across the mammalian RBC membrane.[21] Consequently, the increase in the RBC concentration of ^{35}S relative to the plasma concentration might reflect either a change in membrane permeability or a nonionic binding of the ^{35}S inside the cell. On the other hand, the experiments showing that the transfer of ^{35}S from the plasma to the RBC was considerably less in vitro than in vivo are consistent with the possibility that an active transport mechanism may function during life.

Whether the inspired sulfur that enters the RBC may affect these cells is unclear. Lee[3] reported an increase in the hemoglobin concentration of guinea pigs exposed to 6 to 310 ppm of SO_2 for only one hour. The increase in hemoglobin concentration was roughly proportional to the concentration of the gas between 6 and 20 ppm but the proportionality ceased at concentrations above 20 ppm. The result of long-term exposure of animals to SO_2 have been inconsistent. Volkova[22] found no significant changes in the concentrations of hemoglobin and RBC in rabbits exposed to concentrations below 20 mg/cu m (7.0 ppm) of SO_2 for 3.5 hours/day over a period of 1.5 months. Recently, Battigelli et al[23] reported no detectable change in the hematocrit value of rats exposed to a mixture of SO_2 (1 ppm) and dust (graphite, 1 mg/cu m) for four months. However, Ball et al[4] reported an increase in hemoglobin concentration together with increases in the white blood cell count and the percentage of neutrophils in rats exposed to 1 to 32 ppm of SO_2 for periods up to 13 months. Hayashi[6] reported that an initial increase of RBC was followed by a gradual return to the pre-exposure level in rabbits that were exposed to 100 ppm of SO_2 for ten months.

Our previous data[10] showed that the kidney excreted ^{35}S at a rate approximately proportional to the level of ^{35}S in plasma and whole blood; in addition, the rate of ^{35}S excretion was not influenced by manitol, an osmotic diuretic. These findings would be expected to the extent that ^{35}S is excreted principally as an inorganic sulfate and that the reabsorptive mechanism for the sulfate is readily saturated.[24] Generally, urinary sulfur is present in three forms: inorganic sulfate, ethereal sulfate, and neutral sulfur.[25] We found that 84.4% of the urinary ^{35}S was excreted as inorganic sulfate, 7.6% as ethereal sulfate, while the form of the remainder was not determined.

If it is assumed that all of the $^{35}SO_2$ entering the nasopharynx was taken up by the mucosa in these experiments, then 1% to 6% of the total amount that entered the body was excreted in the urine during the few hours of the study. As shown in the previous report,[10] the amount of ^{35}S present in the circulation, coincident with the peak concentration, ranged from 5% to 18% of the dose. If little or none of the ^{35}S was

excreted in the feces during this period, the remaining three fourths or more of the inspired dose must have been distributed between the site of absorption and the peripheral tissues of the body.

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References

1. Erban L: Influence of the long-term action of low SO_2 concentration on the protein spectrum of the blood serum in rabbits. *Cesk Hyg* **6**:555-559, 1961.

2. Symon K: Air pollution research in Czechoslovakia. *Proc Roy Soc Med* **57**(suppl):988-990, 1964.

3. Lee SD, Danner RM: Biological effects of SO_2 exposures on guinea pigs. *Arch Environ Health* **12**:583-587, 1966.

4. Ball COT, Heyssel RM, Balchum OJ, et al: Survival of rats chronically exposed to sulfur dioxide. *Physiologist* **3**:15, 1960.

5. Ronzani, cited by Greenwald I: Effects of inhalation of low concentrations of sulfer dioxide upon man and other mammals. *Arch Industr Hyg* **10**:455-475, 1954.

6. Hayashi M: A study of SO_2-poisoning. II. The influence of chronic SO_2-poisoning upon the hemogram and myelogram of the rabbit. *J Science Labour* **32**:704-720, 1956.

7. Bystrova TA: Certain aspects of the effect of sulfur dioxide studied with the aid of labeled atoms. *Gig Sanit* **22**:30-37, 1957.

8. Balchum OJ, Dybicki J, Meneely GR: Pulmonary resistance and compliance with concurrent radioactive sulfur distribution in dogs breathing $^{35}SO_2$. *J Appl Physiol* **15**:62-66, 1960.

9. Vasil'eva OG: Distribution and elimination of ^{35}S in animals after inhalation of labeled sulfuric acid aerosol. *Gig Tr Prof Zabol* **1**:39-43, 1957.

10. Frank NR, Yoder RE, Yokoyama E, et al: The diffusion of $^{35}SO_2$ from tissue fluids into the lungs following exposure of dogs to $^{35}SO_2$. *Health Phys* **13**:31-38, 1967.

11. Frank NR, Yoder RE, Brain J, et al: SO_2 (^{35}S labeled) absorption by the nose and mouth under condition of varying concentration and flow. *Arch Environ Health* **18**:315-322, 1969.

12. Daniel SS, Morishima HO, James LS, et al: Lactate and pyruvate gradients between red blood cells and plasma during acute asphyxia. *J Appl Physiol* **19**:1100-1104, 1964.

13. Grogan CH, Robez E: Simple apparatus for concentrating biologic fluids of low protein content. *J Lab Clin Med* **45**:495-499, 1955.

14. Dodge JT, Mitchell C, Hanahan DJ: The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. *Arch Biochem* **100**:119-130, 1963.

15. Folin O: On sulfate and sulfur determination. *J Biol Chem* **1**:131-159, 1905-1906.

16. Kinsman S (ed): *Radiological Health Handbook.* US Dept Health, Education, and Welfare, Public Health Service, 1957.

17. Balchum OJ, Dybicki J, Meneely GR: The dynamics of sulfur dioxide inhalation: Absorption, distribution, and retention. *Arch Industr Health* **21**:564-569, 1960.

18. Well PG: *The Plasma Protein.* Philadelphia, JB Lippincott Co, 1959.

19. Smith LH, Anderson B, Odell TT Jr: Binding of ^{35}S-labeled sulfate in serum of rats as studied by filter-paper electrophoresis. *Proc Soc Exp Biol Med* **90**:360-364, 1955.

20. Navrotsky VK: The effect of small concentrations of sulfur dioxide during chronic poisoning on the immunologic reactivity of rabbits. *Gig Sanit* **24**:21-26, 1959.

21. Omachi A: Sulfate transport in human red cell inhibition by some uncouplers of oxidative phosphorylation. *Science* **145**:1449-1450, 1964.

22. Volkova NV: Effect of low concentration of SO_2 on hemoglobin and erythrocyte in animal's blood. *Tr Leningr Sanitarnogig Med Inst* **58**:126-135, 1960.

23. Battigelli MC, Cole HM, Fraser DA, et al: Long-term effects of sulfur dioxide and graphite dust on rat. *Arch Environ Health* **18**:602-608, 1969.

24. Pitts RF: *Physiology of the Kidney and Body Fluids.* Chicago, Year Book Medical Publishers Inc, 1963.

25. Keele CA, Neil E: *Samson Wright's Applied Physiology,* ed 11. London, Oxford University Press Inc, 1965.