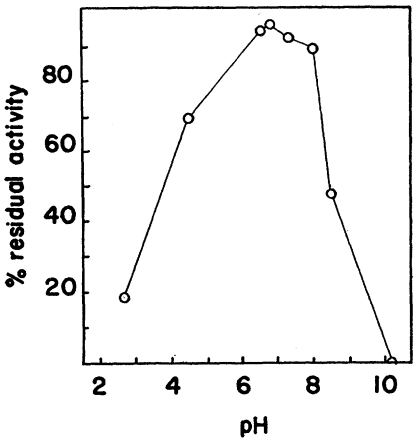

Fig. 2. Influence of *p*H on the stability of paramecin 34 at 37°C. The ordinate represents the residual activity after a 30-minute exposure to a particular *p*H, as explained in the text.

51 at 8.5 and Pn 34 at 6.8. At their respective *p*H optima both substances decay at similar rates.

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Solubility of Carbon Dioxide in Body Fat

In recent studies (*1*) in this laboratory of the distribution of CO_2 in rats that had been exposed to atmospheres rich in this gas, it was found that a solubility coefficient for CO_2 in body fat was required for calculating the distribution of CO_2 in various tissues. The literature, however, contains little quantitative data on the subject (*2*). In 1907 Vernon (*3*) measured the CO_2 contained by samples of fats that had been equilibrated with air, but his data are not sufficient for calculating a solubility coefficient for the gas. Van Slyke, Sendroy, Hastings, and Neill (*4*) described solubility coefficients for CO_2 in whole blood, serum, and salt solutions, noting various factors which affected them. While these authors observed a higher solubility of CO_2 in lipemic sera, which they ascribed to the fat content, they did not measure the solubility of CO_2 in fat directly.

Vibrans (*5*) measured the solubility of CO_2 in cottonseed oil and lard, but no solubility coefficient for CO_2 has been determined for forms of mammalian body fat other than lard. I therefore undertook to determine such a number for the depot fat of three species: rat, dog, and man (*6*).

Depot fat was obtained as follows: Six 200-day-old male Wistar rats were killed by a blow on the head and exsanguinated, and fat from the perirenal, subcutaneous, and retroperitoneal areas was collected and pooled. This pool of tissue was mixed and divided into two lots. Perirenal and subcutaneous fat was taken from a single adult male mongrel dog 2 hours after it had been killed by exsanguination under anesthesia, and both types were combined in a single pool. Human fat was obtained 5 hours after death from the omentum of an 86-year-old woman. The fatty tissue collected was homogenized with sand in buffer solution of *p*H 4.0 at 30 to 35°C. One lot of rat fat was homogenized with buffer of *p*H 7.40. The containers were centrifuged for 30 minutes, and the clear upper layer of melted fat was removed with a pipette and stored at 5°C in a clean, tightly stoppered flask until it was used. Equilibration was carried out within 48 hours after the fat was obtained. No attempt was made to characterize the samples of fat chemically except as follows: water content was zero, for the samples failed to lose weight when they were stored in a desiccator for 48 hours over calcium chloride at room temperature; solubility in a 50/50 mixture of anhydrous ethyl ether and petroleum ether was complete; a melting point was determined for each sample.

Three- to five-milliliter samples of fat were equilibrated in flasks at 38°C for 2.5 to 5 hours. (Equilibration was complete after 1 hour.) A dry atmosphere containing 23.5 to 24.9 percent CO_2 was passed continuously through the flasks. Barometric pressure was noted, and the pCO_2 of the gas phase was corrected accordingly. After equilibration, samples were obtained for analysis by aspirating the fat anaerobically from the equilibration flask with a syringe and needle thrust through a rubber serum stopper. One-milliliter Van Slyke pipettes were filled with the liquid, equilibrated fat directly from the syringe without exposure to air. The specific gravity was determined for each type of fat studied. The volume of fat delivered by the pipette was actually less than 1 ml because of the high viscosity of the material. A suitable correction for fat left on the walls of the pipette was determined in each case on the basis of the specific gravity of the appropriate type of fat. This fat was then added anaerobically to 2.5 ml of previously extracted acidified water in the chamber of the Van Slyke manometric apparatus, and its CO_2 content was measured by the usual procedure.

The factors for the calculation of the total CO_2 content of samples given by Van Slyke and Sendroy (*7*) are based on the solubility of CO_2 in water. In our procedure the fluid phase was not homogeneous, but instead contained two compartments: 1 ml of fat and 2.5 ml of water, each having different solubility coefficients for CO_2. Therefore, new factors were calculated by obtaining a first approximation of the solubility coefficient for fat by use of the Van Slyke and Sendroy factors and then by recalculating the factors, using this number. The new factors were then applied to the original manometric readings.

The results obtained are shown in Table 1. The fats taken from the three species showed certain minor differences. The melting points for rat and human fat were almost identical. That for dog fat was considerably higher. This may have been an artifact, however, for this sample could have been contaminated, and the melting point was determined some time after the equilibration with CO_2 was carried out. Some change in the fat may have occurred during storage in the icebox. The specific gravity of the human fat appeared to be slightly lower than that of rat and dog fat.

Two samples of rat fat were studied. Sample A was extracted from adipose tissue with a buffer at *p*H 4.00, while sample B was extracted with a buffer at

Table 1. Solubility coefficients for CO_2 in depot fat at 38°C.

Species	Melting point (°C)	Specific gravity	Number of samples equilibrated	CO_2		f_{CO_2}
				cm^3/g	cm^3/cm^3 (α)	
Dog	30	0.9155	9	0.9405 ± 0.0140	0.8609 ± 0.0129	0.0556
Rat (A)	19	0.9154	9	0.9738 ± 0.0092	0.8917 ± 0.0077	0.0576
Rat (B)	19	0.9154	10	0.9813 ± 0.0089	0.8985 ± 0.0082	0.0578
Human being	18	0.9110	8	0.9306 ± 0.0483	0.8476 ± 0.0447	0.0550

pH 7.40. Columns 5 and 6 give the averages of the values obtained for each type of fat, expressed as cubic centimeters of CO_2 dissolved per gram of fat and per cubic centimeter of fat (α) at 1 atm pressure of CO_2 at 38°C. The standard deviation of each figure is given. The last column gives the Henry's law coefficient (f) for CO_2 in each type of fat, expressed in millimoles of CO_2 dissolved per kilogram of fat per millimeter of pCO_2 at 38°C.

It is apparent that the solubility of CO_2 in human and dog fat is almost identical. Its solubility in rat fat appears to be somewhat higher, the difference between the two being at the borderline of significance with $p < 0.02$. A comparison of rat fat samples A and B indicates that the pH of the buffer used for the extraction of fat from the adipose tissue has no effect on CO_2 solubility. Why rat fat should have a higher solubility coefficient for CO_2 than dog and human fat is not apparent from these data. Further chemical characterization of the fat samples used might have shed some light on the matter.

All these values closely approximate the solubility coefficients for cottonseed oil and lard calculated from Vibrans' report. This similarity suggested to us that, while differences in the solubility of CO_2 in fats of varying composition taken from a variety of species may occur, the differences are minor and would have little significance in calculations of the distribution of CO_2 in tissue samples. Since fat from various areas of the body was combined in the case of the rats and the dog, these data throw no light on possible differences in the solubility of CO_2 in fat taken from various areas of the same individual. However, in view of the minor differences to be found in the solubility of CO_2 in fats from various species, it seems unlikely that these differences would be very great.

It is of interest to compare the average solubility coefficient of CO_2 in rat fat reported here with those for water and serum reported by Van Slyke (*3*) and for acetate buffer determined by us. (αCO_2 for acetate buffer, 0.183*M*, pH 4.00, at 38°C = 0.458.) Carbon dioxide is 1.67 times more soluble in body fat than in water; 1.76 times more soluble in fat than in serum; and 1.95 times more soluble in fat than in acetate buffer. When ratios are calculated on a weight basis, the value rises as high as 2.14 : 1.

The greater solubility of CO_2 in lipides may be of considerable significance in the manner in which CO_2 is removed from the cells and excreted. It suggests that CO_2 may diffuse across the lipoid cell membrane in a gaseous form and not in the hydrated form H_2CO_3 or as the ion HCO_3^-, both of which one would expect to be relatively insoluble in lipides.

These data indicate that, in an obese individual, a significant portion of the total body CO_2 may be in solution in the fat depots. This fact may be of considerable importance in the calculation of gas exchanges in short-term studies of respiratory function.

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Complement Fixation with Mouse Hepatitis Virus

Viral agents causing hepatitis in mice have appeared during the course of studies of enteric disease of the suckling mouse (*1*), human viral hepatitis (*2, 3*), and mouse-leukemia (*4*). Their presence is a likely consequence of the "blind-passage emergence" of a dormant agent. The agents isolated are more pathogenic for younger mice than for older ones. They appear to induce immunity in the exposed mouse, and serum neutralization tests with neurotropic strains of virus have demonstrated an antigenic relationship among several strains of hepatitis virus (*5, 6*).

Some of the antigenic properties of a strain of mouse hepatitis virus (MHV) have been studied with particular reference to the extraction of complement-fixing antigen from infected liver tissue. This strain has been described extensively by Gledhill *et al.* (*2, 5, 7*) and is the type from which the enhancing agent, *Eperythrozoon coccoides,* has been excluded. When new-born Swiss mice are inoculated intraperitoneally with infective liver emulsion, they die in the following 5 to 6 days and show extensive degenerative lesions in the liver. Livers from 20 to 30 infected mice were pooled and stored at −20°C until they were processed. They were emulsified by a Ten Broeck grinder in Hank's balanced salt solution (10 percent by weight) and centrifuged at 2000 rev/min for 30 minutes, and then the supernatant fluid was centrifuged at 10,000 rev/min for 20 minutes. The latter tissue extract was inoculated intraperitoneally into adult Swiss mice (0.1 ml) twice at 2-week intervals, and the mice were exsanguinated at 2 weeks after the last inoculation. Such inocula killed baby mice when diluted to 10^{-5} (0.05 ml intraperitoneally). Controls consisted of pooled serum collected from mice of the same colony just prior to the study of mouse hepatitis virus infection and from mice inoculated with liver extracts from uninoculated mice.

Antigens were prepared from infected liver tissues by a procedure similar to that described above for the preparation of the immunizing inoculum. In addition, the supernatant fluids were centrifuged at 140,000 g for 1 and 2 hours. Aliquots of antigen were further processed, as follows: (i) desiccation and reconstitution in distilled water; (ii) desiccation and extraction with chemically pure benzene three times at 20-minute intervals, then reconstitution in distilled water to original volume; (iii) mixture with one-third volume of anesthetic diethyl ether at 4°C overnight, and then rendered ether-free by centrifugation and vacuum; (iv) heated at 61°C for 20 minutes.

The complement-fixation technique employed was essentially that described by Bengtson (*8*) in which 0.2-ml quantities each of antigen, serum, and complement were used. The hemolytic system was added after incubation overnight at 4°C. Complement-fixation was determined by visual inspection.

Table 1. Serological detection of mouse hepatitis virus (MHV) in liver extracts.

Serum dilution	Dilution of MHV liver extracts				Antigen dilution
	1:2	1:4	1:8	1:16	1:32
	Positive serum				
1:10	++++	++++	++++	++++	++++
1:20	++++	++++	++	−	−
1:40	++++	++++	−	−	−
1:80	++++	−	−	−	−
	Normal serum				
1:10	−	−			