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The Surface Areas of Proteins. IV. Sorption of Polar Gases[1]

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The sorptions of NH_3, HCl, BF_3 and CH_3NH_2 by egg albumin have been studied and shown to be a bulk property of the protein and not dependent on the state of dispersion of the solid protein. The sorptions are characterized by relatively large evolutions of heat, very slow attainment of equilibrium, hysteresis and, with the exception of NH_3, the appearance of transient constant pressure regions in the gas isotherms. With HCl, egg albumin forms a permanent complex containing a fixed and reproducible amount of HCl; 1.00 ± 0.04 mmole HCl/g. protein. This figure is in good agreement with the acid-binding capacity of the protein calculated by other workers. The complex is insoluble in water and 0.1 *M* HCl but is soluble in 0.1 *M* NaOH. There is also permanent complex formation and loss of solubility with BF_3 but not with the basic gases. It is proposed that aside from the permanently bound gas, the sorption of polar gases involves a "solvation" of polar groups in the protein molecule.

Introduction

The study of the interactions of solid, dry proteins with gaseous reagents offers an attractive method of investigation of the former since the results are not complicated by the role played by solvent interactions. A considerable amount of work along these lines has been done by Bancroft and Barnett,[2,3] who studied in some detail the binding power for NH_3 and HCl of acidic and basic groups, respectively, in organic compounds including proteins. They were able to show that with few exceptions, free carboxyl groups will react stoichiometrically with NH_3 as demonstrated by the appearance of isobaric regions in the gas sorption isotherms (*e.g.*, glutamic acid, *p*-aminobenzoic acid, succinic acid, etc.). Similarly they showed that amino nitrogen will generally react stoichiometrically with HCl (*e.g.*, glycine, glycylglycine, glutamic acid, acetamide, aniline, hexaminetetramine, biuret, etc.). On applying the same methods to the proteins, zein, casein, arachin, edestin, fibrin and gliadin they found no isobars in the ammonia isotherms. On the other hand, all of these proteins, with the exception of zein and arachin, gave one or more isobaric regions in the HCl sorption isotherms, indicating compound formation. However, the equivalent weights computed from the extents of the isobars did not correlate with any of the equivalent weights measured by reactions of the same proteins in solution. Additional work by others[4,5,6] has added but little to the conclusions of these early papers.

These results were thought sufficiently interesting to warrant further investigation. In particular it was important to be able to separate the effects of purely surface adsorption from bulk sorption, a question which did not concern the earlier workers. In a series of recent papers[7,8] a set of techniques have been described which make it possible to distinguish between bulk properties and those properties attributable to surface adsorption.

In the present paper a report is made on the results of an investigation of the sorption of HCl, NH_3, BF_3 and CH_3NH_2 on egg albumin. As will be shown the sorption of these gases is independent of the surface areas of the proteins and is related to the specific nature of the protein. However, the sorption is by no means as simple or unambiguous as implied by the conclusions of Bancroft, *et al.*

Experimental Procedure

Egg Albumin.—The egg albumin used in the present work was obtained from two different sources. The major fraction was from a sample prepared by Dr. David A. Ellis who purified it by standard techniques from raw egg white. Its properties and the details of its preparation have been described in a previous paper.[7] The second sample which was used for only a few of the runs, was obtained from Armour and Co. in the form of a white powder.

Both samples were spray-frozen from 2% aqueous solutions into liquid nitrogen and vacuum-dried at low temperatures to produce very finely-dispersed, fluffy powders. Samples having lesser dispersion were obtained by lyophilizing a 6% aqueous solution. It has been shown[7] that these treatments do not denature the protein insofar as solubility and electrophoresis patterns may be used as criteria. The surface areas of these samples, determined by application of the BET[9] theory to their low temperature nitrogen adsorption isotherms, ranged from 5.1 to 21 sq. meters/gram.

Purification of Gases.—Nitrogen and helium gases were used to measure surface and dead space in the system, respectively. Their purification and properties are described in a previous report.[7]

The polar gases used were HCl, NH_3, BF_3 and CH_3NH_2. The sources for these gases were commercial cylinders put out by the Matheson Co. The purity was listed as above 97% in each case. The gases were transferred to storage bulbs on a high vacuum system after having been put through a number of isothermal bulb-to-bulb distillations and thoroughly degassed by pumping at low temperatures. The final samples used were checked for purity by measuring their vapor pressures at temperatures near their boiling points. In every case they were found to agree to within 1 mm. with the values recorded in Landolt–Bornstein.

Sorption Isotherms.—The apparatus used for the sorption measurements is similar to that described previously.[7] Corrections were made for the non-ideality of gases using the Berthelot equation and the final values tabulated in terms of weight of gas sorbed per gram of protein.

The following baths were used for temperature control: −78° (Dry Ice and ether); −196° (liquid nitrogen); −34° ($MgCl_2$ brine and ice); 0° (ice); room temperature (water). It was found that these temperatures could be maintained to within 0.1° over a 24-hour period using a dewar container the top of which was covered with glass wool. Temperatures were measured in each case with appropriate gas thermometers.

Heat Measurements.—An attempt was made to measure crudely the heat liberated during sorption. For this purpose a normal sample cell was sealed at its lower end to a capillary U-tube. A fine gage, copper–constantan thermo-

(1) The major part of the experimental work contained in the present paper has been taken from the Master's Thesis of Jerrold M. Seehof, submitted to the Graduate School of the University of Southern California, January, 1950.

(2) W. D. Bancroft and C. E. Barnett, *J. Phys. Chem.*, **34**, 449, 753, 1217, 1930, 2433 (1930).

(3) W. D. Bancroft and C. E. Barnett, *Proc. Natl. Acad. Sci.*, **16**, 118, 135 (1930).

(4) Belden, *J. Phys. Chem.*, **35**, 2164 (1931).

(5) Parks and Melaven, *ibid.*, **40**, 471 (1936); **41**, 1101 (1937).

(6) Tsai and Hsiao, *J. Chinese Chem. Soc.*, **2**, 87 (1934).

(7) S. W. Benson and D. A. Ellis, THIS JOURNAL, **70**, 3563 (1948); **72**, 2095 (1950).

(8) S. W. Benson, D. A. Ellis and R. Zwanzig, *ibid.*, **72**, 2102 (1950).

(9) S. Brunauer, "The Adsorption of Gases and Vapors. Vol. I. Physical Adsorption," Princeton University Press, Princeton, N. J., 1945.

couple was inserted through the capillary tube and a vacuum seal effected by means of a low melting methyl methacrylate resin. The end of the thermocouple was fixed to be approximately in the center of the powder sample. A similar cell has been described earlier.[8] Voltages were read with a Leeds and Northrup double scale portable potentiometer. Later it was found convenient to use an automatic recording unit (General Electric Co. "Autopot" and Angus recorder). Both systems were calibrated against mercury thermometers.

Sorption Isotherms.—Each sample was dried prior to use by evacuation at room temperature under high vacuum. Twenty-four hours was sufficient to achieve constant weight, the residual pressure being less than 1×10^{-4} mm. A dead space measurement was made with He and the surface area was then measured by means of a −196°, N_2 adsorption isotherm. The samples were reevacuated prior to making the sorption measurements with polar gases.

Data

Ammonia.—Sorption isotherms were measured with NH_3 at 25° and −34°. In Fig. 1 are shown plots of 3, 25° runs and 1, −34° run. The reproducibility was good for samples from the same source. For a sample from a different source, the data fall on a parallel curve only slightly displaced from the curve of the first sample. It may be noted that the −34° isotherm is also parallel to the isotherm at 25°.

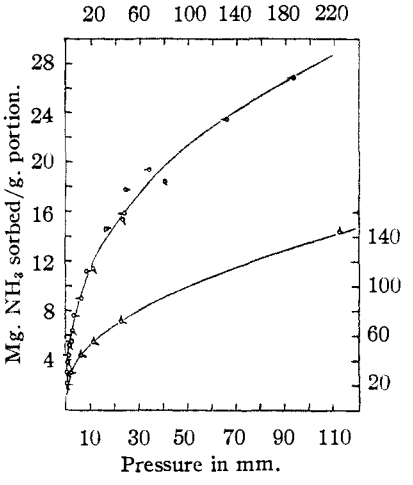

Fig. 1.—Sorption of ammonia on egg albumin: upper curve, sorption at room temperature; lower curve, sorption at −34°. Use right hand ordinates and upper abscissa.

At each pressure a long time was required for attainment of equilibrium, the time increasing with increasing sorption and also with decreasing temperature. At the higher partial pressures 12–24 hours were required per point. A possible reason for the difference in the 25° isotherms may be the longer times allowed for equilibration in the higher isotherm (Fig. 1).

Attempts to study the desorption curves were abandoned because of the prohibitively long equilibrium times required. However it was found that at 25° a hysteresis loop formed on desorption could be scanned back to the original sorption curve. Most of the NH_3 came off on pumping for 12 hours. 0.15 mmole NH_3/g. protein remained and was only finally pumped off after an additional 36–48 hours pumping. From very careful time studies it is estimated that the values recorded in sorption are within 10% of true equilibrium values.

It was found that the data for all runs could be represented to within 4% deviation by a Freundlich type isotherm; $P = a^{-1}W^n$ or

$$\log P = n \log W - \log a$$

in which P is vapor pressure, W is specific weight of NH_3 sorbed and a and n are constants which may depend on temperature. The data from Fig. 1 are shown plotted in this manner in Fig. 2. The best fit to these data is given in Table I.

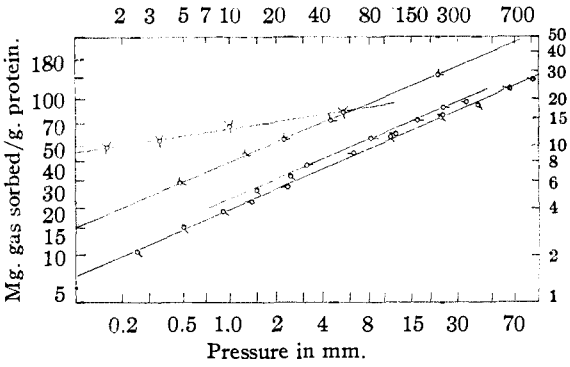

Fig. 2.—Freundlich plot of sorption of ammonia and methylamine on egg albumin: Ov, -O and O- refer to ammonia at room temperatures (use lower abscissa and right hand ordinates); Ȯ- refer to ammonia at −34° (use upper abscissa and left hand ordinates); Ǫ refer to desorption of methylamine at 0° (use upper abscissa and left hand ordinates).

TABLE I

FREUNDLICH CONSTANTS FOR NH_3 SORPTION[a]

Temp., °C.	n	log a
21	2.330	1.390
18[b]	2.396	1.594
−34	2.393	2.811

[a] P in mm.; W in mg. NH_3/g. protein. [b] Egg albumin from different source. Longer equilibrium times allowed.

It can be seen that the exponent, n, is very nearly the same for both temperatures. The partial molal, differential heat of sorption of NH_3 calculated from the approximate Clausius–Clapeyron equation is 7.5 ± 0.5 kcal./mole and is independent of pressure.

Methylamine.—For purposes of comparison, the sorption isotherms of NH_3 were compared with those of the stronger base, methylamine. A run made by Dr. David A. Ellis on the latter is shown plotted in Fig. 3 (sorption and desorption). At roughly equivalent, reduced temperatures (0° for methylamine and −34° for NH_3) the equilibrium times for the two gases were about the same over the isotherms. On a molar basis the NH_3 is the more strongly sorbed although the sorption curves have the same shapes (except at the lowest pressures). The sorption points for methylamine do not lend themselves to an empirical plot. The desorption points fit a Freundlich isotherm with constants: $n = 6.160$; $\log a = 10.200$ (plotted in Fig. 2).

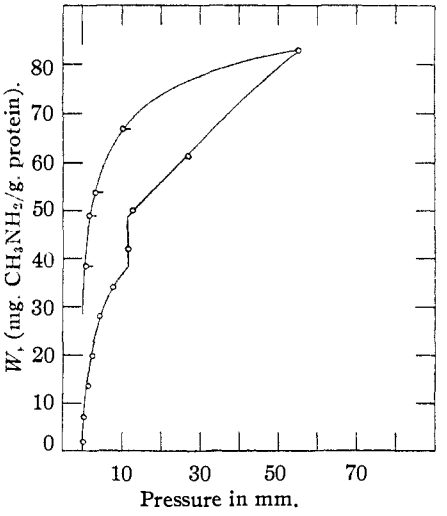

Fig. 3.—Sorption and desorption of methylamine on egg albumin at 0°: O, refer to sorption; O-, refer to desorption.

A significant feature of the methylamine sorption which does not appear in the NH_3 sorption is an abrupt change in slope at a pressure of 11.5 mm. (indicated as an isobar in Fig. 3).

Hydrogen Chloride.—A large number of HCl sorption isotherms were measured both at room temperature and at −78°. Figure 4 is a composite graph of the former. The results fall into two categories. The first indicated by the upper curve (Fig. 4) is a composite of 5 runs made on fresh protein samples. The second (lower curve) is a composite of 3 runs made on samples previously exposed to HCl and pumped for a week to constant weight. The two curves are parallel above a pressure of 5 mm. and there is no effect of surface area for either group.

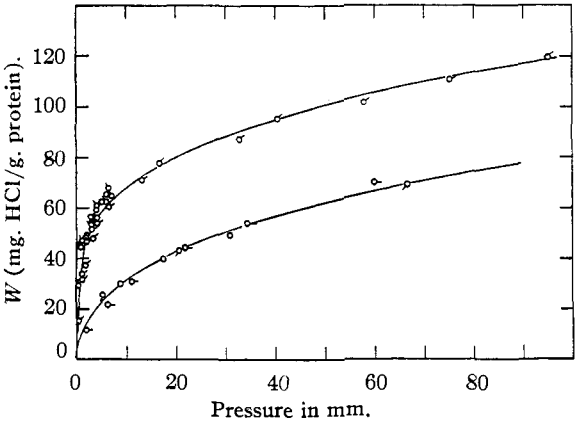

Fig. 4.—Sorption of hydrogen chloride on egg albumin at room temperature: upper curve is composite of 6 different runs made on fresh protein; lower curve is composite of 3 different runs made on HCl–protein complex.

Figure 5 shows the results of three runs at −78°. They show less spread than the room temperature results and again there is no effect of surface area. In addition there is no difference between fresh or rerun protein samples.

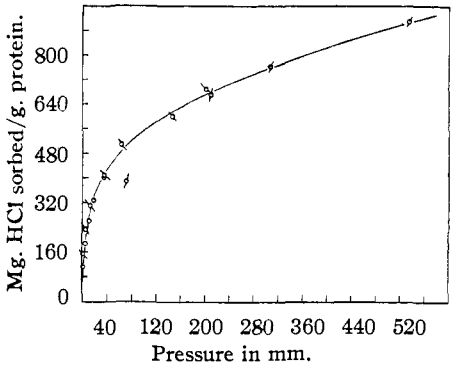

Fig. 5.—Sorption of hydrogen chloride on egg albumin at −78°. Composite of three different runs: ϕ and O refer to fresh protein; ⊖ refer to HCl–protein complex.

Equilibrium was established in the HCl runs in about half the time necessary for the corresponding NH_3 runs. However the same general behavior was noticed, the time increasing with amount sorbed and also with decreasing temperatures.

The most significant difference between the HCl and NH_3 isotherms lay in their behavior at low pressures and in desorption. In Fig. 6 is shown a set of 4 different runs made at low pressures. There are persistant isobars appearing at pressures of 0.10, 1.3 and 3.4 mm. On some curves isobars appear at other pressures as well, but the former always appear. The isobars correspond to a total uptake of about 20 mg. HCl/g. protein although the individual sections are not reproducible in length. It was possible to trace a small hysteresis loop in desorption but again the times were so long that no attempt was made to follow the entire isotherm.

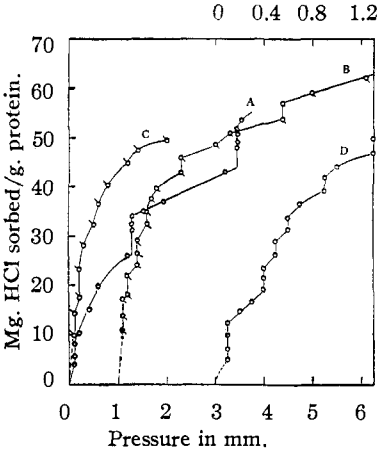

Fig. 6.—Sorption of hydrogen chloride on egg albumin at room temperature; low pressure region: curves A and C have same origin (use lower abscissa); curve B has same coördinates but origin for abscissa is shifted by 1 mm. as shown by dotted line.

After evacuation for 3–7 days the protein exposed to HCl reached a constant weight which indicated 36 mg. of permanently bound HCl per gram protein. This was repeated several times and found to be reproducible to ±1.2 mg. HCl/g. Equilibration of this HCl-protein complex with water vapor at 25° and subsequent re-evacuation did not remove any of the HCl although the bound water could be quantitatively removed.[10] It is interesting to observe that the sorption curves for samples containing bound HCl had none of the low pressure isobars but did parallel at higher pressures the behavior of the sorption curve obtained with fresh protein. The isotherms were separated as shown in Fig. 4 by a constant amount, corresponding to 36 mg. HCl/g. protein which is an additional check on the amount of irreversibly bound material. It was further found that samples of protein which had been used for NH_3 isotherms showed the same HCl isotherms as fresh protein samples.

Since there seemed to be an unaccountable anomaly between the sorption and desorption runs with HCl, a new run was made in which the behavior of the pressure in the region of the isobars was observed. If, as in the original runs, periods of time of about 20–30 minutes were allowed for sorption, the isobars appeared. However, when times of the order of 10–24 hours were allowed, they were not observed. At pressures below 20 mm., the volumes sorbed change on standing by about 2% in two hours and an additional 4% in 18 hours, so that there appears to be a difference of some 6% between the sorptions recorded here at low pressures and "true" equilibrium values.

As a final check on the nature of the processes leading to the formation of irreversibly bound HCl, measurements were made on a fresh protein, samples of which were allowed to remain in contact with HCl for 24 hours at pressures ranging from 3–100 mm. and then evacuated for 24–200 hours. In each case the results were the same for the amount of HCl bound, 36 mg./g. protein.

The data for low and high temperatures can be fitted very nicely by Freudlich type isotherms (Fig. 7). The maximum deviation from the curve at low temperatures is 4%. The room temperature curves fall into two parallel lines for the fresh protein and protein–HCl complex. The low pressure isobars appear as displaced sections of the fresh protein curve. The constants for these equations are given in Table II.

The partial molal differential heat of sorption, calculated from the Clapeyron equation is about 12 kcal./mole HCl sorbed and virtually independent of amount sorbed. For the fresh protein, the value is 6 kcal./mole, decreasing

(10) This HCl-protein complex, containing large amounts of sorbed water, required more than ten days to reach the original and constant weight. This is in direct contrast to the behavior of fresh protein, which is effectively dried in 24–30 hours. The powdery structure of the complex collapsed on water sorption and the final dried sample had only about 20% of its initial volume.

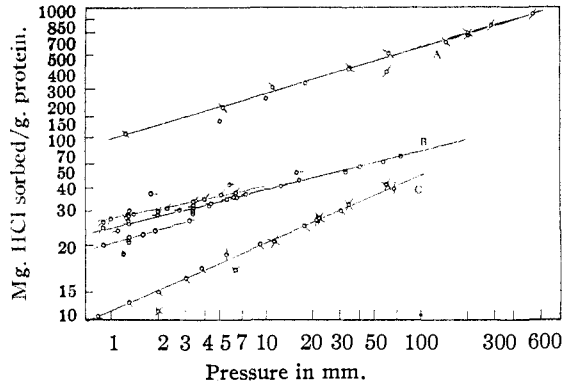

Fig. 7.—Curve D has abscissa indicated in upper part of graph. Freundlich plots of sorption of hydrogn chloride on egg albumin: curve A, composite of 3 runs at −78°; curve B, composite of 6 runs at room temperature on fresh protein; curve C, composite of 3 runs on HCl–protein complex at room temperature.

TABLE II

FREUNDLICH EQUATION CONSTANTS FOR HCl SORPTION

Temp., °C.	n	log a
−78.5	3.442	7.450
21.0	4.120	6.536
21.0[a]	2.321	2.463

[a] For protein containing bound HCl. This curve is given by the constants of the preceding curve if we use for w, the weight of HCl sorbed plus bound HCl. The equation is then: $\log P = 4.120 \log (W - 36) - 6.536$.

slightly with increasing sorption. Since the fresh protein is probably not in true equilibrium, the latter values should not be given serious consideration.

Boron Trifluoride.—The BF_3 isotherm shown in Fig. 8 was measured by Dr. Ellis (sample at −78°). Equilibrium times were from 10–24 hours. Isobars are observed at pressures of 1.6 and 3.5 mm. From the desorption curve, a value of 170 mg. BF_3/g. protein was calculated for permanently bound BF_3. This is higher than that for HCl and is to be expected in view of the high acid strength of BF_3. No further measurements were made on the isotherms because of the long equilibrium times.

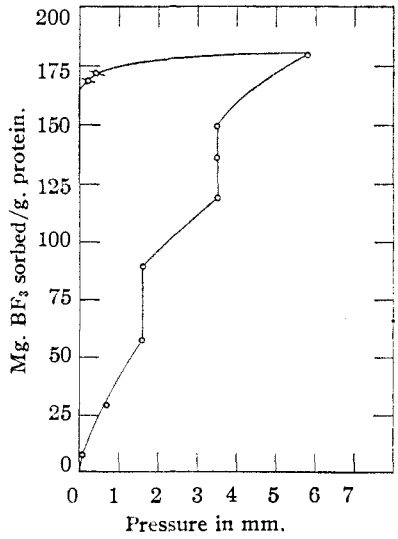

Fig. 8.—Sorption and desorption of boron trifluoride on egg albumin at −78°: upper curve is for desorption, lower curve sorption.

Protein Denaturation.—Proteins that had been exposed to NH_3 showed no changes in physical appearance or solubility in water. Samples exposed to HCl however had contracted slightly in volume and were insoluble in water, 0.1 M HCl (contrast to fresh protein) but soluble in 0.1 M NaOH. The BF_3 complex was also insoluble in water. It thus appears that HCl addition causes denaturation of the protein. This is different from the reaction in aqueous solution.

Heat Effects.—It was found by use of the apparatus described earlier that a temperature rise of 40° could be measured within 20 seconds after admitting HCl to a protein sample. This was the same for samples previously exposed to NH_3 sorption and reëvacuated. If we assume that the specific heat of the protein is 0.33 cal./g.-°C. we can calculate from the weight of the sample (0.16 g.) and the amount of HCl sorbed (1 cc. S.T.P.) a value of 10 kcal. of heat liberated per mole HCl sorbed. Repeat runs showed that this was the same for a range of 10–30 mg. HCl sorbed per gram protein. These values are in good agreement with the 12 kcal./mole HCl estimated from the Clausius–Clapeyron equation and tend to give the numbers further reality.

From the temperature rises found with NH_3 (about 15°) a similar calculation yields a value of 3 kcal./mole of NH_3 sorbed which is in much poorer agreement with the value from the Clapeyron equation (7.5 kcal./mole). This may be, unfortunately, all that can be expected in the way of relative agreement in view of the small amounts of heat and the quite large heat losses.

These direct heat measurements are certainly minimum values for the system, since the assumptions made would tend to decrease the calculated heat. The principal error is undoubtedly due to heat loss through the walls and along the thermocouple wires. It was observed that the latter was the controlling factor and principal source of error. The estimated heat conductivity of the protein–gas system is extremely low and a simplified calculation of the ratio of the heat conductivities of this system to the heat conductivity along the leads gave a value of 0.2.

Discussion

The foregoing results seem consistant with the following model for the protein–polar gas interaction: (1) A rapid, exothermic attachment of polar gas to the most accessible, conjugate groups of the protein solid. This takes place at low pressures and may be irreversible for the strong acids (HCl, BF_3) and bases. (2) A much slower, exothermic reaction between the polar gas and less accessible groups in the solid. This may also be irreversible and can take place at low pressures. (3) A slow, reversible "solvation" of polar groups within the protein probably accompanied by a swelling of the solid. This takes place only at higher pressures. (4) A denaturation (indicated by loss of solubility) accompanying the irreversible sorption which is probably due to the local heating of the protein.

This is of course a completely different model than that proposed for the interaction of proteins with non-polar gases, the latter being attributable entirely to the superficial area of the protein solid.[7] A similar, though more detailed model has been proposed by Pauling[11] to account for the sorption of water vapor by proteins. Water displays the same type of behavior shown by the polar gases studied here.[8] The differences in behavior between the polar and non-polar gases, in protein sorption may be summarized as follows: (a) The amount of polar gas sorbed is independent of the superficial area of the protein. (b) At comparable partial pressures, the amount of polar gas sorbed is from 10^2–10^4 times greater than the amount of

(11) L. Pauling, THIS JOURNAL, **67**, 555 (1945).

non-polar gas. (c) Equilibrium is reached within 2–5 minutes with the non-polar gases, within 30 minutes–24 hours or more for the polar gases. (d) Isosteric heats of sorption are much greater than the heat of liquefaction for the polar gases whereas they are close to the heat of liquefaction for the non-polar gases. (e) There is considerable hysteresis on desorption with all polar gases and also some irreversible binding with some of these gases. The non-polar gases show no hysteresis or irreversible binding. (f) The polar gas isotherms do not fit in a sensible manner any of the physical adsorption isotherms. When pressed into such a fit they give absurdly high values for "surface area." Thus the BET equation yields a value of 2,000 meter^2/gram for the HCl isotherm at $-78°$.

The evidence for points (1) and (2) in the proposed model comes from the direct evidence noted in the previous section. The slowness with which the irreversible addition takes place seems accountable only in terms of interactions involving inaccessible regions of the protein. That there is solvation (point 3) seems reasonable in view of the direct observations of swelling observed with water[12] and with NH_3.[13–16] It is further indicated by the large amounts sorbed at the higher pressures. Thus with HCl at a partial pressure of 0.7 ($-78°$) there are 915 mg. HCl sorbed per gram of protein (Fig. 7). This corresponds to 25 mmoles HCl/g. protein which is twice the total number of mmoles of nitrogen groups present according to the Kjeldahl analysis.[17] Similarly the value of 225 mg. NH_3/g. protein sorbed at $P/P_0 = 0.19$ (at $-34°$), is far in excess of any reasonable value for the number of acidic groups present.[17] Such "solvation" would also explain the fact that the data lend themselves to a Freundlich isotherm.[18]

The large heat evolution observed with HCl (and expected for BF_3) could easily be responsible for the observed denaturation.[19] Simple addition of HCl to the protein should not induce insolubility since the addition of HCl in aqueous solution in larger concentrations produces no such effect. The details of the denaturation are of course difficult to infer. It seems unlikely that exposure of new basic groups is involved since such changes would be expected to manifest themselves in large variations in the isotherms and also in the amount of HCl sorbed permanently. Belden[4] however has observed that HCl darkens gelatin at higher pressures and increases the amount of amino nitrogen.

A feature which seems difficult to account for is the transient isobars observed with HCl. The pressures at which they appear are reproducible but their lengths are not. Parks and Melaven[5] working with hide substances (presumably impure collagen) and HCl also found isobars which disappeared in time. For some of their sorption points they waited as long as 24 days to reach equilibrium.

On the other hand, the reproducibility of the composition of the HCl–protein complex is rather striking and we have attempted to find a correlation between this acid-binding capacity (1.00 ± 0.04 mmoles HCl/g. protein) and the analytical information available for egg albumin. From the work on the maximum acid-binding capacity in solution[17] we find the figure of 0.91 ± 0.02 mmole/g. protein, taken from the papers of Cannan, Kibrick and Palmer.[20] In view of the differences between the two techniques this may be taken as good agreement.

The nature of this irreversible addition is more complex. Intuitively we might expect to find the HCl bound to the free basic groups in the protein (*e.g.*, amines). If these are present as cations (RNH_3^{+1}) then the HCl could still react with the zwitterion partners ($RCOO^{-1}$). The known amino groups which could react directly or indirectly in this way amount to only 0.91–0.93 mmole/g. protein.[21] However, the formaldehyde and nitrous acid reactions both indicate a free amino group content of 0.51–0.55 mmole/g.[22] Since lysine is the only known group which could account for these latter reactions there appears to be a genuine discrepancy between the composition and titration values. If we use the formaldehyde and nitrous acid results in place of the lysine we would have 0.99 to 1.03 mmoles/g. of acid-binding groups. Alternatively, if we assume that all of the free carboxyl groups are in ionic form then the known analysis[17] would give 1.02 mmoles of anions capable of binding HCl.[23] Despite these difficulties it is striking that a fairly simple, gas phase titration is capable

(12) White and Eyring, *Textile Research J.*, **17**, 523 (1947).

(13) Taft, *J. Phys. Chem.*, **34**, 2792 (1930); **35**, 578 (1931).

(14) McChesney and Miller, THIS JOURNAL, **53**, 3888 (1931).

(15) McChesney and Roberts, *ibid.*, **60**, 1935 (1938).

(16) Miller and Roberts, *ibid.*, **56**, 935 (1934); **58**, 309 (1936); **61**, 3554 (1939).

(17) E. J. Cohn and J. T. Edsall, "Proteins, Peptides and Amino Acids," Reinhold Publishing Corp., New York, N. Y., 1943, p. 341, also Chap. 20.

(18) J. Zeldowitch, *Acta Phys. Chim. U. R. S. S.*, **1**, 961 (1934), has shown that sorption will follow a Freundlich plot if the sorption sites have energies of sorption which are distributed with an exponential frequency. This is probably a fair assumption for the polar groups in a solid protein.

(19) In this regard it is striking that fresh protein and protein–HCl complex have the same isotherms at $-78°$. This is in direct contrast to their behavior at room temperature where the latter is lower by 36 mg. HCl and parallel to the isotherm of the former. It may very well be that at these low temperatures the irreversible binding process is so slow that it does not make its appearance. This would be expected if there were any appreciable activation energy required for the binding. It is difficult to check this hypothesis experimentally, by exposing a sample of protein to HCl at $-78°$ and then desorbing since the desorption process requires prohibitively long times at these temperatures. Other types of experiments are however in progress to ascertain the nature of the process. The authors wish to thank one of the Referees for bringing this point to their attention.

(20) Cannan, Kibrick and Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941).

(21) G. R. Tristram, "Advances in Protein Chemistry," Vol. 5, 1949, p. 137. Quotes: Histidine -0.151 mmole/g.; Arginine -0.329 mmole/g.; Lysine -0.432 mmole/g. An almost identical set are obtained by J. C. Lewis, N. S. Snell, D. J. Hirschman and Heinz Fraenkel-Conrat (*J. Biol. Chem.*, **186**, 23 (1950)), who give the respective values: 0.155, 0.339, 0.438. The totals are 0.912 and 0.932, respectively.

(22) R. A. Kekwick and R. K. Cannan, *J. Biochem.*, **30**, 235 (1936).

(23) Some caution must be exercised in trying to interpret such results since it has been reported that gliadin forms a reproducible, strong complex with acetic acid in solution—0.25 mmole acid/g. (W. B. Sinclair and R. A. Gortner, *Cereal Chem.*, **10**, 171 (1933)) and α-cellulose does the same (R. A. Gortner and J. J. McNair, *Ind. Eng. Chem.*, **25**, 505 (1933)) although there are no basic groups in the latter.

of giving such reproducible results on acid-binding capacity.[24]

Further studies are now under way in this Laboratory to investigate the feasibility of using measurements of permanently bound gas to assign acid- or base-binding capacity. Work on water sorption has shown[8] that equilibrium times in sorption may be reduced considerably by utilizing cells packed with metal wire (silver, platinum, aluminum, etc.) to improve thermal conductivity. Similar effects are being studied with other polar gases. The authors would like to suggest to other workers that apparatus be designed to give the maximum heat conductivity in the sample–thermostat system.

(24) In the case of BF_3, it should be noted that the length of the isobars in the sorption curve correspond to 0.93 mmole BF_3/g. protein which is very close to the figure cited for acid-binding capacity. The irreversibly bound BF_3 corresponds to about 2.5 mmoles BF_3/g. protein which does not as yet lend itself to simple interpretation.

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[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, OF THE UNIVERSITY OF CALIFORNIA]

The Relation of Photosynthesis to Respiration[1]

BY J. W. WEIGL,[2] P. M. WARRINGTON[3] AND M. CALVIN

The gas exchange of barley leaves has been studied in a closed system. Partial pressures of oxygen, carbon dioxide and added radiocarbon dioxide were measured simultaneously during periods of illumination and darkness. The following conclusions were reached: In strong light respiratory carbon dioxide originates primarily from endogenous sources and only to a very slight extent from recently assimilated carbon. In the dark recently photosynthesized compounds are actively oxidized in a fairly constant ratio to endogenous respiration. Photosynthesis proceeds at a measurable rate even at the lowest CO_2 pressures observed (0.03 mm.). There is no evidence for a "threshold" concentration of carbon dioxide for the reaction; at the lowest concentrations reached, respiration exactly equals assimilation. In the one experiment which could be evaluated, the mean rate of respiratory CO_2 evolution in strong light was found to be less than that in the dark. Internal rephotosynthesis of respiratory carbon may have been sufficient to account for this effect. Under the conditions of these experiments the assimilation of $C^{14}O_2$ was found to be about 17% slower than that of $C^{12}O_2$.

The relation between photosynthesis and respiration in green plants is, to date, inadequately understood. Until recently, it was not even certain whether, in the light, there occurs any respiratory evolution of carbon dioxide simultaneously with the assimilation of carbon dioxide from the air, or whether, perhaps, the path of carbon in photosynthesis is merely the reverse of that in respiration. The reason for this uncertainty is that the over-all reactions which may be written for these two processes are opposite

$$\text{Photosynthesis: } CO_2 + H_2O \longrightarrow (CH_2O) + O_2$$
$$\text{Respiration: } O_2 + (CH_2O) \longrightarrow H_2O + CO_2$$

where (CH_2O) represents carbohydrates, which are typical photosynthetic products and respiratory substrates.

Numerous investigators have attempted to distinguish between these simultaneous and opposite reactions, and to measure the rate of respiration in the light. The older literature has been reviewed by Weintraub[4] and Rabinowitch.[5] More recently, Kok,[6] van der Veen,[7] Gabrielsen[8] and Warburg, Burk, *et al.*,[9] have made contributions to the problem. The present investigation has attempted to further clarify the relation of photosynthesis to respiration, with emphasis on the role of carbon dioxide in these reactions.

Tracer carbon-14 has made possible a new approach which is, in principle, quite direct. One may place leaves in a closed system, allow them to photosynthesize in radioactive carbon dioxide, and follow continuously, by means of non-destructive methods of analysis, the concentrations of radioactive and inactive carbon dioxide in the gas phase. If simultaneous photosynthesis and respiration involve different chemical or physiological paths, at least the initial respiratory carbon dioxide will be inactive; the rate of reduction of the original specific activity of the radioactive carbon dioxide supplied should be a quantitative measure of the rate of respiration.

Since in these experiments we have been mainly concerned with the exchange of carbon dioxide in the gas phase, we have used the terminology

Photosynthesis: Assimilation of carbon dioxide from the gas
Respiration: Evolution of carbon dioxide into the gas

These definitions have certain shortcomings, resulting from the nature of the measurements. For example, non-photosynthetic carbon dioxide fixation is included with photosynthetic assimilation. However, tracer uptake in the dark was found to be very slow (less than 1% of the photosynthetic rates in runs 14 and 28 and never over 5% in other experiments); hence, non-photosynthetic fixation in the light was probably not very important.

The definitions also imply that even in strong light all respired carbon leaves the cells as carbon dioxide, is mixed with the entire gas phase, and

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(4) R. L. Weintraub, *Botan. Rev.*, **10**, 383 (1944).

(5) E. I. Rabinowitch, "Photosynthesis," Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1945, Chapter 20.

(6) B. Kok, *Enzymologia*, **13**, 1 (1947); *Biochim. et Biophys. Acta*, **3**, 625 (1949).

(7) R. van der Veen, *Physiol. Plantarum*, **2**, 217 (1949).

(8) E. K. Gabrielsen, *Nature*, **163**, 359 (1949).

(9) O. Warburg, D. Burk, V. Schocken, M. Korzenovsky and S. B. Hendricks, *Arch. Biochem.*, **23**, 330 (1949); D. Burk, S. B. Hendricks, M. Korzenovsky, V. Schocken and O. Warburg, *Science*, **110**, 225 (1949).