and 2.5 to 4.0 moles per liter at 250°. Their pressures were uniformly lower than ours by an average of 1.8%.

We also made a comparison of the observations of Sage and Lacey at the densities 5, 6 and 7 moles per liter and at their temperatures 400 and 460°F. The agreement was good. At the lower temperature their pressures were higher than ours by +0.49, −0.22 and +0.55%; while at the higher temperatures the deviations were +0.57, +0.31 and +0.05%.

The second virial coefficient $B_V$ in the equation

$$pV = RT\left(1 + \frac{B_V}{V} + \frac{C_V}{V} + \ldots\right)$$

was determined graphically at each temperature from plots of $V[(pV/RT) - 1]$ against $1/V$. These values are shown in Table III.

TABLE III

SECOND VIRIAL COEFFICIENTS OF NORMAL PENTANE

$$B_V = \text{Limit } \frac{1}{V} \longrightarrow 0 \; V\left(\frac{pV}{RT} - 1\right)$$

| Temp., °C. | 200 | 225 | 250 | 275 | 300 |
|---|---|---|---|---|---|
| $B_v$, l./mole | −0.405 | −0.350 | −0.311 | −0.274 | −0.244 |

We are very grateful to the United States Rubber Company for a fellowship held by one of us (D.R.D.).

CAMBRIDGE, MASSACHUSETTS

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, CATHOLIC UNIVERSITY]

# Sorption of Ammonia by Silk Fibroin

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The sorpion of ammonia by silk fibroin has been measured at −77 and −63°. There is a marked hysteresis in the sorption isotherms, the loop closing at zero pressure. The B.E.T. monolayer point for adsorption occurs when one ammonia molecule is held for every two peptide links in the adsorbent, whereas the monolayer point on desorption occurs at one ammonia per peptide link. Analysis of the hysteresis loop suggests that the dissipative process is due in part to viscous retardation of the swelling of the fiber, and in part to a shifting of sorbed molecules to new sites of lower energy that are exposed as a result of the swelling.

The interesting thermodynamic problems presented by a system that is completely reproducible yet completely surrounded by irreversibility have been discussed in a recent paper by Bridgman.[2] Examples occur in the stress–strain loops of plastically deformed solids, and in the metastable states produced by quenching alloys subject to order–disorder transitions. Another noteworthy example is to be found in the hysteresis of sorption isotherms. We shall describe in this paper a study of the system ammonia–silk fibroin, at −77 and −63°, in which the phenomenological aspects of the sorption hysteresis are especially clear. Previous work with polar gases on fibrous proteins is exemplified by the water–silk system at 25°,[3] though, probably owing to the higher temperature, the irreversible effects were much less evident in this case. Also, in a recent paper, Benson and Seehof[4] report evidence of hysteresis in the ammonia–egg albumin system.

## Experimental Details

Raw silk was degummed by the procedure of Sookne and Harris[5] to remove the sericin from the fibroin, and dried under vacuum at 25° to constant weight. The ammonia, of 99.5% stated purity, was freed from condensable gases by repeated pumping under high vacuum, with intermittent thawing. It was stored in a sodium coated bulb.

The sorption apparatus was of the volumetric type[6] with mercury cut-off valves instead of stopcocks. Pressures were read with a cathetometer to 0.05 mm. from a wide bore mercury manometer. A fibroin sample of about 20 mg. was used. The sample tube was surrounded by a low temperature thermostat that maintained temperatures constant within ±0.2° over periods of a month or more.

Before a sorption run, a new fibroin sample was pumped at 70° and $10^{-6}$ mm. for 24 hours; samples previously exposed to ammonia were restored to their original condition by pumping at 70° and $10^{-6}$ mm. for 72 hours. Dead space volumes were determined with helium. The ammonia vapor pressures were taken from the Landolt-Börnstein Tables.

After admission of a charge of ammonia to the fibroin, adsorption was followed by the decrease in pressure, and the "equilibrium value" was defined by a change in pressure of less than 0.02 mm. per hour, corresponding to from $10^{-4}$ to $5 \times 10^{-4}$ mole $NH_3$ per gram of fibroin per day. The sorption at different pressures was followed by changing the volume of the system stepwise, with the addition of more ammonia as needed. The approximate times $t_e$ required to reach equilibrium at different relative pressures $a = p/p_0$ and temperatures $T$, were as follows: (1) $T = -77°$, $a > 0.22$, $t_e = 50$ hours for both adsorption and desorption; (2) $T = -77°$, $a < 0.22$, $t_e = 50$ hours on adsorption, 72 hours on desorption; (3) $T = -63°$, $a > 0.9$, $t_e = 24$ hours; (4) $T = -63°$, $a = 0.2$ to 0.9, $t_e = 50$ hours for both adsorption and desorption; (5) $T = -63°$, $a < 0.2$, $t_e = 100$ hours for adsorption and $t_e$ for desorption increased from 96 to 144 hours with successively lower $a$. As a rule about 80% of the total adsorption or desorption occurred during the first half of the time, $t_e$.

## Description of the Isotherms

The experimental isotherms are shown in Fig. 1 (−77°) and Fig. 2 (−63°). At this point it is necessary to define the *main adsorption and desorption curves*. An example of each of these is shown in the −63° isotherm, the main adsorption and desorption curves being, respectively, the lower and upper bounding curves. They were obtained by exposing the initially "dry" fibroin to ammonia atmospheres of increasing relative pressure up to a maximum of 0.99, and then desorbing from this maximum pressure to the lowest pressure measurable. At −77° the desorption was started at $a = 0.93$; hence the upper bounding curve at this temperature is not the main desorption curve, which

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[1] Abstracted from a portion of the dissertation submitted by Patricia A. Marshall in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] P. W. Bridgman, *Rev. Modern Phys.*, **22**, 56 (1950).

[3] A. C. Goodings and L. H. Turl, *J. Textile Inst.*, **31**, T69 (1940).

[4] S. W. Benson and J. M. Seehof, THIS JOURNAL, **73**, 5053 (1951).

[5] A. M. Sookne and M. Harris, *J. Research Natl. Bureau Standards*, **23**, 303 (1939).

[6] H. Frey and W. J. Moore, THIS JOURNAL, **70**, 3644 (1948).

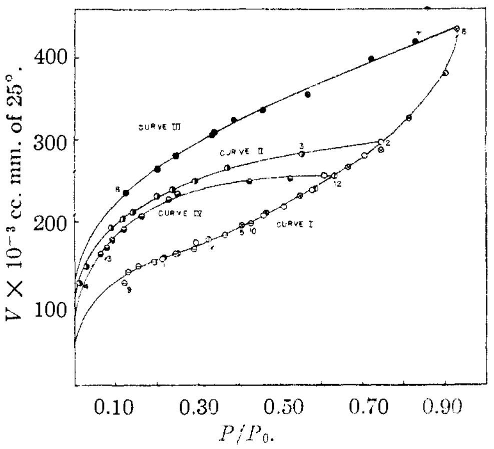

Fig. 1.—Isotherm of ammonia on silk fibroin at −77°.

| Run | Sample | |
|---|---|---|
| ○ = 1 | A | Adsorption |
| ◑ = 1 | A | Desorption |
| ⊗ = 2 | A* | Adsorption |
| ● = 2 | A* | Desorption |
| ⊖ = 3 | A** | Adsorption |
| ⦶ = 4 | B | Adsorption |
| ◒ = 4 | B | Desorption |

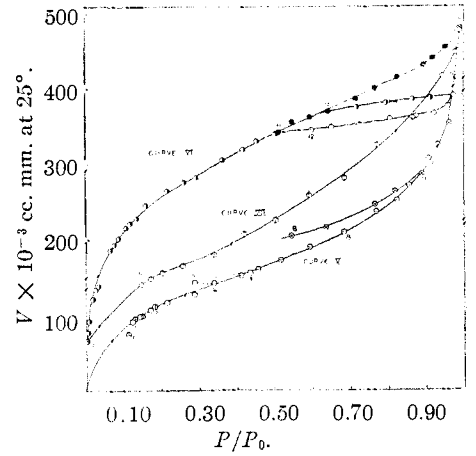

Fig. 2.—Isotherm of ammonia on silk fibroin at −63°.

| Run | Sample | |
|---|---|---|
| ⊕ = 1 | C | Adsorption |
| ○ = 2 | D | Adsorption |
| ⊖ = 2 | D | Adsorption |
| ⊗ = 3 | E | Adsorption |
| ● = 3 | E | Desorption |
| ⦶ = 3 | E | Adsorption |
| ◑ = 3 | E | Desorption |
| ⌀ = 3 | E | Adsorption |

would lie above the experimental upper curve and gradually approach it at low relative pressures. The main adsorption and desorption curves enclose a certain area; any sorption point $(n,a)$ that lies within this area can be obtained by a suitable adsorption–desorption history.

The marked hysteresis of the sorption isotherms is immediately evident; we are not acquainted with any previous example in the literature in which this hysteresis is so well displayed. For example, at the point of greatest difference between the two curves, the desorption value at −63° is 97% greater than the adsorption value, compared to the 8% difference found for water on silk at 25°.[3]

It will be noted that there appears to be no residual permanent sorption as the desorption curve is traversed toward zero pressure. Although it was necessary to lengthen progressively the equilibrium time, to a maximum of six days for point 15 at $a$ = 0.003 in the −63° isotherm, the trend of the curve is unmistakably toward zero sorption at $a$ = 0. The completely reproducible character of the isotherms should also be emphasized. If the sorption history is interrupted at any point, and the fibroin degassed for 72 hours at 70° and $10^{-6}$ mm., points on a new adsorption curve fall on the main adsorption curve of the previous run. Likewise, reproducibility is obtained with fresh samples of fibroin. From the behavior of the main adsorption and desorption curves, we may therefore conclude that the observed hysteresis is not caused by irreversible chemical reaction or chemisorption,[7] and also that it is not caused by a permanent change in the adsorbent. The present example falls therefore in the class of *dynamic hysteresis* rather than that of *static hysteresis*.[8]

Some of the scanning loops obtained will now be described. Let us consider first the −63° isotherm. The adsorption was followed to $a$ = 0.435 (point 4) by decreasing the volume of the system. At this point, the sample tube was closed from the gas buret, and a new charge of ammonia added to the latter. Before the sample cut-off was reopened, the volume of the system was adjusted so that the first equilibrium point would fall below point 4. In order for this to happen, some ammonia would have to desorb from the fibroin. Thus the next point (5) occurred at $a$ = 0.287. Although the system was kept at constant volume for 72 hours, *i.e.*, 24 hours longer than the equilibrium time, point 5 did not fall on the main adsorption curve. When the volume of the system was now decreased, the next point (6) fell on the main curve at $a$ = 0.338. This sequence illustrates rather well the fact that the main adsorption curve represents a reproducible irreversible path between successive states of metastable equilibrium.

The main adsorption curve was then followed to point 7 at $a$ = 0.907, when an accident to the thermostat necessitated the substitution of a new fibroin sample. The new sample was exposed to a single large charge of ammonia, with the intention of picking up the main adsorption curve at about $a$ = 0.5. The large charge, however, resulted in somewhat more adsorption than was obtained from the same amount of ammonia divided into a number of smaller charges, leading to the point 8, after 72 hours. A plausible explanation of this behavior is that when the adsorbent is exposed to the relatively high ammonia pressure, the rate of sorption on all sites is increased, and certain less active sites become covered, which would not be covered by the usual addition of the ammonia by small increments. Thus a point like 8 might be considered as similar to a point reached by desorption from a point 8′. The adsorption curve from point 8 rejoined the main curve at point 9 ($a$ = 0.884), and sorption continued without further incident to point 10 at $a$ = 0.988.

Desorption was now followed along the main desorption curve to $a$ = 0.508, point 11. At this point a remarkable loop consisting of the path 11–12–13–14–11 was traversed. This loop has so small an area that it approximates to a reversible path between the main adsorption and desorption curves. This sort of scanning loop, found at high relative pressures, is quite different from those at lower relative pressures, which will be seen to have much larger areas. A possible explanation of this fact will be advanced later in the paper.

Desorption was now continued from point 14 to point 15 at $a$ = 0.003. An equilibrium time of 6 days was required for point 15, but the desorption was stopped at this point owing to limitations of the pressure measurement and not of the experimenter's patience. A new adsorption curve, beginning with point 16 and extending all the way to point 17 at $a$ = 0.988, was obtained, and it is significant that the main adsorption curve was not reached until the highest pressures.

The history of the −77° isotherms will now be recounted. With a fresh fibroin sample, the main adsorption curve was followed from point 1 ($a$ = 0.213) to point 2 ($a$ = 0.745).

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[7] This explanation has been used to account for sorption hysteresis in water–graphite systems by C. Pierce and R. N. Smith, *J. Phys. Colloid Chem.*, **54**, 784 (1950).

[8] These terms are used in the sense defined by J. M. Burgers, "First Report on Viscosity and Plasticity," North Holland Publishing Co., Amsterdam, 1939.

Desorption was followed along curve 2–3–4 to $a = 0.012$, at which point the fibroin was subjected to the 70° pumping, preparatory to a new adsorption run. The new points fell along the main adsorption curve from point 5 at $a = 0.408$ to point 6 at $a = 0.931$.

The desorption curve 6–7–8 cannot be identified as the main desorption curve, but it is probably identical with the main curve below a relative pressure of about 0.5. This surmise is based on the fact that in the −63° isotherm, the desorption from $a = 0.972$ reached the main curve at $a = 0.642$. The definition of the main desorption curve as the one beginning from $a = 0.99$ is, of course, arbitrary, but at $a = 1.00$ liquid ammonia condenses on the fibroin, and an irreversible shrinkage of the fibroin takes place.

At point 8, the fibroin was again pumped at 70°, and a new series of adsorption points was obtained along the main curve from 9 to 10. A new sample was then introduced, and the adsorption points from 11 to 12 were recorded, all on the main curve. The study was terminated with a desorption curve from 12 to 13. It will be noted that these desorption curves that originate from relative pressures below about 0.8 do not reach the main desorption curve until the lowest relative pressures.

## Analysis of the Experimental Data

**Application of the B.E.T. Equation.**—The main adsorption and desorption isotherms are all of type II in the B.E.T. classification. The data from these isotherms were plotted according to the B.E.T. equation.[9] The equation was used in the form

$$a/n(1 - a) = 1/n_m c + (c - 1)\, a/n_m c$$

Here $n$ is the number of moles sorbed at relative pressure $a$, and $n_m$ is the number of moles of adsorbate needed to cover the first set of sites. When $a/n(1 - a)$ was plotted against $a$ for the ammonia–fibroin data, the results in Fig. 3 were obtained; good straight lines occurred up to a relative pressure of about 0.25. The values of $n_m$ were calculated in the usual way, from the slopes and intercepts[10] of the B.E.T. plots, with the results shown in Table I. To facilitate comparison with the isotherms in Figs. 1 and 2, the values of $v_m$ in cc. of $NH_3$ at 1 mm. and 25° are included.

TABLE I

AMMONIA SORBED AT THE B.E.T. "MONOLAYER POINTS"

| Curve | $n_m$ (mole $NH_3$ per g. fibroin) |  | $v_m$ |  |
|---|---|---|---|---|
|  | −77° | −63° | −77° | −63° |
| Adsorption | $6.6 \times 10^{-3}$ | $5.7 \times 10^{-3}$ | $122 \times 10^3$ | $106 \times 10^3$ |
| Desorption | $1.2 \times 10^{-2}$ | $1.2 \times 10^{-2}$ | $218 \times 10^3$ | $227 \times 10^3$ |

Silk fibroin is a protein composed mainly of glycine, alanine and serine residues. The proportion of acidic and basic side chains is only about 2 mole per cent.[11] The number of peptide links per gram of fibroin was calculated from the reported residue weights to be $1.2 \times 10^{-2}$ mole. This is equal to the number of moles of ammonia held by the fibroin at the desorption monolayer point, and to almost exactly twice the number at the adsorption monolayer point. It is not unreasonable, therefore, to conclude that the B.E.T. monolayer point on desorption occurs when each peptide link binds one ammonia molecule, whereas the monolayer point on adsorption occurs when one ammonia molecule is bound to two peptide links.

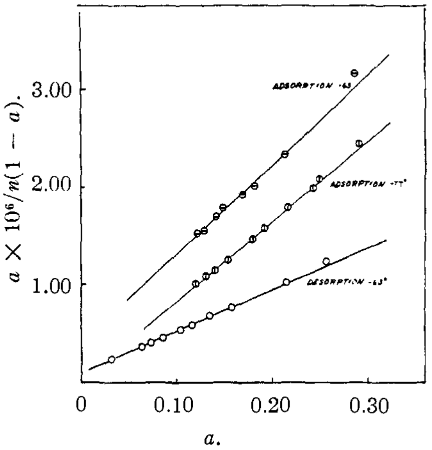

Fig. 3.—B.E.T. plot for ammonia on silk fibroin.

There is increasingly strong evidence that the structure of silk fibroin can be represented by the 3.7 residue helix recently proposed by Pauling, Corey and Branson.[12] In this helical structure, the residues are stereochemically equivalent and the conjugated amide system is planar. The —C=O group is positively directed, and the –N–H group is negatively directed along the helical axis, which is taken as the direction corresponding to the sequence –CHR–CO–NH–CHR– in the peptide chain. Each carbonyl and imino group is hydrogen-bonded to the third amide group beyond itself along the helix. When a model is made of this structure, it is readily seen that there is only one reasonable location for the ammonia at the adsorption monolayer point. One hydrogen of an ammonia molecule can be bonded between oxygens of two adjacent peptide links. The distance between two successive oxygens is 3.03 Å.[13] which is sufficient to fulfill the steric requirements for the proposed situation of the sorbed ammonia.

It is possible to see in a qualitative way how the ammonia held at the desorption monolayer point has increased to one molecule per peptide link. With increasing sorption, there is a radial swelling of the fibrils, due to the increased lateral separations of individual peptide chains. At some stage in this process, the ammonia molecule held between two peptide links may shift to one or the other, and another ammonia occupies the neighboring site that is left open. When the desorption curve is traversed, there is an extra potential energy barrier to be surmounted before the peptide chains can regain their original close packed configuration, the ammonia molecules on each peptide link tending to prevent the shrinkage, or intermolecular hydrogen-bond formation. Since on desorption there are twice as many sites of almost uniform free energy, doubling of the monolayer sorption is observed.

**The Sorption Hysteresis.**—The time required to reach sorption equilibrium in this system was usually about 50 hours for each experimental point,

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[9] S. Brunauer, P. Emmett and E. Teller, THIS JOURNAL, **60**, 309 (1938).

[10] The slopes and intercepts were obtained by the method of least squares. The very small positive intercept at −77° indicates some experimental uncertainty.

[11] D. Jordan Lloyd and M. Garrod, *Trans. Faraday Soc.*, **44**, 441 (1948).

[12] L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci.*, **37**, 205 (1951).

[13] R. B. Corey, personal communication.

or about 1000 hours for an entire main adsorption or desorption curve. The hysteresis in the isotherms indicates, in the most general terms, that there must be occurring within the system some dissipative process whose relaxation time or retardation time is of the same order of magnitude as the time scale of the experiments.

As we have seen, the sorbed ammonia is held not only on the outer surfaces of the fibrils, but also on the peptide links of the individual molecular chains. An individual silk fibril is about 1000 Å. in diameter and thus contains about $10^4$ peptide chains in its cross-section. It might perhaps be thought, therefore, that diffusion into the interior of the fibril is responsible for the observed hysteresis, since on adsorption less active sites near the outside might become covered at the expense of more active sites in the interior, so that the relaxation process would be a slow shift of ammonia from the less active sites of higher potential energy to those of lower potential energy, with the dissipation of the energy difference as irreversible heat. This mechanism may be rejected on two grounds: (1) The observed stoichiometric ratio of ammonia to peptide links at the adsorption monolayer point suggests that equilibrium throughout the fibril is achieved even at low relative pressures. (2) The diffusion coefficients of water vapor into keratin fibers is $D = 10^{-9}$ to $10^{-7}$ cm.$^2$ sec.$^{-1}$ at 20°.[14] The higher values occur at higher contents of sorbed water. The activation energy is about 5000 cal., so that an estimated $D$ at $-70°$ would be not less than about $10^{-11}$. The corresponding time for a mean diffusion path of 500 Å. into the fibril would be about 1 sec. If ammonia in fibroin does not behave much differently from water vapor in keratin, a reasonable supposition, we can disregard slow diffusion as a factor in these experiments.

The hysteresis in keratin–water and cellulose–water systems has been often ascribed to processes of mechanical relaxation in the fibers.[15] As the fibril sorbs vapor, it swells. The resistance to swelling is at first an elastic response, so that the work of swelling is stored as the potential energy of an elastic body under internal stress. Now, however, some change may gradually occur in the swelled fiber, which results in a relaxation of the stress and the gradual dissipation of the strain energy into heat. This picture corresponds to the well known Maxwellian model of a spring coupled in series to a viscous element (dashpot). A little reflection will show that it cannot be applied in the present case, since it necessarily results in a permanent plastic deformation of the stressed material, so that the hysteresis loop cannot close at zero stress. Actually the observations with water vapor on wool tend to display this permanent deformation, so that the model has been applied with some justification in that case.

In the ammonia–fibroin system, if we wish to explain the hysteresis by mechanical processes in the fibrils, we must employ a model consisting of a parallel coupling of the elastic and viscous elements (Voigt model). This model corresponds well to our physical picture of the swelling fiber; it behaves like a spring whose stretching can proceed only at a finite rate because of the necessity to break cross linkages. These consist probably of interchain hydrogen bonds. The breaking of the bonds is facilitated by the presence of the ammonia, which acts like an oily lubricant for a rusty spring. In more chemical language, we might speak of an ammonolysis of the cross links. Such a swelling would have a characteristic retardation time (or spectrum of times), and if this time is commensurate with the time scale of the experiment, hysteresis will be observed. Such a model is consistent with the observed absence of residual strain at zero pressure. It is not, however, entirely consistent with the fact that each isotherm point appears to represent a fairly good metastable equilibrium. If one extrapolates the residual adsorption rate at "equilibrium," one would not reach the desorption point corresponding to the same relative pressure even after a time equal to the entire period of the experiment. The observed large hysteresis loop requires a dissipative process whose retardation time is comparable to the equilibrium time, but if this were in fact the case, we should not expect to find such a good "equilibrium."

The difficulty can be resolved by considering a somewhat different model in which the dissipation is a result of the swelling of the fiber but is not directly coupled with it. The clue to the situation is found in the fact that the monolayer point on desorption contains twice as many ammonia molecules as the monolayer point on adsorption; thus there are more active sites on desorption than on adsorption. The swelling of the fiber that accompanies the sorption of ammonia releases new sorption sites of low energy, a sort of autocatalytic process. The relaxation is then the slow shift of molecules from higher energy sites occupied at lower pressure to lower energy sites uncovered at higher pressure. Of course, much of the change in energy can occur with little if any motion of the sorbed molecules. This reshuffling of the sites can proceed simultaneously with new adsorption or desorption, and thus the difficulty of the good apparent equilibrium is avoided.

This model is similar to one discussed by White and Eyring.[16] It might at first seem to be a Maxwellian model, but it is a doubly degenerate one, corresponding to an isolated spring and isolated dashpot. It therefore cannot be distinguished in its effects from the Voigt model previously described, and it leads to no permanent deformation. It is likely that both processes contribute to the hysteresis, with the effect of the White–Eyring process predominating.

The area of the hysteresis loop is a measure of the frictional dissipation of irreversible heat. The area of the complete $-63°$ loop is 13.8 cal. per gram of fibroin. Since the average residue weight is 77, this corresponds to 1063 cal. per peptide linkage.

**Derived Thermodynamic Quantities.**—Although the laws of thermodynamics which will be used here apply strictly only under equilibrium condi-

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[14] G. King, *Trans. Faraday Soc.*, **41**, 479 (1945).

[15] W. W. Barkas, *ibid.*, **42B**, 137 (1946); A. B. D. Cassie, *ibid.*, **41**, 458 (1945).

[16] H. J. White and H. Eyring, *Textile Res. J.*, **17**, 523 (1947).

tions and no strict thermodynamic significance can be ascribed to the apparent thermodynamic values obtained for the irreversible sorption data, it is interesting to see the nature of the apparent heat and entropy curves obtained when thermodynamics is applied.

The apparent "heats of sorption" are average heats over the range −77 to −63° for the process

$$n\mathrm{NH_3}\ (\text{liquid}) + \text{protein} \rightarrow \text{protein}\cdot n\mathrm{NH_3}$$

The apparent (net) heats of this process were obtained from the relation

$$\Delta H = \frac{RT_2T_1}{T_2 - T_1} \ln \frac{a_1}{a_2}$$

where $a_2$ is the relative pressure at the upper temperature $T_2$ necessary to produce the same amount of sorption as at the relative pressure $a_1$, of the lower temperature $T_1$.

These apparent average (net) "heats of sorption" may be the sum of three quantities: the heat of sorption on certain sites, the heat of swelling of the fibroin, and an ill defined heat of rearrangement. This last term arises from the fact that the "identity" of the sites occupied by the ammonia may not be the same at the two different temperatures even if the number of sites occupied is the same.

The results of the thermodynamic calculations are shown in Figs. 4 and 5. A noteworthy feature of the apparent "heat" curves in Fig. 4 is the definite leveling of the curve at the apparent monolayer point (which for adsorption coincides with the limit of validity of the B.E.T. plot). This break in the heat curve may simply be the point at which the fall in the heat of adsorption with coverage becomes equal to the rise in the heat of swelling with increasing coverage. In the absence of data on the elastic constants of the swelled fibroin, further analysis of the thermodynamic data must be postponed.

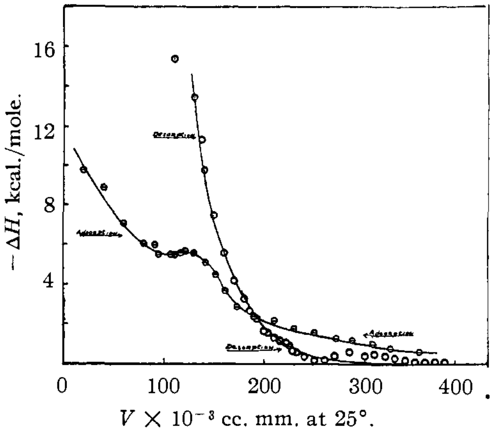

Fig. 4.—Plot of apparent (net) "heat of sorption" *vs.* volume adsorbed per gram of silk computed from the Clausius–Clapeyron equation over the range −77 to −63°.

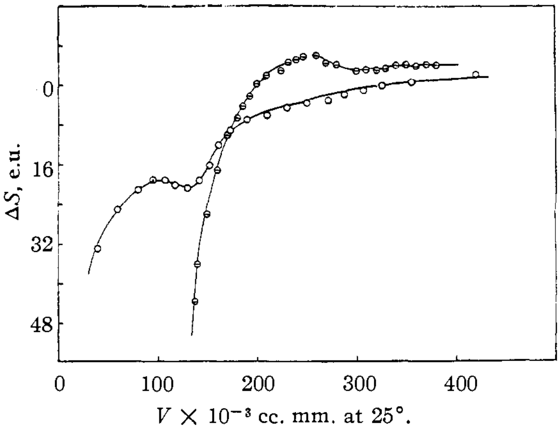

Fig. 5.—Apparent differential standard "Entropy of Sorption," ○ = adsorption at −63°; ⊖ = desorption at −63°.

**Acknowledgments.**—We wish to thank the Research Corporation for a Frederick Gardner Cottrell Grant used for the purchase of equipment. We are indebted to Dr. Milton Harris for a gift of silk and to Dr. Hugh M. Hulburt and Dr. Barbara Van Tassel for valuable discussions and comments.

WASHINGTON, D. C.