## 590. *On Detergent–Protein Interactions.*

By E. D. Goddard and B. A. Pethica.

Calorimetric measurements of the heat of interaction of sodium dodecyl sulphate and bovine serum albumin have been carried out on both sides of the isoelectric point. The results have been interpreted in terms of previously reported stoicheiometric complexes. The heat data are compared with the theoretical calculations of the heats of binding.

In many previous publications from this laboratory (Doty and Schulman; Matalon and Schulman, "Lipo-Proteins," *Discuss. Faraday Soc.*, 1949, **6**, 21, 27; Elkes, Frazer, Schulman, and Stewart, *Proc. Roy. Soc.*, 1945, *A*, **184**, 102) it has been shown that under suitable conditions soluble surface-active molecules penetrate an insoluble monolayer, causing large changes in its surface properties. In order to investigate the energetics of this type of association, it was decided to measure in bulk phase the heats of interaction between molecules similar to those studied by the penetration technique. The results obtained will be published later in full, but

one of interest will be quoted here: on the acid side of the isoelectric point, serum albumin very readily penetrates the negatively charged monolayer, sodium behenyl sulphate, but on the alkaline side only very slightly. This was to be expected on the basis of inter-ionic forces of attraction and repulsion. However, it is known that serum albumin and sodium dodecyl sulphate interact in solution on the alkaline side of the isoelectric point, giving soluble complexes. Therefore attention was turned to a calorimetric investigation of this association, as well as of the precipitation on the acid side.

In recent years, a large number of investigations on the interactions of proteins and anions have been published (Murray Luck, *Discuss. Faraday Soc.*, 1949, **6**, 44). The interactions have been studied by ultra-filtration, dialysis equilibrium, electrophoresis, and absorption-spectrum techniques. An approach to the evaluation of the energetics of these interactions was first made by Klotz (*Arch. Biochem.*, 1946, **9**, 109) on the basis of the law of mass action, and modified so as to allow for the electrostatic contribution to the free energy of binding of successive anions to the protein (Scatchard, Scheinberg, and Armstrong, *J. Amer. Chem. Soc.*, 1950, **72**, 535, 540; Klotz, Walker, and Pivan, *ibid.*, 1946, **68**, 1486). A further modification to the simple theory was put forward to allow for the heterogeneity of reaction sites on the protein, a Gaussian distribution of the intrinsic binding constants being proposed for the combination of serum albumin and alkyl sulphates (Karush and Sonenberg, *ibid.*, 1949, **71**, 1369). Karush (*ibid.*, 1950, **72**, 2705), however, showed that two simple types of adsorption site could account for the interaction of an azo-dye with albumin. On the basis of these various interpretations of the protein–anion equilibrium, a number of values of the heats, free energies, and entropies of the binding processes have been given. No direct measurements of the heats of these interactions have been made. It is the purpose of this communication to provide data on one such interaction, *viz.*, that between bovine serum albumin and sodium dodecyl sulphate, which has been extensively studied by other methods (Putnam, *Adv. Protein Chem.*, 1948, **4**, 79).

## Experimental.

The calorimeter (Fig. 1) consists of a silvered Dewar vessel provided with an observation window, and closed by a well-fitting thick cork. The cork carries a glass stirrer (*A*) driven by a governor-controlled induction motor at 60 r.p.m. The protein solutions were placed in the vessel, and the detergent solutions in a partly immersed bulb (*B*) firmly held in position by a collar (*C*). *D* and *E* represent thermistors and heater respectively; *F* is the breaker support (B.14 joints). The vessel was placed in a water thermostat at 25°. Mixing was effected by breaking the bulb (*B*). Several breaking techniques were tested, and the following practical points emerged: (i) no alteration in liquid levels must occur, and the meniscus inside and outside the bulb should coincide, and be at the wide part of the bulb; (ii) the top of the bulb should be thick, in order to prevent cracking of the glass above the liquid; (iii) the breaker is best operated from within the bulb (*B*), and its level should remain constant; (iv) the re-entrant gossamer area of the bulb is best set opposite the stirrer for rapid mixing on breaking; (v) the thermistors should be set as far as possible from the stirrer and bulb in order to avoid variable eddy currents.

Two glass-protected thermistors (type F, Standard Telephones and Cables Ltd.; Sillars, *J. Sci. Instr.*, 1942, **19**, 81) were used, of total resistance 3700 Ω at 24°. The thermistors were one arm of a Wheatstone bridge, which had arms in the ratio 10 : 4. With this ratio, the variation of resistance of the thermistors was read on the balance arm as 370 Ω/°c. at 24°. This could be read to 0·1 Ω, giving a sensitivity of the instrument of $2{\cdot}7 \times 10^{-4}$°.

The materials used were crystallised bovine serum albumin (Armour), and pure sodium dodecyl sulphate (Verley). The high purity of the sulphate was confirmed by observing that the surface tension–concentration curve showed no minimum. The buffers used were: (*a*) pH 6·8: 0·025M-$Na_2HPO_4$, 0·025M-$NaH_2PO_4$, 0·1M-NaCl; and (*b*) pH 4·3: 0·1M-$CH_3{\cdot}CO_2Na$, 0·2M-$CH_3{\cdot}CO_2H$. 110 Ml. of protein solution and 20 ml. of detergent solution were used in each experiment. At pH 6·8, 1·0 g. of the protein was used, and at pH 4·3, 0·5 g. After assembly, the system was left for 3—4 hours at 23·5°, to reach thermal equilibrium. Resistance readings were then taken until a steady drift with time was observed for 15—30 minutes, then the reagents were mixed. The new drift was measured until steady, whereupon the calibrating current was passed. The energy generated by the heating coil was measured by means of a standard ammeter and voltmeter. A typical run is represented in Fig. 2.

## Results and discussion.

The results of the heat determinations are shown in Fig. 3 for pH 4·3 and 6·8. The heats of reaction are small, and the temperature measurements are subject to considerable error at the lower end of the scale. The results are all subject to a possible error of $\pm 0{\cdot}05$ cal. per g. of protein, which represents the sensitivity of the instrument. The reliability of the bulb-breaking technique was tested in a series of eight blank runs. Six showed no temperature change on breaking and two a temperature change corresponding to a negative heat (expressed as cals./g. of protein) of 0·18 and 0·20 cal. It appears then, that there is an occasional systematic error

in one direction, and it is therefore possible that individual determinations are low by approximately 0·20 cal. This is of importance mainly at the lower detergent : protein ratios. In view of the very small temperature changes involved, we feel that these results are worthy of record, and would be difficult to improve.

Examination of the results at pH 6·8 shows a double inflection at detergent : protein ratios of 0·23 and 0·45. These inflections correspond to the electrophoretically homogeneous complexes between sodium dodecyl sulphate and serum albumin reported by Putnam and Neurath (*J. Biol. Chem.*, 1945, **159**, 195) and denoted as $AD_n$ and $AD_{2n}$, $n$ being approximately 55 in terms of molecules of sodium dodecyl sulphate per albumin molecule (a molecular weight of 70,000 being assumed for albumin). Diffusion experiments on these two complexes gave values of molecular weights which approximated to those expected on the assumption that all the detergent was bound to the protein (*idem, ibid.*, 1945, **160**, 397). These workers found that sodium dodecyl sulphate in excess of the $AD_{2n}$ was bound, but that these higher complexes dissociated during electrophoresis. It is generally assumed that the detergent is bound to the

FIG. 1.

*The calorimeter.*

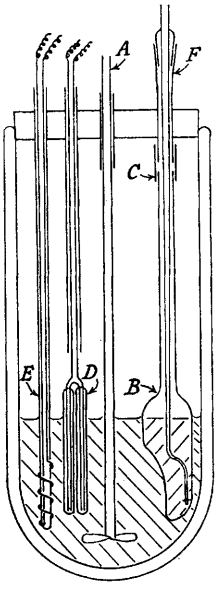
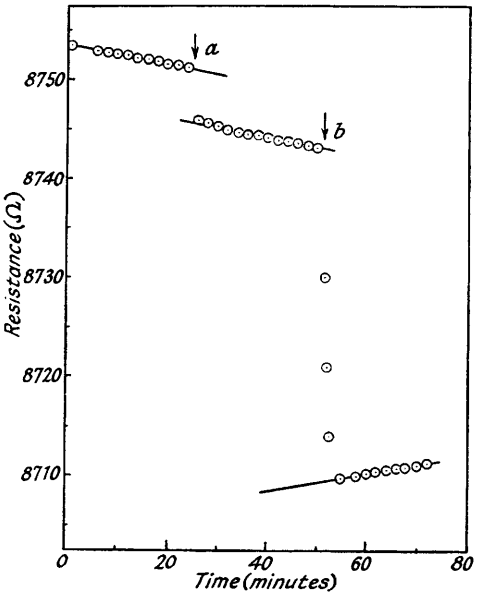



FIG. 2.




*Resistance–time readings of a typical run. Reagents mixed at* (a), *and heater switched on for* 2 *minutes at* (b). *Reaction of* 1·00 *g. of serum albumin and* 1·00 *g. of sodium dodecyl sulphate at* pH 6·8.

cationic groups of the protein. Several analyses have given the number of these cationic groups as 100—110 per serum albumin molecule (Tanford, *J. Amer. Chem. Soc.*, 1950, **72**, 441; Stein and Moore, *J. Biol. Chem.*, 1949, **178**, 79; Brand, *Ann. N.Y. Acad. Sci.*, 1946, **47**, 187). Lundgren (*Adv. Protein Chem.*, 1949, **5**, 305) found that extraction of egg albumin–dodecyl benzenesulphonate mixtures with 60% acetone removed detergent in excess of that bound to the cationic groups of the protein. The remainder could only be removed by addition of inorganic salt to the mixture. The experiments reported here at pH 6·8 support these explanations of the albumin–sodium dodecyl sulphate reaction, the heats of reaction falling into three groups : (*a*) a linear portion up to the complete formation of $AD_n$, with an enthalpy change on formation of $AD_n$ from protein and detergent under these conditions of −1250 cals./mole of sodium dodecyl sulphate; (*b*) a " plateau " corresponding to the reaction $AD_n + nD = AD_{2n}$, $-\Delta H = 230$ cals./mole of sodium dodecyl sulphate; (*c*) a further rise, corresponding to the further binding of detergent, probably reaching a maximum around 1·5 g. of detergent per g. of protein. The initial part of the third stage gives a heat of binding ($-\Delta H$) of 960 cals./mole of sodium dodecyl sulphate. From viscosity data (Neurath and

Putnam, *J. Biol. Chem.*, 1945, **160**, 397; Putnam and Neurath, *J. Amer. Chem. Soc.*, 1944, **66**, 1992), it is concluded that irreversible denaturation occurs on binding of the sodium dodecyl sulphate beyond the $AD_n$ stage (see also Lundgren and O'Connell, *Ind. Eng. Chem.*, 1944, **36**, 370).

The results of the investigation of the range of precipitation of serum albumin with sodium dodecyl sulphate (Putnam and Neurath, *J. Amer. Chem. Soc.*, 1944, **66**, 692) show that maximum precipitation occurs in the range from $AD_n$ to $AD_{2n}$ at acid pH. The re-dissolution of the initially formed precipitate in the region of detergent excess can be explained in terms of the building up of a second detergent layer on the protein, with the polar groups directed outwards (Pankhurst, " Surface Chemistry," Butterworth, London, 1949, p. 109).

The interpretation of the heat data at pH 4·3 is difficult, since heats of precipitation are involved over the range of detergent : protein ratios from 0·1 to 0·5, and the heats of solution of the complexes $AD_n$ and $AD_{2n}$ are unknown. Several important results emerge. First, that

FIG. 3.

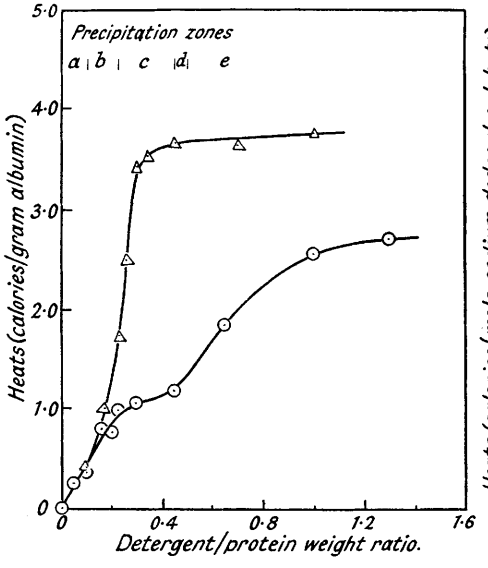



FIG. 4.

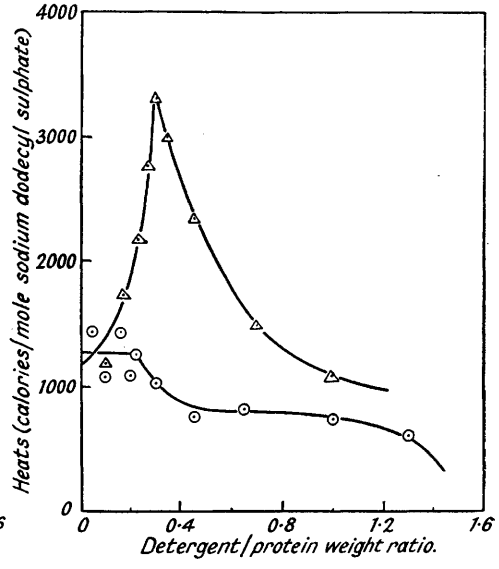



FIG. 3.

*The heats of interaction of serum albumin and sodium dodecyl sulphate.* ⊙ *At* pH 6·8; △ *at* pH 4·3. *The heats are given as* $-\Delta H$, *in cals. per g. of albumin. The precipitation zones at* pH 4·3 *are taken from Putnam and Neurath's data.* (a) *and* (e), *no precipitation*; (b) *and* (d), *partial precipitation*; (c), *complete precipitation.*

FIG. 4.

*The heats of interaction of serum albumin and sodium dodecyl sulphate.* △ *At* pH 6·8. ▲ *At* pH 4·3. *The heats are given as* $-\Delta H$ *in cals. per mole, of sodium dodecyl sulphate.*

where precipitation is small (up to a ratio of 0·1), the heat of reaction is independent of pH from pH 4·3 to pH 6·8. It would appear from Fig. 3 that the heat of interaction at pH 4·3 in region (*e*) is compensated by the approximately equal heat of solution of the precipitate, and the overall heat measured in this range remains constant. In Fig. 4 the results are plotted as overall heats of reaction per mole of sodium dodecyl sulphate versus the detergent : albumin weight ratio. At pH 6·8, this method of plotting confirms the three stages of the interaction. At pH 4·3 a sharp maximum occurs at a ratio of 0·3 in the range of maximum precipitation of the protein, in which denaturation occurs.

Estimations of the heat of binding to serum albumin have been made for a number of anions on the alkaline side of the isolectric point. Klotz and Urquhart (*J. Amer. Chem. Soc.*, 1949, **71**, 847) give $\Delta H$ −2100 cals./mole for methyl-orange, and −2000 for azosulphathiazole. Scatchard, Scheinberg, and Armstrong give a positive $\Delta H$ of 430 ± 540 cals./mole for the chloride ion (*ibid.*, 1950, **72**, 535), and a zero heat for the thiocyanate ion (*ibid.*, p. 540). Karush and Sonenberg (*ibid.*, 1949, **71**, 1369) found for sodium decyl sulphate that the heat of binding

($-\Delta H$) is 2000 cals./mole. They report the surprising result that the heats of binding of sodium octyl and dodecyl sulphates are zero. Our direct measurements give $-\Delta H$ 1250 cals./mole for dodecyl sulphate over the range studied by Karush and Sonenberg. A possible source of the discrepancy is that in Karush and Sonenberg's work the long-chain sulphates were always below the micelle points. In ours the detergent was always above the micelle point before the reaction in the $AD_n$ range and below it after the mixing. A correction should therefore be made for the heat of formation of the sulphate micelles. A number of tests were made to measure the heat of formation of sodium dodecyl sulphate micelles, by dilution of a micellar solution of the salt in the calorimeter. The number of micelles destroyed in the dilution process could be calculated from a knowledge of the critical micelle point (Corrin and Harkins, *ibid.*, 1947, **69**, 687). A mean result of our experiments gave a heat of formation of the micelles from free molecules ($-\Delta H$) as 250 cals./mole of sodium dodecyl sulphate, but this could not be statistically distinguished from zero. Data were collected by Stainsby and Alexander (*Trans. Faraday Soc.*, 1950, **46**, 587) on the temperature dependence of critical micelle points of a number of soaps. From these a value of $\Delta H = -250$ cals./mole of sodium dodecyl sulphate was calculated for the formation of micelles at 24°. Correction of the heat of formation of $AD_n$ for the destruction of detergent micelles in our experiments would, in any case, change the value previously quoted to $\Delta H = -1500$ cals./mole of sulphate, thereby increasing the divergence of these results from those of Karush and Sonenberg. We conclude, therefore, that it is difficult to reconcile Karush and Sonenberg's calculations with our direct measurements.

Since the free energy of binding of sodium dodecyl sulphate to serum albumin is large ($-\Delta G = 7$—$10$ kcals. for the first detergent molecule bound; Murray Luck, *loc. cit.*; Karush and Sonenberg, *loc. cit.*), the binding of the detergent is accompanied by a large increase in entropy, *viz.*, *ca.* 18—28 e.u. per mole of dodecyl sulphate for the first molecule bound. This entropy increase is usually ascribed either to disorientation in the protein or to loss of water of solution on the formation of the ionic detergent–protein bonds. A critical re-evaluation of the thermodynamic treatment of protein–ion equilibria seems desirable in view of the results presented here.

We are indebted to Dr. J. H. Schulman, O.B.E., for helpful discussions and advice, and to the Elsie Ballot Trust for a scholarship to one of us (E. D. G.).

Department of Colloid Science, Cambridge.
Department of Pharmacology, The Medical School,
Hospitals Centre, Birmingham, 15.

[*Received, May 18th*, 1951.]