blue-stained protein bands. This also applied to the zones of the protein fractions separated in cellulose acetate. Esterase bands 7, 13, 15 and 16 were located in the prealbumin fraction, while Bands 10, 11 and 12 mostly in the α_1-globulin fraction, and Bands 2, 3, 4, 5 and 8 in the α_2-globulin fraction. Band 6 and, partly, Bands 2, 4 and 8 were in the β-globulin fraction and Band 1 mainly in the γ-globulin fraction.

In a concentration of $10^{-3}M$, which was found to be effective, eserine inhibited the activity in Bands 2, 3, 4, 5, 10, 11 and 12; with lower concentrations of eserine Bands 3 and 12 were only partially inhibited. In a concentration of $10^{-4}M$, iso-OMPA inhibited Bands 2, 3 and 11, presumably non-specific cholinesterases. In the concentration of $10^{-5}M$ only the band 11 was sensitive to the inhibitor. Even in the highest concentrations used ($10^{-3}M$) 284 C 51 failed to cause any inhibition of the esterase bands; this suggests that acetylcholinesterase is entirely absent from the rat serum.

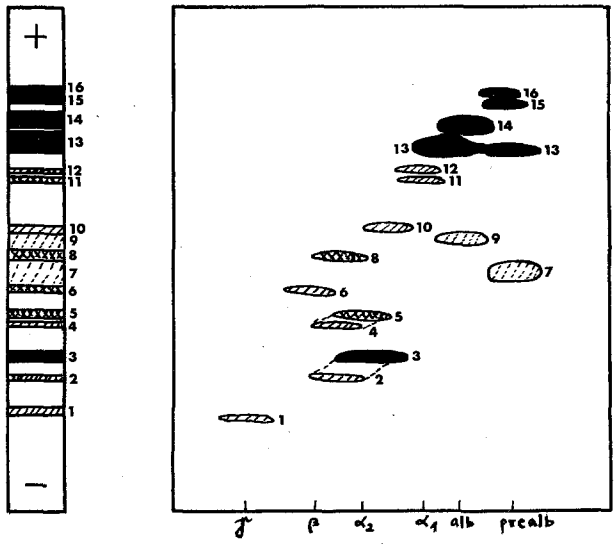

Fig. 3. Diagrammatic presentation of the esterase bands found. The same numbering is used in the text. The corresponding serum protein fractions are marked at the bottom of the two-dimensional separation.

With the two-dimensional step-gradient acrylamide electrophoresis, it was possible to correlate the individual serum proteins and esterase to the α-, β- and γ-globulin bands in the same way as was previously established by SMITHIES[12], using the less discriminating paper and starch system. One-dimensional gel electrophoresis gave very limited information of the distribution of esterases in the classical serum protein fractions. The observation that the esterase bands did not always correspond to the areas of stainable protein is in agreement with earlier observations by HUNTER et al.[7] on separations with paper and starch.

Zusammenfassung. Isoenzyme von Serumesterasen wurden zuerst mit Zelluloseazetat und dann mit einem Gradientgel von Polyakrylamid mit zweidimensionaler Elektrophorese bei der Ratte untersucht. Ein neues Band, das eine starke Albuminesteraseaktivität aufweist und unempfindlich gegen $10^{-3}M$ Physostigmin ist, wurde somit gefunden. Von 17 Esterasebändern waren 7 empfindlich auf Physostigmin, und 3 wurden auch durch Iso-OMPA gehemmt, während 284-C-51 gar keinen Effekt zeigte.

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Mercury Binding to Human Haemoglobin

Mercury solutions, both as inorganic mercury salts and as organic mercury complexes, have found wide application in the estimation of sulphydryl groups in proteins[1–6]. These methods generally use either an amperometric back-titration of mercury after reaction with the -SH groups or a spectrophotometric determination. For these systems the organic mercury derivatives, such as '*p*-mercuribenzoate' have proved more successful then inorganic mercury which suffers from the disadvantage of its divalency since it can react with either one -SH group to form RSHgX or two to form $(RS)_2Hg$[1,2]. This has lead to conflict concerning the number of available sulphydryl groups present.

The degree of denaturation of the protein, especially with haemoglobin, markedly affects the estimated number of thiol groups. Strict adherence to the control of pH and composition is essential and even ammonia based buffers are reported to yield erroneous results due to the formation of metallic ammines[6]. However, the introduction of *tris* buffers has substantially improved the confidence placed in experimental results[7].

The work described here offers an alternative approach to the study of haemoglobin sulphydryl groups and their reaction with mercury using a technique analogous to that previously reported in connection with the interaction of lead with haemoglobin[8].

Method. Whole blood was collected by venepuncture, the cells separated and washed 3 times with physiological saline. The erythrocytes were then haemolysed by repeated freezing and thawing and the stroma removed by centrifugation[9]. The supernatant was removed, diluted $\times 2$ with *tris*-maleic acid buffer, pH 7.0 and samples taken for

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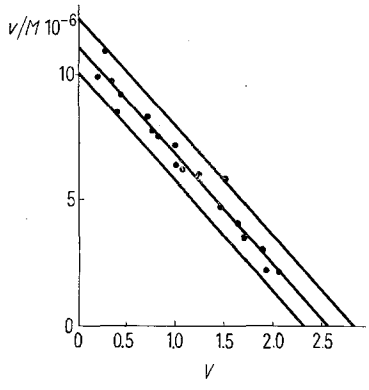
the determination of haemoglobin[10] and total protein[11].

Mercury solutions of concentrations varying from 5 to 500 ppm were prepared from AR grade mercuric chloride dissolved in *tris*-maleic acid buffer pH 7.0. These solutions were labelled by the addition of Hg-203 solution to give a specific activity of 10 μCi/μg Hg. Aliquots of the haemoglobin and mercury solutions were mixed in sealed dialysis tubing bags (Visking) and centrifuged for 2 h at 2,500 *g* in a temperature controlled centrifuge at 25 °C[8].

Initial studies, omitting haemoglobin, showed marked binding of mercury to the bag. Preliminary treatment of the bags with successive washes of 0.1 *N* HCl 0.1 *N* sodium hydroxide solution and finally several changes of boiling distilled water was necessary to reduce the mercury binding to an acceptable and consistent level.

After centrifugation, aliquots of the ultrafiltrate and the residual haemolysate were obtained and the mercury content determined by measurement of the γ-emission of Hg-203 using a Hewlett-Packard auto-γ-spectrometer. The mercury bound to the haemoglobin was determined by difference.

Findings. The results were subjected to analysis by the method of SCATCHARD[12] and are given in the Figure. The intercept on the abscissa indicates that the haemoglobin molecule has a mean value of 2.5 sites available for mercury binding. From the intercept on the ordinate the uncorrected association constant (K_u) for the mercury haemoglobin complex was calculated and gave a value for log K_u = 6.65. Iodoacetate ($10^{-2}M$) blocked the binding of mercury to the haemoglobin molecule thus confirming the addition of the metal to the available -SH groups.



Plot and regression line for v/M against v, where v denotes moles of bound mercury per total moles of haemoglobin and M is the molar concentration of free metal (after SCATCHARD[12]). The 95% confidence limits are included.

The stability constant needs to be corrected for reduction in concentration of free metal by the competing *tris* and chloride complexes[13,14]. The corrected value is such that log K = 19.6. This value agrees well with that obtained by other workers[14].

Discussion. The work described offers an alternative approach to the study of meal binding in biological systems. It has the advantage of simplicity and rapidity compared with the dialysis technique and does not suffer from the problem of electrode contamination experienced in polarography. The method can be adapted to the study of other proteins and metals and although a radioactive tracer determination was involved in this study, conventional spectrophotometric or atomic absorption analysis could also be used.

The mean value obtained for the number of mercury atoms combining with the haemoglobin molecule is in good agreement with those obtained by other workers[1,3,15,16] and appears to confirm the observation[2] that reaction of the native, as opposed to the denatured, protein represents an equilibrium involving all the sulphydryl groups present. The linearity of the plot in the Figure indicates that the binding sites are all equivalent and equally available for mercury.

Zusammenfassung. Mittels ^{203}Hg als Hg-Chlorid wurden die freien SH-Gruppen des Hämoglobins in einem stromafreien Hämolysat titriert und festgestellt, dass die durchschnittliche Zahl freier, reaktiver SH-Gruppen pro Molekül 2,5 betrug.

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A Technique for Establishing Parabiosis between Houseflies (*Musca domestica* L.)

Exchange of 'milieu interne' of living animals by the technique of parabiosis affords unique opportunities for varied experimentation. WIGGLESWORTH's[1] and WILLIAM's[2] work with *Rhodnius* and *Platysamia* respectively, using this technique, are classics in the annals of entomological research. BEAMENT[3] detected blood-borne paralyzing agents in stress-prostrated American roaches by using this technique. COLHOUN[4] has also used this approach for studying insecticidal action.

In the course of an investigation[5] of the nature of resistance mechanisms against the insecticide dieldrin, an attempt was made to effect a parabiotic link between houseflies, *Musca domestica* L. Experiments conducted for establishing successful parabiosis between flies are related below.

The animal used as the 'primary' was deprived of its wings and its mesothoracic tergum was clipped neatly so as to expose the dorsal longitudinal muscles lying underneath (Figure A). A small drop of physiological saline (ASPERN and VON ESCH[6]) was deposited in the incision to keep it moist. The animal was then immobilized on a block of wax using staples (Figure B). The fly used as

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