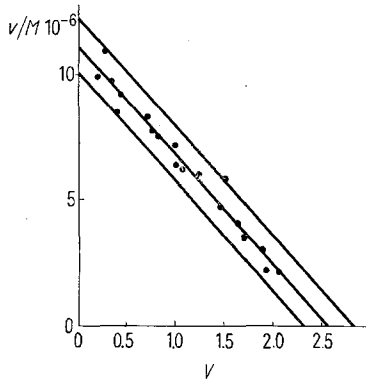
the determination of haemoglobin[10] and total protein[11].

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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[17] Lead-203 was kindly supplied by the M. R. C. Cyclotron Unit, Hammersmith, London. D. B. is a Wellcome Senior Research Fellow in Clinical Science.

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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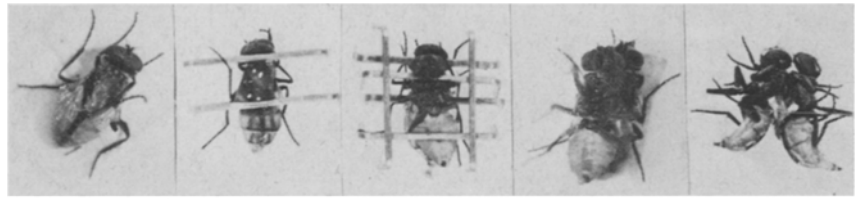

Steps in the establishment of parabiosis between houseflies. A) Fly with incised mesothoracic tergum. B) 'Primary' immobilized by staples. C) 'Primary' and 'Secondary' with their thoracic openings in close apposition, held firm and immobile by a grid of staples. D) Top view of a parabiotic pair. E) Side view of a parabiotic pair.

Duration of survival of parabiotic pairs of different strains of houseflies

Experiment No.	Flies (parabionts)		No. pairs	Pairs surviving/time after union (h)				
	1	2		1	4	8	12	24
1	R[a]	R	22	22	22	22	22	18
2	S[b]	S	10	10	10	10	10	7
3	R	S	15	15	15	15	15	11

[a] Resistant (U2) strain. [b] Susceptible (WHO/SRS/*Musca domestica*/1) strain.

the 'secondary' was similarly incised and placed on the immobilized primary in such a manner that the incisions of the 2 animals were opposed to each other. Once the flies had been thus placed back to back, more staples were gently pressed on the venter of the secondary, en compassing the 2 animals in a grid of staples (Figure C).

Upon the removal of the staples after about 30 min, the 2 individuals remained attached together (Figure D and E) and were not separated inspite of hectic movements of the flies. An examination of these individuals under the binocular microscope showed that they were firmly held together and no part of the incision in either was visible. No exudation of tissue or fluid could be detected in any of the several pairs examined. Manual separation of the pairs under the binocular microscope revealed that the dorsal muscles of the 2 partners had become firmly wedged to each other. This contiguity between the 2 individuals was so firm in some cases that separation caused the dislocation of a block of muscle strands of one or both individuals. Exposed dorsal muscles of a recently separated pair were freely bathed in haemolymph showing that actual parabiosis had been established – mere mechanical adhesion being ruled out as incised flies permitted free exposure to air developed a dark brown, fairly brittle scar tissue.

Establishment of parabiosis was further confirmed by demonstrating the actual exchange of haemolymph between the fly parabionts. For this, flies were paired as described and a union of 1 h allowed. One of the partners of each pair was injected with 1 µl of 0.1% Bromophenol blue solution in Insect Ringer through its pleuron. The flies were permitted to resume activity while still paired and examinations of both the injected and the corresponding uninjected partner were made at different time intervals after injection. Presence of the dye in the muscles and haemolymph could be made out as early as 5 min after the injection. By the 15th min after injection, the dye was visible in the alimentary canal of both the injected and the uninjected parabionts. After 20 min, the distribution of the injected dye in the 2 animals was identical, leaving no doubt about the parabiotic nature of the link.

Once parabiosis had been established by these methods, the success of such operations was determined by keeping the parabiotic pairs on sucrose and water under constant observation and noting their mortality or survival at different time intervals after the operation. All those individuals which were injured during operation or which got apart as a result of their own movements within 1 h of the operation, were discarded. Pairs which did not fall apart within 1 h after the parabiotic operation would not separate from each other later. These were regarded as the successful parabionts.

Observations of such operated individuals belonging to 2 different strains of flies are given in the Table. It was noted that all successful parabionts were viable for at least 12 h after the operation, whereafter some of them died. Even so, nearly 70% of the parabionts could readily survive up to 24 h after the parabiotic operation.

These observations indicated that the technique of parabiosis described above could be most profitably employed for suitably designed short term in vivo experiments. Thus, the technique was found quite suitable for examining the production or otherwise of neurotoxins other than the insecticide in dieldrin-treated flies by using two strains showing a gross difference in sensitivity to the toxicant in question. The results of this study will be published in detail elsewhere later.

Zusammenfassung. Es wird eine neue, sehr einfache Methode zur Erzeugung parabiotischer, 12–24 h lang lebensfähiger Fliegenpaare (*Musca domestica* L.) beschrieben.

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[7] The author wishes to thank Prof. K. N. SAXENA for encouragement.

Corrigendum

J. HANNERZ: *Discharge Properties of Motor Units in Man*, Experientia *29*, 45 (1973). At the top of the Figure 1 the time bar is too long, so that the frequencies of the motor units seems to be twice as high as these given in the text; the time bar (100 msec) should therefore be reduced at 4 mm.