handled, but no altering substance was added; it was used as control. All these red cell aliquots were successively resuspended in autologous plasma and stored at 4°C for 5 days, as described elsewhere[4]. Each sample was then divided into 6 separate aliquots of 5 ml: 3 of these were immediately centrifuged and the remaining 3 were incubated at 37°C for 3 h in a Dubnoff shaker moving at a rate of 50 oscillations/min. K^+ concentration was measured in the plasma of all the aliquots, before and after incubation of the blood, with a Beckman DU flame photometer. K^+ uptake by the cells was calculated by difference, according to the method of KAHN and ACHESON[5], determining the hematocrit value before and after incubation.

Results are summarized in the Table. It is seen that during the incubation both the control and treated red cells removed measurable quantities of K^+ from the plasma against a concentration gradient: hence by an active mechanism. This phenomenon was demonstrable in spite of the concomitant increase of hemolysis produced – especially in treated cell samples – by warming and shaking, which caused a passive shift of K^+ from the lysed cells to the plasma.

K^+ uptake by untreated and AET- or cysteine-treated red cells incubated at 37°C

Sample No.	K^+ uptake (mEq/l of cells ± S.D.[a])		
	Control	AET[b]	Cysteine[b]
1	+ 5.0 (± 0.08)	+ 4.8 (± 0.11)	+ 5.3 (± 0.03)
2	+ 6.7 (± 0.03)	+ 6.0 (± 0.07)	+ 5.6 (± 0.12)
3	+ 3.3 (± 0.08)	+ 5.2 (± 0.07)	+ 4.3 (± 0.23)
4	+ 5.2 (± 0.10)	+ 3.5 (± 0.00)	+ 3.0 (± 0.00)
5	+ 5.4 (± 0.12)	+ 2.5 (± 0.05)	+ 3.8 (± 0.00)
6	+ 3.4 (± 0.00)	+ 4.4 (± 0.11)	+ 7.0 (± 0.00)
7	+ 2.5 (± 0.04)	+ 2.3 (± 0.00)	+ 3.0 (± 0.03)

[a] S.D. = standard deviation of the mean of 3 determinations.
[b] Red cells treated with the altering substance.

The analysis of variance showed that the differences of K^+ uptake between the three groups (control and treated red cells) were not significant ($P > 0.2$).

The results of our experiments show that the enzymatic abnormalities occurring in normal erythrocytes treated with AET or cysteine do not impair the cation transport mechanism that acts at the cell surface. Apparently the two altering substances are not specific inhibitors of the membrane sulfhydryl groups, as are mercury, *n*-ethylmaleimide and *p*-mercuribenzoate, whose inhibitory effect on the cation transport mechanism of the red cell has been demonstrated[6–8].

Riassunto. È stato studiato il meccanismo di scambio cationico di membrana di emazie umane normali trattate con composti sulfidrilici (AET e cisteina). Si è osservato che, durante l'incubazione di 3 h a 37°C, effettuata dopo 5 giorni di soggiorno a 4°C, le emazie normali trattate con i suddetti composti rimuovono attivamente dal plasma normali quantitativi di K^+. I risultati vengono brevemente discussi.

G. D'AMICO, G. SIRCHIA,
S. FERRONE, and M. ROMAGNONI

Istituto di Clinica Medica dell'Università di Milano (Italy), June 21, 1965.

[4] G. D'AMICO and P. P. FOÀ, Exper. *15*, 144 (1959).
[5] J. B. KAHN JR. and G. H. ACHESON, J. Pharmacol. exp. Therap. *115*, 305 (1955).
[6] R. WEED, J. EBER, and A. ROTHSTEIN, J. gen. Physiol. *45*, 395 (1962).
[7] R. F. SHEETS and H. E. HAMILTON, J. lab. clin. Med. *52*, 138 (1958).
[8] H. S. JACOB and J. H. JANDL, J. clin. Invest. *41*, 779 (1962).

A Preliminary Study of the Immunoelectrophoretic Properties of Pregnant Mares Serum (PMS) Together with its Application to the Diagnosis of Pregnancy in the Mare

In this study the specificity of the reactions between pregnant mare serum (PMS) and a developed antisera have been examined by electrophoretic and immunoelectrophoretic techniques. The knowledge gained has been applied to determine the presence or otherwise of a pregnancy in a small group of 8 mares.

On clinical examination a skilled practitioner can usually diagnose pregnancy in the mare between the 38th and 44th day after coitus. ELMENDORFF, LORAINE, and WALLEY[1] and ANTONIADES[2], by the use of various biological pregnancy tests, were able to verify the presence of pregnancy at approximately 40 days, while WIDE and WIDE[3], who used a hemagglutination inhibition technique (HAI), were able to demonstrate that the diagnosis of pregnancy was possible using this technique. They did not, however, test the sera before the 44th day, hence their report is not helpful in determining how early an immunological test will detect the presence of chorionic gonadotrophin in the serum of the mare.

Materials and methods. Electrophoretic and immunoelectrophoretic studies: Agar-gel electrophoretic and immunoelectrophoretic studies were carried out using the techniques as described by MCCARTHY, PENNINGTON, and CRAWFORD[4].

Hemagglutination inhibition assay design: These were identical with those described by MCCARTHY et al.[4] using PMS for the control solutions.

Preparation of antisera: Rabbits were immunized with a solution containing PMS and Bentonite in a dose level

[1] H. V. ELMENDORFF, J. H. LORAINE, and J. K. WALLEY, J. Endocr. *25*, 107 (1962).
[2] H. U. ANTONIADES, *Hormones in Human Plasma* (J. and A. Churchill, London 1960).
[3] M. WIDE and L. WIDE, Nature *198*, 1017 (1963).
[4] C. MCCARTHY, G. W. PENNINGTON, and W. S. CRAWFORD, J. Obstet. Gynaec. Brit. Commw. *71*, 1 (1964).

of 500 IU PMS and 0.5 mg Bentonite for each injection. The immunizing courses of injections were carried out at twice weekly intervals over a period of three weeks. The antisera titres at the end of this period varied between 1/100 and 1/6400. It may be concluded that PMS absorbed on bentonite is reasonably antigenic in that high titres of antibody can be produced.

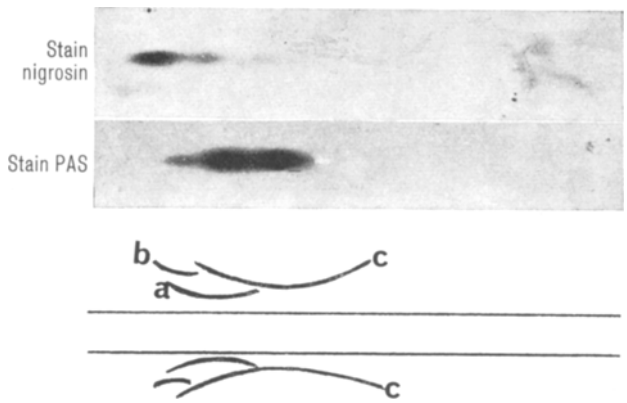

Fig. 1. Development of precipitation lines between PMS and antisera.

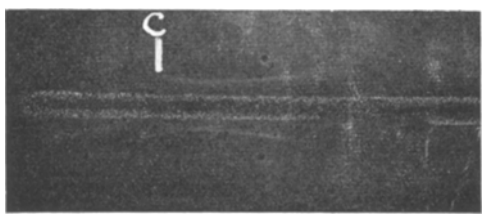

Fig. 2. Development of precipitation lines between PMS and antisera, following absorption of the antisera with normal horse serum.

Preparation of blood cells: The cells were prepared by the BUTT, CROOKE, and CUNNINGHAM[5] modification of LING'S[6] method. Pyruvic aldehyde was used to prepare the cell envelope, the cells being coated with PMS.

Collection of clinical specimens: Blood from 8 mares was withdrawn by intravenous puncture. The sera was separated by centrifugation. PMS was extracted by the method suggested by MISCHELL, WIDE, and GEMZELL[7]. In order to determine the presence of a pregnancy, specimens were collected at 3 day intervals from the 13th to the 54th day following coitus.

Results. It will be observed from Figure 1 that three precipitation lines could be developed between PMS and the antisera. Two of these lines (a and b) were similar to lines which could be developed between normal horse sera and the antisera. The sera was doubly diluted to compensate for the absorption which occurred with normal horse serum.

It can also be observed that line c is centred on the main glycoprotein fraction of PMS. Equine gonadotrophin is known to be a glycoprotein (ANTONIADES[2]).

When an equal volume of antisera was absorbed with an equal volume of normal horse serum, and incubated for 1 h at 37°C, the two most positively situated lines (a and b) disappeared. The remaining line (c) was therefore taken to be the antibody to equine gonadotrophin (Figure 2). The result of electrophoretic studies are shown in Figure 3. Staining reactions both with nigrosin (for protein) and periodic acid-Schiff (PAS) (for glycoprotein) have been undertaken. When nigrosin stain has been used, the horse serum can be seen to have separated into two positively migrating fractions albumin and α-globulin, and one negatively migrating fraction γ-globulin. Protein is also visible on both sides of the origin and is probably the β-fraction. The electrophoresed PMS shows

[5] W. R. BUTT, A. C. CROOKE, and F. J. CUNNINGHAM, Biochem. J. *81*, 596 (1961).
[6] N. R. LING, Biochem. J. *77*, 12 (1960).
[7] D. R. MISCHELL, L. WIDE, and C. A. GEMZELL, J. clin. Endocr. *23*, 125 (1963).

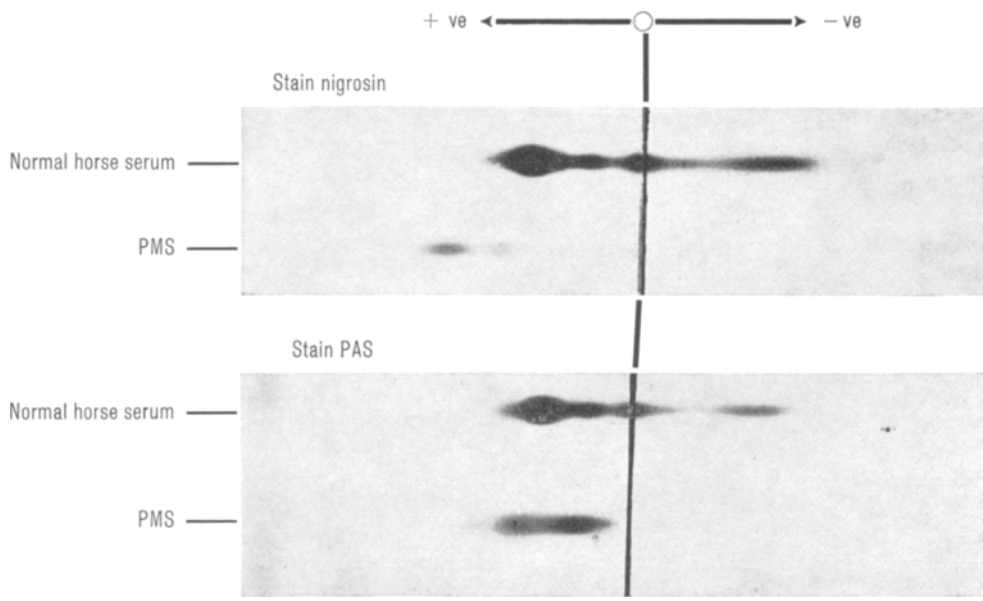

Fig. 3. Electrophoresis of normal non-pregnant horse serum and pregnant mares serum (PMS) stained for protein and glycoprotein.

two positively migrating fractions, one at the albumin position and one on the positive side of the albumin position.

When the electrophoresed pattern is stained with PAS it may be observed that the PMS has separated into 3 glycoprotein fractions. One on the positive side of the albumin position, one at the albumin position, and one between the albumin and the α-globulin position.

Clinical studies. Of the 8 mares originally selected for study 3 became pregnant. Using the HAI technique previously described, a positive result was recorded in all 3 at the 44th, 47th, and 53rd day following coitus.

Specimens of sera, taken from each mare were retested three days after the initial positive result. The result in each case remained positive.

Specimens of urine taken at this time were also positive, the concentration of gonadotrophin present being 7000, 21,000 and 29,000 IU/l.

No false positive results were recorded at any time in the five mares who were not pregnant.

Discussion. Antisera to PMS has been frequently produced by other workers (PIGEON, CLEGG, and COLE[8]; ZONDEK and SULMAN[9]; HAMBURGER, NIEMAN, and SORENSEN[10]). WIDE and WIDE[3] used Freunds adjuvant combined with PMS to raise antisera to the hormone. Theirs is the only other report to date on the use of such antisera for the diagnosis of pregnancy in the mare.

The disappearance of two of the precipitation lines (a and b) after absorption would indicate that the parent antigens of these two non-specific antibodies are present in normal horse sera. It is probable that more than one volume of normal horse serum is necessary to absorb each volume of antisera. Following these studies five volumes of normal horse sera were routinely used for each volume of antisera.

To date no reports of immunoelectrophoretic studies on PMS have been published. This study would indicate that the PMS used (Leo) contains two protein and three glycoprotein fractions. It is obvious that the use of the relatively impure preparations of PMS which are currently available will produce a number of extraneous antibodies. It is possible, however, that these can be absorbed by the use of non-pregnant mares serum. It was hoped that the diagnosis of pregnancy in the mare could be achieved using an HAI method at an earlier stage than the methods currently available. From the positive results achieved in this preliminary study it would appear unlikely that this will prove possible, and that the 40th day following coitus is the earliest time at which chorionic gonadotrophin can be detected in the serum of the pregnant mare[11].

Résumé. Une méthode pour la préparation d'un antisérum de haut titre contre le PMS est décrite. La spécificité a été établie par immunoélectrophorèse. On a mis au point une méthode à la HAI par laquelle le diagnostic de la grossesse peut être fait, chez la jument, entre le 42ème et le 54ème jour après le coït.

C. MCCARTHY and G. W. PENNINGTON

Mater Misericordiae Hospital, Dublin (Ireland) and the Sheffield and Region Endocrine Investigation Centre, Jessop Hospital for Women, Sheffield (England), July 12, 1965.

[8] H. PIGEON, M. T. CLEGG, and H. H. COLE, Acta endocr. (Kbh) *35*, 253 (1960).

[9] B. ZONDEK and F. SULMAN, Proc. Soc. exp. Biol. Med. *36*, 708, 9368 (1937).

[10] C. HAMBURGER, A. NIEMAN, and A. SORENSON, Acta endocr. (Kbh) *26*, 286 (1957).

[11] Acknowledgments: We would like to thank Dr. A. DARRAGH of Leo (Ireland) Ltd., for supplying the PMS. We must also express our gratitude to Capt. T. ROGERS of the Airlie Stud, Lucan, Co. Dublin, for his interest in this work and for the collection of the specimens used in this investigation.

Nuclear Apparatus and Binary Fission in *Spirostomum dharwarensis* n. sp.

Spirostomum dharwarensis n. sp. is a freshwater, moderate-sized ciliate about 600–800 μ long (Figure 1); it is named after the locality from which it was collected. The number of micronuclei is exactly seven. They are small bodies, each 2.0 μ in diameter. They are distributed along the macronuclear band. Sometimes one or two are found adhering to it. In vegetative phases both types of nuclei show a brilliantly positive Feulgen reaction, thereby revealing a considerable amount of DNA present in them.

Spirostomum dharwarensis has the following unique features: it has only seven micronuclei, the micronuclear division is of the synchronous type and the macronuclear division during binary fission is completed far in advance of the cytoplasmic division (Figure 2). In these features *S. dharwarensis* differs from all other species hitherto described under the genus *Spirostomum* (SESHACHAR and PADMAVATHI[1]). During binary fission, it is significant to observe that the macronucleus shows increasingly poor reaction to the Feulgen dye during its condensing process, while the reaction shown by the micronuclei to this dye is not so poor. It may be inferred from this that in *S. dharwarensis* during the nuclear division a considerable amount of DNA disappears from the macronucleus while the micronuclei lose very little of this genetic material.

SESHACHAR and PADMAVATHI[2] regard the synchronous and selective types of micronuclear divisions in ciliates as some biochemical mechanisms acting under the external or internal stimuli. WADDINGTON's[3] cytoplasmic chemodifferentiation hypothesis and HAMMERLING's[4] physiological gradients hypothesis represent other ex-

[1] B. R. SESHACHAR and P. B. PADMAVATHI, J. Protozool. *3*, 145 (1956).

[2] B. R. SESHACHAR and P. B. PADMAVATHI, Curr. Sci. *9*, 281 (1956).

[3] C. H. WADDINGTON, Symp. Soc. exp. Biol. *2*, 145 (1948).

[4] J. HAMMERLING, Arch. Entmech. Org. *131*, 1 (1934).