Table II. Comparison of the actual error due to haemolysis in IgG, IgM and IgA determinations with the calculated CARAWAY percentage error estimation for different values of plasma haemoglobin concentration

Haemoglobin (g/dl)	PCV	% Error of IgG	IgM	IgA	CARAWAY
0	39.5				
1	36.7	3.9	2.3	3.5	2.9
2	34.0	7.5	5.5	6.0	5.7
3	31.2	11.6	7.7	8.3	8.3
4	28.3	14.9	10.3	10.4	10.7
5	25.6	19.0	13.0	12.4	13.1
6	22.8	22.3	15.3	14.4	15.7
7	20.0	25.8	18.0	17.3	17.4
8	17.3	29.3	20.3	18.7	19.4
9	14.4	33.5	23.0	21.3	21.3
10	11.5	37.7	25.8	23.6	23.1

plasma immunoglobulin levels was found, indicating that trisodium citrate has no significant effect upon the single radial immunodiffusion method and that both serum and plasma immunoglobulin estimations give comparable results.

When a correction factor, derived from the ratio of haemolyzed to unhaemolyzed PCV was applied to the observed immunoglobulin value, a corrected immunoglobulin estimation was obtained which did not vary significantly in plasma throughout the whole range of possible haemolysis.

An expression based upon the relative intracellular and extracellular concentrations of a substance has been formulated by CARAWAY[11] which gives an indication of the percentage error due to haemolysis when measuring the concentration of that substance. The percentage error of extracellular fluid contamination using CARAWAY's formula is determined from the plasma haemoglobin concentration, and is given by:

$$\text{Percentage error} = \frac{3\,h\,(c/s - 1)}{1 + 0.03\,h}$$

where c = concentration of substance in red blood cell, s = concentration of substance in plasma, h = haemoglobin concentration in plasma (g/dl).

The estimation of plasma constituents using CARAWAY's formula gives calculated percentage errors which are in close agreement with actual percentage errors, determined experimentally for many plasma constituents[6], and extending the use of this formula to our study, it shows good agreement between calculated and actual percentage errors produced by haemolysis (Table II).

The results of the present work have a direct relevance to the determination of plasma and serum immunoglobulins in clinical diagnosis and research. Frequently blood specimens sent to a laboratory for analysis, arrive with varying degrees of haemolysis. It is not always possible to reject such specimens because of the clinical state of the subject especially in debilitated patients or neonatal infants. The extra error introduced into the results when using such haemolyzed samples should be acknowledged and wherever possible corrected. The present work indicates some of the limitations of measuring immunoglobulins in haemolyzed sera and provides a method for possible correction of such estimations.

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Native Resistance of *Peromyscus maniculatus* to *Nematospiroides dubius* Infection

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Summary. Because of its native resistance, *Peromyscus maniculatus* cannot be a natural host for *Nematospiroides dubius*.

Nematospiroides dubius, a trichostrongyloid parasite of mice, was first described by BAYLIS[2] when he isolated it as a parasite of the woodmouse, *Apodemus sylvaticus*. EHRENFORD[3] rediscovered and isolated this nematode from the deer mouse, *Peromyscus maniculatus* in 1954 and investigators have maintained *N. dubius* in laboratory mice since that time. Other reports of natural infections of *P. maniculatus* with *N. dubius* are in the literature[4,5]. However, BABERO and MATTHIAS[4] found only 4 adult worms in 3 infected *Peromyscus* mice. Indeed, FORRESTER[6] was unable to detect any *N. dubius* infections in 231 *P. maniculatus* mice that were examined. Also FORRESTER and McL. NEILSON[7] reported no success in attempts to establish patent infections of *N. dubius* in *P. maniculatus* with conventional methods or with 350 rads of γ-radiation to immunosuppress the mice prior to parasitic exposure.

Attempts to infect *P. maniculatus* with *N. dubius* utilizing both laboratory-reared and wild-trapped mice of varying ages were also unsuccessful in our laboratory even when larvae with enhanced virulence were used[8]. However we now report that infections of *N. dubius* can be established when *P. maniculatus* mice are immunologically suppressed with a steroid hormone.

Methods and materials. Peromyscus maniculatus mice were trapped in the area surrounding Fort Collins, Colorado. Young *Peromyscus* mice were obtained by breeding from the trapped *Peromyscus* stock in our own laboratory; outbreeding was carefully maintained. Mice were housed in disposable plastic mouse cages and bedding consisted

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of wood shavings. Purina laboratory chow and tap water were available to the mice ad libitum. Room temperature was constant at 22 °C and a 12-h photoperiod was maintained.

Nematospiroides dubius exposure was accomplished by stomach intubation of the desired number of larvae. Eggs of *N. dubius* were cultured to infective larvae from the fecal pellets of 'source' mice according to the method of CROSS and SCOTT[9].

Prednisolone acetate administered i.p. at a dose of 1 mg/day for 4 days prior to exposure with virulent *N. dubius* larvae was used to immunosuppress the mice. After exposure, prednisolone (1 mg) was administered every other day for 10 days to maintain immunosuppression.

Results and discussion. Fecal floats for eggs, using NaCl-specific gravity of 1.20, were positive on day 10 post-exposure and remained positive as long as steroid treatment continued (see Table). However, fecal floats became negative within 10 days after the termination of steroid treatment.

Infectivity of *N. dubuis* for steroid-treated and non-steroid-treated *P. maniculatus*

Group	No. of mice	Treatment	Results
12-weeks	5	Exposure with 200 infective larvae	No infection
12-Weeks	5	Exposure with 500 infective larvae	No infection
12-Weeks	4	Exposure with 800 infective larvae	No infection
8-Weeks[a]	10	Exposure with 200 infective larvae	No infection
8-Weeks[a]	10	Steroid treatment-exposure[b] with 200 infective larvae	Infection established range (120–180 worms recovered as adults)
12-Weeks[a]	10	Steroid treatment-exposure with 200 infective larvae	Infection established range (120–180 worms recovered as adults)

[a] Groups were repeated with the same results. [b] 1mg/day i.p. for 4 days prior to challenge, then on alternate days for a period of 10 days.

From this work and that of previously mentioned investigators, we conclude that *P. maniculatus* is not a natural host for *N. dubius*. Indeed, it is the only mouse strain of the many tested in our laboratory that demonstrated this state of increased resistance. In this regard, *P. maniculatus* very much resembles the rat, as infection in this rodent can only be established with the use of steroids[10]. The exact mechanism of steroid action is unknown; however the mouse is categorized as a steroid sensitive animal[11]. Also, a sharp decrease in circulating lymphocytes, half that of normal levels, was seen in *P. maniculatus* mice immunosuppressed by prednisolone. These histological events are very similar to those seen in some viral infections of mice[12]. This evidence leads us to speculate that *N. dubius* infection occurs naturally only when the *Peromyscus* mouse is sufficiently immunosuppressed, perhaps by a concurrent viral or parasitic infection. Support for the latter speculation comes from the work of COLWELL and WESCOTT[13] who showed that *N. brasiliensis* infection of mice was prolonged by a concurrent infection of *N. dubius*. Apparently any natural resistance of the mouse against infection with *N. brasiliensis* was suppressed by the presence of *N. dubius*.

We believe that the inability to infect *P. maniculatus* with *N. dubius* is due to the increased natural resistance of the mouse and not to an adaptation of the parasite because of laboratory passage as speculated by some authors. One possible reason for the increased native resistance of this mouse is the tendency to avoid inbreeding as evidenced by our work and that of others[14]. We have already demonstrated that inbred strains have a decreased capability for immunization[15]; therefore, the lack of inbreeding in *P. maniculatus* may account for the high degree of native resistance to *N. dubius* infection.

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Undulating Tubules in Lymphocytes of an Apparently Healthy Human Donor

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Summary. So- called 'undulating tubules' were found in the blood lymphocytes of an apparently healthy 33-year-old male. Undulating tubules have been noted to occur frequently in kidney cells and blood lymphocytes of patients suffering from collagen diseases and especially from SLE. They have been suggested to be a possibly pathognomonic finding in such diseases. Our result seems to contradict such an association.

In the course of systemic electron microscopic examinations of blood of patients with leukemia, we observed unusual tubular inclusions in the circulating lymphocytes of a seemingly healthy control patient. Since similar structures have been noted in lymphocytes or other cells in several diseases, especially in collagenosis, our finding seems to merit a brief report.

Peripheral blood was drawn from the cubital vein of a 33-year-old man, who was seemingly in good health and had no history of collagenosis or recent viral diseases. Blood smear examination was normal.

The lymphocytes were separated as follows: 1. defibrination of the blood by stirring for 10 min at room temperature; 2. 4-fold dilution with phosphate-buffered