THE IN VITRO ACTION OF HYDROCORTISONE ON LEUCOCYTE MIGRATION*

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The experiments described in this report are an extension of previous observations that certain adrenal steroids, when added *in vitro*, are able to influence the ameboid migration of leucocytes (Ketchel and Favour, 1953 *a*). Although other investigators have noted effects on leucocytes of adrenal steroids added *in vitro* (Martin *et al.*, 1954, Leahy and Morgan, 1952), the capillary tube method of measuring leucocyte migration has proved sensitive enough to permit dose-response relationships to be determined.

The capillary tube method was first used by Wright (1915), and later by Holst (1921), Ketchel and Favour (1953 *b*, 1955), and O'Neill and Favour (1955). This consists of centrifuging small capillary tubes filled with blood so that a buffy coat of leucocytes is formed at the interphase between the packed red cells and the plasma. The distance which the leucocytes migrate into the plasma can then be measured. Ketchel and Favour (1955) showed that the migration of leucocytes in a blood sample from a healthy individual tends to remain fairly constant from day to day. However, 4- or 5-fold differences may occur in the average migration rates of normal individuals. It was also noted that acute and chronic illnesses are accompanied by wide fluctuations in leucocyte migration. The major control of leucocyte migration is mediated by factors in the plasma. Fractionation of the plasma indicates that two protein fractions oppose one another in their effect—Cohn's fraction II enhances leucocyte migration, and Cohn's fraction III inhibits leucocyte migration.

Methods

The details of studying leucocyte migration by the capillary tube method have been described in a previous publication (Ketchel and Favour, 1955). In the present experiments, heparinized venous blood was separated into cells and plasma by centrifugation. The cells were washed in Hanks's solution (Hanks and Wallace, 1949), and 0.2 ml. of washed cells

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and 0.3 ml. of plasma were combined to form the cell suspension used in the experiment. 0.1 ml. of Hanks's solution containing appropriate concentrations of the steroid[1] under study was added to a series of aliquots of the same cell suspension.

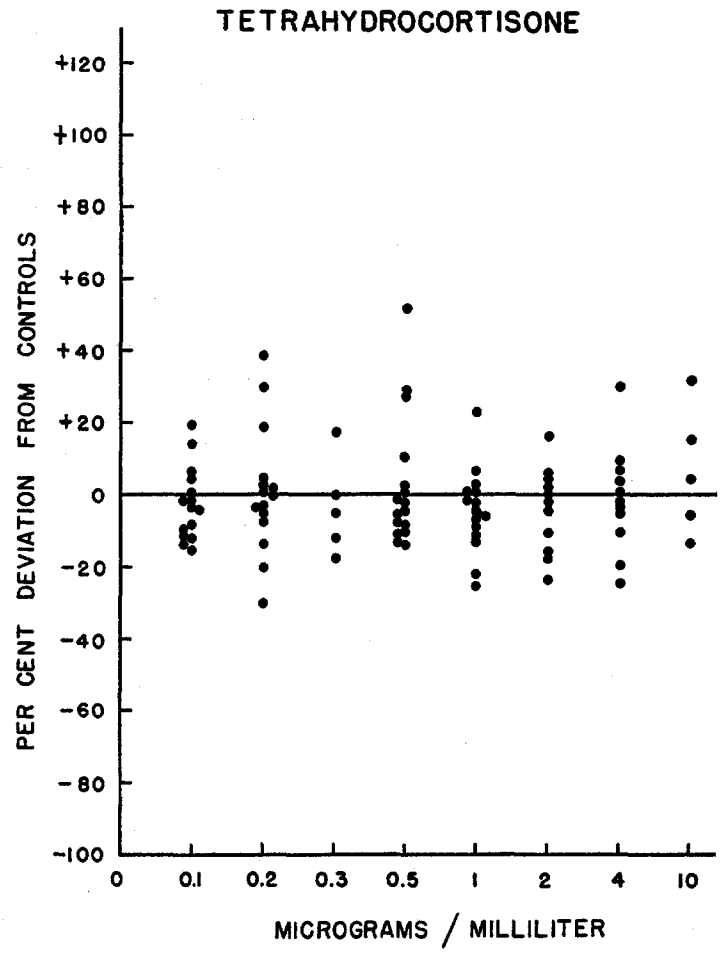

FIG. 1. The influence of specific concentrations of tetrahydrocortisone on *in vitro* leucocyte migration. For each experiment the controls and a range of concentrations of hormone were compared, using aliquots of the same blood. Each point is an average value of ten capillary tubes. In general, the points tend to group around the 0 per cent mark, indicating that no change in leucocyte migration is caused by tetrahydrocortisone.

After the selected hormone was added, the suspension was drawn into thin-walled capillary tubes of the type ordinarily used for melting point determinations. Each tube was sealed and centrifuged into a system of three components—a basal layer of packed red cells, an inter-

[1] Kindly supplied by the Upjohn Company, Kalamazoo, Michigan, and by Merck and Company, Rahway, New Jersey.

mediate "buffy coat" of leucocytes, and an uppermost layer of plasma. Ten tubes were prepared for each hormone concentration tested. The tubes were incubated in a vertical position at 37°C. overnight. The leucocytes in the buffy coat move by ameboid action through the

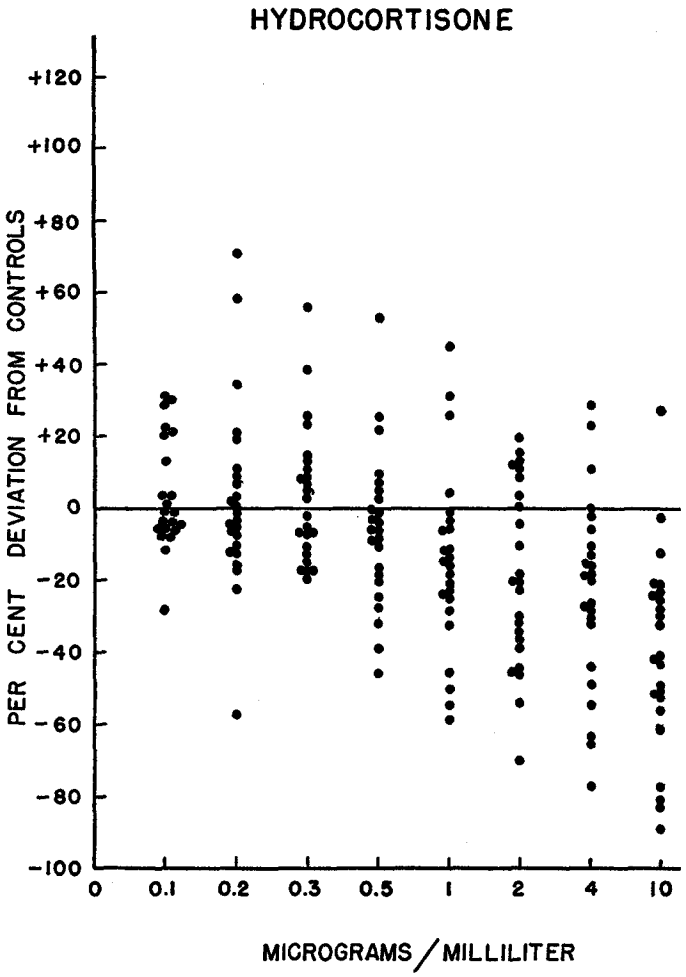

FIG. 2. The influence of specific concentrations of hydrocortisone on leucocyte migration. At lower concentrations there appears to be an increase in both positive and negative deviations from zero. As the concentration is increased to 10 μg./ml., almost all the points fall below the 0 per cent mark.

plasma clot or along the glass wall of the tube. It should be emphasized that this is an active process, with the cells migrating against gravity. At the completion of the incubation period the tubes were examined under a microscope equipped with an ocular micrometer, and the distance which the leucocytes had moved along the tube away from the buffy coat was determined. The results of the 10 determinations made for each hormone concentration were averaged for analysis.

RESULTS

The results of 11 experiments in which the effect of added tetrahydrocortisone was observed are shown in Fig. 1. In this and the following figures, the control migration, that with no steroid added, was called 100 per cent and the

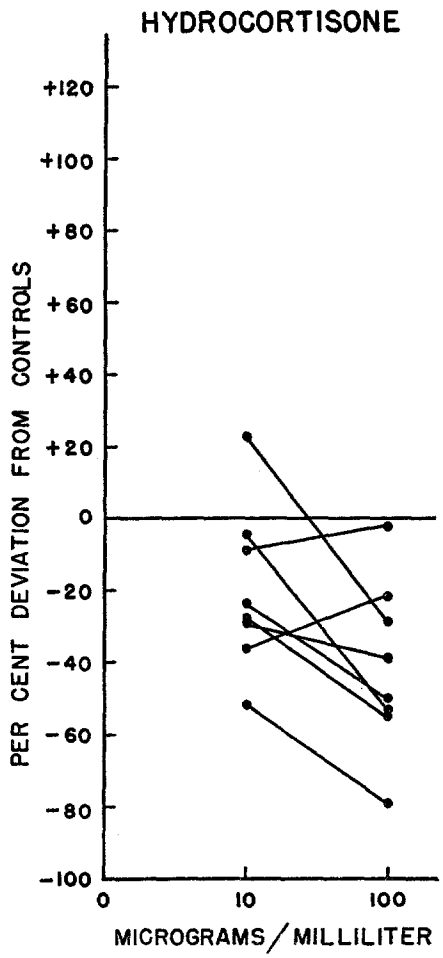

FIG. 3. Effect of high concentration of hydrocortisone on leucocyte migration. More inhibition occurred at 100 μg./ml. than at 10 μg./ml. in 6 out of the 8 experiments.

effects of specific concentrations of hormone were calculated as a per cent change from the control. Aliquots of the same cell suspension were used throughout each experiment. Tetrahydrocortisone was chosen as a control steroid because, while its molecular structure closely resembles that of hydrocortisone, it has no known physiological activity. In the concentrations tested, no significant alteration in the leucocyte migration rate was observed.

Fig. 2 summarizes the results of 24 experiments recording the effects on leucocyte migration of a graded series of hydrocortisone sodium succinate (solucortef, Upjohn Co.) concentrations. At 0.1 μg./ml. no significant alteration in leucocyte migration is noted. With an increase in hormone concentration, a greater frequency of both positive and negative deviations appears. At 2 μg./ml., the average deviation is more negative than at lower concentrations. Higher concentrations of hormone cause a further slowing of cell migration. At 10 μg./ml. most of the determinations showed an inhibition of cell migration as compared to the control. The same results were obtained in a second series of experiments in which the free alcohol form of hydrocortisone was used. This

TABLE I

Variance Analysis of Hydrocortisone Experiments

	Source	Sum of Squares	Degrees of freedom	Mean square	F.
1.	Total	138,929	191		
2.	Trials	48,862	23	2,124	6.7*
3.	Doses	38,969	7	5,567	17.6*
4.	Regression, straight line	31,061	1	31,061	98.0*
5.	Residual	7,908	6	1,318	4.2‡
4(a)	Regression, log of dosage	37,277	1	37,277	117.6*
5(a)	Residual	1,692	6	282	1.9
6.	Remainder	51,089	161	317	

* $p < 0.001$.
‡ $p < 0.01$.

latter series indicates that this effect is not a unique property of the sodium succinate form of hydrocortisone.

Fig. 3 gives the results of a series of experiments in which 10 μg./ml. and 100 μg./ml. were added to the cultures. In 6 out of 8 experiments more inhibition occurred with 100 μg./ml. than with 10 μg./ml. The application of the "t test" to these data shows that the difference is significant at the 0.001 level. While the degree of inhibition can be increased, 100 per cent inhibition was not observed even with very high doses.

Table I records the results of a variance analysis on the data shown in Fig. 2. Line 3 of Table I divides the variation into the series of concentrations of hydrocortisone. This is divided in lines 4 and 5 into a straight line trend and residual variation around the trend. Although the straight line trend is much larger, the residual variation is still significant at the 0.01 level. Accordingly,

this analysis was repeated using the logarithms of the doses rather than arithmetic values. On the basis of this analysis, the variation assigned to regression is even larger, and the residual is no longer significant. Thus the dose-response

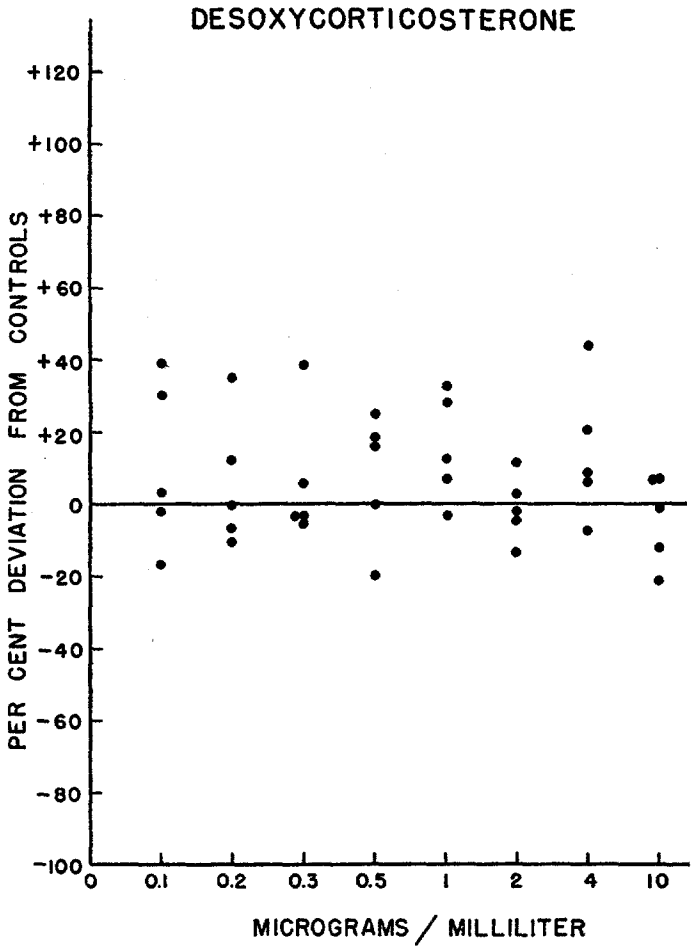

FIG. 4. Effect of desoxycorticosterone on leucocyte migration. No effect is noted at concentrations up to 10 μg/ml.

curve for the effects of hydrocortisone on leucocyte migration can be best expressed by a logarithmic plot.

The effects of desoxycorticosterone on leucocyte migration are shown in Fig. 4. In this series of experiments it is apparent that desoxycorticosterone has no effect on leucocyte migration as measured by this method.

DISCUSSION

By use of a quantitative *in vitro* measurement of leucocyte migration, the cellular effects of three closely related steroids have been observed. Leucocyte migration was not influenced by tetrahydrocortisone or by desoxycorticosterone; hydrocortisone, however, decreased cell migration in proportion to the concentration used. The decrease in cell migration can be better expressed as a logarithmic function than as an arithmetic function of hydrocortisone concentration.

Two lines of reasoning suggest that the hydrocortisone was acting on the cells as a hormone. First, tetrahydrocortisone and desoxycorticosterone, although similar to hydrocortisone structurally, were not effective. Secondly, the concentration of free hydrocortisone in human blood is placed at 0.04 to 0.19μg./ml. (See Gold, 1957), not markedly different from the amounts which were effective in these experiments.

SUMMARY

Hydrocortisone inhibits the ameboid migration of human leucocytes when added *in vitro*. The dose-response curve for the reaction between this steroid and leucocytes can be best expressed by a logarithmic plot of the steroid concentrations.

Tetrahydrocortisone and desoxycorticosterone had no effect on *in vitro* leucotyte migration.

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