rabbit fetal placental extracts, namely serotonin, histamine, acetylcholine, nucleotides, and inorganic ions. The evidence points to a substance of low molecular weight and probably a polypeptide similar to known polypeptides which are active on smooth muscle such as bradykinin, kallidin, and substance P(4). The presence of this powerful substance in fetal placenta of rabbit poses a problem in relation to the physiology of gestation, a matter of conjecture at present. Further studies on the substance, especially in relation to its activity on the uterus, are planned.

Summary. 1) A hypotensive, smooth muscle-contracting principle has been identified in the placenta of the rabbit and of several other species. It is dialysable, stable to heat at neutral pH for 3 minutes at 100°C, and unstable in alkali and acid. It has been separated chromatographically and by use of inhibitors from acetyl choline, serotonin, and histamine. The active principle is hydrolysable with liberation of free amino acids and with resulting loss of activity on guinea pig ileum and blood pressure. It has been tentatively identified as a polypeptide. 2) On intravenous injection the active principle causes a fall in blood pressure, cardiac dilatation, intestinal and uterine peristalsis, and death from respiratory failure. It is found predominantly in the fetal part of the placenta and at all stages of pregnancy in the rabbit from 16th day to term.

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Influence of Prochlorperazine on Hydrocortisone Inhibition of *in vitro* Leucocyte Migration.* (24639)

MELVIN M. KETCHEL AND SOMERS H. STURGIS (Introduced by L. K. Diamond)
(With technical assistance of Aurelia C. Koros and Nancy S. Crowell)
Depts. of Surgery and Gynecology, Harvard Medical School and Peter Bent Brigham Hospital, Boston

Addition of hydrocortisone to human blood has been shown by Ketchel, Favour, and Sturgis(1), to inhibit amoeboid migration of leucocytes in capillary tubes. Several lines of reasoning suggest that hydrocortisone was acting in its capacity as a hormone, rather than in non-specific manner. These reasons are, (1) similar chemical structures, such as tetrahydrocortisone and desoxycorticosterone, were not effective; (2) hydrocortisone was effective in concentrations similar to those which occur in blood; and (3) hydrocortisone from several manufacturers, including both alcohol-free hydrocortisone and hydrocortisone sodium succinate, were effective, thus decreasing the possibility that a toxic contaminant of the hydrocortisone could be responsible for the effect. During these experiments, it was noted that leucocytes of a patient being treated with prochlorperazine† were unaffected by concentrations of hydrocortisone, which had been effective before the patient was given prochlorperazine. This chance observation led to *in vitro* experiments, here described, which attempted to clarify the effect of prochlorperazine on response of leucocytes to hydrocortisone.

Methods and materials. In the capillary tube method of studying leucocyte migration (Ketchel and Favour(2)), small bore capillary tubes are partially filled with blood, and end

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† Compazine, Smith Kline and French Co., Philadelphia, Pa.

TABLE I. Sample Protocol for Combining Leucocytes with Hydrocortisone and Prochlorperazine.

Mixing vial No.	Cells (ml)	Plasma (ml)	Final conc. (μg/ml) of Prochlorperazine (.1 ml added)	Hydrocortisone (.1 ml added)	Hanks' sol.
1	.2	.3	100	100	
2	"	"	10	"	
3	"	"	1	"	
4	"	"	.1	"	
5	"	"	100		.1
6	"	"	10		"
7	"	"	1		"
8	"	"	.1		"
9	"	"		100	"
10	"	"			.2
11	"	"			"

Mixing vials 1 through 4 received both hydrocortisone and prochlorperazine. Mixing vials 5 through 8 received prochlorperazine alone. Mixing vial 9 received hydrocortisone alone; mixing vials 10 and 11 received no hydrocortisone and no prochlorperazine.

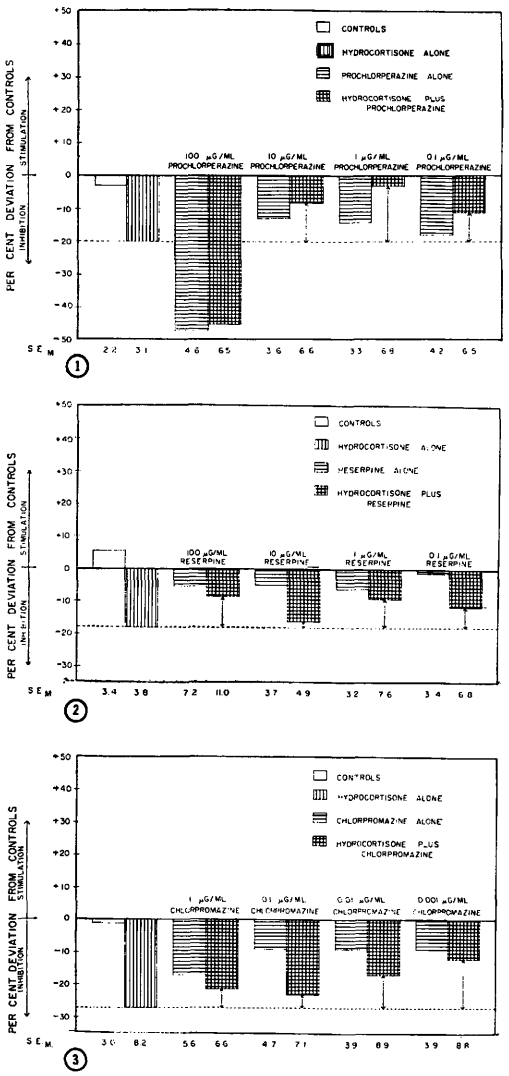
of each tube sealed. Centrifugation results in a system with red cells at bottom of capillary tube, a "buffy coat" of leucocytes over the red cells, and clear plasma at the top. When the capillary tube is incubated vertically, leucocytes move by active amoeboid motion through interstices of clotted plasma or along the walls of capillary tube. The distance which leucocytes move away from the original buffy coat in a standard incubation period, is measured with ocular micrometer in microscope. Details of preparing capillary tubes, were the same as described in earlier papers: Ketchel and Favour(2); Ketchel, Favour, and Sturgis (1). Hydrocortisone sodium succinate‡ and prochlorperazine were dissolved in Hanks' Solution (Hanks and Wallace(3)), and dilutions made so that addition of 0.1 ml to cell suspension would make the desired final concentration. Hanks' solution without added hydrocortisone or prochlorperazine was added when necessary to keep volume relationships constant (Table I). The same cell suspensions, plasma, and other reagents were used throughout each experiment, and the order of preparation of capillary tubes from the mixing vials was randomized. Ten capillary tubes were prepared from each mixing vial, and the results averaged. Mixing vial 10 was called the control, and average migration from capillary tubes prepared from it, was compared with average migration obtained from capillary tubes prepared from the other mixing vials. Inclusion of mixing vial 11, identical to mixing vial 10, provided information on variability of the method.

‡ Solu-Cortef, Upjohn Co., Kalamazoo, Mich.

Results. The results of 11 experiments, to determine effects on leucocyte migration of prochlorperazine and hydrocortisone alone and in combination, are shown in Fig. 1. In this and following figures, results are indicated as average per cent deviation which occurred in capillary tubes made from experimental mixing vials, as compared to capillary tubes made from control mixing vials which contained no prochlorperazine or hydrocortisone. A series of capillary tubes from a second control mixing vial was included in each experiment to determine variation in experimental procedure, and average percent deviation which occurred in capillary tubes made from each second control mixing vial is indicated in the Figures as "controls." By including this second control, percent deviation of controls could be compared statistically to per cent deviation which occurred with hydrocortisone or prochlorperazine. Dotted line in Fig. 1 represents deviation which occurred when hydrocortisone alone was added to the cells, and arrows indicate change from hydrocortisone inhibition which occurred as each concentration of prochlorperazine was added to hydrocortisone in the tubes. In Fig. 1, average per cent deviation of second controls from the first was $-3\% \pm 2.2$. Average deviation caused by hydrocortisone was $-20\% \pm 3.1$. This inhibition caused by hydrocortisone is significant at the 0.01 level.

As further shown in Fig. 1, addition of 100 mg/ml of prochlorperazine to blood inhibited leucocyte migration by almost 50%, and this was neither increased nor decreased by addition of hydrocortisone. Addition of 10 μg/ml of prochlorperazine caused an average deviation from controls of $-13\% \pm 2.2$. The combination of 10 μg/ml of prochlorperazine and 100 μg/ml of hydrocortisone reduced average deviation to $-7\% \pm 6.6$.

The effect which occurred at concentration



Mixing vials 1 through 4 received both hydrocortisone and prochlorperazine. Mixing vials 5 through 8 received prochlorperazine alone. Mixing vial 9 received hydrocortisone alone; mixing vials 10 and 11 received no hydrocortisone and no prochlorperazine.

FIG. 1. Effects of prochlorperazine and hydrocortisone alone and in combination on leucocyte migration. Dotted line represents inhibition caused by addition of hydrocortisone alone, and arrows represent decrease in inhibition resulting from addition of specific concentrations of prochlorperazine to tubes containing hydrocortisone.

FIG. 2. Effects of reserpine and hydrocortisone alone and in combination on leucocyte migration. Dotted line represents inhibition caused by addition of hydrocortisone alone, and arrows represent decrease in inhibition resulting from addition of specific concentrations of reserpine to tubes containing hydrocortisone.

of 1 µg/ml of prochlorperazine is especially noteworthy This concentration caused average deviation from controls of −14% ± 2.6. Hydrocortisone alone, at a concentration of 100 µg/ml, caused average deviation from controls of −20% ± 3.1. Combination of 1 µg/ml of prochlorperazine and 100 µg/ml of hydrocortisone caused an average deviation from controls of −3% ± 0.8. Thus inhibition which occurs with either prochlorperazine or hydrocortisone alone is greatly reduced when the 2 are used in combination. A t-test comparing deviations from controls of capillary tubes containing hydrocortisone alone with those containing hydrocortisone plus 1 µg/ml of prochlorperazine shows that the difference is significant at the 0.05 level. Addition of 0.1 µg/ml of prochlorperazine caused an average deviation from controls of −18% ± 4.2, and the combination of hydrocortisone plus 0.1 µg/ml of prochlorperazine reduced the deviation to −11% ± 6.5.

It thus appears that inhibitions caused by prochlorperazine and by hydrocortisone are not additive. Indeed, at all concentrations tested, inhibition caused by hydrocortisone and prochlorperazine together was less than that caused by either one alone, and at a concentration of 1 µg/ml, prochlorperazine was able almost completely to reverse inhibition caused by hydrocortisone.

A further experiment was carried out to determine if a reaction was occurring directly between hydrocortisone and prochlorperazine, or whether each compound modified the reactions of the cell to the other. Earlier experiments (Ketchel and Sturgis; unpublished observation) showed that 1000 µg/ml of hydrocortisone was able to completely inhibit leucocyte migration. It was reasoned that if the reaction between hydrocortisone and prochlorperazine was a stachiometric one in which the leucocytes were not involved, then the effects of very large amounts of hydrocortisone could be reversed by addition of comparatively large

FIG. 3. Effects of thorazine and hydrocortisone alone and in combination on leucocyte migration. Dotted line represents inhibition caused by addition of hydrocortisone alone, and arrows represent decrease in inhibition resulting from addition of specific concentrations of thorazine to tubes containing hydrocortisone.

amounts of prochlorperazine. A series of cell suspensions containing 0.1, 1, 10, and 100 μg/ml was prepared, and hydrocortisone to attain a final concentration of 1000 μg/ml was added to each. In no case were lucocytes able to migrate in capillary tubes prepared from these cell suspensions, suggesting that reversal of hydrocortisone inhibition which occurred in experiments of Fig. 1 was not caused by a stachiometric reaction between hydrocortisone and prochlorperazine.

A similar series of 15 experiments was carried out with reserpine§ substituted for prochlorperazine (Fig. 2). Reserpine alone, even in concentrations as high as 100 μg/ml, was only slightly inhibitory to leucocyte migration. While at each concentration tested, average inhibition of reserpine plus hydrocortisone was less than that obtained with hydrocortisone alone, the differences did not have statistical significance. It is interesting to note that although statistical significance was not demonstrated, the average inhibition was less at all concentrations, when reserpine was included with hydrocortisone than with hydrocortisone alone. It seems clear that the inhibitions caused by hydrocortisone and by reserpine are not additive.

A third series of experiments was carried out using chlorpromazine hydrochloride‖ (Fig. 3). Thorazine alone was effective in inhibiting leucocyte migration in concentrations as low as 0.0001 μg/ml. As with reserpine, inhibition obtained by combining thorazine with hydrocortisone was less than that obtained with hydrocortisone alone although these differences were not large enough to be statistically significant. As with compazine and reserpine, inhibitions caused by thorazine and hydrocortisone are not additive.

Discussion. The present data indicate that prochlorperazine, at optimal concentrations, is able to reverse the inhibitory affect on leucocyte migration caused by hydrocortisone. Also, at other concentrations of prochlorperazine, and at all concentrations of reserpine and thorazine tested, the inhibitory effects are not added to those caused by hydrocortisone.

The importance of adrenal steroid hormones in the reaction of organisms to stress, is now well accepted, although the biochemical pathways through which the physiological responses are produced are poorly understood. Whatever the cellular mode of action of hydrocortisone, the present experiments suggest that it is interfered with by prochlorperazine, and perhaps by other tranquilizing agents.

Conclusions. 1. Addition to blood of 100 μg/ml of hydrocortisone or of 0.1 to 10 g/ml of prochlorperazine results in inhibition of leucocyte migration. Addition of both compounds together results in less inhibition than is obtained with either compound alone. 2. Reserpine at concentrations of 0.1 μg/ml to 100 μg/ml is less inhibiting to leucocyte migration than is prochlorperazine. Addition of both reserpine and hydrocortisone to blood results in less inhibition to leucocyte migration than when hydrocortisone alone is added. 3. Thorazine at concentrations of 0.001 μg/ml to 1 μg/ml is inhibitory to leucocyte migration. Addition of both thorazine and hydrocortisone to blood, results in less inhibition to leucocyte migration than when hydrocortisone alone is added.

§ Serpasil, Ciba Pharmaceutical Products, Summit, N. J.

‖ Thorazine, Smith, Kline and French, Philadelphia, Pa.

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