Some Properties of Hypotensive Smooth Muscle Contracting Principle in Placenta of Rabbit.* (24638)

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In experiments on placental transfer of insulin in the rabbit(1) damage to the placenta was frequently followed by rise in maternal blood sugar and development of a shock-like state ending in death. An attempt was made to investigate the hyperglycemic phenomenon by preparation and intravenous injection of saline extracts of the placenta. No consistent effect on blood sugar of adult rabbits was noted. However, extracts proved to be very toxic, producing gasping respiration, marked intestinal and uterine peristalsis, sustained fall in blood pressure, and death, apparently from respiratory failure. On opening the chest after death, the lungs were pale and bloodless, the heart grossly dilated, especially the right chambers, and ventricular fibrillation was frequently observed. These effects resembled those of histamine and serotonin. A preliminary characterization of the active principle of rabbit placental extracts has been carried out. It has been shown that most activity is found in the fetal part of the placenta and that it is probably due to a polypeptide.

Methods. Investigation of some physical and chemical properties of the active extract showed that it was stable to heat at a neutral pH for 3 minutes at 100°C. Longer periods of heat at neutral pH were not tried. The extract was unstable to alkali and acid (1 hour at 100°C in 0.2 N HCl or NaOH), completely dialysable with about half the activity appearing in the dialysate in 24 hours when there were equal volumes inside and outside the sac. All activity was lost on ashing at 600°C for 24 hours. Standardization of extraction procedure was then established as follows: fetal placentae were separated from decidual tissues, weighed, and placed in graduated cylinder. Distilled water was added to make final volume 3 times that of the wet placentae and the whole homogenized in Waring blendor. The crude extract was then boiled for about 2 minutes. Each cubic centimeter of crude extract was equivalent to 0.33 g of wet placenta. The boiled extract was then placed in dialysis sac and dialysed against an equal volume of distilled water for 24 hours. Each cubic centimeter of dialysate was equal to about 0.166 g of wet placenta.

Results. The dialysate was assayed on an isolated loop of guinea pig ileum in an oxygenated bath (20 ml) of Tyrode's solution. Two-tenths ml of the dialysate (equivalent to 0.0332 g of wet placenta) caused a sustained contraction. The type of contraction produced by this amount of extract was slower and more sustained than that produced by histamine; also, the extract was washed out from the tissue with much greater difficulty. Contraction produced by the extract was not blocked by an amount of atropine (1 μg/ml, final concentration in bath) sufficient to inhibit the effect of acetylcholine (0.05 μg/ml). Similarly, the contraction was not blocked by an amount of benadryl (0.5 μg/ml) or pyribenzamine (1.0 μg/ml) sufficient to inhibit the effect of histamine (0.05 μg/ml), nor by an amount of lysergic acid diethylamide (1.0 μg/ml, enough to inhibit the effect of serotonin (0.05 μg/ml).

When tested in anesthetized rabbits (3 to 4 kg) and a dog (10 kg), the dialysate caused a fall in blood pressure after intravenous injection. One ml of extract caused a fall of about 60 mm of mercury in the rabbit and 8 ml caused a fall of about the same amount in the dog. There was a tendency for the blood pressure to remain at shock levels in the rabbit after a single injection but not in the dog. Extracts of other tissues (liver, lung, uterus, and maternal placenta) from adult rabbits extracted under identical conditions and equivalent in wet weight to the placental extract, were found to be without effect on the guinea pig ileum and on blood pressure of rabbit and dog. The hypotensive action of placental extract in the rabbit was easily differen-

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tiated from that of histamine, serotonin, and acetylcholine by use of the aforementioned antagonists.

Further purification of the dialysate from placental extracts was carried out by paper chromatography. Ten to 30 ml of the dialysate were evaporated to dryness under vacuum and the residue taken up in a small volume of distilled water. The latter was then placed on strip of Whatman paper No. 1 and run as a descending chromatogram using butanol saturated with 10% ammonium hydroxide as solvent. After 20 hours the strip was dried and cut into horizontal strips 2 cm wide beginning at origin and extending to solvent front. Each strip was then placed in a test tube and eluted with 2 ml of Tyrode's solution. The contents of each tube were then assayed on guinea pig ileum. A rather precise separation of the active placental principle was accomplished consistently by this procedure and was regularly found in the same eluates after 20 hours on the chromatogram paper. Two-tenths ml was active in causing contraction of the ileum and, as before, the effect was not blocked by benadryl and pyribenzamine nor by atropine and lysergic acid diethylamide. The eluates which were active on guinea pig ileum were also effective in lowering blood pressure of rabbit. These results suggest that the hypotensive placental factor and the guinea pig ileum-contracting factor are identical.

Paper chromatograms were developed in ultra-violet light at 260 mμ on Kodak contact standard (specification 87) photographic paper. No absorbing material was found in areas associated with the action on guinea pig ileum, indicating that the principle does not contain nucleotides or other ultra-violet absorbing materials. When histamine and the dialysate from the placental extract were tested on the same chromatogram there was separation but the separation was not sharp. The Rf value of the dialysate ranged from .38 to .54 while that of histamine was constant at .54. Serotonin had an Rf of .62 and there was complete separation from the active principle of the placental extract. Extracts were subjected to paper chromatography and spectrophotofluorometric analysis by the method of Udenfriend, Weissbach, and Clark (2). Both methods proved negative for serotonin. Histamine could not be identified on the chromatograms using the method of Urbach(3). The absence of free reducing groups was shown when chromatograms were sprayed with benzidine reagent with negative results.

Paper chromatograms of dialysate from the placental extract were sprayed with 0.125% ninhydrin in acetone and heated 5 minutes at 100°C. No ninhydrin-reacting material was observed in the area of the chromatogram associated with activity on guinea pig ileum, showing that the activity was not related to presence of free amino acids. Eluates containing active materials from paper chromatograms of plancental extract were made with 4 ml of distilled water and divided into 2 portions. One portion was hydrolysed with 6 N hydrochloric acid for 48 hours at 100°C; the other was stored at 4°C without acid. Both aliquots were then tested on paper chromatograms, as described above, and after control analyses on guinea pig ileum, were sprayed with ninhydrin (0.125% in acetone), and heated 5 minutes at 100°C. There was no contraction of guinea pig ileum caused by the hydrolysed portion of the chromatogram but abundant ninhydrin color was present. The unhydrolysed portion of the chromatogram was still active and no ninhydrin color was present in the acvtive area. These results show that amino acids are liberated on hydrolysis of the active principle.

No significant change in activity of placental extracts was observed from 16th to 27th day of pregnancy in the rabbit. A dialysable, heat stable principle, active on rabbit blood pressure and guinea pig ileum was observed in extracts of the placenta of cow, guinea pig, and pre-eclamptic human patient. The contractile response of the ileum with cow and guinea pig placental extracts was of the rapid type, however, and more closely resembled the effect of histamine, while that of human was of slow and sustained type. Further characterization of the active principle of these placentae has not been carried out.

On evidence presented above it seems possible to rule out with certainty all of known compounds with activities similar to those of

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)

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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.