GUINEA PIG KININOGEN–KININ SYSTEM IN PREGNANCY
AND UNDER HORMONAL INFLUENCE

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Evidences on the participation of the kininogen-kinin system in pregnancy and labour have been provided by the work of Armstrong and Stewart (1), Gomes (2), Werle (3), Martinez et al. (4), Erdös et al. (5), Periti and Gasparri (6), Centaro et al. (7), Meirelles (8) and many others, showing the formation of kinins during labour, a fall in bradykininogen (BKg), an increase of the destroying enzyme (kininase), and of the esterolytic activity, in plasma from pregnant women.

Studies on laboratory animals are scarce; Wiegenshausen et al. (9) described increase of BKg during pregnancy in rabbits and rats, with a subsequent fall during labour, and return to normal values in the puerperium.

We decided to take guinea pigs as experimental animals, because of the relatively large period of pregnancy (average 67 days) and higher (than in the rat), values of the BKg level in plasma. We have taken as parameters of investigation BKg levels, the protein content, the esterolytic activity (on TAME), and the kininolytic activity (kininase) using bradykinin (BK) as substrate.

MATERIAL AND METHODS

The BKg contents were determined by Diniz et al. (10) micro-method, with slight alterations, omitting the final alcohol extraction. After the treatment with trypsin, the volume is adjusted with saline to the required level and the material tested
directly upon a trypsin desensitized piece of guinea pig ileum. The results are given in units of BK·g, calculating the units as corresponding to 0.5 μg of synthetic BK.

Total plasma protein (P) was measured by the biuret method (11) and expressed in mg/ml.

The esterolytic activity (EA) was estimated by the variation of pH in an incubate at 37°C of a fixed amount (50 μmol) of TAME (tosyl arginine methyl ester) in presence of 20 mg of plasma protein in Tris-HCl as buffer, at pH 7.8 in a final volume of 5.5 ml. The pH determinations were recorded with a titrating potentiometer (TTT Radiometer - Copenhagen) with a single glass electrode (GK 2021 B) from the volumes of 0.0067N of NaOH added to keep the pH at the constant level of 7.8. The readings were done at increasing intervals of time (in min) to a zero kinetic reaction. To improve the kinetic of the reaction, a 0.02M of calcium chloride and 0.1M of potassium chloride were added to the incubation mixture. The esterolytic activity is expressed in pmol of TAME hydrolysed by mg of protein per hour.

The kininase activity (KA) was measured in test samples containing 3.0 μg of BK as substrate, 5.0 μl of plasma and the volume completed to 3.0 ml with Tris-HCl buffer at pH 7.4.

Aliquots were taken every 6 minutes for 18 to 24 minutes, and tested directly upon a piece of guinea pig ileum in a 10 ml chamber, at 37°C. Kininase activity is indicated as μg of BK inactivated in one minute per mg of plasma protein.

Pregnant guinea pigs were obtained by exposing the females in oestrus to contact with males for a period of 48 hours and then kept in separate cages, for the duration of the experiment. Water and food were provided as usual. To control the hormonal state, vaginal smears were routinely done before and after the initiation of the experiment.

Blood samples were taken by cardiac puncture using a 2.5% solution of sodium oxalate as anticoagulant in the proportion of 0.1 ml per 0.9 ml of blood.

For the experiments with estradiol and aldosterone, immature guinea pigs were used. Estradiol was administered as the benzoate, in peanut oil in the dose of 0.2 mg/animal, for a period of 20 days. During the first 15 days the injections were mainly sub-cutaneous and then in the last 5 days were alternated by the sub-cutaneous