ally low value of serum FSH of saline controls at 24 h and therefore has no meaning. Elevated serum LH and normal prolactin in the potassium canrenoate-treated rats are similar to the results with spironolactone[8,9].

It was shown that spironolactone increases serum progesterone and lowers serum testosterone[10,11]. Spironolactone is believed to suppress testicular and adrenal androgen production by destroying the heme of cytochrome P-450, thereby inhibiting the enzymatic step involved in the conversion of progesterone to testosterone[12,13]. We could not confirm the previous report[5] that potassium canrenoate decreased serum testosterone levels and the cause of this discrepancy may be due to the difference in the species examined. The negligible loss of the heme of cytochrome P-450 by potassium canrenoate[13] may support the failure of potassium canrenoate to inhibit the androgen production. Potassium canrenoate may increase serum LH by its inhibitory effect on the androgenic receptor, because the possibility was shown that potassium canrenoate exerts its peripheral anti-androgenic effect via competition for the androgenic receptor[14]. The relative unresponsiveness of serum FSH to chronic potassium canrenoate may be due to the differential release of LH and FSH by endogenous LHRH. The normal serum prolactin levels in the rats chronically treated with potassium canrenoate indicate that the occurrence of gynecomastia due to hyperprolactinemia is unlikely during the chronic potassium canrenoate therapy. Although we could not obtain evidence suggesting the inhibition of the synthesis of testosterone by potassium canrenoate, the increased LH levels observed in our study suggest the anti-androgenic action of potassium canrenoate and therefore the possibility that sexual disorders similar to those due to spironolactone therapy may occur during chronic potassium canrenoate treatment.

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Formation of indolyl-3-acetylaspartic acid in rats

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Summary. Indolyl-3-acetylaspartic acid (IAAsp) was detected in the urine of rats given indolyl-3-acetic acid (IAA) i.p. It was ascertained that the conversion of IAA into IAAsp could be carried out not only in plants but also in animals.

Indolyl-3-acetylaspartic acid (IAAsp) was first described as being present in the pea plant by Andreae[2] in 1955. Thereafter, its presence in many kinds of plants has been reported[3,4]. The significance of IAAsp formation was interpreted as the detoxification of higher concentrations of indolyl-3-acetic acid (IAA)[2]. IAA is generally known to be present in animal body as a metabolic product of tryptophan[5], however, the presence of IAAsp has not been known. In the present report the presence of IAAsp in the urine of rats injected with IAA is dealt with.

Materials and methods. Male rats (Wistar King strain) weighing 290–350 g were used as test animals. Rats were allowed to take water ad libitum, but no food was given for 18 h before the administration of IAA or aspartic acid. The blood samples were obtained by heart puncture using heparinized syringes, and pooled from 10 animals for 1 determination. Plasma and erythrocytes were separated by centrifugation of the heparinized blood 3 h after injection of the compound. The erythrocyte sediment was washed with saline solution 3 times and then hemolyzed in 3 times its volume of distilled water. The hemolyzate solution was used as an erythrocyte sample. Urine samples were collected 24 h after injection, from animals kept in metabolism cages. Extraction of IAA and IAAsp were made following the method of Andreae and Good[2], and the determination of these compounds was carried out using a modification of the gas chromatographic method of Seely and Powell[6]. 10 ml of plasma or 40 ml of the hemolyzate solution was acidified to pH 4 with H_3PO_4 and then extracted with 3 times its volume of n-butanol 3 times. Extraction from 10 ml of urine was also made by the same procedure. The butanol solution was washed with a small volume of water and extracted with a small amount of 0.1 N $NaHCO_3$ solution 3 times. The bicarbonate solution was acidified with H_3PO_4 to pH 2.6 and extracted with 10 ml of n-butanol 3 times. The butanol solution was washed with a small amount of water and then concentrated to 5 ml. 0.3 ml of it was applied to a sheet of Merck silica gel 60 plate, and 2-dimensional TLC was carried out with the following sol-

Table 1. IAA and IAAsp contents in urine 24 h after administration of IAA and Asp to rats

Compound applied	IAA (μmoles/animal)	IAAsp (μmoles/animal)
None	0	0
L-Asp 500 mg/kg (s.c.)	0	0
IAA 500 mg/kg (i.p.)	78.20 ± 12.59*	0.009 ± 0.002
IAA 500 mg/kg (i.p.) + L-Asp 500 mg/kg (s.c.)	89.62 ± 11.41	0.015 ± 0.006

* ± SE of 5 determinations.

Table 2. IAA concentration in plasma and erythrocytes after i.p. injection of L-Try (0.3 mmoles/kg)

Concentration of IAA (nmoles/ml)	Time (h) after injection of L-Try 0	0.5	1	3	6
Plasma	0.06	1.20±0.40	1.08±0.17	0.80±0.23	0.51±0.11
Erythrocytes	0.06	0.91±0.34	0.74±0.11	0.46±0.06	0.63±0.17

* ±SE of 5 determinations.

vent mixtures; n-BuOH:CH_3COOH:H_2O (40:10:15, v/v) and methylacetate:propyl alcohol:25% NH_4OH (45:35:20, v/v). The R_f values of authentic IAA and IAAsp were 0.76 and 0.50 with the former solvent, and 0.36 and 0.05 with the latter, respectively. The regions equivalent to the R_f values of IAA and IAAsp of the chromatogram were extracted with 10 ml of 80% ethanol aqueous solution 3 times and the extracted solution was evaporated to dryness at 40 °C. The residue was dried well under a stream of N_2 and 50 µl of trifluoroacetic anhydride (TFAA) was added to the residue and allowed to react for 60 min at 48 °C. Excessive TFAA was removed with a stream of N_2 and the trifluoroacetylated sample was dissolved in 1 ml of methanol containing 10 ng of α-benzene hexachloride (α-BHC). 1 µl of the solution was used for gas chromatographic analysis with a Shimazu Model GC-4BPF gas chromatograph equipped with an electron capture detecter and fitted with a 200 cm glass column, 0.3 cm in diameter. The column packing consisted of 1.5% silicon OV-17 on Gas-Chromosolve W. The column temperature was 200 °C, and the determination temperature was 250 °C. N_2 (40 ml/min) was used as carrier gas.

Results and discussion. When 500 mg (2.85 mmoles) of IAA per kg animal was injected i.p., 78.20±12.59 µmoles of IAA and 0.009±0.002 µmoles of IAAsp were detected in urine samples within 24 h, although none of them was found in the urine of the control animals treated with no IAA. Furthermore, when IAA and aspartic acid (Asp) were simultaneously given, i.p. in the former and s.c. in the latter, IAA and IAAsp were detected more, but when Asp was exclusively applied, they were not detected, as shown in table 1. Weissbach et al.[5] reported that IAA was formed from L-tryptophan enzymatically by guinea-pig kidney and liver extracts, and also by intestinal bacteria. Urinary excretion of IAA was observed by Gordon et al.[7] in germ-free mice to which L-tryptophan was given i.p. However, the formation of IAAsp has never been observed in IAA- or tryptophan-treated animals. In the present experiment, 0.3 mmoles (61.2 mg)/kg of L-tryptophan was injected i.p., IAA was found in both plasma and erythrocytes of the treated rats 30 min after application, but IAAsp was not detected as shown in table 2. In 24-h urine samples, after the application of tryptophan, IAA was detected in quantities as much as 0.088±0.009 µmoles/animal, and IAAsp was not found by the method used, although very small amounts of IAAsp might be present. Further studies on the significance of IAAsp formation are now in progress.

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Studies on the role of the mast cell in local calcergy

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Summary. The treatment of animals with disodium chromoglycate and/or cytochalasins which inhibit mast cell degranulation has no influence on the development of local calcergy induced in the mouse by the s.c. injection of lead acetate.

In a study of the pathogenesis of local calcergy it has been reported by Bridges and McClure[1] that 5 h after the injection of the lead acetate the dermal mast cells have degranulated and the adjacent capillaries are dilated and surrounded by deposits of calcium and phosphate ions. In order to explore the role of the mast cell in this reaction, experiments were performed in which animals were given s.c. injections of lead acetate and also treated with substances known to stabilize mast cells and inhibit their degranulation.

A total of 201 male adult white mice was used (mean weight 30±2.1 g) and 4 experimental procedures were performed. In the 1st experiment 15 animals were used in 3 groups of 5. Each of the 1st group was given a single s.c. injection of lead acetate (PbAc) in 0.1 ml of solution into the lumbar region. The 2nd group was given similar injections and in addition, each animal was given an i.p. injection of 0.3 mg of DSCG (disodium chromoglycate, 'Intal', Fisons Ltd, Leics., England) in 0.1 ml of solution (10 mg/kg b. wt) immediately after the s.c. injection. The 3rd group was similarly treated except that the i.p. dose of DSCG was 3 mg/0.1 ml (100 mg/kg b. wt).

In the 2nd experiment 42 mice were used in 14 groups of 3. Each animal was given an s.c. injection of lead acetate and groups of animals were given either 0.3 mg DSCG/0.1 ml or 3 mg DSCG/0.1 ml by i.p. injection. These latter injections were given 1 h before and 1, 2, 3, 4, 5, 6, and 8 h after the injection of lead acetate.

In the 3rd experiment 72 mice were used in 18 groups of 4. Each animal was injected with PbAc 100 µg/0.1 ml into the lumbar region. Groups of animals were injected (into the same site as PbAc) with cytochalasin A (extract of *Helminthosporium dermatoideum*, Sigma Chemical Co., St. Louis, Mo.). This was made up in twice deionized water in 2