Figure 2 shows a highly significant salivary-gland enlargement during 7–8 days of the animals treated with the capsule containing the 8-mg-solution.

The salivary-gland weights of the rats treated with a capsule containing 2 mg IS were not distinguishable from the controls. Nevertheless, moderate cell-enlargement and mitosis could be seen in the glands of these animals, while substantial cell-proliferation and mitotic activity during 6 days occurred in the animals treated with the 8-mg-solution.

Discussion. The method of giving daily injections of catecholamines has the disadvantage that single doses are catabolized by the animal within a few hours (the half-life of catecholamines in plasma estimated e.g. by Cohen[4] being 1–2 min; Labhart[5] described it to be approximately 10 min) while a low but constant IS-level can be maintained for some days by our method of application. Therefore, if given by single daily injections, a 10fold dose[6] seems to be needed to cause the same effect on salivary-gland enlargement as shown in figure 1.

Although the application of the capsule containing 2 mg of IS has comparatively small visible effect on our target-object, rather striking changes could be seen concerning myocardial histology and some metabolic parameters as will be shown in a following communication. These facts lead us to the opinion that the IS-level causing salivary-gland enlargement can be much lower than hitherto supposed, if it is only kept up for some time.

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Androgenic properties of gibberellic acid in the chick comb bioassay

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Summary. Gibberellic acid stimulates the growth of the comb in the male chicks, but is ineffective in females. Estrogen prevents gibberellin-induced stimulation.

Gibberellins, potent plant hormones, are known to be relatively harmless for animals[1]. There exists evidence that gibberellins might be biologically active in animal organisms. Young animals seem to thrive on the addition of gibberellins to their diet. Gibberellic acid (GA_3) increased the growth of tadpoles[2] as well as the metamorphosis of locusts[3]. Chicks[4] and pigs[5] grew better on fodder containing GA_3 and improved their utilization of feed for a more efficient weight gain. When male mice and rats were injected daily with GA_3 shortly after weaning, their weight gain was consistently higher than that of control animals[6]. The thyroid and adrenal glands were especially well developed and the urinary gonadotropin excretion increased by 50%. An earlier study[7] described experiments with wheat germ oil in which the plant material possessed estrogenic, androgenic and gonadotrophic activity as assessed by a series of biological tests. The ketonic fraction of wheat germ oil was specifically indicated as biologically active. It increased the chick comb weight and the seminal vesicle weight in castrated rats. In other experiments with castrated rats[8] GA_3 partially restored the weight of the prostate and was particularly effective in restoring the size of the levator ani muscle. We report here that GA_3 is also effective in the chick comb growth test which is widely used for assessment of androgenic activity.

White leghorns were divided into groups of 25 birds and injected s.c. every 3 days with 0.2 ml of different concentrations of GA_3 and/or estradiol (E_2) starting 3 days after hatching and ending on the 35th day. The compounds were dissolved in 1:1 glycerol-saline (0.9%) mixture with the addition of 0.5% $NaHCO_3$. Total dose from 10 injections amounted to 20 mg, 2 mg and 0.2 mg GA_3 alone, or in

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Chick comb weight after gibberellin (GA_3) and estradiol (E_2) treatments

Treatment[a]	Males No. of birds	Comb weight (mg)[b]	Females No. of birds	Comb weight (mg)[b]
Control	13	1026 ± 468	12	157 ± 26
0.2 mg GA_3	10	1507 ± 1237	15	152 ± 21
2 mg GA_3	13	1927 ± 860[d]	11	158 ± 40
20 mg GA_3	16	1393 ± 352[c]	9	177 ± 49
0.2 mg E_2	11	523 ± 289[d]	14	162 ± 81
0.2 mg GA_3 + 0.2 mg E_2	11	486 ± 307[d]	14	153 ± 40
2 mg GA_3 + 0.2 mg E_2	14	407 ± 357[d]	11	160 ± 26
20 mg GA_3 + 0.2 mg E_2	11	531 ± 446[c]	13	146 ± 30

[a] Total dose of GA_3 and E_2 listed. [b] Average comb weight ± SD. The difference between control group and treatment is significant to [c] $p < 0.05$, or to [d] $p < 0.01$ by t-test for unequal sample sizes.

combination with 0.2 mg E_2. Body weights were recorded every week. At the end of the experiments the birds were killed, their sex was determined, and their combs were excised and weighed.

There were no significant differences in the body weight between the treated and control groups in our experiment. However, GA_3 increased the comb weight in male chicks, with 2 mg GA_3 being the most effective treatment (table). Estrogen inhibited comb growth in males and was equally effective when injected together with different concentrations of GA_3. Female chick combs did not show any effects of the GA_3 or estrogen injections although androgens normally increase both the male and female chick comb weights[9].

Our experiment confirmed the weak androgenic properties of GA_3 which seems effective in stimulating the growth of the comb in the male chicks and the sex accessory organs in male rats (prostate[8], seminal vesicle[7]). The anabolic activity of GA_3, as noted in the enhancement of body growth[2,4-6] and the levator ani muscle[8] is also reminiscent of androgen action.

However, GA_3 is even more effective in stimulating the growth of the female reproductive organs. It was found to increase the uterus weight in immature[10] and ovariectomized[11] rats and mice. GA_3 apparently acts synergistically with estrogen[10]. In the traumatized uterus of immature, estrone primed rats, GA_3 caused a weight increase of the stimulated horn in a progesterone-like response[10]. Female sex organs are known to be stimulated by androgen treatments[9]. Ovariectomized rats, after estrogen priming, produce significant progestational changes in the uterus within 2 weeks of daily testosterone administration. Progesterone itself has weak androgenic properties, being able to restore partially the prostate weight in castrated male rats[12].

In summary, gibberellic acid apparently possesses some androgenic properties and its action in animal organisms resembles that of progesterone. Further studies are necessary to elucidate the mode of action of GA_3 and its possible interaction with other hormones in male and female organisms at different stages of development.

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DISPUTANDUM

Absence of β-exotoxin in Thuricide® preparations.

A reply to C.B.S.R. Sharma et al.

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Summary. The biological insecticide Thuricide® is produced from *B. thuringiensis*, Berliner, var. *kurstaki* (serotype 3a, 3b), a bacterial strain which does not synthesize exotoxin. Thus, our product is devoid of any C-mitotic or mutagenic potentiality such as is to be found in exotoxin.

Some months ago in this journal, in a paper entitled 'The Exotoxin of *Bacillus thuringiensis:* a New C-Mitotic Agent'[1], some statements were made in relation to the microbial insecticide Thuricide®, a product from Sandoz Ltd, which are incorrect and misleading. In the summary is said: '... an exotoxin from *Bacillus thuringiensis*, a constituent of the microbial insecticide thuricide (sic) has been found...' and further in the text '...there seems to be a need for caution in the extensive use of commercial preparations of *B. thuringiensis* as a microbial insecticide on crop plants.'

First of all, the authors omitted to mention the strain of *Bacillus thuringiensis* (B.t.) they used to produce the exotoxin. This is a very important point in view of the fact that there are, among the numerous serotypes of B.t.[2] some which synthesize exotoxin (e.g. serotype 1), and some which do not (e.g. serotype 3a, 3b)[3]. As a matter of fact, Sharma et al. stated that exotoxin was obtained according to the method of Kim and Huang[4], who used a culture of *B. thuringiensis* var *thuringiensis* belonging to serotype 1.

The biological insecticide Thuricide®, however, is produced from *B.t.* Berliner, var. *kurstaki* (serotype 3a, 3b), a bacterial strain which does not possess the capability to synthesize the thermostable β-exotoxin, also called Thuringiensin A[3]. In this place, it seems necessary to keep in mind that the active principle of Thuricide® is not exotoxin, but a crystalline, high-molecular weight protein, called δ-endotoxin, which is associated with the B.t. spores. Thus, this product is unlikely to be able to possess C-mitotic or mutagenic potentiality, which is related to exotoxin.

Furthermore, the authors used the term 'thuricide' as a common name. Thuricide®, however, is a registered trade mark, the property of the firm Sandoz Ltd., Basle (Switzerland), and reserved for the specific range of products based on *Bacillus thuringiensis*, Berliner, var. *kurstaki* (serotype 3a, 3b). Therefore, the origin of the product or the B.t. strain used should be specified in any future publication dealing with these subjects.

It should also be mentioned that the safety of Thuricide® products for vertebrates (including man) has been consistently demonstrated by extensive toxicity and pathogenicity studies[5,6] which led to their full exemption from residue tolerance for use on food and forage crops by the FDA, now the EPA. As requested by this authority, each batch of Thuricide® is, apart from the other usual quality control procedure, routinely checked for exotoxin as well as for its pathogenicity to mammals. On the basis of the favourable outcome of all these studies, Thuricide® products have been successfully registered also in various other countries in recent years.

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