In this study SMS 201 995 lowers the basal levels of PP, secretin, motilin, pancreatic glucagon and insulin and also effectively suppresses the postprandial release of PP, gastrin, secretin, GIP, pancreatic glucagon and insulin. Hormone suppression was prolonged. SMS 201 995 was found to have a similar pattern of suppression of gastrointestinal hormones, compared to that previously reported for other analogues[15].

SRIF and its analogues have been shown to suppress hormonal hypersecretion in patients with insulinomas, glucagonomas, gastrinomas and VIPomas[14,15] and to inhibit pentagastrin, noradrenaline and alcohol stimulated flushing in patients with carcinoid syndrome, associated with good symptomatic remission[13,16]. However, a major problem in its therapeutic use is its short half-life. In view of the longer duration of action of SMS 201 995 it may prove to be more useful than previous analogues and valuable as an adjunct to surgery or chemotherapy in the treatment of pancreatic endocrine tumors. Ongoing investigations in our department on eight patients with pancreatic endocrine tumors show that SMS 201 995 administered s.c. twice per day suppresses peptide secretion from these tumors resulting in dramatic remission of symptoms[17].

In conclusion the new somatostatin analogues SMS 201 995 is an effective inhibitor of the release of gastroenteropancreatic hormones. Because of its longer duration of action it is likely to be more useful therapeutically in patients with endocrine pancreatic tumors.

1 Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R., Science *179* (1973) 77.
2 Schusdziarra, V., Horm. Metab. Res. *12* (1980) 563.
3 Siler, T.M., Yen, S.S.C., Vale, W., and Guillemin, R., Clin. Endocr. *38* (1974) 742.
4 Bloom. S.R., Mortimer, C.H., Thorner, M.O., Besser, G.M., Hall, R., Gomez-Pan, A., Roy, V.M., Russel, R.C.G., Coy, D.H., Kastin, A.J., and Schally, A., Lancet *1* (1974) 1106.
5 Rivier, J., Brown, M., and Vale, W., Biochem. biophys. Res. Commun. *65* (1975) 746.
6 Brown, M., Rivier, J., and Vale, W., Science *196* (1977) 1467.
7 Vale, W., Rivier. J., Ling, N., and Brown, N., Metabolism *27*, suppl. 1 (1978) 1391.
8 Bauer, W., Briner, U., Doeptner, W., Haller, R., Huguenin, R., Marbach, P., Petcher, T.J., and Pless, J., Life Sci. *31* (1982) 1133.
9 Bloom, S.R., and Long. R.G., Radioimmunoassay of gut regulatory peptides. W.B. Saunders Co. Ltd, London 1982.
10 Sheppard, M., Shapiro, B., Primstone, B., Kronheim, S., Berelowitz, M., and Gregory, M., J. clin. Endocr. Metab. *43* (1979) 50.
11 Vale, W., Rivier, C., Brazeau, P., and Guillemin, R., Endocrinology *95* (1974) 968.
12 Redding, T.W., and Coy, D.H., 56th Meeting of the American Endocrine Society, Atlanta, 1984 (Abstract 198).
13 Long, R.G., Peters, J.R., Bloom, S.R., Brown, M.R., Vale, W., Rivier, J.E., and Grahame-Smith, D.G., Gut *22* (1981) 549.
14 Long, R.G., Peters, J.R., Bloom, S.R., Brown, M.R., Rivier, J.E., Barnes, A.J., Mallinson, C.N., Vale, W., and Christofides, N.D., Lancet *2* (1979) 764.
15 Adrian, T.E., Barnes, A.J., Long. R.G., O'Shaughnessy, D.J., Brown, M., Rivier, J., Vale, W., Blackburn, A.M., and Bloom, S.R., J. clin. Endocr. Metab. *53* (1981) 675.
16 Frolich, J.C., Bloomgarden, Z.T., Oates, J.A., McGuigon, J.E., and Rabinowitz, D., N. Engl. J. Med. *299* (1978) 1055.
17 Wood, S.M., Kraenzlin, M.E., and Bloom, S.R., Gut, in press (1985).

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Ecdysteroids: possible candidates for the hormone which triggers salivary gland degeneration in the ixodid tick *Amblyomma hebraeum**

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Summary. Following engorgement, female ixodid ticks secrete a 'tick salivary gland degeneration factor' (TSGDF) into the hemolymph. Here we show that the arthropod ecdysteroid hormones, ecdysone and 20-hydroxyecdysone, induce degeneration of tick salivary glands maintained in organ culture. The effective dose range in vitro is 30–300 ng/ml, a range reported to be physiological for this species following repletion. In addition, infusion of 20-hydroxyecdysone in vivo induces salivary gland degeneration. We therefore propose that TSGDF may be an ecdysteroid.
Key words. Ecdysone; 20-hydroxyecdysone; ticks; salivary gland degeneration.

Female ixodid ticks commonly increase their b.wt 100-fold or more by imbibing blood of the mammalian host[3]. The blood meal is hyposmotic to tick body fluids (290 mOsm vs 360 mOsm[4]); thus, osmoregulation during this period is accomplished by the secretion of a copious, slightly hyposmotic saliva back into the host's circulation[4,5]. The nutrient portion of the blood meal is also concentrated as a result of salivary fluid secretion.

Following detachment of the tick from the host, secretory ability of the salivary glands is reduced[6]. This loss of function is due to autolysis[7] and is triggered by a blood-borne factor which we have called 'tick salivary gland degeneration factor' (TSGDF)[6]. Salivary gland degeneration will not proceed in female *Amblyomma hebraeum* unless they have fed beyond a weight of approximately 0.30–0.40 g[8]. Degeneration is blocked by severing the opisthosomal nerves; we have thus suggested that stretch receptors in the abdomen signal the CNS to initiate salivary gland degeneration, once a critical weight has been attained[8]. The majority of females will not feed to the critical weight unless they have mated. However, those virgin ticks which do feed beyond the critical level nevertheless fail to resorb their salivary glands[9]. The latter suggests that, in addition to promoting full engorgement, mating exerts a direct influence on salivary gland degeneration. We recently showed that a chemical factor, distinct from TSGDF, must be transferred from the male to the female during copulation in order for salivary gland degeneration to proceed[8]. The identity of the male factor is, as yet, unknown.

Recently, ecdysone and 20-hydroxyecdysone have been identified in the hemolymph of *Amblyomma hebraeum*[10]. Ecdysteroid titres in the hemolymph rise from approximately 10 ng/ml to over 300 ng/ml during the period that salivary gland resorption occurs[11]. Here we propose that TSGDF is an ecdysteroid.

Materials and methods. 1) Ticks. Ticks (*Amblyomma hebraeum* Koch) were obtained from a laboratory colony maintained in darkness, at 26 °C, at a relative humidity of 95%. An equal number of males and females were confined to the backs of rabbits as previously described[4].

2) Organ culture. We used a modification of the 'backless tick explant' organ culture method described by Bell[12]. Briefly, female ticks were removed from the host and surface sterilized by submersion for 1 min in 0.1% thimerosol (Sigma) followed by submersion for 1 min in 70% ethanol. The ticks were transferred to a sterile air cabinet (horizontal laminar flow), and glued (cyanoacrylate glue; alpha aron; Tagosei Chemical Co. Japan) ventral side down to the bottom of a disposable petri dish and covered with sterile tissue culture medium (TCM 199; Gibco). The dorsal cuticle was removed along with the gut diverticula and discarded. The explants were rinsed with three changes of sterile TCM 199, covered with approximately 5 ml of culture medium, and held for 4 days at 26 °C, 95% relative humidity.

3) Assay for fluid secretory competence. Fluid secretory competence was assayed as previously described[8]. Briefly, salivary glands were removed from the explants (or dissected out of intact ticks) after the main salivary ducts had been ligated with 8-0 silk (Davis and Geck) and severed distal to the ligature. The glands were rinsed in TCM 199 and blotted on filter paper for 15 s. After a further 15 s, wet weights were recorded to the nearest 20 µg (typical weights for salivary glands ranged from 3 to 11 mg). The glands were incubated in agitated TCM 199 containing either 1 µM or 10 µM dopamine (Sigma), as indicated for each experiment. These concentrations are known to elicit maximal rates of fluid secretion[13]. After 10–15 min incubation, the glands were again blotted and weighed, and the net gain in weight taken as an index of fluid secretory competence.

4) Infusion of small ticks. A Harvard µl-syringe pump was custom-fitted by the company with a two-tier platform bearing slots for 12 syringes. The solution to be infused was taken up in 1-ml all glass syringes fitted with 30-gauge needles. After selecting the infusion rate, the pump was run for at least 15–20 min before mounting the ticks, in order to achieve a stable rate of fluid delivery. Prior to mounting it on the infusion apparatus, each tick was injected with a priming dose of the infusion medium (2 µl/100 mg b.wt), by means of an 'Alga' µm-syringe unit (Wellcome Reagents Ltd), as previously described[14]. To mount the tick, the needle was introduced into the hemocoel through the articulation between the scutum and capitulum. On pushing the tick gently forward, the needle tip glided easily a few mm into the hemocoel without rupturing the gut diverticula. An adjustable platform was then moved into position to support the ticks. All ticks were infused for 22–24 h at 3.5 or 4.9 µl/h. However, their weight gain was much less than would be indicated by these figures, because much of the fluid injected was excreted by the salivary glands. Thus, the ticks which received 3.5 µl/h (84 µl/day) gained only 21 ± 8 mg (mean ± SEM, n = 10), and the ticks which received 4.9 µl/h (118 µl/day) gained only 42 ± 6 mg (n = 27). Any tick which attained a net weight greater than 0.28 g following infusion was discarded. 20-Hydroxyecdysone (1 mg/ml) was dissolved in 70% ethanol, and this stock solution diluted to the final infusate concentration in sterile TCM 199 containing 200 units/ml penicillin G and 200 µg/ml streptomycin sulphate (Gibco). Since the highest concentration of ethanol in the infusate was 0.7%, vehicle injected controls were infused with that concentration of ethanol. It was necessary to infuse a vehicle rich in nutrients, such as TCM 199. Infusion of isosmotic saline or water vehicle was detrimental to subsequent salivary gland function. To avoid serious bacterial infection, it was also imperative to maintain sterility when infusing TCM 199. At the conclusion of each infusion session, the rate of fluid delivery for each syringe was measured directly. Calibrated µl capillary tubes (containing a small column of liquid paraffin to prevent evaporation) were placed over the needles, and the infusate collected for a few h. The doses of 20-hydroxyecdysone infused into the ticks were 0, 0.07 ± 0.002, 0.68 ± 0.02, 3.62 ± 0.04, 7.15 ± 0.34 and 10.9 ± 0.5 nmoles/g b.wt.

5) Experimental protocol. For experiment 1, ticks over 0.40 g (henceforth called 'large ticks') were put into culture at 0, 12, 24, 48, and 96 h after removal from the host. Fluid secretory competence (in 10 µM dopamine) was assessed 96 h after the ticks were removed from the host. For experiment 2, ticks which weighed 0.18–0.30 g (henceforth called 'small ticks') were cultured for 4 days in the presence of a mixture of ecdysone and 20-hydroxyecdysone (1/1; w/w). The concentrations tested were 10, 30, 100, 300 and 1000 ng total ecdysteroid/ml. Control ticks were cultured in sterile TCM 199 containing 0.07% ethanol (the highest concentration of ethanol in the vehicle). Fluid secretory competence was assayed in 10 µM dopamine. For experiment 3, small ticks, which normally do not suffer salivary gland degener-

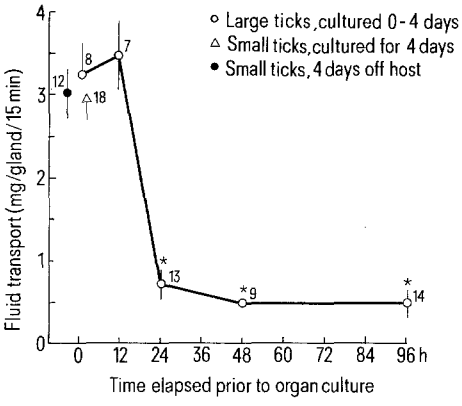

Figure 1. Fluid secretory ability of salivary glands at 96 h post removal following various treatments. For this experiment, 10 µM dopamine was used to assay fluid secretory competence. ○, Large ticks cultured for 0–4 days; △, small ticks cultured for 4 days; ●, small ticks 4 days post removal (these data being taken from Harris and Kaufman[8]). Salivary gland degeneration did not occur in ticks put into culture up to 12 h after removal from the host. However, degeneration proceeded virtually to completion in ticks placed into culture 24 h or more after removal from the host. In this and other figures, SEM is shown when it exceeds the dimension of the symbol. * denotes a significant ($p < 0.05$) loss of secretory competence as compared to ticks put into culture at time 0.

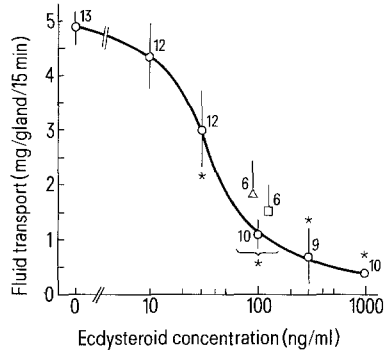

Figure 2. Secretory ability of salivary glands from small ticks cultured in the presence of ecdysone and 20-hydroxyecdysone. A dose dependent loss of secretory function was induced by the ecdysteroids. ○, Glands cultured with an equal mixture (w/w) of ecdysone and 20-hydroxyecdysone; △, glands cultured with ecdysone; □, glands cultured with 20-hydroxyecdysone. * denotes a significant loss ($p < 0.05$) of secretory ability as compared to glands from ticks cultured in 0.07% ethanol in TCM 199. For this experiment, 10 µM dopamine was used to assay fluid secretory competence.

ation within 4 days, were infused with 20-hydroxyecdysone during the first 24 h post-removal from the host, as described in detail above. 3 days after the termination of infusion, fluid secretory competence (in 1 μM dopamine) was tested.

6) Statistical analysis. All treatments were compared by one-way analysis of variance followed by a Student-Newman-Kules test[15]. Differences were considered significant at the $p < 0.05$ level. Data are expressed as mean ± SEM (n).

Results and discussion. Normally, salivary glands from engorged female *Amblyomma hebraeum* lose irreversibly over 95% of their secretory ability by 4 days post-removal from the host (12.93 ± 1.50 mg/gland/15 min (n = 10) for day 0 vs 0.31 ± 0.10 mg/gland/15 min (n = 11) for day 4)[8]. However, the salivary glands of small mated ticks lose only about 73% of their fluid secretory competence during the same period (11.62 ± 1.11 mg/gland/15 min (n = 11) for day 0 vs 3.10 ± 0.26 mg/gland/15 min (n = 12) for day 4)[8], and retain this level of secretory ability for at least 11 more days (3.12 ± 0.19 mg/gland/15 min (n = 6) for day 15)[8]. Much of the lost secretory ability is regained if such ticks are allowed to reattach and recommence feeding[8]. Salivary glands from small ticks cultured for 4 days secreted 3.00 ± 0.34 mg/gland/15 min (n = 18; fig. 1). This rate was not significantly different from the value of 3.10 ± 0.26 mg/gland/15 min (n = 12) previously reported for small ticks 4 days post-removal from the host (Harris and Kaufman[8]; also shown in fig. 1). Thus, salivary glands fare very well during 4 days of organ culture.

After 4 days of organ culture, salivary glands from large ticks secreted 3.15 ± 0.48 mg/gland/15 min (n = 8), a rate not significantly different from that found in small ticks cultured under the same conditions (see above and fig. 1). Thus, under organ culture conditions, TSGDF either was not released in large ticks or else it was diluted to below threshold concentration by the large volume (5 ml) of culture medium. Figure 1 shows, however, that if organ culture is initiated 24 h after removal of large ticks from the host, salivary gland degeneration proceeds normally. In other words, even though loss of secretory competence is only partial in engorged ticks by 24 h post-detachment (7.00 ± 0.63 mg/gland/15 min, n = 7)[8], further progression of degeneration cannot be halted by removing TSGDF from the bathing medium.

Figure 2 illustrates the effect of ecdysteroids on the secretory competence of salivary glands from small ticks kept in organ culture. Salivary glands taken from control ticks (cultured with 0.07% ethanol) secreted 4.88 ± 0.32 mg/gland/15 min (n = 13), a value significantly higher ($p < 0.05$; t-test) than that observed for the salivary glands of small ticks cultured without ethanol (3.00 ± 0.34 mg/gland/15 min, n = 18; fig. 1). Thus, very low concentrations of ethanol might actually improve the viability of tick tissues maintained in organ culture.

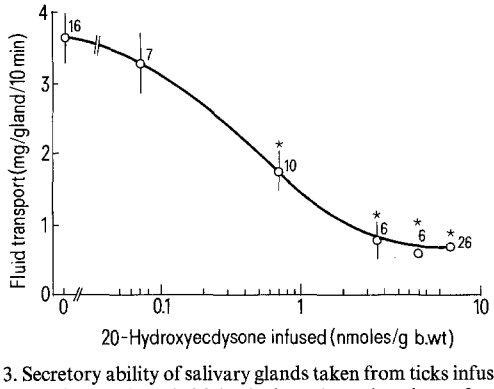

Figure 3. Secretory ability of salivary glands taken from ticks infused with 20-hydroxyecdysone for 22–24 h. A dose dependent loss of secretory ability resulted from the infusion of 20-hydroxyecdysone. * denotes a significant ($p < 0.05$) loss of secretory function as compared to salivary glands taken from ticks infused with vehicle. For this experiment, 1 μM dopamine was used to assay fluid secretory competence.

Salivary glands exposed to as little as 300 ng ecdysteroids/ml culture medium degenerated to the same degree (0.73 ± 0.50 mg/gland/15 min, n = 9) as salivary glands from large unoperated ticks 4 days off the host (0.44 ± 0.16 mg/gland/15 min, n = 14). Ecdysone or 20-hydroxyecdysone alone was equipotent to the mixture (fig. 2). As shown in figure 1, salivary glands from large ticks kept in organ culture for 4 days do not degenerate. However, salivary glands from such ticks, cultured in the presence of 100 ng ecdysteroid/ml, secreted 1.38 ± 0.36 mg/gland/15 min (n = 6). This was not significantly different from the secretory ability of salivary glands taken from small ticks cultured under the same conditions (1.12 ± 0.29 mg/gland/15 min, n = 10; fig. 2). Thus, ecdysteroids induce degeneration of salivary glands from small and large ticks maintained in organ culture.

Constant infusion of 20-hydroxyecdysone in vivo also caused a dose dependent loss of secretory competence (fig. 3). The hormone was infused only during the first 22–24 h, since salivary gland degeneration is irreversible 24 h after initiation (see fig. 1). Small ticks infused with as little as 0.68 nmoles 20-hydroxyecdysone/g b.wt lost a significant degree of secretory function compared to vehicle injected controls. Thus, 20-hydroxyecdysone triggers salivary gland autolysis in vivo. The hormone must be infused over a relatively long period of time. Earlier attempts in our laboratory to induce salivary gland degeneration by single or double bolus injections proved relatively ineffective (Kaufman, unpublished data). This might have been due to the short half life of 20-hydroxyecdysone in tick hemolymph, as observed in some other arthropods. For example, in the dipteran, *Sarcophaga peregrina,* about half the molting hormone activity of injected, radiolabeled 20-hydroxyecdysone was lost 1 h after injection[16]. Thus, large, but infrequent, doses are probably soon reduced to ineffective titres.

The doses of ecdysteroids used in our in vitro experiment were well within the physiological range found in *A. hebraeum* ticks during the period that salivary gland degeneration normally occurs; hemolymph ecdysteroid titres rise from approximately 10 ng/ml, to over 300 ng/ml at the initiation of egg production[11]. Our data strongly suggest that an ecdysteroid is the 'tick salivary gland degeneration factor'.

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3 Balashov, Y.S., Misc. Publ. ent. Soc. Am. *8* (1972) 161.
4 Kaufman, W.R., and Phillips, J.E., J. exp. Biol. *58* (1973) 523.
5 Tatchell, R.J., Nature, Lond. *213* (1967) 940.
6 Harris, R.A., and Kaufman, W.R., J. Insect Physiol. *27* (1981) 241.
7 Till, W.M., Memoirs of the Entomological Society of South Africa, No. 6, (1961) p. 1.
8 Harris, R.A., and Kaufman, W.R., J. exp. Biol. *109* (1984) 281.
9 Kaufman, W.R., in: Current Topics in Vector Research, p. 215. Ed. K.F. Harris. Praeger Scientific, New York 1983.
10 Diehl, P.A., Germond, J.E., and Morici, M., Revue suisse Zool. *89* (1982) 859.
11 Diehl, P.A., Pers. communication, Univ. Neuchâtel, Switzerland.
12 Bell, L.J., Acta trop. *37* (1980) 319.
13 Kaufman, W.R., J. exp. Biol. *64* (1976) 727.
14 Kaufman, W.R., Am. J. Physiol. *235* (1978) R76.
15 Zar, J.H., Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J. 1974.
16 Moribayashi, A.F., and Ohtaki, T., J. Insect Physiol. *24* (1978) 279.

0014-4754/85/060740-03$1.50 + 0.20/0