Effect of ZR-515 Methoprene on *Eucelatoria* sp.

	No. of larvae parasitized	No. of puparia obtained	No. of flies emerged
Larvae treated with JH analogue and parasitized	25	82	Nil
Untreated and parasitized larvae	25	74	72

controlled conditions. *Eucelatoria* sp., originating from the USA, was obtained through the Indian station of the Commonwealth Institute of Biological Control, Bangalore, and mass-multiplied in this laboratory on *H. armigera* for use in biological control programmes.

For experimental purposes, healthy *Heliothis* larvae in their penultimate larval stage were taken. In one group, the JH analogue was topically applied to the larvae at the dose of 1 µl (= 1 mg, in olive oil) per larva. They were fed on the artificial diet and the effect on metamorphosis was observed. In another group, the treated larvae were parasitized by *Eucelatoria* and the effect of the JH analogue on the final emergence of the parasitoid was observed. Untreated *Heliothis* larvae of the same age group and untreated larvae parasitized by *Eucelatoria* were used as controls. 5 larvae were taken each time with 5 replicates. The results are shown in the table.

When the JH analogue was applied to *Heliothis* larvae, the majority of them failed to pupate. The larval life was prolonged to over 10–15 days after the controls pupated and the larvae were much larger in size than the normal ones. They died as larval-pupal intermediates with either partial pupation or with pupal cuticular patches on the body. A few of those which pupated did not develop into adult moths.

When the treated larvae were parasitized, they died on the 2nd or 3rd day after parasitisation as in the controls, and the puparia of the parasitoid were obtained. However, the emergence of flies was nil even after 3 weeks, while flies emerged from the untreated parasitized controls within 1 week.

Wilkinson and Ignoffo observed no apparent effects on adult emergence, length of life and sex-ratio of *Apanteles rubecula* when a JH analogue was applied to its host, *Pieris rapae*[6]. However, topical application of a JH analogue to 6th instar larvae of *Choristoneura fumiferana* (Clem.) adversely affected the development of the tortricid and its parasites *Glypta fumiferanae* (Vier.), *Meteorus trachynotus* Vier., and *Phryxe pecosensis* (Tns.)[7]. Similarly, JH analogue treatment of *Liriomyza sativae* not only inhibited adult emergence of the host but also its parasite, *Opius dimidiatus* (Ashm.)[9]. The data in the present experiment also indicate that Methoprene applied to *Heliothis armigera* adversely affects the emergence of its parasite, *Eucelatoria* sp., as in the case of another tachinid endoparasite, *P. pavida* when Methoprene is applied to the parasitized host, *Galleria mellonella* L.[8].

1 The author is grateful to the Plant Protection Adviser to the Government of India for encouragement and facilities provided; to Dr A.D. Pawar, Directorate of Plant Protection, Quarantine and Storage, Faridabad, and Dr T. Sankaran, Entomologist-in-charge, Commonwealth Institute of Biological Control, Indian Station, Bangalore for useful suggestions and for critically going through the manuscript; also to Prof V.J.A. Novak, Czechoslovak Academy of Sciences, Prague, for the kind supply of the JH analogue.

2 W.R. Bransby-Williams, E. Afr. agric. For. J. *38*, 170 (1972).
3 J. Benskin and S.B. Vinson, J. econ. Ent. *66*, 15 (1973).
4 A.A. Guerra, D.A. Wolfenbarger and R.D. Garcia, J. econ. Ent. *66*, 833 (1973).
5 M.A. El-Guindy and S.I. Bishara, Z. ang. Ent. *81*, 75 (1976).
6 J.D. Wilkinson and C.M. Ignoffo, J. econ. Ent. *66*, 643 (1973).
7 I. Outram, Envir. Ent. *3*, 361 (1974).
8 J.L. Riviere, Entomophaga *20*, 373 (1975).
9 K.M. Lema and S.L. Poe, Fla. Ent. *61*, 67 (1978).
10 Sudha Nagarkatti and Satya Prakash, Tech. Bull. Commonw. Inst. biol. Control *17*, 169 (1974).

Renin in mouse but not in rat submandibular glands

B.J. Morris, R.T. de Zwart and J.A. Young[1]

Department of Physiology, The University of Sydney, Sydney (N.S.W. 2006, Australia), 11 January 1980

Summary. Renin was found in the submandibular glands of male Quackenbush mice in concentrations higher than has been reported for any tissue of any strain or species. However, no renin-like activity could be detected in glands from male and female Wistar rats using either pH 5.8 or 7.4 for assay and a radioimmunoassay specific for renin's reaction product, angiotensin I. Rabbit submandibular glands contained renin.

The submandibular (submaxillary) gland of the male mouse would appear to contain the highest concentration of renin (EC 3.4.99.19) of any tissue of any species[2–7]. The high concentration is due to an action of testosterone[5]. Submandibular renin has been isolated and is immunologically and biochemically similar to renal renin[7]. However, the physiological function, if any, of submandibular renin in the mouse is not known. Rat submandibular glands have also been reported to contain high concentrations of renin-like activity[8–10]. Renin is usually determined indirectly in terms of the initial velocity of its hydrolysis of angiotensinogen to liberate the decapeptide angiotensin I. In the studies of rat glands[8–10] a bioassay was used. In bioassay, the reaction product, angiotensin I (AI), is quantified by i.v. injection of AI which is converted into the pressor octapeptide angiotensin II (AII) in an anaesthetized rat. The assay does not therefore differentiate between AI and AII. This is particularly important as rat submandibular glands contain a serine protease, tonin,[11,12] which can hydrolyze AII directly from angiotensinogen, without formation of AI. Furthermore the studies of rat glands used pH 5.5[8], 5.8[9] and 6.5[10] during incubation of samples with angiotensinogen and recent evidence indicates that at pH less than 6.5 (but not at pH 7.4) tissue cathepsin D can hydrolyze AI from angiotensinogen and so give the illusion of renin activity[13–15]. The present study used a radioimmunoassay specific for AI and assay pH values of 5.8 and 7.4 in a re-examination of the question of renin in rat submandibular glands.

Materials and methods. Adult male and female Quackenbush and Balb/C mice, Wistar rats and Castle Hill white

Renin in submaxillary glands

		Renin (pmoles AI · h^{-1}/tissue) Assay pH 5.8	7.4
Mouse	♂ (n=14)	$(13.6\pm0.6)\times10^8$	$(7.9\pm0.2)\times10^8$
	♀ (n=14)	1430± 120	1010± 110
Rat	♂ (n=26)	<1	<1
	♀ (n=20)	<1	<1
Rabbit	♂ (n=8)	2560± 330	1970± 180
	♀ (n=12)	209± 15	107± 10

rabbits were used. The animals were killed by a blow on the head (mice and rats) or by i.v. pentobarbitone (rabbits). The submandibular glands were rapidly removed on to ice, rinsed in cold 0.9% NaCl, blotted dry, weighed and frozen. Next day the tissue was thawed and each gland was homogenized in cold, distilled water (1:10, wt/vol.) using a Potter-Elvehjem homogenizer and particulate matter was removed by centrifugation at $1000\times g$ for 10 min. Samples of 10 or 50 µl, diluted if necessary, were incubated in glass Wasserman tubes at 37 °C with 200 µl plasma from nephrectomized sheep (2500 pmoles angiotensinogen/ml). Each incubation mixture contained, in addition, 10 mM EDTA, 1 mM 2,3-dimercapto-1-propanol, 2 mM 8-quinolinol, as angiotensinase inhibitors, and 50 mM sodium phosphate buffer of either pH 5.8 or 7.4. The reaction was terminated by adding 2.1 ml of cold, distilled water and placing the tubes in a boiling water bath for 3 min. After centrifugation at $1000\times g$ for 10 min the amount of AI in 10-, 50-, 100- and 200-µl portions of supernatant was determined using an established radioimmunoassay technique[16] in which AI antiserum[17] (1:48,000), having <0.3% cross-reactivity with angiotensin II, and ^{125}I-labelled AI (10^4 cpm of 10^3 µCi/µg; New England Nuclear, Boston, MA), made up in 0.1 M Tris, pH 8, 0.5% BSA, were incubated in a total volume of 1.2 ml for 16–20 h at 4 °C and then free AI was separated from antibody-bound AI by absorption with dextran-coated charcoal[18] (0.1 M Tris, 1% Dextran T-10, 2.5% activated charcoal). [Asp^1, Ile^5] AI (0.015-2.0 pmoles; Beckman Instruments Inc., Palo Alto, CA) was used as standard.

Results and discussion. The submandibular gland of the male Quackenbush mouse contained of the order of a billion pmoles AI · h^{-1}/g tissue of renin activity using sheep angiotensinogen as substrate. This exceeds values, which we have derived, of $2\text{–}5\times10^7$ pmoles AI · h^{-1}/g from studies of C/Novo[4], NMRI[4,5], Charles River[5] and Swiss Webster[6,7] strains using rat[4,5], hog[6] or (1-14) tetradecapeptide[7] renin substrate and assay pH's of 5.5[5], 6.4[6], 6.8[7] and 7.5[4]. The high renin levels in the Quackenbush mouse would appear to be peculiar to this strain; in glands from male Balb/C mice renin was found to be $2.9\pm0.8\times10^5$ pmoles AI · h^{-1}/g (n=12) (assay pH=7.4). The high values we obtained were indicated in several assays performed on different days and represent the highest tissue levels of renin reported. Values at pH 5.8 exceeded those at pH 7.4 as pH 5.8 is close to the pH optimum of the renin-angiotensinogen reaction. The K_m of the reaction of mouse submandibular renin with sheep angiotensinogen was 600–800 pmoles/ml, suggesting that a higher affinity for substrate did not contribute to the high values. In marked contrast no renin-like activity could be detected in submandibular glands of male and female Wistar rats at either pH and with either sheep or rat angiotensinogen as substrate. This finding was confirmed in 5 repetitions of the assay and using tissue preparations obtained weeks apart. In plasma of rats we have found renin values of 5–30 pmoles AI · h^{-1}/ml with the assay described. In submandibular glands of rats the published bioassay values for renin-like activity in terms of pmoles angiotensin · h^{-1}/g tissue, calculated by us for comparison, are approximately 600 (♂ Wistar/NIH)[8], 100 (♀ Wistar/NIH)[8], 9000 (♂ Wistar/Kyoto)[8], 1000 (♂ Sprague-Dawley)[8], 1×10^4 (♂ and ♀ spontaneously hypertensive/NIH or /Kyoto)[8], 6×10^5 (♂ Wistar)[9] and 2×10^4 pmoles angiotensin · h^{-1}/g (♂ Sabra)[10]. Although these measurements were made using assay pH's of less than 6.5, in the present study we found no measurable activity at pH 5.8, indicating that cathepsin D was not responsible. Furthermore, by rat pressor bioassay[19] of samples incubated at pH 7.4 we found pressor material equivalent to 1×10^4 pmoles angiotensin · h^{-1}/g tissue (♂ Wistar). The possibility of renin-like activity reported in the rat[8–10] being due entirely or in part to tonin is supported by the similarity in their location, in the pressor activity of their reaction products, in their pH optima and in the effect of enzyme inhibitors[9,11,12], although it has been stated that tonin is not active in plasma[11]. Furthermore, we have found that addition of 5 mM phenylmethylsulphonyl fluoride or 0.32 mg/ml soybean trypsin inhibitor to the boiled, rat submandibular/sheep plasma incubates did not alter the pressor response. Moreover, an identical pressor response was observed after blockade of angiotensin receptors with 1 nmole [Sar^1, Ala^8] angiotensin II injected i.v. 5 min prior to injection of the sample. These results suggest that some other pressor factor other than angiotensin II may be present in the incubates. The rabbit also contains activity resembling renin in its submandibular glands and the findings of 10 times more in the male than in the female suggest that, like the mouse, this renin is under androgenic control.

1 We wish to express our sincere thanks to Dr E. Hackenthal for his suggestions. This study was supported by the National Heath and Medical Research Council of Australia.

2 E. Werle, R. Vogel and L.F. Göldel, Arch. exp. Path. Pharmak. *230*, 236 (1957).
3 H. Turrian, Helv. physiol. Acta *18*, 259 (1960).
4 J. Bing and P. Fårup, Acta path. microbiol. scand. *64*, 203 (1965).
5 W.J. Oliver and F. Gross, Am. J. Physiol. *213*, 341 (1967).
6 T.S. Chiang, E.G. Erdös, I. Miwa, L.L. Tague and J.J. Coalson, Circulation Res. *23*, 507 (1968).
7 S. Cohen, J.M. Taylor, K. Murakami, A.M. Michelakis and T. Inagami, Biochemistry *11*, 4286 (1972).
8 W. de Jong, W. Lovenberg and A. Sjoerdsma, Biochem. Pharmac. *21*, 2123 (1972).
9 E. Hackenthal, Chr. Koch, Th. Bergemann and F. Gross, Biochem. Pharmac. *21*, 2779 (1972).
10 Y. Gutman, M. Levy and J. Shorr, Br. J. Pharmac. *47*, 59 (1973).
11 R. Boucher, S. Demassieux, R. Garcia and J. Genest, Circulation Res. *41*, Suppl. II, 26 (1977).
12 N.G. Seidah, R. Routhier, M. Caron, M. Chrétien, S. Demassieux, R. Boucher and J. Genest, Can. J. Biochem. *56*, 920 (1978).
13 R.P. Day and I.A. Reid, Endocrinology *99*, 93 (1976).
14 E. Hackenthal, R. Hackenthal and U. Hilgenfeldt, Biochim. biophys. Acta *522*, 574 (1978).
15 B.J. Morris and I.A. Reid, Endocrinology *103*, 1289 (1978).
16 J.R. Stockigt, R.D. Collins and E.G. Biglieri, Circulation Res. *28/29* Supp. II, 175 (1971).
17 G. Moore, A. Lutterodt, G. Burford and K. Lederis, Endocrinology *101*, 1421 (1977).
18 V. Herbert, K.-S. Lau, C.W. Gottlieb and S.J. Bleicher, J. clin. Endocr. Metab. *25*, 1375 (1965).
19 W.S. Peart, Biochem. J. *59*, 300 (1955).