is a direct measure of turbidity, increased first and then decreased. Many workers have reported the pattern of ADP-induced platelet aggregation. The pattern of chitosan-induced aggregation resembles the ADP-induced pattern. Thus, chitosan can act as a normal agonist like ADP. The initial increase in optical density was reported to be due to shape change of platelets¹⁰.

In the present study we observed increased platelet aggregation in diabetic condition. This can be explained by the findings of many workers. In 1972, Kwaan et al.¹¹ found out that there is significant increase in ADP-induced platelet aggregation in diabetic samples compared to controls. Aggregation in washed platelets from rats with diabetes was enhanced; this results in prolongation of thrombogenesis¹². Platelet aggregation in response to ADP in washed platelets was increased in diabetic animals compared to controls. This is due to increased platelet size in diabetic animals¹³. In vitro platelet hyperaggregation was found induced with epinephrine in diabetic condition¹⁴.

The observed significant decrease in the number of platelets in diabetic rats can be explained as follows. Zucker explains in his study that in severe atherosclerosis and in diseases such as thrombosis and diabetes, in addition to the growth factor, two other substances, platelet factor-4 and \(\beta \) thromboglobulin are released in large amounts, resulting in their rapid depletion from the circulation¹⁵. So the observed decrease in platelet number in both sexes of animals during diabetes may be related to altered secretory function of platelets. The decreased platelet number directly affects the platelet plug formation, which is an essential step involved in blood clotting. But from a visual observation we found that chitosan enhances wound healing in diabetic condition. Our result also showed that chitosan cannot normalize platelet aggregation in diabetic conditions. These two results point out that there is no relation between chitosan-induced platelet aggregation and wound healing. Biagini et al. 16. reported that in patients undergoing plastic surgery, the donor sites were treated with N-carboxybutyl chitosan, which enhanced wound healing. This N-carboxybutyl chitosan leads to formation of regularly organized cutaneous tissue and reduces anomalous healing. Although chitosan was not found to help in normalizing the altered platelet aggregation, it helps in wound healing. Thus, chitosan-induced enhanced wound healing in diabetes observed in the present study may be due to organization of newly formed tissue and thus help in damage repair.

In conclusion this study presents the effect of the bioactive polymer chitosan in wound healing in diabetes mellitus.

- Chitin and Chitosan (ed. Anthonsen, T. and Sandford, P.), Elsevier publ., 1989, I edn, pp. 813-819
- 4 Makino, N., Dhalla, K. S., Elimban, V and Dhalla, N. S., Am. J. Physiol, 1987, 253, 202-207
- 5. Domino, S. E., Repaske, M. G., Bonner, A., Kennedy, M. E., Wilson, A. L., Brandon, S. and Limbird, L. E., in *Methods Enzymol*, 1992, 215, 181-200.
- 6 Frojmovic, M. M., Milton, T. G. and Duchstel, A., J. Lab Clin. Med., 1983, 101, 964-976.
- 7. Jamaluddin, M. P. and Krishnan, L. K., J. Biochem. Biophys. Methods, 1987, 14, 191-200.
- 8. Junod, A, Lambert, A. E, Stauffacher, W. and Renold, A. E., J. Clin Invest., 1969, 48, 2129-2139.
- 9 Born, G V. R., Nature, 1962, 194, 927-929.
- 10. Hantgan, R. R., Blood, 1984, 64, 896-906.
- Kwaan, H. C., Colwell, J. A., Cruz, S., Suwanwela, N. and Dobbie,
 J. G., J. Lab. Clin. Med., 1972, 80, 236-239.
- 12. Takıguchi, Y., Wada, K., Matsuno, H. and Nakashima, M, Thromb. Res., 1991, 63, 445-456.
- 13. Dunbar, J. C., Reinholt, L., Henry, R. L., Mammen, E., Diabetes Res. Clin Pract., 1990, 9, 265-272.
- 14 Toth, L., Szenasi, P., Jambor, G., Kammerer, L. and Romics, L., Diabetes Res. Clin. Pract., 1992, 2, 143-148.
- 15. Zucker, M. B., Sci. Am., 1980, 242, 86-103.
- 16 Biagini, G., Bertani, A., Muzzaarelli, R., Domadei, A., DiBenedelto, G., Belligolli, A., Riccotti, G., Zucchinic, R. C., *Biomaterials*, 1991, 3, 281-286.

ACKNOWLEDGEMENTS This work was supported by a grant from Department of Atomic Energy, BARC, India. PSP thanks CSIR for a fellowship.

Received 19 June 1995; revised accepted 15 July 1995

Permeability of cuticle of *Helicoverpa* armigera (Hübner) larvae to deltamethrin

A. P. Padma Kumari, A. Phokela and K. N. Mehrotra

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110 012, India

Permeability of the cuticle of *H. armigera* larvae to deltamethrin has been studied in three populations originating from Delhi, Sirsa and ICRISAT. Delayed penetration of topically applied ¹⁴C-deltamethrin was observed in the Sirsa population. This suggests that the permeability of the cuticle plays an important role in imparting resistance in the Sirsa population, whereas in Delhi and ICRISAT populations it is not very important.

REDUCED permeability of the cuticle may be a factor conferring resistance in insect pests to insecticides $^{1-6}$. Helicoverpa armigera (Hübner) has acquired resistance to synthetic pyrethroids (SPs) in major parts of India $^{7-10}$. Recently, by a very indirect method some evidence was presented to suggest that the cuticle may, perhaps, play some role in manifestation of resistance to SPs in H.

^{1.} Ditzel, J. and Rooth, G., Diabetes, 1955, 4, 474-478.

^{2.} Horne, W. C., Anderson, G. M. and Cohen, D. J., Am. J. Hematol, 1991, 38, 48-53

^{3.} Olsen, R., Schwartzmiller, D., Wepner, W. and Winandy, R., in

armigera populations of Australia¹¹. This led us to reinvestigate the permeability of cuticle to *H. armigera* larvae obtained from three widely separated areas, Sirsa (Haryana), Delhi, and ICRISAT, Andhra Pradesh. We report here that the reduced permeability of the cuticle plays a significant role in imparting resistance to *H. armigera* populations originating from Sirsa.

Final instar larvae of H. armigera used in the present study were collected from chickpea fields in Delhi, from cotton fields in Sirsa (village Balasar) on cotton and from weeds at ICRISAT. The Sirsa population had been subjected to local practices of plant protection prevalent in that area. But all the populations were conditioned on artificial diet¹⁰. For studying permeability, 0.1 µg ¹⁴C-deltamethrin, about 80,000 Bq in one microlitre of methanol, was applied on the thoracic dorsum of the final instar larvae (weighing 350–375 mg), using an electrically operated microapplicator. Larvae treated for different time intervals were held in scintillation counting vials without any food. At intervals of 10 min, three larvae were washed individually with 5 ml of the scintillation mixture (0.55% w/v 2,5-diphenyl oxazole in 2:1 toluene and methyl cellosolve). These washings, having the remaining deltamethrin on cuticle, were estimated in a liquid scintillation counter¹². The percentage of the remaining activity on the thoracic dorsum when plotted (on log scale) against time gave a straight line, suggesting that deltamethrin followed a first-order kinetics in permeating the cuticle. The $t_{0.5}$ value was computed for all the three populations.

The permeability of cuticles in Delhi and ICRISAT populations did not differ and was almost the same as that observed earlier 10 . However, the permeability was significantly different in populations originating from Sirsa. The computed $t_{0.5}$ value was 42.00 min for the Delhi population and 39.96 min for the ICRISAT population. In contrast to this, $t_{0.5}$ was 71.91 min for the Sirsa population (Table 1). This indicated that 14 C-deltamethrin had taken almost twice the time to penetrate the cuticle of the Sirsa population than the other two. The permeability of the cuticle of all the three populations was observed to be monophasic and followed first-order kinetics (Figure 1), which was similar to that reported earlier for insect cuticle and animal integuments $^{13-15}$. It may also be mentioned that in a set of experiments on

Table 1. Permeability of the cuticle of final instar larvae of H. armigera

Location	t _{0.5} (min)	Regression equation	r ²
Delhi	42 00	Y = 2.0 - 0.00716x	0 9445
ICRISAT	39 96	Y = 2.0 - 0.00754x	0 6789
Sirsa	71 91	Y = 20 - 0.00419x	0.9456

Note $t_{0.5}$ is the time required for penetration of 50% of the applied dose. All values were significant at 5% level.

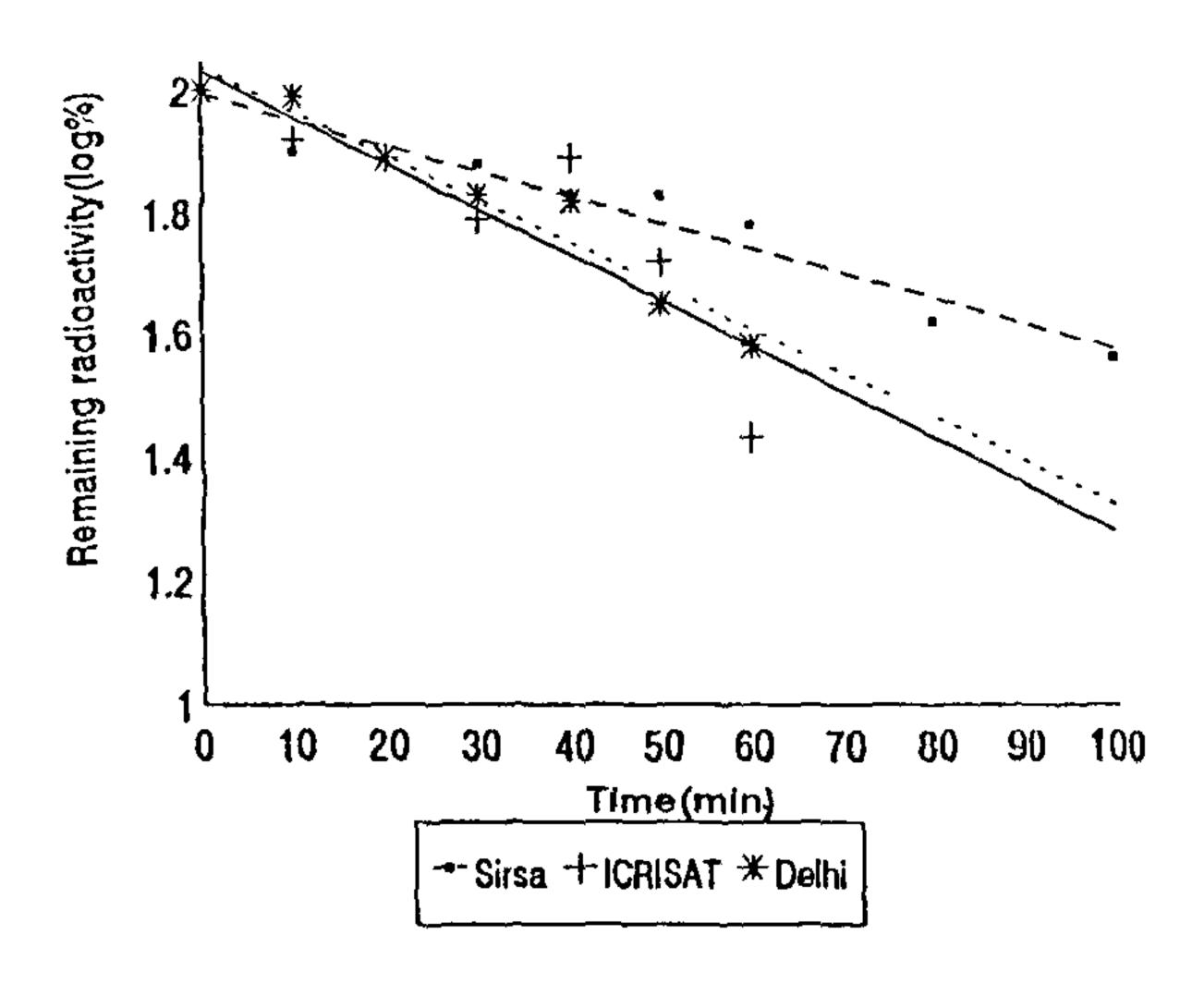


Figure 1. Permeability of *H. armigera* larval cuticle to ¹⁴C-delta-methrin.

the metabolism of ¹⁴C-deltamethrin it was found that metabolism was high in the microsomal fraction of cuticular homogenates of the Sirsa population than in the same of the Delhi population, the ratio being about 2:1 between Sirsa and Delhi. These results, therefore, suggest that poor permeability of deltamethrin along with high rate of metabolism in the cuticle itself was important in imparting resistance to the Sirsa population. It is likely that H. armigera populations have developed resistance to SPs by different mechanisms in different geographical/agro-ecological zones of the country. It is also possible that in the Sirsa population the cuticle is an important factor contributing towards resistance. Thus, the present studies suggest that 'PEN' mechanism, as suggested by Gunning et al. 16 in Australian strains, may play a prominent role in imparting resistance to SPs in the Sirsa population.

¹ Chang, C. K. and Jordan, T. W., Perticide Biochem. Physiol., 1982, 17, 196-204

^{2.} Delorme, R., Fournier, D., Chaufaux, J., Cuany, A., Bride, J. M., Auge, D. and Berge, J. B., Pesticide Biochem. Physiol., 1989, 33, 37-45.

^{3.} Golenda, G. F. and Forgash, A. J., Pesticide Biochem. Physiol., 1989, 33, 37-45.

^{4.} Noppun, V., Saito, T. and Miyata, T., Perticide Brochem. Physiol., 1989, 33, 83-87.

^{5.} Little, E. J., McCaffery, A. R., Walker, C. H. and Parker, T., Pesticide Biochem, Physiol., 1989, 34, 58-68.

^{6.} Rees, H. H., Insect Biochemistry, Chapman and Hall, 1977, p. 63.

^{7.} Dhingra, S., Phokela, A. and Mehrotra, K. N., Natl. Acad. Sci. Lett., 1988, 11, 123-125.

^{8.} McCattery, A. R., Maruf, G. M., Walker, A. J. and Styles, K., Brighton Crop Protection Conference, 1988, vol. 4C-14, pp. 433-438.

⁹ McCaffery, A. R., King, A. B. S., Walker, A. J. and El Nayir, H., Pesticide Sci., 1989, 32, 383-384.

- 10. Phokela, A. and Mehrotra, K. N, Proc. Indian Natn. Sci. Acad, 1989, B55, 235-238.
- 11. Kennaugh, L., Pearce, D., Daly, J. C. and Hobb, A. A, Pesticide Biochem. Physiol., 1993, 43, 234-241.
- 12. Ishaaya, I and Casida, J E, Pesticide Biochem. Physiol, 1980, 14, 173-184.
- 13. Buerger, A. A., J. Theor. Biol., 1966, 11, 131-135.
- 14. Buerger, A. A., J. Theor. Biol., 1967, 14, 66-73.
- 15. Hadgraft, J. and Brian, K. R., Pesticide Sci., 1990, 30, 81-89.
- 16. Gunning, R. V., Easton, C. S., Balfe, M. E. and Ferris, I. G. Pesticide Sci., 1991, 33, 437-490.

ACKNOWLEDGEMENTS. We thank Roussel Uclaf, Procida, France, for providing labelled deltamethrin. This article is a part of the Ph D thesis submitted by the senior author to the Post-Graduate School, IARI, New Delhi.

Received 3 August 1994; revised accepted 14 July 1995