Stigmasterol, m.p. 168–169°, $[\alpha]_D^{30}$ − 49·5° ($CHCl_3$), $C_{29}H_{48}O$; its monoacetate, m.p. 140°, $[\alpha]_D^{30}$ − 54·5° ($CHCl_3$), $C_{31}H_{50}O_2$, was identified by comparison with authentic samples.

β-Amyrin, m.p. 195–196°, $[\alpha]_D^{30}$ + 87·5° ($CHCl_3$)-$C_{30}H_{50}O$; its monoacetate, m.p. 237–238°, $[\alpha]_D^{30}$ + 83·5° ($CHCl_3$), $C_{32}H_{52}O_2$ and benzoate, m.p. 230–231°, $[\alpha]_D^{30}$ + 99·0° ($CHCl_3$), $C_{37}H_{54}O_2$, was identified by comparison with authentic samples.

β-Sitosterol, m.p. 136–137°, $[\alpha]_D^{30}$ − 35·0° ($CHCl_3$), $C_{29}H_{50}O$; its monoacetate, m.p. 126–127°, $[\alpha]_D^{30}$ −32·0° ($CHCl_3$), $C_{31}H_{52}O_2$ and benzoate, m.p. 142–143°, $C_{36}H_{54}O_2$, was identified by comparison with authentic samples.

The authors wish to thank the Council of Scientific and Industrial Research, New Delhi, for the award of fellowships to E.K.S.V. and R.R.K.

Dept. of Chemistry, Andhra University, Waltair 530 003, India,
March 9, 1979.

CH. BHEEMASANKARA RAO.
E. K. S. VIJAYAKUMAR.
R. RAMA KRISHNA.

1. *The Wealth of India*, CSIR, New Delhi, 1962, **6**, 142.
2. Torrent, M. and Maria, T., *Rev. R. Acad. Farm.* (*Chem. Abs.*), *Barcelona*, 1976, **14**, 39.

CARDIOTOXICITY OF IMMUNE SPLEEN CELLS TREATED WITH SPECIFIC ANTIGEN OR ANTISERUM

IN our previous study, we have shown that the spleen cells immunized against the cardiac antigen produce cardiac damage on passive transfer to normal rats[1]. The cardiac injury produced by passively transferred cells may be due to receptors (immunoglobulin ?) on the surface of the sensitized lymphocytes. In the present study, an effort was made to investigate the effect of blockade of these receptors with specific cardiac antigens or antibody on their cardiotoxicity.

The study was carried out on albino rats (150 ± 20 g) and albino rabbits (about 2 kg). The details of the preparation of rat heart extract (HE) and its anticardiac antisera in rabbits have been described earlier[2]. The method for the purification of cardiac antigen I (CAI) and cardiac antigen III (CAIII) have also been described[1].

The rats were immunized by giving two injections of HE (containing 3 mg protein) mixed with complete Freund's adjuvant in the foot pads at 10 days interval. The animals were sacrificed 10 days later, the spleens were removed aseptically and a single celled suspension was prepared in MEM (Hanks base) medium[3, 4]. The suspension of the immune spleen cells (2×10^8 cells/ml) was mixed with an equal amount of an undiluted anticardiac antisera or the antisera diluted two or four folds. The mixture was incubated at 37° C for 90 min and then at 4° C for 18 hours. The cells were washed thrice and were reconstituted to the original volume. For control, the normal spleen cells were also coated in the same manner. One ml of the cell suspension (2×10^8 cells) was inoculated intraperitoneally. In each group, 10 animals were taken. The cells from one animal were transferred passively in two healthy rats. The normal and immune spleen cells without coating were also transferred passively.

In another set of experiments, 2×10^8 immune spleen cells were mixed thoroughly with 10 mg of cardiac antigens (HE, CAI, or CAIII). Similarly, normal spleen cells were also coated with antigens for controls. The mixture was incubated at 37° C for 4 hours; the cells were washed thrice and were reconstituted to the original volume. The cells were inoculated i.p. in groups of 10 rats each as above. After 48 hours the animals from both the experiments were sacrificed and the hearts were studied hitologically for the cardiac injury as described earlier[1, 5].

The results of the cardiotoxicity produced by the passive transfer of immune spleen cells coated with anticardiac antiserum have been summarized in the Table I. The cardiotoxicity of the immune spleen cells was significantly increased on coating them with anticardiac antisera. The cardiac injury produced by passive transfer of untreated immune spleen cells was more or less similar to that produced by normal spleen cells coated with antiserum. Normal untreated spleen cells did not produce any damage.

The cardiotoxicity produced by the passive transfer of immune spleen cells coated with CAI, CAIII or HE, have been summarized in Table I. The extent of cardiac damage was significantly reduced when the immune spleen cells were coated with the CAIII. In the rats receiving immune spleen cells coated with CAI, 25% of the rats showed an increase in cardiotoxicity, whereas 75% of the rats showed no change in the cardiac damage. In the rats receiving immune spleen cells coated with CAIII, 75% of the rats showed decrease in cardiac damage, whereas in the 25% of the rats, there was no damage; 63% of the rats receiving spleen cells, immunized with HE showed decrease in cardiac damage, whereas 37% of the rats showed no change in the cardiac damage. The difference between the cardiac damage produced by immune spleen cells and immune spleen cells coated with CAI and HE was not significant ($p > 0.05$) butt he difference between the cardiac damage produced by the immune spleen cells and immune spleen cells coated with CAIII was highly significant ($p < 0.01$).

Receptors for homologous or heterologous IgG on the surface of lymphoid cells have been demonstrated[6, 7].

TABLE I

Cardiotoxicity of passively transferred immune spleen cells coated with specific antiserum or antigen

Type of cells	Grade of cardiac injury	P value
Normal spleen cells	0±0	
Immune spleen cells	75±10	
1 *Cells treated with antiserum*		
Immune spleen cells + antiserum dilution		<0·05
–undiluted	153±25	
–1 : 2 dilution	143±27	
–1 : 4 dilution	156±31	
Normal spleen cells + antiserum dilution		<0·01
–undiluted	70±14	
–1 : 2 dilution	90±14	
–1 : 4 dilution	80±28	
2 *Cells treated with antigen*		
Immune spleen cells	75±10	>0·05
Immune spleen cells + CAI	85± 9	<0·01
Immune spleen cells ± CAIII	52±15	>0·05
Immune spleen cells + HE	57±17	

Type of cells	Grade of cardiac injury	P value
Normal spleen cells	0±0	
Immune spleen cells	75±10	
1 *Cells treated with antiserum*		
Immune spleen cells + antiserum dilution		<0·05
–undiluted	153±25	
–1 : 2 dilution	143±27	
–1 : 4 dilution	156±31	
Normal spleen cells + antiserum dilution		<0·01
–undiluted	70±14	
–1 : 2 dilution	90±14	
–1 : 4 dilution	80±28	
2 *Cells treated with antigen*		
Immune spleen cells	75±10	>0·05
Immune spleen cells + CAI	85± 9	<0·01
Immune spleen cells ± CAIII	52±15	>0·05
Immune spleen cells + HE	57±17	

it has been shown that cytophilic IgG binds to cells through the Fc piece of the molecule, whether the cells involved are B-cells[8] or T-cells[9]. Dickler[10] has shown receptors on lymphocytes for Fc portion of immunoglobulin molecule. Thus, coating of antibodies on the spleen cells is possible, as shown in the present study.

It was noted that the uncoated immune spleen cells produced cardiac damage of 75 points while intensity of the cardiac damage by immune spleen cells coated with anticardiac antisera was almost double. When normal spleen cells were coated with anticardiac antisera, they produced cardiac damage of the same magnitude as that by uncoated immune cells. Both the cell mediated and the humoral antibodies are known to be cardiotoxic[11] and a summation of their effect was seen in the present study when immune spleen cells and antisera were combined.

The cardiotoxicity of immune spleen cells was also affected by coating of cardiac antigens. Cardiac pamage by passive transfer of immune spleen cells coated with CAI, CAIII and HE was 85 ± 9, 52 ± 15 and 57 ± 17 points respectively. Thus, there was reduction in cardiotoxicity of antigen coated immune spleen cells. Coating with CAIII and HE significantly reduced the cardiotoxicity of immune spleen cells ($p < 0·01$). Thus, it appears that the receptors on the lymphocytes which are immunoglobulin in nature[12] have been blocked by the antigen thereby diminishing their ability to cause cardiotoxicity. It has been reported that T-lymphocytes have receptors for the T-dependent antigen only[13]. CAIII has been found to be a thymus dependent antigen[14]; therefore the reduced ability of CAIII coated cells to produce cardiotoxicity represents blockade of T-lymphocytes by the antigen.

Department of Pathology and Bacteriology,
K.G. Medical College,
Lucknow 226 003,
January 27, 1979.

S. M. NATU.
U. C. CHATURVEDI.*
ASHA MATHUR.

* For correspondence and request for reprint.

1. Natu, S. M. and Chaturvedi, U. C., *Ind. J. Med, Res.*, 1977, **65**, 554.
2. Chaturvedi, U. C. and Mehrotra, R. M. L., *Ibid.*, 1967, **55**, 21
3. —, Tandon, P., Mathur, A. and Kumar, A., *J. Gen. Virol.*, 1978, **39**, 293.
4. —, — and *J.Inf. Dis.* 1978, **13**8, 181.
5. — and Mehrotra, R. M. L., *Ind. J. Med. Sci.*, 1969, **23**, 83.
6. Dickler, H. B. and Kunkel, H. G., *J. Exp. Med.*, 1972, **136**, 191.
7. Basten, A., Miller, J. F. A. P., Sprent, J. and Pye, I., *J. Exp. Med.*, 1972, **135**, 610.
8. Paraskevas, F., Lee, S. T., Orr, K. B. and Israels, L. G., *J. Immunol.*, 1972, **10**8, 1319.
9. Santana, V. and Turk, J. L., *Immunology*, 1975, **2**8, 1173.
10. Dickler, H. B., *Adv. Immunol.*, 1976. **24**, 167.
11. Chaturvedi, U. C. and Mehrotra, R. M. L., *Nature*, 1967, **216**, 267.
12. Mitchison, N. A. *Cold Spring Harb. Symp. Quant. Biol.*, 1967, **32**, 431.
13. Ganfield, D. T., Stern, M. and Maurer, P. H., *Fed. Proc.*, 1977, **36**, 1186.
14. Chaturvedi, U. C., Natu, S. M., Kumar, A., Mathur, A. and Sharma, R. K., *Ind. J. Med. Res.*, 1978, **67**, 124.

A NOTE ON THE OCCURRENCE OF MICRO-STYLOLITE SEAMS IN SULPHIDE-CHERTY ROCK AT DARIBA-RAJPURA DEPOSIT, UDAIPUR DISTRICT, RAJASTHAN

STYLOLITE SEAMS marked with pyrite have earlier been reported by Pandya and Solanki[1] and Chauhan[2] from the syngenetic sedimentary sulphide deposit of Dariba (24° 57′ : 74° 08′)-Rajpura (24° 58′ : 74° 08′). Stylolite seams with galena are now being reported for the first time.

A number of small-scale stylolite seams in meta-chert (Chert and its recrystallised form) have been noted. Fine grained galena is the principal mineral which has crystallised in the seams, but at places it is seen co-existing with pyrite. Figure 1, shows three distinct stylolite seams, and three diagenetic cross fractures which are filled with galena.

There appears to be two distinct periods of formation of fracture-filled galena. The fracture which lies at the intermediate position (Fig. 1) is the last to form since it is not affected by any of the stylolite seams and crosses all the seams. Galena, for this fracture-filling, has been supplied by the broad band of sphalerite and galena. The band itself is made up of about 90% of sphalerite and 10% of galena which is scattered throughout the band. One of the fractures (left in Fig. 1) displaced by the top stylolite seam (No. 3) is displacing intermediate seam (No. 2), and has no effect on the bottom-seam (No. 1). Galena for this fracture must have been supplied by the seam No. 1.

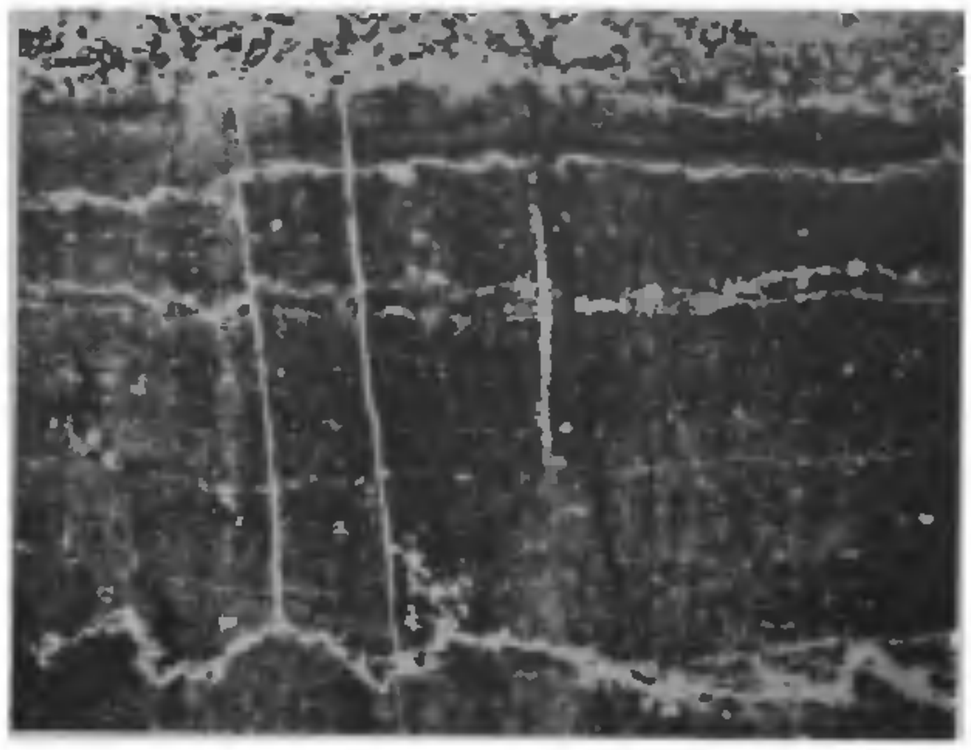

FIG. 1. Stylolite seams (marked with galena) and diagenetic cross fractures filled with galena, in meta-chert. Fracture (left) is displaced by stylolite seam (top) and is displacing the seam which lie at the intermediate position.

Top—A broad band of sphalerite and galena. Microphoto (negative), × 5. White: galena; white with grey tint : sphalerite; black : quartz.

These features confirm that galena and pyrite were present before the formation of the stylolite seams.

Department of Geology, University of Rajasthan, Udaipur 313 001, — M. K. PANDYA.

Department of Metallurgical Engineering, M. R. Engineering College, Jaipur 302 004, — S. L. SOLANKI.

and

Department of Geology, University of Rzjasthan, Udaipur 313 001, — T. K. PANDYA.

January 15, 1979.

1. Pandya, M. K. and Solanki, S. L., *Seminar on Recent Advances in Precambrian Geology and Mineral Deposits with Special Reference to Rajasthan* (Abstract), 1975.
2. Chauhan, D. S., *Jour. Geol. Soc. Ind.*, 1977, **9**, 69.