

to the medium improved the growth of the plantlets. Three or four-leaved plantlets with a good root systems were transferred to small paper cups with pre-sterilized soil compost (figure 4). The procedure of transferring plantlets to soil conditions is being improved to obtain sustained growth and survival of transplants.

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INTERACTION OF PLATELETS AND PLASMA PROTEINS WITH HEMA GRAFTED POLYURETHANES

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THROMBUS formation induced by blood polymer interaction is of key concern in the area of medical implants. Although several investigators^{1,2} have looked into the nature of various polymer surfaces for developing a non-thrombogenic surface, the exact mechanism of thrombus formation still remains a mystery. Adsorption of proteins is considered as the primary process to occur upon contact with blood and subsequent interactions with blood cells leading to thrombus formation. We have attempted to study the competitive adsorption of proteins from the protein mixture 25 mg% albumin, 15 mg% γ -globulin and 7.5 mg% fibrinogen on various surfaces. The relative preference for albumin with an interrelation to platelet adhesion has been examined in this report. The information may help in making the selection of a surface towards its biomedical use.

Fabrication of the vascular grafts³ and their treatment with HEMA for different periods of time were reported earlier⁴. Parts of the samples were irradiated in air and the remaining under N₂ atmosphere at a dose of ~ 0.275 M Rads (⁶⁰Co source). Platelet adhesion and protein adsorption on these surfaces were studied as described earlier^{2,5} using isolated platelets in Tyrode solution from citrated calf blood.

Albumin, fibrinogen and γ -globulin (Human, Sigma Co., USA) were taken in the ratio 25 mg%, 7.5 mg% and 15 mg% respectively in a phosphate buffer of pH 7.4. Labelled proteins albumin (Amersham

Table 1 Platelet adhesion study

Time of exposure to HEMA	Irradiated in air platelet count/mm ² \pm s.d.	Irradiation under N ₂ atm. platelet count/mm ² \pm s.d.
Bare Irradiated	8.7 \pm 2.0	6.7 \pm 1.8
5'	7.5 \pm 1.5	7.0 \pm 2.0
10'	—	7.5 \pm 1.5
15'	5.0 \pm 2.5	5.0 \pm 2.2
20'	—	6.5 \pm 2.2
Bare (non-irradiated)	9.5 \pm 2.7

International, England) and fibrinogen (Amersham International, England) were added to the protein mixture, in amounts which gave moderate surface count with precision. Adsorption was carried out at air-water interface^{2, 5} for a period of 3 hr considered sufficient to achieve equilibrium of proteins on the surface. After adsorption, the samples were thoroughly rinsed with the buffer and were counted in a γ -counter (ECIL, India). The hot protein mixture (0.5 ml) was taken to evaluate the protein concentration on the surface.

The surface concentration was determined assuming that the labelled and the unlabelled proteins adsorb to the same extent. The results reported in table 2 as surface concentration ($\mu\text{gm}/\text{cm}^2$) was computed using the relation⁵

$$\lambda = C_p R_f / A R_s$$

where C_p is bulk concentration (μgm), R_f the count rate of surface, A the area of surface (cm^2) and R_s count rate per ml of protein solution.

Some difference is noticed between the HEMA exposed PEUU samples irradiated in air and in nitrogen atmosphere. It has been observed that the amount adsorbed as shown in tables 2 and 3 alone, does not give the complete picture of an artificial surface towards its blood compatibility. Therefore molar ratio of fibrinogen/albumin, (F/A) at equilibrium² is evaluated, as fibrinogen induces thrombosis, while albumin reduces it. So this ratio is important to evaluate the relative advantage of one surface over the other. Platelet adhesion is also an important parameter for a choice towards biomedical application as indicated in table 1.

Taking molar ratio into account, the time of exposure to HEMA for 15' before irradiation in air seems

Table 2 Adsorption of Albumin and Fibrinogen $\mu\text{gm}/\text{cm}^2$

Time of exposure to HEMA	Irradiated in air		Irradiation under N ₂ atm.	
	Albumin	Fibrinogen	Albumin	Fibrinogen
Bare irradiated	0.44	0.53	0.25	1.07
5'	0.43	1.03	0.35	2.18
10'	0.40	0.96	0.26	1.76
15'	0.58	0.94	0.32	2.02
20'	0.51	0.90	0.25	1.70
Bare (non-irradiated)	0.26	2.18	—	—

Table 3 Desorption of Proteins as a function of time in $\mu\text{g}/\text{cm}^2$

Desorption time	Irradiated in air					Irradiated under N ₂ atm.				
	Irradiated Bare	Albumin				Irradiated Bare	Albumin			
		5' HEMA	10' HEMA	15' HEMA	20' HEMA		5' HEMA	10' HEMA	15' HEMA	20' HEMA
1'	0.38	0.39	0.39	0.43	0.41	0.22	0.32	0.27	0.28	0.20
15'	0.32	0.39	0.38	0.44	0.40	0.21	0.23	0.24	0.26	0.19
45'	0.32	0.31	0.34	0.38	0.35	0.18	0.25	0.25	0.26	0.23
3 hr	0.26	0.29	0.25	0.36	0.35	0.19	0.27	0.24	0.25	0.24

Desorption time	Fibrinogen									
	Irradiated Bare	5' HEMA	10' HEMA	15' HEMA	20' HEMA	Irradiated Bare	5' HEMA	10' HEMA	15' HEMA	20' HEMA
1'	0.47	0.85	0.82	0.75	0.77	0.93	1.32	1.05	1.23	1.21
15'	0.37	0.67	0.62	0.58	0.53	0.72	1.21	1.03	1.16	0.96
45'	0.30	0.59	0.57	0.53	0.47	0.67	1.05	0.77	1.06	0.90
3 hr	0.26	0.54	0.50	0.43	0.42	0.59	0.80	0.61	0.86	0.85

Table 4 Molar ratio for Fib. to Alb. on various surface

Time of exposure to HEMA and Irrad.	Molar ratio Irrad. in air F/A	Irrad. under N ₂ atm. F/A
Bare (irradiated)	0.23	0.83
5'	0.46	1.20
10'	0.47	1.29
15'	0.31	1.22
20'	0.34	1.31
Bare (non-irradiated)	1.6	—

to possess lowest ratio as compared to the other HEMA exposed samples (table 4); the relative number of fibrinogen molecules is smaller on this surface to encourage platelet adhesion. In our results bare-irradiated polymer surface shows even a lower value. Since the molar ratio (F/A) is lower in air irradiated cases as compared to the nitrogen atmosphere, such surfaces should be studied in *in vivo* experiments for further understanding the nature of these surfaces.

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RECORD OF LATE PERMIAN AMMONOID CYCLOLOBUS FROM ZOJILA LA, KARGIL DISTRICT, JAMMU AND KASHMIR

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THE authors in the course of detailed geological studies in the Zoji La-Minamarg section along

Srinagar-Leh road, collected ammonoid fossil *Cyclolobus* sp. of late Permian age at Zoji La and 2.5 km ESE of it, in the Kargil district, Jammu and Kashmir (figure 1).

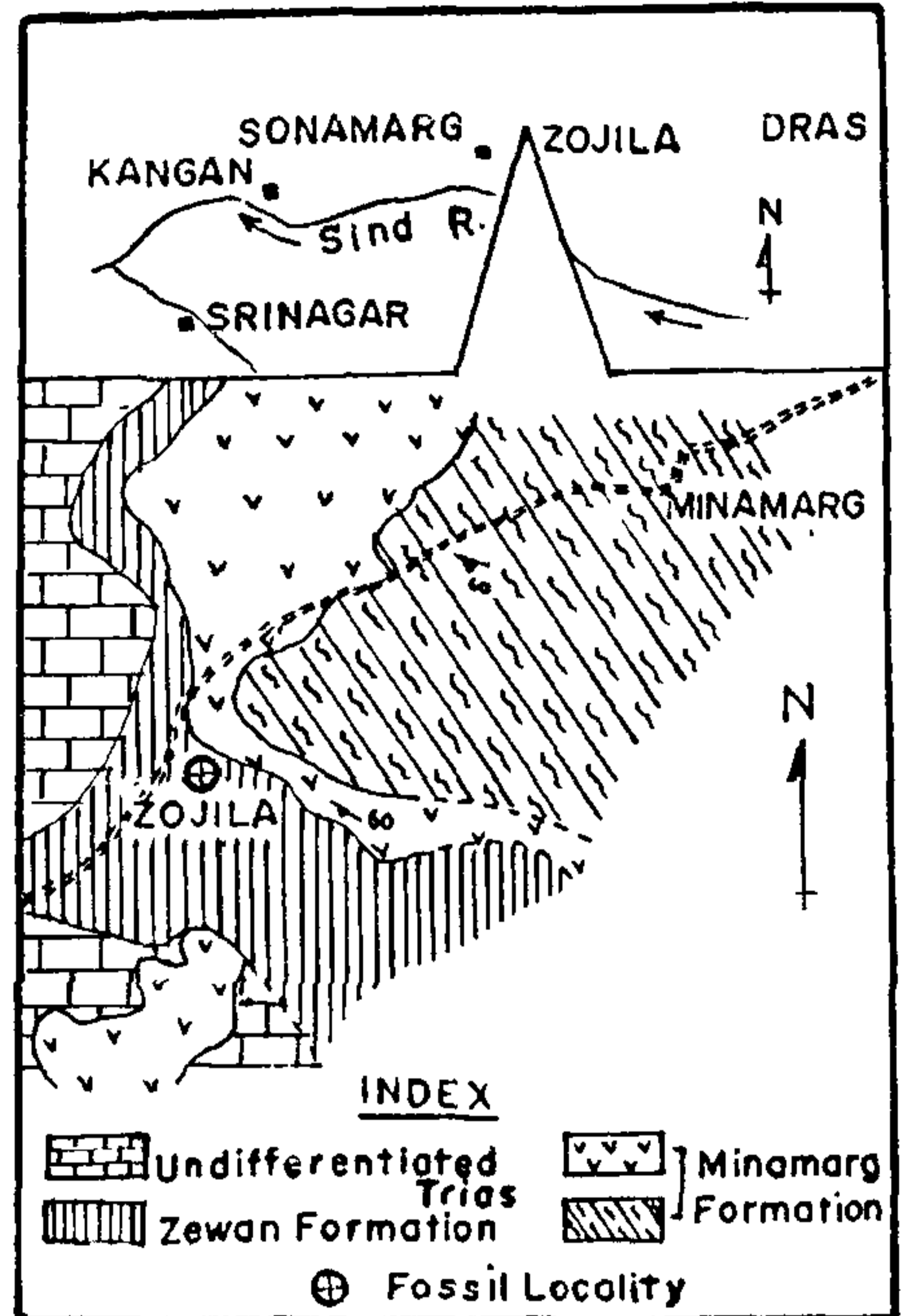


Figure 1. Fossil locality and geological map.

The rocks exposed at Zoji La have been variously correlated and considered as Middle Trias by Middlemiss¹ and Raina², Permo-Carboniferous (Mughalpur Formation) by Raina *et al*³, and Permo-Trias (Zoji La Formation) by Shah *et al*⁴.

The present studies have revealed the following lithostratigraphy in the area:

Undifferentiated Triassic	Massive limestone Interbedded limestone and shale.
Zewan Formation (Upper Permian)	Dark grey phyllite with thin limestone. <i>Cyclolobus</i> cf. <i>C. walkeri</i> . Brownish grey sandstone
	Unconformity