and sometimes in the range of 0.273 to 0.391 K cal/mole at pH-values 4.6 and 3.5 respectively. The entropy changes are in the range of 7–17 cal/mole/deg within which the entropy values of beryllium-TG reaction lie. The thermodynamics of this interaction is comparable to that of other ion-protein interactions[18–20].

The positive values of entropy probably indicate that the transfer of beryllium ion from the solvent to protein is accompanied by the release of water of hydration from macromolecule and the ions of buffer, and that the configuration of folded molecule changes into an unfolded one[21]. The decreased enthalpy at lower pH-values may be attributed to larger electrostatic repulsion between cationic protein and the beryllium ions and therefore lesser release of water of hydration[22]. The temperature dependence of the present interaction indicates that all the 84 carboxyl groups[10] are not equally available for interaction with beryllium ions; presumably, a large fraction of these groups remains buried in the interior of folded regions of polypeptide chain. However, with a rise of temperature the folded polypeptide chain is perturbed and the masked carboxyls exposed for binding with beryllium ions. The results discussed suggest that any environmental effect altered the small ion binding affinity of the macromolecular system.

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HAEMOCYTE RESPONSE AS A POSSIBLE AND NEW PARAMETER IN THE PRELIMINARY SAFETY/TOXICITY EVALUATION OF BIOMATERIALS

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HAEMOCYTES (blood cells) of insects do respond to foreign bodies that enter into their haemocoele[1–3]. In the light of the above observation and in our search for simple and less costly alternative test systems, haemocyte response of insects was investigated as a possible new parameter in the toxicity/safety screening of biomaterials.

Eight different formulations of polyvinylchloride sheets, on which routine toxicological data available (4 nontoxic and 4 toxic samples) were selected as test samples for the present study. Tygon tubings (PVC, Norton Company, USA) were used as control samples. Test and control samples were cut into small strips and were sterilized by gamma radiation. The common house pest, *Periplaneta americana* was chosen as the experimental animal. These animals were immobilized by ether and the samples were implanted into the haemocoel through an incision made between the second and third abdominal sternite of the animal.

Table 1 *Comparison of U.S.P. toxicity screening tests results with that of haemocyte response test*

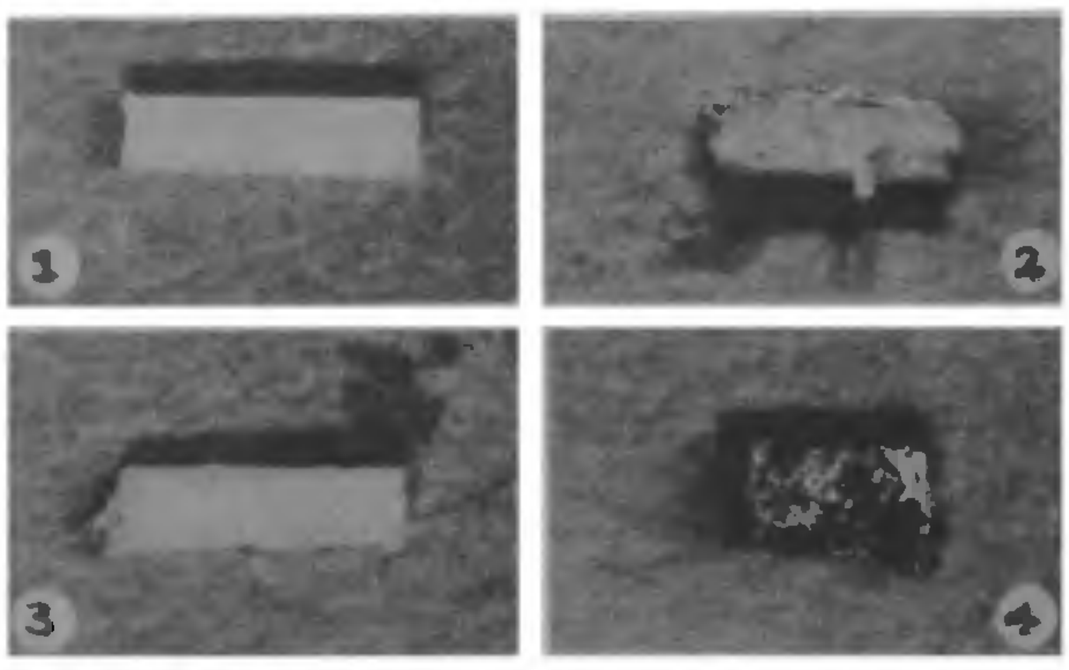
Samples	*In vivo* test and results			Test in *P. americana* and results
	Intramuscular implantation (rabbit)	Acute systemic toxicity (mouse)	Intracutaneous irritation (rabbit)	Implantation in haemocoel
0052-PVC-sheet	T	T	—	T
0063-PVC-sheet	T	T	I	T
0070-PVC-sheet	T	T	—	T
0073-PVC-tubing	T	T	I	T
0087-PVC-sheet	NT	NT	NI	NT
0088-PVC-sheet	NT	NT	NI	NT
0091-PVC-sheet	NT	NT	NI	NT
0092-PVC-sheet	NT	NT	NI	NT

T, toxic; NT, non-toxic; I, irritant; NI, non-irritant; dash indicates not done.

Test samples were implanted on the left side and control samples on the right side of the haemocoel of the experimental animal. Six animals were used for each period of observation per sample. Observation of the recovered samples under a stereo-dissection microscope revealed that there was discernible difference in the haemocyte response elicited by toxic and nontoxic test samples.

Test and control samples recovered at 1, 2, 3, 7 and 30 days after implantation were found to be encapsulated by haemocytes. The haemocyte capsules formed around the control and nontoxic samples recovered after the above noted intervals appeared whitish in colour, translucent and were similar and comparable. The difference between the capsules formed around a 3-day-old and 30-day-old control sample was that, the latter capsule was little more opaque (figures 1 and 3). Haemocyte response towards control and toxic samples showed well-marked differences. The haemocyte capsules formed around the toxic samples were thicker than those formed around the control samples, implanted in the same animal. Capsules formed around the toxic samples had a darker shade when compared with those formed around the control samples and this phenomena increased with the duration of the implantation period (figures 2 and 4).

The importance of the above observation is that the haemocyte response system was able to identify toxic and non-toxic samples and these results were consistent with those obtained in rabbit intramuscular implantation test (U.S.P.)[4] and acute systematic toxicity test in mice (U.S.P) using the same samples (table 1). Efforts are underway to study the structure, size and morphology of these cellular capsules to standardize this new test system, which may be effectively used to identify potential non-toxic candidate materials from a large number of proposed samples and to elucidate the degree of toxicity of the toxic samples.



Figures 1–4. 1, 3. Control sample recovered 3 days and 30 days respectively after implantation. **2, 4.** Test sample recovered 3 days and 30 days respectively after implantation.

19 December 1985; Revised 26 March 1986

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