II. MATERIALS

1. ANIMALS

MICE : Swiss albino mice bred in the Animal House of Chittaranjan National Cancer Research Centre, Calcutta, were used for different studies relating to antitumor activities of lympho-reticular cells and for inducing transplantable ascites tumors. The mice used were 7-8 wks. old males and were of an average body weight of 20-25 gms.

RABBIT : Adult Belgian rabbits supplied by the Animal House of Chittaranjan National Cancer Research Centre, Calcutta, were used for immunization purpose.

2. TUMOR

Methylcholanthrene induced ascitic fibrosarcoma (AFS) grown in Swiss mice were serially transplanted in male, adult Swiss mice and were used as target cells to assess the antitumor activity of the lymphocytes and macrophages.

3. CULTURE MEDIUM

Tissue culture medium, Rosewell Park Memorial Institute - 1640(RPMI-1640) (Bios India) was used throughout the study in various assay procedures. (Composition in the appendix-1).

4. BUFFERS AND REAGENTS

The following buffers and reagents were used in the study, composition of which are given in the appendix :

- Phosphate Buffer Saline 0.15(M) pH 7.2
- Phosphate Buffer 0.10(M) pH 7.4
- Tris Ammonium Chloride Buffer 0.15(M) pH 7.2
- Normal Saline 0.14(M) pH 7.0
- Sodium Phosphate Buffer 0.10(M) pH 7.5
- Acetate Buffer 0.20(M) pH 5.0
- Alsever's solution pH 7.0
Protein reagents:
  Reagent A
  Reagent B

5. STAINS AND FIXATIVE

The stains used were Giemsa, Wright's stain, and Leishman's stain; all were obtained from Glaxo Laboratories, Bombay, India.

6. FINE CHEMICALS AND REAGENTS

(a) N-2 Hydroxyethyl Piperazine N'2-ethanesulfonic Acid (HEPES)

Molecular weight 238 (Sigma Chemical Company, U.S.A.); was used to stabilise the buffering action in the culture medium. The chemical was stored with dessicant at room temperature.

(b) Ethylenediamine tetra-acetic Acid (EDTA)

Used for detaching adhered cells; was obtained from Glaxo Laboratories, Bombay, India.

(c) Dodecyl Sodium Sulfate (SDS)

The detergent was obtained from the Sigma Chemical Company, U.S.A. and was used for disintegration of the cells.

(d) Mangiferin

\((1,3,6,7 - \text{tetra hydroxyxanthone - } c_{2}\-\beta\-\text{D-Glucoside})\) is widely distributed in higher plants. The glucosylxanthone was isolated in substantial amounts from Mangifera indica Linn. (Anacardiaceae) by S. Ghosal, Dept. of Pharmaceutics, Benaras Hindu University and was kindly supplied by him.

(e) \(\beta\-\text{Nitrophenol Phosphate (PNPP)}\)

\(\beta\-\text{nitrophenol phosphate was obtained from the Sigma Chemical Company, U.S.A. and was used as substrate for the enzyme acid phosphatase. The chemical was stored dessicated below 0°C.}\


(f) **Lectins**

The lectins PHA, purified from kidney beans, and Con-A purified from *Canavalis ensiformis* (Sigma Chemical Company, USA) were used for assaying mitogenesis of splenic lymphocytes. The lectins were stored dessicated at 4°C.

(g) **Antibiotics**

Penicillin-Streptomycin solution (Gibco-Bio-Cult, Glasgow, Scotland) stored at -20°C, was used as additive for the culture medium.

(h) **Radioactive Isotope**

$^{51}$Cr-Sodium chromate ($Na_2^{51}CrO_4$) and $^3$H-Thymidine were supplied by Bhabha Atomic Research Centre, Trombay India. The specific activity of $Na_2^{51}CrO_4$ (half life 28 days) ranged between 500 to 750 $\mu$Ci/ml. The specific activity of $^3$H-Thymidine (half life 12 years) was very high (12.6 mCi/ml). The isotopes were stored in the Institute’s hot room.

(i) **Sera**

1. Mouse serum: Mouse serum was collected from Fibrosarcoma (ascites) tumor bearing Swiss mice on 6 to 7 days after tumor inoculation.

2. Fetal Calf Serum (FCS): This was obtained from the Commonwealth Serum Laboratories, Melbourne, Australia; was heat inactivated at 56°C and stored in sterile condition at -20°C. This was used for enriching the culture medium.

In addition to the above chemicals, the common laboratory chemicals and reagents were also used.

7. **ANTIGEN**

1) Tumor antigen (TA): This was prepared from the AFS tumor cells grown in the peritoneal cavity of Swiss mice.

2) Normal Tissue Antigen (NA): This was prepared from pooled organs of normal male Swiss mice.

Preparation of these antigens are discussed in the Method Section.
8. SHEEP BLOOD

Sheep blood samples mixed with anti-coagulant were collected from the Institute of Postgraduate Medical Education and Research, Calcutta, and were stored in Alsever's solution at 4°C for a period of two weeks. The sheep red blood cells (SRBC) required for different studies were separated from the whole blood.