MATERIALS
II. MATERIALS

1. Animals:

Mice: Swiss mice bred in the Animal House of Chittaranjan National Cancer Research Centre, Calcutta, were used for various studies relating to morphological and functional properties of cells of the monocyte-macrophage lineage arising in the peritoneum and for inducing transplantable ascites tumors. The mice used were 6 to 8 weeks old male and were of an average body weight of 20-25 grammes.

Rabbit: An adult Belgian rabbit supplied by the Animal House of Chittaranjan National Cancer Research Centre, Calcutta, was used for immunization purpose.

2. Tumor:

Sarcoma-180 (S-180) ascites cells, grown in Swiss mice were used as target cells in different assay procedures to assess the functional activity of the macrophages.

3. Sheep Blood:

Sheep blood samples mixed with anti-coagulant were collected from Indian Institute of Chemical Biology, Calcutta, and was stored in Alsever's solution at 4°C.
Whenever required sheep red blood cells (SRBC) were separated from the whole blood.

4. Culture Medium:

Tissue Culture Medium, Rosewell Park Memorial Institute - 1640 (RPMI-1640) (Bios India) was used throughout the study in the assay procedures (Appendix- ).

5. Buffers and Reagents:

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

- Phosphate Buffer Saline, 0.15M, pH 7.2
- Phosphate Buffer, 0.01M, pH 6.5
- Tris Ammonia Buffer 0.15M, pH 7.2
- Normal Saline 0.14M, pH 7.0
- Alsever's Solution pH 7.0

6. Stains and Fixative:

The Stains used were Giemsa, Wright's Stain, Leishman's Stain (obtained from Glaxo Laboratories, Bombay, India; Fast Blue B Salt: O- Dianisidine, tetrazolized, (obtained from Sigma Chemical Company, USA).

Buffered Formaldehyde, pH 6.6, was used as fixative. Its composition is given in appendix.
7. Chemicals:

**Percoll:** Pharmacia Fine Chemicals, Sweden, used as medium for density gradient centrifugation of the peritoneal cells. This was stored in sterile condition at 4°C.

**N-2 Hydroxyethylpiperazine N2-ethanesulfonic Acid (HEPES):** molecular weight 238, (Sigma Chemical Company, USA) was used for buffering the culture medium. The chemical was stored with desiccant at room temperature.

**Ethylene diaminetetraacetic Acid (EDTA):** used for detaching adhered cells was obtained from Glaxo Laboratories, Bombay, India.

**Sodium Lauryl Sulfate/Dodecyl Sodium Sulfate (SDS):** (Sigma Chemical Company, USA) was used for disintegration of the cells.

**Tannic Acid (Tannin):** Obtained from Fluka AG, Switzerland, was used for coating SRBC for the Passive Haemagglutination Assay.

**Lectins:** Con A, purified from Canavalis ensiformis and WGA purified from Triticum vulgaris were obtained from Sigma Chemical Company, USA. The lectins were dessicated at 4°C.
Polyinosinic Polycytidylic Acid: Sodium salt of Polyinosinic Polycytidylic Acid (Poly I: C.), molecular weight 100,000 (Sigma Chemical Company, USA) was used for modulating macrophage functions and was stored dessicated below 0°C.

Levamisole: (L(-) 2,3,5,6-Tetrahydro 6-Hydrochloride phenyl-imidazo (2, 1-b) thiazole) (LMS), molecular weight 240 (Sigma Chemical Company, USA) was also used as a modulator. The chemical was stored below 30°C.

Sugars and aminosugars: D(+), Mannose, D(+), Glucose, N-Acetyl Glucosamine (NAGlc), and N-Acetyl Galactosamine (NAGal) were used as probes for surface structure of the macrophages involved in various antitumor activities. Mannose and Glucose (Glaxo Laboratories, Bombay, India), molecular weight 180, each, were stored at room temperature, whereas NAGlc and NAGal (Sigma Chemical Company, USA), molecular weight 221, each, were stored dessicated below 0°C.

2-Naphthyl Acetate (Sigma Chemical Company, USA) was used as the substrate for enzyme reaction. This was stored dessicated below 0°C.
8. **Antibiotics**:

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

9. **Radioactive Isotope**:

Sodium Chromate, $\text{Na}_2^{51}\text{CrO}_4$, was supplied by Bhabha Atomic Research Centre, Bombay, India. The specific activity ranged from 500 to 750 μCi/ml.

10. **Sera**:

Mouse Serum: Mouse Serum was collected from -

1) Normal Swiss mice and (ii) S180 ascites tumor bearing Swiss mice, at 6 to 7 days after tumor inoculation.

Rabbit Serum: Serum was collected from rabbit immunized with sheep red blood cells.

Foetal Calf Serum (FCS): This was obtained from 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.

11. **Antigen**:

Tumor antigens were prepared from S-180 ascitic tumor cells grown in peritoneal cavity of mice.