living organism and is essential in the development of new drugs and in monitoring environmental pollutants. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter. Bioassay is a procedure for the determination of the concentration of a particular constitution of a mixture.

Bioassays are procedures that can determine the concentration of purity or biological activity of a substance such as vitamin, hormone, and plant growth factor. While measuring the effect on an organism, tissue cells, enzymes or the receptor is preparing to be compared to a standard preparation. Bioassays can be classified in two types:

- Qualitative
- Quantitative

Qualitative bioassay is used for assessing the physical effects of a substance that may not be quantified, such as abnormal development or deformity. Example of a qualitative bioassay includes Arnold Adolph Berthold's famous experiment on castrated chickens. This analysis found that by removing the testes of a chicken, it would not develop into a rooster because the endocrine signals necessary for this process were not available.

Quantitative bioassays involve estimation of concentration/potency of a substance by measurement of the biological response it produces. These bioassays are typically analyzed using the methods of biostatistics.

Principles of Bioassay

- Bioassay involves the comparison of the main pharmacological response of the unknown preparation with that of the standard.
- The reference standard and test sample should have same pharmacological effect and mode of action, so that their DRC curve run parallel and their potency ratio can be calculated.
- The test solution and standard should be compared for their established pharmacological effect using a specified pharmacological technique.
- The method selected should be reliable, sensitive, and reproducible and should minimize errors due to biological variation and methodology. (Animals should of same species, sex and weight and number of animals should be large enough to permit statistical analysis.)

Need of Bioassay

1. Bioassays not only help to determine the concentration but also the potency of the sample. Potency denotes activity of the compound, i.e. if a

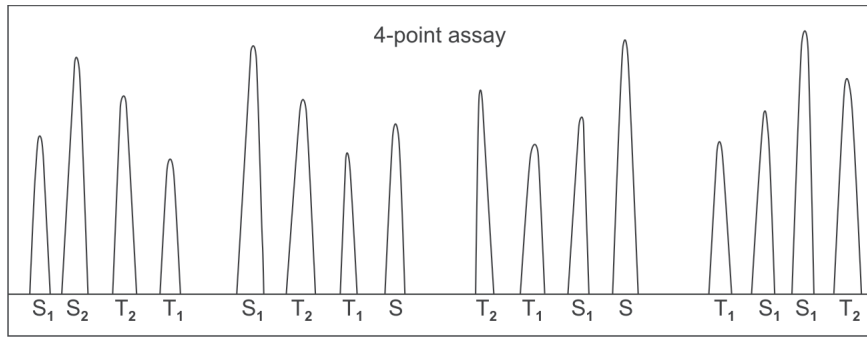

Fig. 1.3: Dose–response curve for 4-point bioassay method

- *Four-point assay* (*2 + 2 dose assay*) [For example: Acetylcholine (ACh) bioassay]
  - LDR curve plotted with varying concentration of standard ACh solutions and given test solution
  - Select two standard doses $s_1$and s_2 from linear part of DRC (let the corresponding response be S_1, S_2)
  - Choose two test doses t_1 and t_2 with response T_1 and T_2 between S_1 and S_2, such that:

$$s_2/s_1 = t_2/t_1 = 2/3$$

  - Record 4 data sets as:

s_1	s_2	t_1	t_2
s_2	t_1	t_2	s_1
t_1	t_2	s_1	s_2
t_2	s_1	s_2	t_s

Figure 1.3 illustrates the dose–response curve for 4-point bioassay.

STATISTICS IN PHARMACOLOGY

Pharmacology routinely employs statistics to help summarize data and, more importantly to test hypotheses.

Data and variables: 'Data' is a collective term for information gathered under various headings or variables. Variables may be related to demographic characteristics, disease specific parameters, such as the presence of coronary disease, or severity of breathlessness; or treatment response variables, such as reduction in pain and improvement of disease.

Science citation index: A citation index is a kind of bibliographic index, an index of citations between publications, allowing the user to easily establish which later documents cite earlier documents. A form of citation index is first found in 12th-century Hebrew religious literature. Legal citation indexes are found in the 18th century and were made popular by citators such as Shepard's Citations (1873). In 1960, Eugene Garfield's Institute for Scientific Information (ISI) introduced the first citation index for papers published in academic journals, first the science citation index (SCI), and later the social sciences citation index (SSCI) and the arts and humanities citation index (AHCI). The first automated citation indexing was done by CiteSeer in 1997. Other sources for such data include general purpose academic citation indexes include:

- Web of Science by Clarivate Analytics (previously the Intellectual Property and Science business of Thomson Reuters).
- Scopus by Elsevier available online only, which similarly combines subject searching with citation browsing and tracking in the sciences and social sciences.
- Indian citation index is an online citation data which covers peer reviewed journals published from India. It covers major subject areas such as scientific, technical, medical, and social sciences and includes arts and humanities. The citation database is the first of its kind in India.

Bibliography

1. Coventry University Harvard Reference Style Guide By Lisa Ganobcsik Williams and Catalina Neculai, Pg. No. 7.
2. Curtis, MJ, Bond, RA, Spina, D, Ahluwalia, A, Alexander, S, Giembycz, MA, and Lawrence, AJ (2015). Experimental design and analysis and their reporting: new guidance for publication in BJP. British journal of pharmacology, 172(14), 3461–3471.
3. Ghosh, M (2007). Fundamentals of experimental pharmacology. Indian Journal of Pharmacology, 39(4), 216–216.
4. Library Services Help Sheet, London South Bank University, Perry Library and Learning Resources Pg. No. 2.

A continuous-flow system was successful, however, with stages of *P. inui* formed after 35 days in culture infective for monkeys.

Rodent Malaria

Among the rodent malarias, the erythrocytic stages of *P. berghei* and *P. chabaudi* have been cultivated *in vitro*. Static cultures of *P. berghei* are developed. In static cultures, conditions may rapidly develop that lead to inhibition of parasite growth and development. Stirring may facilitate rupture of erythrocytes as in passage of infected cells through host capillaries. The use of BME basal medium or BME plus William's medium E gave better growth of and incorporation of radioactive amino acids into *P. chabaudi* than did RPMI 1640 medium. No growth of simian or rodent malarias has been accomplished in cell-free systems.

Avian Malaria

Among the avian malarias, the erythrocytic stage of *P. lophurae* has been cultivated *in vitro*. Parasitic stages of *P. lophurae* freed from host cells by immune lysis were grown extracellularly in duck erythrocyte extract. Degenerate forms of the parasite, reduced incorporation of labeled amino acids, and decreased number, size, and density of food vacuoles appeared with time (about 3 days) in cultured forms.

Sporogonic Stages Ookinete Development

In vitro ookinete formation from *in vivo*-produced gametocytes of *P. berghei* in MEM and other tissue culture media was examined by Weiss and Vanderberg (96). Although MEM gave optimal results, the number of ookinetes formed was 1% or less of the number of macrogametocytes introduced. A better percentage of ookinete formation (up to 44%) can be obtained by comparing HEPES-buffered RPMI 1640 and MEM media, from *in vivo* and *in vitro*-produced *P. berghei* gametocytes. RPMI 1640 gives consistently higher ookinete yields than MEM. Development from gametocyte to oocyst occurred in an acellular medium consisting of RPMI 1640 or Grace's medium with 10 or 20% fetal bovine serum or 5% silkworm serum. The presence of insect cells (Aedes or Toxorhynchites spp.) increased by sixfold the number of oocysts produced compared to the acellular cultures. Cultures, however, did not develop beyond the oocyst stage.

Sporozoite Development

In vitro development of sporozoites of *P. relictum*, an avian parasite, can be done in culture medium consisting of a phosphate and bicarbonate buffered