2. Liver diseases — hepatic necrosis and CCI administration.
3. Bone diseases:
   (a) Rickets
   (b) Osteomalacia
   (c) Hyperparathyroidism
   (d) Osteogenic sarcoma.

For the dog, serum alkaline phosphatase activity can be used as a liver function test.

Serum Glutamic Pyruvic Trnsaminase and Serum Glautamic Oxaloacetic Transaminase. (SGPT & SGOT)

GOT is distributed widely in many tissues but liver , myocardium and skeletal muscles are rich in this enzyme. When there is damage to the cells of these tissue, the enzyme, escapes into the blood and so the SGOT enzymatic activity increases. Glutamic-pyruvic transaminase is present in high concentrations in the liver of cat and dog but little is present in livers of horse, cattle and pigs. So in liver necrosis of dogs and cats the GPT escapes into the blood and so the SGPT activity increases.

Advantage of this is taken to assess the health of the liver in dogs and so this is one of the liver—function testsd in this animal.

Principle

When SGOT is added to a mixture of alpha-ketoglutaric acid and aspartic acid, the enzyme catalyses the transfer of alpha-amino group of aspartic acid and the alpha-ketoglutaric acid and thus oxaloacetic and glutamic acids are formed.

Similarly, SGPT catalyses the transfer of alpha-amino group of alanine and alpha-ketoglutraic acid when added to a mixture or these two amino acids, pyruvic acid and glutamic acid are formed.

Reagents Required

0.1 M phosphate buffer: Dissolve in a liter volumetric flask 13.97 gms of K_2HPO_4 and 2.098 gm NAH_2PO_4 and make upto the mark with distilled water. This has a pH of 7.4.

Stock alpha-ketoglutaric acid solution: In a 100 ml volumetric flask, dissolve 0.292 gm of alpha-ketoglutaric acid in 20 ml of distilled water, adjust the pH to 7.4 with 1 N NaOH and then make upto the mark with distilled water.

1 N NaOH (Approximate): Dissolve 41 gm of NaOH in a litre of distilled water.

Buffered aspartic acid solution: Dissolve 2.955 gm of DL aspartic acid and in 20 ml of 1 N NaOH, adjust pH to 7.4 and make up the volume to 100 ml with 0.1 M phospgate buffer.

GOT substrate Dilute 1 volume of stock alpha-ketoglutaric acid solution with 9 volumes

Procedure

Add 0.1 ml of serum to 6 ml of Ferric chloride precipitating reagent and gently shake. Filter through No 42 filter paper. Prepare final standard by mixing 2 ml of cholesterol working standard (0.1 mg per ml) and 4 ml of Ferric chloride blank solution. To 3 ml each of filtrate, final standard and Ferric chloride blank solution add 2 ml concentrated sulphuric acid slowly. A ring is formed at the interphase. Shake gently. Cool. Compare at 470 mu.

Result :

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \quad \frac{\text{Concentration of standard}}{\text{Volume of unknown}} \times \frac{100}{1} = \text{mg. of cholesterol}$$

Regeants

1. **Stock Ferric chloride colution:** 840 mgs of Ferric chloride is dissolved in a few ml of glacial acetic acid and diluted to 100 ml with acetic acid.

2. **Ferric chloride precipitating reagent:** Stock Ferric chloride solution is diluted 1 in 10 with glacial acetic acid.

3. **Ferric chloride blank:** 1.7 ml of stock Ferric Chloride diluted to 20 ml with glacial acetic acid.

4. **Cholesterol stock standard:** 100 mg pure dry cholesterol is dissolved in 100 ml of glacial acetic acid.

5. **Working standard:** 2 ml of the cholesterol standard mixed with 1.7 ml of stock Ferric choride solution and diluted to 20 ml with glacial acetic acid.

6. **Final standard:** This is prepared by mixing 2 ml of working standard and 4 ml of Ferric chloride solution — just before use.

Cholesterol exists as free cholesterol and cholesterol esters when combined with fatty acids. In the blood serum, total cholesterol ranges, among animals from 50 to 250 mg per 100 ml.

It is the liver that is responsible for the maintenance of homeostasis of serum cholesterol. Cholesterol estimation is indicated in suspected hepatic and thyroid dysfunction.

Hypercholesteremia or Hypercholesterolemia

Increased blood cholesterol values are found in the following conditions. Chronic and acute nephritis, chronic nephrosis, Diabetes mellitus, hjigh lipiud intake, pregnancy, cholelithiasis, hypothyroidism, biliary obstruction and corti-sone therapy.

IMMUNOLOGICAL TEST

A test kit for pregnancy diagnosis in the mare is available from Denver Chemical Manufacturing Co. Stanford. Connceticut, USA. (Detailed description of the test is available in the article. Immunological pregnancy test for Mares by Solmon WJ and Hoff. G (1969). J.Am. Vet. Med. Assoc, 155. 42).

The test is called Hemagglutination Inhibition test. It is based on the presence of Pregnant Mare Serum Gonadotrophin (PMSG) in pregnant mare between the 40th and 120th days of pregnancy. Sheep red blood cells are coated with PMSG. Test kits generally have latex particles coated with PMSG. The antibodies added to the test serum are neutralized by the PMSG present in the positive test serum. So the PMSG coated RBCs settle slowly to the bottom of the test tube, as a ring. The coated RBCs with the negative test serum agglutinate with PMSG antibody and settle fast to the bottom of the test tube as a layer. The antibody is obtained from rabbits (serum) immunised with PMSG. Neutralizer against non specific serum agglutinins is also added while conducting the test.

PREGNANCY DIAGNOSIS USING ULTRASONIC EQUIPMENT

In sheep and sows, pregnancy can be diagnosed by passing ultrasonic waves and receiving them back. The time lapse in between sending and receiving the pulse gives a clue for pregnancy. The ultrasonic Doppler instrument is inserted per rectum. A high accuracy in the diagnosis is reported.

RADIOIMMUNOLOGICAL ASSAY FOR PMSG

This test is very complicated and though accurate, can be conducted only in a well equipped laboratory. This is mentioned here to kindle the enthusiasm and curiosity of interested students.

Radioactive iodinated PMSG (Hot) is agglutinated with antibody PMSG and gamma globulin from heterologous species. The Hot PMSG is replaced by using the principle of competitive binding with known quantities of uniodinated hormone (cold). The hot hormone is separated from the bound complex and radio activity is measured on a gamma counter. A standard graph is prepared. The PMSG from the test is used now to replace the hot PMSG and decrease in radioactivity is measured. This gives an accurate measure of serum level of PMSG.

RADIOGRAPHIC METHODS

X-rays are used for diagnosis of pregnancy in dogs and cats. The calcified fetal skeleton is radio-opaque and ao can be easily seen . In the bitch this is useful to diagnose pregnancy after 45th day. Radiographic diagnosis of pregnancy is possible in ewes also, from the 70th day of pregnancy onwards.

sulphate (SAS) is added to a final concentration of 40% and the precipitate is washed twice in 40% SAS and finally dissolved in 2 ml of normal saline and dialysed against 0.1M Phosphate Buffer saline (PBS) (pH 6.8) to remove the ammonium sulphate. After estimation of protein, it is directly used for conjugation with the enzyme.

(b) The above immunoglobulin is further purified by gel filtration in Sephadex 0.200 column and the break through peaks are pooled, concentrated by vacuum dialysis and used for conjugation after estimation of protein.

II. Conjugation of Immunoglobulin with the Enzyme

1. Protein is estimated and to 5 mg of Ig in PBS (pH 6.8) 12 mg of crytalline horse radish peroxidase enzyme is added and 0.05 ml of 1 % aqueous glutaraldehyde is added in drops
2. Conjugation is allowed to take place at room temperature for 2 hours or at 4°C overnight by constant mixing.
3. Excess of glutaraidehyde is removed by dialysis against threee changes of PBS (pH 6.8).
4. The conjugate is centrifuged at 5000 rpm for 10 minutes and diluted 1:10 and stored at 20°C in small aliquotes.

III. Detection of Rinderpest Viral Antigens in Infected Tissues

1. Buffy coat smears, mouth impression smears and lymph nodes impression smears collected from naturally infected Rinderpest (RP) cases can be used to detect RP viral antigens (Smears from buffy coat of MD cases, brain impression smears from Rabies etc. can be used also for diagnosis of those diseases).
2. The smears and cover slips are fixed in methanol or cold acetone and pre-treated with 10% H_2O_2 to remove the endogenous peroxidase activity.
3. The antibody conjugate is added top the smears and incubated at 37°C for 1 1/2 to 3 hours in a humidified chamber.
4. Wash thoroughly in 0.1 M PBS (pH 6.8) to remove excess of conjugate.
5. Add the substrate reactants: freshly mixed (a) 9 ml of benzidine (50 mg/200 ml) + 1 ml of 40% ammonium chloride + 1 ml. of 5% EDIA (pH 6.0) + 2-3 drops of 3% H_2O_2.

OR

(b) 0.05% 3'-3' diaminobenzidine tetra chloride in Tris-HCI buffer, pH 7-6 with 0.1% H_2O_2

OR

(c) Freshly prepared mixture of 15 mg of Alpha naphthol, 22 mg of P-Phenylne-diamine in 20 ml of 0.07 M PBS, pH 7.2 containing 0.17 M NaCl.

6. Incubate for 5-10 minutes at room temperature and wash in PBS thoroughly.
7. Dry mount and examine under low and high dry lens of a light microscope.
8. Duplicate smears, blocked with untagged antiserum, are used as controls in addition to the normal smears of the corresponding cells and tissues.
9. The cells/tissues containing the viral antigens appear blue or blue black in color.