MATERIALS AND METHODS
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The present investigations were an attempt to explore the effects of different dietary levels of iodine on the metabolic and developmental aspects of albino rats. Various animal experiments were planned and conducted with deficient, normal and excess iodine in the diets on weanling and adult animals, which were matched for age and weight.

The animals were housed individually in previously washed galvanized iron cages. Food and distilled water for drinking were given ad libitum throughout the experimental periods. Animals were weighed initially and thereafter once in a week using triple beam balance (Ohaus, USA).

Preparation of diets

The basal diet was prepared by mixing corn starch and casein. The corn starch and casein were obtained from the local market (Darshan Chemicals). The casein was found to contain 85.0 g % protein, therefore 12.5 g of casein was mixed with 74.5 g of starch to have 10.0 g protein in the mixture. The diet was mixed with vitaminized oil, vitamin mixture and salt mixture at the time of feeding.
The vitamin mixture added at 17 level was prepared as recommended by Oser and Oser (1956). Consisting of water soluble vitamins of 0.6 mg Thiamine Hydrochloride, 1.2 mg Riboflavin, 0.4 mg Pyridoxine Hydrochloride, 5.0 mg Niacin, 4.0 mg Calcium-d-pantethenate, 2.5 mg PABA, 100 mg Inositol, 200 mg Choline Chloride, 1.0 mcg Biotin, 1.0 mcg Folic acid and 1.0 mcg Cyanocobalamine. The groundnut oil was supplemented with fat soluble vitamins to give 200 IU of Vitamin A, 20 IU Vitamin D, 12 mg Vitamin E and 100 mcg Vitamin K in 10 g of oil. The salt mixture was added at 27 level and was prepared as suggested by Hawk and Oser (1964) (Appendix-1).

EXPERIMENTAL PLANS

I. Twentyseven albino rats weighing about 75-85 g were grouped into three groups. Consisting of nine animals in each group (three males and six females). The composition of diets fed is given in the Table No.7. All the animals of the three groups, i.e. low iodine diet (LID), normal iodine diet (NID) and high iodine diet (HID) were fed the respective diets for a period of 47 weeks, after which they were sacrificed.

During the period of 47 weeks the animals were bred twice to obtain progeny and to observe the effect on reproduction. The obtained progeny were sacrificed at different intervals of feeding the respective diets.
Table No.7: Composition of the diets

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>I (LID)</th>
<th>II (NID)</th>
<th>III (HID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>74.5</td>
<td>74.5</td>
<td>74.5</td>
</tr>
<tr>
<td>Casein</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Salt mix</td>
<td>2.0*</td>
<td>2.0**</td>
<td>2.0***</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>100.0</td>
<td>100.00</td>
</tr>
</tbody>
</table>

* Iodine content of the diet 42.2 mcg/100 g diet
** Iodine content of the diet 102.2 mcg/100 g diet
*** Iodine content of the diet 342.2 mcg/100 g diet.
II. Twentyfour albino rats weighing 70 g were required for the experiments. The animals were grouped into four groups consisting of six animals in each. Group-I was fed low iodine diet (LID), group-II was fed normal iodine diet along with 0.5% sodium perchlorate, group-III was fed normal iodine diet (NID) and group-IV was fed high iodine diet (HID). The composition of diet is given in Table No.8. The animals were fed respective diets for the experimental duration of nine weeks after which they were sacrificed.

III. Fortytwo albino rats weighing about 70-73 g were grouped into seven groups each consisting of six animals. Groups-I, II, III and IV were fed in the same manner as mentioned earlier in experiment II. The iodine content of the diet for groups-V, VI, and VII was increased to 10, 15 and 20 times more than the normal iodine diet. Thus three more groups were taken and fed high iodine diet. Composition of the diet is given in Table No.9. The experiment was terminated after eleven weeks of feeding.

IV. Fortytwo rats weighing between 50-60 g were divided into four groups. Group-I was fed normal iodine diet with 0.5% Sodium Perchlorate through drinking water, group-II was fed normal iodine diet (NID), group-III was fed high iodine diet (HID, 20 times more) and group-IV was fed high iodine diet (HID, 25 times more). All the groups contained different number of
Table No.8: Composition of the diets

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>I</th>
<th>II NID+NaClO₄</th>
<th>III NID</th>
<th>IV HID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>74.5</td>
<td>74.5</td>
<td>74.5</td>
<td>74.5</td>
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<tr>
<td>Casein</td>
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<tr>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
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</tr>
</tbody>
</table>

Sodium Perchlorate (NaClO₄)

- --
- 0.5% Through drinking water
- --
- --

Iodine content (mcg/100 g of diet)

- 42.2
- 102.2
- 102.2
- 342.2

* devoid of KI to make it low iodine diet.
Table No. 9: Composition of the diets

<table>
<thead>
<tr>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
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<tbody>
<tr>
<td></td>
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<td>NID</td>
<td>HID</td>
<td>HID</td>
<td>HID</td>
<td>HID</td>
</tr>
<tr>
<td>Ingredients (g)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>12.5</td>
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<td>100.0</td>
<td>100.0</td>
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<td>100.0</td>
</tr>
<tr>
<td>Sodium Perchlorate (NaClO₄)</td>
<td>--</td>
<td>0.5% through drinking water</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Iodine content (mcg/100 g diet)</td>
<td>42.2</td>
<td>102.2</td>
<td>102.2</td>
<td>342.2</td>
<td>642.2</td>
<td>942.2</td>
<td>1242.2</td>
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</table>

* devoid of KI to make a low iodine diet.
animals. The composition of the diets is given in Table No. 10.

Four animals from each group were sacrificed after 18 weeks of feeding and the rest of the animals were continued the experimental feeding for a period of 31 weeks. On completion of 31 weeks feeding the animals were sacrificed.

V. Another generation study was carried out to observe the effects of feeding excessive iodine in the diet and Sodium perchlorate on reproduction and the progeny obtained.

Twentysix animals were grouped into four groups similar to that mentioned in experiment IV. Each consisting of 4, 8, 8 and 6 animals respectively. All the groups consisted of males and females in equal numbers. The diet composition is given in the Table No. 10. The animals were bred and the pups obtained were sacrificed on the 15th day and 20th day.

VI. A long term study was planned to observe the effect of feeding Sodium Perchlorate (Goitrogenic substance) to rats. Twelve animals were divided into two groups having six animals in each consisting of 2 males and 4 females respectively. Group-I was fed sodium perchlorate (0.5%) through drinking water along with normal iodine diet and group-II was fed normal iodine diet (NID). Animals were fed for an experimental duration of twelve months, after which they were sacrificed.
Table No. 10: Composition of the diets

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>I (NID+NaClO₄)</th>
<th>II (NID)</th>
<th>III (HID)</th>
<th>IV (HID)</th>
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<tr>
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<td>74.5</td>
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<tr>
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<td>2.0</td>
</tr>
<tr>
<td>Sodium Perchlorate (NaClO₄)</td>
<td>0.5% through drinking water</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Iodine content (mcg/100 g of diet)</td>
<td>102.2</td>
<td>102.2</td>
<td>1242.2</td>
<td>1542.2</td>
</tr>
</tbody>
</table>
Food intake:

The animals were served known amount of diet daily. The left over diet was removed every day and collected in brown paper bags and dried in an oven at 60°C to make it moisture free, as 8–10 ml of water was added to every 10 g of diet to make it edible. After drying it was weighed to calculate the actual food intake.

Food consumed g per week = Food given - (left over in the bowl + spilled food)

Autopsy Procedure:

At the end of each experimental period the rats were weighed and sacrificed under mild anaesthesia. Blood was collected from the heart directly into clean plain dry and heparinized tube. Blood collected in heparinised tubes were used for estimation of Haemoglobin and Glucose content. The blood collected in plain dry tubes were allowed to clot. The clot was removed and the remaining fluid was centrifuged under refrigeration for obtaining serum which was stored in the freezer until it was analysed.

Tissues were excised and blotted free of blood and tissue fluids on a filter paper, cleared off extraneous tissues and weighed on a preweighed aluminium foil. A known amount of liver tissue was homogenised and centrifuged, which was used for various estimations.
The parameters recorded and analysed during the entire period of study are as follows:

a) Physical parameters

i. Food intake
ii. Weight gain
iii. Tissue fresh weights

b) Biochemical parameters

1. Blood
   i. Haemoglobin content
   ii. Glucose content

2. Serum
   i. Thyroxine (T₄) levels
   ii. Triiodothyronine (T₃) levels
   iii. Thyrotropin (TSH) levels
   iv. Aspartate Glutamate transaminase enzyme activity (AST, EC: 2.6.1.1)*
   v. Alanine Glutamate transaminase enzyme activity (ALT, EC: 2.6.1.2)
   vi. Lactate dehydrogenase enzyme activity (LDH, EC: 1.1.1.27)
   vii. Creatine kinase enzyme activity (CK, EC: 2.7.5.2)**
   viii. Total protein content
   ix. Total cholesterol content
   x. HDL-cholesterol content
   xi. Triglyceride content

3. Liver
   i. Total protein content
   ii. Total Cholesterol content.

* Serum HDL-cholesterol content and serum AST, ALT and LDH enzyme activity were estimated in experiments IV, V and VI.

** Serum Creatine kinase enzyme activity was estimated in experiment VI.
BLOOD HAEMOGLOBIN

Haemoglobin content of blood was estimated by the Chemkit supplied by Ranbaxy Diagnostics based on the Cyanmethaemoglobin method (Van Kampen and Zijistra, 1961).

Twenty microlitres of blood was added to 5.0 ml of Cyanmeth reagent, mixed thoroughly and after 3.0 minutes the colour developed was read at 540 nm against the reagent blank.

Blank: Cyanmeth Reagent as such.
Standard: Range of standard series 5.0-15.0 gm/dl was treated same as above and a calibration curve was prepared. Curve given in the Appendix-2.

Calculation:
Blood Haemoglobin gm per 100 ml = O.D. of sample x 36.77 (factor)

BLOOD GLUCOSE

Glucose content of blood was determined by the Glucose Oxidase method using O-Dianisidine given by Huggett and Nixon (1957).

Hundred microlitres of blood was added to 1.0 ml of 0.05 M Sodium Hydroxide. To this 0.1 ml of 10% Zinc Sulphate was added, mixed well and allowed to stand for 10 minutes then centrifuged and supernatant collected.
Two hundred microlitres of the supernatant was taken and 4.0 ml of Enzyme Dye Reagent was added. Incubation was carried out at 37°C for 45 minutes. Colour developed was read at 430 nm against blank.

Blank: 0.2 ml of distilled water and 4.0 ml of Enzyme Dye Reagent, mixed and used as blank.

Standard: 100 microlitres of glucose standard (100 mg%) treated same as sample.

Calculation:

\[
\text{Blood glucose mg per 100 ml} = \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times 100
\]

**SERUM TRIIODOTHYRONINE (T₃)**

Triiodothyronine levels of serum was estimated by the Enzyme Immunoassay (EIA) kit supplied by BioMerieux, France. The assay is based on a competitive reaction principle (Kaplan, 1985 and Ruffie, 1984).

The assay is performed in two steps.

Step 1: IMMUNOLOGICAL

Hundred microlitres of serum was added in tubes coated with anti-T₃ antibodies. 400 microlitres of Enzyme Conjugate was added and allowed to react. The tubes were incubated for 2 hours.
at 15-20°C. Then the tubes were repeatedly washed with wash solution.

Step 2: ENZYMATIC

To the tube 500 microlitres of Chromogen Substrate is added and incubated for 30 minutes at 15-20°C. On adding 2.0 ml stopping reagent a yellow colour developed is read at 492 nm against the reagent blank.

Blank: 500 microlitres of Chromogen Substrate and 2.0 ml of stopping reagent in EIA tube.

Standard: Range of standard series 0-7.8 ng per ml supplied was treated the same as above.

Calculation: A standard curve was prepared by plotting the standard absorbance against the standard concentration. The sample absorbance was read off the concentration of Triiodothyronine on the curve and expressed as ng per ml of serum (standard curve is presented in Appendix-3).

SERUM THYROIDINE (T₄)

Total Thyroxine levels of serum was estimated by the Enzyme Immunoassay (EIA) kit supplied by BioMerieux, France. The
The assay is based on a competitive reaction principle (Kaplan, 1985 and Ruffie, 1984).

The assay is carried out in two steps.

Step 1: IMMUNOLOGICAL

Twenty microlitres of serum was added in tubes coated with anti-T4 antibodies. 500 microlitres of Enzyme Conjugate was added and allowed to react. The tubes were incubated for one hour at 15-20°C, then the tubes were repeatedly washed with wash solution.

Step 2: ENZYMATIC

To the tube 500 microlitres of Chromogen Substrate was added and incubated for 30 minutes at 15-20°C. On adding 2.0 ml of stopping reagent the yellow colour developed was read at 492 nm against reagent blank.

Blank: 500 microlitres Chromogen Substrate and 2.0 ml of stopping reagent in EIA tube.

Standard: Range of standard series 0-24.86 mcg per 100 ml supplied was treated the same as above.

Calculation: A standard curve was prepared by plotting the standard absorbance against the standard concentration.
The sample absorbance was read off the concentration of Thyroxine on the curve and expressed as mcg per 100 ml of serum (standard curve is presented in Appendix-4).

**SERUM THYROTROPIN (TSH)**

Thyrotropin (TSH) levels of serum was estimated by the Enzyme Immuno Assay (EIA) kit supplied by BioMerieux, France. The assay is based on the sandwich technique using two monoclonal antibodies (Azais and Kaplan, 1985).

The assay is performed in two steps.

**Step 1: IMMUNOLOGICAL**

Two hundred microlitres of serum was added in tube coated with anti-TSH antibodies. 200 microlitres of enzyme conjugate was added and allowed to react. The tubes were incubated for 2 hours at 15-25°C with continuous shaking at 350 rpm. Then the tubes were repeatedly washed with wash solution.

**Step 2: ENZYMATIC**

Three hundred microlitres of Chromogen Substrate was added to the tube and incubated for 30 minutes at 15-25°C. On adding 1.0 ml of stopping reagent yellow colour developed was read at 492 nm against the reagent blank.
Blank: 300 microlitres of Chromogen Substrate and 1.0 ml of stopping reagent in EIA tube.

Standard: Range of standards 0-40 micro IU per ml supplied was treated the same as above.

Calculation: A standard curve was prepared by plotting the standard absorbance against the standard concentration. The sample was read off the concentration on the curve and expressed as micro IU per ml of serum (standard curve is presented in Appendix-5).

**SERUM TOTAL PROTEIN**

Total Protein was estimated by the Biuret Method given by Reinhold (1953) with little modification. Exactly 20 microlitres of serum was added to 2.5 ml of working Biuret reagent, incubated at 37°C for 15 minutes. The colour developed was read at 540 nm against the blank.

Blank: Working Biuret reagent as such

Standard: 20 microlitres of standard (8.0 gm% Sigma Protein Standard) was treated same as sample.

Calculation:

Serum protein g per 100 ml = \( \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times 8 \)
LIVER PROTEIN

Protein content of liver was determined by the method of Lowry et al. (1951).

Ten percent liver homogenate was prepared in 0.9% of KCl by homogenizing at 3000 rpm in Remi homogenizer. 0.2 ml of the homogenate was precipitated with 10% TCA, mixed and centrifuged at 5000 to 10000 rpm for 10 minutes.

The supernatant was decanted and the precipitate dissolved in 10.0 ml of 0.1 N NaOH. 0.2 ml of the above solution was used for protein estimation. Total volume made to 0.6 ml with distilled water. To this 3.0 ml of Lowry C reagent was added and was allowed to stand for 10 minutes at room temperature. Later 0.3 ml of Lowry E diluted reagent was added and cyclomixed. Exactly after 30 minutes the colour developed was read at 750 nm against blank.

Blank: 0.6 ml of distilled water treated same as sample.

Standard: A standard series was prepared having 40, 80, 120 and 160 mcg per tube and treated same as sample.

Calculation:

Liver protein g per 100 g of tissue =

\[
\text{Calculation:} \quad \text{Liver protein g per 100 g of tissue} = \frac{\text{Conc. of std.} \times \text{O.D. of sample} \times \text{Vol. made} \times \text{O.D. of std. aliquot taken} \times \text{sample taken}}{	ext{Liver wt.} \times 1000 \times 1000}
\]

82
SERUM TOTAL CHOLESTEROL

Total cholesterol content of serum was determined by the method of Zlatkis, Zak and Boyle (1953) with a little modification.

Fifty microlitres of serum was precipitated with 2.0 ml of Acetone - Ethanol mix (1:1) shaken well and centrifuged. Supernatant was collected in another test tube. The above procedure was repeated once again to extract maximum lipids. The collected supernatant was evaporated completely at 80-90°C in water bath. After complete evaporation 3.0 ml of glacial acetic acid was added and tube was boiled for a minute. On cooling 2.0 ml of colour reagent was added. Tube was cooled and colour read at 570 nm against the blank.

Blank: 3.0 ml of glacial acetic acid mixed with 2.0 ml of colour reagent.

Standard: Range of standard series 50-200 microgram per ml was prepared and treated the same as sample.

Calculation:

Serum total cholesterol mg per 100 ml =

\[
\frac{\text{Conc. of Std. (mcg)}}{\text{O.D. of std.}} \times \frac{\text{O.D. of sample}}{\text{sample taken}} \times \frac{\text{100}}{\text{1000}}
\]
LIVER TOTAL CHOLESTEROL

Total cholesterol content of the liver tissue was determined by the method given by Varley (1969).

Approximately 0.4 to 0.5 g of fresh liver tissue was taken in 1-2 ml of 0.9% saline solution. Homogenized at 3000 rpm in Remi homogenizer and volume made upto 10 ml with acetone-ethanol mixed (1:1) and kept in cold conditions. Then centrifuged for 10 minutes at 3000 rpm, supernatant collected and volume made upto 10 ml with acetone-ethanol mixture.

Known amount of the supernatant was taken in a test tube and evaporated completely at 80-90°C in water bath. The remaining procedure carried out was similar to that of serum total cholesterol estimation.

Calculation:

Liver total cholesterol mg per gm of tissue:

\[
\text{conc. of std. (mcg)} \times \frac{\text{O.D. of sample}}{\text{O.D. of std.}} \times \frac{\text{total vol. made}}{\text{aliq. taken}} \times \frac{1}{\text{liver taken(g)}} \times \frac{1}{1000}
\]

SERUM TRIGLYCERIDE

Serum triglyceride was estimated by the Autopak kit supplied by Miles India Ltd., based on the enzymatic DHBS colourimetric method (Fossati, 1982 and Eggstein et al., 1974).
Ten microlitres of serum was mixed with 1.0 ml of the working reagent, incubated for 15 minutes at room temperature. The colour developed was read at 505 nm against reagent blank.

Blank: Reagent as such.

Standard: 10 microlitres of triglyceride standard (200 mg/dl) was treated same as sample.

Calculation:

\[
\text{Serum triglyceride mg per 100 ml} = \frac{\text{O.D. of sample}}{\text{O.D. of std.}} \times 200
\]

HDL-CHOLESTEROL

HDL-cholesterol content of serum was estimated using the Autopak Kit supplied by Miles India Ltd., Baroda, based on the phosphotungstate method (Lopes-Virella et al., 1977; Allan et al., 1974; Richmond et al. 1973; Castelli et al. 1977; Miller et al. 1977; Friedewald et al. 1972).

Two hundred microlitres of serum was precipitated with 0.2 ml of precipitating reagent. Mixed well and centrifuged at 3500-4000 rpm for 10 minutes. Clear supernatant was separated immediately and cholesterol content was estimated.

Twenty microlitres of supernatant was added to 1.0 ml of HDL-cholesterol reagent, incubated at room temperature for 30 minutes. The colour developed was read at 505 nm against reagent blank.
Blank: Reagent as such.

Standard: 20 microlitres of HDL-cholesterol (50 mg/dl) was treated as above with precipitation.

Calculation:

**Serum HDL-cholesterol mg per 100 ml =**

\[
\text{Conc. of std (mcg)} \times \frac{\text{O.D. of sample}}{\text{O.D. of std.}} \times \frac{\text{Total volume made}}{\text{Aliq. taken}} \times \frac{\text{sample taken}}{1000} \times 100
\]

**SERUM AST AND ALT (EC: 2.6.1.1 & EC: 2.6.1.2)**

Aspartate-glutamate transaminase (AST): Serum AST was estimated by the Autopak kit recommended by the Scandinavian committee on enzymes supplied by Miles India Ltd., Baroda (SCE, 1974; Penttila et al., 1975 and Hafkenscheid et al. 1979).

Hundred microlitres of serum was added to 1.0 ml of assay mixture and the change in extinction was read at 340 nm at every 30 seconds interval for 2 minutes.

Alanine-glutamate transaminase (ALT): Serum ALT was also estimated as above.

Units of Expression:

I.U. per litre of serum. The change in extinction per minute was multiplied by factor 1749 to convert U.V. reading into I.U.
SERUM LACTATE DEHYDROGENASE (LDH) (EC : 1.1.1.27)

Lactate dehydrogenase activity from serum was estimated by the U.V. method with L-lactate and NAD as given by Anne Vassault (1981) in Methods in Enzymatic Analysis.

Fifty microlitres of serum was added to 2.5 ml of Tris/NaCl/NADH reagent and mixed thoroughly. To this 0.5 ml of Tris/NaCl/Pyruvate was added, mixed and the absorbance was read exactly after 30 seconds at 339 nm for 2 minutes. Against distilled water as blank. The assay mixture contains Tris, 80 mmol/lt; NaCl, 2000 mmol/lt; NADH, 0.2 mmol/lt and Pyruvate, 1.06 mmol/lt.

Calculation:

Serum lactate dehydrogenase activity IU/Lt. = \( A \times 9682 \) (factor) 

SERUM CREATINE KINASE (CK) (EC: 2.7.3.2)

Thirty microlitres of serum was allowed to react with 1.0 ml of reagent. The absorbance was read at 340 nm against distilled water at every 30 seconds interval for 2 minutes.

Calculation:

Serum Creatine Kinase IU/Lt. = Mean absorbance x 5520 (factor)
Statistical formulas used in the treatment of data (Gupta, 1984).

1. Arithmetic mean ($\bar{X}$)

$$\bar{X} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Where $X = \text{individual values}$

$n = \text{size of the sample}$

2. Standard deviation (SD)

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{X})^2}{n-1}}$$

Where $(x_i - \bar{X}) = \text{deviation of the individual value from the arithmetic mean}$

$n = \text{size of the sample}$

3. Standard error (SE)

$$SE = \frac{SD}{\sqrt{n}}$$

Where $SD = \text{standard deviation}$

$n = \text{size of the sample}$

4. 't' test for difference of means

$$t' = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where $\bar{X}_1 = \text{arithmetic mean of the first sample}$

$\bar{X}_2 = \text{arithmetic mean of the second sample}$

$S_1 = \text{standard deviation of the first sample}$

$S_2 = \text{standard deviation of the second sample}$

$n_1 = \text{size of the first sample}$

$n_2 = \text{size of the second sample}$
Reagents used in analytical procedures.

Sr. Name of reagent and method of preparation
No.

1. Acetic acid glacial.

2. Acetone-Ethanol mix: one volume of Acetone mixed with one volume of Ethanol.

3. ALT assay mixture: Consists of Alanine buffer, NADH, LDH and Alpha-ketoglutarate.


5. Anti-T₄ tubes: Polystyrene tubes coated with anti-T₄ antibodies (rabbit).


8. AST assay mixture: Consists of Aspartate buffer, NADH, MDH and Alpha-Ketoglutarate.

9. Biuret Reagent:

Stock: 45 gm of Rochelle Salt (Sodium Potassium Tartarate) was dissolved in 400 ml of 0.2 N Sodium Hydroxide, 15 g of Copper Sulphate was added and dissolved completely. To this 5 gm of Potassium Iodide was added and volume made upto 1 litre with 0.2 N Sodium Hydroxide.

Working: 200 ml of stock Biuret reagent was diluted to 1
litre with 0.2 N Sodium Hydroxide containing 5 gm of Potassium Iodide.

10. Cholesterol standard:
   Stock: 100 mg of pure Cholesterol dissolved in 100 ml of acetic acid.
   Working: 10 ml of stock diluted to 100 ml with Acetic acid.


13. Colour reagent:
   Stock: 10 g of Ferric Chloride dissolved in 100 ml of Acetic acid.
   Working: 1.0 ml of stock diluted to 100 ml with concentrated Sulfuric acid.


15. Cyanmeth reagent: Ready to use.

16. Enzyme Dye Reagent: 125 mg of Glucose Oxidase, 5 mg of Peroxidase and 0.5 ml of 1% O-Dianisidine (in 95% ethanol) per 100 ml of the phosphate solution (pH 7.0).

17. Glucose standard: 100 mg per 100 ml of 0.3 % benzoic acid.

18. Haemoglobin standard: 60 mg Haemoglobin per 100 ml

19. Heparin (1.0 g%): 100 mg of Heparin salt was dissolved in 10.0 ml of distilled water.
20. HDL-cholesterol reagent: Cholesterol Esterase, Cholesterol oxidase, Peroxidase and the chromogen 4-aminophenazone phenol.

21. HDL-cholesterol precipitating reagent: Phosphotungstic acid and Magnesium Chloride.

22. HDL-cholesterol standard: 50 mg per 100 ml

23. Lowry A reagent (2% Sodium Carbonate): 2.0 g of Sodium Carbonate dissolved in 100 ml of 0.1 N NaOH. Prepared freshly.

24. Lowry B reagent: 1% Copper Sulphate solution and 2% Sodium Potassium Tartrate solution. Mixed in equal volumes freshly.

25. Lowry C reagent: Lowry A reagent & Lowry B reagent mixed in 50:1 ratio freshly.

26. Lowry E reagent: 50 g of Sodium Tungstate, 12.5 g of Sodium Molybdate, 350 ml of distilled water, 25 ml of 85% Phosphoric acid, 50 ml of concentrated HCl (12N) were refluxed for 18 hrs and solution was cooled. To this 75 g of Lithium Sulphate, 25 ml of distilled water and 5 to 6 drops of Bromine was added. The mixture was boiled for 15 minutes to remove excess Bromine. Cooled at 30°C and diluted to 500 ml with distilled water, filtered and stored in brown bottle.
27. Diluted Lowry E reagent: One part of the stock diluted with two parts of distilled water.

28. Potassium Chloride (0.9% solution): 0.9 g of Potassium Chloride (KCL) dissolved in 100 ml of distilled water.

29. Protein standard: 8 gm% standard protein from Sigma Chemical Company, U.S.A.

30. Protein standard: 0.1 ml of protein standard (Sigma) 8.0 g % was diluted to 10 ml with 0.1N NaOH.

31. Saline solution (0.9% NaCl): 0.9 g of Sodium Chloride dissolved in 100 ml of distilled water.

32. Sodium dihydrogen phosphate solution (0.5 M): 76 g of NaH$_2$PO$_4$.2H$_2$O per litre adjusted to pH 7.0 with NaOH.

33. Sodium Hydroxide (0.05 M): 2 gm of NaOH was dissolved in 1000 ml of distilled water.

34. Sodium Hydroxide (0.2 N): 8 gm of Sodium Hydroxide (NaOH) dissolved in 1000 ml of distilled water.

35. Sodium Hydroxide (0.1 N): 4.0 g of Sodium Hydroxide (NaOH) dissolved in 1000 ml of distilled water.

36. Stopping reagent: 1.8 N H$_2$SO$_4$.

37. Sulfuric acid concentrated.

38. $T_3$ buffer: Solution of Tris, NaCl, ANS, Sodium Salicylate and Bovine Albumin.

39. $T_4$ buffer: Solution of Tris, NaCl, ANS, Sodium Salicylate and Bovine Albumin.


42. T₃ standards: 0-7.81 ng/ml, human sera + Triiodo-L-Thyronine.

43. T₄ standards: 0-24.86 mcg/100 ml, human sera + L-Thyroxine.

44. Trichloroacetic acid (10% TCA solution): 10 g of Trichloroacetic acid (TCA) was dissolved in 100 ml of distilled water.

45. Triglyceride standard: 200 mg per 100 ml.

46. Triglyceride working reagent: Enzymes, buffer, chromogen and stabilizer.

47. Tris/NaCl solution (Tris 81.3 mmol/lt; NaCl, 203.3 mmol/lt). Dissolve 4.92 g of Tris and 5.95 g of NaCl in 400 ml of distilled water. Adjust pH 7.2 at 30°C with 5 mol/lt HCl. Make upto 500 ml with distilled water.

48. Tris/NaCl/NADH solution (Tris, 81.3 mmol/lt; NaCl, 203.2 mmol/lt; NADH, 0.244 mmol/lt, pH 7.2). Dissolve 0.017 g of NADH, disodium salt in 100 ml solution of Tris NaCl solution.

49. Tris/NaCl/Pyruvate solution (Tris, 81.3 mmol/lt; NaCl, 203.2 mmol/lt, pH 7.2; Pyruvate 9.76 mmol/lt). Dissolve
0.107 g of Sodium Pyruvate crystallized monoSodium salt in 100 ml of Tris/NaCl solution.

50. TSH standards: Calf serum concentration between 0-40 micro IU/ml.

51. Wash solution: Sodium Phosphate, Sodium Chloride, Tween 20 (Sodium Merithiolate).

52. Zinc Sulphate (10%): 10 g of ZnSO₄·7H₂O was dissolved in 100 ml of distilled water.