MATERIAL & METHODS
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SUBJECT OF STUDY:

The study was conducted on a series of 90 patients of either sex ranging between 15 - 60 years of age, admitted at the A.I.R. Medical College Hospital, Jhansi for elective operation from various surgical specialities. The actual operative duration lasted from 45 to 90 minutes (excluding the duration prior to commencement of surgery itself). The patients selected were from A.S.A. Grade I or II.

SELECTION OF PATIENTS:

Only patients, fulfilling the following criteria, were selected for the present study:

1. Patients were between 15 - 60 years of age.
2. Patients were of either sex (Male/Female).
3. The history of the patient did not suggest any disorder, other than that for which the patient was being kept for surgery.
4. On detailed clinical examination of the patient, there was no evidence suggestive of some systemic, metabolic, endocrinal, hepatic, renal, cardiovascular or neurological disorder.
5. The anticipated duration of surgery was within 45 - 90 minutes.
6. The patient did not regularly take any drug likely to influence the levels of sugar or FFA in the blood (particularly hypoglycaemics, hormones, corticosteroids, alpha- and beta- blocking drugs and drugs causing hyper- or hypocholesterolaeemia), at least not within 15 days preceding the operation.
7. The patient did not receive any dextrose-water, dextrose-saline, plasma-expander solution or blood transfusion during the 24 hours preceding the operation.
8. The patient, (if subjected to any previous anaesthetic-surgical procedure), did not show any unusual response.

INVESTIGATIONS:

Every patient was investigated for the following:

1. Total leucocyte count.
2. Differential leucocyte count.
3. Erythrocyte Sedimentation Rate.
4. Urine for sugar and albumin.
5. Urine for microscopic examination.
7. Blood urea (if indicated).
8. Serum cholesterol (wherever desired).
9. Liver function tests (wherever indicated).
12. Intravenous pyelography (where indicated).
14. Plasma free fatty acids (FFA) in the serial blood samples.

PREMEDICATION AND PREPARATION OF THE PATIENTS:

The anaesthetic and operative procedures were carefully explained to all patients. They were properly assured in order to allay any anxiety or apprehension, they might have entertained. Special care was taken to avoid any undue alarm on the part of patients.

All the patients were given lorazepam 2 - 4 mg orally in the night preceding the operation. Thereafter, they were allowed nothing by mouth for about 5 - 7 hours prior to operation.

All the patients were given injection atropine 0.65 mg
intramuscularly 45 minutes before the induction. No other premedication
was given.

ANAESTHESIA:-

Anaesthesia was administered to all the patients in following
way:-

(A) **INDUCTION**:- Pre-oxygenation of the patient for 5 minutes
followed by the sleep dose of 2.5% solution of Thiopentone
sodium and injection Succinylcholine 1 mg/kg were given. After
intermittent positive pressure ventilation of 1 - 2 minutes
all the patients were intubated either oroendotracheally or
nasoendotracheally.

(B) **MAINTENANCE**:- Patients were maintained on any one of the following:-

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Di-Ethyl Ether  Trichloroethylene  Halothane

( 6% - 10% )  ( 0.5% - 1.0% )  ( 0.5% - 1.0% )

An anaesthetic gas mixture of oxygen and nitrous oxide
in the ratio of 40 : 60 with a total flow of 6 - 8 litres/minute
(equal to the minute volume of the patient) along with the
particular inhalational anaesthetic agent was administered to
the patient, using a Macmillan's semi-closed circuit. Sub-epinephrine
doses of non-depolariser muscle relaxant were used, if needed.

Although no blood gas analysis was done, every care
was taken to avoid hypoxia, hypercarbia, excessive tachycardia
or excessive tachypnoea. Hyperventilation was also avoided.
Similarly adequate analgesia and anaesthesia was maintained through
out the whole procedure.

(C) **REVERSAL:** At the end of operation, the patients were reversed from anaesthesia by gradually decreasing the concentration of inhalational anaesthetic and Nitrous oxide, while increasing that of Oxygen. Then patients were allowed to breathe 100% Oxygen for 5 minutes. Patients were shifted to Post-operative ward, when they could maintain a proper airway on their own and the protective reflexes had returned.

**INTRAVENOUS FLUID ADMINISTRATION DURING WHOLE PROCEDURE:**

Patients were transfused only with 0.9% physiological saline (Sodium Chloride) solution. 5% dextrose-water or dextrose-saline solution were avoided, because they may alter the sugar or FFA level in the blood. The solution was infused at the minimum rate and a maximum of 1000 ml fluid was infused.

**WITHDRAWAL OF BLOOD SAMPLES:**

Serial blood samples were taken for the estimation of sugar and FFA in the blood. Samples were withdrawn from the ante-cubital vein (or some suitable vein) by a wide bore needle, under strict aseptic precautions, as under:

(a) First sample was taken 45 minutes after premedication and just before induction of anaesthesia.

(b) Second to fifth samples were taken after induction of anaesthesia at 5, 15, 30 and 45 minutes respectively.

(c) Sixth and seventh samples were taken after start of surgery at 20 and 45 minutes respectively.

The samples were stored at 0°C and analysed for blood sugar and FFA at the earliest possible time.

The mean values of the first samples (taken just before the
induction of anaesthesia) served as controls, with which the subsequent serial mean values of these parameters in each group were compared statistically.

**DETERMINATION OF BLOOD SUGAR LEVELS**

Modified Folin Wu's technique was adopted which is most widely used upto the present time, especially in hospital laboratories. The estimations were done at the earliest possible time to overcome the error which occurs due to reduction of blood glucose on storage.

The basic principle of this technique is — precipitation of blood proteins, reduction of alkaline cupric sulfate to cuprous oxide, and estimation of the amount of such reduction colorimetrically.

**Reagents:**

1. Sodium tungstate 10% — made by dissolving 10 gms. of Na₂W₄O₁₁·2H₂O in water and solution was made to 100 ml.
2. Sulphuric acid 2/3 N. (0.66 N).
3. Alkaline copper sulfate — pure anhydrous sodium bicarbonate 40 gms. was dissolved in 400 ml. water in a litre flask. To this 7.5 gms. of tartaric acid was added and when the latter had dissolved, 4.5 gms of crystallised copper sulfate was added to it. The solution was mixed and the volume was made upto one litre.
4. Phosphomolybdic acid — Molybdic acid 35 gms. and sodium tungstate 5.0 gms. were placed in a 500 ml. beaker and to it 200 ml. of 10% sodium hydroxide was added which was followed by addition of 200 ml. of water. This solution was boiled for about 30 minutes to remove ammonia present in the molybdic acid (volume was reduced to about 350 ml.) The solution was cooled and then 125 ml. of 85% phosphoric acid was added and the solution was diluted to 500 ml.
5. Standard glucose solution:—

Stock: 1% solution of dextrose in a 0.25% benzoic acid was made by dissolving 10 mg. of anhydrous dextrose per ml. of 0.25% benzoic acid.

Working solution: 10 ml. of stock solution was diluted to 100 ml. with 0.25% benzoic acid (1 mg/ml.).

Procedure:

Estimations were carried out in duplicates by taking 0.2 ml. blood along with blank and standard working glucose solution (1 mg = 1 ml.). 3.2 ml. of glass-distilled water was taken in a test-tube to which 0.2 ml. blood was added. To this 0.3 ml. sodium tungstate (10%) and 0.3 ml. sulfuric acid (2/3 N) was added (precipitation of blood proteins takes place). This solution was allowed to stand for about 20 minutes, was then mixed well and centrifuged for 10 minutes. 1 ml. of the supernatant was taken in another test-tube and in case of standard and blank, 1 ml. working standard glucose solution and 1 ml. distilled water respectively were taken and 1 ml. alkaline copper sulfate was added in each tube (Reduction of alkaline cupric sulfate to cuprous oxide takes place). Then the tubes were kept in boiling water for six minutes. 1 ml. phosphomolybdic acid was added in each tube and the tubes were kept again in boiling water for two minutes. To this, 9.5 ml. of glass distilled water was added to make the total volume to 12.5 ml. in each tube. The optical density was measured using blue filter in a colorimeter.

Calculations:

\[
\text{Blood sugar in mg/dl} = \frac{\text{Readings of the unknown (test)}}{\text{Readings of the standard}} \times 100
\]

(In each analysis, two standard solutions were treated simultaneously to reduce the error.)
DETERMINATION OF FREE FATTY ACIDS IN SERUM

Millian Novak's technique (1965) was employed for the estimation of free fatty acid in serum. The basic principle of this technique is — extraction of free fatty acid from the serum, esterification of them by the help of cobalt reagent and then their estimation colorimetrically with the help of an indicator.

Reagents :

COBALT REAGENT :

Solution A :-

Cobalt nitrate-acetic acid — potassium sulfate, was prepared by adding to a solution of K₂SO₄ (saturated while boiling, stored in contact with excess crystals, and filtered before use), 6 gms. of Co(NO₃)₂ • 6 H₂O and 0.8 ml. of acetic acid to give a total volume of 100 ml. at 37°C.

Solution B :- a standard Na₂SO₄ solution, was prepared by adding sodium sulphate to boiling water, kept at 37°C overnight.

Preparation of cobalt reagent - Tristanolamine, 1.35 volume was made upto 10 volumes with solution A. Solution B, 7 volumes was added and the mixture was shaken. This reagent was not stable and was prepared fresh for every series of analyses. Solution A and B were kept at 37°C.

INDICATOR :

Stock solution : 0.4% alpha-nitroso-beta-naphthol in 96% ethanol was prepared by dissolving 0.4 gms. of it in 100 ml. of 96% ethanol. This stock solution 4 ml. was diluted with 46 ml. ethanol before use.
DOLE'S EXTRACTION MIXTURE:

This mixture was prepared by mixing isopropyl alcohol 40 parts, heptane 10 parts, and 1.0 N H₂SO₄ 1 part (all solvents redistilled).

CHLOROFORM : HEPTANE: 5 : 1 (V/V) was made up using redistilled chloroform and heptane.

STANDARD PALMITIC ACID SOLUTION (0.05 M): This was prepared by dissolving palmitic acid 1.3 gms. in Dole's extraction mixture 100 ml. and was stored at 0.0°C.

PROCEDURE: Estimations were carried out in duplicates along with blank and palmitic acid standard.

To 2.5 ml. of Dole's extraction mixture in one of the glass-stoppered tube, 1 ml. serum was added. The liquids were mixed by vibration, care being taken not to allow them to reach the stopper. The test tubes were cooled for 10 minutes in a bath of melting ice. To this 3 ml. of Heptane was added followed by 4 ml. of glass-distilled water. The contents of the tube were then thoroughly mixed. After the phases had separated, 2 ml. was drawn from the upper heptane phase and transferred to another stoppered centrifuge tube. 4 ml. of Chloroform-Heptane was added to this tube followed by 5 ml. of freshly prepared cobalt reagent and the solution was thoroughly mixed for 3 minutes. The mixture was centrifuged for 15 minutes at 2500 rpm and 4 ml. of the upper chloroform-heptane phase was transferred to a test tube containing pinch of anhydrous sodium sulfate. 3 ml. aliquot of the above dehydrated chloroform-heptane mixture was transferred to a test tube containing 3.5 ml. of the indicator solution --- alpha-nitroso-beta-naphthol. The samples were treated simultaneously with the standard solution and blank.
Values were read 30 minutes later at 500 millimicrons in a spectrophotometer.

**CALCULATIONS:**

Standard solution: 1.3 gms. of palmitic acid \((C_{15}H_{31}COO)\) per 100 ml. Dole's extraction mixture or 5.07 mEq/litre.

\[
\text{FFA in mEq/litre} = \frac{\text{Reading of Unknown (test) solution}}{\text{Reading of standard solution}} \times 5.07
\]

In each analysis, two standard solutions were treated simultaneously to reduce the error.

In general, free fatty acid and blood sugar determinations were performed on each specimen; however to test reproducibility, triplicate determinations were performed at intervals throughout the study.