The significance of proper materials and methods which form an essential adjuvant to a planned work, needs no emphasis. For the purposes of description the following serial order has been arbitrarily chosen with a view to give a sequential impression in the assessment of a developing integrated picture. What follows in the following pages is, therefore, an attempt to put the bits of building stones in a proper link.

I. Selection of animals.

II. Preoperative care of the animals.

III. Surgical techniques used.

1) Anaesthesia.

2) Implantation of electrodes and stimulation through these.

3) Electrolytic lesions.

4) Surgical ablations.

5) Preparation of gastric pouches.
IV. Post-operative care of the animals.

V. Study of different visceral systems in anaesthetised and unanaesthetised states.

1) Cardiovascular System.
   (i) Heart rate.
   (ii) Blood pressure.
   (iii) Changes in blood flow through hand.

2) Respiratory system.
   (i) Rate.
   (ii) Depth.

3) Body temperature.

4) Gastrointestinal system.
   (i) Collection of gastric contents.
      (a) Aspiration method.
      (b) Gastric pouches.
   (ii) Estimation of volume, free and total acidity and pepsin contents of gastric secretion.
   (iii) Gastric motility.
   (iv) X-ray after barium meal.

5) Blood Chemistry.
   (i) Estimation of blood sugar.
   (ii) Estimation of blood sodium and potassium.
   (iii) Estimation of blood proteins.

6) Endocrinal activities.
   (i) ACTH activity.
   (ii) Gonadotrophic activity.

7) Other visceral and somatic activities.
VI. Post-mortem studies.

1) Macroscopic.

2) Microscopic.

I. SELECTION OF ANIMALS

Selection of cats and monkeys as the animals of choice has been made for obvious reasons. The domestic cats being familiar to most civilized people have assimilated themselves well with our civilization. Monkeys on the other hand hold quite an important position in the comparative neurology being next to man in evolution. Thus the results obtained from such studies can be safely applied to both the morphological and more so to the functional aspect of the nervous system of human species. Also both these animals show a uniform configuration of the skull with little variations in the location of the different parts of the brain in the individual animals. It is, therefore, easy to adapt them for the stereotaxic studies. The stereotaxic instrument available in the department could be used for cats and monkeys.

II. PREOPERATIVE CARE OF THE ANIMALS

Monkeys weighing between 3 to 4 Kgs. and cats weighing between 2 to 3.5 Kgs. were used for this study. Before undertaking any experimental procedures they were kept in the animal rooms for a few days. This helped them to get familiarised with the type of food and the surroundings. The room temperature was maintained between 75°F - 80°F. The wire mesh cages for
the cats measured 2'x2'x1½' (Fig.9) whereas the ones used for
monkeys were 2'x2'x2½' and had side bars (Fig.10).

The animals were fed ad libitum. The cats were given
minced meat and milk whereas monkeys were fed on soaked grams,
chappaties and seasonal vegetables or fruits.

During this period of acclimatisation every possible effort was made to watch the behaviour of the animals. Various
studies were conducted on them on a number of days the details of which are described later. Operative procedures were then
carried out on these and the studies on visceral responses conducted in the post-operative period. The animals were kep for about 1½ to 3 months, and then sacrificed after the completion of such studies.

III. SURGICAL TECHNIQUES USED

The stereotaxic apparatus, originally devised by V.Horsley
and H.Clarks (1908) with their later modifications and was used for most of the procedures which follow. It enables one to direct a protected stimulating or electrolysis producing needle to any desired part of the brain with a fair amount of accuracy.

The rigid rectangular frame of this instrument fitted with the aural bars and the nasal and orbital plates of the frontal bar (Fig.11) holds the head of the animal in such a way that the external auditory meatuses and the centres of the lower margins of the orbits lie in one horizontal plane called the basal plane. Parallel to this but 10 mm. nearer the vertex lies the horizontal section plane. Beginning with this basal plane, the frontal section plane is defined as perpendicular to the horizontal and passing through the centres of both auditory meatuses. The sagittal section plane bisects the cranium perpendicular to the
Fig. 9
Type of cage used for cats.

Fig. 10
Type of cage used for monkeys.
other two section planes. The zero point of the stereotaxic instrument is the point where the horizontal plane cuts the sagittal and frontal planes. All the measurements are made in both directions from each of these three section planes counted as zero. The whole encephalon is divided by the three section planes into eight segments designated as right and left frontal, occipital, temporal and cerebellar. Each of these segments, finally subdivided into cubic millimeters, presents on its inner aspect three rectilinear surfaces corresponding to the three section planes.

To obtain the desired three dimensional co-ordinates of any given structure in the brain it is necessary to select an ideal and standard sized animal of the different species and make a series of measurements of the actual positions of structures in its brain and mark them on the graph paper. The same formed the first part of the present study.

After fixing the head of the animal in the Horsley-Clarke apparatus, colored needle tracts were produced in the exposed brain at different points in the anteroposterior and para-sagittal direction with reference to the zero of the instrument. The brain was then taken out of the base of the skull and fixed in 5% formalin. It was then cut into lamellae and the co-ordinates of different limbic structures were then measured in the anteroposterior, parasagittal and vertical directions, keeping in view the co-ordinates at which the electrodes were inserted. These co-ordinates were later used both for implantation of electrodes and production of localized electrolytic lesions. For surgical lesions on the other hand, one had to be guided by the general
Fig. 11

Horsley–Clarke stereotaxic apparatus used in the present study.

Fig. 12

Head of the animal fixed in the horizontal frame of the stereotaxic apparatus.
surface anatomy of the brain.

1) Anaesthesia:

All the operative techniques i.e. implantation of electrodes, electrolytic lesions, surgical ablations and preparation of gastric pouches were performed under the effect of Pentobarbitone Sodium (M & B). This preparation used intra-peritoneally in doses of 0.8 - 1.0 c.c. of a 5% solution / Kgm. of the body weight worked quite satisfactorily.

For recording of blood pressure, respiration, E.K.G. etc. in anaesthetised states the animal was again anaesthetised with intraperitoneal pentobarbitone sodium.

2) Implantation of electrodes for stimulation:

A multilead electrode with 3 - 4 leads was made from straightened insulated copper wires, one of which (No.36) was thicker than the other three (No.30). The insulation of tips of these wires was removed for a distance of 0.5 to 1 mm. and these were then joined together at a distance of 2 mm. from each other with 'Plexiglas'. The different leads of this multilead electrode could be easily defined from their respective lengths projecting on the other end i.e. shortest being number one and the longest being number four. The head of the animal was fixed in the horizontal frame of the stereotaxic instrument (Fig.12). Under aseptic precautions a midline scalp incision was made. The periosteum was scrapped and with the help of the guide electrode desired points were marked on the skull. These points were then bored with a 2 mm. trephine needle of an electric burr. Multilead electrodes, guided through these holes, were left in the desired position and the holes were sealed with
successive applications of dental cement. The projecting portion of the electrode was covered with polyethylene tubing, tied to the occipital crest and was then taken out under the skin at the posterior end of the neck through a separate transverse incision. In one animal 3 - 4 such multilead electrodes could be implanted in different limbic regions and these were numbered at the back. The scalp incision was closed and a collar band was applied round the neck to protect the electrodes and keep them in position. Through these implanted multilead electrodes, bipolar stimulation of different regions was carried out in anaesthetised and unanaesthetised waking animals, by means of a Grass electronic square wave stimulator and stimulus isolation unit (Fig.13). For observation of some of the responses cats were allowed to move about freely whereas monkeys had to be tied down to a special stand made for the purpose. Different parameters of stimulation were tried but most commonly 30 - 60 per sec. square wave pulses of 0.2 - 1 m. sec. duration were used. The intensities of stimulations which produced the different responses from different regions ranged from 0 volt to 10 volts but on an average majority of the responses could be elicited with 3 - 8 volts.

3) Electrolytic lesions:

A unipolar straight nichrome wire about 0.12 mm. in diameter was used for making localized electrolytic lesions. This wire was uniformly coated by black Japan by two to three successive coatings leaving one end without such a coating. About 0.5 mm. of the tip of the electrode on the coated side was later cleaned and insulation removed by grinding it with a stone. This also sharpened the end to a tapering point so that
Fig. 13
Set up for stimulating the animal through the implanted electrodes.

Fig. 14
Set up for producing electrolytic lesions.
it could pierce the dura mater when guided through the brain.

With the head of the animal fixed in the Korsley-Clarke apparatus desired points were marked on the scalp after making a midline incision. Small craniotomy holes about 1½ - 2 cm. in diameter were made on either side depending upon the location of limbic regions to be ablated. Every possible care was taken to leave the dura intact and check the bleeding. As the lesions had to be bilateral and symmetrical only one region was destroyed in one animal. The unipolar electrode was guided stereotaxically to the desired points. Another electrode applied to the body of the stereotaxic instrument served as an indifferent or earth electrode. At each point in the selected region electrolysis was produced by the passage of a direct current of 3 milliamperes for 30 seconds (Fig. 14). The extent of the lesion was regulated by producing such electrolysis at adjoining points in the anteroposterior, para-lateral and vertical scales. To get an exact idea of the lesion produced the milliammeter had been previously calibrated in such a way that a passage of current of 3 milliamperes for 30 seconds produced a lesion about 1½ cm. in diameter. The scalp incision was then closed and animal freed from the apparatus.

4) Surgical ablations:

For these procedures though the stereotaxic apparatus was not required for accurate localization, the rigid frame of the instrument was of great help to keep the head of the animal fixed for producing gross lesions in the limbic structures of frontal lobes. For approaching the limbic regions of temporal lobes the head was fixed in a different animal holder fixed to
the operation table. This enabled the head of the animal to be kept on one side and at the desired angle (Fig. 15). After fixing the animal an elliptical scalp incision arching towards the body was made over the frontal bone of the skull to approach the frontal limbic structures. For temporal lobectomies which were done in two stages, similar incision was made around the root of the pinna and about 3 - 4 cm. away from it. Big craniotomy holes were made at the desired points with the help of electric burr and the bone nibbling forceps. The dura was then cut across and taken aside in such a way that it could be stitched after the lesions were made. The bleeding points in the dura and pia mater were cauterized. Large selected areas were cauterized and then bilaterally aspirated with the help of a suction pump carrying a fine, long curved glass or metal cannula with an internal bore of 2 - 3 mm. (Fig. 16). Bleeding from these deep seated regions was controlled by pouring and sucking the fluid through warm saline-soaked cotton plugs which also served as a protection for the underlying brain substance.

To approach the orbital surfaces of the frontal lobes the frontal poles on either side were raised from the base of the skull, while for approaching the anterior cingulate gyri the cerebral hemispheres were retracted apart. The temporal lobe structures on the other hand were lifted from the middle cranial fossa and the cannula guided along to reach the selected regions.

Before stitching the dura and the scalp incision back it was made sure that there was no bleeding from any region.

5) Preparation of gastric pouches:

Gastric pouches of the modified Thomas type were
Fig. 15
For surgical lesions head of the animal is fixed in a mouth holder attached to the operation table.

Fig. 16
Shows the suction pump being used for production of surgical lesion. On the extreme right is the cautery transformer seen.
prepared in some of the cats. This was very kindly taught to workers in the Neurophysiology Research Unit of the Indian Council of Medical Research by Professor S.J. Nasset of Department of Physiology, Medical School Rochester, N.Y., U.S.A. when he was in India as a Visiting Professor. The stomach was taken out through a left paramedian abdominal incision, and held by a pair of Allis's tissue forceps along the greater curvature (Fig. 17). A small hole was made in the most dependent part on the greater curvature and the stomach was everted through it. Considering the size of the pouch and positions of oesophageal and pyloric openings a thin strip of mucous membrane was cut all around (Fig. 18). Keeping the margins inverted, the inner edges of the cut mucous membrane were then stitched by continuous stitches using 000 chrome cat gut. The submucosa in between the two edges of mucous membrane was stitched by continuous silk stitches and this provided an additional support in between the two halves of the stomach (Fig. 19). The outer edges of the mucous membrane which were kept everted were then stitched by chrome cat gut 0.0. The everted edges were next pushed back through the hole.

The stomach was thus divided into two completely separate portions, one in continuation with the oesophageal and pyloric openings and the other forming a pouch with a hole in which a metal cannula especially made for such a purpose was tied (Fig. 20). The other end of this cannula was taken out of the abdominal skin through a stab incision on the left of the abdominal incision and the abdomen was closed (Fig. 21). The gastric pouch was daily irrigated with saline and the animal was put on additional salt to compensate for the loss.
Fig. 17

Stomach of the cat held by Allice's tissue forceps along the greater curvature.

Fig. 18

The strip of mucous membrane cut all round the gastric wall.
in the secretions. After a week or so when the animal completely recovered from the operative trauma, it was again anaesthetised and multilead electrodes were implanted in different regions of limbic system. The animal usually recovered consciousness in 6 - 8 hours and was left undisturbed for another 3 - 4 days to regain its normal activity before starting collection of gastric juice for biochemical analysis.

IV. POST OPERATIVE CARE

As a routine all the operated animals were given 70 - 100 cc of intraperitoneal saline and an injection of procaine Penicillin. Proper care was taken about the environmental temperature. Usually the animals recovered in 6 - 8 hours time and started taking their feeds by mouth. Those who did not take anything by themselves had to be fed by stomach feeds for a few days till they started feeding on their own. As a precautionary measure Penicillin was given for 3 - 4 days after the operation in addition to the daily aseptic dressing of the operated area. Any excessive accumulation of C.S.F. or blood clots whenever present were drained off through a small opening in the stitched incision.

V. STUDY OF DIFFERENT VISCERAL SYSTEMS

Attempts were made to study the various autonomic, visceral, metabolic, endocrinal, behavioural and somatic activities for 1 - 1½ months both before and after the operative procedures. As for the stimulation studies these estimations were done before, during and after the stimulation of a particular area.

The following components of different systems were studied.
Fig. 19
Inner mucous and submucous coats stitched.

Fig. 20
Gastric pouch with the cannula.
The methods used for such a study apply to all occasions such as before and after the operative procedure and also before, during and after the stimulation.

1) Cardiovascular System:

(1) Heart Rate

(a) By palpation - The heart rate was counted just by feeling of the heart beat by placing the palm of the hand over the cardiac apical region.

(b) By cardiotachometer - This method was used only for some of the unanaesthetised animals before and after stimulation of different limbic regions. A Waters C - 224 cardiotachometer offers a method of continuously recording heart rate. Its wide range from 40 - 240 beats per minute makes its use helpful for such studies. Electrodes were securely taped flat to the left and right side of the chest with neutral going to the back of the neck of the animal. The electrode cable when plugged into the input of the instrument gives a direct reading on the sensitive meter which needs previous calibrations (Fig. 23).

(c) By E.K.G. - The E.K.G. was recorded simultaneously along with the respiration and blood pressure on a Grass 3-channel E.K.G. machine (Fig. 23) in anaesthetised monkeys and cats before, during and after stimulation. Heart rate was calculated from these graphs and correlated with the other responses.

(ii) Blood Pressure

(a) By sphygmomanometer - A special small rubber arm cuff was made for animals and blood pressure recorded by a sphygmomanometer by palpation and auscultation in conscious monkeys before and after surgical and electrolytic lesions.
The cannula in the gastric pouch projecting through the abdominal wall.

Fig. 21

Monkey with the Cardio-tachometer.

Fig. 22
(b) **Recording on kymograph** - The animal anaesthetised with intraperitoneal Pentobarbitone Sodium was tied to the operation table. Trachea and femoral or carotid artery were exposed and cannulae introduced. Records were taken before, during and after the stimulation of different regions using different parameters. Short periods of rest were given to the animal in between the stimulations.

In another set of experiments unanaesthetised waking animals were studied. The operative procedures were conducted by first bringing the animal under with a few whiffs of ether. The wounds were infiltrated locally with novocaine. After the effect of anaesthesia was over the records were taken before, during and after stimulations. Animal had to be kept in lying position by holding the limbs whenever it attempted to get up. To eliminate this factor the series of experiments done later were done on animals immobilised with flaxedil given in doses of 1 mg/kg of body weight intravenously. The respiration in such animals was maintained with a respiratory pump.

(c) **Recording on E.E.G. machine through a transducer and balance demodulator unit** - This was done in some of the animals of the anaesthetised series. The femoral artery was exposed and the arterial cannula introduced. The other end of the cannula was connected to a Statham P 23 pressure transducer through an attached plastic tubing filled with 10% citrate solution. The blood pressure was thus recorded through this transducer on a Grass 3-Channel E.E.G. machine, fitted with a balance demodulator unit. The pre and power amplifiers of the E.E.G. machine provided enough amplification to get a good record of blood pressure.
Monkey with the attached electronic gadgets and electro-encephalograph for recording of electrocardiogram, hand plethysmography, blood pressure, respiration and gastrointestinal motility.

Fig. 23

Shows the details of respiratory belt, hand plethysmographic cup and electrodes for electrocardiogram applied to the monkey's body.

Fig. 24
(iii) Changes in blood flow through hand

Special types of metal plethysmographic cups to fit the animal's hand were obtained. These were fitted over the whole hand of the animal and made air tight by the application of plasticine and paste made up of zinc oxide and plaster of paris (Fig. 24). One of the tubes leading from one side of the cup was attached to a Statham transducer which in turn was connected to the Grass 3-channel E.E.G. machine through a bridge. Thus the changes in hand volume could also be recorded on the same machine.

A simultaneous recording of E.E.G., changes in hand volume and blood pressure on this electronic device enabled one to have an integrated picture of the activity of cardiovascular system.

2) Respiratory System:

(i) Direct visual counting of the chest movements and assessment of depth.

This method was adopted both for monkeys and cats in the unanaesthetised state before, during and after stimulation; and before and after surgical and electrolytic lesions.

(ii) Recording of respiration

In unanaesthetised waking animals, a tracheal cannula was introduced under ether and local novocaine anaesthesia and the respiration recorded on a kymograph through a Marey's tambour before, during and after stimulation.

In another set of experiments on the anaesthetised animals, respiration was recorded electronically. A specially made belt with the strain gauge in it was tied over the lower part of the chest (Fig. 24). The leads from this were taken on
to one channel of the B.E.G. machine through a bridge. The amplitude of the recorded respiratory waves could be adjusted with the pre and power amplifier control knobs of the B.E.G. machine.

3) Body Temperature

Rectal temperature was recorded in some of the animals throughout the pre and post operative periods under observation.

4) Gastro-intestinal System

(1) Collection of gastric contents

(a) By aspiration - 14-16 hour's fasting animal was held in position by the animal attendant and a Ryle's tube or rubber tubing of the size of a Ryle's tube was introduced through the central hole of the wooden mouth gag held in between the jaws. The other end of the tube was attached to a 10 - 20 cc record syringe and the contents were withdrawn completely before and at the end of one hour's stimulation.

(b) Collection of gastric contents through gastric pouch.

The cat with the gastric pouch and implanted electrode was starved for 14 - 16 hours. For collection of gastric juice, it was made to stand on a specially prepared metal stand and supported by leather straps. The juice was collected into separate containers at 1 hourly intervals for a total period of 3 - 3 hours. This formed the control. On other days after collecting the first sample of gastric juice, any one region of limbic system was stimulated through the implanted electrodes with the help of a Grass square wave stimulator for a period of one hour. The parameters of stimulation used were, - frequency 30/sec., pulse duration 0.2 - 1 m. sec. and voltage varying
from 2 - 8 volts. The gastric juice was again collected over a half hourly period for a total of 3 hours.

(ii) Estimation of volume, free and total acidity and pepsin content of gastric secretion.

The half hourly gastric contents thus collected through gastric pouches were separately measured and analysed for their free and total acidity and peptic activity. On few occasions when the contents were too little they had to be pooled up together for final analysis. The juice was strained through a fine cloth and residue examined for mucus or blood.

(a) Determination of free and total acidity - The free and total acidity was measured by titrating against 0.01 N NaOH using Topfer's reagent and Phenolphthalein as indicators. The result was expressed in number of millilitres of 0.1 N NaOH necessary to neutralise 100 ml. of gastric juice.

(b) Measurement of peptic activity - The peptic activity was estimated by Cole's method and results expressed in units. The unit of pepsin is defined as the quantity of pepsin required to clot 5 ml. of calcified milk in 100 seconds. A test tube containing 5 ml. of freshly prepared calcified milk was kept in waterbath at 38°C for five minutes. To this was added 0.2 ml. of gastric juice and test tube shaken thoroughly. The tube was immediately replaced in the waterbath and stopwatch started. At intervals the tube was removed and watched for the appearance of precipitate. The time taken for the appearance of precipitate was noted.

To prepare calcified milk 10 ml. of 10% calcium chloride (5.35%) was added to 50 ml. of fresh milk and the volume made up to 100 ml. by diluting with distilled water.
(iii) Recording of gastric and intestinal motility

In some of the animals with implanted electrodes, gastric motility was recorded graphically either on the kymograph or on an "A. I." machine. The animal was anaesthetised with intraperitoneal pentobarbitone sodium. A balloon connected with an attached tubing was introduced into the stomach through mouth. The other end of the tube was attached to a manometer and Marey's tambour through 3 way glass connector. The whole system was filled either with colored water or air and the motility recorded on the kymograph before, during and after stimulation.

In some experiments the tubing was attached through a pressure transducer to the balance demodulator unit of the "A. I." machine and the movements were recorded electronically.

To record intestinal movements a tube with an attached balloon was introduced under laprotony into the lumen of the particular segment of the intestine after making a small opening in its walls. The opening was next closed by purse string sutures to keep the balloon and the tube in position and movements recorded kymographically or electronically as before.

In the unaesthetised group of animals the operative procedures were carried under ether anaesthesia, infiltrating the wound margins with novocaine. The recording was done as already described above.

(iv) X-ray after Barium meal

Some animals developed ballooning of the gastro-intestinal tract after surgical lesions of some of the limbic structures, and this was confirmed by x-ray examination after Barium meal.

5) Studies on Blood Chemistry

In monkeys blood sample was easily obtained through
the superficial dorsal leg veins. In cats on the other hand attempt was made to collect the sample from the femoral vein through intact skin and in most of them this proved quite satisfactory. In those cases where this was not successful, blood was withdrawn directly by heart puncture.

For blood sugar estimations the blood sample was collected in oxalated bottles and for electrolytes and protein estimation it was taken in dry sterilized centrifuge tubes. This procedure was repeated at 15 - 20 days interval at least for two times before and three times after the production of electrolytic and surgical lesions. For expressing the results, a mean of these values taken separately for pre and post operative period was taken into consideration. For the stimulation studies samples were taken before and immediately after one hour's stimulation.

(i) Blood sugar estimation

Blood sugar was estimated by the method of Folin and Wu (1920). The protein-free blood filtrate is heated with alkaline copper solution using a Folin-U tube to prevent re-oxidation. The cuprous oxide formed is treated with phosphomolybdic acid solution. The blue color thus developed is compared with that of a standard in a colorimeter. The results are expressed in mgm. per cent.

(ii) Serum electrolytes.

In the earlier stages of these studies only blood sodium was estimated. The method followed was that of Noyons as modified by King (1947). The sodium in protein free filtrate is precipitated as sodium zinc uranyl acetate.
This precipitate when treated with potassium ferrocyanide gives a plum red colour which is compared colorimetrically with that of a standard sodium chloride solution. The results are expressed in mgm. per 100 ml. of plasma.

With the availability of Beckman flame photometer during the later part of the study, both sodium and potassium were estimated using the flame photometer technique.

(iii) Blood proteins.

Serum proteins were estimated by the Nesslerization method of King (1947). In one group of animals only total proteins was estimated. In another group both total protein and albumin fractions were determined. Difference between the two gives mainly the globulin fraction. The principle involved both for total protein and albumin fraction is the same. Protein is precipitated with zinc sulfate and sodium hydroxide. The precipitate and the fibrin clot are further digested with sulphuric acid and selenium dioxide. The protein nitrogen is estimated colorimetrically as ammonium sulphate with Nessler's solution.

In the later series serum proteins were estimated by paper electrophoresis technique. For evaluating the results qualitatively the depth of the colour and the separation into various bands on the paper strips were taken into consideration. The quantitative estimation was done by extracting the individually dyed portion of various colored fractions in a Soxhlet Extractor and determining colorimetrically the dye uptake. The dye uptake is proportional to the protein fractions separated.

6) Endocrinial activities

(1) A.C.T.H. activity

The role of hypothalamus in the pituitary-adreno-cortical responses had already been worked out (Anand
et al., 1954; Anand and Dua, 1953). In the present studies, attempts were made to find out the effect of stimulation and surgical and electrolytic lesions involving the limbic structures of frontal and temporal lobes on the ACTH response both in monkeys and cats. The direct counting of the eosinophils in the circulating blood was used as the index of the rate of secretion of adrenal cortical hormones. These were counted by the method of Randolph (1944) modified to stain only eosinophil cells. A diluting fluid containing 0.05% phloxine in 50% propylene glycol was freshly prepared every few days by a 1:1 aqueous dilution of 0.1% stock solution in 100% propylene glycol stored in a dark bottle in the refrigerator. The stock solution was changed every month. The blood sample was diluted 1 in 20 in a W.B.C. pipette and after thorough mixing, drops from the middle portion of the pipette, were counted in a Levy counting chamber.

In the group of animals prepared for surgical and electrolytic lesions, the counts were done before and one and four hours after injecting 5 ml. of 10% saline subcutaneously which worked as a stressing agent. The procedure was followed both during the pre-operative and post-operative periods.

In another set of experiments for stimulation studies, the eosinophil counts were done immediately before stimulation, immediately after one hour of stimulation and four hours after the start of stimulation.

(ii) Gonadotrophic activity.

Study of sexual behaviour of the animals has been taken as an indirect evidence of the gonadotrophic activity. Study of this particular response was carried out in detail by
one of the colleagues in the department (Chinna, 1960). The various methods used and the criteria adopted for the assessment of different types of sexual responses are being mentioned here just in bare outlines.

The animals with surgical or electrolytic lesions were observed for any abnormal sexual behaviour and compared with such a behaviour observed pre-operatively. Changes in behaviour were also observed during the periods of stimulation of different limbic structures through implanted electrodes. The behaviour was studied while the animals were kept in isolated cages and also when they were left with other animals of the same or different sex and even of different species. Sometimes dummy animals were also used. Their presenting and mounting reactions and their behaviour towards each other formed an important part of study for the interpretation of sexual responses. In few monkeys with well developed hyper-sexual response castrations were done to see the role of endocrines in such a response. The animals were watched for a period of 1 - 1½ months even after castration.

7) Other visceral and somatic responses.

Throughout the studies, and especially during stimulation of different limbic regions, a record was kept of various somatic responses like retraction of head, blinking, movements of limbs etc., details of which are given later in results. Some of the responses like retraction of nictitating membrane, pupilodilation, pupillo-constriction, piloerection, urination and defecation give a good indication of the sympathetic and parasympathetic activity. A careful watch was thus always kept on these animals during stimulation to look for these
animals during stimulation to look for these responses.

VI. POST MORTEM STUDIES

Towards the end of the study at the time of sacrifice the animal was deeply anaesthetised. Slow perfusion with saline followed by 5% formal saline was carried out through the common carotid artery. This helped in hardening the brain in situ and was an easy way of sacrificing the animal. The implanted electrodes were cut near the surface of the brain leaving the deeper embedded part in position. With great precaution the brain was taken out after detaching it from various attachments.

After proper fixation of brain, the portions of the electrodes left behind at the different implanted sites, were taken out. Histological sections were later prepared to confirm the exact location of implanted electrodes or the electrolytic lesions. In some animals gross frontal sections were cut to study the needle tracts and the regions stimulated. The extent of surgical lesions of brains was made out by macroscopic examination.

The gastrointestinal tract especially the stomach and small intestine were examined for any ulceration or perforation macroscopically. Some of the ulcerated areas were studied histologically.