PROCEEDINGS OF THE THIRD CONFERENCE ASSOCIATION OF PHYSIOLOGISTS AND PHARMACOLOGISTS OF INDIA, JANUARY 1958

Abstracts

1. Studies On Carbohydrate Metabolism In Scorbutic Guinea Pigs.

S. BANERJEE. CALCUTTA

Scurvy is associated with disturbance in carbohydrate metabolism. In scurvy diminished glucose tolerance has been reported in many species of animals. Banerjee and Ghosh observed a decreased liver and muscle hexokinase activity in scurvy. The decreased turnover rate of phosphorelated intermediates of carbohydrate metabolism has been observed in scurvy. Banerjee has also observed degenerative changes in the islets of Langerhans and diminished insulin content of pancreas in scurvy.

There are evidences that insulin is involved in the intermediate metabolism of carbohydrates at the level of Kreb's cycle. In the present work effect of injection of insulin on the glucose tolerance test and liver and muscle glycogen contents in scorbutic guinea pigs have been studied. As a preliminary study, the tissue contents of alpha-ketoacids, citric, malic, and lactic acid of normal, scorbutic and insulin treated scorbutic guinea pigs have been estimated.

Scorbutic guinea pigs showed lowered glucose tolerance. After insulin treatment glucose tolerance showed considerable lowering of blood sugar 150 and 180 minutes after glucose feeding although it still remained significantly higher than that of normal. Insulin treatment also brought about the shifting of peak of blood sugar value from 150th. to 90th. minute period after feeding glucose.

Glycogen content of liver and muscle diminished in scorbutic guinea pigs which was restored by treatment with insulin. This indicates that prolonged insulin treatment can correct to a great extent the disturbed carbohydrate metabolism in scurvy.

It is reported that diabetes is associated with an altered pyruvate metabolism with resultant high blood pyruvate level. Since scurvy simulates diabetes in many aspects of metabolism of carbohydrate it was surmised that scurvy may also be associated with an alteration in pyruvate metabolism. Except in blood, no significant change in the concentration of alpha-ketoacids in

tissues of scorbutic guinea pigs was observed. Possibly scurvy is not associated with any derangements in metabolism of ketoacids.

An increased content of citric acid in tissues of scorbutic animals was observed which was brought to normal level by treatment of insulin. This defect in citric acid metabolism may be either due to an accelerated rate of its synthesis or to a metabolic block below the level of citric acid in the Kreb's cycle. Insulin corrected this defect which indicates that insulin, in some way, facilitates citrate metabolism in scurvy possibly by activating the aconitase enzyme.

Malic acid content of tissues of scorbutic guinea pigs is increased. Insulin treatment lowered the level of malic acid to normal in blood, liver and cardiac muscle. The observation that treatment with insulin lowers both the levels of citric and malic acid to normal indicates that lack of insulin is an important factor in the deranged carbohydrate metabolism in scurvy.

A greatly increased lactic acid content of tissues was observed in scorbutic guinea pigs. Treatment with insulin lowered the lactic acid value even below normal. This accumulation of lactic acid in scorbutic tissues may be attributed to decreased glycogen formation. It is, therefore, likely that glycogenesis is retarded in scurvy due to lack of insulin and that exogenous insulin effectively promotes glycogen formation.

---

2. Effect Of Gastraic Secretion On Plasma Chlorides.

J. S. SAKSENA, S. NARAYANA AND S. V. VENKATARAMA RAO, BANGALORE.

Earlier work by the authors showed that the salivary chlorides and urinary chlorides fall when acid secretion in the stomach is stimulated by a test feed or by giving insulin injection. The present study was undertaken to determine how plasma chloride level is affected by acid secretion in the stomach.

Arterial blood samples were collected in dogs and rabbits through a piece of polythene tubing into oxalated centrifuge tubes having a water repellent coating. In addition to two fasting samples, 15 minutes blood samples were collected for about 2 hours after giving 4 to 6 units of crystalline insulin to stimulate secretion of gastric juice. The chloride content of the plasma obtained from the above samples was determined by Vanslyke and Sendray's method.

The plasma chlorides registered a fall on giving insulin which was maximum about 1 hour after the insulin injection. In view of the earlier

observations on saliva and urine, this fall is attributed to the loss of chloride ions in the stomach.

In view of practical difficulties, it is not considered possible to substitute plasma choride estimation in arterial blood as a clinical test to replace gastric analysis.

---

3. Plama Insulin Estimation and Its Significance in Diabetes Mellitus.

RANITA AIMAN AND R.D. KULKARNI, Poona.

Since certain diabetics are resistant to the effects of insulin, deficiency of insulin cannot be the only cause of diabetes mellitus. Hence it is necessary to estimate plasma insulin in normal and diabetic persons. Rat diaphragm method is presently being used for this purpose. We used 1 in 10 dilution of plasma with a view to obtain actual insulin concentration of the plasma.

Plasma insulin estimation was done in five normal adults and ten diabetics. Plasma insulin concentration of normal persons ranged between 0.7 to 6.3 milliunit per ml. Two diabetics whose disease was diagnosed recently had plasma insulin in the normal range while others hadless. Four of these latter who had moderate insulin in the plasma responded favourably to BZ-55 and one who had negligible insulin in plasma failed to respond. Two diabetics in the younger age group showed the presence of insulin antagonising factors in excess in their plasma.

For the elucidation of pathogenesis of diabetes mellitus single insulin estimations are of no value. Repeated estimations over a prolonged period of normal and diabetic persons are necessary. This preliminary work is reported here only to stress the need and scope of such a study.

---

4. Ventilatory Function Studies in Female Children and Adults*

H. D. SINGH & S. PRABHAKARAN, MADRAS

Employing a Collin's Respirometer, vital capacity (V.C.), Timed Vital Capacity (T. V. C.) and Maximum Breathing Capacity (M. B. C.) were determined in 95 healthy female subjects (70 between 17—29 years and 25

between 6—16 years). The mean values with the ranges and S. D. were given in a preliminary note. In this report, the results of statistical correlation of the values and physical measurements are presented. The regression equations may be useful for calculating the expected values of V. C. and H. B. C. for Indian subjects in the respective age groups.

17—29 years: V. C. in c. c. 1. $2288 + 31 \times$ Ht. (Height in cms.)
2. $542 + 1343 \times$ B. S. A. (Body Surface Area in Sq. meters)

M.B.C. Liters/min. 1.—73 $+1.14 \times$ Ht.
2. $62.9 + 18.44 \times 2$ sec. T.V.C. in litres.

6—16 years: V.C. in c.c. 1.– 2014.5 $+26.59 \times$ Ht.
2.– $689.8 + 2207.84 \times$ B.S.A.
3. $276 + 125.44 \times$ age.

M. B. C. Liters/min. 1.– $128.9 + 1.447 \times$ Ht.
2.– $46.9 + 107.93 \times$ B.S.A.
3. $7.6 + 5.4 \times$ age.
4.– $7.05 + 48.2 \times 2$ sec. T.V.C.

5. Pharmacological Properties of Dihydroxyphenyl ethylamines.

R. S. GREWAL

Barger and Dale considered the chemical configuration of B. phenyl ethylamine as optimal for sympathomimetic action. The effects of dihydroxy substitution at 2:3; 2:5 and 3:5 position on the ring in phenyl ethylamine were studied. The action of these compounds was studied on blood pressure and nictitating membrane of spinal cat and also after the administration of ergotoxine and cocaine. All the three compounds had pressor effect and this was "tyramine type" rather than "adrenaline type" and all these compounds showed tachyphylaxis. Their pressor activity was compared with tyramine.

The mean figure for the ratio of equiactive doses of 3, (8,5) dihydroxy phenyl ethylamine to tyramine on blood pressure was 1.15 to 1 and in the case of 2, 3 dihydroxy phenyl ethylamine 1.32:1 and in case of 2:5 dihydroxy phenyl ethylamine it was 2:1

In causing contraction of the nictitating membrane 2, 3 dihydroxy phenyl ethylamine was twice as active as tyramine while 2: 5 and 3, 5 dihydroxy phenyl ethylamines were eqiuactive in contracting the nictitating memberane but were much weaker than tyramine.

The pressor effect of all the three dihydroxy phenyl ethylamines were reversed by ergotoxine. Cocaine abolished their effect on nictitating membrane and reduced their pressor effect.

All the three dihydroxy phenylethylamines were substrates of amine oxidase.

6. SAR of C. N. S. Depressant Quinazol-4-Ones.

K. N. SAREEN, K. KISHOR AND L.M. PANDE, LUCKNOW

In several communications from this department, a number of 2-alkyl-3-aryl-quinazol-4-ones have been shown to possess clinically useful hypnotic, anticonvulsant and antipyretic activities. Investigation of the structure-activity relationship (SAR) of this entirely new series of central depressants was undertaken to resolve these properties and to enhance their potency without a corresponding increase in the undesirable side effects. This paper deals only with the SAR of their hypnotic action.

3,1-Benzoxazin-4-ones (alkyl anthranils), 3,1-benzoxazin-2, 4- diones, unsubstituted quinazolines and their di- and tetrahydro products, and the 3-alkylquinazolines and the quinazol-4-ones represented by such natural products as rutaecarpine, evodiamine, febrifugine and isofebrifugine (dichroines), vasicine and arborine seem to be devoid of any significant hypnotic activity. 2-Alkyl- and the 2,3-dialkyl-4-ones are also inactive. The hypnotic activity seems to appear first in 3-phenyl-4-one and reaches a substantial level in 2-methyl-3-phenyl-4-one and the 2-ethyl-3-phenyl-4-one. Higher alkyls at C^2 lead to undesirable side effects.

Alteration of the polar factors around N^3. The ortho-methyl substitution in the phenyl ring (o-tolyl) of 2-methyl-3-phenyl-4-one increases the hypnotic potency while the same substitution in the para position, which apparently should have a similar electron availability at N^3, has been found to produce, due to some inexplicable reasons, a complete block in the hypnotic activity. A similar deactivating influence has also been observed in some other hypnotics belonging to the α-hydroxyamide series. A meta-methyl, on the other hand, and methoxyl, bromo and the nitro groups in the phenyl nucleus maintains the activity, though the nitro compounds are toxic. α-and the β-Naphthyls and the higher polycyclic aromatic nuclei at N^3 also inactivate the compounds. The effects of -OH, NH_2, COOH, SO_3H and their derivatives, the higher alkyls, the benzyl and the unsaturated allyl and crotyl radicals is under investigation.

Ring opening and shortening. The opening of the C^2-N^3 or the N^3-C^4 bonds and the ring shortening to imidazoles (cf. barbiturates and the hydantoinates) also lead to inactivation.

Alteration of the polar factors around C^2. The 'reversed phenyl' compounds like 2-phenyl-3-hydro, methyl, benzyl and the phenyl and the 2,4-diones like benzouracils are non-hypnotics.

Alteration of steric factors around C^2. The modification of the steric factors associated with the picolinic C^2-methyl, which is coplaner with the—$N=C^1$— fragment of the pyrimidine half, by its conversion into benzylidene,salicylidene, vanillidene, veratrylidene and the 1-phenyl-1,3-butadienyl compounds also leads to inactivation of the hypnotic activity.

7. On The Pharmacology of The Antibiotics From Fusaria*

M. O. TIRUNARAYAN and M. SIRSI, BANGALORE

Investigations on the isolation and characterisation of five antibiotic principles from the genus *Fusarium* have been carried out. In general, it has been observed that the genus produces three distinct types of antibiotic compounds,—the naphthoquinone, the cyclic peptide and the pyridine carboxylic acid derivative. The antibacterial spectrum varies with individual types of the antibiotics, but the general nature of activity of these appear to be quite similar.

Culture studies on the production of these antibiotics have revealed that in all the cases glucose levels beyond 3 percent were found to elicit maximum activity. However, the nitrogen sources varied in the production of maximum activity.

All the five antibiotics have been found to be stable to slight variation in pH, autoclaving in acid pH, and with definite melting points. The effects of blood, gastric extract and intestinal mucosa on the activity of the antibiotics against *Mycobacterium lacticola* 0-11 have been studied. The results of such investigations indicate that javanicin, fusaric acid, oxysporin and enniatin are not appreciably affected by the presence of blood, while the activity of sambucinin is considerably decreased.

Vigorous acid hydrolysis of sambucinin, enniatin-B and oxysporin yielded the same amino acid, N-methyl-l-valine, identified by paper chromatography. For a large number of antibiotics of this type, Cook and associates have obtained the same amino acid, N-methyl-l-valine and α-hydroxy-isovaleric acid, although differences in molecular formulae between the compounds existed.

Among the antibiotics studied, only javanicin and fusaric acid appeared to be inocuous, exerting no pharmaco-dynamic properties. They did not influence the blood-pressure and respiration in the anaesthetised dog to any appreciable extent, nor did they possess any spasmolytic activity.

*The communication forms part of a Thesis for which Ph.D. degree of the Madras University has be enawarded to M. O. Tirunarayan.

Enniatine-B, oxysporin and sambucinin were found to be highly toxic when administered intravenously to anaesthetised dogs. These three compounds affected blood-pressure and respiration to a great extent, and there appeared to be a sudden circulatory collapse when these compounds were administered at 5 mg./kg. body weight intra-venously.

However, the compounds were found to be non-toxic to mice when administered either intra-muscularly or intra-peritoneally in oil. In view of the relative insolubility in water, intra-venous toxicity studies with mice could not be performed.

Enniatin-B, oxysporin and sambucinin were found to have a direct effect on the smooth muscle of the intestine of the guinea-pig. Although these compounds did not produce any contraction, the response of the musculature to acetyl-choline and histamine was completely blocked by the drugs. Only after a certain period, sometimes extending to about an hour, the specimens of intestinal strips recovered after repeated washing and showed once again the original response to the spasmogens.

All the antibiotics studied, except fusaric acid which was available in very low quantities, were tested for their effect on avian malaria (*Plasmodium gallinaceum* infection in the chick) and one of these, javanicin, showed a very slight effect, although the birds were not able to survive for more than a few days after the death of the untreated birds. Practically, all the antibiotics studied have been found not to exert any antimalarial activity.

Some of the antibiotics have been tested for their effect on progressive tuberculous infection in the mouse. It has been observed that javanicin and sambucinin did not possess any tuberculostatic property *in vivo*. Enniatin-B showed slight activity. Oxysporin, the antibiotic from *F. oxysporum* Schlecht, has been found to possess fairly high activity in murine tuberculosis, and the activity was comparable to that of streptomycin.

Some aspects of the nature of bacterial inhibition by the various antibiotics have been investigated. A number of vitamins, amino acids, and miscellaneous compounds have been tested for their effects on the inhibition caused by the antibiotics. Promising results have been obtained, particularly in this connection, from studies on javanicine, fusaric acid and enniatin-B. It has been observed that riboflavine was able to overcome to a great extent the inhibitory effects of javanicine on *Staphylococcus aureus*. In the same organism, fusaric acid was found to be antagonised both by pyridoxine and nicotinic acid, enniatin-B by inositol and choline.

These investigations further emphasise the nature and mode of action of antibiotics produced by the genus *Fusarium*. These investigations also provide further stimuli for future investigations in the field of antibiotics, especially those derived from *Fusarium* sp.

8. Activity of The Hypothalamic Centres Under The Effct of Changes in Blood Chemistry·

B. K. ANAND, BALDEV SINGH, AND S. DUA, NEW DELHI.

Experimentally it has been already demonstrated that the destruction as well as the electric stimulation through implanted electrodes of the two centres in the hypothalamus (lateral hypothalamus—'Feeding Centre'; medial hypothalamus—'Satiety Centre') lead to marked changes in feeding behaviour of the animals. The influence of the higher nervous regions over these centres has also been demonstrated.

As it had been suggested by some that the activity of the hypothalamic centres may be influenced by changes in blood chemistry, experiments have been undertaken to test this hypothesis. A start was made in this direction by studying the influence of changes in blood glucose over the activity of these hypothalamic centres recorded by electroencephalograph.

So far experiments have been carried out on 8 monkeys and 8 cats. Electrodes were implanted in the 'Feeding Centre', in the 'Satiety centre', as well as in other regions of the hypothalamus in these animals. The electroencephalographic activity through these implanted electrodes was recorded in these animals who were not given any anesthesia. After recording the normal activity from the various hypothalamic regions, changes in blood sugar were produced either by intravenous injection of glucose saline solution, or by intravenous injection of insulin, on different days. Electroencephalographic activity was again recorded through these implanted electrodes and this compared with the activity recorded from these regions before changing the blood sugar.

It has been observed that the activity of the 'Feeding centre' is slightly decreased after hyperglycaemia (4 experiments) and the activity of these feeding centres is increased after producing hypoglycaemia (3 experiments). On the other hand the activity of the 'Satiety centre' is slowed after the production of hypoglycaemia (4 experiments) but no change could be detected in the activity of these after hyperglycaemia (3 experiments). In other experiments where the record was taken from hypothalamic regions other than the feeding and satiety centres, no change in the activity could be induced either by hyperglycaemia or by hypoglycaemia. These experiments suggest that changes in blood glucose definitely change the activity of these hypothalamic centres. Whether only change in blood glucose determines the activity of these centres, or whether the other changes in blood chemistry also have their influence over the activity of these centres, has not been tested so far. The experiments will be continued to test that hypothesis.

Another very interesting observation which has been made during recordings taken from the hypothalamic regions, both in monkeys as well as in cats, has been that in most of these animals the activity recorded from the left hypothalamus is definitely much more marked as compared with the activity recorded from the right hypothalamic regions. The explanation of this left hypothalamic preponderance can only be surmised at this stage.

---

9. Enzymatic Reactions Under High Pressures

G. P. TALWAR, NEW DELHI

Life is supposed to have originated in seas. In 1887, the oceanographic expedition aboard "Talisman" brought back living creatures caught at a depth of 12,000 meters in the sea. At this depth these creatures will be constantly subject to a hydrostatic pressure of about 1,000 atmospheres.

Earlier Van Muschenbroeck and Achard had reported that animals maintained under an atmospheric pressure of 2-3 kg/cm^2 lived longer than those at normal pressure.

Recently Bresler and Collab. (1947-52) in Russia have claimed to have affected the synthesis of Proteins and polysaccharides from their hydrolytic products under high hydrostatic pressures. Their work has been denied by several other workers.

We thought it interesting to conduct a systematic study of the effect of hydrostatic pressures on several isolated enzymatic systems. The results could be summarised as follows:

(i) Low hydrostatic pressures (upto 1500 atmospheres) accelerate the rate of enzymatic reactions.

(ii) Relatively higher pressures (2500-6000 atmospheres) slow down the reaction rate without totally denaturing the enzyme.

(iii) Very high presures (of the order of 8000 kg/cm^2) stop the enzymatic reactions, causing an irreversible denaturation of the enzyme.

(iv) The extent of acceleration, retardation or inactivation of a particular enzymatic reaction varies greatly with the pH of the system.

(v) The acceleration under low pressures is due to the fact that the process of activation of the complex ES entails a diminution of volume.

(vi) It has been seen that in conditions of excess of substrate $\triangle V = -13.7$ ml/mole for the hydrolysis of denatured serum albumin by trypsin. For the same system $\triangle V$ in conditions of non excess of the substrate $= -2.9$ ml/mole.

(vii) From the role of pH in the influence of pressure on the reaction starch—amylase, it is deduced that the molecule of salivary amylase carries a negative charge in its active state.

---

10. Animal Hæmoglobins & The Occurance and Function of Minor Protein Components in The Hæmolysates of Human and Animal Red Blood Cells.

K. V. GIRI, BANGALORE

The paper relates to two of several aspects which have recently claimed our attention :

1. The electrophoretic mobility and heterogeneity of animal hæmoglobins with special reference to the hæmoglobins of the buffalo blood; and
2. The demonstration of the occurrence of minor protein components in the hæmolysates of red blood cells, and their possible role in relation to hæmoglobins.

Among the twelve animal species investigated (horse, elephant, buffalo, guinea-pig, man, rat, rabbit, sheep, cow, goat, dog and camel) the horse hæmoglobin has the highest electrophoretic mobility, while the camel has the lowest. There are appreciable variations in the electrophoretic mobilities of the different species. Approximate mobilities of the hæmoglobins were calculated by comparing the position of the hæmoglobins with respect to the protein components of human serum, after electrophoresis in agar gel. Only buffalo blood showed the presence of two hæmoglobins. Of the two hæmoglobin components in buffalo blood, the minor component (β) has a lower electrophoretic mobility and is about 37 percent of the concentration of hæmoglobin. The two hæmoglobins have been separated by fractional precipitation with ammonium sulphate and their amino acid composition has been determined by circular paper chromatography. An appreciable difference in the amino acid composition is indicated. An interesting observation is the absence of isoleucine in both the hæmoglobins. Alkali denaturation experiments also indicate the heterogeneity of the buffalo hæmoglobin.

In the course of these investigations on hæmoglobins, an interesting observation was made on the occurrence of minor protein components in the

hæmolysates of human and animal red blood cells. The possibility that these minor protein components present in the hæmolysates, serving the purpose of winding themselves between the hæmoglobin molecules should be considered. If this suggestion is supported by further experimental observation which is now in progress in our laboratory, a new light will be thrown on the role of these proteins in the interaction of hæmoglobins and in the phenomenon of sickling of red cells. There are several points arising from our investigations on these minor protein components which require clarification by further investigation, namely :

1. Whether these proteins come from the surface of the red blood cell or interior ;
2. The identity or non-identity of these proteins with those of serum and hæmoglobin ;
3. The nature and amino acid composition of these proteins after isolation in pure form ;
4. The effect of these proteins on the sickling phenomenon ; and
5. Their occurrence and concentration in various animal hæmolysates and in human hæmolysates from normal as well as pathological blood cells.

It is planned to extend the investigation on the minor protein components present in red blood cell hæmolysates on the lines outlined above.

In view of the significant differences observed in the mobilities of the hæmoglobins, the possibility of the application of agar electrophoresis of hæmoglobins in medicolegal examination of blood stains should be explored.

The work reported here was carried out in collaboration with Messrs. N. C. Pillai and G. J. Satyanarayana Rao.

---

11. Experiments on Sensitivity to Acetylcholine and Adrenaline and Nor-Adrenaline in Denevated Salivary Glands.

J. C. PAUL AND J. C. DAVID

Parasympathetic stimulation of the parotid gland in monkeys elicited copious secretion. The secretory response on the denervated side was sluggish, but the total basal secretion remained unaltered. The normal spontaneous flow of saliva is caused by a peripheral action and is maintained by a threshold level of ACh. When the threshold is exceeded automatic secretory blockade is demonstrable in the gland, the effect being enhanced by denervation. The denervated gland responds by

exhibiting a supersensitivity to ACh, adrenaline, nor-adrenaline and pilocarpine. This phenomenon has been attributed to a decrease in the specific ChE content or amine oxidase content of the denervated parotid gland.

---

12. Stress Responses in A Case of Anorexia Nervosa

BALDEV SINGH, B. K. ANAND, C. L. MALHOTRA AND S. DUA, NEW DELHI

A female patient aged 18 years was admitted with history of complete stoppage of eating for the last one month and in a state in which she seemed to be completely out of contact with her surroundings. She could be aroused only with painful stimuli. She had another attack of similar nature last year. She was tested for stress responses with 5 cc. of 10 per cent saline given subcutaneously, as well as subcutaneous injection of 1 cc. of adrenaline. Her eosinophil count, which was very low even before subjecting her to stress, could not be further reduced after stress. E.E.G. revealed overactivity of hypothalamic region with absence of alpha rhythm and of bang response. The patient was therefore put on Largactil 25 mg. thrice a day and the investigations were repeated after 10 days of the Largactil therapy. The eosinophil count showed a rise towards normal levels and this registered a fall after exposure to stress and adrenaline. E E G. also showed less dominance of hypothalamic activity. Alpha rhythm and bang response both had returned. The patient also became quite alert and rather violent during nasal feeds.

As the satiety centre is quite adjacent to the hypothalamic region involved in stress response, anorexia nervosa in this patient is suggested to be a symptom complex due to a vicious circle of psychosis associated with extreme stress reaction with hypothalamic overactivity and most probably anterior pituitary deficiency.

We are extremely grateful to Dr. S. Padmavati for having admitted this case into her wards at the Lady Hardinge Medical College Hospital and allowing us to do the investigations.

---

13 Role of Quinine in Hemolysis

R. S. GREWAL

The action of quinine dihydrochloride on rabbit & human erythrocytes was studied in vitro. It was observed that quinine dihydrochloride produced

hemolysis of rabbit erythrocytes upto a concentration of 1 in 600 and of human erythrocytes upto 1 in 700. The action of quinine dihydrochloride in concentrations of 1 in 10,000 (1/15 times hemolytic concentration) on hemolysis produced by various hemolytic agents like bile salts, saponin and digitonin was studied in vitro. It was observed that quinine in this concentration significantly increases the degree of hemolysis produced by these hemolytic agents. Similarly quinine when injected intravenously in a rabbit in doses of 35 mg/kg increased the susceptibility of erythrocytes to hemolytic action of saponin. Intravascular hemolysis following the administration of quinine is seen clinically in cases of black water fever and on the basis of this experimental work it is suggested that quinine precipitates these hemolytic episodes by rendering the erythrocytes more susceptible to the action of tissue lytic factors

---

14. Photoelectric Colorimeteric Method For Estimating The Degree of Hemolysis in A Blood Sample.

R. S. GREWAL

The method is based on the principle of estimating the total hemoglobin in a completely hemolysed blood sample and then estimating the hemoglobin in the supernatent after affecting hemolysis by the addition of varying concentrations of hemolytic agents. The degree of hemolysis can then be expressed as percentage of total hemoglobin which is taken as 100 percent hemolysis. The use of liberated hemoglobin to express the degree of hemolysis is justified because there is a direct relationship between the number of red blood cells destroyed and the amount of hemoglobin set free as hemolysis is an all or none phenomenon.

The haemoglobin is estimated photoelectric colorimeterically by Acid hematin method.

---

15. Effectiveness of several Indigenous and Synthetic Drugs in Ectopic Ventricular Tachycardia following Acute Myocardial Infarction

R. B. ARORA, NEW DELHI

The occlusion of the anterior descending ramus of the left coronary artery of the dog's heart by a two stage technique (Harris and Kokernot 1950), results in the development of ectopic ventricular tachycardia which begins after a delay of 4 1/2 to 8 hours, reaches a maximum between 12-20 hours after occlusion and then declines gradually, disappearing by the 4th postoperative day. One advantage of this method is that it allows for the evaluation of the antiarrhythmic activity of a drug in unanaesthetized animal, against an arrhythmia which is aetiologically similar to a type of ventricular arrhythmia often occurring in man. Secondly due to its persistent and long lasting character, it permits the estimation of duration of drug action, and repeated drug tests. Thirdly it allows for the elucidation of certain gross toxic effects of the drug in the unanaesthetized animals.

Most of the drug tests were carried out on the first postoperative day, when the ectopic activity was maximum, without anaesthesia or sedation. The test drug was infused intravenously in 20 ml, of saline in five minutes through an indwelling venous catheter. A drug is considered to be effective if it produces more than 50 percent reduction in ectopic rate, maintained for more than 19 minutes. In addition, reduction in total heart rate is considered an essential feature of a suitable drug.

Considering the manifold advantages of this method, it was thought expedient if not imperative to try some drugs of promise, found useful by other experimental methods, before passing them on to the physician for clinical trial. Five antimalarials (Chloroquin, plaquenil, isopentaquin, paludrine, Camoquin) ; five tranquilising agents (Chlorpromazine, rauwiloid, reserpine, benactyzine and meprobamate) and jatamansone (a Ketone isolated from essential oil of Nardostachy's jatamansi) have been investigated and compared with quinidine. Six experiments were done for each drug.

Chloroquin, plaquenil, chlorpromazine, benactyzine, rauwiloid, meprohamate and jatamansone are superior to quinidine in combating this arrhythmia and deserve clinical trial.

16. Ventricular Excitabilty under Hypothermia

C. L. MALHOTRA, B. K. ANAND, BALDEV SINGH, AND P. K. DAS, NEW DELHI

The changes in ventricular excitability, and role of acetylcholine and glutathione metabolism of heart were investigated in relation to the production of ventricular fibrillation during hypothermia. Hypothermia was produced in 33 dogs under ether anaesthesia by surface cooling method. It was usually not necessary to continue anaesthesia below 28°C. In 16 dogs (Group A) no external stimuls was given to the heart while in 17 dogs (Group B) left ventricle was stimulated either mechanically or electrically (by square waves of 3-5 volts, 60/sec. frequency, 0.5 m. sec. duration, for one second) without opening chest, at different temperature levels during hypothermia. In Group A, none of the hearts fibrillated though the temperature was reduced to as low as 8° in some (average 14.2°C). In this group, during hypothermia there was progressive hypotension and bradycardia, and at temperatures below 12°C, periods of asystole lasting for 1/2 minute were seen in some. E. C. G. showed increased P-R interval, rarely A - V block, bundle branch block and intraventricular block, broad based low voltage QRS, various deflections and distortions of T, appearance of Ta wave and sometimes extrasystoles mostly of ventricular origin. Extrasystoles appeared below 20°C and were more common (5 out of 8) in dogs where no respiratory aid was given than those in whom artificial respiration was given (1 out of 8 dogs). With the help of artificial respiration temperature could be reduced to even 8°C, otherwise respiration ceased at 11°C to 15°C.

In group B, 11 out of 17 dogs developed ventricular fibrillation following stimulation at tempreatures between 18°C and 35°C (7 dogs fibrillated between temperatures of 21°C and 26°C) though generally, the same stimuli only produced ventricular extrasystole at higher temperatures. In 9 of these expriments acetylcholine and glutathione of heart was estimated at the temperature when heart fibrillated or otherwise between 15°C and 17°C. The investigations show the importance of external excitation as the cause of ventricular fibrillation. The work is in progress.

---

17. Acid Control of the Pylorus

J. D. PATHAK, BARODA

Since Cannon (1911) pronounced the acid control of the pylorus, the theory has undergone many reverses. That strong acid delays the gastric

emptying has been amply proved by several workers but whether the acid opens the door in mild concentrations has not been substantiated.

Effects of Hcl on gastric evacuation were studied in 75 experiments performed on 3 Medical Subjects under standard condition by Hunt's method. The data are statistically examined. The volumes of gastric contents recovered at the end of 20 minutes have been plotted against concentration of Hcl in the 750 ml. meal that was put into the subject's stomach. The results indicate a dual behaviour of Hcl in the stomach.

1. In concentration upto 10 m. ef. 1., Hcl favours the emptying of the meal from the stomach.
2. With stronger concentrations of Hcl in the meal there is progressively greater retention of gastric contents.
3. The Hcl in the meals depresses the acid secretory activity of the stomach and with high concentrations the contribution tends to be even alkaline in one subjeet.

The results seem to support Cannon's theory of acid control of the pylorus.

---

18. Quantitative studies on Spermatogenesis in Liver Injury

J. C. SACHDEV, AND R. C. SINGH, INDORE

Adult male albino rats weighing between 150 to 200 grams, were maintained on synthetic diet containing 20 percent casein a supplement of vitamin and salts. The animals ware sacrificed at the end of the experimental period of 30 days when their testes and liver tissues were removed. Testes were fixed in Helly's fluid; sectioned and stained by "Periodic acid Fuchsin Sulphurous acid" and counter stained by Haematoxylin.

A quantitative analysis of spermatogenesis based on Roosen Runge classification (1950) was under-taken on these sections and the percentages of the different stages were determined.

Results of average values of different stages in this group of normal rats are represented below :

Stages.	I	II	III	IV	V	VI	VII	VIII
Percentage	3.8	4.8	14.3	5.3	8.7	34.7	12.7	16.2

Preliminary studies on spermatogenesis in liver injuries induced by hepatoxic agents has revealed interesting deviation from the normal on macroscopic and microscopic studies. Increase in the percentages of stage–I, II & III- at the expense of the other stages has been noticed. Further detailed studies regarding the correlation of spermatogenesis with liver injury are in progress.

---

19. Excitation, Inhibition, Conduction, Contraction and the Production of Action Potentials in Unstriated Muscle in the Absence of Electrolytes in the External Medium.

INDERJIT SINGH AND A. K. ACHARYA, AGRA

The stomach muscle of the frog. *Rana tigrina,* has the remarkable property that it can be excited by electric current and acetyl-choline, and inhibited by adrenaline, if immersed in a hypotonic solution of sucrose (Singh and Singh, 1943; Singh, 1944; Singh and Bhatt, 1957). During such contraction, diphasic action potentials are produced. Chemical analyses by the flame photometer show that more thoroughly the sodium is washed out of the interspaces, the better the muscle behaves (Singh and Acharya, 1957). Even a trace of sodium now abolishes the contrations and the action potentials. This shows that the contraction is not due to retention of sodium in the interspaces; as a matter of fact, if the muscle does not give good contractions in the sucrose solution, the presence of sodium should be suspected, and further washing carried out. The optimum temperature for these responses is 30-32°C, and the muscle is rather sensitive to temperature changes; at lower temperatures, the preliminary depressant action of sucrose is prolonged.

---

20. Morphine Inhibition of Cholinergic invervated structures

J. C. PAUL AND J. C. DAVID, VELLORE

The effect of ACh, nicotine and histamine were examined on rabbit ileum. Addition of morphine, atropine and pentolinium tartrate produced varying degrees of inhibition. The morphine inhibition was never complete and was not increased by increasing the concentration of morphine. Large doses of ACh (500 μg.) depress the gut and exert some type of blockage. It is

suggested that morphine depresses the intestinal motility by reducing ACh release from cholinergic nerve endings, the anatomical substratum of the action being possibly on the Cajal cells.

---

21. Observations on the relative percentage of Hæmoglobins A. F. and E. in Hæmoglobin E Heterozygotes.

J. B. CHATTERJEA, SUSHIELA SWARUP AND S. K. GHOSH, CALCUTTA.

Hæmoglobin E heterozygotes were examined in two series. The first series consisted of AE individuals as obtained during family studies on patients suffering from Hb E – thalassaemia disease. The second series consisted of similar individuals isolated during a survey for abnormal hæmoglobins. Haemoglobin F was estimated by the alkali denaturation method of Singer *et al* (Blood 6 : 413, 1951). Hæmolysates were examined by paper electrophoresis following standard technique. The mean values for hæmoglobins A, F and E were 61.5 percent (range 37 to 70), 2.6 percent (range 0.75 to 3.6) and 38.5 percent (range 30 – 63) respectively. Implications of the findings are discussed in the light of clinical and hæmatological data.

---

22. Preliminary Observations on the effect of Alkaloids of Rauwolfia Serpentina (R. S. Total) and Reserpine in blood sugar in cats.

M. L. CHATTERJEE, CALCUTTA

In recent reviews by Bein and Lewis, there was a reference to the causation of hyperglycaemia in rabbits and in dogs after reserpine. Bein quoted also Schuler for having communicated personally a contrary view. It was considered therefore worthywhile to investigate on this point and also to compare the effect of reserpine and that of the total alkaloids on blood sugar in cats. It was seen that in anaesthetised cats intravenous reserpine (0.15 mg./kg.) caused a marked hyperglycaemia in all the four animals tested. On an average an increase of a maximum of 55 percent above the initial level was attained at about 2 hours 15 minutes after reserpine, (the range of increase being between 22-105 percent). On the other hand, the total alcoholic extract of Rauwolfia serpentina was found to produce a progressive depression of blood sugar level. After about 3 hours and 15 minutes of a dose of 0.5-1 mg./kg.

intravenously there was on an average a maximum fall in blood sugar level by 31.7 percent (the range being between 25 percent and 46 percent)below the initial value. Simultaneous records of blood pressure changes showed that there were more or less a similar degree and percentage of fall of blood pressure, in both the groups of animals examined.

---

23. Antidiuretic action of Gymnema sylvestre (Preliminary findings)

M. L. CHATTERJEE, CALCUTTA

Controversy exists regarding the antidiabetic action of this indigenous plant. No study appears to have yet been made to find whether the said or such unsubstantiated claim for antidiabetic action of this and other indigenous plants could have originated in Ayurvedic times for the possible beneficial antidiuretic effect in cases of polyuria. The present preliminary investigation was therefore carried out on water loaded rats (5 ml/100 gm). Watery extract of leaves of Gymnema sylvestre (1 gm. leaves in 50 ml of water) given orally in the same manner as the plain water load (control) was found to decrease comparatively of the percentage of water excretion. This finding appears to be interesting in view of the claim for antidiabetic effect of this indigenous plant.

In 3 different cross over tests with 2 different groups of rats (6 in each group), there was less of excretion in the treated group compared with the control (water load group) inspite of the variation in the excretion on different days of experiments.

---

24. Study of the Pharmacological Actions of Aegelin.

M. L. CHATTERJEE AND M. N. GHOSH, CALCUTTA

The alkaloid Aegelin (from leaves of Aegle marmelos) is an organic amine having a cinnamoyl group which is likely to be dissociated in the digestive tract releasing the parent amine to produce a sympathomimetic action. The leaves have been used in asthma in certain parts of India. Pharmacological investigation of aegelin was therefore taken up.

1. *On blood pressure*. (A) *Orally* a suspension of agelin (1.5 mg. to 1 gm. per kg.) in anaesthetised cats, produced no significant alteration in B. P.

neither before nor following adrenaline.

(B) *By intravenous injection* an alcoholic solution of aegelin (1mg./kg.) produced stimulation of respiration and contraction of denervated nictitating membrane but no definite change in the action of adrenaline on B. P. nor on nictitating membrane., nor on the action of acetylcholine (muscarinic and nicotinic) on B. P. in chloralosed cat.

2. *On isolated perfused hearts.* Aegelin caused some depression of amplitude but increased the rate in guinea pigs and rabbits. Adrenaline action was not modified.

3. *Isolated guinea pig ileum.* Aegelin (1 to 10 mg) did not alter the responses due to histamine or acetylcholine when N/10 HCl (later neutralised by N/10 NaOH); propylene glycol; chloroform and ethyl acetate were used as vehicles.

4. *On the bowel movements.* Aegelin (0.5 to 2 Gm./kg. given with food) appeared to show some laxative action in two kittens (6 experiments). There was an increase in both the frequency and the quantity of the stool passed. From the nature of the stool and the interval between the drug and bowel movements the action appears to be on the large intestine.