EXPERIMENTAL PANCREATIC DIABETES IN THE MONKEY.

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A.H.Neufeld.

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A C K N O W L E D G M E N T.

The work reported was carried out under the direction of Dr. J.B.Collip, Professor of Biochemistry, McGill University. I am much indebted to Professor Collip for the privileges which he has afforded me. I also wish to express my thanks to Professor D.L.Thomson and to Assistant Professor H. Selye for their many helpful suggestions.

A.H.Neufeld.

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Introduction.

In recent years statements have appeared in the literature which indicate that oestrin has a depressing action on the so-called "diabetogenic" function of the pituitary. This study was undertaken to demonstrate the action of oestrin, if any, on the diabetic monkey.

A preliminary report on this work will be presented at the meeting of the Federation of American Societies for Experimental Biology in Memphis, Tenn., April, 1937.

EXPERIMENTAL PANCREATIC DIABETES.

Mehring and Minkowski, in 1885, made a detailed study of the relationship of the pancreas to diabetes. They definitely established that the removal of the pancreas of dogs is followed by a condition resembling diabetes mellitus in man. Later studies have shown that the course of this disease varies in different species. In the dog, hyperglycaemia develops within twenty-four hours after operation, ketosis supervenes. The characteristic symptoms of the condition are polyurea, polydypsia, loss of weight, good appetite. Finally, coma sets in and the animal dies in convulsions.

THE PITUITARY AND DIABETES.

Houssay and Biasotti (10) first demonstrated a functional relation between the pituitary and carbohydrate metabolism. They showed that pancreatectomy in the hypophysectomised toad or dog is not followed by the usual symptoms of pancreatic deprivation. The glucose excretion is lowered in these animals, acidosis stops completely, and the customary fatty degeneration of the liver does not occur. Such animals survive for

long periods. The injection of anterior pituitary preparations into these animals returns them to an extreme diabetic state. These findings have been confirmed by a number of authors (2,6,13,8). Long and Lukens (14) have recently shown that adrenalectomy in a depancreatised animal produces a condition analogous to that of a depancreatised-hypophysectomised animal.

Concerning the manner in which the pituitary exerts its effect on carbohydrate metabolism very little is known. Houssay and his coworkers believe that the pituitary effect is a direct one. They consider that the improved condition of such animals may be caused by an increased sugar utilization. Long considers that the adrenal cortex is an intermediate factor.

Burn and Ling (5) have made a pituitary preparation which, when injected into normal rats, produces a rise in ketone bodies in blood and urine. Anselmino and Hoffmann (1) have produced supporting evidence. Thus it would appear that the anterior pituitary secretes a substance - "the diabetogenic hormone" - which in some fashion elevates the blood sugar, and a second substance - "the ketogenic hormone" - which stimulates ketone bodies formation.

THE EFFECT OF OESTRIN ON PITUITARY FUNCTION.

Investigations have shown that oestrin exerts a suppressing influence on growth, gonadotropic and lactogenic activities of the anterior pituitary (15, 17,18,24). However, evidence has been presented to indicate a release of luteinizing hormone from the pituitaries of oestrin-treated rats (9,11,16,22). These findings suggested the possibility of suppressing the "diabetogenic" factor in the anterior pituitary with oestrin and thus producing a condition analogous to the modified form of diabetes observed in hypophysectomised animals. Barnes et al (3) made a study of this in the depancreatised dog, Nelson and Overholser (19) in the monkey. Their results indicate that oestrin treatment produces only a mild glycosuria and hyperglycaemia, while the cessation of treatment precipitates a severe glycosuria and hyperglycaemia. The survival period of such animals was at least ten days longer than the control.

Clinical application of oestrone treatment in diabetes has been made (12,20,21). The results obtained, however, are not convincing.

EXPERIMENTAL PROCEDURE.

The animals used for this work were sub-adult and adult female and male monkeys (Macaca Mulatta). They were kept in special metabolism cages, receiving the usual colony ration of carrots, cabbages and purina. Twenty-four hour samples of urine were collected and analyses carried out for the total reducing sugars by a micro-modification of Benedict's copper titration method (4), and acetone bodies by Van Slyke's method (23).

Blood samples were taken in the morning before the animals were fed and sugar determinations carried out by the method of Folin and Wu (7).

Pancreatectomy was performed by Dr. Selye. Three animals were kept as controls throughout the course of this investigation.

THE EFFECT OF OESTRIN ON THE DIABETIC MONKEY.

The oestrin used was a crude preparation in oil, containing about 1000 γ of oestrin per c.c. The animals under treatment were given 1 c.c. of this preparation daily by subcutaneous injection.

Seven animals were used. One female received oestrone for ten days prior to pancreatectomy. The remaining animals were not pre-treated. To carry them over the first operative shock, they received insulin

for several days after operation. This was followed by a 2 to 5 day period of combined insulin and oestrin treatment. Finally, insulin was discontinued and only oestrin given for 10 to 18 days. Then all treatment was stopped.

The completeness of pancreatectomy was indicated by the blood and urine sugar findings and assured at autopsy.

Monkey No. 3, receiving oestrone prior to pancreatectomy, died on the fourth day after operation. She refused to take food. On the last day she showed a marked hyperglycaemia (450 - 500 mg. per 100 c.c.) and a high ketosis (1.75 gm.). Apparently a developing acidosis resulted in death.

Monkeys Nos. 2 and 5 showed the longest survival period. Monkey No.5 remained in good condition until the 127th day after operation when she developed bronchial pneumonia and died two days subsequently. Her weight decreased from 4.7 to 4.2 kg. Monkey No.2 was depancreatised on October 6th, 1936, and has remained in excellent condition up to the present time. Her weight has decreased from 4.8 to 4.2 kg. Throughout the period of observation these two animals have shown a consistent hyperglycaemia (200-600 mg. per 100 c.c.) and glycosuria (2-23 gm. per kg. in 24 hrs.) with no noticeable response to oestrone treatment. The ketosis showed a slight increase

during the first 3 to 4 days following the operation. Then it returned to the normal level and remained there. Graph I shows the fluctuations in blood sugar, urine sugar, ketosis and body weight over the entire post-operative life of monkey No. 5.

Similar to monkeys Nos. 2 and 5, the remaining four animals showed no change in glycosuria, hyperglycaemia or ketosis during and after oestrin treatment.

Monkey No. 1 showed a urine sugar of 2 - 7 gm. per kg., and a blood sugar of 300 to 400 mg. in 100 c.c. A slight rise in ketosis was observed during the first two days after operation, reaching a maximum of 0.45 gm. on the second day. By the fourth day this had decreased to the normal level and remained there subsequently (0.01 - 0.09 gm. in 24 hrs.). The animal died on the 19th day following pancreatectomy, from biliary obstruction caused by post-operative adhesions.

Monkey No. 4 showed a fasting blood sugar of 250-300 mg. in 100 c.c. The urine sugar varied between 2 and 13 gm. per kg. The ketosis increased progressively up to the tenth day (4.83 gm.) and then gradually decreased to the normal value of 0.03 gm. on the 21st day. The animal died on the 38th day after operation. Her weight decreased from 5 to 4 kg.

Monkey No. 6 showed a blood sugar of 250 - 500 mg. in 100 c.c. and a urine sugar of 2 - 10 gm. per kg. The ketosis remained at the normal level of 0.02-0.09 gm. in 24 hrs. She died on the 26th day following pancreatectomy. Her weight decreased from 4.7 to 3.8 kg.

Monkey No. 7 showed a blood sugar of 250 - 300 mg. in 100 c.c. and a urine sugar of 2.5 - 13 gm. per kg. The ketosis reached a maximum of 0.35gm. on the third day after operation and then returned to the normal level. She died on the 21st day following pancreatectomy. Her weight decreased from 4.75 to 3.45 kg.

The animals receiving oestrin treatment until shortly before death (monkeys Nos. 1,4,6 and 7) showed typical pre-mortal symptoms, namely a loss of appetite and diarrhoea. Enlarged, brown-coloured adrenals were observed at autopsy showing degenerative changes in the cortex. Any suspected pancreatic remnants were studied histologically and proved negative. The pituitaries and thyroids appeared normal. One animal (monkey No.6) showed a severe ulceration in the caecum.

PANCREATIC DIABETES IN UNTREATED MONKEYS.

Eight monkeys were depancreatised. Of these, four animals were given insulin for varying periods following the operation, and then treatment was discontinued. The remaining four served as untreated controls.

Three of the four untreated control animals (monkeys Nos. 13,14 and 15) died from 3 to 6 days following the operation. They refused to take food. Autopsy findings established the cause of death as post-operative pneumonia. The livers of these animals showed fatty degeneration, the liver fat ranging from 14 to 18 %. The fourth untreated animal died on the 36th day following pancreatectomy. She showed extreme muscular atrophy. Her liver fat was 12.5 %. It is obvious that these animals died from post-operative pulmonary complications rather than pancreatic deficiency.

Monkeys Nos. 8 and 9 were given insulin following pancreatectomy for 17 and 12 days respectively. They showed a marked hyperglycaemia (100 - 600 mg. in 100 c.c.) and glycosuria (2 - 34 gm. per kg.). Their ketosis was similar to that of the oestrone treated animals. They showed a gradual decrease in weight, 4.7 to 3.3 kg. for monkey No. 8 and 4.5 to 3.15 kg. for monkey No. 9.

Monkey No. 8 was sacrificed 92 days following pancreatectomy for analyses. The results are given in Table I. Monkey No. 9 was depancreatised on November 25th, 1936, and has remained in good condition up to the present time.

Monkeys Nos. 10 and 11 were given insulin for four days after pancreatectomy. Both showed a high blood sugar (230-400 mg. in 100 c.c.) and urine sugar (2-18 gm. per kg.) but no ketosis. Their weight decreased from 7.6 to 4.7 and 4.6 to 3.4 kg. respectively. Monkey No. 10 died 76 days following the operation. At autopsy tubercular lesions of the lungs were found. Monkey 11 died 77 days following the operation. No definite lesions were found. Both animals showed normal adrenals and a normal liver fat.

A brief summary of the results is given in Table II.

DISCUSSION.

Contrary to previous findings, it has been shown that oestrin has no effect on the hyperglycaemia and glycosuria of the diabetic monkey.

A constant finding in untreated diabetic monkeys is the absence of ketosis. The cause of this, whether a species variation or some other factor, must remain unanswered for the present. We might mention, however, that in our attempts to produce a ketosis in normal and depancreatised monkeys with alkaline extracts of the anterior pituitary and other "ketogenic" preparations made by Dr. Collip, and known to possess ketogenic properties, we were unsuccessful.

The first few days following pancreatectomy, monkeys show a hyperglycaemia, glycosuria, a developing acidosis, and a fatty degeneration of the liver. Within a few days after operation, however, the latter two symptoms disappear. Apparently the animals quickly adapt themselves to the condition of pancreatic deficiency. The results obtained appear to indicate a certain amount of utilization of sugar by these animals.

The depancreatised monkey is hypersensitive to insulin (cf. Graph II). When fasted for 34 to 70 hours a severe hypoglycaemia ensues (22 mg. in 100 c.c.) while normal controls show only a slight drop in blood sugar (50-60 mg. in 100 c.c.) (cf. Graph III).

It appears that changes in the sugar metabolism following pancreatectomy in the monkey are similar to those obtained by simultaneous pancreatectomy and adrenalectomy, or pancreatectomy and hypophysectomy, in animals such as the dog and the cat.

SUMMARY.

The supposition that oestrin has a depressing action on the so-called "diabetogenic hormone" of the anterior pituitary has not received any confirmation. The untreated depancreatised monkey exhibits a hyperglycaemia, glycosuria, but no ketosis and may survive the operation indefinitely if complications do not set in.

The depancreatised monkey is hypersensitive to insulin. Severe hypoglycaemia ensues when such animals are fasted for 34 to 70 hours.

Changes in carbohydrate metabolism obtained in the depancreatised monkey are similar to those shown by the depancreatised-hypophysectomised, and the depancreatised-adrenalectomised dog or cat.

REFERENCES.

1. Anselmino, K.J. and Hoffmann, F. - Klin. Wochschr., 12: 1435, 1933.
2. Barnes, B.O. and Regan, J.F. - Endocrinol., 17: 522, 1933.
3. Barnes, B.O., Regan, J.F. and Nelson, W.O. - J.Amer. Med. Assoc., 101: 926, 1933.
4. Benedict, S.R. - J.Amer.Med. Assoc., 57: 1193, 1911.
5. Burn, J.H. and Ling, H.W. - Quart. J.Pharmacol., 6: 31, 1933.
6. Collip, J.B. - J. Mount Sinai Hosp., 1: 28, 1934.
7. Folin, O. and Wu, H. - J.Biol. Chem., 41: 367, 1920.
8. Hartman, F.A. and Brownell, K.A. - Proc.Soc.Exp.Biol. and Med., 31: 834, 1934.
9. Hohlweg, W. - Klin. Wochschr., 13: 92, 1934.
10. Houssay, B.A. and Biasotti, A. - Endocrinol., 15: 511, 1931.
11. Lane, C.E. - Anat. Rec., 61: 141, 1935.
12. Liebhart, St. - Ginek. polska, 14: 617, 1935.
13. Long, C.N.H. and Lukens, F.D.W. - Proc.Soc.Exp.Biol. and Med., 32: 326, 1934.
14. Long, C.N.H. and Lukens, F.D.W. - Proc.Soc.Exp.Biol. and Med., 32: 743, 1935.
15. Moore, C.R. and Price, D. - Amer.J.Anat., 50: 13, 1932.

References - Cont.

16. Nelson, W.O. - Anat. Rec., 64: 52, 1935.

17. Nelson, W.O. - Endocrinol., 18: 33, 1934.

18. Nelson, W.O. - Endocrinol., 19: 187, 1935.

19. Nelson, W.O. and Overholser, M.D. - Endocrinol., 20: 473, 1936.

20. Polonovski, M., Warembourg, H. and Driessens, J. - Compt. rend. soc. biol., 121: 1451, 1936.

21. Rathery, F., Kourilsky, R. and Laurent, Y. - Compt. rend. 187: 255, 1928.

22. Selye, H., Collip, J.B. and Thomson, D.L. - Proc.Soc. Exp. Biol. and Med., 32: 1377, 1935.

23. Van Slyke, D.D. - J.Biol. Chem., 32: 455, 1917.

24. Zondek, B. - Lancet, 230: 10, 1936.

TABLE I.

BLOOD AND MUSCLE ANALYSES IN THE DIABETIC MONKEY.

Liver glycogen	2.01 % in terms of reducing sugars.
Muscle glycogen	306 mg. % in terms of reducing sugars.
Heart muscle glycogen	388 mg. % " " " " "
Blood sugar	452 mg. in 100 c.c.
Blood chloride	477 mg. in 100 c.c.
Blood calcium	8.8 mg. in 100 c.c. serum
Blood inorganic phosphorus	2.4 mg. in 100 c.c.
Blood cholesterol	215 mg. in 100 c.c.
Liver fat	11.02 %

TABLE II.

SUMMARY OF RESULTS OF EXPERIMENTS ON MONKEYS.

No.	Blood sugar mg./100c.c.	Urine sugar gm./kg.	Ketosis gm./24 hrs.	Decrease in Weight kg.	Survival period days
OESTRONE TREATED MONKEYS					
1.	300 - 400	2- 7	0.00-0.45	-	19
2.	250 - 600	2-23	0.01-0.20	4.8-4.2	160+
3.	450 - 500	1- 2	0.01-1.7	-	4
4.	250 - 300	2-13	0.03-4.8	5.0-4.0	38
5.	150 - 650	1-19	0.00-0.25	4.7-4.2	129
6.	250 - 500	2-10	0.02-0.09	4.7-3.8	26
7.	250 - 300	2-13	0.02-0.35	4.75-3.45	21
UNTREATED MONKEYS (GIVEN INSULIN FIRST FEW DAYS).					
8.	100 - 600	2-19	0.00-0.30	4.7-3.3	92
9.	150 - 500	2-34	0.00-0.31	4.5-3.15	110+
10.	250 - 400	2-12	0.04-0.8	7.6-4.7	76
11.	230 - 400	2-18	0.01-0.20	4.6-3.4	77
UNTREATED MONKEYS.					
12.	200 - 400	2-19	0.01-2.1	3.95-3.10	36
13.	230 - 350	1- 3	0.06-1.5	5.0	6
14.	270 - 500	2	0.27-1.5	5.45	3
15.	360	1- 2	0.30-1.8	6.05	5
NORMAL CONTROL MONKEYS.					
	70 - 110	0.00	0.00-0.08	6.7	
	70 - 110	0.00	0.00-0.08	5.0	
	65 - 100	0.00	0.00-0.09	3.5	

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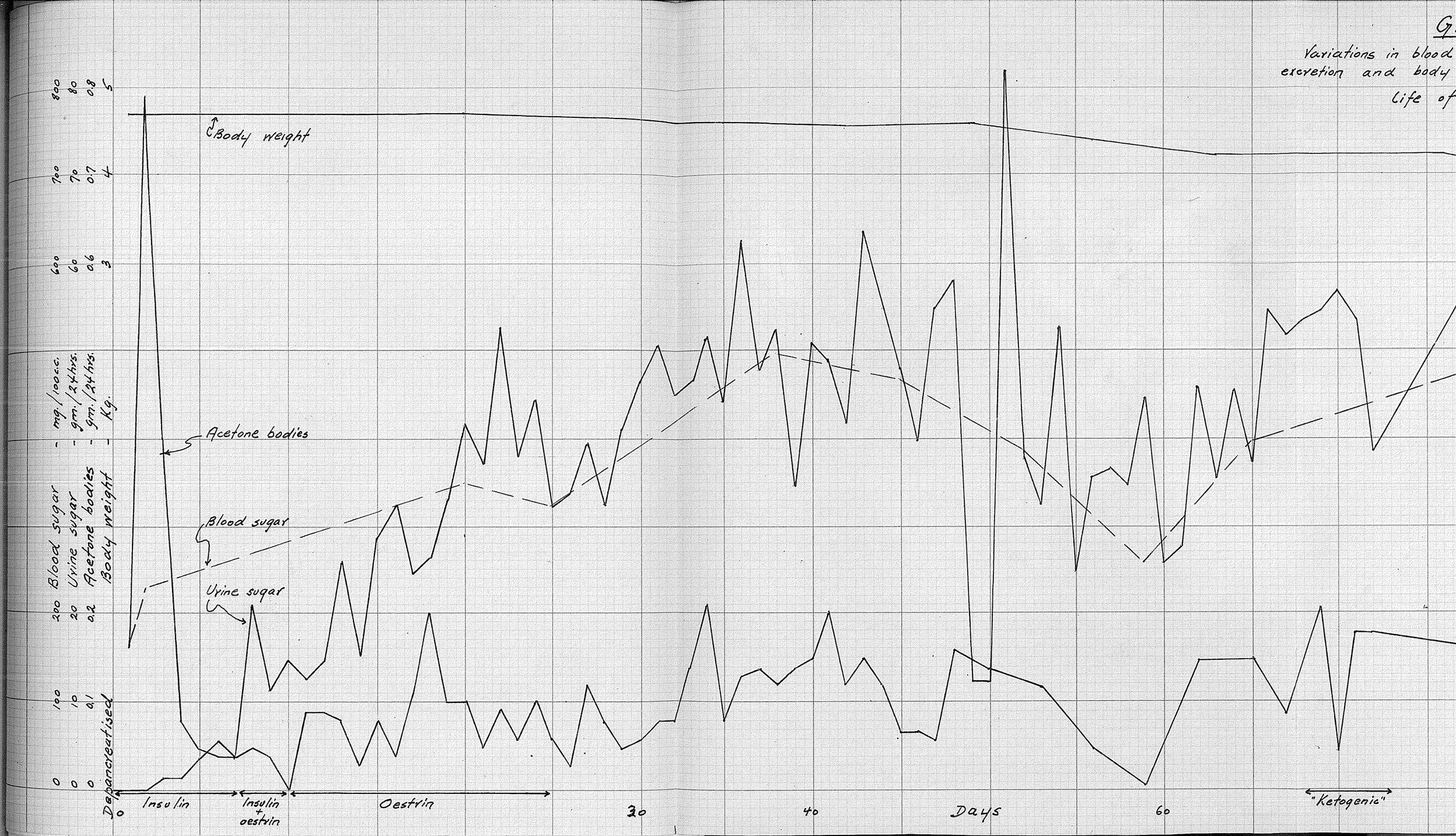
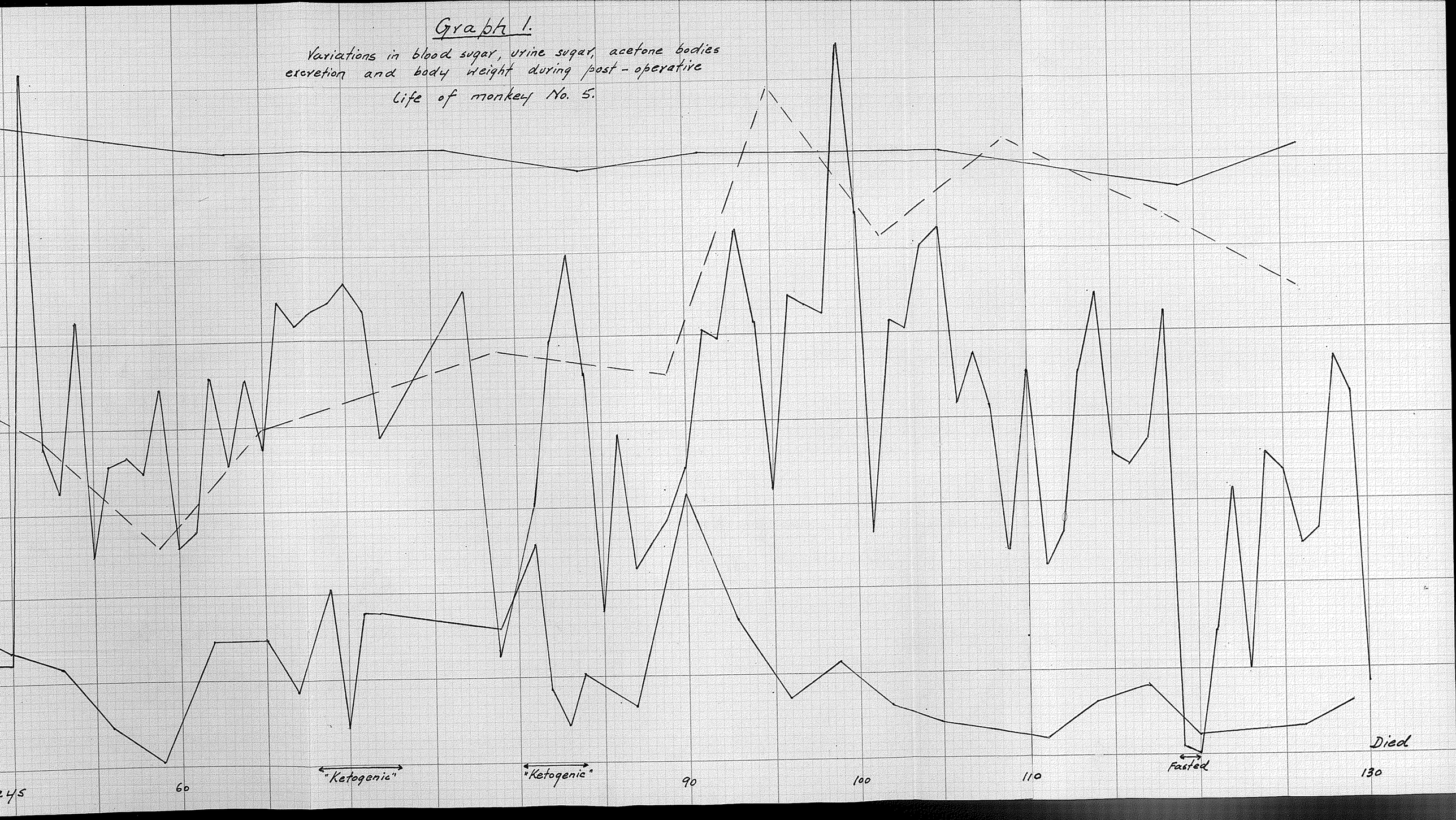
Variations in blood
excretion and body
life of



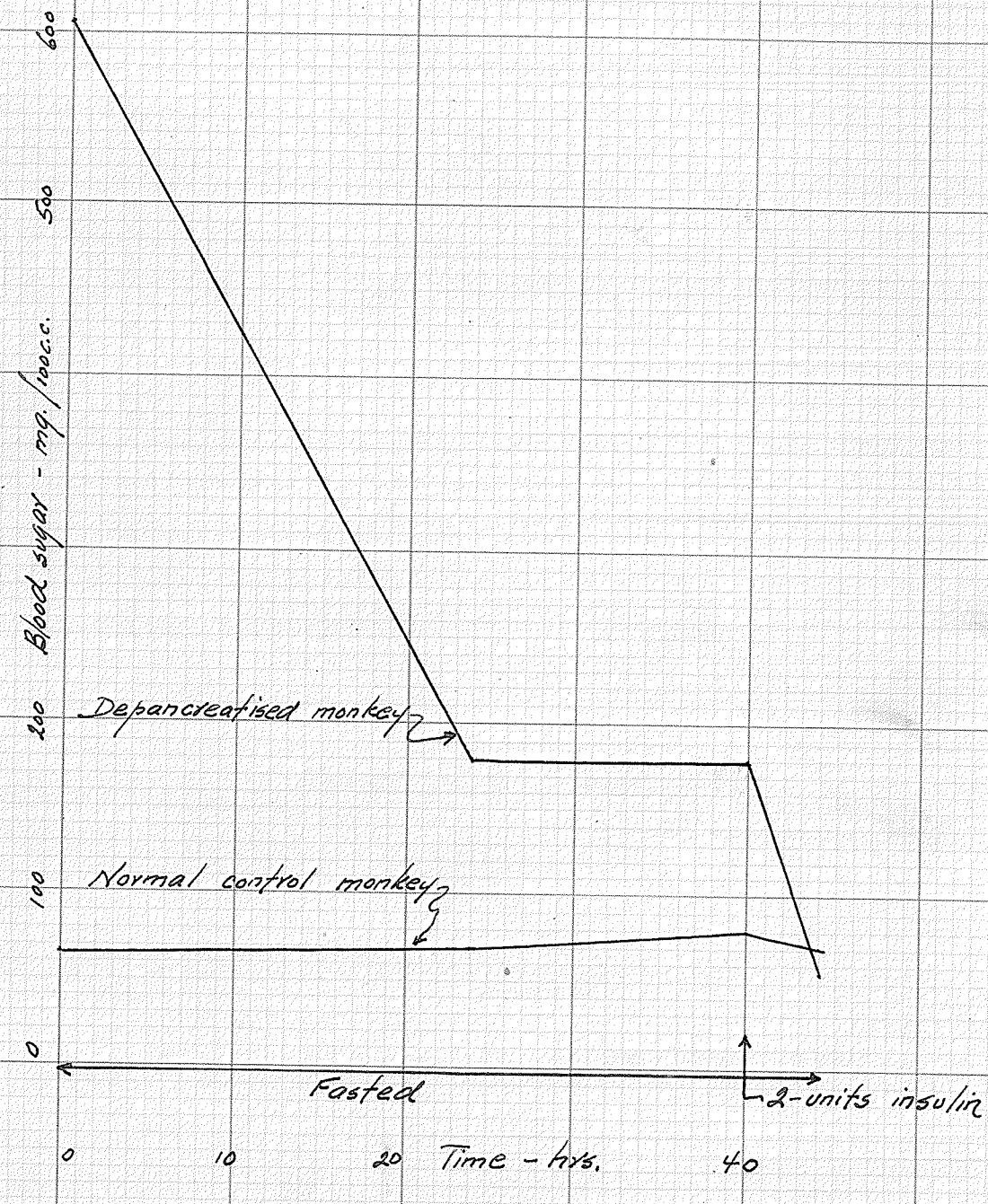
Graph 1.

Variations in blood sugar, urine sugar, acetone bodies excretion and body weight during post-operative life of monkey No. 5.



Graph II.

The effect of insulin on the blood sugar of the depancreatised monkey compared with that of the normal control.



Graph III

The effect of fasting on the blood sugar of the depancreatised monkey compared with that of the normal animal.