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The Metabolic and Hormonal Responses to Glucose Infusion in Anaesthetized Normal and Diabetic Dogs Controlled by an Artificial B-Cell

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Summary. The metabolic response to glucose infusion in anaesthetized normal and pancreatectomized dogs has been assessed. Normoglycaemia was achieved in the diabetic dogs with an external artificial B-cell which administered insulin into the peripheral circulation. No differences were found in the levels of blood glucose, glucagon, lactate, pyruvate and plasma non-esterified fatty acids, either in the fasting state or in response to glucose infusion. However, compared to normal animals normoglycaemic diabetic dogs had significantly elevated circulating levels of insulin and alanine at all times. Fasting levels of the same hormones and metabolites were also measured in conscious dogs. Blood pyruvate levels were higher, and plasma non-esterified fatty acid levels lower, in the anaesthetized animals. There were also minor but consistent changes in blood glucose and plasma insulin while glucagon, lactate and alanine levels were unaffected by anaesthesia. In conclusion, controlled barbiturate anaesthesia has relatively minor effects on the metabolic and hormonal status of the dog. The metabolic and hormonal response to glucose infusion in pancreatectomized dogs treated with an artificial B-cell was almost entirely normalized, except for peripheral hyperinsulinaemia and hyperalaninaemia.

Key words: Glucose, artificial pancreas, insulin, glucagon, lactate, pyruvate, alanine, free fatty acids, anaesthesia, metabolic response, insulin infusion, diabetes.

Absolute insulin deficiency produces abnormalities in the metabolism of protein, fat and carbohydrate. Subcutaneous administration of insulin improves but does not normalize these derangements, particularly with respect to blood glucose. Normoglycaemia can be restored [1, 2] if insulin is infused by an artifical B-cell. Under these conditions the opportunity arises for examining the concurrent concentrations of certain hormones and several intermediates of fuel metabolism during meal absorption [3, 4] and exercise [5, 6]. In the present study, we infused glucose into normal and diabetic dogs and examined the responses of the circulating concentrations of glucose, insulin, glucagon, lactate, pyruvate, alanine and non-esterified fatty acids. Glycaemia was normalized in the diabetics using an artificial B-cell [7] which administered insulin into the peripheral circulation. In addition the effect of anaesthesia alone on the fasting concentrations of these hormones and intermediary metabolites was assessed.

Materials and Methods

1. Animal Preparation and Blood Sampling

Six non-obese male beagles, 1 to 2 years old and weighing 12.2 ± 0.2 kg were fasted for 12 h prior to each experiment. All were studied as anaesthetized controls. Five were included in the post-pancreatectomy protocols. Five other dogs weighing 12.4 ± 0.5 kg were similarly fasted and served as conscious controls. Dogs were fed a weight maintaining diet (600 g/day) consisting of equal weights of dog chow (Master Premium Dinner, Maple Leaf Mills Ltd., Toronto, Ont.) and canned dog food (Meal Mix, Derby Pet Foods Ltd., Toronto, Ont.), given at 0900 h. Calories were distributed as 38% carbohydrate, 33% protein and 29% fat. General anaesthesia was induced with an IV 1:1 mixture of pentobarbitone (Nembutal Sodium Injection, Abbott Laboratories, N. Chicago, Ill.) and thiopentone (Pentothal Sodium, Abbott Laboratories, Montreal, Que.) totalling 25 mg/kg body weight. Anaesthesia was maintained throughout the experiment by a slow infusion (≈ 25 mg/h) of thiopentone into the saphenous vein. The level of anaesthesia was monitored by heart and respiration rates and blood gas analysis. The flow of anaesthetic was adjusted to main-

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Table 1. Amounts of glucose and insulin infused in diabetic dogs during the baseline glucose infusion, and recovery phases of the experiments

Phase (time)	Glucose mg	Insulin mU[a]
Baseline (−40→0 min)	0	174
Glucose infusion (0→60 min)	6840	1608
Recovery (60→180 min)	0	550

[a]1mU = 41 ng insulin

tain these variables close to normal. To compensate for blood loss 0.154 mol/l saline and thiopentone were infused at about 1.5 ml/min throughout the experiment. Body temperature was monitored, and maintained by external heat applied with a heating lamp or electric blanket. A dual-lumen catheter (Abjad Industries, Toronto, Ont.) was placed in a superficial jugular vein and provided blood diluted 1:1 with 0.154 mol/l saline containing 25 U/ml heparin for continuous glucose monitoring. Another catheter was similarly placed to permit the sampling of undiluted blood for the later determination of hormones and intermediary metabolites. Blood (1.5 ml) from the second jugular vein was collected at −40, −10, 0, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min directly into 1.5 ml centrifuge tubes containing 0.15 ml heparin for the later determination of insulin. At −30, 0, 30, 60, 90, 120 and 180 min blood (2 ml) was distributed into 5 ml tubes containing a total of 0.2 ml of a 1:1 mixture of aprotinin (Trasylol 10000 KIU/ml, FBA Pharmaceuticals Ltd., Pte. Claire, Que.) and EDTA (24 mg/ml ethylene-diamine-tetra-acetic-acid in double distilled water, BDH Chemicals, Poole, England) for the later estimation of glucagon and non-esterified fatty acids (NEFA). At the same times blood was taken into capillary tubes for hematocrit determination. At 0, 60 and 180 min blood (2 ml) was collected into chilled tubes containing perchloric acid (PCA, 10% W/V), in a volume equal to that of blood, for the later determination of lactate, pyruvate and alanine concentrations. The total volume of blood in these samples was 41 ml. Continuous glucose estimation required a further 3 ml/h whole blood. At time 0 glucose challenge was begun.

In the conscious control animals samples were drawn from an external jugular vein at 0800 and 0900 just prior to feeding. Minimal stress was induced by the use of a chronic exteriorized indwelling silastic catheter. Blood was distributed as above into tubes containing heparin, EDTA/aprotinin, or cold perchloric acid.

Blood was collected on ice and centrifuged with minimal delay at 4 °C and the supernatants frozen at −20 °C until assay.

2. *Analytical Methods*

The continuous blood glucose analyzer was calibrated against a Beckman glucose analyzer (Beckman Instruments, Fullerton, Cal.) to read equivalent plasma glucose concentration as previously described [8]. Total time delay was 2 min.

Plasma insulin was determined using an anti-porcine insulin anti-serum (from Dr. Peter Wright, Minneapolis, Minn.), a dextran coated charcoal separation of free from bound hormone [9], and a pork insulin standard (Novo Research Institute, Copenhagen, Denmark). Glucagon was determined on unextracted plasma with antiserum 30K (obtained from Dr. R. H. Unger, Dallas, Tex.), purified pork glucagon standard, and ^{125} I-labelled pork glucagon (Novo Research Institute, Copenhagen, Denmark) and the same dextran coated charcoal separation technique. The precisions of the insulin and glucagon assays were approximately ± 10% over the range 0.125 to 4 ng/ml and 20 to 2000 pg/ml respectively. Lactate, pyruvate and alanine were analyzed by microfluorometric adaptations of standard enzymic methods [10] employing an Aminco Fluoro-microphotometer (American Instrument Co., Division of Travenol Laboratories, Silver Springs, Md.). NEFA levels were estimated by a radio-chemical microtechnique [11].

Porcine neutral crystalline zinc insulin (Connaught Laboratories, Ltd., Toronto, Ont.) for infusion was diluted in 0.154 mol/l saline to 17 mU/ml. No loss of insulin was detectable in the absence of added protein. The precision of the saline-insulin dilution was verified by immunoassay as was the immunologic identity of the infused insulin and the standards in the assay conditions used (data not shown).

3. *Blood Glucose Control*

Glycaemic regulation in the diabetic dogs was obtained with an artificial B-cell which used the same continuous glucose analyzer as outlined above [8], and in which the chosen algorithms and constants [12, 13] were previously defined. Insulin was infused into a saphenous vein at a rate responsive on a minute-by-minute basis to both the blood glucose level and its rate of change. Insulin infusion was with a pulsatile pump (Lambda Pump, Harvard Instruments Ltd., Millis, Mass.).

4. *Glucose Infusions*

Glucose infusions were for 60 min into the saphenous vein at a rate of 10 mg/kg/min. All challenges started after a 40 min baseline period of constant glycaemia and were followed by a 120 min recovery period. A precalibrated channel of the multi-channel peristaltic pump in the glucose analyzer was used for glucose infusion.

5. *Pancreatectomy and Management of Diabetes*

Diabetes was induced by surgical removal of the pancreas [7, 13]. Subcutaneous injection of daily doses of equal amounts of NPH and crystalline zinc porcine insulin (Connaught Laboratories Ltd., Toronto, Ont.) were given between experiments. The dose of 18 to 24 U insulin was based on fasting and 2 h postprandial blood glucose levels, determined 5 times weekly. A total of 12–14 capsules of exocrine pancreatic enzyme supplements (Cotazym, Organon Ltd., Montreal, Que.) was given with meals. All dogs maintained their presurgical weights (±1 kg) with this treatment and bowel function was not different from normal dogs.

On the day prior to an experiment the diabetic animals were fed and injected at 0900 and 1700 h. At each time they were given half the usual meal and half the usual total subcutaneous dose of insulin. Intermediate acting insulin was not given. Meals not finished by 2000 h were removed. In all cases on the morning of the experiment the animals were hyperglycaemic (300–400 mg/dl), suggesting that little residual insulin was present. Concurrent plasma insulin levels were less than 0.08 ng/ml. Normoglycaemia was restored over 1–3 h by a combination of continuous intravenous infusion and boluses of 0.5 to 1.0 U insulin, and maintained for at least 40 min prior to glucose challenge. During the latter baseline period insulin was given at a relatively constant basal rate by the artificial B-cell. The amounts of glucose and insulin infused in the diabetic dogs during the baseline, glucose infusion, and recovery periods are summarized in Table 1.

Table 2. Fasting hormone and metabolite levels in conscious normal and anaesthetized diabetic dogs

		Plasma glucose mg/dl	Plasma insulin ng/ml	Plasma glucagon pg/ml	Blood lactate μmol/l	Blood pyruvate μmol/l	Blood alanine μmol/l	Plasma NEFA μmol/l
Conscious	(n)[a]	(15)	(14)	(13)	(13)	(13)	(13)	(13)
normals	mean	90	0.42	80	744	49	295	758
(A)	SEM	±2	±0.02	±12	±72	±6	±29	±75
	max	105	0.59	163	1236	103	484	1157
	min	76	0.30	29	412	22	125	306
Anaesthetized	(n)	(16)	(16)	(11)	(12)	(12)	(12)	(12)
normals	mean	98	0.33	54	873	81	281	550
(B)	SEM	±2	±0.03	±5	±77	±10	±25	±63
	max	113	0.49	89	1273	152	457	982
	min	85	0.12	33	417	39	156	287
Anaesthetized	(n)	(12)	(11)	(12)	(12)	(12)	(12)	(12)
diabetics	mean	101	0.53	60	990	117	732	606
(C)	SEM	±3	±0.03	±9	±216	±19	±111	±78
	max	116	0.71	134	2279	224	1504	1098
	min	90	0.31	26	318	42	415	249
2 tailed t-tests[b]								
B vs A	p	***	*	NS	NS	*	NS	*
C vs B	p	NS	***	NS	NS	NS	*	NS

[a] Is the number of experiments
[b] Levels of significance: * denotes $p<0.05$ – ** denotes $p<0.01$ – *** denotes $p<0.005$ – NS denotes $p>0.05$

Statistical comparisons were made using the F-test for homogeneity of variances at the 5% level of significance, and then the unpaired, or where appropriate the paired, t-test to examine the significance of differences in, or changes from, the fasting levels in each animal.

Results

1. Fasting Levels

Fasting glucose, insulin, glucagon, lactate, pyruvate, alanine and NEFA levels are presented in Table 2.

The fasting glucose levels in the anaesthetized normal dogs were 8 mg/dl higher than those in the conscious normal group ($p<0.005$), blood pyruvate levels were 30 μmol/l higher ($p<0.02$), while insulin was 0.08 ng/ml lower ($p<0.05$), and plasma NEFA were lower by 200 μmol/l ($p<0.05$). Plasma glucagon, blood lactate, and blood alanine levels were unaltered by anaesthesia.

The fasting levels of corresponding variables in the anaesthetized diabetic dogs showed two major changes compared to anaesthetized normal dogs: plasma insulin levels were 0.2 ng/ml higher ($p<0.005$) while alanine levels were significantly and markedly elevated in the diabetic animals ($p<0.05$). Glucose, glucagon, lactate, pyruvate and NEFA levels were unchanged.

2. Response to Glucose Infusion

Changes from fasting levels in blood lactate, pyruvate and alanine are shown in Table 3 for the normal and diabetic dogs. The data represent the mean increments from fasting observed 60 and 180 min after commencement of the 60 min glucose infusion. An apparent initial rise at 60 min and a subsequent fall at 180 min in both the lactate and pyruvate levels were not statistically significant, due mainly to large inter- and intra-individual differences in both groups of anaesthetized animals. Alanine was not affected by the glucose infusion in the normals but showed a gradual decline in the diabetics which was significant at 180 min ($p = 0.025$).

The glycaemic response (Fig. 1) to glucose infusion was similar in the normal and diabetic animals increasing to plateaus of 170 ± 8 mg/dl and 155 ± 8 mg/dl respectively at 30 min, and returning to baseline values 15 min after the infusion was stopped. Glucose concentration was significantly lower in the diabetics only at 30 min ($p = 0.025$). Plasma insulin was elevated in the diabetic animals on the artificial B-cell at all times (Fig. 2). The transient decline in glucagon (Fig. 3) which occurred in both groups was significant at 30 and 60 min. Plasma NEFA concentrations (Fig. 4) were significantly below fasting levels at 30, 60 and 90 min ($p<0.01$),

Table 3. Increments from fasting in blood metabolite levels with glucose infusion in anaesthetized normal and diabetic dogs

		Δ[b]Lactate μmol/l		ΔPyruvate μmol/l		ΔAlanine μmol/l	
	min	60	180	60	180	60	180
Anaesthetized	(n)[a]	(12)	(12)	(12)	(12)	(12)	(12)
normals	mean	+271	−91	+15	0	−14	−42
(A)	SEM	±151	±98	±11	±9	±24	±32
	max	+1303	+565	+77	+52	+146	+126
	min	−420	−652	−70	−61	−158	−231
Anaesthetized	(n)	(12)	(12)	(12)	(12)	(12)	(12)
diabetics	mean	+314	−180	+32	−29	−105	−245
(B)	SEM	±203	±204	±17	±17	±69	±93
	max	+1886	+1208	+124	+97	+222	+274
	min	−751	−1267	−55	−122	−637	−867
2-tailed t-test							
A vs B	p	NS[c]	NS	NS	NS	NS	NS

[a] Is the number of observations
[b] Δdenotes change
[c] NS denotes $p>0.05$

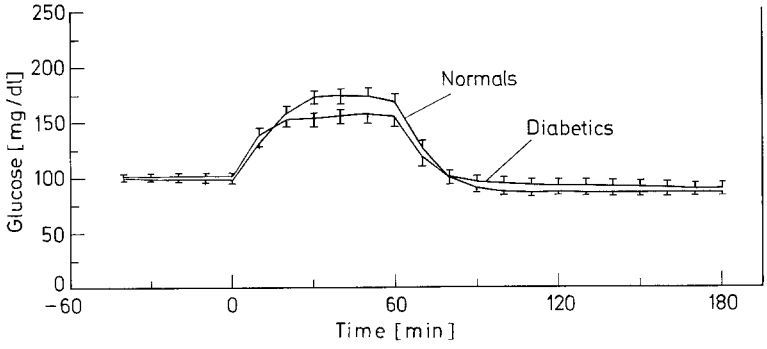

Fig. 1. Mean ± SEM of plasma glucose concentrations. Anaesthetized normal and pancreatectomized dogs before, during and after a 60 min glucose infusion of 10 mg/kg/min started at time 0. Blood glucose was controlled in the diabetic animals using an artificial B-cell and was significantly lower in the diabetics only at 30 min ($p<0.05$)

and returned to pre-infusion concentrations at 180 min. There were no significant differences at any time between the two groups in glucagon and NEFA levels.

Discussion

The present study was undertaken to examine the hormonal and metabolic response to glucose infusion in the diabetic dog when controlled by an artificial B-cell, as compared to sex and weight matched normal dogs. Since the glucose challenge was performed in anaesthetized dogs we measured the effect of barbiturate anaesthesia on the fasting levels of all the hormones and intermediary metabolites studied. Anaesthesia alone resulted in a small but significant increase in glucose and pyruvate while insulin and NEFA showed small decreases. Lactate, alanine and glucagon were unaffected by anaesthesia. Thus the hormones and metabolites measured do not reflect any major physiological alteration occurring with anaesthesia in normal fasting dogs.

The fasting levels of glucose, lactate, pyruvate, NEFA and glucagon in the anaesthetized diabetic dogs receiving insulin by the artificial B-cell were similar to anaesthetized normal animals. However, the peripheral insulin and alanine concentrations were significantly elevated. This observation that the attainment of normoglycaemia with the artificial B-cell results in peripheral hyperinsulinaemia has previously been documented in dogs [12–14] and man [15], and is at least in part related to the peripheral venous route of insulin administration. In contrast portal insulin infusion in the anaesthetized [16] and conscious [17] diabetic dog normalized blood glucose without peripheral hyperinsulinaemia both in the fasting state and during glucose infusion. Whether

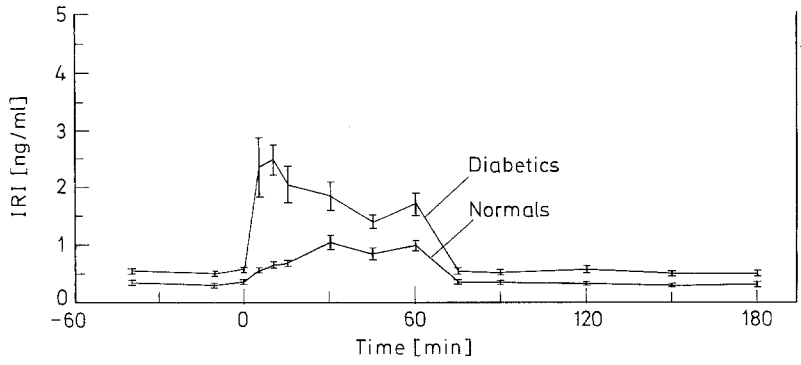

Fig. 2. Mean ± SEM of plasma insulin (IRI) concentrations. Anaesthetized normal and pancreatectomized dogs before, during and after a 60 min glucose infusion of 10 mg/kg/min started at time 0. Plasma insulin in the diabetics was significantly higher than in the controls at all times ($p<0.05$)

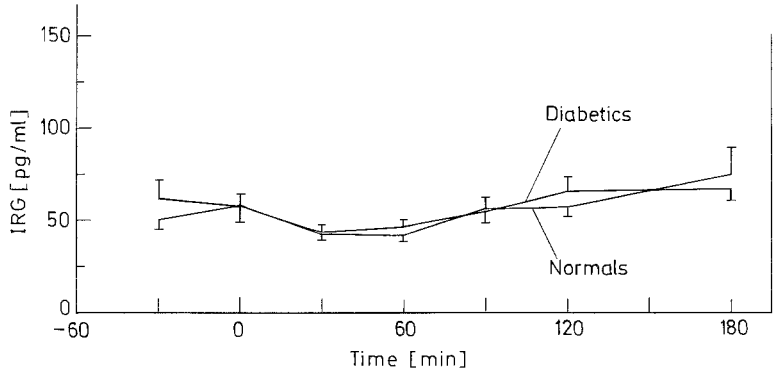

Fig. 3. Mean ± SEM of plasma glucagon (IRG) concentration. Anaesthetized normal and diabetic dogs before during and after a 60 min glucose infusion of 10 mg/kg/min started at time 0. Decrements from the fasting glucagon levels were significant ($p<0.05$) at 30 and 60 min in the pancreatectomized but not in the normal dogs

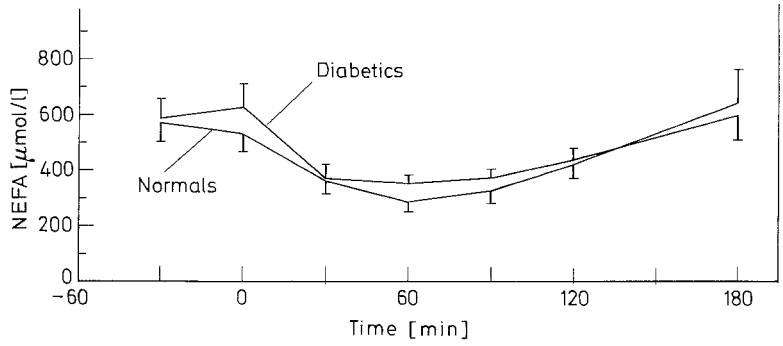

Fig. 4. Mean ± SEM of plasma non-esterified fatty acids (NEFA) concentrations. Anaesthetized normal and diabetic dogs before, during and after a 60 min glucose infusion of 10 mg/kg/min started at time 0. Decrements from the fasting plasma NEFA levels were significant ($p<0.01$) at 30, 60, 90 and 120 min in both the diabetic and normal dogs

the normoglycaemia so produced reflects an abnormal balance in glucose turnover due to high peripheral or low hepatic insulin levels is not clear. That hepatic metabolism is not entirely normalized is suggested by the elevated alanine concentrations.

Glucose production in the post-absorptive state is primarily from hepatic glycogenolysis and gluconeogenesis. In diabetics gluconeogenesis is accelerated as a result of insulin deficiency [18]. Alanine, pyruvate and glycerol are important gluconeogenic precursors and their rate of hepatic extraction has been shown to be accelerated in insulin deficiency [19, 20]. It is thus surprising that in the present study alanine levels were elevated two fold, perhaps reflecting a blocking effect of the hyperinsulinaemia on the liver of these dogs. Mild but persistent hyperalaninaemia (560 ± 66 μmol/l) was also observed in diabetic dogs infused chronically with adequate peripheral insulin to maintain post-absorptive normoglycaemia (unpublished observations). The precise dynamics of these observations could be elucidated by measuring alanine turnover. It is of interest that pyruvate was also elevated although this was not statistically significant.

The anaesthetized normal and diabetic animals showed remarkably similar metabolic responses to glucose infusion. The increase in blood glucose occurred rapidly reaching a plateau at 30 min and

quickly returning to baseline with cessation of the infusion. The fall in NEFA levels reflected the rise in insulin concentration, the antilipolytic effects of which lead to the inhibition of NEFA mobilization from adipose tissue. It is noteworthy that anaesthesia in normal dogs lowers both insulin and NEFA levels, a situation which clearly implies increased NEFA utilization. In spite of the mild (0.2 ng/ml) hyperinsulinaemia in the diabetic dogs there were no differences in the fasting plasma NEFA levels in the anaesthetized animals. Whether this is the result of reduced lipolysis and/or increased utilization is not clear. The fasting lactate, pyruvate, and alanine concentrations varied considerably in normal and diabetic anaesthetized dogs before the glucose infusion and the results are presented as increments from fasting values. Although lactate and pyruvate increased while alanine decreased in the normal and diabetic dogs these changes were not significant because of large inter-individual variability. Alanine concentration continued to decline in the post infusion period and reached significantly lower levels ($p = 0.025$) at 180 min. The diabetic dogs were hyperglycaemic and underinsulinized prior to the start of the experiment. Under these conditions, favourable for gluconeogenesis, alanine production must have been accelerated particularly in view of the concurrent hyperalaninaemia. With the restoration of normoglycaemia by peripheral insulin infusion, gluconeogenesis was rapidly reduced but the accelerated alanine production apparently required several hours to subside. Alternately the liver of the dog may be exquisitely sensitive to insulin so that alanine uptake was reduced. The final alanine levels reached however were above normal and similar to those in fasting diabetic animals made normoglycaemic by long term peripheral insulin infusions, as mentioned above.

The metabolic response to the glucose infusion was in general similar in the normal and diabetic animals but the peripheral insulin concentrations obtained with the artificial B-cell were 2–3 × higher. As discussed above this must in great part be related to the peripheral route of insulin administration.

Although in the pancreatectomized dogs the pancreatic source of glucagon had been removed fasting glucagon concentrations were similar to the normal dogs and decreased in similar fashion with glucose infusion. This observation is consistent with other studies [21–23] which indicate that nonpancreatic glucagon in the dog is immunologically and biologically similar to pancreatic glucagon, and responds in a similar physiological manner.

In summary the present study demonstrates that barbiturate anaesthesia did not cause major hormonal or metabolic disturbance. The metabolic and hormonal response to glucose infusion in diabetic dogs controlled by the artificial B-cell was almost entirely normalized, except for peripheral hyperinsulinaemia and hyperalaninaemia.

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