CHAPTER 10

EFFECT OF ACETYLCHOLINE, CHOLINE CHLORIDE, INSULIN, GLUCAGON AND DEXAMETHASONE ON THE GLYCAEMIC LEVELS IN VAGOTOMIZED PIGEONS

The nature of neural control of liver function and glucose homeostasis is only getting slowly understood. The antagonistic actions of parasympathetic and sympathetic nerves are also seen in their effects on glucose homeostasis and liver functions (Lautt, 1983). While parasympathetic fibres induce glucose uptake, the sympathetic actions induce liver to release glucose (Shimazu, 1983). The parasympathetic action is mediated by acetylcholine and the sympathetic action by norepinephrine. To a certain extent, ACh mimics the action of insulin especially in the induction of liver cells to take up more glucose (Beyner and Geelen, 1982). Vagal stimulation increased glycogen deposition in the liver (Mondon and Burton, 1971) through the activation of glycogen synthetase. Vagotomy reduced glycogen deposition in the liver, and acetylcholine injection restored the rate of deposition (Mondon and Burton, 1971). In birds, vagotomy lowered the glucose level in blood but when injected with glucose hyperglycaemia prevailed for a longer time (Verma, 1982); a situation similar to diabetes in mammals. Vagus is now known to be involved in several glucoregulatory processes: (1) efferent fibres affect the liver directly (2) efferent fibres influence the insulin secretion from B cells and inhibit

the A-cell secretion (3) affernt fibres signal the glycaemic state of the liver to hypothalamus (Lautt, 1980; Lautt, 1983; Shimazu, 1983; Rohner-Jeanrenald et al., 1983). Moreover, ACh is shown to act synergistically with insulin in the uptake of glucose by liver cells (Mondon and Burton, 1971; Pilo and Patel, 1978; Beyner and Geelen, 1982; Niijima, 1969). Apart from these actions acetylcholine secreted by the nerve endings may counteract the action of catecholamine and other hormones such as glucagon and glucocorticoids.

In the present study, ACh, choline chloride, insulin, glucagon and dexamethasone were injected into vagotomized pigeons to understand whether or not the action of these on the glycaemic level has any correlation with vagal innervation.

MATERIALS AND METHODS

Adult domestic pigeons (Columba livia) weighing around 200-250 gms, maintained in laboratory conditions with standard diet were used for experiments. Birds were divided into 5 groups and each group was divided into 5 subgroups having 5 birds each.

The 5 subgroups were (1) Normal over night starved

(2) Sham operated 48 hr. starved

(3) Vagotomized 48 hr. starved

(4) Sham operated 72 hr. starved

(5) Vagotomized 72 hr. starved.

Each main group was injected with hormones as shown below

Group (1) Acetylcholine (15 mg/animal)

(2) Choline chloride (1.5 mg/animal)

(3) Insulin (1 unit/animal)

(4) Glucagon (1 mgm/animal)

(5) Dexamethasone (0.04 mg/animal).

After injection, the blood samples were drawn from wing veins at the time of injection (0 hr.) and at regular intervals of 30 min. (i.e. 30, 60, 90 and 120 min.) and glucose was estimated by Folin and Malmoros Method (1929) (Chapter 1).

RESULTS

ACh Injection (Table 10-1; Fig. 10-1)

In the normal pigeons (overnight starved) ACh injection produced slight decrease at 30 min. and at all the rest of the intervals no significant changes were observed. In sham operated 48 hr. pigeons, ACh produced an increase at 30 min. and a decrease from 90 min. onwards. In vagotomized 48 hr. starved pigeons also, ACh produced an increased glucose level compared to that at the pre-injection period. In sham operated 72 hr. starved pigeons, ACh produced no significant change in blood sugar level. In vagotomized 72 hr. pigeons, ACh administration brought about

Fig. 10-1. Effect of acetylcholine administration on the glycaemic level in normal, sham operated and vagotomized pigeons.

Fig. 10-2. Effect of cholinechloride administration on the glycaemic level in normal, sham operated and vagotomized pigeons.

FIG. 10-1

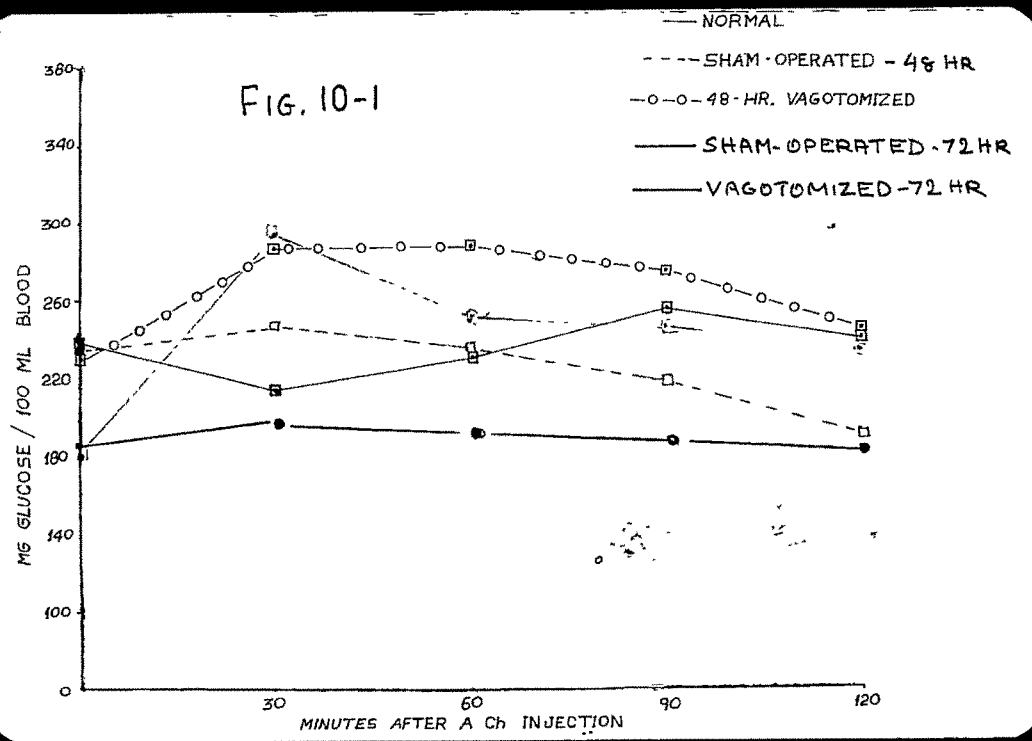

FIG. 10-2

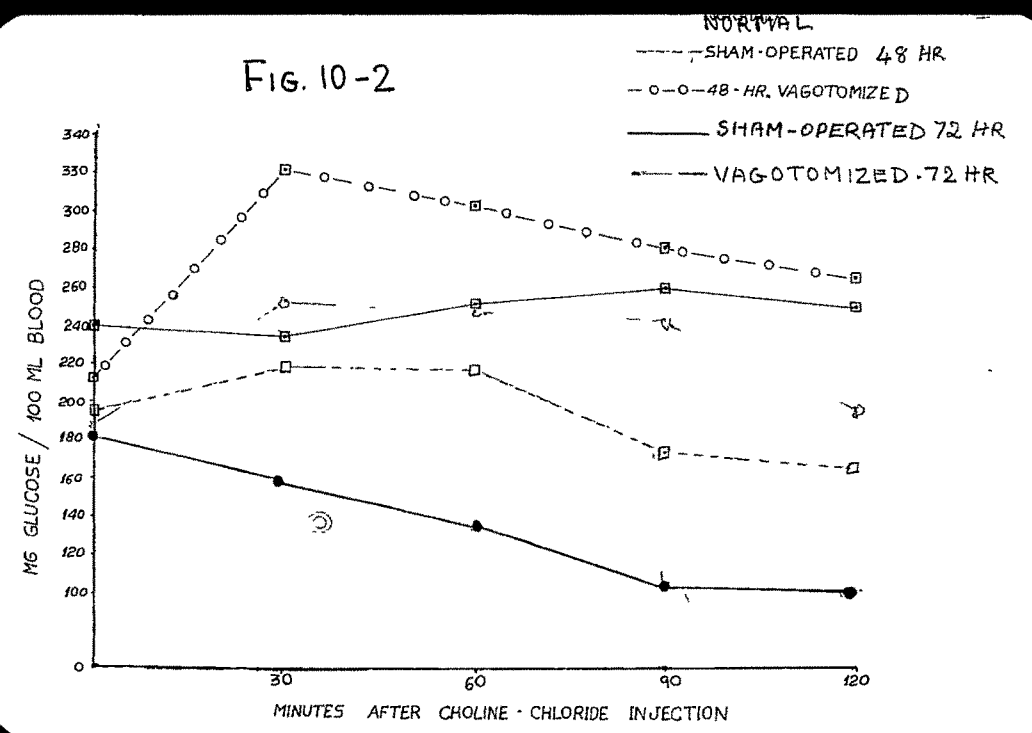

Fig. 10-3. Effect of insulin administration on the glycaemic level in normal, sham operated and vagotomized pigeons.

Fig. 10-4. Effect of glucagon administration on the glycaemic level in normal, sham operated and vagotomized pigeons.

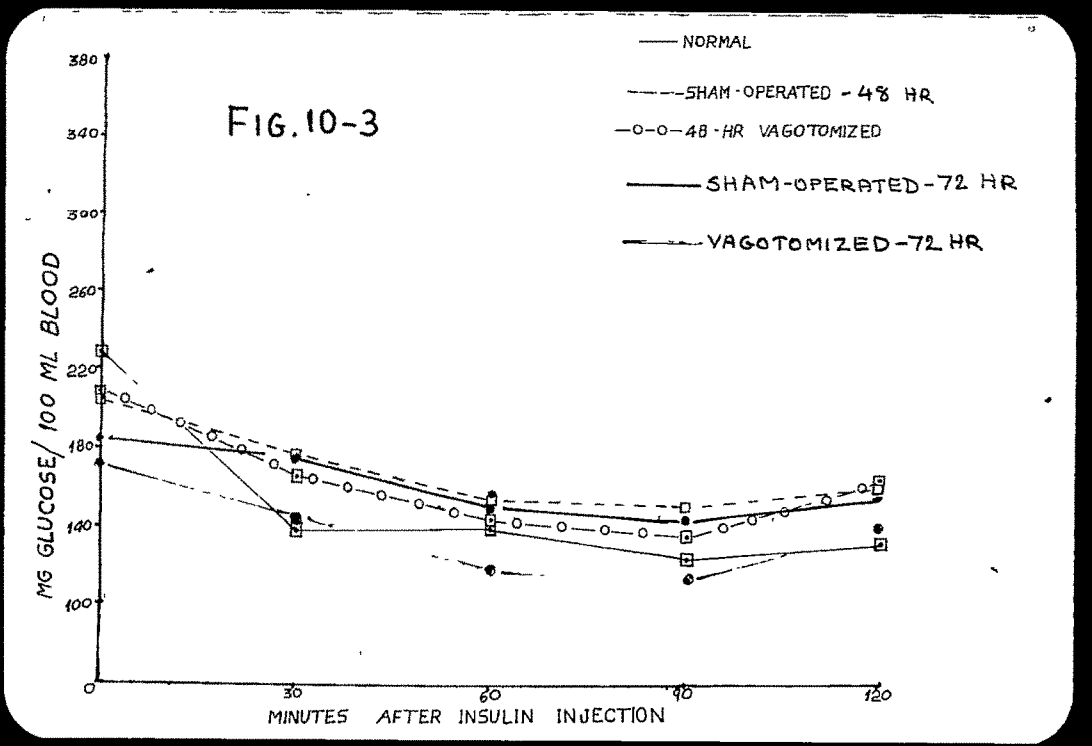

FIG. 10-3

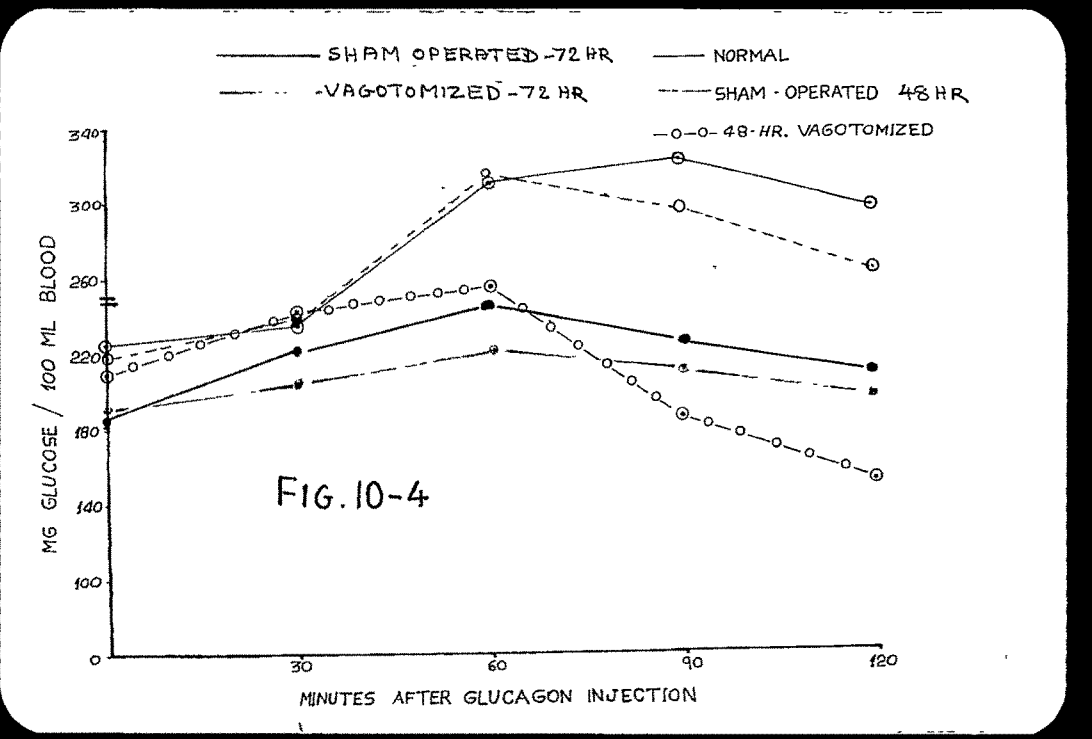

FIG. 10-4

Fig. 10-5. Effect of DXM (dexamethasone) administration on the glycaemic level in normal, sham operated and vagotomized pigeons.

FIG. 10-5

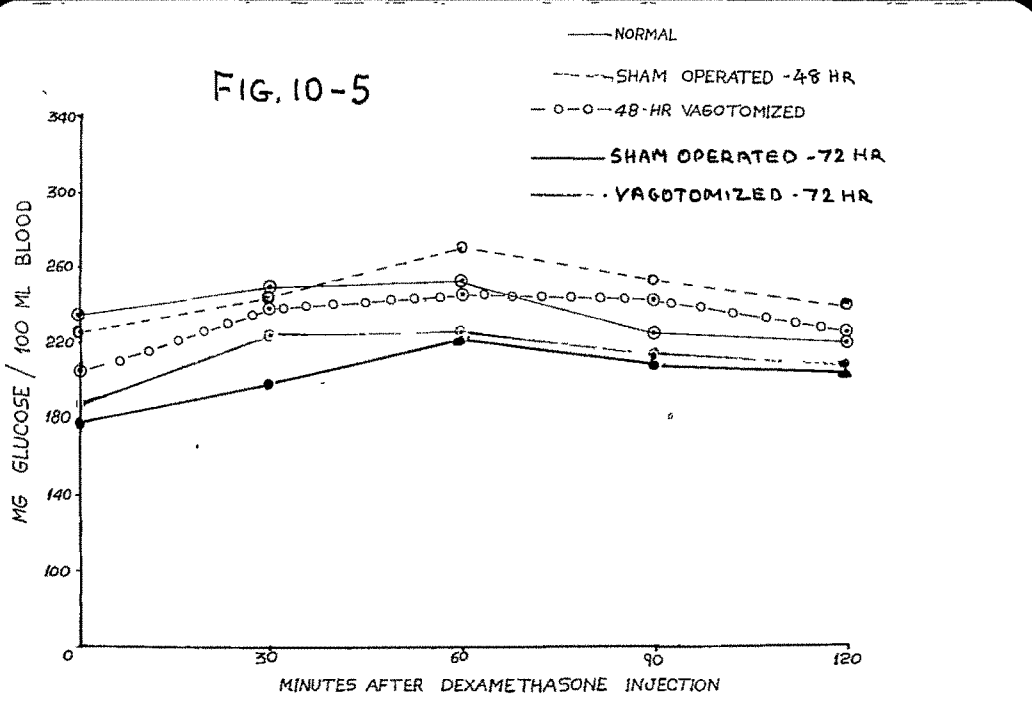
NORMAL
SHAM OPERATED -48 HR
48-HR VAGOTOMIZED
SHAM OPERATED -72 HR
VAGOTOMIZED -72 HR
MG GLUCOSE / 100 ML BLOOD
340
300
260
220
180
140
100
0
30
60
90
120
MINUTES AFTER DEXAMETHASONE INJECTION

Table 10-1

Effect of acetylcholine administration on glucose tolerance in normal, sham operated and vagotomized pigeons (Mean ± S.E.M)
(Glucose - mg/100 ml blood)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vagotomized	72 hr Sham operated	72 hr Vagotomized
0	238.63 ± 6.08	235.33 ± 2.4	230.78 ± 3.34	181.11 NS ± 0.86	181.39 ± 3.02
30	214.36*** ± 2.63	247.52* ± 3.77	286.2**** ± 3.62	192.25 NS ± 1.599	292.83**** ± 6.43
60	231.04 NS ± 9.699	235.397 NS ± 3.31	287.15**** ± 7.84	94.06 NS ± 12.90	253.78**** ± 16.39
90	255.64 NS ± 12.25	217.89* ± 5.55	274.88**** ± 2.59	192.34 NS ± 1.23	247.33**** ± 9.30
120	240.58 NS ± 10.18	190.22* ± 10.18	244.72 NS ± 5.29	180.08 NS ± 7.07	238.94**** ± 2.92

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$

Table 10-2

Effect of choline chloride administration on glucose tolerance in normal, sham operated and vagotomized pigeons (Mean ± S.E.M) Glucose mg/100 ml blood)

Interval in Minutes	Normals	48 hr Sham operated	48 hr Vago-tomized	72 hr Sham operated	72 hr Vago-tomized
0	239.32 ± 1.83	195.45 ± 2.81	212.73 ± 7.41	184.42 ± 0.26	191.45 ± 2.46
30	234.65 NS ± 9.79	217.68**** ± 4.49	320.44**** ± 6.28	158.44* ± 9.0	257.81*** ± 9.92
60	249.89 NS ± 5.97	216.36** ± 16.0	303.4**** ± 2.63	137.37**** ± 1.22	243.52*** ± 8.14
90	259.05* ± 7.87	175.41** ± 6.09	279.29**** ± 5.38	106.2*** ± 4.37	240.35**** ± 4.78
120	251.33 NS ± 5.21	166.89**** ± 3.76	265.66**** ± 1.72	93.77**** ± 6.12	188.76 NS ± 0.896

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$

Table 10-3

Effect of insulin administration on glucose tolerance in normal, sham operated and vagotomized pigeons (Mean ± S.E.M)
(Glucose - Mg/100 ml)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago-tomized	72 hr Sham operated	72 hr Vago-tomized
0	228.29 ± 2.29	212.4 ± 5.32	208.96 ± 0.36	181.38 ± 3.07	173.54 ± 7.04
30	135.42 **** ± 4.45	177.60 **** ± 3.15	166.17 **** ± 6.34	167.36 *** ± 7.56	140.06 *** ± 1.59
60	137.74 **** ± 8.03	151.5 **** ± 2.42	140.58 **** ± 4.07	146.60 **** ± 1.45	118.54 **** ± 1.84
90	121.33 **** ± 7.85	147.2 **** ± 5.86	131.81 **** ± 3.22	141.00 **** ± 2.92	114.09 **** ± 3.08
120	126.97 **** ± 7.52	156.49 **** ± 3.25	159.03 **** ± 2.31	151.44 **** ± 4.12	134.36 **** ± 3.65

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$.

Table 10-4

Effect of glucagon administration on glucose tolerance in normal, sham operated and vagotomized pigeons (Mean ± S. E. M)
(Glucose - Mg/100 ml blood)

Intervals in Minutes	Normal	48 hr Sham operated	48 hr Vago-tomized	72 hr Sham operated	72 hr Vago-tomized
0	225.03 ± 4.43	219.17 ± 5.52	210.87 ± 0.63	188.58 ± 7.38	197.03 ± 1.53
30	235.07 NS ± 13.64	236.67 NS ± 9.14	240.68*** ± 6.47	223.75 NS ± 8.01	209.6*** ± 3.06
60	309.33*** ± 6.16	302.17*** ± 12.38	254.29**** ± 6.51	252.25 NS ± 32.72	214.6**** ± 2.56
90	319.92**** ± 3.62	294.33*** ± 9.87	184.32**** ± 5.01	227.14 NS ± 9.18	210.4*** ± 3.78
120	295.45**** ± 2.38	261.67** ± 9.80	149.93**** ± 9.68	218.33 NS ± 7.49	197.95 NS ± 0.89

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$.

Table 10-5

Effect of dexamethasone administration on glucose tolerance in normal, sham operated and vagotomized pigeons (Mean ± S.E.M) (Glucose - Mg/100 ml blood)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago-tomized	72 hr Sham operated	72 hr Vago-tomized
0	235.99 ± 1.76	225.40 ± 0.71	206.20 ± 1.36	177.93 ± 2.73	190.084 ± 6.41
30	249.75 NS ± 6.76	246.48**** ± 3.898	238.75*** ± 8.68	196.62* ± 5.895	224.68 NS ± 13.52
60	252.67**** ± 0.43	269.65*** ± 10.18	245.52**** ± 5.22	217.38**** ± 3.12	225.65* ± 10.81
90	226.92* ± 2.96	253.94* ± 17.24	244.4**** ± 3.85	213.68*** ± 7.12	212.74 NS ± 13.595
120	222.34**** ± 0.96	239.68**** ± 0.798	226.00* ± 6.78	207.22 NS ± 4.99	209.15 NS ± 3.97

NS - Not significant, * $P<0.05$, ** $P<0.02$, *** $P<0.01$, **** $P<0.001$.

significant increase of blood sugar level right from the 30 min interval onwards.

Choline chloride injection (Table 10-2;Fig.10-2)

The injection of choline chloride into normal over night starved pigeons caused no significant variation in the glucose level except at 90 min. whence a slight increase was observed. Sham operated 48 hr. starved pigeons showed an initial significant increase (at 30 and 60 min) and then the glucose level decreased to a below normal level. In vagotomized 48 hr. starved pigeons, choline chloride caused a highly significant increase in glucose level at all intervals. In sham operated 72 hr. starved pigeons, choline chloride caused a considerable decrease right from 30 min. onwards, the level tapering off to a very low level by 120 min. In vagotomized 72 hr. starved pigeons, the glucose level showed an increase at 30, 60 and 90 min. after the injection of choline chloride but by 120 min. the level decreased to the pre-injection level.

Insulin injection (Table 10-3;Fig.10-3)

Insulin exhibited hypoglycaemic action in all groups of pigeons, whether sham operated or vagotomized. The decrease of glucose level in the blood was significant at all intervals in all groups.

Glucagon injection (Table 10-4; Fig.10-4)

In normal over night starved pigeons, glucagon injection produced hyperglycaemia from 60 min. onwards. This action lasted even at 120 min. In sham operated 48 hr. starved pigeons, glucose level showed no significant variation upon glucagon injection. In vagotomized 48 hr. pigeons, glucagon administration produced an increase in glucose level at 30 and 60 min. but thereafter the level fell drastically below pre-injection level. Sham operated 72 hr. pigeons showed no significant variation at any interval after glucagon administration. In vagotomized 72 hr. pigeons, glucagon injection produced an increase in the glycaemic level by 60 min. and the pre-injection level was attained only at 120 min.

Dexamethasone injection-(DXM) (Table 10-5; Fig.10-5)

This synthetic corticosterone in normal pigeons produced an increase in the glucose level by 60 min., but caused a decrease at the successive intervals. In sham operated 48 hr. pigeons, DXM increased the glycaemic level significantly at all intervals. Vagotomized 48 hr. pigeons responded to DXM very similarly as the sham operated 72 hr. pigeons with similar increase in the glycaemic level at all intervals. Although vagotomized 72 hr. pigeons too showed more or less similar response to DXM, the increase was significant at 60 min. only.

DISCUSSION

The most significant observation was that, ACh when injected into vagotomized pigeons increased the glucose level significantly and this increase lasted even at 120 min. Thus in vagotomized pigeons ACh acts as hyperglycaemic agent. This effect was more or less same in 48 hr. or 72 hr. vagotomized pigeons. In normal (over-night starved) pigeons ACh produced little or no effect. But in sham-operated 48 hr. starved pigeons the ACh injection produced a hypoglycaemic response only at 90 and 120 min., while in sham operated 72 hr. starved pigeons ACh produced virtually no effect. In other words, when vagal innervation is intact, addition of ACh produced a change in the glycaemic level in the pigeons only after 60 min. of injection. This response again depends on the duration of starvation; 72 hr. starved pigeons showing hardly any response to ACh injection. The data show that the action of extraneous ACh when vagus was intact was different from that when vagus was absent. Pigeons with intact innervation tended to show a slight hypoglycaemia in response to ACh administration only in 48 hr. starved group but not in over-night starved or 72 hr. starved groups, indicating that other factors (such as glucagon, catecholamine or corticosteroids) must be counteracting the action of ACh. The hyperglycaemic action of ACh in vagotomized pigeons could be due to the increased release of glucagon and/or catecholamines, due to increased sympathetic tone, or due to starvation. It could be also due to the prevailing inhibition of insulin secretion from the B cells of islets because of acute starvation. Probably the direct action of ACh on the liver cells

to take up more glucose was also not possible due to the increased sympathetic tone. The other disturbance in the vagotomized pigeons was the absence of afferent input from liver to hypothalamus (Lateral Hypothalamic Area-LHA) which controls the parasympathetic system. In the absence of such afferent inputs from the liver the parasympathetic activated insulin secretion also would not take place. Overall effect of ACh administration in vagotomized pigeons, essentially could be, the production of hyperglycaemia as afferent inputs from liver, hypothalamic control, parasympathetic influence over liver function and insulin secretion are all disturbed due to vagotomy; and addition of ACh only resulted in stimulating further the sympathetic tone. The absence of vagus and starvation might be causing a high sympathetic tone as well as higher plasma levels of glucagon, catecholamines and corticosterone.

With choline chloride injection, the glycaemic responses shown by over night starved or vagotomized pigeons were same as with ACh. However, in sham operated pigeons, the choline chloride produced significant hypoglycaemia by 90 min. (in 48 hr. starved) and by 30 min. (in 72 hr. starved). Although ACh produced such hypoglycaemia at 90 min. in sham operated 48 hr. starved pigeons, the action of choline chloride, in this respect was more potent than ACh. Beyner and Geelen (1982) have clearly shown that choline chloride induced more glycogen deposition than ACh. They incidentally, suggested that choline chloride must be the physiological agent as ACh gets degraded quickly by AChE into choline chloride and acetate.

Insulin injection in the normal (over night starved) and sham operated 48 and 72 hr. starved pigeons produced as expected a hypoglycaemic response. The glycaemic level, however showed a tendency to return to pre-injection level by 120 min. The vagotomized pigeons' response to insulin was the same as over night starved or sham operated ones thereby indicating that vagal nerves are not involved in the recovery from insulin induced hypoglycaemia. A similar observation was also made by Dolnikoff et al. (1983) in vagotomized dogs. They have also observed that recovery from insulin induced hypoglycaemia did not occur in central nervous system inactivated dogs or in dogs subjected to concurrent transection of spinal cord between C_4 and C_5 and bilateral vagotomy, while, either spinal transection or severance of vagi alone did not prevent full recovery from hypoglycaemia.

Most interesting result was obtained when glucagon was injected into vagotomized pigeons. The normal (over night starved) and sham operated pigeons responded to glucagon injections as predicted, with hyperglycaemia which even lasted beyond 120 min. and maximum hyperglycaemia was observed by 60 min. However, in vagotomized pigeons (both 48 and 72 hr.) the recovery from hyperglycaemia occured by 90 min. Pigeons with intact nervous elements showed no apparent hurry in bringing normoglycaemic level while in vagotomized pigeons the recovery was quick. In view of the fact that recovery from insulin induced hypoglycaemia and glucagon induced hyperglycaemia did happen in vagotomized pigeons,

it could be reasoned that sympathetic modulation by hypothalamic centres is the main mechanism in birds (or at least in pigeons) that regulates blood sugar level which could take place even in the absence of vagus. When blood sugar level increases sympathetic activity (tone) is down regulated by the hypothalamic centers and when hyperglycemia occurs the sympathetic fibres are activated by the hypothalamic centers.

Synthetic corticosterone (DXM) administration produced a milder hyperglycaemia in normal (overnight starved), sham operated and vagotomized pigeons. Normoglycaemia was, however, established by 120 min. after DXM injection. Since, responses were similar in all groups of pigeons, it could be concluded that vagal fibres are not involved in the modulation of glucocorticoid actions on the liver.