

CHAPTER 11

THE EFFECT OF CHOLINE CHLORIDE ADMINISTRATION ON THE GLUCOSE HOMEOSTASIS (CONTROL OF GLYCAEMIA) IN VAGOTOMIZED PIGEONS

The action of acetylcholine (ACh) on glucose uptake and glycogen deposition by the liver in birds and mammals is elucidated by the work of Ottolenghi *et al.* (1971), Akpan *et al.* (1974), Pilo and Patel (1978) ^{and} Beyner and Geelen (1982). Vagal stimulation also increased the rate of glycogen synthesis in the liver (Shimazu and Fujimoto, 1971). Vagotomy on the other hand decreased hepatic glycogen deposition (Mondon and Burton, 1971). Action of vagal nerve on glucose uptake and glycogen deposition is mostly mediated by acetylcholine, although neuropeptides such as VIP may also be involved in effecting parasympathetic influence as suggested by Shimazu (1983). Vagal nerve stimulation not only increased glycogen synthesis in the liver but also reduced glucose output, probably by counteracting the sympathetic action. Acetylcholine has specific action on the liver cell membrane whereby ionic permeability is increased resulting in the activation of sodium pump, extrusion of sodium, influx of K^+ , ATP utilization, lowering of cAMP and ultimately the entry of glucose into the cell (See Pilo and Patel, 1979). Since AChE is localized on the liver sinusoidal linings, ACh secreted by cholinergic nerve endings is quickly degraded into acetate and choline chloride. In view of this fact, Beyner and Geelen (1981) suggested

that choline chloride may be the factor that is effecting glucose uptake and glycogenesis. In their in vitro experiments with isolated hepatocytes, choline induced maximum glycogen deposition. Since choline chloride injection was found to be more potent in altering glycemic levels in pigeon (Chapter 10), it was thought worthwhile to study the effect of choline chloride injection alone or with glucose on the glycemic level as well as on the hepatic biochemical profile.

MATERIALS AND METHODS

Adult pigeons were divided into 3 groups. Each group was divided into 5 sub-groups as follows.

- (a) Normal overnight starved
- (b) Sham operated - 48 hr. starved
- (c) Vagotomized - 48 hr. starved
- (d) Sham operated - 72 hr. starved
- (e) Vagotomized - 72 hr. starved

Vagotomy was performed as described in Chapter 1. Sham operation was carried out under identical conditions.

Each group consisting of such 5 sub-groups was subjected to following intravenous injections.

- Group 1 - Glucose (70 mg/100 gm body weight)
- Group 2 - Choline chloride (15 mg/animal)
- Group 3 - Glucose + Choline chloride (70 mg/100 gm body weight + 15 mg/animal)

The birds were sacrificed at regular intervals of 0, 30, 60, 90 and 120 min. after intravenous administration of glucose, choline chloride or glucose + ^{choline}chloride. Blood samples were taken by cardiac puncture for blood sugar estimation. The liver was quickly removed and processed for estimations of glycogen, glycogen-synthetase and acid phosphatase. The methods followed for these estimations are described in Chapter 1.

RESULTS

GLUCOSE TOLERANCE TEST (Tables.11-1, 11-2 and 11-3;Fig.11-1)

Glucose alone (Table 11-1; Fig.11-1)

In the normal overnight starved pigeons, glucose injection produced a peak plasma glucose level at 30 minutes, and by 90 minutes normoglycaemia was reestablished. In sham operated (48 hr.) pigeons a more or less similar glucose tolerance was observed. Vagotomized (48 hr.) pigeons showed a hyperglycaemic response that continued to increase upto 90 min. Sham operated 72 hr. pigeons showed a sharp ^{glucose} peak at 30 minutes which then decreased gradually. The vagotomized 72 hr. pigeons also showed a steep rise of glucose at 30 minutes and this high level remained so even by 120 minutes.

Choline chloride alone - (Table.11-2;Fig.11-1)

In normal (overnight starved) pigeons, choline chloride injection produced a gradual increase of glucose level upto 90 minutes. In sham operated (48 hr.) pigeons the increase lasted

FIG.11-1. EFFECT OF GLUCOSE, CHOLINE CHLORIDE OR GLUCOSE + CHOLINE CHLORIDE ADMINISTRATION ON GLUCOSE TOLERANCE IN NORMAL, SHAM OPERATED (SHAM) OR VAGOTOMIZED (VGX) PIGEONS.

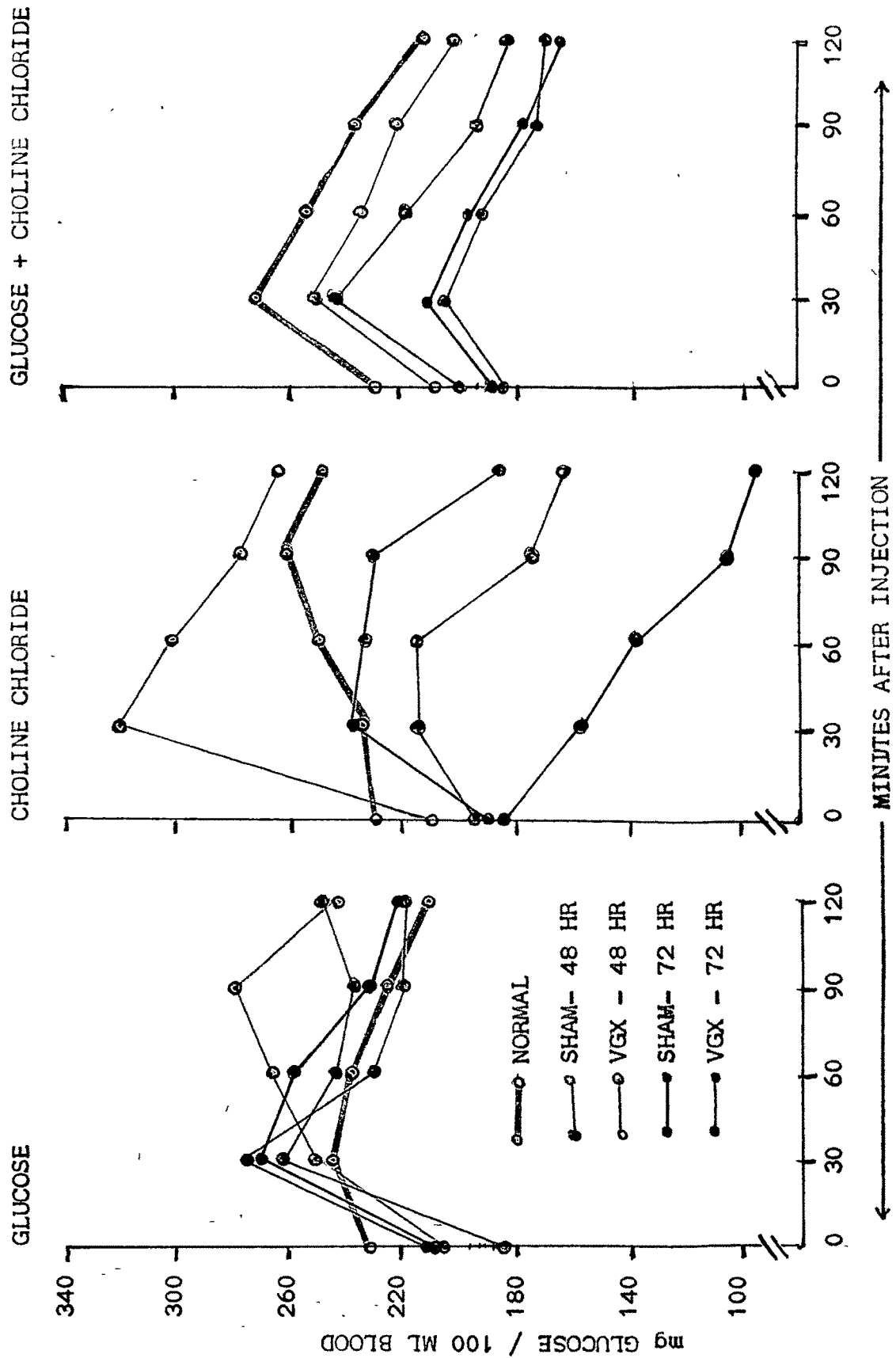


FIG. 11-2. EFFECT OF GLUCOSE, CHOLINE CHLORIDE OR GLUCOSE + CHOLINE CHLORIDE ADMINISTRATION ON GLYCOGEN CONTENT OF THE LIVER OF NORMAL, SHAM OPERATED (SHAM) OR VAGOTOMIZED (VGX) PIGEONS.

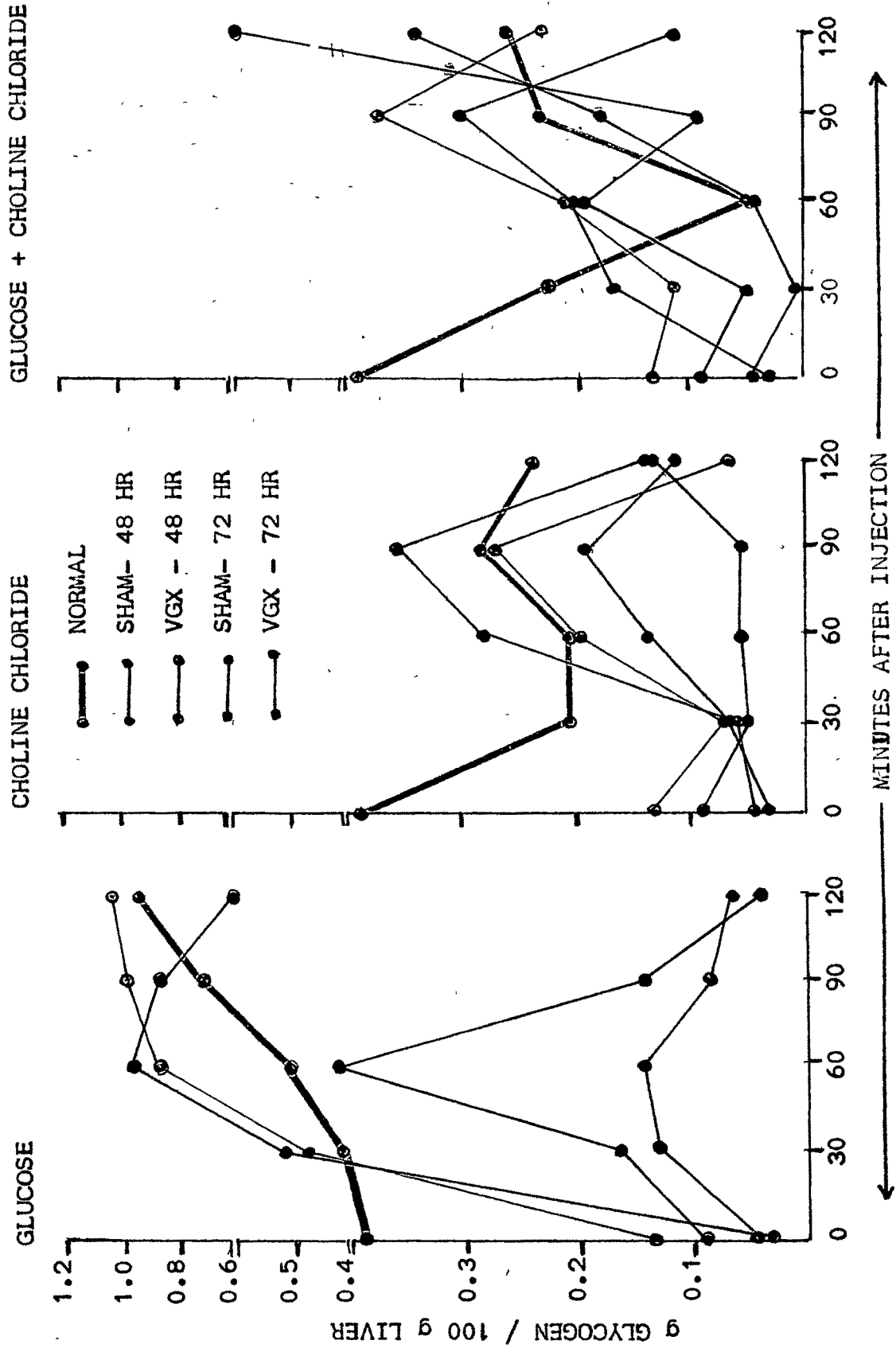


FIG. 11-3. EFFECT OF GLUCOSE, CHOLINE CHLORIDE OR GLUCOSE + CHOLINE CHLORIDE ADMINISTRATION ON GLYCOGEN SYNTHETASE ACTIVITY IN THE LIVER OF NORMAL, SHAM OPERATED (SHAM) OR VAGOTOMIZED (VGX) PIGEON.

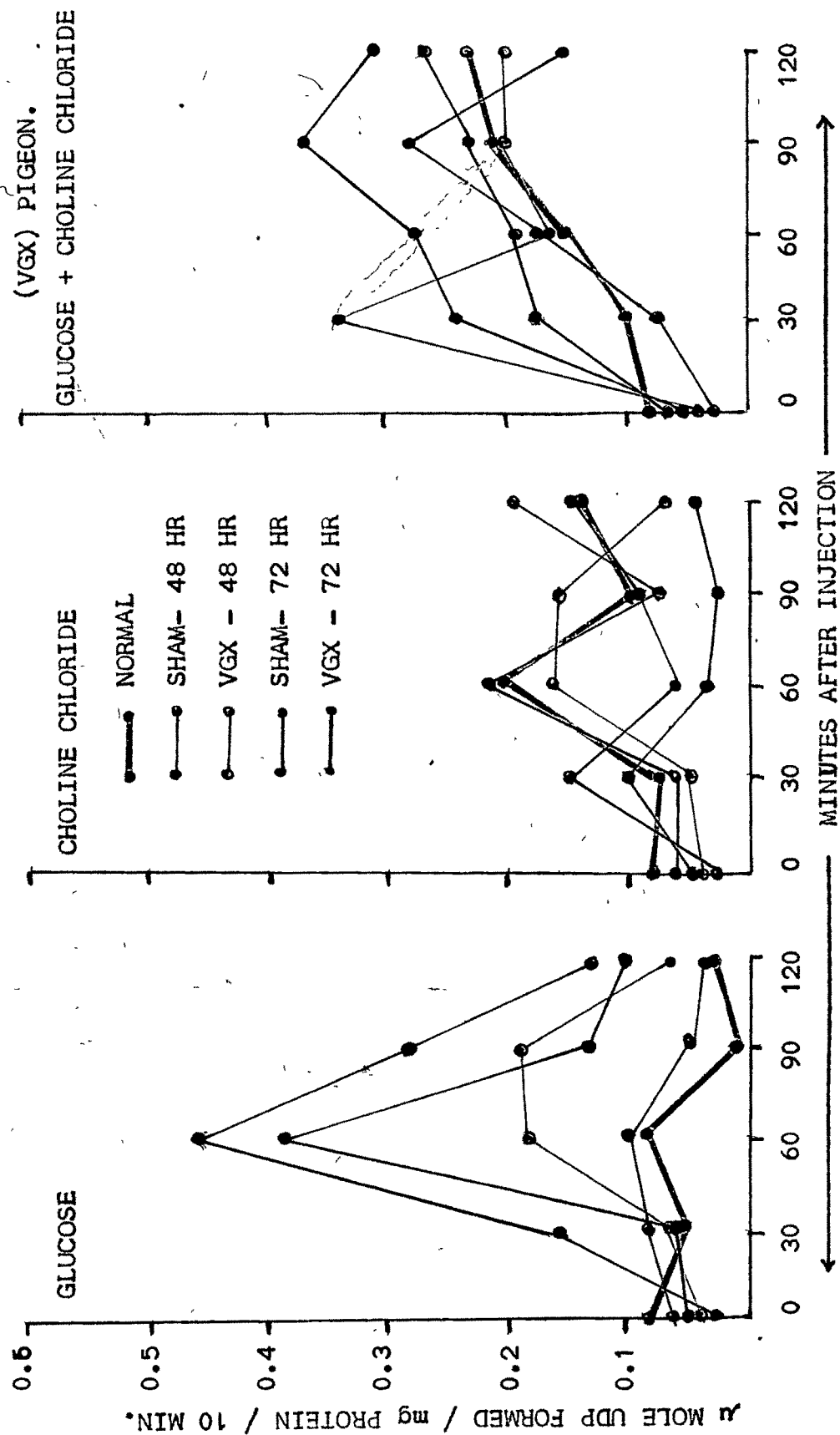


FIG. 11-4. EFFECT OF GLUCOSE, CHOLINE CHLORIDE OR GLUCOSE + CHOLINE CHLORIDE ADMINISTRATION ON ACID PHOSPHATASE ACTIVITY IN THE LIVER OF NORMAL, SHAM OPERATED (SHAM) OR VAGOTOMIZED (VGX) PIGEONS.

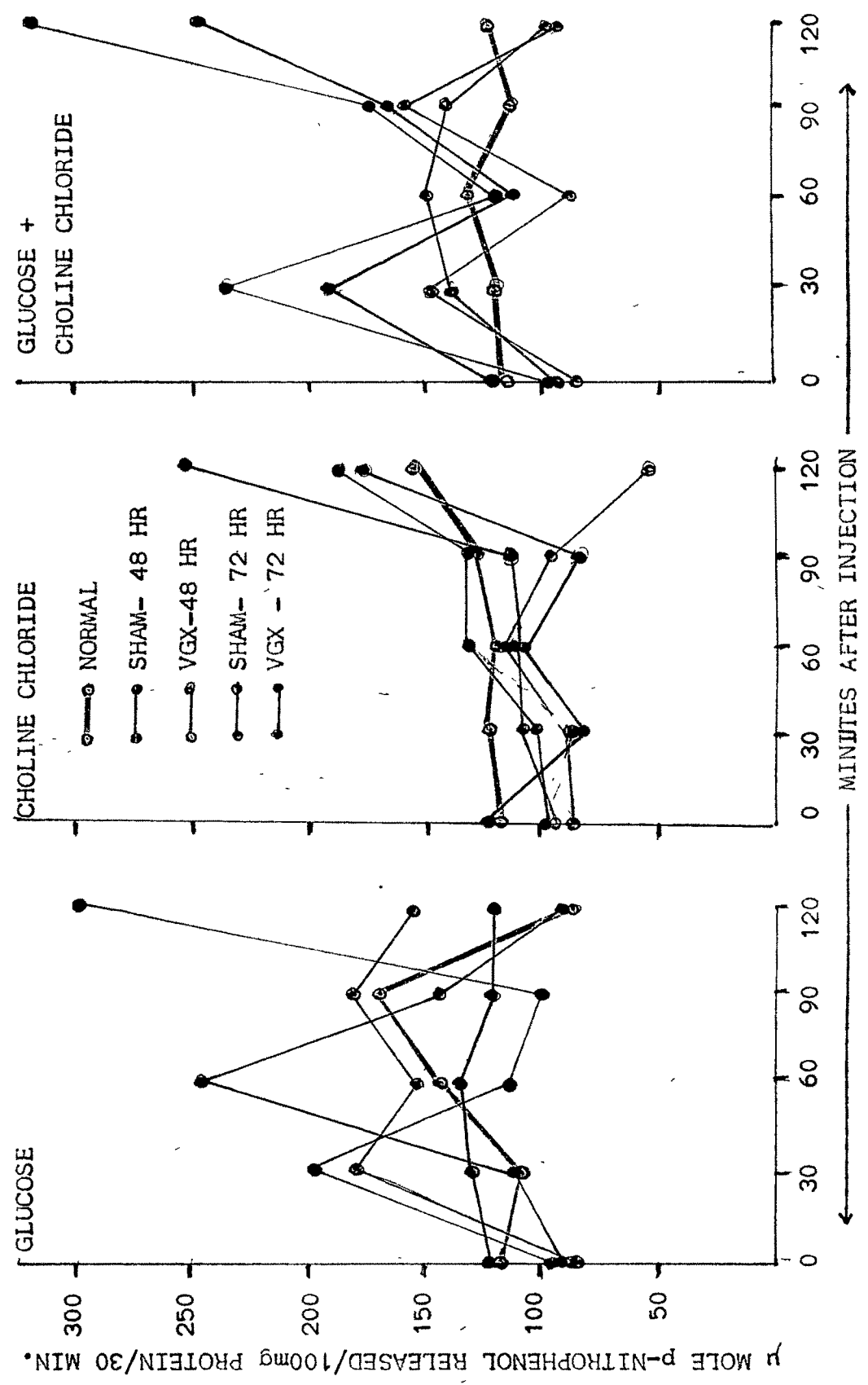


Table 11-1

Effect of glucose administration on the glucose tolerance in normal, sham operated and vagotomized pigeons (Mean $\pm$ 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	233.26 $\pm$ 1.56	210.95 $\pm$ 1.761	211.6 $\pm$ 10.41	206.83 $\pm$ 5.67	185.71 $\pm$ 0.68
30	245.95 $\pm$ 2.59	276.2 $\pm$ 2.21	253.51 $\pm$ 2.98	273.06 $\pm$ 3.53	265.77 $\pm$ 3.14
60	239.71 $\pm$ 3.41	230.42 $\pm$ 3.06	266.4 $\pm$ 2.62	259.21 $\pm$ 7.95	243.0 $\pm$ 5.61
90	226.69 $\pm$ 2.21	221.22 $\pm$ 4.07	279.73 $\pm$ 5.13	233.27 $\pm$ 2.0	236.04 $\pm$ 3.5
120	212.05 $\pm$ 1.47	207.09 $\pm$ 3.21	242.52 $\pm$ 4.25	221.266 $\pm$ 3.11	246.95 $\pm$ 6.787

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

Table 11-2

Effect of choline chloride administration on glucose tolerance in normal, sham operated and vagotomized pigeons (Mean $\pm$ 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	239.32 $\pm$ 1.83	195.45 $\pm$ 2.81	212.73 $\pm$ 7.41	184.42 $\pm$ 0.26	191.45 $\pm$ 2.46
30	234.65 NS $\pm$ 9.79	217.68 $\pm$ 4.49	320.44 $\pm$ 6.28	158.44 $\pm$ 9.0	257.81 $\pm$ 9.92
60	249.89 NS $\pm$ 5.97	216.36 $\pm$ 16.00	303.40 $\pm$ 2.63	137.37 $\pm$ 1.22	243.52 $\pm$ 8.14
90	259.05 $\pm$ 7.87	175.41 $\pm$ 6.09	279.29 $\pm$ 5.38	106.20 $\pm$ 4.37	240.35 $\pm$ 4.78
120	251.33 NS $\pm$ 5.21	166.89 $\pm$ 3.76	265.66 $\pm$ 1.72	93.77 $\pm$ 6.12	188.76 NS $\pm$ 0.896

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

Table 11-3

Effect of glucose + Choline chloride administration on glucose tolerance in normal, sham operated and vagotomized pigeons

(Means $\pm$ 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	232.13 $\pm$ 2.18	201.8 $\pm$ 4.77	209.27 $\pm$ 2.06	189.01 $\pm$ 2.81	184.2 $\pm$ 3.5
30	273.23*** $\pm$ 11.096	246.5*** $\pm$ 9.35	250.3*** $\pm$ 4.32	211.81*** $\pm$ 5.37	211.51*** $\pm$ 4.77
60	253.78*** $\pm$ 4.41	220.1* $\pm$ 5.29	235.05*** $\pm$ 3.3	197.4*** $\pm$ 1.2	193.43 NS $\pm$ 4.73
90	238.74 NS $\pm$ 6.03	195.4 $\pm$ 1.43	223.4** $\pm$ 3.39	178.4 NS $\pm$ 2.42	173.9 NS $\pm$ 2.67
120	213.8*** $\pm$ 1.53	183.95* $\pm$ 2.87	202.05 NS $\pm$ 2.54	167.6 NS $\pm$ 2.29	169.3*** $\pm$ 2.426

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

Table 11-4

Effect of glucose administration on glycogen content of the liver of normal, sham operated and vagotomized pigeons (Mean $\pm$ S.E.M.) Values are expressed as mg glycogen/ $\approx$ 100 mg tissue.

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	0.39943 $\pm$ 0.0711	0.0482 $\pm$ 0.00592	0.1270 $\pm$ 0.0083	0.092 $\pm$ 0.04	0.0349 $\pm$ 0.00123
30	0.1129 $\pm$ 0.029	0.1129 $\pm$ 0.029	0.0474 $\pm$ 0.0047 NS	0.161 NS $\pm$ 0.011	0.524 NS $\pm$ 0.052
60	0.519 NS $\pm$ 0.035	0.141 NS $\pm$ 0.029	0.838 NS $\pm$ 0.08	0.428 ** $\pm$ 0.05	0.946 *** $\pm$ 0.18
90	0.724 NS $\pm$ 0.01	0.088 NS $\pm$ 0.016	0.975 NS $\pm$ 0.027	0.139 NS $\pm$ 0.027	0.863 ** $\pm$ 0.09
120	0.971 NS $\pm$ 0.025	0.054 NS $\pm$ 0.021	1.027 NS $\pm$ 0.248	0.04 NS $\pm$ 0.002	0.593 * $\pm$ 0.06

NS - Note significant, * P 0.05, ** P 0.02, *** P 0.01, **** P 0.001.

Table 11-5

Effect of choline chloride administration on glycogen content of the liver of normal, sham operated and vagotomized pigeons (Mean $\pm$ S.E.M)

(Values are expressed as Mg glycogen/100 mg tissue)					
Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	0.39943 $\pm$ 0.0711	0.0482 $\pm$ 0.5822	0.127 $\pm$ 0.1033	0.092 $\pm$ 0.04	0.0349 $\pm$ 0.0948
30	0.206 $\pm$ 0.029	0.057 $\pm$ 0.004	0.056 $\pm$ 0.011	0.053 $\pm$ 0.008	0.053 $\pm$ 0.0069
60	0.202 $\pm$ 0.031	0.277 $\pm$ 0.024	0.202 $\pm$ 0.002	0.057 $\pm$ 0.008	0.135 $\pm$ 0.009
90	0.283 $\pm$ 0.034	0.352 $\pm$ 0.094	0.266 $\pm$ 0.033	0.053 $\pm$ 0.008	0.190 $\pm$ 0.009
120	0.234 $\pm$ 0.02	0.111 $\pm$ 0.006	0.063 $\pm$ 0.009	0.130 $\pm$ 0.010	0.117 $\pm$ 0.015

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

Table 11-6

Effect of glucose + choline chloride administration on glycogen content of the liver of normal, sham operated and vagotomized pigeons (Mean $\pm$ S.E.M)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	0.39943 ± 0.0711	0.0482 ± 0.005822	0.1270 ± 0.1033	0.092 ± 0.04	0.0349 ± 0.00948
30	0.218 NS ± 0.037	0.008 NS ± 0.002	0.108 NS ± 0.03	0.053 NS ± 0.004	0.164 NS ± 0.014
60	0.041 NS ± 0.008	0.045 NS ± 0.004	0.205 NS ± 0.085	0.197 NS ± 0.055	0.198 NS ± 0.03
90	0.226 NS ± 0.011	0.177 NS ± 0.024	0.373 NS ± 0.10	0.087 NS ± 0.008	0.301 NS ± 0.015
120	0.257 NS ± 0.025	0.341 NS ± 0.014	0.221 NS ± 0.042	0.601 NS ± 0.033	0.1262 NS ± 0.04

NS - Not significant

Table 11-7

Effect of glucose administration on glycogen synthetase activity in the liver of normal, sham operated and vagotomized pigeons (Mean $\pm$ S.E.M).

(Values are expressed as μ mole UDP formed/mg protein/10 minutes)

Interval in Minutes	Normal	48 hr Sham operated	48hr Vago-tomized	72 hr Sham operated	72 hr Vago-tomized
0	0.0782 $\pm$ 0.0456	0.0632 $\pm$ 0.0523	0.0362 $\pm$ 0.0093	0.0483 $\pm$ 0.02	0.0258 $\pm$ 0.0129
30	0.0497 NS $\pm$ 0.005	0.079 NS $\pm$ 0.007	0.059 NS $\pm$ 0.019	0.0654 NS $\pm$ 0.011	0.155* $\pm$ 0.009
60	0.079 NS $\pm$ 0.013	0.099* $\pm$ 0.014	0.181** $\pm$ 0.011	0.387*** $\pm$ 0.02	0.464*** $\pm$ 0.034
90	0.018* $\pm$ 0.003	0.049 NS $\pm$ 0.004	0.187** $\pm$ 0.003	0.134* $\pm$ 0.018	0.282* $\pm$ 0.045
120	0.028 NS $\pm$ 0.005	0.033 NS $\pm$ 0.009	0.056 NS $\pm$ 0.005	0.104* $\pm$ 0.003	0.128* $\pm$ 0.003

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

Table 11-8

Effect of choline chloride administration on glycogen synthetase activity in the liver of normal, sham operated and vagotomized pigeons (Mean $\pm$ S.E.M)

(Values are expressed as μ Mole UDP formed/mg protein/10 minutes)

Interval in Minutes	Normal	48 hr sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	0.0782 $\pm$ 0.0456	0.0632 $\pm$ 0.0523	0.0362 $\pm$ 0.0093	0.0483 $\pm$ 0.02	0.0258 $\pm$ 0.0129
30	0.069 NS $\pm$ 0.009	0.066 NS $\pm$ 0.023	0.043 NS $\pm$ 0.006	0.103* $\pm$ 0.006	0.149* $\pm$ 0.041
60	0.211** $\pm$ 0.031	0.231*** $\pm$ 0.013	0.161* $\pm$ 0.012	0.032 NS $\pm$ 0.002	0.061 NS $\pm$ 0.005
90	0.094 NS $\pm$ 0.005	0.073 NS $\pm$ 0.01	0.159* $\pm$ 0.016	0.025 NS $\pm$ 0.004	0.092 NS $\pm$ 0.004
120	0.144 NS $\pm$ 0.004	0.195 NS $\pm$ 0.043	0.068 NS $\pm$ 0.009	0.042 NS $\pm$ 0.001	0.147* $\pm$ 0.027

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

Table 11-9

Effect of glucose + Choline chloride administration on glycogen synthetase activity in the liver of normal, sham operated and vagotomized pigeons (Mean $\pm$ S.E.M)
(Values are expressed as μ mole UDP formed/mg protein/10 minutes)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	0.1782 $\pm$ 0.0456	0.0632 $\pm$.0523	0.0362 $\pm$.0093	0.0453 $\pm$.02	0.0258 $\pm$.0129
30	0.108 NS $\pm$.003	0.174 ** $\pm$.004	0.329 *** $\pm$ 0.194	0.243 ** $\pm$.064	0.073 NS $\pm$ 0.012
60	0.174 NS $\pm$ 0.008	0.190 ** $\pm$ 0.004	0.159 ** $\pm$.012	0.277 ** $\pm$.04	0.172 ** $\pm$ 0.005
90	0.219 NS $\pm$ 0.012	0.228 *** $\pm$ 0.012	0.207 ** $\pm$.048	0.3693 *** $\pm$ 0.069	0.281 *** $\pm$ 0.033
120	0.232 ** $\pm$ 0.04	0.267 *** $\pm$ 0.019	0.196 ** $\pm$.015	0.317 *** $\pm$ 0.017	0.154 * $\pm$ 0.012

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

Table 11-10

Effect of glucose administration on acid phosphatase activity in the liver of normal, sham operated and vagotomized pigeons.
(Values are expressed as μ mole P-nitrophenol released/mg protein/30 minutes. Mean $\pm$ S.E.M.)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	115.7 $\pm$ 4.96	94.5 $\pm$ 4.56	88.3 $\pm$ 4.87	91.25 $\pm$ 3.26	119.8 $\pm$ 4.25
30	108.66 NS $\pm$ 4.92	111.41 NS $\pm$ 5.93	181.76** $\pm$ 30.99	198.15* $\pm$ 34.399	128.34 NS $\pm$ 2.295
60	140.71* $\pm$ 9.68	248.37*** $\pm$ 142.93	152.45** $\pm$ 5.72	112.39 NS $\pm$ 15.58	134.795 NS $\pm$ 1.80
90	171.69*** $\pm$ 9.48	142.93* $\pm$ 9.22	180.13** $\pm$ 8.85	100.55 NS $\pm$ 6.77	117.58 NS $\pm$ 16.95
120	92.33 NS $\pm$ 4.87	93.08 NS $\pm$ 3.72	156.78 NS $\pm$ 4.197	300.41*** $\pm$ 17.5	117.51 NS $\pm$ 7.09

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

Table 11-11

Effect of choline chloride administration on acid phosphatase activity in the liver of normal, sham operated and vagotomized pigeons.

Values are expressed as μ mole p-nitrophenol released/mg protein/30 minutes.

Interval in Minutes	Normal	48 hr sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	115.7 + 4.96	94.5 + 0.456	88.3 + 0.487	91.25 + 3.26	119.8 + 4.25
30	122.87 NS + 9.3	108.67 NS + 12.16	87.45 NS + 4.06	107.77 NS + 6.65	84.32 NS + 9.68
60	120.51 NS + 8.88	119.49 NS + 12.33	115.89 NS + 17.24	133.12* + 14.77	112.31 NS + 3.74
90	126.87 NS + 7.57	119.53 NS + 4.79	98.63 NS + 2.02	131.18* + 11.05	84.83 NS + 4.45
120	155.03* + 2.74	255.34*** + 4.59	51.92 NS + 4.59	187.94** + 5.26	174.37** + 9.02

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

Table 11-12

Effect of glucose + Choline chloride administration on acid phosphatase activity in the liver of normal, sham operated and vagotomized pigeons.

Values are expressed as μ mole P-nitrophenol released/mg protein/30 minutes. Mean $\pm$ S.E.M)

Interval in Minutes	Normal	48 hr Sham operated	48 hr Vago- tomized	72 hr Sham operated	72 hr Vago- tomized
0	115.7 $\pm$ 4.96	94.5 $\pm$ 4.56	88.3 $\pm$ 4.87	91.25 $\pm$ 3.26	119.8 $\pm$ 4.25
30	121.94 NS $\pm$ 4.03	139.98* $\pm$ 11.75	149.20** $\pm$ 11.20	238.62*** $\pm$ 10.30	192.65*** $\pm$ 12.55
60	130.67 NS $\pm$ 7.90	150.72** $\pm$ 10.75	98.80 NS $\pm$ 4.42	117.74 NS $\pm$ 3.48	110.47 NS $\pm$ 1.80
90	113.47 NS $\pm$ 3.65	141.98* $\pm$ 2.51	158.40** $\pm$ 2.02	169.69** $\pm$ 11.18	164.43** $\pm$ 38.72
120	123.03 NS $\pm$ 8.2	98.65 NS $\pm$ 2.05	93.12 NS $\pm$ 3.69	320.20*** $\pm$ 10.24	247.53*** $\pm$ 4.25

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

upto 60 minutes only. In vagotomized 48 hr. pigeons the rise of glucose level was very sharp at 30 minutes and thereafter a gradual decrease was seen. In sham operated 72 hr. pigeons, the glucose level decreased gradually till 120 minutes. The vagotomized 72 hr. pigeons registered a peak by 30 min.

Glucose + Choline chloride - (Table 11-3; Fig. 11-1)

Interestingly all groups showed a more or less similar pattern. Glucose level showed a peak at 30 minutes and by 90 minutes all reached the pre-injection glycaemic level.

GLYCOGEN IN THE LIVER (Tables 11-4, 11-5 and 11-6; Fig. 11-2)

Glucose injection alone (Table 11-4; Fig. 11-2)

When glucose alone was injected, normal overnight starved pigeons showed a gradual increase in glycogen deposition, while sham operated (both 48 hr. and 72 hr.) pigeons showed an increase upto 60 minutes and then the glycogen content began to decrease. Vagotomized 48 hr. pigeons showed sharp increase at 60 min., and then gradual increase. Vagotomized 72 hr. ^{pigeons} showed a sharp increase till 60 min., and then the level began to fall.

Choline chloride injection - (Table 11-5; Fig. 11-2)

The response of all groups of pigeons to choline chloride was more or less similar. After an initial slight decrease, the glycogen content in all groups showed an increase upto 90 min.

and then decreased to the pre-injection level.

Glucose + Choline chloride injection (Table 11-6; Fig. 11-2)

The normal (overnight starved), sham operated (both 48 hr. and 72 hr.) and vagotomized (48 hr. and 72 hr.) pigeons showed an increase in ~~60~~ glycogen content in the liver by 90 minutes. However, in vagotomized pigeons (48 hr. and 72 hr.), the level dropped by 120 minutes whereas in the rest, the glycogen level continued to increase.

GLYCOGEN SYNTHETASE (Table 11-7, 11-8 and 11-9; Fig. 11-3)

Glucose Injection (Table 11-7; Fig. 11-3)

When glucose alone was injected, glycogen synthetase in the liver of normal (overnight starved) and sham operated (48 hr. and 72 hr.) and vagotomized (48 hr. and 72 hr.) pigeons showed an increase by 60 min. In vagotomized (48 hr.) pigeons the increase seen by 60 minutes remained so even at 90 minutes and then decreased to preinjection level. Both sham operated (72 hr.) and vagotomized (72 hr.) ^{pigeons} showed a sharp increase by 60 minutes and then an equally sharp decrease *thereafter*.

Choline Chloride Injection (Table 11-8; Fig. 11-3)

Both normal (overnight starved) and sham operated (48 hr.) pigeon liver exhibited an increased glycogen synthetase activity by 60 minutes following choline chloride injection. The response

Choline chloride (Table 11-11)

The normal (overnight starved) pigeon liver showed a significant increase only by 120 minutes. The sham operated 48 hour and 72 hour pigeon livers showed a similar but more pronounced increase by 120 minutes. Vagotomized 48 hour ^{Pigeon liver} showed a slight increase at 60 minutes but by 120 minutes the activity decreased below pre-injection level. On the other hand, vagotomized 72 hour pigeon liver showed an increase in acid phosphatase activity at 60 minutes as well as at 120 minutes.

Glucose + Choline chloride (Table 11-12)

The normal overnight starved pigeons showed no significant response in the hepatic acid phosphatase activity following combined glucose + choline chloride injection. Sham operated 48 hour pigeon liver showed a parabolic response peaking at 60 minutes. Vagotomized 48 hour pigeon liver showed two peaks, one at 30 minutes and the other at 90 minutes. 72 hour sham operated and vagotomized pigeons showed a more or less identical response, a sharp peak at 30 minutes and again a sharp increase at 120 minutes.

DISCUSSION

The response of overnight (48 hr. and 72 hr.) starved pigeons to glucose, choline chloride or glucose + choline chloride administration showed much variation. Vagotomy in certain cases aggravated the response and in certain cases eliminated it. Since

a very clear picture of response in all cases was not discernible due to individual variations, only general and rather apparent responses could be discussed here.

The response of vagotomized pigeons to glucose injection was a sustained hyperglycemia. This has also been reported by Verma (1982). The level of hyperglycemia was less in 72 hr. vagotomised pigeons. Choline chloride administration without glucose in vagotomised pigeons elevated Δ glucose level in the blood. The possible explanation that could be extended to this paradoxical response, is that choline chloride injection either activated sympathetic activity and/or effected a release of glucagon. In response to choline chloride injection the sham operated pigeons showed a gradual decrease in blood sugar level. The striking response of glucose + choline chloride administration was that all groups showed a very orderly and identical pattern of glucose tolerance. This fact clearly establishes the stimulatory effect of choline chloride either directly on the liver to take up glucose, or indirectly by inducing Δ insulin release from the β cells, when a glucose load was provided simultaneously. Thus, in the absence of hyperglycemic condition, Δ choline chloride either does not potentiate glucose uptake by liver cells or does activate a sympathetic mediated glucose release in vagotomised pigeons. In other words, the adverse effect of vagotomy (prolonged hyperglycemia following glucose loading) could be effectively prevented by a simultaneous choline chloride administration.

The beneficial effect of choline chloride injection was also seen in glycogen deposition. In glucose + choline chloride injected pigeons, glycogen deposition more or less coincided with decreasing glycemic level in the blood. Similarly, glucose, choline chloride or glucose + choline chloride injection brought about an increase in the glycogen synthetase and acid phosphatase activities, although temporal variations were encountered in different experimental groups. However, glucose + choline chloride administration induced a sharp increase in the activities of these enzymes, which are involved in glucose uptake and glycogen deposition much earlier thereby facilitating glucose removal from the blood.

As mentioned earlier, it is difficult to find explanation to the responses of most of the enzymes studied ~~here~~ due to high variations. Although some of the changes were apparent, they were found statistically non-significant. Unless the sample sizes are increased, this problem will remain.