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THE EFFECT OF INSULIN, GLUCAGON AND PROLACTIN ON LIPID SYNTHESIS AND RELATED METABOLIC ACTIVITY IN MIGRATORY AND NON-MIGRATORY FINCHES

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Abstract—1. The effects of insulin, glucagon and prolactin on lipid synthesis were investigated in house sparrows (*Passer domesticus*) and white-crowned sparrows (*Zonotrichia leucophrys gambelii*).

2. The incorporation of glucose-U-C^{14} and acetate-1-C^{14} into abdominal fat pad lipid was measured in *in vitro* and *in vivo* experiments.

3. None of these hormones accelerated lipid synthesis, even though lipogenesis is sensitive to changes in carbohydrate metabolism (i.e. glucose uptake) in this tissue.

4. Insulin, a potent stimulator of lipid synthesis in mammalian adipose tissue, had no effect on the metabolism of finch fat pads.

5. Glucagon and prolactin inhibited the synthesis of fatty acids from acetate. Only glucagon has the necessary potency to be of physiological significance.

6. It is hypothesized that a relatively high glucagon output inhibits lipid synthesis during non-migratory periods. During migratory periods the output of glucagon falls, allowing lipid synthesis to proceed at a more rapid rate.

INTRODUCTION

MIGRATORY birds manifest at least three different physiological responses under the influences of long spring daylengths: (1) gonadal recrudescence, (2) migratory behavior, and (3) lipid deposition. The first of these, gonadal recrudescence, is caused by increased secretion of gonadotrophic hormones by the anterior pituitary (for review, see Marshall, 1961). Migratory behavior and lipid deposition are independent of the gonadal response (Lofts & Marshall, 1961; Morton & Mewaldt, 1962) and of one another (King & Farner, 1963). The immediate physiological changes which lead to these latter responses are unknown.

Premigratory accumulation of fat occurs very rapidly, often within a period of 5–10 days, and may amount to 25 per cent or more of body weight (for details, see Farner, 1960; Odum, 1960; Wolfson, 1960). Two hypotheses have been proposed whereby long daylengths might cause increased lipid synthesis. First,

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hyperphagia might be induced by stimulation of a "hunger center" or inhibition of a "satiety center" in the hypothalamus (Brobeck, 1955; Soulairac, 1958). Food eaten in excess of daily requirements would be stored as fat and glycogen without a qualitative change in intermediary metabolism. Farner *et al.* (1961) have shown, however, that liver and muscle glycogen decrease during photostimulated lipid deposition. This observation implies a qualitative change in intermediary metabolism favoring lipogenesis. Therefore, an alternative hypothesis is that the photostimulated increase in the rate of lipid synthesis occurs as a result of a hormone-mediated change in intermediary metabolism. Increased utilization of substrate for lipid synthesis would stimulate the appetite center in the hypothalamus (Mayer, 1955) and hyperphagia would follow.

The purposes of this investigation were (1) to determine the effect of hormones on lipid synthesis in adipose tissue and liver of passerine birds and, if possible, to relate these effects to photostimulated lipid deposition, and (2) to compare the hormone effects observed in mammals (for reviews, see Langdon, 1960; Winegrad, 1962) to those observed in passerine birds.

Insulin, glucagon and prolactin were the principal hormones tested in this investigation. Insulin was thoroughly tested because it accelerates lipid synthesis in mammals (Langdon, 1960). Glucagon was tested because a preliminary experiment showed that it was a powerful inhibitor of lipid synthesis. Lipogenesis in the isolated rat epididymal fat pad also is inhibited by glucagon (Orth *et al.*, 1960). Prolactin was tested because it accelerates lipid synthesis in rat adipose tissue (Winegrad *et al.*, 1959) and because it causes fat deposition in white-crowned sparrows (Meier, personal communication).

MATERIALS AND METHODS

The house sparrow (*Passer domesticus*), a non-migratory bird, was chosen for these studies because of its year-round abundance. Data from four experiments on the white-crowned sparrow (*Zonotrichia leucophrys gambelii*) and one experiment on the slate-colored junco (*Junco hyemalis hyemalis*), both migratory species, have been used for comparative purposes. One experiment was performed on Sprague-Dawley rats (100–180 g) and two on Holtzman rats (350–400 g).

Care of animals

House sparrows were captured in the vicinity of Ann Arbor, Michigan. They were maintained in $2\times2\times2$ ft or $2\times4\times6$ ft flight cages on a 14 hr daily photoperiod (except natural photoperiod from 24 July to 2 September 1963*) at room temperature. The diet consisted of millet, cracked corn, chick-starter mash, grit, cuttlebone and mineralized salt, given *ad libitum*. Water, to which a small amount of ABDEC vitamins (Parke-Davis) had been added, was available at all times. A minimum of 5 days was allowed for the birds to adjust to the diet and photoperiod before using them for experiments. All rats were fed *ad libitum* on Ralston Purina rat chow.

* The daylength was 14·5 hr on 24 July and decreased to 13 hr by 13 September.

The white-crowned sparrows were captured near Pullman, Washington, and shipped by air express to Ann Arbor. The slate-colored juncos were captured near Ann Arbor. The white-crowned sparrows and juncos were caged and maintained as noted above for the house sparrows except that they were kept on natural photoperiods. The experiment with juncos was performed in June after these birds had attained maximum premigratory obesity and had begun to lose their fat. The experiments with white-crowned sparrows were performed in October. All but a few birds had attained peak fall premigratory fatness and were in a static phase of obesity.

In vitro *procedures*

After decapitation the abdominal fat pad (ventral bilobed strip of mesenteric adipose tissue which runs caudad from the stomach and pancreas to the cloaca where it splits into two lobes which pass dorso-anteriorly along the body walls for about 1 cm), or epididymal fat pad when rats were used, was removed and cut into two pieces. One piece served as a control, the other as experimental. Each piece was weighed on a torsion balance to an accuracy of ± 1 mg and placed in the incubation medium. Tissues were incubated for 3 hr at 40°C in Krebs Bicarbonate Buffer, pH 7·3–7·4 (Cohen, 1957), containing glucose at a concentration of 2 or 4 mg/ml. The gas phase was 95% O_2 and 5% CO_2. Acetate (sodium salt), when present, was at concentrations of 0·02 mM or 0·5 mM. Either glucose-U-C^{14} or acetate-1-C^{14} (Nuclear Chicago Corporation) was added to the medium in tracer amounts in order to measure the rate of lipid synthesis. Hormones were dissolved directly in the buffer or added to the reaction vessels in 0·05 ml of saline. Total volume in the reaction vessels was 2 or 3 ml if the hormone was dissolved directly in the buffer and 2·05 or 3·05 ml if the hormone was added to reaction vessels in saline solution. Equal volumes of solvent without hormone were used in the control vessels.

In one experiment bovine serum albumin at a concentration of 3 g/100 ml was added to the incubation medium to act as a free fatty acid acceptor (Reshef *et al.*, 1958). The albumin (Nutritional Biochemical Corporation, Cohn Fraction V) was dissolved in bicarbonate buffer and dialyzed against the same buffer without albumin for 24 hr at 7°C with two changes. Free fatty acids (FFA)* released into the medium during the incubation were extracted by the method of Dole (1956). Aliquots of the heptane extract were evaporated to dryness and counted and titrated as indicated below. Non-incubated medium, containing albumin, was extracted similarly and served as a zero-time control.

In vivo *procedures*

After a blood sample was taken from the leg vein, the birds were injected intramuscularly with 0·1 cm^3 of a hormone or control solution and intraperitoneally

* The following abbreviations are used in this report: CoA—coenzyme A; FFA—free fatty acid; glucose-U-C^{14}—glucose uniformly labelled with carbon^{14}; NADPH—reduced nicotinamide adenine diphosphonucleotide phosphate.

with about 2 μc of acetate-1-C^{14} (10–20 μmoles). Next, they were placed in darkened metabolism chambers for 2 hr. At the end of the incubation period a terminal blood sample was collected by decapitation. The abdominal fat pad and the liver were removed and analyzed as indicated below.

Hormone preparations

Insulin (Lilly, Iletin U-40), glucagon-free insulin (Lilly) and glucagon (Lilly, glucagon hydrochloride) were diluted with physiological saline before use. Ovine crystalline prolactin (Squibb, lot No. 53273–002R, 22·5 IU/mg) was dissolved directly in the buffer.

Extraction of fatty acids

Fat pads from the *in vitro* experiments were blotted carefully and placed in 10% alcoholic KOH. Fat pads from *in vivo* experiments were placed directly into the alcoholic KOH. After saponification was complete (e.g. 1 hr at 100°C), the mixture was acidified with 3 N HCl to a thymol blue endpoint and then extracted with 5, 10 or 20 ml of petroleum ether depending on the size of the tissue. The resultant extract of fatty acids was then washed with distilled water. One aliquot of this extract was dried on a planchet and the radioactivity counted (standard error: ± 2 per cent) in a Nuclear Chicago gas-flow geiger counter equipped with a "micromil" window. All samples were corrected for self-absorption (Broda, 1960). A second aliquot was evaporated to dryness in a 10 ml Erlenmeyer flask. The residue was dissolved in 95% ethanol and titrated with 0·02 N NaOH to a phenophthalein-thymol blue endpoint. Re-extraction of the acidified saponification mixture showed that 97 per cent or more of the fatty acids were removed during the first petroleum ether extraction.

Liver pieces from the *in vivo* experiments were homogenized in 1 : 1 chloroform–methanol and centrifuged. The supernatant was evaporated to dryness and saponified (1 hr at 100°C) with 10% alcoholic KOH. The alkaline saponified mixture was extracted once with a large volume (50 ml or more) of petroleum ether to remove the non-saponifiable lipids. This extract was discarded. The saponified mixture was then acidified with 3 N HCl to a thymol blue endpoint and extracted with 5 ml of petroleum ether. This extract, which contained the fatty acids, was washed once with distilled water. Aliquots of the extract were counted or titrated as noted previously.

A zero-time control was used to compensate for the slight contamination of the isolated fatty acids by glucose-U-C^{14}. A weighed piece of adipose tissue was rinsed in iced medium (containing all components including the radioactive label) and transferred to alcoholic KOH without blotting. Saponification and extraction were performed as indicated above. Counts in the fatty acid fraction isolated from this tissue were subtracted from those isolated from the incubated tissues. Using a similar technique contamination from acetate-1-C^{14} was found to be less than

0·1 per cent of the total counts recovered from the fatty acid fraction of the incubated tissues. It was disregarded in experiments employing acetate.

Glucose determinations

Glucose concentration was measured in aliquots of the incubated and unincubated medium in order to determine glucose uptake by the isolated tissues. The medium was diluted 1 : 100 with distilled water and glucose determinations performed with glucose oxidase (Teller, 1956) (Glucostat-Worthington Biochemical Corporation). For the *in vivo* experiments blood was collected from the leg vein into heparinized capillary tubes or over citrate after decapitation. The blood was deproteinized by Folin–Wu reagent (Folin & Wu, 1919), and glucose was determined on 0·5 or 1·0 ml. of the supernatant using the anthrone reagent (Morris, 1948).

Analysis of data

In experiments utilizing paired abdominal fat pads *in vitro* the data were tested for significance by the Wilcoxon Matched Pairs Test (Siegel, 1956). The *in vivo* data were tested for significance by the Mann–Whitney Test (Siegel, 1956).

RESULTS

Effect of insulin in vitro

Insulin has been tested for its effect on glucose uptake, fatty acid and triglyceride synthesis from C^{14}-labeled glucose, and fatty acid synthesis* from C^{14}-labeled acetate in isolated avian adipose tissue (Tables 1–11). Doses used varied from 1·7 mU to 0·5 U per ml of medium, encompassing and exceeding the range of effective doses for the rat epididymal fat pad (Doisy, 1963). The major results are as follows:

1. Insulin had no detectable effect upon fatty acid synthesis from labeled glucose in the abdominal fat pad ($p > 0{\cdot}05$) (Table 1).

2. The synthesis of triglyceride likewise is not affected ($p > 0{\cdot}05$) by insulin (Table 2). Since the synthesis of fatty acids is not influenced by insulin (Table 1), the lack of insulin effect on triglyceride synthesis indicates that the hormone does not affect glyceride-glycerol synthesis.

3. The effect of insulin on fatty acid synthesis from labeled glucose also was tested in furcular adipose tissue, a subcutaneous fat body located near the clavicle (Table 3). The incorporation of glucose carbon into fatty acids of both minced and intact furcular fat was equally unaffected by insulin ($p > 0{\cdot}05$).

The rate of incorporation of labeled glucose into fatty acids of the abdominal and furcular fat pads is very slow. The radioactivity isolated in the fatty acid fraction was considerably less than twice the background level. For this reason subsequent experiments were conducted using a small amount of acetate-1-C^{14} as the label and non-labeled glucose as substrate.

* The terms fatty acid synthesis and triglyceride synthesis as used in this paper mean the rate of incorporation of labeled glucose or labeled acetate into fatty acid or triglyceride.

4. Table 4 shows the effect of insulin on the incorporation of acetate into lipid. Insulin had no effect on fat pads taken from untreated birds ($p > 0{\cdot}05$) (Table 4(a)). The insensitivity of bird fat pads to insulin resembles the greatly diminished

TABLE 1—THE EFFECT OF INSULIN ON INCORPORATION* OF GLUCOSE CARBON INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

Insulin 0·3 U/ml		Exptl. as % control
With Insulin	Without Insulin	
78·9	130	61
107	84·5	127
68·0	60·9	112
49·6	43·6	114
246	310	79
26·5	23·1	115
10·3	5·8	178
	Mean	112
	S.E.	14

* mμmoles glucose C/100 mg wet wt./hr; glucose concentration 4 mg/ml.

TABLE 2—THE EFFECT OF INSULIN ON INCORPORATION* OF GLUCOSE CARBON INTO TRIGLYCERIDE IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

Insulin 0·5 U/ml		Exptl. as % control
With Insulin	Without Insulin	
437	698	63
538	114	472
523	162	323
68·3	8·0	854
1211	754	161
82·4	115	72
88·4	89·9	98
	Mean	291
	S.E.	109

* mμmoles glucose C/100 mg wet wt./hr; glucose concentration 2 mg/ml.

sensitivity to insulin of fat pads taken from diabetic rats (Winegrad & Renold, 1958). Insulin therapy restores the normal fat pad response to insulin *in vitro* in diabetic rats. However, fat pads from house sparrows pretreated with 0·1 U of insulin twice daily for 7 days were still insensitive to insulin ($p > 0{\cdot}05$) (Table

TABLE 3—THE EFFECT OF INSULIN ON INCORPORATION* OF GLUCOSE CARBON INTO LIPID IN ISOLATED FURCULAR ADIPOSE TISSUE FROM THE HOUSE SPARROW

(a) Minced tissue			(b) Intact tissue		
Insulin 1·7 mU/ml		Exptl. as % control	Insulin 30 mU/ml		Exptl. as % control
With Insulin	Without Insulin		With Insulin	Without Insulin	
241	150	161	290	352	82
82·8	73·8	112	189	143	132
60·5	62·5	97	276	265	104
105	95·7	110	44·4	125	36
81·2	59·2	137	152	61·4	248
75·4	63·7	118	86·7	84·7	102
29·1	45·5	64	82·7	107	77
	Mean	114		Mean	112
	S.E.	12		S.E.	25

* mμmoles glucose C/100 mg wet wt./hr; glucose concentration 2 mg/ml.

TABLE 4—THE EFFECT OF INSULIN ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

(a) Normal birds			(b) Insulin pretreated† birds		
Insulin 0·3 U/ml		Exptl. as % control	Insulin 0·5 U/ml		Exptl. as % control
With Insulin	Without Insulin		With Insulin	Without Insulin	
10·7	11·0	97	41·8	86·0	49
5·68	7·79	73	11·2	90·9	12
8·31	15·2	55	24·1	29·5	83
3·71	2·94	126	74·9	184	41
5·56	4·77	117	25·3	18·8	134
1·48	3·20	46	61·4	74·2	83
	Mean	86		Mean	67
	S.E.	13		S.E.	17

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM; glucose concentration 4 mg/ml.

† 0·1 U of insulin twice daily for 7 days.

4(b)). Glucagon-free insulin also had no effect on the incorporation of acetate into lipid ($p > 0{\cdot}05$) (Table 5).

5. Two experiments were designed to test the effect of insulin on glucose uptake in house sparrow abdominal fat pads. Insulin had no effect ($p > 0{\cdot}05$) when

TABLE 5—THE EFFECT OF GLUCAGON-FREE INSULIN ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

Insulin 0·5 U/ml		Exptl. as % control
With Insulin	Without Insulin	
17·3	11·5	150
5·61	5·70	98
23·2	12·2	190
3·25	2·18	149
11·6	5·00	232
3·42	5·76	59
1·51	1·61	94
	Mean	139
	S.E.	23

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM; glucose concentration 2 mg/ml.

TABLE 6—THE EFFECT OF INSULIN ON GLUCOSE UPTAKE* IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

(a) Insulin 0·5 U/ml†		Exptl. minus control	(b) Insulin 0·5 U/ml‡		Exptl. minus control
With Insulin	Without Insulin		With Insulin	Without Insulin	
+1·42	+1·23	+0·19	+2·64	−0·74	+3·38
+0·48	+0·46	+0·02	+0·42	+0·75	−0·33
+0·40	+0·42	−0·02	+2·01	+2·14	−0·13
+0·62	+1·04	−0·42	+0·67	+0·40	+0·27
+4·10	+2·11	+1·99	+5·82	−1·85	+7·67
+1·93	+1·41	+0·52	+1·60	+1·93	−0·33
+0·90	+0·90	0·0	−3·22	−1·39	+1·83
	Mean	+0·33		Mean	+1·77
	S.E.	0·30		S.W.	1·11

* μmoles glucose/100 mg wet wt./hr.
† Glucose concentration 2 mg/ml.
‡ Glucose concentration 4 mg/ml.

the concentration of glucose in the medium was 2 mg/ml (Table 6(a)) or when it was 4 mg/ml (Table 6(b)). Slices of smooth muscle from the house sparrow gizzard also were tested for a glucose uptake response to insulin. Table 7 shows that insulin caused a mean increase in glucose uptake of 0·22 μmoles/100 mg wet wt./hr in this tissue. This increase was significant ($p < 0·02$).

TABLE 7—THE EFFECT OF INSULIN ON GLUCOSE UPTAKE* IN GIZZARD SLICES FROM THE HOUSE SPARROW

Insulin 0·5 U/ml		Exptl. minus control
With Insulin	Without Insulin	
+1·13	+0·85	+0·28
+0·90	+0·69	+0·21
+0·92	+0·69	+0·23
+0·80	+0·62	+0·18
+0·86	+0·63	+0·23
+0·94	+0·77	+0·17
	Mean	+0·22
	S.E.	0·02

* μmoles glucose/100 mg wet wt./hr; glucose concentration 2 mg/ml.

TABLE 8—THE EFFECT* OF INSULIN ON GLUCOSE UPTAKE† AND INCORPORATION‡ OF GLUCOSE CARBON INTO LIPID IN THE ISOLATED RAT EPIDIDYMAL FAT PAD

(a) Glucose uptake			(b) Glucose incorporation		
Insulin 0·5 U/ml		Exptl. minus control	Insulin 0·03 U/ml		Exptl. as % control
With Insulin	Without Insulin		With Insulin	Without Insulin	
+1·44	+0·14	+1·30	19,000	1890	1010
+0·34	+0·04	+0·30	22,700	947	2400
+1·26	+0·21	+1·05	17,600	1320	1330
+0·32	+0·04	+0·28	16,700	1410	1180
+0·76	−0·11	+0·87	17,900	1020	1750
+0·32	0·00	+0·32	21,200	752	2820
+1·09	+0·21	+0·88	15,100	811	1860
	Mean	+0·72		Mean	1760
	S.E.	0·16		S.E.	250

* Two separate experiments.
† μmoles glucose/100 mg wet wt./hr.
‡ mμmoles glucose C/100 mg wet wt./hr; glucose concentration 4 mg/ml.

6. The lack of an insulin effect in house sparrow adipose tissue is in marked contrast to the effects noted in the rat (cf. Langdon, 1960). In order to verify techniques and procedures, two experiments were performed on isolated rat epididymal fat pads. In these experiments insulin caused an increase in glucose uptake of 0·72 μmoles/100 mg wet wt./hr (Table 8(a)) and increased lipogenesis

TABLE 9—THE EFFECT OF GLUCOSE ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

Glucose 2 mg/ml		Exptl. as % control
With Glucose	Without Glucose	
41·9	21·5	195
8·12	3·92	207
38·8	27·3	142
7·42	4·39	169
41·5	34·9	119
14·6	4·11	355
47·1	23·9	197
	Mean	198
	S.E.	29

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM.

TABLE 10—THE EFFECT OF INSULIN ON INCORPORATION* OF GLUCOSE CARBON INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE SLATE-COLORED JUNCO

Insulin 0·3 U/ml		Exptl. as % control
With Insulin	Without Insulin	
16·5	8·2	201
12·2	12·2	100
8·8	12·3	72
7·8	7·3	107
11·5	6·4	180
5·8	5·7	102
7·5	8·0	94
	Mean	122
	S.E.	18

* mμmoles glucose C/100 mg wet wt./hr; glucose concentration 4 mg/ml.

from labeled glucose by 1760 per cent (Table 8(b)). Both of these increases were significant ($p < 0·02$).

7. In the rat epididymal fat pad lipid synthesis from labeled acetate is two and one half or more times greater in the presence of glucose than in its absence (Winegrad & Renold, 1958). The results in Table 9 demonstrate a similar effect in house sparrow adipose tissue. In this tissue glucose caused a twofold increase in the incorporation of acetate into tissue lipid ($p < 0·02$).

TABLE 11—THE EFFECT OF INSULIN ON GLUCOSE UPTAKE* AND INCORPORATION† OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE WHITE-CROWNED SPARROW

(a) Glucose uptake			(b) Acetate incorporation		
Insulin 0·5 U/ml		Exptl. minus control	Insulin 0·5 U/ml		Exptl. as % control
With Insulin	Without Insulin		With Insulin	Without Insulin	
+0·50	+0·26	+0·24	4·91	8·45	58
+5·80	+6·65	−0·85	18·6	22·4	83
+1·15	+0·72	−0·43	5·45	7·30	75
+0·24	+0·14	+0·10	1·97	1·26	156
+0·23	+0·27	−0·04	4·15	3·52	118
+0·31	+0·16	+0·15	3·26	2·96	110
+0·24	+0·13	+0·11	5·05	5·40	94
	Mean	+0·02		Mean	99
	S.E.	0·05		S.E.	12

* μmoles glucose/100 mg wet wt./hr; glucose concentration 2 mg/ml.
† mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM.

8. The response to insulin *in vitro* was tested in the intact abdominal fat pads of two other passerine species. In the slate-colored junco insulin had no effect ($p > 0{\cdot}05$) on the synthesis of fatty acids from labeled glucose (Table 10). Neither glucose uptake nor fatty acid synthesis from labeled acetate was affected ($p > 0{\cdot}05$) by insulin in fat pads from the white-crowned sparrow (Table 11).

Effects of glucagon in vitro

Glucagon causes several changes in the metabolism *in vitro* of rat epididymal fat pads. These effects include accelerated glucose uptake, increased release of free fatty acids and glycerol, and inhibition of lipid synthesis (Orth *et al.*, 1960; Hagen, 1961). The effect of glucagon on three of these phenomena, glucose uptake, free fatty acid release and lipid synthesis, was tested in the isolated house sparrow abdominal fat pad. The results are as follows:

1. Tables 12 and 13 show the effect of four different glucagon concentrations on lipid synthesis from labeled acetate. Significant inhibition ($p < 0{\cdot}02$) was observed when the glucagon concentration was 50, 5·0 or 0·5 μg/ml but not when the dose was 0·05 μg/ml ($p > 0{\cdot}05$). The minimum effective dose, however, is probably lower than 0·05 μg/ml for many individuals, but this is difficult to demonstrate due to the variation encountered. The degree of inhibition as percent of the control value is plotted against the logarithm (base 10) of the glucagon dose in Fig. 1. This dose–response curve is essentially linear over the range of doses tested.

TABLE 12—THE EFFECT OF GLUCAGON ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

Glucagon 0·05 μg/ml		Exptl. as % control	Glucagon 0·5 μg/ml		Exptl. as % control
With Glucagon	Without Glucagon		With Glucagon	Without Glucagon	
3·05	4·33	70	14·2	21·4	66
19·5	24·9	78	7·80	8·38	93
3·55	5·12	69	4·50	6·76	67
2·74	2·67	103	9·78	46·3	21
16·9	16·0	106	10·6	27·3	39
3·54	5·52	64	6·07	6·38	95
5·69	10·3	55	4·42	21·1	21
			3·79	7·80	49
			14·5	15·9	91
			5·89	9·46	62
			1·21	5·00	24
			9·75	18·1	54
	Mean	78		Mean	57
	S.E.	7·3		S.E.	7·9

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM; glucose concentration 2 mg/ml.

TABLE 13—THE EFFECT OF GLUCAGON ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

Glucagon 5 μg/ml†		Exptl. as % control	Glucagon 50 μg/ml‡		Exptl. as % control
With Glucagon	Without Glucagon		With Glucagon	Without Glucagon	
1·98	8·08	25	1·33	258	0·5
6·50	39·3	17	4·54	43·8	10
7·43	19·6	38	1·55	16·2	10
4·42	4·85	91	2·43	118	2·1
0·824	3·10	27	2·41	100	2·4
0·816	2·72	30	4·78	58·1	8·2
1·25	1·82	69	1·59	53·6	3·0
	Mean	42		Mean	5·2
	S.E.	10		S.E.	3·7

* mμmoles acetate C/100 mg wet wt./hr; glucose concentration 2 mg/ml.
† Acetate concentration 0·02 mM.
‡ Acetate concentration 0·5 mM.

TABLE 14—THE EFFECT OF GLUCAGON ON THE INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED RAT EPIDIDYMAL FAT PAD

Glucagon 0·5 μg/ml		Exptl. as % control
With Glucagon	Without Glucagon	
2·07	3·38	61
1·33	2·64	50
1·87	4·92	38
0·602	3·35	18
1·55	5·32	29
0·895	2·42	37
0·796	3·64	22
	Mean	36
	S.E.	5·7

* mμmoles acetate C/100 mg wet wt./hr; glucose concentration 2 mg/ml.

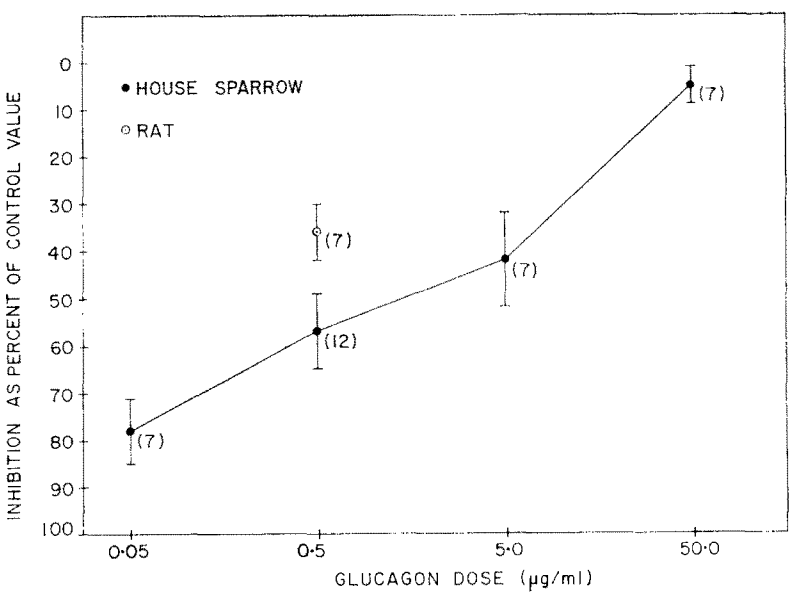

FIG. 1. A dose-response curve showing the effect of glucagon on incorporation of acetate-1-C^{14} into fatty acids by house sparrow and rat adipose tissue incubated in the presence of glucose. The numbers in parentheses indicate the number of paired tissues which were used at each dose. The vertical lines are the standard errors of the means. For further explanation see text and Tables 12–14.

2. The effect of 0·5 μg/ml of glucagon *in vitro* on lipid synthesis from acetate was tested on the rat epididymal fat pad. This dose of glucagon caused a 64 per cent inhibition of lipid synthesis ($p < 0{\cdot}02$) (Table 14). Lipid synthesis in the rat epididymal fat pad, therefore, is more sensitive to glucagon than lipid synthesis in the house sparrow abdominal fat pad (Fig. 1).

3. When a suitable FFA acceptor is present in the medium, FFA will be released from the tissue into the medium (Reshef *et al.*, 1958; Ball & Jungas, 1961). Tables 15 and 16 show the results of an experiment in which bovine serum albumin, Fraction V, was added to the medium at a concentration of 3 g/100 ml. Glucagon (25 μg/ml) had no effect ($p > 0{\cdot}05$) on glucose uptake (Table 15(a)) but caused an 85 per cent increase in FFA release ($p < 0{\cdot}05$) (Table 15(b)). This

TABLE 15—THE EFFECT OF GLUCAGON ON GLUCOSE UPTAKE* AND FREE FATTY ACID RELEASE† IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

(a) Glucose uptake			(b) Free fatty acid release		
Glucagon 25 μg/ml		Exptl. minus control	Glucagon 25 μg/ml		Exptl. as % control
With Glucagon	Without Glucagon		With Glucagon	Without Glucagon	
+0·15	+0·08	+0·07	0·42	0·30	140
+0·29	+0·22	+0·07	0·82	0·58	141
+0·14	+0·06	+0·08	0·43	0·20	215
+0·43	+0·69	−0·26	1·40	0·41	341
+0·04	+0·06	−0·02	0·43	0·25	172
+0·13	0·00	+0·13	0·50	0·22	227
−1·44	+0·84	−2·28	0·48	0·79	61
	Mean	−0·31		Mean	185
	S.E.	0·33		S.E.	33

* μmoles glucose/100 mg wet wt./hr; glucose concentration 2 mg/ml.
† Albumin concentration 3 g/100 ml; μequivalents fatty acid/100 mg wet wt./hr.

dose of glucagon caused a 62 per cent inhibition of total lipid synthesis from acetate ($p < 0{\cdot}02$) (Table 16(c)). The rate of incorporation of labeled acetate into albumin-bound fatty acids and tissue lipid (Table 16(a) and (b)) was inhibited to the same degree as total synthesis.

4. For comparative purposes, the effect of glucagon (5 μg/ml) *in vitro* was tested on the abdominal fat pad of the white-crowned sparrow (Table 17). Glucose uptake was unaffected ($p > 0{\cdot}05$) by this dose, but there was a 48 per cent inhibition of the incorporation of labeled acetate into lipid ($p < 0{\cdot}02$). In the house sparrow abdominal fat pad under the same conditions, 5 μg/ml of glucagon, caused a 58 per cent inhibition (Table 13(a)) of lipid synthesis from labeled acetate.

TABLE 16—THE EFFECT OF GLUCAGON ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

(a) Albumin-bound fatty acids*			(b) Tissue lipid			(c) Total incorporation		
Glucagon 25 μg/ml		Exptl. as % control	Glucagon 25 μg/ml		Exptl. as % control	Glucagon 25 μg/ml		Exptl. as % control
With Glucagon	Without Glucagon		With Glucagon	Without Glucagon		With Glucagon	Without Glucagon	
0·423	1·04	41	1·75	5·89	30	2·18	6·92	32
0·447	2·36	19	1·37	6·88	20	1·83	9·24	20
0·323	0·941	34	1·71	1·93	89	2·03	2·87	71
0·979	4·71	21	2·28	17·9	13	3·26	22·6	14
1·25	1·42	88	4·17	5·07	82	5·43	6·49	84
0·320	1·18	27	0·838	3·40	25	1·16	4·58	25
1·89	4·60	41	4·02	2·40	17	5·91	28·6	21
	Mean	39		Mean	39		Mean	38
	S.E.	8·8		S.E.	12		S.E.	11

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM; glucose concentration 2 mg/ml.

TABLE 17—THE EFFECT OF GLUCAGON ON GLUCOSE UPTAKE* AND INCORPORATION† OF ACETATE INTO THE ISOLATED ABDOMINAL FAT PAD OF THE WHITE-CROWNED SPARROW

Glucose uptake			Acetate incorporation		
Glucagon 5μg/ml		Exptl. minus control	Glucagon 5μg/ml		Exptl. as % control
With Glucagon	Without Glucagon		With Glucagon	Without Glucagon	
+0·13	+0·15	−0·02	0·851	1·54	55
+0·15	+0·18	−0·03	1·13	2·35	48
+0·14	+0·16	−0·02	2·37	2·72	87
+0·15	+0·11	+0·04	1·23	2·40	51
+0·07	+0·02	+0·05	0·839	1·54	54
+0·03	+0·10	−0·07	0·542	1·75	31
+0·03	+0·12	−0·09	1·17	3·07	38
	Mean	−0·02		Mean	52
	S.E.	0·02		S.E.	6·7

* μmoles glucose/100 mg wet wt./hr; glucose concentration 2 mg/ml.
† mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM.

Effects of insulin and glucagon in vivo

Table 18 is a summary of the effects of glucagon and insulin *in vivo* in the house sparrow. Insulin, in doses as low as 0·1 U per bird, caused a significant depression of blood sugar levels, but had no effect on the incorporation of labeled acetate into liver or the abdominal fat pad. Glucagon, at a dose of 5 μg per bird, did not elevate blood glucose levels under the conditions employed in these experiments. A 10 μg dose, however, was highly effective in elevating blood glucose levels. Incorporation of labeled acetate into lipids of the liver was not affected by 5 μg of glucagon. Neither of the glucagon doses tested caused a significant depression of the mean rate of lipid synthesis from labeled acetate in the abdominal fat pad. When the variances of the logarithm (base 10) transformed data were compared by means of an F ratio, the values obtained were $F_{5,5} = 20{\cdot}4$ for the 5 μg dose and $F_{5,6} = 14{\cdot}9$ for the 10 μg dose. The F ratio establishes the probability that the variances of two groups are different (Snedecor, 1956). The variances of the 5 μg and 10 μg groups were significantly different ($p < 0{\cdot}01$) from the variances of their respective control groups.

Effects of prolactin in vitro

In the rat epididymal fat pad, prolactin *in vitro* causes an increase in glucose uptake (Moore & Ball, 1962) and an increase in the rate of incorporation of labeled acetate into lipid (Winegrad *et al.*, 1959). The latter effect is dependent on the presence of glucose in the medium. The effect of prolactin *in vitro*, therefore, was tested on abdominal fat pads from house sparrows and white-crowned sparrows. The results are as follows:

TABLE 18—THE EFFECT OF INSULIN AND GLUCAGON *in vivo* IN THE HOUSE SPARROW

Treatment (per bird)	Change in blood glucose level*		Incorporation of acetate carbon†			
			Liver		Abdominal fat pad	
	With	Without	With	Without	With	Without
Insulin (0·1 U)	+21 ± 11‡	−15 ± 1·6 $p = 0{\cdot}028$§	—	—	—	—
Insulin (1 U)	−41 ± 15	−140 ± 12 $p = 0{\cdot}001$	9·1 ± 2·1	11·7 ± 3·6 $p > 0{\cdot}05$	26·7 ± 7·8	28·8 ± 7·7 $p > 0{\cdot}05$
Glucagon (5 μg)	+31 ± 7·8	+48 ± 20 $p > 0{\cdot}05$	2·65 ± 0·71	2·75 ± 0·71 $p > 0{\cdot}05$	19·1 ± 5·1	8·63 ± 0·64 $p > 0{\cdot}05$
Glucagon (10 μg)	−31 ± 14	+73 ± 23 $p = 0{\cdot}006$	—	—	36·6 ± 14	20·7 ± 1·9 $p > 0{\cdot}05$

* mg glucose/100 ml blood.
† mμmoles acetate C/100 mg non-fat wet wt./hr.
‡ Standard error.
§ Probability associated with no difference (Mann–Whitney).

TABLE 19—THE EFFECT OF PROLACTIN ON GLUCOSE UPTAKE* IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW AND THE WHITE-CROWNED SPARROW

(a) House sparrow			(b) House sparrow			(c) White-crowned sparrow		
Prolactin 1 mg/ml		Exptl. minus control	Prolactin 0·1 mg/ml		Exptl. minus control	Prolactin 1 mg/ml		Exptl. minus control
With Prolactin	Without Prolactin		With Prolactin	Without Prolactin		With Prolactin	Without Prolactin	
+0·08	+0·04	+0·04	+0·03	+0·19	−0·16	+0·34	−0·13	+0·47
+0·15	+0·03	+0·12	+0·21	+0·12	+0·09	+0·25	+0·12	+0·13
+0·24	0·00	+0·24	+0·18	+0·06	+0·12	+0·10	−0·06	+0·16
+0·06	−0·19	+0·25	+0·74	+0·58	+0·16	+0·08	−0·12	+0·20
+0·09	0·00	+0·09	+0·48	+0·08	+0·40	+0·07	−0·04	+0·11
0·00	−0·21	+0·21	+1·12	+1·17	−0·05	+0·01	−0·05	+0·06
−0·17	−0·24	+0·07	+0·29	−0·17	+0·46	+0·09	+0·03	+0·06
	Mean	+0·15		Mean	+0·15		Mean	+0·17
	S.E.	0·03		S.E.	0·08		S.E.	0·05

* μmoles glucose/100 mg wet wt./hr; glucose concentration 2 mg/ml.

1. Prolactin at a concentration of 1 mg/ml accelerated glucose uptake ($p < 0{\cdot}02$) in fat pads from both species (Table 19(a) and (c)); the increase being $+0{\cdot}15$ and $+0{\cdot}17$ μmoles/100 mg wet wt./hr. At a dose of 0·1 mg/ml prolactin had no significant effect ($p > 0{\cdot}05$) on glucose uptake in the house sparrow (Table 19(b)).

TABLE 20—THE EFFECT OF PROLACTIN ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW

(a) Prolactin 1 mg/ml		Exptl. as % control	(b) Prolactin 0·1 mg/ml		Exptl. as % control
With Prolactin	Without Prolactin		With Prolactin	Without Prolactin	
7·90	5·53	143	1·04	1·41	74
3·20	5·22	61	1·56	1·92	81
8·25	5·57	148	2·16	2·78	78
6·77	10·5	64	0·724	0·713	102
7·74	12·1	64	1·34	0·338	396
0·225	0·323	70	4·39	4·67	94
10·2	12·4	82	0·054	0·075	72
	Mean	90		Mean	128
	S.E.	14		S.E.	27

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM; glucose concentration 2 mg/ml.

TABLE 21—THE EFFECT OF PROLACTIN ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE WHITE-CROWNED SPARROW

Prolactin 1 mg/ml		Exptl. as % control
With Prolactin	Without Prolactin	
3·64	2·78	131
0·774	1·17	66
1·90	3·04	63
4·19	3·78	111
3·49	3·35	104
5·76	4·69	123
2·97	3·53	84
	Mean	97
	S.E.	10

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM; glucose concentration 2 mg/ml.

2. When the concentration of acetate in the medium was 0·02 mM, prolactin had no effect ($p > 0{\cdot}05$) on the rate of incorporation of labeled acetate in the fat pads of either species (Tables 20 and 21). However, when the concentration of

acetate in the medium was 0·5 mM, prolactin (1 mg/ml) caused a 54 per cent inhibition of lipid synthesis from acetate (Table 22) in house sparrow fat pads ($p < 0{\cdot}02$). The mean (geometric) rate of lipid synthesis from acetate in control

TABLE 22—THE EFFECT OF PROLACTIN ON INCORPORATION* OF ACETATE INTO LIPID IN THE ISOLATED ABDOMINAL FAT PAD OF THE HOUSE SPARROW WHEN ACETATE IS PRESENT AT A CONCENTRATION OF 0·5 MM

Prolactin 1 mg/ml		Exptl. as % control
With Prolactin	Without Prolactin	
1·39	34·0	4·1
19·5	24·0	81
18·0	35·6	51
7·56	13·4	56
2·74	8·19	33
6·08	11·5	53
	Mean	46
	S.E.	10

* mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·5 mM; glucose concentration 2 mg/ml.

TABLE 23—THE EFFECT OF PROLACTIN ON GLUCOSE UPTAKE* AND INCORPORATION† OF ACETATE INTO LIPID IN THE ISOLATED GLUCAGON-TREATED‡ ABDOMINAL FAT PAD OF THE WHITE-CROWNED SPARROW

(a) Glucose uptake			(b) Acetate incorporation		
Prolactin 1 mg/ml		Exptl. minus Control	Prolactin 1 mg/ml		Exptl. as % control
With Prolactin	Without Prolactin		With Prolactin	Without Prolactin	
+0·09	+0·07	+0·02	0·228	0·825	28
+0·18	+0·11	+0·07	2·91	2·40	121§
+0·14	+0·10	+0·04	0·240	0·727	33
+0·10	+0·09	+0·01	0·173	1·23	14
+0·05	+0·10	−0·05	0·291	0·842	35
+0·08	+0·10	−0·02	0·387	1·20	32
	Mean	+0·01		Mean	28
	S.E.	0·02			

* μmoles glucose/100 mg wet wt./hr; glucose concentration 2 mg/ml.

† mμmoles acetate C/100 mg wet wt./hr; acetate concentration 0·02 mM.

‡ Glucagon present in both experimental and control flasks at a concentration of 5 μg/ml.

§ Not included in mean.

flasks containing 0·02 mM acetate was $50{\cdot}3 \pm 1{\cdot}1$ mμmoles acetate carbon/100 mg non-fat wet wt./hr ($n = 40$). In control flasks containing 0·5 mM acetate the rate was $123 \pm 1{\cdot}2$ mμmoles acetate carbon/100 mg non-fat wet wt./hr ($n = 27$). The difference between the means is highly significant ($p < 0{\cdot}001$).

Effect of prolactin plus glucagon in vitro

Daily injections of prolactin will induce fat deposition in white-crowned sparrows (Meier, personal communication). Since prolactin did not stimulate lipid synthesis *in vitro* in the white-crowned sparrow, the effect *in vitro* of prolactin was tested on glucagon-inhibited fat pads. The prolactin did not block glucagon inhibition (Table 23(b)). On the contrary, prolactin enhanced glucagon inhibition of lipid synthesis in five out of six tissue pairs. The rate of lipid synthesis in both control and experimental flasks of the aberrant tissue pair was at least twofold greater than the rate of lipid synthesis in any other control. This suggests that this tissue was not sensitive to the glucagon which was present in both the control and experimental flasks. Prolactin did not stimulate glucose uptake in the presence of glucagon in these tissues ($p > 0{\cdot}05$) (Table 23(a)).

DISCUSSION

The striking inability of insulin to influence the metabolism of avian adipose tissue *in vitro* is in sharp contrast to the profound effects which insulin has on mammalian adipose tissue (Langdon, 1960; Randle, 1963). In these birds insulin was effective in promoting hypoglycaemia in the whole animal and caused increased glucose uptake by gizzard slices *in vitro*. Therefore, insulin insensitivity may be limited to adipose tissue in these birds.

In mammalian adipose tissue *in vitro* insulin increases glucose uptake, accelerates fatty acid synthesis, and accelerates glyceride-glycerol synthesis. When glucose is absent from the medium, insulin has no effect on fatty acid synthesis (Winegrad & Renold, 1958; Cahill *et al.*, 1959). Furthermore, the addition of glucose to a glucose-free medium accelerates the incorporation of C^{14} labeled pyruvate or acetate into fatty acids (Winegrad & Renold, 1958). It seems certain that the principal effects of insulin on lipogenesis in mammalian adipose tissue are secondary to the accelerated rate of glucose uptake. The insulin-caused increase in glucose uptake probably promotes lipogenesis by increasing the quantity of NADPH or glycerophosphate available for lipid synthesis (Fritz, 1961; Masoro, 1962).

As in mammals, glucose accelerates the incorporation of acetate-1-C^{14} into fatty acids in house sparrow adipose tissue *in vitro* (Table 7). Therefore, the inability of insulin to accelerate glyceride-glycerol synthesis from glucose, fatty acid synthesis from glucose, or fatty acid synthesis from acetate in the presence of glucose in avian adipose tissue is due, at least in part, to its inability to accelerate glucose uptake in this tissue.

The effect of glucagon on avian adipose tissue *in vitro* is similar to its effect in rat adipose tissue. Glucagon accelerates FFA release and inhibits fatty acid synthesis from labeled acetate in both kinds of tissue (Orth *et al.*, 1960; Hagen, 1961). Rat

adipose tissue is more sensitive to the inhibitory effects of glucagon (Fig. 1). Whereas glucagon accelerates glucose uptake in rat epididymal fat pads *in vitro* (Hagen, 1961), it has no effect on glucose uptake in the avian tissues used in this investigation.

Orth *et al.* (1960) have reported that in the rat epididymal fat pad glucagon increases the proportion of newly synthesized fatty acids (i.e. proportion of incorporated radioactive acetate) which are released to the medium. In house sparrow adipose tissue glucagon did not influence the proportion of newly synthesized fatty acids recovered in the medium (Table 16).

The close relationship between fatty acid synthesis and lipolysis (Ball & Jungas, 1961) and the effect of glucagon on both of these systems make it tempting to speculate about a single effect of glucagon which would account for the changes in both systems. By increasing the rate of FFA release glucagon increases the concentration of FFA in the medium (providing a suitable acceptor is present in the medium) and in the tissue (Hagen, 1961). Newly synthesized FFA and FFA released from triglyceride probably form a common pool of fatty acids available for esterification. Therefore, glucagon-elevated levels of FFA should lead to an increase in the concentration of the coenzyme A esters of these fatty acids. It is known that the coenzyme A esters of fatty acids inhibit acetyl CoA carboxylase, the rate limiting enzyme in fatty acid synthesis (Lynen, 1961; Robinson *et al.*, 1963).

If a glucagon-induced acceleration of FFA release is causing all or part of the observed inhibition of fatty acid synthesis, the phenomenon would be more pronounced if no FFA acceptor were present in the medium. Under these conditions all of the FFA released under the influence of glucagon would be retained in the tissue rather than released into the medium (Reshef *et al.*, 1958; Ball & Jungas, 1961), thus causing a greater increase in the concentration of coenzyme A esters of fatty acids and a greater inhibition of acetyl CoA carboxylase. Extrapolating from the dose-response curve for glucagon (Fig. 1), we would expect an inhibition of about 80 per cent by a 25 μg per ml dose when albumin is absent from the medium. The actually observed inhibition in the presence of albumin (62 per cent, Table 16(c)) in fact is less than the predicted inhibition of 80 per cent. This evidence is compatible with the hypothesis that part or all of the inhibition of fatty acid synthesis caused by glucagon is a secondary effect, resulting from an increased concentration of FFA and their coenzyme A esters in the tissue.

Glucagon did inhibit the rate of lipid synthesis in fat pads of intact birds, but the effect was observable only in animals with a relatively high rate of lipid synthesis. This effect is shown graphically in Fig. 2. The control birds of this experiment (Table 18) showed considerable variation in the rate of lipid synthesis in both the liver and the abdominal fat pad. When the rates of lipid synthesis in the liver and fat pad are plotted against one another for each bird, it becomes clear that higher rates of synthesis in the liver are accompanied by higher rates of synthesis in the fat pad in the control birds. The glucagon-treated birds, on the other hand, showed considerable variation in the liver but much less variation

in the fat pads. When the rates of lipid synthesis in the liver and fat pad are plotted against one another for these birds, higher rates of synthesis in the liver are not accompanied by higher rates of synthesis in the fat pads. The decreased

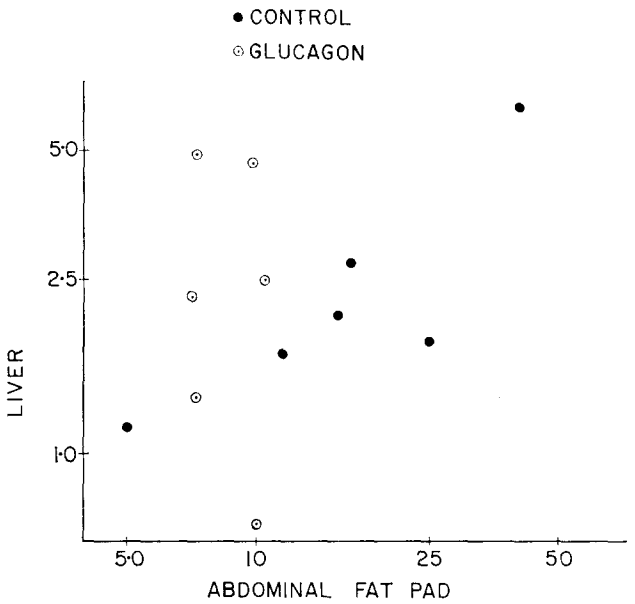

FIG. 2. The relationship between fatty acid synthesis in the liver and fatty acid synthesis in the abdominal fat pad of glucagon-treated (5 μg/bird) and control house sparrows. Each point represents a single bird. Vertical axis: mμmoles acetate carbon incorporated/100 mg liver wet wt./hr ($\log_{10}$ scale). Horizontal axis: mμmoles acetate carbon incorporated/100 mg fat pad non-fat wet wt./hr ($\log_{10}$ scale).

variation observed in the experimental birds can be attributed to inhibition by glucagon.

Prolactin, like insulin, stimulates glucose uptake and lipid synthesis *in vitro* in the rat epididymal fat pad (Winegrad *et al.*, 1959; Moore & Ball, 1962). The effect of prolactin also is dependent on the presence of glucose in the medium (Winegrad *et al.*, 1959). Prolactin *in vitro* causes a small but consistent increase in glucose uptake in avian adipose tissue but does not accelerate lipid synthesis. The acceleration of glucose uptake is four times greater in the rat epididymal fat pad than in avian abdominal fat pads. The failure of the prolactin-induced acceleration of glucose uptake to cause increased lipid synthesis in bird adipose tissue is probably related to the very small increase in the rate of glucose uptake or to the inhibitory effect described below.

In the experiments discussed above the acetate concentration was 0·02 mM. When the acetate concentration was 0·5 mM, prolactin caused a 54 per cent inhibition of fatty acid synthesis from acetate. Fatty acid synthesis was two and

one half times faster when the concentration of acetate was 0·5 mM than when it was 0·02 mM. It is likely that prolactin inhibits an enzymatic step which is rate limiting at the higher concentration but not at the lower concentration. This effect also may explain why the accelerated glucose uptake caused by prolactin does not lead to increased lipid synthesis.

When white-crowned sparrow fat pads were treated with prolactin in the presence of glucagon, prolactin enhanced glucagon inhibition in five of the six tissue pairs. In the sixth pair of tissues prolactin had no effect. In this instance, both the control and the experimental tissue had a much higher rate of synthesis than any of the other five controls. This suggests that glucagon had no inhibitory effect on either the control or the experimental of this tissue pair. The concentration of acetate was 0·02 mM in this experiment; and according to the data presented above, one would not have expected prolactin to be inhibitory. These results are consistent with the hypothesis that glucagon and prolactin inhibit the same enzyme additively. Prolactin alone reduces the activity of the enzyme a small amount, but not enough to make it rate limiting in this system. If glucagon makes the enzyme rate limiting, the additional inhibition caused by prolactin is observable.

It is difficult to evaluate the physiological importance of the hormonal effects described in this investigation. It is significant that none of the hormones tested accelerated lipogenesis. Insulin, which is the most potent hormonal accelerator of lipogenesis in mammals, can be eliminated as a primary factor in the hormonal control of premigratory fat deposition since it had no effect on glucose uptake or lipogenesis in avian adipose tissue under any circumstance. Of those hormones which were inhibitory, only glucagon appears to have the necessary potency to be of physiological importance. The following hypothesis has been formulated on the basis of glucagon inhibition to explain premigratory fattening in birds:

1. Relatively high glucagon levels inhibit the rate of lipid synthesis during most of the year.

2. During the premigratory periods and perhaps the midwinter "fat" periods (a) glucagon levels decrease allowing lipid synthesis to accelerate or (b) some other hormone blocks glucagon inhibition at the tissue level.

Release of inhibition at the tissue level is an attractive hypothesis, but no evidence exists to support it. There is, however, evidence suggesting that glucagon levels are depressed during periods of fattening. In the blackbird (*Turdus merula*) both alpha and beta cells of the pancreatic islets are in a state of minimum activity during winter fattening. (These birds migrate very short distances if at all. They undergo no spring or fall premigratory fattening.) Furthermore, alpha cell activity is lower than beta cell activity at this time and only at this time in the blackbird annual cycle (Epple, 1961).

When migratory white-crowned sparrows are subjected to 20 hr daily photoperiods, a treatment which leads to fat deposition, blood glucose levels are depressed (Farner, personal communication) as would be expected if glucagon output had decreased.

If glucagon output is depressed during the premigratory period, the cause may be altered levels of a specific hormone or a simultaneous alteration in the level of several hormones. Several different hormones can affect the function of the pancreatic islets (Epple, 1961). Future investigations must determine if glucagon output is depressed, and if so, how the output of glucagon is regulated.

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