The Effect of Prolactin on Lipogenesis in the Pigeon. *In Vivo* Studies*

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ABSTRACT: The effect of prolactin on lipogenesis in the pigeon was examined by injecting [U-^{14}C]glucose and [9,10-^{3}H]tristearin into birds which had been untreated or injected daily with 1 mg of bovine prolactin for 5 days. Radioactivity in plasma glucose and in liver, plasma, and adipose tissue total fatty acids (^{14}C and ^{3}H) was determined 0.5, 7, 15, and 30 min after injection of the labeled substances. Radioactive ^{14}C fatty acids appeared promptly and first in the liver of birds from both groups. In those birds receiving prolactin the rate of accumulation of labeled fatty acids was three- to fourfold faster per gram of liver and the liver was nearly double the normal size. Accumulation of ^{14}C fatty acids in plasma and adipose tissue was negligible at 7 min but increased rapidly and linearly with time thereafter. The rate of accumulation in both plasma and adipose tissue of the birds receiving prolactin was markedly accelerated, the increase over the normal being of nearly the same magnitude as seen for the total liver. The fate of the tritiated fatty acids indicates that the liver of birds receiving prolactin had a markedly enhanced capacity to process preformed fatty acids. The results are consistent with the hypothesis that in both normal and prolactin-treated pigeons fatty acids were synthesized predominantly in the liver and released to the blood for transport to adipose tissue where they were picked up and stored. Treatment with prolactin produced an increase in the rate of hepatic fatty acid synthesis and turnover which was reflected in an increased level of plasma fatty acids and deposition of fatty acids in peripheral depots with an increase in body weight.

The studies reported here are an outgrowth of our interest in the ability of many species of birds to synthesize and store large amounts of fat just prior to long-distance migratory flights (Farner, 1960; Odum, 1960). Very little is known about the regulation of this phenomenon. Our first approach to this problem was to ascertain whether in birds, as in certain mammals, adipose tissue serves not only as a storehouse for triglyceride, but also contributes actively to lipogenesis. In studies (Goodridge and Ball, 1966) in which the pigeon was used as the experimental animal, synthesis of fatty acids from glucose or pyruvate by adipose tissue incubated *in vitro* proceeded at a negligible rate and was unaffected by insulin. Low activities of acetyl-CoA[1] carboxylase, citrate cleavage enzyme, malic enzyme, and hexose monophosphate shunt dehydrogenases were found in homogenates of this tissue. These enzymes are abundant in rat adipose tissue (Martin and Vagelos, 1962; Kornacker and Ball, 1965; Wise and Ball, 1964; Ball and Jungas, 1963). On the other hand, activities of these enzymes in pigeon liver were very high and, except for shunt dehydrogenases, 4–20 times higher than those found in rat liver. Studies (Goodridge and Ball, 1967) were next made on the time course of the appearance of ^{14}C fatty acids in liver, blood, and adipose tissue after the intravenous injection of [^{14}C]glucose into unanesthetized fasted-refed pigeons. These studies showed that significant incorporation of glucose carbon into the fatty acids of liver occurred within 3 min after the injection and that the liver content of radioactive fatty acids reached a more or less plateau level at 15 min. Significant appearance of labeled fatty acids in blood and adipose tissue was first seen at 15 min following the injection. Their level in both of these tissues then rose concomitantly and continuously throughout the experimental period of 2 hr while blood radioactive glucose values were falling precipitously. The ratio of counts in glyceride fatty acids to counts in glyceride glycerol remained close to unity in plasma throughout the experiment but rose progressively to a value of 7.0 at 2 hr in adipose tissue. The results of these studies led us to the conclusion that liver is the chief site of fatty acid synthesis from glucose in the pigeon and that adipose tissue derives its glyceride fatty acids from plasma triglyceride and its glyceride glycerol from plasma glucose.

Our attention now has been directed to the possible action that hormones might have upon lipogenic processes in the pigeon. Prolactin was selected as first

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[1] The following abbreviations are used: CoA, coenzyme A; [U-^{14}C]glucose, glucose uniformly labeled with ^{14}C; FFA, free fatty acids.

choice in such a study. Prolactin has long been known to stimulate the enlargement of the pigeon crop sac and its formation of a lipid-rich product called crop milk (Riddle and Braucher, 1931). It also causes increased food consumption and produces gains in total body weight of the pigeon as well as in the weights of specific organs such as the liver, pancreas, and intestine (Bates *et al.*, 1937). Recently, Meier and Farner (1965) have reported that prolactin increases body weight and fat deposition in the migrant white-crowned sparrow (*Zonotrichia leucophrys gambelii*).

The experiments to be presented here show that daily injections into pigeons of 1 mg of bovine prolactin for 5 days caused marked increases in the rate at which pigeons converted glucose to fatty acids. The results were again consistent with those to be expected on the premise that the bulk of fatty acids were synthesized in the liver and released to the blood for transport to adipose tissue where they were picked up and stored.

Methods and Materials

Silver King pigeons (7–8-weeks old, 420–630-g body wt) were maintained on an *ad libitum* diet of Purina pigeon grains and water. The pigeons were housed in a temperature- (22–24°) and humidity-controlled room on a 14.5-hr daily photoperiod. Birds termed "normal" were untreated; those termed "prolactin" received intramuscular (pectoral) injections of bovine prolactin (1 mg/day) for 5 days. Pigeons of both sexes were used and no measurable difference in their response to prolactin was observed.

Unanesthetized pigeons were injected intravenously (alar vein) with 0.5 ml of a preparation containing tracer doses of [U-^{14}C]glucose (7 μc, 90 μg) and [9,10-^{3}H]tristearin (90 μc, 240 μg). This preparation was made by mixing 2.8 ml of 5% bovine serum albumin, 0.2 ml of [U-^{14}C]glucose solution, 0.5 ml of a 1:10 dilution of a coconut oil emulsion (Ediol, Riker Laboratories), and 1.7 mg of dry, tritiated tristearin. A solution of 0.9% NaCl was employed to prepare the aqueous components of the preparation. The final mixture was sonicated at 0–3° until all the tristearin was emulsified. Ediol, which stabilized the emulsion, also contributed 3.5 mg of triglyceride/injection. The emulsion, although stable for at least 4 hr, was always injected within 3 hr after its preparation. After the injections were completed, aliquots of the emulsion were diluted in water and chloroform–methanol (2:1) for determination of glucose and tristearin radioactivity, respectively.

The injected pigeons were placed, individually, in small darkened chambers in a ventilated hood. After 30 sec, 7, 15, or 30 min the birds were killed by decapitation. Blood was collected from the neck in heparinized tubes which were stored in crushed ice for later separation of plasma by centrifugation. Liver and tracheal adipose tissue were removed as rapidly as possible and placed in iced saline. Whole livers were blotted dry, weighed on a torsion balance, and returned to the iced saline. The nitrogen content of small pieces of liver and adipose tissue was determined by a micro-Kjeldahl procedure (Frerichs and Ball, 1962). Total fatty acids were extracted from saponified liver, adipose tissue, and plasma as previously described (Goodridge and Ball, 1967), except that the extracted fatty acids were back-washed with water instead of acidified 33% ethanol. Fatty acids were determined quantitatively by titration. Radioactivity of the fatty acids was determined by dissolving them in 10 ml of toluene–ethanol (9:1) which contained 0.4% 2,5-diphenyloxazole and 0.005% *p*-bis[2-(5-phenyloxazole)]benzene and counting in a two-channel Packard Tri-Carb liquid scintillation spectrophotometer. Corrections for spillage of ^{14}C radioactivity into the tritium channel, quenching, and efficiency of the scintillation spectrophotometer were made in all samples by the addition of ^{14}C and ^{3}H internal standards.

Plasma glucose level and specific activity of the plasma [^{14}C]glucose were determined as previously described (Goodridge and Ball, 1967). Corrections for efficiency of the gas-flow Geiger counter were made by counting known volumes of the same [U-^{14}C]glucose solution in the gas-flow counter and the liquid scintillation spectrophotometer. Plasma FFA was extracted and titrated by the method of Dole (1956).

The bovine serum albumin (fraction V, Mann Research Laboratories) was free of extractable FFA.

TABLE I: The Effect of Prolactin on Total Body and Liver Weight, Liver Fatty Acids, and Blood Fatty Acids and Glucose.

Measurement[a]	Normal	Prolactin
Body weight	–	16 ± 1 (21)
Liver weight	2.0 ± 0.1 (18)	3.6 ± 0.2 (15)
Liver total fatty acids	4.8 ± 0.4 (18)	5.7 ± 0.6 (15)
Plasma total fatty acids	23 ± 1 (24)	37 ± 1 (21)
Plasma free fatty acids	0.61 ± 0.02 (13)	0.87 ± 0.05 (7)
Blood glucose	1.45 ± 0.03 (19)	1.38 ± 0.05 (8)

[a] Body weight is expressed as per cent increase caused by prolactin. Actual body weight for the normals was 500 ± 11 g. (The weight of untreated or saline-injected birds did not change.) Mean initial weight of the prolactin-treated birds was 530 ± 11 g. Liver weight is expressed as per cent of the total body weight; liver fatty acids as microequivalents per milligram of nitrogen; plasma total fatty acids and free fatty acids as microequivalents per milliliter; blood glucose as milligrams per milliliter of whole blood. All values are plus or minus standard error. The numbers of birds in each group are indicated in the parentheses.

TABLE II: The Effect of Prolactin on the Amount and ^{14}C Specific Activity of Glucose in the Plasma after an Intravenous Injection of [U-^{14}C]Glucose.

Measurement	Treatment	Time from Injection to Decapitation (min)			
		0.5	7	15	30
Plasma glucose[a]	Normal	15.8 ± 0.7	16.5 ± 0.6	16.4 ± 0.3	17.3 ● 0.4
	Prolactin	15.2 ± 1.0	17.0 ± 0.4	16.6 ± 1.3	17.8 ± 0.5
Specific activity[b]	Normal	13,000 ± 460	5400 ± 100	3600 ± 230	2400 ± 140
	Prolactin	7700 ± 350	3700 ± 470	2000 ± 250	990 ± 150

[a] Micromoles per milliliter plus or minus standard error. There were six normal and five prolactin-treated birds at each time except 0.5 min which had seven and six, respectively. [b] Disintegrations per minute per micromole plus or minus standard error.

TABLE III: Nitrogen Content[a] of Tissues.

Tissue	Treatment	Time from Injection to Decapitation (min)		
		7	15	30
Liver	Normal	2.54 ± 0.08	2.69 ± 0.09	2.77 ± 0.11
	Prolactin	2.40 ± 0.11	2.39 ± 0.03	2.34 ± 0.10
Adipose	Normal	0.13 ± 0.010	0.18 ± 0.014	0.15 ± 0.007
	Prolactin	0.16 ± 0.019	0.15 ± 0.009	0.13 ± 0.007

[a] Nitrogen (mg) per 100 mg wet wt plus or minus standard error. There were six normal and five prolactin-treated birds at each time.

[U-^{14}C]Glucose and [9,10-^{3}H]tristearin were purchased from New England Nuclear Corp. The bovine prolactin (13 U/mg) was a gift of the Endocrinology Study Section, National Institutes of Health. It was dissolved in 0.9% NaCl with the aid of a few drops of 0.1 N NaOH (final pH 9–10) for injection (0.4 ml/bird).

All of the radioactivity data were corrected for differences in amount of ^{14}C or ^{3}H injected and for differences in body weight at the time of the experiment. Statistical significance of the data was tested by the Mann–Whitney test (Siegel, 1956). Standard errors are provided to indicate the degree of variance in the data.

Results

As shown in Table I increases in body weight and liver weight were observed after prolactin treatment, in agreement with the findings of Bates *et al.* (1937). We also observed a 60% increase in plasma total fatty acids and a 45% increase in plasma FFA in prolactin-treated pigeons. Riddle (1947) observed an increase in blood glucose level and an increase in liver lipids after administration of a prolactin preparation, effects which were not seen in our experiments (Table I). This difference in results may reflect the fact that prolactin preparations of greater purity are available today and that the preparation employed by Riddle may have contained adrenocorticotropic hormone (Bates *et al.*, 1962; Riddle, 1963).

Plasma glucose levels, like whole blood glucose levels, were unaffected by the prolactin treatment (Table II). The slight increases in plasma glucose level (both groups) from 0.5 to 30 min were not significant ($p > 0.5$). ^{14}C specific activity of the plasma glucose was significantly lower in the prolactin-treated birds at all times tested (Table II), suggesting a more rapid utilization of glucose by tissues of the prolactin-treated birds.

The time course for the appearance of fatty acids labeled with ^{14}C into liver, plasma, and adipose tissue is shown in Figure 1. There was an immediate and rapid accumulation of counts in liver fatty acids of both the normal and prolactin-treated birds. In plasma and adipose tissue, accumulation of ^{14}C in fatty acids was slow during the first 7 min but increased rapidly thereafter. At 7 min when very little labeled fatty acid had appeared in the blood or adipose tissue, there were 17 and 26 times more radioactive ^{14}C fatty acids/mg of tissue nitrogen in liver than adipose tissue in the normal and prolactin-treated pigeons, respectively. The data may be expressed in terms of whole liver *vs.* total body adipose tissue by use of the data given in

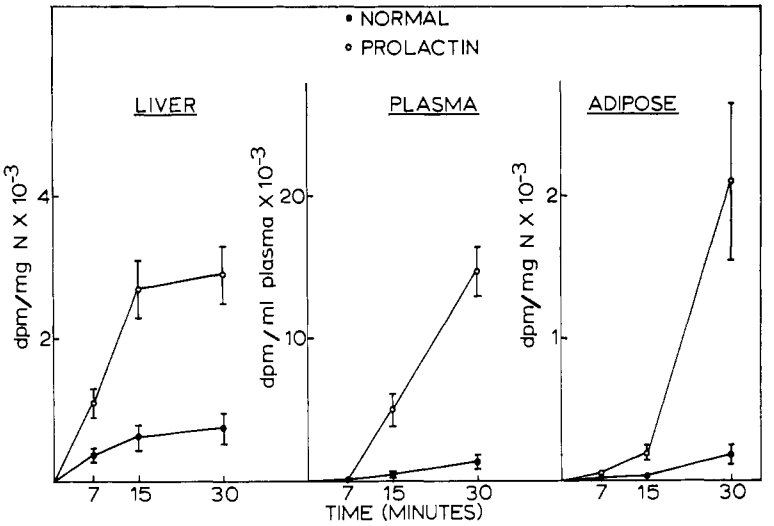

FIGURE 1: The effect of prolactin on the incorporation of [U-^{14}C]glucose into total fatty acids *in vivo*. The data are expressed as dpm in total fatty acids/mg of tissue nitrogen $\times 10^{-3}$. There were six normal and five prolactin-treated birds at each time period. The vertical bars represent the standard error.

Tables I and III along with the assumption that the wet weight of total adipose tissue was 20% of body weight. On this basis the ratio of ^{14}C fatty acids in total liver to total adipose tissue was 31 in the normal and 70 in the prolactin-treated pigeons at the 7-min mark.

Expressed on a nitrogen basis the liver fatty acids from the prolactin-treated birds contained three to four times as much ^{14}C radioactivity as liver fatty acids from normal pigeons at all three time intervals examined (7 min, $p = 0.018$; 15 and 30 min, $p < 0.004$). When it is considered that prolactin administration also nearly doubles the total liver weight (Table I) without appreciable alteration in its nitrogen content (Table III), it is obvious that the liver of birds treated with prolactin convert six to eight times as much glucose to fatty acids as the liver of control pigeons in a given time period.

^{14}C radioactivity of the plasma fatty acids was the same in both groups at 7 min ($p > 0.05$). At 15 min, however, the prolactin-treated pigeons had accumulated 14 times more ^{14}C radioactivity in plasma fatty acids than had the normal birds ($p < 0.004$). At 30 min the difference was 11-fold in favor of the prolactin group ($p < 0.004$). Expressed on a nitrogen basis, at 7 min the fatty acids in adipose tissue from the prolactin-treated birds had twice as much ^{14}C radioactivity as those found in adipose tissue from the normals ($p < 0.004$). This difference would not appear to be owing to changes in the amount of radioactive fatty acids in the plasma since as noted above this value was similar in birds of both groups. It should be recalled, however (Table I), that total plasma fatty acids were on the average 61% higher than normal in the prolactin-treated birds. The difference might therefore reflect a more rapid uptake by adipose tissue of fatty acids at an elevated plasma fatty acid concentration. Another possible explanation is that in the adipose tissue of prolactin-treated birds there was a more rapid *de novo* synthesis of fatty acids from radioactive glucose. At 15 and 30 min the adipose tissue of the pigeons treated with prolactin contain per milligram of nitrogen a quantity of ^{14}C-labeled fatty acids which was, respectively, 6 and 12 times that seen in the normal birds.

A rough estimate may be made as to the percentage of the injected radioactive glucose that was recovered as radioactive fatty acids in liver, plasma, and adipose tissue in these experiments. For this purpose the assumption was made that total adipose tissue represented 20% and plasma 4% of total body weight. If we use the data given in Tables I and III and the values at 30 min, from Figure 1, it may be calculated that in the normal, 1.7%, and in the prolactin-treated birds, 15% of the radioactive glucose carbon injected appeared in fatty acids. However, the average weight of the prolactin birds was 615 g as compared to 500 for the normal. Hence if the percentage conversion for the prolactin groups is adjusted to represent 500 g of body weight then this figure becomes 12.1%. Since the process of fatty acid synthesis from glucose involves the loss of one carbon as CO_2 for each two incorporated into fatty acids, the percentage of total glucose which entered this synthetic pathway becomes 2.6 and 18.2%, respectively. In short, as measured in this manner, the prolactin group converted seven times as much glucose to fatty acids as the control group in the 30-min time period.

As shown in Table IV, the injected [9,10-^{3}H]tristearin was rapidly cleared from the plasma of both normal and prolactin-treated birds. Clearance from plasma of the prolactin-treated birds, however, was more rapid than

TABLE IV: The Effect of Prolactin on the Amount and ^3H Specific Activity of Total Fatty Acids in the Plasma after an Intravenous Injection of [9,10-^3H]Tristearin.

Measurement	Treatment	Time from Injection to Decapitation (min)			
		0.5	7	15	30
Total fatty acids[a]	Normal	25 ± 1.6	24 ± 0.7	22 ± 1.3	20 ± 1.3
	Prolactin	34 ± 2.6	35 ± 3.1	40 ± 1.8	40 ± 1.9
Specific activity[b]	Normal	28,000 ± 1100	860 ± 160	1100 ± 80	1700 ± 230
	Prolactin	11,000 ± 1300	500 ± 30	880 ± 90	1800 ± 190

[a] Microequivalents per milliliter of plasma plus or minus standard error. [b] Disintegration per minute per microequivalent of plasma total fatty acids plus or minus standard error. Numbers of birds in each group same as in Table II.

TABLE V: The Effect of Prolactin on the Fate of Injected [9,10-^3H]Tristearin.[a]

Tissue	Treatment	Time from Injection to Decapitation (min)		
		7	15	30
Liver	Normal	120 ± 26 (30,500)	130 ± 32 (35,000)	140 ± 27 (38,800)
	Prolactin	100 ± 6.4 (53,000)	84 ± 6.9 (44,400)	80 ± 12 (41,400)
Plasma	Normal	20 ± 3.5	24 ± 1.9	34 ± 3.5
	Prolactin	17 ± 1.4	35 ± 3.3	71 ± 3.8
Adipose	Normal	3.7 ± 0.28	4.4 ± 0.28	7.6 ± 0.94
	Prolactin	3.0 ± 0.52	4.0 ± 0.51	12 ± 1.7

[a] Values are given as dpm $\times 10^{-3}$ in total fatty acids/mg of tissue nitrogen or per ml of plasma plus or minus standard error except values in parentheses which are for the total liver.

it was in the normals. At 30 sec, normal plasma fatty acids contained 700,000 dpm/ml of ^3H while prolactin plasma fatty acid contained only 373,000 dpm/ml of ^3H ($p = 0.002$). In both groups plasma fatty acid ^3H specific activity was lowest at 7 min while from 7 to 30 min there was a progressive increase in the specific activity (Table IV). This latter change may have reflected the appearance of the tritiated fatty acids into the lipoprotein fraction of plasma.

There was a very large accumulation of tritium-labeled fatty acids in livers of both groups during the first 7 min of the experimental period. The results for liver are expressed in Table V both in terms of milligrams of tissue nitrogen and of total liver by use of the values given in Tables I and III. At 7 min there was little difference between the normal and prolactin group when results are expressed per unit of liver nitrogen. However, expressed in terms of total liver the normal pigeons contained 16.6% of the injected tritium as compared to a value of 26.4% for the prolactin-treated birds. These results suggest that the more rapid clearance of plasma-tritiated fatty acids in the prolactin group was due primarily to a larger mass of liver rather than to an enhanced rate of uptake per individual cell. We have observed a similar result after injecting an emulsion of [1-^{14}C]tripalmitin (A. G. Goodridge and E. G. Ball, unpublished results). As the experiment progressed there was a slight upward trend to the amount of tritium found in normal livers while in the livers of the prolactin group this trend was downward. Whether the more rapid increase seen in plasma-tritiated fatty acids in the prolactin group in the period 7–30 min as compared to the normal was a reflection of this difference in trend in the two groups is not certain. In any interpretation of these data it must be borne in mind that the tritiated tristearin was injected in the form of an emulsion. The amount present in the liver in this form *vis-à-vis* some other form that may be capable of release into the blood stream at any of the time periods was not known. For example, the bulk of the counts may have become segregated in the Kupffer cells and thus masked changes occurring in what may be termed a more metabolically active pool.

In considering the data on tritiated fatty acid content of adipose tissue presented in Table V the question may

be asked as to what percentage of these values was owing to radioactivity present in the plasma of the tissue. A similar question may be asked concerning the data presented in Figure 1. An approximate answer may be obtained in the following manner. If we take the fat content of adipose tissue as 85% of its wet weight and a value of 0.15 mg of nitrogen/100 mg wet wt (*cf.* Table III) then 1 mg of tissue nitrogen equals 100 mg wet wt of fat-free tissue. From the data of Hausberger and Widelitz (1963) it may be calculated that the blood volume of rat adipose tissue does not exceed 10% of the total volume of the fat free tissue. If this value is assumed to be similar in the pigeon there would be about 0.01 ml of whole blood or 0.005 ml of plasma in an amount of adipose tissue equivalent to 1 mg of nitrogen. Since the values in Table V are given in terms of 1 ml of plasma and 1 mg of adipose tissue nitrogen it may be readily seen that plasma radioactivity contributed less than 5% of the total counts found in adipose tissue. Thus the values for adipose tissue may be taken to reflect a radioactivity of fatty acids present within the adipose tissue cells themselves. These values did, however, tend to reflect those found in the plasma. Thus at 7 min both plasma and adipose tissue values in the prolactin group were lower than the normal. At 30 min the opposite was the case; both values were higher in the prolactin group than the normal.

In the studies presented here we have employed tracheal adipose tissue exclusively. In other studies (Goodridge and Ball, 1967) it was found that the time course and degree of incorporation of glucose carbon into fatty acids are the same for abdominal and tracheal adipose tissue when fasted-refed pigeons are used.

Discussion

In previous studies from this laboratory evidence (Goodridge and Ball, 1966, 1967) was presented which indicated that adipose tissue is not a major site of fatty acid synthesis in the pigeon. The experiments presented here lend further support to this conclusion and also serve to emphasize the magnitude of the role that the liver plays in lipogenesis in the pigeon. The pattern of the time course for the appearance of ^{14}C fatty acids in liver, plasma, and adipose tissue after the intravenous injection of [^{14}C]glucose is compatible with the hypothesis that fatty acids are synthesized in the liver, released to the blood, and then deposited in adipose tissue. This pattern is identical as shown here in normal and prolactin-treated birds or as shown elsewhere in fasted-refed birds (Goodridge and Ball, 1967).

The data given in Table V may be used to calculate the amount of plasma completely cleared of tritiated fatty acid by adipose tissue in a given time interval. The data from the interval 15–30 min are perhaps most suitable for this purpose. In this 15-min interval adipose tissue equal to 1 mg of nitrogen gained 3200 and 8000 dpm in the normal and prolactin groups, respectively. The average disintegrations per minute per milliliter of plasma during this period were 29,000 and 53,000 for the normal and prolactin groups, respectively. Thus, adipose tissue equivalent to 1 mg of nitrogen in the normal and prolactin group cleared 0.11 ml and 0.15 ml of plasma completely of tritiated fatty acids in the 15-min interval. Similar calculations may be made for ^{14}C fatty acids using the data given in Figure 1 for the interval 15–30 min. The net gain in dpm in this situation was 149 and 1910, respectively, for the normal and prolactin groups. The average disintegrations per minute per milliliter of plasma during this period was 840 and 9850 for the normal and prolactin groups, respectively. Thus in this case the clearance values were 0.18 and 0.19 ml of plasma/15 min by an amount of adipose tissue equivalent to 1 mg of nitrogen.

The clearance values calculated from the tritiated fatty acids are 58 and 77% of those calculated from the ^{14}C fatty acids in the case of the normal and prolactin groups, respectively. One interpretation of this difference is that the ^{14}C data yield higher values because total labeled fatty acid accumulation in the tissue in this case was the sum of clearance and *de novo* synthesis. On the other hand, it might be argued that data from the tritiated fatty acids may be low since unlike the ^{14}C data it could represent clearance from two plasma pools, a lipoprotein pool and a pool that is a residue of the injected emulsion, the latter with a lower clearance rate. In any case, the difference between the two sets of calculations is not great enough to invalidate the conclusion that the fatty acids in pigeon adipose tissue were predominantly derived from plasma triglycerides.

At first glance it would appear from the calculated clearance values that no significant difference exists between the rate of uptake of fatty acids by adipose tissue of normal and prolactin-treated birds. However, it must be recalled that the amount of total fatty acids in a given volume of plasma is some 60% higher in the prolactin group (*cf.* Table I). Thus, the amount of fatty acid taken up by adipose tissue of the birds receiving prolactin was greater than normal. Whether this was a consequence of the prolactin treatment or merely of the elevated plasma level of fatty acids is not apparent.

The calculated clearance values for fatty acids lie in the range of 0.11–0.19 ml of plasma/15 min per mg of tissue nitrogen and yield an average value of 0.16 ml. This is equivalent to 0.01 ml/min per mg of tissue nitrogen or 0.015 ml/min per g wet wt of tissue. Herd and Goodman (1966) reported blood flow rates in adipose tissue of rats that ranged from 0.10 to 0.16 ml/min for an average of 0.13 ml/g wet wt. If rates are similar in the pigeon and if we assumed plasma to be 40% of blood volume then about 29% of plasma was cleared of fatty acids during its passage through pigeon adipose tissue.

The results presented here on the rapid and striking changes produced in the lipogenic capabilities of pigeon liver after the injection of prolactin unfortunately furnish no clues as to how these changes are initiated by the hormone preparation. The liver could be the direct target organ of prolactin or of some other hormone whose release is triggered by prolactin. On the other hand, the response of the liver could be an adaptive change to a chain of events initiated by prolactin at a site remote from liver. For example, the primary action of prolactin could have been to increase food consumption. The in-

creases in size of pancreas, intestine, and liver described by Bates *et al.* (1937), and confirmed here for liver, then could all be ascribed to a hypertrophy due to hyperphagia. The increase in weight of these organs along with the deposition of fat engendered by the high caloric intake would account for the rapid increase in total body weight. As will be described in a later publication, we have observed that fatty acid synthesis (*in vitro*) and the activity of certain enzymes involved in lipogenesis, for example, malic and citrate cleavage enzymes, rise markedly in the liver of pigeons treated with prolactin. Similar changes have been observed in fatty acid synthesis and the activity of these enzymes in rat liver and adipose tissue when dietary regime is altered so as to stimulate lipogenic processes (*cf.* Ball, 1966).

Finally, the effects of prolactin upon lipogenic processes in the pigeon may be considered in relation to the rapid and intensive deposition of fat that occurs prior to migration in many species of birds. Meier and Farner (1965) have shown that daily injections of prolactin cause a rapid deposition of fat with a concomitant increase in body weight in the migratory white-crowned sparrow (*Z. leucophrys gambelii*). Thus, the lipogenic effect of prolactin appears to be similar in both the pigeon (a nonmigrant) and the white-crowned sparrow. If prolactin-induced lipogenesis and migratory fattening are similar in nature, then our results with the pigeon and those of Goodridge (1964) with three finch species suggest that attention should be focused upon metabolic changes in the liver rather than adipose tissue in future studies on migratory obesity. Although major increases in lipid translocation *via* the blood and net lipid uptake by adipose tissue almost surely accompany premigratory fat deposition, our results suggest that these effects may be secondary to an increased rate of lipogenesis occurring in the liver.

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