fasting, that is an initial depletion, return to base line, and finally depletion observable 64-88 hours after onset of the fast. It was concluded that a variety of apparently unrelated stimuli could alter the GH content of the pituitary. Taken together with other evidence it appears that GH secretion in the rat is very labile and influenced by a variety of stresses as well as by alterations in the supply of available carbohydrate.

1. Krulich, L., McCann, S. M., Endocrinology, 1966, v78, 759.
2. Greenspan, F. S., Li, C. H., Simpson, M. E., Evans, H. M., ibid., 1949, v45, 455.
3. Krulich, L., Dhariwal, A. P. S., McCann, S. M., Proc. Soc. Exp. Biol. and Med., 1965, v120, 180.
4. Roth, J., Glick, S. M., Yalow, R. S., Berson, S. A., Diabetes, 1964, v13, 355.
5. Knobil, E., The Physiologist, 1966, v9, 25.
6. Smith, G., Katz, S., Root, A., Eberline, W. R., Bongiovanni, A. M., Dhariwal, A. P. S., McCann, S. M., Program of the 48th Meeting of the Endocrine Society, 1966, p35.
7. Friedman, R. C., Reichlin, S., Endocrinology, 1965, v76, 787.
8. Meites, J., Fiel, N. J., ibid., 1965, v77, 455.
9. Franz, J., Haselbach, C. H., Libert, O., Acta Endocrinol., 1962, v41, 336.
10. Deuben, R. R., Meites, J., Endocrinology, 1964, v74, 408.
11. Ishida, Y., Kuroshima, A., Bowers, C. A., Schally, A. V., ibid., 1965, v77, 759.
12. Dhariwal, A. P. S., Krulich, L., Katz, S., McCann, S. M., ibid., 1965, v77, 932.

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Gestational and Dietary Influences on the Lipid Content of the Infant Buccal Fat Pad.* (31206)

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The buccal fat pad contributes significantly to the prominence of the cheek of the newborn and infant. It is well developed in the premature fetus, appears to be one of the earliest sites of well developed fetal adipose tissue deposition, and probably is important in the sucking mechanism.

The preservation of the buccal fat pad during emaciated states of infancy and childhood remains unexplained. Early investigators(1) believed that compositional differences might explain this phenomenon; however, Shattock(2) in 1909 reported that the chemical composition of the buccal pad was identical to subcutaneous fat utilizing iodine values and melting points. Inadequate methodology prevented detailed chemical analysis. Recent technologic advances now make it possible to obtain an accurate fatty acid analysis with relative ease. To determine whether biochemical differences explain the resistance of this tissue to mobilization during emaciated states, this specialized adipose tissue was analyzed and its composition compared to that of abdominal subcutaneous adipose tissue during various stages of intrauterine and post-partum development.

Methods. Obtaining the sample. The buccal pad is easily exposed through a small cruciate incision in the buccal mucosa inferior to the zygomatic bone in a fossa formed by the masseter, buccinator, and platysma muscles. Small pieces of the right buccal fat pad and abdominal subcutaneous fat were obtained at necropsy from unfed infants who expired within 24 hours after birth. Infants of mothers with known metabolic disease were excluded from this study. Comparable specimens were obtained from older children who died from non-metabolic causes.

Preparation and analysis of the sample. Fragments of tissue were pulverized with a glass rod during lipid extraction in a 1:1 mixture of petroleum ether (BP 30°-60°C) and isopropyl alcohol. Fatty acid methyl esters

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were prepared for analysis by gas-liquid chromatography (GLC) by interesterification with methanol-HCl(3). Identification and measurement of all samples was done by elution at 184.5°C from a column of celite particles coated with ethylene glycol adipate polyester. Details of the GLC techniques employed are those described by Farquhar *et al*(4). The percentage composition of the individual fatty acid methyl esters was calculated from peak areas determined by triangulation.

The major lipid classes were identified qualitatively by thin-layer (silica gel) chromatography(5) in 3 samples from a premature infant, a full-term infant, and a 5-year-old child. Chromatoplates were developed with a mixture of petroleum ether (60-70°), ethyl ether, and glacial acetic acid (67.2:32.4:0.4—by volume).

Results. Six fatty acids—myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2)—constituted over 90% of the total number of triglyceride fatty acids identified by GLC. The compositional similarity of abdominal and buccal fat triglyceride is demonstrated in Table I. However, small differences are present. Analysis of the differences utilizing Student's t-test indicates consistent and significantly higher 16:1 (4.29% ± 1.56) values in the abdominal site of the premature and term infants sampled. The increased 18:0 (1.27% ± .90) content of buccal fat throughout gestation is also statistically significant. When compared with normal adult tissue, a general increase in 16:0 and decrease in 18:2 fatty acids observed in fetal adipose tissue during the last trimester of pregnancy(5) is reflected in both buccal and abdominal samples. These changes are most marked in the larger term infants. Adult values are approached during late infancy and early childhood. The *in utero* decline and post-partum rise in 18:2 is shown in Fig. 1.

No qualitative differences in the major lipid classes (triglyceride, cholesterol, free fatty acid, partial glyceride, and phospholipid) could be demonstrated at the 2 sites by thin-layer chromatography. Triglyceride consti-

TABLE I. Abdominal Subcutaneous *vs* Buccal Fat. A comparison of fatty acid composition of adipose tissue removed from abdominal subcutaneous tissue and buccal fat pad in the same subject.

Fatty acid	Wt	750 g	850 g	1300 g	1500 g	3030 g	3560 g	4420 g	Mean difference abd. minus buccal ± S.D.	4590 g 37 days	11 kg 16 mo	18.2 kg 5.5 yr	Normal adult*
14:0	Abdominal	2.3	3.6	4.8	3.4	5.0	2.5	4.4	+.67 ± .87	8.5	7.5	5.7	3.3 ± .1
	Buccal	3.3	2.0	4.1	3.1	3.4	1.7	3.7	.1 < p > .05	4.9	10.8	5.9	
16:0	Abdominal	31.4	37.5	41.1	41.4	37.6	44.9	36.9	+.90 ± 5.09	32.2	35.4	22.4	19.5 ± 2.1
	Buccal	31.6	38.1	35.7	37.9	37.3	53.3	42.1	.7 < p > .6	32.1	28.5	25.4	
16:1	Abdominal	19.7	16.5	13.3	16.2	17.0	11.8	15.4	+4.29 ± 1.56	13.3	6.5	5.1	6.9 ± .1
	Buccal	12.6	12.6	11.4	10.0	12.7	6.9	11.7	p < .001	10.1	6.6	6.4	
18:0	Abdominal	4.2	2.1	4.7	2.9	3.6	4.4	4.5	−1.27 ± .90	5.0	5.1	7.8	4.2 ± 1.1
	Buccal	4.4	3.4	6.6	5.4	5.6	5.1	4.8	p < .01	8.0	4.0	5.0	
18:1	Abdominal	31.9	31.4	29.1	29.3	31.0	33.2	35.3	−3.60 ± 4.70	31.8	35.5	45.6	41.2 ± 4.4
	Buccal	36.2	38.8	33.2	38.6	36.5	29.6	33.5	.1 < p > .05	39.4	38.9	43.7	
18:2	Abdominal	4.1	4.6	2.9	3.2	2.1	2.8	1.6	.57 ± 1.09	1.2	2.6	4.9	11.4 ± 1.4
	Buccal	5.0	2.9	4.5	2.7	2.4	2.2	2.0	.9 < p > .8	1.2	3.0	5.0	
% of total	Abdominal	93.6	95.7	95.9	96.4	96.3	99.6	98.1		92.0	92.6	91.5	
	Buccal	93.1	97.8	95.5	97.7	97.9	98.8	97.8		95.7	91.8	91.4	

* From Hirsch, J., *et al*(6).

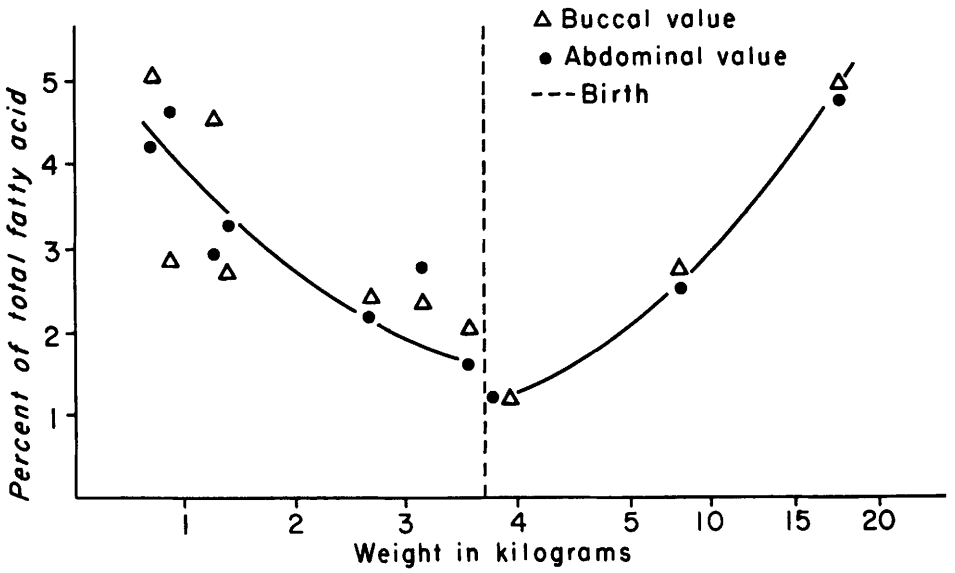

FIG. 1. Changes in 18:2 content of abdominal subcutaneous and buccal fat during *in utero* and post-partum development.

tuted all but trace amounts of the total lipid studied in each case.

Discussion. The fatty acid profile of the 750 g infant is most similar to maternal adipose tissue stores which have been shown to be the same as normal adult tissue(6). However, the fetal changes occurring during the last trimester of pregnancy result at term in a profile of major fatty acids markedly different from maternal composition. The increments seen here in 16:0 and 16:1, and the progressive decline in 18:2 in the sampled sites of each infant are typical of the pattern to be expected with lipogenesis resulting from feeding a high carbohydrate diet(7). In the last trimester of pregnancy there is a 12-fold increase in body fat while the total body weight increases only 3-fold(8). The major fraction of adipose fat in the newborn is a result of this marked lipogenesis late in pregnancy. The composition of this fat suggests that it is made primarily from maternal carbohydrate.

Though the small differences demonstrated in fatty acid composition of triglyceride from infant buccal and abdominal subcutaneous fat are statistically significant, they probably do not reflect intrinsic physiologic dissimilarities. The consistently close relationship of the values of 18:2 at the 2 sites in each member of the series suggests that the metabolic response of the buccal pad measured by intrauterine and post-partum lipogenesis is similar to that of other major body sites of adipose tissue deposition.

Summary. Comparison of the lipid content of the buccal and abdominal subcutaneous fat depots by gas liquid and thin-layer chromatography during gestation and infancy reveals no qualitative difference in major lipid classes, and small unexplained differences in triglyceride fatty acid composition. Similar alterations in composition occur in both tissues during the third trimester of pregnancy. The metabolic response of adipose tissue in these two sites to *in utero* and dietary influences of infancy is probably identical. Compositional differences do not appear to explain the preservation of the buccal pad in starvation states.

1. Scammon, R. E., Anat. Rec., 1918-19, v15, 267.
2. Shattock, S. G., Proc. Roy. Soc. Med., 1909, v2, 207.
3. Stoffel, W., Chu, F., Ahrens, E. H., Jr., Anal. Chem., 1959, v31, 307.
4. Farquhar, J. W., Insull, W., Rosen, P., Stoffel, W., Ahrens, E. H., Jr., Nutrit. Rev., supplement, v17, 11.

5. Mangold, H. K., Malins, D. C., J. Am. Oil Chem. Soc., 1960, v37, 383.
6. Hirsch, J., Farquhar, J. W., Ahrens, E. H., Jr., Peterson, M., Stoffel, W., Am. J. Clin. Nutrit., 1960, v8, 499.
7. Ahrens, E. H., Jr., Hirsch, J., Oette, K., Farquhar, J. W., Stein, J., Trans. Assn. Am. Phys., 1961, v74, 134.
8. Widdowson, E. M., Spray, C. M., Arch. Dis. Child., 1951, v26, 205.

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Progesterone on Blood and Tissue Iron in Albino Rats. (31207)

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Estrogen causes severe anemia in both male and female animals(1,2,3,4). Dutta and Mukherjee(5) have recently observed that natural and synthetic estrogen reduced blood iron levels and increased iron content in the kidney. Nigst(6) has noted a stimulating effect of estradiol benzoate on the blood forming tissues. It has also been observed(7) that administration of either progesterone or testosterone increased blood iron. Estrogen being the dominating gonadal hormone in the female apparently has a tendency to produce an anemic state which is probably balanced by progesterone. From these observations the possible controlling role of progesterone on the mobilization of iron has been sought in albino rats.

Materials and methods. Healthy albino rats, laboratory inbred of both sexes, weighing 180 ± 20 g were selected for this experiment. The animals were placed in individual cages and were pair-fed a diet consisting of germinated Bengal gram *(Cicer arietinum)* and loaf. Water was supplemented *ad libitum*. Average food consumption per day per rat was estimated at 25 ± 2 g.

Animals were divided into 3 groups, each group consisting of 24 rats—12 males and 12 females. One group of animals was treated for 3 days and another group for 10 days with progesterone (2 mg/rat/day). Remaining animals served as controls. Progesterone-treated rats were sacrificed 24 hours after the last injection. From the control group of rats 6 males and 6 females were sacrificed at the end of 3 days and 10 days, respectively, along with 2 different experimental groups of rats. Blood and tissues were collected from each animal for hematological examination and iron estimation. Blood was collected by cardiac puncture in mixed oxalated tubes for hematological examination as well as for total blood iron, hemoglobin(8) tissue iron(9) and plasma iron(10). Total erythrocytes and leucocytes were counted employing standard pipettes and a Neubauer's counting chamber. Differential counts were done on dried smears.

Results and discussion. Progesterone treatment (2 mg/rat/day) for 3 and 10 consecutive days in both male and female rats increased total blood iron level and plasma iron level with a decrease in tissue iron content of bone marrow and liver. Change in the spleen iron content was apparent only in male rats and no alternation in the iron content of kidneys was observed either in male or female rats. No change was observed in total cell count or differential count in the blood from progesterone-treated rats. It has been noted (5) that both natural and synthetic estrogen causes a significant lowering of blood iron level, a rise in iron content of liver and kidney and a fall in spleen and bone marrow iron content. Thus the actions of estrogen and progesterone on iron level maintenance and regulation are almost opposite. The lowering of tissue iron content by progesterone may be the result of tissue iron release which apparently is enhanced by progesterone. This is further corroborated by an increase in transportable iron in the plasma. Progesterone neither stimulated nor depressed the hematological activity as the peripheral blood