

THE CELLULAR DEVELOPMENT OF ADIPOSE TISSUES*

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PREFACE

Let me preface my remarks by saying that much of the data I have included in this paper are from species other than meat animals. This is not to imply that the results from the rat can be applied to the pig or from the pig to beef cattle or so on. It is included mainly for comparative purposes and also because not a great deal of data are available in meat animals. However, thanks to the cooperation of Dr. R. L. Hood and Dr. C. E. Allen (University of Minnesota) and Mr. Y. Lee and Dr. R. G. Kauffman (University of Wisconsin), I have been able to include some of the most current meat animal research on this subject.

INTRODUCTION

From a histological standpoint, adipose tissue is not a very dramatic tissue. Adipose cells have no striations, no Z-lines, no filaments and no processes. They do not contract, secrete or conduct, but they do proliferate and they do expand. The development and consequence of this proliferation and expansion is the subject of this paper.

A knowledge of the cellular composition of adipose tissue is necessary for a meaningful expression of metabolic data. The average adipose cell diameter can vary from less than 20 microns to greater than 160 microns depending on the age and nutritional state of the animal and the location of the adipose tissue sample. Therefore, the expression of metabolic parameters on a per cell basis is much more meaningful than on a weight basis. In addition, many of the functions of adipose tissue metabolism are related to its cellular composition. The insulin responsiveness of human adipose tissue, for example, decreases as adipocyte size increases. Glucose oxidation to CO₂, however, is independent of cell size alterations (Salans, *et al.*, 1968).

Adipose tissue mass can expand by either hyperplasia (cell proliferation), hypertrophy (cell enlargement) or a combination of the two. The biological mechanisms that control cell mitosis and proliferation are probably quite different from those which cause cellular hypertrophy. Consequently, a description of cellular changes and abnormalities of adipose tissue during normal growth and experimental manipulation is essential for the understanding of the etiology of excessive fat accumulation.

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Much of the research reported in this paper has been made possible by the development of an improved technique for the measurement of adipose tissue cellularity (Hirsch and Gallian, 1968). The method involves treating adipose tissue by prolonged fixation with osmium tetroxide followed by separation of the fixed adipose cells by screening (figure 1). The fixed cells are suspended and counted by the use of an electronic particle measuring device called a Coulter counter. Sizing the cells can be accomplished also by use of the Coulter counter or by direct measurements under a light microscope. By the use of this technique accurate measurement of adipose cell size and number can be made on any sample of adipose tissue with one exception. Immature adipose cells with very small amounts of lipid are usually lost in the preparatory process during screening. Consequently, the technique is a measure of mature adipocytes--adipose cell precursors and preadipocytes are not measured.

ADIPOSE CELL DEVELOPMENT DURING GROWTH

The domestic pig is representative of the normal pattern of adipose cell development found in most other species which have been studied. This pattern is demonstrated in figure 2 (Anderson and Kauffman, 1972). Total adipose cell number was extrapolated from the average cells per gram tissue of the outer and middle subcutaneous, since these two depots make up the majority of all carcass fat in the pig (Kauffman and St. Clair, 1965). These data show that changes in total carcass adipose tissue in young pigs (between 1 and 2 months) were due primarily to increases in the number of adipose cells. Between 2 and 5 months, changes were due to a combination hypertrophy and hyperplasia. After 5 months there were no significant increases in adipose cell number; consequently, adipose mass increased solely by the process of cell enlargement. From our work and also that of Hood and Allen (1972a), it appears that a plateau in cell number is reached between an age of 5 and 6 months. After this age, increases in the size of the adipose depot are accomplished almost exclusively by the process of cell enlargement. This is even more evident in figure 6 which shows the cellular development of adipose tissue in the Hormel miniature pig. The total cell number of the miniatures plateaued between 37 and 54 kg. At this weight the miniatures were a little less than 6 months old. The possibility exists in the younger animals (1 and 2 months of age) that a certain percent of the cells were not counted simply because they were too small to be measured. Consequently, we can say with certainty only that porcine adipose tissue increased by a combination of hypertrophy and hyperplasia up to 5 months, after which time it expanded by hypertrophy only.

In the rat, adipose tissue cell number remains constant from 15 weeks to adulthood or the point at which a stable body weight is reached (Hirsch and Han, 1969). Whether or not the plateau in cell number at 5 months is the maximum attainable number of cells in the pig is not certain. It seems quite possible that if the pig were to continue to be fattened that at some point cell size would become so large that hyperplasia would again be necessary.

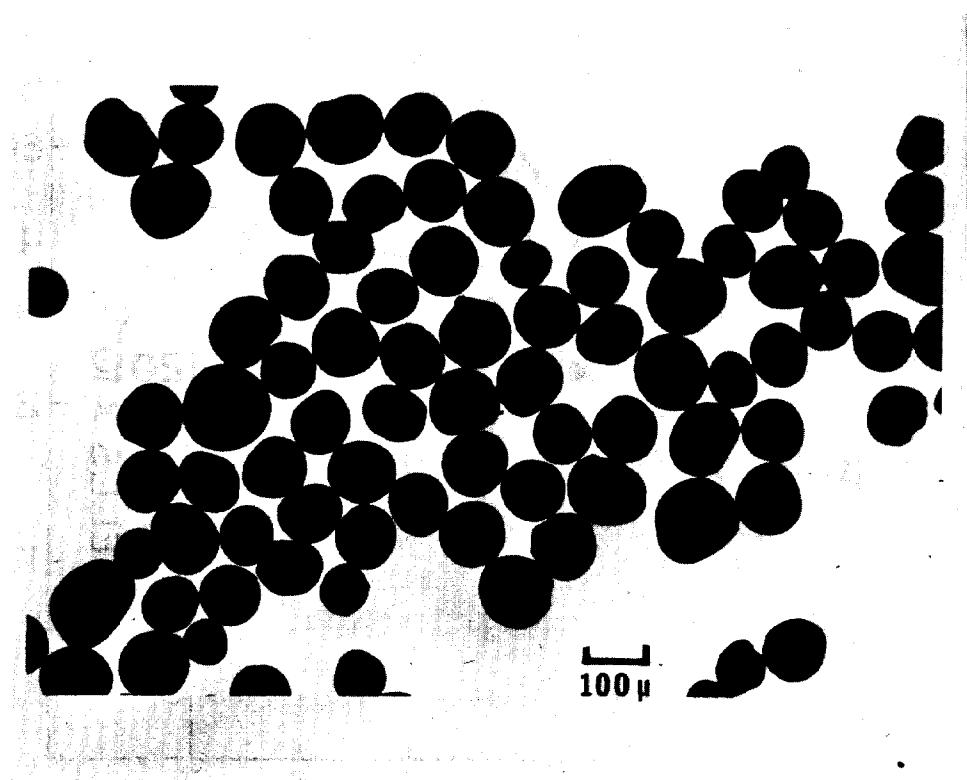


Figure 1. Example of osmium tetroxide fixed adipose cells which have been prepared for counting on a Coulter electronic counter by the method of Hirsch and Gallian (1968).

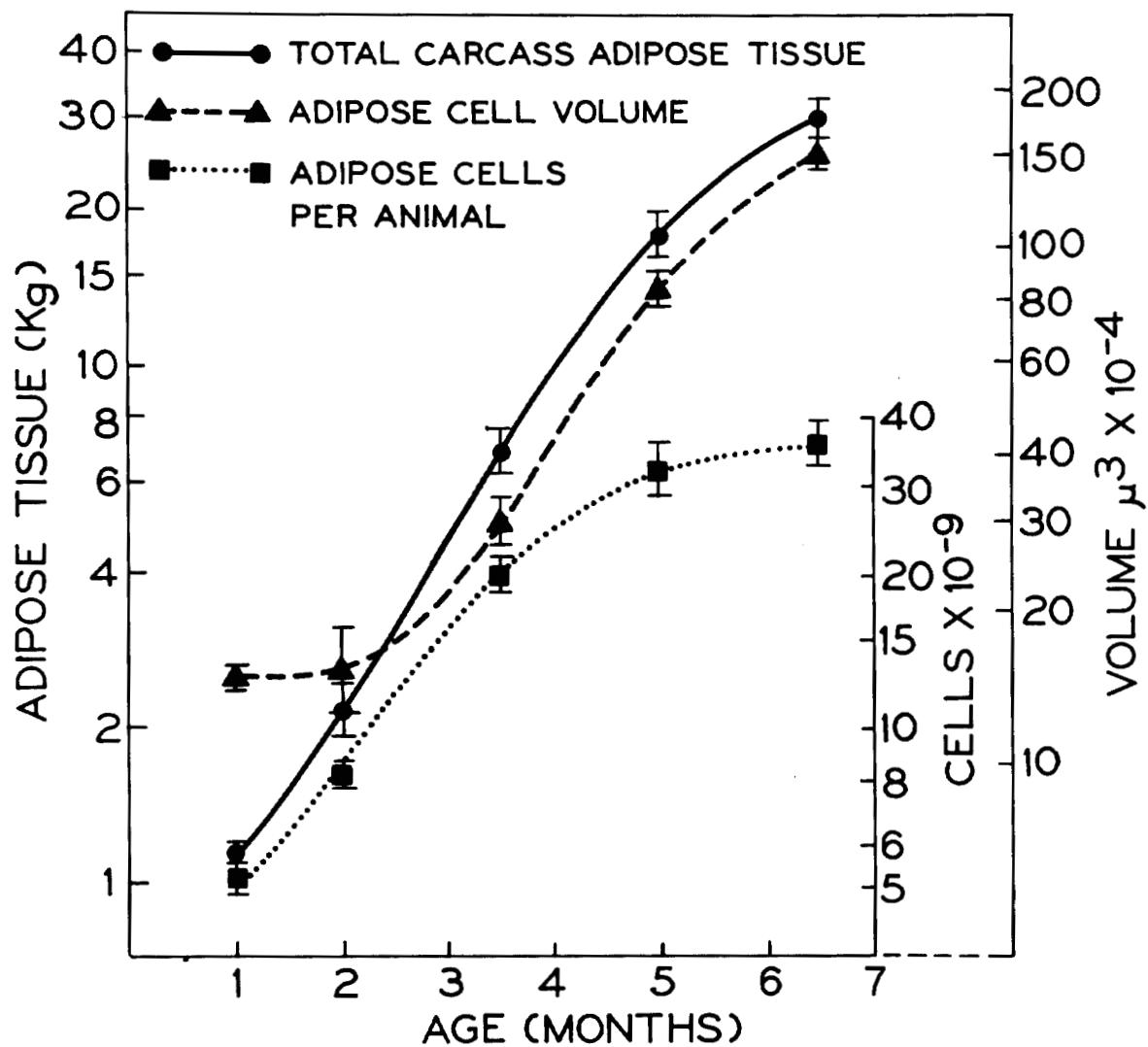


Figure 2. Changes in the accumulated weight of carcass adipose tissue (solid line), the volume of adipose cells from the middle subcutaneous backfat region (dashed line), and extrapolated total adipose cell number (dotted line) as determined by Coulter counter measurements of osmium fixed cells.

Figure 3 shows data to indicate that species can vary significantly in the method of adipose tissue enlargement. Using microscopic measurements on isolated cells from collagenase treated epididymal adipose tissue, DiGirolamo and Mendlinger (1971) found that the rat and hamster had a considerable capacity to enlarge the fat cell size from age 6 weeks to 1 year (8.2-fold and 4.5-fold increase in mean cell volume, respectively) while the guinea pig had only a limited capacity in this respect with only a 1.8-fold increase. Conversely, in the same time interval, the guinea pig showed a marked increase (12.7-fold) in the number of fat cells in the pad, while the rat and hamster had only a two-fold and a 1.4-fold increase. Their results suggest definite species differences in that the guinea pig expands in its epididymal adipose tissue mainly by an increase in the number of fat cells with little change in cell size, while the rat, hamster and pig do so mainly by an enlargement of the individual adipose cells.

CELLULARITY OF THE OBESE

A question of considerable importance in the study of adipose tissue development is how obesity or excessive fat accumulation is expressed in cellular terms. Hirsch and Knittle (1970) in their work with excessively obese human subjects (four times the normal amount of adipose stores) found that both adipose cell size and number was greater in the obese subjects as compared to non-obese; however, adipocyte number had increased to a greater extent (190%) than adipocyte size (40%). The degree of obesity was found to be directly and highly correlated with the number of adipose cells (figure 4a). The correlation coefficient was .8117. Subjects studied varied in their content of body fat from 42 to 103 kg compared to an average of 18 kg in the non-obese. No correlation could be made between the degree of fatness and cell size (figure 4b).

The data suggested that human obesity developed largely as the result of hyperplasia. However, in a further attempt to categorize the data, Hirsch and Knittle used family histories and photos to divide the obese subjects into groups according to the time of onset of obesity. They found that those individuals whose weight problems began before the age of 10 years comprised the majority of the group with the highest cell numbers. Six of the 8 cases (onset at age less than 10 years) had cell numbers in excess of 90×10^9 or 3.5 times the normal level. The group whose history clearly showed adult onset obesity (after 20 years of age) were among the lower cell numbers of the obese subject but were among the largest cell sizes. The data suggest that childhood obesity is associated most frequently with a hyperplastic increase in adipose depots whereas the degree of hyperplasia is much less in adult onset obesity with hypertrophy assuming a greater importance.

Salans *et al.* (1971), provided additional evidence that adult onset obesity is accompanied mainly by hypertrophic changes in the adipocyte. They studied a group of adult male volunteers who made gains of 15% to 25% in their body weight after a prolonged period of high caloric intake. Cell size was approximately doubled in all subjects at the peak of the weight

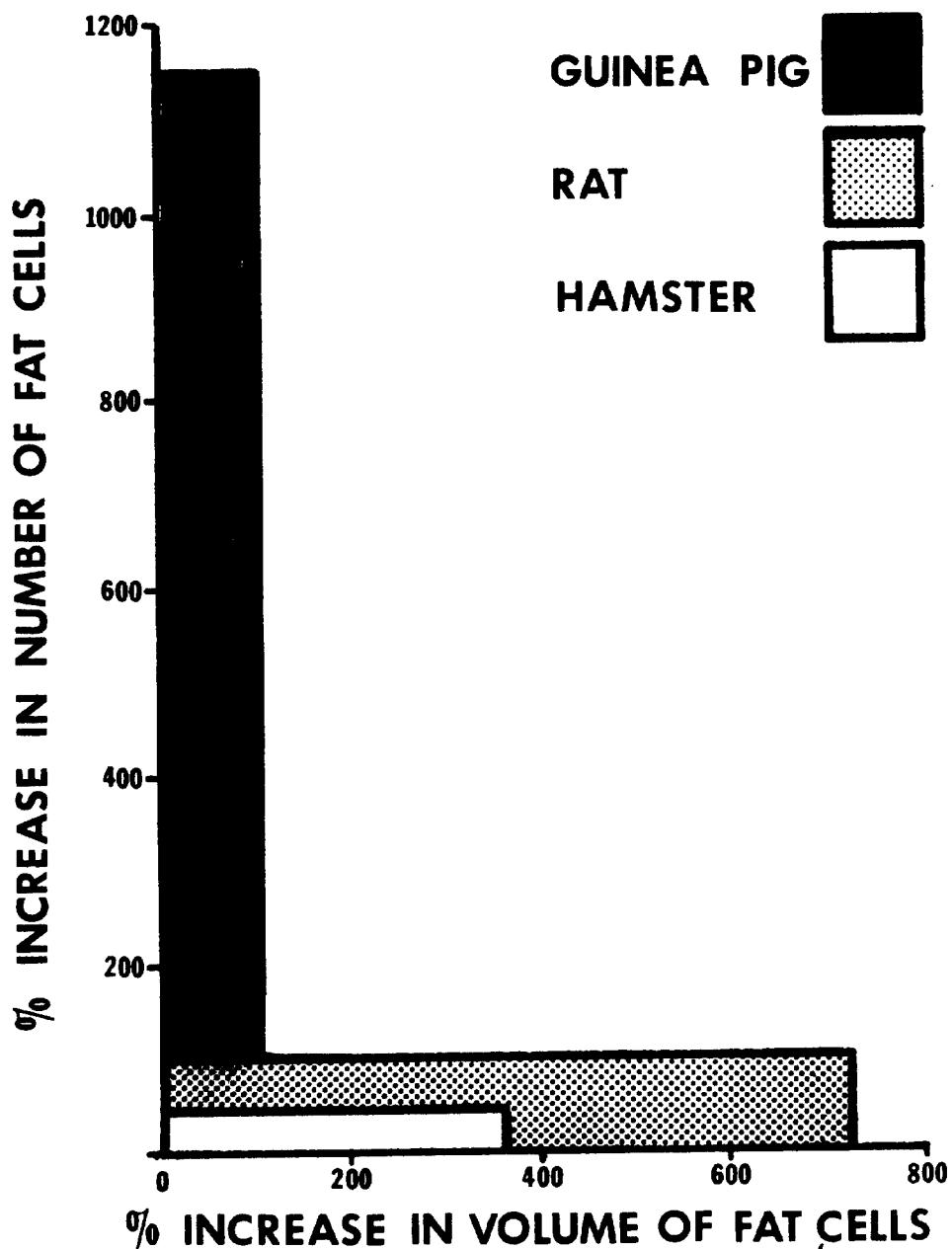


Figure 3. Relative increase in mean fat cell volume and number in epididymal fat pads of rats, hamsters, and guinea pigs fed ad libitum from age 6 weeks to 11-15 months. Values for fat cell volume and number determined in respective groups of 6-wk-old animals were set at 0%. DiGirolamo, et al., Amer. J. Physiol. 221:859 (1971).

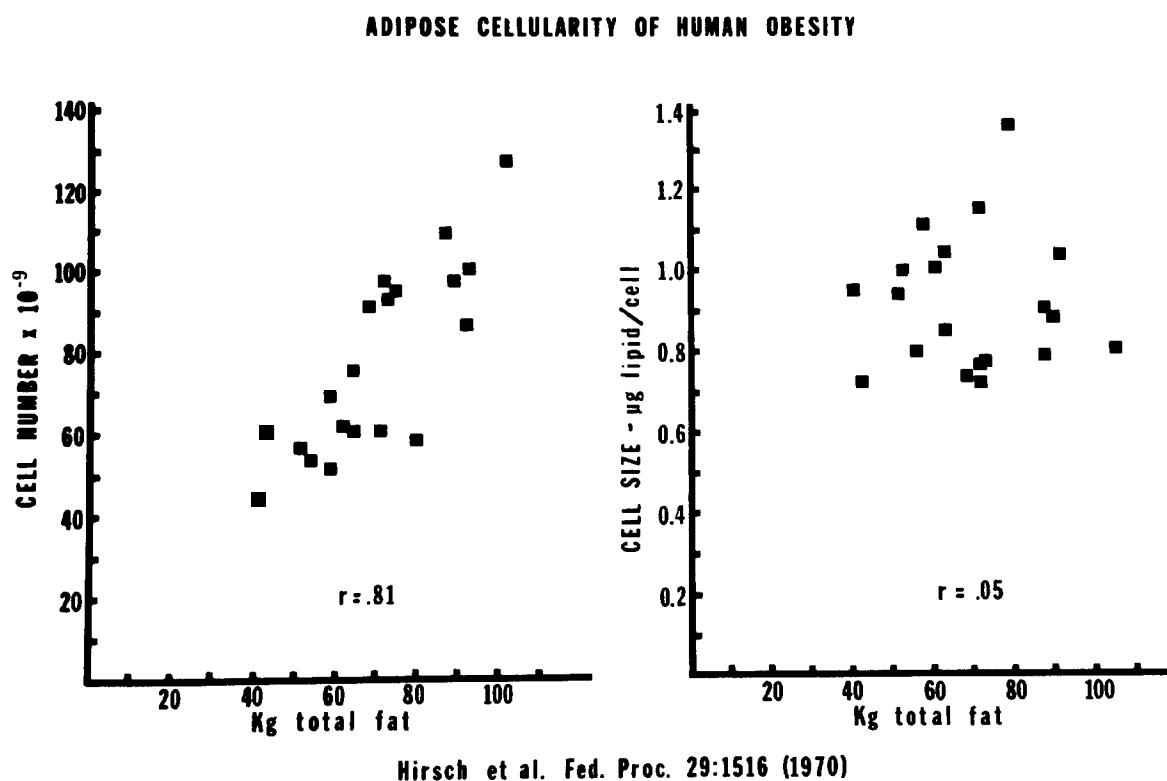


Figure 4. Adipose cellularity of human obesity. Hirsch and Knittle, Fed. Proc. 29:1516 (1970).

gain and then decreased to normal size after reducing to their original weights. Cell number was not significantly changed in any of the subjects. The work of Bjorntorp and Sjostrom (1971) also indicates that with moderate enlargement of the body fat stores (20-40 kg), fat cell size is the dominating factor contributing to increased adipose tissue, whereas, with severe obesity cell number is the more important factor.

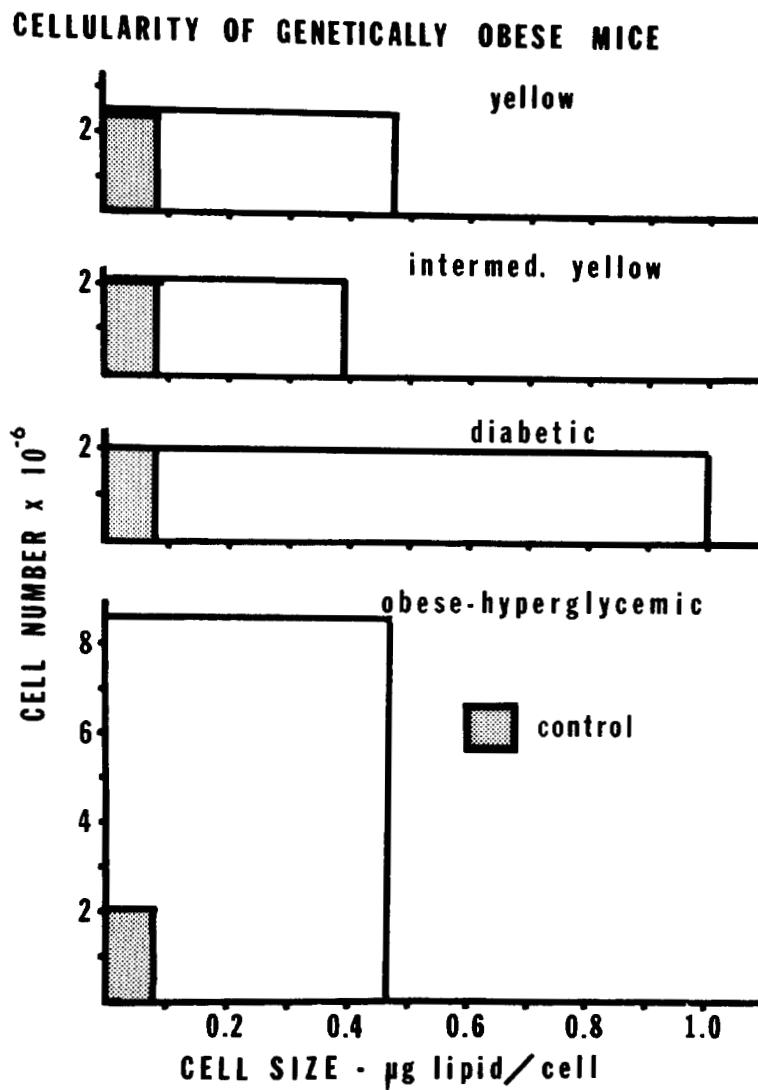
Animal experiments have shown that genetic obesity in the rat and mouse expresses itself either by hypertrophy or by a combination of hypertrophy and hyperplasia (figure 5). In this study, Johnson and Hirsch (1972) compared the cellular composition of four strains of mice, whose obesity had developed from single gene mutations. The four strains of obese mice (yellow, intermediate yellow, diabetic and obese-hyperglycemic) were compared with the non-obese littermates of the yellow and intermediate yellow.

The cellular composition of the subcutaneous adipose tissue from 26 week old females shows that the obesity in the first three strains is manifested strictly by an increase in cell size; cell number was not significantly different from controls. In the obese-hyperglycemic mouse, however, both cell size and cell number were increased. The Zucker "fatty" rat, also a single gene mutation for obesity, exhibits a hyperplastic-hypertrophic obesity similar to that of the obese-hyperglycemic mouse (Johnson *et al.*, 1971). Thus, the two general classifications were suggested: Hypertrophic, which would be a model for adult onset obesity and hypertrophic-hyperplastic, which would be a model for early onset extreme obesity in humans.

The work of Hood and Allen (1972a) suggests that excessive fat accumulation in the pig is of the first type; that is, caused by hypertrophy only with no increases in cell number. The development of adipose tissue in three strains of pigs was studied--muscular Hampshire x Yorkshire cross-bred barrows, fat-type Minnesota 1 x Minnesota 3 crossbred barrows, and the Hormel miniature (figure 6). The pigs were taken to a constant weight rather than a constant age. For the miniatures, the weights were 28, 37, 45 and 54 kg, and for the Hamp x York and the Minn 3 x 1 the weights were 28, 54, 83 and 109 kg.

At 109 kg, the difference in extramuscular carcass fat was 16%. The Hamp x York had 30.6% fat, the Minn 3 x 1 had 46.6%. This difference was due completely to the larger cell volume of the Minn barrows (9×10^5 cubic microns as compared to 5×10^5 for the Hamp x York). Even though the Minn barrows had 16% more fat, the total adipose cell number was less. Consequently, the excessive fat accumulation in the Minn strain can be attributed strictly to hypertrophy.

Further comparisons were made between littermates of varying degrees of fatness. The percent carcass fat was found to be more highly correlated with adipose cell size than it was with adipose cell number (correlation coefficients of 0.44 and 0.15, respectively) (Hood and Allen, 1972a). In human adipose tissue in the range of low to normal body fat, adipose cell size is also the most important factor in determining the amount of body fat (Bjorntorp *et al.*, 1971; Bjorntorp and Ostman, 1971; Salans *et al.*, 1971).



Johnson et al. J.L.R. 13:2 (1972)

Figure 5. Relative contributions of cell size and cell number to adiposity of subcutaneous depots in female genetically obese mice. Controls are non-obese littermates of the yellow and intermediate yellow. Johnson and Hirsch, J. Lipid Res. 13:2 (1972).

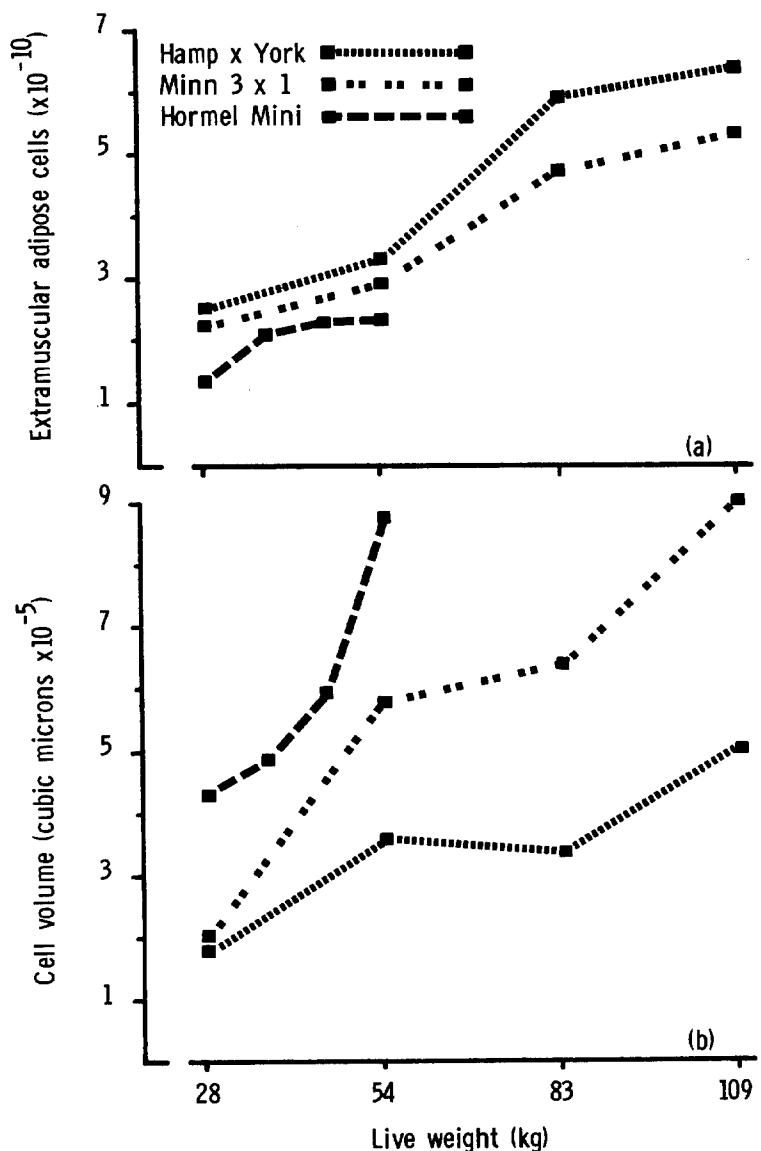


Figure 6. Increase in the number and volume of extramuscular adipose cells during growth of three breeds of swine. Hood, Ph.D. Thesis (1972).

EXPERIMENTAL MANIPULATION OF ADIPOSE CELLULARITY

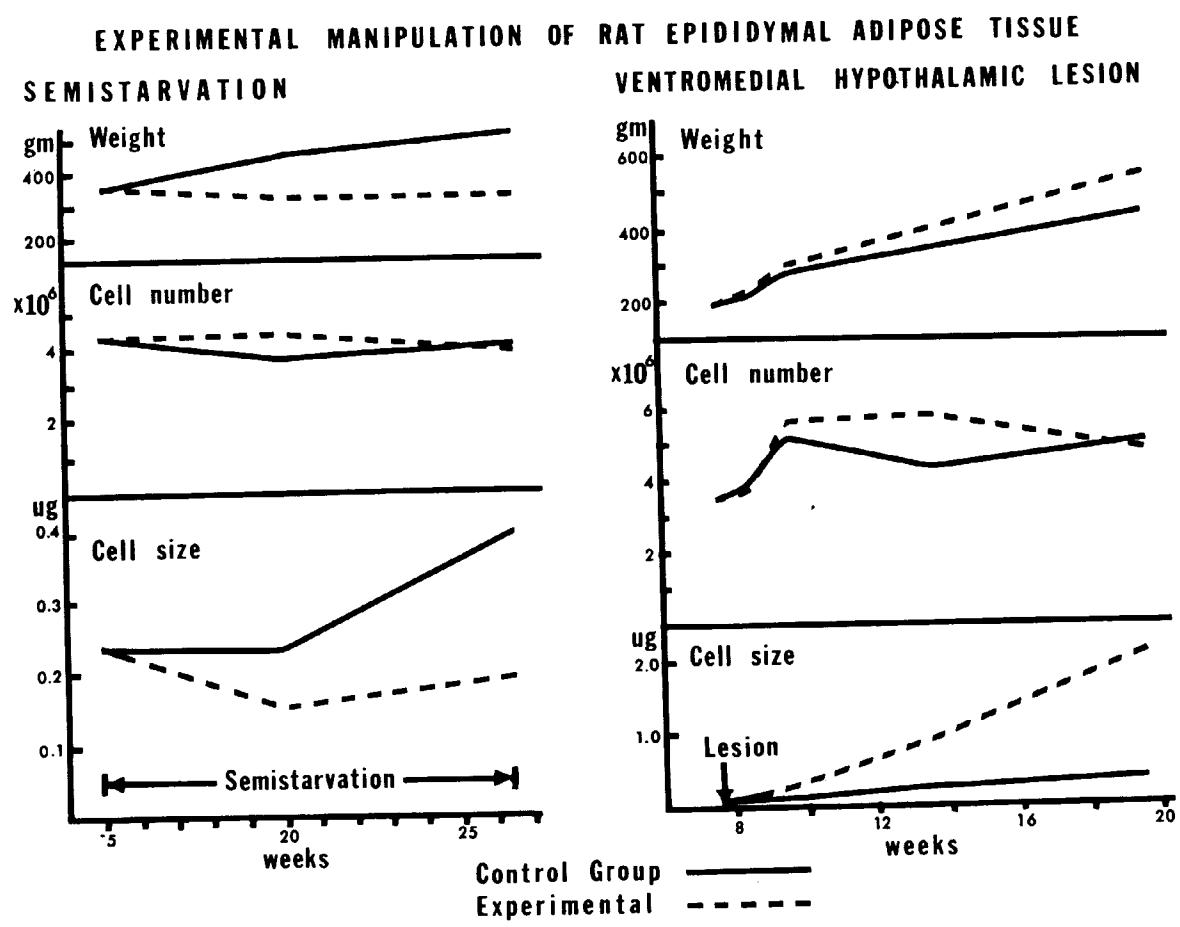
Experimentation to either increase or decrease the adipose tissue mass has resulted, in some instances, in a change in total adipose cell number; but, for the most part, adipose cell number tends to remain unchanged by experimental treatment. For example, manipulation of adipose tissue either by starvation or experimental obesity has resulted in changes in cell size with no appreciable change in cell number. Two examples of such manipulation of rat adipose tissue are shown in figure 7 (Hirsch and Han, 1969).

Figure 7a demonstrates the results of semistarvation on adipose cellularity. The starved animals received one-half the caloric intake of the controls starting at the 15th week of age and continuing for 11.5 weeks. Cell size decreased in the semistarved group during this period; however, cell number was not significantly changed. In figure 7b, adipose mass was increased by destruction of the ventromedial hypothalamic nuclei at 7 weeks of age, resulting in hyperphagia due to the destruction of the satiety center in the hypothalamus. The result was a large increase in body weight as compared to sham-operated controls which was a reflection of the enormous expansion of the adipose tissue. Again, the expansion was accompanied by a six-fold increase in cell size with no significant change in cell number.

Hood and Allen (1972a) obtained similar results when studying the effect of starvation on porcine adipose tissue (figure 8). Changes were measured during growth from 83 kg to 109 kg and then during starvation from 109 kg back to 77 kg or the point where carcass weight of the starved group was approximately equal to that of the 83 kg growing pigs. Carcass fat of the starved Hamp x York pig was reduced to a lower level than that of its 83 kg counterpart (figure 8a). This change was the result of changes in cell volume (figure 8b) with no significant changes in cell number (figure 8c).

The carcass fat in the Minn strain, however, was not reduced to the extent that it was in the Hamp x York barrows (figure 8a). Carcass fat did not go below that which had been determined for the 83 kg growing pig. In fact, perirenal fat in the starved Minn pigs showed no significant reduction in amount indicating an impairment of fat mobilization in the Minn strain. Cell size changes, however, were similar to those of the Hamp x York. That is, average cell size decreased below that of the 83 kg growing pig. Unexpectedly, however, the total adipose cell number continued to increase even during the starvation period. Hood suggested that the most plausible explanation for these results would be that cell number in the Minn barrows had not yet reached a plateau level at 109 kg and at this stage of growth, starvation had no effect in retarding adipose cell proliferation. The explanation is supported by the work of Hirsch and Han (1969) which shows that starvation at 6 weeks of age and prolonged semistarvation at 15 weeks of age had no effect on the adipose cell number attained by the adult rat, and also by the work of Johnson and Hirsch (1972) which shows that obese mice reach a plateau in cell number at an older age than non-obese.

Figure 7. Effect of starvation and experimental obesity on the cellularity of rat adipose tissue. Hirsch and Han, J. Lipid Res. 10:77. (1969).



Hirsch and Han, J. L. R. 10:77 (1969)

EFFECT OF STARVATION ON
PORCINE ADIPOSE TISSUE

■ 83 kg growing pig
■ 109 kg growing pig
▨ 77 kg fasted from 109 kg

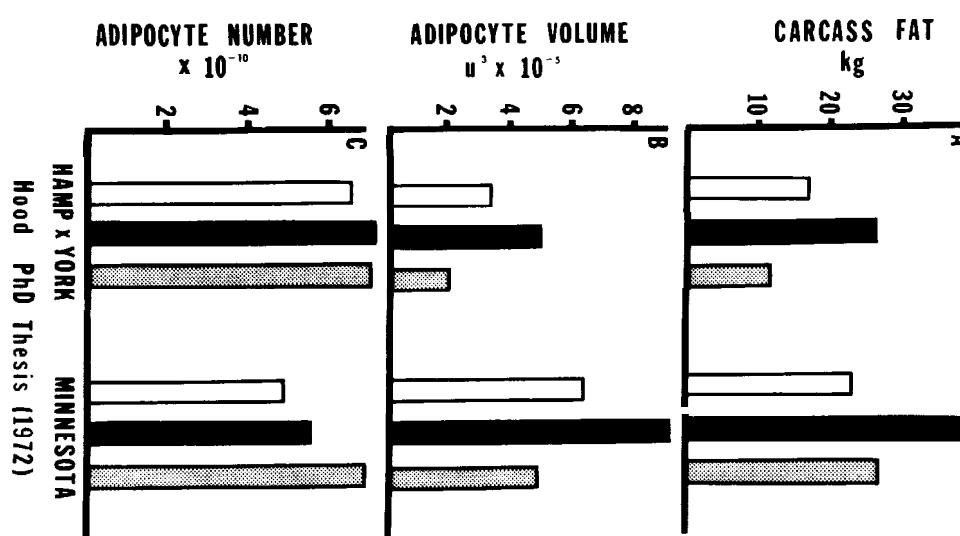


Figure 8. Effect of starvation on porcine adipose tissue. Hood, Ph.D. Thesis (1972).

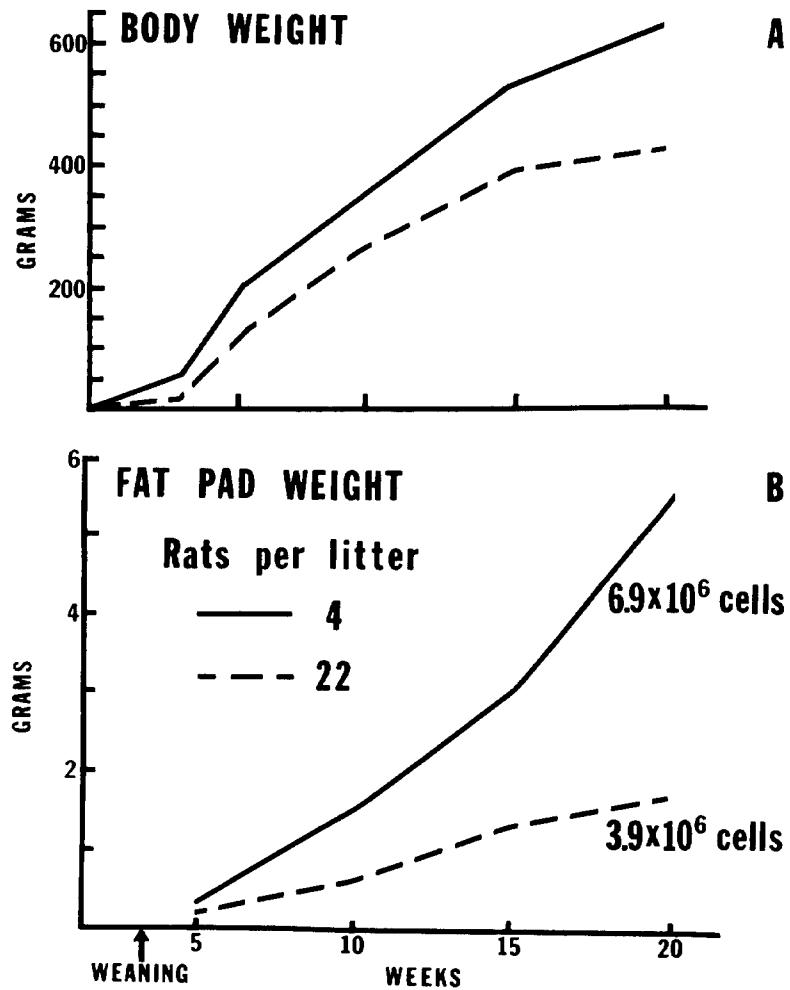
The result that carcass fat in the fasted pig was not decreased below that of the 83 kg pig was also an unexpected finding. During the process of growth from 83 to 109 kg, muscle, bone and other tissues are also expanding; therefore, during the fasting period adipose tissue should be decreased below that which is found in a growing pig of the same carcass weight, if lipid is being used as a preferential source of energy. This was not the case in the Minnesota strain, indicating that other sources of energy were being used, most likely muscle protein. Whether this is the result of an impairment of fat mobilization or an increased capacity of the Minn strain to utilize other sources of energy is not known.

There have been only a few experiments reported in the literature in which experimental manipulation has resulted in a change in adipose cell number. In one of these studies Therriault, *et al.* (1969) determined the cellular development of rats maintained at cold temperatures. The epididymal fat pads of rats that were maintained at near freezing temperatures (5° C) developed over twice as many fat cells as rats that were maintained at room temperature (25° C). The fat cells, however, were much smaller in size and the weight of the fat pad itself was less in the cold acclimated rat. Therriault and Mellin (1971) found that in order to achieve continued hyperplasia throughout the adult stage that the rats must have a continued exposure to the cold environment as well as begin the exposure at a very early age (at a weight of 50-75 g). Removal from the cold stopped the hyperplastic growth and acclimating 300 g rats to the cold did not result in hyperplasia. The experiment was not extended long enough to determine if the excessive adipose cell number of cold exposed rats would result in an excessive accumulation of fat when the rats were returned to ambient temperatures.

In another study, Knittle and Hirsch (1968) determined that nutritional restrictions during the suckling period of rats resulted in a permanent decrease in adipose cell number in the epididymal fat pads. Caloric intake was varied during the suckling period by manipulating litter size. At birth, the litters were redistributed to give some mothers a litter of four (4) and others a litter of 22. Then after weaning, all rats were treated identically and were given free access to standard laboratory chow.

Figure 9 shows the effects of litter size on body weight and epididymal fat pad weight. The caloric restrictions during the suckling periods which were due to increased litter size resulted in a permanent decrease in the fat pad weight (figure 9b) which was proportionally greater than the decrease in body weight (figure 9a). That is, adipose tissue was affected by caloric restriction to a greater extent than other body tissues. Figure 9b shows the cell number comparison of the two groups at 20 weeks of age. The decreased adipose tissue of the 22 per litter group was due to decreases in both adipose cell size and adipose cell number. The fact that adipose cell number was decreased was particularly important from a medical standpoint for two reasons: First of all, excessive human obesity is associated with increased cell number and any treatment that could conceivably decrease cell number may be helpful in the prevention of obesity. Secondly, it was also demonstrated by Knittle and Hirsch in this experiment that the rate at which fat

**EFFECT OF EARLY NUTRITION ON THE
DEVELOPMENT OF RAT EPIDIDYMAL FAT PADS**



Knittle et al. J. Clin. Invest. 47:2091 (1968)

Figure 9. Effect of early nutrition on the development of rat epididymal fat pads. Decreased fat pad weight of the 22 rats per litter group was a result of decreases in both cell size and cell number. Total adipose cell number in the epididymal fat pads is indicated in (B). Knittle and Hirsch, J. Clin. Invest. 47:2091 (1968).

is synthesized in the rat adipose cell does not seem to be affected by cell size. That is, a small adipose cell from the epididymal fat pad of a young rat has the same capacity to synthesize fat as a large fat cell from an older animal. Consequently, it was reasoned that if all adipose cells had similar activity any reduction in the number of adipose cells would result in a reduction in the total capacity of the adipose tissue to synthesize fat. Subsequent to this, however, other work has shown that for human (Smith, 1971) and porcine adipose tissues (Anderson *et al.*, 1972a; Anderson and Kauffman, 1972) adipose cells of different size, different body location and from various ages do not have similar rates of lipogenesis or activities of lipogenic enzymes.

As a result of the rat studies, however, a number of papers appeared in the popular press and as annotations in medical journals (Burch, 1971) in support of the proposition that proper nutrition during childhood may be able to control obesity problems. The proposition is apparently based on the assumption that decreases in caloric intake during early childhood would result in permanently decreased adipose cell number and size which in turn would result in decreased body fat into adulthood.

Work by Lee (1972) has recently shown that this type of caloric restriction in the pig has no effect on adult adipose numbers. Furthermore, excessive fat accumulation in the pig is the result of hypertrophy rather than hyperplasia and the effect that decreased cell numbers would have on the ultimate carcass fat content is uncertain.

The suggestion that adipose cell number may exert a control over adult fat content is tempting to hypothesize. The fact that total cell number is not altered during weight reduction in extremely obese humans (Knittle and Hirsch, 1970) suggests that the increased cell number of excessive obesity is an irreversible process and may account for the tendency for obesity to relapse (Brook, 1971). It has been hypothesized by Masoro (1968) that adipose cells may put out a lipostatic signal to the hypothalamus to effect the feeding response. The evidence for such a control by adipose tissue, however, is limited and there is evidence in humans and other species to suggest that cell number *per se* may not be of primary importance in determining ultimate carcass fat content. Salans *et al.* (1971), found no relation between adipose cell number and the tendency of human subjects to gain weight under voluntarily increased caloric intake. Furthermore, Schemmel *et al.* (1971), found that removal of portions of adipose tissue in four-week-old rats did not exert a significant influence on total fat content of adult rats (34 weeks). Despite the fact that there was no regrowth and no replacement of the excised fat depots, the percent body fat was not decreased as a result of the lipectomy. Cellular measurements of adipose tissue were not made in this experiment but it suggests that a surgical decrease in adipose cell number resulted in no long term effects on total body fat content. A definitive study of cellular changes is needed to further clarify the response of adipose tissue to lipectomy.

ANATOMICAL VARIATIONS IN ADIPOSE TISSUES

Much of the data presented in this paper has been obtained by cellular measurements from single areas of adipose tissue, and then projected to be indicative of the entire adipose tissue mass. It should be remembered in the analysis of the data that the size of adipose cells, the enzyme activities, the protein content, the amount of connective tissue framework and other factors are distinctly different at some locations as compared to others. For example, differences in the fatty acid composition of the layers of subcutaneous fat and perirenal fat have been known for many years (Bhattacharya and Hilditch, 1931) (figure 10). The outer (OS) layer of subcutaneous fat is higher in composition of unsaturated fatty acids than either the middle (MS) and inner (IS) layer or the perirenal depot.

Anderson *et al.* (1972a), compared the lipogenic enzyme activities of adipose tissues from seven anatomical locations including three layers of backfat (OS, MS and IS), intermuscular (IM), perirenal (PR), mesenteric (M) and subcutaneous adipose tissue (L) taken from the lower medial portion of the hind leg--a place where little fat is deposited. The enzymes that were measured have been demonstrated to be involved in fatty acid synthesis--acetyl-CoA carboxylase (CBX), citrate cleavage enzyme (CCE), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) (figure 11). Adipose size and number, as well as lipid and protein content, were also measured in order to establish some of the differences that exist between different areas of porcine adipose tissue. It was found that adipose tissue from areas where fat was deposited very readily (particularly the perirenal region) had higher enzyme activities, larger adipose cells, a less amount of stromal tissue, a greater amount of ether extractable lipid, and a lower concentration of adipose cells per gram of tissue than samples from areas where fat is deposited only sparsely (leg subcutaneous) (table 1).

Anderson and Kauffman (1972) also demonstrated differences in the growth of various adipose tissues (table 2). Simply by measuring the three layers of subcutaneous fat at the 10-11th rib, it was found that between the ages 3.5 and 6.5 months, inner subcutaneous fat (IS) increased in thickness 9-fold (.84 cm), middle subcutaneous (MS) increased 2-fold (.80 cm) while outer subcutaneous (OS) increased only 50% (.36 cm). These differences subjectively correlated with other biochemical measurements that were made on the tissue. For example, MS and IS had substantial increases in enzyme activities between 3.5 and 5 months that were not found in outer subcutaneous and after 5 months the activities remained significantly higher than outer subcutaneous activities. Hood and Allen (1972a) also showed that during starvation the middle layer is decreased to a greater extent than the outer layer. Thus, the data indicate that not all adipose tissues develop at the same rate nor do they respond to treatment to the same degree. This suggests the need for a more thorough study of adipose tissue anatomy in meat animals, particularly the proportions of middle subcutaneous to the less metabolically active outer subcutaneous layer.

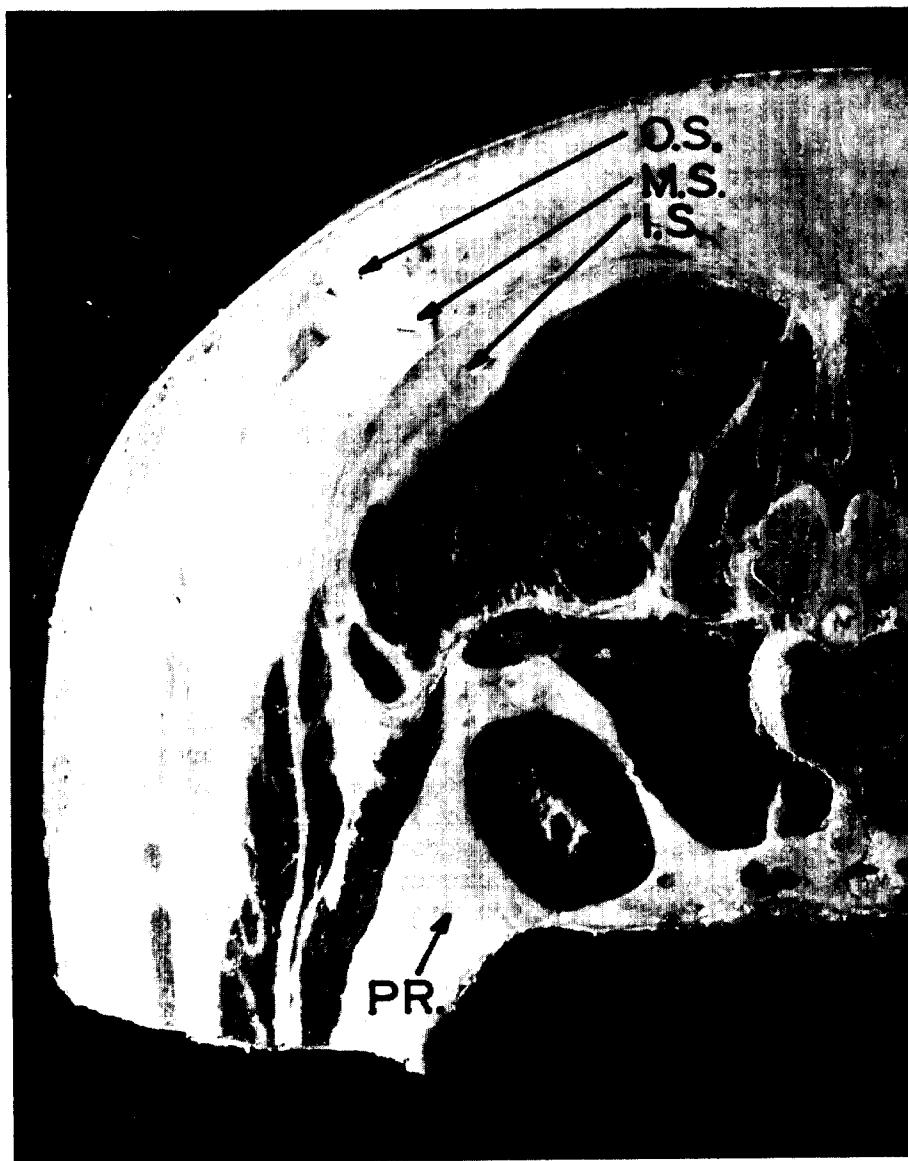


Figure 10. Transverse section through the lumbar region of a porcine carcass depicting outer (OS), middle (MS) and inner (IS) subcutaneous backfat separated by two distinct layers of fascia, and the perirenal adipose tissue (PR) surrounding the kidney.

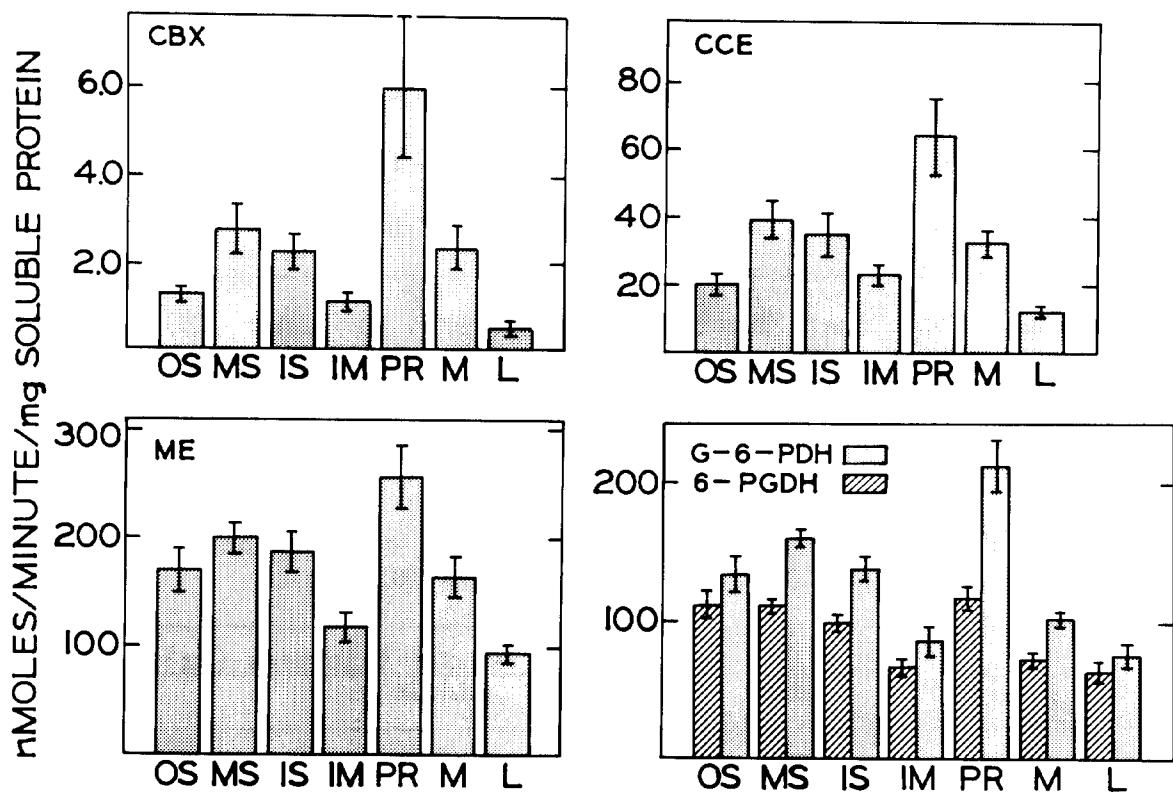


Figure 11. Comparison of enzyme activities in adipose tissue from seven anatomical locations. The anatomical locations are: outer (OS), middle (MS) and inner (IS) subcutaneous backfat, intermuscular (IM), perirenal (PR), mesenteric (M), and leg subcutaneous (L) adipose tissue. Activities of acetyl-CoA carboxylase (CBX), citrate cleavage enzyme (CCE), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), and 6-phosphogluconate dehydrogenase (6-PGDH) are recorded in nmoles per minute per mg soluble supernatant protein \pm S.E.M.

TABLE 1. ANATOMICAL VARIATION IN
PORCINE ADIPOSE TISSUE CHARACTERISTICS

Adipose location	Percent lipid	Cell volume $\mu^3 \times 10^{-4}$	Mg protein per g wet tissue	Cells $\times 10^{-6}$ per g wet tissue
OS	88.8 ± 0.5	125 ± 10.3	2.58 ± .07	.857 ± .063
MS	93.4 ± 0.7	144 ± 2.3	2.42 ± .08	.713 ± .049
IS	87.7 ± 1.8	131 ± 11.5	2.86 ± .09	.810 ± .068
IM	76.3 ± 1.4	76 ± 9.4	4.41 ± .21	1.227 ± .106
PR	96.1 ± 0.4	221 ± 31.9	2.50 ± .24	.601 ± .076
M	84.0 ± 3.7	110 ± 8.7	3.34 ± .27	.918 ± .065
L	70.9 ± 4.2	71 ± 6.5	4.47 ± .37	1.223 ± .072

Adipose tissue locations: Outer (OS), middle (MS) and inner (IS) subcutaneous backfat: intermuscular (IM), perirenal (PR), mesenteric (M) and leg subcutaneous (L). All values are the mean of five animals ± S.E.M.

TABLE 2. CHANGES IN THICKNESS OF THE THREE LAYERS
OF PORCINE SUBCUTANEOUS BACKFAT WITH AGE

Period	Subcutaneous Backfat thickness (cm)		
	Inner	Middle	Outer
1 month	.04 ± .03	.29 ± .03	.44 ± .03
2 months	.04 ± .03	.42 ± .04	.44 ± .04
3.5 months	.10 ± .03	.68 ± .08	.71 ± .03
5 months	.58 ± .04	1.24 ± .17	1.04 ± .10
6.5 months	.94 ± .04	1.48 ± .12	1.07 ± .06

All values are the mean of five animals ± S.E.M.

INTRAMUSCULAR ADIPOSE TISSUE

Another important adipose tissue depot especially in meat animal research is intramuscular fat or marbling fat. While other depots adversely affect retail yield and carcass value (Allen, 1969), marbling fat is needed to maintain certain organoleptic qualities of meat, particularly juiciness and flavor (Blumer, 1963; Kauffman *et al.*, 1964). The lipogenic capabilities of intramuscular fat are basically the same as subcutaneous fat; that is, fat synthesizing enzyme systems are found in both tissues, allowing for *in situ* fat synthesis in intramuscular fat. The enzyme activities, however, are lower in marbling fat than in subcutaneous (Lee and Kauffman, 1971; Chakrabarty and Romans, 1972).

Current research has indicated basic differences between intramuscular fat and subcutaneous fat both with respect to its cellularity and also with respect to the development of its fat synthesizing capabilities. Adipose cells in both bovine (Moody and Cassens, 1968) and porcine (Lee and Kauffman, 1971) marbling are smaller than corresponding subcutaneous fat. Hood and Allen (1972b) studied fat cell size distribution in the marbling fat of four muscles in 470 kg Hereford x Angus steers--Longissimus dorsi, semimembranosus, trapezius and brisket (figure 12). When compared to the perirenal and subcutaneous fat depots of the same steers, the marbling fat contained a bimodal distribution of cells with an abundance of small cells indicating that the intramuscular adipose tissue of these muscles was probably developing by hyperplasia as well as hypertrophy. Neither perirenal nor subcutaneous adipose tissues, however, possessed cells below 70 μ in diameter, suggesting the absence of hyperplasia in these tissues.

Correlation coefficients were calculated to determine the relation between percent intramuscular fat and either cell size or cell number (table 3). With the exception of the LD, average cell volume was not related to percent intramuscular fat. The number of adipose cells per 100 g muscle tissue, however, was significantly correlated to the percent intramuscular fat both within muscles and across muscles, suggesting that increase in cell number is a major contributing factor to increased intramuscular fat in bovine muscle. The presence of small cells suggests that intramuscular fat is later developing than other adipose tissue areas and that final intramuscular adipose cell number had not been reached in the 14 month old steer (Hood and Allen, 1972b).

Another indication that intramuscular fat is a later developing adipose tissue has come from the work of Lee and Kauffman (1972) who have recently determined that the fat synthesizing enzymes in porcine marbling show a different pattern of development from subcutaneous fat. In our initial work with porcine adipose tissue, we found that in the four tissue areas measured (outer, middle and inner subcutaneous and perirenal) the activities of the lipogenic enzymes reached a peak at 5 months (figure 13). The increase was particularly sharp in middle and inner subcutaneous between 3.5 and 5 months, and corresponded to (1) a period of rapid growth of these two depots (table 2); (2) a period when average adipose cell size increased at its most rapid rate (figure 1); and (3) a period when total carcass adipose tissue relative to muscle mass made the largest increase of any period except during suckling (figure 14).

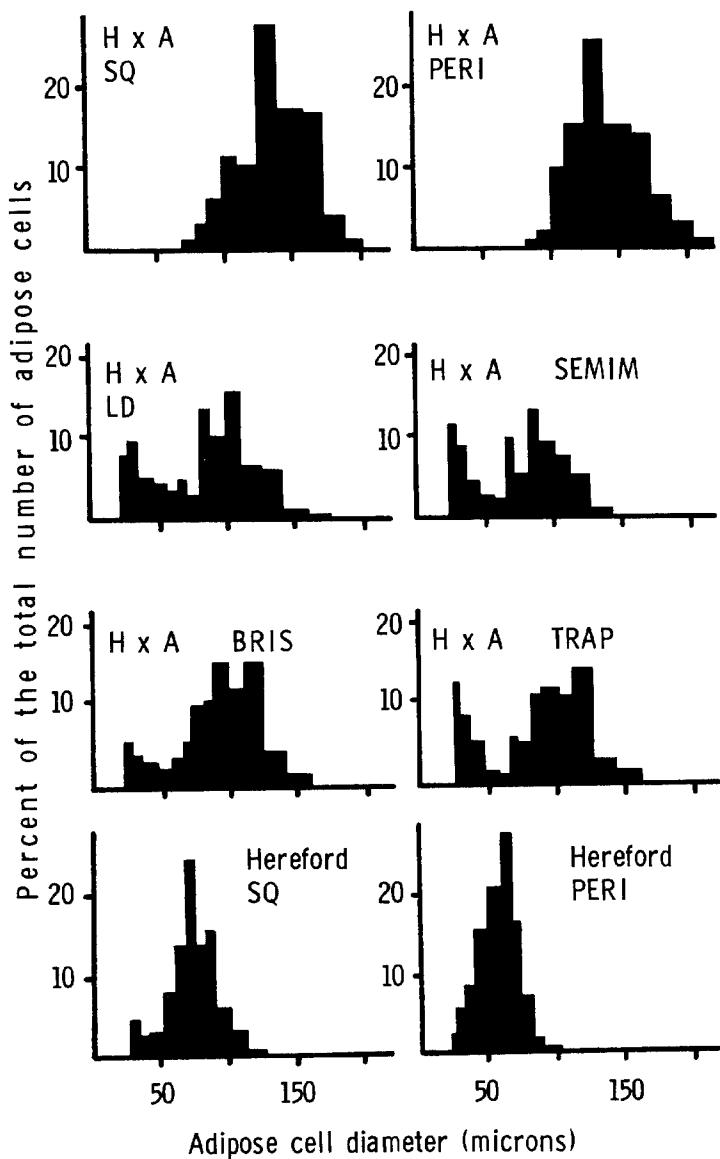


Figure 12. Number frequency distribution of adipose cells from bovine tissue. Hood, Ph.D. Thesis (1972).

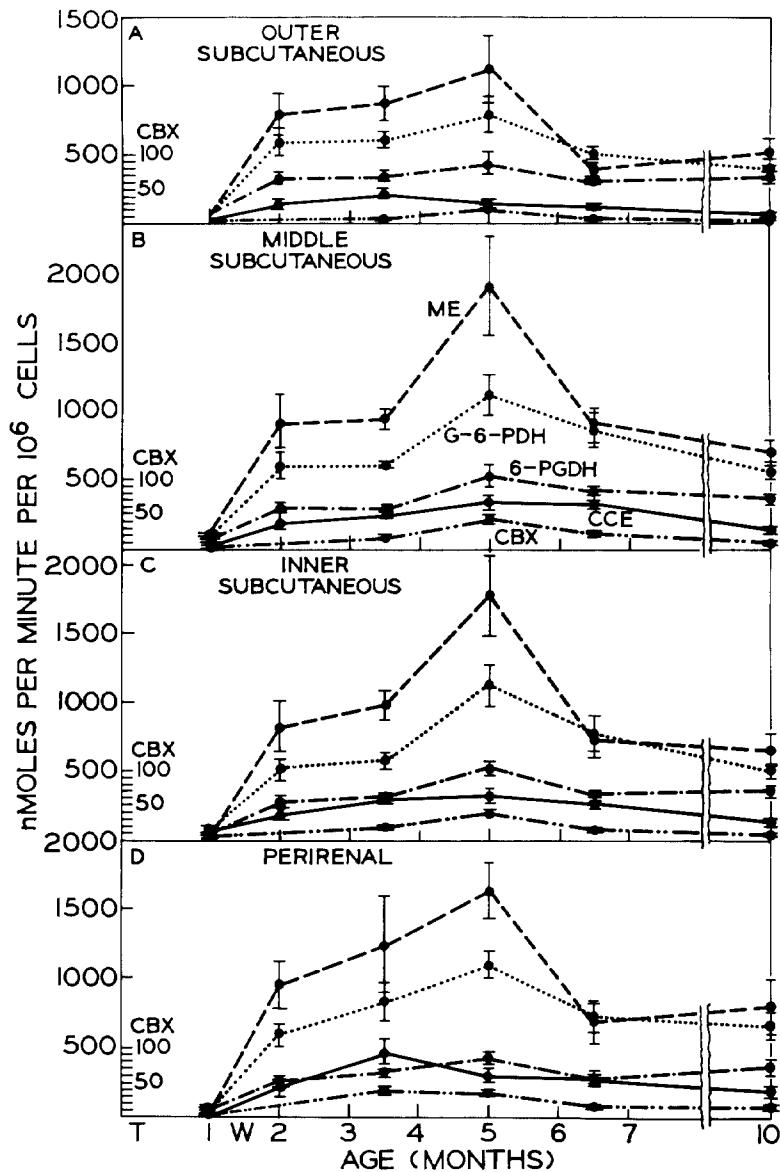


Figure 13. Changes in enzyme activities in porcine adipose tissue during growth. Enzyme abbreviations are the same as in figure 11. CBX activity is plotted according to the smaller scale on the ordinate of each graph.

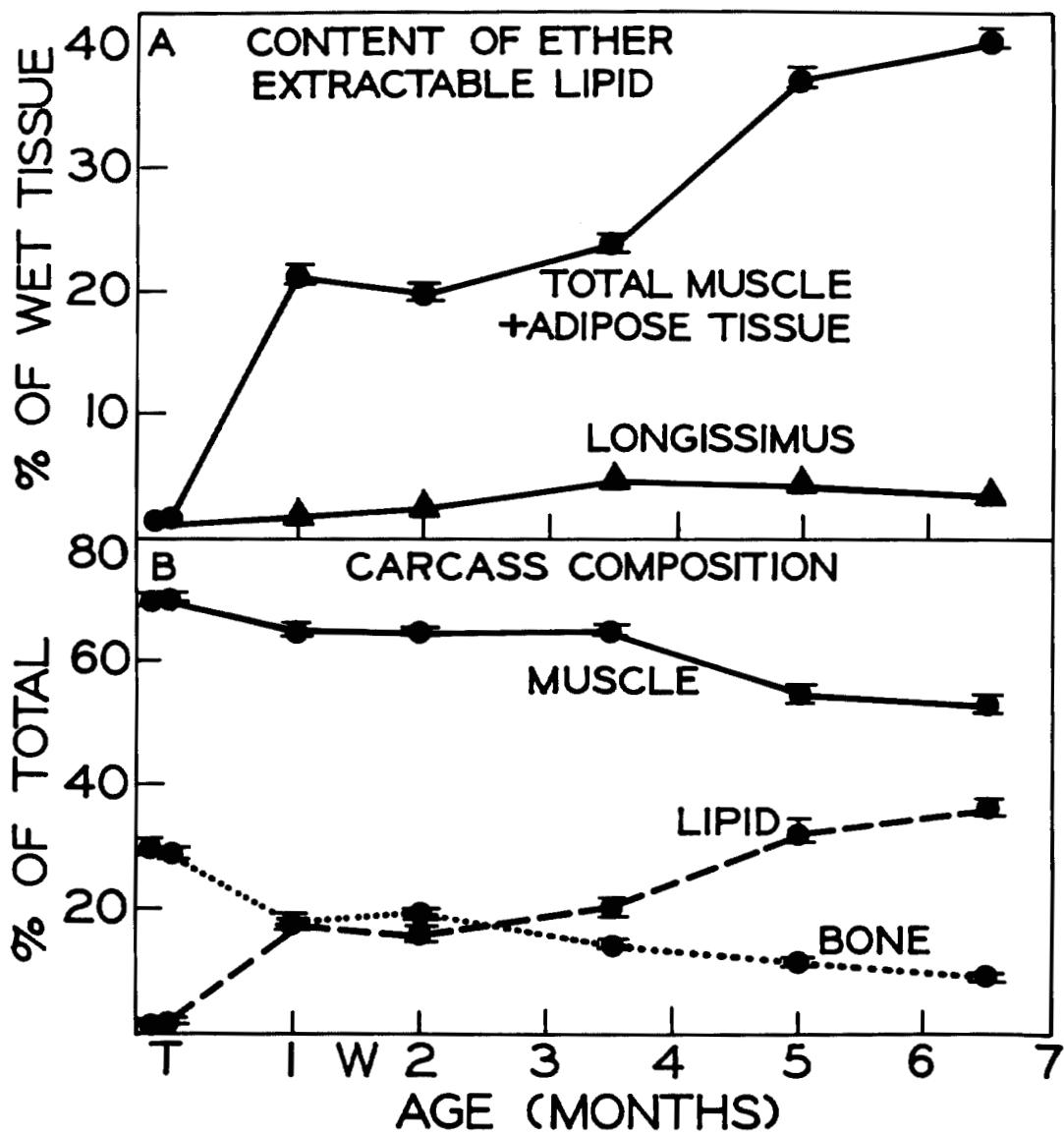


Figure 14a. Changes in the ether extractable lipid content of (1) combined muscle and adipose tissue from the entire carcass and (2) the longissimus during growth of the pig.

Figure 14b. Changes in the proportion of carcass components with growth plotted as the percent of the total carcass. Percent muscle is expressed on a lipid free basis. T and W refer to term and weaning, respectively.

TABLE 3. CORRELATION OF INTRAMUSCULAR FAT
WITH THE NUMBER AND SIZE OF INTRAMUSCULAR ADIPOSE CELLS
FROM 470 kg HEREFORD x ANGUS STEERS¹

	Intramuscular fat (percent) vs.		
	Average cell diameter	Average cell volume	Number of cells per 100g muscle
Longissimus Dorsi	0.79	0.75	0.81
Trapezius	-0.36	-0.37	0.94
Semimembranosus	-0.33	0.08	0.91
Brisket	-0.28	-0.29	0.89
All muscles	0.32	0.35	0.87

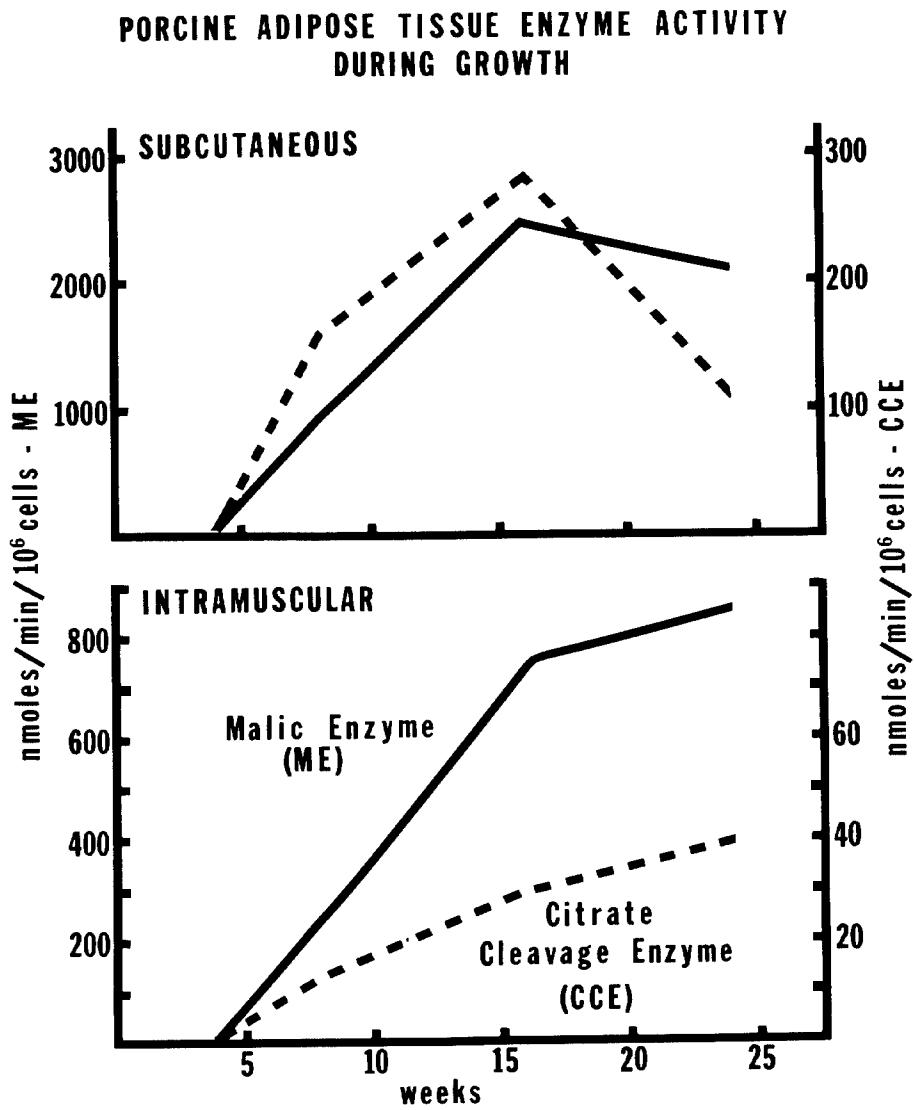
¹ Correlation coefficients are significant P < .01 if r > .87 and P < .05 if r > .75 within muscles and P < .01 if r > .51 and P < .05 if r > .40 across muscles. Hood, Ph.D. Thesis, 1972.

The unique feature of intramuscular fat is that (1) it does not increase in proportion to total body fat (figure 14a) and (2) the activities of lipid synthesizing enzymes do not decrease after 5 months, but instead continue to increase. In the experiments of Lee and Kauffman (1972) subcutaneous enzyme activities decreased after 16 weeks; however, intramuscular fat continued to increase in activity until the end of the experimental period at 24 weeks (figure 15). This again is another indication that intramuscular fat is later developing and is relatively independent in cellular development and metabolism from other areas of adipose tissue.

SUMMARY

The cellular composition and development of adipose tissues in meat animals can be summarized as follows:

(1) The development of adipose tissue in the pig is accomplished by a combination of cellular hyperplasia and hypertrophy up to an age of approximately 5 to 6 months. After this point, significant cell proliferation stops and hypertrophy of existing cells contributes exclusively to increases in adipose tissue mass. In pigs which have a greater propensity to deposit large amounts of adipose tissue, the plateau in adipose cell number may come at an older age. In bovine adipose tissue development, the exact role of hyperplasia and hypertrophy is uncertain, but it appears that hyperplasia is complete in Hereford x Angus steers at 14 months. The cellular development in the ovine species has not been reported.



Lee and Kauffman, 1972

Figure 15. Development of malic enzyme and citrate cleavage enzyme activities in subcutaneous and intramuscular adipose depots of the pig.

(2) By comparisons of fat and lean strains of pigs it appears that excessive fat accumulation in porcine adipose tissue is manifested completely by the process of cellular hypertrophy. Contrary to expectations, the fat strain had fewer estimated carcass adipose cells than the lean strain. Even within litters, the amount of carcass adipose tissue was more highly correlated with adipose cell size than with total cell number. This suggests that cellular hypertrophy rather than hyperplasia is the dominating factor contributing to excessive fat accumulation in the pig. Whether this oversized cell is the result of some abnormality of metabolism within the adipose cell itself or whether it is secondary to other changes in body physiology is yet to be determined. In bovine adipose tissue, however, dissection of the standard rib from 14 month Holstein and Hereford x Angus steers revealed that the lesser amount of adipose tissue in the Holstein rib was the result of decreased cell number as well as a smaller cell size (Hood and Allen, 1972b). Again, ovine data have not been reported.

(3) Experimental manipulations of adipose stores in species other than meat animals have resulted in changes in both adipocyte number and size. Manipulations of adipose tissue in the pig by starvation either during the suckling period or at 109 kg resulted only in reductions in cell size with no permanent reduction of cell number.

(4) Basic differences in the cellular and metabolic characteristics of porcine adipose tissues from various anatomical locations suggest the need for a more thorough examination of adipose variability in other species and a more definitive study of adipose tissue anatomy in the pig, particularly the proportions of outer subcutaneous to the more metabolically responsive middle layer. Studies of the cellular and metabolic characteristics of marbling fat have been reported in this paper with great interest. Differences in the cellular and metabolic development of marbling and subcutaneous adipose tissues offer hope that these two fat depots may be controlled separately to allow for decreased extramuscular fat without sacrificing the intramuscular fat which is needed to maintain meat quality.

REFERENCES

- Allen, E. 1969. Importance of adipose tissue to the meat industry. Proc. Meat Industry Res. Conf. Amer. Meat Inst. Foundation. Chicago, Ill. pp. 1-8.
- Anderson, D. B. and R. G. Kauffman. 1972. Cellular and enzymatic changes in porcine adipose tissue during growth. *J. Lipid Res.* (Submitted).
- Anderson, D. B., R. G. Kauffman and L. L. Kastenschmidt. 1972a. Lipogenic enzyme activities and cellularity of porcine adipose tissue from various anatomical locations. *J. Lipid Res.* (In press).
- Anderson, D. B., R. G. Kauffman and N. J. Benevenga. 1972b. Estimate of fatty acid turnover in porcine adipose tissue. *Lipids.* (In press).
- Bhattacharya, R. and T. P. Hilditch. 1931. The body fats of the pig. I. Influence of ingested fat on the component fatty acids. *Biochem. J.* 25:1954-1964.
- Bjorntorp, P. and L. Sjostrom. 1971. Number and size of adipose tissue fat cells in relation to metabolism in human obesity. *Metabolism* 20:703-713.
- Bjorntorp, P., C. Bengtsson, G. Blohme, A. Jonsson, L. Sjostrom, E. Tibblin, G. Tibblin and L. Wilhelmson. 1971. Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women. *Metabolism* 20:927-935.
- Bjorntorp, P. and J. Ostman. 1971. Human adipose tissue dynamics and regulation. *Adv. Metab. Disord.* 5:277-327.
- Blumer, T. N. 1963. Relationship of marbling to the palatability of beef. *J. Anim. Sci.* 22:771.
- Brook, C.G.D. 1971. Adipose cell size and number in obesity. *Diabetologia* 7:294-295. (Abstract, British Diabetic Association).
- Burch, G. E. 1971. Fat cells, nutrition and obesity. *Amer. Heart J.* 82:839.
- Chakrabarty, K. and J. R. Romans. 1972. Lipogenesis in the adipose cells of the bovine (*Bos taurus*) as related to their intramuscular fat content. *Comp. Biochem. Physiol.* 41B:603-615.
- DiGirolamo, M., S. Mendlinger and J. W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. *Amer. J. Physiol.* 221:850-858.
- DiGirolamo, M. and S. Mendlinger. 1971. Role of fat cell size and number in enlargement of epididymal fat pads in three species. *Amer. J. Physiol.* 221:859-864.

- Gordon, E. S. 1970. Metabolic aspects of obesity. *Advances Metab. Dis.* 4:229-296.
- Hood, R. L. 1972. Adipose tissue cellularity and lipogenic activity in porcine and bovine animals. Ph.D. Thesis. University of Minnesota.
- Hood, R. L. and C. E. Allen. 1972a. Cellularity of porcine adipose tissue: Effects of growth and adiposity. *J. Lipid Res.* (Submitted).
- Hood, R. L. and C. E. Allen. 1972b. Cellularity of bovine adipose tissue. *J. Lipid Res.* (Submitted).
- Hood, R. L. and C. E. Allen. 1972c. Lipogenic enzyme activity in adipose tissue during the growth of swine with different propensities to fatten. *J. Nutr.* (Submitted).
- Hirsch, J. and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* 9:110-119.
- Hirsch, J. and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation and obesity. *J. Lipid Res.* 10:77-82.
- Hirsch, J. and J. L. Knittle. 1970. Cellularity of obese and nonobese human adipose tissue. *Fed. Proc.* 29:1516-1521.
- Johnson, P. R. and J. Hirsch. 1972. Cellularity of adipose depots in six strains of genetically obese mice. *J. Lipid Res.* 13:2-11.
- Johnson, P. R., L. M. Zucker, J.A.F. Cruce and J. Hirsch. 1971. Cellularity of adipose depots in the genetically obese Zucker rat. *J. Lipid Res.* 12:706-714.
- Kauffman, R. G., Z. L. Carpenter, R. W. Bray and W. G. Hoekstra. 1964. Biochemical properties of pork and their relationship to quality. II. Intramuscular fat. *J. Food Sci.* 29:70-74.
- Kauffman, R. G. and L. E. St. Clair. 1965. Porcine Myology. Bul. 715. University of Illinois, College of Agriculture, Agricultural Experiment Station.
- Knittle, J. L. and J. Hirsch. 1968. Effect of early nutrition on the development of rat epididymal fat pads: Cellularity and metabolism. *J. Clin. Invest.* 47:2091-2098.
- Lee, Y. B. and R. G. Kauffman. 1971. Lipogenic enzyme activities in intramuscular adipose tissue of the pig. *J. Anim. Sci.* 33:1144. (Abstr.).
- Lee, Y. B. and R. G. Kauffman. 1972. The development of porcine intramuscular adipose tissue. (In preparation).

- Lee, Y. B. 1972. The effect of growth and environment on the biochemistry of porcine lens and adipose tissue. Ph.D. Thesis. University of Wisconsin, Madison. (In preparation).
- Masoro, E. J. 1968. Physiological Chemistry of Lipids in Mammals. W. B. Saunders Co. Philadelphia, Pa. pp. 243-252.
- Moody, W. G. and R. G. Cassens. 1968. A quantitative and morphological study of bovine longissimus fat cells. *J. Food Sci.* 33:47-52.
- Nutrition Reviews. 1969. Factors affecting the cellularity of adipose tissue. 27:23-24.
- Nutrition Reviews. 1969. Cellularity of rat adipose tissue in relation to growth, starvation and obesity. 27:146-149.
- Nutrition Reviews. 1971. Cellular growth in infantile malnutrition. 29:6-8.
- Nutrition Reviews. 1971. Cellular growth in the fat adolescent. 29:158-160.
- Nutrition Reviews. 1971. Fat cell size and lipid metabolism. 29:188-190.
- Salans, L. B., J. L. Knittle and J. Hirsch. 1968. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. *J. Clin. Invest.* 47:153-165.
- Salans, L. B., E. S. Horton and E.A.H. Sims. 1971. Experimental obesity in man: Cellular character of adipose tissue. *J. Clin. Invest.* 50:1005-1011.
- Schemmel, R., O. Mickelsen, S. A. Pierce, J. T. Johnson and R. G. Schirmer. 1971. Fat depot removal, food intake, body fat, and fat depot weight in obese rats. *Proc. Soc. Exp. Biol. Med.* 136:1269-1273.
- Sigwart, U., L. G. Tedeschi and C. G. Tedeschi. 1970. Factors in adipogenesis. *Hum. Pathol.* 1:399-418.
- Smith, U. 1971. Effect of cell size on lipid synthesis by human adipose tissue in vitro. *J. Lipid Res.* 12:65-70.
- Therriault, D. G., R. W. Hubbard and D. B. Mellin. 1969. Endocrine control of fat mobilization in the isolated fat cells of cold-exposed rats. *Lipid* 4:413-420.
- Therriault, D. G. and D. B. Mellin. 1971. Cellularity of adipose tissue in cold-exposed rats and the calorogenic effect of norepinephrine. *Lipids* 6:486-491.

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JOHN R. ROMANS: Our second speaker is Dr. Lawrence J. Machlin. After receiving his formal education at Cornell and Georgetown Universities, he went to the USDA where he held the position of nutritionist and biochemist. In 1956 he joined the Monsanto Company as a chemist and in 1963 he was given the position of Senior Group Leader, which he presently holds. In addition, he has a position as a lecturer with Washington University in St. Louis and also does some cooperative research with that Institution. Of his several organizations, are the American Institute of Nutrition and the Endocrinology Society. I might just mention that we are very fortunate to have Dr. Machlin here today since the Endocrinologists are meeting out East. He passed up that meeting to be here with us today. He is also a member of the Society for Nutritional Education, the New York Academy of Sciences, The American Society of Animal Science and the Poultry Science Association. Dr. Machlin is author or co-author of 60 scientific articles. He has three patents and three others pending. Among his many accomplishments, one particularly appropriate for this program is the fact that he pioneered the assays for insulin and growth hormone in farm animals. It is a pleasure for me to present to you Dr. Lawrence Machlin, a Senior Group Leader in Physiology at Monsanto Company, St. Louis.

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