mal emergence time in April 1976. By then, *T. alsophilae* had undergone three generations, and we assumed the long-lived original parasites could still be reproductively active. In April, only 13 *O. trychiata* masses were found, and parasitization averaged 99 percent. The absence of larvae anywhere in the area in May confirmed the outbreak had been controlled. It is to be hoped that *T. alsophilae* will maintain itself on any of four species of *Oxydia* or other Geometridae found in Colombia.

Our results strongly support Pimentel's (*1*) contention that parasites from allied genera can be used effectively against native pests. We are convinced that biological control specialists should look in the direction of matching parasites from one host to a host in a nearby taxon. Although compatibility with the pest's environment is repeatedly stressed as a criterion for selecting which parasites to introduce, we found that *T. alsophilae* adapted readily from the North American to the Andean terrain despite considerable differences in climatic conditions between locales. As Anderson (*5*) pointed out, research with egg parasites of forest defoliators has been neglected. Our experience with *T. alsophilae* shows that use of egg parasites offers many control possibilities and requires only imagination, cooperation, and support.

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6. This report grew out of a Food and Agriculture Organization (United Nations) assignment of A.T.D. to Colombia, and the subsequent graduate study of A.E.B. under H. C. Coppel at the University of Wisconsin. We thank C. L. Morris, J. A. Copony, and T. C. Tigner for assistance with the field collection of parasitized fal. cankerworm eggs; D. C. Ferguson, P. M. Marsh, and C. F. W. Muesebeck for their identifications of the insects used in this study; and D. C. Allen, J. F. Anderson, H. C. Coppel, and D. Pimentel for reviewing the manuscript.

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Adipose Tissue Regeneration Following Lipectomy

Abstract. *Surgical removal of subcutaneous fat depots in weanling rats leads to a regenerative response. If the rats are fed a diet high in fat, adipose mass and adipocyte number are precisely restored within 7 months of surgery. Thus, under appropriate experimental circumstances, compensatory hyperplasia will occur in adipose tissues of the rat.*

Obesity is a disorder characterized by an excessive accumulation of adipose tissue. Histologic examination reveals excessively large adipocytes and, often, an excessive number of adipocytes. In the accompanying report we have presented evidence that the size of the adipocyte is a major regulatory feature in the control of adipose tissue mass (*1*). Thus, some disorder of this regulation could be responsible for the large adipocyte size component of obesity. This report deals with the regulation involved in the development of adipocyte number, the other histologic feature of concern in human obesity.

It has been proposed that adipocyte number in normal man and rats is determined during some early critical period or periods of adipocyte proliferation (*2*). During such periods, nutritional manipulations may appreciably alter ultimate adipocyte number. In the epididymal fat pad of the Sprague-Dawley rat the proliferative period ends at about 35 days of age (*3*); food restriction before that age causes a permanent deficit of up to 40 percent in epididymal pad adipocyte number, but food restriction thereafter causes no such deficit (*2*). Surgical excision of part of a proliferating organ has often been used to determine whether cellular proliferation is occurring and the degree to which proliferation can be modified. In many organs such surgery has been found to stimulate a rapid and fully compensatory hyperplastic response, which is regarded as proof for the existence of regulated cellular proliferation. The response to partial hepatectomy is one example which has been extensively studied (*4*). Surprisingly, partial removal of the epididymal fat pad during its proliferative phase does not induce any measurable degree of compensatory hyperplasia or regeneration (*5*). In numerous experiments that we have performed with both rats and mice, we have never seen regeneration of epididymal pads, regardless of the age of the animals at the time of surgery or the extent of the lipectomy (*6*).

This report demonstrates that by excising another site, the subcutaneous inguinal fat depot of the young rat, one can induce a fully restorative cellular response to partial organectomy similar to that seen in other proliferating tissues. The choice of site and timing of excision are both critical.

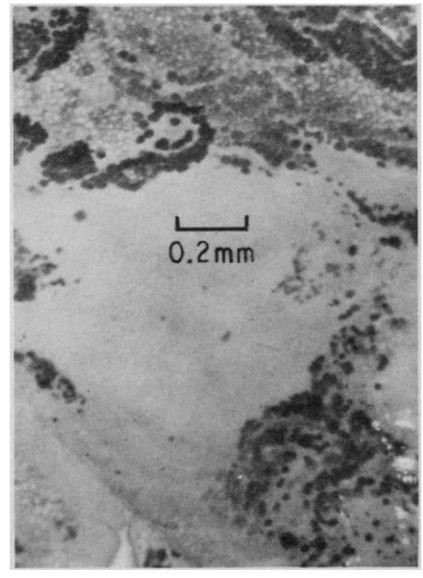

Fig. 1. Photomicrograph, made 4 weeks after surgery, of the outer surface of the collagenous sheath which forms at the site of inguinal area lipectomy. The adipocytes on the sheath surface were stained with oil red O.

We removed both inguinal fat depots from each of 31 3-week-old Sprague-Dawley rats by a surgical procedure previously described (*6*). Twenty-one rats identical in age and mean body weight to the experimental rats served as sham-operated controls. Eight experimental rats were killed immediately after surgery to determine the adipose cellularity of the tissue removed as well as of the remaining subcutaneous tissues. Four weeks after surgery we killed two additional experimental rats and examined their subcutaneous inguinal areas. All remaining rats (21 experimental and 21 sham-operated controls) were killed 7 months after surgery. The subcutaneous, mesenteric-omental, and retroperitoneal depots were carefully dissected, weighed, and sampled for cell number and lipid content determinations. Of the rats kept alive for the entire course of the experiment, eight experimental and eight control rats had ad libitum access to only water and Purina Laboratory Chow (low-fat diet or LF). The remaining 13 experimental and 13 control rats had ad

Table 1. Cellularity of the subcutaneous adipose depots in LF-fed and HF-fed lipectomized 8-month-old Sprague-Dawley rats.

Group	Inguinal depots			Remaining subcutaneous depots			Total subcutaneous depots	
	Cell number $\times 10^6$	Cell size (μg of lipid per cell)	Total lipid (g)	Cell number $\times 10^6$	Cell size (μg of lipid per cell)	Total lipid (g)	Cell number $\times 10^6$	Total lipid (g)
LF-fed								
Lipectomized ($N = 8$)	18.93 ± 2.52*	0.40 ± 0.03†	7.29 ± 0.88‡	41.65 ± 2.67†	0.47 ± 0.05†	18.80 ± 1.54†	60.58 ± 4.19‡	26.09 ± 2.13§
Sham-operated ($N = 8$)	30.60 ± 1.95	0.34 ± 0.03	10.30 ± 0.75	46.30 ± 5.00	0.34 ± 0.03	15.12 ± 1.28	76.90 ± 6.01	25.42 ± 1.80
HF-fed (16 weeks)								
Lipectomized ($N = 13$)	24.98 ± 2.20*	0.71 ± 0.04†	17.08 ± 1.25*	72.92 ± 4.76†	0.67 ± 0.05†	47.02 ± 2.89‖	97.90 ± 6.44§	64.10 ± 4.00§
Sham-operated ($N = 13$)	37.72 ± 3.25	0.70 ± 0.04	25.67 ± 2.01	60.34 ± 4.85	0.65 ± 0.04	38.28 ± 2.87	98.06 ± 7.74¶	63.95 ± 4.65
Cellularity at surgery (3 weeks of age)	7.69 ± 0.23	0.11 ± 0.01	0.82 ± 0.09	9.52 ± 0.75	0.10 ± 0.01	0.98 ± 0.11	17.21 ± 0.93	1.80 ± 0.19

*Significantly different from sham-operated controls ($P < .01$, Student's t-test, one-tailed). †Not significant, two-tailed t-test. ‡Significantly different from sham-operated controls ($P < .05$, one-tailed t-test). §Not significant, one-tailed t-test. ‖Significantly different from sham-operated controls ($P < .05$, two-tailed t-test). ¶Significantly different from LF-fed sham-operated controls ($P < .05$, one-tailed t-test).

libitum access to only water and chow until age 12 weeks, at which time they were switched from chow to a highly palatable high-fat semipurified diet (HF) (*1*), which they received for the remaining 16 weeks of the study.

The inguinal depots which were removed contained 7.69 ± 0.23 million adipocytes, while the remaining subcutaneous tissue (dorsal scapular, axillary, and buttock) contained 9.52 ± 0.75 million adipocytes. Thus, approximately 45 percent of the subcutaneous adipocytes were surgically removed. Four weeks after surgery, new fascial tissue was present in the inguinal areas of the experimental rats. The new tissue was seen to be in the form of a sheath, attached to the cutaneous and muscular tissues at the periphery of the inguinal areas, forming a loose pocket above the underlying muscular layer at each inguinal site. Careful observation of the sheaths with a low-power (6 ×) dissecting microscope revealed that they were free of fat except for several scattered nubbins of adipose tissue on their outer surfaces; that is, the surfaces normally adjacent to the skin. The sheaths were stained with oil red O so that adipocytes could be more clearly observed. With somewhat greater magnification (25 to 50 ×) we were able to see uniformly sized adipocytes (30 to 40 μm in diameter) on the outer subcutaneous surfaces of the sheaths (Fig. 1). The adipocytes appeared to spread laterally as a monolayer away from blood vessels with which they were associated. We estimate that about 30 percent of the outer surface of each sheath was covered with such a monolayer. We found no evidence of adipocytes on the inner surfaces of the sheaths.

By 7 months after surgery we found that complete adipose tissue regeneration had occurred in the HF-fed lipectomized rats. Mean wet weight, depot lipid content, adipocyte number, and adipocyte size of all adipose tissues, including subcutaneous ones, were virtually identical between the HF-fed lipectomized and sham-operated rats. Table 1 summarizes the subcutaneous fat depot analyses and shows that the normal subcutaneous adipose mass and adipocyte number are achieved in the HF-fed lipectomized rats with a somewhat abnormal adipocyte distribution; that is, there are somewhat fewer adipocytes than normal in the inguinal areas and somewhat more than normal in the remaining subcutaneous areas. This abnormal distribution may be due to a problem in precise definition of the borders of the inguinal sites, or some of the regeneration may have actually occurred elsewhere in the subcutaneous adipose tissues. In either case, it is clear that subcutaneous adipose cells can be restored with numerical precision after surgical excision.

It can also be seen in Table 1 that the adipocytes in the regenerated inguinal tissues of the HF-fed lipectomized rats are equivalent in size to other subcutaneous adipocytes as well as to the inguinal adipocytes of controls. The total subcutaneous fat tissue mass of the HF-fed lipectomized rats is thus composed of adipocytes that are equal in both size and number to adipocytes of the controls.

One would predict that if regeneration had not occurred, the subcutaneous fat mass of the lipectomized rats would have contained 45 percent fewer cells than the respective tissue of controls, since the removed inguinal depots contained 45 percent of the subcutaneous adipocytes present at the time of surgery. The clear lack of a deficit in the number of adipocytes in the HF-fed lipectomized rats 7 months after surgery thus means that not only were all their surgically removed adipocytes replaced (about 7 million cells), but also the full replicative potential of the inguinal tissue was realized (about 40 million cells). Furthermore, the number of adipocytes in the internal (nonsubcutaneous) fat depots was essentially identical (46.12 compared to 45.86 million cells) in lipectomized and sham-operated rats, indicating that subcutaneous adipose tissue regeneration is exclusively a site-specific event that does not involve the nonsubcutaneous adipose depots of the body.

In the LF-fed rats some cells were restored, but the total number of subcutaneous adipocytes did not equal that of sham-operated controls. Possibly, more time is needed for regrowth of adipose tissue when the rats are fed LF rather than HF, or perhaps when inguinal lipectomy is performed on 3-week-old Sprague-Dawley rats, LF is insufficient to promote full regeneration. Liebelt *et al.* (*7*) suggested that a neoplastic transformation and thus an increase in the cellular component of the adipose tissue mass might occur in response to certain stimuli (such as HF feeding). The data presented here tend to support such a view with respect to the subcutaneous adipose tissue. The apparent influence of HF feeding on subcutaneous adipocyte number beginning at 12 weeks of age (in terms of the promotion of both full regeneration and a modest adipocyte number increase in HF-fed compared to LF-fed rats) suggests that subcutaneous adipose tissue may be unlike epididymal fat pad tissue in the Sprague-Dawley rat in that it may have the capacity to continue proliferating adipocytes beyond 12

weeks of age. This matter requires further investigation.

Interestingly, the LF-fed lipectomized rats developed as much total subcutaneous adipose mass as their controls by storing a somewhat greater amount of lipid in subcutaneous adipocytes. Such a hypertrophic response to lipectomy has also been observed in other experiments (*1*, *5*, *6*). However, it is now apparent that a hypertrophic response to lipectomy is usually quite limited, and only serves to accommodate new lipid stores that would otherwise have been accumulated in the excised cells. In some animals, such as the Sprague-Dawley rat, the degree of such possible accommodation is probably small, while in others, especially the potentially obese, it may be quite large (*1*).

We conclude from this study that the development of the subcutaneous adipose tissue during the first few postnatal weeks of the rat's life is a precisely regulated event. That is, the proliferation of subcutaneous adipocytes is monitored and adjusted. Subcutaneous adipocyte regulation in the rat is thus similar to the regulation seen in skin and liver, except that adipocyte regulation may terminate at some time shortly after weaning when adipocyte proliferation terminates. That regulation does indeed terminate is suggested by the failure of Kral (*8*) to observe regeneration of inguinal fat in the Sprague-Dawley rat lipectomized at 15 weeks of age. Furthermore, our previous failures to observe subcutaneous adipose tissue regeneration in the NCS/R mouse lipectomized at 12 days of age (*6*) or to observe regeneration of the epididymal fat pad in young rats and mice (*5*, *6*) suggest that there are strain and site variations in the phenomenon of adipose tissue regeneration and thus perhaps in the normal mechanisms or sequences of adipocyte proliferation and development.

This study leads to at least two observations which could be relevant to human obesity. First, assuming a degree of similarity between human and rat, the existence of an adipocyte proliferation regulatory process suggests that the hyperplastic component of human obesity may well be the result of a disorder in that process, as the hypertrophic component of obesity is very likely the result of a disorder in the process that regulates adipocyte size. Determining the nature of such regulatory disorders obviously has a high priority for future study. Second, the observation that the high-fat diet promoted a greater degree of adipocyte regeneration than did the chow diet supports the notion that dietary factors can affect adipocyte proliferation and ultimate adipocyte number (*3*).

In summary, we have observed complete regeneration of subcutaneous adipose tissue in rats which were lipectomized at 3 weeks of age and fed a high-fat diet beginning at 12 weeks of age. Rats fed only a chow diet achieved only incomplete regeneration. The restored subcutaneous adipose tissue mass of the HF-fed rats was equivalent to the subcutaneous adipose mass of controls in terms of both adipocyte number and mean lipid content per cell. Therefore, the proliferative processes which establish the adipocyte population of the subcutaneous fat tissue, and the system which determines average adipocyte size, are both active and precise in their regulation at least until the time of weaning in the rat. How long beyond weaning the regulated response of regeneration will occur and what role dietary factors play in the response are questions which remain to be answered.

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Surgical Removal of Adipose Tissue Alters Feeding Behavior and the Development of Obesity in Rats

Abstract. *Lipectomized and sham-operated rats were fed a high-fat diet to induce hyperphagia and rapid fat accumulation. Lipectomized rats with 25 percent fewer adipocytes were less hyperphagic and accumulated less fat, but their adipocytes remained equal in size to adipocytes of controls. A role for adipocyte size in fat storage regulation and food intake control is postulated.*

The hypothesis that body weight of mammals is regulated received experimental support as early as 1939 (*1*), but the more specific hypothesis of Kennedy (*2*) that body fat mass is regulated was not directly tested until the studies of Liebelt and co-workers in 1963 and 1965 (*3*). In their studies, as well as those of others (*4*), surgical removal of adipose tissue in mice or rats resulted in enlargement of remaining fat depots relative to those of control animals. Such findings were interpreted as demonstrating compensatory growth of adipose tissue and thus that total body fat mass of mice and rats is regulated. The clearest demonstrations of such apparently compensatory growth were in brain-damaged or genetically obese animals. In contrast, in recent experiments with normal rats and mice it was found that removal of various adipose tissue depots does not result in compensatory growth of remaining depots (*5*). Thus, total body fat content of rats and mice is probably not directly regulated, as Kennedy had suggested, but regulation of some other parameter related to total body fat must be responsible for the usual stability of body fat. In this report we present evidence that body fat stability in the adult rat is achieved by means of the regulation of adipocyte lipid content (or adipocyte size), and that such regulation can operate by influencing food intake.

In the young rat, increases in both adipocyte size and adipocyte number constitute normal growth of the adipose mass (*6*), but at about the time of weaning adipocyte proliferation usually ceases. Subsequent to weaning most adipose tissue growth occurs as the result of adipocyte enlargement alone (*7*). If adipocyte size were involved in the regulation of fat storage, one might expect that adipocytes would have a tendency to resist excess enlargement and that adipose tissue growth in rats after weaning