large quantities of the acetates. The amount of acetates individual *F. subsericea* workers received, on the average, was equal to or greater than the entire volatile contents of their own gland reservoirs, and more than enough to cause an alarm reaction. We feel certain that this accounts not only for the disorientation observed in many defenders during the raids but also, to some extent, for the panic and rapid retreat displayed by the slave-species colonies, and the relative ease with which their nests are breached by the slave-makers. The acetates, by virtue of the large quantities dispensed and their relatively low evaporation rate (as compared with that of other common alarm substances, such as undecane and citronellal), are, in effect, "superpheromones." They create penetrating and long-lasting alarm signals. They also serve as offensive "propaganda substances" against the colonies of the slave species, which cannot help but respond to them as alarm pheromones (*4, 6*).

It thus appears that the acetates of the Dufour's gland of *F. pergandei* and *F. subintegra* perform no less than three distinct functions in the life of the slave-maker colonies: as defensive and offensive chemical weapons, as alarm pheromones for communication within the colony, and as offensive "propaganda substances" directed at alien colonies during slave raids.

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Insulin and Microtubules in Rat Adipocytes

Abstract. *Insulin appears to promote microtubule assembly in rat adipocytes. Neither oxytocin nor high concentrations of glucose has this effect. Colchicine inhibits stimulation by insulin of lipid and glycogen synthesis without influencing stimulation by insulin of glucose oxidation. The anabolic effects of oxytocin or high concentrations of glucose are not inhibited by colchicine. The "directive effect" of insulin may involve microtubules.*

Attempts to correlate the diverse metabolic effects of insulin with ultrastructural changes in target cells have met with little or no success. The view, based upon a single study of fat cells (*1*), that insulin stimulates the formation of pinocytotic and cytoplasmic vesicles has not been supported by subsequent investigations (*2*). We now present evidence which suggests that in the fat cells of rats insulin stimulates the formation of microtubules—structures known to be associated with such diverse cellular processes as mitosis, axonal flow, and melanin granule movement (*3*). In addition, we show that characteristic anabolic responses of adipocytes to insulin are inhibited by colchicine, which disrupts microtubules by binding to their constitutent subunits (*4*). We suggest that the directive effects of insulin on anabolic processes (*5*) may depend upon microtubule assembly.

Fat cells were isolated from epididymal fat pads (*6*) from rats which were fasted for 2 to 3 days or which had free access to food. For electron microscopic studies, cells were incubated in Krebs-Ringer bicarbonate (KRB), containing 1 percent purified bovine serum albumin, but not glucose (unless otherwise stated), in the presence or absence of 10 microunits of insulin per milliliter (*7*), a dose that is nearly the maximum one for lipogenesis. The reactions were terminated by addition of glutaraldehyde, cells were postfixed in OsO_4; the cells were then embedded in Araldite (*8*).

Electron microscopic studies of fat cells incubated without or with added insulin suggested that hormone treatment promoted microtubule assembly.

Table 1. Effect of colchicine on basal and insulin-stimulated glucose oxidation and lipogenesis in adipocytes. The assay procedures have been described (*11*). Cells were incubated in KRB containing 1 percent bovine serum albumin and 1 m*M* [1-^{14}C]glucose. Values from a representative experiment are the mean values of three to four incubations ± standard error.

Colchicine concentration (*M*)	Glucose incorporation into CO_2*		Glucose incorporation into total lipid†	
	No insulin	Insulin‡	No insulin	Insulin‡
0	1.54 ± .07	3.40 ± .21	0.65 ± .03	2.25 ± .11
5×10^{-7}	1.47 ± .03	3.50 ± .16	0.74 ± .03	2.03 ± .12§
5×10^{-6}	1.56 ± .09	3.43 ± .14	0.64 ± .02	1.90 ± .17§
5×10^{-5}	1.53 ± .06	3.55 ± .22	0.72 ± .09	1.65 ± .04§

*Units are micromoles of glucose oxidized to CO_2 per gram of total lipid per hour. † Units are micromoles of glucose incorporated in the total lipid per gram of total lipid per hour. ‡ Insulin concentrates of 10 microunits per milliliter. § Effect of insulin (incremental difference between samples incubated with and without insulin) significantly reduced by colchicine ($P < .01$).

Table 2. Effect of colchicine ($5 \times 10^{-5}M$) on adipocyte lipogenesis and glycogenogenesis stimulated by insulin, oxytocin, and high glucose. Results are mean values ± standard error from groups of three to four incubations from a typical experiment. Values are obtained as the difference between basal and treated incorporation rates and represent the effect of the treatment on the anabolic process.

Treatment	Glucose incorporation into total lipid*		Glucose incorporation into total glycogen†	
	No colchicine	Colchicine	No colchicine	Colchicine
Insulin (10 μunit/ml)	4.02 ± .15	3.00 ± .14‡	0.91 ± .08	0.65 ± .08§
Oxytocin (0.5 μg/ml)	1.37 ± .14	1.87 ± .35	<0.01	<0.01
Glucose 10 (μ*M*/ml)	3.59 ± .19	3.50 ± .37	1.51 ± .01	1.50 ± .19

* Lipogenesis was measured as the incorporation of [1-^{14}C]glucose into the heptane-soluble fraction of cells (*11*). The basal rate of lipogenesis in this experiment was 1.23 ± 0.12 μmole of glucose incorporated per gram of total lipid per hour. † Glycogenogenesis was measured by incorporation of [1-^{14}C]glucose into KOH-soluble, ethanol-insoluble material left after removal of lipids with heptane, as measured by Gutman and Shafrir (*15*). Basal rates were <0.01 μmole of glucose incorporated per gram of total lipid per hour. ‡ Effect of colchicine significant, $P < .01$. § Effect of colchicine significant, $P < .05$.

Microtubules were commonly observed at fat cells (from both fasted and fed rats) incubated with insulin for either 10 or 60 minutes (Fig. 1). Microtubules were not detected in untreated control cells prepared from fasted rats and were only rarely seen in untreated cells from fed animals (*9*). These statements are based on examination of at least 100 cells with each type of treatment. Sections were coded so that the microscopist was unaware of the conditions of incubation of the adipocytes prior to fixation. Because of the apparently scattered distribution and irregular orientation of the microtubules and their small diameter relative to section thickness, quantitative measurements were not feasible with this material. Microtubules were not arranged in any readily discernible orientation, except that they were usually perpendicular to a radius of the cell (Fig. 1). After preliminary treatment of fat cells with $5 \times 10^{-5} M$ colchicine for 45 minutes before the addition of insulin, microtubules were not observed (*10*).

If the expression of insulin action depends on microtubule formation, interference with microtubule integrity by colchicine should result in an inhibition of those insulin-stimulated parameters that require microtubule assembly. Table 1 shows the insulin response of fat cells first incubated for 45 minutes with various concentrations of colchicine. In the doses tested, colchicine did not affect the basal levels of glucose oxidation or glucose conversion to lipids. The stimulation of lipogenesis by insulin was inhibited by colchicine; however, colchicine did not affect insulin-stimulated glucose oxidation. Maximum inhibition of insulin-stimulated lipogenesis was achieved with colchicine at concentrations of 5×10^{-5} mole/liter. Colchicine also inhibited insulin-stimulated glycogen formation (Table 2).

Anabolic processes in fat cells can be stimulated by raising the extracellular glucose concentration (*6, 11*) or by adding certain chemical agents. To determine whether stimulation of microtubule assembly in fat cells is a unique feature of insulin action, fat cells were incubated in a "high glucose" (10 mM) medium without added hormones or with added oxytocin (0.5 μg/ml) in standard media with 1.0 mM glucose (Table 2). Oxytocin was tested because it stimulates glucose oxidation and lipogenesis in fat cells (*6, 11*) although its mechanism of action differs from that of insulin (*11, 12*); unlike insulin, oxytocin does not stimulate glycogenogenesis (*13*). Lipid synthesis was increased by incubating cells with oxytocin and by raising the extracellular glucose concentration, but colchicine had no affect on the lipogenic activity

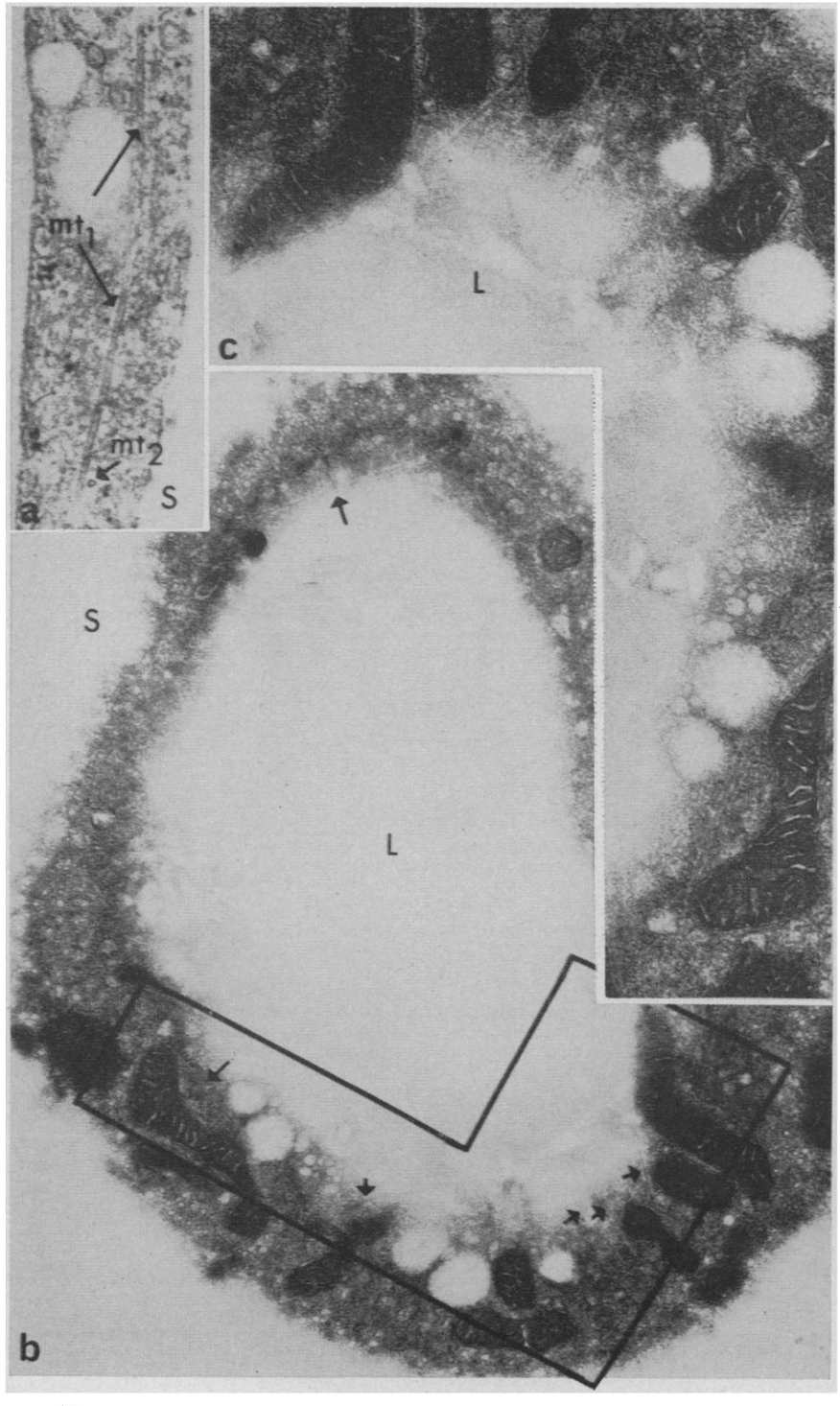

Fig. 1. Sections of adipocytes fixed after incubation with 10 microunits of insulin per milliliter without glucose for 10 minutes. *S* is the extracellular space; *L* is the lipid drop within the fat cell. (a) Section through the central part of an adipocyte, showing cytoplasmic envelope that surrounds lipid. Two microtubules (mt_1) lie nearly in the plane of section; another (mt_2) is transverse to it (×18,000). (b) Fairly thick section near one pole of a cell. Plane of section nearly tangent to lipid droplet. Arrows indicate some of the many microtubules in this section. The tubules appear to form a network tangent to the fat droplet (×18,000). (c) Enlargement of region of adipocyte outlined in (b). Note electron-lucent region around each tubule (×30,000).

of oxytocin or high glucose. Glycogen synthesis in adipocytes was stimulated by 10 m*M* glucose, but not by oxytocin. Although colchicine inhibited insulin-stimulated glycogen synthesis, it had no effect on the glycogenogenic response to high glucose. Microtubules were not observed in electron micrographs of adipocytes incubated for 10 or 60 minutes with either 10 m*M* glucose or with oxytocin (0.5 μg/ml) in the absence of added glucose.

These results indicate that concentrations of insulin within the physiological range induce the appearance of microtubules in fat cells. This structural rearrangement of cytoplasm seems to be specific for insulin because neither oxytocin nor high glucose concentration produces this effect. The finding that colchicine did not influence insulin-stimulated glucose oxidation while it inhibited the effect of insulin to stimulate the biosynthesis of lipid and glycogen, indicates that microtubules are not involved in the primary interaction of insulin with its receptor in the plasma membrane (*14*) or in the consequent increase in glucose transport. The facts that microtubules were not observed in cells treated with oxytocin or high glucose and that colchicine did not inhibit the effects of oxytocin or high glucose suggest that (i) microtubules are not associated with the subcellular machinery involved in glucose metabolism and that (ii) alteration of the basal rate of glucose metabolism by substances other than insulin does not require microtubule assembly.

The fact that colchicine inhibited but did not abolish the effects of insulin on the synthesis of lipids and glycogen must be viewed in the context that the insulin effect to increase glucose uptake was not impaired by colchicine treatment. The partial stimulatory effect of insulin on lipid and glycogen synthesis in the presence of colchicine is the consequence of insulin action to increase glucose transport into the cell, a process that does not appear to involve microtubules. Reorganization of the cytoplasm by microtubule assembly would appear to be essential for a unique effect of insulin to "direct" glucose flow into certain metabolic pathways but not others. Such a "directive effect" of insulin, long recognized in muscle (*5*), appears to be operative in fat cells as well, as these studies with colchicine reveal.

Although microtubules have been described in association with a great number of cellular processes, our report is the first linking microtubule assembly to hormonal regulation of metabolic activity.

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Immunotherapy of Cancer: Regression of Tumors after Intralesional Injection of Living Mycobacterium bovis

Abstract. *Injection of living* Mycobacterium bovis *(strain BCG) into established intradermal tumors caused tumor regression and prevented the development of metastases.*

Immunologic methods are of value in the clinical treatment of epidermal tumors, intradermal metastases of malignant melanoma, and acute leukemia (*1*). Successful treatment of skin tumors in man requires the development of an inflammatory reaction of the delayed type at the site of the tumor. There is no animal model available which adequately reflects this kind of immunotherapy. We now show that tumor nodules in guinea pigs regress at the site of inflammatory reactions provoked by living *Mycobacterium bovis,* strain BCG. This treatment impairs the development of lymph node metastases.

Inbred (Sewall Wright) strain-2 male guinea pigs were obtained from the breeding colony of the National Institutes of Health. The induction of primary hepatomas in guinea pigs by feeding them the water-soluble carcinogen diethylnitrosamine and the antigenic and biologic characteristics of transplantable ascites tumors derived from these primary tumors have been described (*2*). In our experiments, ascites tumor line 10, sixth transplant genera-