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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8. Solutions used for fixation were 2 percent glutaraldehyde and 1 percent OsO_4, each in 0.15*M* sodium phosphate buffer (*p*H 7.5). Portions (50 μl) of packed fat cells, prepared from 2 to 3 g (wet weight) of rat epididymal fat pads, were added to Eppendorf vials containing the KRB and other components of the incubation medium. Incubation volume was 0.5 ml. At the end of the incubation period, 1 ml of cold glutaraldehyde was added to each vial. The vials were centrifuged for 2 minutes in an Eppendorf centrifuge, so that the cells were packed into a pellicle that floated on the surface of the glutaraldehyde-medium mixture. The infranatant was removed by aspiration and the pellicle was fixed in glutaraldehyde for 30 minutes. The pellicles were washed for 15 minutes in 0.15*M* sodium phosphate buffer (*p*H 7.5) and then fixed for 30 minutes in OsO_4. All steps from fixation through the first steps of dehydration were carried out in the cold. The Araldite 502, which was used for embedding, was a gift from the Ciba Corp., Summit, New Jersey.
9. While this manuscript was being prepared, it was reported that microtubules are observed infrequently in (i) adipocytes isolated from rats with free access to food [M. C. Schots, J. E. Stewart, A. S. Garfinkel, C. F. Whelan, N. Baker, M. Cohen, T. J. Hensley, M. Jacobson, in *Drugs Affecting Lipid Metabolism*, W. L. Holmes, L. A. Carlson, R. Paoletti, Eds. (Plenum, New York, 1969), p. 161] and in (ii) insulin- or epinephrine-treated adipocytes from fed rats with free access to food [S. W. Cushman, *J. Cell. Biol.* **46**, 326 (1970)]. These observations are not inconsistent with ours.
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17 November 1970

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-

Table 1. Prevention of growth of lymph node metastases after intratumor injection of BCG. I.T., intratumor injection.

Group	Treatment	Response of guinea pigs to intratumor injection of BCG	
		Intradermal tumor regression*	Palpable lymph node metastases†
1	BCG I.T.	6/8	2/8‡
2	Diluent I.T.	0/8	8/8
3	Excision		8/8

* Ratio of number of animals with intradermal tumor regression to the number of animals tested. † Ratio of the number of animals with palpable lymph node metastases to the number of animals tested. ‡ The difference in incidence of palpable lymph node metastases in groups 1 and 3 is significant by the Fisher exact test at $P < .005$.

tion, was used (*3*). This tumor, a hepatocarcinoma, grows progressively when 10^5 cells are injected intradermally and metastasizes to the lymph nodes draining the site of tumor injection. Guinea pigs die 60 to 90 days after intradermal inoculation of 10^6 tumor cells. The BCG (Phipps strain TMC 1029) was obtained from the Trudeau Institute (*4*). Guinea pigs were immunized with a single intradermal injection of 6×10^6 living BCG organisms. About 28 days after immunization 10^6 line 10 tumor cells were injected intradermally. When intradermal tumor nodules 8 mm in diameter and 3 mm thick were visible, living BCG organisms (12×10^6 organisms contained in 0.2 ml) were injected intradermally into the base of the tumor through a 26 gauge needle; 12×10^6 organisms contained in 0.2 ml were injected subcutaneously into the base of the tumor through a 26 gauge needle. Delayed hypersensitivity reactions were measured 24 hours after injection of BCG organisms. Tumor nodules, and the inflammatory reactions caused by BCG injection were measured twice a week. Papule size is a measure of the tumor nodule and of the inflammatory reaction elicited in the tumor nodule by BCG.

The response of guinea pigs immunized to BCG and unimmunized guinea pigs to intratumor injection of living BCG is illustrated in Fig. 1, A and B. In this experiment, seven immunized guinea pigs received intratumor injections of BCG; six immunized guinea pigs received intratumor injections of saline; eight unimmunized guinea pigs received intratumor injections of BCG and six unimmunized guinea pigs received intratumor injections of saline. Guinea pigs immunized to BCG, developed delayed hypersensitivity reactions at the tumor site when the tumor was injected with living BCG; immunized guinea pigs did not show skin reactions when the tumor was injected with saline; unimmunized guinea pigs did not develop skin reactions at the tumor site when the tumor was injected with living BCG or saline. An indolent inflammatory reaction characterized by edema, induration, necrosis, and ulceration developed in immunized and unimmunized guinea pigs at the site of BCG inoculation. In immunized and unimmunized guinea pigs established tumors regressed at the site of inflammatory reactions evoked by BCG.

The development of metastases was affected by BCG treatment (Table 1). In this experiment guinea pigs were treated 6 days after intradermal injection of tumor cells by excision of the growing intradermal tumor or by intratumor injection of BCG or saline. Guinea pigs treated by tumor excision developed palpable, progressively growing, lymph node metastases 30 days after excision. There was no tumor recurrence at the excision site. Metastases were not detected in guinea pigs that received intralesional injections of BCG; guinea pigs treated with saline developed metastases and had progressive tumor growth. We interpret these findings to mean that tumor cells were present in the regional lymph nodes of all experimental animals on the day of treatment and that BCG treatment prevented the formation of clinically detectable metastases.

Table 2. Response of guinea pigs to intratumor injection of BCG. The results are expressed as the ratio of the number of animals with complete tumor regression to the number of animals tested. The difference between treated and control groups by the Fisher exact test (*7*) is significant at $P < .01$.

Experiment	BCG treated	Control
1	8/12	0/4
2	10/15	0/12
3	6/8	0/7
Total	24/35 (69 percent)	0/23 (0 percent)

Our experience with BCG treatment is summarized in Table 2. Of the 35 animals treated with BCG, 24 are free of tumor; there was marked retarda-

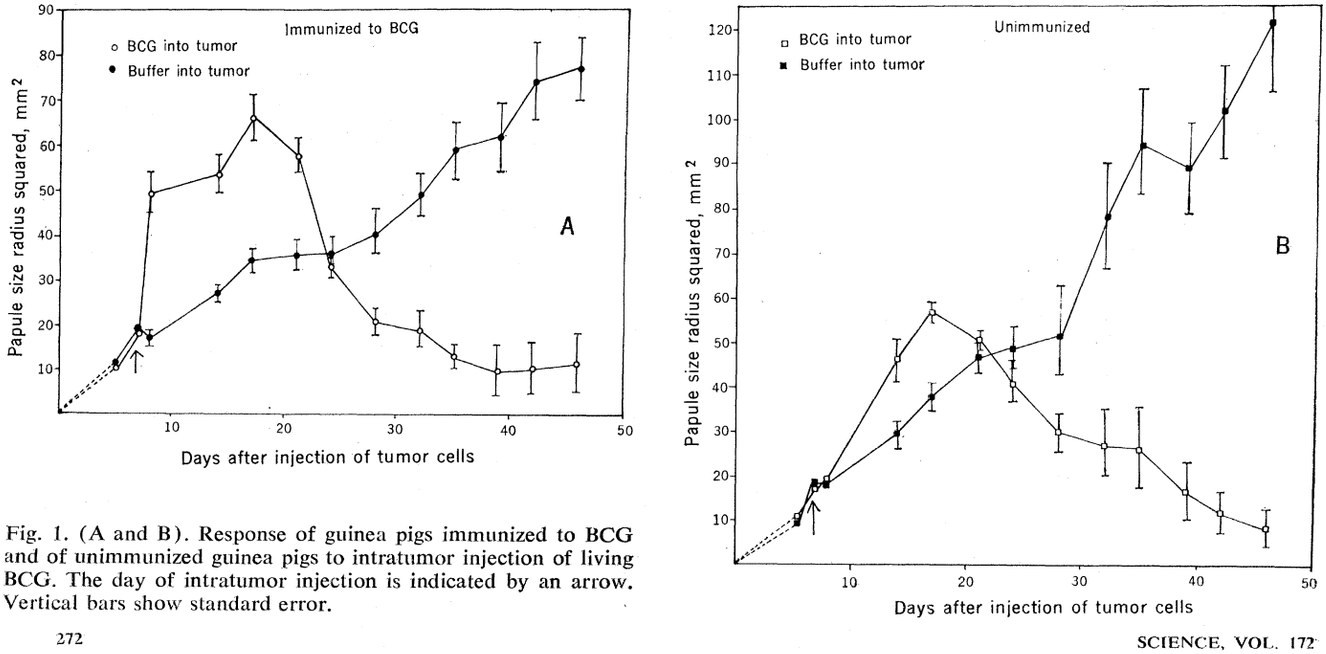

Fig. 1. (A and B). Response of guinea pigs immunized to BCG and of unimmunized guinea pigs to intratumor injection of living BCG. The day of intratumor injection is indicated by an arrow. Vertical bars show standard error.

tion of tumor growth in the remainder of the animals. The trauma associated with injection of saline did not lead to tumor regression. The observation period for experiment 1 is 164 days, for experiment 2 it is 97 days, and for experiment 3 it is 72 days. The tumor is uniformly fatal to guinea pigs that do not show complete tumor regression.

The only complication of BCG treatment was the occasional occurrence of infection with a Gram-negative organism at sites of BCG injection.

This is the first report of animal immunotherapy based on the principle that tumor growth is inhibited at sites of delayed hypersensitivity reactions provoked by antigens unrelated to the tumor. Our work differs from other previous work (*5*) on BCG and tumor growth in several important respects. (i) Our experiments involve treatment of established palpable tumors. Most animal experiments involve BCG-mediated prevention of tumor growth. (ii) Tumors that were 100 mg in size completely regressed as a result of local BCG therapy. The maximum tumor load that could be effectively treated with systemic BCG treatment was 10^5 cells (*6*). (iii) For optimum therapeutic effect contact between BCG and tumor cells was necessary. This critical variable has not been appreciated before. (iv) Treatment with BCG prevented the formation of clinically detectable metastases. Our model may be conveniently used for experiments designed to resolve the question of the nature of the mechanism of immunologic tumor destruction and the question of whether established tumors in sites other than the skin can be controlled by the type of immunotherapy described.

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30 December 1970

Somatosensory System: Organizational Hierarchy from Single Units in Monkey Area 5

Abstract. *The receptive fields of single cells in area 5 of monkey parietal cortex were studied by extracellular recording. Cells were driven primarily by gentle manipulation of multiple joints residing on one or more limbs. Both excitatory and inhibitory convergence were demonstrated. It is postulated that the multijoint receptive fields of area 5 are the result of convergence from single-joint cells of the primary receiving area. An analogy is drawn between the modification of information in the visual and somatosensory systems.*

Previous studies of response characteristics to physiological stimulation of single cells in the somatosensory system (*1–6*) have not suggested an organizational hierarchy analogous to that demonstrated by Hubel and Wiesel (*7*) in the visual system. Indeed, Powell and Mountcastle (*1*) emphasize the fidelity with which response properties of peripheral somesthetic receptors are reproduced by single units in the somatosensory thalamic relay nucleus (VPL) and primary cortical receiving area, S1 (Brodmann's cytoarchitectonic areas 3, 1, and 2). This is in marked contrast to the visual system where messages undergo considerable modification, especially in the cortex.

The receptive fields of VPL neurons are modality specific and entirely contralateral (*2*). Kinesthetic cells respond to movement of one joint in one direction only, and touch cells respond to small areas of stimulation. In S1, cells show similar properties (*3*). Intramodality convergence, that is, convergence of more than one joint, is not reported. Intermodality convergence is extremely rare. In general, within S1, the modalities of touch and kinesthesis are segregated into separate cellular columns. In a later study of S1, Werner and Whitsel (*4*) found no evidence for convergence of either type. In view of the recently demonstrated bilateral projection of S1 to Brodmann's area 5 of parietal cortex (*8*), we investigated receptive field properties in this region to determine whether higher order interaction may exist within the somatosensory system.

Short-term experiments were carried out in six *Macaca mulatta*, weighing 2 to 3.5 kg. Animals were prepared under a short-acting barbiturate (sodium methohexital) and later were studied in the awake, unanesthetized state (paralyzed with gallamine triethiodide and artificially respired). Wound margins and pressure points were infiltrated with a long-lasting local anesthetic preparation (procaine in peanut oil). Adequate fluid and electrolyte intake was given intravenously, and temperature and blood pressure were monitored. Single unit extracellular recording was undertaken with tungsten microelectrodes, insulated except for a 2- to 10-μm tip exposure. Unit activity was led through a field effect transistor source follower to a Tektronix 1A7A amplifier and recorded on magnetic tape with a concurrent voice channel. Joints were manually rotated. Skin was stimulated with blunt probes, brushes, and air blasts. For every cell excited by joint manipulation, care was taken to rule out effects of concurrent touch or tissue deformation. A unit was con-

Table 1. Receptive field properties of cells in area 5.

Properties			Cells (No.)
Modality			
Position sense only			47
S1 properties		5	
Involvement of more than one joint		42	
One limb	6	(+5 from T-P)	
Two limbs	24	(+4 from T-P)	
Three limbs	7		
Four limbs	5		
Touch only			9
S1 properties		2	
S2 properties		7	
Touch-position sense convergence (T-P)			9
Unable to characterize			10
Total			75
Laterality			
Contralateral only			23
Ipsilateral only			3
Bilateral			39
Unable to characterize			10
Total			75