diac output and increased blood flow at 20 minutes and 72 hours, lower at 4 and 24 hours. The adrenals, bone (limb bone, skull, and spine), and bronchial artery flow to the lung (except at 20 minutes and 4 hours) had a significantly higher fraction of output and lower resistance throughout the stress period; the thyroid had a lower fraction of cardiac output and blood flow and a higher resistance during the stress.

After the initial fall, the continued increase in resistance of skeletal muscle (which in these monkeys comprised 43 percent of the animal's weight) was the predominant regional influence contributing to the increase in total peripheral resistance. Because the amount of muscular activity was not systematically evaluated during the experiment it was not possible to evaluate the metabolic requirements in muscle which may have contributed to the blood flow and resistance changes.

The very large increases in the fraction of cardiac output to the hepatic artery throughout the stress period is similar to that found in bled monkeys (*7*). Although these observed distributional changes do not, necessarily, reflect the nutritional demands of tissues, it seems that the maintenance of arterial blood flow to the liver, as to the heart, is an important aspect of increased sympathetic function.

It can be concluded that a combination of behavioral stress, fatigue, and other physical factors in the primate results in a maintained elevation of systemic arterial pressure over a 72-hour period. Initially, the elevated pressure is due to an increase in cardiac output with a balanced pattern of peripheral resistance changes. Subsequently, cardiac output returns toward baseline levels while total peripheral resistance increases, primarily owing to the progressive vasoconstriction in skeletal muscle. Neurogenic, humoral, and local tissue factors are all probably important for the observed hemodynamic changes, but are at present undefined.

A relationship between long-term avoidance conditioning and high systemic blood pressure in the monkey has been previously demonstrated (*8*). The main contribution of this report is to elucidate the possible hemodynamic mechanisms operating to produce this stress-related hypertension, although the duration and the severity of the stress and related physical factors were of a much different nature in the present study. Further, the preparation and the methods described demonstrate the feasibility of studying the contribution of both humoral and autonomic responses to environmentally induced stress in the unanesthetized primate.

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Contraction of Granulation Tissue in vitro: Similarity to Smooth Muscle

Abstract. *Strips of granulation tissue from three different experimental models contract in vitro when treated with substances that induce contraction of smooth muscle. Because the fibroblasts in such tissues have some ultrastructural features typical of smooth muscle, our findings indicate that fibroblasts are able to modulate toward a cell type that is morphologically and functionally close to smooth muscle.*

The process of repair often results in local shrinkage, best exemplified by the contraction of healing wounds. This shrinkage is thought to depend upon contraction of the granulation tissue (*1*), probably of its fibroblasts (*2*). Indeed, cultured fibroblasts extracted with glycerin contract in vitro in response to adenosine triphosphate (*3*), presumably as the result of an effect on actomyosin (*4*).

In an electron microscopic study of fibroblasts in four models of contracting granulation tissue, we were impressed by the fact that many of these cells developed characteristics intermediate between those of "typical" fibroblasts and those of smooth muscle cells (*5*). This suggested that granulation tissue might contract in response to the same stimuli that affect smooth muscle in vitro.

Therefore, we tested the contractility of strips of granulation tissue as it is routinely tested on smooth muscle. The preparations were suspended from one end of a frontal lever (*6*) and attached to the bottom of a bath containing 20 ml of Tyrode's solution maintained at 37°C and bubbled with 95 percent oxygen plus 5 percent carbon dioxide. The lever amplified vertical displacement by a factor of 6; the tungsten recording stylus inscribed on metallized paper advancing on a kymograph (*6*) at a rate of 1 mm per minute.

We first used granulation tissue from Selye's "granuloma pouch" (*7*), produced in 100- to 130-g male Wistar albino rats by the injection of 20 ml of air and then 1 ml of 1 percent croton oil (*8*) in corn oil into the dorsal subcutaneous tissue. A spiral cut around the pouch wall (1 to 2 mm thick) gave a strip of tissue 70 to 90 mm long and 6 to 8 mm wide weighing 700 to 1100 mg.

5-Hydroxytryptamine (5-HT) (1×10^{-5} g per milliliter of Tyrode's solution, final concentration in the bath) caused an immediate contraction of such strips (Fig. 1A), which usually reached a maximum in 1 to 2 minutes; the contraction tended to persist at this level or relax slightly, but did not return to the baseline. The actual shortening of the tissue was of the order of 3 percent. A typical dose-response study is shown in Fig. 1B. In strips left for 1 hour in the bath without oxygenation, the effect of 5-HT was greatly diminished. The age of the granulation tissue also made some difference; a strip from a 7-day pouch failed to contract with 5-HT, and one from an 8-day pouch contracted only slightly. However, strips from pouches 11 to 32 days old reacted uniformly well, despite the older ones being histologically a little more fibrous. Bradykinin (1×10^{-5} g/ml) caused a similar though lesser contraction than 5-HT at the same concentration; the response

to histamine (1×10^{-5} g/ml) was slight and slower to reach its peak. Strong and quick contractions were obtained with angiotensin (1×10^{-5} g/ml) and vasopressin [0.25 international unit (I.U.) per milliliter of Tyrode's solution] (Fig. 1C). Responses to epinephrine (1×10^{-5} g/ml) and norepinephrine (1×10^{-5} g/ml) were moderately good, but their peaks had slower rises. Papaverine (1×10^{-4} g/ml) caused a slow relaxation, whether it was applied to a fresh strip, to a strip under the influence of 5-HT (Fig. 1A), or to one influenced by another active agent; in such cases the relaxation usually progressed to a level well below the baseline. Acetylcholine, tryptophan, and histidine, all at concentrations of 1×10^{-5} g/ml, had no detectable effect on strips of granuloma pouch.

To rule out the possibility that the contractions observed might be somehow related to the granuloma pouch as such, we tested a different type of granulation tissue. Clots obtained from 8 ml of homologous blood were implanted into the peritoneal cavity of 200- to 300-g rats; by 7 days each clot was surrounded by a thin fibrous capsule partially adherent to the omentum and to the pelvic fat bodies. Strips obtained from this capsule between 7 and 12 days responded to 5-HT and papaverine in the same manner as those of the granuloma pouch (Fig. 1D).

To study the relevance of these data for wound contraction, we also tested the granulation tissue obtained from the base of 7- to 12-day-old healing wounds produced on the backs of 100- to 200-g rats by the excision of a piece of skin, including the panniculus carnosus, measuring 2.5 by 2.5 cm. The first results obtained with strips of this wound tissue were disappointing; the contractions were small and unconvincing. Therefore, assuming that the tissue in this case had already become well contracted in vivo, we tried stretching the strips gently and progressively for 1 to 2 hours (increasing their length by 50 to 100 percent) before testing. Good responses were then obtained with bradykinin (Fig. 1E) and angiotensin (Fig. 1F) both at a concentration of 1×10^{-5} g/ml, and with vasopressin (0.25 I.U./ml). Epinephrine (1×10^{-5} g/ml) gave a smaller, but definite, contraction. We never recorded a satisfactory response with 5-HT (1×10^{-7} to 1×10^{-4} g/ml) or with histamine (1×10^{-5} to 1×10^{-4} g/ml). Papaverine (1×10^{-4} g/ml) again consistently produced relaxation of the strips (Fig. 1, E and F).

As controls, we used various rat tissues including dorsal skin with and without the panniculus carnosus, dorsal subcutaneous tissue (from which the granuloma pouch develops), tail skin, and tail tendons. In no case was any contraction or relaxation recorded, even after the preparations had been stretched.

In seeking a chemical basis for granulation tissue contractility, we extracted actomyosin (*9*) from 21-day-old granuloma pouch walls. The yield was 4 mg/g (wet weight), which is comparable to the value (3.5 mg/g, wet weight) we obtained with identically prepared extracts of uteri from pregnant rats. The calcium-activated adenosine triphosphatase activities of these extracts were similar, each splitting approximately 10 nmole of adenosine triphosphate per milligram of protein per minute (*10*).

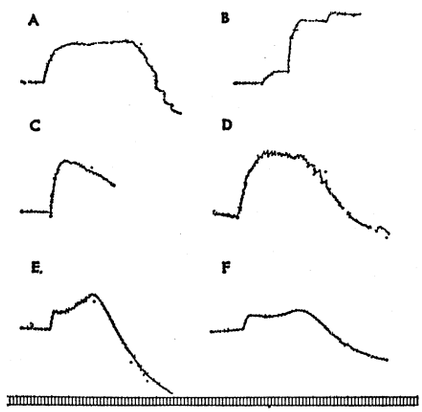

Fig. 1. Effects of various substances on the length of strips of granulation tissue. The scale indicates time, one division representing 1 minute. (A) Contraction of 25-day-old granuloma pouch tissue in response to 5-HT (1×10^{-5} g/ml), followed by relaxation with papaverine (1×10^{-4} g/ml). (B) Contraction of 22-day-old granuloma pouch with graded doses of 5-HT: 1×10^{-7} g/ml, no effect; 1×10^{-6} g/ml, first small rise of tracing; and 1×10^{-5} and 1×10^{-4} g/ml. (C) Contraction of 15-day-old granuloma pouch with vasopressin (0.25 I.U./ml). (D) Contraction of granulation tissue obtained from surface of blood clot, 11 days after intraperitoneal implantation, in response to 5-HT (1×10^{-5} g/ml) and relaxation with papaverine (1×10^{-4} g/ml). (E) Contraction of granulation tissue from base of 12-day-old skin wound in response to bradykinin (1×10^{-5} g/ml) and relaxation with papaverine (1×10^{-4} g/ml). (F) Contraction of 8-day-old skin wound tissue with angiotensin (1×10^{-5} g/ml) and relaxation with papaverine (1×10^{-4} g/ml). All curves are photographs of actual tracings, reduced to about one half.

These experimental data indicate that granulation tissue is a contractile organ resembling smooth muscle. That oxygen is required for its contraction in vitro suggests that a cellular process is involved. The active structures could be the fibroblasts, the blood vessels, free smooth muscle cells derived from the vessels, or a combination of these elements. Since the control preparations, even those that were richly vascular, did not contract under the same conditions of testing, it is unlikely that an effect on blood vessels contributed significantly to our results.

On the other hand, the fibroblasts in the models studied fulfill a number of requirements that would be expected of contractile cells (*5*). They contain an extensive fibrillar system, and their nuclei show deep folds and pinches; in addition, there are intercellular connections in the form of maculae adhaerentes, as well as hemidesmosomes with surrounding material similar to basement membrane; the two latter features, singly or jointly, are essential if cellular contraction is to induce a shortening of the tissue as a whole. It is of interest that modified fibroblasts ultrastructurally similar to those of granulation tissue have been recently described in certain normal tissues such as the chicken aorta (*11*) and rat ovary (*12*); the significance of these is as yet unknown.

The last possibility, that granulation tissue consists of free smooth muscle cells derived from blood vessels, cannot be excluded but it is unlikely for several reasons. First, electron microscopy shows that the extravascular cells in our models of granulation tissue are overwhelmingly of the contractile type (*5*); thus one would have to assume that most of the granulation tissue is built up by the smooth muscle of blood vessels, while the commonest type of connective tissue cell, the fibroblast, merely stands by. Second, a shedding of smooth muscle cells by blood vessels is not recognizable morphologically. Third, fibroblasts cultivated in vitro normally develop an extensive cytoplasmic fibrillar system (*13*). In preliminary studies of such fibroblasts obtained from normal rat dermis, we have observed that the addition of serotonin (1×10^{-5} g/ml) to the culture medium causes cellular contraction within 15 to 20 minutes, whereas tryptophan (1×10^{-5} g/ml) has no effect under the same conditions.

Further work is needed to pinpoint the nature and mediation of granulation tissue contraction in situ during wound healing. However, our results support the conclusion that fibroblasts, under certain conditions, are capable of modulating toward a cell type that is structurally and functionally close to smooth muscle; for these cells the name "myofibroblast" may be appropriate.

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Bilateral Symmetry and Interneuronal Organization in the Buccal Ganglia of Aplysia

Abstract. *Principles of functional organization of the bilaterally symmetric buccal ganglia of* Aplysia *were studied in 20 identified cells used as a reference population. Four of the identified cells (two in each ganglion) are multiaction interneurons, each of which innervates six identified ipsilateral follower cells, mediating cholinergic excitation to one cell and cholinergic inhibition to five others. Bilateral coordination is effected by common inputs to all four interneurons. Ipsilateral pairs of interneurons are electrotonically coupled and produce identical synaptic actions on their common follower population. This apparent redundancy of interneuronal action leads to feed-forward summation, eliciting amplified synaptic output from each interneuron pair.*

The buccal ganglia of the marine mollusk *Aplysia californica* share with the better known abdominal ganglion several advantages for neurophysiological studies (*1*). Chief among these is the presence of large neuronal cell bodies that are easily penetrated by microelectrodes, thus permitting the electrophysiological identification of individual cells. In addition, the two buccal ganglia are symmetric and provide an opportunity for studying the principles of organization of a bilaterally symmetric structure. I have identified 20 cells, including four multiaction, presumably cholinergic, interneurons, in the two buccal ganglia and have used these cells as a reference population for describing the functional interconnections of these ganglia (*2*). The two interneurons in each ganglion receive common inputs and produce identical synaptic actions on their common follower cell population. This arrangement permits neural information to be conveyed through either of two parallel channels. In addition, information can be conveyed through both channels simultaneously, because electrical coupling between the two interneurons tends to cause them to fire synchronously in response to a large common input. Moreover, activity in one interneuron tends to initiate activity in the other.

The two mirror-image buccal ganglia are linked by a commissure and are located on the caudal surface of the pharyngeal bulb (buccal muscle). The ganglia innervate the buccal musculature, the radula, the esophagus, and the salivary glands (*3*). Each of the ten identified cells in one ganglion has a symmetric mate in the other ganglion. The symmetric cell pairs display similar properties, including common synaptic input (*1*). The symmetric cells are not directly interconnected, although functional connections do exist between ganglia. Six of the ten identified cells on each side are innervated by two interneurons (BL_4 and BL_5 on the left, or BR_4 and BR_5 on the right). The actions of each interneuron appear to be confined ipsilaterally; I have not yet found contralateral follower cells.

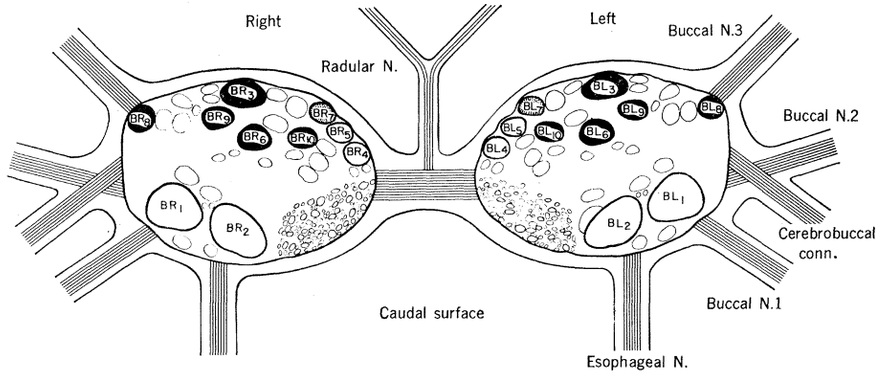

Fig. 1. Schematic drawing of the caudal surface of the buccal ganglia of *Aplysia californica* indicating typical positions of 20 identified cells. Cells are designated by the letters *BL* or *BR*, which indicate location in the left or right buccal ganglion, followed by a one- or two-digit number. Anatomically and functionally symmetric cells are assigned the same number, otherwise the numerical designations are arbitrary. Cells BL_4, BL_5, BR_4, and BR_5 are multiaction interneurons. Each mediates inhibition to five ipsilateral cells, shown outlined in black, and excitation to one ipsilateral cell, shown stippled.