TABLE II. DNA Values in Uteri after Thyroidectomy and Estrogen Stimulation.

	DNA (μg/gm of tissue wet wt.) mean ± SE
Nonthyroidectomized (control)	11,296 ± 1529
Nonthyroidectomized estrogen stimulated	4849 ± 323[a]
Thyroidectomized	10,520 ± 319
Thyroidectomized estrogen stimulated	5007 ± 345[a]

[a] $p < .05$ when compared to appropriate control.

shift results in formation of less ATP's since succinate-to-coenzyme Q does not result in the formation of an ATP. Many investigators find a lowered P/O ratio in hyperthyroidism.

With respect to smooth muscle, no change in enzyme activity or DNA content in bladder or uterus was found with either thyroidectomy or thyroxine injections. Barker (6) found no change in oxygen consumption of gastric smooth muscle after thyroidectomy or thyroxine treatment and Whaley *et al.* (7) found duodenal smooth muscle unresponsive to thyroxine treatment as determined by oxygen consumption measurements. The activity of succinoxidase is unaltered with thyroidectomy in uterine smooth muscle (1). These results support the hypothesis that smooth muscle is not responsive to thyroid hormones.

It appears from the results in Table II that thyroid hormones are not necessary for a response from the uterine muscle tissue to estrogen since activity of the enzyme DPNH–cytochrome *c* reductase increased equally in the thyroidectomized animals as in the nonthyroidectomized animals and the DNA content decreased to the same extent.

Summary. Changes in the activity of DPNH–cytochrome *c* reductase in heart, liver, bladder, and uterus were determined after thyroidectomy and thyroxine treatment. Liver and heart showed decreases in enzyme activity in the hypermetabolic state. Bladder and uterus were unresponsive. Enzyme activity increased and DNA content decreased in uteri after estrogen stimulation irrespective of metabolic status of the animal.

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Barium Stimulation of Esophageal Smooth Muscle* (32955)

JAMES CHRISTENSEN[1] (Introduced by James A. Clifton)
(With the technical assistance of Fredrick L. Barnett)
Gastroenterology Research Laboratory, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52240

Knowledge of the responses of esophageal smooth muscle to drugs should lead to a better understanding of the mechanisms of esophageal movement in deglutition. The pharmacology of esophageal smooth muscle has received little attention. In intestinal smooth muscle, barium induces contraction either through stimulation of preganglionic nerves, postganglionic nerves or by a direct effect on the smooth muscle cells (3–8). In the dog, whose esophagus is mainly striated muscle, barium *in vivo* induces contractions which are abolished by atropine (9). The

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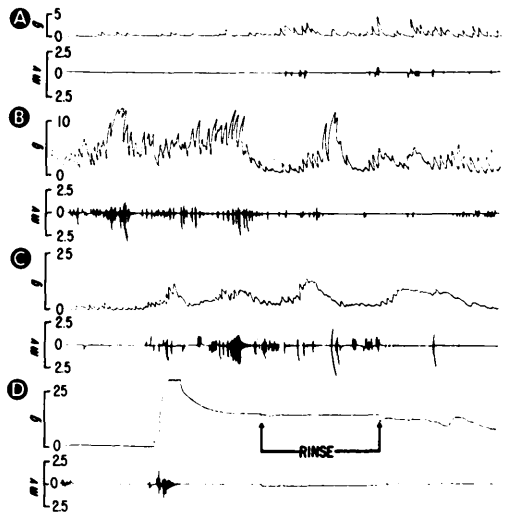

FIG. 1. Motor and electrical responses to increasing concentrations of $BaCl_2$ in one muscle strip. In each pair of tracings the upper trace shows tension and the lower traces shows electrical activity recorded from the surface of the strip. A: The responses to $BaCl_2$, 10^{-4} gm/ml. B: The responses to $BaCl_2$, 5×10^{-4} gm/ml. C: The responses to $BaCl_2$, 10^{-3} gm/ml. D: The responses to $BaCl_2$, 5×10^{-3} gm/ml. In D, a period of rinsing is shown not to reverse the contracted state of the tissue. In each pair of tracings the total length shown is 8 min.

response of esophageal smooth muscle to barium, however, has not been previously reported.

Methods. The entire esophagus was removed from ether-anesthetized adult cats of both sexes. Transverse strips, $1 \times \frac{1}{3}$ cm, cut from the distal 2 cm of the esophagus, were immersed in a bath of Krebs–Ringer solution bubbled continuously with 95% O_2–5% CO_2 and held at 36.5–37.5°C. The Krebs–Ringer solution had the composition previously described (1,2) except that it contained no sulfate. Isometric contractions of the strips were registered continuously with a force-displacement transducer (Grass Instrument Co., FT.03C). A glass-pore salt bridge electrode (1,2) served to detect electrical activity from the surface of the muscle. Both tension and electrical activity were recorded simultaneously on a polygraph. Tension responses were measured by planimetry of the area under the curves of the tension record during the first 5 min of response to barium chloride added to the bath. The following agents, dissolved in Krebs–Ringer solution, were used: barium chloride, atropine sulfate, nicotine sulfate, hexamethonium bromide and dl-isoproterenol. All drug concentrations are expressed as the base; barium chloride concentration is expressed as the salt.

Results. The strips showed no spontaneous or stretch-induced activity. The $BaCl_2$ always caused contractions. The lowest effective concentration was 5×10^{-5} g/ml in 4 experiments, 10^{-4} in one, and 5×10^{-4} in 5. At near-threshold concentrations the response was delayed up to 10 minutes; at near-maximal concentrations it was delayed up to 5 minutes. The onset of response could not be accelerated by stirring the bath. At low concentrations the motor response was powerful rhythmic contractions of variable amplitude occurring in bursts alternating with quiescent periods (Figs. 1 A and B). Bursts of electrical spikes accompanied each contraction. At higher concentrations (Fig. 1C) there was an increase in baseline tension with very prolonged bursts of electrical spikes. These prolonged bursts always showed a uniform diminution of amplitude. At near-maximal concentrations (Fig. 1D) there was always a single prolonged burst of electrical spikes with the characteristic uniform diminution of amplitude accompanying a very powerful and sustained tonic contraction. Washing readily eliminated the response to $BaCl_2$ present in concentrations less than 10^{-3} gm/ml, but washing for 1–2 hours was needed to return the tension to baseline levels when the muscle was exposed to concentrations of 5×10^{-3} gm/ml (10 experiments). Strips exposed to this highest concentration of the salt never regained their former sensitivity to low concentrations of $BaCl_2$. The threshold was often reduced 100-fold.

In 3 strips from each of 5 cats, the response to $BaCl_2$ in concentrations from 1×10^{-5} to 5×10^{-3} gm/ml was recorded alone and in the presence of atropine 10^{-6} gm/ml or 10^{-4} gm/ml. Each cat provided one strip for each of the 3 experimental situations. The mean dose responses of the 3 groups of 5 strips did not differ (Fig. 2). Thus, atropine does not

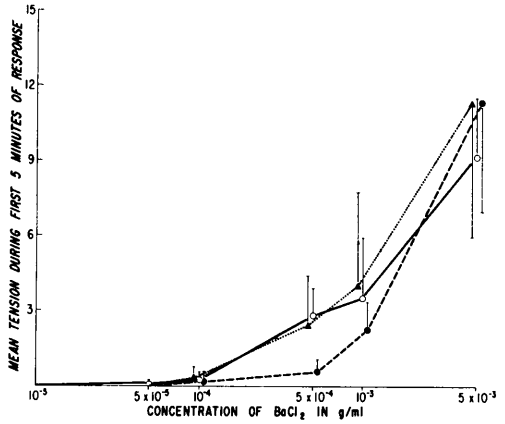

FIG. 2. Mean dose–response curves to $BaCl_2$ alone and with atropine. Each curve is the mean of identical studies of 5 esophageal strips from 5 separate cats. The abscissa shows concentration (log). The ordinate shows mean tension during the first 5 min of response. Brackets show 1 SD from the mean; △ . . . , response to $BaCl_2$ alone; response to $BaCl_2$ with atropine, 10^{-6} gm/ml; ○ response to $BaCl_2$ with atropine, 10^{-4} gm/ml.

prevent the barium response. This observation also probably excludes an action of barium on preganglionic nerve elements. We investigated this more directly by seeking an effect of nicotine or hexamethonium on the response to $BaCl_2$. In 3 experiments with each ganglionic blocking agent in concentrations up to 10^{-4} gm/ml, neither agent affected the response.

Adrenergic α-receptors are excitatory in this muscle, but stimulation of adrenergic β-receptors can inhibit contractions induced by cholinergic drugs. We studied the effect of the selective adrenergic β-receptor stimulant, isopropylnorepinephrine, on the contractions produced by the barium ion. In 6 experiments, concentrations of isopropylnorepinephrine as high as 10^{-5} gm/ml produced no change in the response of the strips to $BaCl_2$.

Discussion. These experiments were intended to answer the following question: Is the excitatory effect of barium in this muscle due to excitation of local nerves or to a direct effect of barium on the muscle? In isolated small intestine from several species barium initiates a peristaltic reflex which is opposed by hexamethonium (3–5,8). In longitudinal muscle of the guinea-pig ileum, barium-induced contractions are prevented by atropine and botulinum toxin, but are unaffected by hexamethonium (3,6). In the presence of hexamethonium and hyoscine, large concentrations of barium can still produce contractions of guinea-pig ileal longitudinal muscle. These observations indicate that barium can cause contraction of intestinal preparations by stimulation of preganglionic or postganglionic cholinergic nerves or by a direct action on smooth muscle. In our preparation of esophageal muscle the barium response is not affected by ganglionic blocking agents or by atropine. Thus, barium is not acting by exciting local motor nerves. This conclusion is supported by the nature of the response to barium as compared to responses to cholinergic drugs and to norepinephrine (2). The long delay in onset, the wide irregular swings in tension and the persisting contracture at high concentrations are never seen with the autonomic drugs. The electrical response of the muscle is also different. The spike bursts associated with lower concentrations of barium resemble those produced by other stimulants, whereas the very prolonged spike bursts of decreasing amplitude associated with higher concentrations of barium ion are unique to the response to barium.

Summary. This study reports the effect of $BaCl_2$ on circular smooth muscle from the distal esophagus of the cat. The magnitude of the contractile response is dose-related; the threshold concentration varies from 5×10^{-5} to 5×10^{-4} gm/ml and irreversible contracture occurs at concentrations of 5×10^{-3} gm/ml or greater. The dose–response curve is not affected by atropine, hexamethonium, nicotine or the β-adrenergic stimulant, dl-isoproterenol. Thus, the response to barium is the result of a direct action on muscle rather than an indirect action through stimulation of motor elements of the esophageal intramural plexuses.

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Effect of Calcium on Bone Marrow Mitosis *in Vitro** (32956)

HELEN J. MORTON
Division of Radiation Biology, National Research Council of Canada, Ottawa, Canada

When calcium salts are injected into animals there is a marked elevation of serum calcium (1,2) and increased mitosis in rat bone marrow (3). Furthermore, postirradiation injection of calcium salts is an effective therapeutic measure since it approximately doubles survival (4). If this effect was due to a direct action on the individual marrow cells, serum prepared in this manner should also increase mitosis in these cells in culture and so permit a more detailed study of the mechanisms involved. Therefore, the serum from calcium-treated rats was tested as the nutrient for rat bone marrow cultures under conditions which permit active erythropoiesis for many days (5).

The present study showed that such calcium-enriched serum has a direct effect on the multiplication of isolated marrow cells *in vitro*. A large proportion of the marrow cells undergo rapid, sequential divisions with a very short generation time, and subsequent maturation of these cells seems to occur in a normal manner. The net result is, therefore, a rapid and dramatic increase in the effective marrow cell population.

Materials and Methods. Complete details of the bone marrow culture system have been published (5,6). Cultures consisted of a suspension of femoral marrow cells from 100-gm rats, grown on a coverslip, in a small petri dish, using 5 ml of isogenic serum as nutrient. They were incubated in an atmosphere of 30% CO_2 plus 70% humidified air at 32°C for optimum erythroid maturation (6).

Calcium and magnesium-enriched serum (Ca-serum, Mg-serum) were prepared by injecting adult (>400 gm) rats with 1.0 ml of a 62.5 m*M* aqueous solution of either element as the chloride. Two intraperitoneal injections were given, at 30 min and 3 min, respectively, before the animal was bled to death under ether anesthesia. Serum prepared in this manner contained between 11.5 and 14 mg/100 ml of calcium, or between 3.6 and 5.0 mg/100 ml of magnesium. Normal rat serum contained 9.5–10.5 mg/100 ml of calcium and 1.2–2.9 mg/100 ml of magnesium. These ion levels were determined by the murexide method (7). In later experiments it was found unnecessary to pretreat rats to obtain Ca-serum. Cultures were prepared in normal serum and 0.5 ml of 1% calcium chloride was added directly to the culture. Both forms of Ca-serum produced the same results.

When total nucleated cells were counted, the cells from the supernatant and the coverslip were combined, treated with saponin to lyse the red cells, and counted in a model B Coulter counter. A microscopic check was also made to ensure that there were no clumps of cells. In these cultures the immature cells remained mainly on the coverslip whereas the mature cells were found mostly in supernatant fluid. The supernatant fluid might therefore be considered analogous to peripheral blood *in vivo* and reticulocytes were determined as the percentage of total non-nucleated cells in the supernatant fluid, using brilliant cresyl blue. Some samples were also stained with Wright's stain as described previously

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