weight = 4.1 ± 2.3 mg. The muscle bath was held at 24 °C, and the substrate was butyrate (10 m*M*). Isometric contractions were ellicited (10 V, 2 ms) and the resulting twitch tension (τ) and time derivative ($\dot{\tau}$) were recorded. In addition, the metabolic activity was monitored by recording changes in the intramitochondrial NADH/NAD ratio using a microfluorometer[5]. Each muscle was subjected to a series of 15 twitches at 7 different frequencies; bovine insulin (10 mU/cc; Sigma Corp.) added and the series repeated.

To combine the results from the individual experiments the ratio of the mechanical response with insulin and the control values at each frequency was formed. These were combined among experiments to obtain the cumulative results. By combining the data in this way, the errors due to inter-animal differences are minimized. Significant differences were determined using the paired *t*-test and the Fisher cumulative χ^2 [6]. The significance level was 0.01.

Results. The net effect of the addition of insulin is given in the table below:

P_{max}: 1.00 ± 0.14	τ: 1.22 ± 0.06
TPT: 0.97 ± 0.04	$\dot{\tau}_{max}$: 1.29 ± 0.05
	$\dot{\tau}_{min}$: 1.38 ± 0.11

Values given are mean ± S.E.M. ratios (insulin/control) for all frequencies. $N = 11$. P_{max} = Maximum change in NADH fluorescence; TPT = time to peak tension; τ = peak twitch tension; $\dot{\tau} = d\tau/dt$.

Insulin did not significantly alter either the metabolic response (P_{max}) or the TPT. However, there is a significant increase (22%) in twitch tension (τ) which is accompanied by a corresponding increase in both the maximum and minimum time derivative of the tension ($\dot{\tau}_{max}$ and $\dot{\tau}_{min}$). The dependance upon frequency for this inotropic effect is shown in the figure. Except at the slowest frequency (0.025 Hz), the increase in both τ and $\dot{\tau}_{max}$ would appear to be constant, independent of frequency. The apparent drop off at $\nu = 0.6$ Hz is due to the muscle becoming hypoxic as indicated by the NADH fluorescence signal[7]. Preliminary experiments ($N = 6$) to determine the temperature sensitivity of this inotropic effect show no change in the functional relation but an increase in the magnitude:

τ_I/τ_C (23 °C) = 1.17 ± 0.04 vs τ_I/τ_C (30 °C) = 1.3. ± 0.03.

Discussion. The data presented here clearly show that insulin is an inotropic agent. This effect would appear to be frequency independent (figure) but the magnitude is sensitive to temperature. The increase in τ is paralleled by a corresponding increase in $\dot{\tau}_{max}$. This mutual increase is reflected by no change in the TPT. These data suggest that the inotropic effect of insulin is due to an increase in the amount of calcium available for release rather than a change in the kinetics e.g. theophylline or epinephrine.

Recent reports[8–10] have indicated that insulin causes release of membrane bound Ca^{++} into the cytoplasm. This effect of insulin could explain the data reported here.

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Reticular Potentials Evoked by Electrical Stimulation of Individual Semicircular Canals[1]

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6 April 1976.

Summary. Responses of pontine reticular formation neurons following single shock electrical stimulation of single semicircular canals were recorded with tungsten microelectrodes in 40 curarized guinea-pigs. The field and unitary potentials obtained from 62 reticular sites, exhibited latency values ranging from 0.3 to 2.5 msec. The early latencies (0.3–0.5 msec) have been interpreted as responses mediated by primary vestibular fibres projecting directly to the reticular substance.

The relationships between the vestibular apparatus and the brain stem reticular formation (RF) have been previously investigated[2–6], showing that the RF is actively involved, especially in the control of eye movements. In fact, electrical stimulation of the whole vestibular nerve elicited evoked responses in the pontomedullary reticular substance[7]. Furthermore, separate thermic stimulations of single semicircular osseous canals, in the guinea-pig, showed different patterns of convergence of the ampullary input on paramedian pontine reticular units[8]. Therefore, electrical stimulation of single ampullae seemed interesting in order to study the possible specificity of representation of a given semicircular canal in the reticular nuclei and to elicit field and unitary potentials evaluating their latencies.

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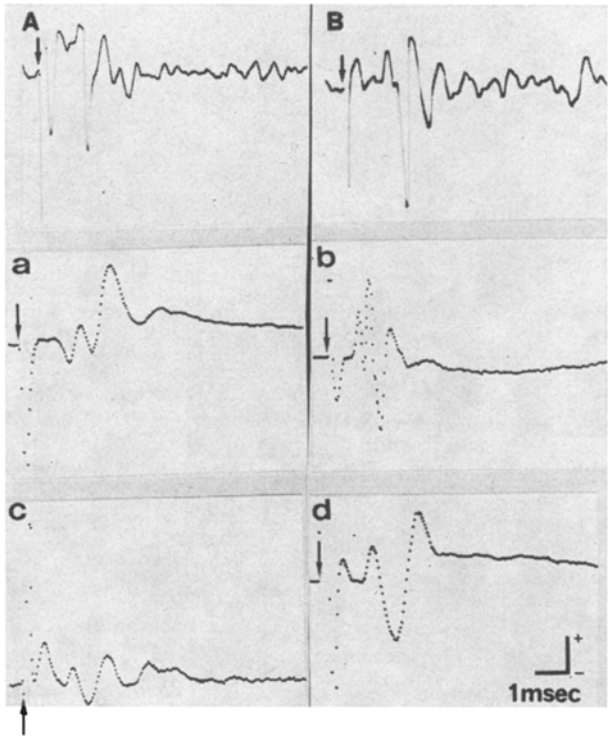
In the present research 40 guinea-pigs were employed. The animals were anaesthetized, tracheotomized, fixed in a stereotaxic apparatus. The ampullae of anterior and lateral osseous canals were bilaterally exposed and a hole (diameter 1 mm) was made in each osseous ampulla, where bipolar electrodes made from 0.5 mm copper wire insulated except at the tip, were introduced and fixed with acrylic dental cement. Rectangular pulses 0.1 msec in duration at a rate of 1/sec for a period of 5–15 sec were delivered through the stimulating electrodes to the ampullary receptors. The lowest stimulation voltage able to induce eye nystagmus beating on the plane of the stimulated canal according to FLOURENS' law[9], was taken as the threshold value (T), which ranged from 1 to 8 V. The stimulation current intensities corresponding to these voltages were calculated to range from 70 μA to 600 μA. The animals were then curarized, maintained under artificial respiration and the dorsal surface of cerebellum was exposed. A tungsten microelectrode (tip diameter 1–8 μm; resistance 300–600 KΩ), connected through a P5 Grass preamplifier to a beam of a Tektronix 565 dual beam oscilloscope and to a channel of a 5480 A Hewlett-Packard signal analyzer, was driven into the pons. The histological controls performed in all the experiments, in order to ascertain the exact position of the recording microelectrode tip, showed that the recorded sites were localized mainly in the nucleus reticularis (n.r.) pontis caudalis, n.r. pontis oralis, n.r. tegmenti pontis, n.r. gigantocellularis and n. r. lateralis.



A, B: reticular unitary potentials evoked by electrical stimulation of ipsilateral anterior canal in A (latency 0.3 msec) and of contralateral anterior canal in B (latency 1.5 msec). a–d: reticular field potentials elicited by individual electrical stimulation of the 2 ipsilateral and the 2 contralateral ampullae. Note the early latency potential (0.4 msec) in c. Each trace represents the average of 32 responses. Calibration: Time 1 msec; amplitude 100 μv. The arrows indicate the stimulus artifact.

The possibility of current diffusion to the neighbouring ampullar receptors and to the auditory labyrinth was taken into consideration. According to TOKUMASU et al.[10], stimulus spread is not significant if voltages 2.5–3 T are used: in fact, the recorded field potentials reached a plateau at 2.5–3 T; only when increasing further the stimulus intensity, the plateau was interrupted by a new phase of growth due to current spread to neighbouring structures. Therefore, high stimulus values were never utilized. As for direct current diffusion to brain stem, it can be excluded, considering that the ampullar stimulation evoked in the vestibular nuclei their typical pattern of reponse (P, N_1, N_2 components) while, in the reticular substance, different field potentials with different shape and longer latencies were observed. Besides, for the reticular evoked responses it was necessary to double the least stimulation voltage needed to evoke the vestibular field potentials[6]. The reticular potentials were also distinct from those of the medial longitudinal fascicle (MLF) which were more circumscribed and of smaller amplitude.

Evoked field potentials (Figure, a–d) elicited by the stimulation of ipsilateral and contralateral vertical and horizontal canals exhibited different wave form, amplitude (from 100 to 400 μV) and polarity. Their onset latencies ranged from 0.3 to 2.5 msec, with most frequent values of 0.8–1.3 msec. Unitary potentials (Figure A, B) were also recorded in a small percentage of cases, presenting the same range of latencies; short latencies (0.3 to 0.5 msec) were observed only in 4 units. The field potentials could be recorded over a dorso-ventral extension of approximately 2000 μm, while the unitary ones for a maximum range of 200 μm. In the reticular nuclei explored, it was neither possible to observe any specificity of representation from a given semicircular canal nor any circumscribed vestibular projection field. As for the reticular field potentials exhibiting latency from 0.6 to 1.0 msec, they could be hypothetized as belonging to axons of secondary vestibular neurons projecting to RF, rather than to reticular cells. The longer latencies (1.2 to 2.5 msec) could suggest the involvement of reticular neurons connected to vestibular nuclei by means of multisynaptic pathways. Difficulties have arisen in the interpretation of the short-latency potentials (0.3–0.5 msec), since even a single synaptic delay in the vestibular nuclei could have not be taken into account. These early potentials could be assumed to be: 1. produced by the field potentials due to the synchronous activation of the VIIIth nerve fibres or 2. generated by the efferent vestibular fibres, stimulated antidromically, or 3. the responses of primary vestibular fibres projecting to the reticular substance.

The first hypothesis can be discarded considering that the unitary potentials were recorded from a delimited area of 200 μm and the histological controls showed that the recording sites were localized in the paramedian pontine reticular formation at a distance of approximately 4 mm from the 8th nerve entrance. The second assumption can also be excluded since the direct efferent reticular bundles arise mainly from cells in the bulbar reticular substance at the sides of the median raphe in its dorsal half[11,12]. On the other hand, our early latency recordings

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were taken from reticular nuclei localized more rostrally and laterally, as shown by histological controls. As for the third hypothesis, CAJAL[13] followed primary vestibular fibres till the reticular formation without being able to trace, however, their sites of termination which were, later, described by BRODAL[3] and further confirmed by CARPENTER[14] and GERNANDT[15]. According to these researchers, these primary fibres would leave the 8th nerve trunk before entering the vestibular nuclei and reach the reticular neurons directly. Primary vestibular fibres have been also reported to project directly to the cerebellar cortex and the fastigial nucleus[13–16]. Another interpretation of these short-latencies responses would be an electrotonic coupling between primary afferent fibres and neurons located either in the medial vestibular nucleus or in the surrounding reticular formation. The existence of this type of gap-junction synapse has been hypothetized in the mammalian abducens motoneurons and in explaining also latencies of 0.58 msec recorded in the MLF following electrical stimulation of contralateral vestibular nerve[17].

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Effect of Prostaglandin E_1 on Rat Gastric Motility and Cyclic Nucleotide Content

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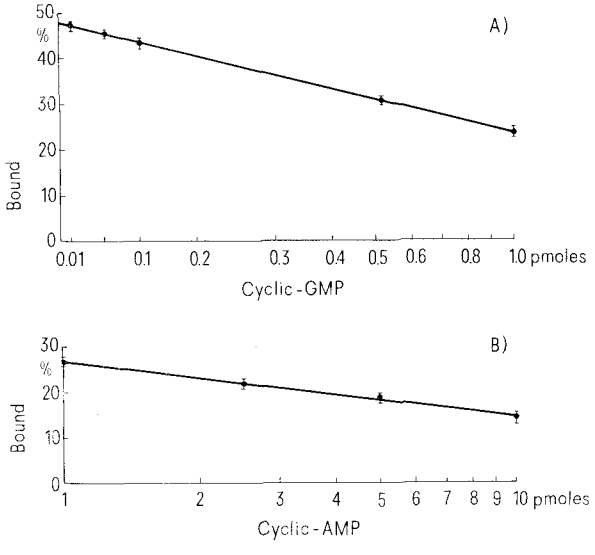
Summary. Administration of exogenous prostaglandin E_1 resulted in an increase in contractility of rat fundic muscle measured in vivo; a significant decrease in fundic tissue levels of cyclic- adenosine monophosphate and a significant increase in cyclic-guanosine monophosphate.

Prostaglandin (PG) interactions with adenosine 3¹,5¹-monophosphate (cAMP) have been studied in association with several different types of hormone systems[1,2] and research involving bi-directionally controlled mechanisms has implicated cyclic guanosine 3¹,5¹-monophosphate (cGMP) as an important regulator molecule which may act in opposition to cAMP[3]. By in vivo studying motility of the rat fundus, we have investigated the relationship between cAMP, cGMP, prostaglandin E_1 (PGE_1) and motility; PGE_1 should potentiate a cAMP decrease with an increase in motility and a decrease in cGMP should parallel a decrease in motility[4].



Standard curves for cyclic-GMP and cyclic-AMP. Each value represents duplicate trials with mean and SEM. A) cGMP; B) cAMP.

Male Holtzman rats 3–6 months of age, weighing 300–325 g were used in the experiment. They were maintained in one large cage with a common source of food (Purina Lab Chow) and water. The construction of the cage did not allow chewable roughage to accumulate. The rats were divided into 2 groups: control group, consisting of 5 rats which received daily i.p. injections of ethanol-Krebs solution solvent[5]; test group, consisting of 5 rats which received daily i.p. injections of PGE_1 (compliments of Dr. J. PIKE, The Upjohn Company, Kalamazoo, Mich., USA), 4.5 mg/kg[6]. Individual rat weight was checked daily to insure injections of proper dose.

A telemetry system[7] was used to monitor stomach motility. After a suitable plane of anaesthesia was induced with ether, a transverse incision accross the midline was made, and the transducer was firmly sutured to the outside fundic area. The body of the transmitter was placed in the peritoneal cavity and lightly sutured to the body wall to prevent displacement.

After implantation, recordings were made by connecting the discriminator output of an FM radio receiver directly to a strip-chart recorder. Three days were allowed for general recovery after which the telemetry system was tested for correct in vivo transmission. Recording was started 4 days after implantation, and gastric motility was monitored for a period of 14 days. The rats were

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