Figure 1 shows the differential uptake of equimolar digitoxin and ouabain into the cells of the gut of *O. fasciatus* over time. The distribution ratio of internal to external substrate concentration (DR) at 4 h is at an equilibrium level of 0.4 for ouabain compared to 17.5 for unequilibrated digitoxin (assuming 80% tissue water content). Low Na^+ buffer effected a decreased uptake of digitoxin; DR at 4 h is 8.7. Similar results were obtained with ouabain; DR at equilibrium is 0.09. A high concentration of K^+ (20 m*M*) had no effect on absorption of either substrate at 2 min, 10 min and 1 h of incubation; nor did an excess of ouabain decrease digitoxin uptake at 1, 2, and 20 min or 2 h. Figures 2 and 3 show that the uptakes of ouabain and digitoxin are nonsaturable and directly proportional to concentration in the ranges shown. *O. fasciatus* fed *A. syriaca* seeds contain cardenolides[6]; these animals showed no significant difference in ouabain uptake from bugs fed sunflower seeds.

Uptake of both ouabain and digitoxin into the gut, as reported in this study, exhibits characteristics of diffusion. The fact that digitoxin, being only slightly water soluble, has a high lipid-water partition coefficient may account for its observed concentration into the gut; ouabain is highly water soluble by comparison. In addition, digitoxin, but not ouabain, is observed to cross intestinal membranes rapidly in vertebrate systems[10]. If metabolism of digitoxin occurs within the cells, this might further account for the inability of this substrate to reach equilibrium even at 4 h.

The preferential absorption of the nonpolar cardenolide digitoxin into the gut of *O. fasciatus* is to be contrasted with the high concentration of polar cardenolides, e.g. ouabain and digitoxin metabolites, found in the dorsolateral glands[6]. Metabolic and selective concentrative processes must bring about this reversal.

[10] T. Z. CZAKY and Y. HARA, Am. J. Physiol. *209*, 467 (1965).

Study of the Characteristics of the Inotropic Effect of Insulin in Rabbit Papillary Muscle[1]

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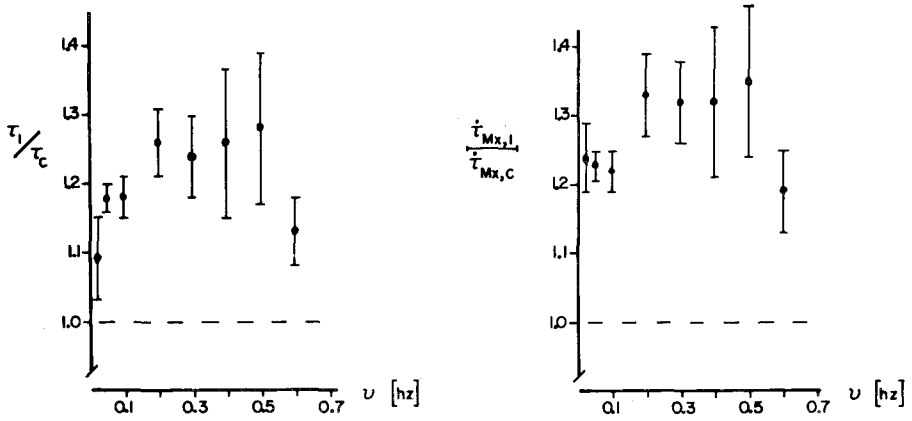
Summary. The effect of insulin was examined with emphasis on the alteration in the force-frequency relation. The results show that insulin does not change the time to peak tension nor the time of contraction. The inotropic effect was significant and did not depend upon the frequency of stimulation. However, there was a definite dependence of the magnitude of the inotropic effect on temperature. Previous studies have indicated that the inotropic effect is not a result of increased substrate availability or changes in cAMP phosphodiesterase activity. These results and those reported here are consistant with the hypothesis that insulin's inotropic effect is due to increases in intracellular Ca^{++}.

Although the dominant action of insulin is in facilitating glucose entry into the cell, recent results indicate that it is also an inotropic agent[2]. Since the preferred myocardial substrate is fatty acids and not glucose, it is doubtful that the inotropic effect is a result of alteration in glucose metabolism. Another explanation for insulin's inotropic could be its effect on cAMP phosphodiesterase (PDE)[3]. Preliminary studies comparing the effects of insulin with other PDE effectors (theophylline and imidazole) argue against this. Since very little insulin penetrates into the sarcoplasm[4], the cause of the inotropic effect might be an alteration of ion transport. The studies presented here were conducted to examine more closely the characteristics of the change in performance due to insulin using the changes in the force-frequency relation.

Methods. Cardiectomies were performed on stunned female rabbits weighing 2–3 kg. A papillary muscle from the right ventricle was tied (6–0 silk suture) and then suspended between two silver stimulating electrodes. The mean muscle diameter (1 g load) was 1.1 ± 0.4 mm,



Effect of insulin on mechanical performance of rabbit pappillary muscle. Abscissa = frequency of stimulation [Hz]; Ordinate = ratio of performance in the presence of insulin (10 mU/cc) vs control. τ = maximum twitch tension; $\dot{\tau}_{max} = (d\tau/dt)_{max}$. Maximum time derivative of the tension. Points represent mean ± S. E. M. (N = 11). Butyric acid (10 m*M*) substrate.

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.

[1] This work was supported by a grant from the U. S. Public Health Service HL-02285.

[2] J. C. Lee and S. E. Downing, Fed. Proc. *34*, 448 (Abstr. 1289) (1975).
[3] Y. T. Woo and J. F. Manery, Arch. Biochem. Biophys. *154*, 510–519 (1973).
[4] P. M. Edelman and I. L. Schwartz, Am. J. Med. *40*, 695–708 (1966).
[5] J. B. Chapman, J. Gen. Physiol. *59*, 135–154 (1972).
[6] A. Hold, in *Statistical Theory with Engineering Applications* (John Wiley & Son; New York 1967), p. 407–410.
[7] B. Chance, (Circ. Res. Suppl. *I 38*, 31–38 (1976).
[8] M. S. Kafka and C. Y. C. Pak, J. Gen. Physiol. *54*, 134–143 (1969).
[9] O. Walaas, E. Walaas and O. Grønnerød, Acta Endocrin. *77*, Suppl. 191, 93–129 (1974).
[10] T. Clausen, J. Elbrink and B. R. Martin, Acta Endocrin. *77*, Suppl. 191, 137–143 (1974).

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

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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.

[1] This work was supported by a grant of CNR.

[2] A. Brodal, in *The Reticular Formation of the Brain Stem* (William Ramsay Henderson Trust, Edinburgh 1958).
[3] A. Brodal, in *Progress in Brain Research* (Eds. A. Brodal and O. Pompeiano; Elsevier Publ. Co., Amsterdam 1972), vol. 37, p. 553.
[4] F. Duensing und K. P. Schaefer, Arch. Psychiatr. Nervenkr. *196*, 265 (1957).
[5] R. Lorente de No', Arch. Neurol. Psychiatr. *30*, 245 (1933).
[6] W. Precht, in *Handbook of Sensory Physiology* (Ed. H. H. Kornhuber; Springer Verlag, Berlin, Heidelberg, New York 1972) vol. 6, p. 353.
[7] B. W. Peterson, M. Filion, L. Felpel and C. Abzug, Expl. Brain Res. *22*, 335 (1975).
[8] L. Petrosini, D. Troiani and E. Manni, Archo. ital. Biol. *113*, 63 (1975).