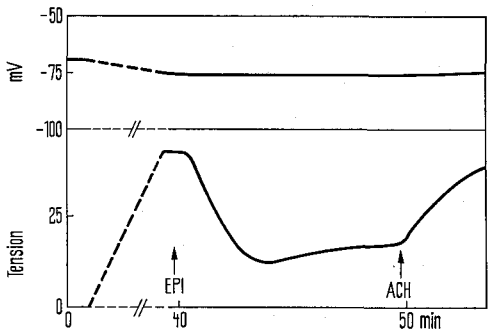
effect of epinephrine and atropine on Ca contractures can be explained if it is further assumed that the contracture depends on a continuous influx of Ca. Since the membrane potential does not change significantly, this influx must be very small or be associated with other ion movements. The increase in contracture produced by acetylcholine can then be explained by assuming that influx of Ca is increased. That epinephrine lowers Ca influx in the frog's ventricle [4] supports this explanation, but the opposite effect has been reported for the heart of other species [5–7].



Effect of epinephrine (10^{-7} g/ml) and acetylcholine (10^{-7} g/ml) on tension (lower line) and membrane potential (upper line) during contracture induced by high Ca (34 m*M*) Ringer solution. High Ca Ringer solution produced a slow increase in resting potential and a contracture. Arrows: application of epinephrine (EPI) and acetylcholine (ACH).

Zusammenfassung. Kontrakturen des Froschventrikels in Ringerlösung mit hoher Ca-Konzentration werden durch Azetylcholin verstärkt, während Adrenalin Erschlaffung herbeiführt. Diese Wirkungen sind nicht mit Änderungen des Membranpotentials verbunden und sind vielleicht die Folge sehr kleiner Änderungen des Ca-Austausches. Bei niederer Ca-Konzentration kann Azetylcholin auch Erschlaffung erzeugen.

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Lack of Effect of *Tris*-(Hydroxymethyl)Aminomethane Upon Myocardial Contractility

The cardiovascular actions of *Tris*(hydroxymethyl)-amino-methane (THAM) have been reported previously [1–7]. This amine has been found to increase ventricular contractile force and the reponse of the heart to catecholamines in acidotic dogs [3]. It has been suggested that the cardiovascular actions of THAM are not dependent on changes in arterial blood pH [6]; however, Wang and Katz found no increase in ventricular contractile force and coronary blood flow when THAM titrated to pH 7.40 was injected to dogs [7].

When THAM is injected in the bloodstream, 2 separate phenomena occur; on one side, there is a decrease in pCO_2 of the blood that is in immediate contact with THAM; later on, after blood has been equilibrated in the lungs, pH will remain high, its value obviously depending upon the amount of THAM injected.

It was our intention to study whether or not THAM has any inotropic action upon isolated heart muscle, in a system in which acid base changes could be avoided.

Methods. The experiments were performed using isolated heart muscle; in 5 experiments, strips of the right ventricle of rat, and in 7 experiments, right ventricular cat papillary muscle, was used. The muscles were mounted in a chamber, thermostatized at 30 °C, through which Ringer solution, previously equilibrated with a gas mixture of 5% CO_2 in O_2, was allowed to flow at a rate of 5 ml/min. The muscle, attached to a force transducer, was stimulated at a rate of 12/min. The isometric developed tension (DT), as well as the rate of rise of tension (dp/dt), and the temperature of the solution, were recorded in a polygraph.

The pH and pCO_2 of the solutions were measured anaerobically with electrodes thermostatized at 30 °C.

The experimental sequence was the following: since the beginning of the experiment, the muscle was immersed in a solution containing $NaHCO_3$ 30 m*M*/l, and equilibrated with a gas mixture with a pCO_2 of approximately 40 mm Hg. The pH of the solution was approximately 7.40. After a steady state was achieved in DT and dp/dt, the solution was changed to a second one in which $NaHCO_3$ was replaced by THAM, in a concentration of 40.8 m*M*/l, that, when equilibrated with a pCO_2 of 40 mm Hg, yielded a pH of about 7.40. The muscle was immersed in this solution for approximately 15 min, and then switched again to the first solution. During the 3 periods samples for pH and pCO_2 and fast recordings were obtained. The results obtained during the first and second exposure to the solution of $NaHCO_3$ were essentially the same, and were averaged.

Results and discussion. Figure 1 shows the effect of changing the solution containing $NaHCO_3$ to another containing THAM at the same pH and pCO_2. In this

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particular experiment, it was associated with a slight decrease in DT and maximum *dp/dt*. The results of all the experiments, as shown in Figure 2, indicate that the replacement of $NaHCO_3$ by THAM was not followed by any consistent change in contractility, as evidenced by the lack of difference in DT, *dp/dt* and time to peak tension (TTP).

The results obtained in this experiments show that THAM does not exert any inotropic influence when extracellular pH and pCO_2 are kept constant. When $NaHCO_3$ is replaced by THAM, there is a decrease in extracellular Na concentration. As a matter of fact, in 7 of the experiments, a 0.15*M* THAM solution was used; in this case Na concentration in Ringer was 27% below normal. In the remaining experiments, a solution of 0.3*M* THAM, which produced a decrease of 13% in extracellular Na, was used. When the experiments using 0.15*M* THAM are considered separately, a slight but statistically significant increase in contractility (DT being $7.14 \pm 2.7\%$ higher than control) is found following replacement of $NaHCO_3$ by THAM. In the experiments in which Na concentration was closer to normal, the variation in DT found when the THAM solution was used ($-0.5 \pm 2.3\%$) was not significant. These findings are in agreement with the reports that low extracellular Na increase myocardial contractility[8–10].

On the other hand, in the experiments in which the 0.3*M* THAM solution was used, a slight increase in osmolarity was produced. Even though it has been reported that changes in osmolarity affect myocardial contractility[11], we think very unlikely that the range of changes in osmolarity of our experiments can elicit a change in contractility. At any rate, the direction of change with the variations in osmolarity produced, would be that of an increase in contractility.

It has been suggested that intracellular pH is one of the major determinants of the changes in myocardial performance that follow acid base alterations[7,11–15]. The possibility exists that THAM, being 27.0% undissociated at pH 7.40, could have entered the cell and increased intracellular pH. The fact that in our experiments THAM was not associated with changes in contractility, suggests the following possibilities: (1) A change from bicarbonate to THAM at constant pCO_2 and pH did not produce significant changes in intracellular pH, or the changes produced were not large enough to elicit measurable variations in myocardial contractility. (2) In spite of the changes in intracellular pH, variations in contractility were not detected when external pCO_2 and pH were maintained constant.

We think that experiments in which an estimate of intracellular pH is made will throw light upon this problem[16].

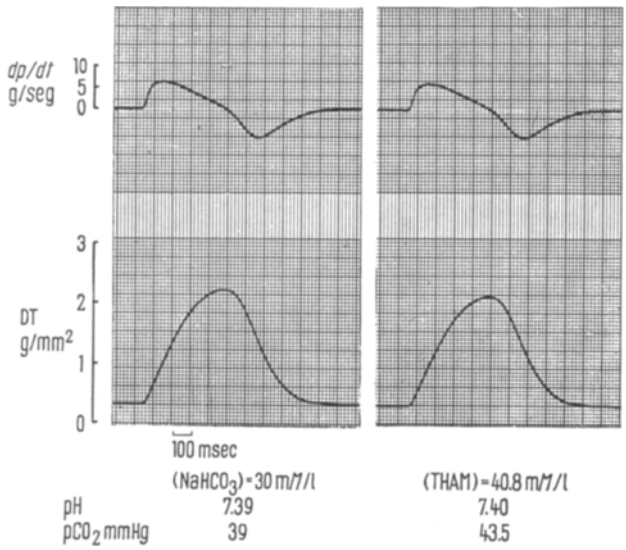

Fig. 1. Effect of a change from bicarbonate to THAM at the same pH and pCO_2 *dp/dt*, rate of rise of the tension in g/seg. DT, developed tension in g/mm²; the cross section of the muscle was calculated dividing the wet weight of the muscle by its length.

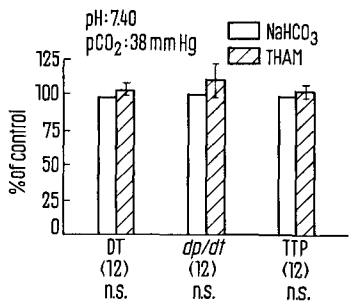

Fig. 2. Effect of a change from bicarbonate to THAM upon developed tension (DT) maximum rate of rise of the tension (*dp/dt*) and time to peak tension (TTP). Bars indicate % of change from the control ± 2 standard errors in 12 experiments. NS, not statistically significant differences.

Résumé. Pour étudier l'action du THAM sur la contractilité, dans une préparation de myocarde isolé, on a réalisé des expériences, où l'on compare l'effet de remplacer une solution de Ringer contenant 30 m*M*/l de CO_3HNa, équilibrée avec une pCO_2 de 40 mm Hg (pH 7,40), par une solution de THAM ayant le même pH avec le même pCO_2. Les résultats ont permis de conclure que le THAM n'a aucune influence inotrope positive sur la contractilité, lorsque le pH extracellulaire et le pCO_2 restent invariables.

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