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**Automaticity and Mechanical Work in Anoxybiontic State of Frog's Heart; Quantitative Estimation of Natural Excitations.**

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A quantitative method was worked out for the registration of the stroke volume of the frog's heart under as nearly normal physiological conditions as possible, with the sole exception that oxygen was rigorously excluded. Carefully purified hydrogen was perfused continuously through the circulatory system, and through the bath which contained the heart, from which the stroke volume was recorded by the Asher recorder. As the pressure was observed in an arterial manometer, the work of the heart could be computed. The system contained venous and arterial valves. The pressure on the venous inflow-side, and the arterial resistance could be varied. The perfusing fluid introduced by Brömser, Barkan and Hahn was used.

In this system automatic beat could be observed for 61 hours, notwithstanding that for long periods the heart had been forced to beat more rapidly and do more work than normally. These observations demonstrate conclusively the possibility of the persistence of automatic excitations without oxygen for a much longer time than is stated in the current literature. If one could exclude the necrobiosis of the small fragments of tissue tied to the canula, the automatic beat would probably last longer.

The principal conditions for the persistence of a good automatic beat are the right composition of the circulating fluid, a periodical renewal of the bath fluid, low pressure on the venous side, an optimal arterial pressure, a constant perfusion of hydrogen, and especially the maintenance of circulation in case the beats begin to fail. Artificial beats of the heart, by a pump arrangement, restore the heart invariably.

The amount of work done by the heart and especially the question of fatigue and restoration was then examined. For over two days the anoxybiontic heart could maintain a fairly normal circulation. Perhaps of still greater importance is the fact that even restoration was possible, notwithstanding the lack of oxygen. By forcing the heart through artificial stimulation to a more rapid rate of beat over a number of hours there appears fatigue. This, from numerous investigations on skeleton-muscles in the Berne physiological in-

stitution, we have called *apparent fatigue*. For as soon as the rate of beat is diminished through change to a less frequent rate, or through letting the automatic excitations act on the heart, the beats are augmented, and in a short time become the same as before. As our method permitted measurement of the work done, it was found that during the state of apparent fatigue less work was done and the minute volume was diminished. Re-establishing a normal rate also raised the work done and the stroke volume to return to normal. The changes from one state to the other could be repeated many times during a period of over 48 hours. The only difference between restoration in absence or presence of oxygen is that in the latter case restoration is still more rapid.

The possibility of restoration of the frog's heart without oxygen marks clearly a deep difference between the biochemical mechanism of the heart and skeleton muscle. The researches of Hill and Meyerhof have taught the importance of oxygen in the phase of restitution for the skeleton muscle. Various researches in the Berne physiological institution have shown that under physiological conditions the skeleton muscle is practically not fatiguable, *viz.*, can be restored from an apparent fatigue through the sole change from a more frequent to a less frequent contraction. All these studies were made on the intact animal. But even for the isolated sartorius muscle, we were able to create physiological conditions, so that after many hours of incessant work the muscle did not become inefficient. But in all these experiments oxygen was necessary. If the bath of the muscle was perfused with hydrogen a slower rate of contractions was not able to restore the muscle.

Incidentally, on studying the work of the heart under lack of oxygen, observations were made on a fundamental problem of normal excitations. As already mentioned, frequently artificial stimulations were applied, by aid of the calibrated Kronecker inductorium. The longer the heart had to work under exclusion of oxygen, the more the excitability towards artificial stimulations was lowered. Sometimes even 13,000 units of the Kronecker inductorium failed to stimulate. But on interrupting the artificial stimulations automatic beats set in, thereby showing that the natural excitations are more potent than the artificial ones. We have here gained, perhaps for the first time, a quantitative method to estimate the equivalent intensity of natural excitations. I prefer to use the term equivalent intensity to avoid for the moment the hypothesis that the potential difference of natural stimuli are the same as those of the alternating current of the Kronecker inductorium. The greater po-

tency of natural stimuli has been observed already in the investigations of Sherrington and Graham Brown, when artificial stimuli were unable to call forth a reflex movement, but the natural stimuli arising in the spinal cord produced movements. In the case of our heart experiments the possibility appears of a fairly quantitative measurement.

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## On the Ionization of Calcium Citrate.

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The hypothesis that citrate ions combine with calcium ions in some way and thus remove them from solution serves to explain a number of observations. Among these may be mentioned the following: non-precipitation of calcium phosphate in alkaline solutions when magnesium citrate is present; the failure of Shipley, Kramer and Howland<sup>1</sup> to obtain calcification *in vitro* even with a Ca x P product of 60 when the calcium is present as calcium citrate; the unpublished observations of Shelling and Maslow in this laboratory on the production, by intravenous injection of sodium citrate, of convulsions clinically similar to those of tetany, but with a normal total serum calcium and a greatly increased dialyzable calcium; and the results reported very recently in the important contributions of Hastings, Murray and Sendroy<sup>2</sup> on the solubility of calcium carbonate and calcium phosphate.

There are no studies, however, which bear directly on the ionization of calcium citrate. We are, therefore, reporting some experiments which give direct evidence in favor of the above hypothesis.

Conductivity titrations were performed using N/200 calcium chloride and N/200 sodium citrate solutions. The conductivity of each solution was measured, that of the citrate being found the greater. Measured volumes of the citrate solution were added to a measured volume of the calcium solution. The conductivity of the resulting mixture was measured after each addition. The first addition of the citrate produced a decrease in conductivity instead of an increase. Further additions of citrate caused further decreases. It was only after an equivalent quantity of citrate had been added