

a transparent square millimeter scale (divided to  $0.5 \text{ mm}^2$ ) on both sides of the strip using a magnifying glass. The average value in  $\text{mm}^2$  was then plotted against the absolute amount of the applied protein. The exact con-

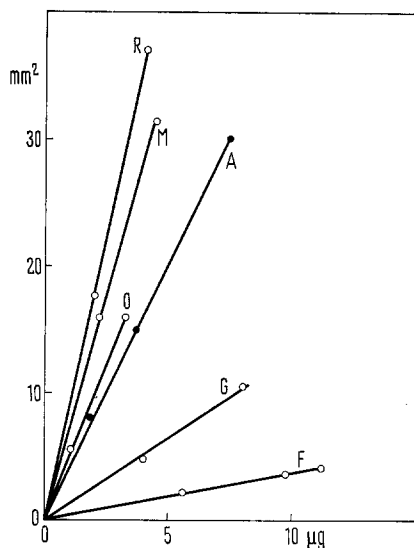


Fig. 3. Calibration curves of proteins. R, ribonuclease; M, myoglobin; O, ovalbumin; A, serumalbumin; G, gammaglobulin; F, fibrinogen. PVC membrane filters Sartorius SM 12801 and  $0.1 \text{ M}$  phosphate buffer with  $0.9\%$  NaCl were used.

centration of the pure protein standards was determined separately by dry weight and spectrophotometrically.

**Results.** The calibration curves (Figure 3) were different for individual proteins and all were linear in the given region with a deviation of the mean not exceeding  $3\%$ . This fact which confirmed all our previous experiences in this field<sup>1,2,4</sup>, makes it possible and reasonable to construct the calibration curve for a given protein (or a standard protein mixture, e.g. serum) by measuring exactly only one dot, i.e. the area corresponding to a given amount of protein and then drawing a straight line through that dot and the zero point of the scale. To ensure regular results it is necessary to avoid the presence of detergents, e.g. the Tweens, of high-molecular polyethyleneglycols<sup>4</sup> and of nonstandard proteins.

It is possible to conclude that this new microtechnique is very accurate, easy to perform, inexpensive and rapid, each estimation taking only a few minutes. It is suitable both for routine or for special ultra-micro estimation of soluble proteins in a broad range of molecular weights and at physiological pH values.

**Zusammenfassung.** Chromatographische Entwicklung von Proteinen auf PVC-Membranfiltern und Flächenbestimmung des adsorbierten Films zur raschen Proteinanalyse wurden beschrieben.

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## A Capacitance Indicator for Infinitesimal Muscular Movements

This report concerns a new way of registering very small muscular movements in several places at the same time, without any mechanical connections between the object and the recording device, by means of a capacitance movement indicator. The simultaneous use of 4 indicators on a Langendorff heart preparation gives a more complete picture of muscular displacements at a higher sensitivity and a higher frequency than is ordinarily achieved with classical methods of registration.

An existing capacitance bridge method was modified, using the heart as one of the two plates in a condenser<sup>1</sup>.

The device is very small ( $20 \times 15 \times 5 \text{ cm}$ ), constructed of inexpensive discrete components and operated by a  $9 \text{ V}$  battery. The sensitive device of the capacitance movement indicator is a bridge with a transformer fed by a  $12 \text{ kc/s}$  oscillator. 2 arms in the bridge are formed by the transformer and the other 2 by the condensers  $C_1$  and  $C_2$ , whose common point is connected to the earth (Figure 1). The bridge is balanced when the 2 condensers are equal. The capacity of one of them can be changed, by heart movements when the heart, which constitutes one of the condenser  $C_2$  plates, produces a change in the distance between these plates. This will also be the case when the earth point, which is the same as the heart and one of the condenser  $C_2$  plates, moves. The bridge is then unbalanced and by measuring this unbalance one may obtain a relative measurement of the movements. Signals due to the unbalance in the bridge are tapped from the center of the transformer and after amplification they are fed to a phase sensitive detector. Changes in the capacity of  $C_1$  or  $C_2$  cause the signals from the detector to change level, in one direction by an increase and in

the opposite by a decrease. The upper registration limit of the device is determined by the filter condensers in the detector, which in the present apparatus permit frequencies as high as  $1500 \text{ Hz}$ . After the detector the signals pass another set of filters which are chosen to suit the frequencies in the pattern to be registered and here an upper limit of about  $350 \text{ Hz}$  is chosen. In these experiments an Ink-Jet oscillograph with an upper limit of about  $700 \text{ Hz}$  was used. The amplitude of the deflections on the recorder depends on the sensitivity of the capacitance movement indicator and the distance to the object. The sensitive plate with its device has to be balanced with an oscilloscope each time its distance to the object is varied. The sensitive endpoint  $T_2$  can be arranged in different ways depending on the size of the object and its movements. A closer approach of the earthed heart towards the sensitive plate can be registered as a positive or negative deflection on the recorder. Geometrically the plate can be of any shape and of any metal. It can be formed as a plate, or a ball, but the cut off wire of the conductor alone will suffice. The conductor to the sensitive plate has to be earthed in order to avoid disturbances. As the device measures its relations towards earth, the cable should preferably be of low capacitance type (about  $30 \text{ pf/m}$ ).

Three types of model experiments were made, 1 with an eccentric plate, 1 with a micrometer screw and 1 with

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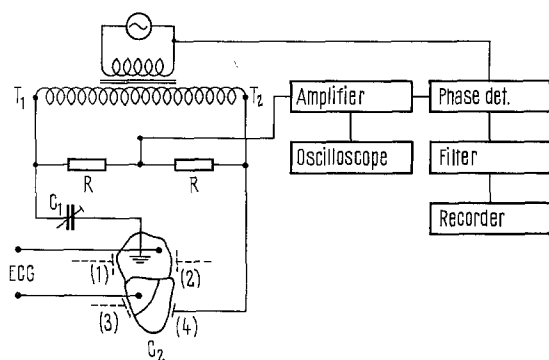


Fig. 1. Block diagram showing one capacitance movement indicator connected. The figures (1)–(4) show the positions for the 4 devices. The heart is earthed through the perfusion fluid.  $T_1$  and  $T_2$  are the arms of the transformer.  $C_1$  and  $C_2$  are the condensers in the capacitance bridge.

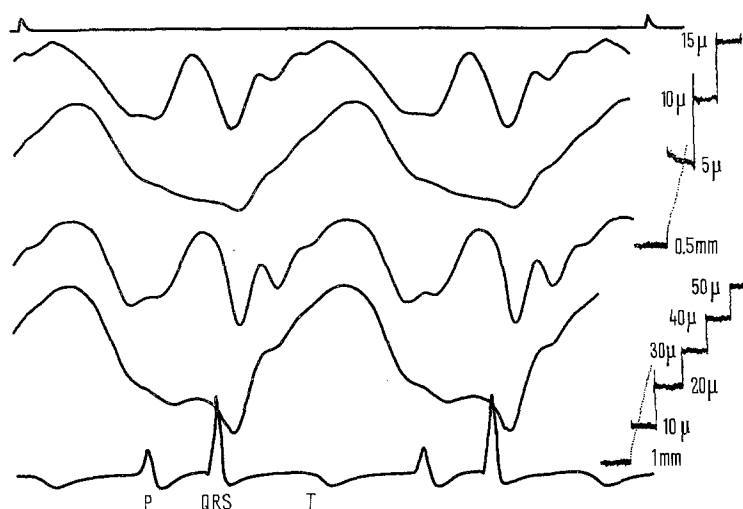


Fig. 2. Left: One selected experiment with a guinea-pig heart perfused in vitro. The first curve comprises 1 sec time marks, the following 4 the excursions of the chambers of the heart and the sixth is the ECG. Right: Calibration of the movements. The distance to the plate is given in mm and the adjustments of the micrometer in  $\mu$ .

an earphone membrane. The first tests were carried out by fastening an 1.75 mm eccentric plate to a motor at a speed of  $670 \times g$ . The result is a true sine function. The tests with the micrometer were carried out to ascertain absolute movements in mm. The closer the plate is towards the object, the greater becomes the amplitude of the deflections on the recorder. The capacitance movement indicator's linearity can be estimated from the intervals between the different adjustments. At smaller distances than 0.5 mm between the object and the sensitive plate, the micrometer screw was not useful because the capacitance movement indicator became too sensitive for this type of experiments. If the sensitive plate is at a distance of 0.5 mm from the object, and adjustment on the micrometer screw of  $\frac{1}{2}$  scaleline, i.e. 5  $\mu$ , is recorded as a pronounced deflection when the capacitance to the movement indicator is adjusted to maximum sensitivity (Figure 2, right). The tests with the earphone membrane were carried out to ascertain the frequency response. The movements of the membrane were controlled under the microscope and found to have an amplitude of about 1  $\mu$  through frequencies from 15 Hz up to 700 Hz. It is possible to amplify the signals a further 100 times by a preamplifier to the recorder.

These experiments show that one is well below cell size and that it should be possible to measure single cell movements in a tissue culture, or other very small biological movements, without mechanical connections and in several places at the same time. With a high speed recorder it is possible to follow a movement sequence, for example, the first movements of the embryological heart in situ, registered simultaneously with the ECG. With the sensitive plate at some distance from the object large movements can be registered with the same accuracy as smaller ones.

The guinea-pig hearts were perfused ad modum Langendorff with Krebs solution at 38.5 °C and 30–40 mm Hg.<sup>2</sup> Figure 2 to the left shows 6 curves from one of these experiments. The first comprises one second time marks. The second reflects the excursions of the right auricle and the third the right ventricle. The fourth curve shows the excursions of the left auricle and the fifth the left ventricle. For convention an upward deflection of the

curve indicates a systolic contraction of the muscle. The sixth curve shows the ECG<sup>3</sup> and, of course, all the curves are in synchrony with each other.

Further evaluation of the curves will be made in a separate publication.

*Zusammenfassung.* Registriermethode für kleine Muskelbewegungen ohne Direktkontakt synchron an mehreren Stellen bei simultanen elektrischen Messungen.

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<sup>2</sup> L. THER, *Pharmakologische Methoden* (Wissenschaftliche Verlagsgesellschaft m.b.H., Stuttgart 1949).

<sup>3</sup> M. J. GOLDMAN, *Principles of Clinical Electrocardiography* (Lange Medical Publications, Los Altos, California 1964).