nung der 2,4-Dinitrophenylhydrazone verlief entweder in der Mischung *n*-Butylalkohol-Äthylalkohol-Wasser (4:1:5) bei aufsteigendem Entwickeln oder in der Mischung *n*-Propylalkohol-Benzol-5 *N* Ammoniak (5:2:2), wo zweimal aufsteigend entwickelt wurde. Aus dem Chromatogramm wurden die 2,4-Dinitrophenylhydrazone der Ketosäuren mit 1 *N* NaOH (2 mal 30 m mit je 5 ml) extrahiert; das Dinitrophenylhydrazon des Glyceraldehyds wurde mit 96%igem Äthanol (10 ml/24 h) eluiert. Die photometrische Bestimmung der Konzentration der Ketosäurendinitrophenylhydrazone wurde bei 520 nm und diejenige des Glyceraldehyd-dinitrophenylhydrazons bei 380 nm mit dem Spektrophotometer 'Spekol ZV' (C. Zeiss, Jena, DDR) durchgeführt. Weil Oxalazetat während der Überführung in das entsprechende 2,4-Dinitrophenylhydrazon grösstenteils zum Pyruvat zersetzt wird, entsprechen die Werte für den Pyruvatgehalt dem Gesamtgehalt des Pyruvats und Oxalacetats. In der Tabelle werden die Konzentrationen (µg/1 g Trockengewicht) der freien sowie der in Bisulfit-Addukten gebundenen Carbonylverbindungen (Glyceraldehyd, α-Ketoglutarat, Pyruvat zusammen mit Oxalacetat) in den Organen der Kontroll- sowie Versuchskeimlinge angegeben. Alle Werte sind Ergebnisse von 2 Bestimmungen.

Der Anteil der als Bisulfit-Addukte anwesenden Carbonylverbindungen im prozentualen Gesamtgehalt der entsprechenden Carbonylverbindung änderte sich nach mehr als 24stündiger Einwirkung des $SO_2$ nur sehr wenig. Höhere Konzentration des Glyceraldehyd-Bisulfits in den Wurzeln als in den Achsen führt zur stärkeren Störung des Saccharidstoffwechsels in den Wurzeln[12]. Die sehr hohe relative Konzentration des α-Ketoglutarat-Bisulfits in den Achsen deutet auf eine bedeutende Störung des Citrat-Cyclus.

Die Entstehung der Bisulfit-Addukte aus Carbonylverbindungen und die Entnahme dieser weiteren metabolischen Umwandlungen können die Einwirkung des $SO_2$ auf den Saccharidstoffwechsel sowie auf denjenigen der organischen Säuren weitgehend erklären. Der Mangel an freien Ketosäuren kann auch den Gehalt einiger Aminosäuren wesentlich verändern[12], weil nur eine verminderte Konzentration der Ketosäuren den Transaminierungsreaktionen zur Verfügung steht. Die Steuerung der Transaminierungsvorgänge durch $SO_2$ haben wir bereits abgeklärt und über unsere Ergebnisse wird in einer nachfolgenden Mitteilung berichtet[18].

*Summary.* The intoxication of 15-day-old green pea seedlings with 1% gaseous $SO_2$ causes an important concentration fall of some free ketoacids (pyruvic, oxalacetic, α-ketoglutaric) and of glyceraldehyde in the roots and shoots. This fall is a consequence of the reaction of these carbonyl compounds with $SO_2$ in the plant tissue under the formation of bisulphite adducts (α-hydroxysulfonic acids), which have been chemically proved and quantitatively estimated.

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## Physicochemical Automaticity at a Mercury-Electrolyte Interface: Associated Electrical Potential and Impedance Changes

There is a growing interest in experimentally observable periodic membrane processes, especially in the context of the origins of rhythmic phenomena exhibited by living organisms[1]. Teorell's[2] classical studies on electro-osmotic flow clearly demonstrated periodic oscillations of potential and volume flow across coarse membranes. Electrical potential oscillations may also be observed in an ultra-thin poly-electrolyte double membrane system under conditions of constant current injection[3]. These are but two examples of physicochemical automaticity, of which the passive iron wire model as a nerve analogue is perhaps the most widely known[4]. We report here on measurements made on an earlier system exhibiting rhythmic behaviour[5,6].

In 1873, Lippmann[5] reported that when a drop of mercury is placed in dilute acid containing potassium dichromate and an iron wire is dipped into the liquid in close proximity to the drop, regular and rapid oscillations of the drop occur which may last for hours. This experiment, often referenced as Ostwald's[7] artificial heart, was most probably first developed in Kühne's laboratory[6,7]. We have reproduced the experimental conditions as described by Lippmann and monitored 3 aspects of the electrochemical activity displayed during this phenomenon. Firstly, stainless steel electrodes were placed in the fluid surrounding the drop and the potential variations were measured by a conventional ECG amplifying system. Secondly, the mercury drop was placed upon a stainless steel contact situated at the bottom of the recessed centre of the dish and the voltage between the drop and the iron needle dipping into the solution recorded. Finally, between these same latter two terminals, electrical impedance was also recorded. All electronic units were dc coupled and of standard design[8]. The 3 events were displayed on a high speed ultraviolet recorder[9]. In our experiments, a 6 mm mercury drop was immersed in 10 ml of a 10% nitric acid solution containing approximately 40 mg of potassium dichromate ($\simeq$ 0.4%). An iron needle was secured on an

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[9] 1508 Visicorder, Honeywell, Denver (Colorado, USA).

adjustable mount to facilitate accurate placement of the point of the needle in relation to the drop.

Figure 1 shows typical variations in surrounding fluid potential (trace a), drop-needle impedance (trace b), and drop-needle potential (trace c), respectively, as the contour of the drop oscillated. We have been unable to trace any published electrograms of this phenomenon, which is almost inconceivable considering that the centenary of LIPPMANN's paper is approaching. Leaving aside the obvious analogies with electrophysiological signals, there are certain interesting features apparent in Figure 1. The impedance record clearly shows that the needle and drop do not actually come into direct contact, in contradiction to LIPPMAN's assertion[5], swinging from a minimum of about 615 ohms to a maximum of 795 ohms, the notch in the descending limb being at approximately 700 ohms; when the needle was brought into contact oscillations stopped and the impedance dropped to virtually zero. The impedance trace shows a rapid increase which corresponds to physical contraction of the droplet and a long decaying phase (decrease in impedance) corresponding to the expansion (and flattening) of the drop and showing the notch which, in turn, coincides in time with a small wave in trace a) and with the reappearance of the voltage (repolarization) between drop and needle in trace c). Analysis of the events in Figure 1c reveals both a bistable nature which is suggestive of the well-known (electrocapillary) dependance of mercury surface tension on electrode potential and polarizing current density[10], and also hysteresis, on which so many oscillatory phenomena depend[3]. The needle becomes positive by some 0.7 to 0.8 volts to the interior of the mercury during the second half of the expansion period, and the needle point becomes black, probably through formation of $Fe_3C$. In addition, the volume-conductor potential (trace a) appears to be the usual differentiation of the signal derived from an oscillating dipole (trace a) and trace c)).

A rudimentary explanation of this phenomenon is as follows. The potassium dichromate decreases mercury surface tension due to repulsive forces in the double-layer at the mercury-electrolyte interface[11] (Figure 2a). As the iron needle is advanced towards the drop, electrode current increases due to decreasing interelectrode impedance (Figure 1b) until a critical current is reached and the iron passivates, causing the mercury potential to fall and the drop to contract (increased surface tension), thus expelling the dichromate film (Figure 2b). The potassium salt then diffuses back to the surface of the drop and increases the mercury potential, resulting in a change of shape. The iron depassivates at a second interelectrode current density (hysteresis) and the cycle is then repeated once the critical passivation current is again exceeded.

This phenomenon exhibits transition kinetics at one interface (activation and passivation of iron) which induces a mechanical change at a proximal boundary (mercury), the events being mediated by variations in electrolyte current and electrode surface potentials. From an interdisciplinary viewpoint, therefore, while agreeing with COLE[12] on the complexities of apparently straight-

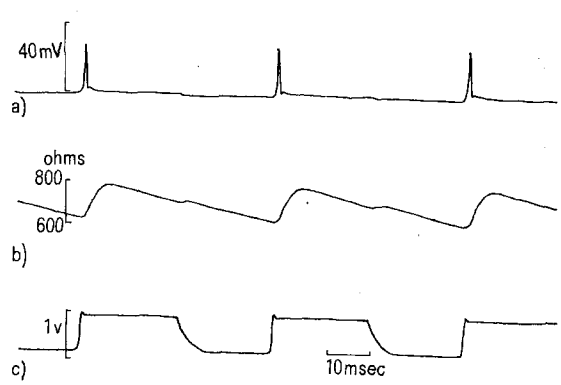


Fig. 1. Simultaneous recordings from an oscillating mercury drop. Experimental conditions as described in the text, temperature 24°C. Trace a) electrogram from bathing fluid; b) impedance between drop and proximal iron needle; c) drop-needle potential (period of 45 msec corresponding to an oscillation frequency of 22 Hz). Doubling of dichromate concentration or acid strength, or reduction of fluid temperature to 4°C had no measurable effect on the repetition frequency.

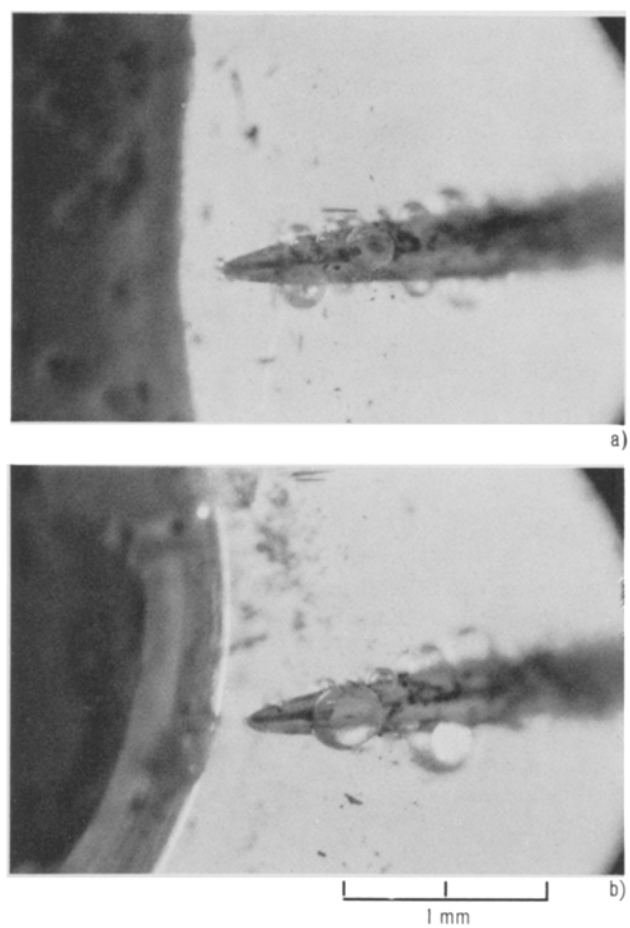


Fig. 2. These plates were obtained through a metallurgical microscope (×40) viewing the drop in a watchglass from below. a) Dichromate in excess of 40 mg was added until a marked flattening of the drop contour was observed. b) On advancement of the needle towards the drop, contraction occurred, expelling the dichromate film and resulting in a marked curvature of the mercury-electrolyte boundary (increased surface tension). Similar conditions in the routine experimental arrangement, as described in the text, resulted in regular oscillations of the drop following the initial contraction.

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forward passivity phenomena, it seems reasonable to assert that models of motion, rhythm and electrical discharge based on physiochemical action, which have been often used in the verbally transmitted culture of physiology, deserve a wider audience and a more careful analysis than they have yet received.

*Zusammenfassung.* Durch Einführung einer Stahlnadel werden andauernde elektrische und mechanische Schwankungen erzeugt und zwar bei wechselnder Oberflächenoxydation und Reduktion eines Quecksilbertröpfchens, das in verdünnter Säure mit $K_2Cr_2O_7$ liegt. Die Schwankungen des Elektropotentials und des Widerstands wurden gemessen und zusammen mit den mechanischen Schwankungen photographiert.

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## Phenobarbital Liver Microsomal Induction in MHV-3 Viral Hepatitis of the Mouse

Mouse hepatitis by MHV-3 Craig virus is characterized by parenchymal damage evolving rapidly towards a difused necrosis about 48 h from virus inoculation[1]. Previous works from this laboratory have shown that the earliest liver cell lesions involve lysosome membranes, while damage of mitochondria and of plasma membranes appears later[2–4]. Protein and RNA synthesis do not seem to be substantially altered even at an advanced stage of the infection[5,6]. The possibility of enhancing enzyme synthesis after phenobarbital treatment of mice in the presence of a progressed liver damage seemed to be of particular interest and has been actually the object of this research.

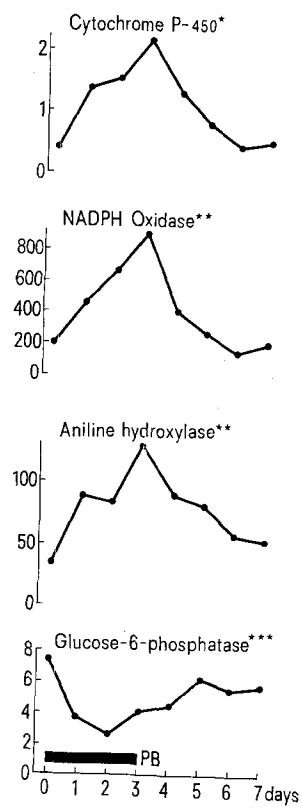


Fig. 1. Microsomal enzymes from mouse liver during and after phenobarbital treatment. Mean values of 2 concordant experiments. * mμmoles per mg of microsomal protein. ** activities expressed as mμmoles of substrate metabolized or product formed per mg of microsomal protein/h. *** μmoles of Pi liberated per mg of microsomal protein/h. ■, Phenobarbital (PB) 40 mg/kg i.p. twice a day.

*Materials and methods.* Male albino Swiss mice weighing 20–25 g were used. Treated animals were given 40 mg/kg i.p. twice a day of phenobarbital (PB) in 0.9% NaCl, while controls received an equivalent volume of 0.9% NaCl.

In the first series of experiments the course of PB induction in normal mice was examined. Groups of 6–7 mice fed ad libitum were killed by decapitation 1, 2 or 3 days after PB treatment and 1, 2, 3 or 4 days after cessation of treatment, as reported in Figure 1.

In a second series of experiments induction by PB of liver microsomal enzymes was studied in the course of hepatitis. Animals were given i.p. 0.1 ml suspension of infected mouse liver containing about 10,000 $LD_{50}$ of the Craig strain of MHV-3 virus. Groups of 6–7 mice were sacrificed at 0, 24 and 48 h of PB treatment, and at 0, 24 and 48 h of infection with all the relative possible combinations. Blood was collected in heparinized tubes and plasma glutamic-oxalacetic transaminase (GOT) was determined according to Tonhazy et al.[7].

Pools of livers from the various groups of animals were weighed, chilled on ice and homogenized with 4 volumes of cold 1.15% KCl. The homogenate was centrifuged at 10,000 *g* for 20 min and then the supernatant fraction was again centrifuged at 105,000 *g* for 1 h in a Spinco Ultracentrifuge. The microsome pellet was finally suspended in 1.15% KCl so that 1.0 ml contained the microsomes from 1.0 g of wet liver.

Protein content was determined according to Lowry et al.[8]. The mean protein content of microsomes was found to be 26.5 mg $\pm$ 4.2 (S.D.) per gram of wet liver. No substantial difference as regards the protein microsomal

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