Influence of high altitude climate on cancer development. In the Table some of the major physiological effects of high altitude treatment have been summarized. WARBURG[21] exposed cancerous rats to a gas mixture, containing 5% oxygen, for 40 h simulating an altitude of 12,000 m. The tumours became markedly but not completely necrotic. The experiment was repeated by CAMPBELL and KRAMER[22] at a simulated altitude of 5,100 m. Rats were kept alive for 2 weeks. The tumours did not regress but the growth was markedly inhibited. SUNDSTROEM and MICHAELS[23] observed regressions in Walker tumours in all experimental series kept at 360 mm Hg atmospheric pressure (about 6,000 m altitude). The average regressional rate as compared with controls was 39%. Under 300 mm Hg it was 45%. The effect is explained by the authors by stimulation of the adrenal function by reduced oxygen pressure at altitude[5], changing the level of corticosteroids in the blood serum. Also the observed increase in calcium in necrotic tumours and the decrease in calcium content of livers during reduced atmospheric pressure may be involved. The importance of the adrenal gland is suggested by the fact that adrenalectomy of tumour-bearing rats stops further regression after low pressure treatment. In fact the tumours start growing again.

Influence of different complex meterological conditions on growth of transplanted tumours. UNGEHEUER, BREZOWSKI, WRABA and RABES[24] studied the causes of fluctuations in the number of rats in pure strains of rats in which no cancer development occurred after tumour transplantation. 737 rats of the Walker carcinoma strain were studied during the period June 1961–June 1963. It was found that the number of non-resistant animals was smallest during quiet high pressure atmospheric conditions. The number increased during the approach of cold fronts and dropped again after the front had passed. The observed differences (67 and 79%) were statistically significant. The effect is greatest when the meteorological stimulus takes place 1 or 2 days after the tumour transplantation.

Possible effect of meteorological factors on viruses. The increasing evidence that leukemia and various types of cancer[25–27] may be caused by certain carcinogenic viruses is another aspect of possible meteorological effects on cancer. Various biometeorological studies (TROMP[3]) have shown that virus development depends on certain critical boundaries of temperature and humidity. Meteorological factors influence also the general resistance of the body against infections and the permeability of membranes. They also affect the antibody level of the serum[28]. It is therefore not excluded that soil, weather and climate could influence carcinogenic processes induced by viruses.

Conclusion. The various physiological mechanisms described, which are triggered by meteorological stimuli, may help to explain the effect of psychosomatic factors on cancer[29]. Whereas in purely psychosomatic research it may be difficult to study the real mechanisms involved, biometeorological studies on the effects of meteorological stress may assist future psychosomatic cancer research.

Various studies suggest the great influence of thermoregulatory processes on the hormonal functions in the human body. Disturbance of these processes may lead to cancer development. Experiments by TROMP and BOUMA have shown the very poor thermoregulatory efficiency of cancer patients. Studies by LEA, LEE, MCVAY, KRASNOW, GLASER, DE SAUVAGE NOLTING and others confirm the effect of meteorological stresses (in particular thermal stresses) on thermoregulatory processes and cancer development. The similarity in physiological processes during meteorological and psychological stresses may facilitate the studies on the influence of psychosomatic factors on cancer.

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PRO EXPERIMENTIS

An Electronic Method for Measuring the Heart Frequency of the Waterflea: *Daphnia pulex*

So far relatively few researchers have explored the possibility of employing invertebrates for testing drugs. Two of us (R.N. and G.N.) students of the HTS in Heerlen, had to prepare a synthesis of phenobarbital and compare the synthetic with a commercial product.

In a preliminary research, *Daphnia pulex* appeared to respond specifically to a number of drugs and hormones. We have found that the following altered the heart frequency of the water flea: oxytocine ADH, cAMP, acetylcholine, triiodothyronine. With phenobarbital there is a sharp decrease in heart frequency. In this study a clear correlation was found between heart frequency and phenobarbital concentration, which allowed us to test the 2 different phenobarbital preparations mentioned above. A special technique was devised to measure the heart frequency.

Materials. The apparatus used is shown in Figure 1a. It consists of: 1. A projection microscope type: Ken-A vision model Tech-A, lamp: 100 W 1A/GE. 20 V 5A/G $16^1/_2$/3.; lenses: objective N.A. 25, ocular 8× and 10×. 2. To maintain a constant temperature, a heat absorber is placed between the animal specimen and the projection lamp. The heat absorber is a saturated solution of $CuSO_4$, chilled with ice, which keeps the specimen temperature constant within 0.5 °C for a maximum period of 10 min. 3. A light dependent resistance (LDR) connected to an amplifier (Figure 2), 4. A source of direct electrical current: 2 nine volt batteries connected in series. 5. An oscillo-

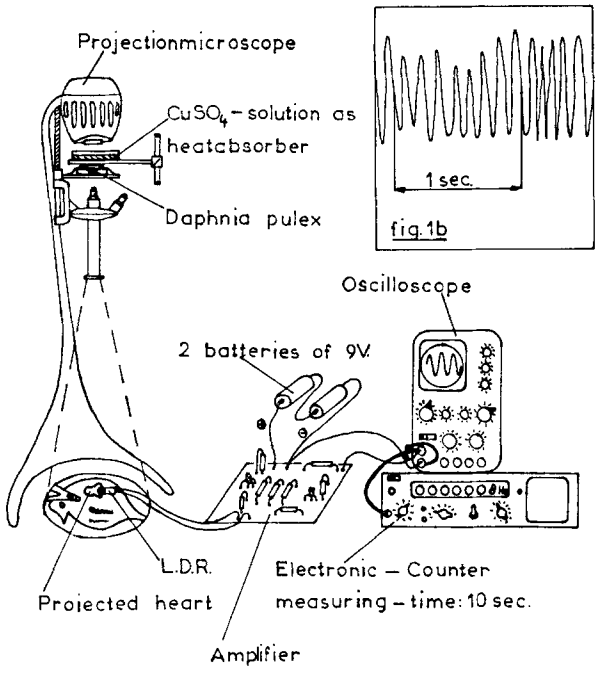

Fig. 1. a) Schematic illustration of the required apparatus. b) A typical 'optical cardiogram'.

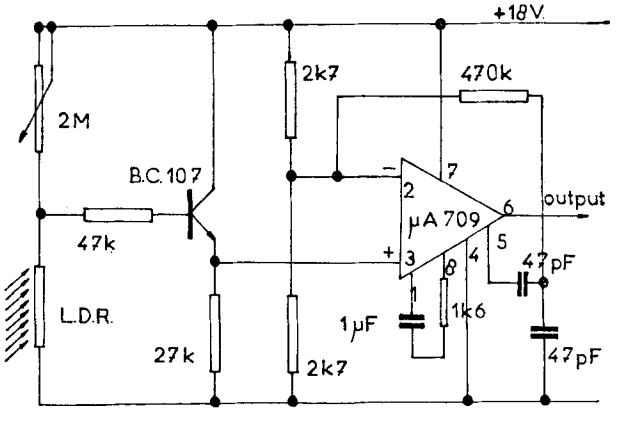

Fig. 2. Electronic diagram for amplification of the optical heartsignal of *Daphnia pulex*.

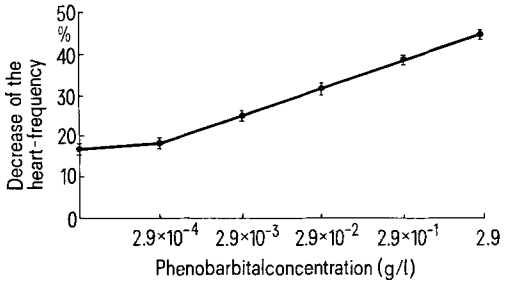

Fig. 3. Relation between the heart-frequency of the waterflea (*Daphnia pulex*) and phenobarbital concentration (data from column 5, Table).

scope: type Philips X-Y G.M. 5639. 6. An electronic counter: type Hewlett Packard 5245 L. Chemicals: phenobarbital obtained from Merck.

Method. For measuring the heartbeat, a *Daphnia* is transferred with a pasteur pipette to a lens glass (a glass slide with a concave excavation in the middle). The preparation is covered with a normal cover glass and pressure is applied to the cover glass until the *Daphnia* is fixed. If the animal is still moving, it is too small and a larger animal is used. Animals measuring 1.8–2.3 mm from the midpoint of the eye to the tail are usually suitable. The slide glass is transferred to the projection table of the microscope and the enlargment is set at 250×. The projection of a part of the heart wall should fall upon the LDR. The exposed area of the LDR is decreased with black tape until a clear signal appears on the oscilloscope of 0.1–1.0 V. The heart frequency is measured with the electronic counter for a period of 10 sec. Measurements can be done for 10 min, after which the heat absorber must be renewed before additional experiments can be done. The heart frequency of each animal is measured for 10 sec intervals from which the mean can be calculated. After measuring the normal heart frequency, the animal is transferred to an experimental solution for 30 min, then the heart frequency is measured again, following the above procedure. 8 animals were incubated in each of the following aqueous solutions: 0.0, 2.9×10^{-4}, 2.9×10^{-3}, 2.9×10^{-2}, 2.9×10^{-1} g phenobarbital/l. For each concentration of phenobarbital one should calculate: 1. The mean heart frequency (without phenobarbital), data are pooled from 8 animals, each *Daphnia* is measured 5 times and during 10 sec ($n = 40$). 2. The mean heart frequency (with phenobarbital) $n = 40$. 3. The mean % decrease in heart frequence. The % decrease =

$$\frac{\text{normal frequence} - \text{frequence after incubation}}{\text{normal frequence}} \times 100.$$

4. Calculated % decrease (% decrease $= 40.4 \pm 5.97$ log concentration) 5. S.D. of the mean % decrease: $S.D._{\bar{x}} = \frac{\sqrt{\sum (x-\bar{x})^2}}{n-1}$; $\bar{x}$ = calculated % decrease. About 1 out of 5 animals cannot stand an accidental rough manipulation (a pipetting effect) or a lateral body compression (a cover glass effect). Any irregular heart frequency at time zero, due to these effects, makes the animal not suitable for a test experiment.

Results and discussion. The Table and Figure 3 shows the average decrease in heart frequency for animals incubated in the different phenobarbital solutions. There is a definite linear relationship between the concentration of phenobarbital (log) and the percentage decrease of heart frequency before and after incubation. The *Daphnia pulex* thus demonstrated a quantifiable physiological sensitivity to phenobarbital which was sufficient for our purposes. However other researchers may prefer to use *Daphnia pulex* rather than rats and mice because the principle advantages are: 1. It is an universally available organism and as a test animal it is easy and inexpensive to maintain. 2. The heart frequency of the animal is large. In our experiments 100% refers to a heart frequency of ca. 500 beats/min for normal animals at temperature 20 °C and a length of 1.8–2.3 mm. Thus any significant change can be measured more accurately. The stroboscopic method[1,3]. is not

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Summary of statistical data

Concentration of phenobarbital (g/l)	Mean frequence without phenobarbital	Heart-beats/min with phenobarbital	Mean decrease (%)	Calculated decrease (%)	S.D. (%)
(1)	(2)	(3)	(4)	(5)	(6)
2.9	494	282	42.9	43.2	3.4
2.9×10^{-1}	494	300	39.4	37.2	2.6
2.9×10^{-2}	504	348	30.8	31.3	2.5
2.9×10^{-3}	498	372	25.4	25.3	4.0
2.9×10^{-4}	510	414	19.0	19.3	2.6
0.0	510	426	17.1	—	3.3

In column (2) and (3) data are pooled from 8 animals, each measured 5 times during 10 sec, hence $n = 40$. Regression line (without zero concentration): % decrease = 40.4 + 5.97 log concentration. S.D. in the coefficient: 0.16. Multiple correlation coefficient: 0.94.

precise (S.D. ± 22%, $n = 4$ for 1 *D. pulex*) and as yet we have no experience with the electro-cardiogram method[2]. 3. The actual experiment is quite simple, as is the electronic apparatus used (Figures 1 and 2). 4. By connecting the output of the amplifier to a recorder, one can obtain an 'optical cardiogram' (see inlay Figure 1b). 5. To draw a comparison between invertebrates and mammals concerning the physiological effects of phenobarbital is to invite more complex questions far beyond the scope of this study. However, proving that the heart frequency of *Daphnia* is useful as a biological parameter in a test for phenobarbital suggests the possibility that *Daphnia* or other similarly 'simple organisms' would be useful in comparitive biological studies of other drugs and hormones.

Zusammenfassung. Neue Methode zur exakten Bestimmung der Herzschlagfrequenz transparenter Wasserorganismen (*Daphnia pulex* 500/min) über einen Mikroprojektor bei Anwendung einer vom Licht abhängigen Resistenz (LDR).

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Zur Synthese der *m*-Aminophenylboronsäure, Effektor in der Affinitätschromatografie von Nukleinsäuren

Affinitätschromatografische Trennverfahren haben in den letzten Jahren auf dem Gebiet der Polynucleotide zunehmend an Bedeutung gewonnen. Hochspezifische Trennungen konnten unter anderem auf Basis der Wechselwirkungen zwischen komplementären Nucleobasen[1] sowie zwischen Oligo- und Polynucleotiden mit hydrophoben Substituenten und tritylierten oder benzoylierten Cellulosen[2] erzielt werden. Um aus einem Gemisch von Mono- oder Oligonucleotiden solche mit terminaler *cis*-Diolgruppe zu trennen, bedient man sich mit Vorteil ihrer Wechselwirkung mit Polymeren, die Boronatgruppen enthalten. Diese Art von Affinitätstrennung wurde zum Beispiel eingesetzt, um 3'-terminale Fragmente von Polyribonucleotiden zu analysieren[3] oder um Desoxyoligonucleotide mit Ribo-Terminus aus einem Gemisch mit Desoxy-Oligomeren gleicher Kettenlänge abzusondern[4]. Die benötigten Säulenmaterialien wurden entweder durch Umsetzung hydrophiler Polymerer mit *m*-Succinylaminophenylboronsäure[5] oder durch Copolymerisation von *m*-Methacryloylaminophenylboronsäure mit geeigneten Vernetzern[6] gewonnen. In beiden Fällen dient *m*-Aminophenylboronsäure als Effektorgruppe. Für diese Schlüsselsubstanz haben wir einen einfachen Syntheseweg entwickelt, der von preisgünstigen Reagenzien ausgeht.

Ergebnisse. Der Weg, den wir zur Synthese von *m*-Aminophenylboronsäure beschritten haben, ist in Schema aufgezeichnet. Als Ausgangsmaterial diente *m*-Bromanilin (I), das zunächst am Stickstoffatom mit Trimethylsilylschutzgruppen versehen wurde[7]. Eine Grignard-Reaktion

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