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### Contractile state and myocardial oxygen consumption of the isolated feline left ventricle

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In isolated cat hearts, perfused with blood from donor cats, a balloon was introduced via the atrium into the left ventricle. Balloon volume could be changed during contraction with a membrane pump. Left ventricular oxygen consumption ( $\dot{M}\dot{V}_{O_2}$  in  $\text{cm}^3 \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ ) was measured during isovolumic and zero load contractions, at different end-diastolic balloon volumes ( $V_{ed}$  in  $\text{cm}^3$ ). Heart rate was kept at 2.5 Hz. In 6 experiments we studied the effect of two different calcium concentrations of the arterial blood. We found for zero load contractions that  $\dot{M}\dot{V}_{O_2} = 3.19 \pm 0.20 \dot{V}_{ed}$  at a  $[\text{Ca}^{2+}]_o$  of 0.91 mM and that  $\dot{M}\dot{V}_{O_2} = 4.71 \pm 0.37 \dot{V}_{ed}$  at a  $[\text{Ca}^{2+}]_o$  of 1.81 mM. Isovolumic contractions, generating pressure gave  $\dot{M}\dot{V}_{O_2} = 2.84 \pm 1.17 \dot{V}_{ed}$  and  $\dot{M}\dot{V}_{O_2} = 4.51 \pm 1.14 \dot{V}_{ed}$ , respectively. These findings imply, that for zero load contractions,  $\dot{M}\dot{V}_{O_2}$  changes little with  $\dot{V}_{ed}$  at low  $[\text{Ca}^{2+}]_o$ . This dependency is somewhat stronger at higher  $[\text{Ca}^{2+}]_o$  but not significantly different ( $p > 0.05$ ; paired t-test). Isovolumic  $\dot{M}\dot{V}_{O_2}$  depends much more on  $\dot{V}_{ed}$ , but this dependency remains also unchanged when  $[\text{Ca}^{2+}]_o$  is increased ( $p > 0.05$ ; paired t-test). In 6 experiments  $[\text{Ca}^{2+}]_o$  was varied over a wide range during zero load contractions at  $\dot{V}_{ed} = 0$ . For  $[\text{Ca}^{2+}]_o$  higher than 5.0 mM no further increase in oxygen consumption was found. We conclude that contractile state, varied by  $[\text{Ca}^{2+}]_o$ , is an independent determinant of myocardial oxygen consumption.

### Structure and function of the sinoatrial node

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The impulse in the sinoatrial node is generated by cells that contain relatively few myofilaments in the rabbit, the guinea-pig and the cat. These so-called primary pacemaker cells give therefore a rather 'empty' impression. The area of relatively low-myofilament density is substantially larger than the primary pacemaker area. The area of earliest discharging cells with identical action potential configuration is considered as the primary pacemaker area. From the primary pacemaker towards the periphery there is a more abrupt transition in electrophysiological parameters than in morphological parameters. There is a large difference in the ratio of collagen to myocytes between the cat on one hand and the guinea-pig and the rabbit on the other hand. In the cat more than 50% of the nodal volume is occupied by collagen. In the rabbit and the guinea-pig more than 50% is occupied by myocytes.

### Electrophysiological disturbances underlying atrial fibrillation

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The activation sequence during acetylcholine induced atrial fibrillation (AF) was studied in isolated Langendorff perfused canine hearts by recording simultaneously from 192 endocardial leads. Series of activation maps were constructed covering 1 sec of persistent AF. These maps clearly demonstrated that AF was based on the presence of multiple circulating wavelets. However, the number of waves at any particular moment during AF varied considerably. New wavefronts were continuously formed by division at areas of temporary conduction block, while other wavelets ceased to exist either due to fusion with other waves or because of extinction at the A-V ring. To characterize this balance between waxing and waning of wavelets during persistent AF, we actually counted the number of

wavelets at intervals of 10 msec. In both atria 6 wavelets were present, varying between 3 and 10. There was no difference between the right and the left atrium. Each atrium accommodated 3 wavelets, ranging between a maximum of 5 and a minimum of 0. The temporary arrest of fibrillation in one atrium did not cause termination of AF, because it was immediately reinitiated from the other atrium. On the other hand it shows that the presence of 3 multiple wavelets is *not* sufficient for the persistence of AF.

### Physiological changes during whole body hyperthermia for the treatment of malignancies

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Since Von Ardenne published his therapy scheme (multistep therapy program) for the treatment of cancer in the early fifties interest for combination of radiotherapy with hyperthermia has been very large. During recent years knowledge of anesthesia and insight into the physiological effects of hyperthermia has reached such a level that vital parameters can be much better interpreted, measured and registered and necessary correction immediately performed adequately. Since 3 years whole body hyperthermia is performed in Rotterdam as a preparation for effective radiotherapy in extensive malignancies. Intensive cardiovascular studies were done in 30 patients during hyperthermia treatment. The patients were anesthetized with nitrous oxide/oxygen, relaxation and then were warmed up for 2 h in a modified Siemens hyperthermiacabin to a temperature of 41.8°C. Hyperthermia induced a great increase in cardiac output and heartwork with decrease of peripheral resistance in the systemic as well as the vascular bed of the lungs. The arterial pulmonalis pressure increased while the systemic blood pressure decreased. The result was a proportionally greater increase in right ventricular work as compared to a much less increased left ventricular work. Oxygen consumption went up with 34% from which a major part was due to the increase of heartwork. Furthermore significant changes of hematocrit, electrolytes and serum enzymes were seen. Based on the data gained in patients and animal experiments it can be concluded that the liver is the limiting organ for treatment with whole body hyperthermia.

### Individual variation in the serum cholesterol response to dietary cholesterol in man and laboratory animals

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Differences in the response of serum cholesterol to dietary cholesterol in rabbits and rats have a genetic basis. In inbred rabbits a 5-fold greater response was found in two hyper-responsive strains than in two hypo-responsive strains. Similar differences in serum cholesterol response between inbred strains of rats were also observed. In order to see whether humans exist with a consistently high or low serum cholesterol response to an increase in cholesterol intake, we have carried out three controlled dietary trials with the same subjects. Although the response in each individual was only partly reproducible from one study to another, the hyperresponders consistently showed a significantly higher serum cholesterol response in the second and third trial than the selected hyporesponders. We conclude that human hyper- and hyporesponders do exist. We have investigated whether differences in responsiveness are related to differences in endogenous cholesterol synthesis. In rats on a low cholesterol diet whole body cholesterol synthesis (measured as faecal excretion of bile acids and neu-

tral steroids plus cholesterol retention minus intake) was about 2-fold higher in a hypo- than in a hyperresponsive strain. In humans, cholesterol synthesis on a low-cholesterol diet, measured as faecal excretion minus intake, was also negatively associated with the response of serum cholesterol to dietary cholesterol. These data suggest that hyperresponders have a limited range for down regulation of cholesterol synthesis after cholesterol loading, and this may partly explain the high cholesterolemic response in such individuals. Supported by grant No. 31.013 of the Netherlands Heart Foundation and an established investigatorship to M.B.K.

### Individual differences in the circadian system response to shifts of the social zeitgeber

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Morning-type (M-type) and evening-type (E-type) persons not only differ in the external phase position of circadian rhythms, but also in the internal temporal order of these rhythms. (G. A. Kerkhof, Inter-individual differences in the human circadian system. A review. *Biological Psychology* (1984) in press). The role which these individual differences play in the response of the circadian system to external disturbances forms the main object of the present study. During a 12-day period – from 6 days before until 6 days following the 1-h changes from East-European Time to Mid-European Time (autumn 1982) and vice versa (spring 1983) – samples of M-type and E-type subjects kept daily records of their hours of sleep, and – as frequent as possible – hourly self-measurements of oral temperature and subjective alertness. The major effects of these clock time changes occurred in response to the advance change (i.e. in the spring). For M-types, the alertness rhythm followed with a delay of 2 days, while the other two rhythms changed their phase instantaneously; for E-types, however, the temperature rhythm was delayed for 3 days.

### On-line registration of physiological data during closed circuit anaesthesia

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Anesthesia with a closed circuit system is discussed since 1924, but has never been so far developed that it could be performed with automatically controlled ventilation. On the basis of the rolling seal rebreathing respirometer we have developed at the Erasmus University Rotterdam the first fully automatically controlled closed circuit system. Volume loss is registered by the piston which does not reach its endposition at the end of expiration and supplemented by an electronically feedback controlled injection of  $N_2O$ , thus the total volume is kept constant. At the same time the inspiratory oxygen concentration is kept constant on a preset value, feedback controlled. The closed circuit system is fully separated from the ventilator system so that any type of ventilator can be used to drive the respirometer externally. As volume and inspiratory oxygen concentration are kept constant the new system offers the possibility to add several parameters to patient monitoring:

1. Exact registration of gas uptake from breath to breath (e.g.  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$ ).
  2. Registration of a constant fraction of inspiratory gases independent of minute volume and gas uptake.
  3. Registration of several important cardiorespiratory parameters based on noninvasive rebreathing techniques, e.g. functional residual capacity, ventilation distribution ratio, cardiac output.
- Further development of the possibilities of the automatically controlled closed circuit system will decisively enlarge the scale of measurable vital physiological parameters of seriously ill patients in the operation room and on the intensive care unit.

### Results of an investigation on the origin of heart sounds with the aid of phono-echocardiography and multi-site phonocardiography

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Origin of heart sounds has been studied from two different points of view. Temporal relationship of heart sounds to valvular motion has been investigated by using combined phono- and echocardiographic techniques: typical discernible vibration patterns have been related with events in echocardiographic tracings, especially those connected with valvular opening and closure. On the other hand, spatial vibratory patterns have been investigated by applying multi-site recording of phono-cardiographic signals at 49 chest wall points, homogeneously distributed at the precordial area. Wave form parameters such as norm (vibration intensity), correlation value (vibration similarity with respect to the signal measured at a chosen reference point) and reconstruction value (vibration similarity with respect to all observed chest wall points) have been calculated for well defined intervals of the heart cycle. These parameters connected with appropriately chosen intervals reveal typical spatial distributions. E.g., phase reversal of the aortic component of the second sound is perfectly indicated by correlation and reconstruction distribution, the latter showing a clear minimum at the chest wall site where the phase reversal occurs. Combining both methods, the first one giving reliable temporal information in connection with valvular motion, the second one supplying information about spatial vibratory distribution and thus allowing discrimination between effects of different physiological vibration sources, will contribute to the understanding of the physical mechanisms of heart sound generation.

### The development of coupling between heart cells in tissue culture

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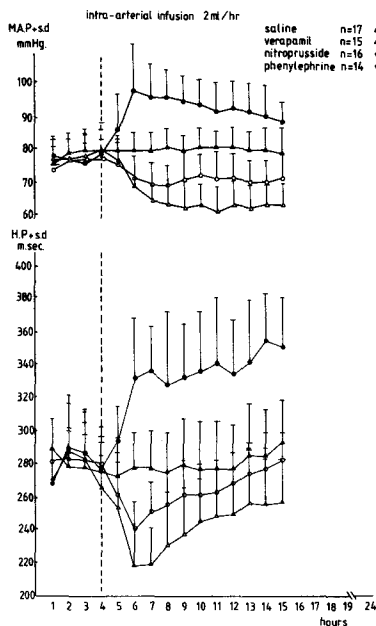
Neonatal rat heart cells, when isolated in tissue culture, beat spontaneously and irregularly. When two such cells grow together they synchronize their rate of beating. We analyzed the emergence of this synchronization of beat rate. Before physical contact between a pair of heart cells is existent each of them beats in its own irregular rate; the degree of irregularity is a function of the mean inter beat interval (IBI): the smaller the IBI, the smaller the degree of irregularity. Once physical contact is made by the growing cells synchronization occurs most often from one beat to another. In some cases a short period of partial synchronization is observed during with 1:2, 1:3, etc. modes of synchronization are present. In virtually all cases studied the common mean IBI of the pair is intermediate between the mean IBI of each of the originally single isolated cells. There is a strong correlation between the mean IBI of the pair and that of the originally fastest beating cell. Sometimes the cellpairs beat at the same rate but not synchronous: the originally faster beating cell leads the originally slower one by some tens of milliseconds. We presume that in these cases the amount of coupling between the two cells is less than between really synchronously beating cells. Contrary to literature reports stating otherwise, these observations tend to support the classical pacemaker hypothesis.

### Temporal adaptation of reflex changes in heart rate during chronic infusion of vasoactive drugs

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The chronotropic effect of calcium entry blockers on the heart depends on the interaction between the direct negative chronotropic effect and the indirect reflex tachycardia due to hypotension. Resetting of the baroreceptor reflex may change the

interaction between direct and indirect effects. In 24 conscious rabbits mean arterial pressure (MAP) and heart period (HP) were measured during 24 h a day and averaged over 1-h periods. MAP was  $82 \pm 6$  mmHg ( $\pm$  SD) and HP was  $274 \pm 15$  msec ( $\pm$  SD). No diurnal rhythm was observed in MAP but HP was significantly longer in the morning than in the evening. The changes induced by intra-arterial infusion of verapamil (5 mg/h) are shown in the figure. For comparison, a hypo-



tensive drug without a direct effect on the heart was infused (nitroprusside; 2 mg/h), as well as a hypertensive drug (phenylephrine; 0.9 mg/h and saline. These results suggest that the effect of phenylephrine on MAP and HP is not just a reflection of the effect of verapamil and nitroprusside. They also suggest that the reflex effect due to hypotension may largely disappear during the period of infusion.

### Alinidine: effect on rate of rise and repolarization of the action potential of guinea pig atrial fibers

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The effect of alinidine on the maximal rate of rise and the repolarization of the action potential of guinea pig atrial fibers was studied. The isolated preparations of the right atrium were driven at 2 to 5 Hz. Alinidine was applied in doses between  $0.7 \times 10^{-5}$  M and  $5.7 \times 10^{-5}$  M. Alinidine delayed repolarization, especially the last part. Already at  $0.7 \times 10^{-5}$  M the effect on repolarization was significant. Higher concentrations further delayed repolarization, but to a lesser extent. The maximal rate of rise ( $V_{max}$ ) was decreased by alinidine; this effect was only significant at  $5.7 \times 10^{-5}$  M ( $p < 0.05$ ). The decrease of  $V_{max}$  was independent of cycle length; therefore it is unlikely that this decrease is caused by an inhibition of the Na/K-pump activity. It seems that there is no common mechanism of action of alinidine responsible for both the delay of the repolarization and the decrease of  $V_{max}$  of the atrial action potential, since the dose/effect ratios of the two parameters differ.

### Skeletal muscle microcirculation

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Perfusion of the terminal vascular bed of the intact tenuissimus muscle preparation of anesthetized rabbits can be studied in

situ by intravital microscopy. Under these conditions perfusion of the resting muscle superfused with a solution containing a minimum of oxygen is comparable with that of an unexposed muscle. Perfusion of the terminal vascular bed is not continuous but periodic in nature. Transverse arterioles (TA), running across the muscle fibers, distribute the blood to their first order side branches (FOS). Spontaneous (rhythmic) changes in diameter with a cycle length of 2–15 sec (vasomotion) in TA and FOS regulate the perfusion of the capillary bed. FOS often completely close during vasomotion cycles, causing a complete cessation of capillary flow. The on-off nature of capillary flow will cause dynamic changes in capillary exchange due to the concomitant variations in capillary pressure. The periodic nature of flow and vascular diameter should also be taken into account, when comparing micro and macrocirculatory flow data. Reduction of arterial pressure through aortic occlusion hardly influences vasomotion frequency and amplitude until vasomotion disappears. At low arterial pressures TA and FOS effectively dilate. Both an increase in tissue pressure and a reduction of arterial pressure causes cessation of capillary flow in the presence of dilation of the arterioles. Thus no critical closure due to low transmural pressure has been observed. When subsequently perfusion pressure was increased capillary flow did not start before an increase of about 7 mm Hg.

### The role of hypophysis in painperception

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Various experimental findings of recent years suggest that the hypophysis and adjoining parts of the hypothalamus play a major role in painperception. A high level of endorphines, endorphine receptors and encephalineric neurons were found in the hypophysis and analgesia induced by stress was accompanied by an increase of hypophyseal endorphine levels. Stress induced analgesia could not be produced after surgical hypophysectomy and when opiate antagonists (e.g. naloxone) were injected before. Pain treatment by injection of alcohol into the hypophyseal region (neuro adenolysis of the pituitary gland-NALP) also works in patients with not hormonal tumors. In monkey experiments it was shown that the effect of pain relief using this technique is based on a increase of neuronal activity in the hypophyseal region simultaneous to an inhibition of the evoked potential in the primary sensory cortex using toothpulp stimulation (TPEP), thus inhibition by activation of the hypophyseal region. In rabbits toothpulp evoked potentials (TPEP) were measured in the hypophyseal region (PR) and in the primary sensory cortex (PSC). After injection of naloxone the TPAP response in the PR decreased while the TPEP response in the PCS increased (increase of painperception). When an exogenic opiate was injected the TPEP response in the PR increased while it decreased in the PSC. In a further set of experiments the response was measured in the PSC after a short termed electrical stimulation (10 V, saw shaped, low current, 10 min) of the PR (pituitary region). The TPEP response of the PSC disappeared nearly fully. The experiments were repeated in monkeys to prove reproducibility of results gained in rabbits in the primate. The same results were found. In the meantime we have treated patients in Japan with electrical stimulation of the pituitary region to treat so far in-treatable terminal cancer pain, with excellent results.

### Blockage of sodium channels by the anti-epileptic drug phenytoin

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Phenytoin is a commonly used anti-epileptic drug, whose capacity to block sodium channels is well established. The degree of blockage not only depends on concentration, but is also modulated by membrane voltage. Phenytoin shares this property with a number of other anti-epileptic drugs and a variety of local anesthetics. A quantitative analysis of this modulation

will help to understand the anti-epileptic properties of phenytoin and its mechanism of action. For this aim, sodium currents in single myelinated nerve fibers of *Xenopus laevis* were investigated using a modified Nonner clamp. Sodium currents were elicited by depolarizing voltage steps, with or without conditioning prepulses. Typically, 8  $\mu$ M phenytoin reduced the amplitude of the sodium current transients 50% for all potentials, when stepped from holding potential ( $-80$  mV). A hyperpolarizing prepulse to  $-120$  mV for 3 sec restored the amplitude to 66% of the control value. The effects of depolarizing prepulses are more difficult to study due to fast and slow inactivation of the sodium conductance. To overcome these problems we applied voltage pulseprograms, which remove fast inactivation after a long conditioning prepulse. Preliminary results show a clear enhancement of blockage following 3 sec depolarizing prepulses, after correction for slow inactivation. These effects of depolarizing conditioning prepulses seem to be very relevant for the anti-epileptic action of phenytoin.

### **The effect of partial lesions of the hypothalamic suprachiasmatic nucleus on behavior and on hormone levels in the rat**

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The hypothalamic suprachiasmatic nucleus (SCN) plays an important role in the circadian control of a large number of neuroendocrine and behavioral processes, at least in rodents. Until recently the SCN has been considered to be a densely populated network of rather uniform cells. However, histochemical studies on the rat have revealed that the SCN has a number of rather distinct subdivisions. So the question arises whether the SCN as a whole exerts its function as a main pacemaker for circadian rhythms or must be considered as a network of mutually coupled high frequency oscillators. In order to find out whether partial lesioning of the SCN may change the control of circadian rhythmicity, an experiment was devised in which lesions of the SCN were made in blinded Wistar rats while simultaneously recording wheel running activity, food and water intake, core body temperature and urine corticosterone levels for several months. Although in some cases the lesion resulted in a permanent and complete disappearance of all rhythmicity, in many animals the rhythmicity returned within a couple of days or weeks after the lesion, but with periods shorter than 24 h. Histological analysis of the brain showed that lesion of the rostral 60% of both SCN was present in all cases in which the period of the rhythm was changed. Complete loss of rhythmicity occurred in animals with a complete destruction of both SCN.

### **Selective lesions in the retina and hypothalamus after neonatal administration of monosodium glutamate in rats. A neuro-endocrine model**

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Postnatal subcutaneous administration of monosodium glutamate (MSG) in rats results in several endocrine deficits, growth retardation and obesity. These phenomena are mainly due to a reduction in the number of dopaminergic and catecholaminergic cells in the hypothalamic arcuate nucleus. MSG also causes degeneration of the inner layer of the retina, a decreased diameter of the optic nerves and the absence of the b-wave in the electroretinogram. However, the massive retinal damage after MSG administration does not result in complete blindness. The projection to the suprachiasmatic nucleus (the retino-hypothalamic tract) is still functional in the entrainment of circadian rhythms to the environmental light-dark cycle. Histochemical analysis shows that administration of MSG to new born rats accelerates a migration process of fluorescent dopaminergic cells from the median eminence towards the arcuate nucleus, which normally occurs from day 22 till 55 after birth, by 2 weeks. Electron microscopic analysis of the optic

nerves at adult age reveals a selective loss of axons with a perimeter between 1 and 3  $\mu$ m as well as of axons above 10  $\mu$ m, the range between 3 and 10  $\mu$ m being spared. This medium size group corresponds with the X-system in the cat.

### **The effect of lesions of the dorsomedian hypothalamic nucleus on the composition and activity of brushborder enzymes of the small intestine in the rat**

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Food intake in the human as well as in the animal is regulated by the brain. This food intake has in normal conditions a circadian rhythm. The internal clock is localized in the suprachiasmatic nucleus. Damage of this nucleus abolishes the periodicity of most of the functions. Damage in the lower central with clock activity makes some functions a-rhythmic. Damage of the dorsomedian- hypothalamus (DMH) does not affect the circadian character of activity and body temperature in the rat but food intake becomes a-rhythmic. Circadian changes are found in the activity of the brush-border enzymes. These changes could not be demonstrated in rats with a DMH lesion. Changing enzyme activity can be caused by either changes in enzyme synthesis, in enzyme degradation or in changes in activation. We study circadian changes in the electrophoretic pattern of brush-border membrane fractions and we paid special attention to the sucrase-isomaltase fractions. We could demonstrate that there is a circadian rhythm in the ratio of low and high molecular weight fractions of the sucrase-isomaltase complex.

### **Effects of variation in the level and time course of retinal outflow on the rabbit's circadian rhythm in food intake**

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Continuous records of the circadian rhythms in food intake were obtained from 13 rabbits of both sexes which were consecutively exposed for 5 months to continuous illumination (200–250 lx), total darkness, LD 1:23, LD 12:12, and were blinded afterwards. It appears that the circadian pattern of food intake differs markedly under these circumstances and that it is governed by at least 2 oscillators. One of these (the Illumination Sensitive oscillator) has a freerunning period  $\tau$  which is only slightly different from 24 h and is not influenced by the level of optic outflow, whereas the other (the Illumination Sensitive oscillator) has, on the average, a  $\tau$  of 24.55 h in DD, of 24.24 h in LL and of 23.85 h after blinding. These observations suggest that the centers controlling the rabbit's food intake rhythm are steadily influenced by the maintained retinal ('dark-') discharge and that the latter is continuously suppressed (inhibited) by constant illumination.

### **Involvement of monkey inferior parietal lobule in preparation of visually cued arm movements**

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We have previously shown that neurons in the region of the arcuate sulcus of the macaque cerebral cortex show pronounced modulation of activity during preparation for specific movements, often related to the trajectory of the arm movement required to reach for a visible target. Subsequent anatomical and electrophysiological experiments suggested a flow of information from the anterolateral part of the inferior parietal lobule (IPL) through the caudal bank of the arcuate sulcus to the primary motor cortex. The present experiment was designed to compare neuronal activity in the IPL with that in the

arcuate area. A macaque monkey was trained to perform the same task as in the previous experiment, namely to reach with the left arm for a food reward when a buzzer was heard. This GO signal occurred at a variable delay (1.0–2.5 sec) after the food became visible in one of three positions. A recording chamber giving access to both banks of the intraparietal sulcus was implanted over the right hemisphere. A total of 37 task-related single neurons were recorded. 15 of these neurons modulated their activity following presentation of the visible target. All these neurons were situated in the IPL. Neurons in areas 1 and 2 modulated only during the GO-triggered movement phase and were often related to somatosensory input from hand or fingers. It is concluded that neurons in the anterolateral part of the IPL, like those in the posterior bank of the arcuate sulcus are concerned with preparation of reaching movements in visible space.

### **Imprecision of fixation during voluntary head oscillations is not an artefact of ocular torsion**

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Recent measurements of the precision of fixation by Steinman, Collewijn et al., using a scleral coil method, have indicated considerable imprecision of gaze during active, horizontal head movements. The effective gain of compensatory reflexes was between 0.95 and 1.05. As the difference from unity is small, it is essential to exclude sources of artefact. One source could be torsion of the eye in space: 1. if the scleral coil is not perfectly perpendicular to the visual axis, ocular torsion will affect the recorded horizontal and vertical eye positions; 2. horizontal head movements may be contaminated by head torsion. As yet we could not measure ocular torsion directly, but we could measure head torsion as well as the horizontal and vertical head and eye position simultaneously. For static conditions compensation in the torsional axis is known to be very small and it may be assumed that ocular torsion equals head torsion. We derived equations relating the angles of coil misalignment and ocular torsion with the measured horizontal and vertical eye position. A numerical correction based upon these relations thus eliminated the torsion artefact. We applied this correction in experiments with human subjects who fixated a target at optical infinity while making different static torsional, horizontal and vertical head deviations and also in a mock experiment. Subsequently, the same corrections were applied to fixation during voluntary head oscillations in horizontal, vertical and torsional directions. Horizontal and vertical head oscillations were both usually accompanied by head torsion (coupling factor about 0.05–0.17). In some cases, the correction of the torsional artefact resulted in a moderate reduction of gaze instability. In other cases, the correction proved counterproductive; this suggests an active counterrolling of the eye in the head. We conclude that: 1. dynamic gaze instability is not accounted for by a torsional artefact; 2. considerable torsional counterroll is likely to occur and should be measured to evaluate gaze instability completely.

### **The dynamics of lid and eye movements associated with blinking and eye closure**

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Reliable data on eye movements behind closed lids are scarce and obviously hard to obtain. Although the traditional notions about upward eye movements during every eye closure including blinks (Bell's phenomenon) are apparently supported by EOG recordings, it has been demonstrated that lid movements cause artifacts which make the vertical EOG completely useless. We studied eye movements during blinks and prolonged eye lid closures ('squares') with a scleral coil embedded in a silicone annulus which adheres firmly to the eye so that true ocular position is recorded under all conditions. Lid movements were recorded with a second coil, connected to the lid by a light weight lever. During a typical voluntary blink the upper

lid moved about 10 mm downward (maximal velocity about 160 mm/sec) and about 3 mm nasally. The total duration of the lid movement was 0.3–0.4 sec. During such blinks the eye invariably moved briefly downwards and nasalwards (with about equal amplitudes) from the primary position. These eye movements typically had an amplitude of 2–3 deg, a maximal velocity of about 75 deg/sec and a duration of 250 msec. Psychophysical experiments with the location of an afterimage of a flash, triggered by the downward movement of one (covered) eye with a coil, confirmed the presence of a downward displacement during a blink of the other eye without a coil. During 'squares' the lid moved down about half as fast as during a blink but the eye moved upwards by about 2–14 deg, depending on the vertical starting position, with a velocity not exceeding about 25 deg/sec. The opening phase consisted of a saccadic, downward eye movement followed by lid opening and subsequently a correction saccade. It is concluded that Bell's phenomenon occurs only when the eye is closed for longer than a brief instant.

### **Paradoxical sleep in the rat: effects of deprivation on body temperature**

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The function of paradoxical sleep is studied by determining the behavioral effects after its deprivation. In contrast with results obtained with the platform technique, deprivation induced by the pendulum technique (van Hulzen and Coenen, 1980) causes only small effects on subsequent behavior. The origin of the platform-pendulum controversy is still unclear. It is known (Ferguson and Dement, 1968) that body temperature increases following platform deprivation. Whether this factor is responsible for the upper mentioned controversy is investigated. One group of Wistar rats ( $n = 9$ ) is deprived with the platform, another with the pendulum technique ( $n = 6$ ). Before, during and after paradoxical sleep deprivation, body temperature is rectally measured 1 h prior and 1 h after onset of the dark period. Immediately following initiation of deprivation, body temperature increases ( $0.8 \pm 0.3^\circ\text{C}$ ). The hyperthermia is maintained for the 72 h lasting deprivation period. There are also indications for disturbance of the circadian temperature rhythmicity. In all these aspects there are no differences between both groups. Following deprivation, temperature of the pendulum rats decreases till base-line ( $37.5 \pm 0.5^\circ\text{C}$ ), while for the platform rats it decreases further. These rats show a prolonged hypothermia of  $0.5 \pm 0.2^\circ\text{C}$  under base-line. Whether this difference in body temperature can explain the large behavioral differences following platform and pendulum deprivation, remains a point for discussion.

### **Visuo-motor behavior after bilateral removal of the visual cortex in the rabbit**

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J. Ten Cate and A. W. H. van Herk (Arch. Néerl. Physiol. 28 (1933) 337–476) described the behavior of rabbits after bilateral removal of the occipital lobe. In an open-field situation the animals were at first unable to avoid obstacles. After several weeks they managed to avoid these objects while running. In studies carried out in animals which were kept in cages after the same operation it was found that pattern discrimination was impaired. Besides that the threshold of brightness discrimination was increased. (M. W. van Hof, J. van Hof-van Duin and J. F. Hobelen, Behav. Brain Res. 9 (1983) 257–262). In the present investigation it was studied whether the defect after bilateral removal of the occipital is task-dependent or influenced by the nature of the environment in which the animals are kept post-operatively. Operated animals were released in a room with a great variety of objects. At first they behaved like 'blind animals', but after some 8 weeks running, climbing and jumping was almost normal. In some of the animals the eyelids were sutured. The initial handicap re-

turned. The other animals were trained in visual discrimination tasks. In spite of their normal open-field behavior they were unable to discriminate striated patterns of different orientation and the threshold of brightness discrimination was increased. In other words, post-operative visuo-motor recovery after bilateral removal of the occipital lobe is remarkably task-dependent.

### **In vitro interconversions of locust hemolymph lipoproteins in relation to hormone-stimulated lipid mobilization from fat body**

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In the locust, mobilization of diacylglycerol from the fat body in response to injection of adipokinetic hormone (AKH) results in elevation of the diacylglycerol level in the hemolymph and is accompanied by the formation of a high molecular weight lipoprotein ( $A^+$ ) from existing hemolymph protein components, involving both the resting lipoprotein  $A_1$  and the non-lipid carrying protein C, as well as the increased diacylglycerol. The effect of AKH on diacylglycerol mobilization from the fat body and the lipoprotein rearrangement in the hemolymph was studied in vitro. In diluted hemolymph, AKH induces both diacylglycerol release from fat body and lipoprotein  $A^+$  formation. In a medium containing isolated hemolymph proteins (lipoprotein  $A_1$  and protein C) both processes occur to some extent without the intervention of AKH; AKH does, however, markedly stimulate these processes. Octopamine, a neurohormone that has been proposed to be released upon stress and to induce some increase in hemolymph lipid level, is shown to have a limited and short-term effect on lipid mobilization from fat body, but failed to induce changes in the lipoprotein pattern, in vitro.

### **Pulmonary stretch receptors and the control of breathing in exercise**

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The partial pressure of  $CO_2$  ( $PCO_2$ ) in arterial blood as a function of the work load remains constant up to moderate exercise and, thereafter, decreases for strenuous exercise. This apparent contradiction with the classical theory of the control of breathing occupied many scientists in the last decades. Recently, however, Sheldon and Green showed that an increase in the  $CO_2$ -flux to the lungs stimulates ventilation, sufficiently, to account for the exercise hyperpnea. Vagotomy abolished this response. Evidently, the pulmonary stretch receptors (PSRs) are involved in this phenomenon. We have developed a model for a pulmonary contribution to the control of breathing based on the properties of PSRs in relation to the inspiratory activity of the respiratory neurons. These properties can be arranged in two positive feedback loops and one negative feedback loop. The positive feedbacks include the well-known vagal augmentation of inspiratory motoneuron activity and the Breuer-Hering deflation reflex, respectively. The positive feedbacks account for the exercise hyperpnea and their ventilatory output is controlled by the negative feedback. This control is based on a) the inverse  $PCO_2$  responsiveness of PSRs, b) the low levels of  $CO_2$  in the airways during inspiration, and c) the impact of PSRs on the inspiratory off-switch. In summary, the pulmonary contribution to the exercise hyperpnea (positive feedbacks) is controlled by the end-inspiratory  $PCO_2$  levels in the airways (negative feedback) that can be shown to be closely related to the  $PCO_2$  in arterial blood (J.F. Green and M.I. Sheldon, J. appl. Phys. REEP 54 (1983) 997-1002; and M.I. Sheldon and J.F. Green, J. appl. Phys., REEP 52 (1982) 1192-1197).

### **Induced alkalosis and physical performance**

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Concerning the effect of induced alkalosis on physical performance conflicting findings have been reported in literature. Since measuring of performance appears to be the most critical point in such studies we have chosen for a design to minimize subjective and environmental factors. 5 subjects performed 18 exercise tests after intravenous administration (double blind) of 0.9% NaCl (control), 0.18 or 0.36 g·kg<sup>-1</sup> NaHCO<sub>3</sub> in a balanced randomized order. The test protocol consisted of 4 1-min 125%  $V_{O_{2max}}$  work bouts on a bicycle ergometer interrupted by 1-min pauses. Endurance of the 5th all out bout was considered as the test performance. Mean endurance were 115, 120 and 140 sec, respectively, however not significantly different due to the large range. HCO<sub>3</sub><sup>-</sup> and pH in blood did differ highly significant throughout the exercise test between the 3 experimental conditions. Blood lactate concentration showed a significant elevation (1-3 mmol·l<sup>-1</sup>) from the 3rd bout in 0.36 g·kg<sup>-1</sup> NaHCO<sub>3</sub> tests compared to control. No differences in  $V_{O_2}$ ,  $V_E$  or heart rate were found. However, analysis of the data after regrouping the tests according to the endurance time resulted in the preceding work periods (bout 1 to 4) in a significantly lower  $V_{O_2}$  (5%) and  $V_E$  (5-8%) of long endurance tests as compared to short ones. Blood lactate concentration showed no difference till the last work bout, but the post exercise values were significantly higher (1-4 mmol·l<sup>-1</sup>) in long endurance tests as compared to the short ones. This finding can be explained by the higher lactate production as result of the longer working time. Summarizing it can be concluded that the effect of an increased buffer capacity of the blood on physical performance is partly masked by a daily fluctuation in work efficiency.

### **Exercise-induced changes in plasma androgen levels in women: changes in the biologically active fraction**

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We investigated changes in total plasma testosterone (T), percentage free T (FT) and precursors of T, androstenedione (A) and dehydroepiandrosterone sulphate (DHEAS) in 6 female marathon runners (MR) and 13 untrained women (UT), all with normal menstrual cycles, during standardized long lasting exercise, in the follicular (FP) and luteal phase (LP) of their menstrual cycles. Blood was obtained every 15 min. Data analysis was performed with an ANOVA among groups with repeated measurements. The results showed:

1. No differences in androgen responses between FP and LP.
2. A significant ( $p < 0.001$ ) linear increase as a function of exercise time in plasma T in UT and MR. The increments being relatively more pronounced ( $p < 0.01$ ) in MR than in UT (41% and 24%, respectively after 43 min of exercise). In the MR plasma T exceeded the baseline values with 105% after 75 min of exercise.
3. A significant ( $p < 0.01$ ) increase in plasma A and DHEAS in MR but not in UT. For DHEAS the highest values were reached after 45 min of exercise and exceeded the baseline value by 41%, for A the highest values were reached at the end of exercise (55%).
4. A significant ( $p < 0.05$ ) rise (12%) in FT in both groups. The free T concentration and biologically active fractions increased by 25% and 77% (UT) and 80% and 225% (MR), respectively.

These results may suggest a role of adrenal androgens in the high incidence of menstrual cycle disorders in athletes.

## The force-velocity relationship of muscle of trained and untrained subjects

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The force-velocity curve (FVC) of the arm flexion movement of untrained males ( $n = 123$ ) and females ( $n = 110$ ), and male athletes ( $n = 48$ ) was established according to De Koning et al. (Int. J. Sports Med. 5 (1984) 43–46). Using a simple mechanical model of the arm, the FVC of a flexor equivalent was derived. The FVC, described by Hill's equation, was defined by the parameters  $F_0$  (maximal static strength),  $v_0$  (maximal unloaded speed of shortening) and  $H$  (a measure for the concavity); maximal power ( $P_0$ ) was calculated also.  $F_0$ ,  $v_0$  and  $P_0$  were standardized for the anthropometrically determined arm cross-sectional muscle-and-bone area (MBA), upper arm length ( $L$ ) and 'muscle volume' (MBAL), respectively. Within the given age range, the level of the curve parameters was almost constant.  $H$  was the same (0.58) for all three groups. Mean values for  $F_0$ ,  $v_0$  and  $P_0$  of the untrained males were 1175 N,  $0.97 \text{ m} \cdot \text{sec}^{-1}$  and 195 W; for these respective parameters, the ratio females/untrained males was 0.69, 0.80 and 0.57 and the ratio athletes/untrained males was 1.30, 1.04 and 1.30. For the standardized  $F_0$ ,  $v_0$  and  $P_0$ , the ratio females/untrained males was 0.92, 0.88 and 0.83 and the ratio athletes/untrained males 0.94, 1.02 and 0.92, respectively. Part of the difference between the groups in standardized  $F_0$  and  $P_0$  may be due to a systematic error in MBA.

## Presence and properties of a light-programmed circadian rhythm in the rabbit's EEG

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13 female rabbits were exposed to a LD 12:12 regimen for 15 months in which their condition was monitored by continuous recording of the food intake pattern. Two experiments during which a.o. the occipital EEG over 0.5–40.0 cps was continually analyzed for 30–50 h, were performed with each animal in the course of 4 months after implantation of the epidural electrodes, in 5 months after removal of the cervical sympathetic nerves and after sectioning of the optic nerves. It appears that in intact rabbits the root mean square value  $\sigma$  of the visual cortex EEG fluctuates as a whole according to a block-shaped circadian rhythm. This rhythm, which is clearly 'programmed' since its time course follows the same of the preceding L:D schedule, has a stable phase with Night Time Potentials and an average  $\sigma$  which is 16% lower than in the phase with Day Time Potentials. After sympathectomy the rhythm is still present, although its amplitude has been reduced with one third by this procedure (and the spectral composition of the EEG has slightly been altered in favor of the lower frequencies). After blinding the EEG rhythm is further reduced in amplitude whereas  $\sigma$  (measured over 24 h) has increased with some 20% and the EEG spectrum has strongly been altered in favor of, again, the lower frequencies (0.5–4.5 cps). The results suggest that in rabbits the EEG of the visual cortex is strongly influenced by the maintained ('dark-') discharge of the retina and by circadian changes in the same.

## Cross-coupling between horizontal and vertical components of oblique saccades

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We have made a detailed study of dynamic properties of horizontal, vertical and oblique saccades in order to distinguish between alternative models of the saccadic system. Eye movements were measured with the double magnetic induction method in two monkeys. It appears that components of oblique

saccades have very well synchronized onsets. Saccade component peak velocity and duration depend strongly on the size of the orthogonal component and cannot be predicted from knowledge of properties of purely horizontal and vertical saccades. Consequently, the idea that orthogonal components of oblique saccades are generated by synchronized but otherwise independent pulse generators, such that components have a fixed relation between amplitude and their dynamic properties, must be rejected. In an attempt to account for the observed cross-coupling between components, we have developed a two-dimensional model of the saccadic system where vectorial error is first converted into a vectorial eye velocity signal by a nonlinear pulse generator. In a subsequent decomposition stage, component related eye velocity signals are generated from this vectorial eye velocity signal. The model gives a very reasonable fit with the data. According to this common source model, component stretching as observed in the data is a natural consequence of the nonlinearity in the proposed vectorial pulse generator.

## Does prematurity affect the development of visual functions?

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The development of visual acuity, monocular optokinetic nystagmus (mon.OKN) and visual component of the threat response was compared in normal fullterm and in prematurely born infants, in order to determine whether extra visual experience affects visual development. Visual acuity was assessed by means of the preferential looking technique: 120 acuity estimates have been obtained in 90 infants born 4–14 weeks prematurely, and ranging in age from 3 to 62 weeks from birth. In the preterm infants, acuity was consistently lower than that of normal fullterm infants of comparable postnatal age. However, when ages were corrected for prematurity, i.e. calculated from the date at which the infant should normally have been born after 40 weeks gestation, acuity values of fullterm and preterm infants overlapped, indicating that visual experience prior to the normal gestational age at birth does not influence acuity development. Mon.OKN and the development of the visual component of the threat response were tested in order to distinguish subcortical and cortical components of visual processing. Fullterm infants showed symmetrical mon.OKN from 12 weeks of age onwards, whereas the visual component of the threat response was positive after 17 weeks. Results obtained in 79 preterms overlapped those of fullterms, but only when ages were corrected for prematurity. Visual development in the preterm infant seems to be related to conceptional or corrected, rather than postnatal age; there is no indication of either retarded or impaired nor accelerated development.

## Stereopsis, vergence and motion perception during dichoptic vision of moving random-dot stereograms

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The precision of vergence eye movements, the limits of fusion with stereopsis and perception of motion in dynamic conditions are only partially known. We investigated the effects of imposed, disjunctive motion. Random-dot stereograms ( $30 \times 30$  deg, distance 1.43 m, elements 18 min arc) were viewed dichoptically by human subjects without a fixed visual frame of reference, with the head stationary. Movements of both eyes were recorded with a scleral coil technique. The patterns seen by the left and right eye were oscillated horizontally in counterphase with a sinusoidal or triangular motion. The ratio between the amplitudes of the movements of the two images was varied systematically. These stimuli induced vergence movements with a residual vergence error which had a similar shape as the stimulus movement and could increase to 2 deg without loss of stereopsis. The limit for stereopsis and fusion

followed a hyperbola in the frequency-amplitude plane of target vergence; stereopsis was maintained for target vergence velocities up to 6 deg/sec. Under this condition only horizontal, and no in-depth motion was perceived. The magnitude of the binocularly perceived motion, signalled by manual tracking of the perceived displacement equalled the algebraic sum of the monocular motion percepts. As a special case, equal but opposite motions of the stereograms induced vergence movements but were perceived as a completely stationary, fused image in stereoscopic depth.

### Influence of training on growth and biological maturation in female athletes

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The purpose of this study is to gain more insight into the relation between physical exercise and growth and pubertal development of female athletes, especially gymnasts and swimmers. Cross-sectionally we investigated differences in onset and progression of puberty, body composition and growth in 1064 girls. Also we determined the relations between the pubertal events and body composition, especially the amount of body fat, or training activities. Furthermore we evaluated growth pattern of these groups until 11 years of age retrospectively ( $n = 362$ ). In addition we tested the hypothesis that these differences in pubertal development is modulated by estron levels in relation to the fat mass ( $n = 98$ ). Finally we investigated the influence of training on growth and sexual maturation of weanling female Wistar rats ( $n = 143$ ). The results reveal that the onset of puberty and the menarche are shifted to a later age in different groups of gymnasts as compared with swimmers and school girls. Relations were found between the parameters of pubertal development and fatmass, body height and training activity from young age on has no direct influence on growth. The results of the animal experiment support these findings. Sexual maturation is shifted towards a later date in rats which are subjected to an intensive training program. No differences in growth patterns between the intensively, moderately and nontrained groups could be detected.

### Regulation of fuel supply to insect flight muscles

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Insect flight is an extremely energy-demanding phenomenon and constitutes an excellent model system for studying metabolic regulation during strong effort. Flight performance is accompanied with a high rate of oxygen consumption in consequence of intense substrate utilization. In the locust both carbohydrate and lipid can be used as flight muscle substrates, lipids providing energy particularly during prolonged flight. Fat, body glycogen and triacylglycerol stores are mobilized under the influence of adipokinetic hormone, which is released from the corpus cardiacum at an enhanced rate upon flight. The hormone stimulates synthesis of trehalose from glycogen and of diacylglycerols (DG) from triacylglycerols. Hormone-dependent elevation of fat body cAMP levels and cyclic nucleotide-induced activation of protein kinase have been demonstrated as well as activation of glycogen phosphorylase. Enhanced DG release causes a rapid elevation of hemolymph DG concentration. Elevated DG are taken up by flight-specific lipoproteins in the hemolymph. The dynamics of loading of DG-carrying lipoproteins in the locust demonstrates a unique remodelling system in which hemolymph (lipo)protein fractions, already existing in the resting state, and fat body DG give rise to new flight-specific lipoproteins, which carry the mobilized DG to the flight muscles. Components of these lipoproteins may facilitate receptor binding or lipoprotein activation.

### Transient morphological and biochemical changes in rat skeletal muscle after exercise

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To investigate the process of muscular degeneration after exercise and its time course, groups of 7 rats ran at submaximal intensity for 1 h on a treadmill. Each group was sacrificed at a different interval after exercise. The right hindlimb was fixated with buffered glutaraldehyde via an aortic canula. After fixation different muscles were dissected and prepared for light- and electron-microscopy. The left limb muscles were frozen in liquid freon and used for histochemistry and biochemistry. In 1–4% of type I fibers in soleus and vastus lateralis muscle and 0.5–1% of type II fibers in rectus femoris segmental degeneration was found, the length of the affected segment varying from 150 to 250  $\mu\text{m}$ . Degeneration was absent in gastrocnemius, tibialis anterior, extensor digitorum longus and biceps femoris muscle. Immediately after exercise signs of degeneration were only detectable at ultra structural level, while 2–3 h later the degeneration became visible at microscopic level. Degeneration and inflammation were most pronounced 24–48 h after running, where-upon regeneration occurred. Blood lactate concentration at rest and immediately after running were  $3.18 \pm 1.32 \text{ mmol} \cdot \text{l}^{-1}$  and  $3.22 \pm 1.45 \text{ mmol} \cdot \text{l}^{-1}$ , respectively. Muscle lactate concentration in soleus muscle of control animals was  $8.82 \pm 3.32 \text{ mmol} \cdot \text{kg}^{-1}$  dry weight, while immediately after and 24 h after exercise these values were  $8.59 \pm 2.71$  and  $16.63 \pm 4.68 \text{ mmol} \cdot \text{kg}^{-1}$  dry weight, respectively. The latter results are suggestive for a disturbance of microcirculation in the affected muscles.

### The clinical value of exercise testing in elderly patients

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Between 1978 and 1983, 545 exercise tests were performed by 338 males and 121 females above 64 years of age. This represents 7% of the total number of 8213 exercise tests. A history of myocardial infarction was present in 53% of the males and 34% of the females, while 12% of patients had previous heart surgery. Exercise was performed on a bicycle ergometer with stepwise workload increments of 10 or 20 W/min. In 28 patients the physician stopped the test because of serious arrhythmias or abnormal blood pressure response. The test was terminated because of fatigue (40%), angina (20%), dyspnea (20%) or leg fatigue including claudication (20%). Peak workload averaged 110 W in males and 80 W in females, which corresponds to 120% of the predicted normal values. Heart rate increased on average from 80 to 130 beats/min. ECG changes compatible with myocardial ischemia were observed in 49% of patients. Although elderly patients constitute a small fraction of the population referred for exercise testing, these findings indicate that the clinical value of the test when performed is similar to that in younger patients. The observation that most patients achieved higher than 'normal' workload may be due to selection by the referring physicians. On the other hand the reliability of the reference values should be questioned.

### Gas exchange via peritoneal perfusion

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Extracorporeal circulation with oxygenators is so far the only possibility to adequately oxygenate and decarboxylate patients with severe lung damage (ARDS or severe lung trauma). Extracorporeal circulation cannot be performed in acute situations in the majority of hospitals because the necessary equipment is

not present. In the present study experiments are described studying the possibilities to increase arterial oxygen partial pressures and decrease carbondioxyde partial pressures by peritoneal perfusion with oxygen transporting fluorocarbon solutions (fluosol DA, 20%). The perfusion is performed in rabbits using a bubble oxygenator and a circulation pump with a pump capacity of 25 ml fluosol p/min. Control measurements of blood gases were performed at different inspiratory oxygen concentrations between 0.16 and 0.5. The measurements were repeated during intraperitoneal perfusion with fluosol. Significant increases of arterial  $PO_2$  were measured at all different  $FIO_2$ 's ( $p < 0.05$ ). At the same time decreases of arterial  $PCO_2$  ( $p < 0.05$ ) were seen in those animals that were not hypoxic ( $paO_2 > 10$  kPa). It was interesting to see that oxygen was even excreted via the lungs in healthy animals that were ventilated with low oxygen concentration during peritoneal perfusion. In conclusion, the experiments have shown that peritoneal perfusion might be a method to increase blood oxygenation and decrease blood  $PCO_2$  in life endangering situations in cases of pulmonary failures.

### Comparison of chemoreflex gains from dynamic end-tidal $CO_2$ forcing and artificial medullary perfusion

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In experiments on 14 with chloralose-urethane anesthetized cats we combined Dynamic End-tidal Forcing (DEF) and Artificial Brainstem Perfusion (ABP) during normoxia ( $PETO_2$  15 kPa) and hypoxia ( $PETO_2$  8 kPa). The experiments were analyzed using a two-compartment model. In DEF the time course of the ventilation upon an isoxic step increase in  $PETCO_2$  of about 1 kPa during 7 min, followed by a step decrease to the initial value were analyzed. In ABP the blood gas tensions of the medulla (central) and the systemic circulation (peripheral) can be imposed independently. The peripheral ( $Sp$ ) and central ( $Sc$ )  $CO_2$  gains and the apneic threshold ( $B$ ) obtained with both techniques were subjected to multivariate analysis. They were not significantly different ( $p = 0.41$ ). Mean values for DEF and ABP with SD for the ratio  $Sp/(Sp+Sc)$  during normoxia were  $0.18 \pm 0.06$  and  $0.18 \pm 0.04$  and during hypoxia  $0.24 \pm 0.06$  and  $0.26 \pm 0.09$ . The corresponding values for  $B$  during normoxia were  $3.7 \pm 0.4$  kPa and  $3.7 \pm 0.5$  kPa and during hypoxia  $3.3 \pm 0.7$  kPa and  $2.8 \pm 0.8$  kPa. The agreement between the two techniques indicates that the DEF technique is very promising for separating central and peripheral  $CO_2$  effects on ventilation.

### Axial dispersion of gases in a model of the bronchial airways

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The longitudinal dispersion of helium (He), methane ( $CH_4$ ), acetylene ( $C_2H_2$ ), butane ( $C_4H_{10}$ ), sulphurhexafluoride ( $SF_6$ ) and octafluorocyclobutane ( $C_4F_8$ ) was measured in a plexy glass model of the human bronchial airways. The model consisted of seven symmetric airway generations (0–6). Length and inner diameter of the different branches of the model were chosen to be equal to those given by Weibel's model A. A constant inspiratory or expiratory bulk flow,  $\dot{V}$ , of room air was applied in the model. The selected values for  $\dot{V}$  were  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1 and 2 l/sec. A small bolus of the aforementioned tracer gases was injected within 1 msec into the bulk flow at the inlet of the model, and the bolus dispersion curve was measured at the outlet by means of the mass spectrometer. The shape of the bolus dispersion curve could be shown to be close to that of a Gaussian function. This allowed us, therefore, to characterize the shape of the bolus dispersion curve by means of the volume variance  $\sigma_v$ .  $\sigma_v$  increased (ca. 50%) between the lightest (He) and the heaviest ( $C_4F_8$ ) tracer gas used. For each tracer

gas, we observed no significant changes in  $\sigma_v$  for the applied expiratory bulk flows. On the contrary,  $\sigma_v$  decreased moderately between  $\dot{V} = \frac{1}{4}$  and  $\dot{V} = 2$  l/sec for inspiratory bulk flows. The overall means value of  $\sigma_v$  was 15.3 ml and 13.6 ml for inspiratory and expiratory bulk flows, respectively.

### The effects of unilateral frontal eye field lesions on eye-head coordination in monkeys

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Unilateral lesions of the frontal eye field in monkeys result in a temporary neglect of the visual field contralateral to the side of the lesion. Mainly from behavioral studies it has been concluded that recovery is complete within 1–2 weeks. Although the involvement of the FEF in eye movement control is apparent from anatomical and physiological data, its precise function still has to be established. Therefore we investigated the effects of unilateral FEF lesions upon eye-head coordination in 3 monkeys. The animals were trained in a computerized apparatus setup to perform a visual search task while eye and head movements were recorded with scleral search coils in a rotating magnetic field using phase angle detection. Immediately after the lesion the animals responded only to targets presented at the side ipsilateral to the lesion. After 1 week the animals gradually increased their behavioral responses towards the contralateral side. After 3 weeks the neglect on the behavioral task had disappeared. The coordination of eye and head in reorienting movements towards the side contralateral to the lesion was permanently changed. The animals seldomly displaced their eyes in the head more than  $20^\circ$  to the contralateral side, while amplitude and velocity of head movements were increased. It is concluded that the FEF plays an important role in the control of voluntary eye and head movements.

### The threshold of tilt discrimination in the rabbit

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Rabbits are able to discriminate vertical vs. oblique striations with an accuracy of  $7-8^\circ$  (M.W. van Hof, Behav. Brain Res. 4 (1982) 315–318). After bilateral removal of the occipital lobe the ability to discriminate striated patterns of different orientation is severely impaired (M.W. van Hof, J. van Hof-van Duin and J.F. Hobbelen, Behav. Brain Res. 9 (1983) 257–262). Up till now no satisfactory explanation of the threshold of tilt discrimination has been given in terms of trigger features of neurons in the occipital lobe (M.W. van Hof, Doc. Ophthalmol. Proc. Series, No. 30 (1981) 74–81). Since several categories of neurons in the visual system of the rabbit respond differently to moving and flickering targets, it could be useful in future electrophysiological experiments to have behavioral information on the rabbit's ability to discriminate stroboscopically illuminated patterns. It was found that stroboscopically illuminated striations (3 per sec) are readily discriminated by rabbits trained with continuously illuminated striations. However, the threshold was found to be in between  $10^\circ$  and  $15^\circ$ . This means that the absence of retinal image movement has only a quantitative effect on tilt discrimination.

### Study on the rheological behavior of blood platelets in vivo

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Because of their role in hemostasis and thrombosis blood platelets should come in contact with the vessel wall and with each other. Study of the flow behavior of platelets in a microvessel using intravital microscopy requires visualization and localization of the individual platelets. In anesthetized rabbits

visualization of platelets, flowing in mesenteric arterioles, was achieved by labeling the platelets in vivo with the fluorochrome acridine red. Using the peak intensity of their flashed fluorescence microscopic images as displayed on video the platelets could be localized objectively within a thin optical section. The median plane of a cylindric blood vessel represents its cross-sectional area because of rotational symmetry. Thus platelet concentration distribution, orientation and velocity profile were studied using only platelets located within a shallow section around this plane. Platelet concentration distribution was found to be non-uniform: the concentration decreased from the wall towards the vessel center. Branch points had a pronounced influence on the concentration distribution. In vessel segments without nearby branch points the average concentration near the wall was about twice that in the center. Platelet orientation is not random: they tend to align themselves with their equatorial plane parallel to the wall. The tendency to alignment increased from the vessel center towards the wall. In addition, evidence was found for tumbling of the platelets. In straight vessel segments platelet velocity profiles had the shape of a flattened parabola, both in systole and diastole, with maximum velocities ranging from 1 to 14 mm/sec.

## Response of vertebral and carotid blood flows to isocapnic hypoxia

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One of the mechanisms proposed to explain the depression of ventilation by central hypoxia is a change in cerebral blood flow. We therefore measured blood flows in one carotid and one vertebral artery of cats, anesthetized with chloralose-urethane, using perivascular electromagnetic flow sensors. During hyperoxia (PETO<sub>2</sub> 55 kPa) the mean carotid and vertebral blood flows were 20 and 2 ml·min<sup>-1</sup>, respectively. The percentage increase in flows upon lowering of the PETO<sub>2</sub> are summarized in the table. The time course of the blood flows following step-like changes in PETO<sub>2</sub> was analyzed with a first order model. For the carotid blood flow the mean time constant for steps into hypoxia was 25 sec and for steps out of hypoxia 18 sec. The corresponding values for the vertebral blood flow were 38 sec and 15 sec. The observed changes in blood flow can explain a major part of the ventilatory depression by central hypoxia.

PETO <sub>2</sub>	14 kPa	9 kPa	6.5 kPa	4.1 kPa
Carotid flow	4%*	17%*	28%*	74%*
Vertebral flow	3%	12%*	39%*	76%*

\*Significantly different from hyperoxic value (p < 0.05).

## Pulmonary extraction of dopamine in the conscious dog

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The pulmonary handling of dopamine was compared to that of adrenaline and noradrenaline in the conscious dog in order to study the intrapulmonary significance of the metabolism for the perfusion. 15 nmol of each catecholamine was injected into the inferior caval vein together with cardiogreen as intravascular indicator. Dilution curves of each substance were derived from plasma samples withdrawn from the pulmonary artery and the aorta. Adrenaline was extracted for 3% which is comparable to the accuracy of the assay. The extraction of noradrenaline and dopamine amounted resp. 13% and 34%. The extraction of dopamine was remarkable, the more so as dopamine is assumed in literature to be unaffected in the pulmonary circulation just as adrenaline. The extraction has to be ascribed to selective uptake of dopamine and noradrenaline

into the endothelial cells. Increased permeability of the capillaries can be excluded as the mechanism of removal from the pulmonary circulation, since each catecholamine appears in the aorta at the same time as cardiogreen. Inactivation within the vascular lumen can also be excluded. Circulating enzymes would metabolize adrenaline similarly as dopamine. A transient change in perfusion of the lung by shunting can be provoked by injection of adrenaline and noradrenaline, which is accompanied by a momentaneous decrease of p<sub>aO<sub>2</sub></sub>. Probably a similar effect of dopamine can be attenuated locally by pulmonary metabolism (F.W. van Schaik, G.M. van Heeswijk, J.M. den Hertog and G.H. Huisman, Pulmonary extraction of dopamine in the conscious dog, Arch. int. Physiol. Biochim. 91 (1983) 215-222).

## Skeletal muscle flow during endotoxin shock

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Endotoxin causes redistribution of the cardiac output. Since a large part of body mass is skeletal muscle (± 40%) changes in its perfusion could have marked effects on perfusion of other organs. We have studied the effects of endotoxin (*E. coli* endotoxin, 1.5 mg·kg<sup>-1</sup>) on muscle blood flow in forelimb, thorax, diaphragm and hindlimb (5 different muscles) and on blood flow in skin. We have used radioactive microspheres in 6 control and 6 endotoxin treated dogs before saline or endotoxin (at t = 0) and at t = 90 and 120 min. Flows in femoral artery and vein were also measured (electromagnetic flowtransducer). Immediately after endotoxin cardiac output, mean arterial pressure and flow in femoral artery and vein fell markedly followed by partial recovery at t = 30. Subsequently hemodynamic variables gradually deteriorated but flow in femoral artery and vein did not. The ratio of flow in femoral artery and vein decreased (by 63% at t = 120; p < 0.05). Apart from the diaphragm, flow to skeletal muscle did not change or increased after endotoxin but decreased in the control group. Percentage of the cardiac output to brachial, intercostal and hindlimb muscle and skin increased after endotoxin (by 163, 167, 111 and 120% at t = 120, respectively; p < 0.05). The 5 muscles of the hindlimb did not respond differently to endotoxin. In spite of diminished arterial flow (from 60 to 40 ml·min<sup>-1</sup>; at t = 120), skeletal muscle flow was thus maintained in the hindlimb probably due to closing of shunts and redistribution of blood away from bone.

## Generation and propagation of epileptiform activity in the hippocampal slice preparation

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For the investigation of epileptiform events in the hippocampal CA1 field, in-vitro slices of the guinea-pig were used. After adding 0.1 mmol 4-aminopyridine to the bathing medium, field potentials were recorded with an electrode array, consisting of 8 semi-microelectrodes at spacings of 0.1 mm. A comparison was made between the spontaneously occurring field potentials (SFP) in CA1 and those evoked by different inputs to the CA1 pyramidal cells, namely alveus, str. oriens and Schaffer collaterals. For this purpose the electrode array was placed in CA1, parallel to the axes for the pyramidal cells. The regularly occurring SEP's presented a similar distribution as the potentials evoked by stimulation of str. oriens or alveus of CA1, but differed from those evoked by stimulation of the Schaffer collaterals. This indicates that in CA1 SFP's are generated in a similar way as field potentials evoked by alveus or str. oriens stimulation. It was also found that SFP's are propagated from CA3 and CA1 at a velocity of 0.16-0.30 m/sec. Therefore pathways in alveus and str. oriens, connecting CA3 and CA1, may be important in propagating epileptiform activity. This was supported by experiments in which different pathways were sectioned.

## Single electrode voltage-clamp experiments on CA1 hippocampal cells in vitro

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The purpose of our study concerning the action of epileptogenic and anti-epileptogenic drugs is to investigate the role of ionic channels in the generation of epileptic activity. Because of the important role of the hippocampus in epilepsy, the hippocampal slice was considered an appropriate model system. However, since the conditions in the hippocampal slice are unfavorable for penetrating a single cell with 2 electrodes, a conventional voltage-clamp system cannot be used. An alternative is to use a single electrode voltage-clamp system, which alternatively measures membrane potential and injects current. We have developed such a system and its performance has first been tested on an electronic equivalent of a neuronal membrane, containing only passive elements. Under optimal conditions and using a 30-megohm electrode a stabilization time of 5 msec could be obtained. It was found possible to study membrane properties of CA1 cells at 35–37°C with relatively low impedance electrodes (40–60 megohms). Although still preliminary, the first experiments on slices treated with 4-aminopyridine suggested that the spontaneous epileptiform discharges induced by this drug are followed by long duration outward currents, whose nature is presently investigated.

## Potassium conductance kinetics in the node of Ranvier

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The kinetics of the voltage dependent potassium conductance (delayed rectifier) in the frog node of Ranvier were studied by means of voltage jump experiments. To eliminate effects of potassium ion accumulation either a double pulse technique or an isotonic KCl solution was used. The activation and deactivation of the potassium conductance can be characterized by a single time constant. However the magnitude of the time constant is quite different for activation and deactivation at the same test voltage. This invalidates the well-known Hodgkin-

Huxley (H.H.) description for the potassium conductance in normal Ringer solution. In isotonic KCl solution the kinetic analysis shows that a fast and a slow process underly constitutives approximately 75% of the overall potassium conductance. Its time constant has a different voltage dependency during activation and deactivation. Therefore in isotonic KCl solution the potassium conductance cannot be described in terms of the H.H. formalism. Description of activating and deactivating potassium currents according to two populations of H.H. channels gave good fits. However it was impossible to obtain acceptable fits of deactivating potassium currents, when the time constants were taken from fits of potassium activation at the same test voltage. This is inconsistent with the existence of two populations of Hodgkin-Huxley  $K^+$ -channels.

## The role of the dorsomedial hypothalamic nucleus in the control of food intake in rats

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The hypothalamic suprachiasmatic nucleus (SCN) is a major pacemaker for the circadian control of many forms of behavior. The SCN has a sparse but widespread efferent projection system with a major bundle extending periventricularly. In order to find out whether we could disrupt selectively the circadian control of one type of behavior only, an experiment was done in which electrolytic lesions of the dorsomedial hypothalamic nucleus (DMH) were made in 20 female rats while continuously recording locomotor activity, food intake, drinking, body temperature and urinary corticosterone. The results of this experiment revealed an undisturbed circadian rhythm in locomotor behavior, in body temperature and in urinary corticosterone. In all animals, however, the circadian pattern of the food intake disappeared. There were no signs of hypo- or hyperphagia, despite the fact that body weight was reduced up to 3 months after the lesion. Microiontophoretic application of kainic acid into the DMH resulted in a similar decrease in body weight. However, there was no disturbance in circadian pattern. We conclude that the DMH area is a specific link between the SCN and the food intake control center, containing at least two populations of neurones involved in the control of circadian rhythmicity as well as in the control of body weight.

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