5 lambs (2 acute, 3 chronic) the balloons were kept inflated, within the lateral ventricles, for a corresponding period of time without any modification of the pulse wave. At the end of the experiments, the animals were sacrificed under deep anesthesia, and then the brains were carefully examined and fixed with 10% formaldehyde solution and sectioned for checking hydrocephalus. Histological studies were also performed in order to analyze the ependyma, the periventricular parenchyma and the CSF-pathways.

Results. Acute experiments. In 2 lambs which received pulses 3 times higher than the normal ones for 3 h, an ipsilateral ventricular dilatation of light degree occurred. The septum pellucidum was always deviated contralaterally. Histologically, the ependyma appeared to be interrupted mostly at the level of the frontal horn. In the only lamb which underwent trial with pulses 5 times higher than the normal, in addition to the anatomical changes described above, necrotic lesions of the periventricular parenchyma were found. In each animal, the subarachnoid spaces were patent.

Chronic experiments. A bilateral ventricular dilatation occurred in all animals (pulses 2–3–4 times higher than the normal). The ventricle containing the pulsatile balloon was larger than the contralateral one; the septum pellucidum was deviated contralaterally or frayed (2 cases). The frontal (figure 3B) and, to a less degree, the occipital horns were mostly dilated. The ependyma was interrupted, especially at the level of maximal ventricular enlargement. In 2 animals there was a dilatation of the IIIrd and IVth ventricles and of the aqueduct. In all cases CSF-pathways were patent at the histological control.

Control experiments. Acute and chronic animals in which the balloons were kept inflated did not show significant ventricular dilatation (figure 3A). At the same time, the ependyma was found normal at the histological examination. When the pulsatile balloon was extraventricular, a necrotic cavity was the only histological finding related to the mass lesion of the balloon; no ventricular dilatation occurred and the ependyma layer was intact.

Discussion. Attention has recently been paid to the intraventricular pulse pressure in the genesis of hydrocephalus on the grounds of findings obtained by prolonged intraventricular CSF-pressure recordings in hydrocephalic patients[18,19]. Abnormal high pulse pressure, either in basal conditions or – more evidently – during physiological sleep, was well correlated with the positive results of the CSF-diversion procedures in patients affected by normotensive hydrocephalus[20,21].

High intraventricular CSF-pulsation without any significant change in the mean intraventricular pressure have been produced in the course of the present investigation. The results of our experiments stress the role of the intraventricular pulsations in the genesis of intraventricular dilatation. This view is supported by the following data:

1. Consistent recurrence of ventricular dilatation in animals which underwent artificial increase of CSF-pulse pressure.
2. Lack of ventricular dilatation in animals with extraventricular pulsating balloons or with intraventricular nonpulsating balloons acting only as a mass.
3. Obvious relationship of the degree of ventricular dilatation to the duration of application of artificial pulse.
4. Asymmetrical dilatation of the lateral ventricle submitted to artificial pulsations.
5. Larger dilatation of the ventricular regions which received the maximum impact of the pulse wave (frontal and, for a less extent, occipital horns).
6. Dilatation of the distal portion of the ventricular system in chronic experiments.
7. Absence of obstructive lesions in the CSF-pathways.

Furthermore, we stress the importance of the above-mentioned method in obtaining an original model of communicating hydrocephalus without producing impairment in the CSF-absorption and/or circulation in the peripheral subarachnoid spaces.

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False interpretation of membrane transport data due to osmotic volume changes of cells

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Summary. It is shown theoretically that in a nonsaturable transport system across a cell membrane, the kinetical analysis yields (erroneously) apparent saturation kinetics if osmotic volume changes of the cell occur and are not taken into account. Experimentally this is illustrated for the case of exit of glycerol from beef erythrocytes.

One of the criteria in current use for the recognition of carrier mediation in membrane transport is saturability, as indicated for instance by a negative abscissa intercept in the double reciprocal plot of rate versus concentration (Lineweaver-Burk plot).

This plot is reliable and valuable if certain requirements are met. They include the condition that what is used for the abscissa of the plot are reciprocal values of actual concentrations and not, as happens occasionally, of amounts of penetrating substance per cell which are taken to represent intracellular concentrations, implying that the cell volume is constant. If concentrations are involved that are sufficiently high to elicit osmotic water shifts and, thereby, changes of the cell volume, then the use of quantities, rather than true concentrations, gives rise to systematical errors. For example, a nonsaturable transport system falsely appears to be saturable or, in the case of a saturable system, the Michaelis constant evaluated from the plot can be substantially in error (too low). It is the purpose of this communication to deal with this type of error, both by quantitative discussion of the underlying kinetical equations and by experimental demonstration.

We consider the case of exit of a substance from a cell through a cell membrane devoid of pumping activity but allowing equilibration, into a large volume of external medium with concentration N of nonpenetrating substances and concentration zero of penetrating substrate. 3 cases will be compared:
a) Carrier mediated transport with Michaelis-Menten-kinetics at constant cell volume V = 1.
b) Transfer with linear kinetics (e.g. diffusion) at constant volume V = 1.

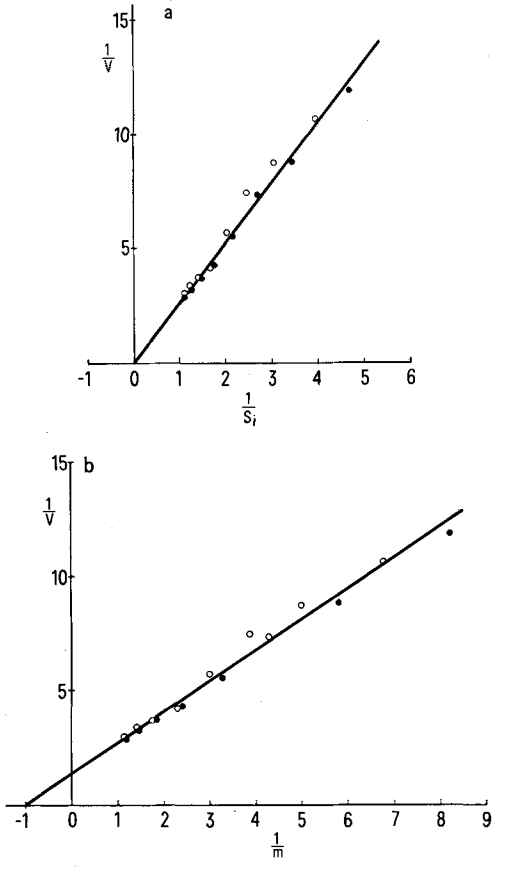

Fig. 1. Data from 2 experiments on exit of glycerol from beef erythrocytes at 37 °C, plotted according to equation 1a in *a* and according to equation 5 in *b*.

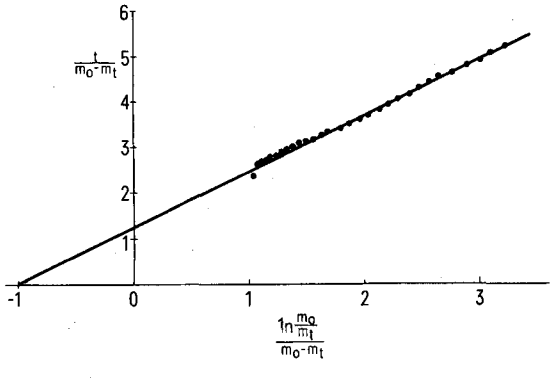

Fig. 2. Data from 6 experiments on exit of glycerol from beef red cells at 37 °C, plotted according to equation 6.

c) Transfer with linear kinetics (e.g. diffusion) with osmotic volume changes.
The following symbols will be used:

S_i = intracellular concentration of a substrate
m_t = intracellular amount of substrate at time t
m_0 = intracellular amount of substrate at time t = O
t = time
v = $-\,d_m/d_t$ = *rate of exit*
V = volume of the cell

Furthermore in case a:

v_{max} = maximal velocity of exit
k_m = Michaelis constant
'k_m' = apparent Michaelis constant (see text)
a = abscissa intercept in the plot of 1/v vs 1/m (see text)

In case b:

P = permeability constant of substrate

Units used will be cell units according to Jacobs (unit of concentration: isotonicity [300 m osm/l]; unit of volume: volume of intracellular water in a cell in its natural environment). The rate equation for case a is thus

$$v = v_{max} \frac{S_i}{S_i + k_m} \tag{1}$$

and can be written in the wellknown double reciprocal form

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{k_m}{v_{max}} \cdot \frac{1}{S_i} . \tag{1a}$$

For case b the rate equation is:

$$v = P \cdot S_i .$$

Since $S_i = m/V$ and since V = 1, in this case m and S_i are interchangeable, implying

$$v = P \cdot m .$$

For case c the rate equation will be likewise equation 2 which can be written

$$v = P \cdot \frac{m}{V} . \tag{3}$$

But here for V the condition for osmotic equilibrium holds:

$$\frac{m+1}{V} = N \text{ or } S_i = \frac{m}{V} = N \frac{m}{m+1} . \tag{4}$$

Combining equations 4 and 3 yields

$$v = PN \frac{m}{m+1} . \tag{5}$$

Comparison of equation 5 and equation 1 shows close formal similarity. If, in case c, the volume is erroneously considered as constant (V = 1, and therefore, m = S_i) evuation according to equation 1 will yield $v_{max} = P \cdot N$ and $k_m = 1$.
To test this conclusion experimentally, experiments were performed on beef red cells with 2 substances the penetration of which is known to follow linear kinetics and is generally assumed to occur by diffusion: glycerol (at 37 °C) and ethylene glycol (at 20 °C). Beef red cells were equilibrated with 1.0 isotonic solutions of these substances in PBS. (phosphate buffered saline) and then suspended in a 1000fold volume of substrate-free PBS. Exit from the cells was followed by recording light scattering[1] and evaluated by means of suitable calibrations to give V

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and S_i or m (from equation 4) as a function of t. Figure 1a shows the result of 2 experiments with glycerol in a double reciprocal plot of v versus S_i according to Lineweaver and Burk[2]. The abscissa intercept is near zero (as predicted) with a value of −0.009. The plot of 1/v versus 1/m (figure 2b), however, yields a negative abscissa intercept of −1.027 and gives a k_m-value of 0.974.

The evaluation according to Lineweaver and Burk requires the estimation of slopes, which decreases the accuracy. Therefore, 2 series of experiments were evaluated with the use of integrated equations. Equation 1a can, for the condition V = 1, be written

$$-\frac{dt}{dm} = \frac{1}{v_{max}}\left(1 + \frac{k_m}{m}\right) \tag{1b}$$

and integrated to give

$$\frac{t}{m_o - m_t} = \frac{1}{v_{max}} + \frac{k_m}{v_{max}} \cdot \frac{\ln (m_o/m_t)}{m_o - m_t} \tag{6}$$

which is formally similar to equation 1a, but can be used without estimating slopes. Figure 2 gives the results of a series of 6 experiments with glycerol at 37 °C evaluated using equation 6. A value of 0.980 ± 0.137 is obtained for the negative abscissa intercept. A similar series of 6 experiments with ethylenglycol at 20 °C yielded a negative intercept of 1.095 ± 0.26. Thus, the prediction made above in the discussion ef equation 5 is well substantiated. If the system under consideration actually is carrier-mediated, with a true Michaelis constant of k_m, then the apparent value of k_m (denoted as 'k_m') as obtained from a double reciprocal plot of 1/v vs 1/m will be lower than true k_m, indicating a false (too light) value for the affinity of substrate to carrier. (The error can be considerable, if the affinity is low, i.e. if k_m is high.) This emerges from substituting equation 4 into equation 1 to obtain

$$v = v_{max}\frac{(m/[m+1])\ N}{(m/[m+1])\ N + k_m} \tag{7}$$

and, in double reciprocal form (after rearranging)

$$\frac{1}{v} = \frac{1}{v_{max}}\left(1 + \frac{k_m}{N}\right) + \frac{k_m}{N\ v_{max}} \cdot \frac{1}{m}. \tag{8}$$

The abscissa intercept (which, in the Lineaver-Burk evaluation, represents $-1/k_m$), then is

$$a = -\frac{1}{'k_m'} = -\left((1 + \frac{N}{k_m}\right) \tag{9}$$

For N = 1 (e.g. isotonic saline), therefore, the value of $1/'k_m'$ is higher by 1.0 than $1/k_m$. The value of k_m, then, is

$$k_m = -\frac{N}{a+1}. \tag{10}$$

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Corticotropin and nonshivering thermogenesis

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Summary. Chronic treatment with corticotropin led to reduced calorigenic effect of norepinephrine in cold acclimatized rats, but potentiated its effect in controls. This inhibitory effect was not due to the observed decrease in corticosterone plasma level, as it was shown by metopirone administration. It is concluded that corticotropin could have a competitive action on receptor sites mediating the calorigenic effect of norepinephrine in nonshivering thermogenesis.

The occurrence of nonshivering thermogenesis (NST) in constant or fluctuating cold-acclimatised rats is related to a hypersensitivity to calorigenic effects of norepinephrine (NE)[2]. In these animals, the increase in calorigenic effect of NA may be related to an increase in lipolytic effect of NE[3,4]. Corticotropin is also known to have a lipolytic action in vivo[5] and in vitro[6,7].

The aim of this investigation was to study the dependence of NE calorigenic effect on corticotropin or corticosterone in rats acclimatized to different temperatures.

Materials and methods. Experiments were performed on Long-Evans male rats. Animals were acclimatized for 2 months to different thermic conditions: a control group was maintained at 28 °C (thermal neutrality), another group was acclimatized to a constant cold at 5 °C (CA). The last group was acclimatized to a nycthemeral fluctuating temperature from 5 °C to 28 °C (Cy). The enhancement of oxygen consumption by NE infusion (4 µg/kg during 15 min) was used as an estimation of NST[8]. Oxygen consumption ($\dot{V}O_2$) was measured at 25 °C using a Beckman analyzer. Infusions of NE were performed through the jugular vein on unanesthetized and unrestrained rats. Several days before experiments, jugular vein was catheterized. Plasma and adrenal corticosterone levels were estimated using fluorometric method of Silber et al.[9].

Results and discussion. When given by acute injection (10–20 IU/kg i.v. or i.p.) or infusions (5 IU/kg during 15 min), no effect of corticotropin (ACTH, Choay) on $\dot{V}O_2$ or on calorigenic effect of NE were observed. Chronic administration (2 IU/kg for 10 days) led to an increase in basal metabolism (20%) and calorigenic effect of NE (50%) in controls (table). In Cy group, basal metabolism was increased (25%) but calorigenic effect of NE was not changed. In CA group, basal metabolism was not changed, but calorigenic effect of NE was decreased (40%).

1 Grateful acknowledgment is expressed to Mrs M. Lopez for her technical assistance.

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