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Obituary Notice. The first named author of the following survey, Professor Dr. WERNER KUHN, died in Basel on the 27th of August, 1963, at the age of 64. Experientia has lost one of its most stimulating and many-sided natural scientific contributors (publications on questions of the physico-chemical constitution of the earth, on ageing, on the structure of the fiber-molecule, on contractility, on separation of substances, and other subjects), one, who to the highest degree, was capable of dealing with fundamental general biological problems and of establishing basic physical-chemical methods, such as artificial muscle fibers, artificial kidneys and artificial swimbladders, which have proved to be of particular value for the understanding of living processes.

H. M.

The Filling Mechanism of the Swimbladder

Generation of High Gas Pressures through Hairpin Countercurrent Multiplication

By W. Kuhn, A. Ramel, H. J. Kuhn, and E. Marti*

1. *Introduction.* Many species of fish possess a gas-filled swimbladder, and it is well known that the fish make use of this organ to control their buoyancy wherever they live, in shallow water at sea level or at great depth in the sea. Thus, in order to retain neutral buoyancy the total pressure of the gases inside the bladder must be 1 atm at sea level or, e.g., 100 atm when living at the depth of 1000 m. But the function of the bladder cannot be restricted to retention of neutral buoyancy alone; the pelagic fish in the top 1000 m frequently make extensive vertical migrations (KAMPA and BODEN[1]), and if they are to retain neutral buoyancy everywhere, they must secrete gas as they descend and reabsorb gas as they rise.

BIOT[2] noted that the swimbladder of fish caught at great depth must have contained gas at very high pressure and that the gas was almost pure oxygen. The first quantitative analyses of swimbladder gases obtained from deep-sea eels were reported by SCHLOESING and RICHARD[3]. A typical example of their results in a specimen caught at 900 m was: oxygen 75.1%, nitrogen 20.5%, carbon dioxide 3.1%, and argon 0.4%. Almost a century after BIOT's experiments, HÜFNER[4] communicated the striking discovery that the swimbladder of white fish (*Coregonus acronius*) from the bottom of Lake Constance at a depth of 60 to 80 m contained 99% nitrogen. Accordingly, the nitrogen tension in the bladder at the depth at which the fish was caught must have been 6 to 8 atm. Similar findings were reported by SAUNDERS[5], TAIT[6], SCHOLANDER, VAN DAM, and ENNS[7], who found virtually pure nitrogen at 10 atm pressure in the swimbladder of several species of fresh-water physostomes captured from the depths of Lake Huron and Lake Michigan. The swimbladder of cyprinids (goldfish), too, is normally filled with nitrogen. The purity of the gas is such that PRIESTLEY, when no nitrogen of comparable purity could be prepared, used to withdraw it from the swimbladder of the carp purchased at the market (quoted in DE FOURCROY[8]).

Recent investigations have confirmed and extended the earlier work. SCHOLANDER and VAN DAM[9] and SCHOLANDER[10] have studied gas taken from the swimbladders of fish living down to 1400 m. They included also analyses for argon. The general picture emerging from these and earlier studies is as follows:

(a) The deeper the fish live in the ocean, the greater the percentage of oxygen becomes; it often accounts for over 90% of total gas.

(b) In the special case of rather low depth (from sea-level to sometimes over 200 m), on the other hand, the nitrogen tension in many cases increases linearly with depth (SCHOLANDER[10] or DENTON[11]). Nitrogen in these cases appears to be secreted as a constant fraction of oxygen secretion. The ratio p_{N_2}/p_{O_2} characteristic for a certain species lies usually between 2 to 15%.

(c) The argon-nitrogen ratio lies reasonably close to 1.18×10^{-2}, the value found in air.

A notable exception to this general picture appear to be the aforementioned coregonids and cyprinids.

To complete our survey of the composition of swimbladder gases, we should recall also the very important experiments on direct stimulation of gas secretion (MOREAU[12]) and analyses of the freshly secreted gas of

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fish living in shallow water. (Deep sea fish are not accessible for such experimentation.) Fish can be induced to secrete gas in several quite different ways (MOREAU[12], JACOBS[13], AKITA[14], and FAENGE[15]). The new gas secreted in all cases was overwhelmingly oxygen. Carbon dioxide, although often found in appreciable amounts, was never the major constituent but varied from a negligible amount to a few percent. After secretion is complete, the fraction of nitrogen (and argon) rises slowly until finally nitrogen accounts for the major part of swimbladder gas. This is assumed to be the result of preferential reabsorption and exchange of oxygen and carbon dioxide between the swimbladder and the surrounding tissue, leaving nitrogen and argon behind. WITTENBERG[16] has made a remarkable further contribution to this type of investigation. With several species of fish, by carefully removing the residual contents of the bladder and collecting only the very freshly secreted gases, he could confirm in five cases the earlier findings that oxygen is the prominent constituent. Although the partial pressures of argon and nitrogen were low, their ratio was appreciably higher than in air, approaching 2.64×10^{-2}, a value reflecting the relatively higher solubility of argon in water. The relative proportions of helium and neon which are less soluble in water than nitrogen were correspondingly reduced. In two species of trout, however, the newly secreted gas consisted of 95–99% nitrogen, and the argon to nitrogen ratio was not different from that in air.

2. *Examination of Current Theories.* With these facts in mind we may examine the present status of theory about gas secretion into the swimbladder. For a detailed discussion the reader is referred to the excellent survey of DENTON[11]. We shall focus attention here only on points which are directly related to our paper or for which we believe a critical account is missing with respect to our concept. There are two crucial questions to answer by any theory which claims to solve the formidable task of explaining gas secretion, *where* and *how*. From MARSHALL's description of abyssal fish[17], there can be no doubt that gases must be brought into the swimbladder through the combined action of a glandular epithelium, the gas gland (MÜLLER[18]) and vascular structures, the retia mirabilia (DELAROCHE[19]). The observed correlation between functional requirements and the extent to which the retia mirabilia are developed in these fishes suggest that the rete must play an essential part in gas secretion. MARSHALL[17] describes in *Lionurus filicauda* six (!) retia each of 20 mm length, and in *Bassozetus taenia* two retia, each 25 mm long. The anatomical structure in the swimbladder associated with gas secretion in these cases must be the gas gland and the retia. Opinions are divided as to the individual contributions of these structures to the overall result of the secreting mechanism. This question, however, is so intimately related to the current ideas of their function that we may proceed to it without delay.

The net result of the process is a transport of gases, as different in their properties as oxygen, carbon dioxide, nitrogen, and argon, *against a high dissolved gas concentration gradient* between the ambient sea-water and the content of the swimbladder. It is therefore a *conspicuous example of an active transport,* that is, a transport requiring free energy for its maintenance. The main difficulty in explaining the operation of the swimbladder thus appears to be the *generation* of high partial pressures rather than the subsequent *maintenance* by compensation of relatively small diffusion losses. Although the latter itself is an astonishing example of biological engineering, this function was already early relegated to the rete, which consists of regular and intimate checkerboard-like intercalations of arterial and venous capillaries, running parallel, and thus constituting an efficient *countercurrent exchanger* (WOODLAND[20], HALDANE[21], HALL[22], JACOBS[23], SCHOLANDER and VAN DAM[24]). The function of the rete in this respect is often compared with an industrial heat exchanger (SCHOLANDER[10,25]). But this comparison, although accounting for one essential property under stationary conditions, misses *a second fundamental* capacity of the rete, *the building up of high pressures* toward the end of the rete, when operating as a *countercurrent multiplier.* An analogous thing, i.e., a preferential transport of heat toward the vertex, never happens in a heat exchanger. Thus the picture of the heat exchanger should be applied with caution. One is led astray if one limits the function of the rete by definition to a mere diffusion barrier, when actually it is meant to be the locus of pressure *generation.* This error occurs frequently in the literature. SCHOLANDER[10] was the first to apply to *this* problem the countercurrent multiplier successfully introduced into biology by KUHN and RYFFEL[26], by HARGITAY and KUHN[27], and further developed by KUHN and RAMEL[28]. But also SCHOLANDER in his pioneering attempt to apply the

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countercurrent multiplier principle to gas secretion, appears to have been unaware of this difference. He formulated the diffusion characteristics of the rete in two basic equations which in this form are strictly applicable only to the *stationary end-state*, in which the swimbladder *is filled* and no *further gases are secreted* (except for diffusion losses). Hence the equations should not be used, as is done later on, to discuss secretion rates.

Despite this inadequacy and the oversimplification in assuming a linear pressure gradient along the capillaries of the rete, when in reality an *exponential pressure function* should be considered (see section 3(c)), SCHOLANDER's paper is still a very valuable account of certain operational characteristics of the countercurrent multiplier system. Prerequisite for such a system to operate, besides a critical minimum of blood flow, is the existence of a minute *'single concentrating effect'*. A small relative elevation of the partial pressures in the efferent as compared to the afferent capillaries of the gases to be secreted would lead to a slightly elevated concentration in the afferent capillaries, if it is assumed that pressure equilibration between the capillaries is possible by diffusion. *This single concentrating effect could then be multiplied by an enormous factor through countercurrent multiplication.* Every substance which *reduces the solubility* of the inert gases in the venous capillaries and particularly *reduces the binding of oxygen* to blood hemoglobin, would be a candidate for this pressure elevation. It is obvious after what is said about the principle features of the countercurrent multiplier, that if this substance were known, a general theory accounting for the simultaneous secretion of all the gases mentioned could be presented. The most likely candidate is lactic acid, which is assumed to be added to the blood at the vertex of the retial capillaries through glandular action of the gas gland (COPELAND[29], FAENGE[15], BALL, STRITTMATTER, and COOPER[30]). Lactic acid would have the combined action of salting-out the physically dissolved gases (O_2, N_2, CO, Ar) and, through acidification of the blood and the Bohr and Root effect, would liberate CO_2 and O_2 from the hemoglobin, respectively. Experiments recently carried out *in vivo* with *Anguilla vulgaris* (KUHN, MOSER, and KUHN[31] and subsequently STEEN[32]) have shown that during the filling period of the swimbladder, and only during this period, the lactic acid content in the vein coming from the rete exceeds the concentration in the artery leading to it by 20 to 60 mg%. It will be seen that the excess lactic acid concentration found in the vein of the rete is in excellent agreement with the value of 45 mg% found to be sufficient for the eel, based on the Bohr and Root effects measured by SCHOLANDER and VAN DAM[24]. To be sure, we are fully aware of the fact that our proposition is by no means complete. Other metabolites not found yet (e.g. NH_4HCO_3) may have perhaps to be given a supporting role in the overall scheme. Salting-out effects were first proposed by KOCH[33] to account for the secretion of inert gases. HALDANE[21], HALL[22], JACOBS[23], and particularly SCHOLANDER and VAN DAM[24], are to be credited with the introduction of the Bohr and Root effects as a possible explanation for oxygen liberation. SCHOLANDER[10], however, was later led to reject the Bohr and Root effects as the basis of oxygen secretion. According to him the essential role of the rete would be a diffusion barrier. SCHOLANDER and VAN DAM[24], in their excellent study on oxygen dissociation of blood, have shown that the Bohr and Root effects at pH 6 were nullified at pressures above 50 atm in some fish which live and secrete oxygen at much greater depths, and therefore could not be the primary pressure reactions. It will be shown in section 4(b) that this is in reality no serious handicap.

In concluding this survey we should mention finally the interesting theories of WITTENBERG[16] and the WITTENBERGS[34]. In further developing the bubble theory of POWERS[35], WITTENBERG has given this theory a solid theoretical foundation. It is argued that secretion of inert gases is a *corollary* of the secretion of oxygen (even in fishes whose bladders contain only nitrogen), and that chemical work is necessary only for the latter process. It is suggested that oxygen is secreted in the form of minute bubbles and that inert gases diffuse into these bubbles during their formation. The oxygen bubbles then would carry with them the inert gases into the swimbladder. The composition of the gases freshly secreted into the swimbladder as predicted by his quantitative treatment is in fairly good agreement with his experimental findings (see above). We cannot follow, however, his reasoning in those cases (e.g. the trout) where his experiments failed to prove the theory. Most recent experiments on *Tinca tinca* (KROHN and PIIPER[36]) appear to support an *active* concentration of inert gases rather than a *passive* carrier mechanism in the case of the cyprinids and coregonids. We believe that a mechanism of inert gas secretion entirely based on the multiplier principle will adequately explain the detailed experimental findings (KUHN and KUHN, in press). The WITTENBERGS[34], although in favour of countercurrent multiplication for the secretion of oxygen, reject the oxyhemoglobin of blood as the immediate source of oxygen on grounds

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of their elegant experiments on simultaneous transport of CO and O_2. An *apparent* partition coefficient for a presumptive common carrier was calculated from the concentration of the gases administered and the gases secreted. It was found to be 5.4 over the entire concentration range investigated. By comparison of the proportions of carboxy- and oxyhemoglobin in the blood with the composition of the gases secreted, they were led to the conclusion that the gases could not have evolved directly from combination with blood hemoglobin. The suggestion was therefore advanced that cellular (active) oxygen transport from the efferent to the afferent capillaries, mediated by a common carrier (another hemoglobin), occurs in the rete mirabile, thus establishing the single concentrating effect required for countercurrent multiplication.

We believe that the experimental findings, though extremely interesting and valuable, do not conclusively ensure this interpretation. There is no reason for the expectation put forward in their paper that O_2 and CO would be secreted into the swimbladder according to the partition coefficient as defined by DOUGLAS and HALDANE[37]. This assumption would only be true if the complexes were decomposed quantitatively, or at least to the same relative extent in the gland near the vertex of the system. ROOT and GREEN[38] have pointed out that pH change in toadfish blood only affects the oxygen-binding capacity and practically not at all the carbon monoxide-binding capacity of this hemoglobin. This criticism does not invalidate the assumption that the apparent partition coefficient of the WITTENBERGS is expressing an intrinsic property of the secretory mechanism. Its interpretation, however, must await a careful mathematical analysis of the Bohr and Root effects on the oxygen and CO-dissociation of toadfish hemoglobin in connection with countercurrent multiplication. See also the remark at the end of section 4(b).

It is the aim of the present paper to elucidate in detail the function of a countercurrent multiplier, applied to both the *generation and the maintenance* of high gas pressures in the swimbladder. A certain minimum of mathematical formulae could not be avoided without impairing clarity. For a more rigorous treatment we refer to KUHN and KUHN[39].

3. *Countercurrent Multiplication in the Case of Nitrogen or Argon.* Countercurrent multiplication is easiest understood in considering first the concentrating of inert gases, e.g. nitrogen or argon. The countercurrent system of the rete (Figure 1) is schematically depicted in Figure 2. An afferent channel Λ_a and an efferent channel Λ_e, each of height a, length L, and width 1 cm (not shown in the Figure), correspond to the *afferent* and *efferent* limbs of the rete capillary system. They are separated from each other by a membrane, M, which is assumed to be *permeable for the nitrogen* (or argon) dissolved in the liquid, and *non-permeable for the solvent* (water) and for the salt. V designates the vertex (bend) of the capillary. B represents the bladder (or a manometer) in contact with V, and G the gland which may produce a defined quantity of electrolytes (e.g. NH_4HCO_3 or lactic acid) and add them to the blood passing through V.

The existence of a single concentrating effect, produced by the addition of salt to a nitrogen solution in water, will now be explained with the aid of Figure 3a, b, and c and the multiplication of it with the aid of Figure 3c–h.

Let the whole arrangement Figure 2 be filled, at the beginning of the experiment, with a solution of nitrogen of concentration c_0. This situation is represented in

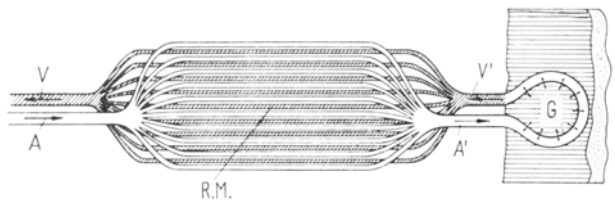

Fig. 1. Schematic drawing of the capillary rete mirabile (R.M.) and the gas gland (G) in relation to the swimbladder wall of the common eel. The blood derived from the A. coeliaca arrives through the A. ductus pneumatici which splits into two branches (A). (Only one is depicted on the diagram.) Each branch then divides into ~120,000 capillaries which run parallel for ~1 cm and assemble again to the artery A' leading to the gas gland G in the swimbladder wall (represented with exaggerated thickness). Here a second capillary network, coating the entire interior surface of the wall, is built up. The capillaries of the glandular network are collected by vein V', which forms a third capillary network of ~80,000 venous capillaries closely intermingled with the arterial capillaries of the incoming artery (A), constituting together the countercurrent system of the rete mirabile (R.M.) of which the eel has a set of two. The venous capillaries finally assemble into the vein V, which is connected with the portal vein leading to the liver.

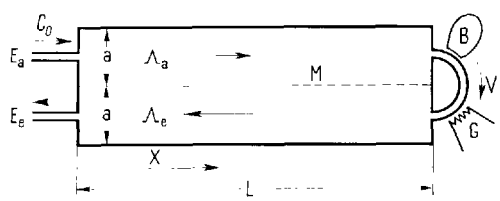

Fig. 2. The hairpin countercurrent system of the rete is schematically depicted. Channels Λ_a and Λ_e correspond to the afferent and efferent limbs of the retial capillaries. G, corresponding to the gas gland, is a dosage device through which either salt and/or, for example, lactic acid can be added to the blood passing at the vertex (V). M represents a membrane permeable for the gas, e.g. N_2 or O_2, and impermeable for the solvent (water) and the substances added through G. E_a and E_e are the places where the blood enters and leaves the system. The gases concentrated by countercurrent multiplication may be transferred to the bladder B in contact with V as soon as the particular partial pressure exceeds the one inside B.

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Figure 3a, the index 0 indicating the existence in all parts of the apparatus of the initial concentration c_0.

(a) *The single concentrating effect.* We now introduce fresh nitrogen solution of concentration c_0 through E_a and let the corresponding volume pass from Λ_a through V into Λ_e. A quantity of salt is added to the solution when it passes through V by means of the gland, which increases the salt concentration of the solution by a small amount Δc_s, leaving the nitrogen concentration (c_0) practically unchanged. At the end of this process, the solution in Λ_a contains nitrogen in the concentration c_0, the solution in Λ_e the same nitrogen concentration and, in addition to this, salt in the concentration Δc_s. This situation is represented in Figure 3b, where hatching indicates the presence of salt in Λ_e. It is known that salt generally decreases the solubility of gases in water.

Let A_a be the solubility coefficient of nitrogen in Λ_a; the partial nitrogen *pressure* p_a of the solution in Λ_a will then be related to the nitrogen *concentration* c_a in Λ_a by the well known equation

$$c_a = A_a \, p_a. \tag{3.1}$$

The solubility decrease in Λ_e produced by salt is expressed in terms of a different solubility coefficient A_e. It is

$$A_e = A_a \, (1 - \varepsilon \cdot \Delta c_s), \tag{3.2}$$

where ε is a parameter specific for the salt, but it is of similar magnitude for any electrolyte (e.g. sodium chloride, sodium bicarbonate or sodium lactate) if only inert gases are considered. It is mainly a consequence of the electric field produced by the ions in the solution.

If p_e is the partial nitrogen pressure of the solution in Λ_e of nitrogen concentration c_e, containing (relative to the solution in Λ_a) an additional salt concentration Δc_s, then, according to (3.2) and in analogy to (3.1)

$$c_e = A_a \, (1 - \varepsilon \cdot \Delta c_s) \cdot p_e. \tag{3.3}$$

using the abbreviation

$$\varepsilon \cdot \Delta c_s = \delta \tag{3.4}$$

and dividing (3.1) by (3.3), we obtain (for small values of δ):

$$\frac{c_a}{c_e} = \frac{p_a}{p_e} \, (1 + \delta) \cdot \tag{3.5}$$

Applying this equation to the situation represented in Figure 3b, where $c_a = c_e = c_0$, we realize that

$$p_e = p_a \, (1 + \delta) \tag{3.6}$$

(generally in the case where $c_a = c_e$), i.e. the nitrogen pressure in Λ_e exceeds the nitrogen pressure in Λ_a. In this case, and always if $p_e > p_a$, a transfer of nitrogen through the membrane will take place until the partial pressures of nitrogen in the gaseous phase above the solution in Λ_a and Λ_e will be equal. The equilibrium state, i.e. $p_a = p_e$ (the thermodynamic equilibrium with respect to nitrogen) will, again according to (3.5), be realized when

$$c_a = c_e \, (1 + \delta) \tag{3.7}$$

(equilibrium condition for $p_a = p_e$).

Notice that (3.7) is independent of the partial pressures and a function only of δ (which is related to Δc_s and ε through equation (3.4)). If Δc_s were 0.02 mol/l and taking ε for nitrogen in an aqueous solution of NaCl as 0.43 l/mol (Critical Tables, vol. III, p. 275) we get for δ a value of 0.0086. According to observations of STEEN (kindly communicated to us by sending the preprints of a publication to appear in Acta physiol. scand. *57* (1963)) there would exist a specific solubility decrease of nitrogen and argon by addition of small quantities of lactic acid to the blood of anguilla vulgaris. If this observation is correct, its effect would

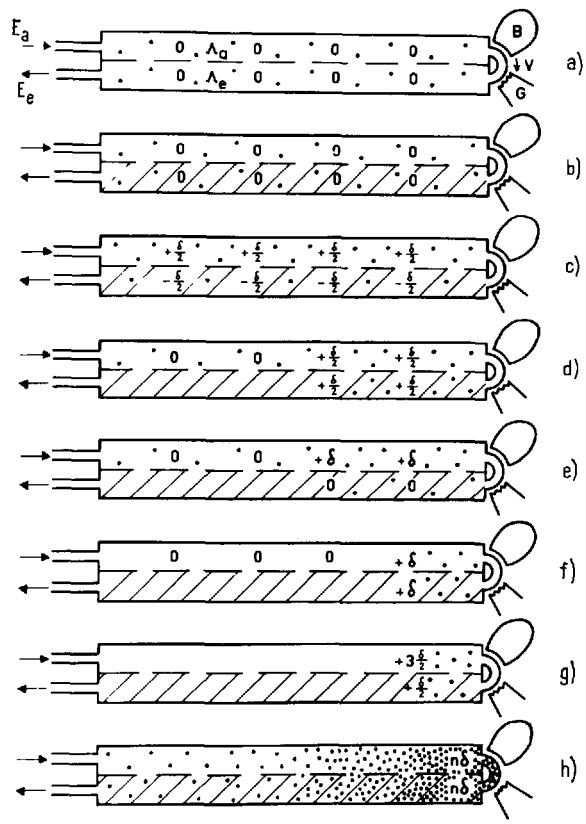

Fig. 3. The continuous process of countercurrent multiplication is split into a logical sequence of single steps, Figure 3a–Figure 3g, which lead to a progressive accumulation of N_2 or O_2 towards the vertex V of the system, and finally to a stationary end state as indicated in Figure 3h. The highest gas concentration and correspondingly the highest partial pressures are obtained at the vertex of the countercurrent multiplier. Hatching of the efferent limb indicates the existence of a salt increment Δc_s, or the presence of a small amount of lactic acid, which leads to a reduced physical solubility or liberation of chemically bound oxygen from hemoglobin. (For further description see text.)

formally be equivalent to a specifically high value of ε, valid for nitrogen and argon in the case of lactic acid addition to the blood. The difference Δc_s assumed of 0.02 mol/l would correspond to a shift by 10% of the normal electrolyte concentration in the blood of the deep-sea eel; e.g. a total hydrolysis of 60 mg% urea would give an NH_4HCO_3-concentration of about this value (0.02 mol/l). A δ of 0.0086 corresponds further to an increase of c_a by 0.86% over the value of c_e. *This concentration ratio moreover will be established independently of the absolute value of c_e as long as equation (3.2) holds, if only the system is given time to reach equilibrium. The equilibrium concentrating factor $(1+\delta)$ will be called the single concentrating effect* for nitrogen, produced by the salt concentration difference Δc_s.

In the case of Figure 3b the transfer of nitrogen from Λ_e to Λ_a will produce a decrease of the concentration in Λ_e and an increase in Λ_a in such a way that in the final state reached, issuing from Figure 3b we will everywhere in Λ_a have $c_a = c_0\,(1 + \delta/2)$, everywhere in Λ_e at the same time $c_e = c_0\,(1 - \delta/2)$, in accordance with equation (3.7). This state is indicated in Figure 3c by the indices $+\,\delta/2$ and $-\,\delta/2$.

(b) *Multiplication of the single concentrating effect.* In order to multiply the single concentrating effect let fresh solution with nitrogen concentration c_0 enter through E_a (Figure 3c) until the left half of Λ_a is restored while a corresponding volume of liquid passes through V from Λ_a to Λ_e. While it passes through V, the NaCl-concentration is again increased by Δc_s through action of the gland G. The situation thus reached is represented in Figure 3d. The nitrogen concentration now is $c_0\,(1 + \delta/2)$ in the right-hand part of Λ_a *and* of Λ_e, the solution in Λ_e containing NaCl in addition to nitrogen. This situation is unstable because $p_e > p_a$ according to (3.6). A transport of nitrogen will again take place from Λ_e to Λ_a until a new equilibrium state is reached with $c_a = c_0(1 + \delta)$ and $c_e = c_0$ in the right-hand part of the system, as indicated in Figure 3e.

After this equilibrium is established again, let some fresh liquid with the nitrogen concentration c_0 enter through E_a into Λ_a while liquid with a nitrogen concentration $c_0\,(1 + \delta)$ passes from Λ_a to Λ_e through V where its salt concentration is increased by Δc_s. This leads to the situation indicated in Figure 3f. Again we find at the right-hand end of the system equal concentrations of nitrogen (this time $c_0\,(1 + \delta)$) with a NaCl concentration Δc_s in Λ_e and none in Λ_a. This again leads to a transfer of nitrogen from Λ_e to Λ_a until, in this part of the system, the nitrogen concentration is $c_0\,(1 + 3\,\delta/2)$ in Λ_a and $c_0\,(1 + \delta/2)$ in Λ_e as indicated in Figure 3g. The continuation of this transport of liquid from Λ_a to Λ_e through V where its salt concentration is increased by Δc_s while its nitrogen content remains unaffected, obviously leads to a continuous increase of the nitrogen concentration and correspondingly according to equation (3.1) of the nitrogen partial pressure in the right-hand part, near the vertex of the hairpin countercurrent system (Figure 3h).

Another comprehensive argument for this establishment of a high nitrogen concentration near V results from examination of the nitrogen *transport balance* in the channels in the case of a *continuous flow* in the hairpin countercurrent arrangement. If $c_{a,x}$ and $c_{e,x}$ are the nitrogen concentrations in Λ_a and Λ_e at a distance x from the origin of the system (Figure 2), $c_{a,x}$ will (if equilibrium with respect to nitrogen is attained through M between adjacent parts of Λ_a and Λ_e) exceed $c_{e,x}$ by the factor $(1 + \delta)$ (equation (3.7). If the cross section of channel Λ_a (and of Λ_e) is $a \cdot 1$ cm^2 and if u (and $-u$) is the linear velocity in Λ_a (and in Λ_e), it is seen that the number of gram molecules of nitrogen which are in the unit of time transported to the right in channel Λ_a exceeds the number of gram molecules which are transported to the left in channel Λ_e. That is, at the point x

$$\frac{dn}{dt} = (c_{a,x} - c_{e,x})\,u \cdot a \qquad (3.8)$$

gram molecules are transported *in total* per unit of time towards the vertex of the system. This must, in the course of time, lead to an increase of the nitrogen concentration in the right-hand part of the system, as depicted in Figure 3h.

(c) *The stationary end state.* The accumulation of nitrogen at the right-hand side of the system (Figure 2 and 3) must, for two reasons, come to an end when $c_{a,L}$ at $x = L$ has reached a certain maximum value.

The first and main reason is due to the time requirement for attainment of the equilibrium condition (equation (3.7)). As we saw in the last paragraph, in order to render possible the gas accumulation towards the vertex of the system, nitrogen has to pass through the membrane M from Λ_e to Λ_a. If the flow rate were high, this transfer of nitrogen could not take place sufficiently rapidly, and the solution would leave Λ_e (at E_e) with the (nitrogen) concentration $c_{a,x=0}$. No multiplication of the single concentrating effect and not even its establishment could occur. Hence, the flow rate must be low in order to facilitate the transfer of nitrogen from Λ_e to Λ_a.

A second reason limiting the concentrating towards the vertex becomes important in the limit of very low flow rate. It is the diffusion of nitrogen in the x-direction (the direction of flow in Λ_a) of Figure 2 or 3 which in the case of solution at rest would abolish any concentration gradient. It can be shown (KUHN and KUHN[39]) that this second effect (back-diffusion in the x-direction) can be neglected in the case of the rete if the linear velocity of the blood in the capillaries exceeds a value of $u = 0.2$ cm sec^{-1}. The actual velocity in the rete appears to be in the vicinity of this value. A value only four times smaller than the actual value

would already lead to considerable back-diffusion and a corresponding decay of the multiplication effect.

The actual concentration distribution along the channels of the hairpin at stationary conditions (neglecting back-diffusion, which is permissible in case of the rete) will now be calculated. It follows from equation (3.8) that no further net transport of nitrogen towards (or away from) the vertex takes place if, for all values of x, the condition

$$c_{a,x} = c_{e,x} \tag{3.9}$$

(stationary end state condition) is fulfilled. Equation (3.9) is seen to be a necessary and also a sufficient criterion for the stationary end state.

From (3.9) in conjunction with (3.6) it follows that

$$p_{e,x} = p_{a,x}\,(1 + \delta) \tag{3.10}$$

(for $c_{a,x} = c_{e,x}$; in the stationary end state). It says that at stationary conditions corresponding to Figure 3h, the partial pressure of nitrogen everywhere in the efferent limb is higher by a factor of $(1 + \delta)$ than the nitrogen pressure in the afferent limb. The inevitable result is a steady flow of nitrogen across the membrane M everywhere from Λ_e to Λ_a.

The quantitative consideration of the nitrogen flow through M, due to the pressure difference (3.10), will now lead to a differential equation for the dependence of $c_{a,x}$ on x. We consider for this purpose a small section of channel Λ_a, extending from x to $x + dx$ (Figure 2). Let γ be the permeability coefficient of this membrane for nitrogen, that is, the amount of nitrogen passing through the membrane at a pressure difference of unity per unit time and unit area. The number of gram molecules of nitrogen entering our volume element per unit time, through membrane M, is then

$$dn = \gamma\,(p_{e,x} - p_{a,x})\,dx = \gamma\,p_{a,x}\,\delta\,dx. \tag{3.11}$$

It was stated in the consideration leading to equation (3.8) that the number of gram molecules of nitrogen which enter per unit of time, the volume element beginning at x *by flow*, will be $a\,u\,c_{a,x}$; the number of gram molecules leaving the volume element at $x + dx$ will similarly be equal to $a\,u\,c_{a,x+dx}$. If in the stationary state $c_{a,x}$ has to remain constant in time, the net number of gram molecules per second entering the volume element has to be zero. Considering the contributions resulting from diffusion through the membrane and from convection, we obtain

$$\gamma\,(p_{e,x} - p_{a,x})\,dx + u\,a\,(c_{a,x} - c_{a,x+dx}) = 0$$

or

$$\frac{dc_a}{dx} = \frac{\gamma}{u\,a}\,(p_{e,x} - p_{a,x}). \tag{3.11a}$$

Finally, expressing c_a and $p_{e,x}$ in terms of $p_{a,x}$ with equations (3.1) and (3.10), we have

$$\frac{dp_{a,x}}{dx} = \frac{\gamma\,\delta}{u\,a\,A_a}\,p_{a,x}. \tag{3.12}$$

This is the differential equation for stationary conditions. Integrating (3.12) between the limits $x = 0$ and $x = L$, we obtain for the partial pressure in channel Λ_a as a function of x

$$p_{a,x} = p_{a,x=0}\,e^{(\gamma\,\delta/u\,a\,A_a)\,x}. \tag{3.13}$$

Equation (3.13) can also be written in a form which reveals another aspect of its physical meaning.

Since γ, L, u, a and A_a are constant parameters for a given set of conditions they can be combined in one general constant, which entirely describes the effectiveness of a given countercurrent multiplier. Put

$$\frac{\gamma\,L}{u\,a\,A_0} = n_\omega \tag{3.13a}$$

and substitute in equation (3.13) to obtain

$$\frac{p_{a,L}}{p_{a,x=0}} = e^{n_\omega\,\delta}\,. \tag{3.13b}$$

The pressure at the end of the rete is expressed here as a function of δ, the parameter of the single concentrating effect obtained with a given concentration increment Δc_s. As δ is much smaller than unity e^δ can be developed in a power series. Taking only the first order term, e^δ becomes $1 + \delta$. It is recognized that this is the single concentrating effect for nitrogen produced by the salt concentration difference Δc_s. Combining these notations we have

$$\frac{p_{a,L}}{p_{a,x=0}} = (1 + \delta)^{n_\omega}. \tag{3.14}$$

The partial pressure increase produced in the countercurrent multiplier equals the increase which would be realized by an n_ω-fold repetition of the single concentrating effect. (Recognize the analogy to the number of plates in distillation.)

(d) *Numerical evaluation of the general pressure function (equation (3.13))*. It is of interest for practical reasons to evaluate equation (3.13) with the actual parameters of the rete of the eel (*Anguilla vulgaris*). The average length of the retial capillaries of this species is approximately 1 cm (DORN[40]). The linear velocity of the blood in the capillaries, u, is $\sim$0.2 cm sec^{-1}, and the average capillary diameter is reported to be $\sim 10^{-3}$ cm. As each afferent capillary in the countercurrent system Figure 1 is in contact with 4 or 6 efferent capillaries etc., the distance over which the exchange of gases (N_2 or O_2) between Λ_a and Λ_e has to take place will in effect be *smaller* than the capillary diameter. For this reason the value of a as defined by Figure 2 has formally been taken as $a = 5 \cdot 10^{-4}$ cm instead of 10^{-3} cm. The permeability factor can be estimated to have, for N_2 or O_2, a value of about $3 \cdot 10^{-14}$ mol

[40] E. DORN, Z. Zellforsch. *55*, 849 (1961).

dyne^{-1} sec^{-1} (KUHN and KUHN[39], p. 430). A_a is Henry's constant in the afferent capillaries; it is close to A_0, the value for pure water. If c_a is expressed in mol/cm^3 and p_a in dyne/cm^2, the value of A_0 for nitrogen from data in the literature is $0.77 \cdot 10^{-12}$ mol dyne^{-1} cm^{-1}.

With these parameters the multiplication factor $p_{a,L}/p_{a,x=0}$ and the pressure $p_{a,L}$ at the end of the rete can be calculated as a function of Δc_s, the increase of the salt concentration in the efferent relative to the afferent capillaries. It is convenient to perform this calculation by means of equation (3.14). The single concentrating factor $1 + \delta$ is obtained with the notation $\varepsilon \Delta c_s = \delta$ (equation (3.4)), and n_ω by means of equation (3.13) and (3.13a). In case of the eel ($L = 1$ cm) the value for n_ω becomes 390; with $L = 2$ cm, n_ω becomes 780 correspondingly. In Figure 4, $p_{a,x}$ is plotted on a logarithmic scale against x, the distance from origin of the retial capillaries. Three sets of conditions were selected corresponding to an increase Δc_s of the salt content by 1%, 10%, and 25% over the normal level of fish blood.

While the limit of osmotic resistance of the blood cells is restricting Δc_s to an upper value somewhere between 1% and 10% (physiological range), there is no such restriction evident for n_ω. The physiological adaptation to the special environmental conditions of the deep-sea during the evolutionary process most likely occurred by increasing the length of the retial capillaries. Hence, one expects according to this reasoning to find retia mirabilia of especially pronounced length in case of abyssal fish. That this in fact is true is convincingly evidenced by the length of the retia of the two abyssal fish mentioned in the introduction.

In order to demonstrate the sensitive dependence of the attainable pressures on the length of the capillaries with otherwise unchanged parameters we have extended the diagram (Figure 4) to a length of 2 cm. Notice that the factor 2 becomes part of the exponent in equation (3.13b).

(e) *Rate of filling.* The high values of the nitrogen pressure obtainable near the vertex of the retial capillaries according to equation (3.13) do not involve, as one might expect, a high rate of filling of the swimbladder with nitrogen. On the contrary, it is seen that the filling rate in case of nitrogen will be extremely low. If $\dot{q}$ is the total volume of blood passing the retial capillaries per unit time, the amount of nitrogen entering the rete at E_a is $\dot{q}\, c_{a,x=0}$ and the amount of nitrogen leaving the rete at E_e becomes $\dot{q}\, c_{e,x=0}$. With the most perfect arrangement it will just be possible to realize the equilibrium condition equation (3.7). The net transport of nitrogen towards the end of the rete thus becomes

$$\frac{dn}{dx} = \dot{q}\, c_{a,x=0} - \dot{q}\, c_{e,x=0} = \dot{q}\, c_{a,x=0}\, \delta \cdot \quad (3.15)$$

It is obvious that for a given rate of flow of blood at best only the fraction δ of the dissolved nitrogen which enters through E_a per unit time can be used to fill the swimbladder. If δ is small the rate of filling of the bladder will be correspondingly small; it could be increased by increasing δ; it cannot, however, be increased by increasing L, the length of the rete. An increase of L helps, according to equation (3.13) only to increase the vertex value of the nitrogen pressure $p_{a,x=L}$. The smallness of δ and the corresponding low rate of filling is an important feature of the addition of salt mechanism of the inert gas filling of the swimbladder. A swift filling with oxygen will be seen to be possible by application of the Bohr- and Root-effects (instead of or besides the solubility change by salt addition) for the production of the single concentrating effect in case of oxygen.

(f) *Experimental verification of salting out effect multiplication.* An experimental test of these conditions, i.e. of the possibility of preparing a high concentration (and partial pressure) of a dissolved gas by multiplication of a small salting out effect in a hairpin countercurrent multiplier, has been carried out recently in this laboratory in collaboration with E. MARTI and P. MOSER (KUHN[41]). In this experiment the gas brought from low to high concentration (and correspondingly high partial pressure) was CO_2, dissolved in water. A solution containing $c_0 = 9.6 \times 10^{-3}$ mole CO_2 per l was introduced through E_a into the afferent limb A_a of the system (scheme Figure 2). The temperature was 20° C throughout the experiment. The CO_2 partial pressure of the solution entering through

[41] W. KUHN, Naturw. *50*, 171 (1963).

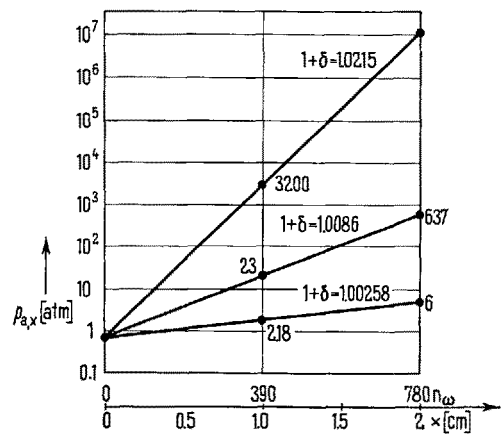

Fig. 4. Theoretical curves calculated with equation (3.14), showing the increase of N_2 partial pressure along the x-direction of Figure 2 at stationary end state. Logarithmic plot of $p_{a,x}$ versus x, the distance from the entrance for values of the single concentrating factor $1 + \delta = 1.00258$, 1.0086, 1.0215 as indicated. Other parameters: $p_{a\,x=0} = 0.8$ atm; $\gamma = 3 \times 10^{-14}$ mol dyne^{-1} sec^{-1}; $u = 0.2$ cm sec^{-1}; $a = 5 \times 10^{-4}$ cm; $A_a \simeq A_0 = 0.77 \times 10^{-12}$ mol dyne^{-1} sec^{-1}.

E_a was, according to the solubility coefficient of CO_2 in water and the CO_2 concentration mentioned, about 192 mm Hg; it was controlled and found constant in the course of the experiment (curve E_a of Figure 5).

At the vertex of the hairpin countercurrent system (near V in Figure 2) 3.4 g K_2SO_4 per 100 g solution was added to the liquid by means of a dosage device G. The partial pressure of CO_2 over an aqueous solution containing 9.6×10^{-3} mole CO_2 and at the same time 0.2 mole of K_2SO_4 (i.e. 34 g K_2SO_4) per l is, according to data in literature and also according to our own measurements, 213 mm Hg at 20°, i.e., 21 mm Hg higher than for a CO_2 solution of the same CO_2 concentration in pure water. This is indicated by the dotted horizontal S (single effect) in Figure 5.

The membrane M, separating the afferent and efferent limbs Λ_a and Λ_e (Figure 2) was in this experiment a rubber sheet, 1.8×10^{-2} cm thick, known to be permeable for CO_2 and impermeable for the solvent (water) and for the salt (K_2SO_4).

Limbs Λ_a (and Λ_e) were spiral channels each with depth $a = 0.1$ cm, width 0.5 cm, and length $L = 394$ cm, engraved in brass plates 25 cm in diameter (see HARGITAY and KUHN[27], Fig. 9, p. 551). Using the data given for the rubber membrane, the values found in literature for the solubility and diffusion coefficient of CO_2 in rubber, and taking into account the contribution of the water layer to the total diffusion resistance occurring in the CO_2 exchange between channel Λ_a and Λ_e, we have in this experiment a permeability coefficient $\gamma = 1.5 \cdot 10^{-13}$ mol dyne^{-1} sec^{-1}.

The CO_2 partial pressure near the vertex of the system (near B in the scheme of Figure 2) was measured with a manometer, indicating the CO_2 pressure in a small gas chamber (2 cm^3) which, *via* a rubber membrane, was in CO_2 exchange equilibrium with the solution passing through V (Figure 2).

The CO_2 pressure actually observed at the vertex of the system in the course of an experiment is shown as a function of time (abscissa) in curve B of Figure 5. K_2SO_4 is added to the liquid passing through V in the time interval 90 to 474 h. The pressure near B equals the pressure near E_a as long as no K_2SO_4 is administered (up to time 90 h). It starts rising as soon as K_2SO_4 is introduced. The rise of pressure is seen to exceed by far the value 213 mm Hg (horizontal S) corresponding to the single salting out effect. The possibility of multiplying the single salting out effect, produced by K_2SO_4 on CO_2 in water, is thus demonstrated. As CO_2 can be withdrawn from the system near B with a pressure of e.g. 385 mm Hg (at time 474 h) while the CO_2 pressure near E_a is 192 mm Hg only, the system is seen to bring about an active CO_2 transport, similar to the active transport materialized in the swimbladder.

The accumulation of CO_2 near the vertex is followed by a decrease down to the value of pressure near E_a as soon as the addition of K_2SO_4 is interrupted. This shows anew that it is the salting out effect which is the basis of the building up of high CO_2 pressures in the system. It can be mentioned that the rate of filling of the gas chamber (chamber plus manometer) near B in Figure 2 as well as the final pressure (to be extrapolated from curve B of Figure 5) is in good agreement with the formulae developed in the preceding paragraph and the numerical values of the parameters explained in this section.

As a further result obtained with this apparatus, we mention that nitrogen as well as a mixture of nitrogen and CO_2 has been subjected to the salting out effect of K_2SO_4 and its multiplication. As the solubilities and diffusion constants in rubber (and in water) are greatly different for N_2 and for CO_2, the γ-values (permeability parameters) are different too in a well defined and known way.

The introduction of these numerical values into the formulae and the experimental results show in agreement one with the other that the salting out effect and its multiplication of the CO_2 and the N_2 concentrations occurs simultaneously and independently, each with its own rate and final concentrating factor. It was found, for instance, that the final concentrating factor for the same salt addition was about 3.6 times higher for N_2 than for CO_2. This is of interest because it allows the general statement that the concentrating factor and the rate of its establishment can be fundamentally different for two gases exposed simultaneously to the same conditions in a hairpin countercurrent system. Attention is drawn to the point that, even in the simple case of the salting out problem, the final concentrating factor ($p_{a,L}/p_{a,x=0}$) according to equation

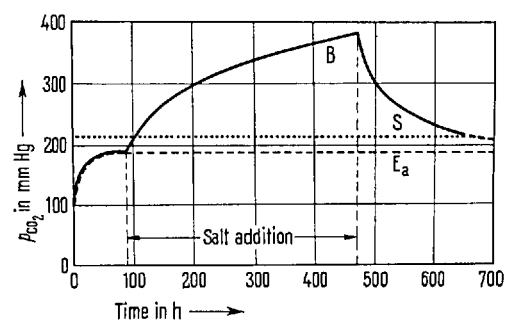

Fig. 5. Experimental countercurrent multiplication of a salting out effect produced by K_2SO_4 on CO_2 dissolved in water. CO_2-partial pressures as a function of time observed at the entrance E_a and the vertex (near B) of an experimental set-up corresponding to Figure 2. 0.2 mol/l K_2SO_4 were continuously added to a solution containing 9.6×10^{-3} mol CO_2 per l when passing through V (Figure 2) during the time interval marked 'salt addition'. Curve E_a (dashed line): CO_2-partial pressure at the entrance E_a of the system. – Curve B (solid line): CO_2-partial pressure measured at the vertex of the system (near B). The CO_2 pressure starts rising at $t \simeq 90$ h as soon as salt is added, and is declining again at $t = 474$ h when salt addition is interrupted. – Curve S (dotted line): CO_2-partial pressure increase from 192 to 213 mm Hg corresponding to single salting out effect of the K_2SO_4 added.

3.13b not only depends on the solubility coefficient A_0 but in addition to this also on the δ- and the γ-value of the gas considered. No comparative prediction should be made based on one of these parameters alone, the combination of all of them being decisive. A similar remark is valid in the case considered in the following section, where the single concentrating effect arises from a combination of the salting out effect with chemical equilibrium changes.

4. *Countercurrent Multiplication in the Case of Oxygen.* After having dealt in the former section with the simple case of *inert gas secretion* and having developed the framework of basic notations for an understanding of countercurrent multiplication, we shall proceed now to *oxygen secretion.* A swift nitrogen filling of the swimbladder is seen (equation 3.15) to be impaired by a low value of δ and, in addition, by a low carrier capacity $c_{a,x=0}$ of the blood for inert gases like nitrogen and argon, due to their low physical solubility. In the case of oxygen, nature has developed a transport mechanism of startling capacity through utilization of a specific oxygen carrier, the hemoglobin to which oxygen is *chemically* bound. The amount of oxygen bound to the hemoglobin of 1 cm³ blood at pH 7.6, room temperature, and 0.2 atm oxygen pressure is $\sim 10^{-5}$ mol cm^{-3}.

The amount physically dissolved under equal conditions is only 3×10^{-7} mol cm^{-3}, however. The binding capacity of the blood under these conditions thus exceeds the capacity for physical dissolution of oxygen (or the inert gases in general) by a factor 33 (!). The hemoglobin of fish blood furthermore bears another striking feature. Not only is the well-known Bohr effect (i.e. the displacement of the oxygen dissociation curve by carbon dioxide or acid) strongly pronounced in the case of fish blood hemoglobin, but there is also a *strongly pH-dependent binding capacity* observed (ROOT[42] and GREEN and ROOT[43]). (For a possible similar specific effect of pH-changes on the solubility in the blood of nitrogen and argon see STEEN[44]. In the case of the black grouper fish a change of carbon dioxide from 5% to 10% or a corresponding change of acidity will displace more than half of the oxygen combined as oxyhemoglobin, even against an oxygen pressure of 80 (or even 140) atm (SCHOLANDER and VAN DAM[24]). Although there are exceptions to these findings (e.g. in the case of the long-nosed eel where above 50 atm the Root effect is minute) one just cannot ignore this striking predisposition of the hemoglobin as a presumptive oxygen carrier for gas secretion into the swimbladder. Moreover, it will come out as a result of our quantitative analysis that the aforementioned implication (minute Root effect in certain fish at very elevated pressures) is no handicap for the generalizing concept put forth in this paragraph of hemoglobin as the oxygen carrier in gas secretion. This is accomplished by the assumption that a solubility displacement (salting out effect) as already demonstrated for nitrogen is, in the case of oxygen, supplementing the Bohr and eventual Root effect. This process, the salting out contribution, is not subjected to the former implications even at highest pressures. It will become predominent at pressures above ~ 50 atm, thus taking over further filling of the swimbladder. Below ~ 50 atm, however, the shape of the pressure function is mainly determined by the Bohr and Root effect.

(a) *The single concentrating effect in the case of oxygen.* The single concentrating effect in the case of oxygen, i.e. a difference at equilibrium between the oxygen concentration $c_{O_2,a}$ and $c_{O_2,e}$ in channel Λ_a and Λ_e of Figure 2, thus can arise through *cooperation of three* effects: (i) the salt effect on the solubility coefficient of oxygen in water, (ii) the Bohr effect, i.e. a shift of the equilibrium $Hb + O_2 \rightleftharpoons HbO_2$ through a decrease of the binding constant K, and finally, (iii) the Root effect, i.e. the reduced total binding capacity of the hemoglobin for oxygen. It is implicitly assumed that these three effects are *simultaneously* provoked by addition of a small amount of *lactic acid* and (eventually) of some *neutral salt* (sodium chloride or ammonium bicarbonate) to the blood when passing the vertex V of the countercurrent system of Figure 2.

The determination of the single concentrating effect as defined in paragraph 3.1 (equation (3.6)), and later of the pressure function $p_{O_2,a(x)}$ at stationary end-state conditions, requires the knowledge of $c_{O_2,a}$ and $c_{O_2,e}$, i.e. the total concentration of oxygen at every level x of channels Λ_a and Λ_e, respectively. The total concentration of oxygen c_{O_2} in either channel will be the sum of two terms:

$$c_{O_2} = c_{O_2,f} + c_{HbO_2}$$

where $c_{O_2,f}$ denotes the amount of oxygen *physically dissolved* per unit volume, and c_{HbO_2} the amount of oxygen *chemically bound* to hemoglobin per unit volume of blood. Let A_a be the solubility coefficient of oxygen in water, $p_{O_2,a}$ the partial pressure of oxygen in Λ_a, and K_a the equilibrium constant for the binding of oxygen to hemoglobin under conditions prevailing in channel Λ_a; A_e, $p_{O_2,e}$, K_e the corresponding expressions pertaining to channel Λ_e; and β the fraction of the total hemoglobin concentration c_{Hb} in Λ_e which remains accessible to oxygen binding after addition of a given amount of lactic acid (Root effect). Making use of equations (3.1) and (3.3) and the law of mass action for oxygen binding to hemoglobin, we obtain for $c_{O_2,a}$

[42] R. W. ROOT, Biol. Bull. Woods Hole *61*, 427 (1931).
[43] A. A. GREEN and R. W. ROOT, Biol. Bull. Woods Hole *64*, 383 (1933).
[44] J. B. STEEN, Acta physiol. scand. *57*, (1963).

and $c_{O_2,e}$, i.e. the total concentration of oxygen in A_a and A_e respectively,

$$c_{O_2,a} = A_a\, p_{O_2,a} \left\{ 1 + \frac{K_a\, c_{Hb}}{1 + A_a K_a p_{O_2,a}} \right\} \quad (4.1a)$$

and

$$c_{O_2,e} = A_e\, p_{O_2,e} \left\{ 1 + \frac{K_e\, c_{Hb}\, \beta}{1 + A_e K_e p_{O_2,e}} \right\} \quad (4.1b)$$

It is noticed that equations (4.1a) and (4.1b) are generalizations of equations (3.1) and (3.3) for the case of nitrogen to the case of oxygen where a salting out effect *and effects on the chemical binding* of oxygen to hemoglobin had to be considered. Indeed, for $c_{Hb} = 0$, identity of the corresponding parallel expressions would result.

In order to obtain the single concentrating effect we follow closely the considerations as outlined in paragraph 3.1. for the case of nitrogen. Transfer of oxygen between channel A_e and A_a through the membrane M will take place until at equilibrium the partial pressures of oxygen $p_{O_2,a}$ and $p_{O_2,e}$ (at corresponding values of x) will be equal. The ratio $c_{O_2,a}/c_{O_2,e}$ under this condition ($p_{O_2,a} = p_{O_2,e}$) is per definition called the single concentrating effect.

It is seen by executing this division that the single concentrating effect produced by salt and lactic acid addition *is not a constant*; it is dependent on the oxygen pressure realized. If p_{O_2} increases with x in Figure 2 or 3, the single concentrating effect will decrease and hence *will be itself a function of* x. From data recorded in literature we obtain for ε (salting out effect of NaCl for oxygen, equation (3.2)) a value of 0.4 l mol^{-1} (LANDOLT-BÖRNSTEIN, *Physikalisch-chemische Tabellen*, Hauptwerk I, p. 71; 5th ed.) and for the solubility coefficient of oxygen in water at 15°C, $A_0 = 1.53 \cdot 10^{-12}$ mol dyne^{-1} cm^{-1}.

If the neutral salt concentration in A_e is increased by $\Delta c_s = 0.02$ mol l^{-1} (as in the case for nitrogen) we shall have (in analogy to equation (3.2.)) $A_e = A_0 \cdot (1 - \varepsilon\, \Delta c_s) = 1.5177 \cdot 10^{-12}$ mol dyne^{-1} cm^{-1}. With these values, $\delta \simeq \varepsilon\, \Delta c_s$ becomes $8 \cdot 10^{-3}$.

The influence of the addition of lactic acid is mainly expressed in the change of K_a to K_e and in β. The information (SCHOLANDER and VAN DAM[24]) that at a partial pressure of 50 mm Hg about 50% of the total hemoglobin present is oxyhemoglobin, leads to a K_a of 1.0×10^7 cm^3 mol^{-1}, and again from data observed by the same authors we conclude that the addition of 5×10^{-3} mol of lactic acid per l brings about a decrease of the equilibrium constant by a factor of 4, i.e. from $K_a = 10^7$ to $K_e = 0.25 \times 10^7$ cm^3 mol^{-1} (Bohr effect).

The decrease of the oxygen-saturation capacity of hemoglobin was formally accounted for by the factor β. β is a constant smaller than unity and was estimated to be ~ 0.9 for addition of 5×10^{-3} mol per l of lactic acid (Root effect). Finally we take from the literature the value for c_{Hb}, the total hemoglobin content per unit volume of blood, to be 10^{-5} mol per cm^3 (FRY[45]).

Putting these parameters together and being interested primarily in the single concentrating effect *at the entrance* of the rete (at position E_a and E_e of Figure 2) where the initial oxygen pressure is 0.2 atm = 2×10^5 dyn cm^{-2}, we obtain from (4.1a) and (4.1b)

$$\frac{c_{O_2,a}}{c_{O_2,e}} = 1.876 \quad (4.2)$$

(in the case of oxygen exchange equilibrium at $x = 0$ and $p_{O_2} = 0.2$ atm). The total oxygen concentration at the entrance of A_a is (at equilibrium) *about twice* the concentration in A_e and the same value of x. Examination of formulae (4.1a) and (4.1b) reveals that this large difference is mainly due to the Bohr effect. If only the salt effect on the solubility were present (i.e. for $c_{Hb} = 0$) we should have independent of pressure and position

$$\frac{c_{O_2,a}}{c_{O_2,e}} = 1.008\,.$$

The concentration increment in A_a relative to A_e at the rete entrance at equilibrium is thus, when all three, Bohr, Root, and salting out effects, are considered, about 87.6/0.8 = 110 times greater than it would be if the salting out effect were active alone. We shall see in paragraph 4(d) that this is the reason for the swift filling rate of the swimbladder in the case of oxygen secretion.

(b) *Multiplication of the single concentrating effect and stationary end state in the case of oxygen.* The basic condition for countercurrent multiplication to occur was seen to be the existence of a single concentrating effect. As a result of this effect the total oxygen concentration at each level in A_a is increased relative to its value in A_e. If the solution is now put in motion, more oxygen is transported through A_a to the right than through A_e to the left, thus leading to an accumulation of oxygen towards the vertex of the countercurrent capillaries. Accumulation stops according to the reasoning of section 3(c) when, under the influence of convection, diffusion and gas transport through the membrane, equation (3.9) is fulfilled. It was shown that the moment this condition prevails at each cross section of the system, a pressure gradient has built up which becomes stationary. The stationary condition is expressed also in the case of oxygen by equation (3.9). This together with equations (4.1a) and (4.1b) allows an evaluation of the oxygen partial pressure $p_{O_2,e}$ in limb A_e and of

[45] F. A. FRY, *Physiology of Fishes* (Academic Press, New York 1957), vol. I, p. 87.

the oxygen partial pressure $p_{O_2,a}$ in limb A_a. The resulting differential equation for $p_{O_2,a}$ can be solved numerically. The reader who is interested in the details is referred to KUHN and KUHN[39], equation (29).

In this section we proceed with a discussion of the numerical results plotted in Figure 6. The relevant parameters were given in section 4(a) and can also be obtained from the legend to Figure 6. Oxygen pressures are plotted on a logarithmic scale against x, the distance from the entrance of the capillaries. Curve a) was calculated under the assumption of simultaneous cooperation of all three effects. In order to differentiate the individual contributions of each effect, three (other) curves are plotted. In curve b) the contribution of the Root effect was omitted ($\beta = 1$). Curves c) and d) illustrate the exclusive contribution due to Bohr or salting out effect, respectively.

Comparison of these curves reveals some striking features of oxygen concentration: in all cases where the Bohr effect is involved there is a steep increase of the pressure at the very beginning of the countercurrent capillaries. A small portion (0.1–0.2 cm) of their total length (1 cm) is sufficient to generate pressures between 10 and 100 atm. In this region the curves smoothly bend and attain a slope which is characteristic for a practically exclusive action of the salting out effect (compare with curve d). At higher pressures (above ~100 atm) further increase of the pressure becomes an exclusive contribution of the salting out effect. The last statement is of greatest significance with respect to the observations of SCHOLANDER and VAN DAM[24], who found the Bohr and Root effect to be nullified at this pressure in some fish which live at great depths. It is thus the answer promised in section 2 to this problem. It is furthermore noticed that the end-values of oxygen pressures which can be realized, despite the moderate conditions introduced, are much higher when Bohr and Root effect are involved; ~2000 atm in curve a), as compared to 4.29 atm in curve d) where only the salting out effect is considered.

The amount of 5×10^{-3} mol of lactic acid per l on which these calculations are based, was introduced in section 4(a) without further justification. In the mean time this value has obtained a solid experimental foundation through experiments carried out *in vivo* with *Anguilla vulgaris* by KUHN, MOSER, and KUHN[31] and also by STEEN[32]. They found that the lactic acid content in the vein coming from the rete exceeds the concentration in the artery leading to it of 2.23×10^{-3} to 6.7×10^{-3} mol/l.

In concluding this section it can be said that countercurrent multiplication of salting out, Bohr and Root effects on the inert gases and oxygen, respectively, either applied in combination or alone, can cover the whole hydrostatic pressure range known to be populated by fish. Although a great variety of observations reported in the introduction can be explained at the present status of the theory, it does, however, require further development in those cases where gas mixtures are secreted. Preliminary attempts on experimental and theoretical grounds have shown that this will be achieved by properly accounting for permeability differences between the gases involved and differences in the sensitivity of physical solubility and chemical binding constants to substances added at the rete vertex.

(c) *Rate of filling with oxygen.* The remark is justified that an excess lactic acid concentration of 5×10^{-3} mol/l in the efferent capillaries is necessary for the eel when living in the deep sea, where a pressure of, for example, 100 or 200 atm has to be built up, but unnecessary for the eel caught in fresh water where the total hydrostatic pressure is 1–2 atm. It is obvious that here a lactic acid concentration of 10 or 50 times less would be sufficient to generate the small pressure required. The situation is, however, different if the speed of filling is considered.

Equation (3.15) applied to the case for oxygen states that the number of gram molecules of oxygen which may per unit time be transferred to the bladder will at best be equal to

$$\frac{dn}{dt} = \dot{q}\left[c_{O_2,a,x=0} - c_{O_2,e,x=0}\right]. \qquad (4.3)$$

Now, with a lactic acid addition of 5×10^{-3} mol/l as assumed for the estimation of the parameters K_e and β, and for $p_{O_2,a} = 0.2$ atm (2×10^5 dyn cm^2), a condition approximately valid at the entrance ($x = 0$) of

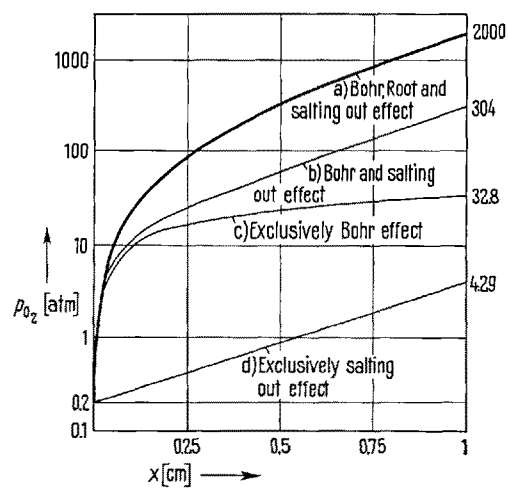

Fig. 6. Theoretical curves showing the increase of the O_2 partial pressure along the x-direction of Figure 2 at stationary end state. Logarithmic plot of $p_{O_2,a,x}$ versus x, the distance from the entrance. – Curve a): $K_a/K_e = 4$; $\beta = 0.9$; $1+\delta = 1.008$. Curve b): $K_a/K_e = 4$; $\beta = 1$; $1+\delta = 1.008$. Curve c): $K_a/K_e = 4$; $\beta = 1$; $1+\delta = 1.0$. Curve d): $K_a/K_e = 1$; $\beta = 1$; $1+\delta = 1.008$. – Other parameters: $p_{O_2,a,x=0} = 0.2$ atm; amount of lactic acid added 5×10^{-3} mol/l; amount of salt added 2×10^{-2} mol/l; $\gamma = 6 \times 10^{-14}$ mol dyne^{-1} sec^{-1}; $A_a \simeq A_0 = 1.5 \cdot 10^{-12}$ mol dyne^{-1} cm^{-1}; $u = 0.2$ cm sec^{-1}; $a = 5 \times 10^{-4}$ cm. Calculated with equation (29) (KUHN and KUHN[39]).

the rete of a fish living in fresh water saturated with atmospheric air, we obtained (equation 4.2) $c_{O_2,a} = c_{O_2,e} \cdot 1.876$. Introducing this into (4.3) we have

$$\frac{dn}{dt} = 0.47\, c_{O_2,a,x=0}\, \dot{q}. \qquad (4.4)$$

That is, 47% or half of the total oxygen content of the blood (sum of freely dissolved oxygen and oxygen bound to hemoglobin) entering the rete can be continuously transferred to the bladder. The pressure under which the gas has to be delivered is not important as long as it is smaller by a factor of 3 or 5 than the maximum pressure attainable at $x = L$ according to the foregoing section. This means: the eel living in the deep sea and the eel living in fresh water can by means of a lactic acid addition of, for example 5×10^{-3} mol/l transport half of the oxygen contained in the rete arterial blood into the swimbladder. If the eel living in the fresh water would reduce the lactic acid addition by a factor of 10 to, for example, 4.5 mg%, the value of $\ln K_a/K_e$ would presumably drop from 1.4 to 0.14; i.e. we would have $K_a = 1.0 \times 10^7$ and $K_e = 0.86 \times 10^7$, while $\beta \simeq 1$. The single concentrating factor $1 + \delta$ would then become ~ 1.04 under these conditions and dn/dt would be $0.04\, c_{O_2,a,x=0}\, \dot{q}$. The speed of filling of the swimbladder would, with a lactic acid addition of 4.5 mg%, be 12 times slower than with 45 mg%.

According to this consideration the addition of $\sim 5 \times 10^{-3}$ mol/l of lactic acid should be mainly required, in the case of the fresh water eel, for the sake of rapid filling.

It is obvious that a similar means of increasing the speed of filling, i.e. of increasing δ, does not exist in the case of nitrogen. This again seems to be the reason for the observed fact that species whose swimbladder is normally filled with a gas containing much more nitrogen than oxygen, after a loss of gas, first show a swift secretion of oxygen, which in the course of some days or some weeks is replaced by nitrogen.

5. *Possible Deviation from some of the Assumptions.* We have made the assumption, both in the scheme for nitrogen and for oxygen, that the membrane M separating channel A_a and A_e of Figure 2 or 3 was permeable for nitrogen (or oxygen), *impermeable* for salt and lactic acid, and impermeable also for water. It can be seen that the assumption as to the impermeability of the membrane for salt (and lactic acid) may be dropped to a considerable extent practically without any deleterious consequences for the single concentrating effect and its multiplication. If, for example, in the case of concentrating nitrogen the membrane M has some permeability for the salt, the addition of salt at the vertex (by the gland G in Figure 2 or 3) would result in a partial transfer of salt from A_e to A_a and thereby in a partial back transport of salt via A_a to the vertex. This process would, in the final stationary end state, result in an increase of the salt concentration towards the vertex, in such a way, however, that the difference Δc_s between the salt concentration in A_e and A_a remains unaltered, being determined by the volume of liquid passing and the amount of salt produced by the gland per unit of time. It has, however, been pointed out that the single concentrating effect is determined by Δc_s and, at least in a first approximation, is independent of the absolute value of c_s. This shows that a partial permeability of M would lead to a certain increase of c_s without a change in Δc_s towards the vertex of the rete, leaving the single concentrating effect and its multiplication unaffected in a first approximation. A corresponding statement holds for lactic acid when concentrating oxygen. It would be of interest to look for an increase of these concentrations and thereby an increase of the molality towards the vertex of the rete; the increase when realized would indicate a certain permeability of M for the salt (and for the lactic acid) which, however, would be of practically no importance for the filling of the bladder.

6. *Thermodynamic Efficiency of Filling the Swimbladder.* It has been pointed out in section 2, and it is evident also from the present discussion of the countercurrent multiplication, that the net result of the process is a transport of gases from a level of low partial pressure in sea water to very high partial pressures at the vertex of the system. It is well known that such a result will never be realized spontaneously; it needs the expenditure of a definite amount of mechanical energy (free energy). We shall now examine the thermodynamic efficiency of the process, i.e. we shall compare the free energy change corresponding to the transport of gas from low to high partial pressure with the mechanical energy actually introduced into the system per unit of time. The latter will be higher than the former, the ratio of the two values being the thermodynamic efficiency. While the mechanical energy corresponding to the gas transport is easily obtained from the amount of gas which is brought per sec from partial pressure $p_{a,x=0}$ at the entrance to $p_{a,L}$, the pressure at the vertex, where it is delivered to the bladder, a closer look at the details of the salting out mechanism is required in order to obtain the mechanical energy which is necessary per unit of time to keep the multiplier system going.

We notice that, when multiplying the salting out effect, a certain amount of salt has continuously to be added to the solution passing the vertex near V (Figure 2) in order to diminish the solubility of, for example, N_2 in A_e relative to A_a. The salt has thus to be added to a solution which contains a high concentration of dissolved N_2 while the solution leaving A_e through E_e has still the higher salt concentration but a very much lower N_2 concentration. It can be shown that the salting out effect produced by the salt on the gas is paralleled by a 'gassing out effect' of the gas

(N_2) on the salt, i.e. the dissolved gas increases the chemical potential of the salt in the solution. The interdependence is quantitatively expressed by Maxwell's cross-relations.

Thus, under stationary conditions, salt has to be brought continuously from the low activity existing at the exit of A_e to the high activity at the vertex. The mechanical energy required for this operation matches both the mechanical energy required for the filling of the bladder and the energy dissipated per unit of time when the dissolved gas passes from A_e to A_a through the membrane, being subsequently transported by convection in A_e and A_a, performing in total the multiplier circular motion described in section 3.2. In a case where the concentrating factor equals 5, the quotient of the free energy corresponding to the filling of the bladder and the mechanical energy actually spent for the salt transport will be about 40%; similar values were previously obtained for the performance of the kidney (KUHN and RAMEL[28]) and in distillation (KUHN, NARTEN, and PETERLI[46]).

If the O_2 concentration is considered, lactic acid instead of salt has to be added near V in Figure 2. In other respects the considerations leading to the thermodynamic efficiency are analogous for N_2 (salting out effect) and for O_2 (Bohr and Root effects). Here also the source of free energy stems from the difference between the chemical potentials of lactic acid added at the vertex and available at the exit of A_e.

Under conditions which are practically realized in the swimbladder, performance coefficients of 70% are in principle possible. Part of the free energy supplied to the system, here also, is lost in the partly irreversible multiplier circular transport process.

The result of this consideration shows that for the attainment of certain aims nature uses means and methods, which though charged with irreversibility, have quite a 'reasonable' efficiency. It is furthermore seen, in particular in the case of oxygen transportation, how nature makes use of chemical free energy to realize an active gas transport. The free energy of lactic acid in this case is converted into osmotic work, i.e. is utilized for gas compression without the use of piston and cylinder. It is understood that lactic acid leaving the multiplier system through E_e will be used in subsequent metabolic processes (e.g. in the liver) and that its chemical potential available for these processes is smaller than the chemical potential realized when this substance (lactic acid) was produced in the gland and added to the oxygen charged blood near V in Figure 2. It is this difference in the chemical potential under which lactic acid has to be produced in the gland, and under which it is available after leaving the countercurrent system, which 'pays' for the high pressure oxygen filling of the swimbladder.

7. *Relation to Active Transport Phenomena.* The net result obtained by the entire arrangement of the rete, the gland and the swimbladder is the secretion of oxygen and nitrogen into the swimbladder with a total pressure of, for example, 100 or 200 atm from a medium which is saturated with a total pressure of 1 atm of these gases only. The filling of the swimbladder thus requires (as mentioned in the introduction) a transport of these gases from a low to a high partial pressure. When considering this net result, we do not hesitate to state that an active transport, i.e. a transport against an existing concentration gradient, has been realized. As this net result has been explained in detail by production in the gland of some lactic acid from glycogen and possibly of some electrolyte (e.g. by hydrolysis of urea, etc.), it can be stated that we are here confronted by perhaps the first highly active transport phenomenon whose mechanism is described by fully controlled physical and chemical processes. The concentration of urea in the kidney is also produced by means of multiplication of a single concentrating effect in a hairpin countercurrent system. In the case of the kidney, however, the single concentrating effect (an active sodium transport through the Henle loop membrane) cannot yet be declared as being fully traced back to well known physical and chemical processes in spite of promising attempts to reach this goal.

Zusammenfassung. Es wird gezeigt, dass die Erzeugung der hohen Gasdrucke, welche in der Schwimmblase von Tiefseefischen beobachtet werden, durch *Vervielfachung* bekannter Konzentrier-Einzeleffekte in der durch das rete mirabile gegebenen Haarnadelgegenstromvorrichtung möglich ist.

Der Konzentrier-Einzeleffekt – eine kleine Erhöhung der Gaskonzentration in den afferenten relativ zu benachbarten efferenten Kapillarelementen – dürfte bei allen Gasen durch eine geringe Erhöhung der Elektrolytkonzentration (Aussalzwirkung) zustande kommen.

Im Falle von Sauerstoff überlagern sich der Aussalzwirkung bei Zusatz von Milchsäure die durch pH-Verschiebung bedingten Bohr- und Rooteffekte, wobei die Substanzen dem Blut am Scheitel des rete durch die dort befindliche Drüse zugefügt werden.

Auf Grund der vorliegenden Berechnungen, die sich auf die beim Aal gegebenen Verhältnisse stützen, würde eine relative Erhöhung des Salzgehaltes um 0,02 *M*/l im Falle von Stickstoff einen Einzeleffekt erzeugen, aus welchem infolge der durch das rete bewirkten Vervielfachung ein Partialdruck von etwa 25 Atm N_2 aufgebaut werden könnte.

Eine relative Erhöhung des Milchsäuregehaltes in den efferenten Kapillaren um 45 mg% (beim Aal *in*

[46] W. KUHN, A. NARTEN, and E. PETERLI, Helv. chim. Acta *40*, 1066 (1957).

vivo experimentell bestätigt) müsste für Sauerstoff einen Einzeleffekt hervorbringen, der bis zu Enddrucken von ~3000 Atm multipliziert werden kann. Die genaue Analyse lässt in diesem Fall erkennen, dass bei niedrigen Partialdrücken der Bohr- und Rooteffekt, und nach Erreichung hoher O_2-Drücke, der Aussalzeffekt den im Gegenstrom vervielfachten Einzeleffekt bildet.

Es wird ein Modellversuch beschrieben, bei welchem die Anreicherung eines Gases durch Vervielfachung des Aussalzeffektes in einer Gegenstromvorrichtung tatsächlich durchgeführt wurde.

Es wird weiter darauf hingewiesen, dass der Vorgang im Gesamteffekt einen *aktiven Transport* darstellt und es wird die Herkunft der für einen solchen Vorgang benötigten freien Energie durch eine thermodynamische Betrachtung aufgezeigt.

Im übrigen wurde versucht, die allgemeinen Prinzipien der Gegenstrom-Multiplikation verständlich zu machen, und bestehende Theorien über die Gaskonzentrierung in der Schwimmblase wurden im Lichte dieser Erkenntnisse einer sachlichen Diskussion und Kritik unterworfen.

Prinzipien cerebraler Organisation*

Von W. R. Hess**

Zur Einleitung. Um dem Leser, welcher mit der Physiologie des Zentralnervensystems nicht näher vertraut ist, die richtige Einstellung zum Titelthema zu vermitteln, ist es geboten, kurz auf Untersuchungen zurückzukommen, welche einen tieferliegenden Abschnitt des Gehirnes, den sogenannten Hirnstamm, betreffen (Hess[1]). Es hat sich um eine experimentelle Analyse der Funktion des sogenannten Zwischenhirnes und angrenzender Hirnabschnitte gehandelt. Dabei kamen unter anderem Symptome zum Vorschein, welche bestimmten Stimmungslagen des Versuchstieres (Katze) entsprechen. Sehr prägnant ist z. B. die Äusserung von Wut, wobei das Tier in direkter Abhängigkeit von elektrischer Reizung in einem genau umschriebenen Gebiet knurrt, faucht oder schneuzt und sich auch die Haare des Schwanzes und des Rückens sträuben. Nähert man sich dem Tier mit der Hand, so riskiert man einen gutgezielten Schlag gegen diese. Bei Fortsetzung des Reizes kann es sogar dazu kommen, dass die Katze eine in der Nähe stehende Person angreifend anspringt. Wer das ganze Bild ins Auge fasst, stellt eine genaue Übereinstimmung mit dem Verhalten fest, welches man zu sehen bekommt, wenn ein Hund auf eine Katze losgeht und diese sich mit Mut und in Wut zur Wehr setzt. – Bei Reizung an benachbarter Stelle reagiert das Tier hingegen mit ausgesprochenem Fresstrieb (Brügger[2]). Ferner kann in analoger Weise ein Verhalten produziert werden, wie es für Durst spezifisch ist. Eine im circumscripten Gebiet des Zwischenhirns gereizte Ziege begibt sich zu einem im Aufenthaltsraum befindlichen Wassereimer und trinkt solange, wie die Reizung dauert. Setzt man diese aus, so hört die Ziege auch mit dem Trinken auf, um bei neuem Reiz wieder zu beginnen. Andersson hat die Bedingungen dieser Wirkung noch eingehender studiert, wobei er Ziegen darauf dressierte, sich bei natürlichem Durst auf Umwegen aus einem gefüllten Gefäss Wasser zu beschaffen. Ein solches Tier reagiert nun auch auf die angelernte Weise, wenn es, obgleich wassergesättigt, im «Durstareal» des Zwischenhirns gereizt wird[3,4]. – Die genannten und noch anderweitige Erfahrungen normaler wie erlernter Verhaltensweisen als Antwort auf künstliche Reizung können nicht anders verstanden werden, als dass durch die elektrische Reizung je nach Angriffsort diese oder jene Gefühle, wie Wut, Hunger, Durst u. s. w. ausgelöst werden, d. h. so, wie durch eine physiologische Reizsituation, z. B. wenn das Bild eines angreifenden Hundes auf die Netzhäute der Augen geworfen wird bzw. der Zuckergehalt des Blutes unter ein gewisses Minimum absinkt oder die Salzkonzentration des Blutes durch grosse Wasserverluste einen bestimmten Schwellenwert überschreitet. Näher besehen sind diese Sachwerthalte sehr bemerkenswert; denn sie besagen nichts anderes, als dass die funktionsspezifischen Sinneszellen, welche das Triebverhalten steuern, nicht zwischen dem sogenannten adäquaten, d. h. dem physiologischen und dem elektrischen Reiz unterscheiden können. Entsprechend kommt es einfach darauf an, dass diese Kontrollorgane in Erregung versetzt werden. Massgebend für die Wirkung ist, wie

* Herrn Prof. A. v. Muralt bei Anlass seines 60. Geburtstages gewidmet.

** Zürich.

[1] W. R. Hess, *Die Methodik der lokalisierten Reizung und Ausschaltung subkortikaler Hirnabschnitte.* Beiträge zur Physiologie des Hirnstammes, I. Teil (Georg-Thieme-Verlag, Leipzig 1932); W. R. Hess, *Das Zwischenhirn, Syndrome, Lokalisationen, Funktionen.* Zweite, erweiterte Auflage (Benno Schwabe Verlag, Basel 1954). – R. W. Hunsperger, Helv. physiol. pharmacol. Acta *14*, 70 (1956).

[2] M. Brügger, Helv. physiol. pharmacol. Acta *1*, 183 (1943).

[3] B. Andersson, Acta physiol. Scand. *28*, 188 (1953).

[4] B. Andersson und S. M. McCann, Acta physiol. scand. *33*, 333 (1955).