of the multiplicity of possible impulse transmissions is to be activated in a concrete case, it is dependent upon the momentary position of the eye because the direction of pull of individual muscles is determined by the momentary position. A short examination of the functional *model of the organization of the oculomotor system* (represented in the 'Motor system as an organization problem', Biologisches Zentralblatt *61* (1941) 546–572, may give further details (fig. 3). S means the visual field with 5 points, B the gaze field with 9 points and M the single eye muscles with their directions of traction. The five spheres in the upper part of the model represent geometrically defined visual rays in the central receptor system. Each of these representatives is in relation with representatives of defined eye positions. The impulse transfer occurs only where visual impulse and position impulse coincide; thus the combination of forces which is adequate to the position of the eyes can be chosen and transferred to the oculomotor nuclei. In explaining his model, Hess said, 'there is strict geometric order of relations'. The spatial position of the representatives, however, is irrelevant: *the model is a quantitatively valid image of an order which manifests itself in performance.* How far the model can be translated into morphological details, is a question per se. W.R. Hess would certainly be very much pleased to see the results of modern morphologic and electrophysiologic research on the oculomotor system realized by the contributions of Akert, Henn and Büttner in Zurich or by the computer model of oculomotor function of Robinson in Baltimore.

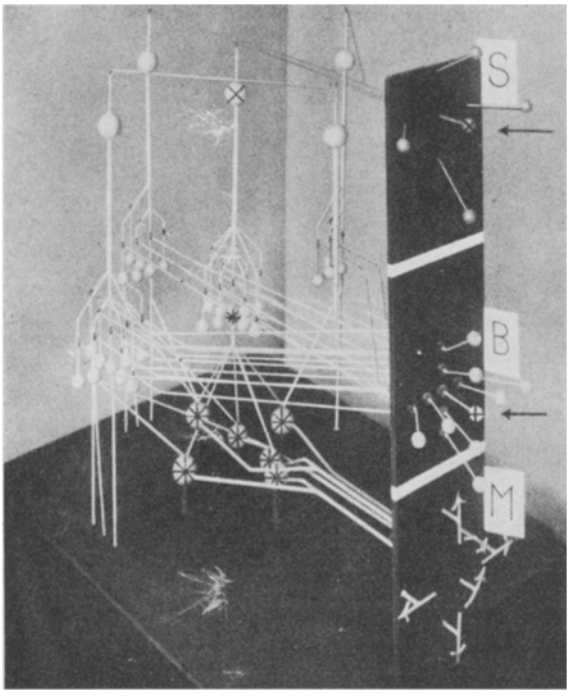

Figure 3.

It was highly fortunate that W.R. Hess – even for a short time – was an ophthalmologist. He has given ophthalmology not only an instrument of great practical value but also many original new insights into basic problems. In recognition of his merits the *Swiss Ophthalmological Society had the pleasure of bestowing on him honorary membership* in autumn 1971. The following words of Hermann v. Helmholtz seem to serve as a 'credo', summarizing the way of thinking and working of W.R. Hess: 'When examining the phenomena of nature it is necessary to think up to the end and not to stop half-way'.

Vascular smooth muscle: Intracellular aspects of adrenergic receptor contraction coupling

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For over three decades (1920–1950), W.R. Hess had been interested in the regulation of smooth muscle, because he recognized that smooth muscle in one of the principle 'effector-organs' of the autonomous nervous system.

I. Synergistic coordination of smooth muscle (Hess[14])

In 1946, v. Euler discovered that the sympathetic nerves exerted their influence on the contractile state of smooth muscle by releasing noradrenaline and adrenaline as transmitters[11]. Hess and his colleagues studied the effect of these substances on isolated smooth muscle. To Hess, it seemed quite puzzling that the same substance, e.g. adrenaline could contract one smooth muscle, while relaxing an other one. While the underlying mechanism for this diversity was not understood, it made, nevertheless, sense to Hess from a teleological point of view. Was it not to the advantage of a dog during 'fight or flight' – he asked – that the increased adrenaline levels in the blood should cause contraction of the spleen and the spleen vessels, while

causing relaxation[9] of the coronary arteries[14]? Another example of such synergistic coordination of different smooth muscles is pupillary mydriasis: Noradrenaline causes contraction of the dilator pupillae, while in the antagonistic muscle, the iris sphincter, it causes relaxation[24,15]. These different reactivities of smooth muscle to the same transmitter (e.g. noradrenaline) may be understood in terms of a functional coordination of the ergotropic-sympathetic effects. As to the possible mechanism accounting for such diversity, Hess[14] remarked: 'Since the same transmitter may cause contraction in one case and relaxation in an other one, one must assume that 1. there may be an intracellular switch mechanism within the smooth muscle cell controling contraction and relaxation, and 2. this intracellular switch must be influenced by certain specific structures of the cell.' What Hess meant by these structures, soon became known as α- and β-receptors[3,4]. Schaeppi and Koella[29] were able to show that the relaxing effect of adrenaline and noradrenaline on the cat iris sphincter is caused by stimulation of β-receptors, while the contracting effect of noradrenaline on the dilator pupillae is due to a stimulation of α-receptors[28]. However, the signal transmission from α- and β-receptors to the contractile machinery (receptor contraction coupling) and the nature of the intracellular controle system postulated by Hess is only now being elucidated.

II. α-Adrenergic activation

Contraction of all kinds of smooth muscles is associated with an increase in intracellular free Ca-ion concentration which activates the contractile machinery[23]; whereas in skeletal muscle, the Ca-ions activating contraction are exclusively released from the sarcoplasmic reticulum; other calcium stores, such as calcium binding sites near the cell membrane, may be also important in smooth muscle. For instance, noradrenaline reacting with α-receptors may induce contraction of arterial smooth muscle by releasing Ca-ions from these calcium sites, but in addition, it may also cause an increased Ca-influx into the cell[6]. The increased Ca-ion concentration then activates the contractile machinery and increases contractile force[12] and actomyosin ATPase activity[30,36]. The muscle fiber relaxes when the increased Ca-ions are taken up again by the sarcoplasmic reticulum[22].

The detailed mechanism by which Ca-ions activate the contractile machinery is only now being elucidated (fig. 1 A). There is now much evidence that the Ca-binding proteins in smooth muscle are different from those in skeletal muscle. In particular, Ca^{++} does not appear to react with troponin C as in the case of skeletal muscle, but with another Ca-binding protein, calmodulin[16]. The calcium-calmodulin-complex then activates a protein kinase known as myosin light chain kinase, since it specifically phosphorylates the 20,000 dalton light chain of smooth muscle myosin[7]. Once phosphorylated, the myosin can react with actin to form contractile linkages[1]. This interaction occurs in a cyclic fashion involving attachment, power stroke and detachment of crossbridges and the splitting of one molecule of ATP in each cyclic interaction. The crossbridge activity causes either force generation and/or shortening of the muscle fibers. Murphy and his colleagues have recently shown that in contracted smooth muscle, myosin is more phosphorylated than in relaxed smooth muscle[8]. The degree of phosphorylation and hence of contraction depends on the balance of the activities of kinase on the one hand and of myosin phosphatase on the other hand. For instance, calmodulin activates - at a given Ca-ion concentration - the myosin light chain kinase and, by doing so, it increases the degree of myosin phosphorylation and

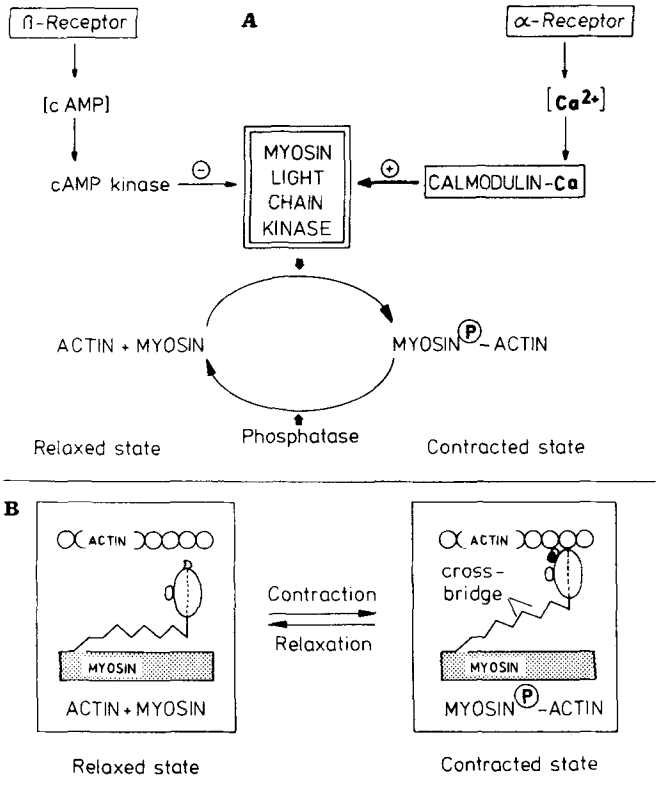

Figure 1. Pathway of α-adrenergic activating and β-adrenergic inhibitory mechanisms in vascular smooth muscle. *A* Adrenaline or noradrenaline may react with α-receptors, cause an increase in Ca-ion concentration leading to the formation of the calcium-calmodulin complex which, in turn, activates the myosin light chain kinase catalyzing the phosphorylation of myosin, a prerequisite of actin-myosin interaction.
Activation of the β-receptor causes β-adrenergic relaxation via stimulation of adenylate cyclase, increase in c-AMP level, activation of c-AMP dependent protein kinase causing phosphorylation and inhibition of myosin light chain kinase. Contraction is inhibited, relaxation occurs. *B* Interaction of myosin crossbridge with actin filament in the relaxed and the contracted state. Contraction occurs, when a myosin light chain (mol.wt 20,000 d) attached to the myosin head becomes phosphorylated by the myosin light chain kinase, thus allowing the attachment of the crossbridge to actin. Relaxation occurs, when this process is reversed by the action of a myosin phosphatase. (Further details see text and table)

force development of isolated contractile structures[37]. On the other hand, if the Ca-ion concentration is reduced, the myosin light chain kinase activity is inhibited, so that now, the balance between phosphorylation and dephosphorylation processes is shifted in the direction of dephosphorylation by the phosphatase: the muscle relaxes.

III. β-Adrenergic relaxation

In many types of smooth muscle including coronary arteries, relaxation induced by adrenaline via stimulation of β-receptors (fig. 1B) is associated with an increased activity of the cyclase and by an increase in cyclic AMP levels[5,32,18]. c-AMP mediates its effects through activation of c-AMP dependent protein kinase, and this activation involves the dissociation of the holoenzyme into its regulatory subunit and its (active) catalytic subunit[17,21]. The latter phosphorylates specific proteins. In the case of vascular smooth muscle, it was recently shown by Di Salvo et al.[35] that β-receptor stimulation by catecholamines leads to increased levels of the active form of c-AMP dependent protein kinase. Incubation of homogenates with activated c-AMP dependent protein kinase causes a phosphorylation of the myosin light chain kinase[34], a partial dephosphorylation of the myosin light chains and an inhibition of the actomyosin ATPases[19] and the contraction of isolated contractile structures[25,27].

Adelstein and Hathaway[2] have suggested a mechanism by which this c-AMP dependent inhibition could be brought about. According to this hypothesis, c-AMP dependent phosphorylation of the myosin light chain kinase would inhibit the interaction of Ca-ions and calmodulin with myosin kinase, thus inhibiting the enzyme. The consequence of this inhibition is that the balance between phosphorylating and dephosphorylating processes may be shifted in the direction of myosin dephosphorylation, and this would cause inhibition of actomyosin ATPase and contraction: the muscle relaxes.

Relaxation would, of course, also be induced if the kinase were inhibited by reducing the Ca-ion concentration. Indeed, it had been suggested that increased c-AMP levels during β-adrenergic relaxation may also stimulate the Ca-uptake by the sarcoplasmic reticulum and thus reduce the intracellular Ca-ion concentration[6].

In these schemes for the mechanism of β-adrenergic relaxation, it had been tentatively assumed that inhibition of myosin light chain kinase would lead to dephosphorylation and relaxation by the action of a myosin phosphatase; (the existence of the latter could recently be demonstrated in vascular smooth muscle, and it could also be shown that addition of this enzyme to isolated contractile structures promotes relaxation[26]. It is clear, however, that inhibition of light chain kinase activity and of contractile processes would not lead to relaxation under conditions in which the phosphatase were inhibited. In view of these possibilities, it is of great importance to find out how smooth muscle myosin phosphatase is regulated.

IV. A critical experiment

As mentioned already, β-adrenergic stimulation of vascular smooth muscle causes an increase in intracellular c-AMP level which, in turn, activates the c-AMP dependent protein kinase by releasing its catalytic subunit. We attempted to demonstrate that cyclic AMP or the catalytic subunit of c-AMP dependent protein kinase inhibits the contractile mechanism[25,27]; in order to do this, the contractile mechanism must be made accessable to these substances. This is achieved by chemically skinning the smooth muscle fibers, i.e. by removing the external membrane by treatment with the detergent triton-X-100. Such chemically skinned fibers are demembranated; even internal membranes, such as those of the sarcoplasmic reticulum are missing, but the fibers retain the structure of the contractile machinery. When suspended in solutions containing Mg-ATP, pH buffers and an ATP regenerating system as well as a very low Ca-ion concentration (below 10^{-8} M buffered with EGTA), these preparations are relaxed. For measurements of contractile responses, the preparations are mounted horizontally to a sensitive electromechanical transducer at one side and to a glass rod attached to an adjustable micromanipulator drive at the other side. When calcium is added to the relaxing solution to increase the free Ca-ion concentration to 1 μM, the preparation contracts and maintains tension, as long as the Ca-ion concentration remains increased. Relaxation is induced by lowering the free Ca-ion concentration again by immersion into relaxing solution. However, the fiber bundle can also be made to relax partially by the addition of cyclic AMP (0.01–0.1 mM). This relaxation is presumably due to the activation of c-AMP dependent protein kinase within the fiber bundle. In any case, a similar relaxing effect and inhibition of contraction is achieved by incubation of fibers with the catalytic subunit of c-AMP dependent protein kinase (fig. 2). While the in vivo action of c-AMP-mediated β-adrenergic relaxation may be partly ascribed to a stimulation of the Ca-reuptake by the sarcoplasmic reticulum followed by a lowering of the free Ca-ion concentration[6], such an action of c-AMP can be ruled out in the case of inhibition of skinned smooth muscle by c-AMP; the internal membrane systems, such as the sarcoplasmic reticulum, had been removed by triton treatment of the fiber bundles, and the Ca-ion concentration was kept constant by a strong buffering system (Ca-EGTA). In conclusion then, c-AMP or the catalytic

subunit of c-AMP dependent protein kinase must have inhibited the contractile mechanism directly, presumably by inhibiting the light chain kinase, as suggested by Adelstein and his colleagues[2]. As shown already, in living smooth muscle, this increase in c-AMP levels and activation of the c-AMP dependent protein kinase is brought about by catecholamines via β-adrenergic stimulation and activation of adenylate-cyclase. It would appear, therefore, that β-adrenergic stimulation inhibits contraction by lowering the sensitivity of contractile structures to the intracellular Ca-ion concentration rather than by lowering the Ca-ion concentration itself. Modulation of Ca-sensitivity by humoral factors, such as hormones, may be a new principle for long term modulation of contractile responsiveness of smooth muscle and changes in smooth muscle tone.

V. Energy costs of smooth muscle regulation

The synergistic coordination of vascular smooth muscle by adrenaline and nor-adrenaline would use up a great deal of our daily energy supply, if the rate of energy expenditure for contraction were as high as in skeletal muscle. Glück and Paul[13] have shown, however, that in vascular smooth muscle, the rate of energy expenditure is about 500 times smaller than in skeletal muscle. From the rate of oxygen consumption and from the aerobic lactate production, it could be calculated that 1 g arterial smooth muscle must split about 1.3 µM of ATP/min, in order to maintain maximum force (20 N/cm^2). This low rate of ATP splitting reflects a high 'holding economy' which can be fully accounted for by the low ATPase activity of smooth muscle actomyosin. Since the rate of ATP splitting by the actomyosin ATPase in 1 g artery is about 20×10^{-9} M/sec and the myosin content is about 20×10^{-9} M/g artery, it follows that the catalytic turnover number of the ATPase is about 1 sec^{-1}. If 1 molecule of ATP is split during a cycle of attachment and detachment of myosin crossbridges, it follows that it takes about 1 sec to split 1 molecule of substrate and just as long to undergo a crossbridge cycle of attachment and detachment. The low energy costs required for contraction of smooth muscle may therefore be largely due to the slow crossbridge cycling; partly, it is also due to the fact that smooth muscle is capable of maintaining similar tension as skeletal muscle, but about 10 times less myosin/g of tissue.

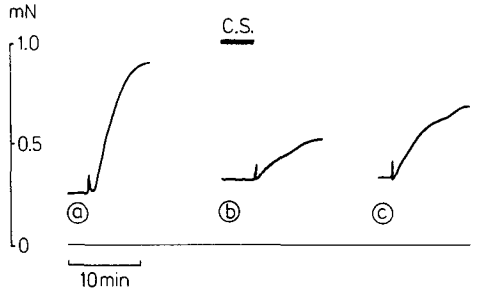

Figure 2. Isometric contraction of skinned (demembranated) vascular smooth muscle after increasing Ca-ion concentration to 0.2 µM Ca^{++} a) before, b) after incubation with 300 U/ml catalytic subunit (C.S.) of c-AMP dependent protein kinase, c) response after washing out catalytic subunit. Skinned vascular smooth muscle was suspended in ATP salt solution containing (mM): K^+ 21; Na^+ 36; Mg (total) 10; EGTA or Ca-EGTA 4; ATP 7.5; imidazole 20; Cl^- 35; azide 1; calmodulin 4 and an ATP regenerating system consisting of 10 mM phosphocreatine and 10 U/ml of creatine phosphokinase. pH of final solution was 6.7; in between contractions, fibers were relaxed in similar solution, where Ca-EGTA had been replaced by EGTA ($Ca^{++} < 10^{-8}$ M). T = 21 °C[25].

Because of the special features of smooth muscle, myosin, in particular its low ATPase activity causing a slow crossbridge cycle, smooth muscle is not only economical but also slow to contract and to relax[23]: During relaxation, crossbridges may detach only very slowly. This very feature may also contribute greatly to the holding economy of smooth muscle, since during relaxation, tension appears to be maintained without 'active state' and with very much less energy expenditure than during contraction[33]. It was this finding which suggested to Siegman[33] that tension during relaxation may be passive and due to non-cycling and non-ATP-splitting attached actin myosin cross linkages. Such a catch state could recently also be demonstrated in isolated contractile structures of skinned guinea-pig taenia coli[31], and it could be abolished by inorganic phosphate in concentrations actually occurring in smooth muscle.

In many ways, this state may resemble the catch state (Sperrtonus) of bivalves which are capable of keeping their valves closed for days without fatigue and with little energy expenditure[23].

VI. Intracellular coordination of smooth muscle

In conclusion then, during the last 30 years, there has been much progress in solving the puzzle about why

Scheme of control sequence in receptor contraction coupling of vascular smooth muscle following stimulation with adrenaline or noradrenaline

β-Adrenergic relaxation[2,34]	α-Adrenergic activation
1. Activation of β-receptor	1. Activation of α-receptor
2. Activation of adenylate cyclase and formation of c-AMP[32]	2. Opening of membrane Ca-channels[6]
3. Activation of c-AMP dependent protein kinase[35]	3. Ca^{++} reacts with calmodulin[16]
4. Kinase phosphorylates and inhibits myosin light chain kinase[34]	4. The Ca-calmodulin complex activates myosin light chain kinase[7]
5. Phosphorylation of the 20,000 dalton myosin light chain inhibited[34]	5. Myosin light chain kinase[7] phosphorylates the 20,000 dalton light chain of myosin
6. Because of inhibition of kinase, myosin becomes partly dephosphorylated by myosin phosphatase[34]	6. Phosphorylated myosin interacts with actin: activation of actomyosin ATPase, force generation[1]
7. Inhibition of actin myosin interaction: relaxation[27,25]	

the same transmitter substance (e.g. adrenaline) may cause contraction of smooth muscle in one case and relaxation in another one. It is now known that activation of α-receptors causes smooth muscle activation by releasing intracellular Ca-ions, activating the contractile machinery within the cell, while activation of β-receptors reduces contractile activity by lowering the responsiveness of the contractile machinery to the intracellular Ca-ion concentration or by reducing the latter (table). A disturbance of this subtle control mechanism might lead perhaps to certain diseased states caused by smooth muscle spasms, such as the spastic forms of coronary disease. It is necessary to point out, however, that the investigations of the intracellular switch of the contractile machinery postulated by Hess are still in an early phase, and there is a great lack of knowledge on such important aspects as the role of leiotonin[10] and of myosin phosphatases[26].

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1 Adelstein, R.S., and Eisenberg, E., Regulation and kinetics of the actin myosin interaction. A. Rev. Biochem. *49* (1980) 921-956.
2 Adelstein, R.S., and Hathaway, D.R., Role of Ca^{2+} and cyclic AMP in regulation smooth muscle contraction. Am. J. Cardiol. *44* (1979) 783-786.
3 Ahlquist, R.P., A study of a adrenotropic receptors. Am. J. Physiol. *153* (1948) 586-600.
4 Ahlquist, R.P., The adrenotropic receptor-detector. Archs int. Pharmacodyn. *139* (1962) 38-41.
5 Bär, H., Cyclic nuleotides and smooth muscle. Adv. cycl. Nucleot. Res. *4* (1974) 195-237.
6 Breemen, C. van, Aaronson, P., Loutzenhiser, R., and Meisheri, K., Ca^{2+} movements in smooth muscle. Chest, suppl. *78* (1980) 157-165.
7 Dabrowska, R., Sherry, J.M.F., Aromatorio, D.K., and Hartshorne, D.J., Modulator protein as a component of myosin light chain kinase from chicken gizzard. Biochemistry *17* (1978) 253-258.
8 Dillon, P.F., Aksoy, M.O., Driska, S.P., and Murphy, R.A., Myosin phosphorylation and the crossbridge cycle in arterial smooth muscle. Science *211* (1981) 495-497.
9 Ducret, S., Die Reaktion der Coronararterie auf Adrenalin. Pflügers Arch. *225* (1930) 680.
10 Ebashi, S., Regulation of muscle concentration. Proc. R. Soc. Lond. *207* (1980) 259-286.
11 Euler, U.S. von, A specific sympathicomimetic ergone in adrenergic nerve fibres (sympathicus) and its relation to adrenaline and noradrenaline. Acta physiol. scand. *12* (1946) 73-95.
12 Filo, R.B., Bohr, D.F., and Rüegg, J.C., Glycerinated skeletal and smooth muscle: Calcium and magnesium dependence. Science *147* (1965) 1581-1583.
13 Glück, E., and Paul, R.J., The aerobic metabolism of porcine-carotid artery and its relationship to isometric force. Pflügers Arch. *370* (1977) 9-18.
14 Hess, W.R., Die funktionelle Organisation des vegetativen Nervensystems. Benno Schwabe, Basel 1948.
15 Hess, W.R., and Koella, W., Experimentelle Studien über die antagonistische Innervation II. Z. exp. Med. *116* (1950) 431-443.
16 Klee, C.B., Crouch, T.H., and Richman, P.G., Calmodulin. A. Rev. Biochem. *49* (1980) 489-515.
17 Krebs, E.G., and Beavo, J.A., Phosphorylation of enzymes. A. Rev. Biochem. *48* (1979) 923-959.
18 Kukovetz, W.P., Pöch, G., Holzmann, S., Wurm, A., and Riner, J., Role of cyclic nucleotides in adenosinemediated regulation of coronary flow. Adv. cycl. Nucleot. Res. *9* (1978) 397-409.
19 Mrwa, U., Troschka, M., and Rüegg, J.C., cAMP-dependent inhibition of smooth muscle actomyosin. FEBS Lett. *107* (1979) 371-374.
20 Müller, E., and Breemen, C. van, Role of intracellular Ca^{2+}-sequestration in β-adrenergic relaxation of a smooth muscle. Nature *281* (1979) 682-683.
21 Nimmo, H.G., and Cohen, P., Hormonal control of protein phosphorylation. Adv. cycl. Nucleot. Res. *8* (1977) 145-266.
22 Raeymaekers, L., and Hasselbach, W., Ca^{2+} uptake, Ca^{2+}-ATPase activity, phosphoprotein formation and phosphate turnover in a microsomal fraction of smooth muscle. Eur. J. Biochem. *116* (1981) 373-378.
23 Rüegg, J.C., Smooth muscle tone. Physiol. Rev. *51* (1971) 201-248.
24 Rüegg, J.C., and Hess, W.R., Die Wirkung von Adrenalin, Nor-Adrenalin and Acetylcholin auf die isolierten Irismuskeln. Helv. physiol. Acta *11* (1953) 216-230.
25 Rüegg, J.C., and Paul, R.J., Vascular smooth muscle: calmodulin and cyclic AMP-dependent protein kinase alter calcium sensitivity in porcine carotid skinned fibers. Circulation Res. *50* (1982) 394-399.
26 Rüegg, J.C., Paul, R.J., and DiSalvo, J., A relaxing factor of vascular smooth muscle, a myosin phosphatase? Biochem. biophys. Res. Commun. *106* (1982) 1126-1133.
27 Rüegg, J.C., Sparrow, M.P., and Mrwa, U., Cyclic-AMP mediated relaxation of chemically skinned fibers of smooth muscle. Pflügers Arch. *390* (1981) 198-201.
28 Schaeppi, U., and Koella, W.P., Innervation of cat iris dilator. Am. J. Physiol. *207* (1964) 1411-1416.
29 Schaeppi, U., and Koella, W.P., Adrenergic innervation of cat iris sphincter. Am. J. Physiol. *207* (1964) 273-278.
30 Schirmer, H., Die Besonderheiten des kontraktilen Proteins der Arterien. Biochem. Z. *343* (1965) 269-282.
31 Schneider, M., Sparrow, M.P., and Rüegg, J.C., Inorganic phosphate promotes relaxation of chemically skinned smooth muscle of guinea-pig taenia coli. Experientia *37* (1981) 980-982.
32 Seidel, C.L., Schnarr, R.L., and Sparks, H.V., Coronary artery: cyclic AMP content during adrenergic receptor stimulation. Am. J. Physiol. *229* (1975) 265-274.
33 Siegmann, M.J., Butler, Th.M., Moers, S.U., and Davies, R.E., Chemical energetics of force development, force maintenance and relaxation in mammalian smooth muscle. J. gen. Physiol. *76* (1980) 609-629.
34 Silver, P.J., and DiSalvo, J., Adenosine 3'-5'monophosphate mediated inhibition of myosin light chain phosphorylase in bovine aortic actomyosin. J. biol. Chem. *254* (1979) 9951-9954.
35 Silver, P.J., Schmidt-Silver, C., and DiSalvo, J., β-Adrenergic relaxation and cAMP kinase activation in coronary arterial smooth muscle. Am. J. Physiol. *242* (1982) H177-H184.
36 Sparrow, M.P., Maxwell, L.C., Rüegg, J.C., and Bohr, D.F., Preparation and properties of a calcium ion-sensitive actomyosin from vascular smooth muscle. Am. J. Physiol. *219* (1970) 1366-1372.
37 Sparrow, M.P., Mrwa, U., Hofmann, F., and Rüegg, J.C., Calmodulin is essential for smooth muscle contraction. FEBS Lett. *125* (1981) 141-145.