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# Actovegin®: a biological drug for more than 5 decades

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## Actovegin®: ein Biologikum seit mehr als 5 Dekaden

**Zusammenfassung.** Actovegin® ist ein biologisches Pharmakon, das von einer natürlichen Quelle stammt: es handelt sich um ein Kälberblut-Hämidialysat. Seine therapeutischen Vorzüge rühren von unterschiedlichen pharmakodynamischen Aktivitäten her, die eine gemeinsame Zielrichtung verfolgen, nämlich die Verbesserung des zellulären Stoffwechsels. Dies resultiert von einer Insulin-artigen Aktivität die von Inositol-phospho-oligosaccharid en mediiert wird. Actovegin® erzielt günstige Effekte in verschiedenen pathophysiologischen klinischen Situationen; hierzu zählen Fehlfunktionen der Blutzirkulation und verwandter Erkrankungen, Hauttransplantationen sowie akute und chronische Wunden. In diesem Übersichtsartikel fassen wir die unterschiedlichen pharmakodynamischen Wirkungen des Kälberblut-Hemidialysats und seine wesentlichen klinischen Wirkungen zusammen.

**Schlüsselwörter:** Actovegin®, Hämodialysat, Insulin-artige Aktivität

**Summary.** Actovegin® is a biological drug manufactured from a natural source: it is a calf blood hemodialysate. Its therapeutic benefits stem from a variety of pharmacodynamic actions that can be summarized to a common goal, i.e. the enhancement of cellular metabolism; this results from an insulin-like activity mediated by Inositol-phospho-oligosaccharides. Actovegin® results in beneficial effects in several pathophysiological clinical settings including malfunction of the blood circulation and trophic disturbances in the brain, impairment of peripheral blood circulation and associated diseases, dermal transplants and acute and chronic wounds. Here, we give an overview of the pharmacodynamic actions of calf-blood hemidialysate and its beneficial effects in a variety of clinical settings.

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**Key words:** Actovegin®, hemodialysate, insulin-like activity

## Introduction

Biological drugs dominate the pipelines of pharmaceutical drug development. However, not only biological products under current clinical development deserve our attention but also concepts and drugs that exist for a reasonably longer time. Among these, the concept of vaccination has been developed in western medicine some 200 years ago in an unequalled success story, regardless of potential deficits and side effects: the victories are numerous (e.g. the erasement of pox after the installation of the world-wide vaccination program in the late 50s by the World Health Organization). Furthermore, emerging concepts drive the use of vaccination against a variety of cancer types, although these therapies have to evolve from a "proof-of-principle" stage to a "proof-of-efficacy" stage. Other biological drugs that stem from older days include monoclonal antibodies (with an awarded Nobel prize to Niels K. Jerne, Georges J.F. Köhler and César Milstein in 1984) as well as a variety of blood products like concentrates of factor VIII and – a hemodialysate of calf-blood called Actovegin.

Chemical-analytical data for Actovegin reveal a clear, aqueous solution of light yellow-brown colour. It contains ninhydrin-positive substances while tests on protein are negative. As a blood dialysate, Actovegin contains typical salts and trace elements. However, since Actovegin is a mixture of complex composed blood dialysate, it is difficult to define a single pharmacologically active ingredient. Low-molecular weight

peptides (2% of the dry matter) and nucleic acid derivatives have been primarily regarded as the active ingredients of the calf-blood hemidialysate; however, several other low molecular weight compounds are known, but the active components are not yet clear (see below).

Clinically, the calf-blood hemidialysate has been registered for the following indications: malfunction of the blood circulation in the brain and trophic disturbances (i.e. ischemic insult, cranio-cerebral injury); impairment of peripheral blood circulation and associated diseases (angiopathy and ulcus cruris); dermal transplants; burns, chemical burns, wound healing problems: torpid wounds, decubitus; dermal and mucosal lesions after radiation (prophylaxis and therapy). Despite this variety of indications, one can clearly depict the connecting activity of calf-blood hemidialysate, the favourable trophic effects that this hemodialysate exerts on cells and organ tissue.

In this review, we will concentrate on the calf-blood hemidialysate Actovegin and its pharmacodynamic effects drawing a picture of an old product that has a wide prescription tradition in a variety of disorders coming back into the focus of clinical reality – and fosters both novel basic and clinical research. After a short introductory overview on how the product is manufactured, we will cover the to-date elucidated molecular mechanism of action. Finally, we will characterise the calf-blood hemidialysate in the clinical setting with a summary of few a clinical studies.

## Manufacturing process

Actovegin is a deproteinated, pyrogen- and antigen-free hemodialysate of calf blood. It is manufactured from calf blood in several steps by ultrafiltration: here, the manufacturer uses different cut off sieves: first, an ultrafiltration step employing a cut off of 6 kD is performed, followed by a vacuum distillation step and removal of the precipitate by filtration (0.45 µm) and titration to pH 6.8. Afterwards, the product is subjected to sterile filtration with prefilters of 0.2 µm and 0.45 µm and stored at 2–6°C for more than 14 days and subsequently filtered (0.45 µm) and again titrated to pH 6.8. After subsequent pH titration steps, the product is again subject to filtration (7 µm and 0.2 µm) and another ultrafiltration step with a 10 kDa cut off, followed by sterile filtration with prefilters of 0.45 µm and 0.2 µm. After another storage period at 2–6°C for more than 56 days, the final precipitate is removed by filtration (0.45 µm) and diluted to a nominal concentration to 200 mg/ml dry weight. Finally, deproteinization is completed by sterile filtration with prefilters of 0.2 µm and 0.45 µm. The analysis of the final product shows that it contains a mixture of natural substances: both inorganic components like common blood electrolytes (e.g. chloride, phosphate, sodium, potassium, calcium, and magnesium, several sources for nitrogen, amino acids, peptides, glucose, acetate and lactate) and organic components like amino acids, a number oligopeptides, nucleosides, glycosphingolipids and products of the intermediary metabolism. Since proteins are removed from the defibrinated blood via ultrafiltration (cut-off 5000 Da), the product is tested negative for the presence of protein by SDS-polyacrylamide gel electrophoresis. It is completely free of proteins and any other contaminating components reflected by its safe usage to date; apart from single occurrences, no common pattern of immunological response has been reported over a treatment period of around 50 years. Furthermore, toxicity tests in mice and other relevant species reveal that calf-blood hemidialysate, after intravenous application, has an acute toxicity of more than 50 times the maximum therapeutic dose. Negative subchronic toxicity testing over a period of three months also lends support that Actovegin is non-toxic and no chronic pathological organic changes have been observed either macro- or microscopically. Since Actovegin is being produced from a biological source, it is undergoing two tests to ensure homogenous activity of the product: this is achieved by determination of the oxygen consumption (given as µl/mg tissue/hour) in the homogenate of guinea pig liver homogenates (as measured in a microspirometer according to Warburg). The target value for Actovegin is a QO2 of 4.0/8 mg substance. A second standardisation method is the uptake of tritium-labelled glucose into the lipid fraction of adipocytes (prepared from epididymic rat adipose tissue). The calf-blood hemidialysate enhances this uptake in an Insulin-like fashion and exhibits at 1 mg/ml incubation medium an Insulin-like activity (ILA) of more than 20 µU.

## Pre-clinical findings

Actovegin has a broad range of activity with the major action based primarly on an organ-independent influence of cell metabolism that leads to an increased oxygen uptake and utility as well as glucose uptake by the cells. By this means, Actovegin exerts an activating effect on energy metabolism beneficially influencing a

variety of clinical settings. Furthermore, Actovegin promotes oxidative metabolism and shifts the redox-balance of the cells into the direction of oxidised substrates. This also leads to an increased availability of energy-rich phosphates like ATP and creatine phosphate.

The preclinical studies leading to the assumption of these positive effects have been reported from a broad repertoire of testing strategies conducted in both *in vitro* (e.g. cell culture) and *in vivo* (i.e. animal studies). Furthermore, it has been shown that Actovegin can reveal beneficial effect on disturbances of the blood circulation. This leads to more effective energy supply to distinct tissue, including the brain. In the following, we will subsume the preclinical reports on the pharmacodynamic activity of Actovegin divided into pertinent subsections according to the leading effect. The different actions of Actovegin can be attributed to the nature of the product: it consists of a mixture of different agents, similar to herbal extracts – where this is considered an advantage.

## Insulin-like activity (ILA) and glucose metabolism

Actovegin has been shown to act in a fashion similar to insulin [1]; this finding is further supported by Parade [2] who described a significant insulin-like effect of Actovegin on glucose uptake in rat lipocytes; the authors conclude from their work that Actovegin contains a physiological substance being responsible for the observed effects. The question on the nature of these active ingredients resulted in a number of different studies: Inositol-phospho-oligosaccharides (IPOs) have been discussed as the important ingredients in Actovegin underlying its ILA and stimulative to the glucose metabolism.

IPOs are released from liver membranes upon insulin stimulation [3, 4] and mimic a wide spectrum of ILA in different cells due to their soluble nature and widespread distribution [5]. This observation was also supported by Mato et al. [6] as well as Kellerer et al. [7]: the latter authors used rat fibroblasts overexpressing human insulin receptor isoforms and incubated with insulin. This produced the release of IPO which were used to determine if IPOs can directly stimulate glucose uptake by glucose transporters: this was the case. Since these experiments relied on cells heavily overexpressing the insulin receptors, analogous experiments were performed with isolated rat fat cells expressing the physiological receptor levels.

Earlier evidence came from animal studies where the levels of glucose were measured in liver homogenates prepared from guinea-pig [8]. Actovegin induced significant differences in glucose values after 60, 90 and 120 min incubation. Furthermore, the ILA reported by Bachmann et al. [1] was not dependent on the presence of insulin, as evidenced by ILA after co-application of insulin antibodies and Actovegin®: here, blood glucose levels in animals that received Actovegin displayed blood sugar levels clearly lower than in the control group. Mohnike et al. [10] describe experiments that evaluate the effect of Actovegin leading to a stimulating effect on glucose uptake and utilization in rat hemidiaphragm. In a follow-up study from the same laboratory, Machicao et al. [11] examined whether IPO isolated from hemodialysate have insulin-like activity in rat fat cells. The authors show that IPOs actions resemble some of insulin's key activities by mimicking the major metabolic effects of insulin in adipocytes [12]. Importantly, inositol monophosphate, glucosamine and mannose completely inhibited the effect of IP oligosaccharides, thus underscoring the specificity and pharmacological activity of these active ingredients of Actovegin [13] (Fig. 1). In *in vitro* assays, using

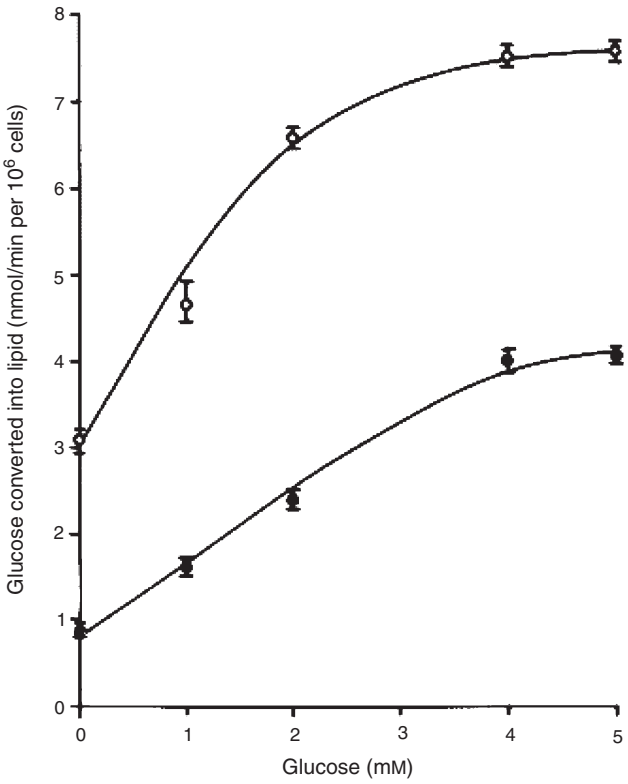


**Fig. 1:** Influence of glucose concentration on basal and IPO-stimulated lipogenesis. Dose-response curves of the activation of lipogenesis by glucose in the absence (filled symbols) and presence (open symbols) of IPO (2 ug/ml). Mean values + S.E.M. of four experiments are shown. Reprinted with permission from Ref. [13]

myocytes (BC3H-1), insulin induced hydrolysis of phosphatidylinositol-glycan that stimulated the generation of inositol-glycan and diacylglycerol [14]. However, these authors failed to obtain evidence that inositol-glycan facilitated the uptake of glucose in these cells; only glucose oxidation and lipogenesis were clearly enhanced.

To further substantiate the idea of ILA of IP-oligosaccharides derived from Actovegin hemodialysate, Obermaier-Kusser et al. [9] used rat adipocytes to measure the influence of IPO on glucose transport in direct comparison to insulin: application of IPO on 3-O-methylglucose transport exerted a significant and dose-dependent effect; in comparison to insulin which stimulated glucose transport 10-fold, IP oligosaccharides stimulated glucose transport 5-fold (Fig. 2).

However, in the same publication, Obermaier-Kusser et al. also measured the binding of cytochalasin B to the plasma-membrane bound glucose carriers; to their surprise, the number of glucose carriers was not enhanced in the presence of IP-oligosaccharides in contrast to the activity of insulin which enhanced the number of active carrier sites by 3–5-fold [9]. They also excluded the possibility that IP-oligosaccharides stimulated the insulin receptor-associated receptor tyrosin kinase and therefore suggested that they do not interact with steps in signal transmission beyond the receptor-kinase level. Therefore, the authors insinuate that a two-step model may be applicable involving not only the activity of the compounds on the trafficking of transporters to the plasma membrane but also a direct way of transport stimulation. Further characterisation of the IP-oligosaccharide subfractions will substantiate the active compound in more detail.

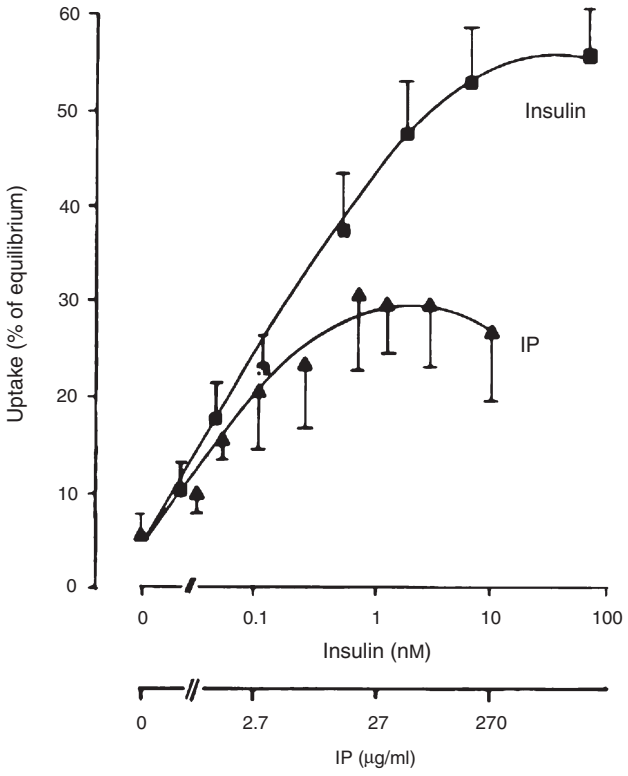


**Fig. 2:** Freshly isolated rat adipocytes [$(4.5–5.5) \times 10^6$ cells/ml] were incubated with insulin or IP-oligosaccharides derived from Actovegin® hemodialysate for 20 min at the concentrations given on the abscissa. The composition of the IP-oligosaccharide fraction was as follows: erythrose 2.54 mM (0.305 mg/ml), ribose 1.49 mM (0.166 mg/ml), arabinose 0.5 mM (0.150 mg/ml), xylose 1.26 mM (0.189 mg/ml), mannose 0.60 mM (0.108 mg/ml), galactose 0.30 mM (0.053 mg/ml), glucose 1.22 mM (0.220 mg/ml), inositol 0.56 mM (0.100 mg/ml). The saccharides were complexed as phosphates or sulphates. The IP-oligosaccharide fraction is acid- and alkali-labile. 3-*O*-Methylglucose uptake was then determined for 4 s as described in the Materials and methods section and is expressed as % of equilibrium. The curves show mean values ± SEM of six experiments. Acetylation of the IP-oligosaccharide fraction decreased the effect of 27 or 270 µg/ml by approx. 50% ($n = 2$) Reprinted with permission from Ref. [9]

In conclusion, direct as well as indirect evidence supports the notion that Actovegin exerts a clear insulin-like effect, which is, nevertheless, distinct from the action of insulin: it is more direct activation of the transport process rather than an enhancement of the trafficking that is stimulated by insulin. Nevertheless, the endpoint of the activities of both insulin and IP-oligosaccharides exerting ILA is the enhancement of glucose utilization which may directly impact on the cellular metabolism and energy balance in distinct cellular systems.

## Oxygen uptake, metabolism and hypoxia

Studies on oxygen uptake, metabolism and hypoxia have been performed in different animal models such as rat, guinea pig and dog. Jager et al. [15] published a study on the first evidence that Actovegin exerts effects on cellular respiration of purified rat liver mitochondria: oxygen consumption was significantly enhanced. de Groot et al. [16] examined the effect of hypoxia on isolated rat hepatocytes using a trypan blue survival test: hypoxic conditions in the presence of Actovegin led to a significantly higher survival. The authors concluded that Actovegin exerted a marked protection/retardation against hypoxic cell injury. Brecht and de Groot [17] tested free amino acids on hypoxic cell injury in isolated, cultured rat hepatocytes. Experiments were performed as described above in de Groot et al. [16]. This report supports the notion that amino acids contained in Actovegin might be underlying for its protective effect against hypoxic damage. In line with these observations, Schäfer [18] analysed the oxygen uptake in rat liver parenchyma cells under the influence of Actovegin in comparison

to placebo and described an enhancing effect by Actovegin on cell respiration (internal report, data on file). Reichel et al. [19] showed that the augmenting effect of Actovegin on oxygen intake and glucose uptake in cellular systems relies on an enhancement of the phosphorylating properties of the cells in that oxygen uptake increases by up to 40% in the presence of Actovegin. Again, this supports the notion that Actovegin enhances cellular activity. In support of this notion, Kuninaka et al. [20] assessed mitochondrial respiration by polarography in a rat liver mitochondria preparation and came to conclusions that support the findings by Reichel et al. [19]: oxidative phosphorylation is significantly augmented by addition of Actovegin.

Another tempting question was the velocity by which Actovegin exerts its effect on intracellular energy supply: Schwabe [21] showed that Actovegin substantially increased intracellular oxidative metabolism and leads to acceleration of not only energy metabolism but also reserve metabolism (accompanied by an increase in glycogen and potassium deposition for instance in the heart and liver tissue).

Pichotka et al. [22] studied the influence on oxygen uptake: Actovegin induced a statistically highly significant increase in the oxygen uptake in a dose-related manner as compared to the value obtained under control conditions. Rammler [23] photometrically determined whether Actovegin exerts an effect on the ATP-concentrations of the brain over 240 min. The results show that Actovegin possesses the potency to increase the ATP in tissue (internal report, data on file). This result was one of the first observations to clearly show that Actovegin has a direct bearing on metabolism of the brain. But not only the brain received beneficial support of its oxygen consumption and metabolism, but also heart tissue as shown by Chanh et al. [24, 25]: these authors studied the influence of Actovegin on modifications of respiration, general metabolism and systemic haemodynamics in a model of experimental hypoxia and described a substantial increase of oxygen uptake and stimulation of the cardiac performance.

Hence, Actovegin possesses the capability to exert ILA and stimulation of cellular metabolism as well as an increase of oxygen uptake and performance of energy production. These effects have been reported in a distinct variety of tissue of various organs lending support to Actovegin being a stimulant agent to support energy-deprived and starved tissues. Therefore, it is tempting to analyze if these effects that replenish energy and oxygen in tissues also lead to growth and replication of cells within tissues. This issue has been addressed by the examination of Actovegin's effects on wound healing, where a direct effect was observed.

## Wound healing and effects on radiation-induced damage

The effects of Actovegin on wound healing have already been observed in the phase of early advent of this hemodialysate. Neinhardt [26] experimentally addressed wound healing processes; he observed that Actovegin®-treatment leads to closing of the wounds approximately 2 days earlier as compared to controls. In a more direct series of experiments, Mochida et al. [27] tested the influence of Actovegin on the tensile strength of an incised abdominal muscle in animals; the authors conclude that Actovegin significantly supports wound healing. This notion is also supported by an *in vitro* study on guinea pig heart endothelial cells by Schönwald et al. [28]: they assessed the growth-promoting effect of Actovegin that synergizes with miscellaneous growth factor effects.

Wound healing heavily relies on the capacity of single cells to migrate into the area of the wound and replication of these cells. Therefore, several studies investigated the migration-stimulative effects of Actovegin in cell culture assays. Miltenburger et al. [29] assess the influence of Actovegin on the functions of fibroblasts and keratinocytes and find that cell migration is highly significantly affected by addition of Actovegin alone, and even more stimulated by co-administration of Actovegin and TGF-$\beta$. Furthermore, the keratinocyte outgrowth is distinctly enhanced in the presence of Actovegin®, hence the trophic possibilities of tissue to actively rebuild tissue integrity.

Not only the trophic and cell migration-stimulative effects have been examined in *in-vitro* settings, but also the functional integrity and activity of immune cells that enhance tissue remodelling and thereby wound healing. Therefore, Actovegin should be capable to also enhance the function of immune cells; several studies examined the possibilities of Actovegin in *in-vitro* assays: Monocyte-derived macrophages play a major role in inflammatory responses in wound healing and tissue remodelling; Actovegin exerts significant effects on human monocytes cultured *in vitro* in the presence of human serum: Spessotto et al. [30] prepared monocytes from buffy coats and assayed monolayer cultures under a microscope for morphology and cell density, as well

as protein content. Cell density was increased in all concentrations of applied Actovegin, as well as the cell protein content. Actovegin partly acts like a substitute, for blood serum favours survival and differentiation of monocytes in culture.

Radiation induced damage in cells and tissues has been regarded as a crucial test case for protective and supportive action of many different pharmacologically active substances. Actovegin has been studied regarding the effects of radiation on whole animals and exerted positive effects in some, but not all, studies. However, the published studies do not unequivocally follow the same protocols. Nevertheless, in most studies, a positive effect of Actovegin could be shown. Many observations point to a possible protective effect of Actovegin against the effects of ionizing radiation. Bauer and Locker [31] investigated the effects of Actovegin on the survival of mice that have been irradiated by a lethal dose of gamma radiation. Application of Actovegin led concentration-dependently to a better survival of the Actovegin®-treated animals after 30 days following irradiation. The control groups showed practically no survival after 30 days. Barth et al. [32] examined the time-dependence of the injection of Actovegin in relation to the damage produced by irradiation. The results reveal an optimal time point for the injection after three hours after the irradiation had taken place. Prophylactic treatment with Actovegin on 6 consecutive days before the radiation did not reveal any preventive effect of Actovegin. In a similar study, Basu et al. [33] examined the radioprotective effects of Actovegin in adult rats; while control animals died within 30 days, the rats treated with Actovegin 1 hour before irradiation had a statistically significant higher survival rate, as already described in the study of Bauer and Locker [31], thereby confirming their results. By contrast, Tamou and Trott [34] describe findings on the occurrence of radiation-induced ulcers in the rectum of rats where the results in the Actovegin group did not differ from control conditions.

Therefore, it seemed tempting to also examine the Actovegin effect on radiation-induced damaged in an *in-vitro* setting. Sigdestad et al. [35] conducted a study aiming at understanding the intracellular effects of Actovegin in irradiated cells in culture. They did not observe any influence of irradiation on cell survival *in vitro*; however, they found that the cells treated with Actovegin exhibited much less single-strand DNA breaks as compared to the control cells. This also supported the effects described earlier that pointed to a beneficial effect of Actovegin on cell survival, also on the level of the cell nucleus.

Importantly and in line with the findings described above, a multi-centre, double-blind, placebo-controlled, randomised, parallel group clinical trial has been recently performed to evaluate efficacy and safety of Actovegin in diabetic type 2 patients with symptomatic diabetic peripheral polyneuropathy [36]. The treatment was associated with a significant reduction of the positive sensory symptoms and a decrease in Vibration perception threshold with clear tendency towards significance as compared with placebo treatment.

## Disturbances of blood circulation

Wolff [37] performed biochemical analyses of rat liver tissue after *in vivo* induction of hemorrhagic shock for 60 min. Actovegin led to a change in the liver metabolites compared to placebo and, after analysis of the data, the author suggests an activating effect for the oxidative metabolism of the cell by Actovegin – in line with the above-mentioned effects. Importantly, hypercholesterinaemia plays a major role in the pathophysiological mechanism leading to thrombosis and thromboembolic events: Giarola [38] addressed the question if Actovegin could exert an effect on the plasma lipids of rabbits with experimentally induced hypercholesterinaemia; the authors reported a positive effect of Actovegin in thromboembolic states. In line with these experiments, Chanh et al. [24] conducted a study in anesthetized dogs where respiration, general metabolism and systemic haemodynamics were tested in the presence or absence of Actovegin. It has been concluded from the results that Actovegin has the potential to directly enhance the cardiac output and improve the performance under pathophysiological conditions. Similarly, Somogyi [39] conducted a study directed to understand the effects of Actovegin on the level of the myocardial cells and experimentally induced intermittent hypoxia. The authors induced myocardial metabolism derangement in dogs by clamping of an aorto-coronary bypass. Animals treated with Actovegin showed a successful prevention of the morphological and biochemical changes. The authors concluded from their study that the administration of Actovegin protects myocardial tissue from severe hypoxic damage. These findings were fully supported by Eichler and Völker [40]: they assessed *in vitro* in explant cultures from chicken embryonic heart tissue in a paradigm of experimental myocardial failure that the addition of Actovegin reactivated or compensated and/or regenerated the myocardial tissue. Furthermore, the

addition of Actovegin to the explant cultures prolonged the time until first symptoms of insufficiency occurred. The authors discuss this effect as stemming from the direct stimulatory action of Actovegin on cell metabolism.

## Effects on the central nervous system

Given the different effect exerted by Actovegin in various organ systems as summarized above, we can expect an immediate beneficial effect in the central nervous system (CNS) as well: in fact, there are several preclinical observations from animal models as well as nerve tissue that support an extrapolation of the systemic effects.

Regarding the cellular metabolism in the CNS, Lanner and Argyropoulos [41] conducted a series of electroencephalographic experiments in rabbits during an experimentally induced temporary ischemia of the brain. They reasoned that a pharmacologically active agent like Actovegin shown to be able to enhance the cellular availability and utilization of glucose should also stimulate the compromised CNS tissue. The authors administered Actovegin over three days intramuscularly, anesthetized the rabbits and started the experiment by ligating the arteries that provide the blood flow to the brain that resulted in an acute ischemia of the brain. The verum group had Actovegin instilled via the carotid artery in comparison to a control group of animals. The results clearly show that Actovegin induced a significantly prolonged revitalization time in the treated animals compared to the untreated control animals. This observation was in line with the report by Krüger and Quadbeck [42]: they treated rats under hypoxic conditions and found as the most effective treatment glucose co-administered with Actovegin; the authors suggest that the effect relies on the facilitating effect exerted by Actovegin on the glucose-uptake in the brain.

Given the strong autoregulation of the vascular beds in the CNS, this effect of Actovegin is even more remarkable since it insinuates a directly stimulant effect of cell metabolism in compromised nerve tissue although it does not directly affect cerebral blood flow. This has also been concluded from earlier experiments performed by Quadbeck et al. [43], where an increase in [$^{14}$C]glucose uptake into the CNS has been directly shown to be augmented by Actovegin. Furthermore, the enhanced glucose content of brain tissue was not the result of a mere underuse of glucose in the brain because glucose utilization as revealed by the resulting stimulation of [$^{14}$C]$CO_2$ production. A follow up study performed by Hoyer and Betz [44] using the complete cerebral ischemia paradigm also corroborated the findings described so far. The authors examined how the time course of metabolic abnormalities, measured as the concentrations of glucose, lactate, creatine phosphate and adenosine triphosphate (ATP) normalized in two year old rats. After complete cerebral ischemia, these markers returned relatively rapidly to baseline values However, after 48 and 72 h postischemic recirculation, the authors noted a prolonged metabolic energy imbalance in cerebral cortex and hippocampus. Application of Actovegin efficiently counteracted these detrimental effects on CNS tissues. These results have also been supported by observations made in rats with kainic-acid induced excitotoxic brain injury: the application of Actovegin resulted in a reduction of the mortality of rats in the course of status epilepticus [45].

Altogether, these results underscore the effectiveness of Actovegin treatment in situations where CNS tissue is compromised, e.g. in brain ischemia where supportive activity in cell metabolism is an absolute requirement; thus, Actovegin is apparently helpful in detrimental postischemic disorders and may endow neuronal survival in these critical conditions.

The effects shown in the different animal experiments have also been demonstrated in a number of clinical trials: Actovegin was tested in a model for hypoxia employed in a placebo-controlled, double-blind clinical trial with crossover design [46]. The authors recorded visually evoked potentials as well as the electroretinogram of probands receiving Actovegin or placebo in a fictitious height of 5500 m above sea level (by using an inspiratory oxygen concentration of 10.5%). Both measurement categories showed dose-dependent changes only after the application of Actovegin®.

In other placebo-controlled, double-blind clinical trial with crossover design, these reports were also supported by subsequent findings [47, 48] in support of clinical efficacy of Actovegin in geriatric patients. In a most recent *in vitro* study, these clinical findings of Actovegin were supported by the following results: Actovegin was found to exert neuroprotective effects on rat primary neurons in a dose-related manner; the findings suggest a role for Actovegin in the protection of neuronal cells against apoptosis and to specifically lower oxidative stress (Elmlinger, Bobrova, Kriebel, Movsesyan, Husum, and McCracken, manuscript submitted).

## Conclusion

Actovegin has proven its efficacy in a variety of different preclinical tests, including effects on cells in *in-vitro* assays as well as in animal tests. Its direct action on cellular metabolism is believed to follow an insulin-like activity on glucose transport into the cells. Hence, inositol-phospho-oligosaccharides most likely underlie Actovegin's mode of action that make this well-tolerated hemodialysate from calf blood a therapeutic choice also – and especially – in the era of biological drugs.

## Conflict of interest

FB and JP declare that there is no conflict of interest, MWE and GL are employees of Nycomed, GN and HHS received an educational grant that supported the writing of the present publication.

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