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EPO, a …. stupefying hormone!

The world of sport has been precipitated into the scientific era with the arrival of a whole new generation of drugs exported from the world of biotechnology. Recombinant EPO is a product of genetic engineering and since its appearance in the sports arena it has broken almost as many sportsmen's careers as their records. Yet, before becoming public enemy number l, this protein helped to improve the lives of millions of people and it does deserve to be rehabilitated. Let us take a closer look at a protein which is the talk of the town.

Drug-taking through the ages

Every year the mild season brings with it its horde of sporting events. But since the summer of '98 when the Festina cycling team admitted to taking EPO – amongst other drugs – new records gave ground for debate more than enthusiasm. Every outstanding performance was accompanied by a whiff of suspicion; the spectre of drugs hovers above every stadium.

Yet drug-taking is not new. Through the ages, man has been fascinated by the prospect of somehow increasing his physical and intellectual capacities. In the beginning, it was a question of survival in a hostile environment, in which he had to prove his superiority over animals or the enemy. It was but a small step from training for war to training for sport. And drug-taking was already the name of the game – so to speak – during the first Olympic Games. The Ancient Greeks believed that to excel in sports bestowed on them the benevolence of the Gods. So taking stimulants to improve their performance was common practice in their time. They also firmly believed that wrestlers gained by eating the flesh of bulls and those competing in the long or high jumps gained by eating the flesh of an antelope!

But over the centuries, the recipes became more sophisticated and the incentives changed. Globalization has encouraged chauvinism and the economic stakes replaced the benevolence of the gods. Amphetamines followed the "wonder potions" of the l900s, themselves directly exported from the horse-riding circles. Amphetamines were developed in the laboratories of Nazi Germany and were prized by airpilots of World War II. They then joined the world of sport when peace came, and continued to be used until the first anti-drug tests were carried out in the 70s. From then on, the successes of the toxicologists who « cheat » the system have alternated with those who develop anti-doping tests. The last 30 years have seen the « rise and fall » of corticoids, anabolic steroids, testosterones and growth hormones. Hormones make really effective drugs; not only are they stimulants but they do indeed increase an athlete's performance. Today, doping during the whole period of training has replaced intermittent doping. Little by little, thanks to biotechnology, wholly artificial substances with no endogenous equivalent are being replaced by substances which perfectly match those produced by our own

organism and as a result makes them very difficult to detect. This is the case of EPO, the latest newcomer and leading drug of the 90s.

The therapeutic properties of EPO

E-P-O : three letters that immediately bring to mind cycling, the Tour de France and drugs. It is a bad reputation that tends to make one forget that EPO is a natural substance produced by our bodies and was first used for its therapeutic properties. EPO (or erythropoietin) is, in fact, a protein or more precisely a hormone, i.e. it is a substance produced by a gland and released into the bloodstream, which in turn conveys it to the organs on which the hormone acts. It is produced mainly in the kidneys (although a little in the liver) and acts on the bone marrow, where it stimulates the production of erythrocytes, otherwise known as the red blood cells. The red blood cells are the most important cells in the bloodstream; their function is to transport the oxygen molecules that we inhale. For this, the cells are literally filled with a protein called « hemoglobin » which is an oxygen carrier. One of these proteins alone is capable of linking four oxygen molecules. When the blood courses through our lungs, the hemoglobin molecules gather the oxygen molecules and convey them to every cell in our body. The oxygen is in turn exchanged for carbon dioxide molecules – virtually the waste product that our cells produce whilst fulfilling their various functions. Once delivered to the cells, the oxygen will supply the energy they need to function. Once shipped back to our lungs via the red blood cells, the carbon dioxide molecules are exhaled and another deep breath replenishes the oxygen supply.

However, nothing lasts forever in this world and red blood cells are no exception. After l20 days, they are eliminated by cells – the macrophages – in the liver and the spleen. In this way, l00 billion red blood cells are destroyed on a daily basis, in each one of us! This is why our bone marrow must constantly produce more. A decrease in the quantity of oxygen in our organism, caused by red blood cell insufficiency or high altitude, stimulates the kidney cells to produce more EPO and to supply it to the bloodstream. Once in the bone marrow, the EPO recognizes a protein called a receptor on the surface of the erythrocytes. At this early stage of their development they are not yet producing hemoglobin. Linking up with this receptor triggers a chain reaction within the cell which will multiply and develop to its final stage. Once mature, the resulting erythrocytes are released into the blood stream.

These properties were seized upon by the medical world. Thousands of patients suffering from anemia were given a new lease of life, whether anemia resulted from kidney deficiency (the main source of EPO), chemotherapy or the taking of zidovudine (AZT) for the treatment of AIDS. All these patients were unable to lead a normal life because of reduced physical endurance, great fatigue, headaches and breathlessness.

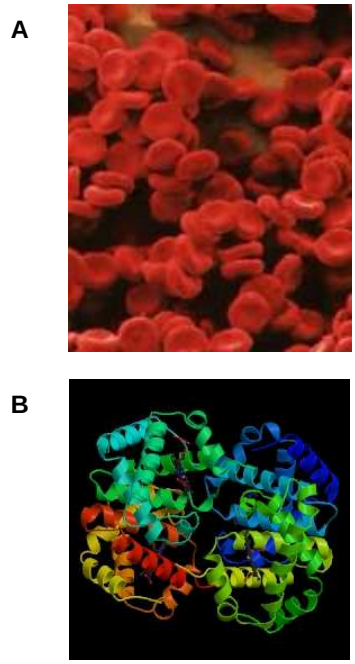

Fig.1 ***A****. Human red blood cells (actual size : 8 microns),* ***B****. Structure of a hemoglobin molecule*

Unfortunately, it took a long time before EPO could be produced in laboratories. On the one hand because the concentration of the hormone in human blood or urine is very low and it was impracticable to produce naturally. On the other hand, the size of the protein and the complexity of its structure made its chemical synthesis far too complicated. So it was not until the 80s that EPO could be produced in sufficient quantities as a result of genetic engineering. The technique introduces a foreign gene inside a cell. Placed in a suitable environment, the cell will multiply to the extent of producing a « cell culture » every one of which will reproduce the foreign protein as well as its own. Currently, this hormone is produced on an industrial scale via cell cultures produced from hamster cells and into which human EPO genes have been introduced. The human protein, produced in large quantities, is then refined. Such EPO, known as « recombinant » EPO, is essentially the same as the physiological EPO. It avoids recourse to painful blood transfusions for anemic patients (not to mention possible complications), which were the only known treatment until then.

Then athletes fell under EPO's charms…

Of course the world of sports could not remain blind to the properties of EPO. Since the hormone increases the number of « oxygen carriers » in the blood system, it also increases the quantity of oxygen in the body. In the event of endurance races, such as cycling, which require large quantities of oxygen, the prospect of oxygen enhancement was more than attractive. Until the advent of EPO, sportsmen could only increase the number of their red cells by natural means, by training at high altitude or, more artificially, by sitting in a hypobar or hypoxic chamber for a certain time or, although quite illegal, by auto blood transfusion. With EPO, the lungs, heart and muscles get so much oxygen that they can function at 200% without feeling fatigue. What is more, lactic acid is slower to develop. Lactic acid is produced by cells, following intense muscular efforts, once oxygen has become scarce. And it is this which is responsible for pain and stiffness in the limbs. As a result, records have been broken one after the other. Ten years after recombinant EPO was introduced on the market, erythropoietin now seems to be sportsmen's first choice for stimulating their muscles in their never-ending search for new records.

But is it wise and without risk to follow such a regime? The secondary effects of EPO have been studied therapeutically, i.e. in patients suffering from anemia. High doses of EPO cause thickening of the blood due to the increase of red blood cells in the system. The resulting lack of fluidity drives the heart to « pump » harder. Consequently, there is a high risk of coronary and cerebral thrombosis, as well as pulmonary embolism. In the long run, EPO intake can lead to auto-immune diseases, high blood pressure (arterial hypertension) or bone marrow cancer. Other troubles have been noted such as palpitations of the heart, muscular pain, skin eruptions, nausea and violent headaches. But the secondary effects of EPO have never been studied on healthy subjects – sportsmen in particular. It is no doubt all a question of dosage; but it is probably a safe bet to say that, with the escalation of drug-taking nowadays, the doses of EPO taken are far from homeopathic. In fact, in the last few years, although it has never been proved, EPO is presumed to be the underlying cause of a certain number of suspect deaths of sportsmen by heart attack.

Screening for EPO: ten years too late

The International Olympic Committee forbade the use of EPO over l0 years ago. Unfortunately, the synthetic hormone differs very little from the one that is naturally secreted in the body. From a chemical point of view, the two molecules are so alike that until recently it had been impossible to differentiate them. An athlete was suspected of doping if his hematocrit –red cell blood count – was high. One small drop of blood was enough for the test but the rate, which normally is about 40%, varies from one person to another and even in the same person after a stay at high altitude for example. So an individual could have a count above the authorized limit of 50% without having taken EPO. Conversely, an injection of physiological serum was enough to dilute the blood and thus lower a high blood count.

Only in the last year has it become possible to differentiate synthetic EPO from physiological EPO and with a sample of urine. For there resides, after all, a minute difference between them. Let us return to our favorite illustration of a pearl necklace to represent a protein. Once produced, the necklace will undergo a certain number of modifications without changing the order of its pearls. Sometimes, « decorations » are added. These decorations vary according to the species and the type of cell from which the protein has come. The resulting 'decorated' proteins are slightly different and termed « isoforms ». In the case of EPO, it is thought that these isoforms are a result of glycosylation, i.e. molecules of sugar are stuck onto the protein. Since the electrical charge of sugar molecules is negative, the recombinant hormone – which contains fewer sugars than the natural hormone – will present a smaller negative charge. Thanks to this difference, the isoforms can be separated by an electric field and their EPO origin will be recognized as endo- or exogenous. There are several brands of recombinant EPO on the market, which vary by their sugar-molecule content. A French team has developed a test so sensitive that it is even able to detect the difference between two brand products : Eprex by Janssen-Cilag and NeoRecorman by Roche.

EPO though is rapidly eliminated by our organism and can only be detected within a limited period of a few days after it has been taken, whereas the improvement of an athlete's performance will last up to two weeks. Consequently, screening is only useful when done outside competition or during sports trials which last several days, such as the Tour de France in which cyclists take injections at each stage. Concurrently, an Australian team has developed an effective but indirect method of tracing EPO by studying altered blood parameters following the intake of exogenous EPO. This test claims to be able to trace exogenous EPO up to l0 days after it has been taken but is unfortunately not wholly reliable.

The question is whether a substance should not be added to the preparation of EPO, which would show up whilst screening. Much in the same way Asterix added a blue coloring to his magic potion

and subsequently caught out Roman athletes who had cheated in the Olympic Games! This is no joke. As a result of American pressure, such a trick was used by Roche in the case of Rohypnol, a drug taken to fight off insomnia but also used as a stupefacient. Unfortunately, modifying the formula of EPO would mean creating a new drug, which would necessarily entail new clinical trials – a long and, above all, costly procedure. For the time being, the various pharmaceutical firms concerned are not very keen. In fact, despite the adverse publicity surrounding EPO doping it would not be to their advantage to see a change in the situation. Indeed, l0 years after its introduction on the market, 80% of EPO sales are destined for illegal use.

In any event, the problem lies elsewhere: EPO screening should have made its appearance over 10 years ago. New forms of EPO have evolved and are much more difficult to detect. One example is « retarded » EPO which is diffused in the organism at a slower rate and can be taken by injection only once a week instead of two or three times. Its concentration is low but constant and its effects more durable. This means that « retarded » EPO will at first not be traceable. Or EPO-like, yet another version of EPO, which represents in effect only the active part of the protein thus avoiding forms of intolerance. Just as effective alternatives to EPO are now available: These are semi-synthetic products such as reticulated hemoglobin or perfluorocarbure (PFC) both excellent oxygen carriers in the blood, which do not increase the blood count and so are undetectable at this stage.

Doping in the future

For some l0 years now, biotechnological techniques have been introduced into the world of sport. The anti-drug campaign certainly can crow over their victory thanks to the discovery of the urine method of EPO screening. But EPO is not the only drug – far from it. Other recombinant and as yet undetectable hormones have been diverted from their original therapeutic use and have spread throughout the stadiums. One is the growth hormone, an other genetically engineered protein, often taken as a « cocktail » with EPO. No one knows exactly the consequences of such a brew in the long run, but it could probably bring on cardio-vascular troubles and cancer.

Obviously methods of control will always lag behind drug-taking and in the same way recombinant proteins are but a foretaste of what these new techniques can offer. Gene therapy could in the relatively near future condition athletes' organisms to produce their own drugs by introducing the corresponding genes in their cells. The resulting hormones would be undetectable from endogenous ones since they would, in every way, be produced in similar conditions. Drug screening methods would then have to change radically. Science fiction? The future will tell. But the fight against drug-taking is certainly overshadowed by the growing progress in biotechnology.

Sylvie Déthiollaz*

*Translation: Geneviève Baillie

For further information

On the internet:

- Doping Info : http://www.dopinginfo.ch/fr/
- Sciences&Avenir : www.chez.com/nethorizon/dopage/Accsa.htm

Illustrations:

- Fig.1A, Adaptation: http://www.interet-general.info/article.php3?id_article=3585
- Fig.1B, Source: PDB ID : 1ABW, Kroeger, K.S., Kundrot, C.E., Structures of a hemoglobin-based blood substitute: insights into the function of allosteric proteins. *Structure* **5** pp.227 (1997)

At UniProtKB/Swiss-Prot:

- Erythropoietin, Homo sapiens (humain): P01588

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More ample information on the subject is available at: infodopage@hospvd.ch

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