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## 1. INTRODUCTION

### 1.1. BACKGROUND

Interrelationship of the trace element content of the cultivated field of the agriculture, the trace element content of the body of the farm animals maintained in these fields, and the areal spread of diseases due to trace element deficiency have been studied for a long time. In spite of the fact that both the soil and its vegetation contain lavishly the elements needed, deficiency symptoms manifest in several animals.

According to the data of the literature, the peat and its humic acid content assist in efficiently incorporating the trace elements into the animal organism which is impossible for the anorganic compounds in the form of the simple metal salts.

The majority of these observations and the experiments with peat preparations were carried out by Dr. Elek Csucska. The basic experiments were followed by long years of research work. An extraction method has been elaborated to release humic acid from calcium huminate. Adding the proper metal ions to humic acid, the product improves the clinical status of the deficiency patients.

### 1.2. DATA FROM THE LITERATURE

The results of the recent research work clearly demonstrated that animals were unable to utilize the metal ions which were introduced in form of anorganic compounds into the organism. References confirmed that the efficiency of anorganic metal salts is very low in the supplementation of the trace elements which are needed by the body.<sup>1</sup>

Similarly, data of the literature demonstrated that peat and peat soil possessed the capacity of metal ion-binding.<sup>2</sup> It was shown that the humic acid component of the peat was responsible for the capacity of keeping trace elements in chelate binding.<sup>3</sup> It became evident, that the

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Kirchgessner, M. et al.: *Z. Tierphysiol. tierernährg. Futtermittelkunde* 54: 184-189, 1989

<sup>2</sup> Rouleau, C. et al.: *Pharmacol. Toxicol.* 74(4-5): 271-279, 1994

<sup>3</sup> Patton, R.S.: *Feedstuff* 62(2): 14-17, 1990

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incorporation of the vital metal ions might be improved by the administration of peat and the extract of peat.

Additionally, it was observed that animal food enriched in humic acid could be curative not only in the deficiency diseases, but it also improved the reproductory function of several domestic animals and their resistance against infectious diseases. Furthermore, it could exponentially increase the utilization of the nutrients and, consequently, the body weight rised.<sup>4</sup> Obviously, what proved to be helpful in the animal organism may possess therapeutic value in humans, too.

Many well documented trace element deficiency diseases are known in the medical practice and the research of the physiological role of the trace elements is still continued.

### **1.3. DEVELOPMENT OF A PARAMEDICINAL PRODUCT**

The research workers of the HORIZON-MULTIPLAN Ltd. and the laboratories involved by the company have investigated the HUMET<sup>®</sup> since 1992. It gained its final composition during this period. Its efficiency was proved in controlled experiments, its further therapeutic indication was unveiled and its safety was tested in toxicity studies.

On the base of these data, the National Institute of Pharmacy (OGYI, Hungary) approved the product of HORIZON-MULTIPLAN Ltd. in 1993 and the production started in 1994.

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<sup>4</sup>Gundel, J. et al. H-M Documentum HUMET-037, 1995

## 2. COMPOSITION OF THE PRODUCT AND THE ROLE OF THE COMPONENTS

In the HUMET®-R Syrup, the vehicle is a humic acid preparate of homogenous origin derived from a geologically young (about 3.000-7.000 years old) plain moorland. This vehicle is completed with several micro and macroelements. This physiologically active product is a complex trace element preparate and its humic acid vehicle is a biologically compatible chelate-former, which assures the good absorption and bioavailability of metal ions added. Supposedly, each of its constituents possesses some physiological effect but the general roboration effect results from their joint action.

### 2.1. STRUCTURE AND CHEMICAL PROPERTIES OF HUMIC ACID

During the last 50 years several research works and theoretical considerations were focused on the chemical structure of the humins. Their common source is the lignin which constitutes the hardly degradable compact frame of the plants. This lignin undergoes a slow microbiological transformation (caused primarily by bacteria and fungi) and chemical changes in the soil. The joint effect of these changes leads to the enrichment of various soils (primary peat and brown-coal) in humic acids. The therapeutically most important two groups of humins are the humic acids and fulvic acids which are distinguished on the base of their acid/base solubility.

According to our present knowledge, the humic acids are chemically multisubstituted polyaromatic heterocyclic macro molecules which incorporate cyclic structures joined by aliphatic carbon chains. This primary structure can fix other organic components, such as carbohydrate, proteins, lipids in physical and chemical binding.<sup>5</sup>

Depending on the status of oxydization, the aromatic and chinoidal structures contain oxo-, hydroxyl-, carboxyl-, amine, - and substituted amine groups which can bind several bivalent metal ions in chelate binding. This chelate-binding capacity has been exploited for a long

<sup>5</sup> Schulten, H.H. and B. Plage: *Naturwissenschaften* 78: 311-312, 1991

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- time in clearing away of the toxic heavy metals from waste water and surface waters running downhill from the area under mining activity.<sup>6</sup>

### 2.2. THE BIOLOGICAL ROLE OF HUMIC ACIDS

Humic acids are not easily definable compounds as it was discussed already in the introduction. Their place of origin may be more characteristic than the simple qualitative chemical analysis or some other investigations. Their biological effects may be very different.

A humin preparate known as derived from the peat (Tolpa Torf preparate, TTP) exerts immuno-modulator effect.<sup>7</sup> The TTP preparate increases the production of tumor necrosis factor (TNF) in human leukocytes and stimulates the synthesis of interferon.<sup>8</sup> It can reconstitute the immune responses of mice which are suppressed by zinc phosphamide.<sup>9</sup> The TTP preparate, given prophylactically, significantly decreased the damage of the gastric mucosa and duodenal ulcer<sup>10</sup> and its regenerative effect was demonstrated also in the liver.<sup>11</sup> Humic acids were stated to influence the function of the endocrine system. The effect on the thyroid function was studied in mice and demonstrated that humic acids antagonize the action of thyroxin; this effect is mediated by blocking the activity of the Na<sup>+</sup> / K<sup>+</sup>-ATP-ase<sup>12</sup>. The antibacterial effect of humic acids antagonize the mutant strain of streptococci producing glucane which is responsible for the development of caries<sup>13</sup>. The anti-viral effectivity of humic acids is also known.

<sup>6</sup> Reide, U. et al.: Patent application EP 04677018

Tomschey, O. et al.: Patent application HU 70335

<sup>7</sup> Baj, Z. et al.: *Acta Pol. Pharm.* 50(6): 481-489, 1993

<sup>8</sup> Ingłot, A.D. et al.: *Arch. Immunol. Ther. Exp.* 41: 73-80, 1993

Jbminska-Domoradzka, B.: *Acta Pol. Pharm.* 50: 501-506, 1993

<sup>10</sup> Brzozowsky, T. et al.: *Acta Pol. Pharm.* 51: 103-107, 1993

<sup>11</sup> Malinski, C. et al.: *Acta Pol. Pharm.* 50: 413-416, 1993

<sup>12</sup> Huang, T.S. et al.: *J. Endocrin Invest.* 17: 787-791, 1994

<sup>13</sup> Japan patent: JP 86-4642860305

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In cell culture, synthetic humin analogues block the human immunodeficiency virus (HIV)<sup>14</sup>, the paralysis<sup>15</sup>, and herpes virus<sup>16</sup> which fact may have an outstanding importance.

The anti-allergic effect (e.g.: in "hay fever") of salts, formed by humic acids with alkali metals, was also demonstrated.<sup>17</sup>

The dermatological efficiency of humic acids is noteworthy. Salts of humic acid formed with ammonia and alkali metals significantly shortened the time of wound healing<sup>18</sup>.

The humic acids were demonstrated to inhibit the reproduction of malignant tumor cells. Thus, they can be useful in the anti-cancer therapy, too<sup>19</sup>.

The humic acids, as natural absorbents of the ultraviolet light, may protect the human skin<sup>20</sup>.

A radiation protective effect of humic acid in rats was demonstrated using its sodium salt. The lethal effect of gamma radiant <sup>60</sup>Co was prevented by 50 percent in animal experiments<sup>21</sup>.

Significant therapeutical opportunities open to the more and more wider utilization of the antiphlogistic effect of the humic acids, especially in the treatment of arthritis.<sup>22</sup>

It has been reported several times that humic acids possess toxic heavy metal binding capacity<sup>23</sup>.

<sup>14</sup> Schneider, J.: *Virology* 218: 389-395, 1996

<sup>15</sup> Eur. Pat. Appl.: EP: 537430 A1 93421

<sup>16</sup> Thiel K. et al.: *Pharmazie* 36: 50-53, 1981

<sup>17</sup> Eur. Pat. Appl.: EP: 530455 A1 930310

<sup>18</sup> Eur. Pat. Appl.: EP: 537429 A1 930421

<sup>19</sup> Juresik, I.: Meeting of Int. Humic Subst. Soc., 6th Meeting, 1994

Editor: Senesi, Nicola; Miano Teodoro p.: 1331-6

Publisher: Elsevier, Amsterdam.

<sup>20</sup> Ger. Offen: DE 93-4318210 930601

<sup>21</sup> Pukhova, G.G. et al.: *Radiobiologiya* 27: 650-653, 1987

<sup>22</sup> Rushev, D. et al.: *Guminovje Veschestva Biosfere*,

Editor: Orlov D.S.: p.: 219, 1993

Publisher: Nauka, Moscow, Russia

<sup>23</sup> Kloocking, R.: Int Meeting of Int. Humic Subst. Soc., 6th Meeting, 1994

Editor: Senesi, Nicola; Miano, Teodoro p.: 1245-57

Publisher: Elsevier, Amsterdam

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The formerly discussed therapeutical applications already introduced and the potential curative indications outline the possibility of further basic research activity.

**2.3. THE HUMIC ACID METAL CHELATE**

For the therapeutic application, the most interesting physiological effects can be attributed to the metal binding capacity of humic acids. Metal binding capacity of humic acids is based in part on the chelate forming. The development of chelate-binding masks the charge of the metal ion. The chelate-bound metal loses its hydrate cover and gets the hydrophilic/hydrophobic characteristics of the chelate-forming compound. Thus, the chelate could theoretically easily pass the hydrophobic cell membrane.

The metal-humic acid interactions are selective. Namely, humic acids, binding the heavy metals (lead, cadmium, mercury) which are toxic, mobilize and eliminate them from the organism, and the same time some vital macro- and microelements are transported by the humic acids into the body to the specific enzymes. The humic acids can affect several biological processes with hitherto unknown mechanism.

Recently, it became evident that selenium (selenite) is essential to the function of antioxidant enzymes (e.g.: glutathion-peroxidase) which are responsible for the elimination of free radicals. This mechanism is important everywhere where increased formation of free radicals is present (radiation effect, tumor, increased degradation of lipid and protein, long-lasting starvation, etc.). In the organism, the lack of selenium causes the deficient function of the muscular tissue and the tumorigenic effect of cadmium or lead increases possibly in humans and certainly in animals, respectively. Sufficient selenium supply can prevent the cardiomyopathy and the muscular dystrophy. The molybden (molybdate) content of the diet assures the cofactor of xanthin oxydase, aldehyde oxydase and sulphite oxydase<sup>24</sup>. The vanadium (vanadate) component inhibits the phosphatases which control the intracellular signal transduction and, thereby, can prolong the duration of the action of hormones. In diabetes, the gene

<sup>24</sup> Rajagopalan, K.V.: *Nutr. Rev.* 45: 321-328, 1987

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expression of certain enzymes changes<sup>25</sup>.  $K^+$  in the preparate is the most important intracellular cation which has central role in the stimulus conduction, and in the maintenance of basic life processes.  $K^+$  deficiency may occur following drug treatment (e.g.: diuretics) or because of some diseases. Beside these metal ions, HUMET®-R contains further six bivalent metal ions which are in chelate-binding with the humic acids.

### 4. BIVALENT METAL IONS AND THEIR PHYSIOLOGICAL ROLE

Several monographs widely discussed the biological role of the bivalent metal ions which may be summarized as follows.

- Iron (Fe) is the basic component of the functional group of hemoglobin and myoglobin transporting the oxygen and of the electron-transporting cytochromes. There are clinical symptoms of iron deficiency (fatigue, headache, stomatitis, gingivitis, loss of appetite, etc.) In chronic deficient state, hypochrome anemia with microcytemia and bone marrow hyperplasia develop. The administration of iron to the organism is influenced by the presence or absence of the other micro-elements. At the same time, the iron intake potentiates the elimination of the toxic lead.
- Magnesium (Mg) is a natural calcium antagonist which influences the metabolism of calcium, phosphorous and sodium. Mg is the activator of the glycolysis, it has significant role in the protein metabolism. It modifies the muscular function, participates in the maintenance of the circulatory homeostasis, decreases the blood pressure (relaxation of the vascular smooth muscle). Mg has a role in the energy metabolism and in the reproductive function. Mg deficiency is manifested in spastic responses.
- Zinc (Zn) is the component of several enzymes. It has a central role in the formation of the steric structure of insulin and in the synthesis of DNA and RNA. The presence of Zn is especially important in lead and cadmium exposition: its administration decreases the toxicity of these metals. Zn deficiency causes

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<sup>25</sup> Brichard, S.M. et al.: Diabetologia 37(11): 1065-72, 1994

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typical symptoms (dermal changes, alopecia, disturbance in the testicular development, sexual retardation, hepato- and splenomegaly, growth disturbances, delayed wound healing, decreased immunological defensive function. The level of Zn may decrease following the adverse effect of corticosteroid or diuretic therapy, in sickle cell anemia, lung tumors or myocardial infarction and in consequence of anticoncipient use.

Copper (Cu) has significant role in the hemopoiesis, cell-oxidation, energy metabolism and in the cerebral catecholamine metabolism. It influences the iron- and zinc balances, and the reproductory functions. Its lack may be one of the causes of infertility and the cadmium toxicity increases. Consistent Cu deficiency evokes anemia, bone marrow alterations, growth retardation, cerebral dysfunction and myocardial destruction.

Cobalt (Co) influences the iron metabolism. It increases the hemoglobin concentration in the red blood cells. Co is the metal component of the prosthetic group of the vitamin B<sub>12</sub>. It is one of the components of  $\beta$ -lysine-isomerase, glycerin dehydro-genase, and methionin aminopeptidase<sup>26</sup>.

Manganese (Mn) actively influences the development of the bones. It is characterized by a less effective absorption due to the competitive antagonism of calcium. It has an important role in the integrity of biomembranes. Out of most, it is the constituent of the farnesil-pysophosphate-synthetase enzyme, which takes place in the synthesis of cholesterol needed to build up the membranes. The manganese containing super-oxyde-dismutase enzyme jointly with Cu-Zn-super-oxyde-dismutase protects the membranes from the harmful effect of super-oxydes. Its absence can cause dermatitis, symptoms similar to diabetes under glucose charging, pigment disturbances of hair, growth problems and infertility.

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<sup>26</sup> Arfin, S.M. et al.: *Proc. Natl. Acad. Sci. USA* 92(17): 7714.18, 1995

### 3. PRECLINICAL INVESTIGATION

#### 3.1. TOXICOLOGICAL INVESTIGATIONS

##### 3.1.1. Acute toxicity studies in rats (with 14-day s post-treatment observation period)

Acute toxicity studies were performed in form of "limit tests" in two species (mouse and rat), in compliance with the GLP regulations, for the determination of the acute oral LD<sub>50</sub> values.<sup>27</sup>

These studies were carried out in male and female Wistar rats and in CFLP mice. At the beginning of the studies, the animals were 5-6 weeks old.

The animals were treated with the humic acid supplemented with trace elements (supplemented humic acid, SHA) which is the active ingredient of the HUMET®-R, in total amount of 40 ml/kg. Related to the standard humic acid prepare containing 15 mg/ml, this 'total amount' corresponds to 600 mg effective dose of humic acid.

The animals were fasted for 18 hours, the active principle of HUMET®-R (SHA) was given (p.o.) via gavage in a volume of 10 ml/kg, twice per 24 hours (10 males and 10 females in every group). Control groups (10 for each gender) were given in physiological saline in the same volume as the active treated ones. Animals were maintained for further 14-day (post-treatment observation period).

The lethality per group, the body weight of the animals during the post-treatment period were studied. The necropsy did not reveal any pathological changes in the several organs.

The single-dose administration of SHA did not cause altered behavioural effects or any other pathological changes.

Acute oral LD<sub>50</sub> value of this trace element prepare could not be properly determined in Wistar rats and CFLP mice. There was no lethality after the doses applied. LD<sub>50</sub>(male) > 600 mg/kg; LD<sub>50</sub>(female): > 600 mg/kg in both species<sup>27</sup>.

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<sup>27</sup> Kovács, M. et al.: H-M Document HUMET 35501, 1996

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3.1.2. Subacute (4-weeks) dietary treatment of rats with HUMET®-R

In the course of a four-week treatment, the effects of HUMET®-R on the hematological parameters, and the body weight gain were studied.

At the end of the observation period, the mass of several organs (lung, liver, spleen, kidney) were registered and the possible macroscopic changes were studied in the several organs.

Treatment was carried out in five groups (n = 10), the animals were given 5, 15, 50, 150, 500 mg/kg/day doses in the different groups. The amount of the HUMET®-R preperate was related to the dried content mixed with the trace element deficient food. Control group was supplied with normal food.

The results obtained in connection with the rats fed in four weeks with food completed with HUMET®-R can be summarized as follows:

It was demonstrated that the humic acid containing preperate HUMET®-R *did not affect* the animals' general physical state (motility, food intake), the weight of the whole body and that of different organs, the hematological and blood chemistry parameters during the four-week diet treatment.

The animals fed with 150 and 500 mg/kg/day doses of HUMET®-R lost their appetite and consequent weight loss developed three weeks after starting the treatment but, the hematological and blood chemistry parameters remained unchanged.

HUMET®-R did not reduce the survival after of the animals in any dose-group; lethality did not occur. There was no significant difference between the control and HUMET®-R treated any dose group of animals in the value of hematological or blood chemistry parameters.<sup>28</sup> No significant difference was shown.

3.1.3. Cumulative toxicity evaluation in rats

Aim of the study was to determine the cumulative toxicity of HUMET®-R.

Study was performed in control and treated Wistar rats (n = 10).

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<sup>28</sup> Gacsályi, A. et al.: *H-M Doc. HUMET 032*, 1995

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The results of the previous studies shown the product to be non-toxic. The LD<sub>50</sub>-value became arbitrarily selected to be 150 mg/kg. Rats were orally treated with 9, 13.5, 20, 30, 45 and 68% of this LD<sub>50</sub> value (13.5, 20.3, 30.0, 45.0, 67.5, 101.3 mg/kg/day), and each was given daily volumes of 5 ml/kg in an overall period of 24 days.

Following the treatment, the total body weight and the relative organ weight (thymus, lung, heart, liver, spleen, kidney, adrenals) were determined. Each of these organs was histologically processed. The hematological parameters were determined and differential blood-count was taken. Serum iron value and level of the thyroid hormones (T<sub>3</sub> - T<sub>4</sub>) were measured, too.

According to the results of this study, HUMET®-R preparate did not evoke any changes related to cumulative toxicity. The decrease of the leukocyte count, the hematocrit, the MCV and serum iron could not be taken for a toxic effects.

Other biologically detectable changes were never observed.<sup>29</sup>

### 3.1.4. Mutagenicity studies

In the Ames test, the mutagenicity activity of the lyophilized HUMET®-R preparate was determined. The study was carried out in several strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1537, TA 1538) both in the presence and absence of liver microsomal fraction, activated with Arochlor 1254, using positive and negative control groups. (Concentrations studied of HUMET®-R were: 469, 938, 1875, 3750, 7500 microgram per plate.)<sup>30</sup>

Results of the study showed that HUMET®-R did not possess any mutagenic activity after the administration of the above concentrations, in this test.

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<sup>29</sup> Dési, I.: *H-M Doc. HUMET 016*, 1993

<sup>30</sup> Oláh, B.: *H-M Doc. HUMET 008*, 1992

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### 3.2. PHARMACOLOGICAL INVESTIGATIONS

In the course of the animal experiments, primarily the biological effects of Supplemented Humic Acid (SHA), being the active principle of HUMET<sup>®</sup>-R Syrup, were studied in being well controlled animal experiments.

#### 2.1. Study of the immunological effects

To date, HUMET<sup>®</sup>-R was studied in so-called non-controlled clinical studies involving volunteers but, most of them suffered from a tumor disease. In these cases the treatment resulted, in part, the cease of growth of the tumor, or its healing. It should be stressed however, that in these studies the administration of HUMET<sup>®</sup>-R syrup was made in the form of adjuvant therapy.

As it is known, the tumors are cellular agglomerate with abnormal reproduction capacity occurring in several tissues. They have the ability, by deceiving the immune system of the body, to proliferate abundantly and finally to destroy it. In this study our purpose was to determine how the growth and proliferation of the tumor cells can be inhibited by the administration of HUMET<sup>®</sup>-R (supplemented humic acid, SHA).

In this study, C57BL (black)6J male mice were used, their mean age was 3 months, the mean body weight 20 g was.

SHA was mixed into the drinking water in a concentration of 1.5 ml/L.

For studying the effect on the immune system, several cell sets were given subcutaneously into the trunk area above the hip. The growth rate of the tumors, injected subcutaneously, and its possible inhibition by the SHA were studied.

The experimental results showed that the tumor could grow *only* in untreated animals inoculated with the least amount of tumor cells ( $10^3$  cell/animal). Such difference was not seen after inoculation with hundredfold higher concentration of the inoculation ( $10^5$  cell/animal) but, the growth of tumors was lower in the treated than in the control

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animals. Among the groups of animals inoculated with one million tumor cells, half of the treated animals were alive 2.5 months after beginning the study. However, already 7 weeks after inoculation, the control animals had to be over-anaesthetized to spare them of suffering due to their enormous tumors.

Subcutaneous tumors developed also in the treated animals but, the animals gnawed out their tumors causing great wounds. The majority gnawed wounds cleanly healed without infection, and the remaining part of the tumor did not grow in most cases.

Such response of animals is usual in the development of inflammatory processes. The study convincingly demonstrated the positive effect of this preparate (SHA) upon the immune system and this can give an explanation for the tumor-inhibitory effect, too.<sup>31</sup>

### 3.2.2. Effect on the iron metabolism

The objective of these studies was to obtain

- Information about the veterinary therapeutic usefulness of HUMET®-R and an iron-chelate preparate in sows and piglets and
- further experimental evidence about the effect upon the iron-deficient anemia of rats treated with SHA and several granulates of solid physical state (this latter investigation was carried out in GLP-conform study).

#### *3.2.2.1. Study of the effect on the iron intake in piglets and sows*

Among the conventional animal breeding conditions, the iron-deficiency anemia of the piglets regularly develops. In the development of iron-deficiency anemia the following factors are involved:

- the very low iron level in the body of the newborn piglet which is the lowest in comparison with the other mammals;
- increasing iron-deficiency due to the rapid growth;
- the low iron level of the milk of the sows which is the unique food for the sucking-pigs.

The pigs, born with body weight of 1.2-1.5 kg and 30-50 mg iron reserve, are provided with totally 30 mg iron during the first 5 weeks of life. At the same time, 120-150 mg daily iron intake would be needed

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<sup>31</sup> Duda, E., Nagy, T., Tubak, V.: *H-M. Doc.* 44-3-11, 1997

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for the synthesis of hemoglobin, and enzymes containing myoglobin or iron (cytochrome, cytochrome-oxidase, catalase, peroxidase, etc.).

For the prevention of iron-deficiency caused anemia of pigs, the *parenteral iron treatment* has become widely used which caused a sudden increase of iron content of the blood and the saturation of transferrin was nearly 100%. However, the hindrance of the parenteral iron therapy is the inhibition of the mechanism which promotes the iron absorption. Natural way of the prevention of iron deficiency is the *oral administration of the iron*. Among the preparations of such type we can find the HUMET<sup>®</sup>-R syrup.

100 ml of HUMET<sup>®</sup>-R syrup was applied on 30 g perlite and this provided 140 mg iron intake for litter consisting of 10 pigs. Under suitable experimental conditions and nutrition, the following conclusion may be drawn:

The highest total body iron content of newborn pigs was found in the off-spring of sows supplied with HUMET<sup>®</sup>-R; the second was the group supplied with iron-chelate. The lowest iron level was stored in the animals of the untreated control group.

The blood hemoglobin content of pigs of sows, which were fed with HUMET<sup>®</sup>, was significantly higher than that of either the control group or the iron-chelate consuming group.<sup>32</sup>

**3.2.2.2. Study of the effect of trace element supplemented humic acid prepartate (SHA) in iron-deficiency rat model**

Pregnant Sprague-Dawley rats were fed with normal, (control) and iron-deficient (Fe < 10 ppm) rodent food during the entire gestation and lactation period. After weaning, the following groups were formed:

control group: feeding with normal food,  
iron-deficiency group: feeding with iron-deficient food  
group fed with SHA: feeding with iron-deficient food  
Reference substance was Aktiferrin syrup.

Doses:	SHA	0.66 ml/kg (i.e.: 3.7 mg Fe <sup>++</sup> /kg)
	Aktiferrin	0.54 ml/kg (i.e.: 3.7 mg Fe <sup>++</sup> /kg )

<sup>32</sup> Gundel, J. et al.: *H-M Doc. HUMET 037*, 1995

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Following the weaning, the hematological parameters of the control and the iron-deficient (anemic) offspring individuals were determined before starting the 21-day treatment with either SHA or the reference substance (Aktiferrin).

Offspring individuals, exposed to iron-deficiency pre- and postnatally, had body weight 60% less than that of the control ones, the red blood cell (RBC) count, mean volume of erythrocytes (MCV), hemoglobin (Hb) hematocrit(Htc), serum iron concentration (Fe), and transferrin saturation (Sat) were significantly lower, the ratio of zinc-protoporphyrine/hem (ZP) and total iron binding capacity (TIBC) were significantly higher.

Both in the offspring individuals treated with Aktiferrin- or SHA, the value of Hb, Htc, MCV reached, the value of RBC, ZP, Fe and TIBC approached the values of the control group. There was no significant difference between the effects of Aktiferrin and SHA.

Conclusively, according to the animal experiments, SHA (HUMET®-R) is outstandingly suitable for the therapy of the iron-deficiency anemia and it is equieffective with Aktiferrin regarding the iron content.<sup>33</sup>

### 3.2.3. Investigation of the cardioprotective effect

Heart failure and several types of arrhythmias due to ischemic heart diseases play central role in the mortality of the cardiovascular diseases.

The aim of the study was to demonstrate the antifibrillatory effect of the supplemented humic acid (SHA) during a reperfusion period following 25 min coronary occlusion in isolated rat cardiac preparation. It was possible to obtain experimental data about cardioprotective action of HUMET®-R Syrup given in repeated-dose long-term administration.

SHA was administered in an oral dose of 10 mg/kg for two weeks. At the end of the treatment, the heart was exteriorized, a canule was inserted into the aorta and perfusion was carried out for 10 min

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<sup>33</sup> Szakmári, É., Hudák, A.: *H-M- Doc. 42-1-11*, 1997

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maintaining constant perfusion pressure, according to Langendorf. During this period, a canule was introduced into the right atria using Neele's method. The value of 'preload' and 'afterload' was kept constant during the entire period of the experiments.

In these experiments, the coronary blood flow, the aortic blood flow, the heart rate, the left ventricular enddiastolic pressure (LVEDP) were measured and the ratio of onset of the ventricular fibrillation (VF) and the first derivative of the upstroke phase of the left atricular pressure (i.e.: contractility:  $dp/dt_{max}$ ) were calculated.

The results of the experiments are shown in the Table 1.

**Table 1: Anti-ischemic effect of 2-week EHA-treatment**

Treatment	CBF ml/min	AF ml/min	HR min <sup>-1</sup>	VF %	+dp/dt <sub>max</sub> kPa/s	LVEDP kPa
before ischemia (n = 8)	22.9 ±0.9	43.4 ±1.5	265 ±6.0	0	1026±45	0.51 ±0.04
after ischemia	20.4 ±0.9	13.3 ±2.5	260 ±3.9	87.5	609 ±53	1.53 ±0.09
SHA treatment 10 mg/kg p.o. for 2 weeks (n = 8)	24.5* ±0.8	24.5* ±2.8	263 ±3.0	12.5*	788* ±36	1.09* ±0.08

p<0.05 X ± S.E.M. (after ischemia or SHA)

A two weeks oral administration of 10 mg/kg SHA could improve all parameters which became pathologic after ischemia. Although the dose-dependent character of the response remained to be studied, the cardioprotective effect of SHA seemed to be proven<sup>34</sup>.

<sup>34</sup> Ferdinandy, P.: H-M-Doc. 39-1-08, 1997

## HUMET®-Syrup

### 3.2.4. Effect of HUMET®-R on the mobilization of a toxic heavy metal in pigs

Experiments were performed in pigs (body weight range was 16.2-18.2 kg at the beginning of the study). HUMET®- R Syrup was given in three different doses 2.5; 7.5; 20 ml/day/pig (i.e.: 1.1, 3.3, 8.8 mg/kg/day humic acid).

The aim of the study was to investigate the effect of the preparate on the elimination of <sup>203</sup>Hg isotope from the organism and vital organs.

The treatment with HUMET®- R Syrup started 5 days before the administration of the Hg-isotope and lasted 11 days after the administration of the isotope. The changes of the elimination from the total body and the fate of radioactivity of feces, urine, and several organs in response to the administration of the preparate.

The results of the study were summarized in the Table 2:

**Table 2: Influence of HUMET®-R on the mobilization of <sup>203</sup>Hg isotope (expressed in percent of Hg activity; 503.9 kBq = 100%).**

Dose mg/kg/day	Feces	Urine	Feces/Urine	Total
Control (n = 4)	52.8 ±2.3	12.1 ±4.4	4.36	64.9±2.8
1.1 (n = 4)	53.8 ±4.0	13.0 ±4.4	4.14	66.9±0.9
3.3 (n = 3)	60.2 ±6.1	15.4 ±7.9	3.91	75.6±1.9
8.8 (n = 4)	67.9 ±9.5	18.1 ±10.1	3.71	86.6±3.5

mean ± S.E.M.

From the above results one can observe that HUMET®-R Syrup increases the amount of <sup>203</sup>Hg in the feces, the urine and, total value, in tendency. Apart from the lowest dose (1.1 mg/kg/day), the same trend was observed in the other organs as well, however, no numerical value was given due to the great standard deviation and the low case number. The effect proved to be dose-dependent. Namely, increasing

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the doses, the biological effect increases. In spite of the non-significant data, it seems evident that one can obtain positive results by performing the experiment with the suitable animal number.<sup>35</sup>

3.2.5. Effect of the humic acid on the regeneration of the hemopoietic system

According to the results of our preliminary experiments, there is a possibility to develop a therapeutical preparate for the prevention or regeneration of the damage of the hemopoietic system by using the humic acid and the endowed humic acid.

It is known that the humic acids are heteropolycondensates containing highly variable components, they are allomelanins which can be found in the soils, in coals and peats which develop during the slow decomposition of plant residues by means of their chemical and biological transformation.

They contain polymerized phenolic macromolecules, and their composition highly depends on the place and time of their origin.

Their chelate-forming with metal ions, especially, their iron-binding activity, are well-known. Their practical application has been discussed since the fifties in the literature.

Our humic acid preparation, which is the basic material of HUMET<sup>®</sup>-R, can affect advantageously the regeneration of the hemopoietic system damaged by <sup>60</sup>Co-gamma irradiation.

We could not find any data or reference in the literature about such biological effectivity of the humic acid.

Detailed examination was performed in animal experiments using several doses of whole-body gamma irradiation to develop a treatment procedure which would be applied in the human therapy, as well. The biological effectivity of the humic acid preparate produced was demonstrated in the following experimental arrangement:

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<sup>35</sup> Sarudi, I., Rétfalvy, T., Lassú, I.: *H-M Doc. 45-1-12*, 1997

## HUMET®-Syrup

### I. Experimental animals

At the beginning of these experiments the animals were randomized according to body weight. They were male animals (b.w.: 190-220 g) of Wistar strain (HUMAN Vaccine and Pharmaceuticals, Co., Ltd., Gödöllő, Hungary). The animals were kept in rooms with controlled temperature ( $23 \pm 3^\circ\text{C}$ ), relative humidity of  $60 \pm 10\%$ , and alternative lighting (light / dark cycle by 12 hours) and in type II plastic cages (5 animals / cage). Drinking water and normal and humic acid enriched foods were provided *ad libitum*.

Rats were adapted to the experimental conditions for two weeks. During the experiments, the general clinical state of the animals was controlled daily.

### II. The active substance applied

The preparate which contained 5-15% humic acid was given by gavage in several doses to the experimental animals.

### III. Whole-body irradiation

Whole body irradiation of the rats was carried out in special plastic restraint cage (40 animals/cage). This dose of irradiation was 7.0 Gray (Gy) (dose intensity: 0.82 Gy/min). The  $\text{LD}_{50/30}$  value: 7.5 Gy, which was characteristic for this rat strain.

### IV. Hematological examinations

On the day 0, 7, 14, 21, and 28 of the experiment the abdominal aorta was prepared for blood sampling in ether anesthesia. Hematological parameters determined were: leukocytes (WBC), red blood cells (RBC), hemoglobin (Hgb), hematocrit (Htc), platelet (TRO), reticulocyte (RET). Determination was made with types PHA-1 and PHA-2 automatic device (made by MEDICOR, Hungary). The measurement error of the system was 1-3%.

### V. Experimental groups

In these experiments, the treatment with several doses of humic acid preparate was performed in groups of 30 animals. On the Figures, the mean values characteristic to the Wistar strain were always demonstrated.

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- Group 1: Whole body irradiation with 7 Gy <sup>60</sup>Co gamma radiation (standard food + drinking water)
- Group 2: 7-days pretreatment with humic acid (240 mg/animal/day active ingredient), then whole-body irradiation with 7 Gy and further four-week treatment with 240 mg/animal/day dose of humic acid
- Group 3: Whole-body irradiation with 7 Gy then, single-dose administration of 240 mg/animal/day dose of humic acid
- Group 4: Seven-days pretreatment with humic acid (90 mg/animal/day), then whole-body irradiation with 7 Gy and further four-week treatment with 90 mg/animal/day dose of humic acid
- Group 5: Whole-body irradiation with 7 Gy then single-dose treatment with 90 mg/animal/day dose of humic acid

The statistical analysis of the Student test was used for the evaluation of the experimental data.

### VI. Results

Evaluation of the hematological and chemical parameters demonstrated, that independently of the treatments applied, each value was in the proximity of the reference value corresponding to the regeneration of the hemopoetic system, at the end of the experiment (28 day).

The hematological parameters of the rats treated in different manner, the changes of leukocytes (WBC) and thrombocytes of the 240 mg/animal/day treated group and those of the 90 mg/animal/day treated group were demonstrated on the Fig 1, Fig.2 and on the Fig. 3, Fig 4, respectively.

#### *The results obtained after treatment with 240 mg/animal/day dose*

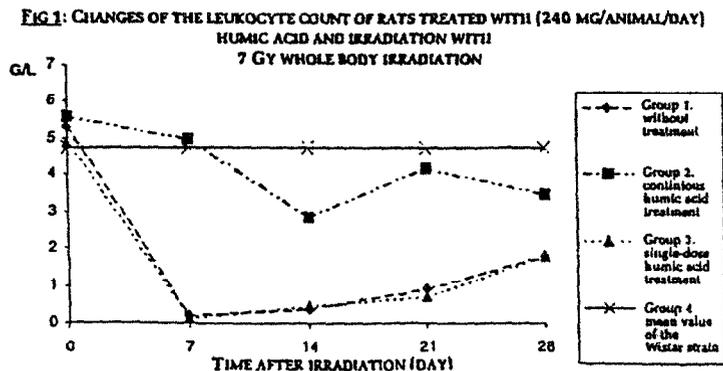
It was stated that the count of leukocytes and thrombocytes significantly decreased one week after whole body irradiation (Group 1, control group) and in animals which were treated with humic acid by single administration (Group 3). In case of the single-dose administered humic acid, only the thrombocyte count showed moderate increase of regeneration during the third week.

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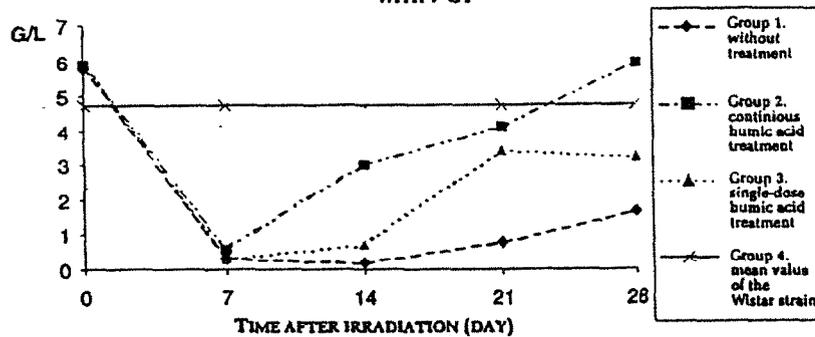
In the animals of the control group (Group1), the regeneration of the counts of both leukocytes and thrombocytes appeared only on the third week after irradiation.

In the animals which were pretreated and following the 7 Gy whole-body irradiation further treated with 240 mg/animal/day dose of humic acid (Group 2), there were no damage of the hemopoetic system. Thus, the count of leukocytes and thrombocytes remained in the proximity of the reference values which were characteristic to the Wistar strain.

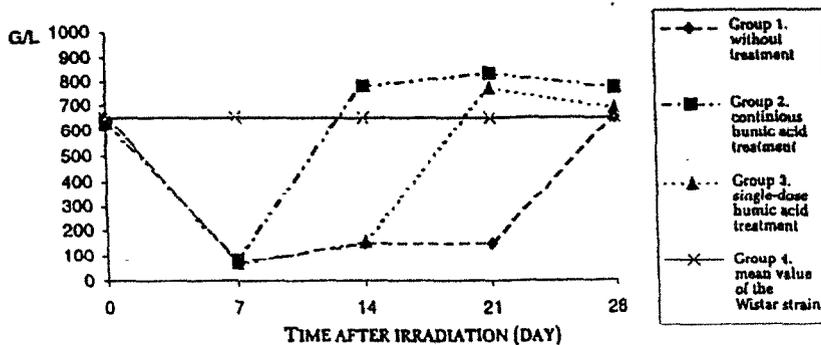
Our experimental results demonstrated that humic acid/humin prepartate given in adequate dose (240 mg/animal/day) and using the appropriate treating arrangement (pretreatment at first, then maintenance treatment after irradiation), could prevent the damage of the hemopoetic system due to high-dose irradiation with ionization irradiation (Fig 1 and Fig 2).



**FIG 3: EFFECT OF HUMIC ACID (90 MG/ANIMAL/DAY) TREATMENT ON THE CHANGES OF THE LEUKOCYTE COUNT OF ANIMALS IRRADIATED WITH 7 Gy**

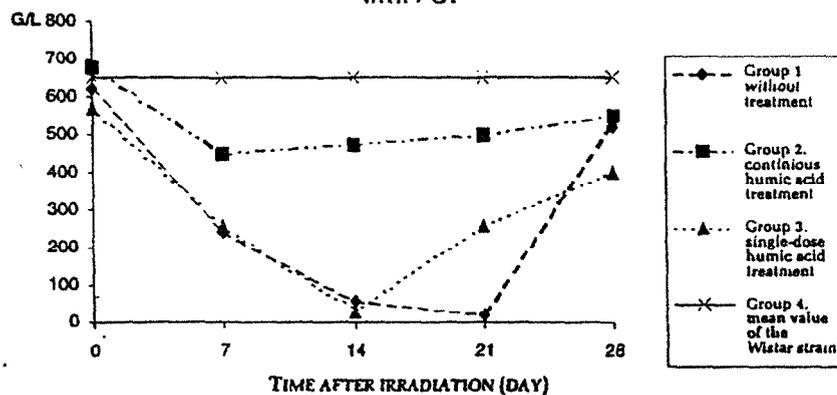


**FIG 4: EFFECT OF HUMIC ACID (90 MG/ANIMAL/DAY) TREATMENT ON THE THROMBOCYTE COUNT OF RATS IRRADIATED WITH 7 Gy**



Conclusively, one can state that after a whole-body irradiation with high-dose <sup>60</sup>Co gamma radiation, the normalization of radiation-caused hemopoietic changes were evoked by several therapeutic doses of humic acid prepartate.

FIG 2: CHANGES OF THE THROMBOCYTE COUNT OF THE RATS  
TREATED WITH (240 MG/ ANIMAL/DAY) HUMIC ACID AND IRRADIATED  
WITH 7 GY



*Results obtained after the treatment with 90 mg/animal/day/dose:*

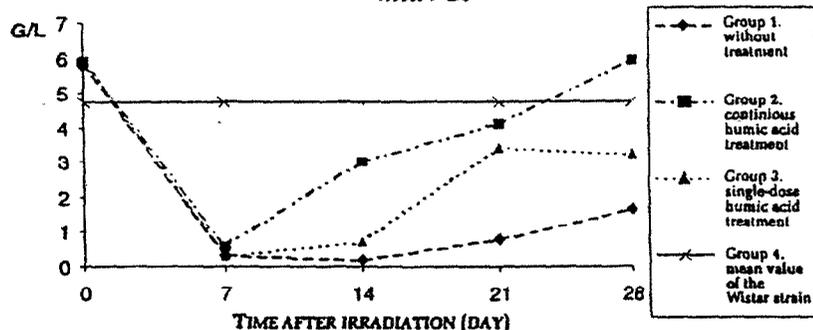
One week after whole-body irradiation, both in single-dose and continuously treated animals, the *leukocyte count* and the *thrombocyte count* significantly ( $p < 0.05$ ) decreased.

Low cell count was measured also in the second week after irradiation in the irradiation control (Group 1) and single-dose treated (Group 5) animals.

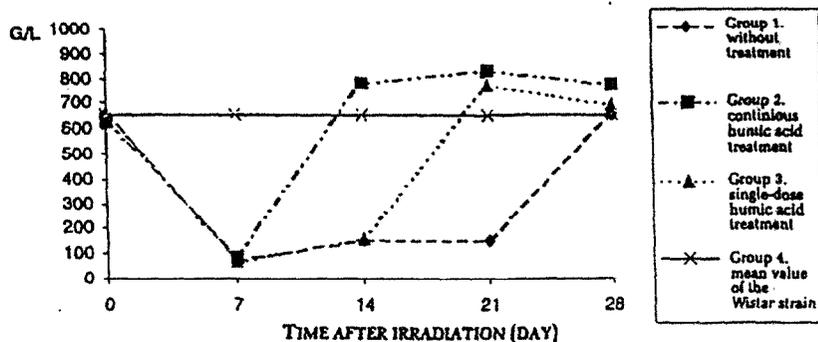
In the animals of the control group which were only irradiated (Group 1) the regeneration of both leukocyte and thrombocyte counts started only after the third week.

In the animals given 90 mg/animal/day humic acid treatment, if humic acid *pretreatment* was used (Group 4), the regeneration of both cell types started intensively already after the first week and till the end of the second week, the values were similar to those of the control animals (Fig 3 and 4).

**FIG 3: EFFECT OF HUMIC ACID (90 MG/ANIMAL/DAY) TREATMENT ON THE CHANGES OF THE LEUKOCYTE COUNT OF ANIMALS IRRADIATED WITH 7 GY**



**FIG 4: EFFECT OF HUMIC ACID (90 MG/ANIMAL/DAY) TREATMENT ON THE THROMBOCYTE COUNT OF RATS IRRADIATED WITH 7 GY**



Conclusively, one can state that after a whole-body irradiation with high-dose <sup>60</sup>Co gamma radiation, the normalization of radiation-caused hemopoietic changes were evoked by several therapeutic doses of humic acid preparate.

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The most advantageous effect was obtained in the animals which were also pretreated (before irradiation) with the preparate of the patent.

The results of these experiments which were demonstrated as examples showed that humic acid/humin preparate might be properly applied for the prevention of the damage of the hemopoetic system of several origin, or for the efficient facilitation of the regeneration of the hemopoetic functions already damaged.

The biological efficacy of the humic acid/humin preparate provided the possible successful application of this therapeutic material in the human therapy in patients who were exposed therapeutically or accidentally to ionization radiation (reactor accident or accidental ionization of patients or staff). Further experiments show that above preparate is suitable for the acceleration of the regeneration of the hemopoetic damage due to chemotherapeutic treatment.<sup>36</sup>

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<sup>36</sup> Naményi, J. et al.: Patent application number: P97 011093

## 4. CLINICAL OBSERVATIONS

### 4.1. STUDY OF THE EFFECTS ON THE SERUM IRON LEVEL

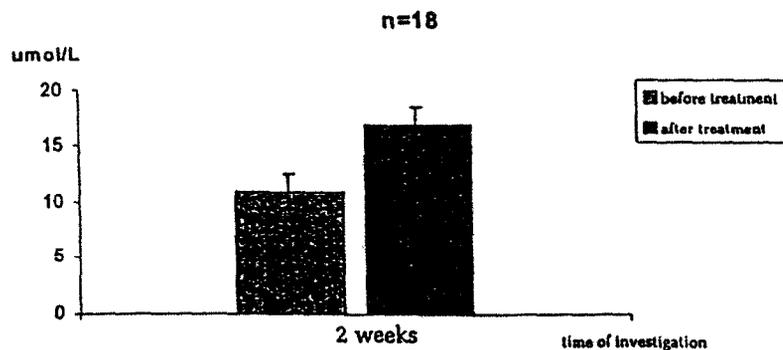
The study was performed in an open clinical trial (Dept. of Pediatrics, Erzsébet Hospital, Hódmezővásárhely, Dr. P. Szűts, Dr. P. szó) in anemic children (age 1-18 years).

Anemia was diagnosed by complete blood count analysis from a blood sample taken before oral administration of HUMET®-R Syrup.

In case of small children, 2x1 ml (for age 1-2 years), 2x2.5 - 5.0 ml (for age 2-3 years old) of HUMET®-R were given maximum for 3 weeks. Therapeutic efficacy was demonstrated by checking the total body count on the weeks 2 and 4 after treatment. Twenty two persons were involved in this study.

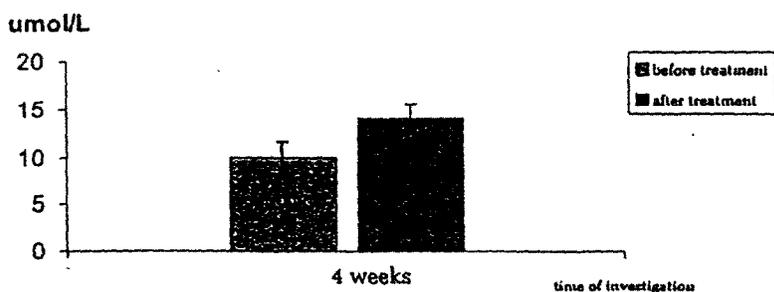
The results are demonstrated in the Figures 5, 6:

**Fig.5:** Effect of HUMET®-R on the serum iron level in children



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**Fig.6: Effect of HUMET<sup>®</sup>-R on the serum iron level in children (n=19)**  
n=19



According to the results of the study, a three-weeks oral treatment with adequate doses of HUMET<sup>®</sup>-R caused significant increase of the serum iron level which was a marked effect on the week 4, too (i.e.: one week after cessation of treatment).<sup>37</sup>

#### **4.2. EFFECT OF HUMET<sup>®</sup>-R SYRUP IN VOLUNTEERS WORKING IN CADMIUM EXPOSITION**

The aim of the study was to study the influence of regular intake of HUMET<sup>®</sup>-R Syrup, on the biological parameters (blood and urine cadmium concentration) of subjects working in cadmium exposition and on their clinical laboratory parameters (hemopoiesis, liver and kidney functions) characteristic to their health status.

The workers involved in the study were employed partly in battery production, partly in over-plating some component with cadmium; the performance of these technical processes needed a very simple activity.

The persons studied worked in cadmium exposition and their blood cadmium level (90 nmol/L) or the urine level (10 nmol/mmol creatinine) remained within the determined biological limit values.

<sup>37</sup> Szűts, P., Koszó, P.: H-M Doc. HUMET 045, 1996

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The patients were examined three-times: record of the basic state (examination No 1) which was followed by a six-week voluntarily intake of HUMET®-R Syrup in daily dose of 10 ml (examination No 2), and on the week 14 after cessation of the treatment (examination No 3).

All patients were symptom- and complaintless and remained capable of work during the entire period of investigation. There was no intermittent disease.

In the course of the study, for characterizing the degree of exposition, the biological exposition parameters of cadmium were measured as follows: the level of cadmium concentration in the blood and urine samples (value of quantitative urine determination was corrected to be the creatinine concentration), the effect on the hemopoiesis (total quantitative blood count, serum iron concentration, iron-binding capacity and saturation), the value of the liver function (serum bilirubin concentration, the activity of aspartateamino-transferase (GOT), alanine aminotransferase (GPT), gamma-glutamyl transferase (GGT), and the values of the kidney function (study of general state, and sediment, quantitative protein concentration of urine, N-acetyl-βD-glucosaminidase NAG) activity, the concentration of serum creatinine, carbamide and uric acid (urate). From the results of the study, the three examinations (basic state, after six-week treatment with HUMET®-R, and on the week 14 after the treatment) of 16 male patients regarding the cadmium concentration of the blood and urine and the results of the other laboratory parameters are demonstrated in the Tables 3.,4.

Table 3: Changes of the blood and urine cadmium content in volunteers

Measurement (n = 16 males)	Blood Cd (nmol/L)	Urine Cd (nmol/mmol creatinine)
Basic state	8.5 ± 5.7 (2.3 - 23.3)	1.0 ± 0.6 (0.3 - 2.6)
6 weeks after HUMET®-R treatment 10 ml/day	7.2 ± 4.8* (2.5 - 18.4)	1.3 ± 0.7* (0.4 - 2.8)
on the week 14 after treatment	6.3 ± 4.6* (1.1 - 17.3)	2.0 ± 1.1* (0.5 - 5.0)

X ± S.D. \*p < 0.05

**Table 4: Effect of HUMET®-R treatment on hematological and urine parameters in cadmium exposition**

Parameter tested	examination No 1	examination No 2	examination No 3	Significant difference p <	
	mean±S.D.	mean±S.D.	mean±S.D.	1 vs.2.	2 vs.3
Red blood cell count (T/l)	5.17 0.17	5.06 0.20	5.01 0.28	0.05	NS
noglobin	152.8	149.1	153.4	0.01	0.05
) )	4.8	6.3	6.2		
Hematocrit (%)	46.1 1.6	44.7 2.3	44.8 1.9	0.01	NS
MCV (fl)	88.8 3.1	87.9 3.4	89.3 3.7	0.01	0.001
Leukocyte count (G/L)	6.6 1.3	6.7 1.8	7.4 1.8	NS	0.01
GPT (U/L)	47.1 36.0	38.6 26.7	41.9 25.1	0.05	NS
Urate (micromole/L)	432.7 110.0	334.6 116.7	340.0 76.6	0.001	NS
Urine protein (mg/mmolcr.)	9.6 17.4	5.9 12.5	6.2 6.8	0. 05	NS

Decrease of the blood cadmium concentration (15 and 13%) and the increase of the urine cadmium concentration (25 and 56%) were significant both in response to the six-week HUMET®-R treatment and at the end of the treatment-free period (week 14). It was noteworthy that at the end of the six-week HUMET®-R treatment the serum GPT activity (from 47.1 to 38.6 U/L), the urate concentration (from 432.7 to 334.6 micromol), the total protein concentration of the urine (from 9.6 to 5.9 mg/mmol creatinine) significantly decreased.

Conclusively it can be stated that the regular daily intake of HUMET®-R Syrup decreased the absorption of the cadmium and increased its urinary elimination. In respect to the outstandingly cumulative potency of cadmium in the human organism, this clinical finding has extraordinary importance from the point of view of the prevention.<sup>38</sup>

<sup>38</sup> Hudák, A. et al.: Orv. Hetil. 138(22): 1411-1416, 1997

#### 4.3. EFFECT ON THE PHYSICAL PERFORMANCE OF THE FIRST-CLASS SPORTSMEN

According to several year of experience, the physical performance of the sportsmen, following extreme load, can be characterized by the parameters as follows

- the blood lactate concentration related to the resting hematocrit value,
- the maximum heart rate,
- the maximum O<sub>2</sub> uptake related to body weight kg,
- and the correlation connection between them.

The effect of three-week continuous daily intake of HUMET®-R prepartate (20 mol/day) was studied in complaintless first-class sportsmen rendered into groups according to the changes related to the resting value of the hematocrit. The blood lactate concentration (LA), the maximal heart rate (HR), the maximal O<sub>2</sub>-uptake related to the kg body weight (RVO<sub>2</sub>) and their correlation connection were studied.

The Htc and Hb values, related to the initial values, depending on the high or low level of the latters, decreased or increased and the mean values remained in proximity of the mean value of the physiological range. LA, HR and RVO<sub>2</sub>, level of the sportsmen, grouped according to the low (<0.43) and high (>0.47) Htc values, were compared. Examining all sportsmen, there was a positive correlation between Htc and RVO<sub>2</sub>, LA and RVO<sub>2</sub> (both Htc and lactate are proportional with the aerobic performance) in low Htc group (value < 0.43), the same correlation was negative in the high Htc group (value > 0.47).

On the base of this three-weeks HUMET®-R treatment one can state that the result of the treatment is the optimalization of the value of Htc and Hb and approximation of the physiological reference values<sup>39</sup>.

<sup>39</sup> Petrekanits, M.: H-MM Doc. HUMET 026, 1992