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## FEATURE ARTICLE

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# Examination of the Immune-Stimulation Effect of Some Plant Homeopathic Drugs

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In medicinal plant research, the period up to around 1970 is characterized by the discovery of strong-acting plant substances similar to heart glycosides, such as ergot, curare or rauwolfia alkaloids. Research of medicinal drugs and phytotherapeutical drugs with less strong or weaker actions has been neglected in particular due to the rise of chemotherapy and under the impression of the great success of antibiotic therapy. Nowadays the main focus of research has shifted. The virtually exhausted reservoir of strong-acting medicinal drugs and the partly negative consequences of chemotherapy, which for a long time was too one-sided and too excessively pursued, have led to a reconsideration of traditional therapy concepts and the revival of preventive medicine.

For medicinal plant research today this means concentrating on all those drugs belonging to the group of weak or weaker-acting drugs. Nowadays this drug group still constitutes the main component of those drugs used in phytotherapy and homeopathy. In contrast to phytopharmacological drugs which usually exist as monosubstances, phytotherapeutic drugs excel due to their large therapeutical range and practically no side effects. But it is exactly these criteria which make it so difficult to prove the action and effectiveness of these preparations, especially as classical pharmacology offers very few in-vitro and in-vivo models with which weak effects can be measured. In addition, many of these preparations are mixed or combination preparations where standardization or adjustment of specific active ingredient content or active values is known to be particularly difficult. We also know from experience that more than a few of these

phytopreparations only become effective after long-term therapy. Appropriate test models to prove this type of active mechanism were unavailable and this is still the case today. And finally we must assume, insofar as one is convinced of the effectiveness of such preparations due to the long empirical application, that the effect of these preparations arises at least in part in a different manner than many of our strong-acting preparations, e.g. via the activation or influence of regulatory systems according to the laws of cybernetics. This applies particularly to homeopathic drugs. It requires no further explanation that such action mechanisms cannot be determined, or are extremely difficult to determine with conventional test models and measuring procedures.

The users maintain that the effectiveness of homeopathic drugs has been proven on humans, but we still do not know today exactly how this comes about. All previous explanations are no more than hypotheses and require exact scientific documentation.

17 years ago we set ourselves the task of proving the action and effectiveness of the preparation group of the so-called reversing agents, agents for non-specific stimulus therapy or influenza treatments.

Some of these preparation groups are nowadays referred to as immune stimulants.

The composition of these preparations reveals the following:

- 1) With only a few exceptions, these are mixed or combination preparations.

- 2) They contain plant extracts, partly in high dilutions or potencies, from the original tincture up to D 12 and higher, whereby the potency range from D 1 - D 6 prevails. These preparations are accordingly to be attributed to the group of homeopathic drugs.
- 3) In addition to plant extracts, some preparations contain inorganic additives, such as sulphur, silicea, graphite, or ferrum phosphoricum, as well as animal toxins such as crotalus, lachesis, naja, apis or acidum formicum.
- 4) Administration is oral (p.o.), intramuscular (i.m.) or intravenous (i.v.).

The following appear very frequently among the drugs used: *echinacea purpurea* and *angustifolia*, *eupatorium cannabinum* and *perfoliatum*, *thuja occidentalis*, *baptisia tinctoria*, *vincetoxicum officinale*, *arnica officinalis* and *calendula officinalis*.

The fact that a part of the plant extracts is applied highly diluted indicates that the effect does not arise or only arises in part due to a direct interaction with pathogenic microorganisms, but primarily due to the effect on the immune system. We have therefore tried, as already reported (Wagner 1983 and 1984a), to achieve proof in three ways:

- 1) by chemical analysis of the drugs contained in these preparations
- 2) by examining injection preparations available on the market in different immunological test systems and
- 3) by controlled studies on healthy and immune-suppressed persons.

The immunological tests used were the granulocyte test (a) (Brandt, 1967, Tympner and colleagues 1978, Stubner 1983), the carbon clearance test (b), (Biozzi and colleagues 1953), the T-lymphocyte transformation test (c), (see e.g. Weir, 1978) and recently the granulocyte bioluminescence test (d), (Allen 1981). Of these tests, a, c, and d are in-vitro tests, whereas test b is conducted on mice. Human granulocytes were used for all in-vitro tests.

In the granulocyte test the phagocytosis index is determined microscopically by counting the phagocytosed yeast particles in comparison to the control sample.

The bioluminescence method compares the quantity of released oxygen and peroxide radicals from granulocytes after pre-incubation with opsonised zymosan and in the presence of a lumigen. A comparative study with aristolochic acid showed that the two methods correlated well, at least in in-vitro tests (Kreutzkamp 1984) (Fig. 1).

The carbon clearance test measures the elimination speed of injected carbon particles from the blood. The elimination speed shown in the form of a regression line is an indication of the efficiency or phagocytosis activity of the phagocytic system (Fig. 2).

In the T lymphocyte test the induction of blastotransformers of normal lymphocytes and their subsequent mitosis rate is measured.

The reason that phagocytosis models were preferred for the tests is due to the fact that macro- and microphagocytosis plays a particularly important role in resisting infection. (Stosel 1974, Lohmann-Matthes 1981).

The tests concerning 1) have so far led to the isolation and structure clarification of some polysaccharides in a series of drugs; these were shown to increase the phagocytosis effect in both in-vitro and in-vivo models (Wagner and colleagues 1984 a and c).

Fig 1: Comparison of the dose-effect graphs of aristolochic acid in the granulocyte and CL test.

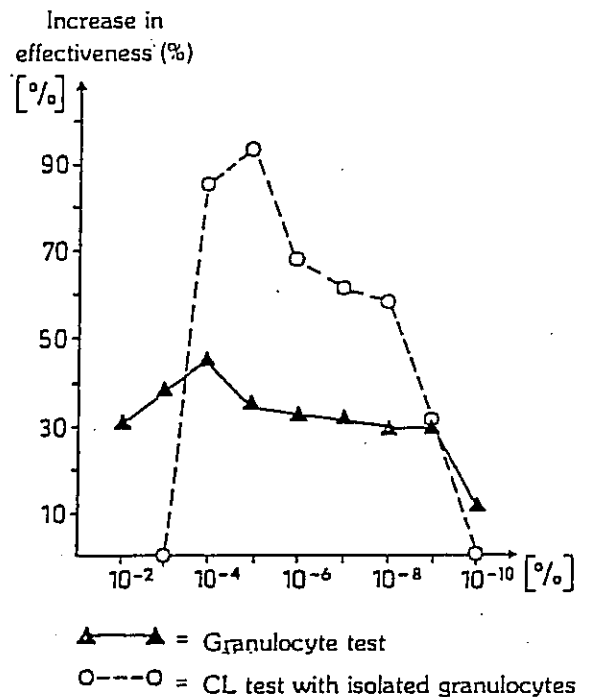
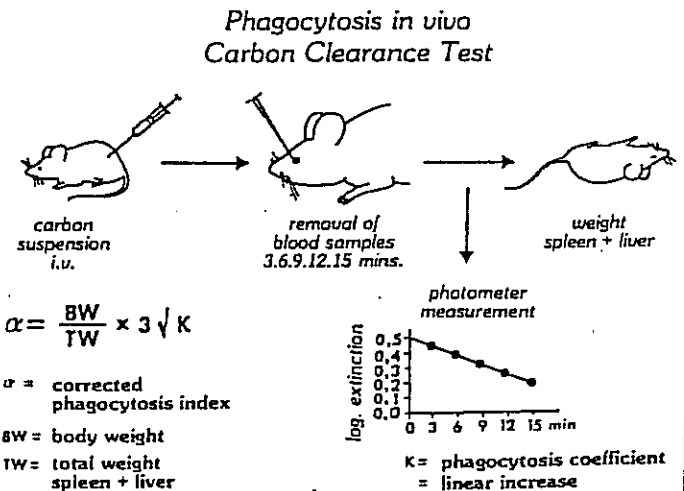


Fig. 2: (from Drews)



The positive results attained in the carbon clearance tests and recently also with bioluminescence measurement were more thoroughly investigated particularly with regard to the echinacea and camomile polysaccharides. The optimum active concentration as determined in these tests lies between  $10^{-2}$  to  $10^{-6}$  mg/ml.

These results correspond to those of Japanese work groups who recognized in particular the fungus polysaccharide lentinan from lentinus edodes, schizophyllan from schizophyllum commune and the protein-bound krestin (PSK) from coriolus vesicolor as effective immune stimulants (Wagner and Proksch 1984b and c). It may therefore now be assumed that particularly structured polysaccharides of certain plants are involved in the immune-stimulating effect of the extracts manufactured from these, at least insofar as a water/alcohol mixture with a high H<sub>2</sub>O content was used in manufacture. Since some extracts are also made with higher-percentage alcohol, there must also be active principles which are more attributable to the lipophilic group of substances. Literature also gives numerous indications of this (Wagner and Proksch 1984b). Thus in the groups of substances: sesquiterpene lactones, alkaloids, chinons, phospholipids and simple phenols, compounds are to be found which are capable of affecting both cellular and humoral immune resistance in the sense of immune stimulation (Wagner 1983). We recently recognized some alkaloids from uncaria tomentosa, especially isopteropodin (Fig. 3), the known emetogenic emetin (Fig. 3), cytotoxic-acting plumbagin (Fig. 3) and some sesquiterpene lactones from eupatorium cannabinum and perfoliatum as potential stimulators of phagocytosis or of the mononuclear phagocytic system (Kreutzkamp and Vollmar 1984). This makes it probable that active ingredients other than the polysaccharides are involved in the immune-stimulating action of echinacea, eupatorium, calendula and other drugs. There are already some preliminary concrete indications in this direction.

Tests of the individual drugs have so far shown the following results:

1. The drugs mentioned contain chemically defined active substances which may be called upon to explain at least a part of the effectiveness described.
2. Most of the compounds tested develop their effectiveness within the concentration range in which homeopathic dilutions or potencies are applied, i.e. concentrations of  $10^{-2}$  -  $10^{-8}$ .

This second result is not entirely surprising. The antigen-acting lipopolysaccharides of gram negative bacteria release an immune response or respectively act pyrogenically at concentrations of  $5 \times 10^{-5}$  ug/ml and lower.

At an even lower concentration ( $5 - 500$  nMol =  $0.10 - 0.25$  ug/ml) phorbol myristate from euphorbium species for example has maximum stimulation on the macrophagenic or granulocyte phagocytosis, determined according to the bioluminescence method (Kensler and Trush 1981). It has long been known that hormones and transmitters are effective in these concentrations, even if in different systems.

Two additional observations are worth mentioning:

The first refers to the reinforcement of the action of one substance by adding a second. In one case, effectiveness could only be determined in-vivo after a clearly inert carrier substance had been added to the test formulation. This phenomenon is reminiscent of Freund's adjuvant for intensification of the immune response (Freund 1956).

The second observation would appear to be of a more fundamental nature. In testing some low-molecular compounds known to be cytotoxic, we detected an immune-stimulating effect at low substance concentrations, whereas, in high concentrations an immune-suppressing effect, i.e. the cytotoxic action described could be observed, measured on Walker-250-carcinoma-ascites cells with the <sup>3</sup>H-Thymidin-Incorporation Test (Kreutzkamp 1984, Weitzel 1967), (see Fig. 4); that is, the same substance is capable of acting as a cytotoxic or an immune-stimulant according to the dose, in

Fig. 3:

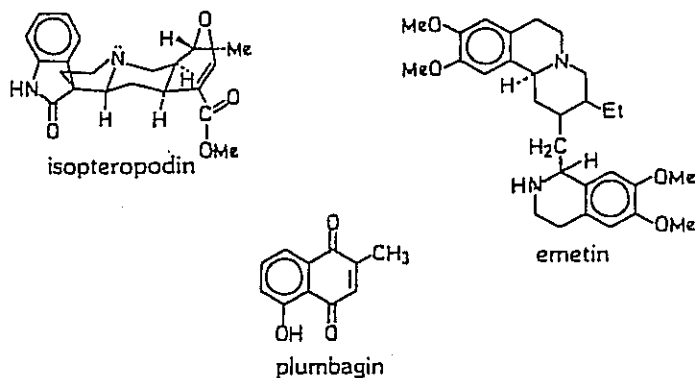
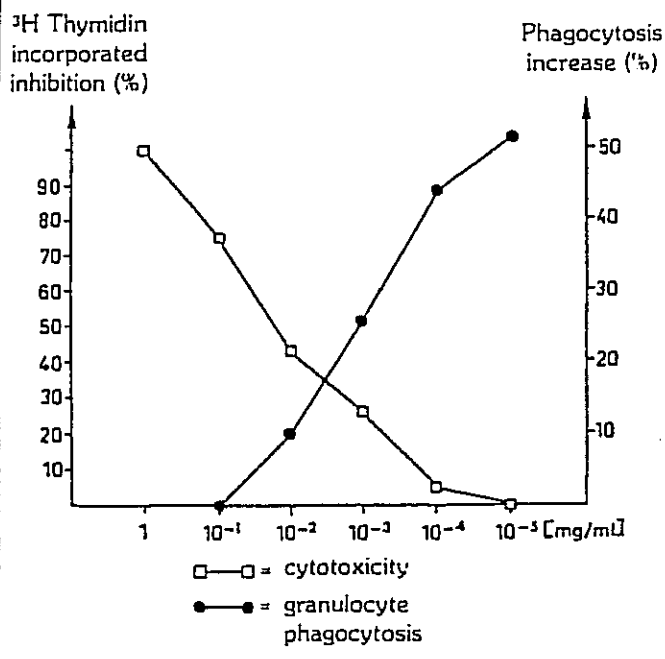


Fig. 4: Cytotoxicity and Granulocyte Phagocytosis of Emetin



higher doses however only as a cytotoxic, while the immune-stimulation released by high dilutions may also mean immune-induced cytotoxicity. Whether or not the homeopathic principle "similia similibus curentur" can be seen here would be a matter for discussion. In stimulation therapy it is certainly known from experience that low doses act in contrast to higher doses, the one as a stimulant, the other suppressive. This principle which is expressed in the famous Arndt-Schulz rule, could apply specially to the application of immune modulators.

These extensive preliminary tests were necessary to be able to conduct the relevant experiments with different preparations. At this point some positively conducted controlled

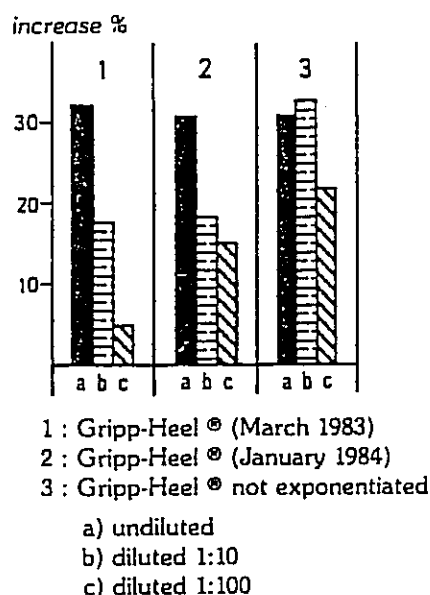
studies on humans were already available with the preparations Tardolyt® (aristolochic acid) (Giss 1980, Kluthe and colleagues 1982), with Echinacin® (Mose 1983) and Esberitox® (Quadripur 1974, 1976, Bockhorst and colleagues 1982, Forth and Beuscher 1981, Blunck 1983). The preparations tested were applied p.o. with the exception of Echinacin (i.m.). The results refer primarily to infection resistance.

In our tests we excluded p.o. preparations for the time being. The first tests were conducted with Engystol® and Gripp-Heel® injection preparations. Both preparations led to an increase in phagocytic activity in the scale of 20-40% both in the granulocyte and carbon clearance tests and also in the bioluminescence test (Figs. 5a - g, Fig. 6, Fig. 7). Dilutions of the preparations at a ratio of 1:10 or 1:100 led, with one exception, to a decline in effectiveness, i.e. the selected extract dilutions appeared to be the optimum application form in almost all cases.

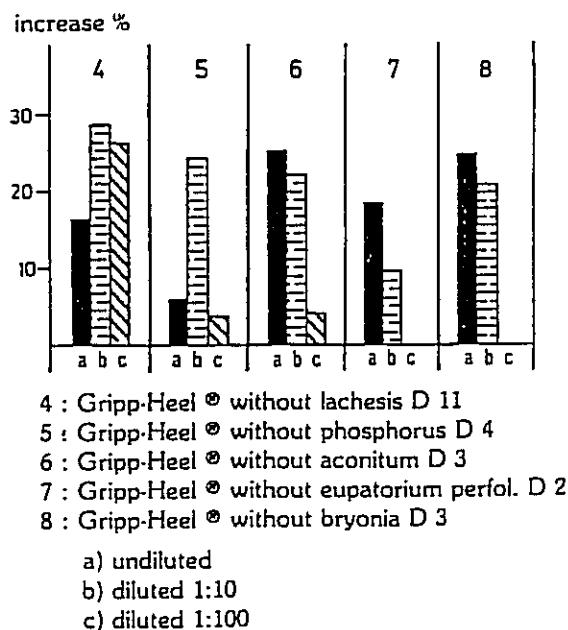
If one interpolates the necessary concentrations for humans from those injection quantities used in the carbon clearance test, it must be considered that mice normally respond much worse than humans (1/5 - 1/10).

In order to ascertain the significance of the combination of the main drug extracts with other extracts and inorganic additives or animal toxins, we tested Engystol and Gripp-Heel preparations in the granulocyte test omitting individual components. This yielded the surprising result that in all "minus-one" preparations the stimulation rate was lower than in unaltered preparations (Figs. 5b, 5d.). It must therefore be concluded that the various additives act positively on immune stimulation in the sense of exponentiation or addition. The greatest decline in effect could be observed when phosphorus was omitted.

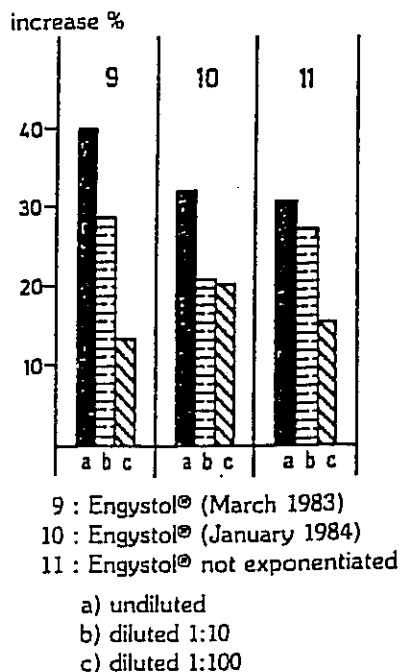
**Fig. 5a: Phagocytosis tests of Heel injection preparations**



**Fig. 5b: Phagocytosis tests of Heel injection preparations**



**Fig. 5c: Phagocytosis tests of Heel injection preparations**



It is interesting to note that a 1 : 1 mixture of Gripp-Heel and Engystol diluted 1 : 10 was 10 - 20% more effective than the two individual preparations (Fig. 5f). This result correlates with practical experience.

Of the following six further preparations, Euphorbium compositum, Bryonia-Injeel forte and Vincetoxicum-Injeel D 10 were approximately equally effective as Gripp-Heel or Engystol in the granulocyte test (see Fig. 5c).

The examination of preparations of other companies (e.g. Lomapharm, Cefak) with similar composition led in part to equally good results. It was however quite clear that all the preparations with extract or original tincture dilutions in the range D 4 - D 8 proved more effective in the in-vitro granulocyte test than preparations with D 1 - D 3 dilutions.

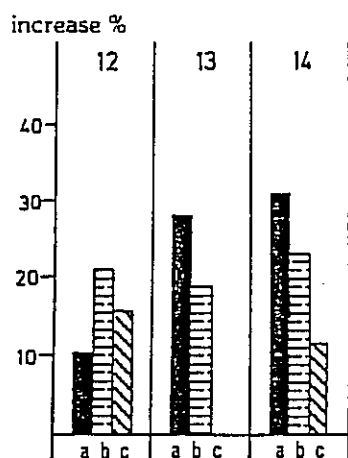
This result allows the assumption that immune stimulation is more likely to be achieved with lower rather than high substance concentrations.

Since eupatorium-type phorbol esters are known as strong inducers of immune responses (Grimm and colleagues, 1980, Abb and colleagues 1979) it was not surprising that we also found corresponding stimulation with eupatorium preparations. (Fig. 5g).

Of the other Injeel preparations tested in the granulocyte test, Vincetoxicum-Injeel D 10 showed the strongest and Sulfur-Injeel the lowest effectiveness. The slight differences in effectiveness so far observed between pure dilutions and potencies have not yet been statistically determined.

After these positive results we then conducted primary orientating experiments on test persons. These served pri-

Fig. 5d: Phagocytosis tests of Heel injection preparations



12 : Engystol® without sulphur D 4

13 : Engystol® without plant ash of vincetoxicum D30

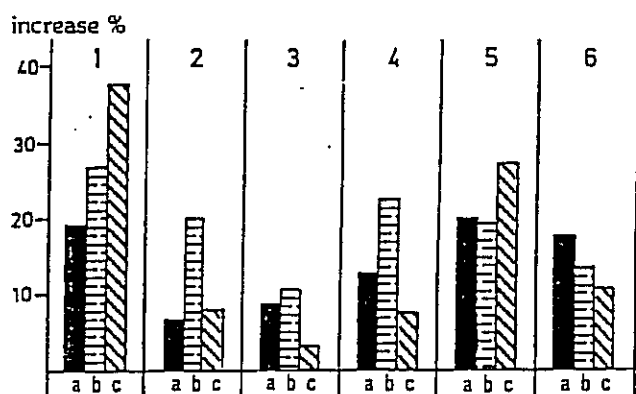
14 : Engystol® without vincetoxicum D 6

a) undiluted

b) diluted 1:10

c) diluted 1:100

Fig 5e: Phagocytosis tests of Heel injection preparations



1 : Bryonia-Injeel forte

2 : Ubichinin-Injeel forte

3 : Sulfur-Injeel forte

4 : Vincetoxicum-Injeel forte D 4

5 : Vincetoxicum-Injeel D 10

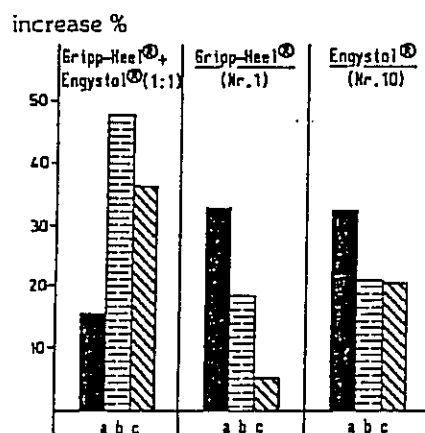
6 : Plant ash of vincetoxicum D 30

a) undiluted

b) diluted 1:10

c) diluted 1:100

Fig. 5f: Phagocytosis tests of Heel injection preparations



a) undiluted

b) diluted 1:10

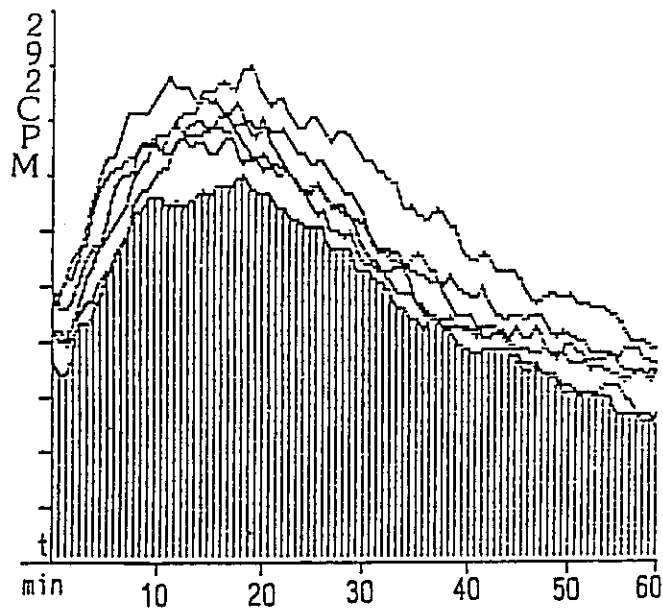
c) diluted 1:100

Fig. 5g: Phagocytosis increase in % \*) acc. to Brandt

Euphorbium - D <sub>6</sub>	undiluted	38.2
Inj.	1 : 10	20.0
	1 : 100	10.4
Euphorbium comp.	undiluted	21.5
Inj.	1 : 10	30.0
	1 : 100	26.7
Euphorbium - Injeel	undiluted	32.9
forte	1 : 10	34.9
	1 : 100	23.9

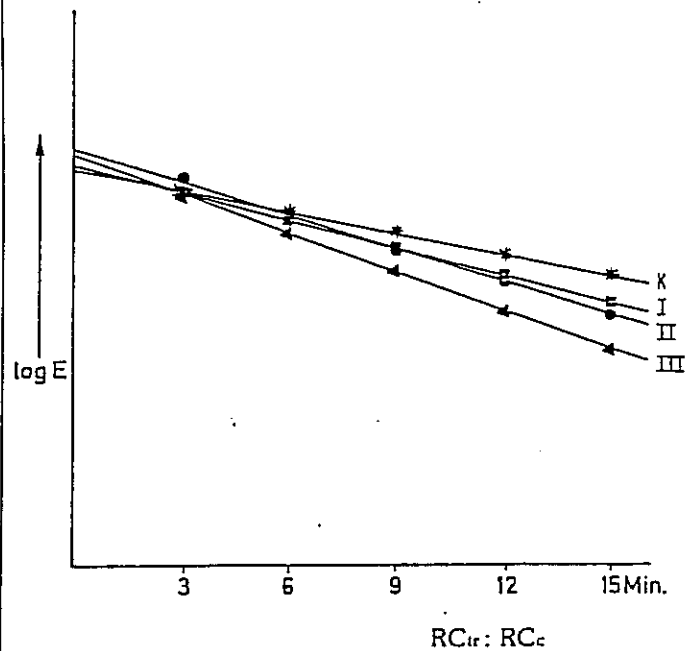
\*) mean values after 3 measurements

Fig. 6: Granulocyte Bioluminescence Test



Channel	Concentration	Peak maximum cpm x 10 <sup>-3</sup>	Biolumi- nescence %
1	control	205	—
2	undiluted	265	29.3
3	10 <sup>1</sup>	257	25.3
4	10 <sup>2</sup>	239	16.6
5	10 <sup>3</sup>	236	15.1
6	10 <sup>4</sup>	227	9.7

Fig. 7: Carbon Clearance of Engystol®, Gripp-Heel® and Engystol®/Gripp-Heel® 1:1 mixture (1ml/kg)



K = Control  
 I = Gripp-Heel® 1.36  
 II = Engystol® 1.53  
 III = Engystol-Gripp Heel 1.96  
 1:1 mixture  
 RCr: RCc: > 1.0 stimulation  
 < 1.0 suppression

marily to determine the most suitable test conditions, such as level of dosage, the injection times to be selected, most suitable application manner and the most reliable measuring method. The 1:1 mixture of Gripp-Heel and Engystol was administered twice each on two different days, on 1st and 3rd days i.v. and p.o., after a preceding double control test. Granulocyte analysis took place on 4 successive days with the Brandt method, and for comparison with the chemoluminescence method. Although these experiments, which have so far only been conducted on 6 test persons, have as yet no value as evidence, it could clearly be seen that the Brandt method would seem to be the most reliable for determining phagocytic activity. It is also our opinion that i.v. injection should be chosen for the first controlled studies. Simultaneous measurement of Ig values should also be aimed for.

**Amendment remarks:**

In the meantime double blank studies have been completed using two preparations, each conducted on 38 test persons, with similarly good results. A report on the results will appear in the near future.

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