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Our premium natural extracts originate from meticulously selected flower pollen found in "Rye." These extracts undergo a unique proprietary production process crafted by Graminex L.L.C. in Ohio, United States. This exclusive process encompasses every stage, from cultivation and harvesting to the creation of high-quality natural extracts, specifically G60 and G63, derived from GBX flower pollen particles. Graminex holds the sole rights to this process and maintains adherence to strict pharmaceutical production standards in alignment with the World Health Organization's requirements.

Our extracts are renowned for their world-class production standards, boasting ORAC certification for exceptionally high antioxidant concentration and CAP-e Test accreditation, which signifies outstanding absorption into red blood cells. Over more than five decades, we have consistently refined and improved our product's efficacy.

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Pollitin - สารอาหารบำบัดเซลล์อ

สารสกัดธรรมชาติคุณภาพสูง สกัดจากเกสรดอกไม้จาก “ข้าวไรย์” ที่มีสูตรลับเฉพาะของ บริษัท (Graminex L.L.C.) ที่รัฐโอไฮโอ้ ประเทศสหรัฐอเมริกา ในการปลูก เก็บ และผลิตสกัดธรรมชาติคุณภาพสูง G60, G63 จากอณูละอองเกสรดอกไม้ GBX, Graminex® เอกสิทธิ์เฉพาะของบริษัท Graminex เท่านั่นที่ผลิตได้เพียงเจ้าเดียวในโลก อยู่ภายใต้การควบคุมมาตรฐานการผลิตยา ตามข้อกำหนดขององค์การอนามัยโลก

จนเราได้รับการรับรองมาตรฐานการผลิตระดับโลกระดับเดียวกับการผลิตยาเพราะ Pollitin ได้รับรองการทดสอบค่า ORAC หรือ ค่าระดับความเข้มข้นของสารต้านอนุมูลอิสระที่สูงมาก และ CAP-e Test หรือค่าความสามารถในการดูดซึมเข้าสู่เม็ดเลือดแแดงในระดับที่สูงจนได้รับ

การขึ้นทะเบียนเป็น “NUTRACEUTICAL” หรือ “โภชนเภสัช สารอาหารบำบัดระดับเซลล์” ที่สามารถแก้ไขปัญหาฟื้นฟูได้ลึกถึงระดับเซลล์ มีฤทธิ์ฆ่าเชื้อแบคทีเรีย และมีผลเสริมสร้างภูมิต้านทานเมื่อเซลล์ต่างๆ ได้รับสารอาหารที่เหมาะสมตามระบบต่างๆ ในร่างกาย ส่งผลให้ร่างกายสามารถต่อสู้กับ เซลล์ที่ผิดปกติภายในร่างกายได้ถึง 95% และยังได้รับรองมาตรฐานการผลิตและประสิทธิภาพจากองค์กรต่างๆ มากมายระดับโลก รวมไปถึงยังได้รับรางวัลการันตีอีกมากมายจาก เอกสิทธิ์สูตรลับพิเศษเฉพาะของ Graminex ทำให้สินค้ามีคุณภาพและเกิดผลลัพธ์ที่ดีและน่าเชื่อถือ จนได้รับการยอมรับระดับสากลอีกด้วย

ตลอดระยะเวลากว่า 50 ปี เราได้มีการวิจัยพัฒนาประสิทธิภาพอย่างต่อเนื่อง มีการวิจัยจากสถาบันทางการแพทย์และเภสัชกรรมรับรองมากกว่า 150 การวิจัย เรามีความภูมิใจอย่างมากในการเป็นผู้ผลิตหนึ่งเดียวของโลกที่ได้ครอบครอง ถือลิขสิทธิ์เอกสิทธิ์กระบวนการผลิตและสูตรเฉพาะ G60 และ G63 จากละอองเกสรดอกไม้ชนิด GBX ที่ไม่มีใครสามารถทำได้ ส่งผลให้ Pollitin เป็นที่ยอมรับจากคนจำนวนมากใน 6 ทวีป 50 ประเทศทั่วโลก และได้รับผลตอบรับที่ดีจากผู้บริโภคในการซื้อซ้ำสินค้าอย่างต่อเนื่องมากกว่า 50 ปี

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TOPPIC

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General immunological properties of fat-soluble (Cernitin GBX) and water-soluble (Cernitin T60) pollen extracts

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The immunological properties of fat-soluble (Cernitin GBX) and water-soluble (Cernitin T60) pollen extracts were examined *in vivo* and *in vitro*. For investigations *in vitro* we used the water-soluble pollen extract (T60), and *in vivo* the fat-soluble form (GBX). The aim of the *in vivo* experiments was to evaluate their effect on IgG antibody production, their capability of rosette formation (E-RFC) and cell indicating IgM-plaqueforming cells (PFC) hemolysins. Also observations were made on the effect of the pollen extract on graft *vs* host reaction, the transplantation barrier and delayed hypersensitivity in relation to sheep red blood cells (SRBC). Its assumed effect on phagocytosis and blastic transformation was assessed *in vitro*. In both *in vivo* and *in vitro* systems the influence of T60 and GBX preparations on the population of T- and B-cells was tested. A relationship was revealed between immunological activity and the evaluated Cernitins. Both the Cernitins examined demonstrated moderate immunoregulatory properties, but the immunosuppressive component was predominant.

Keywords: pollen extracts; immunological properties.

INTRODUCTION

Cernitins (pollen extracts obtained from A.B. Cernelle, Vegeholm, Sweden), contain water-soluble (Cernitin T60) and fat-soluble (Cernitin GBX) substances. The aim of the present work was to examine the effect of Cernitins on immunological parameters *in vivo* and *in vitro.* Previously it had been published that Cernitins given orally or intraperitoneally inhibited or counteracted the elevation of aminotransferase activity and the inflammatory process, necrosis and steatosis of liver cells (Wójcicki *et al.,* 1985).

MATERIALS AND METHODS

Effect on the formation of precipitins (IgG). Investigations were carried out on mice of the 129 Ao/Boy strain. The titre of precipitins was detected (every week) by the Ouchterlony (1949) method modified by Wilson and Pringle (1954). Animals were injected subcutaneously with a 1% solution of ovalbumin, 1 mL/kg three times a day every third day. Cernitin GBX was administered 10 mg/kg i.p. from day 3 once a day, for 21 days.

Effect on plaque forming cells (Mishell and Dutton, 1967). A plaque forming cell test was performed according to Jerne and Nordin (1963). Examinations were carried out on mice of 129 Ao/Boy strain. Cernitin GBX was administered i.p. 10 mg/kg, starting from day 1 and afterwards once a day for 4 days. On day 0 a sensitizing dose of 10% sheep erythrocytes was injected i.p. (0.2 mL per

mouse). On the fourth day the test proper was carried out, using the spleen cells.

Rosette E-forming test. The experiment was carried out according to Bach and Dardenne (1972) on mice of the 129 Ao/Boy strain. Cernitin GBX was administered i.p. 10 mg/kg, starting from the day 1 and afterwards once a day for 4 days.

Determination of T- and B-lymphocytes (Boyum, 1976; Gorer and O'Gorman, 1956; Pasternak, 1969). *In vivo*. Mice of 129 Ao/Boy strain received Cernitin GBX for 4 consecutive days (10 mg/kg i.p.). Lymphocytes were isolated by centrifugation (800 revs per min for 5 min at 4˚C) over a Picoll gradient. T-cells were detected by the cytotoxic test. The percentage of B-lymphocytes was determined by direct immunofluorescence. The number of fluorescent cells which were identified as B-lymphocytes is expressed in proportion to 1000 counted cells. *In vitro.* Cernitin T60 was added at 0.6, 3.0 and 15.0 mg/mL final concentration in Hanks' solution. The detailed procedure of T- and B-lymphocyte determination was described by Hoffman and Kunkel (1976).

Skin graft test (Marckman, 1966; Plużańska, 1969). Investigations were carried out on young mice of 129 Ao/Boy strain. Skin grafts of 1 cm^2 in size were transplanted in the allogenic system of mice of C_{57} B1 strain. Mice-recipients were treated with Cernitin GBX (10 mg/kg i.p.) once a day, starting 1 day before being grafted and until complete graft rejection had occurred.

Graft vs host reation (Ford *et al.,* 1970). The spleens of mice of the B_6 strain were removed and rinsed in cold HBS solution. Local graft vs. host (GvH) reaction was induced by injecting 0.05 mL of a suspension of these cells in HBS solution into the right footpad of Balb/CxB_6 mice. The right (test) and left (control) popliteal lymphnodes were removed and weighed 6 days post injection. Cernitin GBX was administered at a dose of 10 mg/kg i.p. daily for 6 days starting one day before the injection in the footpad. Results were expressed by the difference between the mean weights of the left and right popliteal nodes.

Delayed hypersensitivity test on sheep erythrocytes (SEC) (Papadimitriou *et al., 1983).* 0.2 mL of a 10% SEC solution was injected intravenously into mice of the 129 Ao/Boy strain on day 0. After 4 days, 0.05 mL of a 50% SEC solution was injected subcutaneously into a hind leg footpad: the thickness of the footpad was measured in mm after 24 h and 48 h. 10 mg/kg Cernitin GBX or 50 mg/kg Cernitin T60 were injected i.p. into the mice on day 1 and on every other day afterwards.

Blastic transformation. Experiments were carried out with human venous blood by the isotope method according to Hersh and Oppenheim (1965), modified by Plużańska (1969). Cernitin T60 was added in a Parker solution to the culture in concentrations of 0.6, 3.0 and 15.0 mg/mL 1 h before the application of 20 µg/mL phytohemagglutinin A (PHA) to the culture. The cultures were incubated for 48 h at 37˚C in a 5% CO_2 atmosphere. ^{14}C-labelled thymidine was added and incubation was continued for another 24 h under the same conditions. Radioactivity was determined by scintillation counting and expressed as counts per min.

Phagocytic activity. The investigations were carried out according to Steuden's (1978) method. The test proper was carried out using 0.1 mL of *Staphylococcus aureus* bacteria (5 x 10^8 per mL) and 0.1 mL of Cernitin T60 in concentrations of 0.6, 3.0 and 15.0 mg/mL + 0.1 mL of granulocyte solution obtained from the peritoneal exudate of a guinea-pig which had received beuillon i.p. The cultures were then centrifuged at 3000 revs per min for 30 min and 37˚C. Radioactivity was measured in 1 mL of supernatant in a Beckman scintillation counter, type 3801. Results were expressed as the percentage of phagocytosed bacteria.

Statistical analysis. Statistical analysis of the data was performed using Student's t-test. In all

comparisons, p <0.05 was considered to be significant.

RESULTS

The effect on the formation of precipitins. It was shown that the titre of precipitins was virtually unchanged in animals receiving Cernitin GBX;

the titre oscillated between 1:128 and 1:512, both in mice treated with Cernitin GBX and in the control group.

The effect on the plaque formed cells (PFC) and on the rosette forming cells (RFC). Cernitin GBX injected i.p. into mice affected the number of cells producing hemolysins (PFC) to some degree and also the ability of lymphocytes to form RFC with sheep erythrocytes. However, the results were contrasting: a marked increase in PFC was demonstrated while the number of RFC was reduced. The differences were statistically significant (p<0.05) in both cases (Table 1).

Table 1. Effective of Cernitin GBX on the plaque forming cells (PFC) and the rosette E forming cell (E-RFC)

Treatment	PFC (2×10^6 cells)	%	RFC (2×10^3 cells)	%
Cernitin GBX (10 mg/kg, $n = 10$)	771[a]	323	3.5[a]	19.4
Control ($n = 10$)	220	100	18.0	100.0

[a] $p < 0.05$.

The effect of Cernitin GBX (*in vivo*) and Cernitin T60 (*in vitro)* on the T- and B-lymphocyte subpopulations. Cernitin GBX (10 mg/kg) and Cernitin T60 (0.6-15.0 mg/mL) did not change significantly (p>0.05) either the number of T- and B-lymphocytes or the lymphocytes of the peripheral blood possessing no receptor (null) (Table 2).

Table 2. Influence of the Cernitin GBX (*in vivo*) and Cernitin T60 (*in vitro*) on the T- and B-lymphocyte subpopulations

Treatment	Lymphocyte subpopulations (%) T	B	Null
Cernitin GBX (10 mg/kg, $n = 10$)	64	24	13
Control ($n = 10$)	64	27	11
Cernitin T60 (mg/mL)			
0.6 ($n = 5$)	58	21	21
3.0 ($n = 5$)	63	19	18
15.0 ($n = 5$)	67	12	21
Control ($n = 5$)	60	22	18

Skin graft test and graft host reaction (GxH). In animals receiving Cernitin GBX, the rejection time of the skin graft was somewhat prolonged (p>0.05). The index of GvH was decreased under the influence of this preparation, but in comparison with the control group there was no statistically significant difference (p>0.05) (Table 3).

Table 3. Influence of Cernitin GBX on the skin graft rejection and graft vs host reaction (GvH)

Treatment	Rejection time of the skin graft (days)	GvH index
Cernitin GBX ($n = 10$)	11.0	1.2
Control ($n = 10$)	10.6	1.8

Delayed hypersensitivity to SEC test. *In vivo* Cernitin GBX and Cernitin T60 did not intensify the response to SEC. The index of the increased in footpad thickness was unchanged after 24 h, while after 48 h it was even slightly diminished (p>0.05) in comparison with the control group (Table 4).

Table 4. Effect of the Cernitin GBX and Cernitin T60 on delayed hypersensitivity against SEC

Treatment	Dose (mg/kg)	Index of the increase of thickness of footpad After 24 h	%	After 48 h	%
Cernitin GBX ($n = 10$)	10	4.5	100	3.0	85.7
Control ($n = 10$)	—	4.5	100	3.5	100.0
Cernitin T60 ($n = 5$)	50	4.5	100	3.0	85.7
Control ($n = 5$)	—	4.5	100	3.5	100.0

Blastic Transformation. Cernitin T60 markedly decreased blastic transformation *in vitro* expressed as the number of impulses for [^{14}C] thymidine per min. Nonspecific induction of blastic transformation with phytohemagglutinin (PHA) confirmed, to some degree, the results obtained. But the interaction between Cernitin

T60 and PHA was irregular and depended on the concentration of the preparation. However, the suppressive component was predominant (Table 5).

Table 5. Effect of Cernitin T60 on the blastic transformation (isotope method)

Preparation	Concentration (mg/mL)	Counts per min
Cernitin T60 ($n = 5$)	0.6	1060[a]
Cernitin T60 ($n = 5$)	3.0	857[a]
Cernitin T60 ($n = 5$)	15.0	170
Control ($n = 5$)		227
PHA ($n = 5$)		7108
PHA + Cernitin T60 ($n = 5$)	0.6	4934
PHA + Cernitin T60 ($n = 5$)	3.0	8934
PHA + Cernitin T60 ($n = 5$)	15.0	1648[a]

[a] $p < 0.05$.

Phagocytic activity. The *in vitro* process of *Staphylococcus aureus* phagocytosis by guinea-pig granulocytes of the peritoneal exudates was completely inhibited by Cernitin T60 in concentrations of 0.6-15.0 mg/mL.

DISCUSSION

The definite anti-inflammatory effect of Cernitin extracts was demonstrated by the *Croton* oil-induced edema test (Itch, 1968). In the cotton pellet test, Cernitin T60 showed an anti-inflammatory activity in rats corresponding to the inflammation-inhibiting effect of phenylbutazone. But T60 was completely devoid of toxicity (Glømme and Rasmussen, 1965). It was also possible to confirm the anti-inflammatory effect of Cernitins on carrageenin-induced edema in rats (Dessi, 1971). Cernitins administered orally to rats demonstrated a marked antiinflammatory effect compared to the very active anti-inflammatory agents injected intraperitoneally as controls. Cernitins also inhibited the inflammatory process induced by galactosamine administration to rats (Wójcicki *et al.,* 1985). The results obtained in this experiment show that Cernitin GBX and Cernitin T60 are able to affect the course of the induced immunological processes. Such an effect is, however, defined and conditioned by the test type used and by the dose applied.

The number of RFC was affected by Cernitin GBX in a quite contrasting way. An increase in the number of PFC was accompanied by a reduction in the RFC. One may argue that the preparation examined plays an essential role in immunological processes due to regulation of the reciprocal relationship between both kinds of cells. Thus, Cernitin GBX may have a quite significant immunomodifying function.
The number of RFC was affected by Cernitin GBX in a quite contrasting way. An increase in the number of PFC was accompanied by a reduction in the RFC. One may argue that the preparation examined plays an essential role in immunological processes due to regulation of the reciprocal relationship between both kinds of cells. Thus, Cernitin GBX may have a quite significant immunomodifying function.

T- and B-lymphocytes are the morphological basis of the immunologic process. Cernitin GBX (*in vivo*) and Cernitin T60 (*in vitro*) did not alter the reciprocal relationship between the above mentioned subpopulations of lymphocytes. This could mean that another factor modulating reactivity is present or that the changes are not sufficiently marked as to be shown by the quantitative difference between the subpopulations of T- and B-lymphocytes. These results may be due to an ability to produce lymphokins, mainly interleukin 2 (Wybran and Schandene, 1985) rather than differences in morphological element

Cernitin GBX did not influence the barrier of a graft, although slight changes of graft host reaction were noted. Thus, Cernitin GBX did not change essentially either the graft reaction of the graft host reaction. The delayed reaction of hypersensitivity against SEC was not modified by the examined Cernitins. On the other hand, the blastic transformation was affected by Cernitin T60. It was reduced *in vitro* proportionally to the concentration of Cernitin T60. In relation to phytohemagglutinin a two-phase reaction was observed. Cernitin T60 applied in both low concentration and especially in high concentration, diminished the reaction, while the intermediate concentration was not effective. Thus, our observations confirm the results obtained by Kimura and Inoue (1968) demonstrating the lack of allergenic properties of both Cernitins. Our studies showed, however, that there is a relationship between the immunological system and the Cernitins tested. We can conclude, therefore, that the pollen extracts effectively possess an immunotropic/ immunoregulatory component. They show, *in vitro*, a slight immunosuppressive effect (E-

RFC)—B-lymphocyte antagonism in reaction to blastogenic effect of PHA—and occasionally they act as a stimulator (PEC, blastic index). In some experimental systems they are ineffective (GvH, transplantation barrier, SEC test).

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New immunomodulators with antitumoral properties; Isolation of active naturally-occurring anti-mitotic components of MR>1KD from pollen extract T60

Jean-Claude Jaton, Geneva, April 1994

The *in vivo* assay for inhibitory active components present in a pollen extract served as an efficient guide-line for the purification procedure outlined below (Table 1). The designation of components listed in Table will be fully described in the text and figures. Briefly, the active material exhibits a size higher than 1 kDa but lower than 2 kDa; such material is comprised within Sephadex G-25 SF fraction III. The most active component appeared to be fraction III-b5, as summarized in Table 1. The final purification step was carried out by HPLC on a reverse phase column (molecules b51/b52). Two approaches were attempted.

A. From T60 SF-011 (containing up to 60% maltodextrine)

1. Dialysis of T60 SF-011 vs. dist. water for 48 hr using Spectra/ Por membrane MW CO 1,000

Dissolve 80 g of T60 in 280 ml distilled water. Fill Spectra/Por molecularporous membrane tubing MW CO 1,000 with T60 solution. Fill up to 1/3 of the tubing content. Length of tubing about 45 cm. Six tubings should be prepared. Dialyse vs. 101 H_20 in the cold room (+ 4°C) and change the diffusate (outside fluid) after 24 hr. After 48 hr, remove the bags from the diffusate, open each one and concentrate the "inside bag" content (brown color) to a small volume (100-150 ml) prior to lyophilization. Do not remove the precipitate.

Recovery: on the average, 20 g, i.e. ~25% ~20g A1

This preparation is called A1.

2. Dialysis of "A1" using Spectra/Por membrane MW CO 2,000 vs. water

In this step, the active components are to be found in the diffusate ("outside fluid") since the molecular weight cut off value is 2 kDa (MW CO 2,000). Dissolve 20g "A1" in 100 ml H_20 and distribute the solution (with precipitate) into 4 tubings: each tubing should be filled up to 1/3 of its total content. Dialyse 4 bags vs. 2 1 H_20 (i.e. 2 bags/ 1 I-cylinder) at 4°C for one week. Pool the diffusates from both cylinders every day (21), evaporate (Büchi Rotavapor) to 100 ml and lyophilize.

After one week: the yield of recovered material (slightly yellowish powder) is about 25-30%. This material is designated "A2". 5-6g

Then, the A2 mixture is subjected to Sephadex G-25 SF gel filtration in order to separate active fractions IIIa, IIIb, and IIIc (Fig. 1).

Fraction IIIb was further rerun on a similar gel filtration column and 5 subfractions were obtained, of which III-b5 was found to be the major one (Fig. 2).

Fraction III-b5 was finally purified by H.P.L.C. and 2 major fractions, designated III-b51 and III-b52 were obtained. Typical data are shown for information (Fig 3).

The ratio of b51 to b52 in fraction III-b5 is about 61.7% to 38.2%, respectively, as measured from the area under each peak detected at 280 nm. Fractions b51 + b52 account for about 40% of the total absorbing material at 280 nm.

B. FROM SPISSUM (without maltodextrine).

Dialysis of spissum TA 080 code Nº 207000-17

Expected content: 23 g % (i.e. 115 g/ 500 ml)

Volume: 500 ml

1. First dialysis using Spectrapor membrane CO 1 kDa vs. 10 liters of water.

Eight castings were prepared and filled up to one third of the total volume with undiluted visqueous spissum solution. Dialysis time was 48 hr with 4 changes of water. The content "inside bag" was recovered and lyophilized : 16 g.

Recovery $^{16}/_{115}$ ≈ 14%

This material is designated A1 from Spissum

2. Second dialysis of A1 from spissum using Spectrapor membrane CO 3.5 kDa vs. water

A1 (16 g) was dissolved in 40 ml H_20 and dialyzed vs. 1 liter of water for 24 hr. The outside fluid was changed every day for a period of up to 9 daysin the cold room. The dialysate was evaporated to a small volume and lyophilized every day or every 2 days.

Recovery ("outside bag" content)	Day 1	600 mg
	2	500 mg
	3	250 mg
	4 + 5	400 mg
	6 + 7	200 mg
	8 + 9	200 mg
	TOTAL	2'150 mg

Yield (after 9 days of dialysis) : ~13%

This material was designated A 3.5.

Seperation of 2g of A 3.5 on Sephadex G-25 SF (2.6 cm x 90 cm)

Conditions: as usual

Recovery of fractions:

IIIa : 45 mg

IIIb : 39 mg
IIIc : 22mg

Seperation of fraction III-b on Sephadex G-25 SF

See Fig. 2 for details, which shows the separation into FrIII-b1, b2, b3, b4, and b5; these fractions were derived from T60 SF-O11. Fig. 4 shows similar data but obtained from material derived from spissum TA 080. Fraction b5 is the prominent fraction (80%).

Fractionation of fraction III-b5 on HPLC

Two major fractions, designated b51 and b52 were routinely obtained, either from T60 SF11 (Fig. 3) or directly from spissum TA 080, as illustrated in Fig. 4.

Varian HPLC program

Solvent A 0.1% TFA

Solvent B acetonitrile (ACN)

T (time)	
0 min	5% B (ACN)
20 min	20% B (ACN)
21 min	60% B (ACN)
24 min	60% B (ACN)
25 min	5% B (ACN)

Equilibration time : 10 min

Injection- to injection time : 35 min.

Molecules b51 and b52 may be epimers in solution

Preliminary data from Prof. U. Burger (Univ. of Geneva) suggest that b51 and b52 are interconvertible, as suggested by NMR spectra in D_2O after a few days at 4°C. On the other hand, mass spectrometry spectra of b51 and b52 were virtually identical. Yet, b51 and b52 were separately isolated from preparative HPLC runs and lyophilized. The separation efficiency was better than 98%. An aliquot of b51(in D_2O), and of b52(in D_2O) was injected into HPLC, respectively. The chromatograms are presented in Fig. 5 & Fig. 6.

Conclusions:

1. purified b51 (one single peak) converts into b52 and reciprocally. Rentetion times of both compounds are identical to those of b51 and b52 present in fraction III-b5 (i.e. in pollen extract).
2. minor peaks at 12 min, and the doublet peak around 17 min may reflect degradation products from b51 and b52, which are also visible in fraction III-b5(Fig. 3).
3. the ratio of purified b51, which converts into b52 (Fig. 5) is:

b51 (63.8%) $\longrightarrow$ b52 (36.2%)

4. conversely, the ratio of purified b52, which converts into b51 (Fig. 6) is:

b52 (40%) $\longrightarrow$ b51 (60%).

Thus, b51 and b52 are likely to be present in an equilibrium in pollen extract, i.e. about 60% b51/ 40% b52. Isolated species (either b51 or b52) interconvert and yield the same ratio, as found in pollen extract.

Biologically active molecules from the b series are likely to be glyconjugates.

Microchemical determinations of G-25 SF fraction b5, and of fractions b51/b52 highly suggest that they contain sugar units and an aglucone moiety, which behaves as a phenolic compound. This was based on data which were obtained upon mild hydolosis of b5 or b51/b52 at 100°C for 6 hr in the presence of bidistilled 1.0 N HC1 under high vacuo. TLC patterns of the hydrolyzate unraveled monosaccharide (glucose) and an aglucone, which positively stained with the Pauli reagent. Because rough MS data suggested an atomic mass of 1002.2 kDa for both b51 and b2, the expected composition ob b51/b52 could be aglucone moiety linked to a tetra- or penta-glucose unit. The type of glycosidic linkage (α-or β-) was not evaluated.

The data should be taken with caution, as no high resolution mass spectrometry nor NMR spectra were yet determined for the aglucone. Runs in a 600 mHz NMR machine in Zurich may be helpful for the identification of the sugar moiety, in particular about the α- or β-glycosidic linkages [Prof. U. Burger, March, 1993]. Mass spectrometry data should be available before Eastern 1993 (Prof. J.-C. Jaton, Dr. K. Rose, Dept. of Medical Biochemistry).

Hydolysis of b51/b52 molecules

The method used was based on the recent work of Spiro & Spiro (Anal. Biochem. (1992) 204, 152-157). Conditions were: 3-5 mg of glycoconjugate in 250-330 µl of 1.0 N HCl in a glass hydrolysis tube. Temperature: 100°C for 6 hr under vacuo. Drying down step followed by washing with water and centrifugation of dark brown precipitate. Supernatants were kept at 4°C prior to HPLC fractionation.

HPLC analysis of hydrolyzate from b51/b52

A. <u>Under no vacuum</u>

With a mixture b51/b52 (60%/40%), major peaks 2 and 4 were recorded according to Fig. 7. Hydolysis products will be designated by H.

First run peak 2 b51H	Elution time : 16.45
2nd run peak 2	" " : 16.26
First run peak 4 b52H	Elution time : 17.46
2nd run peak 4	" " : 17.24

B. Under vacuum

Hydrolysis of b52 compound only; detection at 295 nm rather than at 280 nm. See Fig. 8.

Peak 1 = b51H	elution time : 16.16	28% (OD_{295})
Peak 2 = b52H	elution time : 17.69	72% (OD_{295})

Preliminary characterization of biomolecules present in fraction III-a

Pooled fraction III-a was loaded onto a (2.6 x 90 cm) Sephadex G-25 SF column. Two major peaks are apparent and the material under each peak was collected. From 140 mg III-a, a3 accounts for 30 mg and a4, 66 mg (Fig. 9). Analytical runs of fraction a3 and a4 were carried out (Figs. 10 & 11) by HPLC under standard conditions.

Material a3 discloses 3 major fractions, designated A31, A32, and A33. The last eluting peak at 25.63 min is of no interest, because of the washing of the column (Fig. 10). Material a4 is more complex with possibly 3 pairs of compounds (Fig. 11):

a) the pair eluting at 17.71 min + 18.02 min.

b) the major eluting at 18.76 min + 19.51 min.

c) the pair eluting at 20.58 min + 21.77 min.

The last pair (c) may well be the b51/b52 pair (see Fig. 3 for retention times) as fraction a is cross-contaminated by b (Fig. 1).

Preliminary MS data obtained from HPLC purified compounds a31 and a33 (Fig. 10.), and a41 and a42, respectively (Fig. 11).

Disappointing MS spectra (electrospray) were recorded for a31 and a33.

a31: signals at 597/575/439 are the major ones. A minor signal at 940.

a33: major signals at 801/815 and lower masses data.

Conclusions: strange, do not make sense at the moment.

Better, encouraging MS spectra were obtained for a41 and a42.

a41: 1164; 1183; 1002.2; 840; 822

a42: 1164; 1182.9; 1002.1; 840; 822

A41 and a42 appear to exhibit the same MS spectra.

Conclusions:

a) a41/a42 constitute probably a pair similar to the pair b51/b52

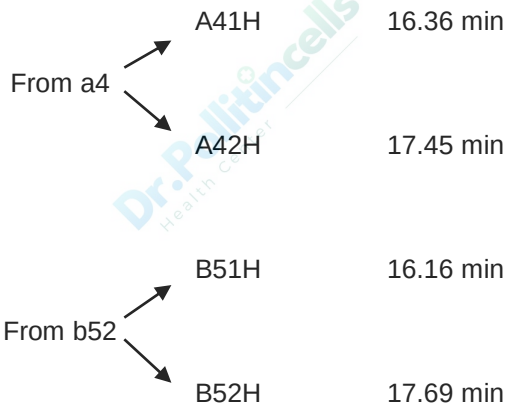
b) the MS spectrum of a41/a42 pair also exhibits degradative material of Mr 1002.2 kDa, i.e. precisely the mass of the pair b51/b52; when one more hexose unit is removed from a41/a42,

one should get 840.2 (1002 -162). If one hydrated hexose unit is removed, one should get 822, which is consistent with what we observed. Thus, I feel that the pair a41/a42 differs from the pair b51/b52 by the addition of one more hexose unit to the b51/b52, yielding a41/a42. Furthermore, hydrolysis results suggest that the aglucone might be the same in the a and b series (see below).

Hydolyzate of a4 (crude a4 according to Fig. 11)

The results are illustrated in Fig. 12, which shows that peak A41H and A42H are present in the same ratio (36% vs. 64%) as found for the hydrolyzate of b51/b52 and that the retention times of A41H + A42H are virtually identical to those of the hydrolysis products of b51/b52.

Retention times from hydrolysis products of a4 and of b52



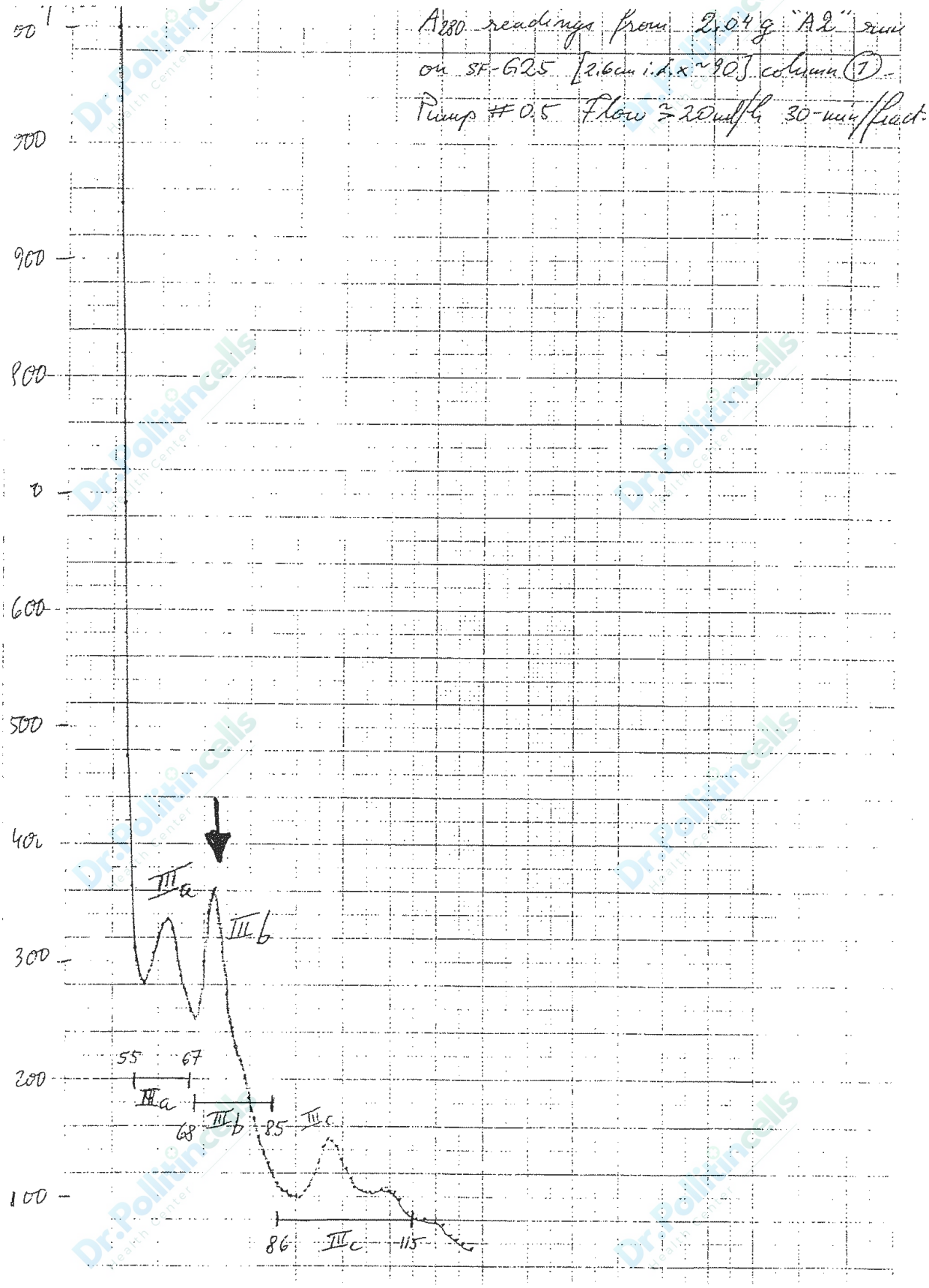
The hydrolyzates from a4 and b52 will be subjected to MS spectroscopy and NMR analyses. The material (~1 mg) was provided on March 29-31, 1993 to Dr. K. Rose (MS) and to Prof. U. Burger (NMR, Sciences II). Very preliminary data from Prof. Burger suggest that that protonic NMR spectra of the hydrolysis products from a41/a42 and b51/b52 are indistinguishable. Thus, the aglucone of a or b is likely to be the same.

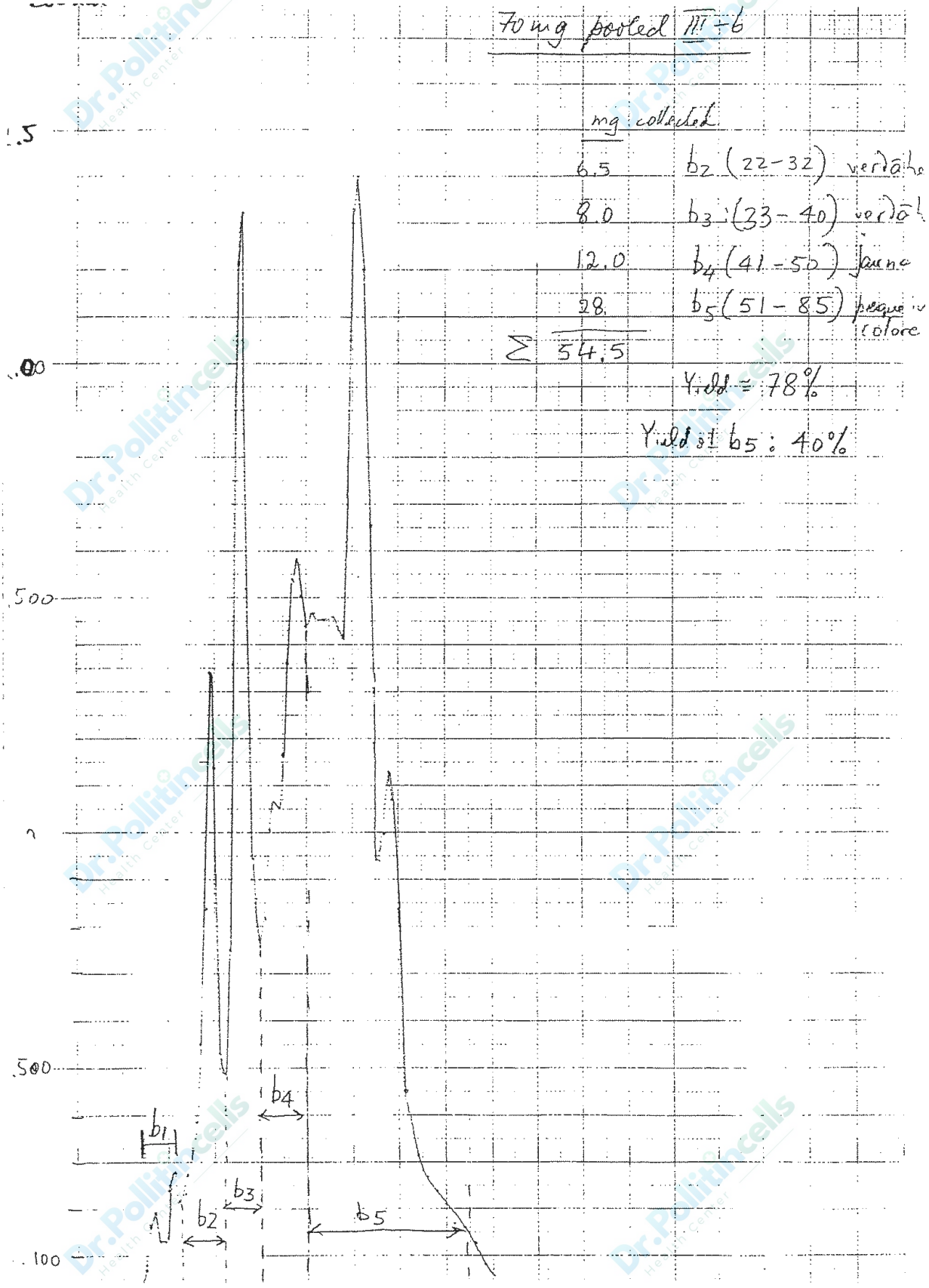
We can, at that time, speculate that biomolecules of the b series (b3, b4 and b5) or of the a series (a3, a4, and a5), which display significant inhibitory activity (Table 1), are all related to each other; they may well differ from each other by the number of hexose units attached to the aglucone moiety.

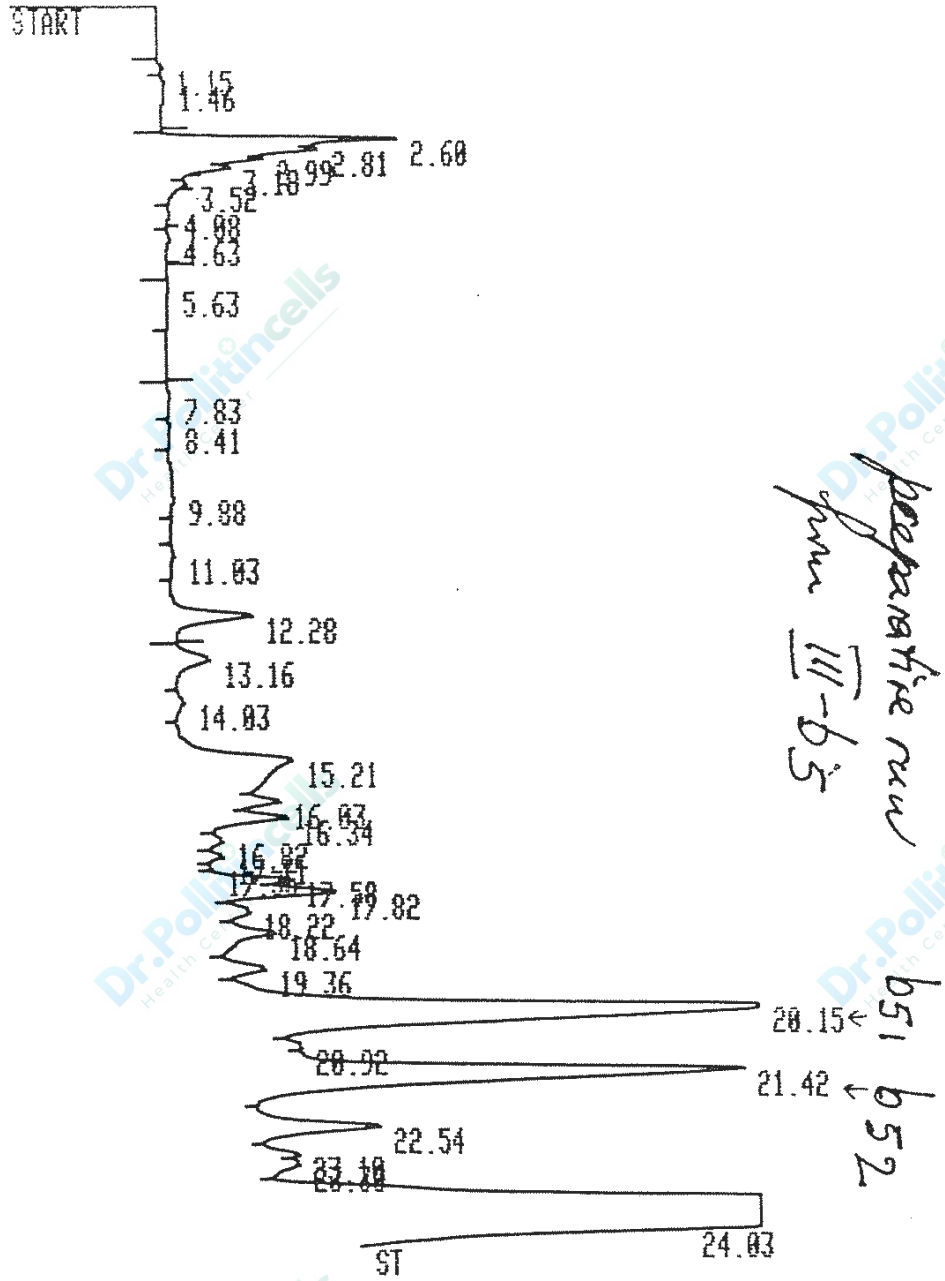
Table 1. *In vitro* bioassay for inhibitory naturally-occurring components from a pollen extract.

As developed by Prof. Sirotnak at the Memorial Sloan Kettering Cancer Center in New York. S180 tumoral cells were injected in the peritoneal cavity of a group of 10 mince (ascite formation); following implantation, mice received the drug to be tested i.p.; after one week, the volume of packed cells is measured, thus giving the % growth of tumor as compared to control. The assay is referred to as the "packed cell volume assay."

	Compounds	IC50 (mg or μg/mouse)
I	SF-011 T60	1.6 mg
	"A1"	0.39 mg + 0.5 mg
	G-25 SF fraction III (a + b + c combined)	< 50 μg
II	SF-011 T60	2.5 mg + 0.5 mg
	III-a3	< 12.5 μg ← weight loss !
	a4	25 μg
	a5	> 50 μg
	III-b3	15 μg
	b4	18 μg
	b5	< 12.5 μg
III	T60 SF-011	2.8 mg
	III-b51 (HPLC purified)	6.5 μg
	III-b52 (" ")	> 20 μg


A280 readings from 2.04 g "A2" run
on SF-625 [2.6cm i.d. x ~30] column (I)
Pump #05 Flow ≈ 20ml/h 30-min/fract.
50
700
900
800
0
600
500
400
300
200
100
III a
III b
55
67
III a
68
III b
85
III c
86
III c
115


70 mg pooled III-6
mg collected
6.5 b2 (22-32) verdāle
8.0 b3 (33-40) verdāle
12.0 b4 (41-50) jaune
28. b5 (51-85) presque incolore
Σ 54.5
Yield = 78%
Yield of b5 : 40%
.5
.00
500
500
100
b1
b2
b3
b4
b5



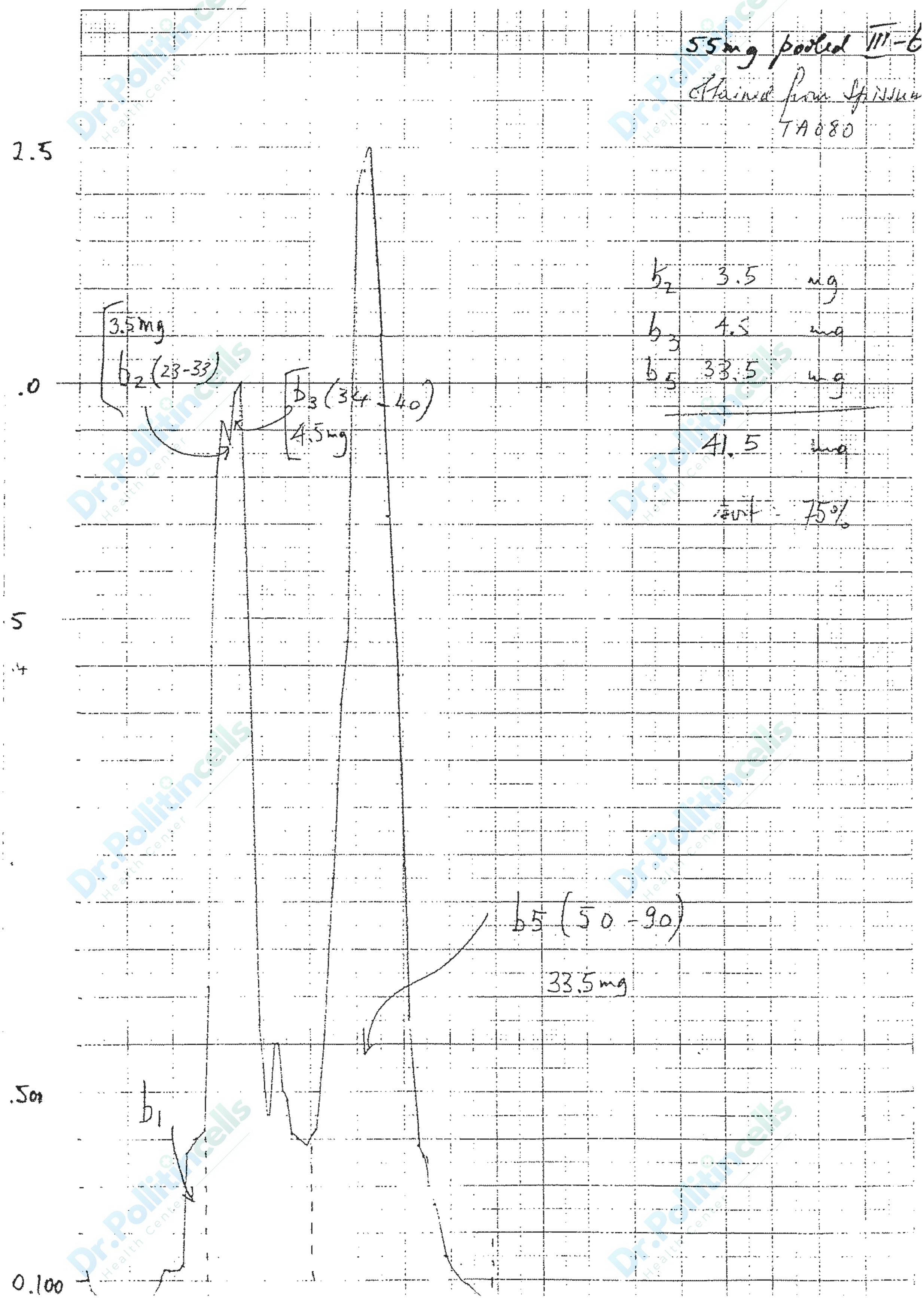
HPLC profile of III-b5 detection UV at 280nm

preparative run from III-b5

b51

b52

Fig. 3



55mg pooled III-6
obtained from
TA080
2.5
.0
5
.50
0.100
3.5mg
b2 (23-33)
b3 (34-40)
4.5mg
b1
b5 (50-90)
33.5mg
b2 3.5 mg
b3 4.5 mg
b5 33.5 mg
41.5 mg
75%

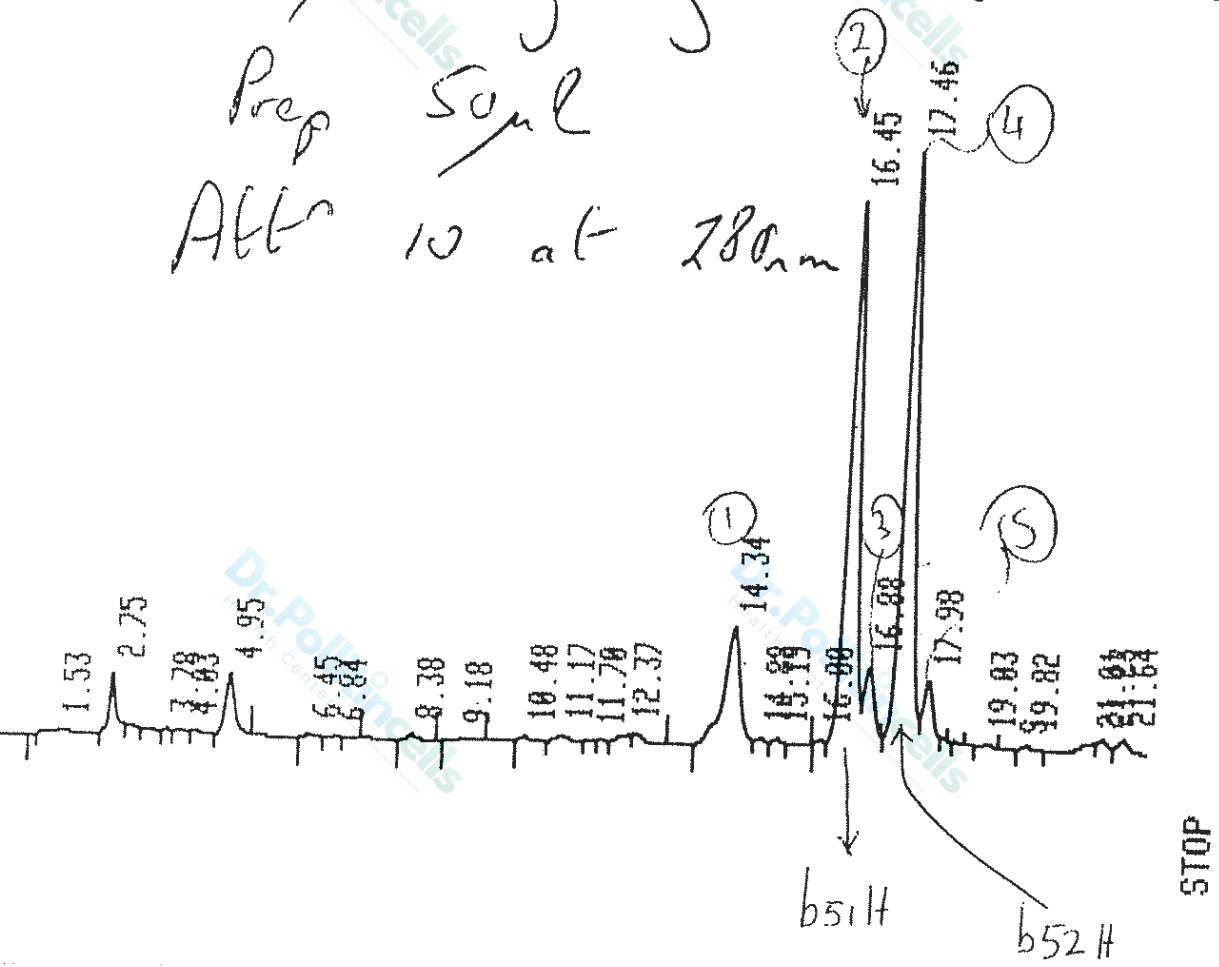
HILC profile of pure b52 ret. 2 wk old ladies
on the same HPLC column → 2 forms in equilibrium

b52 20µl D_2O (tube 12 HN)

50µl hydrolyzed (b51+b52) under no vacuum

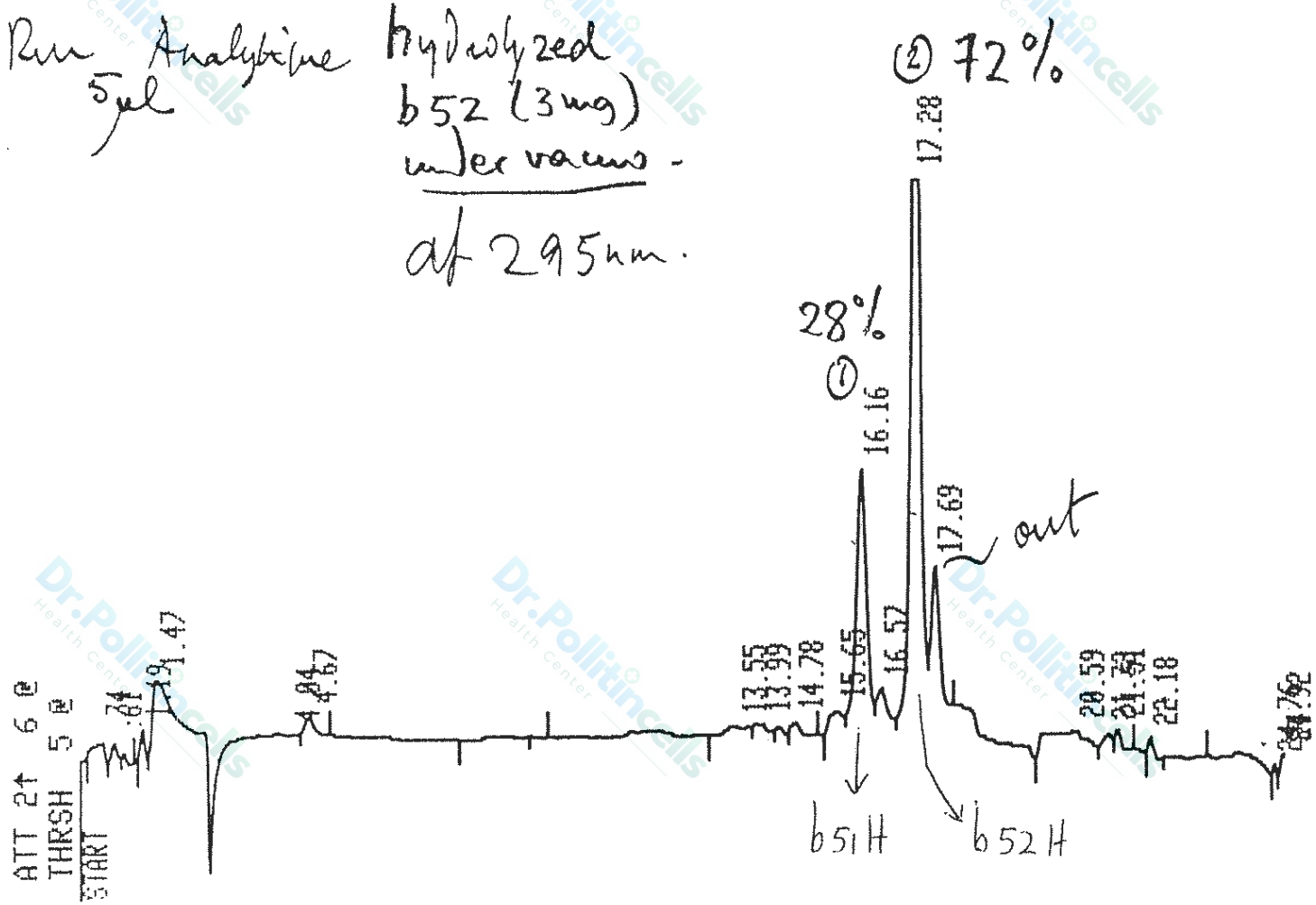
Prep 50µl

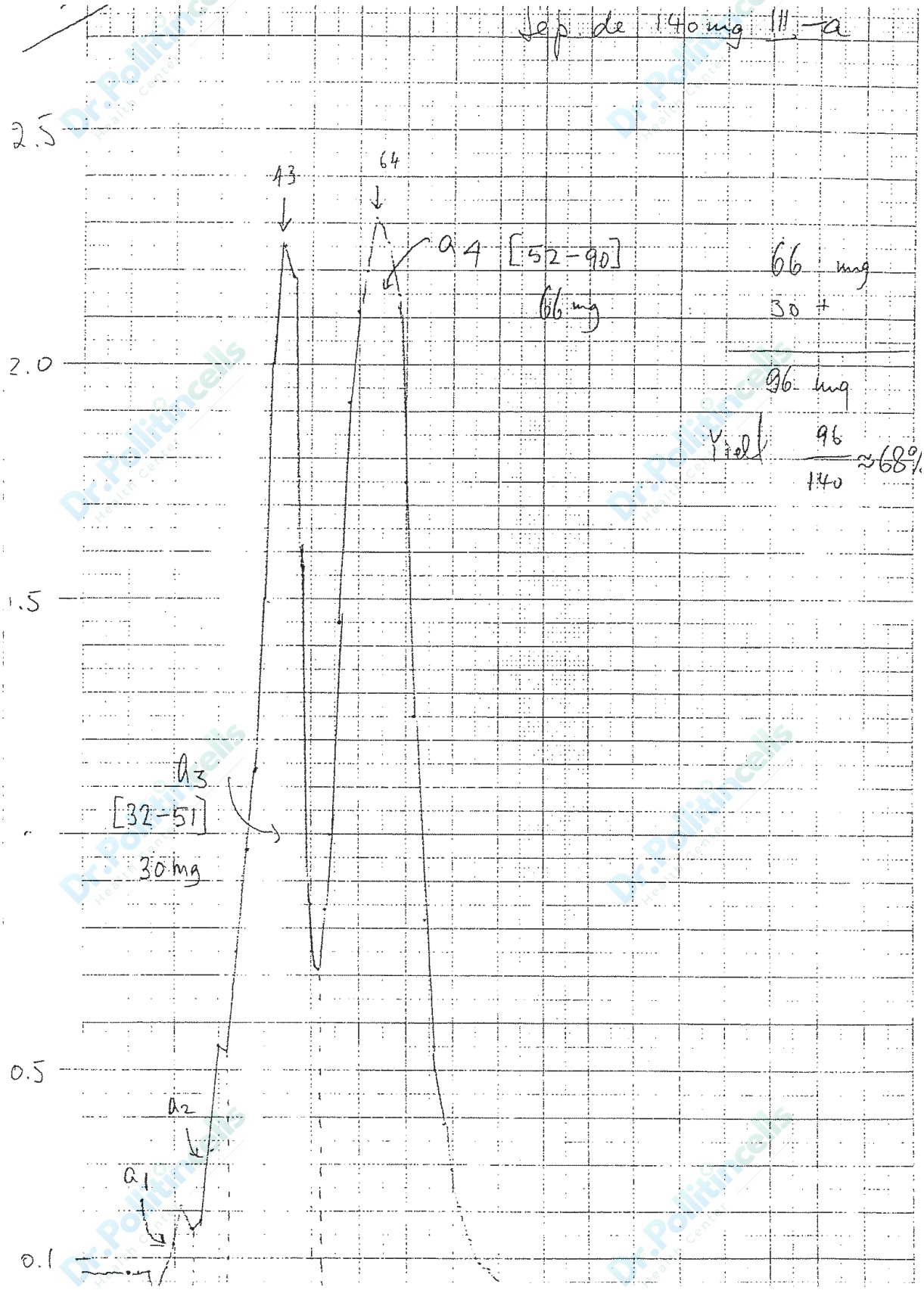
Attn 10 at 280nm

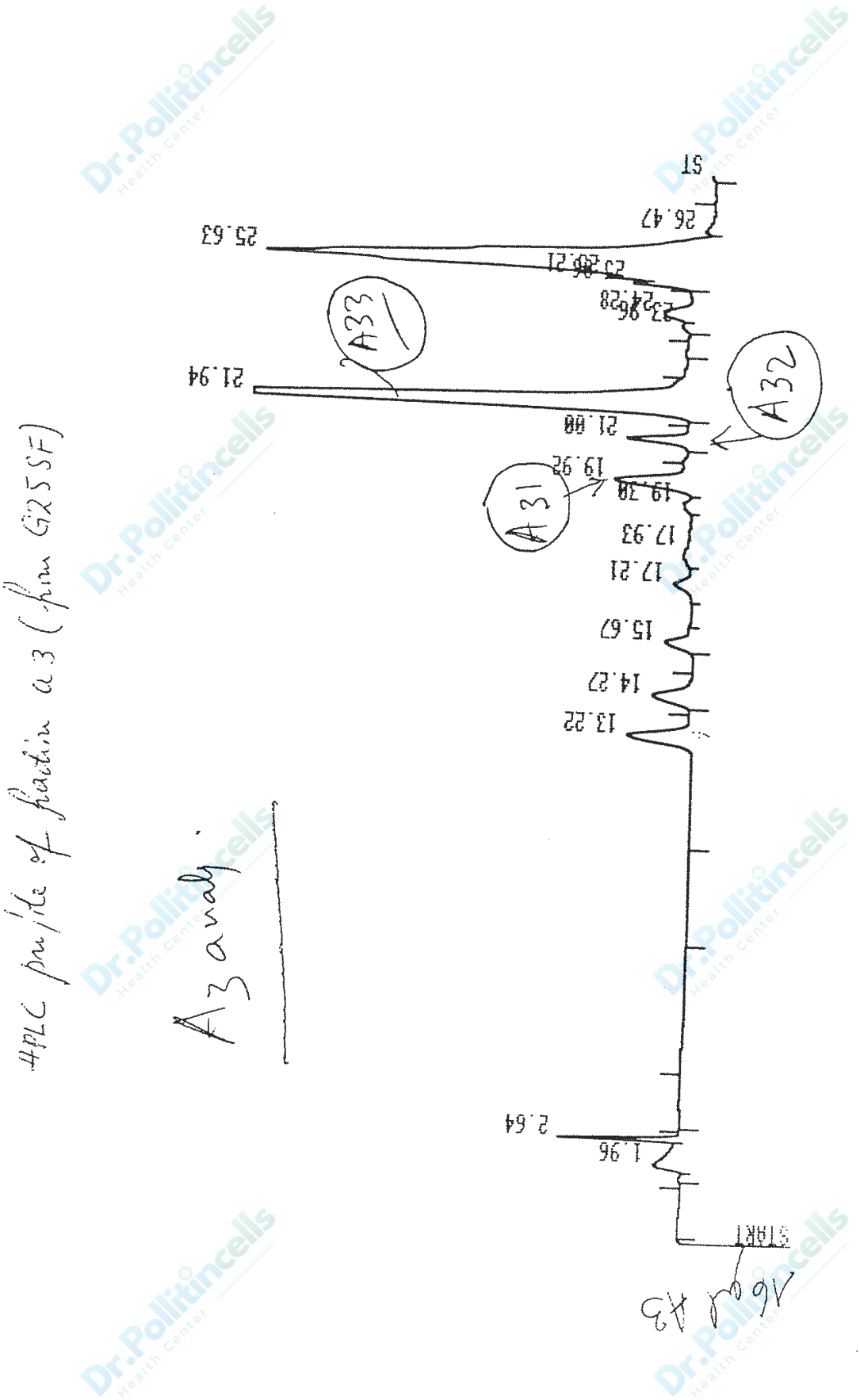


aglucone after hydrolysis at 100°C in 1N HCl

Fig. 8

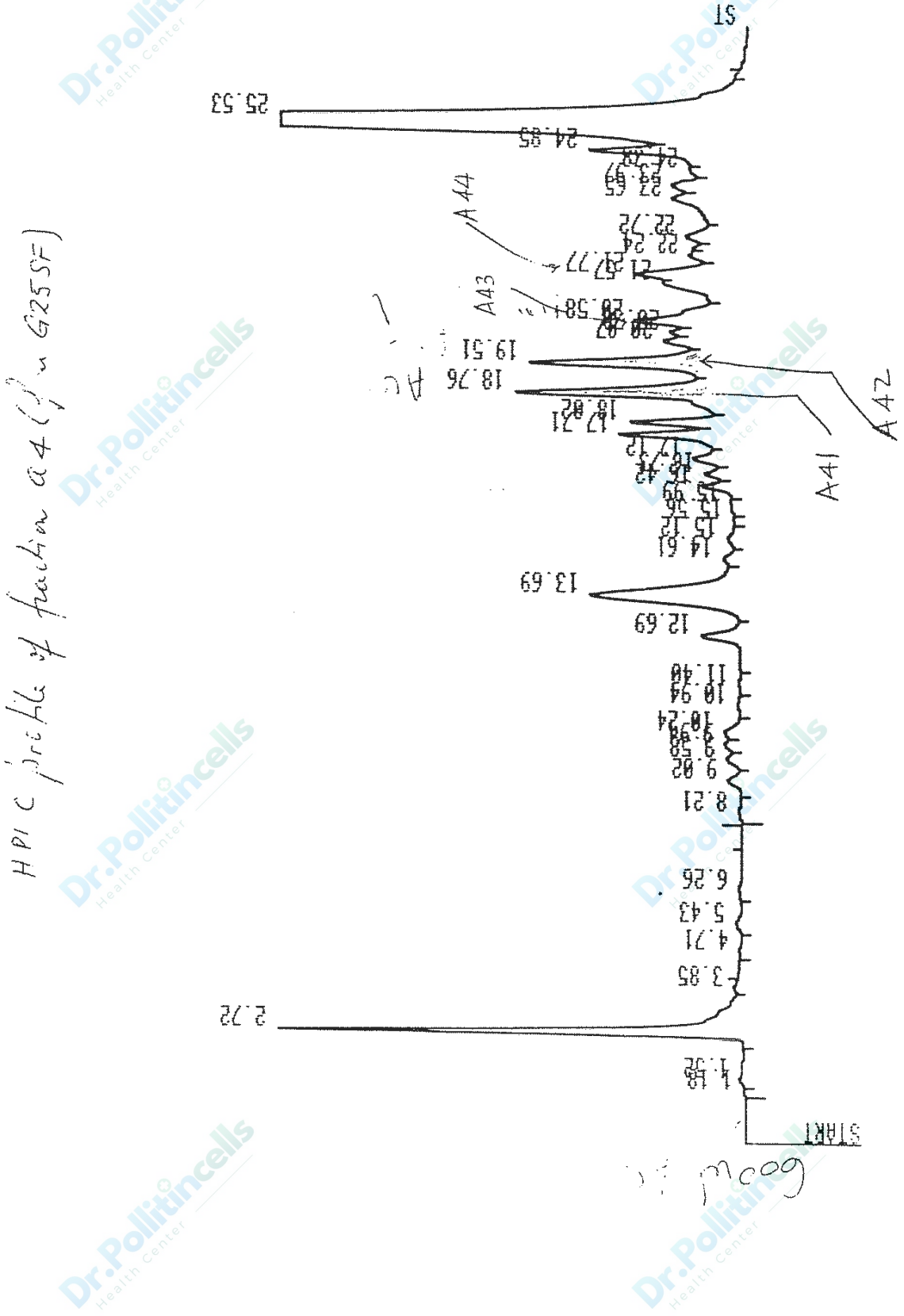

Run Analytique
5µl
hydrolyzed
b52 (3mg)
under vacuum -
at 295nm.
② 72%
① 28%
ATT 21 6 @
THRSH 5 @
START
1.47
4.04
4.67
13.55
13.99
14.78
15.65
16.16
16.57
17.28
17.69
out
20.59
21.53
22.18
b51H
b52H


sep de 140 mg
2.5
2.0
1.5
0.5
0.1
43
64
94 [52-90]
66 mg
66 mg
30 +
96 mg
Yield
96/140 ≈68%
h3
[32-51]
30 mg
h2
h1


HPLC purity of fraction a3 (from G25SF)
A3 analy
Abs 43
START
1.96
2.64
13.22
14.27
15.67
17.21
17.93
19.30
19.92
21.00
21.94
23.96
24.28
25.21
25.63
26.47
ST
A31
A32
A33

HPLC profile of pure b51 after 2 h. boiling
on the Varian HPLC column → 2 forms in equilibrium

b51 (in D_2O) 5μl


HPLC profile of fraction a4 (Jurkat G25SF)
START
2.72
13.69
12.69
A41
A42
A43
A44
ST

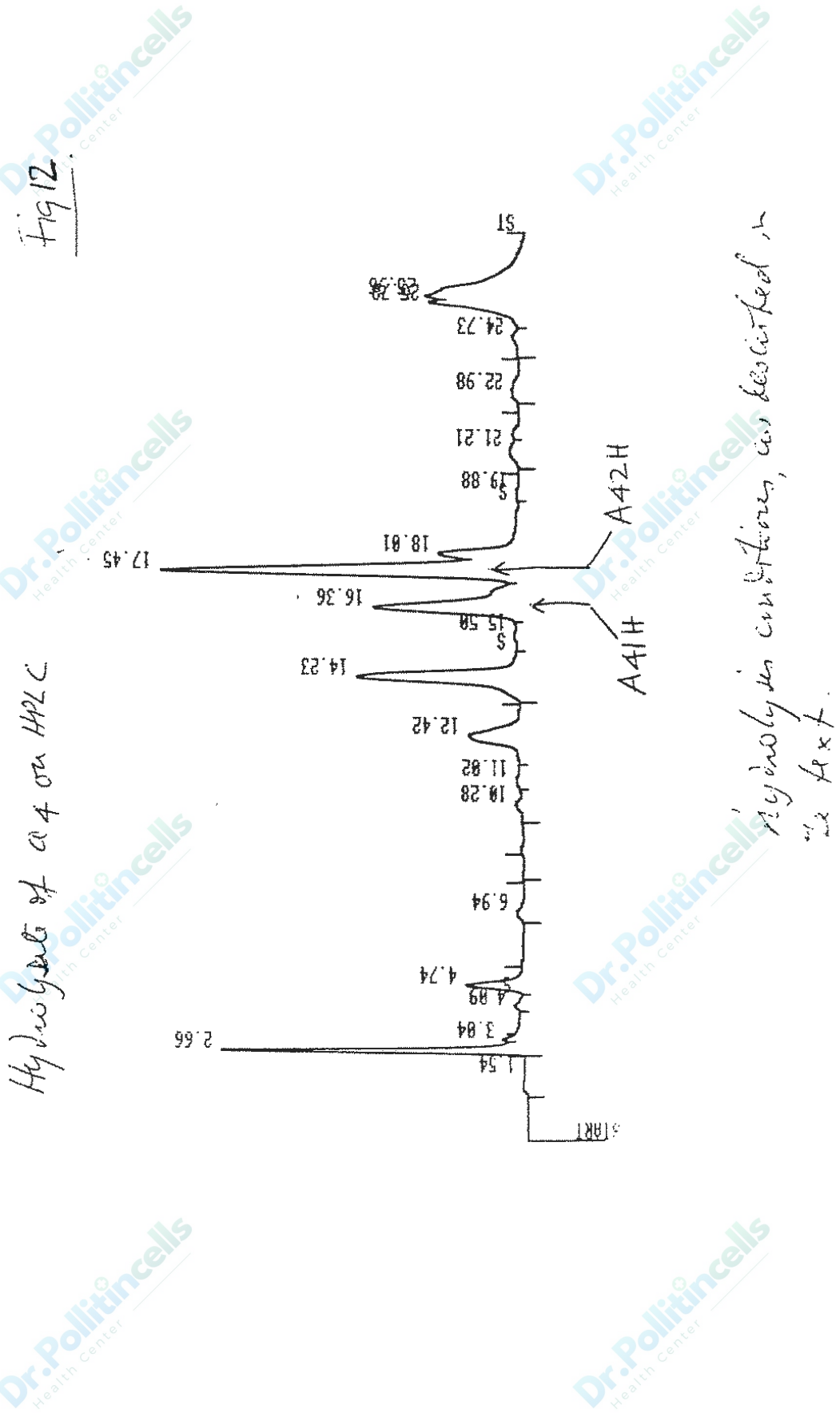


Fig 12.
Hydrolysate of α4 on HT29
A41H
A42H
ST
2.66
3.84
4.74
6.94
12.42
14.23
16.36
17.45
18.01
24.73
22.98
21.21
19.28
11.82
4.89
1.54

Pollen as a Prophylactic against the Common Cold

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Pollen extract has been employed to a considerable extent, since 1955, in the treatment of prostate problems of various kinds (1-5, 8-11).

There would appear to be a widespread opinion that pollen extract also possesses a certain value as a roborant and cold-preventative. This effect has been referred to by Noyes [12] on the basis of a small amount of research material. The roborant effect has also been studied by Glömme [6] in comprehensive experiments on animals.

Critical epidemiological investigations on a large scale have not, however, been carried out. Against this background it seemed desirable to conduct a major field study of the effect of pollen extract on those liable to military service, in connection both with prevention of colds and with general roborant properties.

MATERIALS AND METHODS

The investigation was initiated by the Defense Department Research Institute, and carried out with the consent of the Military Governor in the Sixth Military Area (Upper Norrland), the Chief Physician to the Army, and the State Pharmaceutical Laboratory. The study was carried out on three separate occasions on a total of 775 conscripts in the Sixth Military Region. The designation and size of the groups studied are shown in Table 1.

Table 1. Group division and number of experimental persons

Group	Unit	Number of experimental persons
A	Eng. 3	224
B	Eng. 3	116
C	A 8	99
D	A 8	140
E	Eng. 3 rep-unit	44
F	Eng. 3	152

Group A consisted of newly enrolled conscripts, who were confined to barracks during the whole test period. The object of this was to test the problem during a period in which conscripts, who often come from different environments and different infective situations, are known from experience to be affected by a large number of mixed infections. With regard to Groups B-F, the experiments were carried out in connection with winter field-exercises, under conditions where troops are often exposed to major physical and psychical strains in a period when the danger of infection is great. In other particulars the experiments were carried out on all the field-service groups under substantially identical conditions.

The preparation to be tested, Cernilton, was made available by the manufacturers, AB Cernelle of Vegeholm. The dosage in group A, B and C was one tablet three times daily for 14 days. Two tablets were administered once daily for the same period to subjects of group E and F. The specifications of the preparations tested are shown in Table 2.

Table 2. The specifications of the preparations tested

Specification	Groups	
	A - C	E - F
Cernitin T60 sec. (Extr. pollinis aquos sec.)	60 mg	200 mg
Cernitin GBX$_t$ (Extr. pollinis oleos.)	3 mg	10 mg
Constituentiae et coloris	q. s.	q. s.
M.F. tabl. No. 1		

The experimental model was of the so-called double-blind type. Each unit was divided up into more-or-less equal ,,primar'' research units of 10 - 15 men, generally consisting of personel belonging to the same barrack-room, of smaller working group, with high individual working frequency. With the change distribution of the tablets it was ensured that every ,,primary unit'' was represented by more-or-less an equal number of experimental persons, with Cernilton or placebo-medication. This arrangement was made in order to balance any effect which might arise between the experimental persons within the various ,,primary units'' (infected). The blind tablets and Cernilton tablets had exactly the same taste and appearance.

A leader was selected for each group, whose responsibility it was to see to it that the tablets were taken in the way arranged. The experimental persons were asked to make notes on a special diary card during the whole experimental period concerning their state of health, with special attention to certain subjective symptoms, visits to the doctor, and sickness certification. The group leader was responsible for seeing that this was thoroughly carried out. No doctor participated in this part of the experiment.

RESULTS

The possible prophylactic effect of a preparation against symptoms of the common cold can obviously only be evaluated on the basis of material where there is ,,normally'' a rather high incidence of sickness. Of the six units tested during the relevant experimental periods, symptoms indicative of infection of the upper air passage occurred as indicated in Table 3. The table shows that the frequency of colds was low or very low in groups B and E. These groups have therefore been excluded from following discussion. The incidence of certain symptoms of infection of the upper air passage, divided up in accordance with the investigation group and type of tablet, is shown in Table 4.

Table 4. Incidence of sore throat, coughing, hoarseness, and nasal catarrh within the experimental groups

Symptom	Experimental groups							
	A		C		D		F	
	P	C	P	C	P	C	P	C
Sore throat	21,4	18,8	23,6	12,5	17,3	9,8	17,9	21,2
Coughing	28,0	30,7	35,3	29,2	18,8	11,2	31,3	21,2
Hoarseness	11,1	14,5	11,8	20,9	13,0	7,0	13,5	10,6
Nasal catarrh	37,5	35,0	31,4	29,3	24,6	28,2	32,9	31,7
Basic number	107	117	51	48	69	71	67	85

P – placebo (%), C – Cernilton (%)

The table shows a clear distinction between Cernilton and placebo treated experimental persons in investigation groups C and D in relation to sore throat. The differences are in favour of the preparation, and are significant at the 10% level, Khisquare analysis with correlation for continuity in the present case. Coughing also tends to occur rather less frequently with the Cernilton-treated groups (C, D, and F), though it is only within group F that the results are significant at the 10% level. The figures shown in the table for hoarseness and nasal catarrh symptoms can not be regarded as showing any effect: the difference between Cernilton-series and placebo-series are not significantly different from zero. Symptoms of influenza occurred only to a slight extent, and could not be used to evaluate any possible prophylactic effect.

The relative numbers of persons during the observation period who visited the doctor or were certified sick are shown in Table 5.
Visits to the doctor and sick-certification occurred practically only in groups D and F. There was a clear distinction favourable to the preparation between the Cernilton and placebo treated experimental persons, particularly in group D, but also to some extent in group F. The distinction for group D is significant at the 5% level with respect to visits to the doctor, and at the 1% level with respect to sick-certification.

Table 5. Visits to the doctor and sickness certification within the experimental groups

Visits	Experimental groups							
	A		C		D		F	
	P	C	P	C	P	C	P	C
Visited doctor	0,9	0,0	3,9	4,2	13,0	2,8	7,5	4,7
Certified sick	2,8	0,0	0,0	0,0	17,3	2,8	16,5	10,7
Basic number	107	117	51	48	69	71	67	85

P – placebo (%), C – Cernilton (%)

With respect to all the symptoms discussed here, and also to sick-certification, the experimental persons were asked to indicate for how long the symptoms or the certification had lasted. There was no clear distinction between Cernilton-treated and placebo-treated individuals, although there was a certain non-significant tendency for shorter times observed in the case of the Cernilton groups.

The experimental persons were also asked to give a general opinion about their condition during the experimental period, in particular as to whether they felt more tired or more alertt than usual. The alternative answers were formulated differently in the 1965 and 1966 investigations. In 1965 only the two alternatives ,,more tired than usua'' and ,,more alert than usual'' were given, with the result that the experimental persons were,,compelle'' to choose one alternative or the other, or to leave the question unanswered. In the 1966 investigations a further alternative ,,unchange'' was allowed.

Comparison shows that the experimental persons treated with Cernilton in groups C and D show a higher percent of ,,more tired'' than those with placebo-treatment The frequency ,,more tired" is higher throughout for the placebo-treated persons in all four groups. A summing-up of all the experimental groups gives significance at the 10% level.

Finally, it should be said that only individuals with common cold symptoms in the four groups have been considered. The frequencies of ,,more alert'' and ,,more tired'' amongst the persons showing symptoms of colds are given. The tendency is thus amplified and the effects of Cernilton summed up over the groups then reaches the significance-level of 2.5%.

DISCUSSION

The field experiment carried out has not given an unquivocal result in relation to the prophylactic effect of the preparations used against the common cold. It has been shown that under certain conditions it is effective against some symptoms, that is, sore throat and coughing, in groups C and D. That the corresponding effects could not be deduced from groups A and F indicates the need for great caution in generalizing the results. It lies in the nature of the experiment that the Cernilton-treated and placebo-treated experimental persons are fully comparable within the units because of the,,blind'' randomizing. On the other hand, the four main groups themselves are not comparable on the same basis because of the different risks of being infected by the common cold, or of the type of infection experienced. Thus, fur example, group A consisted of a depot unit, which differs from the exercise units with relation both to the incidence of infection and the extent of strain experienced.

The frequency of visits to the doctor and sick-certification indicate that group D and F may have experienced heavier burdens than the two remaining groups. Here a clear distinction between Cernilton-treated and placebo-treated experimental persons has proved demonstrable both with relation to visits to the doctor and sick-certification.

The roborant effect of Cernilton has been evaluated on the basis of a question about condition. Here also groups C and D, and possibly F, give the clearest indication. It should be observed that the distinction is primarily expressed in a lower frequency of ,,tired'' amongst the Cernilton-treated persons. This occurs, naturally, in relation to the situation of the experimental persons, in which the burdens and the occurrence of common colds gives the least encouragement for individuals to report themselves as ,,more alert'' than usual. The results of the condition-question has also been considered separately for those individuals who declared themselves as suffering from some symptoms of the common cold. The object with this was to obtain a specially afflicted group for which any effect of Cernilton would have been particularly valuable. It is found that the effect in this analysis is most clearly expressed where the frequency of ,.more tired'' is lower throughout for all four units. The effect is most marked in group D, where none of the 26 sick persons in the Cernilton-treated group complain of having been ,,more tired''. The number of sick persons is admittedly relatively low, but the overall tendency gives nevertheless an unequiocally significant picture.

As we have already said, the results should not be generalized, at all events not to the extent that quantitative evaluations of the protective effect are given. It should also be remembered in this connection that the experimental situation for

military personel in training is an extremely specialized one.

It would be expected that in this situation, particularly when those concerned are aware that an experiment is being undertaken, that such persons would be extremely observant about their condition of health, and that tendencies to exaggeration may be found. This would not, however, be the reason for the observed effect of Cernilton, but it would make any quantitative evaluation very hazardous. All that should therefore be said for the present, therefore, is that the preparation under certain conditions combats the symptoms associated with infection of the upper air passage, and might for this reason be a useful prophylactic. The preparation has in addition shown during this investigation a roborant effect, in accordance with the observations already reported by Ask-Upmark [1], Glömme and Rasmussen [6] and Graudal [7].

Further elucidation of the conditions under which this effect arises, or the principle on which it is based, could not be provided by this field experiment, nor was this envisaged when it was undertaken.

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..*(Translation from FEMINA* No.40, 1961)

Prospect on Pollen

Ingrid Ericson

Cernitin, a microbiological digest (soluble extract) of a pollen extract, is today subject to great attention within the medical world. In connection with certain infectious complaints it has proved superior to antibiotics and altogether it offers interesting aspects on the possibilities of our finally obtaining an effective drug which promotes the ability of the body to mobilize forces needed, to kill a bacterial or viral culture.

A Gagarin travels out in space and the sensation makes mankind intoxicated with happiness with the new prospects and worlds of excitement opened up by the blast off. But a few million pollen grains cross our way on their spacetrip and nothing else happens but some of us are horrified at the onset of hay fever. The pollen does not give subject matter for any fascinating faction, does not create mass psychoses or release primitive hero worship—it just exists in its microscopical smallness and quietness. Yet the pollen in fact is a sensational factor maybe of greater dimension than a human space traveller. Maybe the answer to our most urgent question.

That pollen could give us the answer how life actually arose is a romantic theory. Of course, no scientist would dream of expressing thoughts in that direction publicly at the stage pollen research stands today. We can only note that pollen is subject to intensive research in laboratories around the world and that results have not been absent. In other words: quite a lot is proved, and much remains to be proved. And so we laymen are free to read between the lines and theorize—anyway we are sure that we are not concerned purely with utopian ideas.

In a report from the Cernelle Company in Vegeholm, published in Femina No.4, 1957, we rendered an account of how it is possible for a scientist today to pursue a rational pollen research programme. Accordingly it was the founder Gösta Carlsson who realized the desire of the scientist to have an unlimited and stable supply of pure pollen at their disposal, and it is these two who first should receive thanks from mankind. Earlier it was impossible to obtain an accurate analysis of pollen, partly because the material was not available in the large amounts needed and partly because the time available to work on the material was so short, since the pollen grain did not last without the outer membrane. Now however these difficulties have been eliminated and the Cernelle company has outgrown its swaddling-clothes and is striding out into international arenas. They still remain the sole collectors and distributors of pure pollen on a big scale, in the world, but they are now also manufacturing a number of their own drugs, the Cernitin preparations. The ultra-up-to-date laboratories keep their doors open to foreign and domestic research scientists. Tests with the Cernitin preparations and future development are controlled by asst. prof. Hans Palmstierna, Bacteriological Institution, the Karolinska Institute.

WHY JUST POLLEN?

The bees have the ability to do what we cannot, that is to make a sex-determination beforehand. The larvae which are to become queen bees are consistently fed a well-balanced meal consisting of a pollen and honey. Why just pollen? The analysis gave a plain answer on behalf of the bees, pollen is a perfect building material for the cells. All known vitamins occur, more than a score of aminoacids, lipoids or fats and minerals together with enzymes, coenzymes and growth hormones plus a number of substances of a biochemical nature not so far identified. Thus it is seen that the pollen is extremely complicated,

and why it works as it does on the human system is hard to define. But nevertheless the Cernitin preparations are subject to great medical attention thanks to the astonishing results affected with them. The effective part of the Cernitin preparations is made from a standardized mixture of a type of pure pollen, which is extracted by a dissolving agent and then exposed to Autolys (self reaction) combined with controlled digestion by micro-organisms. The Cernitin preparations are thus microbiological digests of a pollen extract. All modern laboratory tests have been done with these, and they have been tested on mice and rabbits and so on. No secondary effects have been proved—not even when they have been tested on pollen allergies. And best of all is, that despite the fact that the pollen has been exposed to such extensive tests so that all secondary effects have been eliminated, none of the effective contents have been destroyed but exist unadulterated in the prepared Cernitin.

EFFECTIVE AGAINST INFECTIONS

Already at an early stage it was found that Cernitin was effective in connection with infections. When the so called "Asian Flu" raged a few years ago one could shorten the fever period considerably by administering preparations by Cernitin, and in hospitals the preventing effects of the pollen preparations in similar cases of risk of infection have also been noted. But Cernitin really came to the attention in the medical world when Professor Erik Ask-Upmark reported his experiences of Cernitin in connection with prostate (Svenska Läkartidningen/The Swedish Medical Journal/1959; 56; 1840, No. 26). The prostate, which especially affects elderly men, has so far been regarded as an illness difficult to cure and not even strong doses of antibiotics have been able to cure it. Often there are frequent relapses with a chronic final stage. On top of that the prostate is painful, very exhausting and psychologically quite upsetting. But it was by a mere chance that Professor Ask-Upmark had his attention directed to the pollen preparations. A man in his fifties fell ill with an acute prostate in May, 1952. It abated after a month but recurred repeatedly at 6-8 week intervals. Chloromycetin was the only thing which could turn the tide of this acute case, but it could not prevent recurrence, despite the fact that at one stage as much as 150 gram chloromycetin was used during a period of two months. Only once did it take nearly three months before recurrence appeared but this, longer interval was after a cystotomy. In May, 1957, the patient started on his own accord to take 6 pollen tablets a day to strengthen him since he felt tired. Since then he has not had more than one recurrence. That was in the beginning and in connection with a journey when he skipped the pollen preparation for a fortnight.

After Professor Ask-Upmark's observations prostate patients at the Urological Clinic in Lund and in few other places have also been treated with pollen preparations and good results have been attained. In these cases a variant of Cernitin, Cernilton, which is available both in fluid form and in pills was used. Cernitin is an ethical drug according to the rules of the Medical Board concerning registering of preparations as medicines. One could ask why an entirely poison free and non habit forming remedy should be available only on prescription, and with Professor Ask-Upmark we can also wonder why all the Cernitin preparations are not on the pharmaceutical benefits scheme.

AN INTERESTING THEORY

What exactly happens in the organism when it comes into contact with Cernitin? Something rather revolutionary must happen since not even antibiotics vindicate themselves in this context. Well, exactly what happens we don't know yet. But with tests on bacteria cultures at least one interesting theory came to light. Two bacteria cultures of the same bacteria flora were used. To one was added antibiotics and to the other Cernitin. As expected the bacteria flora died from the antibiotics—but it happily lived on with the Cernitin. In a few tests the bacteria flora even developed together with the Cernitin. Thus without further ado one can point out the fact that Cernitin does not have the same qualities as antibiotics. Cernitin acts neither checking nor killing a bacterial flora. At this stage we also know that an antibiotic after certain usage creates immunity towards just that type of antibiotic used which also occurs with the sulpha preparations. But this is not the case with Cernitin. It is life promoting in the highest degree, and so far we can at least theoretically suppose that what happens when the organisms comes into contact with Cernitin is that the natural defense mechanism is activated in such

a way that it becomes possible for the organism itself to mobilize the forces needed in order to destroy a bacteria culture. Strengthening the theory about the activising of the organism are the good results obtained within the geriatrics with the aid of Cernitin preparations. In a number of homes for the aged a definite mental activising and a general improvement of the physical health has been noticed after regular doses of Cernilton. The old, who before the treatment with Cernilton had been sitting doing nothing and were disinterested in the world around them, have afterwards been much more alert and shown a considerable interest in their environment.

Considering that we are becoming a community of predominantly old people it would be wonderful if the less comfortable aging symptoms could be made easier. It has also been shown that the body more easily utilises other valuable substances if they are given in combination with Cernitin. Ground bones for instance are very difficult for the body to absorb, but in combination with Cernitin and vitamins A and D the body can utilize up to 85 per cent of the calcium. The Cernident tablet, as it has been called, has given very good results in connection with pregnancy where the possibility of albumin cramps has been eliminated by giving the body sufficient amounts of calcium, phosphorus, fluoride and vitamins. And because of the great value of calcium and fluoride the skeleton and tooth formation the effect of the tablets has of course also been studied by dentists. The Cernident tablets have been shown effective when it comes to preventing tooth decay, and the Medical Board is going to raise the question about the suitability of making Cernident an obligatory supplement to school meals.

Thus the Cernitin preparations have seriously come into the medical limelight here at home, in the rest of Scandinavia and Europe and in both North and South America. The survey done on Cernitin in connection with a test of American football players is considered sensational. Dr. Charles E. Noyes was the leader of the experiment, which was carried out in Maitland, Florida. Thirty football players from Winter Park High School were divided in two groups, one half got the ordinary multivitamin tablets while the other got pollen tablets or Pollitabs, as they are called when sold abroad. The group receiving pollen tablets showed an increase in weight at the end of the season, which is remarkable. Here the weight increase must have meant an activating of the cells in the muscle mass. On top of that it was found that these boys had a greater resistance against infections—according to the statistics only one day's illness because of a cold and seven because of influenza. The corresponding numbers for the ordinary vitamin group were respectively sixteen and eighteen. And while doctors and researchers around the world are busy finding out the possibilities of the already available Cernitin preparations, the research in AB Cernelle Laboratories is still going on under high pressure. Now more can be extracted from the pollen than ever before, and the latest news is a number of fat soluble substances, green in colour and with a local anesthetic effect. The designation is Cernitin GBX 1. So far it only comes in as a constituent of Cernitin healing ointment. Besides the great project which is the collecting of pollen and the manufacturing of medicines, a series of cosmetic preparations based on Cernitin have also been made up under the leadership of pharmacist Erich Paul Tönisson. We are grateful for the low prices of these cosmetics—thus it is possible for all women to give their complexion the biologically correct stimulant it needs for cell regeneration and the normalizing of these cells.

Biologically correct. Well, nothing could be more right than the composition of pollen. All constituents well balanced in nature's own ingenious laboratory in harmony with all cells of the organism. And the scientists have got a field of research so extensive that they seem to shun the closing of the accounts. But their adventure is great, and the more doors opened up to nature's secret chambers the more stupefying the discoveries. But don't let us get quasi-scientifically romantic—the existing facts are sufficient. The prospects on pollen are more sensational than the prospects on the moon. To us earth creatures...

The Use of Cernitin, an Extract of Organic Pollen, to Increase Body Weight and to Increase Resistance Toward Infections

BRIEF DESCRIPTION OF THE PRODUCT

For centuries the nutritional value of naturally occurring pollen has been recognized by scientists throughout the world. For the first time a commercial source of natural pollen has been made available by AB Cernelle of Vegeholm, Sweden, marketed under the trade name POLLITABS*. These tablets contain Cernitin, a microbiological extract of pollen, which is organic, unadulterated, and free of contamination. Prior to the extraction of Cernitin, the pollen is collected by a patented process (not insect-gathered) from unsprayed plants on a large plantation far removed from industrial wastes or other air-borne contamination. During the preparation of Pollitabs, no synthetic active ingredients are added. These food tablets are completely free from side effects and even pollen-allergic persons have taken large doses without any unforward effects.

THE BACKGROUND OF THE STUDY

During the past two years, we have used Pollitabs in our practice for many diversified complaints and syndromes. Certain results have occurred predominately regardless of the purpose for which the tablet was prescribed. Foremost among these have been increased appetite, weight gain, increased vigor and sense of well being, and decreased susceptibility toward infections. Therefore, it was thought that a football team would make a good preliminary control study to more accurately determine two of these factors in an objective manner: i.e. weight gain and resistance to infection.

DESCRIPTION OF THE STUDY

A local high school football team, consisting of thirty active players were selected for this study. The team was divided into two groups; those receiving pollitabs and those receiveing a standard multiple vitamin preparation. The study covers a period of 15 weeks, the first three of which neither Pollitabs nor multiple vitamins were used. It was during this initial 3 week period that each player lost excessive weight, in most cases, representing excess adipose tissue. Beginning at the end of the 3rd week, 15 players were started on two Pollitabs daily and the control group on the multiple vitamins daily. All medication was administered daily and individually by the coach. A record was kept of the players' weights at weekly intervals and the average weight for the group has been plotted on Graph 1. It can be noted that the group receiving the pollitabs regained their pre-season weight after taking the tablets for 7 $^1/_2$ weeks and 4 $^1/_2$ weeks later, at the end of the season, actually showed the Pollitabs group with a 5 $^1/_2$ pound average increase in weight over their preseason level. The group taking the multi-vitamins remained generally constant from the third to fifteenth week, showing no further loss or gain. The opinion has been expressed by impartial former professional players, who have seen this report, that it is almost unheard of for a football player to weigh more at the end of the season than he did before practice started.

CONTRAST OF STUDY

Graph 2 shows a striking contrast between the two groups regarding the number of days lost from the common cold or influenza. Since the two groups were in close contact physically during the study period and since the selection of the players to take Pollitabs was made at random without regard to socio-economic or other factors, it is felt that the results are quite significant.

SUMMARY

A preliminary control study was performed to determine the comparative weight-building properties and infection-resisting properties of a newly available product, Pollitabs, as compared to a standard multi-vitamin.

The results show a marked ability of the Cernitin Pollitabs to produce better weight gain and increased resistance toward infections. It is felt

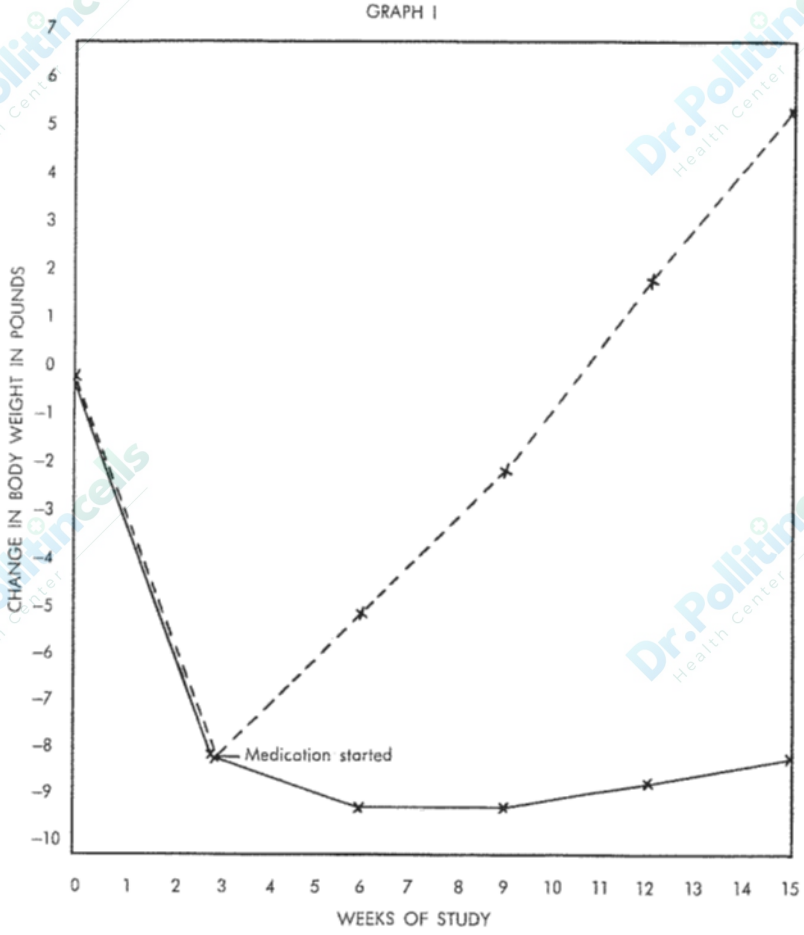
that further studies are definitely indicated and these are being planned.

This study was performed at the Winter Park High School and under the strict personal supervision of Coach Mosher, and under the direction of Charles E. Noyes, M.D.

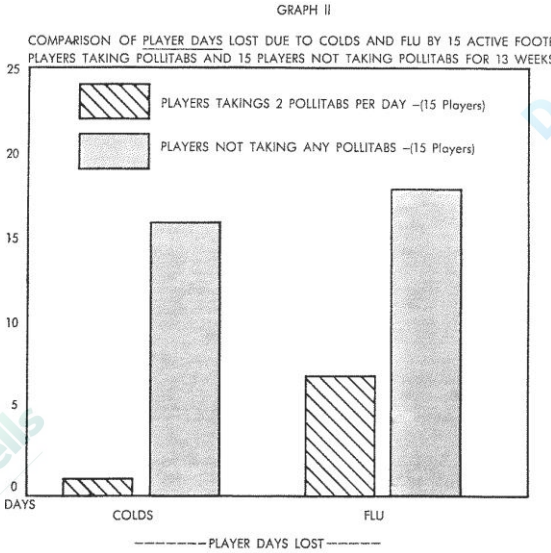
Charles E. Noyes, Jr. M.D.

* The Pollitabs used in this study were furnished by POLL-N-CO., INC., Maitland, Florida.

GRAPH I



GRAPH II



NAME	Pos	NO POLLITABS			WEIGHT with POLLITABS									Days Lost From Colds	Days Lost From FLU	Days Lost INJURIES	Games Missed a/c of Injury	Quarters Played our of 40
dates		822	829	9.5	912	919	926	103	17	24	31	117	19	21	28	125					
1. Gene Mooney	G	154	151	145	146	146	148	151	149	150	152	156	154	153	154	154	1	2	6*	0	37
2. John Cash	E	161	158	152	155	156	156	158	159	161	163	164	165	167	169	170	0	0	0	0	40
3. Max Morris	G	145	139	135	135	136	137	139	139	140	141	143	145	146	149	151	0	2	0	0	36
4. Ray Myers	FB	155	154	152	151	153	155	157	157	157	158	161	161	161	164	165	0	0	5**	1	36
5. Dewey Ramsby	QB	150	146	146	146	147	147	147	147	147	149	152	151	151	153	153	0	0	0	0	34
6. Chan Muller	G	169	165	162	164	166	166	170	172	171	170	172	175	177	177	177	0	0	0	0	36
7. Frank Ferguson	T	225	215	210	210	212	211	213	215	220	221	224	228	227	224	226	0	2	8***	0	39
8. Gregg Holland	HB	161	155	156	156	160	161	160	160	163	163	162	161	165	165	168	0	0	0	0	40
9. Tim Hartung	T	186	179	171	176	175	175	178	181	180	182	182	185	188	189	189	0	0	12****	2	32
10. Chas. Cayll	HB	149	141	138	141	141	142	144	144	144	147	149	152	155	156	157	0	0	5 (A)	1	36
11. Bill Collier	QB	145	141	140	141	142	143	146	146	145	145	147	145	145	144	146	0	0	0	0	40
12. Julius Mullins	T	184	180	176	175	176	178	181	180	180	179	180	182	182	180	184	0	1	0	0	40
13. Dick Thomas	C	150	148	143	146	145	145	146	145	144	146	149	152	154	156	156	0	0	0	0	30
14. Mike Born	E	168	161	159	156	157	157	160	163	163	165	166	167	169	170	173	0	0	5 (B)	1	36
15. Ned Whitner	FB	180	179	172	176	176	178	177	176	178	177	180	186	185	189	188	0	0	20 (C)	4	22

Weights taken weekly.

Boys took 2 Pollitabs per day

* Bruised Shoulder
** Sprained Ankle
*** Bruised Shoulder
**** Twisted Knee

(A) Sprained Back
(B) Broken Neck
(C) Broken Collarbone

15 boys did NOT take Pollitabs — Their record: 16 days lost because of COLDS 18 days lost because of FLU.

Bob Mosher, Asst Football Coach
Winter Park High School, Winter Park, Florida
Tel: Midway 7—3078

MUSCLE SUPPORT:

GRAMINEX Flower Pollen Extract

Metabolic Adaptation of Muscles to Exercise, Vibration and Raised Temperature under the Influence of Cernitin™™

Teresa J. Sawicka, Piotr I, Laszczyca, Bogdan Smylla and Zbigniew Jethon

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Sawicka, T. J., Laszcztca, P., Smylla, B. and Jethon, Z.: Metabolic adaptation of muscles to exercise, vibration and raised temperature under the influence of Cernitin™. Acta physiol. pol., 1984, 35(2), 141-150. Wistar rats were used to study the effects of Cernitin™, i.e. aqueous and oil extracts of pollens, on the metabolic adaptation of the soleus muscle to exercise, vibration and raised ambient temperature. The animals were exposed to selected combinations of these factors for 5 days during 1.5 hour daily. A part of the animals was given orally Cernitin™ in daily doses of 6 mg/mg of body weight for 10 days before the exposure. Among the adaptation changes studied in the soleus muscle, 24 hours after the last exposure, Cernitin™ caused: 1) a reduction of the amount of total protein, 2) an increase in the proportion of soluble proteins in the protein fraction, 3) an increase in the tissue oxygen consumption, 4) an increase of already elevated pyruvate kinase activity, 5) a further rise in ATP level, 6) an increase in lactic dehydrogenase activity, 7) a rise in the activity of cholinesterases. Moreover, it increased significantly the body weight and the weight of the studied soleus muscle. Cernitin™, in combination with certain types of exposure used in this experiment, exerted a catabolic action, increased the rate of anaerobic metabolism and enhanced adaptation to exercise, vibration and temperature. The direction of the adaptation changes depended on the type of exposure to environmental factors.

The adaptation of human organism to the living and working environment becomes often difficult, due to the influence of new noxious environmental factors. This is true particularly of extreme conditions, which exist in certain types of work places. The means used for facilitating the adaptation of man – operator are not always sufficient for reducing the intensity of the harmful effects of the environment, nevertheless they enhance the ability of carrying out work.

One of the factors enhancing adaptation processes are Cernitin™, components of extracts obtained from pollens of flowers. Their favourable effect has been demonstrated on the rate of weight gain [23], course of adaptation to increased ambient temperature [5], resistance to infections [25], and alleviation of the intensity of inflammatory processes [20, 25]. Moreover Cernitin™ have been shown to exert a favourable effect on the capability of performing exercise [1, 11] and the magnitude of training effect [1]. Cernitin™ influence also favourably the well-being and the ability to carry out intellectual and operational work [5].

Exposure to a combination of high ambient temperature and vibration is not infrequent during occupational work. Physical effort of the worker may increase the harmful effects of these factors leading to a rapid decrease of his ability to work. A synergistic action of vibration and physical activity leading to development of vibration-induced lesions is known [9]. Raised ambient temperature antagonizes in this case the effects of vibration [9, 22].

The aim of the present study was to investigate the possibility of using Cernitin™ as an agent

increasing the adaptation effect, that is maintenance of the ability to work under conditions of high ambient temperature and exposure to vibration. The studied problems were limited to the metabolic effects of Cernitin™ in the light of the data known as yet on the mechanism of action of these substances, especially on protein and steroid metabolism [2, 25].

The effects of Cernitin™ were investigated on certain aspects of the exercise metabolism in rats subjected to swimming exercise, sinusoidal vibration and raised ambient temperature.

Materials and Methods

The experiments were carried out on 48 male Wistar rats weighing 190-240 g. The animals were divided into 8 groups in the following way:

Cernilton® was given for 10 days (the rats in groups KC, PC, PWC, PWTC) orally in daily doses of 2 ml. This dose was equivalent to 6

K — control, sedentary group,
KC — sedentary group receiving Cernilton®,
P — group subjected to swimming until exhaustion,
PC — group subjected to swimming and receiving Cernilton®,
PW — group exposed to swimming effort and sinusoidal vibration,
PWC — group treated as PW and receiving Cernilton®,
PWT — group exposed to swimming effort, sinusoidal vibration and high ambient temperature of 37°C during exposure to vibration,
PWTC — group exposed as the PWT group after pretreatment with Cernilton®.

mg/kg of body weight of Cernitin™ T60 (the water-soluble fraction) and 0.3 mg/kg of body weight of Cernitin™ GBX (the lipid-soluble fraction). In accordance with the declaration of the producer this dose corresponded after calculation for 1 kg of body weight daily to: 0.36-0.55 mg of free amino acids, about 0.2 mg of a mixture of various lipids, among them sterols 0.030 to 0.048 mg. The amount of vitamins received by the animals with Cernilton® was of the order of 10^8 ng for thiamin, riboflavin, pyriodoxamine, pantothenic acid, folic acid and inositol, 10^8 ng for niacin, 10^4 ng for ascorbic acid, 10^0 ng for tocopherol, 10^0 mIU for calciferol. Besides that, this dose contained carotens, 10^1 ng, xantophils 10^8 ng, and mineral components such as Ca, K, P, CI, Na, Mg, Al, Fe, Si, Zn, Mn, Cu in amounts found in vegetable tissues ranging from 50 ng to 60 ug. The preparation used was Cernilton® produced by AB Cernelle, Vegeholm 6250, S 2620 Engelholm, Sweden. The rats in groups K, P, PW, PWT received during the same time 2 ml of a 0.9% NaCl solution orally, as placebo.

On the first day after completion of Cernilton® or placebo administration the animals were subjected to swimming effort, vibration and raised ambient temperature for 5 days.

The animals swam without load until exhaustion in water at 32°C, in tanks making impossible passive floating on water surface. Sinusoidal vibration was applied during 1.5 hour daily using a vibration table steered by a vibration generator, at a mean acceleration of 1.11 m/sec^2 and 4 Hz frequency [8].

During the exposure to vibration the animals in groups PW and PWC remained in an ambient temperature of 19-20°C, and the animals in groups PWT and PWTC were exposed to a temperature of 37°C. Twenty-four hours after the last exposure the animals were killed by decapitation. The soleus muscle was taken from the hindpaws. Muscle fragments were homogenized at 0°C in a proportion of 40 mg of muscle tissue for 1 ml of 0.9% NaCI solution. The following determinations were carried out in the homogenate:

1) concentrations of total protein and protein soluble in isotonic saline by the method of Lowry [21],

2) ATP content in the muscle using Eskalab test kit, (in $HClO_4$ homogenate),

3) pyruvate kinase (PK) activity using Eskalab test kit,

4) lactic dehydrogenase activity (LDH) using Eskalab LDH-UV kit,

5) muscle acetylcholinesterase activity (AChE) by Hestrin's method using acetylcholine as substrate [10].

A part of the obtained muscle mass was homogenized in Tyrode's solution until a final proportion of 100 mg of tissue per 1 ml of the solution for determining the intensity of tissue respiration by Warburg's method.

The results were subjected to statistical analysis by the generally accepted methods. The calculated results included the arithmetical mean and standard deviation. The differences were accepted as significant at $p<0.05$.

Results

The rate of weight gain in the rats not receiving Cernilton® was higher than in the controls (group K) during exercise and exercise combined with vibration (groups P and PW) by 19.2% and 9.3% respectively, while combined exposure to exercise, vibration and high ambient temperature decreased the rate of weight gain by 14.2 %. The ratio of the soleus muscle weight to the total body weight decreased significantly as compared with controls: by 9.2% in the group subjected to exercise (P) and by 4.2% in the group subjected to exercise and vibration (PW). The effect of combined exercise, vibration and high ambient temperature appeared as a decrease of the content of total protein in the soleus muscle which was significant only in group P decreasing by about 17% in relation to controls. The concentration of soluble protein in the soleus muscle decreased significantly in all groups in relation to controls: P – 17.9%, PW – 25.0%, PWT – 23.1%. The ratio of the content of soluble protein to that of total protein decreased significantly by 17.5% only in group PW.

Table 1. Metabolic adaptation of soleus muscle to exercise, vibration and high ambient temperature after treatment with Cernitin™™.

		K	KC	P	PC	PW	PWC	PWT	PWTC
AM	g/24h	1.40	2.36	1.67	1.68	1.53	1.52	1.20	1.80
M_m	mg/g b.m.	0.418 0.024	0.375 0.017	0.380 0.021	0.397 0.007	0.400 0.012	0.393 0.025	0.441 0.019	0.401 0.027
PrT	mg/g t.	101.0 11.7	73.6 14.6	83.6 10.5	72.3 14.2	91.9 15.4	56.8 4.1	79.3 34.3	69.0 12.0
PrS	mg/g t.	15.6 2.4	15.2 1.4	12.8 0.9	14.3 2.2	11.7 2.0	13.8 2.2	12.0 1.7	24.2 4.8
VO_2	Nmol O_2 min mg prot.	19.7 5.9	34.2 12.3	40.2 9.3	33.2 20.9	25.6 10.1	64.1 12.1	54.6 25.5	41.8 15.4
ATP	mg/g prot.	16.8 3.3	39.4 7.2	17.3 4.3	22.7 14.6	45.9 30.6	47.4 6.9	32.7 25.4	35.2 10.4
LDH	IU/g prot.	438 78	777 125	444 49	743 156	437 74	1346 146	496 235	1005 162
PK	IU/ g Prot.	6.6 0.6	13.8 0.8	16.8 0.4	14.2 0.6	9.6 0.4	20.7 0.3	10.1 0.5	15.7 0.5
ChE	IU/ mg prot.	21.53 1.69	23.54 2.24	21.03 2.06	23.52 1.99	20.84 2.44	25.03 1.42	19.65 1.90	24.93 2.65

Designations: K – control group, P – swimming, T – high temperature, C – Cernitin™ administration, AM – weight gain, m_m – proportion of muscle mass to body mass, PrT and PrS – total and soluble protein levels in the muscle, ATP – ATP level, LDH, PK, ChE – activity of: lactic dehydrogenase, pyruvate kinase and cholinesterase

The activity of tissue metabolism expressed as oxygen consumption by tissue homogenate showed an increasing tendency during the exposure to these external factors by 104.5% in group P and 179.0% in group PWT ($p<0.01$). The increase of kinase activity in the studied muscle was significant in all groups in relation to controls and it was 154.5% in group P, 45.5% in group PW and 53.1% in group PWT. Attention is called to the agreement between the directions of changes in tissue respiratory activity and pyruvate kinase activity. The ATP content of the muscle calculated for one unit of total protein was 173% above the control level in group PW ($p<0.05$). In the remaining groups the rise was statistically not significant. The activity of LDH and ChE showed no significant changes in the groups of rats not subjected to treatment with Cernitin™, independently of the action of other factors.

A particularly evident effect of Cernitin™ on the weight gain rate was observed in the group of sedentary rats (KC) as compared with group K (a 68.2% increase) and in group PWTC as compared with PWT group (a 50.0% increase). In the remaining groups changes in relation to the control group and between the corresponding groups were below 20%. The ratio of the soleus muscle weight to the total body weight was not significantly changed. The only exceptions were: a decrease of this index by 9.4% in group KC as compared with group K, and a decrease by 9.1% in group PWTC as compared with PWT.

The concentration of total protein in the soleus muscle was decreased by Cernitin™ in all groups amounting in the case of group pairs to the following values: KC/K – 27.2%, PC/P – 13.5%, PWC/PW – 38.2% and PWTC/PWT – 13%.

A reverse relationship is observed in the concentration of soluble protein since Cernitin™ raised this concentration. However, only a rise by 100% between PWTC/PWT groups was significant. This increased the proportion of enzymatic soluble proteins in the total protein pool by 34.3% for KC/K, 29.4% for PC/P, 91% for PWC/PW and 131.0% for PWTC/PWT.

The oxygen consumption by the homogenates of muscles from the animals treated with Cernitin™ was always higher than in controls independently of the exposure, and in the rats in groups KC and PWC Cernitin™ caused a significant increase in tissue respiration by 73.7% for KC/K and 150.0% for PWC/PW. The remaining changes were not significant.

Significant differences in pyruvate kinase activity were observed only between groups KC and K, and PWC and PW. In both these cases this increase was 115.5% and 115.4% respectively.

The ATP content showed an increasing tendency during treatment with Cernitin™, but this increase was significant only for KC/K, being 111.0%.

LDH activity increased in all groups treated with Cernitin™: 77.5% for KC/K, 67.3% for PC/P, 207.2% for PWC/PW, 102.5% for PWTC/PWT.

ChE activity increased significantly in all groups treated with Cernitin™ with the exception of the sedentary group: 9.2% for PC/P, 11.8% for PWC/PW, and 27.0% for PWTC/PWT.

Discussion

Physical exercise, vibration and high ambient temperature produced disturbances in the protein metabolism in the soleus muscle manifesting themselves as a decrease in the amounts of total and soluble proteins and of the ratio of the soleus weight to the total body weight. These changes were associated with increased intensity of tissue respiration and pyruvate kinase activity. The rate of weight gain decreased or increased in different groups without an unequivocal correlation with the metabolism of the studied muscle. This absence of correlation might be explained as due to brief exposure time or to considerable metabolic, functional differences between various muscles and tissues. The muscle fibres of the ST type prevailing in the soleus respond differently than

the FT muscle fibres prevailing in other muscles. Moreover, these responses are specific with respect to the stimulus and hormonal regulation [23, 29]. Metabolic changes observed by other authors induced by exercise [15, 26, 30], raised ambient temperature [28] and vibration [9, 22] were similar to those observed by us. The character of these changes resembled the metabolic changes observed during stress reaction. Less data are found on the combined effect of several stress-inducing factors on the organism. The effect of exercise combined with vibration and the effect of vibration and high temperature studied by us suggested that under these conditions the reaction of the organism was changed. The most characteristic finding was he change in the equilibrium of the anabolic and catabolic processes. The change was manifested as a decrease in protein concentration and an increase of tissue respiration, as well as an increase in ATP level, which was particularly evident after exposure to vibration (PW group). Raised ambient temperature seemed to exert a protective effect on the equilibrium between catabolism and anabolism. The damaging effect of low ambient temperature on humans exposed to vibration has been reported in the literature [9, 22].

The observed absence of changes in LDH activity after the exposures used in this experiment indicates that their intensity was too small to cause mobilization of anaerobic metabolism [31, 34].

In our experiment Cernitin™ were given before exposing the rats to exercise, vibration and high ambient temperature. Thus the effects observed after Cernitin™ administration were due either to metabolic changes caused by them prior to exposure or to the action of tissue deposits of Cernitin™ or their derivatives mobilized by exposure to stress-inducing factors. The rise in the requirements for amino acids, vitamins, steroids, trace elements, and energy in animals subjected to stress-inducing exposure is known [12, 13, 14, 18, 32]. The five-day exposure to stress-inducing factors in this experiment failed probably to exhaust the stores of these substances in the organism, since the work of Karvonen [14] shows that they can cover much longer time periods.

Administration of Cernitin™ to rats caused in all groups an increase in the proportion of the soluble protein fraction and in the activity of catabolic enzymes (PK, LDH, ChE) in the soleus muscle. Cernitin™ potentated also tissue respiration during exercise, vibration and high ambient temperature. Similarly also, a further rise of ATP was observed in the muscle. The increase of the catabolic activity induced with administration of Cernitin™ manifesting itself as a better utilization of the energy of food components [1, 6], enhanced training effect and effort tolerance has been already described in man and rats [1, 25]. Cernitin™ raise also intestinal absorption of food components [33] and in this field their action is contrary to that of vibration which decreases absorption [27]. Increased LDH activity and reduction of post-exercise blood lactate concentration following intake of Cernitin™ have been described by Jethon [13] and Dabrowski [5]. This effect was observed also in our experiment.

The effect of Cernitin™ on protein metabolism in the soleus muscle manifested itself as an increase in the proportion of the soluble fraction of cell proteins with a decrease in the total protein concentration. The suggested intensification of protein catabolism at the expense of protein anabolism, and intensification by Cernitin™ of changes induced by stressors. These results disagree with those obtained by other authors who found decreased catabolism of amino acids and proteins in humans receiving Cernitin™, with decreased loss of nitrogen in the form of urea [11, 13]. The above discussed differences in the metabolic characteristics of muscles [23, 29] and acceleration, or at least stabilization, by Cernitin™ of the weight gain of rats suggest that in muscles belonging to other metabolic type than the soleus muscle, or in other tissues Cernitin™ stimulated the anabolic processes. This supposition is supported by observations of other authors that Cernitin™ enhanced protein

synthesis during healing of wounds or fractures [20, 25] and increased the rate of weight gain [6, 25, 29].

Increased activity of muscle cholinesterases observed after administration of Cernitin™ may be due to the action of these substances on the nervous system. Cernitin™ are known to increase the psychotechnical performance and intellectual ability as well as the sense of well-being [5, 7, 20, 25]. The possibility of intensification of the tropic action of the nerves on the muscles is not ruled out and the activity of acetylcholinesterase (AChE) depends on this action [3, 4, 35]. Another possibility is increased production or change of the turnover half time of enzymatic proteins similar to the hepatic fraction of secretory enzymes, such as pseudocholinesterase. This supposition is confirmed by the parallelism between increasing ChE activity and the proportion of soluble protein fraction.

The mechanism of the action of Cernitin™ on the effects of the tested exposures depends, probably on a synergistic action of the various components of this mixture of substances [2]. This mechanism may be connected with increased availability (through better absorption) of vitamins as precursors of coenzymes and trace elements as activators. Among the literature reports attention is called to the fact that Cernitin™ raise the amount of 17-ketosteroids and 17-hydroxysteroids excreted with urine [7, 25] and cause vacuolization of the fascicular zone of the adrenal cortex [25], without concomitant signs of adrenal cortical hypertrophy [2, 6]. The possibility cannot be ruled out that 27-29-carbon steroids of Cernitin™ undergo metabolic changes in the organism. The possibility of increased production of 21-carbon corticosteroids is suggested by the investigations described by Oudot [25], and mobilization of the production of 18 and 19-carbox sex steroids is suggested by the results of the investigations of Diczfalusy [6]. Chemical analysis carried out by Kvanta [17, 19] showed a similarity between Cernitin™ steroids and certain oestrogens. Thus the changes observed in our experiment may be due to "facilitation" of the hormonal regulation considering the interaction between steroids, and and differences in the sensitivity of the metabolism of various types of muscles and tissues to their action [16, 23, 29].

In summary, the administration of Cernitin™ during exposure of the organism to combined exercise-vibration-thermal stress produces multi-directional changes not always favorable for such metabolic indices as: muscle mass, level of muscle protein or weight gain. Administration of Cernitin™ seems to be advantageous in individuals not exposed to physical work (KC rats) and subjected to thermal stress (PWTC rats) in view of the necessity of maintenance of homeostasis in protein metabolism and growth processes.

However, during exercise and vibration stress Cernitin™ increases the metabolic fitness (PC and PWC rats) which is unquestionably and advantageous effect enhancing adaptation to working environment.

The always-present Cernitin™ effect increasing LDH activity with increased aerobic metabolism suggests a greater anaerobic potential and tolerance.

Conclusions

1. Cernilton® shows an action stimulating the cellular metabolism and when administered to rats exposed to stress it increases the intensity of catabolic processes.
2. Significantly raised LDH activity after Cernilton® suggests intensification of aerobic metabolism, and increased potential and tolerance of anaerobic metabolism.
3. Cernilton® increases also the energy stores of the phosphagen pool of the muscles at rest during exercise stress.
4. The anabolism of proteins in muscles with prevalence of ST fibers (soleus muscle) in animals exposed to stress was increased much less than the overall catabolism of protein. Metabolic changes induced by Cernilton®, particularly those of protein

anabolism, are probably, quite different, in various tissues, and probably the ST muscles respond to this agent differently than FT muscles and various non-muscular tissues.

5. Cernilton® effect differed significantly during various types of stress to which the rats were exposed:
   - during vibration stress the drug caused mobilization of catabolism at the expense of anabolism,
   - during exercise stress it caused no drastic changes in the anabolism-catabolism equilibrium,
   - the exposure to high ambient temperature and other stress types applied in this experiment increased anabolism above the level observed at room temperature, although catabolism activation by Cernilton® was still significant.
6. Changes in the activity of enzymes and weight gain in the rats exposed to thermal stress point to a favorable effect of Cernilton® on thermal adaptation.
7. Changes in cholinesterase activity in the soleus muscle caused by Cernilton® indicate that its effects either the production of proteins similar to hepatic secretory fraction or the tropic action of motor neurons on the muscle and on the metabolism of muscle proteins.
8. In the potential mechanism of Cernilton®, besides the importance of vitamin and amino acid supplements, the effect of this drug should be analyzed on steroid metabolism and the related effects on hormonal regulation which are probably of essential importance in the development of the observed changes.

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MUSCLE SUPPORT:

GRAMINEX Flower Pollen Extract

Physical performance by weightlifters after consumption of nutritive preparations

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In order to increase performance and maintain health, it is common for sportsmen to consume various nutritive preparations containing, for example, vitamins, amino acids, proteins and minerals, particularly iron. It is often considered that the intense physical activity of sportsmen requires an increased intake of such substances, compared with the requirements of those who lead physically less active lives. Systematic evaluation of the effects of these substances has been rather rare. On the contrary, any effects have often been judged subjectively.

The purpose of this experiment is to endeavor to assess how consumption of various nutritional preparations during training affects weightlifters.

Test subjects and procedure

42 weight lifters (aged between 18 and 24) of good but not international standard were divided at random into 6 groups each consisting of 7 persons. One control group was not given nutritive supplements, while each of the other groups received various preparations, as shown in Table 1.

Table 1
Nutritive preparations used:

1. Multivitamin preparations (Polfa®) (A_1, B_1, B_2, nicotinamide, pyridoxine, Ca pantothenate, B_{12}, C, D, E) in approximately double the recommended daily doses.
2. Hemoglobin preparation (Hemoglobin-caps®), 2 g/d
3. Multivitamin preparation (Vitaral®) A_1, B_1, B_2, pyrodoxin, C, D, approximately normal recommended daily dose.
4. Pollen preparation (Pollitabs sport®), Cernelle – 4 tablets daily, pollen extract, Cernitin T60 – 50 mg, Cernitin GBX – 1 mg per tablet.
5. Pollen/ amino acid preparation (“Stark-protein®” – 8 capsules daily, Pollitabs® - 4 tablets daily, Cernelle). Pollen extract, Cernitin T60-50 mg. Cernitin GBX – 1 mg./1 tablet. Amino acid concentrates containing 18 free amino acids, including all the essential ones: 350 amino acids per capsule.
6. No nutritive preparations.

The work capacity of the participants, measured with a standard bicycle ergometer at the beginning of the experiment and then again after 6 weeks at the training camp, at which all the participants undertook approximately the same type of training. The increase in the blood’s lactic acid level after 10 minutes of cycling, with a load of 2 watts per kg of body weight was measured before and after 6 weeks of training. The results were processed according to normal statistical procedures and the Student “t-test.”

Results

Work capacity, expressed in terms of oxygen consumption per kg of body weight, increased in all participants during the training period. There were, however, considerable differences among the groups; differences which, for three of the nutritive preparations, were significant in comparison with the control group (see Table 2).

The increase in lactic acid concentration in the blood after 10 minutes of cycling showed a decline after 6 weeks of training in all groups. However, the decline differed from group to group and diverged significantly from the control group in two of the groups given nutritional supplements (Table 3).

Table 2 Average increase of work capacity after 6 weeks of training.

	Increase %	Significant level compared to control group
1. Multivitamins	93	5%
2. Hemoglobin preparation	84	5%
3. Multivitamins	48	no change
4. Pollen extract	70	no change
5. Pollen extract + amino acids	123	1%
6. Control group	31	--

Table 3 Average decrease in lactate after exercise and after 6 weeks of training.

	mM/1	Significant level compared to control group
1. Multivitamins	2.3	5%
2. Hemoglobin preparation	1.9	5%
3. Multivitamins	1.5	no change
4. Pollen extract	1.7	no change
5. Pollen extract + amino acids	2.6	1%
6. Control group	1.4	--

Summary:

The work capacity of weightlifters, and the formation of lactate in their blood, during a 6-week training period were significantly affected by the administration of various nutritional substances. A multivitamin preparation and a combination of amino acids and pollen show the greatest effect.

Disscussion:

The fact that the increase in lactic acid after exercise decreases and work capacity increases in connection with training is, of course, well-known and self-evident. The differences between the changes noted among the groups must reasonably be attributed to the administration of different nutritional substances. The greatest changes in both variables were noted in the group given the combination of Polltiabs and "Stark-protein" ("Strong" or "concentrated" protein), while Pollitabs alone did not produce any significant difference compared to the control group. The protein administered in the form of "Stark-protein" corresponded to only about 2.8 g of protein per day and could not reasonably have affected in any way the protein balance. The overall effect must therefore be caused by some form of synergism between the amino acids in the "Stark-protein" and the pollen extract.

Of course, on the basis of this relatively small experiment, it is not possible to analyse with certainty the relative importance of the various factors. Nevertheless, the experiment does support the notion that various nutritional additives – particulary the combination of pollen extract and amino acids – can lead to a distinct improvement in performance in connection with training.

In discussions about the importance of vitamins to physical performance, it has sometimes been said that the benefits of vitamins observed among Eastern Bloc performers – as distinct from the results of experiments in the West – could be attributable to a lower vitamin "status" in the Eastern Bloc. All of the weightlifters participating in the experiment enjoyed a balanced, high-vitamin diet. Thus, latter interpretation is not confirmed by the present experiment.

MUSCLE SUPPORT:

GRAMINEX Flower Pollen Extract

The effects of pollen and protein extracts on selected blood factors and performance of athletes

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The use of dietary supplements by athletes searching for the slight physiological edge over their opponents in competition is a fairly common practice. Pollen extract (PE), pure bee pollen (BP), and protein extract (PRE) preparations, extolled for their performance improvement characteristics, have recently been popularized by a number of world class athletes, their coaches, and trainers, and reported in several newspaper and magazine articles. [3 4 5 19 20 21 22] These articles report subjective opinion and heuristic observations and lack the credibility of scientific experimentation. While considerable evidence has been presented that PE is successful in the treatment of prostatis,[1 2 8 26] bleeding stomach ulcers,[10] and increases resistance to colds and respiratory infections, little has been done experimentally to validate claims related to improvement of athletic performance. The daily dosage in the majority of the studies mentioned adhered to the manufacturer's labeled prescription (four to six tablets per day), with the length of the experiments ranging from three to 36 days,[18 26] eight to twelve weeks,[9 18] and as long as ten months.[17]

While Nuttila[22] claimed that the purpose of PE ingestion was to increase red blood cells in athletes and thus facilitate the transport of oxygen, Millar[21] reported that no direct correlation was established between PE ingestion and Hgb increase. However, Nuttila attributed the increase in Hgb concentration found among Finnish runners to the effects of PE and high protein diet.

Steben et al.[27] studied the effects of a BP on selected blood factors and performance of varsity college swimmers and found no significant differences in Hgb and Hct levels among the groups studied. Since evidence presented by Rose[25] indicated the possible incidence of the muscular weakness and lethargy of hypokalamia among collegiate varsity distance runners in spite of otherwise good aerobic fitness, it was conjectured that the addition of potassium (K^+) to the diet in a palatable form would alleviate the condition. Steben found that BP did not significantly improve K^+ levels in the blood over other groups studied.

Fijalkowski[9] found a large, but non-significant, difference in the improvement of work capacity in weightlifters using PE. Steben[27] found that the use of PE did not result in any significant difference in performance of swimmers over that of the control group.

There are also conflicting ideas on the necessity of a PRE in the athlete's diet. Poortmans[24] indicated that protein is not used as a primary energy source when caloric supply is sufficient. It is a generally accepted fact that the major function of protein is to sustain cell growth and

maintain various body tissues. Jormakka[13] indicated a PRE should be a supplement to the low protein diets that are normal in Third World countries. It seems that the presence of high quality protein foods, which contain all of the known amino acids, would be sufficient to maintain cell construction and would not be metabolized as an energy source unless an inadequate caloric intake was present.

The PRE used in this study (reported by the manufacturer A. B. Cernelle, to contain quick absorbable free amino acids and low molecular peptides) was also used in Fijalkowski's 14 day weight training study which attributed the increase Hgb content of the blood to the supplement. However, in his study the experimental group received both the PRE and PE-Pollitab in a dosage "according to the producers instruction" (sic),[9] either of which could have been responsible for the increase. No other studies have been found which compare the use of a protein supplement with changes in performance.

The purpose of this study was to validate and further investigate the question of whether normal training over a period of time rather than food supplements was primarily responsible for improved performance of endurance athletes who ingest normal diets. Specific biochemical parameters of blood serum K, Hgb, and Hct values were selected for investigation of their possible influence on prevention of the tiring effects of hypokalamia and improvement of oxygen carrying ability of the blood, respectively.

Procedure

The placebo double blind experiment was undertaken at Catholic High School of Baton Rouge, Louisiana, with 18 male cross country runners for twelve weeks during the Fall Semester, 1977. The runners were randomly divided into three diet groups. Individuals in the first group orally ingested four PE capsules daily before breakfast. A similar procedure prescribing four placebo capsules was followed by members of group two, while the individuals of group three took four PRE capsules. The rational for the prescription was based on recommended daily dosages suggested on the label of the product bottle and also found in the producer's monographs.

At the beginning and the conclusion of the experiment, blood samples were drawn from each individual for three consecutive days before practice. Serum K^+ levels were analyzed by an Instrument Lab flame photometer, with Hgb and Hct levels determined by a Cotter S automatic Counter. All of the runners took their meals, except for lunch, at home and were advised to dine as normally accustomed.

The runners preceded their formal training with a voluntary summer program of slow, long mileage work. Formal training at the inception of the experiment consisted of 70 miles of over distance type work per week conducted on the road and cross country trails, gradually evolving into 100 miles per week of faster pace work. Near the end of the 12 week experiment, 40 percent of the mileage was negotiated with Fartlek style training and a limited amount of race pace interval type work. Prior to the State Meet, and the conclusion of the season and the experiment, the overall volume was reduced to 60 miles per week. Pre and post performance tests consisted of determining the average velocity for a three mile run conducted on the same surveyed and permanently marked cross country course. Split times were provided to assist the runners in the judgment of pace at the mile and two mile posts in both time trials and provided the information for change in performance.

The data which consisted of the serum K^+ mEq/L, Hgb gm, Hct %, and performance yds/ sec (velocity) for average three mile run performance were statistically treated with a split plot ANOVA with diet and pre-post measures as the main and split plot respectively. Probability was reported at the .05 and .01 levels of significance.

Analysis of samples of materials used in the study were reported to determine concentrations of minerals germane to the blood analysis. If PE

and PRE is reputed to directly or synergistically help an individual become more enduring, then levels of K^+ and Fe, to assist in modifying the possible fatigue effects of hypokalemia and enhance the increased oxygen carrying characteristics of Hgb and Hct concentration respectively, should be found in relatively high concentration (Table 1).

TABLE 1.—*Chemical analysis of diet treatments.*

Sample	Fe ppm/capsule	K^+ ppm/capsule
PE, 365 mg*	100.0	2000.0
Placebo, 350 mg*	30.0	120.0
PRE, 360 mg*	4500.0	280.0

*Mean of the contents of ten capsules.

Findings

An ANOVA (Table 2) with diet and pre and post measures as the main and split plot respectively, found no significant differences among the diet groups in blood levels of K^+, Hgb and Hct. Significant pre and post differences were found in blood levels of K^+ and Hgb. Pre versus post for performance was highly significant. The analysis in performance was nonsignificant and diet x pre and post measure interaction was nonsignificant for all variables.

While the ANOVA found significant pre and post differences in blood levels of K^+ and Hgb, they cannot be attributed to the diet supplements since the analysis found no significance among the diet groups in blood levels of K^+, Hgb, and Hct. The difference in K^+ was negative yet within normal blood level limits (Table 3). An earlier study[27] conducted for eight weeks suggested that an extended experiment might sustain Rose's observation that hypokalemia could appear in endurance athletes at the conclusion of arduous season and might be moderated by the inclusion of K^+ bearing foods in the diet. The results of our study did not support the inclusion of pollen extracts or protein extract food supplements in normal diets for maintenance of K^+ levels and prevention of hypokalemia.

The fact that the analysis found a significant difference in Hgb and not in Hct is difficult to explain. Table 2 reveals that Hct just missed being significant, even though once a rule of evidence is set up concerning significance, a nonsignificant result should not be considered important. An increase of hematocrit to optimal levels should be associated with an increased blood volume and hemoglobin concentration.[7 14 23] The increased hematocrit could then help

TABLE 2.—*Split Plot ANOVA with diet as the main plot; Pre-post measure as the split plot.*

Analysis of variance	DF	Mean square	F
For K^+ mEq/L			
Diet	2	.054	N.S.
Error A	15	.108	
Pre-post measure	1	.188	5.909*
Diet x pre post measure	2	.048	N.S.
Residual	15	.032	
Corrected total	35	.071	
For Hgb gm			
Diet	2	.093	N.S.
Error A	15	.573	
Pre-post measure	1	1.562	7.560*
Diet x pre post measure	2	.070	N.S.
Residual	15	.208	
Corrected total	35	.435	
For Hct %			
Diet	2	1.897	N.S.
Error A	15	5.436	
Pre-post measure	1	5.290	4.514 N.S.
Diet x pre-post measure	2	.041	N.S.
Residual	15	1.172	
Corrected total	35	3.094	
For average velocity yds/sec			
Diet	2	.080	N.S.
Error A	15	.752	
Pre-post measure	1	1.000	75.000**
Diet x pre post measure	2	.010	N.S.
Residual	15	.013	
Corrected total	35	.362	

*$P_{.05}$ 4.45 C 1 and 15 df.
**$P_{.01}$ 6.20 C 1 and 15 df.

TABLE 3.—*A summary of data for diet, blood, and performance measures.*

Diet	Pre-post Measure	No.	Mean velocity (Ŷ) yds/sec	SD	K+ 3.3-5.5 mEq/L	SD	Hgb 14-18 gm	SD	Hct 41-42 %	SD
Pollen	Pre	6	4.700	.548	4.150	.234	13.233	.156	38.817	.987
	Post	6	5.067	.625	4.150	.485	13.783	.999	39.700	2.559
Placebo	Pre	6	4.650	.653	4.250	.234	13.367	.216	38.700	1.410
	Post	6	4.917	.725	4.050	.152	13.617	.426	39.350	1.249
Protein	Pre	6	4.767	.532	4.150	.197	13.750	.720	39.417	2.278
	Post	6	5.133	.609	3.917	.117	14.200	.576	40.183	1.888
Pre-post measure										
	Pre	18	4.706	.517	4.183	.209	13.450	.528	38.978	1.537
	Post	18	5.039	.605	4.039	.291	13.867	.693	39.744	1.833
Diet										
	Pollen	12	4.883	.567	4.150	.348	13.508	.776	39.258	1.824
	Placebo	12	4.793	.644	4.150	.206	13.491	.333	39.025	1.258
	Protein	12	4.950	.553	4.033	.188	13.975	.636	39.800	1.948
Overall means		36	4.872	.593	4.111	.263	13.658	.650	39.361	1.734

oxygen transport, and it is unlikely that any slight increase in blood viscosity would place any restriction on the efforts of cardiac output to assist oxygen uptake capabilities. Conversely, the combination of continued lysis of red blood cells due to vigorous exercise in any individual over a long period of time, even with a normal diet, could be reflected in decreased hemoglobin and hematocrit levels. An examination of Table 3 reveals, with the exception of the post mean values for the protein supplement diet, Hgb and Hct values remained slightly below normal levels. Whether this suggests that teen-age athletes may need to add protein or iron bearing foods to their normal diet is problematical since the study found similar values in subjects who did and did not use the protein supplement.

Summary

R. E. STEBEN, P. BOUDREAUX

The effect of pollen and protein extracts on selected blood factors and performance of athletes.

Volunteer (18) male high school cross country runners were randomly subdivided into three diet groups for a twelve week, placebo, double blind design, nutrition-performance experiment. Diets 1, 2, and 3 supplemented normal diets with daily ingestion of four pollen extract, four placebo, and four protein extract capsules respectively. Blood samples drawn from each individual before and after the experiment were analyzed for serum K, Hgb, and Hc levels. The mean velocity of a pre and post three mile run conducted on the same course was the performance measure. An ANOVA, with diet and pre and post measures as the main and split plot respectively, found no significant differences among the diet groups in blood levels of K, Hgb, and Hct. Significant pre and post differences were found in blood levels of K and Hgb. Pre versus post for performance was highly significant. The analysis to compare diets for differences in performance was nonsignficant, and diet x pre-post measure interaction was nonsignificant for all variables. The findings failed to uncover any advantage in taking pollen or protein extracts for improvement or maintenance of K, Hct, and Hgb blood levels or improvement in performance.

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OTHER SUPPORT:

GRAMINEX Flower Pollen Extract

A study on the effect of digested Pollen Extract* on the frequency of spontaneous lung infections in Rats

Introduction:

In a study carried out at the Norwegian Institute of Work Science, Department of Occupational Health, it was found that addition of a specially digested pollen extract to the food of rats, was preferred, when the test animals were given free choice between three different food mixtures. All of these food mixtures presumably being fully satisfactory combinations of necessary nutritious elements (protein, carbohydrates and fat, vitamins, minerals and trace elements) (1).

There was no change in the original three mixtures during the experimental except for the addition of 1% of Cernilton to one of the food mixtures. It is well-established that rats (as also many other animals) have a pronounced ability to choose a food mixture containing substances, which may prove necessary to them (2, 3). We found those results interesting and they gave a rational basis for further studies as to a possible effect of pollen extract in the form of Cernilton. If being a general roborating substance, it might be possible to explain at least to some degree the results reported on the good results obtained by giving Cernilton tablets to patients with chronic prostatitis and also to patients with infectious diseases e.g. in the upper respiratory tract.

Macroscopic pathological-anatomical lung infections.

In the same report there was by the macroscopical pathological-anatomical examination observed a marked difference as to the frequency of infected lungs in the control animals compared with those which had been given Cernilton in their food. An English edition of the above mentioned report were published in an almost identical form as the first mentioned report in Norwegian (4). Here the author is concluding: “in the tests carried out using self-selection cages, a tendency was detected, which might be interpreted to mean that the Cernitin diet may contain one or more substances that are useful to the living organism, although it is not possible to offer any explanation for the action mechanism of such an assumed effect”.

As to the pathological findings on autopsy of the lungs they were concentrated on the difference in the frequency of findings of gross macroscopical-pathological changes between the two groups: Controls and Cernilton-treated rats.

The following definitions were given as “marked pathological findings” (5):

a) Definite enlargement of the lungs with slow and uncomplete retraction. Muco-purulent excrete from the trachea either spontaneously or by slight pressure on the lung for further examination.

b) Distinct palpable nodules which by section contained large amounts of purulent secret, “infected bronchiectasis”.

c) Well-defined dark red or greyish-red atelectasis which by section contained purulent secretion.

These findings which according to many authors who have studied the lung pathology in rats (5,

6, 7, 8) are remarkably common, and they are of decesive importance when using rats for studying experimentally, affections in their lungs.

Pathological-anatomical changes of the kind mentioned are very frequent when rats reach an age of 18-24 months or more. Mostly one will find such lung pathological changes in 50-75 percent when carefully examining the lungs by autopsy, even though these rats very seldom show obvious clinical symptoms or signs of advanced lung infection before being killed and subjected to autopsy.

By carefully standardizing of all controllable factors, the author has succeeded in keeping the number of such changes at a level at about 10 - 20 percent, when using rats in lung experiments. This has partly been obtained by using antibiotic treatment (given intraperitoneally once a week during the observation period).

In this first experiment the autopsy was carried out as a rutine and there had been no original intention to study especially the lung changes. The four groups of animals used in functional tests, studying the possible influence of Cernitin on spontaneous motoric activity were kept on the diet on 1% Cernilton for a total period of 6-7 months. In this Cernilton-treated group only one animal out of 12 (6 males and 6 females) showed a macroscopic lung change as defined above. In the control group one rat died before end of the experiment, nothing definite was mentioned by the physician, carrying out the routine autopsy, which thus did not indicate lung infection. Of the remainder 11 (5 males and 6 females) there was altogether 6 with macroscopical-pathological lung changes of the kind described above. Because of the often varying findings as to lung infections and the small number of animals in these tests, the author stated: “In the opinion of the author, it is not possible to present any definite conclusions on the basis of the above. These findings may possibly indicate a certain effect, but if a comparison is made with the often widely varying results obtained from otherwise untreated control animals in the same age group as the animals discussed above the results do not permit any definite conclusions to be drawn even though they may be interesting per se and can be considered to motivate continued investigations on the lines proposed here”.

In the above mentioned experiment the difference as to macroscopic lung infections between the control group and the Cernilton-treated group may be calculated statistically (Table 1).

Table 1

Effect on pathological lung changes in preliminary tests with 1 percent Cernilton in the food.

Treatment	Total Number of animals	No. of rats with macr. pathol. anat. lung changes		S.E. of the percentage
		No.	Percent	
Standard food without Cernitin	12	6	50	15.1
Standard food with Cernitin	12	1	8.3	8.2

Difference between Cernilton-treated and standard food-treated animals in percent:

41.3 ± 17.2
T = 2.42
0.05 > P > 0.0

This indicated a statistically, probable significance that the results might not have arisen by chance. If everything being equal except for the addition of Cernilton to the food, there is presumed to be less than 5 percent but more than 2 percent probability for an accidental result of this kind. Nevertheless the small number of animals and the somewhat lower frequency in the treated group than usual and, more important, the higher frequency in the control group, kept on our standard food, convinced the author that it was impossible to draw any other conclusions from these experiments than what was stated above.

Experimental conditions.

As a consequence of this first experiment it was therefore carried out two supplementary experiments, one during the winter 1968-69, and one during the winter 1969-70. These experiments were carried out in another animal stable, where the basic conditions were not so good and carefully controlled as in the animal stable in the first experiment. The first mentioned experiments were carried out where the functional tests took place. This makes it of primary importance to keep all controllable conditions as optimal and constant as possible.

The author holds the view that it might be of interest to find out whether the difference (if it should occur again) would be more or less pronounced when the conditions in the stable as draught, temperature, humidity, quality and care of cages etc. was not kept at the same optimal level as in the first experiment.

Two test series comprising originally altogether 40 animals in each group were carried out. Before starting the differentiation in food-supply (at about 4-5 months of age) there died two animals in the intended control-group and one in the intended Cernitin-group.

Cernitin (given as Cernilton) was added to the food in an amount of one percent. The experiment was started as mentioned above some time after dividing animals comparing each other as to sex and weight and as mentioned above at an age of 4-5 months and it was continued for 6 months.

Results:

During the 6 months of experiment there died 3 animals, all after more than three and a half months: one in the Cernitin-treated group and two in the control group. By autopsy of these rats that was marked lung infections in the one animal belonging to the Cernitin-treated group. One of them dying spontaneously in the control group had a big tumor, in the control group had distinct pathological infections in the lungs.

At the end of the experiment a careful macroscopical pathological-anatomical examination was carried out of all remaining animals, making at that time 38 rats in the Cernitin-treated group and 36 rats in the control group. All rats were of course presented with blind numbers to the examiner (the author). By this autopsy there was found 13 animals with lung changes of the kind defined above in the Cernitin-group, and 21 in the control group.

This may seem to indicate a probable statistical significance in favor of the group being given Cernilton-containing food compared with the standard food mixture. It may, however, be more conclusive when taking into consideration also the animals dying during the latter part of the experiment.

Table 2

Frequency of macroscopical pathological-anatomical lung infections at autopsy at the end of the experiment in Cernitin-treated and control rats.

Treatment	Number of rats at onset	Died spontaneously during experiment	Number of rats at end of experiment	Macr. pathol. anat. lung infections		
				No.	Percent	S.E.
Standard food	38	2	36	21	58.3	± 8.19
Standard food with Cernilton	39	1	38	13	34.2	± 7.91

Difference between controls and Cernitin-treated animals in percent:

23.9 ± 11.39
T = 2.098
0.05 P 0.02

Table 3

Frequency of pathological-anatomical lung infections in animals drying after more than three and a half months (14 weeks) of the experiments or at the autopsy at the end of the experiments (6 months).

Treatment	Number of animals at onset of experiment	Total number of rats with macr. pathol. anat. lung infections		
		No.	Percent	S.E.
Standard food	38	22	57.9	± 7.69
Standard food with Cernitin	39	14	35.9	± 8.01

Difference between controls and Cernitin-treated animals in percent:

22 ± 11.10
T = 1.982
P < 0.05

When making these animals into consideration there is hardly a 5 percent significance any longer.

Comments:

The results of these experiments may be of interest when compared with the first one (Table 1). The less pronounced favourable results in the last studied groups (Table 2 and 3) may indicate that an eventual effect of Cernilton, which most probably may be due to a general roborating effect of the preparation, are unable to prevent the deleterious effect of less satisfactory conditions. This seems according to the author's opinion to strengthen the view that there may possibly be a positive effect because of a general roborating influence e.g. due to the supply of a balanced combination of trace elements, vitamins, and a small amount of essential amino acids which may in itself give the type of effect which we in lack of a more precise expression are mentioning: general roborating effect. That there also is a certain streptolysin inhibitory effect of a hitherto not definitely defined substance in Cernitin is proved (9. 10) but whether this factor has any well-defined effect as to infections in the respiratory tract in rats is yet an open question.

Comparing the results obtained on the frequency of spontaneous lung infections in rats by adding Cernilton to the food, with observation reported as to the positive effect of Cernilton on infectious diseases in man, it also seems to suggest the view that the general roborating effect is the most probable explanation of a positive effect. The effect on chronic prostatitis (11, 12, 13, 14, 15, 16, 17, 18) and on infections in the upper respiratory tract (19, 20, 21, 22).

The statistical evaluation of the results seems to indicate a tendency in the direction that there may be a beneficial effect of Cernilton in these cases of infectious diseases, but the effect is seldom so pronounced that they are given a satisfactory statistical result by evaluation. This of course may be due to the mostly very small groups examined, but it may also be explained because of the very difficult deferential diagnostic problems in many of these infectious diseases.

It seems to the author that the general roborating effect yields a rational explanation for presuming an effect in cases where either the amount of or the balance between the substance which is unsatisfactory in the daily diet. In acute cases or cases where antibiotic—or chemotherapeutic treatment may be indicated, Cernilton is by no means an alternative and may be contraindicated when running the danger that it may be used not as a complement but as a substitute to well-defined indication for antibiotic and chemotherapeutic treatment. The effect of Cernilton may be to support the natural resources of the organism to counteract infections. This will mostly be actual for longterm treatment or as prophylaxis against exacerbations e.g. in chronic prostatitis or as prophylactic agent in very frequently recidiving infections in the upper respiratory tract.

Oslo, 21st January, 1971

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Acute Oral Toxicity Study in Rats with G-63 Food Product

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Abstract

The purpose of this study was to assess the acute oral toxicity produced when the test material was administered as a single dose by the oral route (gavage) to rats.

Male and female Crl: CD (SD) rats were assigned to 3 groups (five/sex/group). Each group was given G-63 Food Product at dose levels of 2000, 3500, or 5000 mg/kg at a dose volume of 20 mL/kg.

The animals received a single dose by oral gavage. The animals were observed for 15 days postdose and then sacrificed and necropsied. No tissues were saved.

Assessment of toxicity was based on mortality, body weights, clinical observations, and macroscopic observations.

All animals survived to termination, gained weight throughout the study, and were unremarkable at necropsy except for one 3500 mg/kg male (Animal No. B04315) that had a jejunum filled with reddish-yellow, slightly viscous fluid, and one 5000 mg/kg female (Animal No. B05018) with white lesions on the left apical lobe of the lungs. These findings were not considered to be treatment related.

All animals were normal at the postdose clinical observations, the daily cageside observations, and the weekly detailed observations except for one 5000 mg/kg female (Animal No. B04323) with a sore/scab on the proximal tail at the Day 8 detailed observations and one 5000 mg/kg female (Animal No. B0518) with a sore/scab on the proximal tail that was sensitive to the touch at the Day 8 and 15 detailed observations.

In conclusion, the maximum tolerated dose when administered via oral gavage to rats as a single dose is greater than 5000 mg/kg.

OTHER SUPPORT:

GRAMINEX Flower Pollen Extract

Basic Study of Cernilton

Immuno-Serological Findings

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March 19, 1968

1. **Introduction**

CERNILTON is a pollen preparation extracted from a mixture of 8 different pollens and contains as its chief ingredient some 20 kinds of amino acids. It also contains small amounts of sugar and glycoside but no sedimentary proteins.

Judging from its method of extraction and the results of analysis, there seems little danger of the preparation having an antigenic or allergenic property. Nevertheless, immunoserological studies are carried out here for the sake of safety.

2. **Materials**

Materials used were Cernitin T-60 and Cernitin GBX which are extracted and purified from crude pollen. The former contains mainly amino acids (no proteins) while the latter mainly lipoid. Both were placed at the authors' disposal by Tobishi Pharmaceutical Co., Ltd. after chemical analysis.

3. **Methods**

1) Antibody-producing Properties of Cernitin T-60 and Cernitin GBX

Animals used were male rabbits weighing about 2.5 kg, which were immunized according to the dosage schedules below. Ten days after the last injection the blood was drawn and serum separated for determination of the antibody titre.

Group A (Cernitin T-60 Administration Group)

1. 2,400 g Rabbit 2 ml/kg of Cernitin T-60 (30 mg/ml) injected intravenously 3 times

weekly for 2 weeks (total dosage 360 mg/kg).

2. 2,500 g Rabbit

3. 2,500 g Rabbit 0.05 ml (15 mg) of Cernitin T 60 solution (300 mg/ml) injected into foot pad at 5 sites (2 times weekly, 4 times all).

4. 2,400 g Rabbit

Group B (Cernitin GBX Administration Group)

1. 2,600 g Rabbit 0.05 ml (36 mg) of Cernitin GBX original liquid injected into food pad at 5 sites (2 times weekly, 4 times all).

2. 2,500 g Rabbit

3. 2,400 g Rabbit

2) Sensitinogenicity of Cernitin T-60 and Cernitin GBX (Anaphylactic Shock: Guinea Pigs)

Cernitin T-60 and Cernitin GBX were injected subcutaneously into the groin in 10 male guinea pigs weighing about 250 g, 2 times weekly 4 times all (each time 15 mg). Two weeks after the last injection the antigens (15 mg/ml) were injected intravenously for observation of anaphylactic shock.

3) Sensitinogenicity of Cernitin T-60 and Cernitin GBX Arthus' Phenomenon: Rabbits)

To the rabbits immunized according to method 1) 0.1 ml of the antigens (15 mg/ml, 3 mg/ml, 0.6 mg/ml) was injected subcutaneously to the back (after shaving off the hairs) 10 days after the last injection, and the presence of reddening and induration was examined.

4) Determination of Antibody Titre

A. Precipitin Reaction: Immunized rabbit-serums obtained under method 1) were activated (56°C, 30 mins) and diluted serially with 5% gum Arabic physiological saline. The precipitin reactions of Cernitin T-60 and Cernitin GBX were then examined routinely.

B. Haemagglutination Reaction: Immunized antiserums obtained under method 1) were studied routinely according to the agglutination reaction test for sensitized corpuscles, using sheepcorpuscles sensitized with Cernitin T-60 and Cernitin GBX, as follows: Antiserums (inactivated) were made subject to adsorption by sheep-corpuscles washed beforehand. Sheep-corpuscles were first treated with tannic acid, sensitized with the antigens (1 mg/ml in case of Cernitin T-60 and 0.1 mg/ml in case of Cernitin GBX, dissolved or suspended in phosphate buffer solution with a pH of 6.2), and then added to the

diluted series of antiserums at a dose of 0.05 ml. Agglutination values were determined after storing the antiserums so obtained at a temperature of 37° C for 2 hours and at room temperature for 20 hours.

C. Gel-Precipitin Reaction: The test was carried out in a routine manner according to Ouchterlony's method. Antiserums obtained under method 1) were used as testing samples while Cernitin T-60 and Cernitin GBX as antigens.

Results

A) Serological Study of Rabbit-Serums Immunized with Cernitin T-60 and Cernitin GBX

Serological study was made of the serums (7 rabbits) immunized according to method 1).

A) Precipitin Reaction (double layer method):

Table 1

		Precipitin Reaction (diluted antiserums)			
		Undiluted Antiserum	X2	X4	X8
Rabbits Group A (T-60 Administration Group)	No. 1	–	–	–	–
	2	–	–	–	–
	3	–	–	–	–
	4	–	–	–	–
Rabbits Group B (GBX Administration Group)	No. 1	–	–	–	–
	2	–	–	–	–
	3	–	–	–	–

Notes: Cernitin T-60 (30 mg/ml) was used as precipinogen for antiserums of Group A (Nos. 1-4). For antiserums of Group B (Nos. 1-3) Cernitin GBX (1.5 mg/ml) was used.

As may be noted from the Table, results were negative in all cases, revealing no antibodies at all.

B. Haemagglutination Reaction:

Results of haemagglutination reaction test carried out according to method 4) are as given in Table 2.

Table 2

		X10	X20	X40	X80	X160	X320	X640
Rabbits Group A (T-60 Administration Group)	No. 1 a	+++	+++	++	+	+	-	-
	b	+++	+++	++	+	-	-	-
	No. 2 a	+++	+++	++	+	+	-	-
	b	+++	+++	+	-	-	-	-
	No. 3 a	+++	+++	+++	++	+	+	-
	b	+++	++	++	+	-	-	-
	No. 4 a	+++	+++	++	+	+	-	-
	b	+++	+++	++	+	-	-	-
Rabbits Group B (GBX Administration Group)	No. 1 a	-	-	-	-	-	-	-
	b	-	-	-	-	-	-	-
	No. 2 a	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-
	No. 3 a	-	-	-	-	-	-	-
	b	-	-	-	-	-	-	-

Notes: a…Corpuscles sensitized with Cernitin T-60
b…Corpuscles sensitized with Cernitin GBX

The agglutination values of the rabbit-serums of Group A (immunized with Cernitin T-60) were 160-320 with Cernitin T-60 sensitized corpuscles. Even with Cernitin GBX sensitized corpuscles the values were as high as 40-80. The rabbit-serums of Group B (immunized with Cernitin GBX) showed no agglutination at all with Cernitin T-60 or Cernitin GBX sensitized corpuscles.

C. Gel-Precipitin Reaction (Ouchterlony's Method):

Precipitin reaction test as carried out according to Ouchterlony's method, with the antiserums (Group A 4 cases, Group B 3 cases) placed in the center and the antigens in the peripheral areas, as shown in the left chart, revealed negative results in all cases with no appearance of precipitation lines.

B) Sensitigenocity of Cernitin T-60 and Cernitin GBX
A. Anaphylactic Shock (Guinea Pigs): Antigens (15 mg/ml) were administered intravenously at a dose of 1 ml to guinea pigs sensitized according to method 2) and observation was made as to the presence of anaphylactic shock.

Table 3

	Shock Injection	Guinea Pigs	Symptoms
Cernitin T-60 Sensitized Group	Cernitin T-60 (30 mg/ml) 1 ml i.v.inj.	1. 240 g	_ (Survived)
		2. 260 g	_ ( )
		3. 250 g	_ ( )
		4. 280 g	_ ( )
		5. 260 g	_ ( )
Cernitin GBX Sensitized Group	Cernitin GBX (15 mg/ml) 1 ml i.v.inj.	6. 250 g	_ ( )
		7. 270 g	_ ( )
		8. 240 g	_ ( )
		9. 250 g	_ ( )
		10. 260 g	_ ( )

As may be seen from the Table, no cases showed anaphylactic shock and all cases survived.

B. Arthurs' Phenomenon (Rabbits): Rabbits were immunized according to method 1). After shaving off the hairs, the antigens (0.1 ml) were administered subcutaneously to the animals at 6 sites and observation was made as to the presence of the symptoms of reddening and induration. Results are given in Table 4.

Table 4

		Cernitin T-60			Cernitin GBX		
		15 mg/ml	3 mg/ml	0.6 mg/ml	15 mg/ml	3 mg/ml	0.6 mg/ml
Rabbits Group A Immunized with T-60	No. 1	1.2 X 1.1 cm	_	_	_	_	_
	2	1.4 X 1.2 cm	_	_	_	_	_
	3	1.0 X 0.8 m	_	_	_	_	_
	4	0.7 X 0.8 cm	_	_	_	_	_
Rabbits Group B Immunized with GBX	No. 1	_	_	_	_	_	_
	2	_	_	_	_	_	_
	3	_	_	_	_	_	_

Note: Figures indicates sizes of reddening (in diameters).

As the results would show, there was observed a slight degree of reddening when Cernitin T-60 was injected subcutaneously at a concentration of 15 mg/ml in rabbits immunized with Cernitin T-60. No bleeding, necrosis or induration was noted, however.

4. **Summary and Conclusion**

Pollen extracts Cernitin T-60 and Cernitin GBX were studied immunoserologically to examine their antigenicity and sensitinogenicity, with results as summarized below.

1) Examination was made as to the antibody-producing properties of Cernitin T-60 and Cernitin GBX using the serums of immunized rabbits. Results were negative in all cases by means of the precipitin

reaction (double layer method) and gel-precipitin reaction (Ouchterlony's method) tests. By means of haemagglutination test the agglutination value was slightly elevated in Cernitin T-60 immunized rabbit-serums but not in Cernitin GBX immunized serums.

2) Observation was made as to anaphylactic shock in guinea pigs strongly sensitized with Cernitin T-60 and Cernitin GBX, but the results were negative in all cases.

3) Observation was also made as to Arthus' phenomenon using rabbits strongly sensitized with Cernitin T-60 and Cernitin GBX. When Cernitin T-60 was used as the antigen and given at a concentration of 15 mg/ml, there was observed a slight degree of reddening in rabbits immunized with Cernitin T-60. At lower concentrations no symptoms were revealed at all. Results were negative in all rabbits immunized with Cernitin GBX.

It may be said in conclusion that both Cernitin T-60 and Cernitin GBX have either no or, if any, an extremely slight degree of antigenicity or sensitinogenicity.