

615714

FORM 1
REGULATION 9

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-1973

APPLICATION FOR A PATENT

We LABORATORIOS SERONO S.A.

of La Calle Genova 17-Planta 6, 28004 MADRID, SPAIN

hereby apply for the grant of a Patent for an invention entitled:

METHOD OF ENHANCING MENTAL FUNCTION

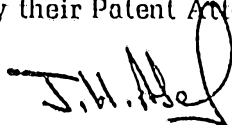
which is described in the accompanying complete specification. This Application is a Convention Application and is based on the Applications numbered: 148,667 and 206,100 for a Patent or similar protection made in U.S.A. (both) on 26 January 1988 and 13 June 1988, respectively.

Our address for service is:

GRIFFITH HACK & CO.
71 YORK STREET
SYDNEY N.S.W. 2000
AUSTRALIA

DATED this 23rd day of January, 1989.

LABORATORIOS SERONO S.A.
By their Patent Attorneys



GRIFFITH HACK & CO.

TO: THE COMMISSIONER OF PATENTS
COMMONWEALTH OF AUSTRALIA

B — APPLICATION BY ASSIGNEE OF INVENTORCOMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952DECLARATION IN SUPPORT OF AN APPLICATION FOR A PATENT

(Name of applicant) In support of an application made by:
LABORATORIOS SERONO S.A.

(Title) for a patent for an invention entitled:
METHOD OF ENHANCING MENTAL FUNCTION

(Full name and address of signatory) I, .Franciso, Rubi, Sandi,
of .La, Calle, Genova, 17-Planta .6, 28004, MADRID, .SPAIN

do solemnly and sincerely declare as follows:

(Full name and address of inventor(s)) 1. I am authorised by the above mentioned applicant for the patent to make this declaration on its behalf.
2. The name and address of each actual inventor of the invention is as follows: ..Ramon, Cacabelos; Francisco, Rubia, Vila, and, Thomas, Ortiz, Alonso.. of Avenida de la Corona B 13 Santa Eugenia de Rivorira
.La, Corona,, Spain ., .Pesadilla, .1, Fuente del Fresno Madrid, Spain and San Bernardo 130 28015 Madrid, SPAIN.....

State whether by assignment of contract of employment) and the facts upon which the applicant is entitled to make this application are as follows:
.....The applicant is the assignee of the said invention from the said inventor.....

(Delete paragraphs 3 and 4 for non-Convention application) 3. The basic application(s) as defined by Section 141 of the Act was (were) made as follows:
Country United States of America on January 26, 1988.....
in the name(s) Ramon, Cacabelos; Francisco, Rubia, Vila, and, Thomas, Ortiz Alonso and in United States of America on June 13, 1988.....
in the name(s) Ramon, Cavabelos; Francisco, Rubia, Vila, and, Thomas, Ortiz Alonso and in on
in the name(s)

4. The basic application(s) referred to in the preceding paragraph was(were) the first application(s) made in a Convention country in respect of the invention the subject of this application.

(Place and date of signing) Declared at .Madrid..... this ..13th..... day of .March..... 1989.

Signed: ..Francisco, Rubio, Sandi.....

Position: ..General Manager.....

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- (71) Applicant(s)
LABORATORIOS SERONO S.A.
- (72) Inventor(s)
RAMON CACABELOS; FRANCISCO RUBIA VILA; THOMAS ORTIZ ALONSO
- (74) Attorney or Agent
GRIFFITH HACK & CO., GPO Box 4164, SYDNEY NSW 2001
- (57) Claim

1. A method of enhancing the mental function of an individual which comprises administering to that individual an effective mental function enhancing amount of a hypothalamic hypophysiotropic hormone.

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COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

Form 10

COMPLETE SPECIFICATION

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TO BE COMPLETED BY APPLICANT

Name of Applicant: LABORATORIOS SERONO S.A.
Address of Applicant: La Calle Genova 17-Planta 6, 28004
MADRID, SPAIN
Actual Inventor: Ramon Cacabelos; Francisco Rubia Vila and
Thomas Ortiz Alonso
Address for Service: GRIFFITH HACK & CO.
71 YORK STREET
SYDNEY NSW 2000
AUSTRALIA

Complete Specification for the invention entitled:

METHOD OF ENHANCING MENTAL FUNCTION

The following statement is a full description of this invention,
including the best method of performing it known to me/us:-

6302A:rk

- 1A -

METHOD OF ENHANCING MENTAL FUNCTION

BACKGROUND OF THE INVENTION

The somatotropinergic system (STS) is the only neuroendocrine axis in which specific stimulatory and inhibitory neuropeptidergic regulators have been demonstrated thus far. In normal conditions, growth hormone releasing factor (GRF) and somatostatin (SS) are the hypothalamic hypophysiotropic hormones responsible for the regulation of growth hormone (GH) secretion.

At the same time, GRF and SS are influenced by central monoaminergic and peptidergic neuromodulators to optimize the function of the STS. See Cacabelos R., Niigawa H. and Hariguchi S., "Hypothalamo-hypophyseal System and Brain Function", J. Clin. Sci. 22:1108-1120 (1986).

Recent investigations suggest that the functional structure of the STS characteristically represented at the peripheral level might exist in the central nervous system (CNS). Furthermore, SS levels are reduced in specific areas of the CNS in patients with senile dementia and are elevated in the neostria-

tum of patients with Huntington's chorea. See Cacabelos R., Niigawa H. and Ikemura Y., "Neuroendocrine Correlates in Senile Dementia of the Alzheimer Type", *Progr. Clin. Neurosci.*, 2:231-247 (1986); Beal M.F., Uhl G., Mazurek M.F., Kowall N. and Martin J.B.

"Somatostatin: Alterations in the Central Nervous System in Neurological Diseases", in Martin J.B. and Barchas J.D. (Ed.), *Neuropeptides in Neurologic and Psychiatric Disease*, pp. 215-257 (Raven Press, New York, 1986); Epelbaum J. "Somatostatin in the Central Nervous System: Physiology and Pathological Modifications", *Progr. Neurobiol.*, 27:63-100 (1986). In addition, an abnormal rise of GH in response to GnRH or TRH has been described in several neuropsychiatric disorders. See Brown G.M., Koslow S.M., and Reichlin S., Ed., *Neuroendocrinology and Psychiatric Disorder* (Raven Press, New York, 1984). It has been speculated that the central regulators of the STS (e.g., GRF, SS) influence higher activities of the CNS. Such speculation led to search for potential therapeutic uses of these neuropeptides in those neurological diseases in which the normal functioning of the STS is impaired.

The neurochemical characteristics of early and late onset senile dementia of the Alzheimer type (SDAT) can be clearly demonstrated in postmortem studies in which it is possible to observe that cholinergic and somatostatinergic deficits are more pronounced in patients with an early onset of the disease. See Rossor M.N., Iversen L.L., Reynolds G.P., Mountjoy C.Q. and Roth M. "Neurochemical Character-

istics of Early and Late Onset Types of Alzheimer's Disease", Br. Med. J., 288:961-964 (1984). In the advanced stages, clinical assessments show that the course of the disease is more rapid and prominent in younger patients.

Since dementia may be attributed to various etiologies of which SDAT accounts for at least 50% of the cases, the search for antemortem markers capable of establishing an early differential diagnosis for identifying potentially reversible or treatable causes of dementia is becoming an extremely important matter.

SDAT is believed to be a multisystem disorder. See Price D.L., Struble R.G., Whitehouse P.J., Kitt C.A., Cork L.C., Walker L.C. and Casanova M.F. "Alzheimer's Disease: A Multisystem Disorder" in: Martin J.B. and Barchas J.D. (Ed), Neuropeptides in Neurologic and Psychiatric Disease, pp. 209-214 (Raven Press, New York, 1986). The most relevant peptidergic abnormalities in SDAT involve a clear deficit in cortical somatostatin and corticotropin-releasing factor (CRF). Other peptidergic and monoaminergic systems are also affected.

THE INVENTION

It has been discovered that the hypothalamic hypophysiotropic hormones, that is growth hormone releasing factor and somatostatin, affect mental function. Specifically, either or both hormones exert significant affects on locomotor activity and improve learning abilities. When the individual is suffering from a neuropsychiatric disorder, the hormones can induce at least partial return toward normalcy. When the individual is not suffering from a neuropsychiatric disorder, e.g. in healthy individuals, the hormones can

effect improvement of the mental function. For instance, significant changes have been observed in such psychometric tests as short term memory and attention tests, as well as in EEG (electroencephalogram mapping). Also because the hormones effect an improvement in individuals suffering from senile dementia of the Alzheimer's type, they can be used to diagnose this deficiency, particularly in the early stages. In particular, it has been found that the GRF induced GH response can be taken as a useful marker for early stages of SDAT.

DESCRIPTION OF THE INVENTION

In accordance with the present invention, the mental functions of an individual are enhanced by the administration of a hypothalamic hypophysiotropic hormone to that individual.

Various commercially available forms of SS and GRF can be employed in the invention. Biologically active fragments can be substituted for all or part of the SS or GRF used. Somatostatin is generally characterized as growth hormone-releasing inhibiting factor. It is compound No. 8561 in Merck Index, 10th Ed. (1983). It is preferred that the 1-14 fragment (i.e., SS-14) be employed herein. Various commercial products containing useful forms of SS may be used herein. Mixtures are operable.

Growth hormone releasing factor is usually described as the stimulatory growth-hormone releasing factor of the hypothalamus that assists in the neuro-regulation of growth hormone secretion. It is compound No. 4416 in Merck Index, 10th Ed. (1983). Preferred

for use herein are the segments or fragments designated as 1-44 and 1-29. Various commercial products containing useful forms may be used herein. Mixtures
5 are operable.

Pharmaceutical preparations used in accordance with the invention will contain one or more of the hormones together with the conventional pharmaceutically acceptable vehicles. Generally, the hormones are
10 administered in an amount from about 1 to 50 mcg/kg. Peptide levels of approximately 1 to 10 mg/kg body weight i.v. and of about 5 to approximately 50 mcg/kg body weight s.c. are operable.

The length of time for which the hormone-containing
15 composition is administered can vary greatly. Depending on the effect desired, daily administration may be preferred. Administration every 6 or 12 hours can also be useful. Useful times of administration depend upon the preferences of the treating physician.

20 In order to demonstrate the effect of the hormones on mental function, various experiments were carried out. Some of the results are set forth in the accompanying drawings. In these drawings:

Figure 1 includes computer recordings of behavioural
25 patterns of rats treated with somatostatin and growth hormone-releasing factor in a maze paradigm.

A. Behavioural pattern of prototype control rat (5 mcg 0.9% saline, i.c.v.) in a maze paradigm.

B. Behavioral pattern of a rat treated with
30 somatostatin (SS-14; 1 mcg, i.c.v.).

C. Behavioral pattern of a rat treated with growth hormone-releasing factor (GRF₁₋₄₄; 1 mcg, i.c.v.).

D. Behavioral pattern of a rat treated with GRF₁₋₄₄ (5 mcg., i.c.v.).

The animals had to learn to escape from the shock area in order to avoid a continuous 1.5 mA electric shock.

Experimental time - 30 minutes.

Figure 2 shows the effects of somatostatin and GRF on locomotor activity and learning in a maze paradigm.

The rats were treated intracerebroventricularly with SS-14 (1 mcg) and GRF₁₋₄₄ (1 mcg) and their behavior was automatically recorded by computer using the maze paradigm shown in Figure 1. The designation p/t denotes pulses per time unit (minute). Locomotor activity was quantified in pulses per minute or cm²/min. Total inputs (I/O) in neutral position (white bars) and shock position (black bars) denote how many times the animals enter neutral and shock areas. The N/S ratio results from dividing I/O in neutral position by I/O in shock position and is interpreted as an index of learning in the 30-minute experimental period. The positional time (seconds) represents how long the animals stay in neutral (white bars) or shock (black bars) areas. N-sp and S-sp (in seconds) indicate the time-latency per movement or input in neutral (white bars) and shock (black bars) areas, respectively.

p<0.005 vs C (control)

$p < 0.005$ (NA vs SA)

Figure 3 shows GRF-induced GH response in elderly subjects () and patients with early () and late onset senile dementia of the Alzheimer type ().

* $p < 0.005$ vs. basal level (0) (means $\pm$ SD)

** $p < 0.005$ vs. control ()

Figure 4 illustrates the correlation between the GRF-induced GH response 60 minutes after injection and the mental performance of patients with early onset senile dementia of the Alzheimer type 24 hours prior to testing.

Example 1

To clarify whether SS and GRF display at the central level the same antagonistic effect that they have at the pituitary level, the effects of these neuropeptides on several behavioral parameters have been studied using the OUCEM-86TM (Osaka University Computerized Electronic Maze). The system is integrated by the following components: a Programmable Electronic Platform (PEP) (620x620 mm) equipped with 48 photo beam sensors (OPX-T30); a Programming Panel (PP) (Model BECM-0062) for paradigm stimulation; a Shock Generator Scrambler (SGS) (Model BSG-1065) to apply continuous or discontinuous electric current to the PEP; a Control Station (CS) (BEC 16-Dio Computer Control Station BCS-1105; Bio Medica, Ltd., Osaka) which processes inputs/outputs as an interface device to automatically computerize behavioral parameters; and a Computer System (NEC PC-9801 VM2) with 1 megabyte

Random Access Memory (RAM) for data processing and experimental setting.

Male Wistar rats (175-200 g) (N = 5-8 rats/group) received intracerebroventricular (i.c.v.) injections of GRF₁₋₄₄ and SS-14 over a range of 0.1 to 10 mcg. Then the rats were studied in the OUCEM under two different paradigms (e.g., open field and maze paradigms).

The animals were placed in the PEP for 30-minute periods and locomotor activity (LA) was automatically recorded as total inputs (I/O) including movements in four-footed position (4F) and in two-footed position (2F), grooming, rearing and jumping activity, while linear (horizontal) movements (4F) are represented as pulses per minute (p/m) or cm²/min.

The i.c.v. administration of SS (1 mcg) provoked quantitative and qualitative changes with a significant decrease in LA (31.16 ± 6.90 p/m vs 20.88 ± 2.82 p/m, $t=3.08$, $p<0.02$) while GRF (1 mcg) induced a hyperkinetic syndrome characterized by compulsive, uncontrolled movements with an LA rate of 47.60 ± 5.35 pm ($t=4.21$, $p<0.005$) (control = 1122 ± 248 cm²/min; GRF = 1713.6 ± 69.5 cm²/min., $t = 18.73$, $p<0.005$). These changes were dose-dependent (SS:ED₅₀ = 1.83 nmol, E_{max} = 6.10 nmol; GRF₁₋₄₄:ED₅₀ = 99.1 pmol, E_{max} = 1.98 nmol; GRF₁₋₂₉:ED₅₀ = 297 pmol, E_{max} = 5.95 nmol).

Example 2

This example shows the effects of the intracerebroventricular administration of SS and GRF on locomotor activity and learning in a maze paradigm.

Using the maze paradigm shown in Figure 1 in which the rats must learn to escape from the shock area in order to avoid a continuous 1.5 mA electric shock, SS (1 mcg, i.c.v.) significantly reduced LA (17.12 ± 5.42 vs 6.01 ± 1.45 p/m, $t=4.42$, $p<0.005$; Figs. 1B, 3) and GRF increased LA in a dose-related manner (26.66 ± 3.15 p/m, $t=3.40$, $p<0.01$ at 1 mcg, Fig. 1C; and 90.2 ± 8.4 p/m, $t=17.51$, $p<0.005$, at 5 mcg, Fig. 1D). The amounts of inputs (I/O) induced by SS was 103.5 ± 29.5 (control = 216.25 ± 55.19 , $t=8.49$, $p<0.005$) in the neutral area (NA) and 77.14 ± 13.5 I/O in the shock area (SA) (control = 298 ± 40 , $t = 1.26$, n.s.) GRF generated 459.35 ± 32.5 I/O in NA ($t=18.13$, $p<0.005$) and 340.5 ± 63.5 I/O in SA ($t=9.07$, $p<0.005$) (Figs. 1C, 3). The NA/SA (N/S) ratios for control, SS- and GRF-treated rats were 0.72, 1.33 and 1.35, respectively (Fig. 2).

Our results clearly show that GRF and SS exert antagonistic effects on LA in novelty conditions in both open field and maze paradigms (Figs. 1, 2). If we consider the N/S ratio as an index of learning, it seems that both SS and GRF improve learning abilities at doses of approximately 6.10×10^{-10} mol and 1.98×10^{-10} mol, respectively.

However, since GRF induced a hyperkinetic syndrome in which the I/O rate as well as the positional time in SA were significantly higher than in the control group, it could be inferred that SS is more effective than GRF in improving learning. Indeed, the GRF-induced locomotor agitation (hyperkinetic syndrome)

constitutes a serious drawback for the animal trying to differentiate and discriminate patterns in the maze (Figs. 1C, D).

Example 3

Twenty female inpatients with primary degenerative dementia (Alzheimer like) aged 67-84 years were divided in two therapy-groups with GRF (1-29) NH₂. All patients were treated with this product for 8 days with either of the following schedules.

I) 250 mcg s.c. twice daily

II) 50 mcg i.v. twice daily

Therapy was started after a 15-day wash-out period for psychotropic drugs.

Evolution of patients was assessed using the MMS Folstein test and Crichton's scale test at 12 hours, 24 hours and 7 days after starting with GRF. The statistical evaluation was made using Friedman test with a risk level $p < 0.01$ (highly significant differences).

Both groups show a significant increase in both scores and clinical findings, more important in the group with i.v. GRF administration than in the one with s.c. administration. The effect starts at 12 hours after administration and remains significant over the whole 7 days of treatment.

The lower scores in the Crichton's scale observed for the I group are probably related with the fact that, since inclusion in either group was dependent on the difficulty of the i.v. puncture, the pa-

tients included in the s.c. group were those whose clinical situation was worst.

During the treatment with GRF the nurse in charge of the patients reported an improvement of the patient's situation regarding sphincter control and general psychomotricity. This improvement returned to the pretreatment situation when the GRF administration was discontinued.

After finishing GRF administration, the same groups have been evaluated with MMS-Folstein test and Crichton's scale test in order to both assess the test's reproductibility and to establish the elimination of the GRF effect thus confirming the significant effect of GRF (1-29) NH_2 in those patients. The variation coefficients were 12% for MMS-Folstein test and 2.7% for Crichton's scale test.

Example 4

The subjects were divided into three groups:

(a) controls (N = 9): 5 females and 4 males (age = 70.10 ± 2.76 years; range = 66-75);

(b) inpatients with early onset senile dementia of the Alzheimer type (EOSDAT) (N = 10): 5 females and 5 males (age 65.00 ± 3.31 years; range = 57-69); and

(c) inpatients with late onset senile dementia of the Alzheimer type (LOSDAT) (N = 10): 5 females and 5 males (age = 75.70 ± 3.77 years; range = 70-82).

All the SDAT patients met the DSM-III criteria for primary degenerative dementia and rigorous diagnostic criteria for SDAT. As EOSDAT patients were considered those whose insidious memory loss or deterioration of cognitive functions began before the age of 60 and had a progressive course of more than 2 years. In LOSDAT, the onset of the disease occurred after the age of 60. The degree of severity of the dementia was assessed with Hasegawa's Dementia Rating Scale (DRS) (Hasegawa K. and Inoue K., "An Investigation of Dementia Rating Scale for the Elderly", Seishinigaku 16:965-969, 1974), Folstein's Mini-Mental State (MMS) Test (Folstein M.F., Folstein S. and McHugh P., "Mini-Mental State: A Practical Method for Grading the Cognitive State of Patients for the Clinician., J. Psychiat. Res. 12:189-198, 1975) and a modified model of the Brief Cognitive Rating Scale (BCRS) and Functional Assessment Stages (FAST) of Reisberg (Reisberg B., Ferris S.H. and De Leon M.J. "Senile Dementia of the Alzheimer Type: Diagnosis and Differential Diagnostic Features with Special Reference to Functional Assessment Staging" in Traber J. and Gispen W.H. (Ed), Senile Dementia of the Alzheimer Type, pp. 18-37, Springer-Verlag, Berlin, 1985). Concomitant pathology other than SDAT was discarded under a careful screening of the subjects with CT Scan, EEG, ECG and complementary laboratory data. Current diagnosis or history of SDM-III major affective disorder or schizophrenia as well as history of endocrine disease were criteria for exclusion. All of

the control subjects were free of psychiatric and endocrine illness. All subjects included had been drug-free for a minimum of 7 days before testing.

A 21-gauge indwelling venous needle with an attached three-way stopcock was inserted into the antecubital vein. Baseline blood samples for GH determination were collected immediately before testing. GRF(1-44)NH₂ was then injected as an intravenous bolus (100 mcg), and blood samples were obtained at 15, 30, 45, 60, 90 and 120 minutes after injection for determination of GH concentration in plasma. The plasma GH levels were measured by radioimmunoassay using a commercial GH-RIA-Kit (Dainabot Co. Ltd. Tokyo). The intra- and inter-assay coefficients of variation were 6.9% and 10.6%, respectively.

Simultaneously to the performance of the GRF test, electroencephalographic and cardiovascular monitorization of the subjects were carried out. Twenty-four hours prior to the GRF test, and 3, 12, 24 and 48 hours after testing, the DRS, MMS, BCRS, and FAST tests were performed to evaluate mental performance and behavior. Additional monitoring of GRF effects was done by global physician assessment and evaluation of the Mihara Nursing Scale (MNS). This test was developed to be used as a non-cognitive parametric indicator involving several items, including quantification of food intake, locomotion, social interaction, and complementary laboratory data.

The results were analyzed statistically by the Student's test, analysis of variance (ANOVA) and the Mann-Whitney test using a NEC PC-9801VM2 computer.

GRF inducted a marked increase in the levels of plasma GH in from 30 to 90 minutes after injection with a maximum peak (15.61 ± 5.71 ng/ml, $t = 6.08$, $p < 0.005$) at 60 minutes in EOSDAT (Fig. 3). This response was absent in both LOSDAT and control subjects, in whom plasma GH increased slightly but never reached a significant level over basal concentrations (Fig. 3).

In the EOSDAT responders, the rise of plasma GH levels after GRF injection showed a great individual variability (range = 5.5-28.75 ng/ml). This response was higher in females (19.74 ± 4.86 ng/ml) than in males (11.52 ± 2.82 ng/ml, $p < 0.005$) (see the Table). No sexual differences were evident in the other two groups. Significant differences in the basal plasma GH concentrations among the three groups were not detected.

TABLE 1. SEX- AND TIME-DEPENDENT RESPONSES OF GROWTH HORMONE TO GROWTH HORMONE-RELEASING FACTOR IN PATIENTS WITH SENILE DEMENTIA AND ELDERLY SUBJECTS

TIME (min.)	CONTROL (females)	CONTROL (males)	EOSDAT (females)	EOSDAT (males)	LOSDAT (females)	LOSDAT (males)
0	1.03 $\pm$ 0.83	1.07 $\pm$ 0.92	1.92 $\pm$ 1.52	1.99 $\pm$ 1.67	1.09 $\pm$ 1.01	0.95 $\pm$ 0.98
15	3.09 $\pm$ 1.58	2.21 $\pm$ 1.87	2.23 $\pm$ 1.90	2.70 $\pm$ 1.92	2.04 $\pm$ 1.01	2.13 $\pm$ 0.94
30	3.68 $\pm$ 2.09	3.16 $\pm$ 2.76	6.26 $\pm$ 2.05	5.49 $\pm$ 1.91	3.65 $\pm$ 2.35	2.81 $\pm$ 1.90
45	4.28 $\pm$ 3.38	5.17 $\pm$ 3.46	14.86 $\pm$ 3.57	6.87 $\pm$ 2.84*	4.68 $\pm$ 3.18	3.61 $\pm$ 3.51
60	2.97 $\pm$ 1.96	3.17 $\pm$ 2.61	19.74 $\pm$ 4.86	11.52 $\pm$ 2.82**	4.97 $\pm$ 3.86	4.09 $\pm$ 2.56
90	2.04 $\pm$ 1.38	1.88 $\pm$ 1.87	11.28 $\pm$ 5.72	7.41 $\pm$ 2.79	4.04 $\pm$ 3.22	3.08 $\pm$ 2.22
120	1.10 $\pm$ 0.95	0.91 $\pm$ 0.87	5.09 $\pm$ 3.01	2.73 $\pm$ 1.71	2.44 $\pm$ 2.05	1.64 $\pm$ 0.62
N	5	4	5	5	5	5
AGE	70.20 $\pm$ 2.78	70.00 $\pm$ 2.73	65.00 $\pm$ 4.25	65.00 $\pm$ 2.00	76.20 $\pm$ 4.48	75.20 $\pm$ 2.00
RANGE	67 - 75	66 - 73	57 - 69	62 - 68	70 - 82	73 - 80
STAGE	I - II	I - II	VI - VII	V - VII	VI - VII	VI - VII

EOSDAT = Early Onset Senile Dementia of the Alzheimer Type

LOSDAT = Late Onset Senile Dementia of the Alzheimer Type

* p<0.01 vs. females

**p<0.005 vs. females

EEG was abnormal in 95% of SDAT patients due to the advanced stage of the disease. Basic electroencephalographic patterns for controls (N = 6) and SDAT subjects (N = 7) prior to the GRF test were 9.27 ± 0.11 Hz/ 47.36 ± 3.36 μ V and 8.08 ± 0.67 Hz/ 40.80 ± 6.06 μ V, respectively. Global EEG evaluation after testing yielded a basic rhythm of 9.05 ± 0.12 Hz ($p < 0.025$)/ 49.67 ± 5.75 μ V for controls and 7.46 ± 0.53 Hz/ 43.69 ± 5.12 μ V for SDAT. Sequential EEG analysis revealed a fall in frequency (8.75 ± 0.25 Hz, $p < 0.005$) and an increase in amplitude (55.60 ± 6.40 μ V, $p < 0.02$) in control subjects and in SDAT patients (7.05 ± 0.90 Hz, $p < 0.05$)/ 52.5 ± 9.89 μ V, $p < 0.005$) in from 15 to 45 minutes after injection, preceding the maximum plasma GH peak.

There was a good correlation of scores between the DRS and MMS tests. Basal scores for DRS/MMS prior to the GRF test were 3.5 ± 1.75 / 3.5 ± 2.65 . After testing, scores increased to 5.5 ± 2.10 / 5.15 ± 3.75 (3 hr), 6.75 ± 3.25 / 5.5 ± 3.65 (12 hr), 4.35 ± 1.95 / 4.5 ± 2.75 (24 hr), and 3.5 ± 1.55 / 4.35 ± 2.75 (48 hr).

Although these differences were not statistically significant, they were clinically relevant. An inverse curvilinear relationship (Fig. 4) between the variables mental performance (x) and maximum GH response to GRF at 60 minutes (y) was observed ($r = -0.83$). A careful evaluation of the BCRS showed that the axes more significantly affected by GRF were Axis 4 (orientation, 30%), Axis 7 (psychomotor, 50%), and Axis

8 (mood and behavior, 40%). According to the MNS, appetite increased in 45% of the patients and in 30% of the controls (2 patients developed a bulimic syndrome), and social interaction improved in 40% of the patients. All these behaviors were transient.

These results demonstrate that GRF induces a significant increase of the plasma GH levels 60 minutes after injection in EOSDAT. This GH response is about 15 minutes delayed with respect to the small peak observed in control subjects.

Furthermore, the GRF-induced GH response slightly correlates with the severity of the disease and is accompanied by EEG and behavioral changes mainly circumscribed to psychomotor functions.

Example 5

Study Protocol and General Procedures

5 healthy volunteers of both sexes aged 18 - 25 years were admitted to this single blind study. Patients underwent the following procedures in a random way:

1. Baseline: registration of EEG (electroencephalogram), psychometric testing (short term memory), assessment of P-300 potential by audiometric testing.

2. The same after placebo (saline).

3. The same after GRF.

Each of phases 1 to 3 was followed by a period of at least one week before starting the next one.

Every patient underwent all 3 phases.

Dosage

GRF: 125 mcg i.v. (approx. 1.5 - 2.5 mcg/kg)

Saline: The same volume as used to dilute

GRF.

Memory test (short term memory)

The memory test consisted in the memorization of a list of neutral words. The investigator read the words and then a dispersion phase ensued consisting in the performance of some arithmetical tasks. The subject was then asked to repeat as many words from the list as he/she could remember.

The words used in each case were different, in order to avoid learning.

Attention test

The aim of this test was to study the evoked potential P-300, known to be related with the attention span required to follow up and memorize a phenomenon.

The stimulus selected for this test was an auditive one since audiometric testing of this potential has proven to be easy to perform and reliable. After a stimulus, a sound in this case, the mechanisms involved in the process of the hearing itself produce some bioelectric activity directly related to the stimulus itself. The perception process is more complex than the mere "hearing" and even after the stimulus has been removed, bioelectric activity related

to it can be observed, as a form of 'echoes'. These 'echoes' are the evoked potentials. P-300 is a positive wave that can be observed 300 milliseconds after the first peak due to the stimulus, and it is known to be related with the conscious processing of it, namely with the attention devoted to it.

The subject hears some beeps of either 1000 or 2000 hertz, given in a random way. He is then asked to say how many high beeps (2000 hertz) were sent.

EEG monitoring

EEG monitoring started 10 minutes after the injection and lasted through to the end of the tests (20 - 30 minutes).

Immediately after completing the first group of tests (i.e. 30 - 40 minutes after injection), the procedure recommenced with new words and beeps, under EEG monitoring.

Duration of the session

Each session lasted about one hour from the injection.

EEG mapping

This was performed with a PATHFINDER device.

A first screening of the maps for each frequency band (alpha, beta 1, beta 2, delta and theta) was performed, as well as for P-300.

Changes were defined as any variation in amplitude (potential) or in topographical distribution

of the bands and were studied subject by subject, comparing the pattern obtained after injection of the products versus baseline recording and versus placebo.

The improvement in performance observed for the psychometric tests correlates with an impressive modification of the EEG patterns, both in amplitude and in the topographical distribution.

RESULTS

The analysis of the results focused on the most apparent changes in performance and/or EEG. After a preliminary Fourier's analysis, maps have been constructed for those frequency bands showing the most evident modifications, i.e., alpha, beta-2 and P-300.

Results of psychometric tests (memory)

The number of memorized words among the five subjects admitted to the study was the following (mean $\pm$ sd):

Baseline	:	8.4 $\pm$ 1.34
Placebo	:	12.4 $\pm$ 3.13
GRF (10 min. after inj.)	:	14.0 $\pm$ 3.0
GRF (30-40 min. after inj.)	:	16.2 $\pm$ 2.38

Subject by subject, results appear as follows:

<u>Subject</u>	<u>Baseline</u>	<u>30-40 min. after GRF</u>
1	9	15
2	7	16
3	10	19

4	7	18
5	9	13

Therefore, GRF enhances memorization capabilities. The importance of this observation is greatly increased by its correlation with the changes observed in the EEG maps, as shown below.

Analysis of bioelectric activity

Alpha waves:

A net increase in energy was observed.

Furthermore, in normal subjects at rest, the peak of alpha activity was mainly located at the right occipital lobe, near the midline of brain. Thirty to forty minutes after GRF, a clear spreading of the alpha activity over the whole brain was observed, suggesting an overall stimulation of brain activity, possibly related to nonspecific stimulation of the activating reticular system.

Beta 2 waves:

In normal circumstances, the main activity of beta 2 waves is mainly located at the left parieto-occipital area. The map after 30 - 40 min. from GRF administration showed the following changes:

1. Overall spreading of beta 2 activity to the whole brain.
2. Increase in intensity at the initial left parieto-occipital focus (intensity more than doubled over the original values) and in extension of this focus.

3. Appearance of a new focus located at the right edge of the frontal lobe.

The left parieto-occipital lobe is known to be related to memory, so that an increase of electrical activity in this area correlates well with the findings in the performance test.

On the other hand, the appearance of a new focus of beta 2 activity at the frontal lobe (integration area) is interpreted as a consequence of the fact that the subjects construct certain logical associations in order to connect the neutral words to each other to form a sentence.

P-300 activity:

Again, a net increase is observed in the P-300 energy 30 - 40 min. after administration of GRF. P-300 is an evoked potential known to be related to attention. This has been interpreted as the ability of the subject to 'devote a more intense attention to stimuli' after GRF than in baseline conditions.

Overall interpretation:

The effect of GRF is interpreted as an 'economizing' action, i.e., GRF favors the concentration of the 'available activity' in the areas where it is most needed.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of enhancing the mental function of an
5 individual which comprises administering to that
individual an effective mental function enhancing amount
of a hypothalamic hypophysiotropic hormone.

2. The method of claim 1 in which the hormone is
(a) somatostatin, (b) growth hormone releasing factor,
10 (c) a physiological active fragment of (a) or (b), or (d)
a mixture of at least two of (a), (b) or (c).

3. The method of claim 2 in which the hormone is
administered intravenously in an amount of 1 to 10
mcg/kg.

15 4. The method of claim 2 wherein the hormone is
administered subcutaneously in an amount of 5 to 50
mcg/kg.

5. The method of claim 2 wherein the individual is
affected by dementia and the effective amount is
20 sufficient to decrease the level of dementia.

6. The method of claim 5 wherein the dementia is
senile dementia of the Alzheimer's type.

7. The method of claim 2 wherein the mental
function is memory and the amount is sufficient to
25 enhance short term memory.

8. The method of claim 2 wherein the mental function is attention span and the amount is sufficient to increase attention span.

9. The method of claim 2 that wherein at least one of the neuropsychiatric and growth hormone response to said administration is monitored.

10. The method of claim 2 wherein said hormone is somatostatin or active fragment thereof.

11. The method of claim 2 wherein said hormone is growth hormone releasing factor or active fragment thereof.

12. A method of detecting senile dementia of the Alzheimer's type in an individual which comprises administering to the individual an amount of a hypothalamic hypophysiotropic hormone effective to enhance the mental functions of a normal individual and monitoring the neuropsychiatric and growth hormone response to said administration.

13. The method of claim 12 wherein the administration amount is from 1 to 50 mcg/kg.

14. The method of claim 13 wherein the hormone is growth hormone releasing factor or active fragment thereof.

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15. The method of claim 14 wherein the
administration is intravenous in an amount from 1 to 10
5 mcg/kg.

16. The method of claim 14 wherein the
administration is subcutaneously in an amount of 5 to 50
mcg/kg.

17. A method of enhancing the mental function of an
10 individual substantially as disclosed herein in
conjunction with any one of the Examples excluding
control tests.

Dated this 26th day of July 1991

15

LABORATORIOS SERONO S.A.

By their Patent Attorneys

GRIFFITH HACK & CO

-On time (contunous)

File name:S-3

Display time

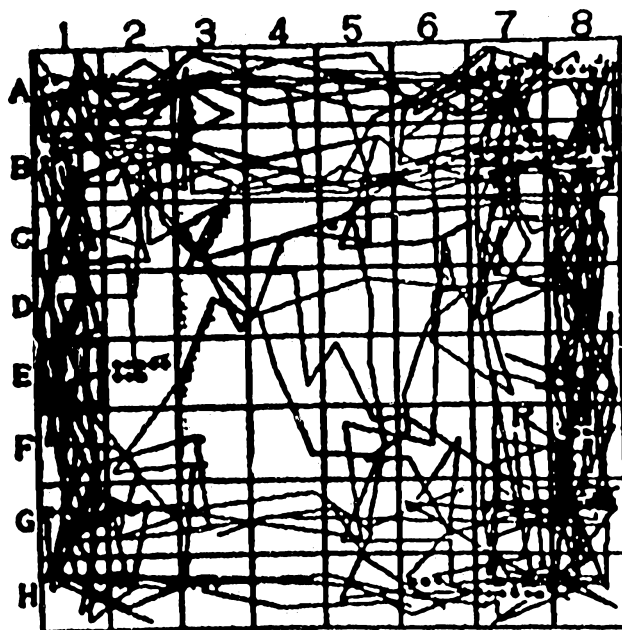
0: 0 to 0:30

Experimental clock

0:30: 0

Shock:OFF

- ◆:two-footed
- :Jump
- :now SHOCK area
- :Feeder location
- :Watering location
- :Starting point
- END! any key hit



f.1=STOP

FIG. 1A.

A

-On time (contunous)

File name:SS-3

Display time

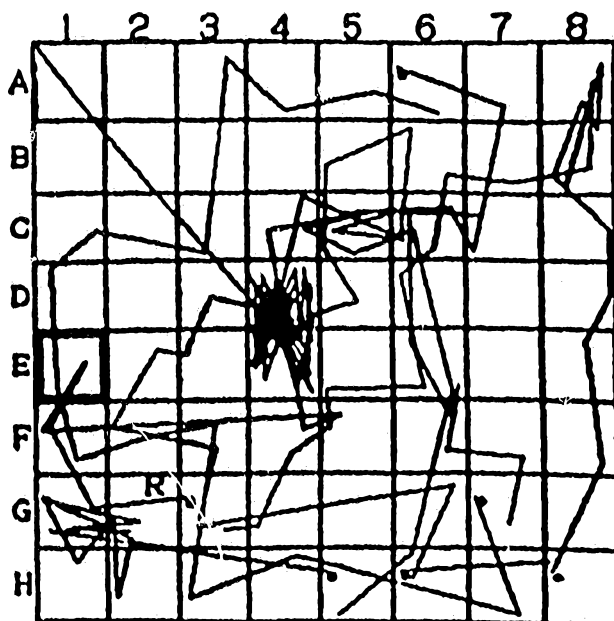
0: 0 to 0:30

Experimental clock

0:30: 0

Shock:OFF

- ◆:two-footed
- :Jump
- :now SHOCK area
- :Feeder location
- :Watering location
- :Starting point
- END! any key hit



f.1=STOP

FIG. 1B.

B

-On time (contunous)

File name:GRF-4

Display time

0: 0 to 0:30

Experimental clock

0:30: 0

Shock:OFF

◆:two-footed

●:Jump

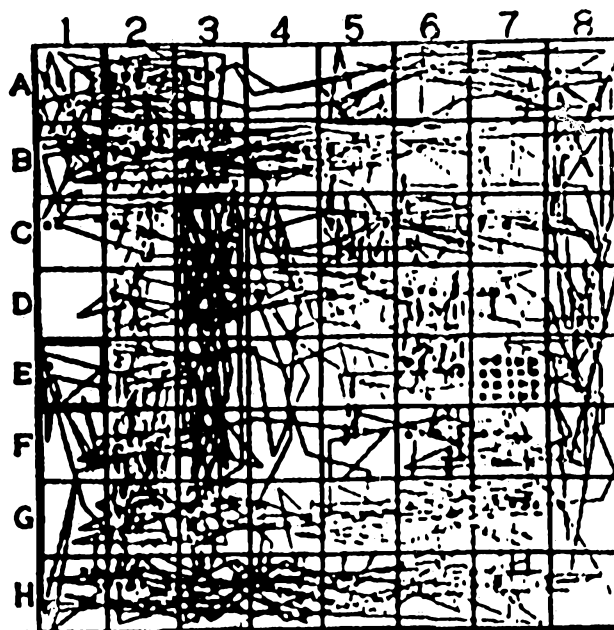
■:now SHOCK area

□:Feeder location

□:Watering location

□:Starting point

END! any key hit



f.1=STOP

FIG. 1C.

C

-On time (contunous)

File name:GRF-2

Display time

0: 0 to 0:30

Experimental clock

0:30: 0

Shock:OFF

◆:two-footed

●:Jump

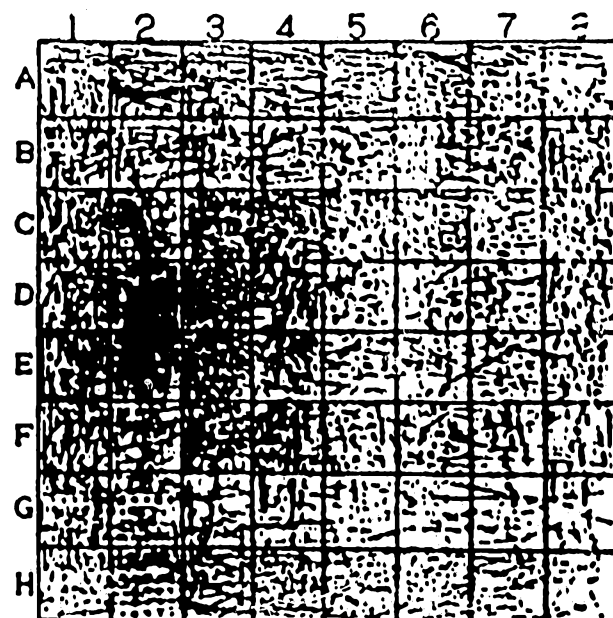
■:now SHOCK area

□:Feeder location

□:Watering location

□:Starting point

END! any key hit



f.1=STOP

FIG. 1D.

D

EFFECTS OF SOMATOSTATIN AND GRF ON BEHAVIOR (SNBM-MAZE)

NEUTRAL POSITION

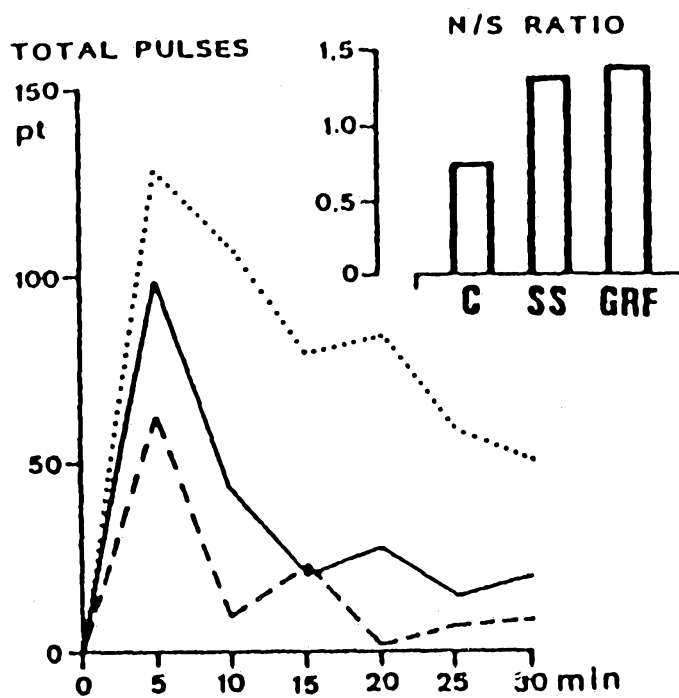


FIG. 2A.

SHOCK POSITION

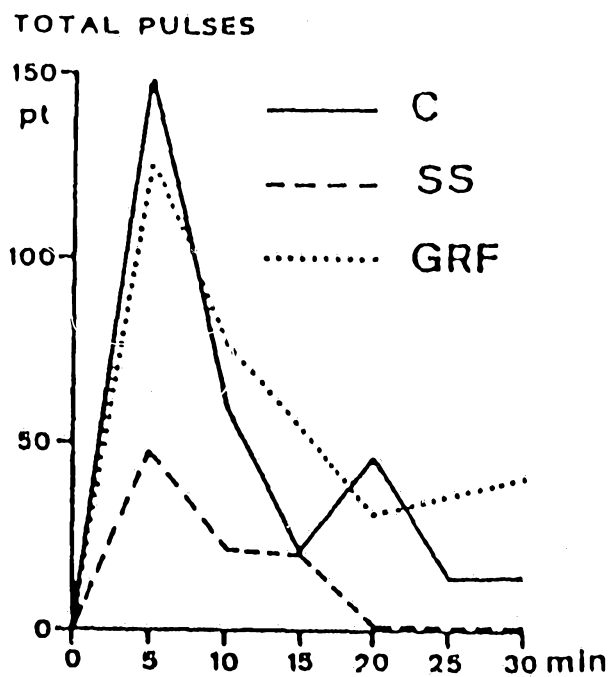


FIG. 2B.

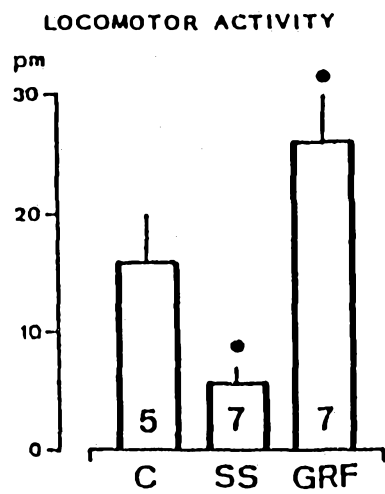


FIG. 2C.

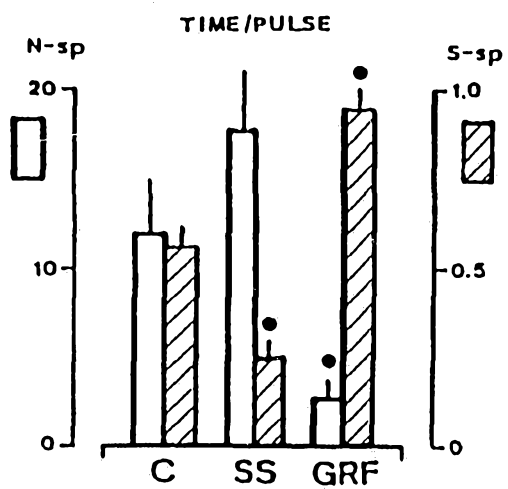


FIG. 2D.

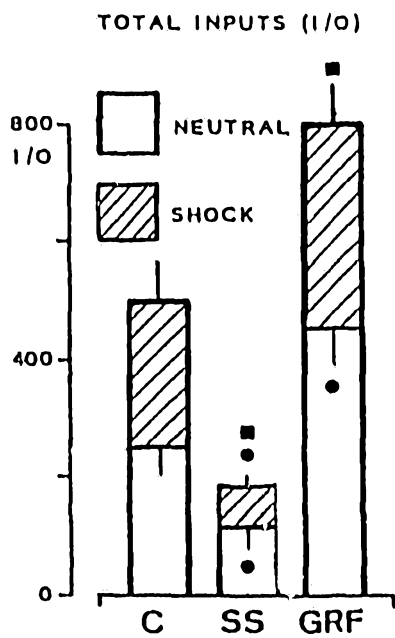


FIG. 2E.

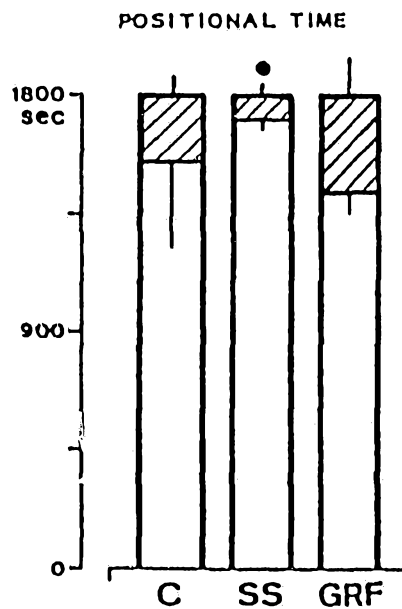


FIG. 2F.

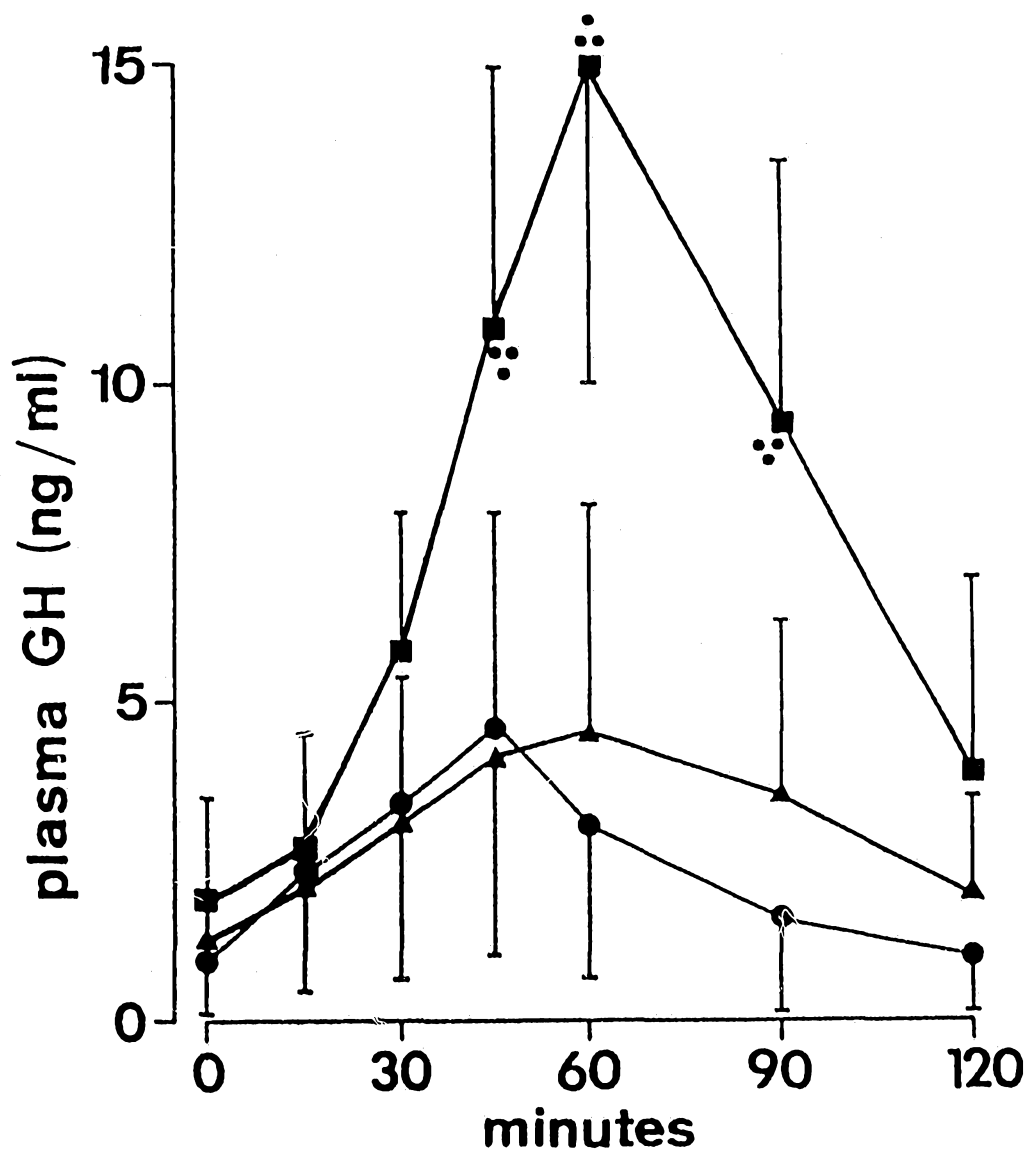


FIG. 3.

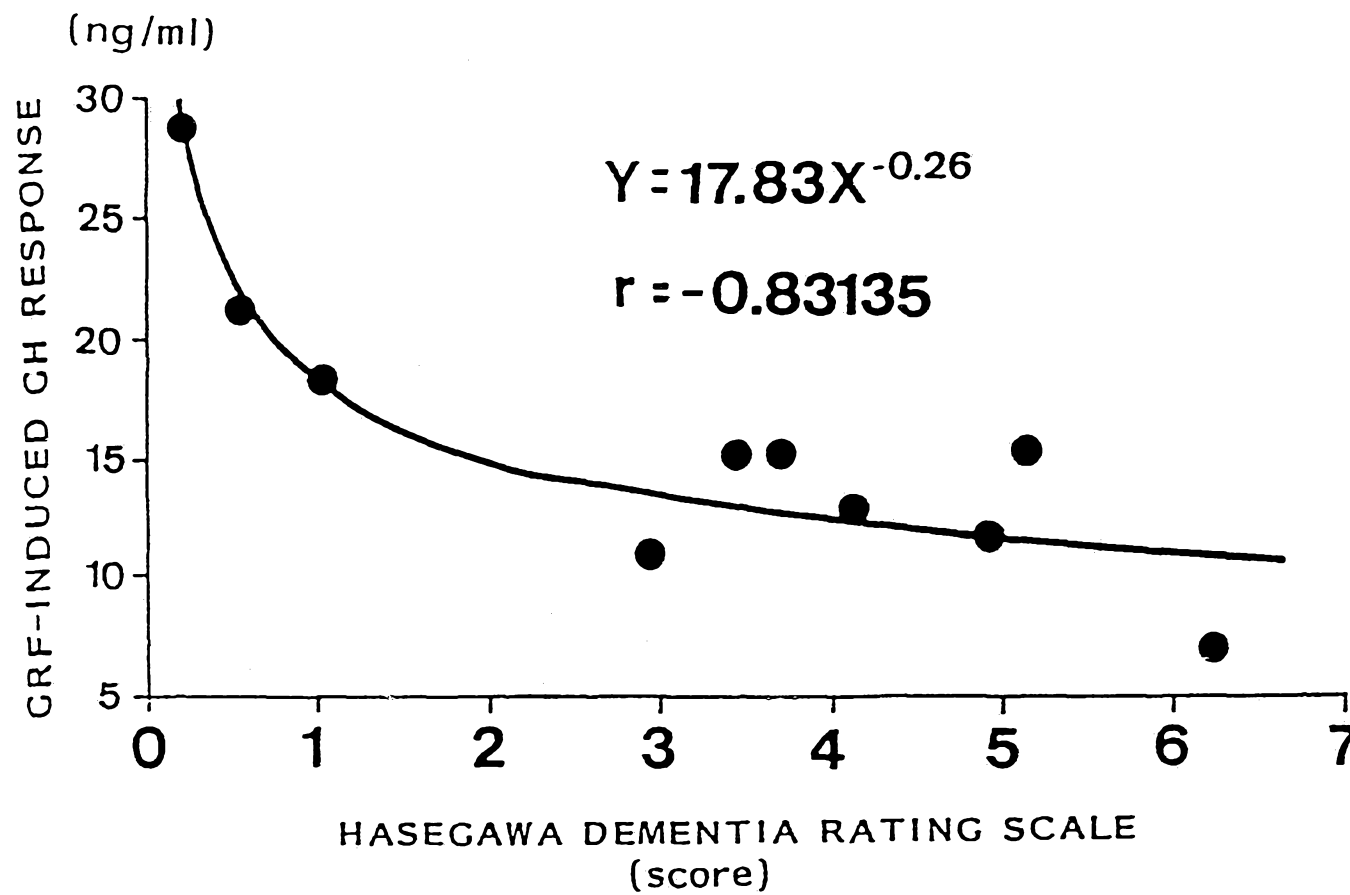


FIG. 4.

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