| 1.0 | 1.25 | 1.1 | 1.4 | 1.6 | 2.0 | 2.2 | 2.8 | 2.5 |

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A
APPLICATION FOR A STANDARD PATENT
OR A STANDARD PATENT OF ADDITION

X. We., LPB Istituto Farmaceutico S.p.A.
Via Dei Lavoratori, 54, CINISELLO BALSAMO, ITALY.

hereby apply for the grant of a standard patent for an invention entitled "Pharmaceutical compositions for the treatment of involutional brain syndromes and mental decay" which is described in the accompanying specification.

*(To be included in the case of a Convention application)
Details of basic application(s) -
Number of basic application .................................................. 48055 A/85
Name of Convention country in which basic application was filed .......... ITALY
Date of basic application ......................................................... MAY 8, 1985

*(To be included in the case of an application made by virtue of section 51)
Number of original application ..............................................
Person by whom made ...........................................................

*(To be included in the case of an application for a patent of addition)
I request that the patent may be granted as a patent of addition to the patent applied for on Application No. .............................................................................. Patent No. ..........................................................

In the name of .................................................................
I request that the term of the patent of addition be the same as that for the main invention or so much of the patent for the main invention as is unexpired.

My address for service is ....................................................
KELVIN LORD AND COMPANY, PATENT AND TRADE MARK ATTORNEYS,
4 DOURO PLACE, WESTERN AUSTRALIA, AUSTRALIA 6005

Dated this ................................................. AUGUST 9th, 1985

To: THE COMMISSIONER OF PATENTS

LPB Istituto Farmaceutico S.p.A.
By their Patent Attorneys
KELVIN LORD AND COMPANY

This form must be accompanied by either a provisional specification (Form 9 and true copy) or by a complete specification (Form 10 and true copy).

* These sections are to be completed only where applicable.
DECLARATION IN SUPPORT OF A CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT OR PATENT OF ADDITION

In support of the application made by LPB ISTITUTO FARMACEUTICO S.p.A.

for a patent for an invention entitled "PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF INVOLUTORY BRAIN SYNDROMES AND MENTAL DECAY"

I/W., GABRIELE BRAGLIA, of Via Del Ronco 40, Carimate, Como, ITALY.

We do solemnly and sincerely declare as follows:

1. I am/we are the applicant(s) for the patent, or am/are authorised by the abovementioned applicant to make this declaration on its behalf.

2. The basic application(s) as defined by Section 141 of the Act was/were made in the following country or countries on the following date(s) by the following applicant(s) namely:

   in ITALY on MAY 8 1985
   by LPB ISTITUTO FARMACEUTICO S.p.A.

3. The said basic application(s) was/were the first application(s) made in a Convention country in respect of the invention the subject of the application.

4. The actual inventor(s) of the said invention was/are Carlo Scolastico of Via Vallisneri 13/B, Milan, ITALY and Gabriele Braglia of Via Del Ronco 40, Carimate, Como, ITALY.

5. The facts upon which the applicant(s) is/are entitled to make this application are as follows:- The Applicant would, if a patent were granted upon an application made by the actual inventors in respect of the said invention, be entitled to have the patent assigned to it.

DECLARED at Cinisello B. this 19th day of July 1985

LPB Istituto Farmaceutico S.p.A.
1. Pharmaceutical compositions for the treatment of brain involutional syndromes of an essential, vascular or traumatic origin, and of the mental decay in the elderly, characterized by the fact that said compositions contain L-alpha-glycerylphosphorylcholine as the active ingredient.

5. Use of L-alpha-glycerylphosphorylcholine for the preparation of a drug useful for the treatment of brain involutional syndromes of an essential, vascular or traumatic origin, and of the mental decay in the elderly.
AUSTRALIA
PATENTS ACT 1952

COMPLETE SPECIFICATION
(ORIGINAL)

FOR OFFICE USE:

Application Number:
Lodged:

Class
Int. Class

Complete Specification Lodged:
Accepted:
Published:

Priority:

Related Art:

Name of Applicant(s): LPB Istituto Farmaceutico S.p.A.,

Address of Applicant(s): Via Dei Lavoratori, 54
CINISELLO BALSAMO
ITALY

Actual Inventor(s): Carlo SCOLASTICO
and
Gabriele BRAGLIA

Address for Service: Kelvin Lord & Co.,
4 Douro Place,
WEST PERTH,
Western Australia 6005.

Complete Specification for the invention entitled:

"Pharmaceutical compositions for the
treatment of involutional brain syndromes
and mental decay"
PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF INVOLUTIONAL BRAIN SYNDROMES AND MENTAL DECAY

The invention concerns pharmaceutical compositions containing, as active ingredient, L-alpha-glycerylphosphorylcholine (possibly combined with other drugs active on the central nervous system), suited for the treatment of brain involutorial syndromes of an essential, vascular or traumatic origin, and of the mental decay in the elderly.

L-Alpha-glycerylphosphorylcholine (I)

\[
\begin{align*}
\text{ACH} & \quad \text{OH} \\
\text{HO-CH} & \quad \text{H} \\
\text{CH}_2\text{O-P} & \quad \text{O} \\
& \quad \text{O-CH}_2\text{CH}_2\text{-N(CH}_3\text{)}_3
\end{align*}
\]

is known to exert a therapeutic activity in patients affected with anomalies in lipid metabolism, acute, subacute and chronic hepatitis, steatosis, and analogous pathologic forms: see in this connection the Italian Patent Application by the Applicant, No. 25706 A/79, filed on 9-14-1979.

L-Alpha-glycerylphosphorylcholine, when given orally or parenterally, has now surprisingly been found to exert a quite interesting activity also on the central nervous system, in terms of an activation of the central cholinergic system, and indirectly of the functionally related dopaminergic system. The pharmacological results are hereinbelow reported.

1. Effects on the Dopamine Turnover

Male rats, given oral or intraperitoneal increasing doses of L-alpha-glycerylphosphorylcholine, were sacrific-
ed 2 hours after the treatment. The striatal concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) were measured by HPLC, using an inverse phase column according to the method of L.J. Felice et al., J. Neurochem., 31, 5 1461-1467, 1978. Table 1 shows that the compound induces a significant increase of the striatal concentrations of DOPAC at doses higher than 80 μMoles/kg. Said increase persists for at least 4 hours (Table 2).

### Table 1

**Effect of the Intraperitoneal or Oral Administration of Various Doses of L-alpha-Glycerylphosphorylcholine on the Striatal Concentrations of 3,4-Dihydroxyphenylacetic Acid**

<table>
<thead>
<tr>
<th>Dose (μMoles/kg) 2 hours before the sacrifice</th>
<th>Administration route</th>
<th>DOPAC ng/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Intraperitoneal</td>
<td>2.45 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>2.40 ± 0.30</td>
</tr>
<tr>
<td>10</td>
<td>Intraperitoneal</td>
<td>2.49 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>2.37 ± 0.21</td>
</tr>
<tr>
<td>20</td>
<td>Intraperitoneal</td>
<td>2.30 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>2.19 ± 0.27</td>
</tr>
<tr>
<td>80</td>
<td>Intraperitoneal</td>
<td>2.87 ± 0.34**</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>2.76 ± 0.24*</td>
</tr>
<tr>
<td>160</td>
<td>Intraperitoneal</td>
<td>3.55 ± 0.25**</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>3.50 ± 0.19**</td>
</tr>
<tr>
<td>240</td>
<td>Intraperitoneal</td>
<td>3.69 ± 0.25**</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>3.65 ± 0.24**</td>
</tr>
</tbody>
</table>

The values represent the means ± SD of 8 animals in each group of treatment.

* P < 0.05; ** P < 0.01 Dunnet's t-test.
TABLE 2

STRIATAL CONCENTRATIONS OF 3,4-DIHYDROXYPHENYLACETIC ACID AT VARIOUS TIME AFTER THE INTRAPERITONEAL AND ORAL ADMINISTRATIONS OF L-ALPHA-GLYCERYLPHOSPHORYLCHOLINE (160 µMoles/kg)

<table>
<thead>
<tr>
<th>Time</th>
<th>Administration route</th>
<th>Intraperitoneal</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.39 ± 0.22</td>
<td>2.50 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>3.75 ± 0.27**</td>
<td>3.20 ± 0.18**</td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>3.96 ± 0.18**</td>
<td>3.84 ± 0.17**</td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>2.88 ± 0.25**</td>
<td>3.30 ± 0.21**</td>
<td></td>
</tr>
</tbody>
</table>

The values are the means ± SD of 8 animals in each group of treatment, and are expressed as ng/mg of tissue.

*P < 0.01 Dunnet's t-test.

2. Effect on the Dopamine Release

The dopamine release was assessed on slices of rat striatum (300 x 300 μ) taken out 2 hours after the oral treatment with L-alpha-glycerylphosphorylcholine (alpha-GPC) (240 µMoles/kg). The slices were preincubated with oxygenated Krebs-Ringer's fluid containing 3 dopamine (0.4 µCi/ml, 10^-7 M) and CaCl2 that favors the tissue uptake. Subsequently, the incubation medium was added with KCl (50 µMoles) as depolarizing agent, and the radioactivity liberated in the medium, during 10 minutes, was assessed by a liquid phase scintillation spectrometer. Figure 1 shows that the in vivo treatment with the compound produces an increase of the capacities of the striatum slices.
prepared from the treated animals, to liberate in vitro dopamine following a depolarizant stimulus.

In Figure 1 the horizontal broken and solid lines indicate the means and the standard deviations, respectively, of the experimental groups. The basal release proved equivalent to 3022 ± 546 and 2904 ± 453 dpm/mg protein/10 min for the control and the treated animals respectively.

3. Investigations on the Binding with the Cholinergic Receptors

The investigation on the cholinergic receptors on in toto rat's brain was carried out using the method of H. Yamamura and S.H. Suyder, Mol. Pharmacol., 10, 861-867, 1974, using ³H-quinonuclyldibenzyate (QNB) as the binding agent. L-Alpha-glycerylphosphorylcholine and the reference compounds were incubated at concentrations ranging between 5 x 10⁻¹⁰ and 5 x 10⁻³ moles for 60' at 25°C. Figure 2, in which the various symbols, have the below reported significances, i.e.:

- (⚫) QNB
- (◇) Choline
- (◆) Alpha-glycerylphosphorylcholine
- (□) Oxotremorine
- (○) Acetylcholine
- (▲) Lecithin

states that L-alpha-glycerylphosphorylcholine cannot significantly bind in vitro with the cholinergic receptor.

This evidence suggests that the in vitro activity on the cholinergic transmission is indirect, mediated possibly by an increased synthesis of acetylcholine.

4. Distribution of ³H(Me)-L-alpha-glycerylphosphorylcholine in the Brain Tissues

Some rats were given orally and intraperitoneally
the labelled compound (20-100 μCi/kg), varying the doses following dilution with the cold drug), specific activity 45 μCi/mg. The experimental animals were sacrificed at various times after the treatment, and the brains dissected in the various areas; blood was also taken out in parallel. The radioactivity, present in the tissues, was counted in a liquid phase scintillation spectrometer.

The data reported in Table 3 state that L-alpha-glycerylphosphorylcholine (80 μMoles/kg), given orally and intraperitoneally, attains significant concentrations in various areas of the central nervous system.

As shown in Table 4, after oral administration a good relationship is found between the administered dose and the quantity of L-alpha-glycerylphosphorylcholine that attains the various brain areas.
### Table 3

**Activity (dpm/g) at various times, and in various brain areas, after intraperitoneal and oral administration of 3H-alpha-glycerylphosphorylcholine (80 μMoles/kg, 100 μCi/kg)**

<table>
<thead>
<tr>
<th>Administration route</th>
<th>Time (h)</th>
<th>Cortex (dpm/g)</th>
<th>Cerebellum (dpm/g)</th>
<th>Striatum (dpm/g)</th>
<th>Hippocampus (dpm/g)</th>
<th>Hypothalamus (dpm/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraperitoneal</strong></td>
<td>0.5</td>
<td>485 ± 188</td>
<td>485 ± 188</td>
<td>426 ± 102</td>
<td>411 ± 123</td>
<td>573 ± 188</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>676 ± 41</td>
<td>629 ± 68</td>
<td>512 ± 85</td>
<td>435 ± 96</td>
<td>1206 ± 532</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>803 ± 101</td>
<td>779 ± 15</td>
<td>638 ± 75</td>
<td>568 ± 141</td>
<td>1155 ± 406</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>688 ± 287</td>
<td>612 ± 329</td>
<td>571 ± 229</td>
<td>450 ± 223</td>
<td>1152 ± 44</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>291 ± 156</td>
<td>232 ± 29</td>
<td>259 ± 6</td>
<td>132 ± 23</td>
<td>570 ± 100</td>
</tr>
<tr>
<td><strong>Oral</strong></td>
<td>1</td>
<td>790 ± 182</td>
<td>659 ± 146</td>
<td>646 ± 123</td>
<td>717 ± 197</td>
<td>1797 ± 326</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1064 ± 179</td>
<td>768 ± 126</td>
<td>794 ± 203</td>
<td>936 ± 69</td>
<td>1524 ± 19</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>564 ± 119</td>
<td>488 ± 129</td>
<td>491 ± 87</td>
<td>420 ± 75</td>
<td>1136 ± 53</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>706 ± 64</td>
<td>685 ± 31</td>
<td>644 ± 100</td>
<td>647 ± 123</td>
<td>953 ± 76</td>
</tr>
</tbody>
</table>

The values are the means ± SD of 8 animals in each experimental group.
TABLE 3

ACTIVITY (dpm/g) AT VARIOUS TIMES, AND IN VARIOUS BRAIN AREAS, AFTER INTRAPERITONEAL ORAL ADMINISTRATION OF 3H-ALPHA-GLYCERYLPHOSPHORYLCHOLINE (80 μMoles/kg, 100 μCi/kg)

<table>
<thead>
<tr>
<th>Administration route</th>
<th>Time h</th>
<th>Activity (dpm/g x 10^-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cortex</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>0.5</td>
<td>485 ± 188</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>676 ± 41</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>803 ± 101</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>688 ± 287</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>291 ± 156</td>
</tr>
<tr>
<td>Oral</td>
<td>1</td>
<td>790 ± 182</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1064 ± 179</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>564 ± 119</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>706 ± 64</td>
</tr>
</tbody>
</table>

The values are the means ± SD of 8 animals in each experimental group.
TABLE 4
ESTIMATED CONCENTRATIONS OF L-ALPHA-GLYCERYLPHOSPHORYLCHOLINE IN VARIOUS BRAIN AREAS, 1 HOUR AFTER THE ADMINISTRATION OF VARIOUS DOSES OF THE DRUG

<table>
<thead>
<tr>
<th>Dose</th>
<th>5 µMoles/kg</th>
<th>10 µMoles/kg</th>
<th>20 µMoles/kg</th>
<th>80 µMoles/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-alpha-glycerylphosphorylcholine (nmoles/g tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>9.1±2.33</td>
<td>13.56±1.24</td>
<td>20.4±6.18</td>
<td>65.3±11.8</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.0±0.20</td>
<td>3.4±0.86</td>
<td>4.1±0.40</td>
<td>24.0±5.34</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>3.0±0.26</td>
<td>4.9±1.13</td>
<td>6.1±1.87</td>
<td>26.0±7.16</td>
</tr>
<tr>
<td>Striatum</td>
<td>4.7±2.27</td>
<td>7.28±2.14</td>
<td>10.7±2.41</td>
<td>23.5±4.47</td>
</tr>
<tr>
<td>Cortex</td>
<td>6.9±0.97</td>
<td>10.2±2.07</td>
<td>12.7±4.14</td>
<td>29.1±6.61</td>
</tr>
</tbody>
</table>

The values are the means ± SD of 8 animals in each experimental group.

(1) Calculated from (dpm/g tissue): (dpm/mole).

The hereinabove reported results induced to extend clinically the investigation, with specific reference to the symptomatic pattern associated with an insufficient cholinergic function, i.e. loss of memory, manias, behavioral disturbances, and so on. L-Alpha-glycerylphosphorylcholine was consequently confirmed to be a real and effective therapeutic agent, as confirmed by the below
CLINICAL TRIALS

1. Investigation on the Brain Syndromes of an Essential Vascular or Traumatic Origin

Markedly positive results were obtained in initial involutional syndromes on which L-alpha-glycerylphosphorylcholine could exert a positive action on the basic neurophysiological mechanisms. Forty patients, affected by a brain syndrome of a vascular nature or of an Alzheimer-type, showed a significant improvement of the neurologic pattern (short- and medium-term memory, cognitive functions) that could also be evidenced by the EEG tracings, altered before the treatment.

L-Alpha-glycerylphosphorylcholine, given to 20 patients with head trauma, produced a significant regression of all neuropsychic symptoms of the post-concussional syndrome (headache, dizziness, balance disturbances): this regression was matched by a normalization of the acoustic evoked potentials of the brainstem (Baer).

2. Investigation on the Mental Decay in the Elderly

The treatment with L-alpha-glycerylphosphorylcholine induced, in 70 elderly patients with mental decay, a clear improvement of the mental tests, pertaining to attention, recent memory and cognitive abilities. Said results are to be referred to the participation of the drug in the activation of the memory function, and particularly of the cortex interneuronal connections impaired in the process of senile decay.

L-Alpha-glycerylphosphorylcholine, assessed in two 30 controlled clinical trials, versus cytidindiphosphocholi-
ne, in 70 patients with an essential or vascular senile decay, proved to be able to improve the psychointellective conditions of the patient, and to attenuate the initially existent neurological symptomatic pattern; the positive recovery of recent memory, time-space disorientation and confusional state shall be specifically underlined. Said results are superimposable to the ones observed with the reference drug.

Moreover, the present invention concerns all industrially applicable aspects associated with the use of L-alpha-glycerylphosphorylcholine as a therapeutic agent to be used in the treatment of involutional brain syndromes of an essential vascular or traumatic origin and in the treatment of the mental decay in the elderly. Therefore, an essential aspect of the investigation covers pharmaceutical compositions containing, as active ingredient, prescheduled and therapeutically effective quantities of L-alpha-glycerylphosphorylcholine, possibly in mixture with excipients of current use in drug compounding, and possibly in mixture with other active ingredients, said compositions being however scheduled for the treatment of the above stated pathologic forms.

Unlimiting examples of pharmaceutical compositions, according to the present invention, are represented by:

25 (a) soft-gelatin capsules, containing 400 mg of L-alpha-glycerylphosphorylcholine, to be taken 2-3 times daily according to medical prescription;

(b) ampuls of 1000 mg of L-alpha-glycerylphosphorylcholine, for intramuscular injection, their contents to be injected once daily;
(c) ampuls of 400 mg of L-alpha-glycerylphosphorylcholine for IV drip infusion, their contents to be injected at the rate of 1-6 daily, according to medical prescription.
The claims defining the invention are as follows:

1. Pharmaceutical compositions for the treatment of brain involutional syndromes of an essential, vascular or traumatic origin, and of the mental decay in the elderly, characterized by the fact that said compositions contain L-alpha-glycerylphosphorylcholine as the active ingredient.

2. Pharmaceutical compositions according to claim 1, to be administered orally.

3. Pharmaceutical compositions according to claim 1, to be given parenterally.

4. Pharmaceutical compositions according to claim 1, said compositions also containing other drugs active on the central nervous system.

5. Use of L-alpha-glycerylphosphorylcholine for the preparation of a drug useful for the treatment of brain involutional syndromes of an essential, vascular or traumatic origin, and of the mental decay in the elderly.

6. Pharmaceutical compositions for the treatment of brain involutional syndromes containing L-alpha-glycerylphosphorylcholine and the use of said compound for the preparation of a drug useful for the treatment of the said syndrome substantially as hereinbefore described.

DATED AUGUST 9, 1985
S.P.A
By their Patent Attorneys
KELVIN LORD AND COMPANY
PERTH, WESTERN AUSTRALIA
The treatment of carotid, vascular or traumatic in the elderly, positions contain active ingre-
and to claim 1,
and to claim 1,
and to claim 1,
and to claim 1,
and to claim 1.

The treatment of brain invol-

ture or traumatic

treatment of

Lentico S.p.A

Veimey

RANY

MALIA
FIG. 2

$^{3}$H-QNB BOUND (% OF THE CONTROL)