RNA TREATMENT OF DEMENTIA

A Double-blind Study

S. MUNCH-PETERSEN, H. PAKKENBERG, H. KORNERUP, J. ORTMANN, E. IPSEN, P. JACOBSEN and H. SIMMELSGÅRD

ABSTRACT

Uridine uptake in the nerve cells of the cortex in mice was demonstrated by autoradiography after oral administration of $^{13}$H-uridine. Following this trial a clinical treatment was carried out. The material consisted of twenty-two mental hospital patients with mild to moderate degrees of dementia. Ten of these patients received treatment by oral administration of about 20 g hydrolyzed yeast-RNA daily over a period of 4 months, while 12 patients received placebo. A psychological investigation and a clinical evaluation were performed prior to, during and immediately after the investigation, as well as 2 months later. Furthermore, a neurological investigation, EEG, determination of serum uric acid, serum creatinine and serum cholesterol were all made simultaneously with the psychological tests. Pneumoencephalography was performed in 12 patients prior to the start of the investigation (7 of these showed moderate, diffuse atrophy, and 5 showed severe, diffuse atrophy). In general, it may be concluded that there was no change in the state of dementia as a result of the treatment given.

In 1957, Cameron et al. reported for the first time the intravenous injection of DNA in senile and arteriosclerotic patients, administered in association with intrathecal hyaluronidase, in an attempt to bring DNA into intimate contact with the brain. Since then, Cameron et al. (1958, 1961 a, b, 1963 a, b and c) in particular, but also other authors, for example Montanari et al. (1961), Kral et al. (1967), Nodine et al. (1967), Goetze-Claren (1969), Odens (1969) and Schinko (1967) have reported on the ingestion of RNA or its break-down products, ribonucleotides, in aged patients suffering from impaired memory, and memory tests in these patients have been evaluated in relation to ingestion of the substances mentioned.

A survey of previous investigations is provided in Table 1.
Table 1.

<table>
<thead>
<tr>
<th>Author</th>
<th>Selection criteria</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameron (1958)</td>
<td>Aged pts suffering from impairment of the retention phase of memory.</td>
<td>23</td>
</tr>
<tr>
<td>Cameron et al.</td>
<td><em>Group I:</em> Pts selected from aged individuals suffering from memory defects who were attending the Allan Memorial Institute of Psychiatry. Those who suffered from mood disturbance, recent cerebral accident or malnutrition were not included. Diagnostic sub-grouping: Brain arteriosclerosis 29, mean age 69.3. Senile dementia 8, mean age 70. Korsakoff's psychosis or presenile dementia 4.</td>
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<td><em>Group II:</em> Aged pts from the Geriatric Ward of a provincial mental hospital, considerably more advanced in their illness than group I. Diagnostic sub-grouping: Brain arteriosclerosis, mean age not stated. The numerical distribution of the two diagnostic sub-groups is not stated.</td>
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<td>Montanari et al.</td>
<td>17 hospitalized pts and 3 out-pts at the clinic for nervous and mental diseases at the University of Ferrara. 14 men and 6 women aged 18–82 years (mean age 54). A heterogeneous group of pts, the majority suffering from cerebral vasculopathy of the type arteriosclerosis with dementia, in some cases combined with Parkinsonism, tetraplegia and carotid-thrombosis. Also pts with oligophrenia, presenile depression and confusion, dysmetabolic encephalopathy and neurasthenia.</td>
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<tr>
<td>Odens (1961)</td>
<td>16 pts in the age group 62–96, all suffering from impaired memory. Two groups, one suffering from arteriosclerosis and the other from impaired memory only. The numerical distribution in the two groups is not stated.</td>
<td>16</td>
</tr>
<tr>
<td>Substance, dosage and duration of treatment</td>
<td>Effect</td>
<td></td>
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<tr>
<td>--------------------------------------------</td>
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| 2-year studies with DNA given up to 4 X daily i.v. and with RNA given up to 2 X daily i.v. | Evaluated by clinical data and memory tests, favourable results were seen in all pts and good results in 50%.
| 1-year studies with RNA (up to 75 g) (usually 10-20 g) given daily orally in capsules. | Evaluated by clinical data and memory tests, improvement was observed to varying degrees in 69% of the pts with brain arteriosclerosis, and a considerably less pronounced improvement in 38% of the pts with senile dementia. Korsakoff and presenile cases were too few in number to permit any general statements to be made.
| The majority of the pts received 2-75 g RNA daily orally, in slowly dissolving pills. 14 pts were given RNA i.v. 50-2000 mg daily. | Evaluated by memory tests, favourable results were not obtained in the senile pts either receiving RNA or in those receiving placebo. Significant improvement to the 0.05 level in the arteriosclerotic cases receiving RNA on one of the memory tests (counting test). It is not stated whether there was a significant difference from the placebo group. Another test (Wechsler memory scale) does not show any significant improvement.
<p>| Brain arteriosclerotic pts: Mean total oral dose: 1219.45 g. Mean total i.v. dose: 163.88 g. Average duration of treatment: 6.48 months. | Evaluated by clinical data and memory tests, no effect was observed in the pts with dysmetabolic encephalopathy and tetraplegia or in a pt with arteriosclerotic dementia and episodic confusion. In the remainder of the pts improvements of various kinds were observed. In all cases as a group there was a 80% improvement. |
| Senile dementia pts: Mean total oral dose: 1309.25 g. Mean total i.v. dose: 32.5 g. Average duration of treatment: 8.75 months. | RNA was given in daily doses of from 30-40 g, which were increased after 4 weeks to 50 g. |
| Group II was divided into sub-group A who received placebo and sub-group B who received RNA (693 g RNA orally over 12 weeks) on a double-blind basis. The numerical distribution of the two groups (RNA and placebo) is not stated. Test results prior to treatment indicate significantly better test values for the RNA group. | |</p>
<table>
<thead>
<tr>
<th>Author</th>
<th>Selection criteria</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameron et al. (1963 a and b)</td>
<td>Presenile and aged pts selected on the basis of established severe memory defect. No pt with other psychotic or neurotic condition or recent cerebral accident was included. The physical condition of all, apart from the normal frailties of age, was normal. Only pts whose deficit was relatively stationary were included. Diagnostic sub-grouping: 4 arteriosclerotics, 7 presenile and 4 senile. Age not stated.</td>
<td>16</td>
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<tr>
<td>Cameron et al. (1963 c)</td>
<td>Brain arteriosclerosis, senile dementia and presenile dementia.</td>
<td>38</td>
</tr>
<tr>
<td>Kral et al. (1967)</td>
<td>17 men and 34 women, 29 in the geriatric ward of a mental hospital, 9 attended the psychiatric geriatric clinic of a general hospital and 7 were members of a Golden Age Club. Ages from 50 to 84 years, with a mean age of approx. 70 years. Diagnoses: 15 senile dementia, 12 arteriosclerotic brain disease, 5 with an amnestic syndrome formed a miscellaneous group and 19 were classified as senescent, i.e. they were clinically free from psychiatric or neurological disorders or chronic neurotic reactions, and did not have chronic brain syndromes of either type. Subjects unable to cooperate due to sensory impairment, aphasia or acute psychotic episodes were excluded.</td>
<td>39</td>
</tr>
<tr>
<td>Nodine et al. (1967)</td>
<td>Patients hospitalized at Harrisburg State Hospital. The Charts of 450 pts with diagnoses of senile or arteriosclerotic brain disease were reviewed. Those over 60 years, 123 pts in all, who were reported not to suffer from gross orientation defects, were interviewed. Of these, 72 were eliminated for diverse reasons: too deteriorated to cooperate, 31; hearing impairment, 11; associated physical disease, 7; other psychiatric diseases, 15; and language barrier and other cases, 8.</td>
<td>33</td>
</tr>
<tr>
<td>Schinko (1967)</td>
<td>All pts admitted for a long period and adjusted to the geriatric department at the Vienna psychiatric hospital and whose condition is stationary and permits testing.</td>
<td>120</td>
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Effect Substance, dosage and duration of treatment

<table>
<thead>
<tr>
<th>Substance, dosage and duration of treatment</th>
<th>Effect</th>
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<tbody>
<tr>
<td>RNA produced from yeast was administered <em>i.v.</em>: a) 10 g RNA dropwise over 6-8 hours, b) 10 g RNA <em>i.v.</em> over 50 minutes. 2 pts received more than 200 g RNA. All others received 50-70 g RNA in 6 applications over a 2-week period.</td>
<td>Evaluated by memory tests, substantial increases in after-treatment values were seen in all tests. The best results were seen in the arteriosclerotic group, the second best in the pre-senile group and the poorest in the senile group.</td>
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<td>10 g RNA prepared from yeast was administered <em>i.v.</em> in the course of 2-8 hours, 1-3 × weekly, a total of 100 g RNA to each pt. 16 pts received RNA solution with a small percentage of small fragments of the molecule (relatively undegraded). 22 pts received RNA solution with 20-30% larger amounts of small fragments of RNA (high degree of degraded material).</td>
<td>Evaluated by memory tests, increases were observed in several after-treatment values. Relatively undegraded solution is better than solution containing a high degree of degraded material.</td>
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<tr>
<td>39 pts received 6 g RNA daily in capsules for a week. A total of 42 g RNA.</td>
<td>None of the various memory tests showed any significant increase from the pretreatment value at the 0.05 level or better.</td>
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*Double-blind study:* 13 of the 39 subjects who received RNA capsules were matched (according to sex, diagnostic category and retention span) with 13 subjects who received only placebo capsules.

One test out of several different memory tests showed a significant increase from the pretreatment value at the 0.01 level in the RNA group. No significant difference between the RNA group and the placebo group.

33 (51) pts were randomly assigned: 17 to RNA (4 omitted), 16 to placebo (3 omitted) and 18 to an experimental agent. RNA prepared from yeast, 10 g orally daily for 4 weeks, a total of 280 g RNA. *Double-blind study.*

Evaluated by clinical data and various tests, no significant difference was found between the RNA group and the placebo group.

| Ribonucleotide (equal parts of AMP-CMP-GMP-ribonucleotide) | 33 pts received 600 mg |
In their first report Cameron et al. (1958) found the best effects were obtained in patients with severe memory impairment and marked confusion. In their later investigations, Cameron et al. (1961 a, b, 1963 a, b) found that patients with moderately advanced memory failure (retention failure) fared better on RNA than did patients with marked memory impairment. This state of affairs was also found by Schinko (1967). Cameron et al. (1961 a, b, 1963 a, b) also found that the best results were obtained in individuals whose memory disturbances were caused by arteriosclerosis, the second best group of results included the presenile, and the third best group the senile. Similar results were also found by Schinko (1967) and, inconclusively, by Odens (1969). Cameron et al. (1958 and 1963 a) found that i.v. administration was markedly more effective than oral administration with respect to the amount and rapidity of improvement. As regards the size of the dose and the time of onset of the effect, Cameron (1958) found that results were usually noted after 4 or 5 daily injections of RNA, and after a period of 2 weeks it was usually not necessary to continue the injections; furthermore, Cameron & Solyom (1961 b) found that while a mean minimal total dose of RNA of 8.2 g intravenously or 539 g orally had to be administered to obtain improvement, some patients improved on as little as 2.9 g intravenously or 30 g orally, or on as much as 19 g intravenously or 1482 g orally. Montanari et al.
Effect Substance, dosage and duration of treatment

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dosage and Duration</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMP, UMP</td>
<td>orally daily in capsules for an average of 30 weeks</td>
<td>Improvement in about 1/2%</td>
</tr>
<tr>
<td>Duration of the treatment</td>
<td>12-36 weeks</td>
<td></td>
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<tr>
<td>18 pts received 1200 mg ribonucleotide orally daily in capsules for 24 weeks</td>
<td>Improvement in %</td>
<td></td>
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<tr>
<td>Ribonucleotide (equal parts of AMP-CMP-GMP-UMP)</td>
<td>69 pts (81 with 12 deaths) received 100 mg ribonucleotide i.m. daily for 4 weeks, and thereafter 1200 mg orally daily in capsules for 22 weeks</td>
<td>Improvement in 52.2%</td>
</tr>
<tr>
<td></td>
<td>Unchanged condition in 21.7%</td>
<td>Exacerbation in 26.1%</td>
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</table>

(1961) found that there was a rapid effect in the course of a few days. Schinko (1967) found that the best effect was obtained with the largest dose of ribonucleotide (see Table 1).

Examining the survey of previous investigations, it will appear that in several, more or less systematically uncontrolled investigations (Cameron et al. 1958, 1961 a, b, c, Montanari et al. 1961, Odens 1969 and Schinko 1967), a more or less beneficial effect of RNA or ribonucleotide has been obtained. Two controlled double-blind investigations have been made (Nodine et al. 1967 and Kral et al. 1967) in which no significant difference was found between the effect of orally administered placebo and orally administered RNA, respectively. The third double-blind investigation was made by Cameron et al. (1961 a, b). Details of this investigation are, however, not sufficiently complete to permit the reader to draw definite conclusions. It is therefore a question whether the apparent improvement demonstrated in the uncontrolled experiments is due to RNA (ribonucleotide) or to quite different circumstances, such as psychological bias, or as Nodine et al. (1967) suggest to the attention given the patients during the investigation, or to the effects of practice in the test situation. Nothing definite has been demonstrated with regard to the relationship between size of dose and effect, but results from several of the uncontrolled investigations might suggest a more favourable effect by employment of larger doses,
and the possibility cannot be ignored that the negative result of the blind tests (Kral et al. 1967 and Nodine et al. 1967) may be due to too small a dosage, a possibility also mentioned by Kral et al. (1967).

On the whole it must be said that on the basis of the investigations made so far, no proof exists that RNA (ribonucleotide) has any favourable influence on the impairment of memory in aged patients. There is thus good reason to re-examine the effect of the substance under controlled conditions and at high doses.

It is a well-known fact that RNA is broken down in the intestinal tract by ribonucleases, for example from the pancreas. Bases, nucleosides and nucleotides are then absorbed. Wilson et al. (1954) have demonstrated that N15-labelled RNA, administered orally, is absorbed in human subjects, and Roll et al. (1949) have shown that nucleosides and nucleotides, administered orally, are included in nucleic acids in rat tissues. Finally, Rubini et al. (1961) have found uptake of H2-thymidine in many tissues, following oral administration. The transfer of RNA precursors from blood to brain has presented several problems. Intravenous administration of H2-5-uridine in mice results in the labelling of nerve cells in the brain on autoradiography (Pakkenberg & Fog 1972). The same holds if tritiated uridine or cytidine is administered intraperitoneally, whereas administration of H3-uracil and H3-guanine only results in very weak labelling (Pakkenberg & Fog 1973).

Hogans et al. (1971) have shown that a considerable part of the RNA-synthesis in the nerve cells of the brain probably involves, among other things, uridine conveyed by the circulation, and not originating by de novo synthesis as hitherto assumed.

It must therefore be assumed that a certain part of orally administered RNA, after enzymatic break-down in the intestinal tract, passes the blood-brain barrier and takes part in the RNA-synthesis by the nerve cells in the brain.

AUTHORS' INVESTIGATIONS

Absorption experiment

Ten white mice, weighing approximately 25 g, received 0.1 mC H3-uridine, specific activity 5 Ci/mM. The uridine was dissolved in water, and 0.1 ml per animal was administered by tube into the ventricle. Twenty-four hours later the mice were sacrificed using chloroform and immediately afterwards were perfused with formalin 4 per cent through the left ventricle of the heart. After imbedding in paraffin, 4 μm thick sections were cut and immersed in Ilford Nuclear Research Emulsion K-5. After exposure at 4° for 70 days, the sections were developed with Amidol for 4 min at 18°. The sections were then stained with haematoxylin-eosin.
Autoradiograms were produced in this way from brain, small intestine, liver and kidney.

Figure 1 shows pronounced activity in all 4 organs. In the brain, over the choroid plexus there is almost the same amount of activity as in the liver, kidney and intestinal tract, though the uridine uptake of the nerve cells is less pronounced. This distribution harmonizes with the findings in previous investigations (Pakkenberg & Fog 1972, 1973), but the uptake in the nerve cells seems to be relatively reduced on oral administration as compared with intravenous or intraperitoneal administration. In the experiments made here, however, we have not made any grain counts, as the amount of uridine administered per animal was much smaller than was the case in previous experiments. This makes a direct comparison impossible. We can conclude, however, that following oral administration of an RNA precursor, a distinct uptake is found in the nerve cells of the cortex.

MATERIAL AND METHODS

Selection of the Patients
In selecting the patients for the investigation it was important that they should not be cases of too advanced dementia, partly because they would then not be able to collaborate in the cognitive tests, and partly because previous experience suggested that there was a greater chance of positive results if the patients had only slight degree of dementia.

To obtain a homogeneous patient material, the aim was primarily to find a group of patients suffering exclusively from senile dementia, but since with this criterion we were only able to find a very limited group, we decided to include patients suffering from both presenile dementia and alcoholic dementia, as well as a few patients suffering from traumatic dementia.

These selection criteria had the advantage that they enabled us to compare the possible effect of ribonucleic acid (RNA) on a group of patients suffering from presenile/senile dementia and on a group of patients with alcoholic dementia, since measurements of cerebral blood flow had demonstrated no reduced values in Korsakoff's syndrome in alcoholics (Ingvar et al. 1969).

A total of 29 patients, long-term cases in St. Hans Mental Hospital, were selected. In 22 of these, the experimental conditions were satisfied in all essentials.

Informed consent was obtained both from the selected patients and from any relatives.

The Patient Material
1) 7 patients with alcoholic dementia, aged 51–65: 6 men and 1 woman.
2) 6 patients with presenile dementia, aged 52–67: 5 men and 1 woman.
3) 7 patients with senile dementia, aged 71–82: 1 man and 6 women.
4) 2 patients with traumatic dementia, aged 58–71: 2 men.

7 of the 29 patients were excluded:
One woman was excluded prior to the investigation on account of too marked presenile dementia.

One man was excluded prior to the investigation because of insufficient cooperation.

One man was excluded at the commencement of the investigation on account of acute psychosis.

One woman, who had undergone resection of the ventricle, was excluded during the investigation because of ventricular dyspepsia.

One man was omitted during the investigation on account of a somatic disease not connected with the investigation.

One man died from cancer during the investigation.

One man with presenile dementia was excluded during the investigation because of diarrhoea.

These last 4 patients were thus excluded during the investigation, and 3 of them were given RNA.

**Procedure and dosage**

The aim was to administer as large a dose of RNA as possible, since previous investigations had suggested a relationship between size of dose and its effect.

We did not venture to administer RNA by the intravenous route, both because of a previously described shock effect, and because RNA had to be given daily over a long period. Hydrolyzed yeast RNA was used with a view to promoting absorption from the intestinal tract. Hydrolyzed RNA consists of an equilibrium mixture of RNA, ribonucleotides and ribonucleosides, but quantitatively only about 5–15 per cent deviations in RNA content are involved.

About 20 g was administered orally per 24 hours, distributed over 3 doses of 40 ml of a slightly sweetened solution, and as placebo a similar sweetened solution, not containing protein. In the course of a period of 4 months a total of about 20×120 g or about 2400 g hydrolyzed RNA was thus administered during the test. Ten of the 22 patients received RNA, while 12 were given the placebo. The distribution was random, and the experiment was carried out as a double-blind test. The conditions for a double-blind test were satisfied by the fact that only the pharmacist who made up the solutions knew which of the patients received RNA and which received placebo.

The statistical methods were established before the commencement of the test. All psychological and clinical data were statistically analyzed before the code was broken (see below).

During the investigation all other medication and treatment was, as far as possible, kept at a constant level.

**Planned Investigations**

Cognitive tests were to be evaluated a total of 4 times with intervals of 2 months, i.e. 1st time immediately before the investigation was commenced, 2nd time during

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*Figure 1. Autoradiograms of A: small intestine, B: liver, C: kidney, D: nerve cells, and E: the choroid plexus from a mouse given 0.1 mC 5-H8 uridine orally 24 hours before perfusion through the heart.*
the administration of RNA, 3rd time at the termination of the administration
4 months after the commencement of the investigation, and 4th time 6 months
after the commencement of the investigation or 2 months after termination of the
RNA administration.

An overall clinical evaluation was likewise to be made on the same four occasions
as the psychological evaluation.

*Psychological Investigation*

Previous investigations did not permit any hypotheses as to how RNA might
affect intellectual functioning, either generally or specifically. The aim of this part
of the investigation was therefore to elucidate whether RNA caused if not an overall
improvement in the intellectual function, at any rate changes in the cognitive
structure. For this purpose, and also with a view to the possibility of measuring
fluctuations in the performance level from one test to another, a quantifiable
omnibus test battery was selected, consisting of: sub-tests from WAIS (Vocabulary,
Picture Completion, Digit Span, Digit Symbol, Block Design), Ruth Andersen's
learning and retention test for visual geometric figures, Word Pair test (three easy
and three difficult word pairs), the Stroop test, Word Mobilisation test, Enumera-
tions (weeks and months), the 100 minus 7 test and Learning Numbers. In con-
nection with the psychological testing, the patients' allo- and autoplastic orientation
was examined.

After the first investigation before the medication, the two actual memory tests
had to be omitted (the geometrical figures and Word Pairs), since they could be
successfully carried through in only 7 out of the 29 cases. Apart from this, a more
or less uniform body of data could be collected from all patients in all four
investigations.

After careful examination of the psychological rationale of the tests, the analysis
of the test data was arranged in a triad: Speed, Attention and Analytical Factor.

The data analyzed from the first psychological test suggested a satisfactory
randomization, since no statistically significant difference was found between the
test results from the two groups. This applied also for the orientation, the various
parts of the cognitive triad, the overall evaluation and the two "hold tests" employed: Vocabularly and Picture Completion.

The investigations were made and the data analyzed by the same clinical
psychologist, *i.e.* by one of the authors (H.K.).

*The Clinical Questionnaire*

Where possible, all 4 evaluations of a patient were done by one and the same
physician and nurse with a view to obtaining the greatest possible comparability.
In each individual investigation, the clinical questionnaire employed was that used
by Blessed et al. (1968).

Answers to the questionnaire were marked "1" in cases where the condition was
evaluated as normal (for the age), and with "0" where the condition was evaluated
as below normal. Each individual questionnaire was filled in without the in-
vestigator being able to make comparisons with previously filled-in questionnaires.

Based on the clinical data entered, an evaluation was made of the changes that
had taken place in the condition of each individual patient from the 1st to the
2nd, the 1st to the 3rd, and the 1st to the 4th investigation, within each of the 6
sub-groups in the questionnaire: changes in the performance of everyday activities,
habits, personality (including interests, impulses), orientation, memory and
concentration. In only 11 out of the 22 patients who carried through the investigation could all 18 evaluations be made. In the remaining 11 subjects, the clinical sections were so incomplete that only 8.7 evaluations could be made on an average. The evaluation was graded as follows: unchanged condition was marked 0, improvement was marked + rising to ++, and deterioration was marked — falling to ---.

The clinical neurological investigation was intended to be carried out in all patients before, during and after the period of treatment, and in cases of abnormal findings, the investigations were to be made by a neurologist, i.e. by one of the authors (H.P.).

Electroencephalography (EEG) was also to be done in all patients before, during and after the period of treatment. The EEG findings were evaluated by Munke Hertel Wulff, M.D., Chief Physician.

Pneumoencephalography (PEG) was to be done in good time before the commencement of the investigation in those patients who were able and willing to collaborate in this examination. The PEG was done at the Radiological Department of Copenhagen Municipal Hospital, and was evaluated by one of the authors (H.P.).

The PEG was thus the only examination which could not be carried through in all the patients; nor did we find it reasonable to encumber very old and weak patients with this test.

The PEG was therefore performed in 12 patients, the result being moderate diffuse atrophy in 7 cases, and severe diffuse atrophy in 5 cases.

Blood Examinations

Serum uric acid, serum creatinine and serum cholesterol, like the clinical tests, but with the exception of PEG, were to be determined immediately before commencement of the investigation, after treatment for 2 and 4 months, and 2 months after termination of the treatment.

Statistical Procedure

The statistical analysis is associated particularly with the results of the psychological investigations, since these were available for all patients and were directly suitable for numerical treatment. Owing to sense impairment in a few patients, certain individual tests could not be performed. The observations lacking were then replaced by a "missing plot technique", in which the results obtained by these patients in other tests were considered. A rank order was calculated for each test result (best result was given lowest rank value No. 1, etc.), both for the absolute scores at each of the four investigation occasions, and for changes in scores from one occasion to any other occasion.

For the rank values found, means were then calculated for the now comparable data as follows: Orientation, Speed, Attention and Analytical factor. Finally, they were all grouped together into one overall evaluation (Table 2).

RESULTS OF THE RNA INVESTIGATION

Psychological-Clinical Results

The mean rank values found for the overall evaluations of the psychological tests, expressing the state at a given time, were examined
Figure 2. *Average rank values for overall psychological evaluation comparing the 3rd against the 1st investigation.* • = RNA. ○ = placebo.

graphically, the results for two different test occasions (a total of 6 possible occasions) being plotted. The records showed that from one occasion to the next, a pronounced stability was found in the mutual rank placing, which must be considered to express both the high degree of reliability of the psychological tests, and the fact that no great changes took place in the condition of the patients, by comparison with the fairly great differences between them.

Figure 2 shows this state of affairs by comparing the 1st and 3rd tests (selected at random among the 6 possibilities).

The alcoholics (4 from the placebo and 3 from the RNA group) did not show signs of being different from the group of diagnosed senile patients (7 from the placebo group and 6 from the RNA group) with respect to changes from one investigation to the next. There was no tendency for the rather small changes in the mutual sequence of the patients to differ between the RNA group and the placebo group.
The mean values found for the change in score from one occasion to another were examined by a Mann-Whitney u-test (Wilcoxon’s two sample test), whereby the RNA group was compared with the placebo group. The results are shown in Table 2.

As is seen, no significant values have been found either in the functions already specified or in the overall evaluation. From the observations made, therefore, the possibility cannot be excluded that the RNA group and the placebo group are identical. However, on closer study of Table 2 (see the stippled area), it must be admitted that considering the limited extent of the material, the results do not exclude the possibility that the RNA treatment may have had a certain effect. If this be the case, the effect seems to set in early for the basal psychological functions of Speed and Attention, and then gradually diminish. In the case of the higher cortical functions, the Analytical factor, this possible effect sets in only after a longer period of medication, and it seems to persist after the withdrawal of the treatment. These tendencies are no more pronounced in the one or the other of the two main diagnostic groups.

In a very few cases, the clinical evaluation pointed to a distinct but transient change in a patient’s condition, either for the better or for the worse. However, there was no evidence that it was the patients from the RNA group in particular who showed improvement. There was no agreement between the clinical evaluation and the results of the psychological tests.

**Neurological Evaluation**

Two cases showed tremor, whereas one patient had a Parkinson’s syndrome. One patient had a polyneuropathy, and 2 patients had

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**Table 2. Analysis of the average rank values for the change in the score for psychological part functions.** Mann-Whitney’s u-test (Wilcoxon’s two sample test) for evaluation of the difference between the RNA group and the placebo group. U-values less than -1.64 are significant at the 5 per cent level (one-sided test).

<table>
<thead>
<tr>
<th>Part function</th>
<th>Changes between tests No.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2-1</td>
</tr>
<tr>
<td>Orientation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Speed</td>
<td></td>
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<tr>
<td></td>
<td>-1.45</td>
</tr>
<tr>
<td>Attention</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.06</td>
</tr>
<tr>
<td>Analytical factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>Overall evaluation</td>
<td></td>
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<td></td>
<td>-0.92</td>
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hyperkinesia. In one patient with severe cerebral atrophy there were multiple neurological deficits. In no case was there any change in the neurological status during or following the treatment.

Results of the EEG Studies

The 10 patients in whom it proved possible to make the examination before, during and after the treatment period, showed the following results:

In 6 patients the EEG was unchanged in all 3 curves, but in only one patient were the curve quite normal.

In 2 patients who had received RNA, there was a change in the curves. In one of these 2 patients the EEG was diffusely abnormal before and after the administration, but slightly abnormal focally during the administration. In the other patient, the EEG became more abnormal during RNA, and this lasted after termination of the administration.

In 2 patients, who had also received RNA, the results were difficult to correlate. In one of these 2 patients, the EEG was perhaps slightly more abnormal in the last curve after the RNA administration. In the other patient, the 2nd and 3rd curves were doubtfully more abnormal focally.

The variations in EEG can hardly be related to the ingestion of RNA. Moderate variations, such as those mentioned here, can always occur in abnormal EEG's. This holds also for focal abnormalities, such as those often seen in elderly subjects, and which may be vascularly determined.

Serum Changes During the Investigation

The serum studies showed normal values in the course of the entire experiment, with the exception of rises in serum uric acid, which was found elevated 2 and 4 months after the commencement of the investigation in all patients who had received hydrolyzed RNA, and only in these patients. The serum uric acid values dropped again to normal at an examination 2 months after the termination of the RNA administration. This finding must undoubtedly be taken to express absorption of the hydrolyzed RNA.

Serum cholesterol was found slightly elevated in only one patient during the administration of RNA, at the termination of this, and 6 months later, whereas previously it had been high normal, but this may presumably be due to chance.

In the patient who had diabetes mellitus, the blood sugar values fell
during the treatment with RNA, and the antidiabetic treatment had to be stopped; this treatment in fact became superfluous, since the blood sugar values remained stable at a good level. After cessation of the RNA administration, the blood sugar values rose again, and antidiabetic treatment had to be resumed.

**Side Effects During the Test**

Side effects in those patients who had received hydrolyzed RNA consisted especially of diarrhoea, which occurred particularly among the oldest patients. As a rule the tendency to diarrhoea could easily be counteracted by drugs, except in 2 patients, one of whom had to be excluded from the test owing to diarrhoea, whereas it was only necessary to curtail the investigation for 10 days in the case of the other patient, who has been included.

In one patient, who had had joint pain previously, periodic joint pain occurred also during the investigation, but this did not necessitate any interruption in this.

Another patient had an attack of arthritis urica during the investigation; this complaint was effectively treated with butazolidine, and this patient was able to carry through the investigation.

**DISCUSSION**

Previous investigations into oral administration of ribonucleic acid or its derivates have provided grounds for believing that they have some effect on dementia, and animal tests have shown that even orally administered ribonucleic acid derivates are taken up in the brain cells. We therefore decided to test the effect of orally administered hydrolyzed ribonucleic acid to patients with slight to moderately severe dementia (of moderate degree). On the one hand, the patients should not suffer from a degree of dementia which would exclude them from collaborating in an extensive psychological investigation, and on the other hand they should be without any physical complaints which might involve changes in their condition during the test period, which extended over 6 months. The comparatively large number of patients excluded must be considered in the light of these conditions. As the conditions had to be adhered to, the number of patients was restricted, but on the other hand the patients could be given intensive examination and treatment. Since previous investigations seemed to suggest a relationship between dosage and effect, doses were employed of about 20 g hydrolyzed ribonucleic acid daily, although this involved a tendency to diarrhoea. Evidence that the hydrolyzed ribonucleic acid
was absorbed was provided by the observation of a considerable increase in serum uric acid, but only in those cases where RNA was administered.

Previous investigations have examined mainly the possible effect of a given RNA treatment on memory in a patient suffering from dementia, in particular with respect to the effect on patients with arteriosclerotic dementia. Unlike the present investigation, these previous investigations have not attempted to elucidate possible changes in the formal organization and structure of thinking. The results suggest that after treatment with RNA for 4 months there is a possible effect, which starts relatively rapidly in the case of basal, underlying mental functions, as for instance speed and attention functions, and not until later is this effect seen in the case of the more complicated, higher forms of mental activity. In future research, it will be of great interest, if a somewhat larger patient material is used, to investigate with a similar experimental design whether RNA has the effect mentioned, in general and particularly for certain patient categories, or only for such categories. The modest patient material and the predominance of patients with senile dementia in the present investigation are decidedly the greatest shortcomings in the elucidation of these circumstances.

CONCLUSION

Allowing for the small fluctuations that are to be expected in the "condition" and collaboration of the patients, neither the psychological nor the clinical overall evaluation was found to provide evidence for an improvement in dementia following administration of RNA. On the other hand, there was a tendency towards rapid improvement in the case of the basal psychological functions, whereas this tendency only appeared after a longer period of time in the case of the higher cortical functions. A more certain evaluation of this conclusion, however, will demand a larger and more differentiated patient material. The present investigation does not justify attempting the administration of RNA as therapy for dementia.

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