ERRATA

In the paper by Demetrios G. Magiros entitled “A Method for Defining Principal Modes of Nonlinear Systems Utilizing Infinite Determinants,” which appeared in volume 46, number 12, pages 1608–1611, the author wishes to make the following corrections:

1. On page 1609, line 2, substitute “anchor spring” for “coupling,” so that the phrase reads “with nonlinear anchor spring.”

2. On page 1609, section 3, lines 1 and 2, for the phrases “$K_1$ and $K_3$ the spring constants of the anchor springs, $K_2 = K_2 + \mu z^2$ that of the coupling,” substitute “$K_2$ and $K_3$ the spring constants of the coupling and the second anchor spring, $\bar{K}_1 = K_1 + \mu z^2$ that of the first anchor spring, . . .”

In the paper entitled “On the Mutagenic Effect of Alkylating Agents,” by Ekkehard Bautz and Ernst Freese, which appeared in volume 46, number 12, pages 1585–1594, the authors wish to have the sentence beginning on line 2 of page 1591 read as follows: “The smallest amount of 7-ethyladenine that could have been detected is 0.04 micromoles,” instead of 0.4 micromoles.

In the paper entitled “Regulation by Coliphage Lambda of the Expression of the Capacity to Synthesize a Sequence of Host Enzymes,” by Michael B. Yarmolinsky and Herbert Wiesmeyer, which appeared in volume 46, number 12, pages 1626–1645, the authors wish to make the following corrections:

Page 1636, line 15, for reference 44 read 54; page 1640, line 3 from bottom, for reference 44 read 54; page 1644, line 8, for reference 11 read 19; and page 1644, line 9, for reference 44 read 45.
the ATP-ADP exchange reaction of oxidative phosphorylation in digitonin fragments of mitochondria. This factor is postulated to be either a "cementing" protein or an intermediate enzyme of the energy-coupling mechanism linking the terminal enzymatic step catalyzing the ATP-ADP exchange, which is DNP-insensitive, with a preceding, DNP-sensitive reaction. Preparations of M-factor also show C-factor activity, i.e., they promote contraction of glutathione-swollen mitochondria by ATP.

* Supported by grants from the National Institutes of Health, the National Science Foundation, the Nutrition Foundation, Inc., and the Whitehall Foundation.
† Senior Research Fellow, National Institutes of Health.
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6 Wadkins, C. L., Fed. Proc., 18, 346 (1959), and investigations to be published.
7 Wadkins, C. L., and A. L. Lehninger, these PROCEEDINGS, 46, 1576 (1960).

ON THE MUTAGENIC EFFECT OF ALKYLATING AGENTS*

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Communicated by Sewall Wright, October 17, 1960

Many alkylating agents have been found to exert a mutagenic effect on a great variety of organisms.1 These agents vary in their composition and structure from simple to very complex molecules, which will be classified here in two main groups, the mono- and polyfunctional agents.

Although, for the most part, polyfunctional agents have been used for mutation and cancer therapeutic studies investigations for a molecular explanation of mutation induction with monofunctional agents are more promising, since their reactions are more accessible to chemical analysis. In such investigations it has been found already that methyl groups attack guanine and adenine on their 7-N-position,2 and also adenine on its 1- and 3-position,3 whereas ethylation has been found to esterify primary phosphate groups in DNA.2, 4

One year ago, it was reported that ethylmethanesulfate causes high mutation rates in T4 phages.5 Therefore, investigation of the mechanism of chemically induced mutations by alkylating agents on bacteriophage T4 seemed to us most promising since the rII region of this phage is the most extensively analyzed genetic system6 and because of the experiences acquired in our laboratories during studies on reversion induction with nitrous acid.7
This paper is concerned with inactivation and with forward and reverse mutation studies on T4 phage by the action of methylating, ethylating, and propylating agents. The mutagenic specificity of ethylethanesulfonate was determined, and, in agreement with the biological results, it will be shown chemically that the main mutagenic effect of ethylation is probably due to the elimination of 7-ethylguanine from DNA.

Materials and Methods.—Phages: T4 wild type (Benzer) and rII-type mutants derived from it.

Bacteria: B = E. coli B (American); K = K 12 (λ). MMS, EES, and PPS were prepared from the corresponding alkylidades and silver sulfate according to the procedure of Kurbatow9 and purified by distillation. (MMS = methylmethanesulfonate, EES = ethylethanesulfonate, PPS = propylpropanesulfonate.)

Inactivation of T4 phage: Various amounts of the three alkylating agents were added to 10 cc 0.5 molar sodium acetate buffer, pH 6.6. The mixtures were shaken vigorously, and after 2 min, 0.1-ml samples of T4 wild type phages were added. After various times of treatment at 37°C, 0.1-ml aliquots were diluted out into 10 ml nutrient broth containing 0.2 ml 50 per cent sodium thiosulfate, incubated for 24 hours at 37°C, and plated on bacteria B. In general, the procedure described by Loveless6 was followed.

Mutation rates were measured, using the same conditions.

Reversion studies: The phage stocks listed in Table 1 were tested by treating the phages with 0.11 M EES in 0.5 M sodium acetate buffer, pH 6.6, at 37°C for 4, 8, and 12 minutes followed by incubation for 24 hours at 37°C and were plated at a concentration of about 5 x 10^6 phage particles on each plate together with 5 x 10^6 bacteria K. This gave a multiplicity of plating 0.1, which was sufficient to show no significant amounts of multiplicity reactivation. The induction of reverse mutations was also tested by preabsorption of the treated phages on bacteria B which had been killed by UV to about 10^-3 survivals. The phages were added with a multiplicity of approximately 0.1, incubated for 8 min, and then plated on bacteria K.

Chemical analysis: Reactions on deoxyguanosine and DNA were performed in 1.0 M sodium acetate buffer, pH 6.6, at 37°C for 24 hours with 0.13 M dimethyl- and diethylsulfate. Using 10 mg deoxyguanosine in 10 ml buffer, the reaction mixture was applied at pH 10 to a Dowex I formate form anion exchange column. A fraction collector (Gilson Medical Electronics) with UV recording system at 254 mμ was employed to detect the absorbed products after elution. Spectra of the absorbing products were taken in a Zeiss spectrophotometer and compared with spectra of 7-methyladenine and 7-ethylguanine (kindly supplied by Dr. Hutchins of the Wellcome Research Laboratories). For the reaction of diethylsulfate with DNA, 0.5 gm of highly polymerized Herring sperm DNA (Sigma Chem. Co.) was suspended in 50 ml 1 M sodium acetate buffer, pH 6.6, and was dialyzed twice at 5°C against 1 liter of 1 M sodium acetate buffer for 24 hours until no further absorbing material could be detected in the dialyzate. To the DNA 0.8 ml of diethylsulfate was added, and the mixture was allowed to react for 24 hours at 37°C. The reaction mixture was then dialyzed against 500 cc 0.01 M saline for 24 hours at 37°C to yield approximately 90 percent of the hydrolyzed purines in the dialyzate. To the dialyzate 0.2 gm of sodium thiosulfate was added to destroy a possible excess of unreacted diethylsulfate, and the dialyzate was concentrated to about 20 ml by evaporation at 30°C in vacuo. The pH was raised to 10 with ammonia and this solution was applied to the same column as described above.

Results.—Inactivation of Phage T4 was measured after treatment with MMS, EES, and PPS followed by incubation at 37°C for 24 hours in the presence of 1% sodium thiosulfate. A plot of the logarithm of phage survivors with respect to time of treatment usually does not give a straight line. At high concentrations of the agent, the negative slope increases, as already observed by Loveless6 with similar alkylating agents, but at low concentrations, the negative slope decreases.

Both forward and reverse mutation rates are much larger with EES than with the other two agents (Figs. 1 and 2). It was found that the mutagen EES gave a 7- to 8-fold increase in the forward mutation frequency (r+ → r) over the spontaneous
level, at 1 per cent survivals, whereas MMS and PPS showed only a slight increase. Similarly the induction of reverse mutation by EES shows a 30-fold increase over the spontaneous level compared with the corresponding induction values of 1.6 for MMS and 2.4 for PPS. Absolute induction rates, the probability of reversion induction per lethal hit and phage—of 0.008 for MMS, 0.165 for EES, and 0.012 for PPS were calculated by a method described elsewhere.6

In another EES inactivation experiment on T4 wild type phage, using different times of incubation before plating, it could be shown (Fig. 3) that the inactivation slope increases with increasing time of incubation after treatment, whereas a control sample of phages without EES treatment showed no inactivation under these conditions. This gives strong evidence that the EES already attached to the phage causes the further inactivation upon incubation. It shows also that dilution into 1 per cent sodium thiosulfate stops further alkylation, since all slopes can be extrapolated to about 1. Plating for mutation rates with these incubation times was carried out in the same manner as described above. Plating without incubation gave about half of the number of clear r-/+ mottled plaques given by plating after 24 hours (Fig. 1). Moreover, mostly mottled plaques were ob-

![Graph](image-url)
served for this direct plating after treatment while further incubation enhanced the frequency of both r and mottled plaques.

Reversion studies: EES, the most efficient of the three agents, was chosen for reversion tests on two classes of rII-type mutants—those induced with 5-bromouracil (N mutants) and those with 2-aminopurine (AP mutants). The results are given in Table 1. The mutants seem to fall into two distinct groups: those capable of being reverted by EES (AP mutants) and those showing little or no response to the agent (N mutants). By plating the inactivated phages directly on bacteria K, for the AP mutants the values of the total mutation induction, \( \alpha/\beta \), vary between \( 4.6 \times 10^{-6} \) for AP 12 and \( 0.065 \times 10^{-6} \) for AP 70. All the N mutants except N 29 and N 19 showed no detectable rate of induction, which is indicated by \( \alpha/\beta < 0.01 \). N 29 happens to be a very reactive mutant and also shows the highest induction with nitrous acid among the N mutants,\(^7\) and is strongly inducible by AP.\(^9\)

Each of five mutant strains of the N and the AP type was tested with preadsorption on bacteria B, and the results are given in the last row of Table 1. The AP

![Graph showing reversion rates.](image-url)

**Fig. 72.—Reversion rates.** Back-mutated wild-type plaques per \( 10^6 \) viable r-mutants of AP 156. MMS = 0.04 M, PPS = 0.07 M, EES = 0.11 M. The value \( \alpha/\beta \) is the increase of revertants per viable phage over the spontaneous level at \( \ln B/B_o = 1 \).
FIG. 3.—Inactivation curves of T4 wild type phages treated with 0.11 M EES, followed by incubation. × = 0 hours’ incubation; 0 = 12 hours’ incubation; + = 24 hours’ incubation.

mutants show an even higher increase in the reversion rates, whereas the N mutants show no effect. Although the increase of reversion rates for the AP mutants on the average is 10 times as high as without preadsorption, the accuracy of these values is much lower, because the increase of the spontaneous background is also 10 to 100 times higher than without preadsorption.

Chemical Analysis.—Alkylation of deoxyguanosine: Samples of 5 mg each of deoxyguanosine were incubated with 0.15 M dimethyl- and diethylsulfate for 24 hours at 37° at pH 6.6 and applied to the column. The deoxyguanosine alkylated
at the 7-N-position was not absorbed by the resin, because of its positive charge, and it could be collected in the first fractions. Elution with 0.2 M ammonium formate buffer pH 9.5 separated the 7-alkylguanine from the unreacted deoxyguanosine. UV analysis at pH 9.5 showed a \( \lambda \) max at 283 m\( \mu \) for both the 7-methyl- and the 7-ethylguanine. Assuming a molar extinction coefficient of \( 8 \times 10^{+3} \) at \( \lambda \) max for all guanine derivatives, the following amounts were detected:

\[
\begin{align*}
7\text{-ethyldeoxyguanosine} & : 0.08 \text{ mg} \\
7\text{-ethylguanine} & : 0.33 \text{ mg} \\
7\text{-methyldeoxyguanosine} & : 0.7 \text{ mg} \\
7\text{-methylguanine} & : 0.28 \text{ mg}
\end{align*}
\]

Comparing these ratios with each other, the hydrolysis of the ethylated deoxyguanosine appears 5 times as efficient as the hydrolysis of the methylated deoxyguanosine.

**Estimation of hydrolyzed purines from DNA:** A large sample of 0.5 DNA was treated with diethylsulfate as described above and was dialyzed, and the dialyzate was applied to the ion-exchange column. Elution with pH 9.5 ammonium formate buffer produced first nonabsorbed positively charged material, followed by a large peak, identified as 7-ethylguanine. A peak of 7-ethyladenine, normally appearing among the first fractions after adjustment of the eluant to pH 8.5, could not be detected. Calculation of the total amount of eluted 7-ethylguanine gave an optical density at pH 9.5 of 0.19 per 100 ml. Compared with the molar extinction coefficient of \( 8 \times 10^{+3} \), 2.2 micromoles = 0.4 mg of 7-ethylguanine were recovered. Starting with 0.5 gm DNA, which contains about 60 mg guanine, the yield was

**TABLE 1**

**Induction of Reversions by EES**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>( K_{o}/B_{o} ) in ( 10^{-4} )</th>
<th>Without Preadsorption</th>
<th>With Preadsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_{o}/B_{o} ) in ( 10^{-4} )</td>
<td>( I )</td>
<td>( a/\beta ) in ( 10^{-4} )</td>
<td>( a/\beta ) in ( 10^{-4} )</td>
</tr>
<tr>
<td>AP 12</td>
<td>0.85</td>
<td>5.4</td>
<td>4.6</td>
</tr>
<tr>
<td>AP 41</td>
<td>0.37</td>
<td>6.2</td>
<td>2.3</td>
</tr>
<tr>
<td>AP 275</td>
<td>1.07</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>AP 114</td>
<td>0.49</td>
<td>2.8</td>
<td>1.35</td>
</tr>
<tr>
<td>AP 72</td>
<td>0.76</td>
<td>1.6</td>
<td>1.24</td>
</tr>
<tr>
<td>AP 83</td>
<td>0.006</td>
<td>43.3</td>
<td>0.26</td>
</tr>
<tr>
<td>AP 156</td>
<td>0.005</td>
<td>31.1</td>
<td>0.165</td>
</tr>
<tr>
<td>AP 70</td>
<td>0.028</td>
<td>2.3</td>
<td>0.065</td>
</tr>
<tr>
<td>N 29</td>
<td>0.04</td>
<td>1.7</td>
<td>0.03</td>
</tr>
<tr>
<td>N 19</td>
<td>0.07</td>
<td>1.2</td>
<td>0.016</td>
</tr>
<tr>
<td>N 24</td>
<td>0.32</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AP 61</td>
<td>0.18</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N 31</td>
<td>0.01</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N 12</td>
<td>0.03</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N 101</td>
<td>0.002</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N 17</td>
<td>0.02</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N 7</td>
<td>0.03</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N 21</td>
<td>0.006</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>r 114</td>
<td>0.20</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>r 131</td>
<td>0.43</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

For each mutant, the spontaneous reversion rates \( K_{o}/B_{o} \), the induction factor \( I = K/B : K_{o}/B_{o} \), and the absolute rates of reversion induction \( a/\beta \) are given per lethal hit for direct plating on bacteria \( K \). \( "K" = \) titer on bacteria \( K \), \( "B" = \) titer on bacteria \( B \). The reversion rates \( a/\beta \) obtained after preadsorption of the phages on bacteria \( B \) are given in the last row of Table 1. \( a/\beta < \) means no detectable amount of induction. The mutant strain AP 61 was previously found to belong to the N-mutant group.7,9
about 0.6 per cent depurination during 24 hours treatment, followed by 24 hours incubation while dialyzing. The smallest amount of 7-ethyladenine that could have been detected is 0.4 micromoles. Thus we conclude that the formation of 7-ethyladenine was at least 50 times less frequent than that of 7-ethylguanine.

Discussion.—It has been found\textsuperscript{9} that bromouracil-induced mutants show higher induction of reverse mutations by 2-aminopurine than by bromodeoxyuridine and vice versa. These observations suggested that most mutants induced by one of the two agents arose by a change of an adenine-thymine (A-T) to a guanine-cytosine (G-C) base pair and that the mutants induced by the other agent arose by a change of a G-C to an A-T base pair. Nitrous acid was hoped to be a suitable agent to decide by reversion tests which one of the two base pairs was changed in the cases of AP mutants and of N mutants; but the small difference in reaction velocity of nitrous acid with the two base pairs\textsuperscript{10, 11} did not allow a definite measurement of the specificity of its mutagenic action.

Ethylation, on the other hand, causes a rather specific mutagenic effect, as was found by Kriegl,\textsuperscript{12} who, using T4 phages, demonstrated that mutants produced by the action of ethylmethanesulfonate (EMS) cannot be induced to revert by the same agent. The results in Table 1 indicate that the specificity of EES turns out to be sufficient to differentiate between the AP mutant having the lowest reversion rate by EES and the highest revertible N mutant. The result of these reversion

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Proposed mechanism of depurination by ethylation. I and II: mesomeric states of 7-ethyldeoxyguanosine. IV: removal of R- caused by state II. R- = deoxyriboyl.}
\end{figure}
studies demonstrates a high specificity of EES which probably can be used to analyze the base-pair change which has been induced by other mutagens. In order to obtain quantitative chemical evidence for the biological specificity of EES, we had to find out first which one of the different reactions of alkylating agents on DNA could be made responsible for the induction of mutation.

Reiner and Zamenhof, treating transforming DNA with dimethylsulfate, have shown that 7-methylguanine and 7-methyladenine were two of several products obtained after hydrolysis. Purines were found to be much more reactive than the pyrimidines. Using diethylsulfate, they found only esterification with primary phosphate groups, which also was reported by Alexander and Stacey. Recently, Brooks and Lawley reported methylation of adenylic acid to give, in addition, 1-methyl- and 3-methyladenine after hydrolysis.

The reaction of ethylating agents with primary phosphate groups in DNA seems not to be responsible for the induction of point mutations, which we consider here, since the subsequent effect could be only a break in the phosphate-sugar backbone similar to the decay of incorporated $^{32}$P which has not yet been found to cause mutations. Moreover, this reaction would not give rise to single-base-pair changes but larger alterations in the genome. Hence, we conclude that one of the reactions with the DNA bases themselves must be responsible for the induction of point mutations.

Regarding the reaction on the 3-N-position of adenine, alkylation would yield an uncharged 3-alkyl-6-iminopurine as product, which could behave like guanine when pairing and simultaneously cause a base-pair change in the DNA molecule. The cause of mutation might thus be answered, but not the question why methylation causes much less mutation than does ethylation, because the reaction of methyl groups with the ring nitrogens in purines is known to go much faster than ethylation. These contradictions made us assume that there must be a further reaction within the alkylated DNA. In our experiments, ethylation of DNA for 24 hours at 37°C, followed by further incubation for 24 hours at the same temperature and neutral pH, yielded 0.6 per cent 7-ethylguanine but no detectable amount of ethylated adenine. Methylation, in contrast, is less effective in breaking the N-glycosidic bond and also is less mutagenic (see Figs. 1 and 2). These observations can be explained by the following theoretical considerations.

Ethylation at the 7-N-position of guanine uses the free electron pair of the 7-N-atom of the imidazole ring to bind the ethyl group, causing a full positive charge as shown in Figure 4, structure I. This structure stays in resonance with structure II, yielding a resonance hybrid. The positive charge, distributed to both N-atoms of the imidazole ring, greatly weakens both the 7-N-ethyl and the 9-N-sugar bonds, so that hydrolysis takes place even at room temperature and neutral pH. Figure 4 shows the following simplified and idealized mechanism. The imidazole ring, ethylated on its 7-N-atom, has gained a positive charge, which is distributed over the ring, yielding to the two extremal states I and II. State I enables the removal of the C$_2$H$_5^+$ group from the guanine nucleotide, yielding back the unchanged DNA. Structure II, however, causes the eventual hydrolysis of the N-glycosidic bond, thus removing guanine and changing the genetic properties of its DNA. Methylation also induces a positive charge in the imidazole ring, but the methyl group is able to carry a part of the charge because of its
electron-inducing capacity; i.e., methyl groups are able to participate in the resonance of the imidazole ring, which simultaneously causes less positive charge at the 9-N-atom and also less hydrolysis of the N-sugar bond.

Some further biological results, which are given in Figure 3, support the theory above. From the curves, it can be seen that the titer of the inactivated phages shows a further decrease after incubation at 12 and 24 hours at 37°C, depending on the number of ethyl groups the phages originally had received. This behavior would be expected, since, during incubation, more and more ethylated guanines get removed from DNA.

Once a DNA molecule, missing a guanine, duplicates, one would expect the incorporation of either a purine or pyrimidine into the new complementary strand, giving rise to both transversions and transitions. It was observed that depurination caused by high temperature and by low pH produces mutations and that these mutations have been produced mainly by transitions.

From this point of view, we can say that the depurination by EES causes also mutation by substitution of adenine for the guanine lost under the action of ethyl groups. Nevertheless, there might be also a certain amount of mutations due to transversions, which could not have been detected in our reversion studies.

If we compare the high inducibility of the AP mutants and the inert behavior of the N mutants with the chemical results, we have evidence for the following conclusions: Following the reaction of ethylating agents on DNA.

1. Liberation of 7-ethylguanine occurs at least 50 times more frequently than of 7-ethyladenine.
2. From our experimental results and the theoretical considerations, we can strongly suggest that the removal of guanine has to be the main cause of mutation induction by ethylethanesulfonate.
3. The tested mutants induced by the two base analogues, 2-aminopurine and 5-bromouracil, arose by the following base pair changes:

\[
\begin{align*}
A & \to G \\
T & \to C
\end{align*}
\]

for most mutants induced by 2-aminopurine

\[
\begin{align*}
G & \to A \\
C & \to T
\end{align*}
\]

for most mutants induced by 5-bromouracil

This latter conclusion is made with the assumption that our reverse mutations are back mutations to the original T4 wild type, which will be discussed in a subsequent paper.

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SOME SINGULAR MIXED PROBLEMS*

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COMMUNICATED BY EINAR HILLE, OCTOBER 14, 1960

1. Introduction.—In this note we shall consider the differential operator

$$L^k = \partial^2 / \partial t^2 + [k/t + \alpha(t)] \partial / \partial t + \gamma(t),$$

where $k$ is complex with $\text{Re}(k) > 0$, $\alpha \in C'(0, b)$, and $\gamma \in C_0(0, b)$. (The case $k = 0$ may be treated similarly.) $C^m(0, b)$ is the space of $m$-times continuously differentiable functions on $[0, b]$ and the number $b < \infty$ is arbitrary but fixed. Let $\mathcal{C}$ be a separable Hilbert space, and $\Lambda$ an arbitrary self-adjoint operator, semibounded below, with domain $D(\Lambda)$ (dense in $\mathcal{C}$). We will consider the problem

$$(L^k + \Lambda) \omega^k(t) = 0; \ \omega^k(\tau) = T \in D(\Lambda); \ \partial / \partial t \omega^k(\tau) = 0, \quad (1)$$

for $t \rightarrow \omega^k(t) \epsilon \mathcal{C} (\mathcal{C})$, $t \rightarrow \omega^k(t) \epsilon \mathcal{C} (D(\Lambda))$, and $0 \leq \tau \leq t \leq b$. $\mathcal{C} (\mathcal{C})$ is the space of $m$-times continuously differentiable functions with values in $\mathcal{C}$, and we assume here that $D(\Lambda)$ has the topology of the graph, under which it is a Hilbert space.

Since we are assuming of $\gamma(t)$ only that it be continuous, it is clear that the case of semi-bounded $\Lambda$ is equivalent to that of $\Lambda$ positive, which we shall suppose from now on.

Problems similar to (1) have been considered by J. L. Lions\textsuperscript{2, 3} and the author,\textsuperscript{4} while the case $\alpha = \gamma \equiv 0$ goes back to the Euler-Poisson-Darboux (EPD) equation which has been treated in particular by A. Weinstein\textsuperscript{5} in a long series of papers. More abstractly the EPD equation is discussed in refs. 1, 6, and 7. In the present note we shall apply a method used in ref. 4 (where $\Lambda$ is a differential operator with constant coefficients and $\mathcal{C}$ is replaced by $\mathcal{S}'$ etc.) to the problem (1). This method is based on techniques of L. Schwartz.\textsuperscript{8} Our results refine in some respects, for $\text{Re}(k) > 0$, those of Lions.\textsuperscript{7, 8}

Using now the von Neumann spectral decomposition theorem (see for example ref. 9), we know there is a measure $\nu$, a $\sigma$-measurable family of Hilbert spaces $\xi \rightarrow H(\xi)$, and an isometric isomorphism $\theta$: $\mathcal{C} \rightarrow H = \int \theta H(\xi) d\nu(\xi)$, such that $\Lambda$ is diagonalized, with $TeD(\Lambda)$ if and only if $\int_0^\infty \xi_0^2 \| \theta T \|^2_{H(\xi)} d\nu(\xi) < \infty$. The problem (1) is then equivalent to seeking $\theta \omega^k(t, \xi) \in D(\Lambda)$ such that

$$(L^k + \xi) \theta \omega^k(t, \xi) = 0; \ \theta \omega^k(\tau, \xi) = T \theta D(\Lambda); \ \partial / \partial \xi \theta \omega^k(\tau, \xi) = 0, \quad (2)$$