29 November 1974, Volume 186, Number 4166 **SCIENCE**

# Methanol as a Gasoline Extender: A Critique

Claimed benefits for methanol-gasoline blends may only be significant for older, rich-operating cars.

E. E. Wigg

The recent sharp increase in crude oil prices, coupled with the current drive toward energy independence in the United States, has suddenly made the development of processes for the large-scale production of synthetic fuels from domestic coal of crucial importance. Methanol, which is one such fuel that can be derived from coal, has projected manufacturing costs (*1*) that, on an energy-equivalent basis, are roughly comparable to the two other likely coal conversion products: synthetic gasoline and substitute natural gas. In addition to the possibility of producing methanol from coal, it has been suggested that methanol might be produced from the currently flared Middle East natural gas (*2*). These two potential sources could result in large quantities of methanol becoming available for fuel usage within the next few years.

One potential use for methanol is as a motor fuel, either in the "pure" form or as a gasoline blending component (*3*). The possible use of pure methanol is complicated by the lack of interchangeability between methanol and gasoline; the two fuels require very different carburetor settings. For vehicles used in captive fleet operations methanol has definite possibilities. However, this would only have a small impact on the overall consumption of motor fuel. Widespread use of pure methanol as a motor fuel is clearly a long-range proposition.

A detailed discussion of the use of pure methanol as a motor fuel is beyond the scope of this article; an evaluation of the use of methanol as an extender of gasoline is of greater interest because this use of methanol could have a greater impact near term. The advocates of this application for methanol point to better fuel economy, lower exhaust emissions, and better performance as compelling reasons for including methanol in motor gasoline at the earliest possible date. Fuel economy and lower emissions stand out as being particularly important factors for consideration since they are receiving a great deal of attention in today's climate of energy shortages and environmental awareness. While these claimed advantages for methanol use appear to be valid in certain cases, recent experiments conducted at this laboratory indicate that they are only significant for the older cars with richer carburetion which are rapidly disappearing from the roads. The main purpose of this article is to put fuel economy and emissions issues, as they relate to methanol-gasoline blends, into proper perspective. A secondary purpose is to draw attention to some product quality considerations that are associated with the use of methanol and have sometimes been overlooked.

## Product Quality Aspects

Before a new product can be put on the market, the manufacturer must be assured that its use will result in a high degree of customer satisfaction. In the case of gasoline, four areas stand out as being important: octane quality (good octane quality is necessary to prevent engine knock), fuel stability, vapor pressure characteristics, and carburetor performance. The use of methanol in gasoline would, in effect, result in a new product with potential impacts on all these areas. Methanol could have a positive effect on octane quality, but it could also introduce some potentially severe problems related to fuel stability, vapor pressure, and carburetion.

The octane blending values (OBV's) for methanol compare favorably with those of some other hydrocarbon blending components. The octane number of a fuel, which represents its rating for antiknock properties, is calculated by multiplying the OBV of each component by its volumetric concentration and summing the results for all the components in the blend. Octane quality can be important because higher octane fuels allow the use of higher compression ratio engines, which in turn give better efficiency.

There are two scales commonly used to define octane quality, research octane number (RON) and motor octane number (MON). Both the RON and MON are determined with test engines having variable compression ratios; the conditions used for determining MON are somewhat more severe than those used for determining RON, and since MON more closely correlates with the requirements of late-model cars, it is the more critical value (*4*). The OBV's for methanol, and several hydrocarbons having good octane ratings, are shown in Table 1.

The OBV of a fuel component is somewhat dependent on the blend to which it is added. The addition of 15 percent methanol to most motor gasolines would increase the RON significantly and increase the MON slightly. For example, a typical unleaded gasoline might have a RON of 93 and a

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MON of 84. If one used the OBV's for methanol given in Table 1, the addition of 15 percent methanol to this blend would result in RON and MON increasing to 97 and 85, respectively. Data (*5*) show that if the octane number were increased by one, the compression ratio of the engine could be increased by about 4 percent, for example, from 8 to 8.3. According to the relationship between compression ratio and fuel economy given by Corner and Cunningham (*5*) this translates into a potential saving in fuel of less than 2 percent.

One of the most serious problems associated with the use of methanol-gasoline mixtures would be phase separation, which relates to the question of fuel stability. Because of methanol's polar character, its solubility in gasoline is limited. Its solubility is greater in fuels with higher concentrations of aromatic hydrocarbons. For typical gasolines, these solubility constraints place the maximum blending concentration at about 15 percent and, if the fuel is kept under anhydrous conditions, no problems should be encountered. However, the phase separation problem becomes critical when the blend contacts even very small quantities of water (*6*). Rapid phase separation occurs, with the polar water-methanol phase settling out at the bottom. Gasoline containing methanol would therefore have to be stored and distributed under anhydrous conditions, and this would be both difficult and expensive in practical terms. Alternatively, it might be possible to blend the alcohol with the gasoline at the time of sale, right at the pump, but this would also be expensive and would require additional equipment. Furthermore, this procedure would not rule out the less likely occurrence of phase separation in the car tank. If this were to occur, the water-methanol layer at the bottom of the tank would be fed to the carburetor and the resulting air-fuel mixture would be beyond the lean flammability limit and would render the car inoperative.

It has been suggested that if, in addition to methanol, other alcohols of higher molecular weight were added to the gasoline, the severity of the problem would be reduced. However, tests conducted in our laboratories have shown that phase separation still occurs in the presence of less than 1 percent water. One other possible option would be the addition of another component to increase the solubility of the water-methanol phase. However, studies (*7*) indicate that the amount of additive required to effect a significant change would be too high to be practical. Further study in this area may prove valuable.

Another critical problem associated with the use of methanol is the possibility of vapor lock occurring in the vehicle fuel system. For example, fuel vapor may get trapped in the car's fuel pump and thus prevent it from operating normally. Vapor lock is dependent on temperature and the volatility of the fuel. The addition of methanol to gasoline considerably increases the volatility of the fuel. As was the case with phase separation, this problem also stems from the fact that methanol is a polar molecule, while gasoline is essentially nonpolar. In this case, the hydrogen bonds between the methanol molecules, which lead to the anomalously high boiling point of the pure liquid, are a less significant factor in the methanol-gasoline blends, since the alcohol molecules have been diluted and separated by the nonpolar gasoline. Consequently, methanol tends to behave more like one would predict from its molecular weight, which is less than that of propane. The addition of small amounts of methanol to gasoline produces quite large increases in vapor pressure, such increases being independent of the volatility of the base gasoline. In Fig. 1, the increase in the Reid vapor pressure (RVP) in a gasoline mixture is plotted against methanol concentration. The RVP is a commonly used measure of gasoline vapor pressure. As the methanol concentration increases for 0 to about 3 percent, there is a sharp increase in the RVP followed by a leveling off of the relationship. This leveling off at higher methanol concentrations suggests that, as methanol dilution decreases, hydrogen bonding between methanol molecules becomes more important.

Table 1. Octane blending values (MON, motor octane number; RON, research octane number) for methanol and some representative hydrocarbons.

| Compound | MON | RON |
|---|---|---|
| Methanol | 91 | 120 |
| *n*-Butane | 89 | 95 |
| Isopentane | 88 | 94 |
| Isooctane | 100 | 100 |
| Toluene | 96 | 110 |
| *m*-Xylene | 99 | 115 |

Table 2. The characteristics of the two fuels used for the experiments.

| Property | Base fuel | Methanol blend |
|---|---|---|
| RON | 98.3 | 101.7 |
| MON | 86.8 | 87.5 |
| RVP (pounds per square inch) | 11.9 | 11.7 |
| Percent distilled at | | |
| 70°C | 30 | 50 |
| 100°C | 48 | 53 |
| 150°C | 87 | 87 |
| Gravity (grams per milliliter) | 0.759 | 0.779 |

Table 3. Equivalence ratios for the 1967 and 1973 cars driven at various steady state speeds with the base fuel. The stoichiometric equivalence ratio is equal to 1.0, and the values greater than this indicate fuel-lean carburetion and lower values indicate rich carburetion.

| Speed (miles per hour) | Car model | |
|---|---|---|
| | 1967 | 1973 |
| Idle | 0.94 | 1.04 |
| 20 | 0.90 | 1.05 |
| 30 | 0.88 | 1.08 |
| 40 | 0.95 | 1.08 |
| 50 | 1.01 | 1.05 |

Table 4 Fuel economy for each of the three cars, measured over the 1975 federal test procedure, a nonrepetitive cyclic driving pattern of 11 miles with the engine initially at ambient temperature (20° to 30°C).

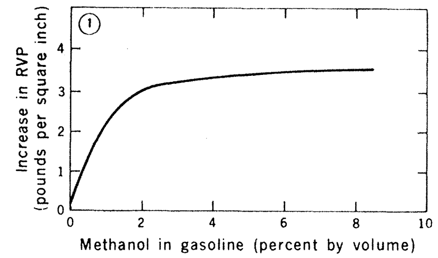
| Car | Fuel economy (miles per gallon)* | | Effect of methanol (%) | |
|---|---|---|---|---|
| | Base fuel | Methanol blend | Volume basis | Energy basis |
| 1967 | 14.3 | 14.4 | + 1 | + 8 |
| 1973 | 11.2 | 10.5 | − 6 | + 1 |
| "1977" | 11.5 | 10.9 | − 5 | + 2 |

* Data calculated from the weight of fuel consumed in a given run.

In addition to its influence on RVP, methanol has a profound effect on the overall distillation curve of the blend. Figure 2 shows curves for a typical gasoline and a 15 percent methanol–gasoline blend. The disproportionately large effect of methanol on the distillation curve for the methanol-gasoline blend results from the fact that methanol forms low boiling azeotropes with hydrocarbons—that is, the methanol depresses the boiling point of the mixture.

It has been found that, for gasoline, the occurrence of vapor lock can be satisfactorily predicted from the fuel volatility characteristics defined by RVP and the percentage of the fuel distilled at 70°C (*8*). Because the addition of 15 percent methanol causes a large increase in both these parameters (Figs. 1 and 2), vapor lock problems would be expected to occur with such a mixture. This problem, which needs further study, might require a reduction in the amounts of some of the low-molecular-weight hydrocarbon components of gasoline, such as butane and pentanes. In fact, if current gasoline vapor pressures were to be maintained, the use of 15 percent methanol blends would require the removal of all the butanes and a significant fraction of the pentanes, amounting to at least 10 percent of the gasoline volume. This means that methanol would become a substitute component instead of an extender. And, because of methanol's relatively low heat content (15 percent methanol is equivalent to only 8 percent gasoline on an energy basis), replacing 10 percent gasoline with 15 percent methanol would actually result in a net loss in total energy available for motor fuel. The unused light hydrocarbons would have to be diverted to alternative applications, where their presence could be of less importance. Furthermore, the removal of butane would mean the loss of a blending component with good MON (see Table 1), and would partially negate any octane advantage provided by the methanol.

A further potential disadvantage of using methanol-gasoline blends is related to the effect the mixture might have on vehicle road performance. Because oxygen accounts for over half of the molecular weight of methanol, its presence in the blend exerts a leaning effect on vehicle carburetion. Because of emission controls, most new cars on the road today are already carbureted near, or in some cases beyond, the lean limit for satisfactory performance. Additional leaning by methanol would be expected to compound this problem. The wider flammability limits of methanol extend its range of satisfactory operation, however, and may partially compensate for the leaner carburetion. However, our tests indicate that the problem still exists, as will be discussed later.



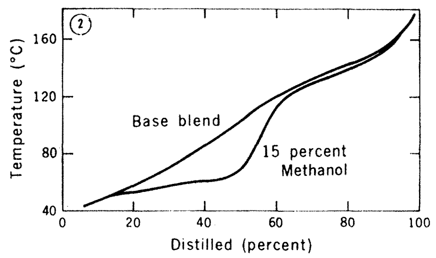


Fig. 1 (left). Effect of methanol on gasoline vapor pressure. The increase in Reid vapor pressure (*RVP*) is measured in pounds per square inch (*13*). Fig. 2 (right). Effect of methanol on fuel distillation characteristics.

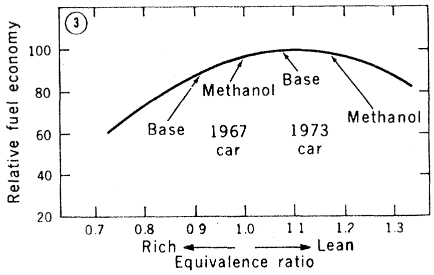


Fig. 3. Relative fuel economy as a function of carburetion.

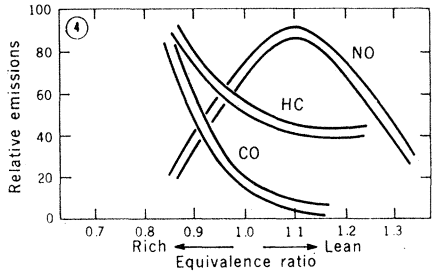


Fig. 4. Relationship between equivalence ratio and exhaust emissions.

## Fuel Economy

For our experiments we used two fuels and three vehicles. The base fuel was a typical unleaded gasoline while the test fuel contained 15 percent methanol, by volume. The two fuels were blended to constant RVP, which required removal of all the butane and about half the pentanes from the base blend prior to the addition of methanol. The important characteristics of each fuel are shown in Table 2.

The three vehicles used were a 1967 model built before any emission control standards were enforced; a 1973 model; and a prototype "1977" model equipped with oxidation catalysts and giving carbon monoxide and hydrocarbon emissions well below 3.4 and 0.41 grams per mile, respectively, the most stringent standards proposed for these pollutants. All three vehicles were made by the same manufacturer and were equipped with V-8 engines. Vehicle weights were similar and for all tests the inertial weight was set at 4000 pounds on the dynamometer.

Vehicle carburetion was found to be the key to explaining most of the observations on fuel economy and emissions. Table 3 shows equivalence ratios calculated from exhaust emission measurements for the 1967 and 1973 cars driven at various steady state speeds. Equivalence ratio is defined as the weight ratio of air to fuel, with the stoichiometrically correct ratio set at 1.0. Fuel-rich carburetion, then, is typified by equivalence ratios less than 1.0 and lean carburetion greater than 1.0. Air injection at the exhaust valves ruled out comparable calculations for the "1977" car. Carburetion differences are extremely important when considering the methanol's influence on fuel economy and emissions, because of the effect of methanol on equivalence ratio.

In Table 4, which shows the fuel economy data for the three cars, each value represents an average of at least two runs. The reproducibility, expressed as the percentage deviation from the average in any set of replicate runs, was about 1 percent. The effect of adding 15 percent methanol to the base gasoline is shown on a volume basis and on an energy basis. Values for the latter are more favorable because the heat content of methanol is roughly half that of gasoline, and they are probably of more interest because methanol fuel manufacturing costs are normally quoted on an energy basis.

The effects of methanol on fuel economy are in good agreement with those that would be predicted from methanol's leaning effect on carburetion. Figure 3 shows the relationship between fuel economy and equivalence ratio as determined for gasoline (*9*). Optimum fuel economy, as far as equivalence ratio is concerned, occurs at about 1.10, that is, when 10 percent excess air is present in the mixture. The equivalence ratio data in Table 3 indicate that with the base fuel, the 1973 car is operating near optimum fuel economy, while the 1967 car is well down the curve on the fuel-rich side. Calculations show that 15 percent methanol results in about a 10 percent increase in equivalence ratio, if one assumes that fuel metering characteristics remain unchanged, a reasonable assumption for the fuels used in this program. From this, one can predict that the leaning effect of methanol should result in a significant improvement in the fuel economy of the 1967 car but very little change in the case of the 1973 car (as shown in Fig. 3). The catalyst-equipped car had carburetion somewhat richer than the 1973 car which should lead to an intermediate effect. This was indeed found.

The inference from these data is that significant reductions in fuel consumption with methanol blends cannot be expected for vehicles operating at equivalence ratios of greater than 1.0 (lean carburetion. By the time any large-scale use of methanol blends could be implemented, this class of vehicles would account for most of the vehicles on the road. From the point of view of economizing on fuel, then, there appears to be little incentive for using methanol in future motor gasolines.

## Exhaust Emissions

Changes in the emission levels of carbon monoxide (CO), hydrocarbons (HC), and nitrogen oxides ($NO_x$) with methanol addition can also generally be accounted for on the basis of the change in equivalence ratio. From Fig. 4 it is evident that the older car, operating on the base fuel at an equivalence ratio of about 0.9, should show substantial decreases in CO and HC and an increase in $NO_x$. The lean-operating car, at about 1.05, should show lesser effects for CO and HC and very little change in $NO_x$ emissions.

The data in Figs. 5, 6, and 7 show that, in general, these changes were found. The only anomaly appears to be that of $NO_x$ emissions for the lean-operating car where a significant decrease was observed. This decrease may be related to methanol's high latent heat of vaporization which could lead to lower peak flame temperatures in the combustion chamber, with attendant lower $NO_x$ emissions. The CO and HC emissions from the catalyst-equipped car were very low for both fuels. The observed increases with the methanol blend were very small on an absolute basis for this car and are not environmentally significant.

As was the case with fuel economy, the beneficial effects of methanol in reducing CO and HC emissions are mainly associated with the older cars. There may be some advantage with newer cars with respect to $NO_x$ emissions, but the importance of this factor requires further investigation.

Vehicular emissions of CO, HC, and $NO_x$ are currently being regulated by law. Aldehydes, a class of organic emission products, do not specifically come under these regulations, although they do make a partial contribution to the HC reading. As a class, aldehydes are about as photochemically reactive in the atmosphere as olefins, the most reactive of the hydrocarbon emission products (*10*). Formaldehyde, the lowest-molecular-weight aldehyde, and a known eye irritant, can be produced from methanol by partial oxidation. It was not surprising, therefore, to find increased aldehyde emissions during tests with the methanol blend. The data in Fig. 8 show 30 percent and 50 percent higher aldehyde emissions for the 1973 and 1967 cars, respectively, with the methanol blend. The aldehyde emissions from the catalyst-equipped car were also higher, but the actual amounts emitted with either fuel were so low that the increase with the methanol blend is probably not meaningful. Analysis of individual aldehydes emitted from the older cars shows that most of the increase in aldehyde emissions was due to formaldehyde; methanol, presumably, was the precursor. It is not clear whether these increases in formaldehyde emission would have a significant impact on the environment.

Table 5. The effect of methanol on the aromatic and olefin content of the hydrocarbons in the exhausts of the three cars.

| Amount of methanol in fuel (%) | Composition (mole %) | |
|---|---|---|
| | Aromatic hydrocarbons* | Olefins |
| | *The 1967 car* | |
| 0 | 28.6 | 17.7 |
| 15 | 34.6 | 20.4 |
| | *The 1973 car* | |
| 0 | 19.3 | 21.6 |
| 15 | 24.3 | 19.3 |
| | *The "1977" car* | |
| 0 | 15.2 | 9.7 |
| 15 | 18.1 | 12.8 |

* Benzene is not included because of its low reactivity.

Table 6. Relative hydrocarbon reactivity in the exhaust from the base fuel and from the 15 percent methanol–gasoline blend.

| Car | Total reactivity | |
|---|---|---|
| | Base fuel | Methanol blend |
| 1967 | 1.00 | 0.78 |
| 1973 | 0.22 | 0.21 |
| "1977" | 0.010 | 0.015 |

Table 7. Car performance prior to warmed-up operation in the 1975 federal test procedure.

| Methanol content (%) | Average number of occurrences per test | | |
|---|---|---|---|
| | Stalls | Hesitations | Backfires |
| | *The 1967 car* | | |
| 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 |
| | *The 1973 car* | | |
| 0 | 1.7 | 0 | 0 |
| 15 | 1.7 | 2 | 1.3 |
| | *The "1977" car* | | |
| 0 | 0 | 0 | 0 |
| 15 | 1.3 | 4 | 0 |

## Hydrocarbon Composition of the Exhaust

The composition of the hydrocarbon fraction in the exhaust is of importance because this governs the tendency of the gases to undergo the photochemical reactions in the atmosphere which produce smog. In general, olefins are more reactive than aromatic hydrocarbons which, in turn, are more reactive than saturates. The effect of methanol on the aromatic and olefin content of the exhaust hydrocarbons is shown in Table 5. It should be noted that the compositional data in the table do not reflect the differences in mass emissions from the three systems. The values show the percentages of aromatic and olefinic compounds in the emitted hydrocarbons.

In each case, the addition of methanol increases the aromatic content of the exhaust hydrocarbon fraction. This is because the fraction of the methanol-containing fuel that produces the hydrocarbons has a higher aromatic content than that of the base blend, the butanes and half the pentanes having been removed to allow matching of RVP between the blends. Methanol itself probably does not produce significant quantities of hydrocarbons.

Two factors play a role in determining olefin content of the exhaust: the hydrocarbon composition of the fuel, and stoichiometry. The addition of methanol to gasoline should reduce the amounts of olefins in the exhaust (*11*) by decreasing the concentration of the exhaust olefin precursors in the fuel. On the other hand, the leaning effect of methanol should produce the opposite effect. In the case of the 1967 car, the higher olefin content of the exhaust suggests that stoichiometric effects predominate, while in the case of the 1973 car the hydrocarbon composition of the fuel is apparently more important. The catalyst on the "1977" car selectively removes the more reactive species.

The reactivity calculations shown in Table 6 put these considerations in perspective. To calculate total reactivity, the concentration of each individual hydrocarbon in the exhaust mixture was multiplied by its reactivity and the results for all the hydrocarbons added together. The reactivity scale used for these calculations is a composite scale that includes contributions from a variety of important smog modifications (*12*).

In calculating total reactivity, one takes into consideration the mass emissions factor. This accounts for the very low values that were obtained for the catalyst-equipped vehicle; the observed increase in reactivity when the methanol blend was used probably has no environmental significance. The total reactivity of the hydrocarbons in the exhaust from the 1973 car showed essentially no change with methanol addition, while those from the 1967 car showed a significant decrease in reactivity. This decrease is due to the decrease in the total amount of hydrocarbons in the exhaust, which more than offset the increase in the reactive character of the emitted hydrocarbons. Thus, in the area of hydrocarbon photochemical reactivity, one can again infer that the addition of methanol to gasoline would be significantly beneficial only for the older cars.

## Car Performance

A qualitative assessment of the effect of 15 percent methanol on car performance was obtained during the fuel economy and emission tests on the three vehicles (1975 federal test procedure described in Table 4). All three cars performed well during warmed-up operation with the methanol blend, and the 1967 car showed no problems at any time. The leaner-operating 1973 and "1977" cars, however, did experience stalling, hesitation, and backfire during the first few minutes of operation from a cold start (Table 7). Stalling was also observed with the base blend in the case of the 1973 car, but hesitation and backfire did not occur.

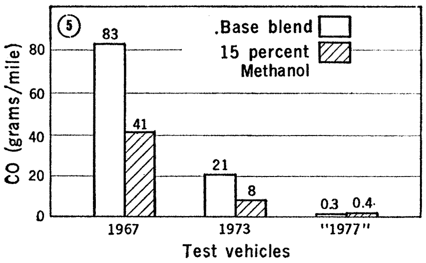


Fig. 5. Effect of methanol on carbon monoxide emissions during the 1975 federal test procedure (*13*).

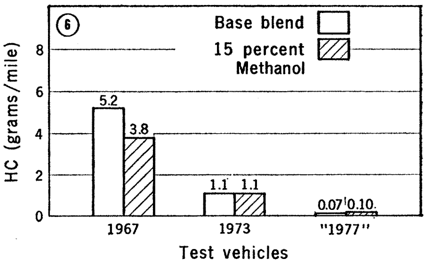


Fig. 6. Effect of methanol on hydrocarbon emissions during the 1975 federal test procedure.

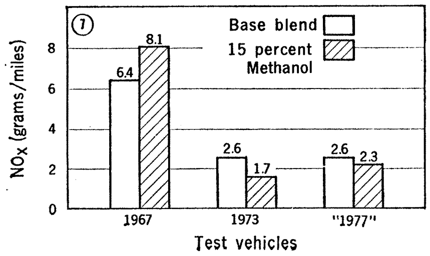


Fig. 7. Effect of methanol on nitrogen oxides emissions during the 1975 federal test procedure.

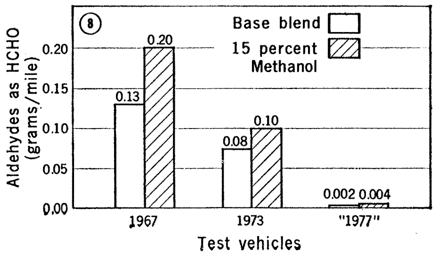


Fig. 8. Effect of methanol on aldehyde emissions during the 1975 federal test procedure.

If the carburetor were provided with a richer fuel-air mixture during startup one would expect the performance problems associated with cold start to be reduced. However, CO and HC emissions would be adversely affected.

It should be pointed out that the 1975 federal test procedure is not a particularly severe test for car performance. Accelerations are not rapid and, in addition, test temperature is specified to be within 68° and 86°F (20° and 30°C). The test temperatures for the runs in this program were between 70° and 80°F. More severe problems would be expected at lower ambient temperatures or under harsher driving conditions.

## Conclusions

The tests conducted with the three vehicles at different emission control levels suggest that, in the area of fuel economy and emissions, potential benefits with methanol blends are related to carburetion and are only significant in the case of the rich-operating cars built before emission control standards were imposed. Theoretical considerations related to methanol's leaning effect on carburetion support this conclusion. Potential advantages for methanol in these areas are therefore continuously diminishing as the older cars leave the roads. At present, these older cars use only about one-fourth of the total motor gasoline consumed and, before methanol could be used on a large scale, this fraction would be much smaller.

The use of methanol in gasoline would almost certainly create severe product quality problems. Water contamination could lead to phase separation in the distribution system and possibly in the car tank as well, and this would require additional investment in fuel handling and blending equipment. Excess fuel volatility in hot weather may also have adverse effects on car performance if the methanol blends include typical concentrations of butanes and pentanes. Removal of these light hydrocarbon components would detract from methanol's role as a gasoline extender and if current fuel volatility specifications were maintained, its use could lead to a net loss in the total available energy for use in motor fuels. Car performance problems associated with excessively lean operation would also be expected in the case of a significant proportion of late-model cars which are adjusted to operate on lean fuel-air mixtures.

If methanol does become available in large quantities, these factors suggest that it would be more practical to use it for purposes other than those related to the extending of motor gasoline, such as for gas turbines used for electric power generation. In this case, the "pure" methanol would act as a clean-burning fuel, having none of the potentially severe product quality problems associated with its use in motor gasoline, while the fuel oil or natural gas currently burned in these turbines could be diverted to other uses.

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13. Conversion factors for nonmetric units used in the text are: 1 mile per gallon equals 0.43 kilometer per liter; 1 pound per square inch equals $6.9 \times 10^4$ dynes per square centimeter; 1 gram per mile equals 0.62 gram per kilometer

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# DNA Ligase: Structure, Mechanism, and Function

## The joining of DNA chains by DNA ligase is an essential component of DNA repair, replication, and recombination.

I. R. Lehman

DNA ligase is an enzyme that can join DNA chains to each other under certain very specific conditions. Although such a ligation activity had long been a feature of models for recombination between genes and for the repair of damage to DNA, the real impetus to search for a DNA joining enzyme stemmed from two experimental findings made in the early 1960's. The first was the discovery by Meselson and Weigle (*1*) and by Kellenberger, Zichichi, and Weigle (*2*) that genetic recombination can occur by the breakage and rejoining of DNA molecules; and the second was the observation by Young and Sinsheimer (*3*), and by Bode and Kaiser (*4*) that a large fraction of linear DNA from bacteriophage $\lambda$ is rapidly converted to covalently closed duplex circles soon after it infects its host bacterium. The extent and vigor of the efforts to find a DNA joining activity is perhaps best conveyed by the independent and nearly simultaneous discovery in 1967 of DNA ligases in uninfected and bacteriophage-infected *Escherichia coli* in no less than five different laboratories (*5–9*).

Although there was a clear and implicit requirement for a DNA ligase in the repair of DNA and in recombination, yet another function became apparent with the report by Okazaki and his co-workers (*10*) that DNA may be replicated discontinuously as short segments which are subsequently joined into the continuous strands that make up the chromosome. As this model for DNA replication has gained acceptance, there has been a corresponding recognition of DNA ligase as an integral part of the cellular replication machinery.

After the discovery of ligases in uninfected and phage-infected *E. coli*, DNA joining activities were observed in a variety of eukaryotic tissues including rabbit bone marrow, spleen, and thymus (*11*), rat liver (*12*), and lily microsporocytes (*13*), so that their widespread distribution is by now well established. I will focus on two of these enzymes: that from *E. coli* and the one induced after infection of *E. coli* with bacteriophage T4. These two DNA ligases are the only ones now available in homogeneous form; they are also the most thoroughly investigated. Both catalyze the synthesis of phosphodiester bonds between directly adjacent 3′-hydroxyl and 5′-phosphoryl termini in duplex DNA. Phosphodiester bond synthesis catalyzed by the *E. coli* ligase is coupled to cleavage of the pyrophosphate bond of diphosphopyridine nucleotide (DPN), alternatively named nicotinamide adenine dinucleotide (NAD) (*14, 15*); the energy for phosphodiester bond synthesis by the bacteriophage T4–induced enzyme (as well as the eukaryotic ligases) is provided by the hydrolysis of the $\alpha,\beta$-pyrophosphate bond of adenosine triphosphate (ATP) (Fig. 1) (*6, 8, 9, 11–13*).

In discussing the structure, mechanism, and function of DNA ligase, I will deal with (i) assay methods, (ii) physicochemical properties and substrate specificity, (iii) chemical mechanisms, (iv) functions in vivo, and (v) use of ligases as reagents in the construction of recombinant DNA molecules in vitro.

### Assay Methods

DNA ligase activity can be measured in a variety of ways: the change in sedimentation coefficient after covalent closure of circles of phage $\lambda$ DNA with two single-strand breaks (nicks) (*5*); covalent linkage of hydrogen-bonded dimers of $\lambda$ DNA as measured by adsorption to hydroxyapatite after de-

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