of *Desulfovibrio*. After prolonged incubation a measurable decrease in the volume of N_2 in active cultures was observed, which could not be accounted for by solubility, diffusion, or other processes involving general gas laws. Consequently, helium and argon were tried as controls, both of which proved to be inert. Further investigations, based upon the decreased ratios of N_2/A, confirmed the fixation of N_2 by H_2-utilizing *Desulfovibrio*.

A pure culture of hydrogenase-producing *Desulfovibrio* was used to inoculate 20 liters of inorganic sea water medium overlayered with 20 liters of gas initially consisting of approximately 80% of catalytically purified H_2. The remaining 20% of the gas was CO_2, N_2, and A, the latter two in a ratio of 1 part of A to 83 parts of N_2. The composition of the gas was determined periodically by means of mass spectrometer analyses.[2]

TABLE 1

CHANGE IN COMPOSITION OF GAS CAUSED BY AUTOTROPHIC SULFATE REDUCERS GROWING IN 20 LITERS OF INORGANIC MEDIUM OVERLAYERED WITH 20 LITERS OF GAS AT 28° C

Component	Composition of gas after		
	2 days (%)	42 days (%)	61 days (%)
H_2	82.93	56.04	47.42
O_2	0	0	0
CO_2	0.51	0.27	0.19
H_2S	0	0	0.1
N_2	16.63	43.14	51.61
A	0.20	0.55	0.68
N_2/A ratio	83	78	76

The growth or reproduction of the bacteria was accompanied by a decrease in the H_2 and CO_2 content of the gas phase, as appreciable quantities of these two components were consumed by the autotroph

$$2H_2 + CO_2 \rightarrow (HCHO) + H_2O,$$

where (HCHO) represents a primary building block of bacterial cell substance, not necessarily formaldehyde. The content of CO_2 in the gas phase was also affected by the pH of the medium, which became more alkaline as sulfate was reduced to sulfide:

$$4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O.$$

As a result of H_2 and CO_2 uptake, the concentration of N_2 and A in the gas phase increased (Table 1), but the decreasing N_2/A ratio indicated that N_2 was being consumed by the autotrophic bacteria. This was confirmed by determining the absolute quantities of each gas in the closed system. The small amount of H_2S appearing in the gas phase is attributable to its absorption by the slightly alkaline medium.

Four other pure cultures of H_2-utilizing sulfate-reducing bacteria incubated at 28° C in mineral salts solution overlayered with a mixture of H_2, CO_2, N_2, and A yielded the results that are shown in Table 2.

TABLE 2

Culture No.	N_2/A ratio after	
	2 days	60 days
45: 268-1	83	67
45: 268-2	83	76
45: 268-3	83	79
45: 268-4	83	55
None (control)	83	83

These observations, coupled with the observed growth of the bacteria in mineral salts solution containing N_2 as the sole source of nitrogen, establish the ability of another physiological group of bacteria to fix N_2. Being widely distributed in marine sediments (*1, 2*), such bacteria may play an important part in the fixation of N_2 in the sea.

The observations help to substantiate the prediction of Lindstrom *et al.* (*3, 4*) that all hydrogenase-producing bacteria fix N_2. These workers employed micro-Kjeldahl and tagged-atom (N_2^{15}) techniques to demonstrate the fixation of N_2 by *Chromatium*, *Chlorobacterium*, and *Rhodospirillum* species. We recommend following the N_2/A ratios of gas mixture, which can be easily and accurately determined by mass spectrometer, as an indicator of the ability of bacteria to fix N_2.

[2] The mass spectrometer analyses were made by the Richfield Oil Corporation, Wilmington, Calif. The writers also acknowledge the valuable suggestions of D. L. Fox, E. D. Goldberg, and N. W. Rakestraw.

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Studies Concerning the Relationship Between Chemical Constitution and Biological Activity in a Group of Reversed Carboxyl (RC) Analogues of Acetylcholine

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This study is directed toward the comparison of parallel derivatives of acetylcholine and its (RC) analogue (*1*) (methyl-β-trimethylammonium propionate, No. 1 in Table 1). In our previous study we noted the high order of muscarinic activity of the (RC) analogue of acetylcholine with respect to depressor effects, smooth muscle-stimulating action, and salivary

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TABLE 1

Compound No.	Structure
1	$CH_3OCCH_2CH_2N^+(CH_3)_3Br^-$
2	$CH_3OCCH_2CH_2N^+(C_2H_5)_3Br^-$
3	$C_2H_5OCCH_2CH_2N^+(CH_3)_3Br^-$
4	$i\text{—}C_4H_9OCCH_2CH_2N^+(CH_3)_3Br^-$
5	$CH_3OCCH_2CH_2N^+(CH_3)_2CH_2CH_2OCCH_3Br^-$
6	$(CH_3)_3N^+CH_2CH_2COCH_2CH_2N^+(CH_3)_2 2Br^-$
7	$(C_2H_5)_3N^+CH_2CH_2COCH_2CH_2N^+(C_2H_5)_2 2Br^-$
8	$(CH_3)_3N^+CH_2CH_2COCH_2CH_2OCCH_2CH_2N^+(CH_3)_2 2Br^-$
9	$CH_3OC\text{—}CH(CH_3)\text{—}CH_2\text{—}N^+(CH_3)_3Br^-$
10	$CH_3OCCH_2CH(CH_3)\text{—}N^+(CH_3)_3Br^-$
11	$CH_3OCCH_2CH_2\text{—}N^+(CH_3)_2(C_2H_5)Br^-$
12	$CH_3OCCH_2CH_2\text{—}N^+(CH_3)(C_2H_5)_2Br^-$

secretion stimulation. With respect to the enzyme cholinesterase (both true and pseudo), the (RC) analogue of acetylcholine showed negligible substrate susceptibility. It was, therefore, of interest to study the effect of including both the acetylcholine structure and its (RC) analogue structure in the same molecule (see compounds Nos. 5 and 6 in Table 1). We found the material (No. 5) to be about 1/100 as potent as acetylcholine in stimulating the guinea pig ileum and in effecting a depressor response in dogs. Owing to the lack of parallelism of the dose response curves for acetylcholine and No. 5, this is no more than a rough estimate. Much more interesting is the fact that No. 5 is hydrolyzed very readily by true cholinesterase (Winthrop-Stearns' preparation) to the extent of 65% that of β-methacholine. Preliminary studies on the hydrolysis products of No. 5 indicate that the enzyme attacks only the acetyl ester end of this molecule.

No. 6 may also be regarded as a double analogue containing both the acetylcholine and its (RC) arrangements holding the carboxyl group in common. This material possesses muscarinic activity (depressor) in low doses (25 γ/kg to 50 γ/kg in dogs) which gradually changes to a pronounced stimulating nicotinic action at higher doses (100 γ/kg to 600 γ/kg), even in the absence of atropine. Its muscarinic activity is potentiated after prostigmine, and we were unable to demonstrate any nicotinic depressant activity associated with this substance. The presence of the carboxy group in No. 6 confers upon it muscarinic activity not possessed by the corresponding di-trimethyl ammonium polymethylene analogues studied by Brown *et al.* (*2*) and Paton and Zaimis (*3*) and found to be potent nicotinic stimulants. The di-triethyl analogue (No. 7), however, is a potent nicotinic depressant, apparently possessing no demonstrable nicotinic stimulant activity, though it blocks a minimal vagal (depressor) stimulus at 0.5 mg/kg.

Similar to the effects in the acetylcholine series pointed out by Ing (*4*), the substitution of one ethyl group for a single nitrogen methyl group in the (RC) analogue series (compound No. 11) decreases muscarinic depressor potency to 1/10, and the substitution of two ethyl groups (compound No. 12) decreases the muscarinic potency to about 1/500—both being compared to the (RC) analogue of acetylcholine itself (No. 1).

The (RC) analogues of α- and β-methacholine (Nos. 10 and 9) are no more than 1/10,000 as potent as the corresponding acetylcholine analogues in regard to depressant, gut-stimulant, and salivary secretory actions. They also exhibit negligible cholinesterase susceptibility similar to the (RC) analogue of acetylcholine. On the other hand, in Nos. 3 and 4, the (RC) analogues of propionyl choline, and isovalerylcholine, respectively, potency relationships are reversed. No. 3 is at least 50 times more potent than propionyl choline as a muscarinic and stimulating nicotinic drug, and No. 4 is still more powerful as a stimulating nicotinic agent (giving a marked pressor effect even in the absence of atropine).

The derivative No. 2 possesses depressor activity in the 1 mg/kg to 2 mg/kg dosage range which is blocked by atropine, revealing a low degree of stimulating nicotinic activity. At high doses (12 mg/kg) it tends to act as a weak ganglionic blocking agent, and at intermediate doses it appears to facilitate ganglionic conduction.

Compound No. 8 is a doubled analogue which may be considered as the (RC) analogue of succinyl dicholine. It possesses weak muscarinic activity, but this is greatly overshadowed by its striking curarelike action (about 85% that of D-tubocurarine chloride by the rabbit head drop method). Indeed the intramolecular distance between the nitrogen heads of this material is almost identical with that of decamethylene di-trimethyl ammonium when each is measured in its most extended form.

All the effects so far mentioned refer only to the total response attained by the various agents in the various test preparations. If one takes rate of response to the same total response, acetylcholine is considerably more active in effecting gut contraction than its (RC) analogue. Thus, to reach a final con-

traction of 73% using the guinea pig ileum, acetylcholine and its (RC) analogue require equal doses, but the (RC) analogue requires 250% longer than acetylcholine, and this difference is even more marked at lower dosage levels. In short, although the dose total-response curves are identical within statistical limits, the dose rate-of-response curves are quite different. This brings up the old question: Which—rate of response or final effect—is a more reliable measure of drug receptor fit? Assuming permeabilities and other factors effecting drug transfer to the receptors to be identical for both agents, then acetylcholine appears to "fit" the receptor more readily, as its effect is more prompt. One might, however, argue that the (RC) analogue "fits" just as well, or perhaps better, but it does not so easily gain access to the receptor machinery, thus accounting for the lag in its relative rate of effect. Arguments of this kind, although didactically fruitful, hardly seem profitable at this stage unless operational procedures clarify the questions concerning transfer.

As noted previously (*1*), the reversal of the carboxyl group of acetylcholine, a modification which leaves over-all molecular dimensions unchanged and also introduces little change in the distance relationships of the oxygen groups to the nitrogen head (*5*), effected relatively little apparent change in the drug-muscarinic and nicotinic receptor relationships. Actually, however, a fuller notion of the extent of this change becomes apparent in the study of parallel derivatives in the two series. Further, this study indicates that moieties imbedded within a molecular matrix continue to manifest their presence in a pharmacologic sense, though the quantitative expression of their activity is greatly modified by the impedance of other groups. For example, in the double analogues Nos. 5, 6, and 8, the presence of methylated nitrogen heads held in a given relationship to oxygen groups was manifested through a greater or lesser degree of muscarinic activity, even though these analogues are far removed, in a whole molecule sense, from acetylcholine. At first this might seem to be a mere statement of the "whole-molecule-fit" concept in other terms. Actually it represents a quite different point of emphasis, in which the working unit is a group of chemical constitutional characteristics abstracted from various active molecular species and in which other molecular features are thought of as perturbing factors.

This mode of abstraction is, of course, well recognized in other fields. For example, in chemistry we ascribe certain reactions as typical of aldehyde, hydroxyl, and amino groups which are in given compounds impeded or modified to a greater or lesser extent by the presence of other constitutional features of the molecule. One finds it helpful to approach given cases as deviating from the general for various specific reasons rather than as isolated instances. In a similar manner one may visualize the receptor as an entity which places certain positive requirements upon drugs for activity but places no definite negative requirements other than that the interaction of the moiety not be impeded. Impedance may take many forms, such as steric interference, the presence of other groups exerting electrostatic field effects that prevent the approach of the molecule containing the moiety to the receptor surface, etc. If, on the other hand, one requires the whole molecule to "fit," then all parts of drugs Nos. 5, 6, and 8 must find appropriate places on the receptor, and this would necessarily need to be true for all materials possessing muscarinic activity. What a variety of materials this receptor would have to be able to accommodate totally! Returning to the moiety-fit point of view, in terms of a historic example, a given lock may be opened by many different keys provided the given projections of the key are present to turn the given tumblers of the lock. If extra projections are present, these need not also have tumblers corresponding to them in the lock. They merely must not get in the way of the projections that do correspond to the tumblers.

In the present instance, although chemically parallel derivatives in the acetylcholine series and the (RC) analogue series are frequently parallel in their pharmacologic actions, the occurrence of striking deviations from parallelism indicates that the interchange of the components of the carboxyl group effects an alteration in the drug-receptor relationships.

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A New Versatile Respirator

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The relative merits of the multitude of artificial respirators for animal experimentation may perhaps best be evaluated on the basis of simplicity of construction, ease of control, and versatility of use. The oldest and most widely used design is the electric motor-driven mechanical pump. Only the most complex and expensive custom-built models have any real degree of ease of control and, even then, almost invariably must be stopped to alter the stroke volume which, in turn, is clearly limited by the dimensions of the cylinder. No commercial model has any provision for altering the ratio of time of ventilation to time of exhaust.

In recent years, a number of respirators have been produced which function by opening and closing a valve in an air supply line; these valves, which may be rotary- (*1, 2*), sleeve-, slide-, or piston-type (*3*), are, in some models, activated by air-driven motors (*1, 2*); others are driven by electric motors through