TABLE 2

SURVIVAL OF MICE EXPOSED TO 1,025 r WITH LEAD PROTECTION OF THE SURGICALLY MOBILIZED SPLEEN WITH REMOVAL OF THE SPLEEN AT VARYING INTERVALS AFTER X-RAY

No. mice used	Spleen-shielding	Interval of splenectomy after irradiation	Survival (%)
24	Yes	Within 10 min before x-ray	0
24	"	Within 5 min after x-ray	0
54	"	1–6 hr after x-ray	66
95	"	2 days after x-ray	39

shielded mice (*2*). Recovery of these tissues in liver- or intestine-shielded animals at 8 days is nearly as far advanced as in the spleen-shielded animals, whereas after lead-shielding of the head, recovery of the tissues is minimal at this interval and nil after kidney-shielding.

The survival of mice in which the circulation to the shielded spleen is clamped off during exposure of the animal to 1,025 r (the clamp is released immediately thereafter) is approximately the same as the survival of animals with spleen-shielding without clamping.

Surgical extirpation of the shielded spleen at intervals after exposure to 1,025 r shows that a beneficial effect on survival, on regeneration of hematopoietic tissue, and on regeneration of the gastrointestinal tract has already been exerted if the shielded spleen has been left intact in the circulation for 1–6 hr (Table 2).

Transplantation of 4 spleens (total weight *ca.* 10 mg) from mice 1–12 days old into the peritoneal cavity of mice immediately after exposure of the recipient adult mice to 1,025 r total-body x-radiation significantly increases the survival of the irradiated mice and hastens regeneration of hematopoietic and gastrointestinal tissue (Table 3). Similar transplantation

TABLE 3

SURVIVAL OF MICE EXPOSED TO 1,025 r X-RADIATION WITHOUT LEAD PROTECTION OF THE SURGICALLY MOBILIZED SPLEEN WITH AND WITHOUT IMPLANTATION OF SPLEENS FROM YOUNG MICE AFTER X-RAY

No. mice used	Spleen implants within ½ hr after irradiation	Survival (%)	Hematopoietic recovery (8 days)
112	None	0	None
63	4 spleens from 1–12-day-old mice	38	Complete
18	2 spleens from 4–5-wk-old mice	45	Not studied
24	4 spleens from 1–8-day-old mice implanted 2 days after irradiation of the recipient	20	" "

of 4 fresh spleens from mice 1–8 days old into adult mice 2 days after exposure to 1,025 r total-body x-radiation likewise enhances survival, but not so effectively as earlier transplantation.

These facts indicate that in contrast to the action of glutathione (*3*), cysteine (*4*), O_2 deprivation (*5*), or cyanide (*6*), which must be administered before or during irradiation in order to reduce the expected radiation mortality, the factor involved in the shielding or transplant experiments is unnecessary during the actual irradiation and is definitely effective after irradiation.

These experiments involving spleen- or liver-shielding or spleen transplants strongly suggest that the factor responsible for recovery from radiation under these conditions is a substance of a noncellular nature. It seems unlikely that (1) cells migrate out from the shielded or transplanted tissue and are responsible for the enhancement of recovery, or (2) that irradiation of tissue produces a "toxin" and that the shielded or implanted tissues exert a direct detoxifying action upon contact with the "toxin." Our present efforts are concentrated on attempts to study the efficacy of simple water extracts of spleen and embryonic tissue on survival and hematopoietic recovery of irradiated mice. The potential implications of these findings in the therapy of radiation injury and in certain other clinical states are obvious.

References

1. JACOBSON, L. O., *et al. J. Lab. Clin. Med.*, **34**, 740 (1949).
2. JACOBSON, L. O., *et al. Ibid.*, **35**, 746 (1950).
3. CHAPMAN, W. H., *et al. Naval Medical Research*, Sept. 1, 1949.
4. PATT, H. M., *et al. Science*, **110**, 213 (1949).
5. DOWDY, A. H., BENNETT, L. R., and CHASTAIN, S. M. *Univ. Calif. (Los Angeles) Pubs. Rept. No. 55* (Jan. 31, 1950).
6. BACQ, A. H., *et al. Science*, **110**, 356 (1950).

Nitrogen Fixation by Sulfate-reducing Bacteria Indicated by Nitrogen/Argon Ratios[1]

Frederick D. Sisler and Claude E. ZoBell

Scripps Institution of Oceanography, University of California, La Jolla

In the course of investigations (*1*) on the utilization of molecular hydrogen by autotrophic, anaerobic, sulfate-reducing bacteria belonging to the genus *Desulfovibrio,* evidence was obtained for the fixation of free nitrogen. In preliminary experiments inverted vials in pairs were placed in glass-stoppered bottles filled with sea water, which served as a suitable mineral medium for the growth of H_2-utilizing sulfate reducers. One vial was filled with H_2 and the other with N_2 as an "inert" control.

It soon became evident, however, that N_2 was not inert in the presence of hydrogenase-producing species

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 514. This investigation was supported in part by a grant from the American Petroleum Institute, Research Project 43A.

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.

References

1. SISLER, F. D., and ZOBELL, C. E. *J. Bact.*, **60**, 747 (1950).
2. ZOBELL, C. E. *Bull. Am. Assoc. Petroleum Geol.*, **31**, 1709 (1947).
3. LINDSTROM, E. S., BURRIS, R. H., and WILSON, P. W. *J. Bact.*, **58**, 313 (1949).
4. LINDSTROM, E. S., TOVE, S. R., and WILSON, P. W. *Science*, **112**, 197 (1950).

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

F. W. Schueler, H. H. Keasling,[1] and R. M. Featherstone

Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City

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

[1] Fellow, American Foundation for Pharmaceutical Education. Portions of the material reported herein were abstracted from a dissertation submitted by H. H. Keasling in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Pharmacology of the College of Medicine and the Graduate College of the State University of Iowa.