yohimbine was a potent antimetabolite of serotonin (*8*), and when it was learned that reserpine is a derivative of yohimbine, it seemed likely that it would have a similar type of action. It may be that some of the pharmacological effects of reserpine arise from an antiserotonin property. The ability of reserpine to displace serotonin from tissues is clearly a type of action possessed by bona fide antimetabolites of serotonin, such as BAS (*9*).

D. W. Woolley
P. M. Edelman
Rockefeller Institute for Medical Research, New York

References and Notes

1. D. W. Woolley, *A Study of Antimetabolites* (Wiley, New York, 1952).
2. E. N. Shaw and D. W. Woolley, *J. Pharmacol. Exptl. Therap.* **116**, 164 (1956); D. W. Woolley and E. N. Shaw, *Science* 124, 34 (1956).
3. D. W. Woolley and E. N. Shaw, *J. Pharmacol. Exptl. Therap.* **121**, 13 (1957).
4. S. Udenfriend, H. Weissbach, C. T. Clark, *J. Biol. Chem.* 215, 337 (1955).
5. G. H. L. Dillard, G. Brecker, E. P. Cronkite, *Proc. Soc. Exptl. Biol. Med.* 78, 796 (1951).
6. V. Erspamer, *Arch. intern. pharmacodynamie* 103, 293 (1953).
7. A. Pletscher, P. A. Shore, B. B. Brodie, *Science* 122, 374 (1955).
8. E. Shaw and D. W. Woolley, *J. Biol. Chem.* 203, 979 (1953).
9. This work was supported, in part, by a grant from the U.S. Public Health Service. One of us (P.M.E.) was on a fellowship provided by State University of New York College of Medicine. We wish to thank the Abbott Laboratories for gifts of serotonin and the Upjohn Company for the hydroxyindoleacetic acid used in this study.

28 October 1957

Unstable Nucleic Acids of Rickettsia mooseri

Cells of *Rickettsia mooseri*, when purified and suspended in isotonic media, rapidly lose certain of their biological properties at 0°C (*1*). This inactivation can be attributed, in part, to a progressive loss of diphosphopyridine nucleotide (DPN) from the rickettsiae into the suspending medium. Incubation of partially inactivated rickettsiae with excess DPN results in a restoration of their various biological functions (*1*, *2*).

Attempts were made, therefore, to determine whether rickettsiae lose other important cofactors besides DPN. Purified suspensions of *R. mooseri* which had been freshly prepared by methods that have been described previously (*3*) were extracted with 5 percent cold perchloric acid in order to obtain the "acid-soluble fraction." In the case of many bacteria, this fraction contains the cofactors and metabolites of low molecular weight (*4*). The rickettsial extracts possessed an ultraviolet absorption spectrum typical of purine and pyrimidine compounds, with a maximum at 260 mμ. Chromatographic analysis performed on lyophilized extracts revealed the presence of four clearly defined ultraviolet absorbing spots, two of which were adenosinediphosphate and DPN. The other two compounds have not yet been identified.

Aliquots of the same purified rickettsial suspension were incubated at 36°C, and samples were taken at intervals over a 3-hour period. In these samples the rickettsiae were sedimented at 22,000*g* for 15 minutes (Sorvall centrifuge model SS-1), and the optical densities (at 260 mμ) of the acidified supernatants were determined. It became apparent that, during 3 hours, more 260-mμ-absorbing materials were liberated than could be accounted for by the total quantity of material extractable at the onset of the experiment. The nucleic acids of the rickettsiae were thought to be a likely source of the excess material liberated.

Rickettsia mooseri was, therefore, analyzed for both types of nucleic acids. Freshly prepared suspensions of the organism were fractionated after the method of Schmidt and Thannhauser (*5*), and analyses of the fractions for phosphorus (*6*), pentose (*7*), and deoxyribonucleic acid (DNA) (*8*) revealed the presence of ribonucleic acid (RNA) (22.6 μg of RNA-phosphorus per milligram of nitrogen) and DNA (6.3 μg of DNA-phosphorus per milligram of nitrogen) in a ratio of 3.5:1, which resembles that reported for many bacteria (*9*).

After these analytical findings had become available, balance studies were performed on the liberation of 260-mμ-absorbing materials from the cells of *R. mooseri*. Six experiments have been performed, and one typical protocol is presented in Table 1. Immediately after preparation, purified suspensions of rickettsiae were diluted with the reagents previously employed in manometric studies on glutamate oxidation (*3*). These mixtures were incubated at 36°C or 4°C, and samples were removed at the times indicated in Table 1. The rickettsiae were sedimented by high-speed centrifugation in the cold, and the sediments were extracted first in the cold and subsequently at 90°C with 5 percent perchloric acid. Supernatants, made 5 percent with respect to perchloric acid, and cold and hot perchloric acid extracts of the sediments were subjected to spectrophotometry and chemical analysis. All spectra (230 to 300 mμ) showed sharp maxima at 260 mμ.

Table 1. Changes in the distribution of ultraviolet-absorbing materials in rickettsial fractions upon incubation.

Time (min)	Optical density (260 mμ)			
	Suspending medium	Rickettsiae extracted with 5% PCA		Total
		Cold	Hot	
	Incubated at 36°C			
0	0.22	0.94	7.32	8.48
60	0.74	0.94	6.79	8.47
120	0.96	1.00	6.46	8.42
180	1.15	1.00	6.28	8.43
	Incubated at 4°C			
0	0.22	0.94	7.32	8.48
60	0.24	1.10	7.13	8.47
120	0.27	1.19	6.98	8.44
1200	0.39	1.35	6.72	8.46

Table 2. Reactivation of the toxicity of *Rickettsia mooseri* after inactivation at 4°C and at 36°C.

Treatment		Toxicity (LD_{50})	(%) Reactivation
Inactivation	Reactivation		
None	None	1:780	
18 hr, 4°C	None	< 1:20	
18 hr, 4°C	1 hr, 36°C, with cofactors*	1:600	77
3 hr, 36°C	None	< 1:20	
3 hr, 36°C	1 hr, 36°C, with cofactors*	< 1:20	0

* The cofactor mixture contained a final concentration of diphosphopyridine nucleotide (0.33 mg/ml), adenosinetriphosphate (0.33 mg/ml), coenzyme A (0.44 mg/ml), and glutathione (3.3 mg/ml), suspended in basal medium.

The results indicate that at 36°C the progressive appearance of 260-mμ-absorbing materials in the suspending medium was matched by a complementary decrease in the nucleic acid (hot 5 percent perchloric acid) fraction. This effect was much less pronounced at 4°C. While incubation at 36°C had little effect on the quantity of material in the cold perchloric acid extracts, it appears that, at 4°C, some of the material derived from the nucleic acids was retained in this fraction. The sums of the three fractions remained constant for the duration of the experiment.

Rickettsial suspensions which had lost toxicity for mice through incubation at 4°C for 18 hours were readily reactivated in a manner similar to that described by Bovarnick (*1*). In contrast, rickettsiae which had been incubated at 36°C for as short a period as 3 hours could not be reactivated by the same procedure. Rickettsial inactivation thus appears to be a complex phenomenon which not only involves the reversible depletion of DPN but may include among other factors the irreversible loss of nucleic acids from the organisms. (See Table 2.)

Ribose and DNA analyses indicated that RNA comprised 75 percent of the nucleic acid components lost by the rickettsial cells. Previous failures to detect RNA in rickettsiae have been ascribed to the loss of this constituent

during purification (*10*). Indeed, the low RNA/DNA ratio (1:3) reported by Smith and Stoker (*11*) for *Coxiella burnetii* may reflect such losses and suggests the physicochemical lability of rickettsial RNA.

The differences between the results obtained at 36°C and at 4°C suggest the participation of enzymatic processes in the loss of nucleic acids from the rickettsiae. Since the omission of glutamic acid did not influence the experimental results, "energy metabolism" does not seem to be of importance. The present findings are perhaps analogous to the degradation of RNA in resting cultures of *Escherichia coli* H, which has been reported by Stephenson and Moyle (*12*).

The loss of nucleic acids from *R. mooseri* upon incubation at 36°C may be one of the reasons for the concomitant inactivation of the biological properties of this organism.

Z. A. Cohn, F. E. Hahn, W. Ceglowski, F. M. Bozeman
Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, D.C.

References

1. M. R. Bovarnick and E. G. Allen, *J. Bacteriol.* 73, 56 (1957).
2. ———, *J. Gen. Physiol.* 38, 169 (1954).
3. C. L. Wisseman *et al.*, *J. Immunol.* 67, 123 (1951).
4. R. B. Roberts *et al.*, *Carnegie Inst. Wash. Publ.* 607 (1955).
5. G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.* 161, 83 (1954).
6. C. H. Fiske and Y. Subbarow, *ibid.* 66, 375 (1925).
7. W. J. Mejbaum, *Z. physiol. Chem. Hoppe-Seyler's* 258, 117 (1939).
8. G. Cerriotti, *J. Biol. Chem.* 198, 297 (1952).
9. E. Chargaff and J. N. Davidson, *The Nucleic Acids* (Academic Press, New York, 1955), vol. II, chap. 16.
10. H. Ris and J. P. Fox, *J. Exptl. Med.* 89, 681 (1949).
11. J. B. Smith and M. G. P. Stoker, *Brit. J. Exptl. Pathol.* 32, 433 (1951).
12. M. Stephenson and J. M. Moyle, *Biochem. J.* 45, vii (1949).

4 October 1957

Barium-140 Radioactivity in Foods

We wish to report the presence of the fission products barium-140 and lanthanum-140 in certain foodstuffs in the United States during periods of nuclear weapons tests. The presence of these nuclides in the amounts observed in no way constitutes a hazard; it carries none of the potential implications associated, for example, with strontium-90. It is of practical concern primarily to those engaged in measurements of radioactivity near the natural levels, and when an accurate summation of total radiation is desired. As in the case of cesium-137 (*1*), measurements of barium-140/lanthanum-140 may also be of value in the study of the fallout process.

Barium-140 has a half-life of 12.8 days, and its daughter lanthanum-140 has a half-life of 40.2 hours. Because of their somewhat similar biochemistry and the short half-life of lanthanum, secular equilibrium is likely to be maintained in biological systems, and both nuclides will follow the chemistry of barium. The biochemical behavior of barium-140 is similar to that of calcium and of strontium-90, but the short half-life of barium-140 and the larger biological discrimination factors against it render it potentially much less dangerous than strontium-90.

The presence of barium-140 was first noted in some deer in New Mexico during the summer of 1956—presumably the result of the United States nuclear test Operation Redwing. It was detected and identified (by its half-life) by means of the Los Alamos human counter (*2*), a large 4π liquid scintillation counter designed especially for the measurement of radioactivity at natural levels in people and foodstuffs. A threefold increase was noted in the gamma activity in the spectral region from 1 to 2 Mev (normally potassium-40 only). This corresponds roughly to a barium-140 activity of 0.03 μc per 70 kg, which is 6 percent of the maximum permissible amount for man, on the basis of the "large population" value (*3*). A cow taken directly from the New Mexico range showed a similar amount of barium-140, but commercial beef did not, perhaps because of different feeding habits or because of the time lag between slaughtering and the appearance of the meat on the retail market. While the apparent potassium-40 activity of milk samples showed a few instances of slight increases during 1956, it was not possible to identify the excess activity.

Barium-140 appeared in several United States milk samples during the months of June, July, and August, 1957, presumably as a result of distant fallout from the test operation in Nevada, Operation Plumb-bob, and perhaps from test operations of the U.S.S.R. Identification of the excess activity in the potassium region of the spectrum was again possible on the basis of the measured half-life. Confirmation of the assignment of the activity to barium-140 was obtained by spectral analysis, for which an 8- by 4-inch sodium iodide crystal in a steel room similar to the installation developed by Marinelli and his coworkers at the Argonne National Laboratory (*4*) was used. All five of the prominent barium-140/lanthanum-140 gamma peaks were identified.

Table 1 summarizes dates and concentrations of barium-140 in powdered milk for those locations at which the barium-140 gamma activity exceeded that of natural potassium-40. While detectable increases in activity in the upper energy

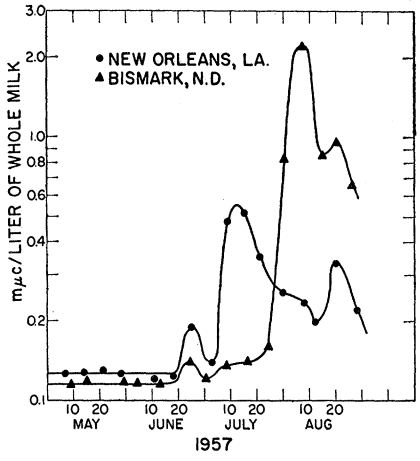

Fig. 1. Typical curves of upper channel activity versus date, for Bismarck, N.D., and New Orleans, La.

channel occurred at other sampling points during the summer months, at none of them did the increase reach this level. Thirty-seven points are routinely sampled.

The dates reported in Table 1 are those on which the samples (50 to 100 pounds of nonfat-dry milk solids) were received at Los Alamos. In general, the delay between production and arrival is about 1 week, and for accurate correlation with meteorological data, the actual production date must be ascertained.

Table 1. Barium-140 peak levels in milk.

Date received (1957)	Estimated barium-140 content (mμc/lit of whole milk)
Bismarck, N.D.	
7 Aug.	2.2
20 Aug.	0.85
Idaho Falls, Idaho	
10 June	0.40
5 Aug.	0.32
26 Aug.	0.54
Payette, Idaho	
19 June	0.12
9 Aug.	0.46
Louisville, Ky.	
21 July	0.27
9 Aug.	0.46
New Orleans, La.	
15 July	0.36
20 Aug.	0.19
Willows, Calif.	
10 June	0.31
Ladysmith, Wis.	
16 Aug.	0.23
Des Moines, Iowa	
9 Aug.	0.18
Ogden, Utah	
19 June	0.06
14 Aug.	0.15
Monroe, Utah	
11 June	0.09
25 July	0.12