Reports

Displacement of Serotonin from Tissues by a Specific Antimetabolite

The mode of action of an antimetabolite seems to be to occupy the specific receptors normally used by the metabolite. The result is a displacement of that metabolite and creation of a deficiency of it (*1*). Actual measurement of this displacement, however, has been made in relatively few cases (such as, for example, the displacement of acetylcholine by antiacetylcholines and of thiamine by pyrithiamine), and for this reason it may be desirable to study the situation with other metabolite-antimetabolite pairs in order to establish the generality of the phenomenon. Because we had available a potent and highly specific antimetabolite of serotonin, we thought it desirable to determine whether this compound would indeed displace serotonin from its sites in animal tissues. This antimetabolite was 1-benzyl-2-methyl-5-methoxytryptamine (BAS), the benzyl analog of serotonin, which has recently been shown to be quite active (*2*), and very specific (*3*) as an antagonist of serotonin in living animals.

One would expect to find that BAS would displace serotonin from tissues rich in this hormone. Actual trial with platelets from rabbit blood showed that this occurred. The serotonin content of the platelets isolated from individual rabbits was measured, and the animals were then given BAS (15 mg/kg, intraperitoneally, daily for 3 days). Platelets were again isolated 1 hour after the last dose of BAS and analyzed for serotonin. The data given in Table 1 show that the BAS lowered the serotonin content.

In order to make sure that the difference observed was not the result of capricious variation from day to day in the serotonin content or in the platelet count and that similar differences might not occur in untreated animals, each rabbit was examined at least twice before the administration of the BAS and at several intervals after this administration. These experiments showed that the values for serotonin content per platelet and for serotonin content per milligram of platelet protein, as well as for serotonin per milliliter of blood (calculated from the analysis of the platelets isolated from the blood), were substantially the same from day to day. After administration of BAS, however, the serotonin content fell to about half the normal values. It was evident that this fall did not occur immediately after injection of the BAS, because blood samples taken 1 hour after the first injection showed no decline in serotonin content of the platelets (in fact, there was a slight rise). It is already known that BAS is characterized by the slow onset of its antiserotonin action, and the present findings illustrate another facet of this property.

The analyses for serotonin were performed by the colorimetric method of Udenfriend *et al.* (*4*) on platelets isolated by the method of Dillard *et al.* (*5*). Platelets were counted in the whole bloods and in the final washed suspensions. About 50 percent of these cells were retrieved. The analytical values given in the last column of Table 1 reflect corrections made for this loss that occurred during isolation.

The validity of the chemical measurements, and of this last point relative to actual content in the whole blood, was checked as follows. Blood from the same rabbits was analyzed by the method described above and also by the pharmacological method for determining serotonin content (*6*), in which the rat uterus is utilized. Whole blood was cytolyzed with distilled water and analyzed directly by means of this pharmacological technique. In order to make sure that the pharmacological effect was due only to serotonin, the dilutions of blood were assayed on a uterus before and after the muscle had been treated with BAS. It is known that BAS specifically blocks the response of a tissue to serotonin. These experiments showed that at least 90 percent of the pharmacological effect was due to serotonin. The quantitative assays showed that the serotonin content of the whole blood of each rabbit was essentially the same as that determined by the colorimetric analysis of the platelets contained therein. Thus, rabbit E gave a value of 10 μg of serotonin per milliliter by the assay of whole blood and 10 μg per milliliter as determined by colorimetric analysis of the isolated platelets with correction made for percentage yield of platelets. Finally, the entire experiment on the lowering of serotonin content by administration of BAS was repeated on three rabbits, but the measurements were made pharmacologically as indicated. These showed that the BAS reduced the average serotonin content of blood from 10.5 to 4.8 μg/ml.

Measurements were also made of the effect of BAS administration on the serotonin content of stomach and small intestines of mice. Both colorimetric and pharmacological methods showed that a slight reduction occurred but that the reduction was not as marked as in the case of rabbit blood. Measurements on the urine of mice treated with BAS showed that excretion of 5-hydroxyindoleacetic acid was not increased but that the urinary excretion of serotonin was markedly increased. Apparently, the BAS inhibited the action of amineoxidase, and consequently the displaced serotonin was excreted largely unchanged.

The finding that there is a displacement of serotonin from tissues by a specific antimetabolite of serotonin may have some bearing on the ideas currently being discussed about the mode of action of reserpine. Pletscher *et al.* (*7*) showed that reserpine displaced serotonin from tissues of animals, and from this finding many hypotheses about the mode of action of the drug have been put forward. We suggest that this displacement of serotonin may be an expression of the antiserotonin activity of reserpine. It had been shown before the structure of reserpine was known that

Table 1. Serotonin content of rabbit blood platelets before and after treatment of animal with BAS. Values shown are the average of determinations on four rabbits.

Yield of washed platelets (%)	Serotonin content (μg per 10^8 platelets)	(μg/mg of platelet protein)	(μg/ml of blood)
No treatment			
47	2.7	16.1	9.4
1 hr after administration of BAS			
44	3.1	20.6	8.2
After 2 days' administration of BAS			
52	1.0	10.0	5.0

All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* **125**, 16 (4 Jan. 1957).

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