

plate method, the bubbler pump⁵ and by the funnel device.⁶ In each experiment four sets of samples were taken at 30-minute intervals. The effect of violet irradiation in lowering morbidity rates or preventing cross infection. If such experiments be attempted it must be borne in mind that certain types

TABLE 1

THE EFFECT OF ULTRAVIOLET FLOOR IRRADIATION WITH FOUR ULTRAVIOLET LAMPS ON AIR-BORNE BACTERIA. LIGHTS ON 10 TO 20 MINUTES AFTER THE SECOND RUN. TIME BETWEEN RUNS ABOUT 30 MINUTES. TEMPERATURE: 31-35° C. RELATIVE HUMIDITY: 53-63 PER CENT. ADDITIONAL FIVE EXPERIMENTS WITH LIGHTS ON AND OFF AND ONE ADDITIONAL CONTROL GAVE SIMILAR RESULTS TO THE ABOVE EXPERIMENTS

Experiment 1							Control					
		Run	No. of observ.	Mean per plate	σ	No/10 c. ft.		Run	No. of observ.	Mean per plate	σ	No/10 c. ft.
Open plate	Lights off	{ 1 2	10 10	20.3 21.1	2.04 1.83		Lights off	{ 1 2	10 10	20.8 17.5	5.40 4.45	
	Lights on	{ 1 2	10 10	4.5 5.4	1.25 2.04			{ 3 4	10 10	20.5 23.2	8.20 2.85	
Bubbler pump	Lights off	{ 1 2	12 12	4.8 4.2	.73 .74	242 210	Lights off	{ 1 2	12 12	4.5 4.3	1.32 .96	225 217
	Lights on	{ 1 2	12 12	1.3 1.9	.38 .44	63 96		{ 3 4	12 12	3.3 4.1	.70 1.19	169 204
Funnel device	Lights off	{ 1 2	2 2	49 43		49 43	Lights off	{ 1 2	2 2	59 50		59 50
	Lights on	{ 1 2	2 2	29 16		29 16		{ 3 4	2 2	47 46		47 46

ultraviolet radiation was determined by starting the lamps after the second set of samples had been taken. As a control two sets of experiments were performed without lighting the lamps so as to estimate the effect of settling without radiation.

The results of these various runs are shown in Table 1. It will be observed that the ultraviolet floor irradiation produced a significant lowering of air-borne bacteria in the experimental chamber.⁷

The results are sufficiently striking to justify the suggestion that floor irradiation be combined with ceiling irradiation in practical tests in barracks or hospital wards to determine the effect, if any, of ultra-

of flooring may prove to be capable of reflecting sufficient amounts of ultraviolet to cause harmful effects.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

SPECTROSCOPIC MICRODETERMINATION OF MUSCLE ADENYLIC ACID

THE absorption spectrum of adenylic acid in the ultraviolet shows a maximum at 2,600 Å, which is characteristic for the adenine group.¹ The deaminated product inosinic acid has its absorption maximum at 2,500 Å. This difference in absorption spectra between the amino and the hydroxy purine nucleotides was described as early as 1932 by Myrbäck, Euler and Hellström² and recently a correspond-

ing difference in absorption spectra has been described for adenine and hypoxanthine.³ Adenylic acid has a much higher absorption than inosinic acid in the range: 2,700-2,600 Å. At 2,650 the absorption of inosinic acid is only 40 per cent. of that of adenylic acid (see Fig. 1). This great difference in absorption spectra has been used in the present studies as a basis for a very sensitive and specific test for Schmidt's deaminase⁴ or for identification and quantitative de-

⁵ S. M. Wheeler, G. E. Foley and T. Duckett Jones, *SCIENCE*, 94: 445, 1941.

⁶ Alexander Hollaender and J. M. Dalla Valle, *U. S. Public Health Reports*, 54: 574, 1939.

⁷ A difference of three times the σ of the series between bacterial counts with lights on and off was considered as a criterion of significance.

¹ Ch. Dhéré, *C. R. Soc. Biol., Paris*, 60: 34, 1906.

* The opinions advanced in this paper are those of the writers and do not represent the official views of the Navy Department.

² K. Myrbäck, H. Euler and H. Hellström, *Zs. physiol. Chem.*, 245: 65, 1932.

³ M. M. Stimson and M. A. Renter, *Jour. Am. Chem. Soc.*, 65: 153, 1943.

⁴ G. Schmidt, *Zs. physiol. Chem.*, 179: 243, 1928.

termination of muscle adenylic acid (adenosine-5-phosphate). If a few micrograms of Schmidt's deaminase are added to a solution of adenylic acid and the absorption at 2,650 Å is determined in the Beckmann spectrophotometer one observes a steady decrease in absorption, proportional to time within the first few minutes, decreasing in rate later. The absorption decreases to less than half (45 per cent.) of the original but seems to come to a standstill before complete deamination has been reached. It has not so far been possible to observe any amination of inosinic acid with ammonia salts.

The deaminase test is performed in the following way. To 10 or 15 µg adenylic acid per ml (5×10^{-5} M) containing 0.05 M succinate buffer pH 5.9 is added to 2 to 5 µg of Schmidt's deaminase, purified through isoelectric precipitation and ammonium sulphate fractionation. The deamination takes place in a quartz vessel, 1 cm in depth, which is exposed to ultraviolet light of the wavelength 2,650 Å. The deamination causes a fall in the absorption and the decrease is read every minute on the absorption scale of the Beckmann spectrophotometer. A measurement of the absorption in the range from 2,400 to 2,800 Å before and after addition of deaminase shows the spectra of adenylic acid and inosinic acid (with traces of adenylic acid) respectively (Fig. 1). A decrease in absorption

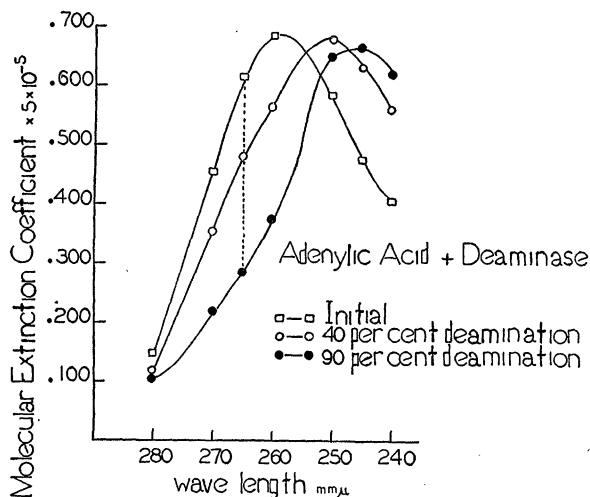


FIG. 1.

at 2,650 Å corresponding to less than 10 per cent. of complete reaction is readily detectable. If the adenylic acid concentration is 5×10^{-5} M, a 10 per cent. decrease corresponds to a liberation of 0.07 µg N.

The formation of adenylic acid when myokinase^{5,6} is added to adenosine diphosphate (2 adenosine di-

phosphate $\rightleftharpoons$ adenosine triphosphate + adenylic acid) can be also demonstrated in the micro test. Addition of purified deaminase to a 5×10^{-5} M solution of adenosine diphosphate (pH 6.2) does not give rise to any change in the absorption at 2,650 Å. If now a few micrograms of myokinase are added the absorption decreases proportionally with the amount of added myokinase, provided deaminase is in excess. Between 40 and 45 per cent. of the adenosine diphosphate is converted to inosinic acid, indicating that more than 80 per cent. of the adenosine diphosphate has been converted into the tri- and monophospho-nucleosides.

The spectrophotometric myokinase test requires one to two µg pyrophosphate P (as adenosine diphosphate) where the hexokinase test requires 20 to 50 µg pyrophosphate P. On the other hand, in the spectrophotometric test both the deaminase and the myokinase act outside their pH optima. The deaminase has a sharp pH optimum at 5.9⁴ the myokinase a broad optimum between pH 7 and 7.5⁶ and neither of the enzymes has any appreciable activity at the pH optimum of the other. At pH 6.2-6.5 both enzymes exhibit a fairly high although not optimal activity.

The deaminase preparations show a slight effect on adenosine. However, adenosine is deaminated 60 times slower than adenylic acid.

Adenylic acid from yeast nucleic acid is not deaminated by the deaminase (*cf.* footnote 4), a fact which in 1928 led Embden and Schmidt⁷ to the differentiation between muscle adenylic acid (adenosine-5-monophosphate) and nucleic acid adenylic acid (adenosine-3-monophosphate).

Thus, the method is specific for muscle adenylic acid (and diadenylic acid⁸). Methods based on adenylic acid as a phosphate transfer system⁹ are specific for adenosine-5-phosphate derivatives but can not distinguish between adenylic and adenosine diphosphate. The micromethod presented here does distinguish between adenylic acid and adenosine diphosphate due to a separation of the deaminase from myokinase.⁶

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⁷ G. Embden and G. Schmidt, *Zs. physiol. Chem.*, 181: 130, 1929.

⁸ W. Kiessling and O. Meyerhof, *Biochem. Zs.*, 296: 410, 1938.

⁹ F. Schlenck and T. Schlenck, *Jour. Biol. Chem.*, 141: 311, 1941.

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⁵ S. P. Colowick and H. M. Kalckar, *Jour. Biol. Chem.*, 148: 117, 1943.

⁶ H. M. Kalckar, *Jour. Biol. Chem.*, 148: 127, 1943.

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