In Table 3 the more probable distribution of the amino acids in myoglobin and hemoglobin of man and horse is

TABLE 3

	Number of amino acid molecules for 4 atoms of iron				Amount of basic amino acid for every atom of iron
	Cystine	Arginine	Histidine	Lysine	
Horse hemoglobin (4)	3	14	39	37	22
" myoglobin (4)	4	8	40	60	27
Human hemoglobin	4	16	35	37	22
" myoglobin	4	8	36	52	24

given, taking as molecular weights 68,000 and 17,000, respectively.

These data show that myoglobin of different animal species contains about half as much arginine and twice as much lysine as hemoglobin of the same animal and that the total amount of basic amino acid radicals is greater in myoglobin than in hemoglobin.

Addendum: Drabkin (*1*) and Theorell (*9*) recently mention having crystallized human myoglobin from cardiac muscle, but the writer has not yet seen these articles.

References

1. Drabkin, D. L. *Amer. J. med. Sci.*, 1945, **209**, 268.
2. Roche, J. *Bull. Soc. chim. Biol.*, 1942, **24**, 1016.
3. Rossi-Fanelli, A. *Boll. Soc. Ital. Biol. Sper.*, 1940, **15**, 484.
4. Rossi-Fanelli, A. *Arch. sci. Biol.*, 1940, **26**, 244.
5. Rossi-Fanelli, A. *Boll. Soc. Ital. Biol. Sper.*, 1941, **16**, 768.
6. Rossi-Fanelli, A. *Boll. Soc. Ital. Biol. Sper.*, 1942, **17**, 206.
7. Rossi-Fanelli, A. *Boll. Soc. Ital. Biol. Sper.*, 1947, **23**, 119.
8. Theorell, H. *Biochem. Z.*, 1932, **252**, 1.
9. Theorell, H. *Arch. Biochem.*, 1947, **12**, 119.
10. Van Slyke, D. D. *J. biol. Chem.*, 1911–12, **10**, 15.

Differential Effects of 2,4-D on Aerobic, Anaerobic, and Facultative Anaerobic Microorganisms

Winfield A. Worth, Jr., and Anne M. McCabe

Department of Pathology,
Duke University School of Medicine

2,4-Dichlorophenoxyacetic acid (2,4-D) is recognized biologically as an auxin (plant hormone) or growth-regulating substance. It is well known that 2,4-D has a selective activity on the growth rate of certain plants. The mode of action of the auxin is still a matter of conjecture, but several workers indicate that 2,4-D is involved in some way in the process of plant respiration (*1, 6*). Hsueh and Lou (*3*) developed this idea more fully in their experiments with germinating rice and barley seeds, which they treated with various concentrations of 2,4-D before allowing them to germinate under aerobic and anaerobic conditions. The germination of barley, a typical aerobic seed, was entirely inhibited, whereas that of rice, a seed known to be able to germinate anaerobically, was only partially inhibited. The results of their experiments seemed to indicate that the rate of germination of the treated barley seeds closely paralleled that of the untreated barley seeds which were grown in an anaerobic atmosphere. It appeared as if oxygen were no longer available to the treated seeds.

That 2,4-D can display bacteriostatic and bacteriocidal properties has been shown by Stevenson and Mitchel (*5*) and Lewis and Hamner (*4*). Dubos (*2*) observed that a number of synthetic, unsaturated, ring-containing acids, including 2,4-D, endowed with auxin activity exerted a bacteriostatic effect on the growth of certain microorganisms. However, no correlation has been made between the effects of 2,4-D on bacteria with respect to the oxygen requirements of bacteria.

The purpose of this report is to demonstrate the effects of different concentrations of 2,4-D on those organisms which vary in their utilization of oxygen.

The organisms were divided into three groups, depending on the amount of oxygen required by them for normal growth. Among the aerobes, or those bacteria which must have free oxygen, *Rhizobium trifolii, R. phaseoli, R. japonicum,* and *Azotobacter chroöcoccus* were selected. These were obtained from the American Type Culture Collection at Georgetown University. The anaerobes, those organisms which will grow only when oxygen is excluded, were represented by *Clostridium welchii, Cl. tetani,* and *Cl. botulinum.* The facultative anaerobes, those aerobic organisms which can grow anaerobically, were selected at random and included *Escherichia coli, Staphylococcus albus,* and *Candida albicans.* The anaerobes and the facultative anaerobes were obtained from clinical material in Duke Hospital. It was necessary to employ different media for the various organisms. A special formula supplied by the American Type Culture Collection was used for *R. trifolii,* while the Waksman medium #79 was used for the remaining aerobes. Brain-heart infusion agar was used for the anaerobic and facultative anaerobic organisms. All of the media employed were adjusted to a pH of 7.4.

Sodium 2,4-dichlorophenoxyacetate, obtained from the J. T. Baker Chemical Company, was used throughout the present study. Solutions of the 2,4-D were prepared and diluted with distilled water to concentrations of 5, 1, 0.1, 0.01, and 0.001% and autoclaved for 15 min. at 18 lbs pressure. Varying amounts of these solutions were measured into a series of tubes of the agars used to give a final volume of 5 cc of agar-2,4-D solution/tube. The final concentrations of 2,4-D, after taking into account the dilution factor of the agar, amounted to 2, 1, 0.2, 0.02, 0.002, and 0.0002%/5 cc of diluent. The number of grams of 2,4-D/5 cc of agar were 0.1, 0.5, 0.25, 0.001, 0.0001, and 0.00001. The agar containing the auxin was poured into level Petri dishes which contained a base of approximately 10–15 cc of the corresponding type of agar.

The organisms were subcultured for 24 hrs on agar slants, which were then washed with a measured quantity of 0.85% saline and streaked with a wire loop on the prepared plates. Each organism was tested against the 6 concentrations of 2,4-D and on control plates containing no 2,4-D. The aerobes were left at room temperature, while the facultative anaerobes were incubated at 37.5° C. The anaerobes were incubated at 37.5° C in Brewer anaerobe jars. Since control plates were employed for each organism, the differences in the temperature do not affect the interpretation of the results obtained. The amount of growth was read at 24-, 48-, and 72-hr intervals. The control of each organism was considered as 4 plus growth, and the remaining plates were compared with the controls. The results are shown in Table 1. Since a human error may enter at this point, a difference of 1 plus in any two recordings should not be interpreted as too significant.

In general, all four of the aerobes tested were inhibited by 2,4-D, particularly in the higher concentrations. At the 48- and 72-hr readings it appeared as if the lower concentrations of 2,4-D increased the amount of growth. No inhibitory effects were observed with the facultative anaerobic organisms, and in the lower concentrations there was an increase in the amount of growth over that on the control plates. The response of *Cl. tetani* resembled that of the facultative anaerobes in that there was an increase in growth with the lower concentrations and no apparent inhibition at higher concentrations. *Cl. welchii* and *Cl. botulinum*, however, vary in their response to 2,4-D, and it is difficult to formulate definite conclusions. There is some slight

TABLE 1

COMPARISON OF AMOUNT OF GROWTH OF MICROORGANISMS ON MEDIA TREATED WITH 2,4-D

	Organism	Time of reading (hrs)	Control	Amount of 2,4-D/5 cc of agar expressed in per cent and grams					
				2% (0.1 gm)	1% (0.05 gm)	0.2% (0.025 gm)	0.02% (0.001 gm)	0.002% (0.0001 gm)	0.0002% (0.00001 gm)
Aerobic organisms	*R. trifolii*	24	4	0	1	3	4	4	4
		48	4	0	2	4	5	5	5
		72	4	1	4	2	5	6	6
	R. phaseoli	24	0	0	0	0	0	0	0
		48	4	0	0	0	0	0	3
		72	4	0	0	1	3	4	4
	R. japonicum	24	0	0	0	0	0	0	0
		48	4	0	0	0	0	0	0
		72	4	0	0	0	1	0 (1)	0 (1)
	A. chroöcoccus	24	4	0	0	0	0	0	2
		48	4	4	4	4	4	4	4
		72	4	4	4	4	4	4	4
Facultative anaerobic organisms	*E. coli*	24	4	4	4	4	5	4	4
		48	4	4	4	5	5	5	4
		72	4	4	4	5	5	5	4
	Staph. albus	24	4	4	5	6	6	6	5
		48	4	4	5	6	6	6	5
		72	4	5	5	6	6	5	5
	C. albicans	24	4	4	4	5	5	4	4
		48	4	4	4	5	5	5	4
		72	4	4	4	5	5	5	4
Anaerobic organisms	*Cl. welchii*	24	4	2	3	3	2	4	3
		48	4	3	3	3	1	4	4
		72	4	4	4	4	2	4	4
	Cl. botulinum	24	4	1	1	3	3	3	3
		48	4	3	1	3	2	4	3
		72	4	3	2	3	2	4	2
	Cl. tetani	24	4	4	5	4	5	4	4
		48	4	3	5	4	2	4	2
		72	4	3	5	4	4	4	4

Key: 0 = no growth, 0 (1) = slight growth, 1 to 2 = poor growth, 3 = good growth, 4 = growth on control plate, 5 to 6 = growth exceeding that on control plates.

inhibition of these two organisms with the highest concentrations, but this result is insignificant when compared with the response of the aerobes. The repeated exposure of these latter clostridia to atmospheric oxygen at the three periods of recording may have affected their rate of metabolism in sufficient degree to cause the irregular effects produced.

From the data accumulated it appears that those organisms which require free oxygen for respiration are "smothered" by 2,4-D. They react in a manner similar to the germinating barley seeds as reported by Hsueh and Lou. Those organisms capable of anaerobic respiration only are not affected to any significant degree by 2,4-D.

References

1. COMMONER, B., and THIMANN, K. V. *J. gen. Physiol.*, 1941, **24**, 279–297.
2. DUBOS, R. J. *Proc. Soc. exp. Biol. Med.*, 1946, **63**, 317.
3. HSUEH, V. L., and LOU, C. H. *Science*, 1947, **105**, 283–285.
4. LEWIS, R. W., and HAMNER, C. L. *Mich. agric. exp. Sta. Quart. Bull.*, 1946, **29**, 112–114.
5. STEVENSON, E. C., and MITCHEL, I. W. *Science*, 1945, **101**, 642–644.
6. THIMANN, K. V. *Collecting Net*, 1940, **15**, 66–69.

The Relative Sensitivities of Bacterial Viruses to Intense Sonic Vibration[1]

THOMAS F. ANDERSON, SHIELA BOGGS, and BETTY C. WINTERS

Johnson Foundation, University of Pennsylvania

It was thought that the multiplication of bacterial viruses might be followed up to the moment of natural lysis by sonically disintegrating infected bacteria at various times after infection. An electron microscope study of the debris might then reveal the various morphological stages in virus proliferation, while counts of infectious particles would permit estimation of the rate of appearance of mature particles. However, preliminary studies of T2 virus, which, because of its easily recognizable tadpole shape, would be ideal for this purpose, showed that it is even more rapidly disintegrated by sonic vibration than the host cells and, therefore, would be unsuited for the proposed investigation (*1*). In an attempt to find suitably resistant viruses we have followed the sonic inactivation of each of a set of 7 viruses (T1–T7) (*5*) active on *E. coli*, strain B. Since an interesting correlation between structure and sensitivity was obtained, we are recording the results here.

Forty-cc filtered samples of each of the bacteriophages were treated separately in the water-cooled cylindrical cup of a magneto-striction sonic oscillator manufactured by the Raytheon Manufacturing Company. Samples were removed at intervals and, together with the untreated control, were assayed for virus activity by the plaque count method. Samples of the host bacteria were treated in an analogous manner and their survivals determined by colony counts.

TABLE 1

PERCENTAGE SURVIVAL OF VARIOUS BACTERIOPHAGES AND OF THEIR HOST, *E. coli* STRAIN B, AFTER EXPOSURE TO INTENSE SONIC VIBRATION

Length of exposure (min)	Bacteriophage							*E. coli* Strain B
	T1	T2	T3	T4	T5	T6	T7	
1	92	73	70	50	30	....	100	80
5	34	1.8	80	0.8	1.6	0.9	60	18
10	30	.07	80	0.009	0.07	0.008	40	1
30	10	..	40	....	....	....	12	0.016
60	1	..	0	....	....	....	1.1	....

In Table 1 are given the results of a typical series of experiments. It is seen that viruses T2, T4, T5, and T6 are more rapidly inactivated than the host bacteria, while the remaining three viruses, T1, T3, and T7, are remarkably resistant to sonic vibration.

These results are interesting in relation to the morphologies of these viruses as seen in the electron microscope (*2*). The resistant viruses T3 and T7 appear to be small spheres 450 A in diameter (*3*, *4*) while T1 is a similar small sphere but with a faint, 1,200-A-long tail attached (*6*). In contrast, the vibration-sensitive viruses T2, T4, and T6 are relatively large, tadpole-shaped structures with frequently pointed heads 600×800 A, consisting of an internal structure and surrounding membrane to which a well-defined tail approximately 1,000 A long is attached (*2*). Likewise, the sensitive virus T5 has a large, round head about 900 A in diameter, also consisting of a membrane surrounding internal structures and with a faint tail some 1,700 A long attached (*2*). It seems likely that the sensitive viruses with their relatively large and complex structures are mechanically disintegrated by intense vibration, while the small, compact viruses are relatively resistant to the shearing forces existing during cavitation of the liquid in which they are suspended.

[1] This work was supported by Contract N6–ori–168, T.O. II between the Navy Department and the Trustees of the University of Pennsylvania and by a grant from the Raytheon Manufacturing Company.

In the oscillator (Type R-22-1) used for this work the vibrating system consists of a stainless-steel diaphragm which forms the base of the specimen cup and laminated nickel strips, the ends of which are attached to the diaphragm. The system is caused to vibrate by oscillating magnetic fields set up by a solenoid surrounding the nickel strips and on which the cup rests. A power supply operating on 110-volt, 60-cycle current and tuned to resonate with the mechanical system provides the driving power which is transmitted to the sample by the vibrating diaphragm. Cooling water flows through a jacket surrounding the cup and sprays over the nickel strips within the solenoid. The average temperature of the specimen is thus held to within a few degrees of that of the cooling water, even during cavitation of the liquid.

References

1. ANDERSON, T. F. *J. Bact.*, 1943, **45**, 303.
2. ANDERSON, T. F. *Cold Spr. Harb. Symp. quant. Biol.*, 1946, **11**, 1.
3. DELBRÜCK, M. *Harvey Lecture Ser.*, 1945–46, **41**, 161.
4. DELBRÜCK, M. *Biol. Rev.*, 1946, **20**, 30.
5. DEMEREC, M., and FANO, U. *Genetics*, 1945, **30**, 119.
6. LURIA, S. E., and ANDERSON, T. F. *Proc. nat. Acad. Sci.*, 1942, **28**, 127.