stands on a removable base; this permits convenient separate manipulation of the countershaft, flask-holder and box.

Before grinding, the bacteria are frozen and dried, either in the lyophil apparatus of Flosdorf and Mudd,[8] or by the desiccator-method.[9] Weighed amounts are then placed in a flask, which is tightly stoppered as previously described, and cooled to —75°C. in the bath used for the mill. If the flask is stoppered before chilling and opened while still cold, there will be a rush of air which will blow the bacterial powder inward from the neck of the flask, instead of into the surrounding atmosphere, an occurrence that will take place if this sequence is not followed.

After being properly chilled the flask is firmly fastened in the holder,† so that it is immersed about an inch in the methyl cellosolve. The top is placed on the box and the motor started. The large masses of dried bacteria are quickly broken up; and the bacteria form a coating over the inner surface of the flask and around the balls. The latter act like hundreds of little hammers which hit and grind the cold, brittle bacteria. It has been found that hemolytic streptococci, in a quantity of 1 gm. dry weight, are effectively disrupted after one to 3 hours' grinding in this cold mill.

This technic has been found superior to grinding bacteria which have been frozen in a thin film on the inner surface of the flask without preliminary drying, a method which is somewhat comparable to that described by Macfadyen and Rowland or by Johlin and Avery. The results of studies of material obtained from desiccated bacteria ground in this mill will be reported later.

9494 P

Photodynamic Action of Methylene Blue on Poliomyelitis Virus.*

L. A. ROSENBLUM, B. HOSKWITH AND S. D. KRAMER.

From the Laboratories of the Infantile Paralysis Commission of the Long Island College of Medicine, and the Jewish Hospital of Brooklyn.

Reitz[1] reviewed the effects of the combined action of light and dyes on a number of bacteria, and found that methylene blue, among other dyes, was a very active photodynamic agent. More recently,

[8] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

[9] Swift, H. F., *J. Bact.*, 1937, **33**, 411.

* This work supported by grants from the Friedsam Foundation and from the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Reitz, A., *Zentralbl. f. Bakt.*, 1908, **45**, 270, 374, 451.

Tung[2] has also studied the photodynamic effect of methylene blue on a number of microörganisms. Perdrau and Todd[4] have reported on the photodynamic effect of methylene blue on a number of viruses including vaccinia, herpes, and canine distemper. Their method consisted of exposing dilute mixtures of methylene blue and the virus filtrate to the radiation of a 100 c.p. pointolite light, at a distance of 20 cm. from the petri dishes which contained the mixtures in 2 mm. layers. The final methylene blue concentrations used varied from 1:10,000 to 1:100,000 and the exposure times ranged from 5 to 30 minutes. After irradiation, the infectivity of the mixtures was tested in animals. Their results indicated that most of the viruses studied were completely inactivated by the above treatment. Perdrau and Todd[4] furthermore observed that although the virus of canine distemper was inactivated, it remained antigenic, and animals treated with this inactivated virus did not develop signs of the disease but showed considerable resistance to infections with the active virus. Shortt and Brooks,[5] however, could not demonstrate any immunity produced by inoculating animals with photodynamically inactivated rabies virus.

This report deals with the photodynamic action of methylene blue on the virus of poliomyelitis.

The methods employed were essentially those of Perdrau and Todd.[3, 4] The methylene blue was Grubler's Methlenbalu, f. Bac. Koch, which was autoclaved when made up to twice the required dilution with saline (See Table I). To insure uniformity only small amounts were made up at frequent intervals.

The virus was obtained from spinal cords of monkeys that were sacrificed at the height of the disease. Cord fragments were ground with sand in physiological saline solution to make 5% suspensions and filtered through Seitz filters. The following manipulations were carried out in a photographic dark room by the light of an orange safelight. Equal amounts of virus suspensions and prepared dilutions of methylene blue were thoroughly mixed. Ten cc. of each of the mixtures was pipetted into sterile petri dishes, thus forming a layer of about 2 mm. at the bottom of each dish. For exposures, the dishes were placed on white paper and the lids slightly raised to prevent a film of water of condensation forming on the upper lids. However, if clouding resulted, the top lids were replaced with dry, sterile ones. Subsequent experiments showed that exposures in a

2 Tung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 328.

3 Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. B.*, 1933, **112**, 288-298.

4 Perdrau, J. R., and Todd, C., *J. Comp. Path. and Therap.*, 1933, **46**, 79.

5 Shortt, H. E., and Brooks, A. G., *Indian J. Med. Res.*, 1935, **22**, 557.

TABLE I.
Photodynamic Action of Methylene Blue on Poliomyelitis Virus.

Date	Monkey No.	Methylene Blue Final Dilution	Time Exposed	Outcome
		Experiment I.		
1/5/37	J249	1:50,000	30 min.	Survived
	J250	"	30 "	Prostrated, sacrificed in 12 days
	Controls			
	J251	"	Not exposed	" " " 11 "
	J252	Saline	30 min.	" " " 7 "
	J253	"	Not exposed	" " " 6 "
		Experiment II.		
2/5/37	J259	1:50,000	45 min.	Survived
	J260	"	45 "	Prostrated, sacrificed in 14 days
	Controls			
	J261	"	Not exposed	" " " 8 "
	J262	Saline	" "	" " " 8 "
		Experiment III.		
4/7/37	J268	1:75,000	60 min.	Survived
	J267	"	60 "	"
	J271	1:100,000	60 "	"
	Control			
	J274	Saline	Not exposed	Prostrated in 10 days; sacrificed in 19 days
		Experiment IV.		
4/21/37	J301	1:75,000	45 min.	Prostrated, sacrificed in 10 days
	J303	"	60 "	Survived
	J302	1:100,000	45 "	Prostrated, sacrificed in 14 days
	J304	"	60 "	Survived
	Control			
	J300	Saline	Not exposed	Prostrated, sacrificed in 6 days

dry, well ventilated room did not produce clouding. The source of light was a new 100 watt bulb placed directly above the plates at a distance of 20 cm. (As indicated in Table I, time and concentrations of dye were varied.) Following exposure, 2 cc. was removed from each plate and kept in a blackened test tube until inoculated intracerebrally into *Macacus rhesus* monkeys. Those animals that succumbed were autopsied and the diagnosis confirmed by histologic study.

The following controls were included: (a) for light, unexposed mixtures of virus and methylene blue, (b) for dye, saline was substituted for methylene blue, (c) for virulence, unexposed plates of equal parts of saline and virus. Experiments in which the virulence controls did not succumb within the incubation period were excluded.

Table I gives the results of the experiments. It will be noted that methylene blue in concentrations from 1:50,000 to 1:100,000 will inactivate the virus of poliomyelitis when exposed to the light of a 100 watt bulb under the experimental conditions. Inactivation was most constantly obtained with dilutions of 1:100,000 of the dye and an exposure of 60 minutes.