HUMAN LEPRA BACILLI EXPOSED TO SUNLIGHT WILL RETAIN THEIR ACID FASTNESS IF THEY ARE FIRST HEATED

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In 1949, Dharmendra and Mukerjee (1) showed that when smears of leprosy tissue rich in bacilli were exposed to sunlight, and subsequently stained by the Ziehl-Neelsen method, hardly any bacilli were seen on slides which had been exposed for eighteen hours. Protection of the smears with pieces of black paper prevented the change, which was not therefore due to the heat of the sun. Further tests showed that the ultra-violet rays produced the change but not the infra-red ones—furthermore the sun's rays had no such effect on rat leprosy bacilli.

In 1951 Mukerji (2) was able to produce similar loss of staining ability after five hours exposure to intense sunlight, also after two hours to ultra-violet and infra-red irradiation and after half an hour to X-rays. Smears from rat leprosy were hardly affected by similar treatment, although "beading" was caused in some of them by exposing them to X-rays of 250 r. for twenty-seven minutes.

It had occurred to the present writer that the neuro-ectodermotropism of the human leprosy bacillus as well as its failure to grow on artificial media and possibly certain other of its properties might be due to the parasitization of it by a bacteriophage, bacterial virus, or inhibitory plasmagene, itself harmful in differing degrees to different people.* It is interesting to note that Richards and Wade (3) as a result of their examination of human leprosy bacilli by phase microscopy wrote:—"In examining the preparations from leprosy lesions we gained a general impression of a mixture of rods apparently in good condition but varied in appearance, others in evidently poor condition grading down to apparent residual or ghost bodies, and conspicuous among them and the background debris free, bright granules which could not be dismissed as mere degenerative fragmentation; some of them resembled bacteriophage in general appearance."

It was considered worth while therefore, despite the very limited apparatus at the writer's disposal to see whether the effect of sunlight in causing the human leprosy bacillus to lose its staining properties could be inhibited, and accordingly some pilot experiments were undertaken. It was found for example that when slides

* Dr. Corcos' evidence for this has been placed before workers familiar with the phage phenomenon, and the general conclusions were that such an explanation is unlikely to be correct.—Editor.

were strongly heated, smear side upwards, in the spirit flame until they were too hot to touch, the effect of sunlight on the staining properties (as demonstrated by the numbers of bacilli afterwards seen) appeared to be less than that on unfixed smears; but it was considered that this effect might be relative and partly due to " weathering " i.e. loss of bacilli from the unheated slides from mechanical causes irrespective of their biological state.

It was also found that bacilli which were possible to stain were present, on examination of fixed smears that had been boiled with water *on the slides*, and that these bacilli were still capable of staining after prolonged exposure to sunlight; however this rather crude method was unsatisfactory because it became obvious that boiling for a sufficient length of time to affect the presumed viability of the bacilli might itself be a potent cause of weathering.

According to Stitt, Clough and Branham (4) many bacilli may be lost from a slide if the usual routine of fixation is followed, and for tubercle bacilli these authors recommend that films be passed four or five times through a flame and fixed with absolute methyl or ethyl alcohol. It was considered however that by applying such methods to the lepra bacillus already on the slide it would not be possible to distinguish any possible killing action of the heat or alcohol from their fixative action.

It was eventually decided to compare the effect of sunlight and darkness upon (virtually) unfixed smears of boiled and unboiled leproma tissue from the same patient and the following experiment was therefore devised.

Methods used.

The only available patient at that time was a woman with severe active nodular lepromatous leprosy who had had two years treatment with hydnocarpus oil. She had however had no treatment at all during the two years prior to the present experiment and her skin smears showed enormous numbers of normally staining bacilli and globi.

From both ears of this patient nodules of approximately the same size were removed. That from the right ear was untreated and smears were made from it directly on to one end of each of fourteen clean microscopic slides which were allowed to dry in the shade, while the nodule from the left ear was boiled for half an hour in distilled water, as for the first stage of making lepromin. The water was poured off and smears were made from the boiled nodule on to the other ends of the slides. All the slides were now drawn *once* rapidly over the flame of a spirit lamp so that they became only just warm to the back of the hand.

Seven of the slides were then exposed to bright sunlight for a total of 24 hours on three successive days while the other seven were kept in a closed slide box except for 20 hours during two dark nights when the slide box was left outdoors with the lid open. Altogether the exposed slides had 24 hours of sunlight and approximately 29 hours in the dark in a closed slide box, while the unexposed slides were in the dark for 53 hours before being stained and examined.

In order to reduce the possibility of acid-fast saprophytic mycobacteria from the materials and apparatus used being mistaken for leprosy bacilli six blank sides were treated with distilled water from the same bottle that contained the water used for boiling the leproma tissue; the water was allowed to evaporate from the slides in the shade and three of them were then placed with the exposed slides, while three were placed with the unexposed ones. They were stained and examined at the same time and by the same methods as the experimental slides.

Immediately prior to examination all the slides were drawn four times through a flame, treated with absolute methyl alcohol and again flamed. They were stained by the Ziehl-Neelsen hot method, decolourized with 5% sulphuric acid and counterstained with methylene blue, a slide rack being used and care being taken to give all slides equal times in carbol fuchsin, acid and counterstain. They were then stood on end on a grooved board and allowed to dry—they were not blotted. Each smear was now carefully examined with the 1/12 oil immersion objective, using first the X5 and then the X12 eyepiece and the scheme shown is used to express the results:—

+ + + + + +—Vast numbers of bacilli in all fields examined.
+ + + + + —Vast numbers of bacilli in 50% of fields examined, large numbers in the other 50%.
+ + + + —Large numbers of bacilli in all fields examined.
+ + + —Large numbers of bacilli in 50% of fields examined. fewer bacilli or none in the other 50%.
+ + —Some bacilli in 50% of fields examined.
+ —Some bacilli in 10% or less of fields examined.
± —Single scattered bacilli present in smear, only identified with difficulty.
– —No bacilli seen.
G—Globi.

(Presence or absence of globi could not be taken into account in expressing bacillary density since it was found that in the smears of the boiled leproma all globi had been broken up and the bacilli

were scattered unevenly about, and, for example smear " Dark—Boiled I." contained vast numbers of bacilli but no globi, whereas smear " Dark—Untreated 3." contained fewer bacilli but some globi.)

	EXPOSED.		DARK.	
	Untreated leproma.	Boiled leproma.	Untreated leproma.	Boiled leproma.
1.	... −	+ + + +	+ + + + G	+ + + + + +
2.	... ±	+ + +	+ + G	+ + + + + +
3.	... −	+ + +	+ + G	+ + + + + +
4.	... ±	+ + + + +	+ + + G	+ + + + +
5.	... ±	+ + + + + +	+ + + + + G	+ + + +
6.	... +	+ + + + +	+ + + + G	+ + + +
7.	... ±	+ + + + + +	+ + + + + G	+ + + + +
	BLANK.		BLANK.	
1.	...	−	−	
2.	...	−	−	
3.	...	−	−	

Discussion of Results.

These results appear to confirm those of Dharmendra and Mukerjee but go rather further in that they seem to show that heating of human leprosy bacilli by boiling them " protects " them from the effect of sunlight. The situation at present then, seems to be that the actinic rays could in theory either be disintergrating the bacilli or be merely rendering them resistant to ordinary staining methods; the point might later be decided by dark-ground, electron microscopy and phase contrast microscopy methods. It is hoped that others will be able to repeat and extend these irradiation experiments, for example, it would be particularly interesting to know what, if any, effect ultra-violet light and X-rays have upon chloroform treated bacilli.

The present writer hopes to undertake further work in order to try and find out for how long boiled lepra bacilli can be exposed to sunlight without losing their acid-fastness. If as he believes, sunlight only affects the staining properties of the living bacilli but not those of dead ones, it should be possible to expose heated bacilli more or less indefinitely without their losing their ability to stain, and some preliminary experiments already strongly suggest that such is indeed the case.

Summary.

The effects of sunlight and darkness upon smears made from boiled and unboiled leproma tissue taken from the same patient are compared. Reasons are given why the results obtained were to be expected.

Acknowledgment.

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