INFECTED AIR-BORNE PARTICLES LIBERATED ON OPENING SCREW- CAPPED BOTTLES

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The problems of the risk of laboratory infection have been investigated over the past few years. Wedum (1953) and Wedum and Reitman (1956) have shown that infected particles are liberated into the air during the performance of many of the procedures commonly carried out in bacteriological laboratories. The techniques used by laboratory workers to perform the same operation often differ widely, and it has been possible to believe that, with a perfect technique, the risk of producing infected aerosols becomes infinitely small, and that laboratory infections arise only from carelessness or in-different technique.

Some of the techniques used in the routine laboratory diagnosis of tuberculosis in Britain were not included in the American investigations, and in particular the danger of infected aerosols arising from the use of screw-capped bottles for bacterial cultures are not mentioned. Partly for the reason that there appeared to be no likely technical variation in the removal of caps from screw-capped bottles and partly because such bottles are commonly used in routine diagnostic laboratories in Britain, the present investigation was confined to the removal of screw caps from bottles containing slope cultures of various organisms.

Methods

A slit-sampler sampling 20 cu. ft. (0.566 cu. m.) of air per minute was used for the detection of infected aerosols. A slit-sampler is usually used to sample air equally from all directions: for the first experiments it seemed desirable to limit the air entering the sampler to air from a fairly narrow sector, since this might be expected to increase the recovery of infected aerosols. For this purpose a paper hood was fitted over the air intake: this had an opening in a vertical plane of approximately 6 by 7 in. (15 by 17.5 cm.) through which air flowed at a velocity of about 70 ft. (21.3 m.) per minute (just detectable on the hand). The bottles were opened about 2 in. (5 cm.) in front of the centre of the opening in the hood. In some experiments the agar plate was allowed to rotate in the usual manner and a number of bottles were opened in rapid succession: in other experiments the infected aerosol formed on opening individual bottles was detected by closing two of the four slits, and starting and stopping the motor so that the infected particles liberated appeared as a line of colonies across the agar plate. This cut down the air flow by about one-half, but did not otherwise interfere with the working of the apparatus.

Infection of the mouth of the bottle was detected by running a sterile loop over the rim and plating on to nutrient agar; an alternative method that was quicker and more economical was to bring the surface of an inverted agar plate down on to the exposed rim of the bottle and then incubate the plate.

Screw-capped bottles of different sizes were used in different experiments; any bottle in which the sloped medium came above the shoulder of the bottle was discarded. The majority of the experiments were carried out using *Chromobacterium prodigiosum* as the test organism, but essentially similar results were obtained with *Staph. aureus* and *Salm. typhimurium*.

First Experiment

A number of preliminary experiments were carried out on groups of 12 or 25 slope cultures in screw-capped bottles. In all of these experiments some infective airborne particles were detected, and in one experiment the colonies appeared on the agar plate in a way that suggested that they had all been liberated at one time. An experiment was therefore carried out in which the bottles were opened individually; at the same time cultures were made from the bottle rims and a record was kept regarding whether the rims were obviously wet or dry (Table I). This was not always easy to determine, since the amount of fluid was often small; some inaccuracies must therefore be expected in this last observation.

TABLE I.—*Infected Aerosols Formed on Opening ½-oz. (14-ml.) Screw-capped Bottles of Slope Culture of Chr. prodigiosum. Each Bottle opened Once Only*

Apparent State of Rim	No. of Bottles with Rim Infected			No. of Bottles with Rim Not Infected			Total
	Aerosol +	Aerosol -	Total	Aerosol +	Aerosol -	Total	
Dry ..	8	1	9	0	65	65	74
Wet ..	35	13	48	1	74	75	123
Total ..	43	14	57	1	139	140	197

It will be seen that the majority of bottles with infected rims produced a bacterial cloud on opening. Bottles with apparently dry rims were rarely infected. The number of colonies recovered varied from 1 to 50 a bottle. The figures in Table I refer to the first opening of each bottle; in some experiments bottles were opened several times. The results were consistent in that bottles with uninfected rims did not produce aerosols. Bottles with infected rims generally produced aerosols, but the number of colonies recovered did not bear any direct relation to the number of times the bottle had been opened. On two occasions bottles with infected rims that had not given a demonstrable aerosol on first opening did so subsequently.

Second Experiment

It seemed desirable to confirm under rather different conditions the observation that infected air-borne particles were formed when bottles with infected rims were opened. Two experiments were carried out in a closed chamber of 300 cu. ft. (8.5 cu. m.) capacity. Twenty-five bottles with infected rims were opened on a bench and the air was sampled by a slit-sampler (20 cu. ft. (0.566 cu. m.) per minute) approximately 6 ft. (1.8 m.) away with its air intake about 2 ft. (60 cm.) above the bench. The slit-sampler was run for four periods of two minutes, after each of which half a minute was spent changing the plates. Bottles were opened during the second two-minute period. The results are given in Table II.

TABLE II.—*Aerosol Formation on Opening 25 Screw-capped Bottles: Rims Infected with Chr. prodigiosum*

No. of colonies per 40 cu. ft. of air (1.13 cu. m.)	Exp. A B	Time in Minutes			
		0-2 (Control Period)	2½-4½ (Bottles Opened)	5-7	7½-9½
0	0	0	11	14	7
0	0	0	9	20	10

Infected particles began to appear when the bottles were opened and persisted until the end of each experiment. Assuming no natural "die away," that recovery by slit-sampler was complete, and that no air was sampled twice, 50 bottles gave rise to $71 \times 300/120$ infected particles = 3.5 per bottle. This is clearly a minimum figure.

Third Experiment

It seemed clear that the infected rim was the source of the aerosol infection on opening the bottle. The following

experiments were carried out to try to find the source of this infection. Half-ounce (14-ml.) screw-cap bottles containing agar slopes were inoculated by the following methods:

Method A.—The tip of a straight wire was infected by touching a colony of *Chr. prodigiosum* and the slope inoculated by a single touch at the bottom. Great care was taken to avoid touching the rim of the bottle.

Method B.—About $\frac{1}{4}$ in. (2 cm.) of a straight wire was infected by passing it through a stab culture. The slope was inoculated as a single streak from the bottom. It was found practically impossible to avoid touching the rim with the wire during this procedure.

Only one out of 24 bottles inoculated by Method A had an infected rim; after inoculation by Method B, 22 out of 24 bottles had infected rims.

The fluid on the rims of uninoculated bottles gave a strong ninhydrin reaction and was presumably a transudate of nutrient agar—that is, nutrient broth. Twelve bottles were inoculated by method B, the rims of alternate bottles were swabbed, and the viable organisms were counted. Four bottles gave a count of fewer than 30, one of 60, and one of 450. After overnight incubation the remaining six bottles were examined in the same way, and all gave counts greater than 4 million. Clearly bacterial growth occurs in the fluid on the rims.

A number of bottles inoculated by method B were flamed after inoculation before the caps were replaced; the bottle necks were held in the Bunsen flame for five seconds. In the control series of unheated bottles, 31 out of 36 had infected rims; of the flamed bottles 4 out of 36 had infected rims. Flaming for periods up to 10 seconds with rapid rotation did not invariably sterilize the necks: a number of bottles were broken as a result of heating.

When Pasteur pipettes were used to inoculate slopes by as scrupulous a technique as possible, 8 out of 24 bottles had infected rims. In another series, flamed as before, 4 out of 24 rims were infected. As a check on individual technique, three competent technicians familiar with this work were asked to inoculate twelve slopes each with a Pasteur pipette; 9 out of 36 bottles had infected rims. Inoculated bottles with uninfected rims were completely inverted, incubated for four hours, and re-examined. All rims were infected.

Further Experiments

Dr. O. M. Lidwell, of the Air Hygiene Laboratory, Colindale, has estimated the size of the infected particles liberated when screw-capped bottles are opened with a new sampling device (Lidwell, in preparation) which separates the airborne cloud into four ranges of particle size. He reports: "In each of two experiments, 25 bottles were opened in our experimental chamber. At least 30%, and probably nearly 50%, of the approximately 60 bacteria-carrying particles recovered from the air are estimated to have had diameters of 4 microns or less. Such particles will be small enough to penetrate into the lung, a fact which may be of consequence when considering risks from handling cultures of *Myco. tuberculosis*. Many other clouds of airborne bacteria consist in the main of much larger particles."

If, when opening the bottles, it was possible to avoid breaking the film of bacterial growth on the rim, no infected aerosol would be produced. The most promising material for sealing bottles is "parafilm" (Gallenkamp); this material forms a good seal on the neck of the bottles with the warmth of the hand, and bottles can be opened by piercing the film with a hot wire and resealed with a fresh piece of parafilm. We have used parafilm on one-half of our routine cultures for *Myco. tuberculosis* for six months with satisfactory results: no attempt was made to sterilize the parafilm, which was applied to the bottles of Löwenstein-Jensen media immediately after they were inoculated with sputum concentrates. The great disadvantage of the material is that, after autoclaving discarded cultures sealed with parafilm, it is difficult to remove the sticky mass from

the neck. Bottles containing slope cultures of *Chr. prodigiosum* inoculated by method B and sealed with parafilm have been opened with a hot wire. Only one infected airborne particle was detected when 30 bottles were opened, although at least 23 had infected rims. In a control series of the same bottles using screw caps, 15 out of 28 gave a demonstrable infected aerosol. In another experiment in the closed chamber of 300 cu. ft. (8.5 cu. m.) capacity, 11 infected particles were detected in three periods of two minutes when 25 screw-capped slope cultures were opened, but none when a similar series of bottles sealed with parafilm were opened with a hot wire.

Discussion

These experiments were carried out to try to assess some of the risks that are involved in handling cultures of *Myco. tuberculosis* in a routine diagnostic laboratory. For much of this work screw-capped bottles are commonly used, and it is clear that opening these bottles when they contain a slope culture may cause infected airborne particles to be liberated. The way in which this comes about appears to be the accidental infection of fluid on the rim at the time of inoculation, and the multiplication of the organisms in this fluid. When the bottle is opened, the film of broth culture on the rim is broken and some of the resulting droplets are small enough to form aerosols. A high proportion of the infected particles formed in this way are of such a size that if inhaled they would penetrate to the alveoli of the lung.

No attempt has been made to demonstrate the formation of infected aerosols on opening bottles containing cultures of *Myco. tuberculosis* because of technical difficulties, but there do not appear to be any reasons why the findings reported above, using *Chr. prodigiosum*, should not apply to cultures of *Myco. tuberculosis*. The rims of a group of cultures of tubercle bacilli were cultured, and it was found that 7 out of 197 were infected and therefore presumably capable of producing an infected aerosol when the bottles were opened.

It is possible with a very careful inoculation technique to avoid infecting the rim of the bottles, but if the bottle is inverted or handled in such a way that the infected water of condensation comes into contact with the cap, the rim will become infected and the result of the careful inoculation technique will be nullified. Competent technicians appear to infect about one-quarter of the bottles they inoculate with a Pasteur pipette; this is a common method of inoculating sputum concentrates when culturing for *Myco. tuberculosis*. Intense flaming of the neck of the bottle after inoculation does not appear to be a complete safeguard, and one must assume that, in spite of the most careful techniques in inoculation and subsequent handling, a proportion of cultures will give rise to infected aerosols on opening.

The use of a plastic seal may offer a solution to this particular problem, but there are other manipulations in the work of a routine diagnostic laboratory which have not been investigated and which may be more important sources of infected air-borne particles.

It is difficult for laboratory workers to assess the risk involved in their own laboratory; the results obtained by other workers, often under rather different circumstances, cannot be repeated because the necessary apparatus for sampling air is not readily available. Cultures made from the rims of inoculated bottles may enable this risk to be estimated, since all bottles with infected rims can be assumed, from the present experiments, to be potential if not actual sources of infected aerosols.

Summary

Infected air-borne particles are liberated when some screw-capped bottles containing slope cultures of *Chr. prodigiosum* are opened.

A mechanism accounting for this observation is suggested.

A possible solution to the problem is discussed.

My thanks are due to Dr. O. M. Lidwell for his determination of particle size and to Dr. R. E. O. Williams for the loan of the slit-sampler and for his help and co-operation in the experiments in the closed chamber.

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TREATMENT OF ADRENAL CORTICAL INSUFFICIENCY

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Prior to 1951 patients with Addison's disease could be maintained only with difficulty on the replacement therapy available. Since cortisone and hydrocortisone have been in use these patients have been able to lead normal lives, and it has also been possible to perform total adrenalectomy in selected cases of malignant disease and Cushing's syndrome. Addison's disease is a rare condition, and it is not surprising that there have been differences of opinion about the place of cortisone in its treatment in relation to deoxycortone and extra salt. However, the performance of total adrenalectomy has provided an opportunity for the study of a larger group of patients who have what is in effect severe Addison's disease. Detailed observations have been made on nine patients with Addison's disease and on 30 after total adrenalectomy. The former have been seen at regular intervals for up to seven years and the latter for up to three years. Particular attention has been given to the place of 9 α -fluorohydrocortisone in the treatment of chronic adrenal insufficiency.

Hormones of the Adrenal Cortex

The two most important hormones produced by the adrenal cortex are hydrocortisone and aldosterone. Hydrocortisone is sometimes referred to as a "glucocorticoid" hormone because it stimulates gluconeogenesis; it has, however, other more important actions. If the level of circulating glucocorticoid drops abruptly, the blood pressure falls, renal function is impaired with reduction of glomerular filtration rate, and sodium tends to leave the extracellular fluid and enter the cells and possibly bone (Hills *et al.*, 1953; Mendelsohn and Pearson, 1955). Cortisone is not secreted by the adrenal glands, but there is interconversion of hydrocortisone and cortisone in the body and the actions of the two are similar. Aldosterone is the main "mineralo-corticoid" hormone of the adrenal cortex. It maintains the body sodium by limiting renal excretion and has a less conspicuous effect in increasing potassium output. Cortisone and hydrocortisone have some mineralo-corticoid action, and in large doses may produce sodium retention and oedema or potassium loss and hypokalaemia.

The other hormones of the adrenal cortex are not necessary for maintaining life. Corticosterone (compound B) has mineralo- and glucocorticoid properties, but in humans it is produced in only very small amounts. In both sexes the adrenal cortex secretes significant quantities of androgens and traces of oestrogens.

Replacement Therapy Available

Hydrocortisone is the most important hormone secreted by the adrenal cortex, and tablets of hydrocortisone or cortisone acetate are essential for adequate replacement therapy. Maintenance of sodium equilibrium is less simple because aldosterone is not yet available. In many patients the weak mineralo-corticoid action of hydrocortisone or cortisone is sufficient provided the salt intake is high. There are two powerful salt-retaining steroids that can be used. Deoxycortone was introduced for the treatment of Addison's disease in 1937 (Simpson, 1938). It must be given by frequent intramuscular injection or implantation of pellets; the sublingual route has been used, but absorption is uncertain. Microcrystalline suspensions of deoxycortone trimethyl acetate may be given by injection at monthly intervals (Thorn *et al.*, 1953). The long-acting preparations (pellets and microcrystalline suspensions) have the disadvantage that their action is not constant throughout the intervals between implantation or injection. An important practical advance in salt-retaining steroids is the fluorine-substituted 9 α -fluorohydrocortisone. Its glucocorticoid activity is twenty times that of the parent compound, whereas sodium-retaining power is increased more than one hundred times (Garrod *et al.*, 1955). Fluorohydrocortisone is readily absorbed from the alimentary canal.

Of the remaining hormones, androgens if indicated can be given in the form of methyltestosterone by the sublingual route. There is no evidence that corticosterone or oestrogen replacement is necessary.

Practical Experience

Hydrocortisone production in a normal subject may be assessed by studies with isotope-labelled steroid (Peterson and Wyngaarden, 1955), or by measurement of urinary glucocorticoid excretion (Moxham and Nabarro, 1956). The adrenal glands of a normal adult secrete 20–25 mg. of hydrocortisone a day, which is equivalent to about 25–37.5 mg. of cortisone acetate. Patients who have had total adrenalectomy and those with severe Addison's disease can usually be maintained on 37.5 mg. of cortisone a day. Because of its rapid absorption and conjugation after oral administration, cortisone should be given as evenly spaced doses of 12.5 mg. When adrenalectomy has been performed for malignant disease 50 mg. of cortisone a day is often prescribed, and the slight excess of glucocorticoid is undoubtedly beneficial in causing an increased sense of well-being and appetite. In some patients it may lead to excessive weight gain.

In many cases the cortisone will also maintain sodium balance provided the salt intake is high. If an excess of

FIG. 1.—Case 1. Total adrenalectomy for carcinoma of breast. Development of sodium-depletion crisis.