to suppose that this experimental design could help to solve the problem of insulin absorption from lipodystrophic sites.

This study has shown that injection of insulin into atrophic fat sites does not appear to alter diabetic control and, similarly, that intramuscular injections of insulin do not cause an increased variation in blood-sugar levels. Instability in diabetic patients therefore appears to be entirely independent of the site of injection of the insulin.

Similarly, in the three patients with hypertrophic lipodystrophy no fixed pattern of blood-sugar levels emerged. In only one patient did a significantly lower blood-sugar level occur when insulin was injected into the hypertrophic area. If this effect is due, as Lamar (1950) suggests, to the occasional flooding of insulin from these hypertrophic depots, then it is surprising that this phenomenon occurred only once in the whole study. It is more likely that this solitary episode of hypoglycaemia was entirely independent of the site of insulin injection.

Summary

Six unstable diabetic patients with severe atrophic lipodystrophy and three patients with severe hypertrophic lipodystrophy were studied to ascertain whether insulin lipodystrophy was a factor in their instability.

The experimental design was an adaptation of one used in insulin therapeutic trials, and each patient acted as her own control.

No pattern of significant differences was observed in the blood-sugar curves obtained from different injection sites, and therefore lipodystrophy cannot be regarded as a cause of diabetic instability.

In view of the belief that intramuscular injections of insulin cause tissue necrosis, it is of interest that no untoward effects occurred from such injections given for four to seven days.

We thank Dr. J. Adler and his assistant for the blood-sugar estimations, and the ward sisters, dietitian, and patients for their willing co-operation in these trials. We also thank Professor R. E. Tunbridge for permission to study his patients.

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The considerable educational activities of the National Association for Mental Health are summarized in its latest Annual Report for the Year 1957-8. Its longest-established course is for school medical officers and is on the ascertainment of educationally subnormal children and mental defectives. Recent changes in the school health services and in the general approach to mental deficiency pointed "to a re-orientation of the course as a whole," but agreement on the precise nature of it in future has not yet been reached. Various refresher courses have also been held, and a new project was a residential course on "Mental Health in the Public Health Field." It was held in April this year, and was attended by ten medical officers of health from the north of England. Students who came from overseas to attend the courses included two from Malta and one from Trinidad who on returning to their own countries will set up entirely new services for the mentally handicapped. Courses for chaplains in mental and mental-deficiency hospitals were found to be useful in enabling doctors and chaplains to discuss problems of concern to both.

CONTAMINATION OF OPERATING-THEATRE AIR WITH *CL. TETANI*

BY

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Cases of post-operative tetanus have been attributed by Robinson, McLeod, and Downie (1946) and by Sevitt (1949) to dust-borne contamination. Both reports imply that such contamination of dust was probably exceptional, in one hospital because of its nearness to grazing pastures, in the other because of the use of plaster hair in repairs to the theatre suite; and in both reports it is suggested that these risks would be reduced by improvements in theatre hygiene, including the use of plenum ventilation with filtered air.

A recent study in this hospital (Lowbury and Lilly, 1958) has shown that *Clostridium welchii* could be isolated from the air at most times, and that these organisms are apparently carried into the hospital with outdoor air; a plenum-ventilated dressing station showed significantly smaller numbers of *Cl. welchii*. In this paper we report a study on the incidence of airborne *Clostridium tetani*. It shows that small numbers of *Cl. tetani* could be found usually in the street and often in the exhaust-ventilated theatres; but none were found in a dressing station which was plenum-ventilated with filtered air. A simple technique for the recognition of toxigenic *Cl. tetani* is described. Some results of examination of dust, soil, faeces, and burns for *Cl. tetani* are also reported.

Methods

Sampling

Bacterial samples from air were collected with a large slit sampler (Bourdillon, Lidwell, and Thomas, 1948), and sometimes also by settle plates, on to nutrient gelatin (20%), which was then melted at 37° C. and distributed into bottles of cooked-meat broth. These, in most of the tests, were heated at 65° C. for 30 minutes before incubation; in later tests the gelatin itself was heated before addition to the cooked-meat broth. After four days' incubation and again after 12 days' incubation at 37° C., Gram-stained films of the cultures in cooked-meat broth were carefully examined for terminal spore-bearing bacilli. When these were present the culture was examined by the methods described below for the presence of *Cl. tetani*.

Samples of faeces and soil were inoculated in cooked-meat broth, which was heated at 80° C. for 10 minutes before incubation. Road dirt, hospital dust, and burn swabs were inoculated in cooked-meat broth, which was heated at 65° C. for 30 minutes before incubation. Gram-stained films were examined, as above, after four days' and after 12 days' incubation.

Isolation and Recognition of *Cl. tetani*

Cultures in cooked-meat broth showing terminal spore-bearing bacilli were subcultured by wire loop to the water of condensation in Fildes (1925) slopes. After anaerobic incubation in a Mackintosh and Fildes jar for 48 hours, the slopes were examined with a hand lens. If a characteristic fine filamentous swarming edge of growth was present, it was subcultured to a second Fildes slope; swarming

growth on this slope, after two days' incubation, was subcultured to cooked-meat broth. All bacilli with round terminal spores were found to swarm in this manner on Fildes slopes. Cooked-meat subcultures were incubated for four days, examined for purity in Gram-stained films, and tested as follows.

(i) Pairs of mice were given injections at the root of the tail of 0.2 ml. of culture to which 2.5% calcium chloride had been added immediately before the injection; one mouse of each pair had received an intramuscular injection of 3 units of antitoxin 24 hours and again one hour before the injection of culture.

(ii) Tubes of sugar media (glucose, maltose, lactose, sucrose, and salicin) containing strips of iron were inoculated and examined for evidence of fermentation after incubation at 37° C. for five to seven days.

(iii) Loeffler's inspissated serum slopes were inoculated, incubated anaerobically for five days, and examined for evidence of digestion.

(iv) Blood-agar plates (with 4% agar), one half of which had previously been spread with 0.1 ml. of undiluted therapeutic tetanus antitoxin, were inoculated in corresponding positions on both sides with the cultures of presumptive *Cl. tetani*; a straight wire was used for the inoculation. Nine cultures and a positive control could be tested on one plate. After two days' anaerobic incubation at 37° C., the plates were examined for growth and for haemolysis inhibited by antitoxin.

Terminal spore-bearing bacilli were described as toxigenic *Cl. tetani* if, after 18 hours, they had killed or caused tetanic spasms in an unprotected but not in a protected mouse. None of the organisms which gave these reactions were found to ferment any of the sugars or to digest a serum slope. On the antitoxin-controlled blood-agar plate—an adaptation of the Nagler plate test (see Lowbury and Lilly, 1955; Lilly, 1958)—toxigenic strains of *Cl. tetani*, including 11 reference strains from the National Collection of Type Cultures, caused good zones of haemolysis on the side without antitoxin, but no haemolysis on the antitoxin-treated side of the plate (see Fig.).

Diagnostic Value of the Antitoxin-controlled Blood-agar Plate ("Bench Mouse")

Fifty strains of terminal spore-bearing bacilli from air subcultured from Fildes slopes were tested both in mice and by the antitoxin-controlled blood-agar plate test, or "bench mouse." All of these strains killed or caused tetanus in unprotected mice, and showed haemolysis only on the side of the plate without antitoxin. One strain was positive in the mouse only on a second test, the first having failed to cause tetanus or to kill the unprotected mouse; it was retested because the "bench mouse" was positive.

Haemolytic strains of streptococci (20 each of groups A, C, D, and G), of *Cl. welchii* (20) and of aerobic spore-bearing bacilli (20) were tested and found to cause haemolysis on both sides of the plate.

"Bench mouse" examined by transmitted light (on left) and by reflected light (on right). The right half of the blood-agar plate was spread with tetanus antitoxin before 10 strains of *Cl. tetani* were spotted in corresponding positions on the two sides. After two days' anaerobic incubation there was growth on both sides, but haemolysis only on the side with no antitoxin.

In view of the identical results obtained by the two methods of testing, we relied on the "bench mouse" for recognition of *Cl. tetani* in the later part of this study.

Air Samples Examined

Samples were taken in the exhaust-ventilated operating theatre on 14 days, in the plenum-ventilated dressing station of the burns unit on 8 days, and in a covered road outside the hospital on 9 days. The ventilation system of the dressing station (Bourdillon and Colebrook, 1946) had Vokes "kompak" and "multivee" filters in series. Samples of approximately 690 cu. ft. (19.5 cubic m.) (30 minutes' sampling) were taken. On six days four such samples were taken in each of the three environments. To obtain some idea of the numbers of *Cl. tetani* present, the gelatin was well mixed after melting and added in four lots each of 15 ml., 7.5 ml., 3.25 ml., and (road samples only) 1.6 ml. to cooked-meat broth. In the other samplings the entire contents of the gelatin plates—that is, 30 minutes' sampling yield—were added to cooked-meat broth. We also examined a series of cultures representing replicates of 32, 16, 8, 4, and 2 minutes' sampling in each environment.

For 23 consecutive days we exposed for periods of six hours settle plates of gelatin for *Cl. tetani* and of a selective medium for *Cl. welchii* (Lowbury and Lilly, 1955) in the theatre and in corridors.

Results

Cl. tetani in the Air of Operating Theatre, Dressing Station, and Road

Table I shows the results of sampling for 30 minutes in each of these three environments on a number of occasions. *Cl. tetani* was isolated from 24 out of 27 samples taken in the road, from 33 out of 50 taken in the operating theatre, and from none of 23 taken in the plenum-ventilated dressing station.

Table II shows the cumulative results of sampling air from all three environments on each of six days by a semi-quantitative technique. Again the air from the road is shown to yield *Cl. tetani* more often than air from the operating theatre, and none were found in the dressing station. The probable number of *Cl. tetani* in these samples cannot be assessed with accuracy. When the Poisson series is applicable, 30% of samples fertile would imply the presence of a mean number of 0.3567 organisms per sample (Fisher, 1946). Approximately 30% of air samples of 345 cu. ft. (9.77 cubic m.) from the operating theatre and of air samples from the road of 86 cu. ft. (2.43 cubic m.) contained *Cl. tetani*. This gives some indication of the range within which counts of *Cl. tetani* might be expected to fall; it is clearly much lower than the counts of *Cl. welchii* in the same operating theatres (usually between 5 and 25 per 100 cu. ft. (1.76 and 8.82 per cubic m.) of air). There was some support for these findings in the results of settle plate culture; 18 out of 23 settle plates from the theatre yielded *Cl. welchii*, but none of the 23 comparable settle plates examined for *Cl. tetani* was positive; 4 out of 15 settle plates from a busy corridor did yield *Cl. tetani*.

The distribution of *Cl. tetani* in air was irregular; when different volumes were examined on the same day, some samples of 690 cu. ft. (19.5 cubic m.) yielded no *Cl. tetani*, but occasionally a sample of 46 cu. ft. (1.3 cubic m.) was positive. The only day on which no samples from the operating theatre yielded *Cl. tetani* was one of continuous rain. The highest proportion of positives was found in dry weather. *Cl. welchii* was previously found to vary with weather in the same way (Lowbury and Lilly, 1958).

TABLE I.—*Cl. tetani* in Samples of Air (690 cubic feet—19.5 cubic metres)

Place	Samples Yielding <i>Cl. tetani</i>	Total Samples	Days
Road	24 (89%)	27	9
Operating theatre (exhaust-ventilated) . . .	33 (65%)	50	14
Burns dressing station (plenum-ventilated) . .	0	23	8

TABLE II.—Cumulative Results of Sampling for *Cl. tetani* on Six Days in Road, Operating Theatre, and Dressing Station

Volume of Sampling Medium Tested (ml.)	Corresponding Volume of Air Tested		Proportion of Samples Yielding <i>Cl. tetani</i>		
	c. ft.	Cubic m.	In Road	In Exhaust-ventilated Operating Theatre	In Plenum-ventilated Dressing Station
15.0	345	9.77	11/24	7/24	0/24
7.5	172.5	4.88	10/24	1/24	0/24
3.25	86.25	2.44	6/24	0/24	0/24
1.625	43.125	1.22	0/8	—	—

Small numbers of air-borne *Cl. tetani* were found in the environment of another city hospital and of a hospital in a rural district. The air of an operating theatre in the latter, which was ventilated by open windows, also yielded *Cl. tetani*.

Other Reservoirs of *Cl. tetani*

Cl. tetani was isolated from 6 out of 7 samples of garden soil, 5 out of 5 samples of ward floor dust, 5 out of 6 samples of street dust, 8 out of 11 trouser turn-ups of laboratory staff, and 10 out of 35 specimens of human faeces. It was not found in any of the 273 swabs from burns which we examined.

Discussion

These studies show that very small numbers of *Cl. tetani* could usually be found in the air of a city hospital; since rather more of these organisms were found in the air outside the hospital and none at all in a dressing station ventilated with filtered air, it appears that contamination was introduced from outside, and was drawn into the operating theatre by exhaust ventilation. The same source of *Cl. welchii* and exclusion of that organism by plenum ventilation with filters have been described elsewhere (Lowbury and Lilly, 1958).

In spite of the regular isolation of *Cl. tetani* from air in these studies, tetanus has not occurred in any patient following an operation in this hospital during the past nine years. The risk of clinical infection from the small numbers of *Cl. tetani* which we found in the air must therefore be extremely remote. From the data presented by Robinson and others (1946), it seems that post-operative tetanus may result from an increase in the amount of environmental contamination with *Cl. tetani*. On the other hand, the rarity of tetanus may simply reflect the sparsity of tetanus bacilli in the environment. It would be useful to know what degree of contamination with *Cl. tetani* is found in theatres in which patients are known or thought to have contracted post-operative tetanus. Spores of *Cl. tetani* can germinate and produce toxin in tissues only under special and, perhaps, exceptional conditions. Although tetanus may follow trivial injury (see Press, 1948), it is nevertheless a rare sequel even of severe lacerated wounds in unprotected patients, and tetanus following rectal operations is a rarity in spite of the relatively frequent presence of *Cl. tetani* in human faeces. As in the case of gas gangrene, we may suppose that self-infection by faecal tetanus bacilli is a possible source of post-operative infection (particularly in abdominal surgery), but, as such cases sometimes occur in small outbreaks, infection from the environment is perhaps more important.

The functions of plenum ventilation in operating theatres are (1) to remove staphylococci and other organisms liberated in the theatre, and (2) to exclude dirt and bacteria from air admitted to the theatre. The latter, as our studies

have shown, include *Cl. tetani*, and *Cl. welchii*, which can be effectively kept out by cotton-wool and fibre filters in series.

Summary

Samples of approximately 690 cu. ft. (19.5 cubic m.) of air from an exhaust-ventilated operating theatre, a plenum-ventilated dressing station, and the road outside the hospital were examined for tetanus bacilli. These were sparsely present in 24 out of 27 examples from the road and 33 out of 50 samples from the operating theatre; none were found in 23 samples from the dressing station.

Cl. tetani was found also in all specimens of hospital floor dust, in trouser turn-ups of laboratory staff, and in 10 out of 35 human faecal specimens; it was not found in any of 273 swabs from burns.

These findings are discussed in relation to the occurrence and prevention of post-operative tetanus.

A simple plate technique (the "bench mouse") for the recognition of toxigenic *Cl. tetani* is described and illustrated.

We thank Mr. W. Cater, A.I.O.T.T., for assistance in air sampling; Dr. R. E. O. Williams for suggestions on the collection of samples; the clinical director, surgeons, and nursing staff of this hospital for their co-operation; and Mr. R. Gill for the photographs.

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CLEANSING OF HOSPITAL BLANKETS

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Several investigators have shown that the usual methods of laundering do not effectively reduce the bacterial contamination of blankets (see Blowers and Wallace, 1955). This unsatisfactory state of affairs is tolerated only because of the great practical difficulties in devising a method of laundering which is cheap, effective, and adaptable to normal laundry procedures.

We were recently asked to report on the bacteriological efficiency of laundering blankets at Guy's Hospital. In the course of this work it was noted that the standard of cleanliness achieved was much better than expected as judged by reports in the literature and by previous observations by one of us (C. G. A. T.) in a similar investigation at another hospital.

It has been suggested that our findings may be of interest to others who are concerned with laundry hygiene.