FURTHER STUDIES IN THE MOUSE INTRA-ORAL INOCULATION TECHNIQUE

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The previous paper presents a description of an original technique for the production of experimental pneumonic lesions in mice and contrasts it briefly with the classical intra-nasal method. The object of the experiments reported here is to compare by means of a radio-active tracer technique, the differential dispersal of an inoculum delivered to anaesthetized mice by the two methods.

MATERIALS AND METHODS

Two batches each of fifteen mice, were inoculated by the intra-oral and intra-nasal routes, respectively, with 0·05 ml. of a solution in saline of di-iodofluorescein labelled with ^{131}I. The activity of this solution was of the order of 1 mC./ml. Details of the intra-oral technique have been described by Epstein (1958) in the preceding paper.

A total of twenty-one mice were inoculated intra-nasally, as six animals spluttered and failing to accept their inoculum satisfactorily, were rejected and replaced by fresh animals. It was not, however, found necessary to reject for this reason, any of the intra-orally inoculated animals. Immediately after inoculation, each animal was placed in a separate glass container and killed 10 min. later. Three fractions were prepared from each animal.

(i) Lungs—larynx, trachea, bronchi and lungs.
(ii) Gut—oesophagus, stomach, jejunum.
(iii) Head.

The head was stripped of its fur and split open sagittally in the mid-line, to expose the nasal cavities and naso-pharynx, in an attempt to minimize *gamma*-ray absorption. The proportion of the inoculum which was present in the three fractions, was assessed by *gamma* counting under conditions of standard geometry, using a shielded G. 10, Pb. Geiger counter. After subtraction of the appropriate background count, all counts were expressed as percentages of the aliquot count obtained under the same geometrical conditions.

RESULTS

The results given in Table 1 indicate the distribution of the inoculum between the three fractions. The figures represent the count rates from the three fractions expressed as percentages of the aliquot count rate, and also the percentages of the aliquot present in the animals at the time of death.

https://doi.org/10.1017/S0022172400037578 Published online by Cambridge University Press

There is a significant difference ($P < 0{\cdot}01$) between the percentages reaching the lungs in the oral and nasal methods of inoculation. The difference between the total animal activity for the two methods is also significant ($P < 0{\cdot}01$).

DISCUSSION

Hoyle & Orr (1945) inoculated intra-nasally a small group of anaesthetized mice with a suspension of ferric ferrocyanide. The animals were sacrificed at intervals after inoculation. The authors found that in those animals killed immediately, a large part of the prussian blue had entered the oesophagus and stomach. The residue was found in the trachea and main bronchi. In animals killed 5 min. later, the dye had reached the lung parenchyma and was clearly visible under the visceral pleura. Holye & Orr did not, however, attempt to deduce even approximately quantitative inferences, as to the mode of distribution of the dye in various sites. The tracer experiments described above were designed to provide this information. Like Hoyle & Orr, our observations were also limited to the immediate fate of the inoculum.

Both the choice of a labelled substance of large molecular weight (584) and the short period of survival of the animals allowed after inoculation, tended to minimize the possibility of significant absorption of the tracer into the blood stream. Such a contingency would complicate the problem, as it would entail a consideration of secondary redistribution of the isotope.

The considerable scatter of results on the total animal and individual fraction activities, is a reflexion of several factors. These include, fluctuations in levels of anaesthesia in different mice and errors inherent in the use of the standard Mantoux syringe. It was not considered desirable to perform inoculations with a precision micrometer syringe, as it was felt that these experiments should reflect standard working conditions.

The results cited in Table 1, clearly indicate that the intra-oral technique is more efficient than the intra-nasal technique, in at least two respects. Expressed as a percentage of the aliquot activity, 78 % of the inoculum was recovered from animals inoculated intra-orally, as opposed to only 45 % in the intra-nasal group. The deviation of these figures from 100 % is believed to be due largely to external loss of the inoculum both during and after inoculation. The lung fractions of the oral group were found to possess some three times the activity of the nasal group. It must be emphasized that these results are biased in favour of the intra-nasally inoculated group, individuals of which, as previously indicated, were selected to include only those apparently accepting their inoculum.

That critical anaesthesia is required for successful intra-nasal inoculation is well known. It was felt, therefore, that the data on low total animal and lung activities in the intra-nasally inoculated animals, are open to criticism, on the grounds that these results may to a certain extent merely constitute a reflexion of the degree of skill of the anaesthetic technique. If this argument be accepted, it follows as a corollary from the high, total animal activity of the intra-orally inoculated animals that critical anaesthesia is not essential to achieve success with this particular

Table 1. *Comparison of activity in individual fractions*

Oral method					Nasal method				
	Percentages of aliquot					Percentages of aliquot			
Mouse	Lungs	Gut	Head	Total	Mouse	Lungs	Gut	Head	Total
1	32	29	22	83	1	6	42	15	63
2	40	23	7	70	2	22	29	18	69
3	41	27	8	71	3	25	21	10	56
4	31	38	9	78	4	31	26	14	71
5	35	38	9	82	5	23	17	11	51
6	0	69	1·5	71	6	0	19	10	29
7	5	109	25	139	7	2	46	16	64
8	33	47	17	97	8	6	27	25	58
9	38	40	9	77	9	0	79	11	90
10	39	50	5	94	10	0·8	25	4	30
11	0·5	36	4	41	11	4	7	6	17
12	64	0·7	13	78	12	3	16	8	27
13	30	49	11	90	13	0·6	10	2	13
14	0·6	70	1·7	72	14	0·7	11	5	17
15	0·6	22	1·1	24	15	0·6	17	6	24
Mean	25·3	43·2	9·6	77·8	Mean	8·3	26·1	10·7	45·3
S.E.	5·0	6·6	2·1	6·5	S.E.	2·8	4·7	1·6	6·2

Table 2. *Distribution of the total animal activity*

Method of inoculation	% of animal activity		
	Lungs	Gut	Head
Intra-oral	31·4	57·5	11·4
Intra-nasal	15·8	58·9	24·8

technique. Table 2 gives the activity of the fractions, expressed as a percentage of the total animal activity, and thus indicates the distribution of the retained fraction of the total volume administered. The differences in activity of the lung fractions in the intra-oral and intra-nasal group cited in Table 1, are sustained although to a lesser degree. This suggests that the poor intra-nasal results cannot be solely accounted for by loss of inoculum due to non-critical anaesthesia. These findings are substantially in accord with the bacteriological studies on aerial contamination described in the preceding paper, which showed that significant droplet dispersal with aerial contamination occurs during intra-nasal, but not during intra-oral inoculation.

SUMMARY

The immediate dispersal of a fluid delivered to anaesthetized mice by the intra-nasal and intra-oral techniques has been studied by means of a radio-active tracer method. The results clearly show that the intra-orally inoculated animal accepts a larger proportion of the total volume inoculated and that a significantly higher fraction of this reaches the lungs than in mice inoculated by the intra-nasal technique.

The authors would like to thank Dr I. Friedmann and Dr H. A. B. Simons for having made this work possible; also, Mr D. Arnott for a generous gift of labelled di-iodofluorescein, and Dr D. A. Osborn and Dr K. Weinbren for helpful suggestions.

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(*MS. received for publication* 15. v. 57)