A POSSIBLE MODE OF TRANSFER OF INFECTION BY SYRINGES USED FOR MASS INOCULATION

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A common mass-inoculation technique (M.R.C., 1945) employs a separate sterile needle for each injection, but does not sterilize the syringe, which contains several doses of inoculum, between injections.

Accidental infections attributable to this practice do not seem to have been described, and experience generally suggests that the technique is, so far as any injection technique can be, a safe one. We do not wish to suggest that the experiments to be described mean that this "separate needle, common syringe" procedure carries a high risk; if a fresh sterile syringe cannot be used for each subject, we think it is the best technique at present available for mass-inoculation purposes.

It seemed, however, worth while to investigate the advantages of using a fresh needle for each injection, partly because of the observations of Hughes (1946), discussed below, and partly because of the association of poliomyelitis with prophylactic inoculation (McCloskey, 1950; Bradford Hill and Knowelden, 1950). It seems unlikely that the transmission of poliomyelitis virus by syringe can account for this association; other hypotheses are more probable in the light of our present knowledge. But, clearly, if there is any risk at all of syringe transmission, the possibility, however remote, should be evaluated as accurately as circumstances permit.

Experiment 1

This first experiment was therefore made with mice and virulent pneumococci. The virulence of a smooth strain of *Streptococcus pneumoniae* Type I was assured by three mouse-to-mouse passages. Twenty-four mice were then inoculated intraperitoneally with diluted peritoneal washings from a dead animal. Next day nine were dead and the remainder were sick. Thirteen which appeared moribund were killed and used immediately in the experiment.

The plan was to fill a sterile syringe with 1 ml. of sterile broth, to inject 0.1 ml. intraperitoneally into one of the recently killed pneumococcal mice, to change the needle and to inject 0.2 ml. of broth into a normal mouse, and then, the needle having been changed again, to inject 0.5 ml. into the same infected mouse's peritoneal cavity; finally, after a third needle change, to inject the remaining 0.2 ml. into a second normal mouse. One normal mouse was therefore injected from a syringe which had delivered 0.1 ml. into an infected animal, and a second normal mouse from the same syringe after delivery of 0.1 and 0.5 ml. into the infected mouse. Two normal mice and one infected mouse were thus injected from one syringe, the needle being changed after each injection.

This procedure was adopted because it was thought that the second injection of 0.5 ml. into the infected mouse might produce a higher intraperitoneal pressure and so a bigger change of reflux of infected fluid into the syringe.

Nine 1-ml. syringes of various makes were used, and 40 needles, sizes 15 and 17. All were oven-sterilized initially; re-sterilization during the experiment was achieved by boiling. In order to minimize as far as possible the risk of cross-infection occurring between the infected and the normal mice, the conduct of the experiment was divided into three tasks, each of which was performed by one operator alone.

The first operator killed the infected mouse by breaking its neck, and pinned it out belly upwards on a cork mat ready for the second operator.

The second operator handled the syringes and made all the injections. He performed the following operations in strict sequence: (1) filled a sterile syringe to the 1-ml. mark with sterile broth; (2) injected 0.1 ml. of broth intraperitoneally into an infected mouse; (3) disconnected the needle from the syringe and placed it in the sterilizer; (4) fitted a sterile needle to the same syringe and then injected 0.2 ml. of broth intraperitoneally into a normal mouse; (5) discarded the second needle into the sterilizer, fitted a fresh sterile needle to the syringe, and injected 0.5 ml. of broth intraperitoneally into the same infected mouse as at stage 2; (6) discarded the third needle to the sterilizer, and, after fitting a fresh sterile needle to the syringe, injected the final 0.2 ml. of broth intraperitoneally into a second normal mouse; and (7) discarded the syringe and needle to the sterilizer.

The sequence of operations was repeated for each of the 13 infected mice, and, in all, 26 normal mice were inoculated in this way. No attempt was made to clean or sterilize the belly walls of the mice. Injections were made in the usual manner. Care was taken not to withdraw the plunger of the syringe after giving the injection. No reflux into the syringes was observed.

The third operator handled the normal mice, holding them for injection, and placing them after injection into cages so that mice inoculated at stage 4 were caged together, labelled "0.1 ml.," and those inoculated at stage 6 were caged together, labelled "0.5 ml."

By the morning after injection, five of the 26 "normal" mice were dead. Fourteen more died in the course of the next 48 hours. The distribution of dead mice between batches is shown in Table I. Blood-agar cultures and

TABLE I.—Experiments with Pneumococcal Mice

Experiment	Batch	No. of Mice Injected	No. of Mice Developing Pneumococcal Septicaemia
1 ..	"0.1 ml."	13	8
	"0.5 ml."	13	11
	"Record" control	5	0
2 ..	"Record" test	10	8
	"Tuberculin" control	5	0
	"Tuberculin" test	10	8

stained films prepared from the heart blood of each dead mouse showed abundant pneumococci. The seven surviving experimental mice were alive and in apparently good health three days after injection.

It appeared from this experiment that a change of needle gave very little protection against the carry-over of infection from infected to normal mice.

Experiment 2

Later, the experiment was repeated, using mice of a different strain and four 1-ml. glass-metal Record syringes together with five 1-ml. all-glass tuberculin syringes. One of the Record syringes was re-boiled during the experiment and used a second time. In this second experiment procedure was simplified, the steps of the operation being

as follows: (1) Record syringes were sterilized by boiling, all-glass syringes by hot-air sterilization; (2) the syringe was filled with 1 ml. of sterile broth; (3) 0.1 ml. of broth was injected intraperitoneally into a normal control mouse; (4) 0.1 ml. of broth was then injected intraperitoneally into a "pneumococcal" mouse killed immediately beforehand by neck-breaking; (5) the used needle was removed and discarded and a fresh sterile needle fitted to the syringe; and (6) 0.1 ml. of broth was then injected intraperitoneally into each of two normal mice.

As they were injected the mice were caged in six separate groups of five mice—"Record" and "tuberculin" controls, "Record" and "tuberculin" first and second test mice.

During the next 48 hours all control mice remained in good health, but 16 of the 20 test animals developed a fatal pneumococcal peritonitis and septicaemia, which was confirmed by culture and smear. The result is shown in Table I.

These mouse experiments were intended to simulate the ordinary technique of mass inoculation carefully performed. As a test of transfer of infection they were, however, very sensitive, because the number of virulent organisms needed to produce pneumococcal septicaemia in the mouse is very small, and the peritoneal cavities of the pneumococcal mice used contained very large numbers of pneumococci.

An attempt has therefore been made to assess the risk of carry-over of micro-organisms in the "separate needle, common syringe" technique of inoculation by means of quantitative experiments using bacterial cultures instead of pneumococcal mice.

Experiments 3 to 5

These were performed with an "artificial mouse" consisting of a length of glass tubing bent at a right-angle, the shorter arm being fitted with a 2-in. (5-cm.) length of rubber tubing, plugged at the far end with a glass rod (Fig. 1). Overnight broth cultures of *Shigella flexneri* (Y6R or NCTC 4832) were pipetted into the tube, filling the rubber and the horizontal glass limb, and rising in the vertical limb to a height which determined the hydrostatic pressure inside the rubber tube. Six syringes were employed in each of these experiments—1-ml. all-glass "tuberculin" syringes in experiments 3 and 4, and, in experiment 5, four 1-ml. "Record" syringes and two all-glass "tuberculin" syringes.

FIG. 1.—"Artificial mouse." A length of glass tubing bent at right angles: the shorter arm is fitted with a 2-in. length of rubber tubing, plugged at the far end with a glass rod.

The pattern of the three experiments was the same. The sterile syringe having been filled with sterile broth, 0.1 ml. was injected through the rubber tube into the "artificial mouse." The needle was then withdrawn. It was changed and not changed on alternate syringes, even-number syringes not having their needles changed, odd-number syringes receiving a fresh sterile needle before the next step in the experiment, which was to discharge 0.2 ml. of broth from the syringe into each of three tubes containing 0.8 ml. of sterile broth. From these tubes suitable dilutions were prepared, and six-plate surface counts (Miles and Misra, 1938) of their contained bacteria were made.

Table II shows the result of these experiments in terms of the number of colony-producing bacilli contained in each of the successive 0.2 ml. volumes of broth discharged

from the syringe after injection of 0.1 ml. into the "artificial mouse," with and without intervening changes of needle.

TABLE II.—Results of Experiments 3 to 5

Experiment	Needle Change	Syringe No.	Bacterial Counts per 0.2 ml. Volume		
			Vol. 1	Vol. 2	Vol. 3
3	Not changed	1	4,000	0	0
		3	22,500	33	0
		5	8,200	8	25
	Changed	2	2,000	791	292
		4	5,700	1,191	225
		6	1,000	200	191
4	Not changed	1	6,165	0	0
		3	180,833	890	316
		5	29,000	2,166	458
	Changed	2	540	83	50
		4	3,583	600	225
		6	0	0	0
5	Not changed	1 "R"	8,750	66	
		3 "R"	5,083	25	
		5	9,333	116	
	Changed	2 "R"	266	58	
		4 "R"	391	25	
		6	0	0	

R = Record glass-metal 1-ml. syringe.

Two of the nine tests in which the needle was changed showed no carry-over of infection, but in the remaining seven change of needle showed very little advantage over the nine tests in which the needle was not changed.

The height of the fluid column in the vertical limb of the "artificial mouse" in these experiments varied between 2 and 6 cm. There was no apparent correlation between height of fluid column and result. However, it seemed possible that the positive pressure against which the first injection was made might be forcing culture along the needle, although no plunger movement was evident. To test this, another experiment was performed.

Experiment 6

In this experiment the procedure was similar except that, instead of injecting through rubber into culture under a few centimetres head of pressure, the first injection was made into a small volume of culture held in a small bottle, the point of the injecting needle being held one or two millimetres below the surface, and the syringe necessarily at an angle with the horizontal, such that the pressure on the broth in the syringe must throughout have been greater than the pressure in the culture around the needle point. The result of this experiment is shown in Table III. One

TABLE III.—Result of Experiment 6

Needle Change	Syringe No.	Bacterial Counts per 0.2 ml. Volume		
		Vol. 1	Vol. 2	Vol. 3
Not changed	1	83,333	1,083	4,083
	3	45,000	1,250	300
	5	31,666	1,916	216
Changed	2	0	0	0
	4	25,000	2,146	893
	6	0	0	0

out of three syringes in which the needles were changed still showed a considerable carry-over of bacteria. In this experiment, as in all the preceding ones, the injection into the infected mouse or culture had been made without emptying the syringe. It seemed possible that, unless the plunger were pushed home against the bottom of the syringe, small plunger movements might cause a reflux into the needle.

Experiment 7

In this experiment injection was not made into the infected material. Instead, each sterile syringe was first washed out with a small volume of sterile broth and

emptied. The point of the needle was then dipped into a culture of *Sh. flexneri* to a depth of one or two millimetres, after which it was withdrawn and the needle was changed. A small volume, up to 1 or 2 ml., of sterile broth was drawn into the syringe through the new needle. Three drops were then expelled on to an agar plate and spread with a sterile spreader.

Sixteen syringes were used, ranging from 1 ml. to 10 ml. in capacity, and of various makes. They were fitted with needles of differing lengths and bore. Next day two of the 16 plates showed numerous colonies of *Sh. flexneri*, the remaining 14 showing none.

This experiment shows that if the needle point is contaminated, even when the syringe is empty and with the plunger pushed home and not moved, and even when there is no appreciable pressure forcing infected fluid back into the needle, it is still possible for the syringe to become contaminated when the needle is changed. It is clear, however, that the frequency of such contamination was less than in those experiments in which the plunger was moved, or in which injections were made into infected fluid under a small head of pressure.

Experiment 8

A final experiment was performed to help separate these two factors of plunger movement and pressure of infected fluid. The "artificial mouse" used in experiments 3 to 5 was employed again, but this time the injecting syringe contained only about 0.1 ml. of fluid, the whole of which

were then discharged on to the surface of an agar plate and spread with a sterile glass spreader.

Next morning, after overnight incubation at 37° C., three of the 16 plates showed no growth, but the remaining plates showed colonies of *Sh. flexneri*, checked by slide agglutination, varying in numbers from six to several hundreds, as is shown in Table IV.

Discussion

Syringes used for intravenous injections often become contaminated with blood (Salaman *et al.*, 1944; Droller, 1945; Mendelssohn and Witts, 1945; Darmady and Hardwick, 1945; Morton, 1948) and may, therefore, transfer infection if used on more than one subject without intervening sterilization. Hughes (1946) demonstrated that the nozzles of syringes used to administer intramuscular injections to human subjects often become contaminated with red corpuscles. He found that the drop of fluid on the end of the syringe nozzle after removal of the needle contained red corpuscles after 15 out of a total of 27 injections made into contracted muscles, and after two out of a total of 12 injections into relaxed muscles.

The experiments here reported show that contaminants on the needle point, even when no injection is made and when the contamination is derived from fluid under negligible hydrostatic pressure, may travel back along the needle and contaminate the syringe when the needle is removed from it. Without further extensive quantitative experiments, it would not be possible to assess the frequency with which this happens; probably the pattern of syringe and of needle would affect the degree of risk.

It is likely, from the experiments reported, that small movements of the plunger and the hydrostatic pressure around the needle tip are factors which increase the risk of syringe contamination; and, of course, both of these factors are involved whenever any injection is given.

If, as appears from experiments 6 and 7, contamination of the needle tip can lead to contamination of the syringe, though the pressure around the needle tip is not significantly raised, then some explanation of the passage of the contaminant back from the needle tip to the syringe nozzle must be sought. The most probable explanation seems to be that offered by Hughes: that withdrawal of the nozzle of the syringe from the socket of the needle may suck fluid back from the lumen and point of the needle to contaminate the point of the syringe nozzle. Measurement of the length and internal diameter of some of the needles used in these experiments indicated that the lumen volume varied between 2.5 and 5.8 c.mm. for small needles of the kind usually employed for subcutaneous and intramuscular injection. These

TABLE IV.—Experiment 8

No.	Syringe Description	Height of Fluid Column		Plate Result: Colonies
		Before Injection (cm.)	After Injection (cm.)	
1	"Tuberculin" 1 ml.	1.5	2.0	++
2	All-glass 10 "	2.0	3.0	13
3	" 2 "	3.0	3.5	++
4	" 2 "	3.5	4.2	6
5	" 5 "	1.5	2.0	0
6	"Tuberculin" 1 "	2.0	2.5	+++
7	" 1 "	2.5	2.9	+++
8	All-glass 5 "	2.9	3.2	26
9	" 5 "	1.8	2.1	+++
10	"Tuberculin" 1 "	2.1	2.6	+++
11	All-glass 2 "	2.6	3.0	0
12	" 2 "	3.0	3.7	0
13	" 1 "	1.5	1.8	+++
14	" 5 "	1.8	2.2	10
15	" 10 "	2.2	2.7	21
16	" 1 "	2.7	3.1	47

++, +++ = Large numbers of colonies, not counted.

was injected through the rubber, so that at the end of the injection the plunger was "home" against the bottom of the syringe.

One "artificial mouse" was used to test four syringes. Sixteen syringes in all, of various sizes and patterns, from 1 ml. to 10 ml. in capacity and fitted with various needles, were tested, using four "artificial mice." The order of procedure was: (1) Sterile broth was drawn into the syringe and discharged until about 0.1 ml. remained in the syringe. (In 1-ml. and 2-ml. syringes this volume could be measured easily. In 5-ml. and 10-ml. syringes it was judged by eye, and checked by the change in height of fluid column noted after its injection into the "artificial mouse.") (2) This terminal volume of broth was then injected through the rubber into the culture of *Sh. flexneri* and the rise in height of fluid column noted. (3) The needle was then discarded, being replaced by a fresh sterile needle. (4) About 0.5 ml. of broth, sterilized in a small individual tube, was then sucked into the syringe through the new needle. (5) Three drops

FIG. 2.—Needle cut in half longitudinally and fitted to syringe.

volumes are probably smaller than the displacement volume of the syringe nozzle in the needle socket. Fig. 2 shows a needle which has been cut in half longitudinally and then fitted to a syringe. The displacement volume of the nozzle in the socket is of the order of 100 c.mm.

It seems clear, then, that the "separate needle, common syringe" technique might transfer infection from one subject to the next if the tissue into which the first injection was made were itself infected. Our experiments indicate, however, that syringe contamination does not always follow removal of a contaminated needle, and therefore the "separate needle, common syringe" technique is safer than the use of one needle and syringe for inoculation of several subjects.

The actual risk of syringe transmission of infection during mass immunization is evidently a very small one, because it depends not only on the imperfection of the syringe technique but also on the probability that muscle, skin, or subcutaneous tissue may harbour an infective agent capable of being transmitted.

Where bacterial infections are concerned the risk is probably very small. An injection made into a subject with an undetected incipient septic lesion might cause, by syringe contamination, lesions in several subjects subsequently inoculated, and all the lesions, including that which in fact caused the infections, might be wrongly attributed to prior contamination of the injection material. Several mass-inoculation accidents, such as the Ring disaster of 1939 (Report, 1939) in which multiple tuberculous abscesses followed inoculations with T.A.F., remain unexplained. Bigger, Blacklock, and Parish (1940) draw attention to the risk of transferring infection from a subject who already has some small infective lesion in his muscle or fascial planes.

Virus infections are more difficult to discuss, for knowledge about the distribution of viruses in tissues is very inadequate. Serum hepatitis is clearly apt to be passed by a faulty injection technique (Hughes, 1946), or even by imperfect sterilization of blood guns used for collection of blood samples in diabetic clinics (Malmros, Wilander, and Herner, 1948). Among neurotropic viruses, the group of encephalitides transmitted by insect bite and due to viruses recoverable from the blood may be important in some parts of the world. Hughes (1944) has drawn attention to various neurological complications which may follow inoculations and which might conceivably be syringe-transmitted. The possibility that poliomyelitis or other neurotropic viruses may exist in relatively high local concentrations in the nerves lying in muscles or in skin or subcutaneous tissues cannot, in our view, be entirely excluded, although on the evidence available this seems an unlikely explanation of the association between poliomyelitis and inoculations.

The hazards of the "separate needle, common syringe" technique may well be greater in countries where malaria and blood-borne viruses are more frequent.

Conclusion

The common practice of giving multiple injections from one syringe is not made entirely safe by using a separate needle for each injection, even when the injection is given into a tissue other than a vein; but this technique is safer than is the use of a single needle for injection of several subjects. The risk probably lies in aspiration of fluid along the needle by suction created when the nozzle of the syringe is withdrawn from the socket of the needle.

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DIPHYLLOBOOTHRIUM INFESTATION AND ANAEMIA IN GREAT BRITAIN

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Diphyllobothrium latum, the fish tapeworm, is uncommon in the British Isles, although cases occur from time to time among the inhabitants of the lake districts of western Ireland. In 1944 Duguid and Sheppard found the trout and sticklebacks of a reservoir in South Wales heavily infested with the plerocercoids of *Diphyllobothrium dendriticum*, and Hickey and Harris (1944) found the adult worm in seagulls in Ireland. Human infestation is virtually unknown in Great Britain, though it is prevalent in other parts of the world, including the United States of America, where it has been introduced by immigrants. In 1924 Passay and Braine referred to an Estonian seaman who expelled a worm in hospital in Aberdeen, and Duguid and Sheppard mention a Belgian soldier who was found to be a carrier. Otherwise no case has been published in this country.

The associated megaloblastic anaemia is remarkable in that it is almost entirely confined to the Finnish race, although there are many other nationalities equally heavily infested with the worm. Even in Finland it is uncommon. Ehrström (1926) stated that it occurred in only one of every 5,000–10,000 carriers, but later workers have found it more frequently. The case recorded below is notable not only on this account but because of its haematological interest and because it raises an important public health problem.

Case Report

A Finnish domestic servant aged 22 had lived in England for two years. Her health had always been good until six to seven months before admission to hospital on July 7, 1949, when she began to notice increasing tiredness, followed later by breathlessness and occasional morning vomiting. She had attended another hospital in May, 1949, where a tentative diagnosis of pernicious anaemia was made and an injection