ENHANCEMENT OF INTRACEREBRAL INFECTION OF MICE WITH *BORDETELLA PERTUSSIS*

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SOME strains of *Bordetella pertussis* multiply continuously after intracerebral injection into a mouse and cause a fatal infection, whilst similar doses of other strains cause only a transient infection. Changes in the bacterial count in the brain after infection with "high-virulence" and "low-virulence" strains were described in a previous paper (Adams, 1970). The pattern of infection with the low-virulence strains could not be accounted for by sensitivity to the bactericidal action of mouse complement or by the occurrence of modulation, but the low-virulence strains appeared to produce a reaction in the mouse brain that rendered it resistant to reinfection by low-virulence but not by high-virulence strains.

Iida *et al.* (1963) showed that treatment of mice with cortisone greatly enhanced the infection produced by the intracerebral inoculation of a low-virulence strain of *Bord. pertussis* but not by that of a high-virulence strain. Lysosomes are stabilised by the addition of hydrocortisone (Fell, 1962; Weissman, 1964). Wolf, Kabat and Newman (1943) and Naidoo and Pratt (1951) showed that the choroid plexus and ependymal cells stain deeply for acid phosphatase, and later Becker *et al.* (1960) showed that the lysosomes of the ependymal cells are concentrated at the apical end of these cells.

The site of infection with *Bord. pertussis* after intracerebral injection in the mouse is on the ependyma of the ventricles (Berenbaum, Ungar and Stevens, 1960; Iida *et al.*, 1962; Hopewell and Adams, 1970). It therefore seemed possible that release of lysosomal enzymes into the brain might play an important role in the course of *Bord. pertussis* infections. Weissman reported that vitamin A and streptolysin O stabilised the lysosome system, and this suggested that these substances might lead to more rapid clearance of infection.

Berenbaum *et al.* and Iida *et al.* (1962) reported that after intracerebral injection of *Bord. pertussis* there was little infiltration of leucocytes into the ventricles until the 3rd day of infection or until the intracerebral viable count was between 10^6 and 10^7 bacteria. This count was higher than that reported by Holt *et al.* (1961) as being necessary for the breakdown of the blood-brain barrier in mice. No breakdown of the blood-brain barrier was noted with low-virulence strains until the viable count in the brain was in excess of 10^6 bacteria, and the viable count with small doses of the low-virulence strains never reached 10^6 bacteria. Also, Iida *et al.* (1966) found that the number of leucocytes in the ventricles of immune mice treated with cortisone and infected with *Bord. pertussis* no. 18/323 was not lower than in untreated control mice. It was therefore thought unlikely that circulating leucocytes played an important role in determining the course of infections with low-virulence strains.

We now report the effects of giving cortisone, vitamin A and streptolysin O,

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and of prior irradiation with low doses of X-rays, on the intracerebral infection of mice with *Bord. pertussis* (see also Adams, 1968).

MATERIALS AND METHODS

The strains of *Bord. pertussis* used, and the method of intracerebral inoculation and of processing the brains were described earlier (Adams, 1970). Theiler's Original strain of mice was used in all experiments. The progress of infection was followed by serial viable counts of homogenised whole brain. The results were expressed as numbers of *Bord. pertussis* per brain, but when no organisms were grown the count was taken to be 50 organisms. The figures in this paper show these numbers, together with geometric mean (GM) of all brain counts on each group of mice.

Other substances were injected into the mice as follows: 2.5 mg cortisone acetate (Cortisyl, Roussel Laboratories) was injected subcutaneously 2 hr before and 1 and 2 days after challenge; vitamin-A alcohol (Koch-Light Laboratories) 2000 IU was injected intracerebrally 2 hr before challenge; and streptolysin O (Burroughs Wellcome and Co.), 1 IU was injected intracerebrally 2 hr before and 1 day after challenge.

For irradiation, the mice were anaesthetised with intraperitoneal sodium pentobarbitone (Nembutal) 60 µg per g body weight and were arranged radially in groups of 6 in the radiation field. Doses were given at 90–95 roentgens (R) per min., from a machine working at 220 kV, 15 mA, with 0.5 mm copper and 1.0 mm aluminium added filtration. Shielding where applicable was with 2.5-mm lead sheet. Exposures were continuously monitored with a Baldwin-Farmer Substandard Dosimeter. Leucocyte counts on blood taken from the brachial artery were made according to Dacie and Lewis (1963). Normal count, about 5000 per µl.

Measurements of lysosomal enzymes were performed on cytoplasmic extracts. These were prepared by adding one brain to 4.5 ml of ice-cold 0.32M sucrose and disrupting it in a Potter-Elvehjem-type homogeniser with a teflon pestle rotated slowly for 5 s. The homogenates were centrifuged at 800g for 10 min., and the resulting supernatant was designated the cytoplasmic extract.

The substrates for enzyme measurements, prepared in 0.05M sodium acetate buffer at pH 5, were 3mM disodium *p*-nitrophenyl phosphate for acid phosphatase, 4mM *p*-nitrophenyl acetamido-2-deoxy-D-glucopyranoside for N-acetyl-β-D-glucosaminidase, 2.5mM *o*-nitrophenyl-β-galactoside for β-galactosidase, and 1mM phenolphthalein glucuronide for β-glucuronidase.

Acid phosphatase, β-galactosidase and N-acetyl-β-D-glucosaminidase measurements were made by the same method but with different substrates. A mixture of 1 ml of the specific substrate, 0.2 ml of the cytoplasmic extract and 0.3 ml of acetate buffer was incubated at 37°C for 15 min. The reaction was stopped by the addition of 1.5 ml of 2.75 per cent. trichloroacetic acid and the mixture was centrifuged at 2000g for 10 min.; 1 ml of the supernatant was added to 2 ml of 0.4M glycine-NaOH buffer, pH 10.5 and the optical density was determined at 420 nm on a Unicam SP600 spectrophotometer. The β-glucuronidase determination was by a similar method, but the reaction was stopped by adding 6 ml of a 0.133M glycine, 0.067M sodium chloride, 0.083M sodium carbonate buffer, pH 10.7, and the mixture was centrifuged at 2000g for 10 min. The optical density of the supernatant was determined at 545 nm.

RESULTS

Effect of treatment with cortisone

Intracerebral infection with approximately 10⁴ viable bacteria of a low-virulence strain (no. B1772) in cortisone-treated mice is shown in fig. 1. The GM of the viable counts in the control and in cortisone-treated mice were similar for the first 24 hr after challenge, but from this time onwards the infection-enhancing effect of cortisone became evident. Whilst the infection in the control

mice declined, the viable counts in the cortisone-treated mice increased 5–10-fold daily. The terminal viable counts with the low-virulence strains in the brains of cortisone-treated mice were in the region of 10^7 – 10^8 bacteria, similar to those obtained with high-virulence strains in normal mice (Adams, 1970).

Fig. 2 shows that cortisone treatment also somewhat enhanced infections with one high-virulence strain (no. G353). From the 2nd day onwards the GM of the counts in the cortisone-treated mice always exceeded those in the control group of animals.

FIG. 1.

FIG. 2.

FIG. 1.—Bacterial counts after intracerebral injection of *Bordetella pertussis* no. B1772, a low-virulence strain, into normal mice and mice given subcutaneous injections of cortisone acetate.

FIG. 2.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. G353, a high-virulence strain, into normal mice and mice given cortisone acetate subcutaneously.

[These and the following figures show individual brain counts and geometric means of all counts for each group of mice.]

Similar experiments were also performed with the low-virulence strains no. B124 and B2288, and with the high-virulence strain no. 18/323. The average time to death with low-virulence strains in cortisone-treated mice was approximately the same as that for equal doses of high-virulence strains in normal mice. The terminal viable counts were also found to be similar, in the range of 10^7 – 10^8 bacteria. When cortisone-treated mice were infected with the high-virulence strains no. 18/323 and no. G353 the average time to death was reduced by 1.4 and 1.5 days respectively. It is to be noted that the “plateau” in the viable count in brains infected with strain no. G353 that is normally seen at the 3rd–4th day (Adams, 1970) did not appear in mice treated with cortisone (fig. 2).

Effect of treatment with vitamin A and streptolysin O

Unexpectedly, it was found that intracerebral injection of both vitamin-A alcohol and streptolysin O enhanced the intracerebral infection with low-virulence strains of *Bord. pertussis*. After an inoculum of 2.1×10^4 viable

bacteria, the GM of the counts in the mice treated with vitamin-A alcohol was approximately 10^6 bacteria at the 4th day, which was almost 10^4 times greater than the count in the control mice, in most of which the brain was "sterile" (fig. 3).

A similar type of enhancement was also noted in streptolysin O-treated mice; the viable count of the 4th day was over 10^3 times greater than that in the control mice (fig. 4).

FIG. 3.

FIG. 4.

FIG. 3.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. B1772 into normal mice and mice given an intracerebral injection of vitamin-A alcohol.

FIG. 4.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. 1772B into normal mice and mice given intracerebral injections of streptolysin O.

Measurement of lysosomal enzymes in mouse brain

In order to find out whether the release of lysosomal enzymes played any part in the pathogenesis of *Bord. pertussis* infection in the mouse brain, measurements were made of the amount of acid phosphatase, N-acetyl β -D-glucosaminidase, β -galacturonidase and β -glucuronidase present in the brain during infection in normal mice and in mice treated with cortisone, vitamin A and streptolysin O. The results obtained with cortisone-treated mice on days 1, 2 and 7 of the infections are shown in table I. Each figure is the mean of enzyme determinations on at least 6 brains. Some difficulty was found in obtaining equal pressures in each homogenisation, and this may account for the wide variation in the amount of free enzyme detected in the control brains. In nearly all of the cases the range of readings in the control mice overlapped the reading in the infected mice, and there was no evidence that cortisone had influenced the amount of free enzyme in the brain in infected mice.

The effect of cortisone, vitamin A and streptolysin O on the amount of acid phosphatase and N-acetyl β -D-glucosaminidase in the brain 2 hr after injection was also measured (table II). There was little difference between the results of the various treatments. Cortisone did not deplete the amount of

TABLE I
Amount of free lysosomal enzymes in the brain of cortisone-treated and untreated mice given intracerebral injections of *Bordetella pertussis**, and in that of normal mice†

<i>Bord. pertussis</i> strain no.	Virulence of strain	Cortisone treatment given	Days since intracerebral challenge	Amount per g wet weight of brain of			
				acid phosphatase	N-acetyl β -D- glucosaminidase	β -galactosidase	β -glucuronidase
B1772	low	no	1	36,670†	11,300‡	1235§	70
B124	low	no	1	35,250	11,800	1380	40
B124	low	yes	1	36,400	12,500	1600	80
18/323	high	no	1	28,400	9100	1300	70
B1772	low	no	2	29,200	10,000	1300	...
B124	low	no	2	41,000	16,000	...	60
B124	low	yes	2	28,600	14,000	...	80
18/323	high	no	2	39,000	16,500	1370	60
B124	low	yes	7	30,000	10,750	...	73
18/323	high	no	7	28,500	13,400	1580	50
None¶	—	no	—	33,140 $\pm$ 3360	12,700 $\pm$ 2500	1310 $\pm$ 250	55 $\pm$ 18

* Average of estimations on 6 or more brains.
† Average and range of estimations on 6 or more brains.
‡ μ g *p*-nitrophenol released per hr.
§ μ g *o*-nitrophenol released per hr.
|| OD units released per hr.
¶ Controls: no injection of *Bord. pertussis* or cortisone.

free enzyme, and treatment with vitamin A or streptolysin O did not lead to the release of an increased amount of enzyme.

Effect of X-irradiation of the whole mouse and of the body only

Preliminary experiments indicated that 400R was below the LD50 dose for the mice and that no deaths occurred in mice observed for 12 days after irradiation. This dose was selected for irradiation of the whole mouse or of the body only. A dose of 1000R was suitable for irradiation of the head when the body was adequately shielded, but irradiation of the body with this dose caused

TABLE II

Amount of free lysosomal enzymes in mouse brain 2 hr after injection of cortisone, vitamin-A alcohol and streptolysin O

Substance injected	Amount per g wet weight of brain of	
	acid phosphatase	N-acetyl β -D-glucosaminidase
Cortisone	26,800*	15,200*
Vitamin-A alcohol	24,800	14,150
Streptolysin O	19,000	12,000
None	27,000	14,950

* μ g *p*-nitrophenol released per hr.

many deaths. Total white blood cell determinations indicated that radiation-scatter was negligible.

In the first place, mice were irradiated with a single dose of 400R either to the body alone with the head shielded or to the whole mouse, and were challenged intracerebrally 3 days later with 3.2×10^4 viable *Bord. pertussis* no. B1772, a low-virulence strain.

A marked difference was observed between the effect on infection of irradiating either the body alone or the whole mouse (fig. 5). From the 2nd day on, the infection in the group in which the whole mouse was irradiated was greatly enhanced, and by the 4th day the mean viable count was nearly 1000 times that in the control group. When only the body was irradiated, no enhancement of intracerebral infection was detected.

Irradiation had greatly reduced the number of circulating white cells in both groups of irradiated mice; in the 5 days after irradiation of the whole mouse the average total WBC was between 190 and 350 cells per μ l and in the same period after irradiation of the body only it was between 310 and 710 cells per μ l. This suggested that these cells were not the main factor determining the course of intracerebral infection.

Effect of irradiation of the head only

Hopewell and Wright (1967) showed that the cellular reaction to cerebral injury was reduced by previous local X-irradiation. This reduction was independent of the time between irradiation and injury, indicating a local origin for brain macrophages, possibly glial cells. If the control of *Bord. pertussis* infections in the mouse brain were mediated through a similar local cellular reaction, irradiation at various intervals before challenge should enhance infection.

FIG. 5.

FIG. 6.

FIG. 5.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. B1772 into normal mice, mice irradiated with 400R to the body only (head shielded) and whole mice irradiated with 400R. Irradiation 3 days before challenge.

FIG. 6.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. B1772 into normal mice and mice irradiated with 400R or 1000R to the head only (body shielded). Irradiation 3 days before challenge.

The heads of mice were irradiated with 400R or 1000R 3 days, 3 wk or 12 wk before challenge with *Bord. pertussis* no. B1772; in each case the mouse's body and limbs were shielded.

When the mice were challenged with 3×10^5 viable *Bord. pertussis* no. B1772 3 days after irradiation, an enhancement of infection was observed from the 2nd day onwards in both the irradiated groups (fig. 6). By the 4th day the viable counts in all irradiated mice were greater than those in the control mice. The total WBC in mice given 1000R showed a small drop 1 and 2 days after infection to $c. 2000$ cells per μl , but after this time there was very little difference between the counts in control and irradiated mice.

Irradiation of the head often led to fatal infection from doses of no. B1772 that caused little mortality in normal mice (table III). The larger radiation dose gave the greater stimulation of infection, 10 of 12 mice died from infection after 1000R irradiation and challenge with 2.4×10^5 viable bacteria, but 400R irradiation and a similar challenge dose led to the death of 7 of 12 mice. Only 1 of the 12 control mice died.

When mice were irradiated 3 wk before challenge with 8.4×10^4 *Bord. pertussis* of no. B1772 the same general picture was obtained (fig. 7). From the

TABLE III

Proportion of fatal infections, and day of death, after intracerebral injection of the low-virulence strain of Bordetella pertussis no. 1772 into mice given irradiation to the head 3 days before, and into unirradiated mice*

Dose of irradiation (R)	Ratio: D/T†	Deaths on day
400	7/12	4, 4, 5, 5, 6, 8, 10
1000	10/12	4, 5, 6, 7, 8, 8, 8, 12, 14, 14
None	1/12	10

* 2.4×10^5 organisms per mouse.

† Number of deaths/number of mice challenged.

2nd day onwards, when the infection in control mice was declining, the bacteria in the irradiated mice were multiplying rapidly. By the 4th day the GM of the count in the brains of the mice irradiated with 1000R and 400R had reached

FIG. 7.

FIG. 8.

FIG. 7.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. B1772 into normal mice and mice irradiated with 400R or 1000R to the head only (body shielded). Irradiation 3 wk before challenge.

FIG. 8.—Bacterial counts after intracerebral injection of *Bord. pertussis* no. B1772 into normal mice and mice irradiated with 400R or 1000R to the head only (body shielded). Irradiation 3 mth before challenge.

4×10^6 and 3×10^5 bacteria respectively, compared with 5.5×10^2 in the control mice. There was a little evidence that by the 4th day the mice irradiated with 400R were showing signs of recovery from the infection.

This observation was confirmed when mice were challenged with 1.3×10^6 viable *Bord. pertussis* B1772 12 wk after irradiation (fig. 8). The counts in the mice irradiated with 1000R remained high after the 3rd day, but the counts in the mice irradiated with 400R declined rapidly at this time and were little different from the counts in the control mice by the 5th day. There may have been some recovery from the damage to the brain caused by low doses of radiation after 12 wk.

TABLE IV

Proportion of actively immunised mice given irradiation to the head or body 3 days previously that died after intracerebral challenge with the high-virulence strain of Bordetella pertussis no. G353

Radiation dose and target	Number of live bacteria injected	Dose of vaccine (number of organisms)	Ratio: D/T*
None	5.6×10^3	2×10^9	0/12
None	1.1×10^4	2×10^9	0/11
None	5×10^3	2×10^9	0/11
400R head	5.6×10^3	2×10^9	0/11
400R head	1.1×10^4	2×10^9	2/11
400R head	5×10^3	2×10^9	0/10
400R body	5.6×10^3	2×10^9	5/10
400R body	1.1×10^4	2×10^9	4/10
400R body	5×10^3	2×10^9	5/9
None	5.6×10^3	none	12/12

* Number of deaths/number of mice challenged.

Effect of irradiation on infections with high- and low-virulence strains of Bord. pertussis in immune mice

The experiments described above clearly showed that some component of the mouse brain is concerned with the elimination of intracerebral infections with low-virulence strains of *Bord. pertussis* in non-immune mice. It was therefore of interest to examine the fate of actively immunised mice that had been irradiated 3 days before challenge with *Bord. pertussis* G353, a high-virulence strain.

Mice were vaccinated intraperitoneally with 2×10^9 heat-killed organisms of a *Bord. pertussis* vaccine and irradiated 14 days later either to the head alone or to the body alone with 400R. The mice were challenged 3 days after irradiation. All the vaccinated but unirradiated mice survived challenge with doses

of the order of 10^4 organisms. Irradiation of the head alone did not significantly reduce the number of mice surviving challenge (30 out of 32), but when the bodies were irradiated with the head shielded, only 15 out of 29 of the mice survived (table IV). A χ^2 analysis of the latter two sets of results gave a value for P of <0.001 . This suggested that the mechanism responsible for the control of intracerebral infection with high-virulence strains in immunised mice was unaffected by irradiation of the head, but was neutralised by irradiation of the body. With low-virulence strains, irradiation of the body did not enhance infection (fig. 5), but irradiation of the head led to a highly fatal disease; vaccination also protected against the effects of irradiation to the head and infection with a low-virulence strain (table V).

TABLE V

Effect of previous irradiation of the head and active immunisation on the proportion of mice dying after intracerebral challenge with the low-virulence strain of Bordetella pertussis no. B1772

Dose of irradiation (R)	Number of live bacteria injected	Dose of vaccine (number of organisms)	Ratio: D/T*
1000	3×10^5	2×10^9	2/12
1000	3×10^5	none	11/12
None	3×10^5	none	1/12

* Number of deaths/number of mice challenged.

DISCUSSION

When mice were pre-treated with cortisone, infections with low-virulence strains of *Bord. pertussis* were greatly enhanced, and mimicked very closely infections with a high-virulence strain in normal mice. Unlike Iida *et al.* (1963) we found that cortisone also enhanced infections with high-virulence strains. Cortisone-treated mice died with slightly higher bacterial counts than untreated mice and at a somewhat earlier time. Cortisone can easily enter the brain. Baron and Abelson (1954) showed that it was a normal constituent of the cerebrospinal fluid in man. Abelson, Baron and Toakley (1955) and Christy and Fishman (1961) showed that the concentration of cortisone in the cerebrospinal fluid was increased 10-fold a few hours after the injection or oral administration of cortisone acetate.

The endyma and choroid plexus contain many lysosomes, and hydrocortisone stabilises the lysosome membrane. Weissman and Thomas (1963) and Weissman, Keiser and Bernheimer (1963) demonstrated the labilising action of vitamin A and streptolysin O on lysosome membranes, and Becker and Sutton (1963) and Sutton (1965) found that vitamin A increased the number and polarisation of lysosomes in the choroid plexus. Weissman and Thomas (1962) demonstrated that endotoxin increased the labilisation of lysosomes and that

cortisone prevented subsequent release of enzymes. If the enzymes were bactericidal for *Bord. pertussis*, release should lead to eradication of infection.

It was found, however, that both streptolysin O and vitamin A enhanced infection, and measurement of lysosomal enzymes during infection and after treatment with cortisone, vitamin A and streptolysin showed no significant increase or decrease in amount of free enzyme. Although the amounts of free β -galactosidase and N-acetyl- β -D-glucosaminidase we detected in the brain in normal mice were in close agreement with those reported by Millson (1965) and Hunter and Millson (1966), these workers recorded approximately a 3-fold increase in the amount of both enzymes in the brain of mice infected with the scrapie agent. Thus mice seem to be capable of producing large amounts of free enzyme in certain circumstances, but a direct effect of lysosomal enzymes in *Bord. pertussis* infection appears to be unlikely.

Whole-body irradiation of mice of the Theiler Original strain with a dose of 400R was non-lethal, but reduced the number of circulating white cells to about one-tenth of the normal value. Enhancement of intracerebral infection with *Bord. pertussis* was not brought about by irradiation of the body, but only when the head was irradiated. Earlier, Berenbaum *et al.* (1960) and Iida *et al.* (1962) failed to find eucocytes in the brains of mice infected with high-virulence strains of *Bord. pertussis* until the intracerebral viable counts were in excess of 10^6 bacteria. Similarly, only an occasional leucocyte was found in sections taken from brains infected with low-virulence strains, and this suggested that leucocytes played little part in controlling low-virulence *Bord. pertussis* infections in normal mice.

The enhancement of intracerebral infection with *Bord. pertussis* by irradiation of the head suggested that a local defence mechanism had been damaged and that the effect persisted for some weeks. After large doses of radiation (1000R), increased susceptibility to infection was still great 12 wk later, but after smaller doses (400R) considerable recovery had taken place in this time. Possible recovery mechanisms of the brain after damage by irradiation are considered elsewhere (Hopewell and Adams, 1970). Hopewell and Wright (1967) showed that with doses of 200–4000R the cerebral glial reaction to injury was diminished, and that this seemed to be independent of the time between irradiation and injury. They suggested that radiation damage was “stored up”, and that when cells were stimulated to divide they either failed to do so or died. Further histological investigations have been carried out to determine the nature of this response in *Bord. pertussis* infections (Hopewell and Adams).

It appears, therefore, that the control of low-virulence infections with *Bord. pertussis* is mediated through the local brain macrophage, the microglial cell. Thus the increased resistance of the brain to reinfection with low-virulence strains (Adams, 1970) may be due to mobilisation of these cells. The enhancing effect of cortisone, streptolysin O and vitamin A on infection may have a similar explanation, but in an inverse sense. Several workers (Foley, Chambers and Adams, 1953; Field, 1957; and Clements, 1958) have shown that cortisone reduced phagocytosis and microglial-cell migration around lesions in the

cerebral cortex. Similarly, Langman and Welch (1966, 1967) showed that pre-treatment of mice and rats with large doses of vitamin A interfered with cell division, and led to the appearance of many abnormal cell types. Streptolysin O, which lyses leucocytes (Hirsch, Bernheimer and Weissman, 1963), may have a similar action on microglial cells.

Other factors appear to be involved in infection with high-virulence strains. Although it was possible to detect some enhancement of infection by irradiation of the head, it is clear that the local microglial response is inadequate to control these organisms. In experiments with high-virulence strains in immunised mice, irradiation of the head made little difference to the number of survivors, but irradiation of the body led to a significantly increased mortality. This suggested that the circulating leucocytes may play an essential role in controlling infections with high-virulence strains in immune mice.

Although irradiation of the head enhanced infection with low-virulence strains in normal mice, most immune mice survived; this indicated that local radiation damage to the brain was of little importance in determining the course of infection in immune mice.

SUMMARY

Intracerebral infection of mice with low-virulence strains of *Bordetella pertussis* was greatly enhanced by the prior administration of cortisone acetate subcutaneously or of vitamin-A alcohol or streptolysin O intracerebrally.

The infections did not appear to stimulate the release of lysosomal enzymes in the brain.

When normal mice were X-irradiated to the head, with the body shielded, subsequent intracerebral infection with low-virulence strains was very much enhanced. Irradiation of the head alone did not affect the survival of actively immunised mice after the intracerebral injection of either high-virulence or low-virulence strains, but irradiation of the body, with the head shielded, significantly reduced the number of survivors after infection with a high-virulence strain.

It appears that a cerebral glial response controls intracerebral infections with low-virulence strains in normal mice, but that circulating leucocytes play a major role in controlling infections with high-virulence strains in immunised mice.

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Addendum

The 11th Report of the International Commission on Radiation Units and Measurements (1968, *Washington, D.C.*) recommends the use of the roentgen (abbreviation R) as the unit of exposure to radiation, and the abbreviation R is used in that sense throughout this paper. For the unit of absorbed dose the Commission recommends the rad.—EDITOR.