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Purified chick embryo cell rabies vaccine: economical multisite intradermal regimen for post-exposure prophylaxis

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SUMMARY

The standard six-dose intramuscular (i.m.) rabies post-exposure vaccine regimen using a new purified chick embryo cell (PCEC) vaccine was compared with two economical multisite intradermal (i.d.) PCEC regimens, a multisite i.m. PCEC schedule and a subcutaneous regimen using a suckling mouse brain (SMB) rabies vaccine manufactured in Thailand. The neutralizing antibody results for the four-site and eight-site i.d. and the standard i.m. PCEC regimens were similar over 3 months. A three-site i.m. PCEC regimen had no advantage. The SMB vaccine gave the lowest antibody levels. Human rabies immune globulin therapy significantly increased the GMT of all groups on day 7, unlike equine antirabies serum (EARS). Both antisera suppressed antibody responses to PCEC on days 14 and 28. Three generalized reactions probably related to EARS were the only serious side effects. An eight-site i.d. PCEC vaccine regimen proved as immunogenic as the routine i.m. schedule and, if implemented as post-exposure prophylaxis, would be the cheapest widely available tissue culture vaccine regimen. The protective efficiency should now be tested in patients bitten by rabid animals.

INTRODUCTION

A new rabies vaccine produced from a Flury-LEP virus strain in primary chick embryo fibroblast cells is now available for human use in several countries, including Thailand. This purified chick embryo cell (PCEC) vaccine has the advantages of high antigenicity and safety expected of a tissue culture vaccine. Six 1 ml doses of PCEC given intramuscularity (i.m.) produce antibody results comparable to human diploid cell strain vaccine (HDCSV) (Barth *et al.* 1984; Bijok *et al.* 1984) at 60% of the cost. PCEC has also proved immunogenic when given by the intradermal (i.d.) route in a pre-exposure regimen (Wasi *et al.* 1985).

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Post-exposure studies in patients bitten by proven rabid animals have confirmed that PCEC affords protection against rabies encephalitis (Ljubičić *et al.* 1985; Wasi *et al.* 1986). PCEC treatment is, however, still too expensive for the millions of people who receive courses of nervous tissue vaccine each year. The cheapest regimen which has been tested post-exposure using tissue culture vaccine is multisite i.d. injections of HDCSV (Warrell *et al.* 1985). This method also induces antibody more rapidly than single-site i.m. injections (Nicholson *et al.* 1979; Warrell *et al.* 1983), which is probably important when passive prophylaxis with immune serum is not available for financial or practical reasons. A similar accelerated antibody response was produced by three doses of PCEC given i.m. at three sites, instead of one site, on the first day (Dr U. Bijok, personal communication).

This study compares the neutralizing antibody response of post-exposure regimens of i.d. PCEC with the three-site i.m. PCEC and with suckling mouse brain (SMB) rabies vaccine which has been used for many years in Thailand. A comparison of immunosuppression by human and equine anti-rabies serum was also carried out.

MATERIALS AND METHODS

Subjects

Healthy volunteers from the hospital staff and veterinary students were randomly assigned to regimens using PCEC vaccine alone or with human rabies immune globulin (HRIG). Patients attending the Queen Saovabha Memorial Institute of the Thai Red Cross Society because of possible contact with rabies, but considered to be at very low risk, were randomly assigned to any regimen, including all SMB vaccine and equine antirabies serum (EARS) groups. All subjects denied previous rabies vaccination and consented to join the study. The ethical committee of the Faculty of Tropical Medicine, Mahidol University, granted approval.

Vaccine regimens

Purified chick embryo cell (PCEC) rabies vaccine (Behringwerke Lot 014, potency 7·5 i.u./ml by the NIH test) was supplied in 1 ml ampoules. The conventional regimen of 6×1 ml doses i.m. into the deltoid (Table 1) was compared with i.d. regimens of 0·1 ml with the first dose given at either 8 sites (deltoid, thigh, abdominal and suprascapular areas) or 4 sites (deltoid and thigh areas) with single-site boosters (deltoid). A three-site 1·0 ml i.m. regimen was given into both deltoids and gluteal muscles.

The suckling mouse brain rabies vaccine (Thai Government Pharmaceutical Organization batch 3, potency $1{\cdot}4 \times 10^6 LD_{50}$/0·03 ml by the Habel test) was used as recommended by the manufacturers: 14 daily doses of 1 ml with three boosters, all given subcutaneously (s.c.) over the abdomen. Vaccine alone was given to groups 1–5 (Table 1).

Human rabies immune globulin (HRIG) (Berirab, Behringwerke Lot 411015, containing 281 i.u./ml) was given in combination with all five regimens of vaccine (groups 6, 8, 9, 11, 12) in a dose of 20 i.u./kg i.m. into gluteal muscles, distant from the site of any vaccine injection.

Table 1. *Vaccine regimens*

Vaccine	Group no. vaccine only	Route	Volume per site (ml)	Number of sites vaccine given						Group no. vaccine plus	
				Day 0	Day 3	Day 7	Day 14	Day 28	Day 91	HRIG*	EARS†
PCEC	1	i.m.	1	1	1	1	1	1	1	6	7
PCEC	2	i.d.	0·1	8‡	—	4	—	1	1	8	—
PCEC	3	i.d.	0·1	4	—	4	—	1	1	9	10
PCEC	4	i.m.	1	3	—	—	1	—	—	11	—
								d 21	d 35 d 91		
SMB	5	s.c.	1	← daily × 14 →				1	1 1	12	—

* Human rabies immunoglobulin. † Equine antirabies serum. ‡ One whole ampoule of vaccine used.

Equine antirabies serum (EARS) (Behringwerke Lot 215005, containing 418 i.u./ml) was given combined with two PCEC regimens: the 1 ml i.m. (Group 7) and the schedule with the smallest amount of vaccine antigen, the four-site i.d. (group 10). A dose of 40 i.u./kg was injected i.m. into the gluteal muscles and, if appropriate, up to half the dose was infiltrated around the bite wound.

Serology

Blood samples were taken from all subjects on days 0, 7, 14, 28 and 91. Serum was coded and stored at −70 °C. Rabies neutralizing antibody was measured blind by the mouse neutralization test, with a sensitivity between 0·1 and 0·27 International Units (i.u.) in different batches. For purposes of calculation, a negative sample was counted as one-tenth of the minimum detectable value of the batch. Geometric mean titres (GMTs) of each group were calculated from $\log_{10}$ values of results in i.u. Comparison between GMTs of two groups was made by an independent t test, while differences between the GMTs of more than two groups were evaluated by Tukey's test of honestly significant difference at the 0·05 level of significance using SPSS/PC+software. Differences between the seroconversion rates and the incidence of side effects were analysed by Fisher's exact probability test when expected frequencies were less than 5, otherwise a 2×2 chi-square test was used.

At each attendance evidence of possible side effects of treatment was sought by inquiry and examination.

RESULTS

One hundred and eighty-four subjects were allocated to 12 treatment groups: numbers 5, 6, 9 and 12 had 16 subjects per group, the rest had 15. There were 102 males and 82 females aged between 10 and 47 (mean 23·5) years. They weighed between 22 and 89 (mean 50·6) kg. The subjects were evenly distributed between groups with respect to these variables. Vaccine was given and blood samples were collected as planned with the following exceptions: one patient was seen on day 15 instead of 14; two came on day 29 not 28; on day 91, 20 patients deviated by 1 day, 4 came 2 days late and 5 came 3–6 days late. These delays were evenly distributed between the treatment groups. Three sera were not assayed due to laboratory problems: two day 14 samples, from groups 1 and 11, and a day 91 sample from group 12. No rabies neutralizing antibody was detected in any sera taken on day 0, with the exception of two which proved negative when subsequently tested by the rapid immunofluorescent focus inhibition test. Neither patient showed an accelerated, secondary type of antibody response. The initial result was considered to be due to non-specific inhibition.

Serological response to vaccine alone

The highest seroconversion rate on day 7 was produced by the three-site i.m. PCEC regimen (Table 2), and although this was statistically similar to the i.d. PCEC regimens, it was significantly greater than the single-site i.m. PCEC regimen ($P = 0{\cdot}035$). No antibody was detected on day 7 in any patient given SMB vaccine.

From day 14 onwards all subjects had detectable neutralizing antibody (Table 3). The GMTs of the two i.d. regimens (groups 2 and 3) were very close throughout

the study and were not significantly different from the standard single-site i.m. regimen (group 1) at any stage. After day 7, only one subject given PCEC vaccine alone had < 0·5 i.u./ml of antibody, and that was in the single-site group on day 14.

Although the GMT of the three-site i.m. PCEC regimen (group 4) was the highest on day 7, it fell below that of the other PCEC groups on day 14. The GMT of 2·0 i.u. was significantly lower than both the i.d. PCEC groups ($P < 0·05$). On days 28 and 91 the GMTs of all groups given PCEC were statistically similar.

The SMB vaccine group (5) had the lowest GMTs on every ocassion. On days 14, 28 and 91 the result was significantly lower than the three highest PCEC regimens ($P = 0·05$). On day 14 two patients had antibody levels < 0·5 i.u./ml.

Serological response to vaccine with immune serum

On day 7 the administration of HRIG significantly increased the GMT of all five vaccine regimens ($P < 0·05$), and the overall seroconversion rate was 94%. EARS treatment did not affect the day 7 results of the two regimens tested: the single-site i.m. and the four-site i.d.

On days 14 and 28 the GMTs of all groups given PCEC with HRIG or EARS were significantly lower than the equivalent group given vaccine alone ($P < 0·05$), with one exception: the suppression of the GMT of the single-site i.m. group with HRIG (group 6) did not reach statistical significance on day 14. EARS suppression of the response to the single-site i.m. regimen (7) resulted in 5 of 15 subjects having < 0·5 i.u. of antibody on day 14 (Table 4). This was also the only group where significant suppression was found on day 91 ($P < 0·05$). Low antibody levels (< 0·5 i.u.) were seen on day 14 in 7 of 15 subjects given three-site i.m. PCEC with HRIG. In one subject in the eight-site i.d. PCEC with HRIG group, the maximum antibody level was 0·4 i.u. throughout the study.

The response to SMB vaccine from day 14 to day 91 was not affected by the addition of HRIG treatment.

Side effects of treatment

The incidence of local and systemic symptoms and signs following each vaccine regimen was remarkably similar, whether or not immune serum was given. The data have therefore been grouped according to the method of vaccination (Table 5). PCEC injected i.m. was more painful than i.d. ($P < 0·000001$) or SMBV s.c. ($P < 0·02$), but i.d. PCEC caused more local irritation, erythema and induration than the i.m. injections ($P < 0·02$). Local side effects were more common in the i.d. group (92%) than in the i.m. PCEC group (66%) ($P < 0·005$).

One patient given SMB vaccine alone had a macular rash around the injection site on days 8–19. Another given i.d. PCEC alone had increasing local reactions with an immediate wheal response with erythema and induration lasting up to 4 days, but no systemic symptoms.

Generalized effects were less frequent. Comparison between vaccine regimens is not possible since the reporting by volunteers and patients is not comparable.

One patient given HRIG and two given EARS complained of buttock pain within 3 days. Three patients treated with i.d. PCEC and EARS showed signs of hypersensitivity. A 21-year-old man was scratched by an apparently healthy stray dog, and half of the EARS was infiltrated around the wound. On day 6 he had

Table 2. *Antirabies antibody seroconversion rates on day 7*

Vaccine regimen	Percentage with antibody		
	Vaccine alone	With HRIG*	With EARS†
PCEC i.m. × 1	7	94	13
PCEC i.d. × 8	27	87	—
PCEC i.d. × 4	20	100	20
PCEC i.m. × 3	47	93	—
SMB	0	94	—

* Human rabies immune globulin. † Equine antirabies serum.

Table 3. *Rabies neutralizing antibody produced by vaccine alone. Values given are geometric mean titres and ranges in international units*

Group no.	Regimen	1 Day sample taken			
		7	14	28	91
1	PCEC i.m. × 1	0·02 < 0·1–0·2	3·1 (1)* 0·3–16·7	10·3 2·1–35.9	4·9 1·4–9·6
2	PCEC i.d. × 8	0·04 < 0·1–0·4	5·4 1·8–16·7	5·8 2·4–13·4	2·7 0·8–10·2
3	PCEC i.d. × 4	0·02 < 0·1–0·3	7·2 1·8–26	7·2 1·5–23·2	2·7 0·9–5·9
4	PCEC i.m. × 3	0·05 < 0·1–0·5	2·0 0·8–9·6	7·3 3·0–25·9	1·8 0·8–4·2
5	SMB	0·02 < 0·1	0·4 (2) 0·2–4·0	3·1 1·4–10·8	1·2 (2) 0·3–7·3

* The number in parentheses indicates the number of samples with antibody levels < 0·5 i.u., excluding day 7 results.

Table 4. *Rabies neutralizing antibody produced by vaccine with rabies immune globulin: geometric mean titres and ranges in international units*

Group no.	Regimen	Day sample taken			
		7	14	28	91
6	PCEC i.m. × 1 + H*	0·3 < 0·1–0·9	1·7 (1)† 0·3–4·7	3·3 1·2–9·1	3·0 0·9–7·4
7	PCEC i.m. × 1 + E‡	0·01 < 0·1–0·1	0·8 (5) 0·3–4·0	3·3 1·1–12·0	2·5 0·7–7·7
8	PCEC i.d. × 8 + H	0·2 < 0·1–0·6	2·1 (1) 0·4–8·2	2·1 (1) 0·4–13·3	1·5 (1) 0·2–8·1
9	PCEC i.d. × 4 + H	0·3 < 0·1–0·8	2·6 0·7–16·7	2·8 1·0–10·7	1·9 0·4–9·1
10	PCEC i.d. × 4 + E	0·02 < 0·1–0·2	2·2 (2) 0·4–13·4	2·8 (1) 0·4–14·9	2·5 0·7–11·3
11	PCEC i.m. × 3 + H	0·3 < 0·1–0·7	0·6 (7) 0·2–1·3	3·4 1·1–13·4	1·0 (1) 0·1–2·4
12	SMBV + H	0·3 < 0·1–0·8	0·9 (2) 0·3–3·6	3·0 0·8–17·6	0·8 (5) 0·3–2·9

* Human rabies immune globulin. † The number in parentheses indicates the number of samples with antibody levels < 0·5 i.u., excluding day 7 results. ‡ Equine antirabies serum.

Table 5. *Suspected side effects of treatment*
(The values stated are the numbers of patients with percentages in parentheses.)

	i.m. PCEC	i.d. PCEC	SMB
Group numbers...	1, 4, 6, 7, 11	2, 3, 8, 9, 10	5, 12
Number of subjects...	76	76	32
Local and regional effects			
Pain at vaccine injection site	37 (49)	8 (10)	7 (22)
Itch at vaccine injection site	8 (10)	49 (64)	9 (28)
Erythema	1	24 (32)	10 (31)
Induration	1	27 (35)	4 (12)
Wheal*	0	1	0
Ecchymosis	0	1	0
Rash*	0	0	1
Inguinal lymphadenopathy	14 (18)	27 (35)	18 (56)
Axillary lymphadenopathy	3 (4)	4 (5)	6 (19)
Buttock pain*	1	1	1
Itch at wound*	—	—	1
Total no. with local effects	50 (66)	70 (92)	25 (78)
Generalized effects			
Feverish feeling	11 (14)	5 (7)	2 (6)
Headache	9 (12)	3 (4)	0
Weakness	5 (7)	4 (5)	1
Influenza-like	2 (3)	1	1
Rash*	0	2 (3)	0
Dizziness	3 (4)	2 (3)	0
Diarrhoea	1	1	0
Total no. with generalized effects	19 (25)	11 (14)	4 (12)
Total no. without signs or symptoms	17 (22)	6 (8)	6 (19)

* See text for details.

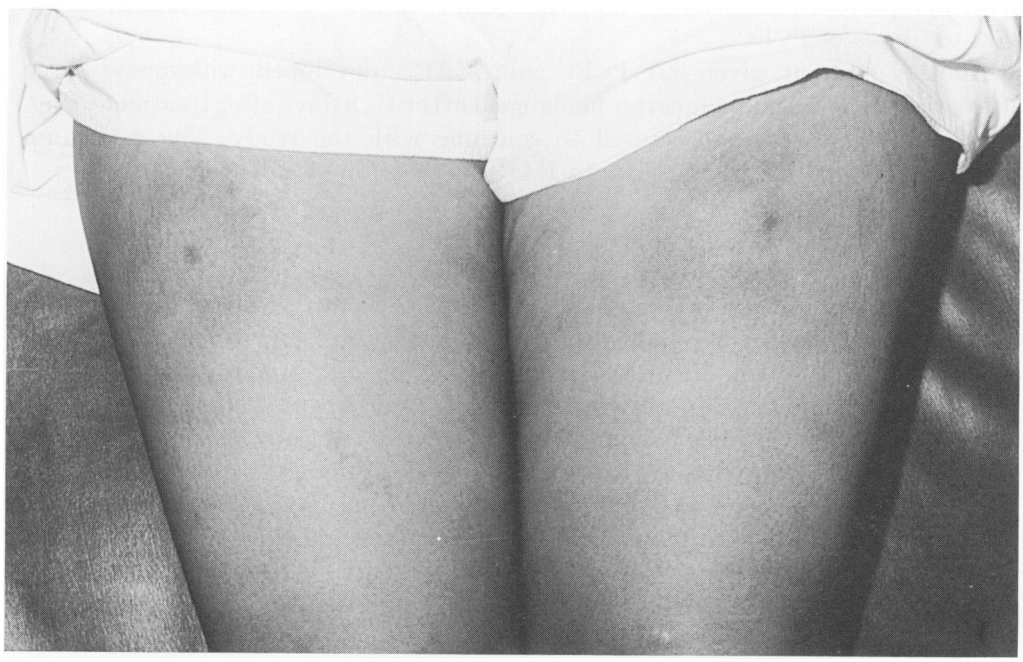

Fig. 1. Thigh intradermal injection sites in a patient with a generalized urticarial erythematous reaction 8 days after receiving PCEC and equine antirabies serum.

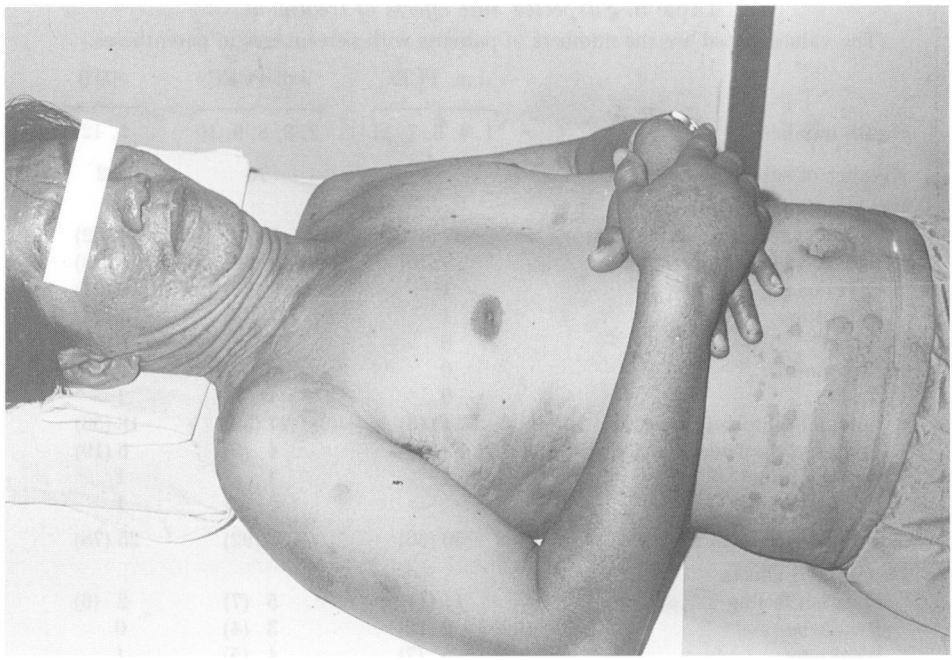

Fig. 2. Forty-five-year-old man with a generalized urticarial reaction and arthritis 9 days after receiving equine antirabies serum and PCEC vaccine.

itching at that site, possible due to the EARS. On days 7 and 8, two women aged 24 and 30 years developed urticarial reactions at their i.d. injection sites (Fig. 1), which spread to become generalized. One also had irritation at the site of EARS infiltration, felt feverish and had arthralgia. Both patients recovered promptly with symptomatic treatment, and the symptoms did not recur following i.d. booster doses of PCEC.

Another patient given i.d PCEC and EARS developed widespread large urticarial plaques and metacarpo-phalangeal arthritis 8 days after treatment (Fig. 2). This 45-year-old man refused to continue with the study. The maximum incidence of generalized reactions to EARS is therefore 3 of 31, or 10%.

DISCUSSION

Multisite intradermal vaccination with PCEC rabies vaccine induces neutralizing antibody levels comparable with the routine i.m. schedule. All i.d. recipients had > 0·5 i.u. of antibody from day 14 onwards, and the suppression of rabies immune serum was similar to that with i.m. treatment. These data resemble the results of identical i.d. regimens with HDCSV (Warrell *et al.* 1984) although the GMTs cannot be compared directly because a different serological method was used. The four-site and eight-site i.d. regimens gave very close results, demonstrating the safety of the eight-site regimen should some of the injections be misplaced subcutaneously or some vaccine wasted. However, one patient given eight-site i.d. PCEC with HRIG attained a maximum titre of only 0·4 i.u. on days 14 and 28, which is similar to the results in 'low-responders' treated with i.m.

PCEC (Bijok, 1985) and indicates that the amount of antigen in the regimen is close to the minimum acceptable post-exposure treatment, especially if immune serum is also used. Further reduction of vaccine dosage, as has been suggested for HDCSV treatment by Harverson (1984) and Monson (1985), risks poor protection in some patients. The eight-site, i.d. regimen has the advantages of reduction in the volume of vaccine used by 70%, and the number of visits to the clinic reduced from six to four.

The response to SMB vaccine is very similar to that reported by Wasi *et al.* (1983), using the same product. Two patients had < 0·5 i.u. of antibody on day 14 and, when combined with HRIG, five had low antibody levels on day 91.

Rabies immune serum treatment should provide the maximum titre of circulating antibody without suppressing the endogenous antibody response to concomitant vaccination. The dose recommended by the CDC (1984) and WHO (1984) is the safest compromise based on available data. In this study HRIG used with PCEC vaccine significantly increased the antibody titre on day 7, but EARS did not. On days 14 and 91 both antisera suppressed the antibody response to PCEC vaccine, but for the standard regimen of single-site i.m. vaccine with HRIG, the reduction was not statistically significant. Relative lowering of the antibody titre following immune serum is unlikely to jeopardize protection against rabies, but levels of < 0·5 i.u. of antibody indicate excess serum or insufficient vaccine. Such a poor result was found in at least one subject in every group with immune serum on day 14, except the four-site i.d. PCEC group with HRIG. This confirms the need for caution against excessive doses of immune serum (Warrell *et al.* 1985) and the need for manufacturers not to underestimate the potency of their products.

The side effects of both i.m. and i.d. PCEC treatment are similar to those following HDCSV injections in a comparable group of Thai volunteers (Warrell *et al.* 1983). It is not possible to be certain whether the PCEC or the EARS was the cause of the urticarial reaction, which was most striking at the thigh i.d. injection sites but was also generalized in two patients. The EARS is the most likely culprit, because later i.d. PCEC injection sites were relatively asymptomatic, so localization to i.d. sites may have been due to a Köebner phenomenon.

If we combine our experience of 101 EARS-treated patients from this and a previous study (using an Institut Pasteur product) (Warrell *et al.* 1985), the complications are: a generalized reaction (serum sickness) in 4%; a local reaction alone in 3% and buttock pain in 3%. Our rate is lower than the 15·6% described by Hosty & Hunter (1953) or 16·3% in the report of 526 cases by Karliner & Belval (1965). Analysis of that large study revealed an increase in reaction rate with age, possibly related to the increase in volume of serum given to adults, among whom 46% had serum sickness. An inexplicable variation in the incidence of reactions occurred during different time periods, which may have been associated with some batches of serum. The low incidence of 4% in this study may be explained by improved methods of purification.

Combining the data from this and our four other studies using HRIG of British or French origin (Warrell *et al.* 1983, 1984; Suntharasamai *et al.* 1986; Chanthavanich *et al.* 1987) no reaction was observed among 235 recipients, some of whom were student volunteers, but 5·5% complained of pain at the injection

site. The lack of serum sickness confirms other reports of minimal complications of HRIG treatment at the usual dosage (Hattwick, Corey & Creech 1976; Nicholson & Turner, 1978; Helmick *et al.* 1982).

The eight-site i.d. PCEC vaccine regimen combined with either HRIG or EARS has proved as immunogenic as the standard single site i.m. course of the vaccine. If it were employed it would be the cheapest available rabies post-exposure treatment using a freely available tissue culture vaccine. The protective efficiency of this regimen must first be confirmed by a trial in patients with proven exposure to rabid animals, such as was performed for the same regimen with HDCSV (Warrell *et al.* 1985).

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REFERENCES

BARTH, R., GRUSCHKAU, H., BIJOK, U., HILFENHAUS, J., HINZ, J., MILCKE, L., MOSER, H., JAEGAR, O., RONNEBERGER, H. & WEINMANN, E. (1984). A new inactivated tissue culture rabies vaccine for use in man. Evaluation of PCEC vaccine by laboratory tests. *Journal of Biological Standardization* **12**, 29–46.

BIJOK, U. (1985). Purified chick embryo cell (PCEC) rabies vaccine: a review of the clinical development 1982–1984. In *Improvements in Rabies Post-Exposure Treatment* (ed. I. Vodopija, K. G. Nicholson, S. Smerdal and U. Bijok), pp. 103–111. Zagreb: Zagreb Institute of Public Health.

BIJOK, U., VODOPIJA, I., SMERDEL, S., THONGCHAROEN, P., NICHOLSON, K., DIETRICH, M. & GONZALEZ DE COSIO, A. (1984). Purified chick embryo cell (PCEC) rabies vaccine for human use: clinical trials. *Behring Institut Mitteilungen* **76**, 155–164.

CENTERS FOR DISEASE CONTROL (1984). Recommendation of the immunization practices advisory committee. Rabies prevention – United States, 1984. *Morbidity and Mortality Weekly Report* **33**, 393–408.

CHANTHAVANICH, P., SUNTHARASAMAI, P., WARRELL, M. J., VIRAVAN, C., LOOAREESUWAN, S., SUPANARANOND, W., KARBWANG, J., WARRELL, D. A., PHILLIPS, R. E. SINHASENI, A., BOONYARATAPHAN, P. & SUREAU, P. (1987). Antibody response to suckling mouse brain rabies vaccines for post-exposure treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 260–263.

HARVERSON, G. (1984). Post-exposure intradermal antirabies vaccine: a cheaper alternative for developing countries. *Tropical Doctor* **14**, 67–70.

HATTWICK, A. W., COREY, L. & CREECH, W. B. (1976). Clinical use of human globulin immune to rabies virus. *Journal of Infectious Diseases* **133** (suppl.), A266–272.

HELMICK, C. G., JOHNSTONE, C., SUMNER, J., WINKLER, W. G. & FAGER, S. (1982). A clinical study of Mérieux human rabies immune globulin. *Journal of Biological Standardization* **10**, 357–367.

HOSTY, T. S. & HUNTER, F. R. (1953). Incidence of reactions to anti-rabies horse serum. *Public Health Reports* **68**, 789–791.

KARLINER, J. S. & BELVAL, G. S. (1965). Incidence of reactions following administration of antirabies serum, study of 526 cases. *Journal of the American Medical Association* **193**, 109–112.

LJUBIČIĆ, M., VODOPIJA, I., SMERDEL, S., BAKLAIĆ, Ž., SVJETLIČIĆ, M. & LOJKIĆ, M. (1985). Efficacy of PCEC vaccine in post-exposure rabies prophylaxis. In *Improvements in Rabies Post-Exposure Treatment* (ed. I. Vodopija, K. G. Nicholson, S. Smerdel and U. Bijok), pp. 95–101. Zagreb: Zagreb Institute of Public Health.

MONSON, M. H. (1985). Practical management of rabies and the 1982 outbreak in Zorzor district, Liberia. *Tropical Doctor* **15**, 50–54.

NICHOLSON, K. G., COLE, P. J., TURNER, G. S. & HARRISON, P. (1979). Immune responses of humans to a human diploid cell strain of rabies virus vaccine: lymphocyte transformation, production of virus-neutralizing antibody, and induction of interferon. *Journal of Infectious Diseases* **140**, 176–182.

NICHOLSON, K. G. & TURNER, G. S. (1978). Studies with human diploid cells strain rabies vaccine and human antirabies immunoglobulin in man. *Developments of Biological Standardization* **40**, 115–120.

SUNTHARASAMAI, P., CHANTHAVANICH, P., WARRELL, M. J., LOOAREESUWAN, S., KARBWANG, J., SUPANARANOND, W., PHILLIPS, R. E., JANSAWAN, W., XUEREF, C., POURADIER-DUTEIL, X. & WARRELL, D. A. (1986). Purified Vero cell rabies vaccine and human diploid cell strain vaccine: comparison of neutralizing antibody responses to post-exposure regimens. *Journal of Hygiene* **96**, 483–489.

WARRELL, M. J., NICHOLSON, K. G., WARRELL, D. A., SUNTHARASAMAI, P., CHANTHAVANICH, P., VIRAVAN, C., SINHASENI, A., CHIEWBAMROONGKIAT, M., POURADIER-DUTEIL, X., XUEREF, C., PHANFUNG, R. & UDOMSAKDI, D. (1985). Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet* i, 1059–1062.

WARRELL, M. J., SUNTHARASAMAI, P., NICHOLSON, K. G., WARRELL, D. A., CHANTHAVANICH, P., VIRAVAN, C., SINHASENI, A., PHANFUNG, R., XUEREF, C. & VINCENT-FALQUET, J.-C. (1984). Multisite intradermal and multisite subcutaneous rabies vaccination: improved economical regimens. *Lancet* i, 874–876.

WARRELL, M. J., WARRELL, D. A., SUNTHARASAMAI, P., VIRAVAN, C., SINHASENI, A., UDOMSAKDI, D., PHANFUNG, R., XUEREF, C., VINCENT-FALQUET, J.-C., NICHOLSON, K. G., BUNNAG, D. & HARINASUTA, T. (1983). An economical regimen of human diploid cell strain antirabies vaccine for post-exposure prophylaxis. *Lancet* ii, 301–304.

WASI, C., CHAIPRASITHIKUL, P., CHAVANICH, L., PUTHAVATHANA, P., THONGCHAROEN, P. & TRISHAHANANDA, M. (1986). Purified chick embryo cell vaccine. *Lancet* i, 40.

WASI, C., CHAIPRASITHIKUL, P., PUTHAVATHANA, P., CHAVANICH, L. & THONGCHAROEN, P. (1985). Immunogenicity and reactogenicity of the new tissue culture rabies vaccine for human use (purified chick embryo cell culture). In *Improvements in Rabies Post-Exposure Treatment* (ed. I. Vodopija, K. G. Nicholson, S. Smerdel and U. Bijok), pp. 85–94. Zagreb: Zagreb Institute of Public Health.

WASI, C., CHAIPRASITHIKUL, P., THONGCHAROEN, P., RUNGPITARUNGSI, V., THARACHAN, C. & LIKANONTSAKUL, S. (1983). Protective antibodies after vaccination with human diploid cell rabies vaccine. *Asian Pacific Journal of Allergy and Immunology* **1**, 125–30.

WHO EXPERT COMMITTEE ON RABIES (1984). Seventh Report. *Technical Report Series* 709, pp. 28–31. Geneva: World Health Organization.