The 17DD yellow fever vaccine from a routine production lot was administered to 58 subjects without a prior vaccination history and to 35 individuals vaccinated three months to twenty-five years previously. Serum samples collected prior to the vaccination and one month later, were assayed for antibody using a standardized plaque reduction neutralization test (PRNT) and expressed in units of the International Reference Preparation (IRP).

In the group of previously unvaccinated subjects, 58/58 (100%) showed seroconversion with geometric mean post vaccination antibody concentrations of >12.6 IU/ml of serum. In the group of previously vaccinated subjects, 12/35 (34%) showed seroconversion with mean post-vaccination antibody levels of >12.6 IU/ml. In this group, seroconversion correlated closely with pre-vaccination antibody levels and no individual (0/20) with levels of 4 IU/ml or greater showed seroconversion. Of the 12 subjects who seroconverted, 10 had been immunized within the previous ten years suggesting primary vaccine failure or a decline in humoral immunity.
INTRODUCTION

Yellow fever vaccine has been produced at the Oswaldo Cruz Foundation since 1937 with progressive improvements in the production technology and product quality. Since 1943, the 17DD strain of the virus has been produced at a constant passage level by the use of a seed lot system. The vaccine is now free of the avian leucosis virus complex and complies with all of the WHO minimum requirements (WHO 1975), including thermostability (WHO 1988).

A dose response study was conducted in young adults in 1988 which demonstrated that as little as 200 plaque forming units of the 17DD virus was sufficient to induce 100% seroconversion (Lopes et al. 1988). The present study was carried out to confirm the continued efficacy of the product.

Yellow fever antibody levels may be determined by a number of methods (Shope & Sather, 1979). Serology has been previously standardized in terms of units of the International Reference Preparation (IRP) of yellow fever antibody (WHO 1990, Lang et al. 1999). In this study, a microtitre plaque reduction neutralization test was employed in conjunction with a standard antibody preparation to define serum antibody concentrations in International Units (IU).

MATERIAL AND METHODS

Volunteers - Ninety-three healthy persons volunteered for this study. A written consent was obtained from each participant. Age and previous vaccination history in terms of the number of doses and interval since the last dose was determined prior to vaccination. At the time of taking the second blood sample, volunteers were questioned regarding pain on injection and reactions following vaccination.

Vaccination - Vaccine lot 942FB002Z-0296 was used in conjunction with saline diluent lot number 943DD015Z-0399. These vaccine and diluent lots had been fully tested and shown to meet all WHO minimal requirements including potency and stability. The vaccine was reconstituted and administered by a professional vaccinator by the subcutaneous route. Three fifty-dose vials were used, each over a 1 hr period and the reconstituted vaccine was held in an ice bath, during the vaccination procedure.

Serum samples - Blood samples were taken by venipuncture prior to vaccination and one month later. Sera were separated and stored at -70°C. Prior to assay, samples were inactivated at 56°C for 30 min.

Antibody determinations - Antibody content of the serum samples was determined by a microtitre plaque reduction neutralization test (PRNT) (Stefano et al. 1999).

Vaccine testing - An additional test was carried out to confirm the thermal stability and potency of this lot of vaccine after reconstitution in the specific diluent. Potency of the reconstituted vaccine was tested by plaque assay in Vero cells immediately and after holding on ice for two hours (WHO 1988).
RESULTS

None of the 93 volunteers considered the vaccine to be painful on injection site. A 26 year-old female who had never been vaccinated reported a local reaction in the form of an induration at the site of injection, which lasted for 3 to 4 days. No systemic reactions were reported.

Following reconstitution of the vaccine and holding on ice, no loss in titre was found after 2 hours. Mean potency by plaque assay was $1.6 \times 10^4$ pfu/dose equivalent to $3.2 \times 10^3$ mouse LD$_{50}$ (MLD$_{50}$). These results confirm earlier stability studies and that the dose administered was in keeping with the WHO minimum requirement of $1 \times 10^3$ MLD$_{50}$ (WHO 1975).

The sensitivity of the PRNT was satisfactory since, in the three tests carried out, 1.26 IU/ml or less of antibody was detected at the first serum dilution tested (1/5). This represents 0.87% of the IRP which contains 143 IU/ml.

Of the 58 previously unvaccinated subjects, 20 showed low levels of plaque inhibition in the pre-vaccination serum sample (0.25 to 1.26 IU/ml). No correlation was found between pre and post-vaccination antibody levels ($p=0.1$) for these 20 serum pairs (Table I). The increase of 4 fold or greater in antibody level occurred in all 58 (100%) subjects in this group, showing a mean increase of >31.6 fold (range 4 fold to 500 fold). Geometric mean antibody content in the post-vaccination serum samples was >12.6 IU/ml with a range of 0.25 to >31.6 IU/ml.

Of the 35 persons with a prior history of vaccination, all showed some level of plaque inhibition in the pre-vaccination serum sample (range 0.25 to >200 IU/ml). Seroconversion in this group correlated closely with antibody concentration in the pre-immunization sample. Thus, 6/6 individuals with initial antibody levels of <1 IU/ml seroconverted showing a mean increase of 20 fold. Where pre-immunization antibody levels were between 1.26 and 3.2 IU/ml, 6/9 (67%) subjects seroconverted with a mean increase of 5 fold. No individual (0/20) with antibody levels of 4 IU/ml or greater in the pre-immunization sample showed seroconversion. Individuals with antibody levels of 4 to 8 IU/ml showed a mean increase of 1.25 fold while those with 10 IU/ml or greater showed a mean reduction of 2 fold.

The number of previously vaccinated subjects in this study were insufficient to permit quantitative correlation between seroconversion rates and the time since the last vaccination. It is notable however, that of the 12 previously vaccinated subjects who showed seroconversion following vaccination, 10 had been vaccinated less than ten years previously (range 0.3 to 8 years).

DISCUSSION

Yellow fever vaccines are highly efficacious and have been extremely successful in controlling yellow fever dissemination in endemic areas.

One month after vaccination administration of the 17DD vaccine all of the previously unvaccinated
subjects showed seroconversion with a fourfold or greater increase in serum antibody. These response rates are in keeping with previous findings for yellow fever vaccines (Lopes et al. 1988, Freestone 1994).

Lang and co-authors (1999) also reported similar seroconversion rates using 17D vaccines from two different manufacturers.

All 35 individuals who had been previously vaccinated showed some level of seropositivity in the pre-vaccination sample. This result is confirmed by the demonstration by Poland and co-workers (1981) of the persistence of neutralizing antibodies in 80.6% of a cohort of veterans. Among the previously vaccinated subjects 12/35 (34%) seroconverted. All 12 seroconverting had pre-vaccination antibody levels of 3.2 IU/ml or less and only 3 subjects in this category failed to seroconvert. None of the 20 subjects with pre-vaccination antibodies at 4 IU/ml or greater showed seroconversion. This finding suggests that antibody levels of 4 IU/ml blocked in vivo replication of the vaccine virus following the parenteral administration of 3.2x10³ MLD₅₀. It was established that yellow fever neutralizing antibody titer e ≥1/10 is accepted as a serological correlate of protection against yellow fever in non-human primates (Freestone 1994) and seroconversion is considered when neutralizing antibodies are above this limit (Lang et al. 1999). In our study, seroconversion was considered as an increase of fourfold in neutralizing antibodies. The use of an in-house reference calibrated from an international reference preparation (National Institute for Biological Standards and Control, Potters Bar, United Kingdom) provided the best available means of assessing protective levels of antibody. By the other hand, definition of protective levels of antibody in units of the International Reference Preparation provides a sound basis for the standardization of yellow fever serology. Lang and co-workers (1999) defined 1 IU/ml as corresponding to 1/10 dilution. From our results, we conclude that it is highly probable that antibody levels of 4 IU/ml would also protect against virulent virus following mosquito transmission.

In the group of individuals vaccinated within the previous ten years (10/12) some had low levels of neutralizing antibodies. Since WHO recommends revaccination at ten year intervals (WHO 1994), this result is disturbing. Four of these subjects had been vaccinated up to two years previously and most probably represent primary vaccine failure. Some authors report weak responses to yellow fever vaccine (Guerra et al. 1997, Stefano et al. 1999) and suggest poor handling and storage of vaccine.

It must be emphasized however, that even when humoral immunity declines, immunological memory may be retained resulting in an anamnestic response on challenge and under these conditions, no clinical disease would be expected. Since a single post-vaccination sample was tested in this study we are unable to differentiate between primary and anamnestic responses.
The antibody response of previously vaccinated subjects in terms of changes in antibody concentrations is of interest. Thus the mean increase in antibody levels is inversely related to the pre-vaccination level (Table II). While large increases by definition occur in the seroconverting individuals, 7/8 of those with high pre-vaccination antibody levels >10 IU/ml or greater show a mean reduction of antibody concentration by 2 fold. This suggests that the vaccine virus is complexed by antibody and eliminated through the excretory pathway. In the intermediate group with pre-vaccination antibody levels of 1.26 a 8 IU/ml a net decrease in antibody occurred in only 2/15 individuals while the remaining 13 showed a mean increase of 1.8 fold. This result indicates that either a weak antibody response occurred to the injected viral antigen or that limited virus replication occurred in these individuals.

REFERENCES


TABLE I

Seroconversion and antibody responses in relation to previous vaccination history with Yellow Fever and pre-vaccination antibody levels.

<table>
<thead>
<tr>
<th>Vaccination history</th>
<th>Antibody levels log_{10} mIU/ml</th>
<th>Geometric mean</th>
<th>Increase</th>
<th>Seroconversion rate*</th>
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<tr>
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<td>Pre-vacc. range</td>
<td>Geometric mean</td>
<td>Post-vacc.</td>
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<tr>
<td>No</td>
<td>&lt;2.4 - 3.1</td>
<td>&lt;2.6</td>
<td>&gt;4.1</td>
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<td>2.7</td>
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<td>&gt;4.0</td>
<td>&gt;0.7</td>
</tr>
<tr>
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<td>3.6 - 3.9</td>
<td>3.8</td>
<td>3.9</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>4.0 - &gt;5.3</td>
<td>&gt;4.3</td>
<td>4.0</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

*: 0.6 log_{10} increase in antibody after one month

TABLE II

Seroconversion in relation to the WHO recommendation for re-vaccination against Yellow Fever.

<table>
<thead>
<tr>
<th>Interval since last vaccination (years)</th>
<th>N° of subjects</th>
<th>Seroconversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N° of subjects</td>
<td>%</td>
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<tr>
<td>0 - 9</td>
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<td>10</td>
</tr>
<tr>
<td>10 - 25</td>
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<td>2</td>
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<tr>
<td>All subjects</td>
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<td>12</td>
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