A note on observations on the Maintainance of Nichol's Train of Treponema Pollida in Rabbits

By

M. D. MUNGALE, Biochemist, Sassoon Hospitals, Poona.

A sensitive test in syphilis serology so far as the clinician is concerned, is one that will enable him to detect all cases of syphilis that presumably need treatment, while a specific test is the one which will prevent non-syphilitic persons being unnecessarily treated for the same. The Standard Serological Tests (S.T.S.), though very useful, have not been either sufficiently sensitive or specific to meet the clinicians requirement. By carrying out specific tests like T.P.T., T.P.I.A., T.P.A. which use specific antigen of Treponemal origin, it is possible to exclude the Biological False Positive cases. One of the Laboratory needs for such tests is to establish and maintain the Nichol's Strain of Theponema Pallida in Rabbit testes to enable specific antigen being prepared. This note contains observations of author on such maintenance of Treponemal strain.

Briefly the technique consisted in selection of V.D.R.L. negative reacting male rabbits of 4 to 6 months age being intratesticullararly innoculated on both the sides at the upper and lower poles of tests with 0.5 ml. of emulsion of the treponemes with 15-20 organisms per field. The innoculum of subsequent rabbits was prepared by extraction of infected testes with 10% normal rabbit serum in saline.

Usually the rabbits developed orchitis on 5th to 7th day and the animal was sacrificed on 10th day when the testes became uniformly indurated, swollen and reddish.

In all, work of maintaining strain was carried out for 16 months and thirtyfour generations of Treponema Pallida were maintained in 112 rabbits. Out of this 23 generations were maintained in Bombay in 75 rabbits and 11 further generations were maintained by using 37 rabbits at Poona. The rabbits of differing breeds were brought from different breeding houses, from Bombay, Poona, Pimpri, etc. and were kept in differing climatic conditions. Hence some observations could be made during these experiments of maintenance of the virulent Strain of Treponema Pallida in Rabbits.

OBSERVATIONS

No. 1 — Initially 12 Rabbits from Haffkine Institute were taken to Madras. These were innoculated in Madras and it was observed that the testicular syphiloma was larger than that produced in Madras in the local Madras Rabbits.

No. 2 — Same difference was noticed in 20 Rabbits from Pathology Dept. Grant Madical College, Bombay, when they were simultaneously compared with 12 rabbits from Haffkine Institute, Bombay.

The size of the infected testes from G.M.C. Rabbits was too small and the number of T. Pallida was comparatively less.

No. 3 — Six rabbits were purchased from Hindustan Antibiotics Ltd. Pimpri. All these animals were comparatively healthier than the Haffkine animals. None of these could be successfully innoculated for maintenance of the strain though they were sero converted by innoculum.

No. 4 — The use of discarded rabbits which were earlier experimented with other Pathogens like Mycotuberculosis was also made in Bombay. After the initial experimentation the animals were observed for one month prior to innoculation with T. Pallida. It was noticed that most of the rabbits took orchitis on 15th to 18th day i.e. the incubation period was longer in these than in the normal rabbits. However the testes yielded the usual concentrantion of treponemes.

No. 5 — Observations similar to the above was noted in Poona, when rabbits discarded after initial experimentation with V. Cholera were used for innoculation.

No. 6 — Whenever rabbbits reacting positively to V.D.R.L. test at a titre less than 1 in 16 were used, the results were also similar to those shown under No. 4 above.

No. 7 — Hares brought from field could not be successfully innoculated for maintenance of strain. They developed after 30 days a dark ground negative sore at the innoculated site which heeled uneventfully. The seronegative reactors turned seropositive after innoculations.

DISCUSSION

Turner discusses similar observations and cites a number of references. The observations could be codified for discussion as below:—

1. **Breed of Rabbit:**—Roshan has reported that Hawana and Dutch breeds of Rabbits were most resistant to infection with Nichol's strain of T. Palladum thhan were English, Himalayan or Rex breeds. The main differences found were in the course of the infection and in the incidence and distribution of generalised lesions. The mean incubation period for the primary orchitis was fairly constant with minor variations which were probably not significant.

The rabbits used in this work were mainly albinos however when 'Hares' were utilised they did not take up the strain although there was sero conversion. This proves that a particular breed of Rabbits is not suitable for Treponemal work.

3. **Health:**—It is generally agreed that experimental treponemal infections are more readily induced in healthy than in unhealthy animals, in the latter, lesions may develop poorly or not at all. However the healtheir animals from Pimpri could not be successfully innoculated for maintenance. The reason for this failure may therefore be

that they had penicillin or antibiotic supplements of innoculations earlier. This was confirmed to be so by later enquiry. Our observations on rabbits used in cholera and tuberculosis work, could possibly be explained by their being relatively less healthy or on grounds of presence of antibody in them which might have interferred to a mild extent.

4. **Food and Housing:**—Ostuji have made experiments and have either prolonged or shortened the incubation period. It is also shown that rabbits when they appear healthier, eat better, gain weight faster and have less diarrohea, the treponemal infections are more readily induced in them. Our observation was that compared to all Animal Houses, the animal-house in Haffkine institute was an ideal one, the animal house in G.M.C. Bombay was in a dark room where light was inadequate, and the animals in Madras were purchased directly from Market. This did show effect on growth of orchitis. The size of infected testes of G.M.C. rabbits was comparatively low. Madras Rabbits also showed that the testicular sphiloma produced is less compared to Haffline Institute Rabbits.

Thus it is observed that when a rabbit gets adequate diet, plenty of air, light and proper upkeep, then the syphiloma is larger.

5. **Influence of Temperature:**—Temperature seems to be one of the environmental factors which affect the course of treponemal infection. Almost everyone who works with experimental syphilis is impressed with the fact that lessions are more frequent and more severe in winter. The work on this has been done by many workers like Brown and Pearce, Yamamoto and Hollaner and Turner. The latter worker found that when 19 rabbits were innoculated with 500 Treponemes at multiple sites on the shaved surfaces of the back, and were maintained in cool environment (18-21°C) each developed complete pattern of 6 lessions within 20 days. On the other hand 18 rabbits with corresponding innoculations maintained in a warm environment (29-31°C) developed lessions poorly or not at all. Our observations are that in Poona, climatic variations are very much more compared to Bombay and Madras (coastal weather). Hence in winter the syphiloma was complete on the 6th Day and in Summer it was complete on 12th day. Thus the seasonal temp. influences the length of incubation period of treponemal infection in rabbits.

6. **Effect of Antibiotics:**—Pathogenic Treponemes are sensitive in greater or less degree to most of the antibiotics now on the market and many of the experiments have been known to have failed owing to the presence of antibiotics in the animal food. Our Pimpri rabbits failed to take up infection on this account.

7. **Influence of Specific Antibody:**—It is known that the presence of cross-reacting antibody against T. Cuniculli may seriously interfere with the work of the experimental syphilis in laboratory. Hardy and Nell have found that when such animals are innoculated with large doses of treponemes for the preparations of agglutinating antigens the harvest of spirochetes tend to agglutinate spontaneously. This means

rabbits which had some antibody do resist T.P. In our experiments it was noticed that the time taken for such rabbits to develop syphilomas was more than the normal ones.

Conclusions:—Work was not preplanned and hence observations are casual and corroborative. A larger series would be necessary if conclusions are to be drawn from observations recorded in this note.

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