

A PRELIMINARY NOTE ON INOCULATION OF MONKEYS
WITH HUMAN LEPROSY MATERIAL
AFTER SPLENECTOMY

BY R. G. COCHRANE, ..

C. G. PANDIT,

AND K. P. MENON

*Lady Willingdon Leper Settlement, Chingleput, and King Institute of Preventive
Medicine, Guindy, Madras*

Recent reports by Adler (1, 2) have indicated that it is possible to infect Syrian hamsters with human leprosy after removal of the spleen. We thought, since the main defense in human leprosy appears to be the cells of the reticulo-endothelial system, that splenectomy might be a vital factor in producing the infection in animals which have hitherto been resistant to it. We therefore decided, as a preliminary experiment, to splenectomize a series of monkeys (*M. sinicus*) and insert into the mesentery a nodule taken from a case of leprosy.

Patients who had subcutaneous nodules which were not fixed to the skin were chosen. In each instance the nodule was excised under local anesthesia, the precaution being taken to dissect away completely the overlying skin before enucleating the nodule, so that there should be no contamination from without. The nodule was placed in Tyrode solution and transferred to the King Institute, Guindy. Within two hours the monkey was anesthetized, the spleen removed (when that was done), the nodule fixed to the splenic stump or omentum, and the abdomen closed. The same stitch which fixed the nodule to the stump was used to close the peritoneum, the nodule thus being brought into close proximity to the abdominal wall.

In total, eight monkeys have been inoculated, but two died before conclusions could be drawn. One (No. 2) died under the anesthetic and the other (No. 8) of malaria, it having previously been given that infection but not adequately treated. Though no outstanding results can be claimed for the other six animals, we have made a few observations which may be of general interest.

MONKEY No. 1. This animal was splenectomized and inoculated in

the manner described by imbedding a nodule in the omentum, on March 2, 1938. Two months later a small ulcer which proved to be a stitch abscess was noticed at the site of the operation wound. Smears were taken from the pus exuding from the abdominal wound and many acid-fast bacilli were found, and in addition globi, both large and small, were noted in many fields. Because the original nodule showed only a few bacilli (one group in 25 fields) it was thought that the development of globi indicated active multiplication of the bacilli. A week later (i.e., two and one-quarter months after the operation) the monkey looked ill and the abdominal wound had broken down further. On both sides of the original incision there were small erythematous lesions which showed a tendency to ulcerate, and there was considerable purulent material exuding from the sinus, which was found to extend to the peritoneum. Lest the animal should subsequently die from a secondary infection, it was decided that it should be chloroformed.

Post mortem examination.—(a) Smears from the abdominal wound showed clusters of acid-fast bacilli in every field examined, and there were also many globi. (b) Scrapings from lesions of the abdominal wall also showed many bacilli in various forms; globi both large and small were present in considerable numbers. (c) Smears from the liver contained acid-fast bacilli, and in one field a definite globus was noticed; in others there were groups of four or five bacilli which appeared to be intracellular. (d) Smears from the kidney also showed acid-fast bacilli, scanty but arranged in clusters.

MONKEY No. 3. This monkey was inoculated on June 4, 1938. As nothing of note developed after three months, it was decided to reinoculate the animal; and on September 14th a nodule was embedded in the subcutaneous tissue of the abdomen. A few weeks later, however, the whole nodule sloughed out. On October 24th the monkey was again inoculated, this time intraperitoneally, the nodule being stitched to the omentum. On December 3rd a superficial ulcer developed, at a distance from the original abdominal wound. Smears from it showed a few acid-fast bacilli in single groups and in clusters. This lesion healed, however, and the monkey is still under observation.

MONKEY No. 4. It was decided to inoculate this animal without splenectomy, and on July 11th a nodule was embedded in the omentum. Apart from a slight discharge from the abdominal wound there was nothing particular to note. The monkey was inspected at intervals, and the nodule was felt up to September 5th, when it had become very much less palpable than at first. Later it was decided to perform splenectomy and reinoculation, and on October 24th the abdomen was reopened. The original nodule was not found, it having apparently been absorbed completely. The spleen, however, was much enlarged. It was removed and another leprous nodule was stitched to the omentum and anchored to the anterior abdominal wall.

Smears from the spleen showed no definite acid-fast bacilli, but here and there in the macrophages there was seen granular, acid-fast material, but whether it was residual from broken-down bacilli or was of other nature could not be determined.

On October 29th the monkey looked ill and was obviously thinner. On the 31st its condition was worse and there was a purulent discharge from the operation wound. Smears showed a few acid-fast bacilli and also staphylococci. The wound was cleansed and dressed with cod liver oil, but the animal died suddenly a few hours later, on the eighth day after splenectomy.

Post mortem examination.—Septic peritonitis, localized on the left side. Smears from the liver showed the presence of acid-fast bacilli, many of which were intracellular. Impression smears of the liver indicated invasion with acid-fast bacilli, which were found in clusters, intracellularly as well as in the connective tissues of the liver. In two or three fields definite globi were seen.

MONKEY No. 5. This animal, inoculated on July 17th, was not splenectomized. The wound healed without difficulty. On October 17th the animal was pale and the abdominal nodule appeared to have increased in size. The condition having greatly improved, splenectomy and reinoculation were done on November 5th, through a lateral incision. The fresh nodule was stitched to the stump of the spleen, which was then fixed to the abdominal wall. On November 7th the animal appeared to be well and was taking its food, but on November 8th it was reported to be ill and it died on the same day.

Post mortem examination.—Pneumonia of the left lower lobe. The first nodule in the omentum was still present. There were no signs of hemorrhage. The nodule which was stitched to the stump of the spleen was intact. Smears taken from the kidneys, liver and bone-marrow were negative for acid-fast bacilli. Scrapings from the old nodule showed a few bacilli in every alternate field, and an occasional globus.

MONKEY No. 6. This monkey, also, was not splenectomized. The nodule was fixed to the omentum and stitched to the anterior abdominal wall on August 22nd. The wound healed but on September 12th there was ulceration and a slight purulent discharge; the nodule was palpable. Smears from the ulcers showed numerous bacilli, with globi and intracellular forms in large numbers. On September 15th the ulcer had healed and there was no discharge or swelling, but the animal was pale. On October 17th a small ulcer developed on the abdominal wall, and material scraped from it showed a few acid-fast bacilli but no definite globi. The general condition of the monkey was not good, but the pallor was less marked. On October 31st the ulcer had healed. On November 5th splenectomy was attempted through a high incision, but because of adhesion of the omentum it was impossible to reach the spleen. Suspicious nodule-like lesions were felt in the omentum and some were removed for examination, but when stained for acid-fast bacilli they were found to be negative.

There was a slight discharge from the abdomen after this second operation. Smears taken from the broken down abdominal wound and from the lower part of the incision showed no acid-fast bacilli. The wound subsequently healed, and on November 28th the spleen was removed through a lateral incision and a fresh nodule was stitched to the stump. Examination revealed no evidence of leprosy infection in the omentum. Multiple smears from the spleen showed no acid-fast bacilli. Between November 28th and

December 1st the monkey was very ill, drowsy, and had to be fed artificially (glucose water), but on December 2nd it showed signs of recovery and began to take food; the old sinus was still discharging. This monkey is still under observation.

MONKEY No. 7. On August 22nd this animal was splenectomized and a nodule was fixed to the omentum, the same stitch being used to close the peritoneum. The wound healed without complications. The nodule was palpable until October 31st. On November 5th the animal was reinoculated. Except for a superficial discharge at the base, the incision wound healed. There was nothing further definite to note until December 3rd, when it was noticed that the nodule which was embedded on November 5th was very much more prominent than at first, and that at its site there was an ulcer from which pus was exuding. Smears of this exudate showed enormous masses of bacilli in globus formation. The bacilli were definite rods, many were beaded and numerous bipolar forms were seen. The lesion subsequently healed and the monkey is still under observation.

DISCUSSION

The experiments reported actually comprise two series. In one (Series A), comprising three monkeys, the animals were first splenectomized and inoculated and later reinoculated. In the other (Series B), also comprising three monkeys, the first inoculation was performed without splenectomy, the subsequent reinoculation being done together with that operation. The observations suggest the following points:

Of Series A, the first animal (No. 1), may be assumed to have been actually infected, two months after the embedding of the nodule. It is to be noted that this one was inoculated only once.

The other two monkeys (Nos. 3 and 7), did not show the same course of events, even though they were reinoculated, one of them twice. These two are still under observation. Apart from a small lesion in monkey No. 3, which developed at a distance from the abdominal incision and subsequently healed, there has been no evidence of infection, but as no examination for internal spread has been made the actual condition is not known. It may be that these monkeys are naturally resistant to infection by *M. leprae*, but only continued observation can determine that question.

In Series B, the negative findings in the omentum and spleen of monkey No. 6 when they were examined two and one-half months after the initial inoculation indicate that no infection had taken place, but we have no evidence of the condition in the liver or kidney.

Monkey No. 5 died three days after splenectomy and reinoculation, so for practical purposes, this animal was really a non-splenectomized one, with only the primary inoculation. The completely negative findings after three months suggest that splenectomy is essential for the spread of infection, but, as before, the question of individual resistance intrudes.

Monkey No. 4, which died eight days after splenectomy and reinoculation, showed evidence of spread in the viscera. Here the question arises as to whether that condition resulted from the first or second inoculation, another point that can only be determined when there are larger numbers of animals available for comparison. The part played by the spleen cannot be established.

CONCLUSION

While these experiments are not in any way conclusive, the following points may be of interest to workers who are conducting animal inoculation experiments in leprosy:

1. There appears to be a possibility of infecting a monkey by performing preliminary splenectomy and embedding a nodule in the peritoneum, preferably fixing it to the splenic stump.

2. There may be a greater chance of success if the animal is splenectomized two months after insertion of the nodule intra-abdominally, with reinoculation at the time of the second operation.

3. These experiments reveal the possibility of individual variability of the factor of resistance, and many monkeys may have to be used before one is found that is actually susceptible to the infection. Monkey No. 1 in our Series A may have been such an animal.

REFERENCES

- (1) ADLER, S. Inoculation of human leprosy into Syrian hamster. *Lancet* 2 (1937) 714.
- (2) ADLER, S. Human leprosy in the Syrian hamster. *Trans. Cairo Cong.* (in press); *abstract in Internat. Jour. Lep.* 6 (1938) 467.