humorale normale, ils auront à leur disposition une quantité beaucoup plus importante d'antigènes tréponèmiques et en conséquence la quantité d'anticorps formée sera plus élevée.

Les réactions de Kline sont en général négatives. Pourtant, dans 2 cas, chez les thymectomisées, on trouve une floculation faiblement positive. Quant au Nelson on le rencontre une fois faiblement positif chez les témoins et une fois douteux chez les souris opérées. Ces derniers résultats sont en accord avec ce qu'on trouve dans la littérature se rapportant à l'étude expérimentale de la syphilis chez la souris: négativité de la sérologie classique[5], positivité exceptionnelle du Nelson.

Il est très vraisemblable que l'absence d'anticorps anti-cardiolipidique correspond à l'absence de lésions déterminées par le tréponème chez la souris ce qui a été signalé également chez le rat[2,10]. Par contre, chez le hamster[10], la sérologie classique est trouvée positive après le test de Nelson car chez cet animal les lésions sont tardives. Pour expliquer le test de Nelson généralement négatif chez la souris après inoculation, il semble qu'on doive admettre une faible immunogénicité de l'antigène spécifique de *T. pallidum* chez cet animal puisque seulement des injections répétées de *T. pallidum* ont permis à McLEOD et MAGNUSON[6] d'obtenir régulièrement des Nelson positifs.

Summary. The purpose of this study was to investigate modifications in the behaviour of a thymectomized mouse towards *Treponema pallidum*, and whether an immunofluorescence technique could take the place of the insufficiencies of classic serology and of Nelson test in syphilized mice.

J. THIVOLET, J. C. MONIER,
M. SEPETJIAN et D. SALUSSOLA[11]

Laboratoire d'Hygiène, Faculté de Médecine, Lyon 8e (France), 7 octobre 1968.

[10] T. FREDRIKSSON, B. HEDERSTEDT et S. ROSENGREN, Acta path. microbiol. scand. *72*, 125 (1968).

[11] Avec la collaboration technique de Mme S. MORAND et Mlle C. BURGY.

Effect of Actinomycin D on the Production of Acute Phase Protein in the Rabbit

Studies on the production of the acute phase protein in the serum of the rabbit (CxRP) have shown that this process is essentially independent of antibody production[1]. Blockage of the reticulo-endothelial system with Thorotrast resulted in a decreased production of CxRP[2]. In rabbits, the liver is the only organ giving evidence of involvement in the synthesis of this protein[3]. Studies on the acute phase protein of the rat[4] have shown that the synthesis of this protein is partially inhibited by actinomycin D or puromycin. However, there are a number of differences between the acute phase protein of the rat and CxRP of the rabbit. Therefore, the present study was undertaken on the effect of actinomycin D on CxRP production.

Mature New Zealand white rabbits were used and their sera were tested to ensure the absence of CxRP. On the basis of previous observations by HURLIMAN[3], rabbits were stimulated to produce CxRP by the i.m. injection (gastrocnemius) of turpentine. Actinomycin D (200 µg/kg) was then administered i.p. to the experimental groups of rabbits at 0, 6, or 12 h after stimulation with turpentine. The control groups of rabbits received the same dosage of actinomycin D alone or the drug solvent (ethanol-saline). Blood samples were taken immediately before the injection of turpentine (zero hour), and at 12-h intervals thereafter over a period of 48 h. Titers of serum CxRP were determined by the capillary precipitation technique[5] against antiserum specific for CxRP.

Results presented on the Table represent the average titers of CxRP in the rabbit serum samples in the different groups. Rabbits receiving turpentine alone showed a high level of CxRP at the 12th h after stimulation. This continued to increase through the 24th h at which time the maximum level was reached and remained constant through the 48th h, as shown graphically in the Figure. Rabbits receiving actinomycin D at zero or 6 h after administration of turpentine showed a significant inhibition of the synthesis of serum CxRP at the 12th h. However the sera of these 2 groups of rabbits showed a sharp rise in titer of CxRP between the 12th and 24th h. Thus the inhibitory effect of the drug on the synthesis of CxRP had a duration of approximately 12 h. In rabbits administered actinomycin D 12 h after the turpentine, their sera showed a significant decrease in the rate of production of their CxRP between the 12th and 24th h, in contrast to the increase in titer noted for the group receiving only turpentine. The actinomycin D control group of rabbits showed a sharp rise in titer of serum CxRP after the 12th h and continued through the 36th h. This rise in serum titer paralleled that of the group of rabbits receiving the drug at the same time as the turpentine. In the group of rabbits receiving the drug solvent, their sera showed a slight rise in titer of CxRP with the maximum level of the protein appearing at 24 h. Preliminary experiments with the use of cycloheximide given to rabbits i.p. (10 mg/kg) showed a degree of inhibition of the synthesis of CxRP similar to that achieved with actinomycin D.

It might be noted that actinomycin D itself stimulated the production of CxRP, which has previously not been reported. Unpublished results by HOKAMA (personal communication) indicated that actinomycin D, injected i.v., had no effect on the production of CxRP, even at

[1] Y. HOKAMA, M. COLEMAN and R. RILEY, Proc. Soc. exp. Biol. Med. *105*, 510 (1961).

[2] S. MONTELLA and H. WOOD, J. exp. Med. *106*, 321 (1957).

[3] J. HURLIMAN, G. THORBECKE and G. HOCHWALD, J. exp. Med. *123*, 365 (1966).

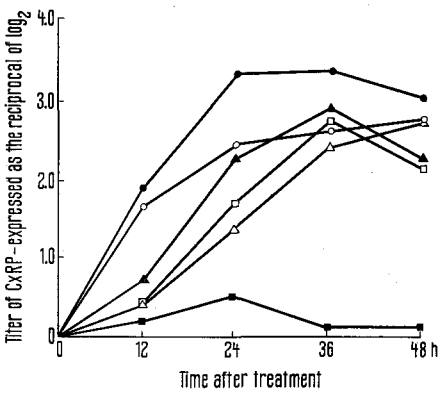
[4] D. DARCY, Br. J. exp. Path. *48*, 608 (1967).

[5] D. SELMAN and A. HALPERN, Angiology *7*, 292 (1956).

Effect of actinomycin D on the production of acute phase protein in the rabbit

Group	Treatment	Total number of rabbits/group	CxRP titers[a] after treatment 12 h	24 h	36 h	48 h
1	Turpentine alone	7	1.9 (0–3.0)	3.4 (2–4.0)	3.4 (3–4.0)	3.0 (2–4.0)
2	Turpentine plus actinomycin D[b] injected at 0 h	6	0.3 (0–0.5)	1.7 (1–2.0)	2.8 (2–4.0)	2.2 (1–3.0)
3	Turpentine plus actinomycin D injected at 6 h	5	0.7 (0–2.0)	2.4 (2–3.0)	3.0 (0)	2.3 (2–3.0)
4	Turpentine plus actinomycin D injected at 12 h	5	1.7 (0.5–3.0)	2.4 (2–3.0)	2.6 (2–4.0)	2.8 (2–4.0)
5	Actinomycin D injected at 0 h	7	0.3 (0–0.5)	1.4 (1–2.0)	2.5 (2–3.0)	2.8 (2–4.0)
6	Actinomycin D solvent injected at 0 h	3	0.2 (0–0.5)	0.5 (0–0.5)	0.1 (0–0.5)	0.1 (0–0.5)

[a] Titers are reported as $\log_2$ of the reciprocal of the highest dilution of serum giving a positive test with the antiserum. Values in parenthesis represent the ranges, other values are averages for the number of rabbits listed. Antiserum used was Goat anti-CxRP from Hyland Laboratories, Los Angeles, California, USA. [b] Actinomycin D was a gift from Merck, Sharp and Dohme Research Laboratories (USA). It was dissolved in a 3.3% solution of ethanol in normal saline, the concentration being 0.02%. It was injected i.p. at a dose of 200 µg/kg.



Effect of actinomycin D on the production of acute phase protein in the rabbit. ● turpentine only; □ turpentine plus actinomycin D injected at 0 hour; ▲ turpentine plus actinomycin D injected at 6 h; ○ turpentine plus actinomycin D injected at 12 h; △ actinomycin D only; ■ actinomycin D solvent.

a level of 1000 µg/kg. However, it was our experience that when actinomycin D was injected i.v. with the following dosages: 200, 400 or 800 µg/kg respectively, all animals receiving 400 or 800 µg/kg died within 12 h, whereas those rabbits injected with 200 µg/kg remained alive for at least 48 h. CxRP titers were not determined on these animals since they had been found to contain detectable amounts of this protein prior to the treatment with actinomycin D.

These data indicate that actinomycin D has an inhibitory effect on the synthesis of CxRP following challenge with turpentine. Similar findings were reported by DARCY[4] on a related rat acute phase protein. However, he found that actinomycin D injected 3 h after the turpentine resulted in a greater response of the acute phase protein after 24 h. DARCY suggested that perhaps a single 'large injury', represented by the injection of the drug and turpentine simultaneously, has less effect than 2 'smaller' but equivalent 'injuries'. However, our results show no significant difference, as noted by CxRP levels in rabbits injected with actinomycin D at 0 or 6 h after turpentine. In addition, we were unable to find any situation where actinomycin D treatment enhanced the stimulation of CxRP to a higher level than the turpentine controls. The lack of increased response in levels of CxRP by the injection of actinomycin D other than simultaneously with turpentine is an additional characteristic difference between CxRP and rat acute phase protein.

Actinomycin D is known to block the DNA-dependent RNA synthesis. Antibody synthesis has been shown to be inhibited in vitro by actinomycin D[6], whereas experiments with intact animals have failed to give consistent results[7,8]. Experiments by SMILEY et al.[9] on the in vitro anamnestic response showed that antibody synthesis was inhibited at moderate concentrations of actinomycin D, whereas at low concentrations antibody synthesis was unaffected or increased even though RNA synthesis was inhibited. They suggested that the mRNA necessary for antibody synthesis was not the limiting factor, either because it was stable or was produced in excess. This might explain the lack of complete inhibition of the synthesis of CxRP in our studies. An alternative explanation might be that CxRP is a subunit of catalase, as has been recently suggested[10,11]. In this instance the CxRP produced when actinomycin D and turpentine are given simultaneously could result from metabolism of catalase[12].

Résumé. La production de la protéine en phase aiguë chez le lapin (CxRP), après injection stimulante de térébenthine, a été arrêtée par l'application i.p. d'actinomycine D. Cette inhibition durait environ 12 h.

J. JENNINGS[13], J. T. SETO and D. A. ANDERSON

Department of Microbiology, California State College at Los Angeles (California 90032, USA), 29 October 1968.

[6] J. UHR, Science *142*, 1476 (1963).
[7] H. NATHANS, S. BIEBER, G. ELION and G. HITCHINGS, Proc. Soc. exp. Biol. Med. *107*, 796 (1961).
[8] J. STERZL, Nature *189*, 1022 (1961).
[9] J. SMILEY, J. HEARD and M. ZIFF, J. exp. Med. *119*, 881 (1964).
[10] Y. HOKAMA and H. CROXATTO, Fedn Proc. *26*, 574 (1967).
[11] Y. HOKAMA, H. CROXATTO, K. YAMADA and E. NISHIMURA, Cancer Res. *27*, 2300 (1967).
[12] This work was supported in part by U.S. Public Health Service grant No. AHT-68-009(A).
[13] Present address: Department of Microbiology, University of Oregon Medical School, Portland, Oregon 97201, USA.