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Experiments on the Loss of *Dictyocaulus filaria* from the Lungs of Infected Sheep. II. The Demonstration of Worms in the Bronchial Mucus

J. F. MICHEL

Central Veterinary Laboratory, Weybridge

Abstract. Experiments are reported which demonstrate that substantial numbers of worms were lost in the bronchial mucus from lambs infected with *Dictyocaulus filaria*. Most of this loss occurred between the 12th and 30th days after infection. The evidence did not suggest that an immune response on the part of the host was involved. The worms passing out of the lungs were uninjured and could continue their development in other lambs to which they were transferred.

It was shown in an earlier communication (MICHEL 1968a) that although experimental infection of sheep with *Dictyocaulus filaria* results in the establishment of worms in the lungs, their number may decrease rapidly and all may have been lost before they reach maturity. The worms might either be destroyed in situ or might pass out of the lungs with the bronchial mucus. Since it was technically possible to recover worms taking this route so that they could be counted and examined, it was decided to make use of the opportunity that this might afford of studying the loss of worms directly. The results of some experiments are therefore reported which demonstrate that an appreciable part of the early decrease in worm numbers is attributable to the loss of worms with the bronchial mucus and that this loss occurs even in circumstances in which a vigorous immune response is unlikely to be elicited.

MATERIALS AND METHODS

The lambs used and the techniques employed were as described in the first paper of this series (MICHEL 1968a). The tracheotomy procedure for the recovery of bronchial mucus was as described elsewhere (MICHEL 1968b).

Worms were separated from the mucus by means of screens. Two screens, both having 400 meshes to the linear inch were employed, one above the other, and the mucus was washed through with the aid of a moderately vigorous jet of water. Even infective larvae are retained by this type of screen, rather less than 10 % being washed through. Of these, 90 % are retained by the second screen so that a recovery of 99 % is achieved.

For transfer to other lambs in experiment 3, worms were recovered from bronchial mucus by means of the Baermann procedure. The mucus was spread on filter papers 17 cm in diameter and these were put face downward in the screens (100 mesh/inch) of Baermann funnels filled with 1.2 % NaCl solution and maintained at 37 °C. After six hours large numbers of worms were drawn off but further worms (which were not used for infection) were present in the stem of the funnel after another 16 hours.

The worms, in 10 ml saline, were administered to the recipient lambs intravenously through a 17 gauge needle.

The length of worms was measured by two methods. Those more than 5 mm long were stretched out in a thin film of water on a microscope slide and measured by means of the vernier of the mechanical stage of a compound microscope fitted with an ocular carrying a hairline. Worms less than 5 mm in length were mounted on slides in a drop of saline under a cover glass and tracings of their mid-line were made by means of a drawing prism fitted to a compound microscope. The tracings were measured with a pair of dividers, the system having been suitably calibrated.

RESULTS

Experiment 1. Three lambs and three yearlings were fitted with tracheotomy tubes and subsequently infected with a single dose of infective larvae of *D. filaria* at the rate of 450 larvae per lb. liveweight. The bronchial mucus was collected from each animal and the

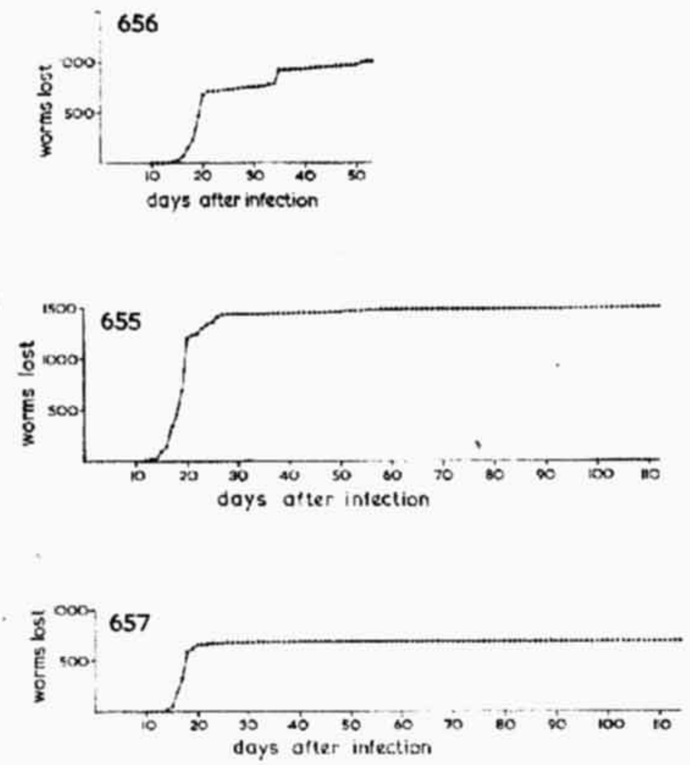

Fig. 1. The cumulative total of worms recovered from the bronchial mucus of lambs in experiment 1.

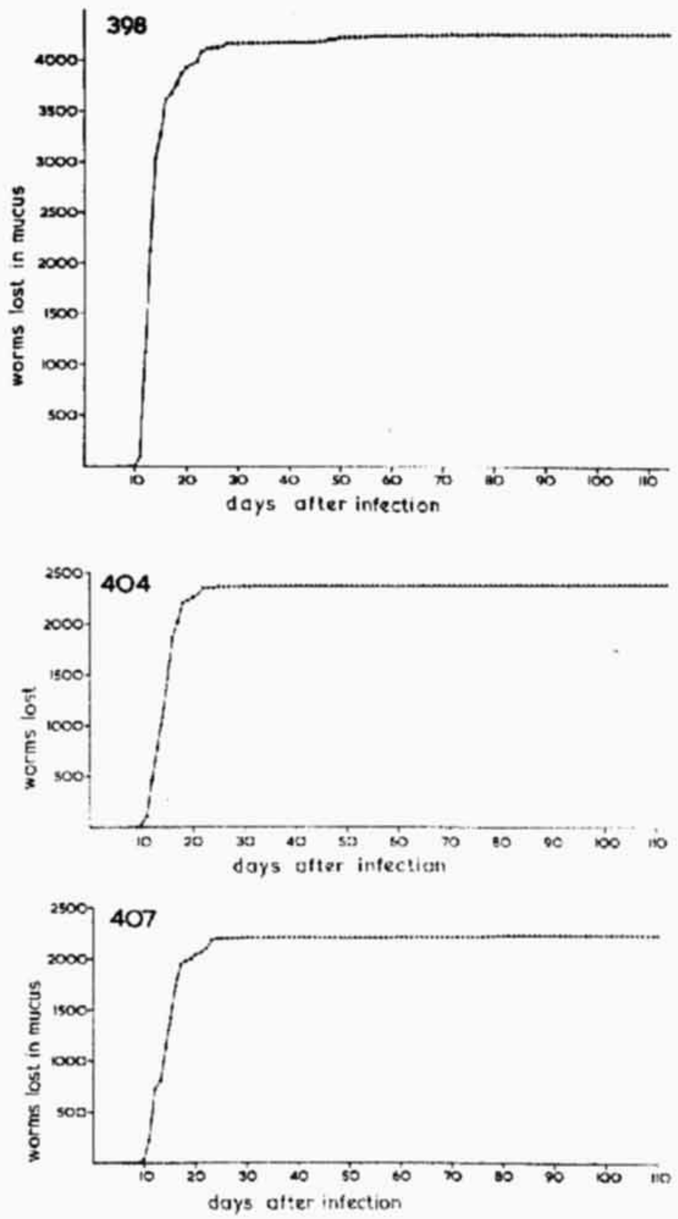

Fig. 2. The cumulative total of worms recovered from the bronchial mucus of yearlings in experiment 1.

worms present in it each day were counted. Cumulative totals of the worms recovered from the bronchial mucus of each animal are shown in figs. 1 and 2. One of the lambs No. 656 died on the 54th day and the remaining animals were slaughtered between the 111th and 115th days. Post mortem worm counts and other details are shown in table 1.

Table 1. Summary of results of experiment 1.

Sheep No.	Age when infected (days)	Weight (lb)	Larvae administered	Worms from bronchial mucus	Worms from mucus × 100 / Larvae administered	Killed days after infection
655	95	57	25,600	1491	5.82	113
656	95	54	24,300	1008	4.15	54 died
657	93	51	23,000	689	3.00	115
398	461	105	47,200	4271	9.05	115
404	459	99	44,500	2372	5.33	113
407	457	93	41,800	2238	5.35	111

Sheep No.	Worms recovered						Total of worms in lungs and bronchial mucus	Total × 100 / Larvae administered
	> 3 cm	> 2 cm	> 1 cm	> 5 mm	< 5 mm	Total		
655	—	—	—	—	—	0	2237	8.74
656	975	99	—	—	—	1074	2586	10.62
657	—	—	—	—	—	0	1034	4.50
398	—	—	—	—	—	0	6407	15.00
404	—	—	—	—	—	0	3559	8.00
407	—	5	3	3	1	12	3369	8.06

Worms began to appear in the mucus about the 10th day, the rate of loss increased fairly strongly and then decreased so that the majority of those lost had appeared by the 30th day. Thereafter the loss continued at a very modest rate for a considerable period. There was no very striking difference in the numbers of worms lost in the bronchial mucus from lambs and yearlings when these are expressed as a proportion of the number of larvae administered. Although no measurements were made, the impression was gained that the worms recovered form the lambs were rather larger than those from the yearlings.

Faecal larval counts are shown in table 2. Appreciable numbers of larvae were passed by two of the three lambs but by none of the yearlings. This would suggest

Table 2. Faecal larval counts of lambs 655, 656 and 657 and yearlings 407, 404 and 398 used in experiment 1.

Day after infection	Larvae per gram of faeces					
	655	656	657	407	404	398
28	0	0	0	0	0	0
31	2.6	10.1	0	0	0	0
36	17.2	162	0	10	28.6	0
42	29.4	316	0	0	0	0.2
44	36.2	280	0.2	0	0.4	0
46	27.2	460	1.2	0	0	0
49	22.8	344	0.6	0	0	0.2
51	66.4	996	0.2	0	0	0
53	182	2702	0.4	2.4	0.4	0
56	260	Died	0	0	0	0
58	268		0	0	0	0
60	282		0	0	0	0
63	160		0	0	0	0.8
65	34		0	0	0	0
67	65		0	0	0	0
70	11.8		1.2	0	0	0
72	12.4		0.6	0	0	0
74	6.8		0	0	0	0
77	6.6		0.2	0	0	0
79	9.4		0	0	0	0
81	8.4		0	0	0	0
84	2.4		0	0	0	0
86	0.6		0	0	0	0
88	1.2		0	0	0	0
91	0.6		0	0	0	0
93	1.0		0	0	0	0
95	0.6		0.2	0	0	0
98	0.2		0	0	0	0
100	0.6		0	0	0	0
102	0		0	0	0	0
105	0		0	0	0	0
107	0		0	0	0	0
109	0		0	0	0	0
111	0		0	0	0	0

Table 4. Faecal larval counts of lambs used in experiment 2

Day after infection	Larvae per gram of faeces					
	1273	1275	1276	1192	1194	1212
21	0	0	0	0	0	0
23	0	0	0	0	0	0
25	0	0	0.1	0	0	0
28	0	0	0	0	0	0
30	0	0	0	0	0	0
32	0	0	0	0	0	0
35	0	0	8.1	0	0	0
37	0.2	0	35.2	0	0	0
39	0	0	41.4	0	0	0
42	5.2	0	52	0	0	0
44	13.2	0	106	0	0	0
46	40	0	42	0	0	0
49	28	0	30	0	0	0
51	15	0	50	0	0	0
53	8	0	28	0	0	0
56	1.6	0	7.8	0	0	0
58	0.4	0	9	0	0	0
60	0	0	4.8	0	0	0
63	0	0	8.6	0	0	0
65	0	0	1.0	0	0	0
69	0	0	0.6	0	0	0
72	0	0	6.2	0	0	0
74	0	0	22.4	0	0	0
77	0	0	3.8	0	0	0
79	0	0	8.8	0	0	0
81	0	0	4.4	0	0.2	0
84	0	0	0.8	0	0	0
86	0	0	0.2	0	0	0
88	0	0	0.2	0	0	0
91	0	0	0.6	0	0	0
93	0	0	0	0	0	0
95	0	0	0	0	0	0
98	0	0	0	0	0	0

Table 3. Summary of results of experiment 2

Sheep No.	Age when infected	Weight (lb)	Larvae administered	Worms from bronchial mucus	Worms from mucus × 100 / larvae administered	Killed days after infection
1273	52	50	3,000	348	11.60	98
1276	49	37.5	2,250	262	11.64	98
1275	49	33.3	2,000	182	9.1	98
1192	198	80.5	4,850	639	13.18	99
1194	198	83.5	5,000	489	9.78	99
1212	163	71.5	4,250	647	15.22	99

Sheep No.	Worms recovered						Total of worms in lungs and bronchial mucus	Total × 100 larvae administered
	> 3 cm	> 2 cm	> 1 cm	> 5 mm	< 5 mm	Total		
1273	3	1	—	—	—	4	526	17.53
1276	11	—		—	—	11	404	17.96
1275	—	—	—	—	—	0	273	13.65
1192	—	4	1	—		5	964	19.88
1194	—	6	2	1	—	9	743	14.86
1212	—	2	2	—	—	4	975	22.94

that a greater number of the worms reached maturity in the lambs than in the yearlings.

Experiment 2. Since both a small infecting dose and the use of young lambs might be expected to decrease the chance of a response on the part of the host, it seemed appropriate to enquire whether the loss of worms in bronchial mucus would still occur if both dose and host age were further decreased. Accordingly another experiment was undertaken using lambs only 50 days old and a much smaller dose of larvae—only 60 per lb. liveweight. To have used even younger lambs would have been very difficult as the tracheotomy procedure demands that the lambs be of a certain minimum size and some time must be allowed for the wound to heal.

Table 5. Plan of experiment

Day	Lamb No.	Treatment
0	2992	infected with 120000 3rd stage larvae
11 – 12	3116	received 421 worms i/v from mucus of 2992
12 – 13	3116	received 1707 worms i/v from mucus of 2992
13 – 14	3019	received 3000 worms i/v from mucus of 2992
18	3116	Killed
20	3019	Killed

Details of the animals used, their worm burdens at the end of the experiment and of the worms recovered in the bronchial mucus are shown in table 3 and in figs 3 and 4. There was no striking difference between the proportion of the worms which

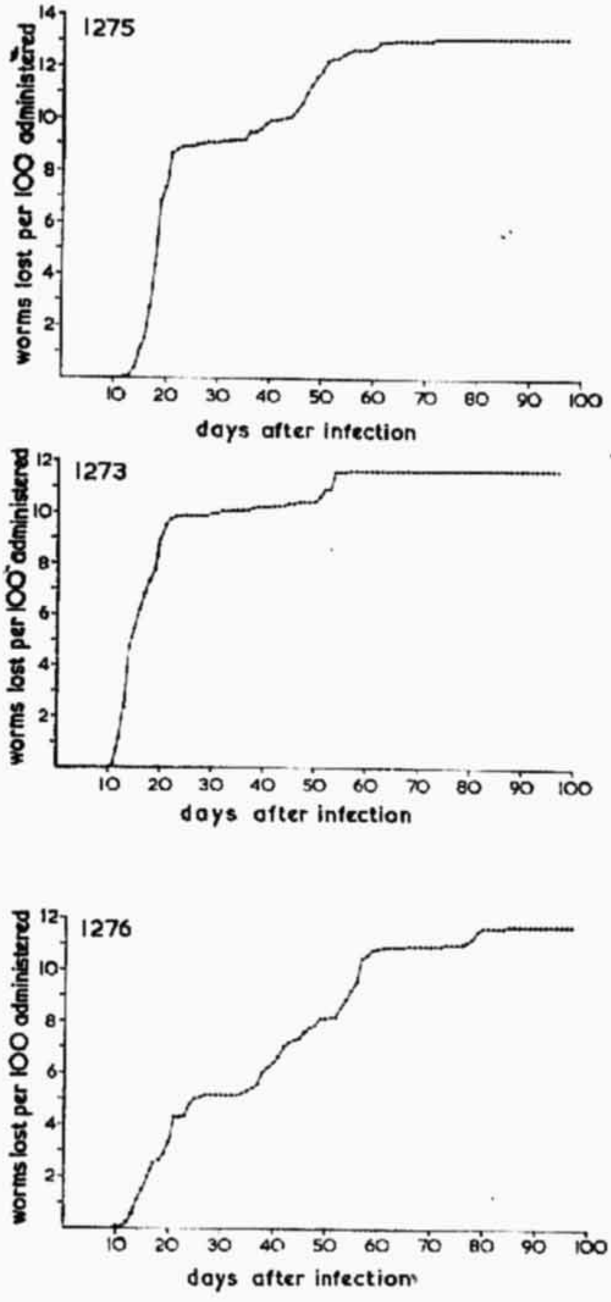

Fig. 3. The cumulative total of worms recovered from the bronchial mucus of young lambs in experiment 2.

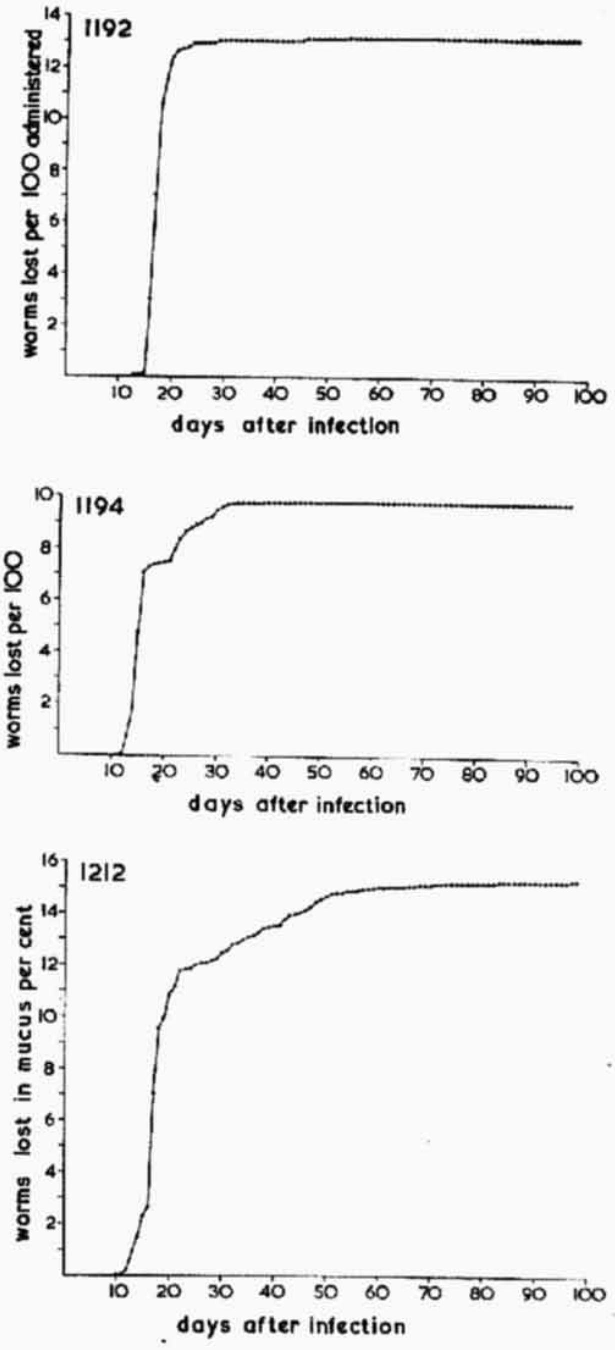

Fig. 4. The cumulative total of worms recovered from the bronchial mucus of older lambs in experiment 2.

was lost from younger and older animals. Faecal larval counts appear in table 4. Larvae were passed in the faeces of two of the younger animals but not in those of the older animals. As will be seen from table 3 the worms remaining in the older animals at the end of the experiment were rather smaller than those in the younger animals and this was also true of the worms recovered from the bronchial mucus.

Experiment 3. It was observed in the course of the foregoing experiments that the worms recovered from bronchial mucus appeared to be alive. This did not mean however that they had not been injured and rendered incapable of persisting in the lungs. The question was investigated by determining whether the worms lost could continue their development in another host. The plan of the experiment is summarised in table 5. A lamb, No. 2992 was fitted with a tracheotomy tube and given a single dose of 120,000 infective larvae. The collection of mucus and recovery of worms from it began on day 10. Those passed on the 11th and 12th days were

Table 6. Mean length (mm) of worms in bronchial mucus of donor lambs and lungs of recipients

	Lamb No.	Mean length of worms on day					
		11	12	13	14	18	20
From mucus of donor	2992	1.33	1.41	1.60		2.38	2.54
In lungs of recipients	3116					3.01*	
	3019						4.52*

*) at autopsy.

especially cleaned and prepared as described above. 421 worms were administered intravenously to a susceptible lamb No. 3116 on day 12 and a further 1707 on day 13. The worms passed by the donor lamb on day 13 were also specially prepared and 3000 were administered intravenously to a second susceptible lamb, No. 3019, on day 14. Lamb 3116 was killed on day 18 and 1070 worms were recovered from its lungs. This represented half of those administered. It was found that the worms had doubled in length in this new host. Lamb 3019 was killed on day 20. Its lungs contained 863 worms, nearly one third of the number administered, and these had more than doubled in length during the six days they had been in their new host. Details of the length of worms recovered from the lungs of the recipients and the mucus of the donor lamb are given in table 6. It will be seen that the worms in the lungs of the recipient lambs were substantially larger than those present in the bronchial mucus of the donor lamb on the same day.

DISCUSSION

It is clear from the results presented, that appreciable numbers of worms are lost in the bronchial mucus from lambs infected with *D. filaria* and that most of this loss occurs before the infection becomes patent. Two thirds of the total flow of mucus is recovered by the technique employed (MICHEL 1968b) and if an appropriate correction is made and the number of worms lost is added to those remaining when the sheep were killed, as has been done in tables 1 and 3, it is evident that this total represents a proportion of the larvae administered similar to that which was found to be initially established in experiments described in an earlier paper (MICHEL 1968a). It is true that this estimate of initial establishment was based on the number of worms present on the 14th and 15th day after infection, when some loss of worms in the mucus may already have occurred, but it may none the less be concluded that a substantial part at least, of the decrease in worm numbers which occurs before the infection becomes patent is attributable to the loss of worms in the bronchial mucus.

The loss occurred equally in lambs and yearlings, even if the lambs were very young and the infections given them very small, conditions in which, in earlier experiments, the decrease in worm numbers had not been seen or had been of limited extent. Four out of six young lambs passed appreciable numbers of larvae in their faeces while none of the older animals did so. This suggests that factors other than the loss of worms in mucus may prevent the infection from becoming patent. It could be that while the rate of loss from young and old hosts was the same, the rate of development of the worms was less in the older animals with the result that the worms reached maturity only in the younger animals. Alternatively worms might be destroyed in situ or lost by some other route.

In this connection it may be relevant that though the output of larvae in the faeces of lamb 655 in experiment 1 fell sharply between the 60th and 70th days, no corresponding increase was seen in the number of worms recovered from the bronchial mucus.

The worms do not appear to grow as rapidly in older animals as they do in young lambs and it could be concluded that the older host presents a less favourable environment for the worms. It seems however that the loss of worms in the bronchial mucus is not necessarily associated with this unsuitability of the host.

That the worms which are lost are not permanently injured emerged from experiment 3 in which worms lost from one host successfully became established in a second host to which they were transferred. The data suggest that in this second susceptible host the worms grew faster than those which remained in the original host. The worms lost from lamb 2992 on the 11th and 12th day and transferred to lamb 3116 were considerable larger on the 18th day than worms lost from lamb 2992 on that day. Similarly, worms lost from 2992 on the 13th day and transferred to lamb 3019 were nearly twice as large on the 20th day as worms present in the mucus of lamb 2992. It follows that unless there was some change between the 12th and 18th days, or the 13th and 20th, in the relationship between the size of worms present in the lungs and of those lost, the worms grew faster in the new lambs to which they were transferred than did those that remained in the original lamb. Experiments to be described in a later paper of this series, indicate that the worms lost in the mucus are, in terms of size, a random sample of those present.

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J.F.M., Central Veterinary Laboratory, Weybridge, England

J. KATHE · H. MOCHMANN: LEPTOSPIREN UND LEPTOSPIROSEN *Part I. Publishing House VEB Fischer, Jena 1967, 482 pp., MDN 115.*

More than 50 years ago *Leptospira icterohaemorrhagiae* was discovered as the pathogenic agent of Weil's disease. Since then numerous species and serotypes of *Leptospira* and the various diseases caused by them have been described. Some earlier monographs on *Leptospira* and leptospiroses became obsolete (Uhlenhuth a. Fromme 1930, Walch-Sorgdrager 1939, Van Thiel 1948, Warfolomejewa 1949, Rimpau 1950, Gsell 1952, Austoni 1953, Zwierz 1957, Alston a. Broom 1958, Kolochine a. Mailloux 1962), because of the vast knowledge obtained on the subject. The present book, edited by the late J. Kathe and his young collaborator H. Mochmann, fills a noticeable gap and may rightly be considered by all scientists concerned with research work on leptospirae as their "Bible". The Editor invited numerous writers who, in recent years, have contributed to knowledge on leptospiroses, to participate in this work. Part I is divided into 5 chapters. Chapter I "Historical development of *Leptospira* research" is written by Kathe. Chapter II "Clinic and therapy" contains articles by Austoni (Padua) on the clinical history and treatment of leptospiroses of man and by Zwierzchowski (Wroclaw) on leptospiroses of domestic and economically important animals. In Chapter III Bruns (Jena) describes the "Comparative pathological anatomy of leptospiroses". Chapter IV contains an article by Popp (Braunschweig) on "Diagnostics of leptospiroses in the laboratory". Most comprehensive is Chapter V on the "Microbiology" of *Leptospira*. Babudieri (Rome) describes the systematics, nomenclature, morphology and serology of *Leptospira* and aquatic *Leptospira*. Füzi (Budapest) deals with the physiology of *Leptospira*, Mochmann (Berlin-Buch) with the susceptibility of *Leptospira* to drugs. The article "Leptospiroses in animal experiments" is written by Kmety, Pleško and Bakoss (Bratislava). Kemenes (Budapest) deals with "Experimental immunity and cross immunity in animals". Each chapter contains a comprehensive list of literature. This new monograph will clearly be very useful. Only the arrangement of the chapters seems slightly illogical, "Pathology" following "Clinical history and therapy" and "Microbiology" being last. In the reviewer's opinion, "Microbiology" should be first. This monograph is heartily recommended to all who are studying *Leptospira* and leptospiroses.

Academician O. Jírovec