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Chemotherapy and Immunity in Leptospira Canicola and L. Icterohaemorrhagiae Infections*.

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Successful control and prevention of leptospiral infections depend upon a) the elimination or treatment of mammals which serve as reservoirs of infection, b) the avoidance of contact with the environment contaminated by the excretions of the reservoir, c) the protection of the susceptible hosts by prophylactic immunization and d) the diagnosis and treatment, including sterilization of the urinary tract. When the Japanese investigators established in 1915 the fact that leptospira are excreted in the urine from the distal portions of the convoluted tubules in the kidneys of apparently healthy field rodents, the problem of leptospirosis became focused on the carriers. Although the published records of 229 cases of leptospiral infections in the United States over a period of 40 years indicates a relatively low incidence, the recent analysis of 78 cases diagnosed in the Detroit area between 1937 and 1946 clearly establishes leptospiral infections as a health hazard of some significance (MOLNER, MEYER and RASKIN, 1948). Poultry handlers, slaughterhouse employees, fish workers, junk peddlers and gardeners constitute some of the occupational groups which work under primitive sanitary conditions and come in contact with water, packing tables, moist earth, rat traps and other objects soiled with the urine of rats. Provided adequate diagnostic facilities are available and the interest of physicians has been aroused, classical leptospiral infections will be recognized. With the adoption of rodent control in food establishments as recommended by the Department of Agriculture, the Food and Drug Administration and various health departments, this source of infection will in time be greatly diminished.

Weil's disease is quite prevalent on the Island of Hawaii; the medical profession is convinced that immunization of cane cutters is probably the only solution of the problem in that area (PATTERSON, 1944).

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The importance of canine leptospirosis, a common and serious disease in dogs, has progressively increased. Serious outbreaks may plight the dog population of cities and counties with a case fatality rate in the clinically recognized canine infections of as high as 85 per cent. Inhabitants of breeding kennels and valuable dogs attending shows are frequently the victims of this infection. Serological surveys have shown that between 3.6 (Italy) and 44 (Belgium) per cent of the dogs the world over have a titer against Leptospira canicola of 1 : 100 and over (RAVEN, 1941). Incidence rates between these extremes have been obtained in the United States. Of 47 apparently normal dogs in San Francisco, 16 (34 per cent) gave serum reactions (1 : 1,000 to 1 : 300,000) indicative of past or subclinical leptospirosis (MEYER et al., 1939). Nineteen per cent of the dogs in Southern California (GREENE, 1941) and 38.1 per cent of 105 rural dogs in Pennsylvania had positive agglutination reactions to L. canicola (RAVEN). With increasing frequency the Leptospira strains specific for the canine species have been isolated from dogs in many parts of the world; it has a cosmopolitan distribution equal to the classical L. icterohaemorrhagiae (VAN THIEL, 1948; COLLIER, 1948).

Ever since 1933 when the first human infection, so-called canicola fever, was identified in Holland, detailed accounts of clinical cases have appeared in medical journals, primarily from western Europe and from the United States. These reports reflect that this disease is being recognized with increasing frequency. Several factors make true estimate of the incidence difficult: Since laboratory diagnostic facilities have been improved the incidence has apparently increased. However, the fact that in the agglutination test which is frequently used in the diagnosis of this disease, Leptospira canicola and L. icterohaemorrhagiae give cross reactions, a warning is extended that these tests must be repeated before decisive results can be obtained. More cases have been reported within the last five years and as a consequence it has probably received more diagnostic attention. It is impossible to determine with any accuracy the number of cases in the Far East; few cases have been reported, but it is likely that more have occurred. Approximately 327 cases are known to have occurred (table I), but this figure does not represent the true incidence.

The mode of transmission from dog to man has been elucidated in several household outbreaks (ROOS, WALCH-SORGDRAGER and SCHÜFFNER, 1937; SENTHILLE, de BAYO and KOLOCHINE-ERBER, 1945). Fondling and other intimate contact as, for example, sleeping with a dog or permitting it to lick the hand which had super-

TABLE I.

Reported cases of canicola fever.

Location	Reported by	Date	Cases	Estimated total
Europe				281
England	Baber and Stuart	1946	1	5
	Laurent et al.	1948	4	
Norway	Aalvik (Minkenhof)	1946	1	1
Denmark	Brammer et al.	1938	1	21
	Borg Petersen (van Thiel)	1938	7	
	Bukh (Rosenbaum)	1940	1	
	Bjørneboe (Rosenbaum)	1941	1	
	Borg Petersen (Minkenhof)		21	
Netherlands	Minkenhof	1948	39	51
	Ruys et al.	1948	50	
	Hlanksma	1949	1	
Germany	Port and Rimpau (Herrlich)	1947	11	32
	Herrlich	1948	1	
	Minkenhof	1948	1	
	Günther-Kühne et al.	1949	20	
	Mackay-Dick and Watts Rimpau	1949	137	5
Austria	Tetzner	1938	1	1
Switzerland	Gsell	1946	9	18
	Minkenhof	1948	18	
France	Bolgert et al.	1945	1	10
	Bolgert et al.	1945	2	
	Minkenhof	1948	5	
	Harvier et al.	1946	1	
	Audoly	1948	1	
	Bergouignon and Kolochine-Erber	1948	1	
	Lereboullet et al.	1949	1	
Italy	Torrini (Günther-Kühne)		1	1
Asia				11
China	Snapper et al.	1940	2	2
Eastern Asia	Cadilla et al.		9	9
South America				7
Argentina	del Sel and Giberti	1947	1	7
	Savino and Rennella (del Sel and Giberti)		6	
West Indies				8
Puerto Rico	Cadilla et al.	1946	1	1
Cuba	Curbello and Marquez	1949	7	7
North America				20
United States	Meyer et al.	1938	2	20
	Ashe et al.	1941	1	
	Bruno et al.	1943	2	
	Tievsky et al.	1944	1	
	Randall and Cooper	1944	2	
	Rosenbaum	1946	1	
	Lindsay and Luke	1949	1	
	Additional known cases		10	
Estimated total				**327**

ficial abrasions or a bite by the family dog initiated canicola fever. When some cases of this type were thoroughly investigated, invariably the existence of a subclinical infection or recovery from clinical Leptospira canicola attack in the dogs was proven; the sera of the dogs were positive in titers of as high as 1 : 30,000 (MEYER and EDDIE).

In view of these epidemiologic observations it required little encouragement by the National Canine Research Foundation to undertake experiments directed toward reducing the losses of valuable dogs in preventing human canicola infections. It is the purpose of this brief report to present observations and conclusions which may be of general value in the control of leptospiral infections.

Antibiotic treatment of leptospira carriers.

Dogs suffering from prolonged canicola leptospiruria acquired as a sequel to classical or subclinical attack constitute the reservoir and the sole spreaders of the infective agent. Leptospiruria establishes itself, according to KLARENBEEK (1938), in 59 per cent of the dogs suffering from clinical infections; it may persist for 18 months (NIEMAND, 1940). The leptospirae discharged in the urine are readily transferred among dogs living together; the habits of licking urine or bringing the nose and tongue in intimate contact with the genitals, the breeding act and possibly contaminated food serve as pathways of infection. Any form of therapy which cures the carrier stage will break the infection chain and ultimately assist in the control of canine leptospirosis.

At the Hooper Foundation, every one of 12 dogs experimentally infected by intraperitoneal injection of a culture of Leptospira canicola or L. icterohaemorrhagiae, having survived the illness, started shedding a large number of leptospirae on the 12th day thereafter. The number of leptospirae was occasionally as high as 13 million living organisms per milliliter of urine. One dog held under observation shed leptospirae which were readily demonstrable in the dark field after 4 months and at that time 0.5 ml. of the urine injected intraperitoneally killed a hamster in 7 days.

Penicillin and streptomycin inhibit the growth of the majority of leptospirae in vitro (PETERSEN and SCHMIDT, 1945); however, exceptions have been reported and it is not unlikely that different types may possess a variable susceptibility to the antibiotics (AUGUSTINE et al., 1944; ALSTON and BROOM, 1944). Furthermore, on the basis of experiments on guinea pigs, it is generally agreed

that penicillin administered early in the course of the infection is destructive of the leptospirae in the blood stream but, as a rule, fails to injure the parasites in the renal tubules (SHIH LU CHANG, 1947; WYLIE and VINCENT, 1947; PETERSEN and SCHMIDT, 1945). BRUNNER (1948) found that Leptospira canicola infections are amenable to penicillin treatment as late as the 6th day after intranasal instillation of the organisms which, in untreated animals, usually ends fatally on the 9th day. He, however, successfully developed hamsters (Mesocricetus a. auratus) into L. canicola carriers by infecting the animals intranasally and treating them from the 3rd day on with 250 units of penicillin in oil and wax every 12 hours. Thus, he concluded that penicillin may save the life of a dog, but may allow the development of carriers. However, he recommended penicillin since it might be useful in the prophylaxis of canine leptospirosis. For example, a dog exposed to carriers either at a dog show or through the breeding act could be protected with penicillin from a fatal infection (8 units/gram body weight every 12 hours for 4 days, given intramuscularly).

The significance of the observation reported by HEILMAN (1945) that hamsters infected with Leptospira icterohaemorrhagiae and treated with streptomycin showed no parasites in the livers and kidneys when examined 28 and 33 days after therapy became apparent with regard to carriers when comparative studies were carried out with L. canicola. At the Hooper Foundation, hamsters infected by various routes and treated during the acute phase of the disease recovered, and leptospirae were not demonstrable in the livers and kidneys of the treated animals by darkfield examination or by culture. In an additional experiment on hamsters the superior destructive action of streptomycin on the leptospirae in the convoluted tubules was conclusively demonstrated (BRUNNER, 1949). Hamsters became renal L. canicola carriers when infected intraperitoneally and treated with penicillin on the 60th and 72nd hours after infection. Throughout all the experiments herein described, the antibiotics were administered in oil and wax. Thirty-six of the 40 hamsters survived, 9 were sacrificed on the 32nd day and proved by cultures to be carriers. Beginning on the same day, 18 of the hamsters were treated with 5 mg. of streptomycin every 24 hours for 3 days. On the 2nd day after the last injection of streptomycin, the 18 treated and 9 other controls were sacrificed. The kidneys of the controls yielded leptospirae demonstrable by darkfield examination and culture, while the organs of the treated hamsters were sterile.

These results justified experiments on dogs. One of 4 puppies, all of which had survived an infection and proven repeatedly to shed large numbers of leptospirae in the urine, was treated intramuscularly with streptomycin, 700 mg. on the 1st day, 500 mg. daily on the 5 following days. When the neutral or alkaline urine was examined a few days after cessation of the treatment, leptospirae could not be demonstrated while the shedding of the parasites continued in the untreated. The 3 remaining carrier dogs (5 weeks after inoculation of the culture) were then treated intramuscularly with streptomycin (40 mg./kilogram for 4 days). On the 5th day of treatment the urine proved free from leptospirae by darkfield examination and culture. Repeated examinations yielded the same negative findings. Autopsies, conducted on the dogs 11 days after treatment had been discontinued, confirmed the absence of leptospirae in the kidney emulsions which had been inoculated intraperitoneally into young hamsters.

Finally, one dog proven a shedder of Leptospira icterohaemorrhagiae and one, of L. canicola were treated with streptomycin for 3 successive days (40 mg./kilogram a day, intramuscularly). Two days after the last treatment neither urine nor kidney tissue yielded leptospirae, while a control dog likewise infected with L. icterohaemorrhagiae was proved still to harbor organisms in the kidney.

These observations prove that *chronic renal infections with leptospirae in dogs may be cured successfully with streptomycin.* In the majority of cases, human and animal leptospirosis is treated at a stage when the parasite has largely disappeared from the blood. Obviously, penicillin is then of relatively little value; however, on account of its superior leptospiricidal action, streptomycin, when used in large doses, prevents relapses and the prolonged localization of the leptospirae in the kidneys. Treatment with this antibiotic does not influence the degree and duration of the cholemia, the degree of nitrogen retention or the rate of disappearance of albuminuria. In this respect the results differ in no way from those obtained with specific antiserum, although it is known that serum treatment does not prevent the development of carriers. Therefore, the apparent panleptospiricidal properties of streptomycin should prove particularly useful in the treatment and prevention of the leptospiroses.

Aureomycin has recently been used in experimental studies similar to those carried out with streptomycin (BRUNNER and MEYER, 1949). Hamsters were treated with 1 mg. of the drug every 8 hours for 3 or 5 days. Its therapeutic value appeared to be similar to that of streptomycin, but it has the advantage of oral administration.

Prophylactic inoculation against leptospiral infections.

If circumstances prohibit the eradication of the vector of the infection, active immunization might be practised. Such environmental conditions, as exist in mines, sewers, and harvest and cane fields, make attempts at rodent extermination difficult and frequently valueless. Owners of dogs are eager to protect their animals, as are the owners of certain fox farms who vaccinate their animals with killed cultures to minimize the losses due to leptospiral infection with the classical rat strain.

In reviewing the previous work on active immunization of animals and man against infection with Leptospira icterohaemorrhagiae, it was noted that homologous strains produced better immunity than heterologous strains, and that living nonvirulent forms may have some additional immunizing property which is absent from the killed form (SMITH, 1937; VAN DER POEL and JOEDO, 1937). These observations prompted VAN THIEL (1938) to immunize 5 individuals with varying doses of a living strain of L. icterohaemorrhagiae which had become avirulent for guinea pigs. In all experimental subjects atypical Weil's disease developed and one became seriously ill. The experimenter therefore concluded that apathogenicity for guinea pigs did not assure avirulence for man, and consequently the vaccination with living avirulent strains thus far available is inadvisable.

Large-scale immunizations with phenol-killed leptospiral cultures are based on the early demonstration by IDO et al. (1916), NOGUCHI (1918), BAERMANN and ZUELZER (1928), and the small group of tests by UHLENHUTH and ZIMMERMANN (1934), ESSEVELD (1937) and DAS GUPTA (1942). They are all reported as successful. The results obtained by WANI (1933) in the coal mines located in the Province of Fukuoka are particularly impressive. A heat-killed (twice at 56° C. for 30 minutes at 24 hour intervals), phenol-preserved (0.5 per cent) antigen containing 30 to 75 million leptospirae per milliliter was used for the inoculations and a dose of 2 ml. followed by another of 3 ml. was given. Among the 10,268 vaccinated miners the resulting morbidity rate was 0.13 per cent (11 cases) and among the unvaccinated, 1.12 per cent (457 cases). Thus, the morbidity rate was less than one ninth.

A glance at the literature from Indonesia conveys the impression that the postinoculation antibody titer reflects the protection afforded by a vaccine. The number of inoculations or the antigenic mass is considered important (ESSEVELD, 1937; SMITH, 1937). DAS GUPTA recognized individual inability to produce titers of over 1 : 300 and VAN THIEL (1938) held the opinion that even the

lowest antibody level (1 : 4) is adequate to indicate an effective immunization.

Consideration of these reports suggested a) that a potent immunizing agent must be prepared from preferably virulent cultures, b) it must be concentrated to reduce the number of injections and c) the leptospira must be inactivated by physical means to preserve the immunogenic potency. Protection experiments at the Hooper Foundation have been conducted on hamsters and dogs.

The antigens are prepared as follows: well-grown cultures of virulent Leptospira canicola or L. icterohaemorrhagiae (60,000 to 100,000 organisms per cubic millimeter) in Schüffner's modification of Verwoort's medium containing 10 per cent rabbit serum are subjected to high-speed centrifugation for 1 hour at 14,000 r.p.m. Very few organisms remain in the supernatant fluid. The centrifugate consisting of liver-leptospira is resuspended in 3 per cent dextrine solution, shell frozen at once and lyophilized. The organisms are invariably killed. The lyophilized antigen is dissolved in saline; it may be frozen after use and kept on dry ice.

Chemically killed antigens are prepared by treating the cultures with 1 or 2 per cent formalin solution before high-speed centrifugation. The formalin-killed centrifugate is resuspended in saline and kept in the refrigerator.

The challenging dose, a virulent culture of leptospirae injected intraperitoneally 12 to 54 days after the second antigen injection, was invariably very high; 0.25 cc. of the culture containing about 20 million organisms constitutes up to 25,000 lethal doses for hamsters, as experiments with L. canicola have proved.

The first experiment with hamsters, summarized in table II, proved the superiority of the frozen and lyophilized antigen over a formalin-killed culture. With a total dose of as little as 0.001 ml. antigen (2 injections at 6 day intervals), 88 per cent of the hamsters inoculated with the lyophilized antigen and only 33 per cent injected with the formalin-killed antigen proved protected. Larger amounts of antigen irrespective of the method of killing gave complete protection.

In the second experiment (table III) it was shown that 80,000 virulent leptospirae killed by freezing and drying protected 8 of 10 hamsters, while 180,000 similarly killed organisms of a culture which had lost its pathogenicity for hamsters or guinea pigs immunized 6 of 10 hamsters.

These observations indicate that an avirulent culture of L. canicola may be used in the preparation of a lyophilized antigen provided it is recognized that the antigen mass or dose must be larger than that employed with a virulent culture. The avirulent

TABLE II.

Immunization experiments with leptospira antigens.
(Lyophilized and Formalin-Killed.)
L. canicola 1126.

Hamsters	Antigen		Day of death after challenge * with L. canicola 1126						Total deaths
	Type	Dose, ml. (given twice)	4th	5th	6th	7th	8th	13th	
9	Lyophilized	0.01							
9		0.001							
9		0.0001			1				1
9	Formalin-killed	0.01							
9		0.001							
9		0,0001	3	1		1		1	6
27	Controls		24	2			1		27

* Challenged on the 15th day after the last injection of antigen.

TABLE III.

Immunization experiments with leptospira antigens.
(Lyophilized Virulent and Avirulent L. canicola.)

Hamsters	Antigen		Day of death after challenge* with L. canicola 1126			Total deaths
	Strain	Dose, ml. (given twice)	4th	5th	6th	
10	1126 (virulent)	0.01				
10		0.001				
10		0.0001	1		1	2
10	R (avirulent)	0.01				
10		0.001				
10		0.0001	2	1	1	4
10	1126 (virulent, oil and wax)	0.01				
10		0.001				
10		0.0001	3	2	1	6
30	Controls		30			30

* Challenged on the 12th day after the last injection of antigen.

culture offers advantages in form of a) ease of cultivation and b) heavy yield (300,000 leptospirae per cubic millimeter; 3 million after concentration) with reduced risk to the persons preparing the vaccine.

In the experiments reported herein, hamsters were successfully immunized with an avirulent strain of L. canicola originating from Holland against challenge with a virulent L. canicola strain isolated from a dog in San Francisco. Analogous experiments were carried out with L. icterohaemorrhagiae. As an immunizing agent an avirulent strain of L. icterohaemorrhagiae, type AB, originating from Holland was used. The hamsters were challenged with a virulent strain of L. icterohaemorrhagiae isolated from rats in San Francisco. The protection reached was irregular and even with a dose of 0.1 cc. of the concentrated antigen, only 4 of 6 hamsters were protected, and 2 of 6 with 0.01 cc., as well as with 0.001 cc. All the 13 controls died. Preliminary experiments with dogs gave similar results. These results may be due either to an immunologic difference between the two serologically identical strains, or to the somewhat lower susceptibility of hamsters and dogs to infection with L. icterohaemorrhagiae. A heat-killed vaccine prepared with the Isige strain of L. icterohaemorrhagiae recently obtained from Japan and used in the dose of 1 ml. in 2 doses protected only 1 of 6 hamsters. Cross-immunization experiments in which L. canicola antigens were used to protect against the heterologous L. icterohaemorrhagiae and, vice versa, showed that hamsters fully protected with 0.005 ml. of L. canicola antigen against a homologous strain exhibited in 5 of 20 animals immunity against a heterologous classical rat strain. The immunity conveyed to hamsters by the L. icterohaemorrhagiae antigen proved inadequate and the cross-protection against infection with L. canicola was of a very low order.

BROOM (1949) in experiments with L. canicola and L. icterohaemorrhagiae immunized hamsters with a 0.5 per cent phenol vaccine. He used doses of 1.0 cc. twice and 0.5 cc. three times of an unconcentrated vaccine, and challenged by intraperitoneal injection of 0.5 cc. of a culture of the same strains as those used for the antigen. He seemed to have worked with approximately the minimal protective dose for L. icterohaemorrhagiae. Of 20 hamsters, 18 were protected with the two-stage immunization; all of 15 with three antigen injections. He also reported some protection with L. canicola antigen against L. icterohaemorrhagiae, and none with L. icterohaemorrhagiae antigen against L. canicola.

NOGUCHI in 1918 immunized guinea pigs with 4 strains of L. icterohaemorrhagiae of American, European and Japanese origin. For full protection against all the strains, three injections of 0.5 cc. of a phenol-killed (0.4 per cent), five times concentrated culture was necessary. Injections of 0.05 cc. protected against the strains

used for immunization, and in some instances against the other strains. Doses of 0.005 cc. protected against the same strain in 2 cases and did not protect against any of the other strains. The challenging dose in all experiments was small, several lethal doses, ranging from 0.005 to 0.00005 cc. of a 3 week old culture with different strains.

In the light of the experiments of NOGUCHI, BROOM and the data presented in this paper, it seems that a dependable immunization of hamsters, guinea pigs, dogs and probably also human beings against infection with L. icterohaemorrhagiae requires relatively large doses of antigen. This is certainly the case if two or more strains are concerned. Smaller doses may be sufficient with certain strains and identical challenging strain, but the immunologic response may vary in different animals and thus depend on the susceptibility of the entire group.

Our results with L. canicola with hamsters and dogs, using a Dutch strain for antigen and challenging with a Californian strain, seem to put the antigenic quality of L. canicola on a different level. Comparatively small amounts of antigen gave good protection in hamsters and dogs.

In a series of experiments the immunity of young dogs (about 6 to 10 weeks old) injected with different leptospiral antigens in a two-stage program at 6 day intervals was challenged by intraperitoneal injection of virulent Leptospira canicola or L. icterohaemorrhagiae (100 to 500 million organisms) maintained at a high virulence through continuous animal passage. This very great number of organisms in the challenging dose is thought to exceed overwhelmingly the number possible in the natural infection of dogs; hence, these doses constitute a severe test of the immunity afforded by the vaccine. The significant data are presented in tables IV and V.

Six dogs immunized with 0.5 ml. of lyophilized avirulent Leptospira canicola were apparently solidly protected against the challenge which caused leptospiruria in all the 5 control dogs. The agglutination titers which rose sharply in the controls were only slightly elevated in the vaccinated dogs (from a titer of 1 : 100 to 1 : 300 or to 1 : 1,000). It is not unlikely that the leptospirae of the challenging injection were promptly destroyed by the antibodies present in as low a titer as 1 : 30.

Two puppies injected with a polyvalent mixture of Leptospira canicola and L. icterohaemorrhagiae lyophilized antigen (0.25 ml. of L. canicola and 0.25 ml. of L. icterohaemorrhagiae) resisted an injection of L. canicola, but of 2 dogs treated in a like manner,

TABLE IV.

Immunization experiment with leptospira antigens.
(Lyophilized Avirulent L. canicola U IV)

Dogs	Antigen	Serum agglutination titers				Proven infection
		Before challenge	Day after challenge* with L. canicola 1126			
			3rd	7th	15th	
Immunized						
1	0.5 ml. of L. canicola U IV given twice	100	100	100	300	
2		100	300	300	100	
3		30	300	100	30	
4		100	100	300	1000	
5		30	30	10	300	
6		100	100	100	100	
Controls						
7		10	300	10000	3000	+
8		10	1000	1000	10000	+
9		0	10	100	3000	+
10		0	1000	10000	3000	+
11		10	100	1000	3000	+

* Challenged on the 25th day after the last injection of antigen.

TABLE V.

Immunization experiments with leptospira antigens.
(Lyophilized Avirulent L. canicola U IV and
Avirulent L. icterohaemorrhagiae A 20)

Dogs	Antigen		Challenge organism	Homologous serum agglutination titers						Proven infection
	Type	Dose, ml. (given twice)		Before challenge	Day after challenge*					
					3rd	6th	9th	12th	18th	
Immunized										
1	L. can.	0.5	L. ict. 917	10	100	+†				+
2	L. can./L. ict.	0.5	L. ict. 917	10	100	300	1000	1000	300	+
3	L. can./L. ict.	0.5	L. ict. 917	10	100	100	300	100	100	
4	L. can./L. ict.	0.5	L. can. 1126	30	30	300	100	100	100	
5	L. can./L. ict.	0.5	L. can. 1126	30	100	300	100	100	100	
Controls										
6			L. ict. 917		100	+†				+
7			L. can. 1126			+†				+
8			L. ict. 917		100	100	1000	1000	3000	+
9			L. ict. 917		100	300	1000	1000	1000	+
10			L. can. 1126			100	+†			+

* Challenged on the 30th day after the last injection of antigen.
† Animal died.

only one resisted a challenge with L. icterohaemorrhagiae. The infected animal had a definite rise in the agglutinin titer and became a leptospira shedder. Of importance is the single observation that 0.5 ml. of lyophilized L. canicola antigen failed to protect against a L. icterohaemorrhagiae challenge infection. It is well known that in the experiments of ESSEVELD (1937) the protection by L. canicola against L. icterohaemorrhagiae was only relative.

The foregoing experiments have demonstrated that immunization of dogs with killed lyophilized avirulent Leptospira canicola cultures gave excellent protection against the homologous, but not against the heterologous, L. icterohaemorrhagiae type. Some protection with increased dosage is possible, as indicated by experiments with hamsters and guinea pigs mentioned above. There is suggestive evidence that the immunogenic potency of L. icterohaemorrhagiae antigens is relatively low, and effective protection in all probability can only be expected with increased dosage. Furthermore, dogs exposed to both dog and rat leptospiral infections must be injected with an antigen prepared with both biotypes. In view of the poor protective qualities of a recent lot of leptospiral vaccine obtained from Japan used for hamsters, the injurious effect of heat and chemicals on the immunogenic properties of leptospiral vaccine requires renewed investigation. Finally, the duration of the immunity conveyed by antigens is subject for further investigation.

The canicola antigen, on subcutaneous injection of 2 doses of 2 cc. each was well tolerated by one of the authors of this report (BRUNNER). The first injection was followed by a slight local reaction; the second, by a mild systemic reaction, largely due to the fact that the antigen used then contained rabbit serum. The agglutination titer rose to 1 : 5,000 by the 24th day after the second injection.

From the standpoint of public health and prophylaxis, the studies on leptospiral immunity in hamsters and dogs artificially induced by the subcutaneous injection of lyophilized antigen have definitely proved that the protection created was nearly complete and consequently prevented the creation of carriers. An antigen and a method of immunization which accomplishes this goal becomes a practical procedure which deserves wider recognition.

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Summary.

The problem of canine leptospirosis is analyzed on the basis of the literature and our own studies, with the intention of making a contribution to chemotherapy and active immunization. A total of 178 cases of canicola fever in human beings are now known to have occurred, hence the growing importance of the disease is stressed.

In a number of experiments with young hamsters, penicillin proved its value as a chemotherapeutic agent, but later the organisms were still shed in the urine (carrier stage). Streptomycin, on the other hand, was fully effective in the treatment of hamsters and dogs that were chronic carriers, in doses of 40 mg. per kilogram body weight per day. Aureomycin showed comparable results with a dose of about 50 mg. per kilogram per os every 12 hours.

Active immunization of hamsters and dogs is described. Cul-

tures of leptospirae are centrifuged and a ten times concentrated killed antigen prepared by deep freezing and drying at the same time in a vacuum (lyophilization). This antigen proves to be more effective than a formalin-killed preparation. Avirulent L. canicola yields cultures which are three times denser than those of virulents strains. Antigens prepared from the avirulent strains proved nearly as immunogenic as the ones made from virulent cultures. Immunization of hamsters with two injections of < 0.001 cc. and dogs with < 0.5 cc. of the L. canicola antigen resulted in a good protection of the animals against infection with a virulent homologous strain of different origin. Analogous experiments with L. icterohaemorrhagiae antigen proved that protection of hamsters against massive infection with a second virulent strain of the same biotype was inadequate (dose of up to 0.1 cc.). It is assumed that antigenically different, but serologically identical L. icterohaemorrhagiae strains exist, and that this could probably be compensated by increasing the vaccine dosage.

Résumé.

Le problème de la leptospirose canine est analysé en se servant des publications et de nos propres recherches à ce sujet, dans l'intention de perfectionner les méthodes de chimiothérapie et d'immunisation active. On a connaissance d'un total de 178 cas de fièvre à L. canicola chez l'homme, et, en conséquence, on souligne l'importance du développement de la maladie.

Dans un certain nombre d'expériences sur de jeunes Cricetus cricetus, la pénicilline s'est montrée active comme agent chimiothérapique, mais dans la suite, des organismes furent encore éliminés dans les urines (phase porteur de germes). La streptomycine, d'autre part, fut complètement effective dans le traitement des C. cricetus et des chiens qui étaient porteurs chroniques, à la dose de 40 mg. par kg. de poids corporel par jour. L'auréomycine a montré des résultats comparables, à la dose d'environ 50 mg. par kg. per os, toutes les 12 heures.

L'immunisation active des C. cricetus et des chiens est décrite. Des cultures de leptospira sont centrifugées et un antigène inactivé et concentré dix fois, est préparé par congelage poussé et dessication dans le vide simultanément (lyophilisation). Cet antigène se montre plus efficace que les préparations inactivées à l'aide de formol. Le L. canicola non virulent produit des cultures qui sont trois fois plus denses que celles des espèces virulentes. Les antigènes préparés à partir des espèces non virulentes présentent un pouvoir immunisant presque semblable à celui des antigènes préparés à partir des cultures virulentes. L'immunisation des C. cri-

cetus avec deux injections inférieures à 0,001 cc. et des chiens avec moins de 0,5 cc. de l'antigène du L. canicola, a produit une bonne protection des animaux contre une infection avec une espèce homologue d'origine différente. Des expériences semblables avec l'antigène de L. icterohaemorrhagiae ont prouvé que la protection des C. cricetus contre une infection massive avec une deuxième espèce virulente biologiquement semblable, était insuffisante (doses jusqu'à 0,1 cc.). L'on suppose que des espèces de L. icterohaemorrhagiae d'un pouvoir antigénique différent, mais sérologiquement identiques, existent, et que cette différence pourrait sans doute être compensée par une augmentation de la dose de vaccin.

Zusammenfassung.

Das Problem der Leptospirosis beim Hund wird auf Grund der Literatur und eigener Untersuchungen mit der Absicht analysiert, einen Beitrag zur Chemotherapie und aktiven Immunisierung zu leisten. 178 Fälle von Canicolafieber beim Menschen werden zitiert und auf die steigende Wichtigkeit der Krankheit hingewiesen. In einer Anzahl von Experimenten mit jungen Hamstern wird gezeigt, daß Penicillin als Chemotherapeutikum in der akuten Phase der Krankheit wirksam ist, daß aber die Organismen später trotzdem im Urin ausgeschieden werden (Dauerausscheider). Streptomycin hingegen ist voll wirksam in der Behandlung chronischer Ausscheider (Hamster und Hunde), mit einer Dosis von 40 mg i.m. per kg Körpergewicht p. d. Aureomycin ergab vergleichbare Resultate mit Streptomycin bei einer Dosierung von ca. 50 mg/kg per os alle 12 Stunden.

Aktive Immunisierung von Hamstern und Hunden wird beschrieben: Leptospirenkulturen wurden zentrifugiert und ein 10fach konzentriertes abgetötetes Antigen durch Tiefkühlen und gleichzeitiges Trocknen im Vakuum (lyophilization) hergestellt, das einem Formolpräparat in der Wirksamkeit überlegen ist. Antigene, aus den dreimal dichter wachsenden avirulenten L. canicola-Kulturen hergestellt, zeigten sich fast ebenso wirksam wie die aus virulenten Organismen. Immunisierung von Hamstern und Hunden mit 2 Injektionen von $< 0{,}001$ cc. bezw. $> 0{,}5$ cc. des L. canicola-Antigens ergab guten Schutz gegen Infektion mit einem virulenten homologen Stamm verschiedener Herkunft. Analoge Experimente mit einem L. icterohaemorrhagiae-Antigen ergab keinen genügenden Schutz von Hamstern gegen massive Infektion mit einem zweiten virulenten Stamm des gleichen Biotyps (Dosierung bis 0,1 cc.). Es wird angenommen, daß antigenetisch verschiedene, aber serologisch identische L. icterohaemorrhagiae-Stämme bestehen, was vielleicht mit Erhöhung der Vaccine-Dosis kompensiert werden kann.