protein through a sulfhydryl group. 2) This vitamin may be maintaining the sulfhydryl enzymes in a reduced state as proposed by Dubnoff(12,13). 3) Vit. B_{12} may be concerned with the maintenance or production of high-energy phosphate bonds.

Summary. 1. Molybdenum at relatively high concentrations inhibited the growth of *Lactobacillus leichmannii.* 2. This inhibition was prevented or reduced by additions of vit. B_{12}, cysteine, glutathione or BAL. 3. Reducing agents *per se* had no effect on the inhibition. 4. The possible interactions of molybdenum, vit. B_{12}, and sulfhydryl compounds on the growth of the microorganism have been discussed.

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Leptospiral Macroscopic Agglutinating Antigens (20393)

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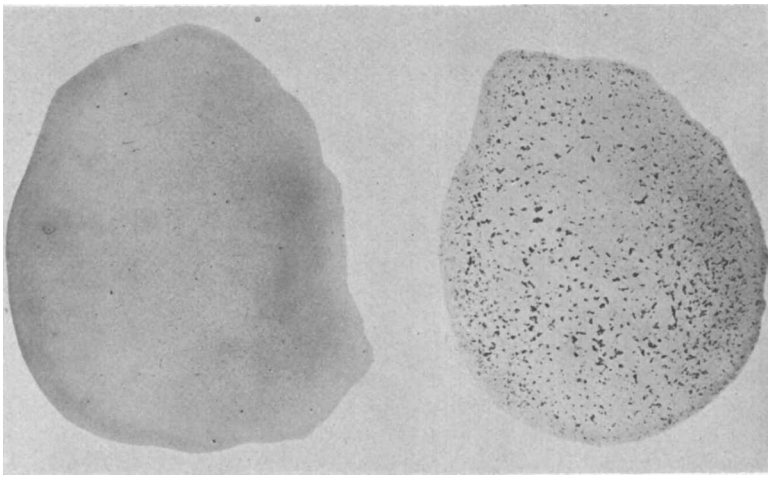
The laboriousness of microscopic agglutination technics for the demonstration of leptospiral antibodies has severely limited their use in the laboratory diagnosis of leptospirosis. The search for simpler and more rapid methods, and for more stable antigens, has led to the development of a number of leptospiral macroscopic agglutinating antigens(1-4), but experience with antigens of these types has not been wholly satisfactory. Their sensitivity, stability, and specificity were not of the same order as that of the more widely used microscopic agglutinating antigens(5,6). Further, visible reactions were often granular in character and difficult to detect. Dye precipitation in stained antigens frequently confused interpretation. The potential usefulness of leptospiral macroscopic agglutinating antigens, however, warranted further studies directed toward overcoming these deficiencies. The influence of electrolytes upon stability of bacterial suspensions, and upon antigen-antibody reactions, has repeatedly been demonstrated(7-9). In particular, the sensitivity of bacterial agglutinating antigens has been shown to be markedly affected by changes in pH and ionic strength. Information about the various optimal conditions for leptospiral agglutinating reactions has not been reported in the literature available to the authors, so that initial studies were directed toward their determination. The adsorption of leptospiral agglutinating antigens onto inert particles had likewise not previously been reported, and it appeared reasonable to determine whether or not this procedure might result in the formation of more readily discernible aggregates in the agglutination reaction.

Macroscopic agglutinating antigens were prepared from nine leptospiral serotypes. The sensitivity, specificity, and stability of these antigens were investigated with immune sera prepared in rabbits and with sera from cases of human, equine, bovine, porcine, and canine leptospirosis.

Methods. 1) *Propagation of Leptospires.*



Negative Positive

FIG. 1. Positive and negative macroscopic agglutinating reactions.

Strains representing nine leptospiral serotypes were cultivated in Stuart's liquid medium (10) at 30°C for 10 to 14 days. The organisms were harvested at their maximum population density as determined by darkground microscopic examination. Leptospires were recovered by centrifugation at 43,300 x g for 45 minutes in a Spinco Model L refrigerated centrifuge, twice washed in one-tenth the original culture volume of distilled water, and again resuspended to one-tenth the original culture volume in distilled water. 2) *Standardization of Leptospiral suspensions.* Previous studies with freeze-dried leptospires (11) indicated optimal agglutinating antigen densities to be of the order of 0.5 mg of dried leptospires per ml. Such suspensions had 24.5% transmission at a wavelength of 600 mu on the Coleman Model 6A Spectrophotometer. This turbidity was also found optimal for non-freeze-dried leptospiral agglutinating antigen suspensions. Accordingly, the suspensions of washed leptospires, obtained above, were standardized to this transmission by the addition of distilled water. The standardized suspensions were incubated in the water bath at 56°C for four hours to kill the organisms. 3) *Determination of optimal pH.* Stained 0.1 M sodium acetate buffer solutions at pH 3.2, pH 4.7, pH 5.7 and pH 6.2, each containing 0.05 g of Crystal Violet (94% dye content) per 30 ml, were prepared. 0.2 ml of the buffers were added to 5.0 ml aliquots of the heat-killed leptospiral suspension. Serial 2-fold dilutions of homologous immune serum in distilled water were prepared through the range 1:5 through 1:2560. Equal volumes (ca 0.03 ml) of serum dilution and of antigen were mixed on a glass plate and incubated at room temperature for 10 minutes. Observations were then made with the unaided eye and reactions recorded as complete (+), incomplete (±), trace (T), or absent (−). The antigen adjusted to pH 5.7 was completely agglutinated by a 1:640 dilution of serum whereas antigens adjusted to pH 3.2, 4.7 and 6.2 resulted in endpoints of 1:20, 1:160 and 1:160 respectively. Based on these results, antigens were subsequently adjusted to pH 5.7. 4) *Determination of optimal ionic strength.* Stained acetate buffers at pH 5.7 with the same dye content as above were prepared at molarities of 0.025, 0.05, 0.10, 0.15 and 0.20. Each buffer in 0.2 ml quantity was added to 5.0 ml of heat-killed leptospiral suspension. These buffered suspensions were used as antigens in titration of homologous immune serum as described above. Antigens prepared at molarities of .05 and .10 were completely agglutinated at a serum dilution of 1:640 while molarities of 0.25, .15 and .20 gave endpoint titers of 1:160. Antigen prepared with 0.1 M stained acetate buffer at pH 5.7 was then used in the examination of homologous serum dilutions prepared in distilled water and in 0.85% NaCl solution. Re-

sults reveal the endpoint agglutinating titers of the serum diluted in distilled water and saline to be 1:640 and 1:160 respectively. 5) *Adsorption onto cholesterol platelets.* To each 5.2 ml of the buffered antigen was added 0.1 ml of a 1% solution of cholesterol (Pfanstiehl ash-free) in absolute ethanol. The cholesterol was added slowly with vigorous agitation of the flask containing the antigen. No effort was made to control the size of the platelets critically. The resultant precipitate was dispersed by shaking. 1.0 ml of glycerol was then added and the mixture again shaken. Comparison of cholesterolized and non-cholesterolized antigens in the examination of immune sera showed the former to possess approximately 4 times the sensitivity of the latter. 6. *Serologic technics.* a) *Macroscopic agglutination.* Serial 4-fold serum dilutions from 1:2.5 through 1:2560 were prepared in distilled water. A glass plate, ruled in one inch squares, was employed in a test box having oblique illumination, a black non-reflective background, and a hinged cover. One drop (ca 0.03 ml) of each serum dilution to be examined was placed on a separate ruled square. An equal quantity of antigen was added to each serum dilution; the antigen and serum dilution were mixed and spread over an area approximately 2 cm in diameter. Further mixing was assured by gently rocking the glass plate for the first minute of the test period and again at 3-minute intervals until the end of the 10-minute period. Agglutination was observed with the unaided eye and recorded as complete, incomplete or absent. Titers were expressed as the reciprocal of the highest dilution in which complete agglutination was observed. Inhibition in the region of antibody excess was frequently encountered in the examination of high titer sera, and, for this reason, all sera to be examined were carried through the dilution schema mentioned above. b) *Microscopic agglutination-lysis.* Parallel examination of serums with this method were conducted as described elsewhere(12).

Results. Cross agglutination reactions. Antisera prepared in rabbits against 18 leptospiral serotypes were examined with the 9 leptospiral macroscopic agglutinating antigens prepared. The results of these examinations

TABLE I. Macroscopic Cross-Agglutination Patterns of Leptospiral Serotypes.

Antiserums	Antigens 4	5	6	8	11	12	13	15	18
1. *L. andaman*									
2. *semerang*									
3. *hyos*									
4. *djatzi*	T								
5. *pomona*		T	4	16		16			4
6. *djasiman*		16	T	16			16		16
7. *sentot*			16						
8. *autumnalis*, Akiyami A				T			16		
9. *autumnalis*, FBF				16					
10. *australis*, A, Ballico									
11. *ballum*					T		16		
12. *Canicola*, Ruebush						T	16		16
13. *icterohemorrhagiae*, Wijnberg							T		16
14. *australis* B, Zanoni							16		
15. *grippotyphosa* Moscow V								T	
16. *alexi*									4
17. *medanensis*									
18. *pyrogenes, Salinem*									T

T = Titer of homologous strain; 4 = ¼ titer; 16 = 1/16 titer.

are shown in Table I. Essentially similar cross reactions are observed in the agglutination-lysis antigen-antibody reactions(13). There is, however, somewhat greater serotype specificity shown by the macroscopic agglutinating antigens.

Comparison of macroscopic agglutination examinations with microscopic agglutination-lysis examinations. Six hundred and twenty-three bovine, 47 porcine, and 50 canine sera were examined by both macroscopic agglutination and microscopic agglutination-lysis technics. The same strains of the same serotypes of leptospires were employed as antigens with both technics. The results of these examinations are summarized in Table II. The table discloses that the degree of correlation between these methods at significant antibody levels is of the order of 70.0% Correlation approaches 80.0% when sera with high antibody levels are examined.

Serial serum specimens from proved cases of Leptospirosis pomonae were examined. The results are summarized in Table III. Significant rises and falls in antibody levels are demonstrable with this technic.

Stability. No loss of sensitivity has been

TABLE II. Examination of Bovine,* Porcine,* and Canine† Sera by Macroscopic and Microscopic Agglutination Technics.

Microscopic agglutination titer	Macroscopic agglutination titer																		No. sera		
	0			5			20			80			320			1280			5120			
	‡B	P	C	B	P	C	B	P	C	B	P	C	B	P	C	B	P	C	B	P	C	
102400	13	0	0	3	0	0	4	0	0	8	0	0	20	4	0	13	0	0	4	0	0	65	4	0
25600	5	0	0	13	0	0	3	2	0	5	2	0	4	0	0	9	0	0	0	0	0	39	4	0
6400	11	0	0	22	1	0	6	3	0	1	1	2	2	1	0	1	0	0	2	0	0	45	6	2
1600	14	0	0	41	2	2	8	3	8	5	1	2	0	0	0	0	0	0	0	0	0	68	6	12
400	41	2	1	23	1	5	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	66	3	7
100	39	4	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	4	2
0	291	20	27	5	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	299	20	27
No. sera	414	26	28	109	4	9	26	8	9	19	4	4	26	5	0	23	0	0	6	0	0	623	47	50

* *L. pomona*, S-91 (Gochenour, W. S., Jr., Johnston, R. V., Yager, R. H., and Gochenour, W. S., *Am. J. Vet. Res.*, 1952, v13, 158) employed as antigen with both technics.
† *L. canicola* Ruebush (Randall, R. and Cooper, H. K., *Science*, 1944, v100, 133) employed as antigen with both technics.
‡ B = Bovine sera; P = Porcine sera; C = Canine sera.

TABLE III. Examination of Serums from Cases of Leptospirosis Pomonae.

Source	Specimen	Macroscopic agglutination titer	Microscopic agglutination titer
Human	1st	0	0
	2nd	320	200
Bovine	1st	80	1600
	2nd	1280	102400
Exp. equine	P*	0	0
	69 day	80	500000
	329 "	5	800
" rabbit	P	0	0
	7 day	20	800
	14 "	80	6400
	21 "	1280	6400

* P = Pre-infection.

observed following storage at 5°C for 15 months. Of interest, however, is the observation that these antigens do not attain their maximum sensitivity until approximately 48 hours after preparation.

Discussion. The optimal conditions for specific agglutination in this system appeared to occur at pH 5.7 and at ionic strengths less than 0.10. Preparation of the antigen as described, and the use of distilled water as a diluent for the sera examined, afforded a convenient approximation to this condition. Macroscopic agglutinating antigens prepared in this manner can play a useful role in the laboratory diagnosis of leptospirosis. The simplicity of the technic of the test, and the stability of the antigens, recommend this procedure to the small laboratory. The lack of correlation between macroscopic agglutination and microscopic agglutination tests in the examination of low titer sera from cattle is a limiting factor in the employment of antigens of this type for serological surveys of bovine leptospirosis. The high antibody levels attained by cattle during the acute phase of leptospiral infection, however, is such that these two technics should be equally reliable for the diagnosis of acute leptospirosis. Perhaps the greatest value of these antigens lies in their utilization in field surveys of outbreaks of acute leptospirosis in domestic animals. Here, where time may be of the essence in the application of measures to limit and control infection, rapid diagnosis of acute cases may be of paramount significance.

Summary. A method for the preparation of macroscopic leptospiral agglutinating antigens is described. Antigens of this type have been prepared from 9 leptospiral serotypes. Cross reaction patterns are essentially comparable to those observed in the agglutination-lysis technic. Antigens so prepared are sensitive, specific, and stable under protracted periods of storage, and produce easily discernible reactions in the presence of antibodies.

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Relative Biological Effectiveness of Radium and Other Alpha Emitters in CF No. 1 Female Mice. (20394)

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Estimates of permissible levels of radioactive isotopes in man depend, in part, upon a comparison of the radiotoxic hazards of these elements and of radium. Radium has become the common denominator for experimental animals and man because of the information available from the study of individuals who were exposed accidentally or who were intentionally treated with it. The data at hand from a series of experiments in progress make possible comparisons a) of the effects of radium and some other alpha emitters on the survival of mice, and b) of their carcinogenic potentialities for the mouse skeleton.

Procedures. The dose ranges of the radioisotopes used are presented in Table I. Radium 226, polonium 210, and thorium 228 were injected as chlorides; plutonium 239 (plus 4) and the 2 uranium isotopes were given as nitrates. Except for uranium 232 and thorium 228, the doses were selected to range from rapidly lethal levels to levels below tolerance. The materials, in isotonic salt solution at pH 2-4, were injected into a lateral tail vein of CF No. 1 female mice that were 9-10 weeks old. Complete protocols and details concerning the handling of these duration-of-life experiments will be presented when the studies are finished. Briefly, all animals were autopsied, tissues were taken for histological examination, and roentgenograms were prepared. Whenever possible, animals were sacrificed when moribund.

TABLE I. Materials and Range of Doses.

Isotope	Range of doses (μc/kg)	No. of animals
Radium 226	.6 –4170	675
Plutonium 239	.04– 50.4	765
Polonium 210	.46– 100	645
Uranium 233	.1 – 100	675
" 232	.1 – 10	135
Thorium 228	6.6 – 360	75
Controls		450
Total		3420