the 2 sera. This finding could have practical application in co-precipitation technics.

1. Schiller, A. A., Schayer, R. W., Hess, E. L., *J. Gen. Physiol.*, 1952, v36, 489.

2. Kabat, E. A., Mayer, M. M., *Experimental Immunochemistry*, Charles C. Thomas, Springfield, 1958.

3. Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., Metcalf, T. G., *Am. J. Path.*, 1958, v34, 1081.

4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.

5. Maurer, P. H., Thorpe, R. M., *J. Immunol.*, 1960, v84, 318.

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Experimental Histoplasmosis in Large Domestic Animals.* (26014)

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Previous studies from these laboratories have been concerned with pathogenesis of histoplasmosis in mice(1-4), dogs(5,6), and monkeys(7). The present report describes experimental disease in horses, cattle, sheep, and swine. Although naturally occurring clinical histoplasmosis is rare in these species(8), skin-test and other evidence(9) suggest that, as in man, asymptomatic infection may be more common. To our knowledge, experimental infection of these species with *H. capsulatum* has not been reported.

Materials and methods. Eight healthy young adult animals, 2 of each species and one of each sex, were used. After completion of base line studies, each was inoculated intratracheally(10) with 5.0 ml of saline suspension of yeast phase *H. capsulatum*† containing 2.0 ml of packed cells from 3-day cultures grown on brain heart infusion (Difco) blood agar. Pontocaine (Winthrop), 2.0 ml, was administered intratracheally 5 minutes prior to inoculation of yeast cells to depress reflex coughing. Animals were observed daily for clinical signs of illness, and blood samples for cultures, complete blood counts, and serologic studies were taken 7, 10, 17 and 24 days after inoculation. Serologic response was studied by collodion agglutination(11) and complement fixation(12) tests at these intervals, then weekly for about 3 months. Skin tests were performed 31 and 73 days after inoculation, using histoplasmin prepared and standardized in infected dogs by previously described methods(13-14). Seven of 8 animals were sacrificed about 3 months post-inoculation, and complete autopsies performed. Tissues taken at autopsy and all blood samples were treated and cultured by the method of Conant(15).

Results. Table I summarizes findings in 8 large domestic animals inoculated intratracheally with *H. capsulatum*. All except the female sheep exhibited clinical evidence of infection and all produced antibodies and developed positive skin tests as compared to pre-inoculation antibody-negative sera and negative skin tests. Fever was first observed in 7 of 8 animals 3-5 days after inoculation, and persisted for about 1 week except in the male sheep and swine, in which 3 weeks of fever was observed. Symptoms varied from slight cough in the 2 swine to definite respiratory symptoms in the male sheep characterized by frequent cough, dyspnea, dry rales, tachypnea and hyperpnea; rales and hyperpnea persisted in this animal up to time of sacrifice at 88 days. A positive blood culture was obtained from the male sheep 7 days after inoculation, and *H. capsulatum* was seen in lung lesions at autopsy. In other animals, symptoms subsided within a few days after the end of the febrile period. A positive blood culture was also obtained from the female swine 7 days after inoculation. No significant

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† Strain G 13—an isolate from spontaneous histoplasmosis in a dog.

TABLE I. Response of Large Domestic Animals to Viable Yeast Phase *H. capsulatum* Given Intratracheally.

Animal	Sex	Signs and symptoms*	Blood culture P.I.D.† pos.	Reciprocal of antibody titer HCA‡	YPCF‡	Skin test P.I.D. pos.	Disposition		Histoplasma seen in tissue
Horse	♀	FC	—	80	160	31-73	Died	110§	+
"	♂	FCDR	—	320	1280	31-73	Sacrificed	87	—
Cattle	♀	FCRT	—	10	160	31-73	"	91	—
"	♂	FCDR	—	10	320	31-73	"	92	—
Sheep	♀	None	—	40	320	31-73	"	88	—
"	♂	FCDRTH	7	80	1280	31-73	"	88	+
Swine	♀	FC	7	10	80	31-75	"	88	—
"	♂	FC	—	10	80	31-73	"	88	—

* F, C, D, R, T, H = Fever, cough, dyspnea, râles, tachypnea, hyperpnea.
† P.I.D. = Post-inoculation day.
‡ HCA = Histoplasmin collodion agglutination; YPCF = Yeast-phase complement fixation; pre-inoculation titers all negative.
§ Rechallenged I.T. on 75th P.I.D. Rechallenged I.V. on 98th P.I.D.

hematologic changes were seen in any of the animals during observation period.

The female horse was rechallenged, intratracheally, 75 days after first inoculation. No clinical signs of infection were seen after rechallenge and no increase in antibody titer occurred. The animal was challenged again, intravenously, with the same dose used intratracheally, on 98th day. After 3 days it became depressed, markedly febrile, hyperpneic, anorectic, and began to lose weight. Death occurred 12 days after intravenous challenge. Histopathologic studies revealed presence of diffuse granulomatous pneumonia superimposed upon foci with calcified centers and extensive peripheral fibrosis of more than 12 days standing. Bodies identified as *H. capsulatum* were seen in giant cells in both types of lesion. The tubercle-like foci appeared to be the result of earlier intratracheal inoculation, whereas the diffuse, active pneumonia was apparently produced by the intravenous challenge.

Gross and histopathologic findings compatible with histoplasmosis were obtained also in both sheep and in the female swine. The male sheep also had multiple focal granulomatous orchitis, with lesions similar to those produced in the lungs and seen in other species with histoplasmosis. Microscopic pathology was less specific in both cattle, the male horse, and the male swine. *H. capsulatum* was not cultured from any of the animals at autopsy and in only 2 cases (Table I) were organisms seen in tissue sections.

Discussion. The above results indicate that horses, cattle, sheep and swine are susceptible to *H. capsulatum* given intratracheally. However, clinical recovery of the animals, paucity of viable organisms in post-mortem tissues, and localization of lesions by fibrosis point to natural ability to cope with the infective agent. In contrast, dogs similarly inoculated in these laboratories(5) developed acute disseminated, fatal histoplasmosis and the organism was readily isolated from the blood and from tissues taken at autopsy. Intravenous challenge in a horse resulted in death 12 days later. This route of inoculation also produced rapidly fatal infection in monkeys (7).

These experiments provide proof that large domestic animals insensitive to histoplasmin can convert to skin-test positivity after exposure to *H. capsulatum,* a finding which supports results observed in the survey of farm animals with histoplasmin. Menges(9) skin-tested horses, cattle, sheep and swine in Missouri and found 73%, 12%, 34% and 2%, respectively, to be reactors.

Summary. Intratracheal inoculation of yeast phase *H. capsulatum* produced non-fatal infection in horses, cattle, sheep and swine as determined by clinical response, development of positive skin tests, production of antibodies, or histopathologic changes. Intrave-

nous inoculation caused fatal infection in a horse.

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1. Campbell, C. C., Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 469.
2. Schaeffer, J., Saslaw, S., *ibid.*, 1954, v85, 223.
3. Saslaw, S., Schaeffer, J., *ibid.*, 1955, v90, 400.
4. Saslaw, S., *ibid.*, 1956, v91, 412.
5. Farrell, R. L., Cole, C. R., Prior, J. A., Saslaw, S., *ibid.*, 1953, v84, 51.
6. Saslaw, S., Maurice, G. E., Cole, C. R., *J. Lab. and Clin. Med.*, 1955, v46, 948.
7. Saslaw, S., Carlisle, H. N., Sparks, J., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 342.
8. Menges, R. W., Furcolow, M. L., Hinton, A., *Am. J. Hyg.*, 1954, v59, 113.
9. Menges, R. W., *Cornell Vet.*, 1954, v44, 21.
10. Blake, F. G., Cecil, R. L., *J. Exp. Med.*, 1920, v31, 403.
11. Saslaw, S., Campbell, C. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 559.
12. Saslaw, S., *J. Lab. and Clin. Med.*, 1948, v33, 811.
13. Howell, A., *Pub. Health Rep.*, 1947, v62, 631.
14. Cole, C. R., Prior, J. A., Saslaw, S., *J. Am. Vet. Med. Assn.*, 1950, v116, 135.
15. Conant, N. F., Smith D. T., Baker, R. D., Callaway, J. L., Martin, D. S., *Manual of Clinical Mycology*, W. B. Saunders Co., Philadelphia, 1954.

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Mucopolysaccharides of Aorta at Various Ages.* (26015)

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Changes in the patterns of mucopolysaccharides (MPS) of cartilage, nucleus pulposus, skin and aorta, concomitant with ageing, have been described in past years. In general, these studies indicated a decrease of total MPS per unit weight with chronological age coincident with an increase in collagen content. The most profound changes were encountered in human costal cartilage(1). In this tissue, a steady decrease in total MPS was observed. Keratosulfate increased in the first two decades and remained constant thereafter. Chondroitin sulfate steadily decreased with time and chondroitin-4 sulfate (ChS-A) was replaced by chondroitin-6 sulfate (ChS-C). Similar changes have been described in degenerating versus normal nucleus pulposus(2). The pattern of age changes in the MPS of skin has also been described.

While these studies were based on isolation and chemical and enzymatic characterization of the isolated fractions, the reported studies of ageing of aorta have been based on analysis of the whole organ for hexosamine, uronic acid, sulfate, and identification of monosaccharides by paper chromatography after acid hydrolysis, as for example in the work of Dyrbye and Kirk(3,4,5). Those authors found until age 60 no change in ratio of galactosamine to glucosamine, and after 60 a slight increase. Similarly, the sulfate content of the tissues was found not to change till age 60. More recently Kirk has reported a crude MPS fraction of aorta to have the anticoagulant activity of 1% standard heparin(3) while Yü and Blumenthal found approximately 4%(6). Jorpes(7) and others have obtained heparin in small quantities from aorta.

The MPS of bovine and human aortae have been isolated and characterized. In bovine abdominal aorta of 1- to 2-year-old cattle, total isolated MPS was approximately 1% of defatted dry weight. Of this total, ChS-A represented 45%, hyaluronic acid 23%, and ChS-B and heparitin sulfate approximately 16% each(8).

The present study was undertaken to com-

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