slight rise in resistance could be achieved with 14 daily transfers of an originally highly susceptible strain of *E. coli*. Wright *et al.*(8) subjected 6 strains of *E. coli* to 20 subcultures in presence of polymyxin B and demonstrated increased resistance (12.5 to >400 μg/ml) in only one of them. Two of 4 strains showed marked increases in resistance by repeated subculture in either polymyxin or colistin and this was accompanied by complete cross-resistance to the heterologous drug. One of the same 2 organisms also yielded one-step highly resistant mutants, which also showed complete crossresistance.

Summary and conclusions. Polymyxin B and colistin, 2 closely related polypeptide antibiotics, had similar antibacterial activity against a large number of pathogenic bacteria. Polymyxin was somewhat more active than colistin against 55% of susceptible strains, on the average 3.4 and 4 times as active against most strains of *N. gonorrhoeae* and *H. influenzae*, respectively. Highly resistant variants developed on repeated subculture of a strain of *A. aerogenes* and one of *Ps. aeruginosa* on agar containing either of these antibiotics; complete crossresistance to the other antibiotic developed in each instance. Single-step mutants of the same strain of *A. aerogenes*, highly resistant to each antibiotic, were readily obtained by seeding a large inoculum on the surface of agar containing 100 or 200 μg/ml of that antibiotic; these mutants were completely cross-resistant to the heterologous antibiotic. Antibiotic-dependent variants were not encountered.

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Received November 16, 1959. P.S.E.B.M., 1960, v103.

Experimental Histoplasmosis in Monkeys.* (25512)

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Previous studies from this laboratory have been concerned with pathogenesis of histoplasmosis in mice(1-4), dogs(5), and other large animals(6). The present report compares infectivity of *Histoplasma capsulatum* for *Macaca mulatta* when administered by intratracheal (IT), intranasal (IN), intragastric (IG) and intravenous (IV) routes.

Materials and methods. Young adult monkeys were inoculated by various routes above with 5 ml of saline suspension of yeast phase *H. capsulatum*† containing 2 ml of packed cells from 4-day cultures grown on brain heart infusion (Difco) blood agar. Eight monkeys were inoculated IT(7), 4 IN(8), 3 IG(9) and 2 IV (long saphenous). Blood cultures, taken 4, 7, 14, and 25 days after inoculation when possible, and all tissues taken at autopsy were treated and cultured by the method of Conant (10). The serologic response of monkeys was followed by collodion agglutination(11) and complement fixation(12) tests. Periorbital subcutaneous tests(13) for sensitivity to histoplasmin were carried out before inoculation and on surviving animals 6-7 weeks after inoculation.

Results. Table I summarizes the findings in 17 monkeys inoculated with *H. capsulatum* by various routes. All 8 monkeys inoculated IT exhibited clinical evidence of infection.

* Supported by Nat. Inst. Health grant.

† Strain G13—an isolate from spontaneous histoplasmosis in a dog, originally obtained from Charlotte Campbell Army Medical Center, Washington, D.C.

TABLE I. Response of Monkeys to Viable Yeast Phase *H. capsulatum* Given by Various Routes.

Monkey No.	Route	Symptoms*	Blood culture, day pos.	Histoplasmin skin test	Antibody response	Disposition: Died, days	Disposition: Sacrificed, mo	Histoplasma isolated at autopsy
1	Intratracheal	G-acute	ND†	ND	—	7		Liver, spleen, mesenteric, bronchial and axillary nodes. Placenta but not fetus.
2		GR-acute	—	+	+		4	None
3		G-acute	ND	ND	—	7		Lung, spleen, bronchial node. Placenta but not fetus.
4		GR-acute	—	+	+		4	None
5		"	—	+	+		6	"
6		GR-mild	4, 7	+	—		4	"
7		"	—	+	+		24	"
8		"	—	+	—		4	Bronchial node
9	Intranasal	C	—	+	—		4	*Idem*
10		GR-mild	7	+	+		24	None
11		C	—	+	—		4	Bronchial node
12		C	—	+	+		6	None
13	Intragastric	TD	—	—	—		4	"
14		"	—	—	—		4	"
15		"	—	+	—		4	"
16	Intravenous	G-acute	4	ND	—	8		Lung, liver, spleen, mesenteric & bronchial nodes.
17		"	7	ND	—	10		*Idem*

* G = Generalized symptoms—fever, weight loss, anorexia, lethargy, abnormal coat. R = Respiratory symptoms—cough, dyspnea, trachypnea, wheezing. C = Cough only. TD = Transient diarrhea.
† ND = Not done.

Two pregnant monkeys (Nos. 1,3) in this group died 7 days after inoculation. Positive cultures were obtained at necropsy from both animals, from various tissues including placentae but not the fetuses. Of the remaining 6 monkeys, 1 (No. 8) of 4 sacrificed at 4 months yielded a positive culture (bronchial node); 1 (No. 6) yielded positive blood cultures at 4 and 7 days, and 2 (Nos. 2,4) exhibited serologic and skin test evidence of infection. Monkeys 5 and 7 sacrificed after 6 and 24 months, respectively, showed skin test and serologic evidence of infection, but cultures were negative.

A mild generalized infection was observed in 1 (No. 10) of 4 monkeys inoculated IN, while the other 3 exhibited cough only (Table I). Positive blood cultures were obtained on 7th day in No. 10 which subsequently showed a positive skin test and serologic evidence of infection; when sacrificed at 24 months, no positive cultures were obtained. Two monkeys (Nos. 9,11) developed positive skin tests but no serologic evidence of infection; however, when sacrificed at 4 months both yielded positive cultures from the bronchial nodes. The 4th monkey in this group (No. 12) developed positive skin test and circulating antibodies but had negative cultures when sacrificed at 6 months.

The 3 monkeys inoculated IG showed no clinical evidence of infection although a mild transient diarrhea was observed during first 24 hours. All blood cultures were negative, and none exhibited demonstrable antibodies. One monkey developed a positive skin test. No positive cultures were obtained when sacrificed at 4 months.

Both monkeys inoculated IV died 8 and 10 days later after an acute fulminating disease process. Positive blood cultures were obtained from both animals ante-mortem, and *H. capsulatum* was isolated from various organs at autopsy. Neither demonstrated anti-

bodies during this short period.

Discussion. The above results indicate that monkeys (*M. mulatta*) are susceptible to infection by yeast phase *H. capsulatum,* administered intravenously, intratracheally and intranasally but not by the gastric route. The clinical course of the illness, appearance of organisms in the blood stream, development of sensitivity to histoplasmin, recovery of organisms at autopsy, and especially the character and extent of antibody response, point to the fact that infection was indeed established. DeMonbreun(14) produced infection in 2 rhesus monkeys inoculated intravenously with yeast phase organisms. Hill and Marcus(15) however, were unable to infect monkeys (*M. irus*) with *H. capsulatum.* These workers inoculated 6 monkeys intratracheally with yeast phase cells in mucin suspension and noticed no gross evidence of infection. Skin tests remained negative, and no antibodies were produced. Intracardial inoculation in 5 monkeys also failed to produce obvious infection, although 3 of 5 became sensitive to histoplasmin and all developed antibodies. Differences in results of various studies are undoubtedly referable to either differences in the strain of *H. capsulatum* used, size and nature of inoculum, or monkey species employed.

In the present study, 2 monkeys inoculated intratracheally died of fulminating disease 7 days after inoculation. The fact that both were pregnant females may have had some bearing on the severity of infection. *H. capsulatum* was recovered from various organs of both animals and from the placentae, but not from either fetus. The organisms were apparently unable to pass the placental barrier. These observations are of interest as they relate to histoplasmosis in pregnant humans.

Our study supports the belief that the respiratory tract is the most common, or likely portal of entry of the organism in spontaneous histoplasmosis. The failure to infect monkeys by introducing the inoculum directly into the stomach seems to minimize the importance of water or other ingested material in pathogenesis of this infection.

Summary. Severe disease was produced in monkeys by yeast phase *H. capsulatum* administered intravenously and intratracheally. Monkeys inoculated intranasally exhibited a more mild disease process. No significant clinical signs of illness were seen in monkeys inoculated by the gastric route.

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Received November 16, 1959. P.S.E.B.M., 1960, v103.