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Studies on Animal Transmission of Lymphogranuloma Inguinale.

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Recent researches have shown that Lymphogranuloma Inguinale as described in the continental countries, and Climatic Bubo, as observed in the tropical and subtropical regions, are identical diseases caused by a filtrable virus which can be transmitted to various animals (Findlay[1]). Although this disease has "so often been described in the United States in the past few years that it can no longer be considered as rare" (Stannus[2]), only little experimental demonstration of the causative virus has been yet brought forward (Grace and Suskind[3]). In our present report the possibility of animal transmission of local virus strains was studied. Seven cases of Climatic Bubo observed and diagnosed in the clinics of the Charity Hospital of New Orleans were used for this purpose. All the patients were colored laborers who had spent all their lives in New Orleans or its immediate vicinity, so that the possibility of an infection with a foreign virus, as it is so commonly observed among sailors, was very remote. The diagnosis of Climatic Bubo or Lymphogranuloma Inguinale was made clinically from the appearance of the inguinal buboes and was confirmed in every case by the positive intracutaneous reaction of Frei and the typical microscopic picture of the excised gland.

For the purpose of the transmission of the virus a 20 per cent emulsion of the excised lymph gland was prepared with sterile physiological saline solution and injected into various animals, except in one series of experiments in which the pus of the bubo

[1] Findlay, G. M., *Trans. Royal Soc. Trop. Med. and Hyg.*, 1933, **27**, 35.

[2] Stannus, H. S., *Trop. Dis. Bull.*, 1934, **31**, 437.

[3] Grace, A. W., and Suskind, F. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 71.

was used. The sterility of the emulsions was tested by smear and culture and in some cases the emulsion was passed through a Berkefeld filter. White mice, monkeys (*Macacus rhesus* and *Hapale penicillata*), guinea pigs, chickens and frogs were used in our experiments. The gland emulsion or the diluted pus was injected intracerebrally, intraperitoneally and subcutaneously into the inguinal region and into the prepuce. The intracerebral dosage in mice, chickens, and frogs varied between 0.01 and 0.03 cc., and in monkeys was 0.2 cc. For the subcutaneous and intraperitoneal injections correspondingly larger amounts of virus emulsion were used. The inoculated animals were kept under observation and killed at intervals of one week to one month, to secure virus for further passage.

For the passage from animal to animal a 20% brain emulsion of the infected animal proved most satisfactory. Virus transmission with the emulsion of other organs succeeded with the spleen and heart blood of the infected animals, but failed with the liver and kidney. A brief summary of our results is listed in Table I.

TABLE I.

Material	Diagnosis — Frei Reaction	Diagnosis — Biopsy	Transmitted to	Results
L20 (Bubo)	Positive	Positive	Mice, monkeys, guinea pigs	Pos. 11 passages
L21 "	"	"	Mice	" 2 "
				Neg. 3 "
L24 "	"	"	"	Pos. 3 "
L26 "	"	"	"	" 7 "
L26 (Pus)	"		Mice, monkeys	Negative
L27 (Bubo)	"	"	"	Pos. 7 "
L31 "	"	"	"	" 2 "
L32 "	"	"	"	" 2 "

In each of the 7 cases of Climatic Bubo, the virus could be transmitted into animals and, up to the time of this report, as many as 11 successful passages could be effected. The virus of case L21 lost its virulence after the third successful passage. Although the gland emulsion of L26 proved infective for mice and could be kept through 7 passages, inoculation with the pus which had been obtained by sterile puncture of the gland gave negative results.

The most reliable animal for the transmission of the virus proved to be the white mouse. Symptoms of disease begin to manifest themselves in the inoculated mice towards the end of the first week. They lose their appetite and the fur takes on a ruffled appearance. Later paralysis of the extremities, and occasionally convulsions appear, and in a large number of cases, spontaneous death occurs. The majority of the mice in our experiments, however, were killed as soon as the first cerebrospinal symptoms became manifest, since

one to 2 weeks seemed to us the optimum interval for continued passage of the virus. The meninges of the successfully inoculated animals showed at autopsy a marked injection and the brain appeared soft and edematous. In the positive cases microscopic study of the brain showed as early as one week after inoculation typical round cell infiltration of the meninges and of the perivascular spaces of the brain. A focal increase of large glial cells in the cortical zone of the brain in the vicinity of the inflammatory lesion could be observed in many instances.

The *Macacus rhesus* proved to be the less useful of the 2 species of monkeys used in our experiments. The common Marmoset (*Hapale penicillata*) developed within 1-2 weeks extensive paralysis of the extremities with convulsions, and the autopsy showed the same type of meningo-encephalitis as in the mouse brain. It was interesting to note, however, that as the rule the histological changes in the brain and in the meninges of the monkeys were less severe than in the mice, although the latter showed less marked cerebral symptoms than the monkeys.

Half the guinea pigs inoculated in the groin developed within 5 to 7 days a palpable enlargement of the inguinal lymph nodes and at autopsy showed purulent material in the gray and swollen glands. While the histological picture in many aspects resembled tuberculosis, occasional small abscesses with polymorphonuclear leucocytes were found as well as a hyperplasia of the endothelial cells.

Chickens and frogs inoculated with similar material did not show any symptoms, nor were any gross or microscopical lesions found in the brain at one week and 2 week intervals.

Successful transmission of the same strain of the virus to animals of a different species was possible at will, provided that the species was susceptible to the disease. In our laboratory the virus of L20 could be transmitted from mouse to monkey, from monkey to mouse, back again to monkey and finally from monkey to mouse without losing its virulence.

Conclusions.—The causative virus of 7 cases of Climatic Bubo has been successfully transmitted to mice, monkeys and guinea pigs. Inoculation of chickens and frogs was not successful. The white mouse and the common marmoset proved to be the most useful experimental animals for transmission of the disease. Transmission of the virus from one susceptible species to another was possible and could be repeated at will without apparent change in its virulence. From these experiments it seems reasonable to conclude that the virus of the disease commonly found in the colored population of New Orleans is identical with the virus of the disease described in tropical and continental countries.