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Rapid Multiplication of *Mycobacterium tuberculosis* in Chinese Hamsters.

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The laboratory diagnosis of tuberculosis by the usual guinea pig inoculation method is admittedly sensitive and reliable in experienced hands and at times almost indispensable. On the other hand, the method contains several defects, one of which is the length of time generally required to arrive at the desired information. If a method is available which could decrease the time factor without interfering with the sensitivity of the susceptible animal tissue it would appear to be ideal for diagnosis. Negre and Bretey[1] found that the accuracy of guinea pig inoculation could be enhanced for diagnostic purpose if the animals were subjected first to a prolonged series of injection with an acetone extract of acid-fast bacilli. Such a procedure, while admittedly useful under special conditions, does not appear to decrease the time factor. Pickof[2] reported that by injecting the suspected specimen into an area of the guinea pig skin previously filled with a suspension of Silica powder diagnosis of acid-fast bacilli could be made within 10 to 12 days. It appears that such a procedure may well be applied to the Chinese hamster, an animal which has been found to be equally if not more susceptible to the tubercle bacilli than the guinea pig.[3-6] Apart from this the initial low cost, the small cost of maintenance and the abundance of this animal in this part of the country have much to recommend their use. In the present communication we have attempted to compare the usefulness of Chinese hamsters to that of the guinea pig under experimental conditions for the rapid diagnosis of *Mycobacterium tuberculosis* and for the differentiation between human strains and possibly other acid-fast bacilli is described.

The technic described by Pickof[2] was closely followed with the exception that silica powder was replaced by kaolin (unpurified). In

1 Negre, L., and Bretey, J., *Am. Rev. Tuberc.*, 1938, **38**, 531.

2 Pickof, F. L., *Am. J. Clin. Path.*, 1939, **9**, 339.

3 Korns, J. H., and Lu, Y. C., *China Med. J.*, 1927, **41**, 234.

4 Korns, J. H., and Lu, Y. C., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 807.

5 Korns, J. H., and Lu, Y. C., *Am. Rev. Tuberc.*, 1928, **17**, 279.

6 Lu, Y. C., *Ibid.*, 1929, **20**, 938.

the first experiment it was considered of interest to compare the relative susceptibility of the 2 species of animals to tuberculosis. For this purpose 11 guinea pigs weighing about 300 g each and 15 hamsters were inoculated with a virulent human strain of *Mycobacterium tuberculosis*. The suspension of the organism was made by prolonged grinding of an accurately weighed amount of moist culture grown on Lowenstein medium for 4 weeks. Then it was suspended in saline in proportion of 1 per 1000 of saline. Serial dilutions were made from this and the dilution showing 1 bacillus per 10 fields when viewed under high magnification (970 ×) was used. The inoculum consisted of 0.1 cc of the suspension for both kinds of animals, inoculation being made on each animal, one on each lateral aspect of the trunk. It is of interest to note that inoculations could be easily made into the delicate loose skin of the hamsters. Examination of all animals were made at the end of 2 weeks. In order to obtain as much material as possible for examination animals were anesthetized, the skin was incised and the caseous material formed at the site of inoculation was smeared on glass slides. Employing such a procedure it was found that the agreement between the 2 species of animals was complete and that the acid-fast bacilli could be demonstrated in practically all the specimens although there were marked variations in the number of organisms from both species of animals. This indicates that the number of animals employed and the number of sites injected are important factors upon which a successful diagnosis of any given specimen depends. As far as this experiment goes the results indicate that Chinese hamsters are just as useful as guinea pigs for the diagnosis of tuberculosis by the Pickof technic.

Since hamsters have been demonstrated to be suitable, it was considered of interest to determine the earliest time at which positive results may be obtained. The size of the inoculum and the bacterial suspension were the same as those used above. Fifteen hamsters were used. Examinations were made on the 5th, 8th, and 11th day, 5 animals being examined each time. It was found that positive findings were obtained on the 8th day in about 50% of the specimens examined although the number of acid-fast bacilli found was significantly less than those observed in the first experiment in 2 weeks. On the 11th day the specimens from all the animals were positive for acid-fast bacilli. The results of this experiment complement those obtained in the first experiment insofar as the usefulness of hamsters for the rapid diagnosis of tuberculosis is concerned.

In the third experiment it was considered of interest to determine whether the method is applicable to other acid-fast bacilli such as

Mycob. bovis, Ravenal strain; *Mycob. tuberculosis* strain H37A, BCG, *Mycob. avium* strain 531, and *Mycob. smegmatis.* The above organisms were obtained from the Phipps Institute and represent virulent, attenuated, and saprophytic strains from various animal sources. Ten hamsters were used for each organism. Three- to 4-week-old cultures grown on appropriate solid medium of all the cultures except *Mycob. smegmatis* were used. In the case of the latter 10-day-old culture was employed. Subcultures from the original slant were made to ensure viability. Suspension and standardization of the inoculum for each organism were made in the same manner as experiment I with the exception that the dilution containing approximately 1 bacillus per every other field was used. The amount of inoculum and the technic of inoculation were essentially the same as those employed in the previous experiments. Examinations made at the end of 2 weeks revealed 2 distinct differences. On the one hand it was found that no acid-fast bacilli could be demonstrated in any of the specimens obtained from animals inoculated with BCG, *Mycob. avium* and *Mycob. smegmatis.* On the other it was found that organisms could be demonstrated in the specimen obtained from animals inoculated with *Mycob. bovis* and *Mycob. tuberculosis* H37A. In case of the former organism acid-fast bacilli were easily found in all the specimens while demonstration of the latter was more difficult. This difference in the ability of proliferation of the 2 organisms in the animal tissue may in some measure be accounted for by the greater virulence of the bovine organism. The results indicate that under the conditions of the experiment, a differential diagnosis can be made in hamsters insofar as the difference between human and bovine strains on the one hand and the avian and non-pathogenic acid-fast bacilli on the other is concerned. The application of this method to clinical materials is being studied.

Conclusion. Chinese hamsters are suitable for the rapid diagnosis of tuberculosis. Positive findings may be obtained in from 10-14 days. Non-pathogenic acid-fast bacilli gave negative results.