siderable cross fixation is not the rule. Similarly, the complement fixation results are not comparable with what is found in non-vaccinated individuals infected with murine typhus, for here again the homologous complement fixation titer is higher than the heterologous with little crossing (see Table II).

In contrast to the complement fixation results, the rickettsial agglutination usually showed a higher murine titer. However, as in the complement fixation results cross agglutination is marked and in many instances there is only a 2-fold difference in titer. The same type antibody response was observed in the cases reported irrespective of whether the epidemic typhus vaccine was administered two years or one week before the present attack of murine typhus. Insofar as no such reaction has been observed in vaccinated subjects who subsequently became infected with atypical pneumonia, measles, infectious hepatitis, smallpox, pneumonia, meningitis, or other virus, bacterial, protozoal infections, it is our belief that the unusual serological findings reported are to be attributed to the previous immunization with rickettsial products followed by an infection with murine rickettsiæ.

It is of interest to observe, as far as can be judged from the description of the symptoms and course of the disease, that the previous administration of the epidemic typhus vaccine had little or no effect upon the severity of the recent attack of murine typhus.

Summary. Twelve cases of murine typhus occurring in individuals who previously received epidemic typhus vaccine are reported. It appears that the previous administration of the epidemic typhus vaccine did not affect the course or severity of the murine disease. The serologic response was different from that found in non-vaccinated cases of epidemic or murine typhus. The Weil-Felix reaction does not seem to be affected, for a rise in titer or high titers were obtained in a majority of the cases. The difference occurred in the specific antibody response. While the epidemic complement fixation titers were higher than the murine titers, a number of specimens showed equal titers or considerable cross fixation. The murine agglutination titers were usually higher than the epidemic titer with considerable cross agglutination. This type of reaction has not been found in individuals who had received epidemic typhus vaccine and who subsequently became infected with bacterial, viral, or protozoal infections. Since many of our soldiers who have received epidemic typhus vaccine may subsequently develop murine typhus either abroad or after return to this country, it is obvious that we may expect to have a considerable number of future cases presenting the serological pattern described in this paper.

15047

Use of Vaginal Smears in Mating the Golden Hamster.

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Various procedures have been adopted in different laboratories for breeding the golden hamster, *Cricetus* (*Mesocricetus*) *auratus* Waterhouse. The general procedure involves placing the male with one or more females for varying lengths of time, then removing the male and watching for signs of pregnancy. The male may be housed with the female during 2 or 3 estrous cycles,[1] or for 7 or 8 days,[2] or the male may be placed in the cage with several females after 6:30 p.m. and, if mating activity is observed, the reacting animals are left together over night.[3] If no mating activity

* Contribution No. 73.

[1] Peczenik, O., *J. Endocrin.*, 1942, **3**, 157.

[2] Sheehan, J. F., and Bruner, J. A., *Turtox News*, 1945, **23**, 65.

[3] Bond, C. R., *Physiol. Zool.*, 1945, **18**, 52.

TABLE I.
Pregnancy Record of Golden Hamsters Caged Together in Proestrus or Estrus and for 48 Hours Thereafter.

No. of the mating	Total matings	Pregnancies		Matings per pregnancy
		No.	% pregnancies	
1st	72	39	54.2	1.85
2nd	18	8	44.4	2.25
3rd	6	2	33.3	3.00
4th	2	0	0.0	—
Total	98	49	50.0	2.00

occurs it is assumed that the female is not in heat and the male is removed immediately. These methods are designed primarily to minimize the time the male and female are caged together, thus keeping at a minimum injuries due to unsocial behavior. The methods are predicated on the probability that during the time of cohabitation the female will pass through at least one period of estrus during which fertilization might occur.

The purpose of the present paper is to report a simple and effective procedure for mating the golden hamster at the time the female is known to be approaching ovulation. The procedure depends on the inspection of vaginal smears from the females chosen for breeding.

When, at any time, it is desired to increase the size of our colony, vaginal smears are made from a sufficient number of the females in our breeding colony to produce the required number of litters. Samples of the vaginal contents are obtained with a sterile platinum loop previously moistened with physiological saline and introduced high in the vaginal lumen. The loop is then passed through a small drop of saline on a microscope slide and the smear is examined unstained. A male is then placed in the cage with each of those females revealed, by inspection of the smears, to be in proestrus or estrus. The animal is presumed to be in proestrus when the smear reveals a predominant number of large squamous cells the nuclei of which are not visible and when, in addition, typical high power fields (X440) exhibit not more than 2 leucocytes. A few oval epithelial cells with prominent nuclei may also be present. The animal is considered to be in estrus when field after field exhibits no leucocytes and when squamous cells alone are present. Illustrations of typical proestrous and estrous smears may be found in an earlier paper.[4] The male is withdrawn from the cage of the female 48 hours after being placed therein. No observation of the cohabiting couples is necessary. The date of introduction of the male is recorded and the cage containing the female is inspected at the time delivery is due. The earliest likely date of delivery will be the 14th day after the male is placed with the female. The latest likely delivery date will be 18 days after the male is placed with the female, *i. e.*, 16 days after the male is removed. Kupperman, Greenblatt, and Hair[5] have shown that in their colony 5% of the cases deliver on the 14th day, 85% on the 15th day, and 11% on the 16th day after the observation of spermatozoa.

Ninety-eight matings were made according to this procedure in all months of the year (Table I). "First mating" is herein defined as the first time that a male is introduced into the cage of a virgin female, or the first time a mating is attempted with a given female after a pregnancy. "Second mating" refers to the second attempt to obtain a pregnancy in a given female which has had no previous litter, or the second attempt to obtain a pregnancy after the previous delivery. From the table it will be noted that first matings resulted in pregnancies in 54.2% of the cases, while 50% of all matings resulted in pregnancies. We have found it uneconomical to main-

[4] Kent, G. C., and Smith, R. A., *Anat. Rec.*, 1945, **92**, in press.

[5] Kupperman, H. S., Greenblatt, R. B., and Hair, L. Q., Abstract, *Anat. Rec.*, 1944, **88**, 441.

tain the female in the breeding colony after the third unsuccessful mating.

The procedure herein outlined has several advantages over other methods: 1. The number of injuries sustained by either member of a pair as a result of unsocial behavior is reduced to a minimum. 2. The procedure may be employed to produce litters at the approximate future date and in the approximate number required. 3. The date of delivery may be predicted within a few days. 4. The procedure may be carried out at any convenient hour of the night or day and it is unnecessary to watch for mating responses or to further observe the animals until the time of parturition draws near.

Peczenik[1] states that the female may be recognized as being in heat when a thick mucus may be squeezed from the vagina. This is not a reliable criterion for determining the proper time for mating among our animals. The animals are still receptive when this thick mucus smear first occurs high in the vagina. At this time the animal is in metestrus, a phase characterized by the presence almost exclusively of elongated squamous cells in abundance and occupying from 4 to 8 hours only. This particular smear picture has not been widely observed. Peczenik states that at this time the animal has already ovulated. It may be that these few hours are the precise time when matings prove fertile. Kupperman, Greenblatt, and Hair[5] state that in their colony mating occurs without exception at this particular time ("in the transitional period between the cornified cell stage and the thick, dense smear of nucleated and cornified cells"). Nevertheless thick mucus may be squeezed from the vagina during early diestrus as well, when matings will not prove fertile. To test the criterion of thick mucus discharge as indicative of the proper time for mating we attempted 18 matings at the time of the cycle when the thick mucus is first evident. The males were left with the females for 48 hours and then withdrawn. We obtained one litter only by this method. In the latter case the vaginal smear of the female indicated estrus on the date the male was removed.

Enders[6] has successfully employed the microscope as an adjunct to breeding mink. We have found the method herein described most efficacious in maintaining the size of our hamster colony, and providing litters on the approximate date when they are required while at the same time eliminating animal injury which often results from unsocial behavior.

Summary. In the experiments herein reported vaginal smears have been utilized in determining the proper time for mating golden hamsters. A male hamster is placed in the cage with each female hamster which is found to be in either proestrus or estrus upon inspection of its vaginal smear. The male is caged with the female for 48 hours and is then removed. Injury due to unsocial behavior of the animals is minimized by this procedure. Forty-nine pregnancies were secured from a total of 98 matings in the present experiments. One pregnancy in 2 matings is considered to be a satisfactory conception rate for this animal under laboratory conditions.

[6] Enders, R. K., *Am. Fur Breeder*, 1939, **11**, 6.