tioned that this greater yield would also be expected on the hypothesis that SF is required solely for "extrinsic thromboplastin." The work of Flynn and Coon(1) suggests that this hypothesis is correct. These workers sedimented "extrinsic thromboplastin" by centrifugation and then washed it by suspension in saline and recentrifugation; they showed that this washed material was active in converting purified prothrombin to thrombin in presence of calcium. The method of preparation of prothrombin was such that it is unlikely to have contained SF. However, this evidence is not entirely conclusive for some free SF not utilized in the initial formation of "extrinsic thromboplastin" might have been adsorbed onto this complex.

When factor VII is excluded from both incubation mixture and substrate clotting tubes in thromboplastin generation test, normal yield is obtained, and there is also normal rate of evolution. Therefore, the finding that absence of factor VII from incubation mixture and substrate tubes affects rate of "extrinsic thromboplastin" formation although having little or no effect on yield is interesting. SF is essential for "intrinsic thromboplastin" generation, primarily affecting yield, so that finding that this factor also primarily affects yield of "extrinsic thromboplastin" was not surprising.

Summary. Previous work showing that factor V, serum, brain and calcium react together to form an active prothrombin conversion factor ("extrinsic thromboplastin") is confirmed. The active components in serum in respect of this reaction include both factor VII and SF but not PTC. SF appears to influence yield while factor VII primarily determines rate.

ADDENDUM. Although SF appears to affect yield rather than rate of formation of both "extrinsic" and "intrinsic" thromboplastin formation, work of Fisch and Duckert (*Thromb. Diath. Haem.*, 1959, v3, 98) appearing since this paper was submitted indicates that SF acts as an enzyme in "intrinsic thromboplastin" formation.

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An Attempt to Recover WEE from Nasal Mites of Sparrows. (24857)

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Bird mites collected from various species of birds harbor the virus of Western Equine Encephalitis (WEE)(1,2,3), and some investigators have shown that transmission of St. Louis encephalitis virus occurs when infected mites were allowed to feed on susceptible chickens(4,5). Inasmuch as no experiments on nasal mites were reported in the literature an attempt was made to recover WEE virus from inoculated sparrows and nasal mites harvested from them.

Materials and methods. Adult English sparrows of both sexes were captured from roosting places at night when flight activity was at a minimum. These birds were banded and bled from the heart for pre-inoculation neutralization test (reference sera); inoculated subcutaneously with 0.1 ml of WEE virus containing 140 chick LD_{50}; and turned loose in screened flight cage approximately 8′ x 12′ x 6′. The WEE virus (3742-c2) was isolated from a pool of *Culex tarsalis* mosqui-

toes collected in July 1954 at Greeley, Colo., and had been passed 2 times in day-old chicks. Forty-eight hours after inoculation, the birds were again bled from heart to establish whether or not WEE virus was in the circulating blood. Some sparrows did not survive this second bleeding so nasal mites were harvested from nasal pasages for inoculation into susceptible chicks. Wet chicks were used as indicator of presence of virus and also in neutralization test as described by Chamberlain *et al.*(6). Twenty days after inoculation, sex determination was made after they were bled from heart. These blood samples were tested for presence of virus and neutralizing antibodies. Nasal mites of genera *Ptilonyssus* and *Speleognathus* were collected and pooled for inoculation into "wet" chicks. Mites were prepared for inoculation by grinding with pestle in mortar with a small amount of sterile alumdum and 5 ml of a 10% normal buffered horse serum. This material was centrifuged at 1500 x g and the supernate removed for inoculation. Because of limited amount of blood obtained on first bleeding, sera were screened at one dilution only (against 12 chick LD_{50}). Following exsanguination at 20 days postinoculation, a screening was made at 2 levels, 150 chick LD_{50} and 1650 LD_{50}, again because of limited amount of sera.

Results. There was a total of 489 *Ptilonyssus* mites and 245 *Speleognathus* mites recovered from nasal passages of 44 sparrows. Although 40 of 44 had circulating WEE virus in the blood, none passed into the gut of mites at detectable levels using a very sensitive test animal (wet chicks). Those birds which did not have viremia had preinoculation neutralizing indices of greater than 12 with one exception, and this bird had both virus and antibodies. All inoculated birds tested, *i.e.*, 36 of 44, developed neutralizing indices of greater than 1650 after 20 days with one exception and this was greater than 150 but less than 1650.

Sex distribution of 30 females and 13 males, with one sex unrecorded at autopsy, did not demonstrate special susceptibility or resistance to either mites or WEE virus even though 3 males of the 13 had no mites and only 1 of 30 female sparrows was free of mites.

Discussion. Nasal mites which feed from epithelium of mucous membranes of nasal passages of sparrows probably are not vectors or reservoirs of WEE virus. The experiment described here adds evidence to agree with the conclusions of Reeves *et al.*(7), Chamberlain and Sikes(8) and Sulkin *et al.*(9) that ectoparasitic mites are not important reservoirs or vectors of encephalitis viruses.

There appeared to be excellent correlation between presence of WEE antibodies and failure to find virus after inoculation. It is unfortunate that precise levels could not be determined on the small amount of sera from each bird. As might be expected, those birds which were successfully infected developed antibodies. Quite by chance one bird was found to have both antibodies and virus present.

If it were possible to remove these mites and infect them by direct inoculation into the gut and return them to an unparasitized bird host, perhaps more information might be gathered.

Summary and conclusion. 1. Twenty days after 44 English sparrows were each inoculated with 140 chick LD_{50} amount of WEE virus, the collected nasal mites were devoid of virus using Chamberlain's "wet" chick method of isolation. 2. Nasal mites of the English sparrow are probably not important vectors or reservoirs of Western encephalitis virus.

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