and food source in early spring; *C. thoracicus* began activity after males of the other 2 species had disappeared, although females were present. Attracted insect that flew upwind toward the pheromone were netted for identification. For pheromone analysis, bees collected at nesting sites were cooled over ice in the field. Whole heads or excised mandibular glands of at least 10 bees per test were extracted with methylene chloride. The extracts were analyzed on an LKB 9000 gas chromatograph-mass spectrometer utilizing an SE 30 capillary column. The first component, eluting at 140 °C, had a characteristic mass spectrum (MS) corresponding to a monoterpene alcohol with a molecular ion at m/z 154 and a base peak at m/z 71, and was identified as linalool (3,7-dimethyl-1,6-octadiene-3-ol). This component was identical to authentic linalool in its MS and GC retention time. 2 other components, eluting at 155 °C were identified as both isomers of citral, neral (3,7-dimethyl-2 trans-octadiene-1-al) and geranial (3,7-dimethyl-2-cis-octadiene-1-al) based on their MS and comparison with authentic compounds. These components were identified in both males and females of the 3 species in an approximate ratio of 3:1:1 (linalool:neral:geranial).

Field tests utilizing synthetic compounds were conducted at an aggregation of 100,000 nests of *C. thoracicus*. Assays with individual components and blends revealed these bees were attracted *only* to the mixture of the 3 components at the ratio found in their mandibular glands; citral or linalool alone were not attractive.

Neral and geranial have been previously reported in secretions of various bees. In *Trigona* it serves as trail and alarm pheromone[3]. In honey bees, it is produced by the Nassanoff gland and attracts foragers[15]. In *Andrena* spp., it is produced by the mandibular glands and may assist aggregation[7]. It is also reported in the colletid bee, *Hylaeus* (=Prosopis) but biological activity was not demonstrated[16,17]. Linalool, however, has not yet been reported from any Hymenoptera and constitutes a novel component of mandibular gland secretion. In the bark beetle, *Ips pini*, accompanied by cis-verbenol and ipsdienol, it serves as an aggregation pheromone[18]. The *Colletes* mandibular gland pheromone is released when the mandibles are used, as in pollen collection or nest excavation. Thus it may serve to assist feeding and nest aggregations, which are often seen in nature.

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Isolation of pathogenic treponemes from hare

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Summary. A presumably new species of pathogenic treponemes was isolated from a lesion on the male organs of hare (*Lepus* sp.) and the scrotum of a rabbit (*Oryctolagus* sp.) was infected with this treponeme. Lesion developed on the scrotum after a 110-day incubation.

Monographs on the infectious diseases of wild animals deny the occurrence of natural treponematosis in the hare (*Lepus* sp.)[1,2]. Mention was made of hare syphilis without any epidemiological proof[3]. Jaksits reported spiral organism (*Spirocheta pallida* subgenus *treponema*) in china ink preparations from lesions of the hare[4]. We have demonstrated serological reactivity in the hare[5] with the antigenes used for human serological tests for syphilis[6]. Having found the antibodies[7], we looked for the antigen and we succeded to demonstrate treponemes in the skin lesions of the scrotum of hares with dark field microscope.

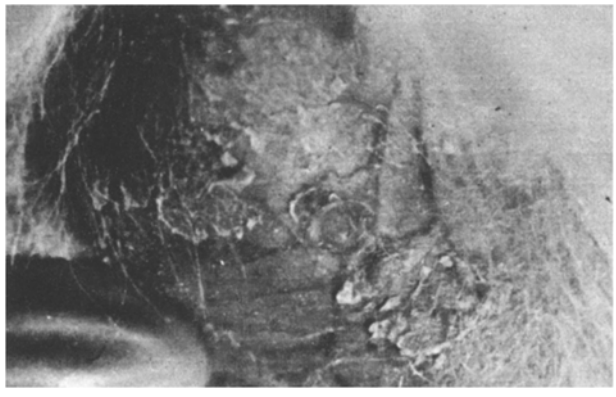
In the present paper, we describe the skin symptoms of hare treponematosis and the successful experimental infection of rabbits with its pathogen.

Material and methods. About 1400 trapped hares were examined and in 15 cases a superficial ulceration or haemorrhagic crust without induration on the surface of genitals was found. Only 5 of them were positive on dark field microscopic examination. These were isolated. Their genital lesions were cleaned mechanically and washed with sterile saline. Then the cleaned surface was scarified and the juice was collected in a syringe and injected into the scrotum of seronegative New Zealand white rabbits. The challenged rabbits were isolated and the scrotum was controlled every 3rd day. The proof of treponeme infection was given by dark field microscopic examination.

Results and discussion. In 1 case, superficial lesions of about 5 cm diameter developed after 110 days on the surface of scrotum of the rabbit around the prick of the injection needle (figure). Removing the crust a great number of treponemes was demonstrated in dark field microscope. From the juice obtained by punction of the testes, treponemes could not be demonstrated.

The morphology of the treponemes isolated from hares and from the challenged rabbits was compared to that of *Treponema pallidum*. The treponemes from the lesion are

somewhat longer (17–20 μm) with the same curve and characteristic motility as *T. pallidum*. In the serous discharge obtained from the eroded lesion, the number of microbes was about 9×10^7 per ml. The pathological phenomenon in the hares was highly hyperaemic genitals. Some of them had scars and skin lesion. Induration was established neither on the natural infected hare nor on challenged rabbits. The isolation of treponemes from genital lesions of hares and the positivity of their serological test for syphilis speak in favour of a natural treponematosis of the hare.



Lesion developed on the scrotum of a rabbit, after a 110-day period of incubation. Challenge was carried out with the suspension of treponemes isolated from the genitals of a hare *(Lepus sp.)*.

In no far as hunters have never been infected, and treponematosis has never been described on the hands of hunters, it is questionable whether this treponeme is pathogenic for the human. As the serum of hare immobilized treponemes in Nelson (TPI) test an antigenic relationship may exist between *T. pallidum* and treponemes of hares. We must consider the long incubation period after experimental infection of the rabbits and the lack of sexual intercourse between rabbits and hares. It may be presumed that this treponeme detected in the hare is not identical with *Treponema coniculi*, or it may be a variant adapted to the hare. If the future examination gives proof that the isolated treponeme is a new species, the nomination *Treponema leporis* could be suggested, because it was isolated from *Lepus sp.*

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On the inhibitory effect of 2-amino-4,6-dichloropyrimidine on growth of Vaccinia virus[1]

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Summary. 2-Amino-4,6-dichloropyrimidine prevents maturation of Vaccinia virus. Proteins synthesized in the presence of the drug are not assembled into virions.

Several dichlorinated pyrimidines have been found to be endowed with a suppressive effect on the growth of Polio I, Coxsackie B_1, Vaccinia and Herpes simplex 1 virus, while being inactive on Newcastle disease, Vesicular stomatitis and Encephalomyocarditis virus[2]. It has also been demonstrated that the inhibitory effect of dichloropyrimidines on Polio virus is due to irreversible impairment of the ability of structural virus proteins to assemble complete particles[3]. Preliminary data emerging from ongoing research indicate that the growth of Vaccinia virus is inhibited by a similar mechanism.

Material and methods. 2-Amino-4,6-dichloropyrimidine (ADCP) was furnished by Fluka, Nonidet P40 by BDH, ^{3}H thymidine (21 Ci/mmole) and ^{14}C leucine (280 mCi/mmole) by Amersham. 5-Fluoro-2′-deoxyuridine (FUdR, by Fluka) and Cycloheximide (Calbiochem) were used as reference inhibitors of DNA and protein synthesis, respectively. Vaccinia virus was kindly provided by NIH; human aneuploid HEp 2 cells were furnished by ATCC (Rockville). Experiments were carried out on 16-h-old cell monolayers (2×10^6 or 3×10^7 cells per sample, according to the experiment), which were infected with 20 infectious units (IU) per cell at 20 °C for 1 h. Cells were then washed 3 times with Hank's balanced saline solution (BSS, pH 7.3) and incubated at 37 °C in Eagle's minimum essential medium (MEM) supplemented with 2% calf serum (pH 7.3). ADCP was added to the medium at 100 μg/ml, corresponding to $\frac{2}{3}$ of the maximum non-cytotoxic dose (MNCTD)[2].

Infectious virus yield was determined by plaque assay[4], starting from whole cultures (2×10^6 cells/sample) which were frozen and thawed 3 times (−70 °C and +20 °C) and freed of cell debris at 3000 rpm for 5 min. In order to obtain primary virus plaques easily detectable at low magnification (×50), infected subcultures were incubated in liquid Eagle's MEM for 30 h at 37 °C before addition of the same medium solidified with 1% agar (Noble, Difco). Plaques were counted 16 h later. This method was found to have an error of about ±30%.

Synthesis of virus DNA and proteins, as well as their incorporation into complete particles, were followed in cells (3×10^7/sample) infected and incubated as above, which were labelled with ^{3}H thymidine (2 μCi/ml) and ^{14}C leucine (0.5 μCi/ml). Cells were detached from the glass by 0.25% trypsin (Difco 1:250) in Hank's BSS, suspended in the same buffer containing 2% calf serum and centrifuged at 1000 rpm for 5 min. Pellets were then resuspended in 0.5 ml of sodium phosphate buffer (1 mmole, pH 7.4) containing 0.75% Nonidet P40 and incubated in an ice-bath for 15 min, with 3 intermittent strokes (5 sec each) in Vortex. Nuclei were then pelletted at 1000 rpm for 5 min and discarded. 0.05 ml of the cytoplasmic extracts thus obtained, precipitated with 10% trichloroacetic acid (TCA) at 4 °C for 10 min and redissolved in Toluene (0.5 ml,