ABSTRACTS

RECOVERY OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS FROM WILD BIRD MITES. The western equine encephalomyelitis virus has recently been isolated from mites in the nests of the yellow-headed blackbird and the English sparrow. From a deserted nest of a yellow-headed blackbird approximately 1,000 mites were collected. These were divided into 4 groups before being ground up and suspended in 30 percent serum broth. After centrifugation the supernatant liquid was used to inoculate mice by the combined intracerebral and intraperitoneal routes. These animals showed convulsions and other signs of encephalitis in 3 to 4 days. The brains of these animals were bacteriologically sterile, so after 3 serial passages identification was undertaken. The material was then demonstrated to be pathogenic for hamsters and guinea pigs. By challenge inoculation of Western equine encephalomyelitis immune guinea pigs and by neutralization tests in mice with specific Western strain antisera, 3 of the 4 suspensions were definitely identified as the Western equine encephalomyelitis virus. Similarly, a collection of mites from the nest of an English sparrow was divided into 2 groups and so treated. Here the virus was isolated from 1 of the groups.


RIBOFLAVIN DEFICIENCY IN THE DAIRY CALF. Evidence indicates that the adult ruminant does not require the vitamin B complex in its diet, due to extensive synthesis of the vitamin in the rumen. However, little experimental work has been done to establish which of the vitamin B complex would be needed if rumen synthesis did not take place. In order to study this problem, young ruminants, in which the rumens were not yet functional, were used for experimental study.

The experimental animals were 2 male Guernsey calves and 1 male Holstein calf. The experiment was started when the calves were 48 hours old, and had been receiving colostrum. The animals were fed on an experimental diet which consisted of casein, lard, wheat germ oil, salts and the essential vitamins with the exception of riboflavin. After the calves had been on the diet for 2 weeks, the gingival surface of the cheeks and the mucosa of the tongue became hyperemic, and the gums bled upon the application of slight pressure. Lesions were observed at the oral canthi and along the edges of the lips. As the experiment continued, the condition of the calves become worse. The salivary secretion became copious, tenacious, chalky-colored, and excessive in amount. In addition, excessive lachrymation was a characteristic symptom. The gains in weight were irregular, the hair appeared rough and dull and the animals...
shed excessively. The calves scoured and the navel region became irritated and inflamed.

After 5 weeks on the diet, 1 of the experimental animals was given 5 mg. of riboflavin by intravenous injection. On the following day, the animal’s appetite increased and salivation decreased. This improvement was only transitory, however, as the animal showed anorexia and increased salivation the second day after injection. The condition of the animal became critical, so the diet was supplemented with 5 mg. of riboflavin per day. Within 3 days after supplementation, the anorexia disappeared, hyperemia of the buccal mucosa disappeared, excessive salivation stopped, and the animal started to gain weight. Ten days later the lesions in the corner of the mouth, along the edges of the lips, and around the navel had healed. New hair began to grow in those areas from which hair had been lost.

The other 2 animals were kept on the experimental diet for 6 weeks. When riboflavin was added to the diet, the response was the same as in the first calf.

During the course of the experiment, examinations of the eye were made with an ophthalmoscope. Neither vascularity of the cornea nor opacity of the lens was noted.

Weekly urine samples were taken and tested for the presence of riboflavin. During the first week, after the animals had been on a diet of colostrum, the amount of riboflavin was high. This decreased sharply until the third week, when no riboflavin was synthesized in the animal’s body. After riboflavin was added to the diet, the animal excreted only a portion of it, giving further evidence that riboflavin is needed by the animal.

The animals were sacrificed at the close of the experiment, but post-mortem examinations failed to reveal any internal gross lesions. Sections from the brachial and sciatic nerves, stained with hematoxylin and eosin stain, showed no microscopic lesions.
Results of the experiment indicate the bovine species requires riboflavin, supplied either in the diet or by rumen or intestinal synthesis.


**Infection of the Bovine Udder with Yeastlike Fungi.**

Ten instances of infection of the bovine udder by yeast-like fungi have been observed during a 6 year period in a herd of approximately 120 purebred Guernsey and Holstein-Friesian cows. The causative organisms from 3 cases were studied in detail and were found to clearly belong to the genus Trichosporon of the family Mycotoruloideae.

In 6 of the observed 10 cases clinical onset of mastitis was evidenced by either a swelling of the gland or a macroscopic abnormality of the secretion, while in the remaining 4 cases the presence of fungi was observed in samples of milk prior to any evidence of clinical mastitis. Macroscopic abnormality of the secretion lasted from 3 to 8 days, whereas fungi, which reached a maximum count of 137,000 per cc. were present for 9 to 48 days. Artificial infection of a cow that was in the seventh month of lactation and free of udder infection was successfully carried out. Fungi were present in the milk as early as the second day following inoculation, and clinical symptoms were in evidence on the sixth day.

That the organism isolated from mastitis is a species of Trichosporon is evidenced by their formation of mycelia, budding cells, and oidia. The physiological activity and a consideration of the animal and pathological source of the organisms indicate that they probably represent a new species.


**Mechanism of the Invasiveness of Cancer.**

An attempt has been made to explain the invasiveness of malignant tumors, which have the ability to infiltrate adjacent tissues and become locally disseminated, in contrast to benign tumors which remain restricted to their site of origin. It was thought that physical and chemical differences must exist between benign and malignant neoplastic cells that permit the former to remain localized and the later to permeate adjacent normal tissues.

Under the assumption that these physical and chemical differences could be demonstrated experimentally, a series of investigations were made. The first experiment was concerned with demonstrating the adhesiveness of cells. This was determined by measuring the bend in a previously calibrated microneedle when the needle was subjected to the strain of detaching one cell from another. It was found that the mean force required to separate 50 pairs of normal squamous epithelial cells was 1.42 mg. Similarly, the value of adhesiveness of 50 pairs of cells from skin papillomata was 1.25 mg. The mean force required to separate 50 pairs of cells from squamous cell cancers of the lip was only 0.47 mg.

This physical difference affords the first requisite for an understanding of invasiveness. If cells are feebly attached to one another, facilitating complete separation, such cells are free to wander into adjacent tissues by ameboid movement.

Attempts were made to find a chemical explanation of this reduced adhesiveness. Normal epithelial cells were subjected to various alterations in the chemical composition of the medium in which they were immersed. In this way, it was shown that an absence of calcium from the media caused a reduction in the adhesiveness of the cells. Other investigations have revealed that cancerous tissue is abnormally low in calcium.

The ameboid movement of cancer cells was then studied. After the chemical basis for the separation of cancer cells from each other had been found, the only additional requisite is the ability to move. Epithelial cells from human carcinomas...
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were observed in tissue culture. It was found that cells frequently became detached from outgrowing sheets of epithelium and these cells were actively ameboid. These cells progressed some distance from the parent cluster of cells and, by their proliferation, built up new colonies of cells.

A third characteristic investigated was the role of spreading factors in the malignancy of tumors. Not only is invasion of surrounding tissues facilitated by ameboid movement of tumor cells, but also by a substance which is secreted by the tumor that makes the surrounding tissues more permeable to the tumor cells. These substances, such as hyaluronidase, are known as spreading factors.

Experiments to test this hypothesis were of two kinds. First, human tumors were analyzed to determine whether they contained greater amounts of these spreading factors than did normal tissue. Extracts were made from a variety of normal and tumor tissues by grinding, freezing, and thawing the tissue and extracting it with sodium acetate. The extract was then assayed, but the results were inconclusive. Some of the malignant tumors contained spreading factors, but in most instances, the amount was not great, and in some it was lacking. However, significant amounts of spreading factors were obtained from tumors in some instances, so that support is lent to the hypothesis that spreading factors may facilitate the invasion of cancer cells.

In the second set of experiments, hyaluronidase was injected into transplantable sarcomas in mice and into virus-induced papillomata in rabbits, in an attempt to increase the invasiveness of these tumors. Results in these experiments were again inconclusive. The negative results force the conclusion that spreading factors of the hyaluronidase type, though they may be found in malignant tumors, are not essential to invasiveness.

Of the 3 factors concerned in the invasiveness of tumor cells, the first 2—decreased adhesiveness and ameboid movement—are of greater importance for invasive growth. It is interesting to note the most invasive of the normal cells—the macrophages, polymorphonuclear leucocytes, and lymphocytes—all detached cells, rarely show any evidence of adhesiveness and all possess great ameboid activity. The cancer cell possesses these same attributes, coupled with its capacity for unlimited proliferation.


**Nutritional Significance of Inositol and Biotin for the Pig.** Biotin and inositol are concerned with food utilization in pigs, since a lack of these 2 vitamins results in smaller gains with a normal amount of food consumption. Pigs fed on a closely controlled basal ration, which contained the vitamins thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, and choline demonstrated no beneficial effect on growth or food utilization when either inositol or biotin was added.

When sulfathaladine, which is very effective in decreasing intestinal synthesis of vitamins, was added to the ration for 6 weeks, a 40 percent decrease in daily growth resulted and a syndrome of deficiency symptoms, such as hair loss, sore feet, and a dark brown exudate on the skin, was observed. The addition of biotin to the basal ration plus sulfathaladine prevented the syndrome described above and increased the growth by 57 percent.

Similarly, the administration of inositol to a lot of pigs, fed the basal ration plus sulfathaladine, alleviated most of the deficiency symptoms that were described above. A possible explanation of the ability of inositol to alleviate biotin deficiency may be found in the stimulating ability of inositol on certain organisms in the intestinal tract which synthesize biotin.

Although no beneficial results are obtained from supplemental feeding of biotin or inositol under conditions of normal intestinal vitamin synthesis, they are 2 factors concerned with the prevention of deficiency symptoms and with the efficiency of food utilization by the pig.