teriid mastigophoran 14 μ long with four flagella, from Burley Lagoon, near Gig Harbor, Washington; and (4) an unidentified brown mastigophoran 8–10 μ long with two long flagella, from Rosedale, Washington.

On February 28, 1946, seven 5-cc. samples, taken in a random manner from each of the cultures, were removed to 1-dram shell vials for experimentation. The cultures filled the vials to a depth of about 38 mm. Two of each series, the first and the last removed, were used as controls. The other 5 vials were subjected at the rate of 132 r/minute to doses in roentgens of 1,000, 10,000, 25,000, 50,000, and 100,000. X-rays were produced at 200 kv. and 20 Ma. with filters of 1 mm. of aluminum and 0.5 mm. of copper. The target distance was 13 inches as measured to the middle of the depth of the culture in the vials. The vials were arranged in a cluster of 4-inch maximum diameter. On March 1 a second series of cultures was also exposed to the same amount of radiation, but in order to shorten the time of exposure the rate was increased to 530 r/minute by halving the target distance to 6.5 inches.

The number of swimming brown flagellates was determined by the use of blood-counting chambers immediately after the removal of the last vial from the X-ray machine. The dose that was immobilizing to 50 per cent of these organisms within 24 hours appeared to be about 50,000 r, while 100,000 r was almost 100 per cent effective. Viability was not readily ascertained in the other species. After irradiation the cultures were transferred to larger vials of 6-dram capacity, and 12 days later were subdivided, about two-thirds of each being removed to an 8-ounce jar. Several days later, when the culture had evaporated to about half of its original volume, the loss was compensated by adding sea water containing 0.1 per cent of milk. Population densities in the cultures were determined at irregular intervals by counting the number of organisms per unit volume of culture, using ruled blood-counting chambers.

TABLE 1

SUMMARY OF FIRST MONTH'S COUNTS EXPRESSED AS PERCENTAGE OF HIGHEST COUNT

Dose in r	*Chlorella*	*Nitzschia closterium*	Carteriid	Brown mastigophoran
0	100	100	80	29
0	83	72	100	100
1,000	94	67	67	66
10,000	75	34	61	37
25,000	58	20	46	0
50,000	43	12	14	0
100,000	23	8	81	0

The results shown are based on counts made only during the first month of the experiment. For the first 45 days there was general agreement in the effect upon all four organisms. Later tendencies of the counts extending to 5 months were erratic or obscure, possibly because of contamination of cultures. The concentrations of each organism at the various dosage levels are compared in Table 1. To arrive at these figures for a species, the counts in each irradiation group were averaged and then expressed as percentage of the highest average count for that species. The carteriid counts at the 100,000-r level were high and suggest faulty technique, but other counts were in general inversely related to amount of exposure above 1,000 r. The counts of cultures exposed to 50,000 r and 100,000 r were low. Counts of cultures exposed to 1,000 r were not unlike the controls in that in 9 cases these were greater than, and in 10 cases less than, the controls. On this same basis of 19 counts for each culture the times that the 10,000 r treatment exceeded the control were twice; for 25,000-r, once; for 50,000 r, none; and for 100,000 r, once.

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The Contagious Nature of a Lymphoid Tumor in Chickens

NELSON F. WATERS

U. S. Regional Poultry Research Laboratory, East Lansing, Michigan

Nearly all treatises on tumors stress the point that little foundation exists in the theory that a tumor, or any agent responsible for a tumor, is communicable in nature. Arrayed against this theory of noninfection is the ever-increasing amount of data suggesting a virus etiology for certain tumors. The specificity of certain of the viruses and the indisputable evidence of infection, direct or indirect, that is associated with virus diseases, lead one to continue the search for a virus or virus-like agent which causes tumors and which is transmissible by contact with infected animals. Again, the knowledge which has been gained in an overall study of other diseases leads one to believe that, in the genesis of specific types of tumors, some orderly pathogenic stimulus is necessary to incite, directly or indirectly, neoplastic growth. Likewise, the myriad of seemingly spontaneous tumors occurring in the animal kingdom exists because certain necessary requirements of host-pathogen-environment relationship have been fulfilled.

In strains of mice susceptible to pulmonary tumors, it is not unusual to find that approximately 90 per cent of the animals at 18 months of age show "spontaneous" tumors (*3*). A description of these indicates such a similar host reaction that it is difficult to believe they were not activated by a common agent and that this agent, through some as yet unexplained manner, can find a portal of entry from one animal to another. Bittner (*2*), Andervont (*1*), and others have shown that mammary adenocarcinomas in mice were due primarily to the relationship between the genetic influence, the hormonal influence, and the milk influence in certain individuals. Discovery of an agent responsible for mammary tumors in mice was the result of carefully designed objective experimentation which included the development of host animals of similar genetic potentialities.

Among the important factors working against any experiment attempting to prove the communicable nature of agents responsible for tumors are the following: (1) If a tumor agent were transmitted by contact from one animal to another, the length of time which elapses before a tumor is grossly visible is generally too great to ascertain that such contact had any relationship to the tumor eventually formed. (2) It is almost impossible to be sure that the experimental animals are actually free of any tumor agent prior to the time of exposure. (3) There are not available enough experimental inbred animals which

are uniformly free of, but susceptible to, the tumor agent studied. Until these and many other variables are controlled, the chances of proving contact transmission are slight indeed.

Research at the U. S. Regional Poultry Research Laboratory, carried on in active collaboration with a number of experiment stations within the region, is involved in an attempt to control lymphomatosis in the domestic fowl. Lymphomatosis is a naturally-occurring malignant disease of chickens characterized by the formation of lymphoid tumors which may be found in nearly all tissues of the bird's body. A review of the literature indicates that the lymphoid tumors associated with this disease are generally considered to be neoplastic entities.

Initial studies at this Laboratory started in 1939, at which time only hatching eggs were introduced onto the premises. Chickens obtained from these eggs were housed in completely new buildings. Exacting quarantine and sanitary measures provided for in all experimental work have been successful over a period of 8 years in preventing all common infectious diseases except lymphomatosis and all parasites of chickens except coccidiosis.

The first naturally occurring case of lymphomatosis at the Laboratory was observed about 40 days after the first chickens were hatched. During the first 600 days of life about 22 per cent of the chickens in this first population developed gross lymphoid lesions typical of the disease. For the past 7 years 20–40 per cent of the birds in each population have shown gross evidence of naturally occurring lymphomatosis. The presence of lymphomatosis in the first population, and the more or less complete absence of all other infectious diseases and parasites, provided very strong evidence of egg transmission, but it was not positive enough to remove all doubt. Accordingly, experiments were designed to provide more proof of such transmission.

The procedures followed consisted in selecting the eggs from certain hens and incubating, hatching, and rearing the resultant chickens by groups of families (a family consists of progeny of one sire and dam) in complete confinement and under extremely rigid quarantine (*8*). Altogether, 22 families of chickens were tested, and at the end of a 300-day period 9 of these (64 birds) had one or more individuals (a total of 16 per cent) showing gross lymphoid tumors, while 13 families (92 birds) showed no evidence of tumors. Sib controls of each of these 22 families were incubated, hatched, and reared with the main population of 1,074 chickens, of which approximately 34 per cent developed gross lymphoid tumors at 600 days of age. The 47 exposed sib controls of the 9 families showed 32 per cent with tumors; the 83 exposed sib controls of the 13 families, 21 per cent.

A second test consisted in selecting 4 females and 1 male, all sibs, from 1 of the 22 families showing no tumors. A mating (maintained in strict pen isolation) of these sibs produced 4 families consisting of 91 progeny which were incubated, hatched, and reared by families each in individual pen isolation. Control sibs and half sibs (121 in all) of these 4 families were incubated and reared with the mixed infected population consisting of 1,059 chickens. At the end of 300 days none of the isolated chickens in the 4 families showed gross lymphoid tumors, and 1 family of 17 chickens is still alive at 1,800 days of age. In contrast, 32 per cent of the exposed sibs and 20 per cent of the main population developed gross lymphoid tumors.

In a third test 4 female sibs were selected from among the progeny of the second-test chickens. Similar to the second test, these 4 females were mated to a brother, the resultant progeny again being incubated and reared by families, each in complete pen isolation. In this test at about 200 days of age, certain birds from 1 of the 4 families were diagnosed, upon gross necropsy, as showing questionable lymphomatosis, and the entire family was summarily destroyed. Of the remaining 3 families, consisting of 72 chickens, all were alive and apparently free of tumors at 350 days, at which time 23 birds were killed and no tumors found. The 49 remaining in this sample were killed at about 500 days of age, and only 1 bird showed gross lesions which could have been typical of lymphomatosis. About 44 per cent of the control sibs (165 birds) of these third-test families had gross lymphoid tumors at 500 days of age when exposed to a mixed infected population (1,014 birds), 35 per cent of which had developed gross tumors during the same period.

From these experiments several important conclusions relative to the communicability of this disease are justified: (1) Certain families of chickens highly susceptible to lymphomatosis can be reared either completely or relatively free of lymphoid tumors as well as all other recognized diseases and parasites (*8*); (2) the families of chickens showing no tumors were highly susceptible to lymphomatosis (*5*); (3) lymphomatosis is apparently a communicable disease, and the agent presumably responsible for it can be transmitted readily from bird to bird (*6, 8*); and (4) the egg is incriminated as a carrier of the agent responsible for the disease (*6, 8*).

The suggestion that a chicken tumor having the properties commonly associated with neoplasia is caused by an agent which is contagious is unique in the history of neoplasia as found in other animals. This raises the question as to whether the lymphoid tumors which characterize lymphomatosis in chickens do represent malignant neoplastic growth as described in other animals. Shimkin (*4*) cautions: "Agreement that the condition represents a malignant neoplasm is desirable, although it is realized that the criteria for malignancy are far from satisfactory and far from being sharply defined."

The experimental techniques used at this Laboratory to obtain inbred chickens relatively free of lymphoid tumors provide thought-provoking information on natural transmission, both by way of the egg and by bird-to-bird contact, and also information on the genetic resistance of the host (*5, 7*). How valuable free, susceptible chickens could be to oncological studies in the fowl is self-evident. If inbred chickens highly susceptible to specific lymphoid tumors, when reared by families in strict pen isolation, develop relatively few or no tumors, it can only be assumed that the actuating causes which promote tumor growth were not prevalent either in the chickens or in the environment surrounding them.

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