THE EFFECT OF STRESS ON THE PARASITISM OF MALLARD DUCKLINGS BY *ECHINURIA UNCINATA* (NEMATODA: SPIRUROIDEA)

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Mallard ducklings inoculated with a known number of infective third-stage juveniles of Echinuria uncinata were subjected to various stressors (crowding, low protein diet, heat exposure, lead shot ingestion) or injected with cortisone acetate. At necropsy, the numbers and sizes of nematodes retained by treated ducklings were compared to those of infected non-treated controls. In addition, treated ducklings were examined for signs of the General Adaptation Syndrome (G.A.S.).

Mallard ducklings stressed by crowding or injected with cortisone retained significantly more and larger nematodes at necropsy than infected non-treated controls and exhibited marked signs of the G.A.S.: an involution of the bursae of Fabricius and thymus glands, hypertrophy of the adrenal glands and a retardation of growth.

Deficient diets also produced signs of the G.A.S. but no correlation with parasitism was apparent. Both heat exposure and lead shot ingestion gave uncertain G.A.S. signs and again no correlation with parasitism was apparent.

Normally, numerous eosinophils infiltrate the granuloma in response to the nematode infection. Stress inhibited this reaction and may have contributed to the retention of a larger number of nematodes which were, on the average longer than those from controls.

Post-natal bursectomy did not result in a significant inhibition of antibody synthesis though bursectomized ducklings stressed by crowding produced less antibody than bursectomized controls.

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Echinuria uncinata (Rudolphi, 1819) Soloviev 1912, is a nematode parasite which infects waterfowl at the junction of the proventriculus and the ventriculus. Lesions, described as granulomas (Shevtsov and Zabello, 1964 in Austin, 1970) are found at this point. The degree of infection and the severity of the lesions vary considerably and the hypothesis was proposed that extremes of environmental factors or "stressors" play a significant role in the severity of this infection.

Extremes of any environmental factor affect the individuals in a population in a variety of ways producing what is loosely described as "stress". Parasitologists have attempted to elucidate the role of "stress" in diseases caused by parasites. The difficulty has been to measure "stress", for it occurs in a wide variety of manifestations differing qualitatively and quantitatively in each host. These difficulties were partly overcome by the working hypothesis of Selye (1950) known as the General Adaptation Syndrome (G.A.S.). By measuring "stress" in terms of changes in size of the organs of an animal's lymphatic tissue and adrenal glands he provided a basis for the measurement of "stress".

This study will examine the disease echinuriasis in mallard ducklings in terms of the interaction of "stress", as defined by Selye (1950) and the numbers and growth of the nematode, E.uncinata. This will be done through five separate studies. The objectives of these five studies are as follows:

(i) To assess the effects of several stressors on the host

and on the parasite load.

Stress will be measured by comparing weights of bursae of Fabricius, thymus glands, adrenal glands, the keel/sternum (K/S) ratio and weight gain of "stressed" and control ducklings. The K/S ratio (or emaciation index (E/I) Cornwell, 1966 is a measure of the pectoral muscle depth relative to keel height (Crichton, 1969). Measurements of "stress" will then be correlated with the numbers and sizes of nematodes retained by ducklings in the stressed and control groups.

(a) To elucidate the effects of crowding as a stressor on echinuriasis in ducklings.

Titman (1967) noted a marked social breakdown accompanied by abnormal behaviour in a dense population of breeding mallards. Neave and Wright (1968) found that the size of adrenals in ruffed grouse increased as population size increased, suggesting that natural crowding is a stressor. Ducklings were crowded into small pens to determine the effect of crowding on the lymphatic organs and adrenals and to find out if stress signs were correlated with increased parasite burdens.

(b) To assess the effect of injections of cortisone on parasite burdens of ducklings in a natural setting.

Injections of cortisone acetate produce signs of the G.A.S. in fowl in the form of bursal (Glick, 1957; 1967; Huble, 1958) and thymic involution (Huble, 1958) as well as a retardation of growth (Dulin, 1955; Baum and Meyer, 1960) and changes in circulating leukocytes (Glick; 1958; 1961). Injections of cortisone acetate provided a convenient method of assessing the effect of increased

adrenocortical function on parasite burdens of ducklings allowed to become infected naturally.

(ii) To examine the relationship between the involution of the bursa and echinuriasis.

The bursa involutes during stress or following injections of cortisone acetate (von Faber, 1964b). Chicks injected by Glick (1967) with 2.5 mg cortisone acetate twice a day for the first five days after hatching had significantly reduced bursae and exhibited a reduced antibody response to bovine serum albumin. The delay in normal antibody response in chicks injected with cortisone was explained in part, by an interference with normal bursal development (Glick, 1967). Ducklings in the present study were bursectomized at two days of age to examine if bursal involution during stress interferes with antibody production and results in a lowered resistance to E. uncinata.

(iii) To determine if histological differences occur in the granulomas produced in non-stressed and stressed birds.

The granulomas are the focal points of Echinuria infections. Gross and histological examination of granulomas may reveal differences in the reaction of the host to the parasite which may aid in the interpretation of the relationship between stress, host response and nematode success.

LITERATURE SURVEY

The General Adaptation Syndrome

Stress as defined by Selye (1956) is the state manifested by a specific syndrome which consists of all the nonspecifically induced changes within a biologic system. A stressor, on the other hand, is the agent which produces stress (Selye, 1956). The specific syndrome by which stress manifests itself is called the general adaptation syndrome (G.A.S.). Prominent characteristics of the G.A.S. are: adrenal enlargement accompanied by an increased production of the adrenocorticoids, shrinkage of the lymphatic organs and loss of body weight (Selye, 1956).

The G.A.S. can be elicited by a wide range of noxious stimuli; for example, injection of foreign substances, cold, heat, x-rays, trauma, pain, forced exercise, starvation, toxic substances and disease (Selye, 1950).

The G.A.S. follows a triphasic course. These three phases were designated respectively: the "alarm reaction" (AR), the "stage of resistance" and the"stage of exhaustion" (Selye, 1956).

The AR is subdivided into the shock and countershock phases. The shock phase is characterized by a number of catabolic changes including the depletion of the adrenal cortical hormones. If the shock phase is survived, then the animal passes into the countershock phase. In this phase the adrenal cortex enlarges and produces more adrenocorticoids. The thymus and other lymphatic structures degenerate and a general reversal of the changes that take place during shock occur (Selye 1956).

The stage of resistance represents the sum of all the non-specific reactions elicited by prolonged exposure to stress (Selye, 1950). In this stage the animal has adapted itself to the stressor. Adrenocortical activity gradually falls to a level only slightly above normal (Selye 1956).

If the action of the stressor is prolonged the animal passes into the stage of exhaustion. The adrenal glands become enlarged and depleted of their supply of adrenocorticoids. The thymus glands and other lymphatic structures atrophy and many of the catabolic changes occurring during the shock phase reappear. The stage of exhaustion terminates in the death of the organism (Selye, 1950; 1956).

The G.A.S. is an adaptive response to a stressor. Resistance is low during the AR, high during the stage of resistance and low in the stage of exhaustion (Selye, 1950). The adaptive hormones of the pituitary-adrenal system are required for adaptation to stress. They maintain life during the AR until the body gains time necessary for the development of specific adaptive phenomena. During the subsequent stage of resistance corticoid production is unnecessary (Selye, 1956).

Various factors alter response to stress. Both the production of adaptive hormones and their effect on individual target organs are greatly influenced by age, heredity, previous exposure to stress and nutritional state (Selye, 1950). Exposure to any one stressor may either increase or decrease resistance to another stressor depending upon the circumstances (Selye, 1952).

Pathways of response to stress were described for mammals (Etkin, 1964; Selye,1956). Stimuli received by the receptors of the central nervous system are passed to the perifornical area of the hypothalamus.

This in turn transmits impulses to the median eminence where a neurosecretory product, corticotropin releasing factor (C.R.F.), is secreted into the primary plexus of the hypophysial portal system. C.R.F. is carried by this portal system to the sinuses of the adenohypophysis. C.R.F. excites the glandular cells to secrete adrenocorticotropic hormone (ACTH) which is carried by the blood to the adrenal glands and elicits the secretion of the adrenocorticoids.

Stress in Fowl

Stress in fowl has been reviewed by von Faber (1964b), who indicated that fowl react to stressors in a similar manner to that of mammals except that they do not develop gastrointestinal ulcers. The main signs of stress in poultry are: enlargement of the anterior pituitary and of the adrenal glands, cholesterol depletion of the adrenals together with an increased output of corticosterone, atrophy of the thymus, bursa and spleen, changes in numbers of circulating leukocytes, increase in blood citric acid and retardation of growth. The regression of the bursa is the most sensitive indicator of stress in young birds (von Faber, 1964b).

Known stressors in fowl include cold, limitation of feed and water, surgical trauma, handling, restraint and crowding. Administration of ACTH and cortical steroids produce many of the signs of stress in fowl (von Faber, 1964b).

Only two references could be found concerning stress in waterfowl: both feather eating (von Faber, 1964a) and various petroleum oils produced adrenal enlargement in ducks (Hartung and Hunt, 1966).

Stress and Parasitism

Waterfowl mortality from helminth parasites has often been attributed to a weakening of the host's resistance to infection by adverse environmental factors. Pre-breeding stress, stress of migration (Trainer and Fischer 1963), nutritional deficiencies (Herman et al., 1955), adverse conditions of habitat, competition for existance (Herman, 1954), starvation, cold, disease (Gower 1938), and possibly pollution (Cornwell, 1966) have been suggested as contributing to lowered resistance to parasitism.

Laboratory experiments showed that various agents lower resistance of animals to infection. Nutritional deficiencies result in significantly greater numbers of Ascaridia galli (Schranck, 1788) in chickens (Zimmerman et al., 1926; Ackert and Beach, 1933; Riedel and Ackert, 1950) and to decreased resistance of rats to typhus (Fitzpatrick, 1948). Survival of Trichinella spiralis (Owen, 1835), Raillet, 1895 was significantly greater in mice stressed by intermittant periods of mild electrical stimulation, loud noise and bright lights (Robinson, 1961). Cold stress increased the numbers of Entamoeba in ground squirrels (Noble, 1966). Fighting, heat exposure, noxious stimulants, noise, crowding, darkness and extreme confinement caused a significant increase in numbers of Trichomonas in ground squirrels (Noble 1961; 1962). Chickens subjected to continuous light, continuous darkness, or partial feed restrictions exhibited a significant increase in numbers of A. galli and incidence of infection (Johnson, 1969). Noble (1971) showed that cortisone, ethylene glycol and stress of restraint significantly increased the development and spread of leishmanial infection.

Mechanisms by Which Stress and Cortisone Injection Decrease Host Resistance to Infection

Inflammation can be regarded as one of the most important protective mechanisms of the body against injury (Metchnikoff, 1891). Stress and injections of cortisone or adrenocorticotropic hormones decrease the inflammatory response (Selye, 1950) and inhibit granuloma formation (Funk and Jensen, 1967). Cellular responses that occur during inflammation are inhibited by stress or injections of cortisone. Daily cortisone treatment of mice (0.5 mg) for 30 days after an initial infection with T. spiralis resulted in almost complete suppression of the normal cellular infiltration of the musculature in response to the invading worms (Coker, 1956). The resistance of the rat to Nematospiroides dubius (Baylis, 1926) was overcome by the use of cortisone (5 mg/ 100 g body weight, daily for 20 days) which suppressed the connective tissue reaction around the developing larvae and allowed the worms to escape encapsulation (Cross,1960). Daily cortisone injection (30 mg/kg body weight) effectively suppressed the cellular response and encapsulation of Litomosoides carinii (Travassos, 1911) Chandler, 1931 larvae in the pleural cavities of white rats. Eosinophil response was suppressed to levels near those of uninfected rats (Olson, 1959). Rats immunized to juveniles of Nippostrongylus muris (Yokogawa, 1920 ; Lane, 1923)but not given cortisone showed intense inflammatory responses in skin sections, with many larvae trapped in nodules. Those injected with cortisone (2 mg daily for several weeks) showed virtually complete suppression of the cellular response in the skin. A greater number of worms was recovered from injected rats and appeared to be related to the suppression of the cellular response (Weinstein, 1953).

Several investigators found that stress or injections of cortisone significantly reduce antibody production to antigens. Chickens injected intramuscularly with 7.5 mg cortisone acetate twice a day for the first five days after hatching exhibited a significantly reduced antibody response to bovine serum albumin (Glick, 1967). A single subcutaneous injection of cortisone acetate (400-500 mg/kg body weight) depressed the serum haemolysin response of adult Swiss mice to sheep erythrocytes when administered near the time of antigen injection. Cortisone depressed both 19S and 7S haemolysin when given prior to antigen (Elliot and Sinclair, 1968). Briggs (1963) found that daily cortisone treatment for 20 days (50 mg/kg body weight, halved after 5-7 days) suppressed the antibody response in rats to L. carinii. Exposure to high environmental temperature before a primary antigenic challenge caused an inhibition of the development of the primary immune response in fowl (Thaxton et al., 1968). High primary titres of antibody produced by chickens to sheep red blood cells, bovine serum albumin, or polyvalent K Salmonella pullorum were depressed within 12 hours after four 30-minute exposures to temperatures of 41.7°C to 43.3° C (Thaxton and Siegel, 1970).

The Bursa of Fabricius and Antibody Production

In the chicken the bursa of Fabricius develops before the 12th. day of embryonation as a follicular epithelial organ from the proctodeal portion of the cloaca. Numerous small epithelial follicles appear along the plicated epithelium of the gut wall and develop into lymphoid follicles between the 13th and 21st day of embryonation. Shortly after hatching the follicles of the bursa are well developed elements and contain both

cortex and medullary components (Good and Finstad, 1967).

In the mallard duck the weight of the bursa increases rapidly after hatching and attains a maximum at eight weeks in males and six weeks in females, then steadily decreases in weight after 12 weeks in males and 16 weeks in females (Ward and Middleton, 1971) and eventually disappears.

The bursa of Fabricius plays an important role in antibody production during the first few weeks of life (Chang et al., 1955; Glick et al., 1956). The age at which a bird is bursectomized has a direct effect on the degree of interference with antibody production (Chang et al., 1957; Mueller et al., 1960; 1962; Graetzer et al., 1963; van Alten et al.,1968; Cain et al., 1969). Data on the age at which bursectomy causes complete inhibition of antibody response are conflicting. These differences depend on many variables such as mode of bursectomy (surgical or hormonal), the age at which the bird is immunized, the nature of the antigen, the mode of immunization and the method of antibody detection (Rose and Orlans, 1968).

Antibody response was reported absent or reduced in chickens surgically bursectomized between hatching and five weeks of age when challenged with Salmonella type O antigen (Chang et al., 1955), sheep red blood cells (Chang et al., 1957; 1958; Pierce et al., 1966; van Alten et al., 1968), bovine serum albumin (Mueller et al., 1960;1962;Graetzer et al., 1963; Carey and Warner, 1964), Eimeria tenella (Challey, 1962), Salmonella typhimurium (Chang et al., 1959), Brucella abortus (Papermaster et al., 1962; Claffin et al., 1966), T_2 coliphage virus (Papermaster et al., 1962), human O erythrocytes (Isakovic et al., 1963; Jankovic and Isakovic, 1966), and human red blood cells (Jankovic and Leskowitz, 1965). Bursectomy does not affect antibody production to Newcastle disease virus (Cho, 1963; Sadler and Edgar, 1968).

Surgical bursectomy after five weeks of age had little or no effect on antibody production to Salmonella type O antigen (Chang et al., 1957) or bovine serum albumin (Mueller et al., 1960).

Hormonal bursectomy by injecting the egg with testosterone proprionate or 19 - nortestosterone at 5 or 12 days of incubation completely inhibits antibody production to bovine serum albumin (Mueller et al., 1960; Papermaster et al., 1962; Carey and Warner, 1964; Pierce and Long, 1965), several standard antigens (Warner and Szenberg, 1962), Plasmodium lophurae (Longnecker et al., 1969), Eimeria tenella (Long and Pierce, 1963; Pierce and Long, 1965), diphtheria toxoid (Cooper et al., 1966), T_2 coliphage virus and Brucella abortus (Papermaster et al., 1962). Clafflin et al. (1966) consistently demonstrated specific antibody was produced to B. abortus in chickens either hormonally bursectomized at five days of incubation or surgically bursectomized at hatching. Removal of the bursa, nevertheless, caused a quantitative depression in the ability of the animals to execute an antibody response.

To completely clear the system of antibody forming cells, bursectomy must be followed by X-irradiation. Chickens so treated are agammaglobinaemic and have neither 19S nor 7S gamma-globulin synthesis and fail to form antibodies even after repeated stimulation (Cooper et al., 1966; 1967). Chickens bursectomized at hatching and X-irradiated the same day showed a complete inhibition of antibody response to Brucella abortus and bovine serum albumin (Cooper et al., 1965; Cooper et al., 1966a).

On the basis of weight gain and mortality it was demonstrated that chickens without bursae are far less resistant to Salmonella typhimurium (Chang et al., 1959), Eimeria tenella (Challey, 1962; Long and Pierce, 1963) and Escherichia coli (Sadler et al., 1969) than non-operated controls. Infections with Plasmodium lophurae developed more rapidly,

incubation exhibited a reduced 7S immunoglobin synthesis but a normal 19S immunoglobin response. Chicks bursectomized on the 21st. day (at hatching) showed both normal 7S and 19S immunoglobin synthesis. Bursal support of development of capacity for 7S immunoglobin occurs after the integrity of the 19S immunoglobin synthesizing system has been established. Similar results of low 7S and normal 19S immunoglobin synthesis of bursectomized chicks were noted by a number of investigators (Cooper et al., 1965; 1968; Ortega, 1965; Pierce and Long, 1966). Further evidence that the development of a competent immunoglobin producing cell system is a sequential process was supplied by Cooper et al. (1967). These investigators found a difference in nucleic acid ratios of bursal lymphoid cells at different ages of embryonic life suggesting a sequential maturation of stem cells of the bursa of Fabricius.

The cells of the bursal follicles do not appear to participate directly in immune responses or antibody synthesis (Dent and Good,1965; Dent and Peterson, 1966). Plasma cells do not develop in the bursa of Fabricius and throughout its functional life the structure seems to maintain an aloofness from the immunological function of the lymphoid tissue (Good and Finstad, 1967).

There is evidence that lymphoid cells of the germinal centers in the chicken spleen are derived from lymphoid cells of the bursa of Fabricius. These cells seem to represent intermediate stages in plasma cell differentiation. Within germinal splenic centers in the chicken, cells are found which are indistinguishable by ultrastructural criteria from bursal lymphocytes. Germinal center cells show a range of fine structural features from those typical of the bursa itself to an intermediate type with haemocytoblast characteristics and finally to cells with

definite preplasma cell morphology (Jankovic and Mitrovic, 1967; Cooper et al., 1967). The cellular makeup of the tonsilla caecalis and Peyer's patches closely resembles that of the germinal centers of the spleen. A large number of mature and immature plasma cells are normal features of these structures. Neonatal bursectomy was followed by a profound decrease in the numbers of germinal centers in the caecal tonsils and Peyer's patches (Jankovic et al., 1963; and Mitrovic, 1967). X-irradiated, bursectomized chickens injected with autologous bursal lymphocyte suspensions exhibited germinal center plasma cells, and development of gamma-globulin synthesis whereas non-injected bursectomized X-irradiated birds did not (Cooper et al., 1966a; 1967). The number of germinal centers of spleen and caecal tonsils were reduced in chickens bursectomized at 19 and 21 days of incubation (van Alten, 1968). Szenberg and Warner (1962) found that the spleen was one half normal size in chicks hormonally bursectomized on the 12th day of incubation. Jankovic et al. (1963) found evidence that extirpation of the bursa brings about a depletion of plasmacytic cells in the spleen, and spleen sections from 19-nortestosterone treated chicks showed reduced lymphoid follicle development (Papermaster et al., 1962).

It is likely that cells influenced by the bursa are present in peripheral lymphoid organs by the time of neonatal bursectomy (Cooper et al., 1967). Isakovic and Jankovic (1964) found evidence supporting this, as hyperimmunization of both control and bursectomized birds was followed by abundant proliferation of plasmacellular elements in the caecal tonsils and spleen.

Strong evidence suggests that a dissociation of immunological function exists between the bursa and the thymus, the bursa being responsible for producing precursor cells that eventually produce antibodies (see

above) while the thymus is necessary for cellular immune competence (Warner and Szenberg, 1962; 1963; Isakovic et al., 1963; Longnecker et al., 1966; Cooper et al., 1966; Cain et al., 1968; Glick 1969; 1970).

What has so far been concluded is that an identifiable, specialized central lymphoid system (thymus, bursa) and a peripheral system of germinal centers (spleen, caecal tonsils, Peyer's patches) exist which can undergo rapid expansion in response to repeated antigenic stimulus (Good and Finstad, 1967). Cells of the bursa of Fabricius early in life migrate to the germinal centers of the fowl spleen, caecal tonsils and Peyer's patches where, upon antigenic stimulation, they proliferate and become immunologically competent plasma cells. Similarly, cells from the thymus migrate to the peripheral germinal centers whereupon they attain cellular immune competence upon antigenic stimulation (Warner and Szenberg, 1962; Papermaster and Good, 1962; Cooper et al., 1965. Good and Finstad (1967) described two separate systems of germinal centers in the spleen of the fowl, one made up of plasmacellular elements (from the bursa) and other of small lymphocytes (from the thymus).

Biology of Echinuria

Austin (1970) studied the biology, pathogenicity and occurrence of Echinuria uncinata (Rudolphi, 1819) Soloviev, 1912 (Nematoda,Spiruroidea) at the Delta Waterfowl Research Station. Definitive hosts of E. uncinata are usually the Anatidae and the most important intermediate hosts are Daphnia magna and D. pulex. Eggs passed in the faeces of infected ducks are ingested by Daphnia spp., the larvae hatch and penetrate the gut wall into the haemocoel where they develop into the third stage

infective juveniles in 28 days at 15°C and 10 days at 20-24°C. Ducks become infected by ingesting Daphnia spp. harboring infective third-stage juveniles. In the mallard, Echinuria moults to the fourth-stage at 10 days to the fifth-stage at 20 days and the females begin to oviposit at 40 days. Juvenile ducks are more susceptible to E. uncinata than adults. Ducklings infected at one week with a standard inoculum have larger and more numerous nematodes than those infected at three months. Susceptibility varies in different species. Shovellers are refractory to E. uncinata while mallards are among the most susceptible species.

Echinuria causes the formation of granulomas at the junction of the proventriculus and ventriculus in ducks and at the junction of the proventriculus and ventriculus and proventriculus and oesophagus in geese. Mortality due to echinuriasis is believed to be a result of a compaction of the oesophagus when the granulomas occlude the lumen of the gut (Cornwell, 1963).

Austin (1970) recorded the range of body length of adult E. uncinata as 6.7 - 20.0 mm for females and 4.6 - 11.6 mm for males.

The peak abundance of Daphnia is in summer and autumn. Austin (1970) found that the numbers of Daphnia harboring infective third stage juveniles of E. uncinata peaked in August and gradually decreased until November when no Daphnia were found.

MATERIALS AND METHODS

GENERAL PROCEDURE

Daphnia Collection and Nematode Recovery

Infective third-stage juveniles of E. uncinata were obtained from naturally infected Daphnia spp. which had been collected with a dip net from the Delta Waterfowl Research Station Pond. Daphnia were crushed and placed in Baermann funnels. An hour and a half later small quantities of filtrate containing nematodes were drawn off from the bottom of the funnels. Nematodes recovered were counted and transferred with an eyelash brush to a 15 mm Syracuse dish.

Rearing of Ducklings

Experimental ducklings used throughout the study were the Delta Waterfowl Research Station strain of the game farm mallard. Day-old ducklings were transferred from incubators to brooders where they were kept until four weeks of age. The pen in which the ducklings were reared was specially built for experimental parasitology (pen specifications can be found in Austin, 1970). Water was drawn from a covered well free of Crustacea. Each day the pen was cleaned and the water changed.

Inoculation with Nematodes

The standard inoculum was 150 infective juveniles of E. uncinata in 0.5 cc of water. This was force fed to the duckling using an eyedropper inserted at the base of the bill 5.0 cm into the oesophagus. Contact with the glottis was avoided as a precaution against regurgitation of the inoculum.

Injection of Chemicals

Injections of cortisone (Cortone, sterile cortisone acetate suspension, M.S.D. Std., each cc = 50 mg)and saline (sterile, isotonic) were made into the breast muscles with a 0.1 cc and 5.0 cc disposable syringe respectively.

Bursectomy and Sham Bursectomy Procedure

Ducklings were bursectomized within two days of hatching. Birds were anaesthetized 10 minutes prior to bursectomy with 0.1 cc of 7% Avertin solution per 10 grams of body weight, injected intraperitonially with a 1.0 cc tuberculin syringe (Appendix I Fig. 1). The down was shaved between the ventral tip of the uropygium and the anus (Appendix I Fig. 2). The skin was swabbed with alcohol, allowed to dry, and incised from the base of the uropygium to within 2.0 mm of the anus (Appendix I Fig. 3). The bursa was grasped with a pair of small sterile, curved forceps, pulled through the incision so that the base was clearly visible (Appendix I Fig. 4), tied off at the base with cotton thread (Appendix I Fig. 5) and excised immediately distally to the ligature (Appendix I Fig. 6). The wound was sutured with a 9.0 mm wound clip (Appendix I Fig. 7). The clips were removed within two weeks of the operation. The same procedure was used for sham bursectomy except that the bursae were neither tied off nor excised.

Identification of Ducklings

Each duckling was identified by colored wires tied around its leg. Webbing between the toes was notched for identification if ducklings were treated soon after hatching.

Necropsy Procedure

Ducklings were weighed and then killed by decapitation so that as little blood as possible was retained in the organs. An incision was made in the skin extending from the anus to the base of the neck to expose the breast muscles and thymus glands. Measurements to determine the K/S ratios were taken. The body cavity was opened to expose the viscera. The thymus, adrenals, spleen and bursa were removed and fixed in either F.A.A. (85 parts of formalin, 15 parts of alcohol and 5 parts of acetic acid) or 15% formalin. The granulomas that were formed at the junction of the proventriculus and ventriculus in response to the nematode infection were counted and measured.

Collection and Weighing of Organs

The thymi, adrenals, spleens and bursae were removed from ducklings at necropsy, preserved in F.A.A. or 15% formalin, then dried in an oven at 97°C for 24 hours and weighed to the nearest 0.1 mg.

Measurement of the K/S Ratio

The keel sternum (K/S) ratio (Crichton,1969) or emaciation index (E.I.) was developed by Cornwell (1966) to quantify the degree of emaciation of waterfowl.

Measurements of the K/S ratio were made by selecting a standard point 2.5 cm from the anterior end and 1.0 cm to the right of the keel. At this point, the perpendicular distance from the ventral edge of the keel to the sternum was measured in millimeters with a calibrated probe. This measurement served as the denominator of a fraction whose numerator was the depth of the muscle at the same point. The K/S ratio is presented as a decimal value.

Collection and Measurement of Nematodes

Nematodes were removed from the granulomas, counted, straightened

in hot 0.05% acetic acid, and stored in vials containing 15% formalin. Three thousand three hundred and eleven nematodes were measured by projecting their image onto a calibrated sheet of graph paper. Lengths were determined by counting the number of units along an imaginary line running the length of the nematode. Damaged nematodes were not measured.

The Index of Parasite Load

When comparing numbers and sizes of nematodes retained by stressed and control ducklings in Parts III and IV of this thesis, it became apparent that neither the numbers of nematodes nor the lengths of nematodes alone presented a completely accurate measurement of nematode success. Therefore, an index was derived which combined the numbers of nematodes with the lengths of nematodes retained by each duckling.

The index of parasite load was derived by determining the sum of the lengths of male and female nematodes retained by each duckling. The mean of this value was then calculated for the stressed ducklings and for the control ducklings to give the mean of the total length of nematodes from ducklings in each group.

Histology

Sections of granulomas were cut at seven microns and stained following standard histological procedures.

Keeping of Records

Data obtained from each duckling were recorded on a standard data sheet (Appendix II).

Statistical Analyses

All statistical analyses (Student's t-test, 1 tail analysis; means) were done using a table -top model Olivetti computer (Olivetti-Underwood programma 101). All recorded differences in comparisons of the effect of stress on organ weights and nematode burdens were statistically significant at the 5% level unless stated otherwise.

Immunological Techniques

Collection and treatment of blood:- ducks were decapitated and blood collected in a beaker. Clotted blood was transferred to labelled vials, kept at room temperature for two hours, then transferred to a refrigerator and held overnight at 4°C. Blood samples were centrifuged for half an hour to separate serum from the coagulum, treated with 0.001% merthiolate solution, and stored at 4°C.

Processing and storage of antigens (*E. uncinata*): - nematodes were removed from granulomas and frozen immediately in glass vials. When required, nematodes were thawed and crushed in a tissue grinder to a creamy consistency. An equal volume of saline was added and the mixture centrifuged for 30 minutes at 1,500 G at 4°C. The supernatant was decanted into vials and used immediately.

Preparation of agarose slides: - new slides were washed thoroughly in soap and water, rinsed in distilled water, dried in an oven, dipped in 0.3% solution of agarose, and dried again. Two ml of hot 1.0% agarose solution was placed on each slide, spread to the edge with a pipette and allowed to set at room temperature. Wells were punched in the agarose using a cork borer with the aid of a template (Appendix III). Sera and antigens were placed in separate wells and incubated in a humidifier at room temperature for 36 hours. Slides were then

immersed in saline for 10 hours and left in a refrigerator at 4°C after which they were washed in three changes of saline per day for three days at room temperature. They were then washed in distilled water for seven hours and dried overnight in an oven. Slides were stained in amido black and light green SF, mounted in D.P.X., and examined for the presence of precipitin lines.

Grading of precipitin reactions: - for examination, slides were placed on a white background and precipitin lines graded according to the following scheme: o=no reaction, 1= weak reaction, 2 = moderate reaction, 3= strong reaction. Slides were graded by two persons and results compared.

EXPERIMENTAL PROCEDURE

Each experiment consisted of groups of at least 10 treated and 10 control ducklings of the same age and approximately the same sex ratios. Five-week old ducklings were stressed three days prior to the standard inoculum and for the duration of the experiment. Inoculations were limited to four to five ducklings each day over a five day period, due to the time required to collect juvenile nematodes. Necropsies were spread over a corresponding five day period in the same sequence in which the ducklings had been inoculated. Necropsies were performed 42 days after inoculation. Treated and control ducklings were maintained in separate enclosures 6.08 m x 1.52 m of which 3.05 x 1.52 m was pond. The procedures used to stress ducklings will be outlined in the descriptions of the individual experiments.

PART I: STRESS SURVEY EXPERIMENT

INTRODUCTION

A series of preliminary experiments was carried out in the summer of 1969 to determine the effects of various stressors (crowding, heat exposure, deficient diet and lead shot ingestion) or injections of cortisone both on the parasitism of mallard ducklings by E. uncinata and on the lymphatic structures and adrenals.

CROWDING STRESS

Procedure

Ten control ducklings and eleven ducklings that were stressed by crowding each received an inoculum of 50 infective juveniles of E. uncinata. Inoculations were begun when ducklings were 42 days of age. Necropsies were performed 35 days post infection.

Crowded ducklings were placed in 0.61 m x 1.22 m enclosure having a wire screen base (Fig.4). The enclosure was placed in the pen in a position that allowed ducklings access to 0.61 m x 0.46 m of water area. Control ducklings were maintained in a 1.52 m x 6.08 m enclosure of which 1.52 m x 3.05 m was pond.

Observations

Crowded ducklings retained a greater number of nematodes than controls and had a larger number of granulomas, smaller K/S ratios, bursae and thymi (Table I). The mean weights of crowded and control ducklings at necropsy were 1043 $\pm$ 18 gm and 1095 $\pm$ 30 gm respectively.

TABLE I

Crowding Experiment I (Stress Survey): comparison of the means of the numbers of nematodes, numbers of granulomas, organ weights and K/S ratios of crowded and control ducklings

	Crowded	Control	d.f.	t	P<
Numbers of nematodes	15.4	6.0	19	2.563	0.01
Numbers of granulomas	3.8	2.5	19	1.423	0.10
Bursa weights *	2.021	2.814	19	2.472	0.025
Thymus weights *	5.723	7.433	19	2.265	0.025
K/S ratios	0.84	0.91	18	2.436	0.01

* Expressed as a ratio of body weight X 10^{-4}

Crowded ducklings showed both morphological and ethological differences in comparison to the controls. Plumage of ducklings stressed by crowding was dishevelled and had lost its hydrophobic characteristics (Fig.4,5). Wetting of feathers was followed by prolonged vigorous preening and violent shivering. Crowded ducklings were often observed attempting to escape the enclosure. They were extremely aggressive and had bare patches on their backs as a result of pecking and feather pulling. Plumage of control ducklings was normal, only two had noticeable patches of feathers missing.

Discussion

This experiment will be discussed in conjunction with the other experiments on crowding in Part III of this thesis.

DEFICIENT DIET

Procedure

Ten ducklings were given ad libitum a diet consisting of corn meal that contained approximately 8 1/3% protein (Dowd and Dent,1945). ten control ducklings were given a diet containing approximately 31% protein. All ducklings were given the standard inoculum of nematodes at 35 days of age and necropsied 51 days later.

Observations

Ducklings given a diet of corn meal did not retain a significantly greater number of nematodes than controls though they had smaller weight

TABLE II

Deficient Diet Experiment (Stress Survey): comparison of the means of numbers of nematodes, numbers of granulomas, mean organ weights, weight gain and K/S ratios of ducklings given a deficient diet and controls.

	Deficient Diet	Control	d.f.	t.	P<
Number of nematodes	27.4	15.5	18	1.392	0.10
Number of granulomas	4.0	2.1	18	1.672	0.10
Bursa weights *	1.823	2.449	18	2.660	0.01
Thymus weights *	3.646	6.624	18	2.882	0.005
Weight gain	285.2	464.9	18	3.570	0.005
K/S ratios	0.81	0.85	17	1.207	0.10

* Expressed as a ratio of body weight X 10^{-4}

gains, bursae and thymi. The mean number of granulomas and K/S ratios did not differ significantly (Table II). Mean weights of treated and control ducklings at necropsy were 950 ± 51 gm and 1099 ± 42 gm respectively.

Discussion

There is evidence that the protein requirement for normal development of ducklings is approximately 19 per cent of total food intake (Horton 1932). Ducklings grew at a much faster rate when fed a ration containing 19 per cent protein than when fed one containing 12 per cent. Hamlyn et al. (1934) obtained somewhat better growth in ducklings at four weeks of age when rations were fed containing approximately 20 per cent rather than 17.5 per cent protein. Scott and Heuser (1951) reported better early growth in ducklings supplied rations containing 17 to 19 per cent instead of 15 per cent protein.

The protein content of the diet given treated ducklings in the present experiment (8 1/3 per cent) was far below that indicated as being necessary for normal development. Although the diet produced signs of the G.A.S. the deficiency may not have been severe enough to significantly reduce the resistance of the ducklings to E. uncinata.

In numerous studies on the effect of the host's diet on the well-being of the parasite, the consensusof opinion from the literature suggests that a deficiency in the host's diet makes the host more susceptible to parasitic infection (Noble and Noble, 1961).

Although there was no differences in the number of parasites in malnourished ducklings and controls, numerous investigators have found that fowl on a deficient diet retain significantly more and significantly larger Ascaridia spp. than those on a control diet (Zimmerman et al., 1926; Ackert et al., 1927; 1931; 1933; Sadun, 1949; Riedel, 1950; Larsh, 1950).

Herman et al.(1955) attributed repeated epizootics of Amidostomum anseris (Zeder,1800) in wintering Canada geese to low protein content of winter forage, though their experiments did not support this.

In general, protein deficiency and malnutrition increases host susceptibility to many infectious diseases. Severe deficiency of both protein and of certain essential amino acids or vitamins reduces antibody formation and phagocytic activity in animals (W.H.O. expert committee on nutrition, 1965).

HEAT EXPOSURE

Procedure

Ten control ducklings were maintained in a 1.52 m x 3.05 m enclosure at normal seasonal ambient temperatures. Eleven ducklings exposed to high environmental temperatures were maintained in a 1.52 m x 3.05 m enclosure heated by brooder lamps to temperatures ranging from 35-43°C. The standard inoculum of nematodes was given to each duckling at 52 days of age and necropsies performed 45 days after inoculation.

Observations

The mean number of nematodes retained by ducklings exposed to high environmental temperatures did not differ significantly from that by controls. Ducklings exposed to high environmental temperatures had significantly smaller weight gains and bursae and significantly larger thymus glands than control ducklings. The mean number of granulomas, K/S ratios and weights of adrenals of the two groups did not differ significantly (Table III). Mean weights of treated and control ducklings at necropsy was 929 $\pm$ 28 gm and 1140 $\pm$ 37 gm respectively.

Ducklings held in the heated enclosures panted almost constantly

TABLE III

Heat Exposure Experiment (Stress Survey): comparison of the means of numbers of nematodes, numbers of granulomas, organ weights, weight gains and K/S ratios of ducklings exposed to high temperature and controls.

	Heat Exposed	Control	d.f.	t	P<
Number of nematodes	18.9	16.0	19	0.280	0.10
Number of granulomas	2.5	4.5	19	1.626	0.10
Bursa weights *	1.533	2.015	19	1.760	0.05
Thymus weights *	4.658	3.316	19	1.792	0.05
Adrenal weights *	2.075	2.124	19	1.626	0.10
Weight gain	55.63	169.4	19	3.915	0.005
K/S ratios	0.77	0.82	18	0.757	0.10

* Expressed as a ratio of body weight X 10^{-4}

and aggregated in corners or along the edges of the enclosure where temperatures were slightly lower. When ducklings were released periodically to clean their enclosure, they beat their wings rapidly with a fanning motion, possibly to dissipate heat from their bodies.

Discussion

No conclusive statement can be made as to whether or not ducklings exposed to high environmental temperatures showed signs of the G.A.S. Bursal weight and weight gain data suggest that the ducklings were in fact stressed, however, thymic, adrenal and K/S ratio data suggest that they were not.

Till and Subhas (1968) found that high environmental temperatures (30°C) do not affect the weights of spleens and bursae or the ascorbic acid content of the adrenal glands of chickens. Adrenal cholesterol of chickens held at 43°C at four regular 30 minute intervals was not affected, though primary immune responses were impaired (Thaxton et al., 1968; Thaxton and Siegel, 1970).

Heat exposure at temperatures of the present experiment or those used by previous investigators apparently does not produce significant signs of the G.A.S. in fowl.

A possible explanation for the lack of stress signs in ducklings in the present experiment may be their acclimation to the constant high environmental temperatures and attainment of the "stage of resistance", in which case no stress symptoms would be discernable (Selye, 1956).

LEAD SHOT INGESTION

Procedure

Fourteen ducklings were each force-fed a total of 17 No.5 lead

shotgun pellets (an average of 2 per week) over a period of 49 days. Ten untreated controls were maintained in a separate enclosure. Inoculations with a standard inoculum were begun when ducklings were 39 days of age, one week prior to initial lead shot administration. Ducklings were necropsied 66 days after receiving the standard nematode inoculum.

Observations

Ducklings treated with lead shot had significantly smaller weight gains than controls. The number of granulomas and the number of nematodes retained by ducklings treated with lead shot did not differ significantly from that of control ducklings. No significant differences were noted in K/S ratios, or the weights of bursae, thymi or adrenals of the two groups (Table IV). Mean weights of treated and control ducklings at necropsy was 1009 ± 36 gm and 1044 ± 35 gm respectively. Only eight of the 14 treated ducklings retained lead shot in their gizzards or showed signs of lead poisoning (i.e. gizzard and intestinal tract stained green).

Discussion

Ingested lead shot are toxic to waterfowl (Irby et al., 1967; Cook and Trainer, 1966; Trainer and Hunt, 1965) though the toxicity depends upon the number of shot ingested (Jordan and Bellrose, 1950; Cook and Trainer, 1966), the length of time pellets are retained (Irby et al., 1967, the age of the bird (Elder 1954), and the hardness of food eaten. Jordan and Bellrose (1951) found that lead shot was more toxic if ducks were fed a diet of whole corn than if fed soft plant material, presumably because the shot were eroded more rapidly.

Six of the 14 ducklings in our experiment contained no lead shot in their gizzards at necropsy. This shot could have been passed soon after

TABLE IV

Lead Shot Ingestion Experiment (Stress Survey): comparison of the means of numbers of nematodes, numbers of granulomas, mean organ weights, weight gain and K/S ratios of ducklings given lead shot and controls.

	Treated	Control	d.f.	t.	P<
Number of nematodes	18.7	12.1	22	0.664	0.10
Numbers of granulomas	3.5	2.1	22	1.184	0.10
Bursa weights *	0.917	1.099	22	1.214	0.10
Thymus weights *	0.991	1.126	22	0.555	0.10
Adrenal weights *	2.086	1.787	22	1.697	0.10
Weight gain	197.9	261.5	22	1.734	0.05
K/S ratios	0.87	0.90	22	1.540	0.10

* Expressed as a ratio of body weight X 10^{-4}

being administered or ground down by the action of the gizzard. It is suspected, however, that these shot were passed rather than eroded as no signs of lead poisoning were present.

None of the ducklings in this experiment fed lead shot died. Those retaining shot had one to 12 of the original 17 shot that were administered over the 49 days. Many of the shot were highly eroded. Ducklings may be more resistant to lead poisoning than adults as suggested by Elder (1954) as year-old mallards fed 8 lead shot each retained from two to eight shot each and were dead within 15 days (Grandy et al., 1968, Irby et al., 1967).

CORTISONE INJECTION

Procedure

Twenty-two ducklings were held until they were 74 days old. Ten were injected with cortisone (50 mg/kilogram body weight) at irregular intervals (days, 1,3,5,7,10,13,17,22,26,31,38,47,54,64,72). Ten ducklings injected with 0.5 cc of saline 0.9% w/v (sterile, isotonic)during the same period with the same frequency, were held as controls. Each duckling was given the standard nematode inoculum. Both groups were held together in a 6.08 x 3.15 m enclosure for 43 days, then brought to the university and maintained in a 3.15 x 3.15 m enclosure for 41 days.

Observations

Ducklings injected with cortisone retained an average of 21.7 nematodes, control ducklings were completely free of nematodes with the exception of one duckling which retained a small nematode in a gland of the proventriculus. Injected ducklings had a significantly larger number

of granulomas, smaller bursae and thymi, smaller K/S ratios and less weight gain than controls. Adrenal weights of the two groups did not differ significantly (Table V). Mean weights of treated and control ducklings at necropsy was 963 ± 33 gm and 1104 ± 36 gm respectively.

Ducklings injected with cortisone lost weight rapidly during the initial period of treatment. To prevent mortality of ducklings, injections were made less frequently for the remainder of the experiment.

At the time of necropsy, control ducklings had completed the development of their adult plumage. Injected ducklings on the other hand, had not completed their moult and retained much of their juvenile plumage.

Discussion

Injections of cortisone produced four observable signs of the G.A.S. in mallard ducklings, namely; bursal and thymic involution, weight loss, and retarded development of the breast muscles.

Both treated and control ducklings exhibited a weight loss during the experiment but the loss was significantly greater in injected birds. Weight loss in control ducklings may have been due to the disturbance of transporting them from Delta to the University of Manitoba and maintaining them in an indoor enclosure.

Injections of cortisone prolonged infections of E. uncinata in mallard ducklings. Injected ducklings still retained relatively large infections at necropsy (mean = 21.7 nematodes) while controls were virtually free of nematodes (mean = 0.1 nematodes). E. uncinata appears to elicit both a cellular (Part II) and humoral immune response (Part V) in mallard ducklings. In addition, Austin (1970) found that resistance of mallard ducklings appears to increase with age. Ducklings in the present experiment were 158 days old at necropsy and had been infected 84 days previously.

TABLE V

Cortisone Injection I Experiment (Stress Survey): comparison of the means of numbers of nematodes, numbers of granulomas, organ weights, weight gain and K/S ratios of ducklings injected with cortisone and controls.

	Cortisone	Control	d.f.	t.	P<
Number of nematodes	21.7	0.1	18	2.818	0.005
Numbers of granulomas	5.2	1.3	18	3.280	0.005
Bursa weights *	0.2443	0.7266	18	2.490	0.025
Thymus weights *	0.238	1.378	18	2.771	0.01
Adrenal weights *	0.244	0.727	18	2.490	0.025
Weight gain	-115	-52	18	1.924	0.05
K/S ratios	0.79	0.87	18	3.355	.005

* Expressed as a ratio of body weight X 10^{-4}

Resistance of control ducklings was sufficiently great to overcome their infections during this period. Injections of cortisone apparently inhibited the development of resistance in the treated ducklings resulting in the retention of relatively large nematode burdens.

Corticoid activity is known to rise sharply as the AR develops, but during the stage of resistance it falls to a level slightly above normal (Selye, 1956). Therefore, injecting synthetic glucocorticoids would, in effect, maintain the animals in a constant AR stage.

GENERAL DISCUSSION - STRESS SURVEY EXPERIMENT

The stress survey study clearly showed that crowding and cortisone injection produce signs of the stress response in mallard ducklings and result in greater nematode burdens. Although deficient diet was a stressor, its relationship to parasitism was not clear due to the variability of nematode numbers.

Heat exposure and lead shot ingestion were difficult to regulate, they produced variable responses and did not result in greater nematode burdens. Further use of these agents in the present study was discontinued.

It was concluded that further investigation of the relationship between stress and the parasitism of mallard ducklings by E. uncinata should involve the use of crowding and cortisone injection.

PART II STUDY OF GRANULOMAS

INTRODUCTION

Cursory examination of granulomas from ducklings in the stress survey study revealed a varying amount of caseous material within the cavity containing the nematodes. The caseous material was more abundant in granulomas of control ducklings, though the relationship between the numbers and sizes of nematodes and the amount of caseous material was uncertain.

Purpose of Study

(1) To determine the nature of the caseous material within the granulomas.

(2) To determine if there is a difference in the quantity of caseous material in granulomas of stressed and control ducklings.

(3) To determine if there is a relationship between the amount of caseous material and the numbers and sizes of nematodes.

This study was carried out in two ways; a histological examination of granulomas of stressed and control ducklings and gross observations of the amount of caseous material in granulomas of stressed and control ducklings in the bursectomy, sham-bursectomy and crowding II experiments.

Procedure - Histology Experiment

Six ducklings stressed by crowding and seven controls were given approximately 200 E. uncinata infective juveniles. Inoculations were made when duckling were 104 days of age and necropsies performed

32 days later.

At necropsy granulomas were removed, fixed in F.A.A., sectioned at 7 microns and stained routinely with haematoxylin and eosin.

PROCEDURE - Gross Examination of Granulomas

The amount of caseous material in granulomas of stressed and control ducklings in the bursectomy, sham-bursectomy and crowding II experiments was graded according to the following scheme: 0= no caseous material, 1= small amount of caseous material, 2= moderate amount of caseous material, 3= large amount of caseous material. The amount of caseous material in granulomas of stressed and control groups in the three experiments was then compared to the numbers and sizes of nematodes retained by each group.

Observations

Histological examination revealed that the caseous material consisted of large aggregations of eosinophils in various stages of disintegration. Granulomas of control ducklings contained fragments of dead nematodes interspersed in the cellular mass and living nematodes, usually smaller than those of stressed ducklings were found around the periphery (Fig. 11). Histological examination of granulomas from stressed ducklings revealed little cellular material was present and nematodes larger than those from control ducklings were found throughtout the cavity (Fig. 10).

Gross examination of granulomas from ducklings in the bursectomy, sham-bursectomy and crowding II experiments revealed that the amount of caseous material differed greatly between stressed and control groups. Granulomas from control ducklings contained large amounts of caseous material while those of stressed ducklings contained little, and were

filled with an opaque fluid (Fig. 1). Comparisons of the numbers and sizes of nematodes retained by stressed and control ducklings in the crowding and bursectomy experiments revealed that stressed ducklings retained significantly more and significantly larger nematodes at necropsy (Tables VI, VIII).

Discussion

The presence of caseous material within the granulomas of control birds may be the result of an immunological response by the host, as large amounts of this material are correlated with a high proportion of nematode stunting and mortality. This observation is supported by the fact that in mammals the cellular immune responses are inhibited during stress (Coker, 1956).

The observations on the inhibition of the cellular response by stress or by injections of cortisone are similar to those of other investigators. Coker (1956) found that daily administration of 0.5 mg of cortisone to mice for 30 days after an initial infection with T.spiralis resulted in almost complete suppression of the normal cellular infiltration of the musculature in response to invading worms.

Mice subjected to both avoidance learning and sound stress showed a highly significant decrease in their ability to form foreign body granulomas in response to subcutaneously implanted cotton pellets. Granulomas in stressed mice were smaller with a general inhibition of capsule formation and cellular infiltration as compared to control mice (Funk and Jensen, 1967). It was also shown that the resistance of rats to N. dubius could be overcome by the use of cortisone, which suppressed the connective tissue reaction around the developing worms (Cross, 1960). Parker (1961) found that treatment with cortisone rendered guinea pigs

less resistant to N. brasiliensis and permitted nearly 11 times more larvae to complete the skin to lung migration as compared to controls. Normally the larvae are trapped by the inflammatory response of the host, but cortisone suppressed this cellular response, permitting more larvae to complete their migration to the lungs.

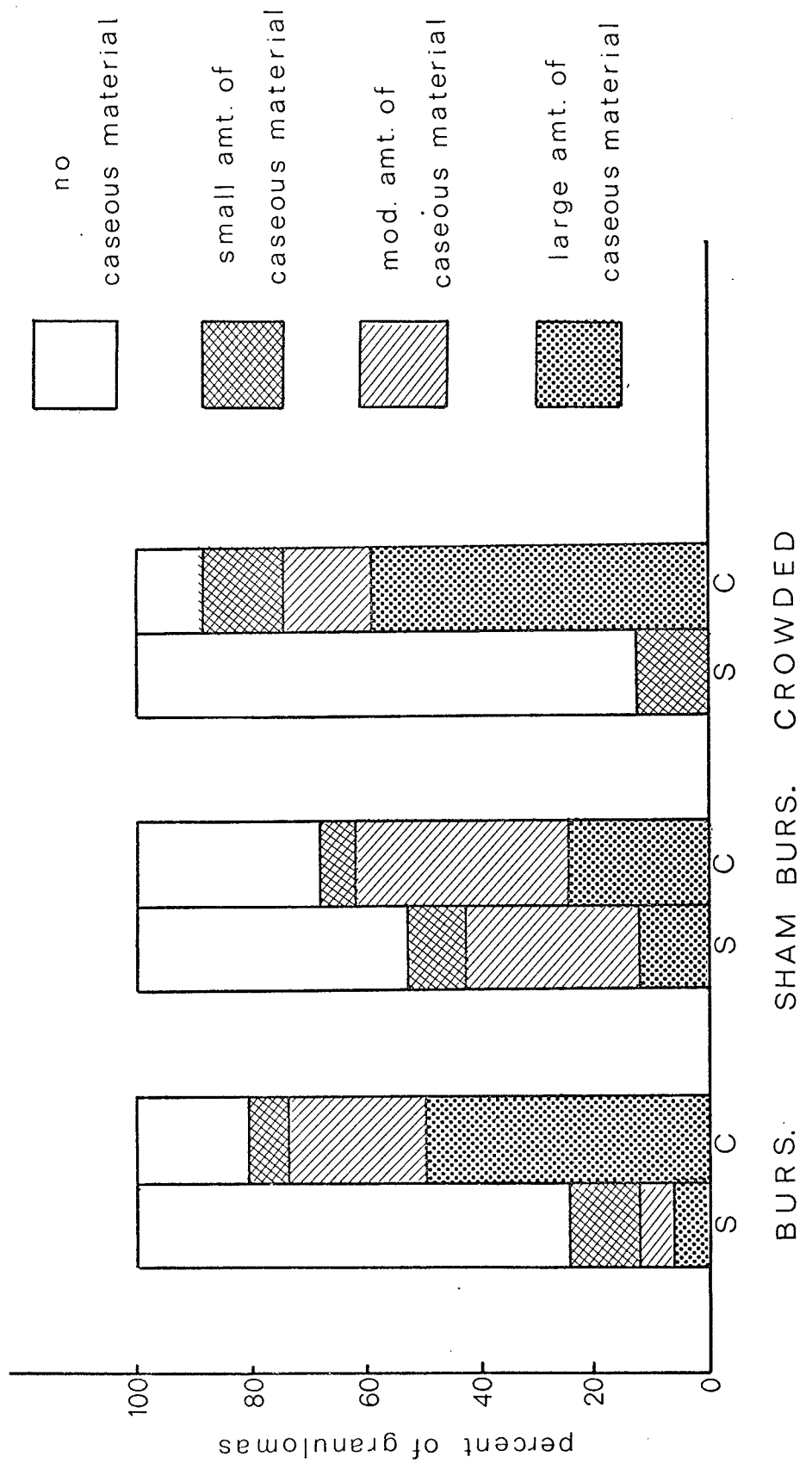


Fig 1. Histograms representing the amount of caseous material in granulomas of stressed (S) and control (C) ducklings in the Bursectomy Sham-Bursectomy and Crowding Experiments.

PART III CROWDING EXPERIMENT II

INTRODUCTION

Titman (1967) described the behavioural disturbance of a crowded population of breeding mallards. The abnormal behaviour resulted in considerable juvenile mortality. Titman interpreted this as an inherent mechanism acting to control the growth of this population. Observations of overcrowding in mice indicated that lowered resistance to disease may be an additional factor contributing to mortality and hence the control of animal populations (Christian, 1968).

Procedure

Ten ducklings stressed by crowding and 12 controls were given the standard nematode inoculum. Crowding began when ducklings were 34 days old, one day before inoculations were begun, and for the duration of the experiment. The area available to each crowded duckling was .07 m^2, controls had 0.9 m^2 each. Ducklings were necropsied 42 days after inoculation.

Observations

Ducklings stressed by crowding retained a greater mean number of nematodes and longer nematodes than controls (Table VI Fig. 12,13), and had a larger mean number of granulomas (Table VI). Granulomas of control ducklings contained a larger amount of caseous material than those of stressed ducklings (Fig. 1). Mean weight gain and mean K/S ratios of crowded ducklings was less than that of controls. Mean weights of bursae and thymi of crowded ducklings were less and mean weights of adrenals were greater per unit body weight than those of controls (Table VI Figs.4,5). Mean weights of stressed and control ducklings at necropsy were 920 $\pm$ 23 gm

TABLE VI

Crowding Experiment II: comparison of the means of the numbers of nematodes, lengths of nematodes, worm burden indices, organ weights, weight gain and K/S ratios of ducklings stressed by crowding and controls.

	Crowded	Control	d.f.	t.	P<
Number of nematodes	78.6	35.7	20	3.690	0.005
Length, female nematodes (mm)	11.49	7.69	380	23.363	0.005
Length, male nematodes (mm)	9.13	7.20	845	21.777	0.005
Worm burden ** index, female nematodes	368.01	100.50	20	5.348	0.005
Worm burden ** index, male nematodes	417.69	136.37	20	4.551	0.005
Numbers of granulomas	6.2	4.8	20	1.832	0.005
Bursa weights *	1.171	1.742	20	3.451	0.005
Thymus weights *	2.151	6.123	20	5.549	0.005
Adrenal weights *	2.946	1.714	20	4.014	0.005
Weight gain (g)	241	393	20	3.769	0.005
K/S ratios	0.65	0.81	20	6.819	0.005

*Expressed as a ratio of body weight X 10^{-4}

** Mean of the total length of female and of male nematodes from crowded and control groups of ducklings.

and 1090 ± 31 gm respectively.

Observations on plumage and aggressive behaviour of ducklings stressed by crowding were similar to those reported of crowded ducklings in the stress survey experiment.

Discussion

There is evidence that crowding is a stressor in wild populations of fowl. Neave and Wright (1968) found that adrenal weights of breeding ruffed grouse increased with increased population density. Titman (1967) studied the behavioural aspects of overcrowding in a breeding population of mallard ducks. He observed a breakdown in social behaviour resulting in fighting, rape, a high frequency of nest parasitism, reduced broodiness among females and abandonment of young. A high mortality of ducklings was noted, apparently due to exposure and lack of parental care. It can be assumed that such a situation was stressful for the birds.

In the present experiment, ducklings maintained under crowded conditions exhibited signs of the stress response similar to that described in fowl (von Faber, 1964b). These signs were correlated with the retention of significantly more and larger nematodes than that of controls. This demonstrated a direct relationship between stress by crowding and increased parasite loads in mallard ducklings.

Titman's (1967) study was made at the Delta Waterfowl Research station in 1966 and 1967. At this time 250 ducks utilized the research station pond and a much smaller (but unknown) number utilized the back marsh. In 1962 an estimated 30% of the ducks at the research station were reported to have died of echinuriasis (Cornwell, 1963). No observations were made on the behaviour of this population but it is known that an estimated 400 ducks utilized the Delta pond and the back marsh that year. In light of data

obtained in the present experiment and that of Titman (1967) a stressful situation may have occurred on the pond at the time of Cornwell's study (1963). Crowding may have lowered the resistance of the ducks to infection resulting in the epizootic of E. uncinata.

The effect of crowding on parasitism was described by Noble (1961; 1962) who found that crowding and fighting in ground squirrels resulted in an increased number of Trichomonas. Weinmann (1967) found that crowding and fighting hindered the development of acquired immunity and increased the infection rate of immune mice with Hymenolepis nana.

Crowding of mallard ducklings involves at least three functional stressors: (1) confinement in a small space; (2) feather wetting and shivering; (3) social disturbance (pecking, feather pulling) due to crowding.

If an animal develops a specific resistance to a particular stressor its resistance to another stressor may be lowered (cross sensitization) (Selye, 1950). As crowding involves at least types of stressors, this may provide an explanation for the marked effect of crowding in mallard ducklings.

An alternative explanation is that typical symptoms of the G.A.S. manifest themselves only during the alarm reaction and the stage of exhaustion (Selye, 1956). Ducklings may not have adapted to the crowding stress (stage of resistance), or perhaps they were only stressed when they were fighting, or when they were wet. If such was the case, the marked signs of the G.A.S. noted in crowded ducklings may have been a result of intermittent stages of shock (AR). The large parasite burdens retained by the crowded ducklings could be

due to the increased adrenal cortical activity during the AR, as adrenal hormones decrease an animal's resistance to disease (Selye,1956).

PART IV BURSECTOMY AND SHAM BURSECTOMY

INTRODUCTION

The bursa of Fabricius is important in antibody production in young fowl (Glick et al., 1956). A major manifestation of the G.A.S. in fowl is an involution of the bursa of Fabricius (von Faber, 1964b). It was suspected that the greater parasite load exhibited by stressed ducklings may be due to an inhibition of antibody response because of bursal involution.

The following experiment tests the use of crowding to induce stress and the importance of the bursa in antibody production to E. uncinata.

Procedure

Twenty-four bursectomized and 20 sham bursectomized ducklings were held until they were five weeks old. Twelve of the former and 10 of the latter were stressed by crowding and the remaining 12 bursectomized and 10 sham bursectomized ducklings were held as controls. Each duckling was given the standard nematode inoculum. Ducklings were necropsied and blood samples taken for antibody tests 42 days after nematode inoculation.

Observations

Bursectomized ducklings that had been stressed by crowding retained a greater mean number of nematodes which were longer than those of controls, and had a larger mean number of granulomas (Table VII, Figs 14,15). Granulomas of control ducklings contained more caseous material than those of controls (Fig 1). Crowded ducklings had smaller mean K/S ratios

and weight gain than controls. Mean weights of thymi of crowded ducklings were less and mean weights of adrenals were greater per unit body weight than those of controls (Table VII). None of the bursectomized ducklings regenerated the bursa of Fabricius. Mean weights of stressed and control ducklings at necropsy were .964 ± 28 gm and 1124 ± 36 gm respectively.

The mean number of nematodes retained by the sham bursectomized ducklings stressed by crowding was not significantly greater than that of controls. The mean lengths of female nematodes retained by crowded ducklings were significantly greater than those of controls though no difference was noted in lengths of male nematodes from the two groups. The mean number of granulomas of the two groups did not differ significantly (Table VIII Figs. 16, 17). Little difference was noted in the amount of caseous material in the granulomas of crowded and control ducklings (Fig 1).

Mean weight gain and mean K/S ratios of crowded ducklings were less than those of controls. Mean weights of bursae and thymi of sham-bursectomized ducklings stressed by crowding were less and mean weights of adrenals were greater per unit body weight than those of controls (Table VIII) mean weights of stressed and control ducklings at necropsy were 982 ± 28 gm and 1090 ± 29 gm respectively.

Antibody Response

Bursectomy did not prevent antibody production to E. uncinata (Figs. 18,19). There was no significant difference in antibody production of bursectomized and sham bursectomized control ducklings. Bursectomized ducklings stressed by crowding produced significantly less antibody than bursectomized control ducklings though there was no significant difference in antibody production of sham bursectomized control ducklings and those stressed by crowding.

TABLE VII

Bursectomy Experiment: comparison of the means of the numbers of nematodes, lengths of nematodes, worm burden indices, organ weights, weight gain and K/S ratios of bursectomized ducklings stressed by crowding and bursectomized controls.

	Crowded bursectomized	Control bursectomised	d.f	t.	P<
Number of nematodes	72.8	14.7	22	7.237	0.005
Length, female nematodes (mm)	11.21	8.22	422	12.077	0.005
Length, male nematodes (mm)	9.18	7.32	542	11.790	0.005
Worm burden ** index, female nematodes	372.54	54.13	22	7.412	0.005
Worm burden ** index, male nematodes	374.17	61.72	22	6.018	0.005
Numbers of granulomas	5.3	2.8	22	2.965	0.005
Thymus weights *	4.014	5.999	22	2.318	0.025
Adrenal weights *	2.731	1.666	22	4.244	0.005
Weight gain (g)	352	511	22	4.079	0.005
K/S ratios	0.74	0.80	22	2.861	0.005

* Expressed as a ratio of body weight X 10^{-4}

** Mean of the total length of female and of male nematodes from crowded and control groups of ducklings.

TABLE VIII

Sham Bursectomy Experiment: comparison of the means of numbers of the nematodes, lengths of nematodes, worm burden indices, organ weights, weight gain and K/S ratios of sham bursectomized ducklings stressed by crowding and sham bursectomized controls.

	Crowded sham bursectomized	Control sham bursectomized	d.f.	t.	P<
Number of nematodes	63.4	37.6	18	1.675	0.10
Length, female nematodes (mm)	11.08	10.35	376	4.031	0.005
Length, male nematodes (mm)	8.94	8.61	531	3.621	0.005
Worm burden ** index, female nematodes	296.54	147.94	18	1.786	0.05
Worm burden ** index, male nematodes	329.39	192.84	18	1.587	0.10
Numbers of granulomas	6.1	4.5	18	1.092	0.10
Bursa weights *	1.354	2.196	18	3.771	0.005
Thymus weights *	5.204	6.900	18	2.618	0.01
Adrenal weights *	0.254	0.201	17	2.438	0.025
Weight gain (g)	316	411	18	1.786	0.05
K/S ratios	0.75	0.81	18	2.388	0.025

*Expressed as a ratio of body weight X 10^{-4}

**Mean of the total length of female and of male nematodes from crowded and control groups of ducklings.

Discussion

Both stress and injections of cortisone lower the resistance of animals to a number of infectious organisms (Noble, 1961; 1966a; 1966b; Robinson, 1961; Weinmann and Rothman 1961; 1967; Briggs, 1963; Soave, 1964; Ogilvie, 1965; Sherman and Ruble, 1967). In the present experiments mallard ducklings stressed by crowding or injected with cortisone retained a significantly larger number of nematodes at necropsy than the controls. These ducklings also exhibited significantly smaller bursae and thymi than controls. These structures are necessary for the attainment of immune competence in fowl (Glick, 1960; 1970; Warner and Szenberg, 1962; 1963; Warner, 1967), though little is known of their function in ducks.

The bursa, thymus and spleen have been collectively termed immunobiological tissue (I.B.T.) (Glick, 1969). Immunodepression by injections of cortisone may result from interference with the function of this tissue. Elliot and Sinclair (1968) found that injecting mice with cortisone results in a significant decrease in the production of antibody due to a depletion of lymphocytes in the blood and lymphoid tissue. Glick (1967) noted that chickens injected with cortisone produce less antibody to bovine serum albumin. There was an involution of both spleens and bursae with a reduction of germinal centers in the former and bursal follicles in the latter.

The plasmacytic germinal centers in the spleen are populated by cells originating in the bursa (Cooper et al., 1967; Jankovich and Mitrovic, 1967). Involution of this structure could conceivably cause a reduction in the number of cells migrating to the spleen and result in a decreased ability to produce antibody.

Antibodies are believed to play an important defensive role in

nematode infections (Thorson, 1953; 1956; 1963; Jackson, 1969). It was suspected that the decreased resistance of stressed ducklings in the present experiments was due to an inhibition of antibody synthesis because of bursal involution.

Experiments with bursectomized ducklings were designed to answer three questions: (1) if the bursa is necessary for the production of antibodies against E. uncinata, (2) if antibodies are functional in the immunological defence of the host against this parasite (3) if stress significantly inhibits antibody synthesis.

If bursectomized control ducklings did not produce antibodies to E. uncinata and had significantly greater nematode burdens than intact ducklings capable of antibody synthesis it could be concluded that (i) the bursa of mallard ducklings is necessary for the development of a competent antibody producing system and (2) antibodies function in the immunological defence of mallard ducklings against E. uncinata.

In addition, if sham bursectomized control ducklings produced more antibody than sham bursectomized ducklings stressed by crowding one could determine if stress inhibits antibody production to E. uncinata.

On the otherhand, if bursectomized ducklings stressed by crowding did not produce antibody to E. uncinata, and retained significantly greater nematode burdens than bursectomized controls, it could be concluded that stress affects resistance to E. uncinata by some other mechanism than antibody synthesis.

Bursectomy at two days of age failed to prevent the synthesis of antibodies by mallard ducklings to E. uncinata (Fig. 19). Consequently it was impossible to obtain much of the information that this experiment was designed to provide. Although antibody synthesis of sham bursectomized ducklings stressed by crowding did not differ significantly from that of

controls, a difference did occur in antibody production of the bursectomized stressed and control groups suggesting that stress inhibits antibody production in mallard ducklings.

A qualitative technique of antibody determination was used but a difference in the intensity of the precipitin bands permitted the grading of the strength of the response of each duckling.

Although these data suggest that stress inhibits antibody synthesis and results in decreased resistance to nematode infections, they do not exclude the possibility that lowered resistance of stressed ducklings may be due to some other factors. Another possible explanation for the lowered resistance of stressed ducklings to E. uncinata is considered in Part II of this thesis.

Bursectomized ducklings in this experiment produced antibodies in response to E. uncinata. The first of three possible explanations is that precipitin bands on the agar slides were not the result of antibody-antigen reactions but were due to some other unexplained phenomena, a possibility suggested by Gardner and Rosenberg (1969). If this was true then non-infected ducklings should show a similar response to that of an infected duckling, but serum from a non-infected control duckling did not produce precipitin lines with E. uncinata antigen. Therefore, the precipitin lines produced by serum from infected ducklings and E. uncinata antigens apparently were the result of an antibody-antigen reaction.

The second possibility is that the bursa plays no role in the production of antibody to E. uncinata. This explanation seems improbable considering the present information on the function of the bursa (Chang et al., 1955; Glick et al., 1956; Glick, 1957; 1970; Mueller et al.,1960;1962; Cooper

et al., 1967; Jankovic and Isakovic, 1966).

The third possibility is that bursectomy was performed too late to prevent a sufficient number of cells, capable of proliferating in response to prolonged antigenic stimulation by E uncinata), from migrating to the germinal centers of the spleen, Peyer's patches and caecal tonsils. There is considerable evidence to support this explanation:

The bursa of Fabricius of fowl begins to develop on the 12th. day of incubation. Shortly after hatching the follicles of the bursa are well developed elements containing cortex and medullary components (Good and Finstad, 1967). The bursa is well developed at hatching and it is likely that bursa-influenced cells are present in peripheral lymphoid organs at the time of neonatal bursectomy (Cooper et al., 1965). In addition, neonatal bursectomy reduces antibody response to single antigenic stimulus, though normal antibody response has been reported in neonatally bursectomized chicks upon repeated antigenic stimulation (Jankovic and Isakovic, 1966; van Alten, 1969). It was also reported that hyperimmunization of both control and bursectomized chicks was followed by abundant proliferation of plasmacellular elements in the tonsilla caecalis and the spleen (Isakovic and Jankovic, 1964).

Ducklings in the present experiment were bursectomized on the day after hatching and were infected for 42 days when blood samples were taken. It is conceivable that this prolonged antigenic stimulus caused the proliferation of cells that had migrated to the spleen, Peyer's patches and the caecal tonsils prior to bursectomy, resulting in the production of detectable amounts of antibody.

No significant difference was noted in numbers and sizes of nematodes or in antibody production of sham bursectomized crowded and

control ducklings. In addition, though sham bursectomized stressed ducklings did exhibit signs of the G.A.S. they were not as marked as those of crowded ducklings in the bursectomy or crowding II experiments. The failure of these ducklings to show significant differences in antibody production or in nematode burden may simply be that they were not as stressed as ducklings in the other experiments involving crowding as a stressor.

PART V CORTISONE INJECTION II

DELTA POND EXPERIMENT

INTRODUCTION

To study the effects of stress on parasitism in a natural setting three major requirements would have to be met.

(i) The ducklings would have to be maintained in an area having large numbers of parasites.

(ii) To properly evaluate the effect of stress on parasitism it would be necessary to have a preponderance of one species of parasite which would be used as an indicator.

(iii) It would be necessary to maintain stressed and control ducklings under exactly the same conditions to allow equal opportunity for each to ingest parasites.

The Delta Waterfowl Research Station pond fulfills the first two requirements because of the natural setting and the high incidence of a single species of parasite Echinuria uncinata.

Injections of cortisone acetate were used to fulfill the third requirement. Although it was not expected that injections of cortisone would imitate the natural conditions of stress, corticoid production increases during stress and participates in the overall response to all stressors (Selye, 1956). Therefore, the purpose of this experiment was to determine the effects of increased cortical activity on nematode burdens, using a synthetic adrenal hormone. In addition, injections of cortisone were used successfully to induce signs of the G.A.S. in ducklings of the stress survey experiment and proved to be the most convenient method of maintaining treated and control birds under exactly the same conditions.

Procedure

Twenty-five ducklings were maintained in an outdoor enclosure on the Delta Waterfowl Research Station pond. Ducklings became infected naturally by ingesting Daphnia spp. harbouring infective juveniles of E. uncinata.

Twelve control ducklings were injected once every three days with 0.5 cc of saline. Thirteen ducklings were injected once every three days with 25 mg of cortisone acetate per kg body weight. Injections were continued for a period of 42 days. Ducklings were weighed every third injection and dosages adjusted accordingly.

Observations

Necropsy revealed that ducklings injected with cortisone had both a greater mean number of nematodes and a greater mean number of granulomas than controls. The mean weight gain and K/S ratios of injected ducklings were smaller and the mean weights of bursae and thymi per unit body weight were less than that of controls. Mean weights of adrenals per unit body weight of the injected ducklings did not differ significantly from that of controls (Table IX). Mean weights of stressed and control ducklings at necropsy were 931 ± 25 gm and 1178 ± 40 gm respectively.

Discussion

Mallard ducklings injected with cortisone developed signs of the G.A.S. and had larger numbers of nematodes than controls.

Hyperinfection may not be a normal occurrence but may be the result of a factor or factors which affect the resistance of mallard ducklings to E. uncinata. This study demonstrated experimentally that injections of cortisone can contribute to hyperinfection of ducklings with E. uncinata in a natural setting.

TABLE IX

Delta Pond Experiment (Cortisone II): comparison of the means of the numbers of nematodes, organ weights, weight gain and K/S ratios of ducklings injected with cortisone and controls.

	Cortisone	Control	d.f.	t.	P<
Number of nematodes	180.5	72.2	23	2.133	0.025
Numbers of granulomas	7.0	4.7	23	3.090	0.005
Bursa weights *	1.422	2.261	23	4.558	0.005
Thymus weights *	2.194	6.294	23	6.531	0.005
Adrenal weights *	1.657	1.826	23	1.227	0.10
Weight gain (g)	295.8	506.4	23	5.062	0.005
K/S ratios	0.76	0.80	23	2.332	0.025

* Expressed as a ratio of body weight X 10^{-4}

The first cortisone experiment (stress survey study) showed that birds given the standard nematode inoculum retained infections longer than controls. In the present experiment ducklings injected with cortisone had a significantly greater number of nematodes than controls. This suggests that stressed ducklings are unable to rid themselves of their nematodes as rapidly as normal birds and accumulate a larger number of nematodes because the turnover may simply not be as great. This assumption is supported by the observation that the granulomas of control ducklings contained large amounts of caseous material interspersed with dead nematodes indicating that old infections are overcome even when new infections are being acquired.

Although corticosterone has been identified as the major adrenocortical hormone of the avian adrenal gland (Sandor et al., 1963; Nagra et al., 1960), the synthetic mammalian adrenocorticoid, cortisone acetate, was used successfully in this experiment to induce signs of the G.A.S. in ducks. Injections of cortisone acetate reduced the size of the bursae and thymi and retarded growth of mallard ducklings. Similar signs have been reported for chickens injected with cortisone acetate (Glick, 1957; Huble, 1958; Gavora and Kondra, 1970).

The amount of cortisone injected into ducklings in the present experiment (25 mg of cortisone / kg body weight, once every three days) produced signs of the G.A.S. of similar magnitude as that of crowding ducklings in a 0.61 m x 1.32 m enclosure (Tables VI and VII).

Injected ducklings may have contained a larger number of nematodes than is indicated by our data. Two kinds of granulomas appeared in these ducklings. One was a closed granuloma, the other was an open granuloma. Several of the ducklings treated with cortisone had granulomas of the latter type. Although it is possible that they may have formed open,

it appeared as though these granulomas had formed in a manner similar to the closed granulomas and may have contained large numbers of nematodes, but at some time during development these granulomas opened up allowing their contents to pass into the lumen. Only a few nematodes were found under the caseous material lining the cavity.

GENERAL DISCUSSION

It is established that parasitism of mallard ducklings by E. uncinata is affected by both crowding and injections of cortisone. Crowded ducklings have greater numbers and larger nematodes than controls. The numbers and sizes of nematodes are correlated with the amount of caseous material present in the granulomas. Caseous material is more abundant in granulomas of control ducklings than in stressed birds.

The larger sizes and greater numbers of nematodes are correlated with greater numbers of granulomas in stressed ducklings resulting in a more severe pathogenesis. Hansen et al.(1957) found that gizzard worms, Amidostomum anseris, were present in larger numbers in geese that had ingested lead shot (presumably because of the stressful effect of lead shot on the host). The pathology was more pronounced in hosts with lead shot than in geese that had no lead shot.

Injections of cortisone, deficient nutrition, and crowding result in an involution of the bursae and thymi of mallard ducklings and (with the exception of cortisone treatment) an enlargement of the adrenals (Tables VI-IX). Stressed ducklings also showed such outward signs as poor plumage, muscular weakness, and emaciation. Such signs were described by investigators reporting severe outbreaks of helminthiasis in waterfowl (Shaw, 1924; Herman et al., 1955; Cornwell, 1963). If these signs are a result of stress as indicated by the present experiments, stress factors may have been involved in these outbreaks. It is therefore, recommended in future studies of severe helminthiasis in

waterfowl to consider the presence of other factors such as shortage of food, pollution and crowding.

Stress and injections of cortisone are associated with a depression of immunological responsiveness (Glick, 1967; Elliot and Sinclair, 1968; Thaxton and Siegel, 1970) and lowered resistance to parasitism (Noble, 1961; 1962; Parker, 1961: Weinmann and Rothman, 1961; 1967). Decreased resistance following immunosuppressive treatment of hosts is an indication of the immunological nature of such resistance (Sollod,1971). Therefore, the differences observed in nematode burden of control ducklings and those injected with cortisone or stressed by crowding support the hypothesis that the resistance of ducklings to E. uncinata is immunologically mediated.

Arrested development occurs in many helminth infections. Many types of arrested development are undoubtedly due to the expression of an immune response (W.H.O. 1965). This further suggests that the resistance to E. uncinata is immunologically mediated, as control ducklings in the present experiments usually had, at necropsy, smaller nematodes than stressed ducklings.

Mallard ducklings produce antibodies to E.uncinata. Bursectomized ducklings stressed by crowding produced significantly less antibody and had significantly greater nematode burdens than controls. In addition, crowded ducklings had granulomas containing far less caseous material than controls. The cellular nature of this caseous material suggests the involvement of a cellular immune response to E. uncinata.

This investigation indicates, therefore, that both a cellular and humoral immune response is elicited by E. uncinata in mallard ducklings and that stress inhibits both responses and results in the retention of greater numbers and larger nematodes.

Mortality of wild ducks due to echinuriasis has been attributed to occlusion of the gut by the granulomas (Cornwell, 1963). In the present study the standard inoculum of 150 infective E. uncinata juveniles was not considered to cause a severe infection. The ducklings raised on the Delta pond had larger granulomas than those fed a standard inoculum. In the most heavily infected individuals the gut was enlarged and no compaction occurred (Figs. 6, 7, 8, 9). This suggests that compaction of the oesophagus may only occur in severe infections.

CONCLUSIONS

I Crowding is a stressor in mallard ducklings and results in enlargement of the adrenals, a reduction in the size of bursae and thymi and a retardation of growth.

II Mallard ducklings given a standard inoculum of nematodes and stressed by crowding retain a significantly larger number and significantly longer nematodes at necropsy than controls.

III A diet deficient in protein produces signs of the G.A.S. in mallard ducklings given a standard inoculum of E. uncinata, but does not cause a retention of a greater number of nematodes.

IV Neither lead shot ingestion nor heat exposure produce signs of the G.A.S. in mallard ducklings given a standard inoculum of E. uncinata and do not cause a retention of a greater number of nematodes.

V Caseous material consists of aggregations of eosinophilic cells in various stages of disintegration.

VI Granulomas of ducklings stressed by crowding contain less caseous material than those of controls.

VII Large amounts of caseous material are associated with nematode stunting and mortality.

VIII Mallard ducklings bursectomized within two days of hatching and given a standard inoculum of nematodes do not produce significantly less antibody than non-bursectomized ducklings given the same inoculum.

IX Injecting mallard ducklings with cortisone acetate results in a reduction in the size of bursae and thymi and a retardation of growth.

X Mallard ducklings injected with cortisone and given a standard inoculum of nematodes retain a significantly larger number of nematodes at necropsy than controls.

XI Mallard ducklings injected with cortisone and allowed to become infected naturally harbor a significantly greater number of nematodes than controls.

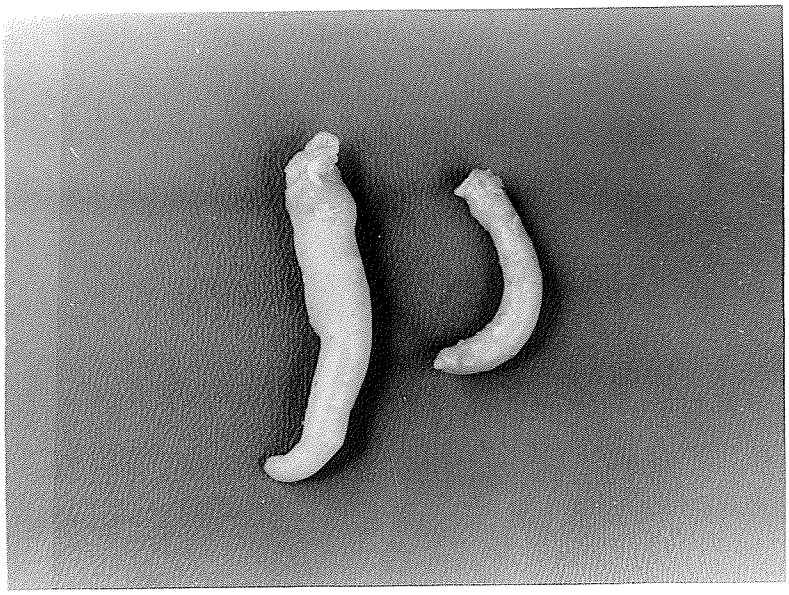
XII Bursectomized ducklings stressed by crowding and given a standard inoculum of E. uncinata produce less antibody than bursectomized controls given the same inoculum.

Fig. 2 Stressed ducklings in crowding enclosure.

Fig. 3 Control ducklings.

Fig. 4 Bursae from control (left) and stressed (right) ducklings Crowding II Experiment.

Fig. 5 Thymi from control (left) and stressed (right) ducklings Crowding II Experiment.



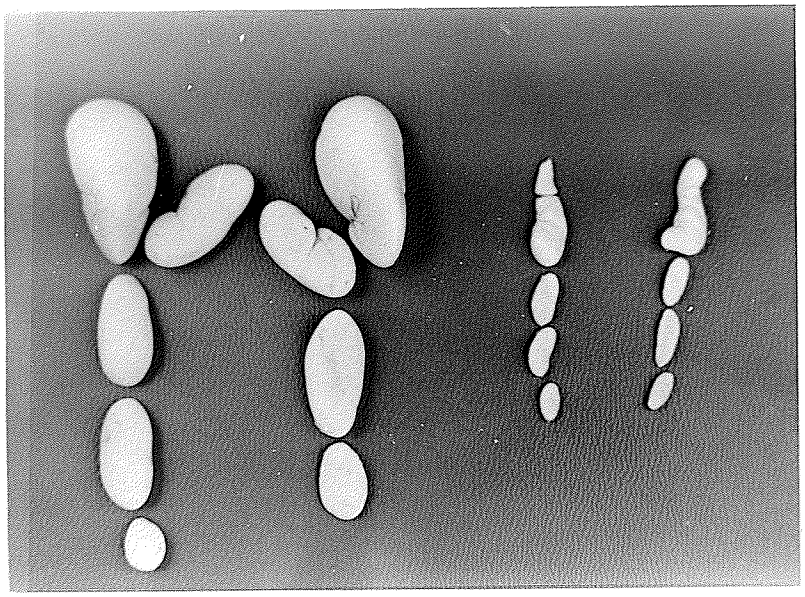


Fig. 6 Proventriculus and gizzard of duckling heavily infected with *E. uncinata*.

Fig. 7 Proventriculus and gizzard of non-infected duckling.

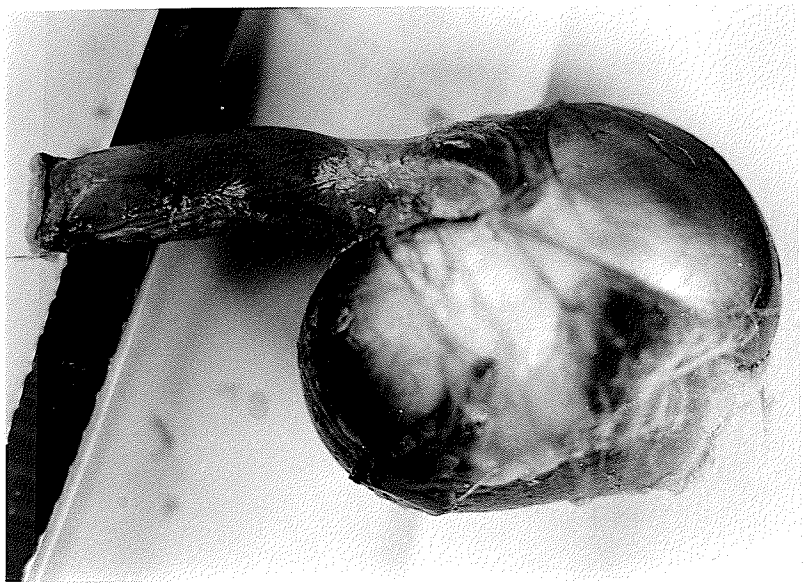
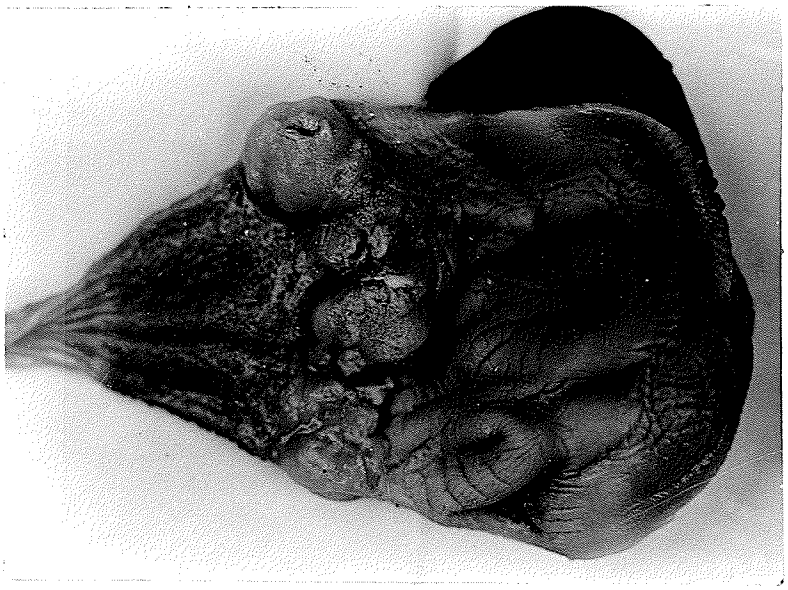


Fig. 8 Proventriculus and gizzard of duckling heavily infected with E. uncinata, cut open to expose the granulomas and the increase in size of the junction of the proventriculus and the gizzard.

Fig. 9 Proventriculus and gizzard of non-infected duckling cut open to show the normal condition of the junction of the proventriculus and the gizzard.



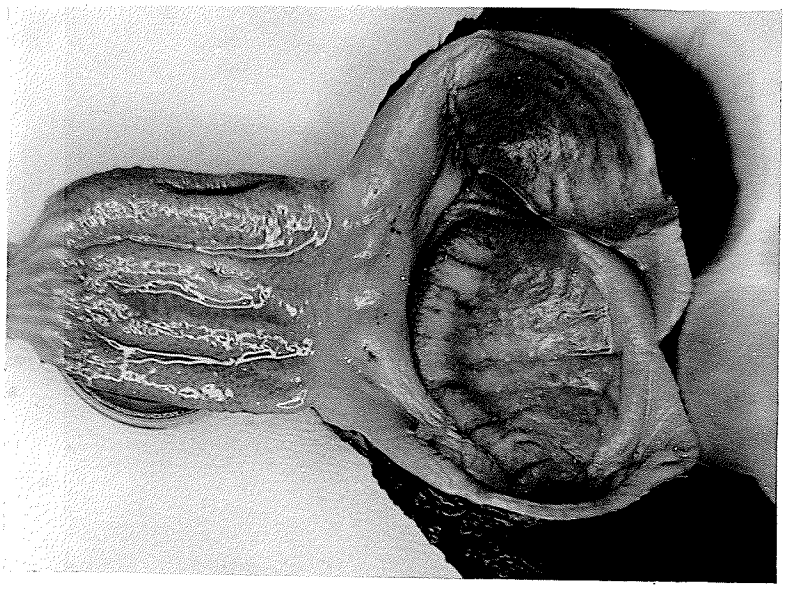
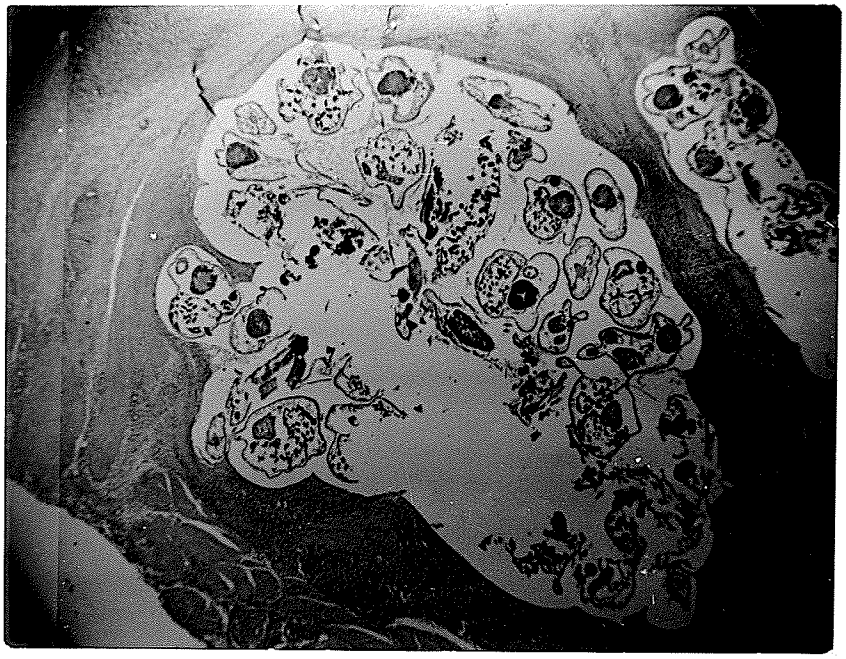
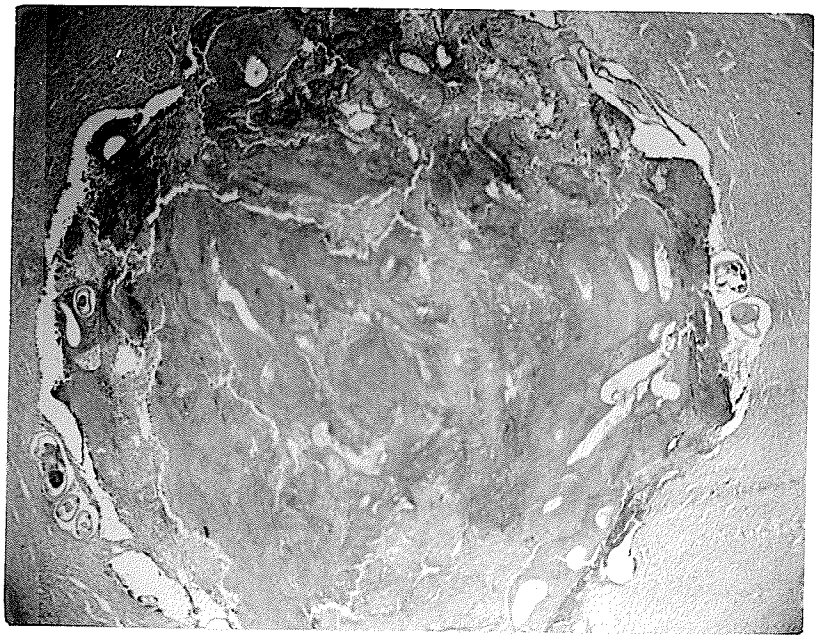


Fig. 10 Histological cross section of a granuloma from a stressed duckling showing cavity containing nematodes (X30).

Fig. 11 Histological cross section of a granuloma from a control duckling showing the caseous core with stunted nematodes around the periphery (X30).





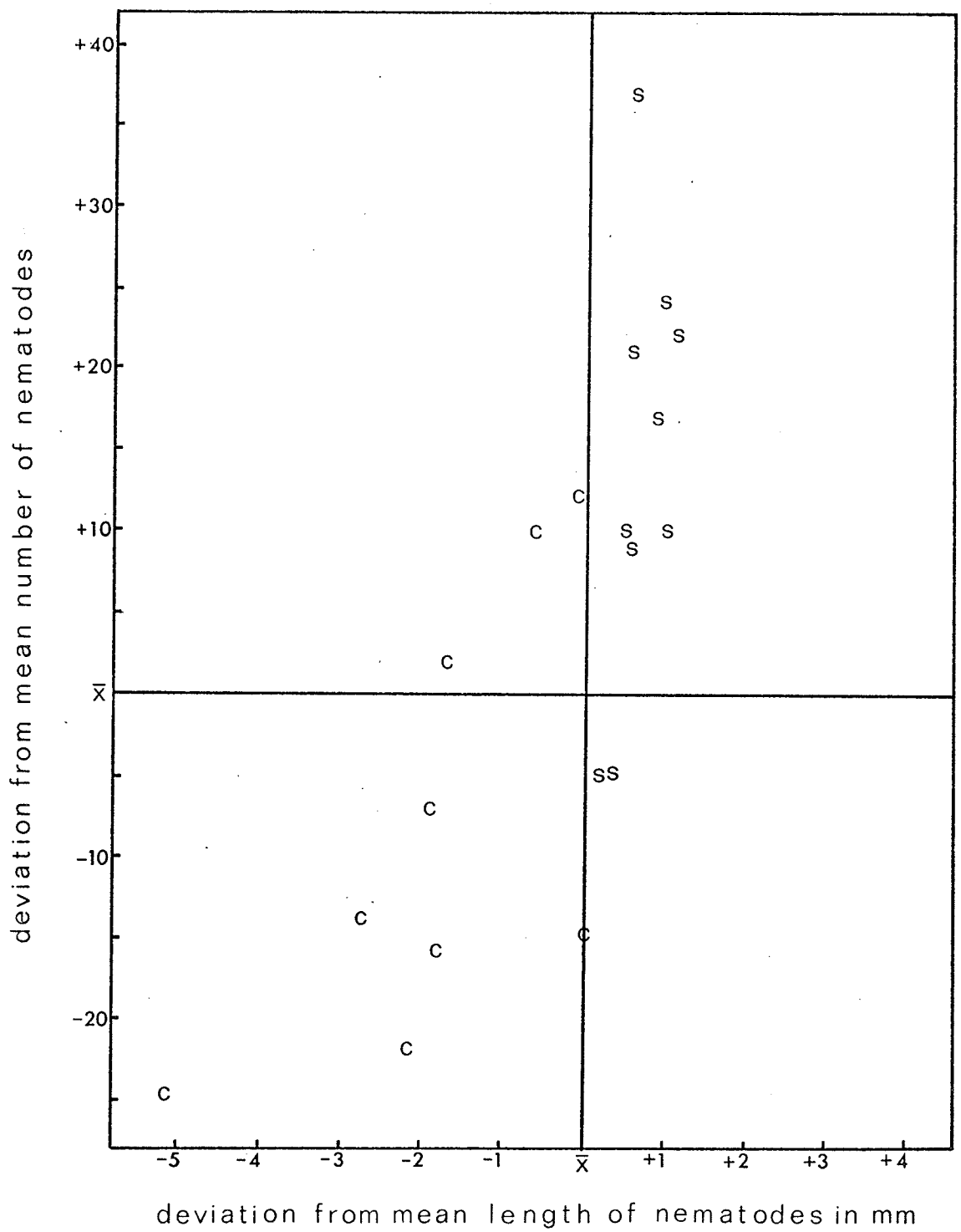


Fig.12 Crowding II Experiment. Distribution of number of female nematodes and mean length measurements for each stressed and control duckling

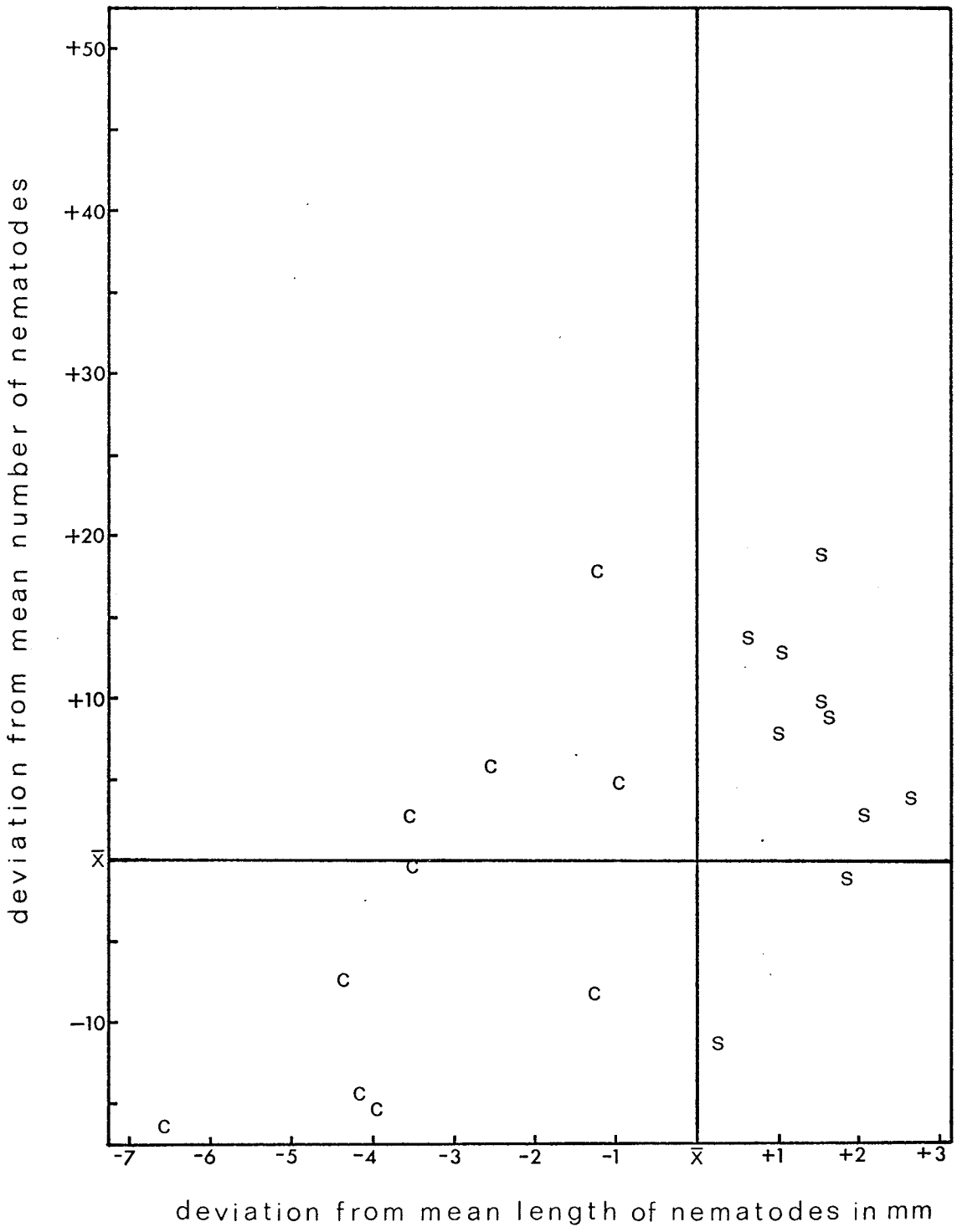


Fig .13 Crowding II Experiment. Distribution of number of male nematodes and mean length measurements for each stressed and control duckling

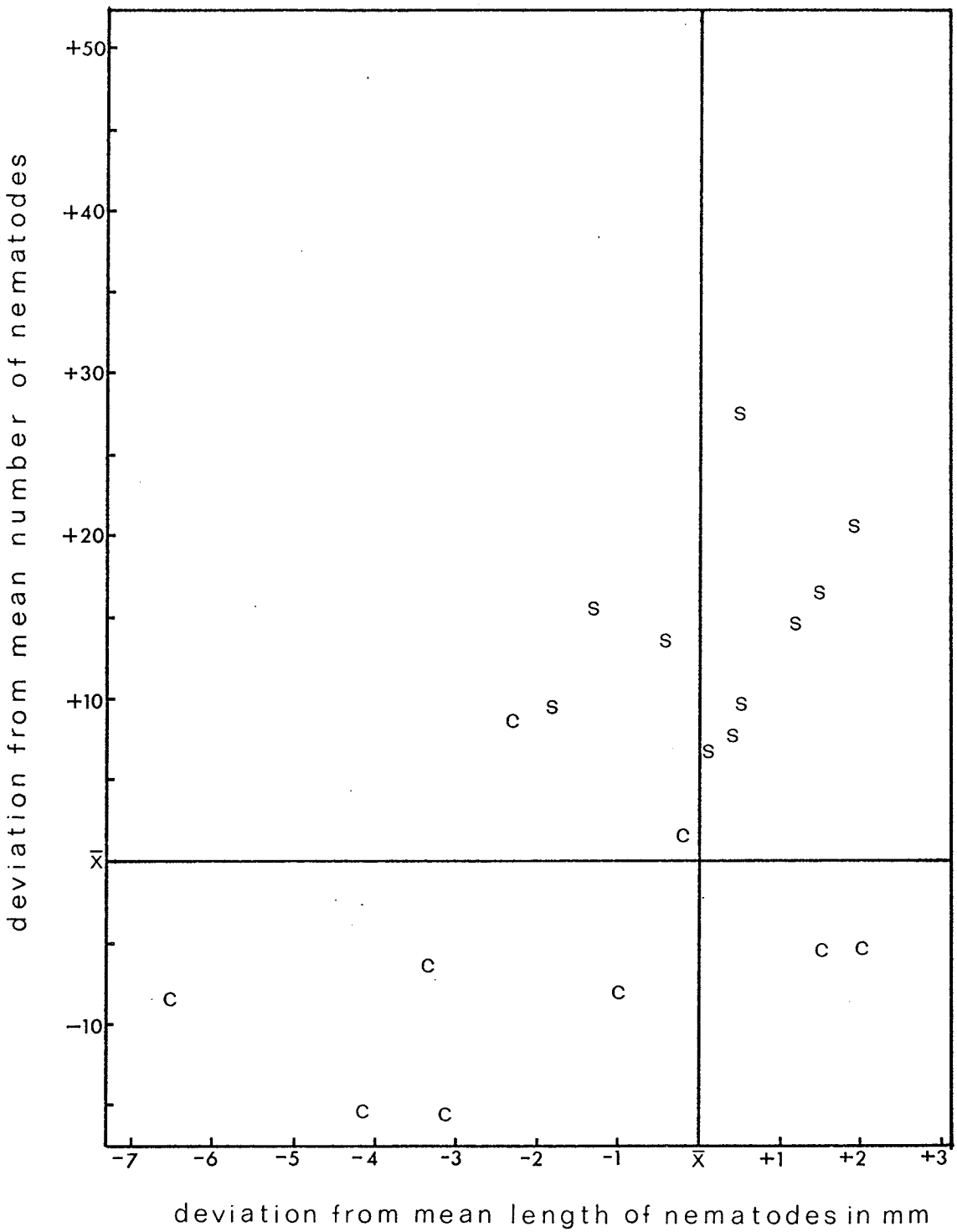


Fig.14 Bursectomy Experiment, distribution of number of female nematodes and mean length measurements for each stressed and control duckling

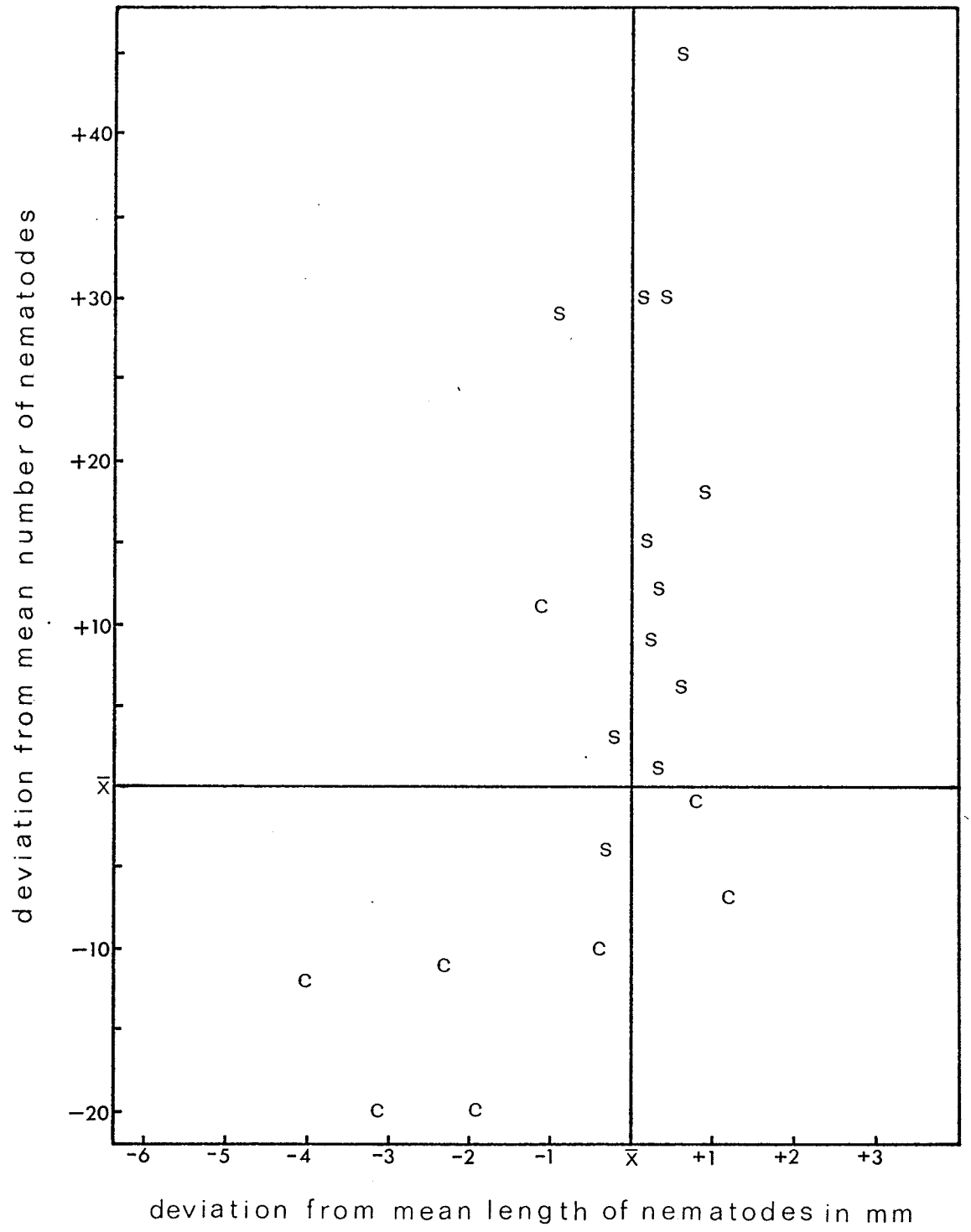


Fig.15 Bursectomy Experiment, distribution of number of male nematodes and mean length measurements for each stressed and control duckling.

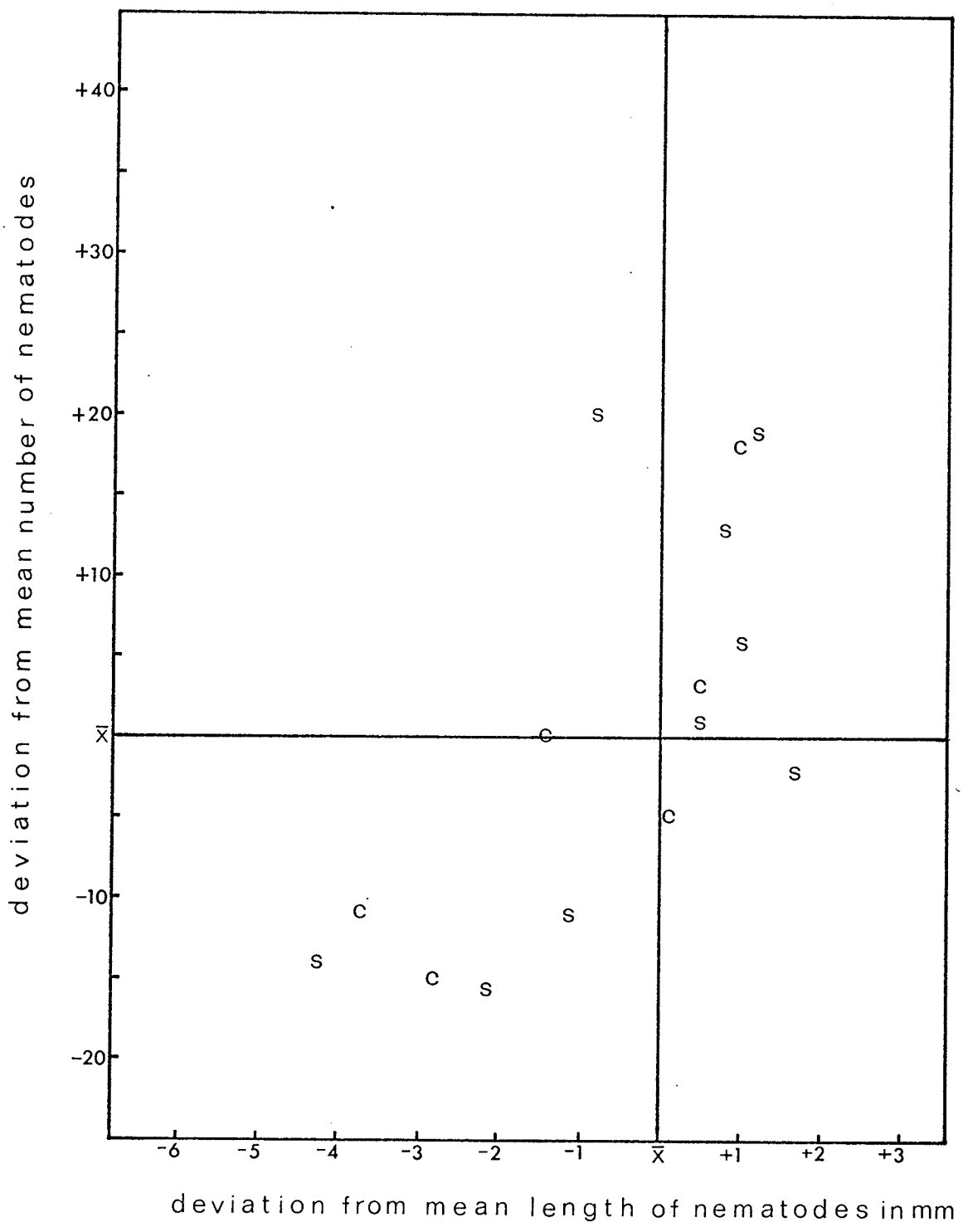


Fig.16 Sham Bursectomy Experiment, distribution of number of female nematodes and mean length measurements for each stressed and control duckling.

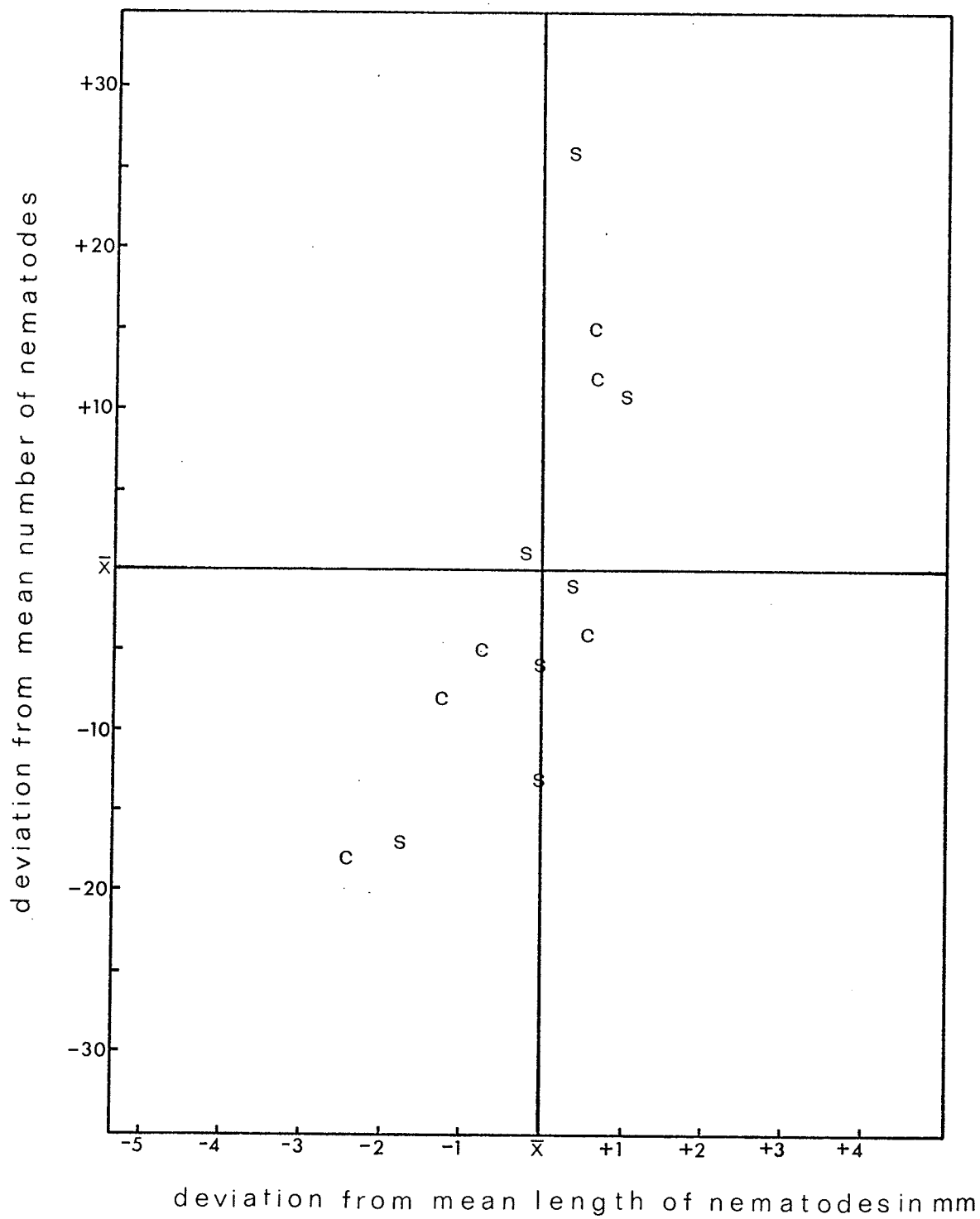


Fig.17 Sham-Bursectomy Experiment. Distribution of number of male nematodes and mean length measurements for each stressed and control duckling.

Fig. 18 EXPERIMENT III Grading of intensity of precipitin bands by two observers (Grades 1 and 2) giving the mean grade for bursectomized crowded (Group I), bursectomized control (Group II) sham bursectomized crowded (Group III) and sham bursectomized control (Group IV).

GROUP I BURSECTOMIZED, CROWDED

SERUM SAMPLE NO.	GRADE 1	GRADE 2
1	0	0
2	0	0
3	1	1
4	1	2
5	1	1
6	0	0
7	1	0
8	1	1
9	1	2
10	1	2
11	1	1
12	1	1
TOTAL	9	11
MEAN	.75	.92

GROUP III SHAM BURSECTOMIZED CROWDED

SERUM SAMPLE NO.	GRADE 1	GRADE 2
26	1	2
27	0	1
28	3	3
29	3	3
30	3	3
31	0	0
32	3	3
33	3	3
34	0	0
35	0	0
TOTAL	16	18
MEAN	1.60	1.80

GROUP II BURSECTOMIZED CONTROL

SERUM SAMPLE NO.	GRADE 1	GRADE 2
13	1	1
14	3	3
15	3	3
16	1	1
17	3	2
18	2	2
19	1	1
20	2	2
21	3	2
22	3	2
23	2	2
24	2	2
25	1	2
TOTAL	27	25
MEAN	2.08	1.92

GROUP IV SHAM BURSECTOMIZED CONTROL

SERUM SAMPLE NO.	GRADE 1	GRADE 2
36	3	3
37	2	2
38	0	0
39	2	3
40	0	0
41	1	2
42	2	3
43	3	3
44	3	3
45	3	3
TOTAL	19	21
MEAN	1.90	2.10
NON-INFECTED CONTROL	0	0

Fig. 19 Statistical analysis (Mann-Whitney test) of grades assigned to intensity of precipitin bands resulting from antibody-antigen reactions, graded by two observers.

BURSECTOMY STRESSED AGAINST BURSECTOMY CONTROL

Observer 1	Observer 2
T= 106.5	T= 96
P 0.01	P 0.01
To.01= 109	To.01= 109

i.e. control shows greater antigen - antibody reactions

SHAM BURSECTOMIZED STRESSED AGAINST SHAM BURSECTOMIZED CONTROL

Observer 1	Observer 2
T= 109	T= 101.5
P 0.05	P 0.05
To.05= 81	To.05= 81

i.e. no difference in antibody - antigen reactions

BURSECTOMIZED CONTROL AGAINST SHAM BURSECTOMIZED CONTROL

Observer 1	Observer 2
T= 135	T= 117
P 0.05	P 0.05
To.05= 88	To.05=88

i.e. no difference in antibody - antigen reactions

BURSECTOMIZED STRESSED AGAINST SHAM BURSECTOMIZED STRESSED

Observer 1	Observer 2
T= 115	T= 124.5
P 0.05	P 0.05
To.05= 85	To.05= 85

i.e. no difference in antibody - antigen reactions

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APPENDICES

APPENDIX I

PHOTOGRAPHS OF BURSECTOMY PROCEDURE

Fig 1. Injection of anaesthetic

Fig 2. View of region between anus and tip of uropygium after being shaved of down


10 20 30

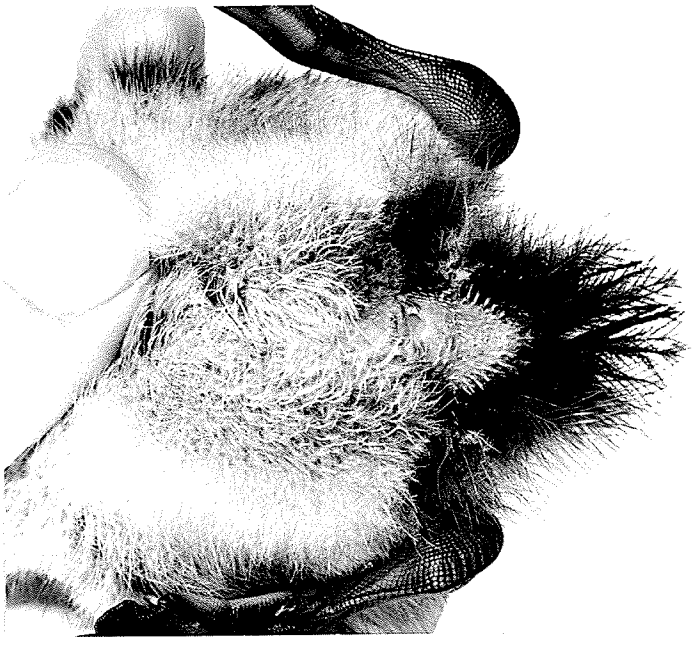


Fig 3. Incision made at base of uropygium ventral to the anus

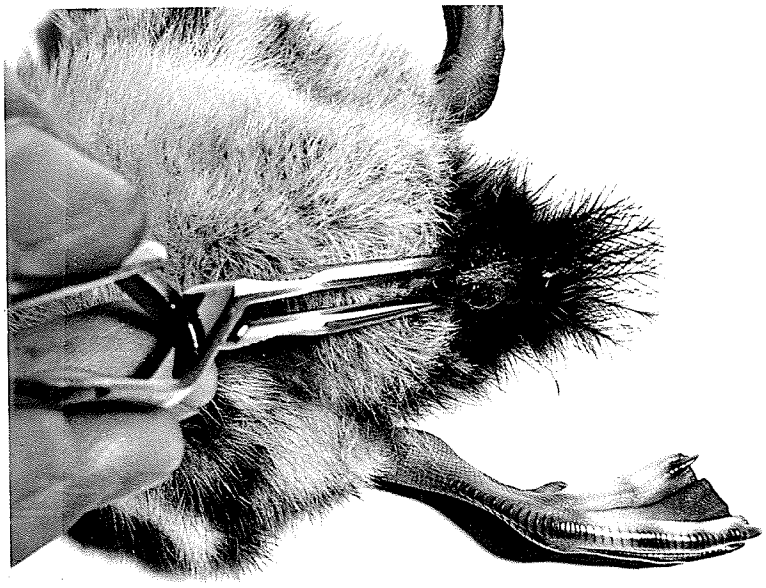
Fig 4. Bursa of Fabricius protruding through the incision

Fig 5. Placing ligature at base of bursa

Fig 6. Excision of the bursa

Fig 7. Wound clip being applied to close the incision

Fig 8. Position of 9 mm wound clip at completion of surgery



APPENDIX II

DATA SHEET

FOR DUCK

EXAMINATIONS

EXPERIMENTAL DATA

Exp. No._____ Start Date_____ Aut. Date_____

Spec. Date_____ Code_____ Pen No._____

Sp._____ Age_____ Sex_____

Wt._____ Weight Difference
Jar & Vial No._____ Slide No._____

K.S. Ratio_____ Adrenal Wt._____ Bursa Wt._____

Thymus Wt._____ Remarks _____

Nematode Sp._____ Date inf._____ No. of Larvae_____

No. of Mature_____ Days stress/inf._____ Source_____

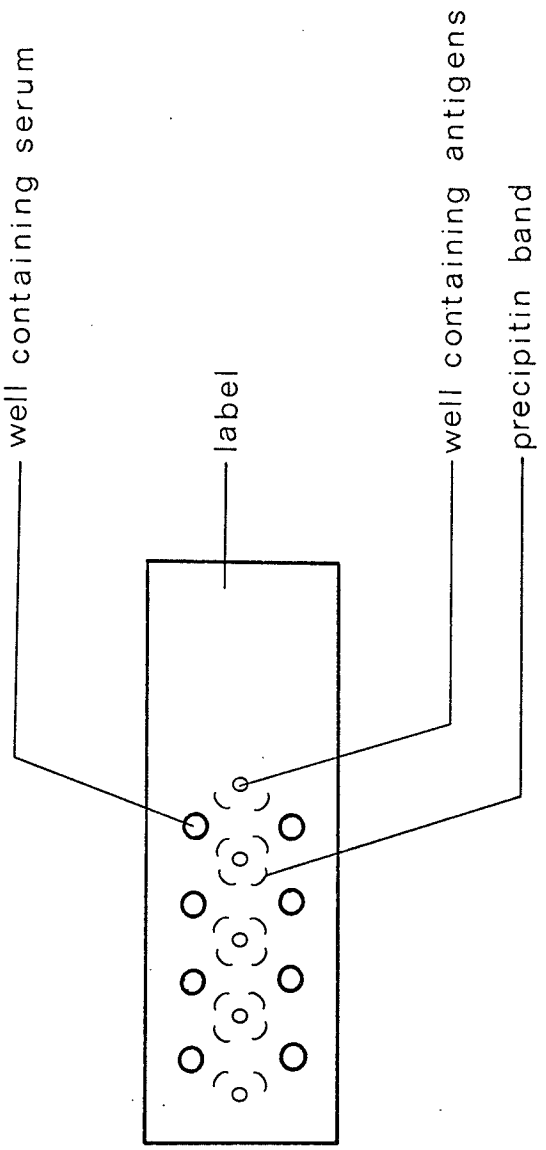
Remarks_____

Other Parasites_____

Remarks_____

APPENDIX III

Diagram of agarose slides used in antibody determinations, showing position of wells containing antigens and serum, as well as positions of precipitin bands


well containing serum
label
well containing antigens
precipitin band