Studies on

THE PATHOLOGY OF AVIAN LEUKOSIS COMPLEX IN POULTRY

THESIS

Submitted to the Faculty of

Veterinary Science,

Magadh University,

In Partial Fulfilment Of The Requirements For The Degree Of

M. Sc. (VETERINARY)

SATYA DEV SHARMA

B. V. Sc. & A. H.

Post Graduate Department of Pathology

BIHAR VETERINAY COLLEGE, PATNA.

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Prof. P.B. Kuppuswamy,
B.A.,G.M.V.C., B.V.Sc.,
P.G.(Newzealand), M.S.(Missouri),
Principal & Head of the Department.

Post-Graduate Department of Pathology,
Bihar Veterinary College,
PATNA.

This is to certify that the entire work presented in the thesis entitled " Studies on the Pathology of Avian Leukosis Complex in Poultry" is the bonafide work of Shri Satya Dev Sharma, a candidate for the degree of M. Sc. (Vet.) with Pathology as his major subject, which was carried out under my supervision and guidance.

P.B. Kuppuswamy
(P.B. KUPPUSWAMY)
31/12/67

31st DECEMBER, 1967.

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(SATYA DEV SHARMA)

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INTRODUCTION

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I N T R O D U C T I O N

Our country is passing through a great crisis owing to acute shortage of food. Increased production of food of animal origin would help in solving the food crisis to a great extent. Late Prime Minister Mr. Lal Bahadur Shastri considered food as a more potent weapon than conventional armaments and was an untiring advocate of adequate production and utilization of animal protein rich foods like fish, eggs and poultry meat to relieve pressure on cereals.

Food, to all intents and purposes is a more powerful weapon of defence than conventional armaments. A well-fed soldier will certainly rout his semi-starved adversary. Even after 20 years of Government sponsored and sided "grow more food" campaign, we are no-where near self-sufficiency in food.

Though U.S.A. and other countries are willing to help us to tide over our food difficulties by supplying large quantities of wheat etc., we should not become dependant on the generosity of other countries. We must endeavour to produce more food-grains, cereals, millets, vegetables, fish, meat, eggs, poultry meat etc. so that we may be self sufficient in respect of our food requirements so that we may be independant in the true sense.

Rising standards of living, growth of education among the peoples of the country and increasing population have created new demands for more variaegated diets and for better

nutrition. Among the finest of the popular, nutritious and relatively inexpensive dishes are poultry and eggs - one of nature's few perfect foods. Rich in protein, they lend themselves to many different ways of preparation so much so that even vegetarians have taken to eggs and poultry.

Increased production of poultry can help meet the demand for more food for feeding millions of underfed mouths. If modern scientific methods of poultry farming and production are adopted, poultry raising will be by far the most fruitful and speedy method of producing food items at competitive costs.

Poultry is considered as one of the major converters of grains and other materials including waste products which are not usually used for human consumption, into highly valuable protective proteinous food.

Although India is the original home of the jungle fowl (Gallus columbae), the poultry industry in this country has not yet developed as in advanced countries like U.S.A.

In Australia, the poultry industry now ranks sixth in terms of farm income from rural sources, provides employment to over 100,000 persons out of a total population of 12 million and represents a capital investment of over £ 20,000,000. The total number of poultry is estimated to be about 25 million, in addition to which some 20 million broilers and half a million turkeys are raised annually. The total annual egg production in Australia is estimated at nearly 200 million dozen eggs, the gross value of these being £ 20.7 million.

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In India, the poultry population was 74 million in 1951 and it has increased to 117 million in 1961 which perhaps produces little over 2,000 million eggs annually, and it contributes to the country's economy over Rs. 100 million. Significant differences will be noticed when comparing these figures with that of the Australian poultry production where 25 million birds are yielding nearly same number of eggs. Another feature worthy of note is that a population of 12 millions in Australia are consuming 1,700 million eggs annually, whereas a population of 450 million in this country are consuming only about 5 eggs per person per year which is extremely low. Also the yearly consumption of poultry meat in India is about 0.31 lbs. as against 10 lbs. in Australia and 29 lbs. in the U.S.A. These differences should be sufficient for us to think seriously about applying scientific methods to improve our poultry production which would go a long way in meeting the needs of our growing population as a valuable supplement to our diet.

Considering the anticipated increase of human population, it has been estimated that in another 15 years, 600 million people would require 1 lakh million eggs per annum to provide 1 egg per head per day for 50% of the population who would be potential consumers of eggs in India. The above target would require increase in the number of laying hens from present day 45 million to 1000 million laying hens. It would also be necessary to increase the production capacity of poultry to at least 100 eggs per annum from the present estimate

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of 60 eggs per hen per annum. In view of the recent heavy demands of eggs and poultry meat in India, poultry keeping has now been taken up on commercial or semi-commercial lines in some of the progressive states in India providing livelihood and resources of income to the people.

As poultry are susceptible to several fatal diseases, the control of these diseases is highly essential for the successful poultry production. Poultry diseases not only cause heavy mortality but also are responsible for colossal economic loss owing to retarded growth, poorly finished sale birds and decreased egg production. More than 50% of the birds die annually and out of this 40% die of bacterial and viral diseases (Naidu - 1959). Since the fowls are more vulnerable to diseases, without proper disease control measures being instituted, some of the flourishing poultry concerns are known to have failed miserably.

Unfortunately, with the introduction of foreign breeds of fowls, new diseases hitherto alien to the soil have made their unwelcome appearance and present a serious threat to the growing poultry industry. In the wake of large scale poultry farming in the country, the hazards of diseases have increased manifold owing to intensive conditions under which birds are raised. Further, close confinement provides quite favourable conditions for rapid spread of infections.

Among the various diseases that are taking a heavy toll of life in our poultry farms, the disease entity "Avian

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Leukosis Complex" occupies a prominent place.

Disease control has unique importance in the successful execution of any livestock improvement scheme. If not adequately protected, the best breeds of fowls evolved through years of labour may be wiped off by the ravages of an infectious disease and thus, the very roots of the development programmes in the expanding poultry industry may be cut. The rational approach, therefore, is to evolve suitable methods of diagnosis and control against the diseases which are still responsible for heavy losses on the poultry farms. For achieving this end, a thorough study of the pathology and pathogenesis of the diseases like Avian Leukosis Complex, that has hitherto defied proper control, is very essential.

The importance of the "Hazards to poultry industry due to"Avian Leukosis Complex" was discussed in the 12th Animal Diseases Conference held at Poona (India) under the auspices of the Indian Council of Agricultural Research. The conference noted that within recent years the problem of Avian Leukosis Complex has considerably increased and stressed the need of more diagnostic laboratories and better facilities for collecting concrete data on various aspects of this complex problem.

A precission diagnosis with "red and green light" decisiveness is essential. It requires no emphasis to have a fair knowledge of the "how and why" of the disease. Manifestation of clinical symptoms, lesions and related histopathological changes and the haematological picture lead to a clearer

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conception of a disease.

The present study was, therefore, undertaken with a view to study the occurrence of the condition; its clinical manifestations, haematological significance, gross and microscopical patho-anatomical changes due to Avian Leukosis Complex and efforts were made to correlate the various findings with the ultimate object of evolving suitable diagnostic methods against this hazardous malady.

DEFINITION OF AVIAN LEUKOSIS COMPLEX:

According to Dorland's Medical dictionary (1964) - page 247 "Leukosis" is the term applied to the proliferation of leukocyte-forming tissue, including myelosis and lymphadenosis. Such proliferation forms the basis of Leukemia.

According to Jungherr and Hughes (1965), the term "leukosis" includes those diseases which are characterized by autonomous proliferation of essential blood-forming cells. Therefore, the term Avian Leukosis Complex has largely replaced the older name Leukemia principally because changes in the circulating blood, as implied by the name, are not an invariable pathologic feature.

Avian Leukosis Complex is an insidious disease of fowls caused by filtrable viruses, manifested by one or a multiple of conditions, characterized by the presence of immature blood cells in the affected tissues where they are not usually seen. Avian Leukosis Complex defies early diagnosis by any of the known methods. Affected birds become

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unthrifty, their yield is reduced to the minimum and terminate their life often fatally.

ETIOLOGY OF AVIAN LEUKOSIS COMPLEX:

Many theories have been postulated to explain the etiology of cancer namely irritation, chemical, hormonal and viral theories. However, these individual theories have failed to explain many types of cancers and therefore the actual mechanism of causation of cancer is still a mystery. As regards the etiology of Avian Leukosis Complex a vast literature has accumulated since the first report of its transmissibility by Ellermann and Bang (1908) by means of cell free filtrate. Similarly fowl paralysis or neural lymphomatosis was shown to be transmitted in the same manner by Vander Walle and Winkler - Junius (1924). Subsequently a number of viral agents have been shown to be responsible for different cancerous conditions by various workers.

Much work has been done from time to time to elucidate the etiology of Avian Leukosis Complex and a variety of factors were suspected to be of etiological importance and finally the viral etiology seems to hold good. It now appears that at least two groups of viruses or agents are responsible (Burmester et al, 1966) for these diseases. Outstanding distinguishing characteristics are - (1) whether or not the virus grows in chicken embryo fibroblast and induces resistance and (2) whether or not the virus causes the formation of a groups specific complement fixing antigen.

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An extremely interesting treatise on the virus host relationship in avian neoplasms explains the importance of research on poultry which may eventually elucidate the cause of certain forms of cancer in man and animals (Schmittle,1967).

A thorough study into the etiological factors of this disease is bound to throw light on the etiology of at least some forms of cancer or leukaemic conditions in man and animals.

CLASSIFICATION OF AVIAN LEUKOSIS COMPLEX:

It may be observed that various names were used to denote the different conditions and in some instances each one of them was described as a separate entity. However, as the picture obtained is essentially one of proliferation (neoplastic) of blood cells the term Avian Leukosis Complex was used as a comprehensive term.

The proposed classification and nomenclature by Biggs (1961, 1963) has taken into account the etiology, epizootiology as well as the pathology. The term "lymphomatosis" has formerly been used for all lesions of similar morphology irrespective of etiologic differences. It is not employed in this classification, to avoid confusion associated with its use in diseases of different etiology. In its place two terms are employed - "Lymphoid leukosis" for the lymphoid proliferations caused by the RIF - positive viruses and "Marek's disease" for all the proliferative lymphoid lesions of the nervous system including the oculi, the viscera, the skeletal

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muscle, and the skin, caused by RIF-negative agents.

The term "acute leukosis" has been applied to outbreaks of neural and visceral leukosis in young birds. Skin and muscle leukosis has been reported to occur in broiler flocks. (Burmester et al., 1966).

Sevoian (1967) has recently proposed, the following open-ended flexible classification, where in types and subtypes of causal agents may be added as needed.

The Avian Leukosis Complex			Also Non-leukotic
Lymphoid	Myeloid	Erythroid	Tumors
Type 1* (RIF-type) Subtype A Subtype B etc.	Type 1* Subtypes*	Type 1* Subtypes*	Type 1*
Type II (JM-type)			
Type III (T-type)			

* Types and subtypes of causal agents added to each category as needed.

It is considered that the framework of this hemopoetic classification is pathologically inclusive and that it is etiologically open-ended. This classification is purposely open-ended to accomodate changes and/or additions of an infinite number of types and subtypes of agents. Arbitrarily, the RIF-type avian tumor viruses, having an inhibitory effect upon the growth of Rous virus in-vitro fibroblasts and

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having a common antigen as determined by Cofal test (Sarma et al.,1964), has been designated as Type I, with respective subtypes, and placed in appropriate cellular categories. This group of viruses can stimulate the lymphoid, myeloid, and erythroid blood lines. In addition, they may cause fibrosarcomas, endotheliomas, osteopetrosis, and nephroblastomas. Therefore, a seperate category of non-leukotic tumours considered pathologically peripheral to the confines of the leukosis classification was made to designate types of viruses demonstrated to have such multipotent oncogenic spectra. A second major category of agents causing lymphoid tumors (so called Marek's disease) is that group which is represented by JM (Sevoian et al., 1962) and B-14 (Biggs et al., 1963) types. A third type of virus causing leukosis in poultry is represented by T-virus (Sevoian et al., 1964). This virus has been propagated in tissue culture and has not been shown to possess RIF activity (Sevoian, 1966; Sevoian et al., 1964).

ECONOMIC IMPORTANCE OF A.L.C.

The avian leukosis complex is known in poultry flocks throughout the world. In America it is one of the most costly poultry diseases. In America, acute Marek's disease (Acute leukosis) has been reported to be one of the most serious causes of mortality and condemnation of carcases (Gordon, 1967). Kestevan (1965) estimated a financial loss

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of $ 6500,000 annually due to this disease in U. S. A. This works to about 30% of the total loss from all the diseases of poultry. In Great Britain the annual loss due to Marek's disease and leukosis has been estimated by the same worker as $ 40,000,000 or £ 13,000,000 approximately or about 5% of the total income from poultry products. It is quite conceivable that similar losses might be occurring in other countries which are advanced in poultry industry. In our country there is no authentic report available about the loss due to Avian Leukosis Complex for want of data on its incidence. In the absence of such information it can be foretold that the losses would work out to be significantly high in India also. The economic importance of this disease becomes much greater as stated by Coles (1954) in that the deaths occur when the birds reach sexual maturity and are to be fed and maintained till then unnecessarily.

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HISTORICAL ACCOUNT AND GEOGRAPHICAL DISTRIBUTION OF AVIAN LEUKOSIS COMPLEX.

HISTORICAL ACCOUNT AND GEOGRPHICAL DISTRIBUTION OF AVIAN LEUKOSIS COMPLEX.

Avian Leukosis Complex has a global spread and exists wherever American and Australian stock have been imported. Its existence in U. K. too has been reported as due to smuggling of eggs from American sources by breeders. It is known in almost all breeds of fowls throughout the world and probably no commercial flocks are free of all leukosis virus. This disease had been reported from different countries e.g. U. S. A., England, Italy and Germany etc. It has also been definitely established that this malady has taken a strong foot-hold in our country as well, though the extent of its spread and the economic loss sustained due to this disease has not yet been fully assessed.

Roloff (1868) first reported one case of lymphosarcomatosis according to Chubb and Gordon (1967), Leukosis in the common fowl was first recorded in Italy by Caparini in 1896, according to Olson (1940). Caprini (1896) described the histological changes of three enlarged livers which were observed at Veterinary Institute at Naples. Moore (1895-96) quoted by Butterfield (1905) reported an epidemic in fowls in U. S. A. under the designation of "Infectious Leukaemia". Butterfield (1905) from the University of Michigan described the histopathological findings of the livers of three hens which he diagnosed as "Aleukaemic lymphadenosis". In the same

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year he reported five cases in which there was an evidence of leukaemic state in addition to the infiltration of visceral organs with lymphoid cells and opined, "Probably the occurrence of a leukaemic and aleukaemic lymphadenoid hyperplasia (or sarcomatosis) in hens is not frequent". Marek (1907) quoted by Engelbreth-Holm (1942), described a disease in fowls, in Hungary, characterised by paralysis of legs and wings and variable enlargement, due to extensive mononuclear infiltration, of the peripheral nerves and of the posterior root ganglion and named it "Neuritis Interestitialis". In 1907 Koch and Robinowitsch and Kon quoted by Olson (1940) observed spontaneous occurrence of fowl leukosis in nature. Warthin (1907 quoted by Lee et al. (1937) described both Leukaemia and aleukaemic lymphocytoma of the chicken and remarked, "Leukaemic and aleukaemic forms of lymphocytoma frequently occur in the fowl and in all respects are analogous to similar conditions in man". He was of the opinion that the two conditions were either genetically related or merely different stages of the same process without showing any evidence of an infective causal agent and he believed that their nature would lead one to classify them under malignant neoplasms.

According to Lee et al. (1937), the pionmer work of the successful transmission of fowl leukosis with a filtrable agent, to healthy fowls, was reported by Ellermann and Bang in 1908-1909. Hirschfeld and Jacoby (1912) quoted by Olson (1940) confirmed the transmissibility of the original strain

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of Ellermann and Bang. Ellermann (1920) quoted by Campbell (1954b) introduced the term "leukosis" for a group of diseases characterised by neoplasia of the haemopoietic tissues. Later Ellermann and his associates (1922, 1923) quoted by Jungherr (1952) recognised three general forms of avian leukosis, namely leukaemic or aleukaemic myeloid leukosis, intravascular lymphoid i.e. erythroid leukosis, and extravascular lymphatic leucosis, all of which were considered to be transmissible and caused by the same filtrable virus. Schmeisser (1915) recorded spontaneous occurrence of myeloid leukaemia in the fowls. Macfie (1941) described a peculiar type of disease in the fowls of Nigeria characterised by droopiness, diarrhoea and paralysis of legs and wings, stupor and death and appearance of various shaped cytoplasmic inclusion bodies entirely composed of chromatin in the cytoplasms of white blood corpuscles and red blood corpuscles. Adler (1925) recorded a disease of fowls in Palestine characterised by various forms of chromatic inclusions in the leukocytes and the disease was diagnosed as leukaemia by Ury on histopathological studies. But Adler did not rule out a mixed infection. Bedson and Knight (1912) and Bayon (1929) described an anaemic disease of fowls in England suspected to be anaemic form of fowl leukosis. Engelbreth Holm described in one of the cases 1.5 millions leukocytes per c.m.m. with 96% small lymphocytes. Furth (1935) has also recorded highest count of lymphocytes as 140,000 per c.m.m.

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According to Pappenheimer et al. (1926) Marek (1907) first described a condition in fowls which showed clinical signs of paresis. Kaupp (1921) quoted by Pappenheimer et al. (1926) reported the occurrence of outbreaks of fowl paralysis in different states of U. S. A. Furth (1931a) for the first time, reported myeloblastic form of avian leukosis in his transmission experiments with viral strains from cases of erythroleukosis. Potel (1939) classified this as allied to leucaemias. Furth (1934-35) considered neurolymphomatosis as allied to leukosis and sarcomatosis but differentiates this condition from others in liver as neurolymphomatosis and Hepatolymphomatosis. Fenestermacher (1936) also differentiates lymphocytoma and fowl paralysis. Jarmai (1935) and Stubbs (1934) also described 3 forms, lymphatic, Myeloid and erythroleukosis. Emoto and Myamoto (1932) in their studies on fowl paralysis observed that this affects principally the nerves and eyes. Gray (1936) recorded staphylococcus aureus, Str. viridans and Bacillus pyocyaneus in affected nerves.

Mathews and Walkey (1929) suggested the term lymphadenoma for visceral form which is essentially extra vascular form of Ellermann. Feldman (1932) and Feldman and Olson (1938) supported this but called this condition Lymphocytoma. Johnson (1932) was however of the opinion that the two can not be seperated and he proposed the term Lymphomatosis for visceral and neural subdivisions.

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Pugh (1927) and Reinhardt (1930) described an uncommon hypertrophic osteopathy in poultry under the terms diffuse periostitis and hyperplastic ostitis. Jungherr (1935) and Jungherr and Landauer (1938) suggested the term osteopetrosis gallinarum and found that certain strains of lymphomatosis carry hypertrophic osteopathic potentialities.

Davis et al. (1947) on the basis of transmission trials established lymphoid leukosis (Visceral lymphomatosis) as a seperate entity distinct from neural lymphomatosis, a view supported by the workers of the United States Bureau of Animal Industry Regional Poultry Research Laboratory (1951).

According to Olson (1940) the disease was reported in 1908 by Ellermann and Bang, in Germany by Luttschwager (1931), in England by Bedgar and Knight (1924), in Sweeden by Maghusson (1915-16), in Italy by Battaglia and Leinati (1929), in Hungary by Jarmai (1930), in Russia by Yakimoff and Rastegaieff (1929), in Holland by Te Hennepe (1927), in Japan by Matsubara (1929), in United States by Warthin (1907), in France by Oberling, Guerin and Boic (1932), in South Africa by Jackson (1936), in Brazil by Rais, Noberga and Revis (1936), in Newzealand by Fitch (1938).

In India the disease was reported from various States. From Bombay it was reported by Mohey Deen (1933) quoted by Kaura (1941), who concluded fowl paralysis (Wryneck) to be due to calcium deficiency. From Hyderabad Mahajan (1934) reported the disease as polyneuritis in fowls. From Mysore

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State Krishnienger (1937) and Naidu (1938) described it as neurolymphomatosis gallinarum. Kaura and Iyer (1937) described paralysis of legs, wings, head and neck in poultry during the course of experimental infection with Ranikhet (New Castle) disease virus. From Central Provinces and Berar (now Madhya Pradesh) Bachan Singh (1940) described it as leg weakness in poultry due to nutritional definiency. Iyer (1941) established a true lymphomatous condition in a hen. Iyer and Hashmi (1945) recorded its incidence in all common breeds of fowls in almost all the provinces. Kuppuswamy (1947) recorded this disease for the first time in Bihar State. Since then, various other workers such as Rao et al. (1950), Das (1953), Krishnan (1960), Awadhiya (1964), Kansal (1966), Sharma et al. (1967), Awadhiya (1967), Babras (1967) and Soman (1967) have also contributed to the existing knowledge from different parts of India.

Much work has been done on various aspects of the disease and numerous investigators have given detailed reports of the etiology, transmission, propagation on chick-embryos, clinical manifestations, gross lesions, histopathological changes, haematological changes and incidence etc. It is not within the scope of this thesis, to review the literature on the various aspects of the disease. However, a review of literature on the aspects that have a direct bearing on the present investigation have been reviewed in the respective chapters ahead.

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MATERIALS AND METHODS.

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MATERIALS AND METHODS

(A) INCIDENCE:

The report on the incidence of Avian Leukosis Complex was prepared for a period of three years (1965-1967) from the data available in the Poultry Extension Centre, Biharsharif, Patna.

The same data have also been used to find out the sex, breed, age and seasonal incidence etc.

The data available in the Pathology Department of the U. P. College of Veterinary Science and Animal Husbandry, Mathura, was also studied with the kind permission accorded by the Professor and Head of the Department of Pathology at the above institution and figures for the past 8 years (1960-1967) have been included to have a comparative idea of incidence of Avian Leukosis Complex at different places.

The other set of data given is in respect of the necropsies conducted by the author, to find out the incidence of Avian Leukosis Complex at different centres.

(B) HAEMATOLOGY:

Sixty female birds between the age of 5-18 months were used for this study. Out of this 32 fowls were White leghorns and 28 Rhode island reds. These birds were supplied from the Poultry Extension Centre, Biharsharif, Patna.

The blood of these birds was examined for different

haematological values viz. changes in blood picture, clotting time, total and differential counts, haemoglobin estimation, packed cell volume, erythrocyte sedimentation rate, and the buffy layer.

Some fowls showing one or more of the unusual haematological values suggestive of avian leukosis complex viz. unusually high total leucocyte count in myeloid leukemia (Furth, 1931), high lymphocyte count in paralytic birds (Bayon, 1931) and increase in the buffy layer in lymphoid leukosis (Sato, 1960) were killed. Thus, an attempt was made to confirm and correlate the findings of these workers with the histopathological studies. The different organs of 5 birds were examined for the presence of gross lesions and tissues from abnormal as well as from apparently normal organs were collected for histophatological studies in order to correlate these findings. The other healthy fowls which were used in this investigation acted as controls.

METHOD OF COLLECTION OF BLOOD:

(a) ANTICOAGULANT:

Small sterile empty penicillin vials were filled with 0.25 ml. of the anticoagulant mixture consisting of the following components:-

Potassium oxalate	...	...	0.8 gm.
Ammonium oxalate	...	...	1.2 gm.
Neutral Distilled water .		...	100 ml.

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These tubes were prepared in advance with the anticoagulant and were evaporated to dryness by placing them in hot air oven at 60°c. The mixture of oxalates does not change the volume of the cells so that no correction for the readings is necessary. These amounts of the two exalates are for 2.5 ml. of blood (Wintrobe, 1956).

(b) COLLECTION OF BLOOD:

The blood required for the study was taken from wingvein. The feathers covering the wing vein region were plucked and the skin was sterilised with rectified spirit. The bird was then put on its side on a stool and was secured by an attendant by holding the legs and wings on one hand and the neck on the other. A sterile hypodemic needle mas used to puncture the wing vein and the blood automatically flowed and was collected directly in to the penicillin vial having the dried anticoagulant mixture as prepared above. Approximately 2 ml. of blood was collected from each bird and were mixed properly with the anticoagulant.

According to Osgood, Haskins and Trotman, cited by Kolmer (1951) oxalated blood may be used for the following examinations, if the latter are made within the designated time after the collection of blood:-

Haemoglobin estimation	...		24 hours.
Red Cell count -	...	...	24 hours.
Thrombocyte count	...	...	1 hour.

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Red Cell volume.	...	...	3 hours.
Colour index	...	...	24 hours.
Volume index	...	...	3 hours.
Saturation index	...	...	3 hours.
White Cell volume.	...	...	24 hours.
Smear for differential count		...	1 hour.
Sedimentation rate	...	...	3 hours.

The various techniques and methods used for determining the different blood values are detailed below:-

(a) CLOTTING TIME:

The method adopted by Varma (1960) was used during the entire period of this study. The Pasteur's capillary pipette was used for studying the clotting time. The skin of the wing vein was sterilised with rectified spirit. The vein was pricked with a pin and the blood was allowed to flow out. The tip of the pipette was placed over the blood and the blood automatically flowed through the capillary tube. When the colume of blood reached about 3"-4", the other end was broken, and thus the blood was taken into the capillary tube. The time at which the blood entered the capillary tube was noted. The capillary tube was then kept at 37°c. At an internal of 10-15 seconds, a small portion of the capillary tube was broken off to see whether the blood has clotted. This was repeated until a thin thread of clot was stretched between the two broken ends. The time at which the thread started to form was also noted and thus

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the clotting time was determined.

(b) HAEMOGLOBIN:

The haemoglobin estimation was carried out by Sahli-Hellige haemoglobinometer, throughout this study. Sahli (1895), quoted by Wintrobe (1956), suggested that blood be diluted with N/10 hydrochloric acid, the haemoglobin of the blood being thus converted to acid haematin which is dark brown in colour.

The empty graduated tube was filled with N/10 hydrochloric acid upto the level of the 10 mark. Blood was drawn into the measuring pipette upto the 20 c.m.m. mark and was carefully added into the tube containing the N/10 hydrochloric acid. The mixture was thoroughly shaken, care being taken not to form air bubbles. The resulting dark brown fluid was then diluted with neutral distilled water until a match with the brown glass standard was attained. The haemoglobin value was read directly from the scale in percentage and in gms./100 c.c., by noting the height of the column of the diluted acid haematin.

To match the solution and the standard, the haemoglobinometer was held upto a good source of indirect natural light. The level in the mixing tube opposite to the lowest meniscus of the diluted acid haematin was read. This gives the percentage of haemoglobin. The end point is generally very sharp and so care was taken when water was to be added.

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When a point was reached at which the solution appeared to match the standards, the reading was taken. Then again water was added in a drop and the solution was mixed and a second reading was taken. If at the second reading the solution was too light, then the first reading was taken.

(c) DIFFERENTIAL LEUCOCYTE COUNT:

Properly made and well-stained blood smears are essential for the white cell differential count. The slides were scrupulously cleaned from grease and dust.

The tip of comb of the birds was sliced off with the help of a small scissors. The first drop of blood oozing out from the cut portion was discarded. The second drop of blood was taken on a slide to make the smear. In all cases, two smears were prepared. The drop of blood was taken over the middle of the slide about 10 m.m. from the end. The thin edge of another slide was applied over the drop of blood and was used as a spreader. The blood was spread along the edge of this spreader. A slight lateral movement accelerated this process. The spreader, at an angle of about 40° to the slide, was then pushed along with the blood, following it along the slide. The film was then allowed to dry by moving it in the air.

(d) STAINING TECHNIQUE:

For staining, the stock solution of Giemsa stain was diluted with an equal quantity of buffer solution, pH 6.8.

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The stain solution was made up fresh every time.

The slide was fixed in methyl alcohol for three minutes and then flooded with the stain. The stain was allowed to act for 30 minutes in order to take up proper staining.

(e) CRITERIA OF DIFFERENTIATING THE LEUKOCYTES:

The following pictures were observed to differentiate the various types of leukocytes:-

LYMPHOCYTES:

The cytoplasm is weakly basophilic and is confined to a narrow rim bordering one side of the nucleus. In the case of larger lymphocytes this constituted the major portion. The nucleus is usually round and in a few cases slightly indented on one side.

HETEROPHILS:

They are characterised by the presence of acidophilic rod shaped crystalline granules in a clear colourless cytoplasm. The nucleus is lobulated with thin connecting threads. The number of lobes are usually two to three.

MONOCYTES:

Some times little difficulty was experienced in differentiating monocytes and large lymphocytes. Monocytes are large cells with relatively more cytoplasm than the large lymphocytes. The cytoplasm has a blue tint. The nucleus is kidneyshaped and in a few cases irregular in outline.

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EOSINOPHILS:

They are of about the same size as the heterophils. They are characterised by relatively large spherical granules whose colour is dull and in contrast to those of the heterophils. The cytoplasm is faint bluish grey tint.

BASOPHILS:

They are of about the same size as the heterophils. The nucleus is usually marked by the mass of granules in the cells, and is lobulated. The cytoplasm is clear and colourless. Dark staining basophilic granules are abundant.

(f) TOTAL RED AND WHITE CELL COUNT:

Throughout the study, the method described by Natt and Herrick (1952) was used. The diluent suggested by Natt *et al.* (1952) consisted of the following:-

Sodium chloride (Nacl)...	...	3.88 gms.
Sodium sulphate (Na_2So_4)	...	2.50 gms.
Disodium hydrogen phosphate ($Na_2HPo_4.12H_2O$).	...	2.91 gms.
Potassium hydrogen phosphate	...	0.25 gms.
Formalin (37%). ...	...	7.50 ml.
Methyl violet 2B ...	...	0.10 gm.

The above chemicals were dissolved in distilled water in the order prescribed above and diluted to a total volume of 1000 ml. in a volumetric flask. After standing

overnight, the solution was filtered through fine filter paper (Whatman No.2). The pH was adjusted to 7.3.

The Thoma red-blood-cell-counting pipette was filled with the blood to the 1.0 mark and the diluting fluid was sucked to the 101 mark. The mixture was allowed to stay for 5 minutes. The blood sample which was thus diluted 1:100 was then shaken in order to obtain an equal cell distribution. All the counts were made by means of Improved Neubaner haemocytometer.

The counting chamber and the coverslip were perfectly cleaned from grease and dust or threads of cotton. The coverslip was placed on the counting chamber so that it covered the entire ruled area. It was ensured that the contract between the counting chamber and the coverslip was well established. The first few drops of the diluted blood were discarded by blowing into the pipette. The pipette was then held in an inclined position and a small drop of diluted blood was just placed at the junction of the coverslip and counting chamber. The drop instantaneously passes under the coverslip by capillary attraction. Every care was taken to ensure that no air bubble was included in the chamber. The cells were then allowed to settle for about 2 minutes before they were counted under high power objective. The erythrocytes were counted in 16 small squares at the four corners each and of the central one of the counting chamber, thus making the area of 80 (16x5) small squares. The leukocytes were counted in

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all the 400 (16x25) small squares. The number of erythrocytes counted was multiplied by 5000 while the number of leucocytes in the 400 small squares were multiplied by 1000 to give the total red and white cell counts respectively. All the cells touching the upper and the left hand line of each square were included for counting white those touching the lower line and the right hand line of each square were not counted.

The nucleus of the oval erythrocytes stained violet and the cytoplasm stained the same colour but to a much less intensity. Since the shape of the erythrocyte is so characteristic that no difficulty could be experienced in differentiating the same from the leucocytes. The other white blood cells stood distinctly with their distinctly violet stained nuclei while the cytophasm took up very light stain.

(g) SEDIMENTATION RATE & PACKED CELL VOLUME:

Throughout this study, the sedimentation rate and packed cell volume were estimated by Wintrobe and Landsberg-method, as suggested by Wintrobe (1956), with the aid of the Wintrobe Haematocrit tube.

The haematocrit tube was filled exactly to the 10 mark at the right side of the scale, with the oxalated blood, by means of a pipette drawn from a glass tubing to such a length that it can be passed to the bottom of the tube. The pipette was passed to the bottom of the haematocrit tube and an uniform pressure was applied to the bulb of the rubber teat. Thus the blood was forced out as the pipette was withdrawn.

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This was done at such a rate that no bubbles of air formed in the tube. The tube was then closed by means of a rubber stopper to check evaporation of blood.

The haematocrit tube was then allowed to stand vertically and the readings were noted at the end of two hours.

After the sedimentation rate was noted, the haemotocrit tube was centrifuged for 30 minutes at 3000 r.p.m. The tubes were taken out of the buckets and the readings were noted. The packed cell volume was expressed in percentage. The reading of "Buffy layer" was also taken and expressed in m.m/100 ml. of blood.

(C) HISTOPATHOLOGY:

The materials for study in the present investigation were mostly collected from the routine autopsies conducted on the birds received from the Poultry Extension Centre, Biharsharif, Patna; Central Poultry Farm, Patna and Poultry Dressing Plant, Patna. Some autopsies were also attended in the department of Pathology, U.P.College of Veterinary Science and Animal Husbandry, Mathura. In all cases a complete autopsy was done. Some of the tissues received from the field for histopathological examination were also utilised in the study. Materials from birds sacrificed on haematological examination were obtained from the Poultry Extension Centre, Biharsharif, Patna. A total of 1530 birds were examined at autopsy. The different organs of 90 birds which were suspected for A.L.C. were examined

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for the presence of gross lesions and tissues from affected as well as some of the normal appearing organs were collected for histopathological studies.

The tissues so collected were categorised as follows:-

1. "P" represents the tissues collected from routine autopsies of birds received from Poultry Extension Centre, Biharsharif, Patna; Central Poultry Farm, Patna and Poultry Dressing Plant, Patna. It also includes the tissues collected from routine autopsies of birds received from various sources in the department of Pathology, U.P. College of Veterinary Science and Animal Husbandry, Mathura.

2. "SP" indicates the tissues received from various other sources as specimens for histopathological examination. No history was available.

3. "H" denotes the tissues collected from birds utilised for haematological studies and sacrificed for collection of tissues for histopathological study.

Necropsy procedure recommended by Carter (1962) cited by Sharma (1964) was followed for the routine postmortem of the birds. The general condition of the body and other abnormalities, if any, were noted. The carcasses were laid on the table with their ventral side up. A longitudinal incision was given in the skin of the ventral part and the skin was stripped digitally from the beak to the vent. The abdomen was

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opened by removing the ventral wall. The breast was removed by cutting it forward on both the sides through the ribs and humero-scapular articulations with the help of a bone cutter. The organs were first observed in situ and then examined systematically by removing from the neck to the cloaca. Gross lesions observed in the organs were noted. Materials for histopathological study were collected.

Tissues for histopathological examination from most of the fowls suspected to have died of Avian Leukosis Complex, tentatively diagnosed on the basis of gross examination of different organs, were fixed in 10% formol-saline solution. The tissues were categorized in series "P", "SP" and "H". The number prefixed indicated the serial number of the case.

The technique recommended in the Laboratory Mannual of Special Staining technics (1953) was followed. As a routine, the tissues after fixation were washed in running stream of water for 24 hours to remove the fixative. The tissue pieces were then dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin-wax. Sections were usually cut at 5 microns.

For staining Harris's haematoxylin and alcoholic solution of Eosin Y were used as a routine in all the sections. The sections were deparaffinised in xylol, carried through descending grades of alcohol to water. The sections after being stained by haematoxylin and removing the excess of haematoxylin by 1% acid alcohol, were dehydrated through

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ascending grades of alcohol, being counterstained by alcoholic solution of eosin during the process of dehydration. The sections were then cleared in two changes of α α xylol and mounted in Depex.

For demonstration and differentiation of the various stages of blood cells in the tissue sections, Jenner - Giemsa's stain was followed as recommended by McIsraels (1955), cited by Sharma (1964). The staining technique is represented as follows:-

1. The sections were deparaffinised.
2. After hydrating the sections were kept in 50% alcohol.
3. Treated the tissue sections with Jenner's stain diluted 50% with buffer solution for 3 minutes.
4. Poured off the Jenner's stain and without washing added Giemsa's stain, ten drops to 5 c.c. buffer solution for 7 minutes.
5. Washed in tap water.
6. Differentiated in 1 in 1500 acetic acid until pink.
7. Dehydrated by several changes of chemically pure acetone.
8. Passed through 2 to 3 changes of normal butyl alcohol.
9. Cleared in xylol.
10. Mounted in synthetic neutral xylol miscible medium, Depex.

Neclei - blue; cytoplasm - pink to rose.

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Composition of stains and buffer solution used in this technique of staining were as follows:-

Jenner's stain - 0.3% solution of Jenner's dye in Methyl alcohol, undiluted.

Giemsa stain - 10 drops of stock solution to 5 cc. of buffer solution.

BUFFER SOLUTION OF pH 6.4.

Potassium acid phosphate - 2.7231 gm.

N/10 Sodium hydroxide solution - 64 ml.

Distilled water - to make 1000 ml.

Some of the sections were also stained with Mann's eosin-methyl blue to detect the presence of any inclusion body, if possible. The steps of the technique were as follows:-

1. The sections were deparaffinised and brought to water through descending grades of alcohol.
2. Stained for 8-10 hours in a coplin jar.
3. Washed in water.
4. Differentiated in absolute alcohol to which 2-3 drops of N/1 sodium hydroxide per 100 ml. were added.
5. Washed rapidly through several changes of absolute alcohol.
6. Cleared in xylol.
7. Mounted in Depex.

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Inclusion bodies, red blood cells, oxyphil cytoplasm and granulas - Re; Nuclei and other tissue elements-blue.

Compostion of the staining solution used in this technique of staining was as follows:-

1% aqueous methyl blue	...	...		35 ml.
1% queous eosin -	...	...	...	45 ml.
Distilled water -	...	...	...	100 ml.

Besides the staining techniques mentioned above, the undermentioned staining techniques were employed for staining the paraffin cut sections whenever the necessity arose for confirming a particular substance:-

1. Van Giesson's stain - for collagenous fibres.
2. Giemsa's stain - for different cellular elements and micro-organisms.
3. Lendrum's stain - for inclusion bodies.

The classification of different types suggested by Biggs (1961) was followed in the present investigation.

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REVIEW OF LITERATURE & PRESENT OBSERVATIONS ON THE INCIDENCE OF AVIAN LEUKOSIS COMPLEX.

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REVIEW OF LITERATURE & PRESENT OBSERVATIONS ON THE INCIDENCE OF AVIAN LEUKOSIS COMPLEX.

REVIEW OF LITERATURE:

Breig (1919) recorded 13% incidence in Denmark. Haupt (1928) recorded 5% incidence of 1700 autopsies in Berlin. Reinhardt (1929) recorded 10% incidence in Leipzig. Hare (cited by Furth 1931) recorded 4% incidence. Hanneppe (cited by Furth 1931) found 7% incidence in Holland. Bornstedt (cited by Jarmai 1934) found 11% incidence in Prussia. Luttschwager (1930-1931) recorded an incidence of 16% in Hanover. Feldman and Olson (1933-1934) recorded an incidence of 10-15% in Minnesota. Jackson (1936) recorded 97 leukosis cases (48.5%) out of 200 cases of neoplastic conditions met with in fowls. Schaff (1936) found lymphogenous leukaemia in 6-7% Jhonston and Wilson (1939) recorded 26% incidence in Parkhead and 9.36% incidence in Chistery during autopsy. Goss (1940) recorded an incidence of 5.7% fowl paralysis, 7.7% other forms and 6.1% poultry neoplasms in 7408 cases. Olson and Bullis (1942) recorded 10.9% leukosis and 5.8% neoplasms in 2304 cases. Campbell (1945) recorded 8.6% leukosis and 6% other neoplasms in 2063 cases.

There appears to exist a close relationship between population size, incidence of Avian Leukosis Complex and total mortality. The results of the analysis of mortality in poultry by Daudley *et al.* (1941), Wilson (1944), Gordon

(1953) showed that the incidence of leukosis steadily increased in England and Wales with the increase in total poultry population from 47.4 million in 1949 to 63.5 million in 1952 and the mortality ranged from 16.23% in 1948 to 25.8% in 1952. Gordon (1953) reported that the mortality rate from leukosis decreased from 1938 to 1943 and during this period the poultry population decreased to nearly half. A further report by Chubb and Gordon (1957) revealed that since then the poultry population increased to nearly 100 million in 1957 and the incidence of the disease being established to nearly 40-50% of adult mortality.

Waters and Pricket (1946) observed only 3 forms and did not meet with any osteopetrotic forms. They got 192 cases of visceral form, 39 of neural form and 6 cases of ocular form out of 3257 autopsies. Coles (1954) observed 23.2% of cases of lymphomatosis in 10,000 autopsies. Waters (1954) observed out of 8850 autopsies 20.9% visceral, 6.5% neural and 0.5% Ocular forms. Coles (1955) reported that out of the total mortality, losses from avian leukosis were 57.1% in 1950-51, 48.9% in 1951-52, 46.2% in 1952-53 and 24.8% in 1953-54. Ishitani et al (1955) recorded an incidence of 18.7% leukosis in 353 autopsies. Nightall (1956) found that out of 6,647 carcasses examined 1,190 (17.9%) were affected with visceral and 450 (6.8%) with neural form of avian leukosis complex. Jordan (1956) observed 13.3% visceral, 2.9% neural

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forms of leukosis and 1.2% other neoplasms in 4459 autopsies. From the figures from the postmortem records at the Ministry's laboratories of Weybridge and Lasswade, Blaxland (1956) reported an average mortality of 23.4% due to leukosis or lymphomatosis out of about 9,000 adult fowls received at Weybrige (England) every year for 4 years and 24% out of approximately 6,000 fowls examined every year at Lasswade (Scotland).

Chubb and Gordon (1957) summarized in a tabulated form the incidence of various forms of leukosis from the previously published reports of Jackson (1936), Olson and Bullis (1942), Campbell (1945), Nightall (1956) and Jordan (1956) and pointed out that out of a total of 1937 fowls in which neoplasia was established, 1517 (78.5%) had some manifestations of the leukosis complex.

In India, Iyer and Hashmi (1945) observed that in one flock the incidence of avian leukosis was 25% of the total losses during 3 years. Viraraghavan and Chandrasekharan Nair (1965) reported the total overall autopsy incidence of 13.9% during the period of 1951-61. Awadhiya (1967) reported the incidence ranging between 0.7% to 3.9% of the total mortality during the period of 5 years from 1955 to 1959 in the poultry farm of Veterinary College, Mathura. Sharma et al (1967) reported the incidence of avian Leukosis Complex at Indian Veterinary Research Institute, Izatnagar Poultry Farm about 0.14% during 1965-66 while 1.9% during 1959-60. Babras

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(1967) reported 10-20% mortality on account of avian leukosis in various poultry farms of Maharashtra State rearing Arbor Acres Queens. Awadhiya (1967) concluded that all the mainfestations of Avian Leukosis Complex have been encountered in this country but the incidence of lymphoid leukosis was the highest. Work carried out at Mathura revealed that the percentage incidence of lymphoid leukosis was 78.22% as compared with other forms of the disease.

(a) BREED INCIDENCE:

Daudley et al (1941) found no significant difference in the incidence of Avian Leukosis Complex in different breeds but there did appear to be a difference in the various types of the disease. The visceral form was most common in Barred Rocks, and neural form in white leghorn. Davis et al (1947), on analysing nearly 14,000 postmortem records in U.S.A. showed a higher incidence of visceral lymphomatosis in Barred Plymouth Rocks than White Playmouth Rocks, White leghorns and Rhode Island Reds. The average incidence of neural and ocular forms was highest in White leghorsn, slightly less in Rhode Island Reds and White Plymouth Rocks, and considerably less in Barred Playmouth Rocks.

Campbell (1945) quoted by Nightall (1956) analysed the results of the postmortem findings of Royal (Dick) Veterinary College over 5 years (1940-1944). He examined 2063 birds and divided his results into breeds as follows:-

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Brown leghorns	- 24.1%	Incidence above average.
Buff Rocks	- 38.8%	
Crosses.	- 20.0%	
White leghorns	- 19.6%	
Rhode Island Reds	- 17.7%	Incidence below average.
Black leghorns	- 17.4%	
Others.	- 16.6%	
White Wyandottes	- 10.3%	Incidence lowest.

From the epizootological study based on six years (1945-46 to 1950-51), Das (1951) showed that the natural incidence of Avian Leukosis Complex was significantly higher in Rhode Island Reds and White leghorns than in the 'Deshi'. He reported that the indigenous breeds were comparatively more resistant to natural infection than exotic breeds.

Nightall (1956) observed that the incidence of Avian Leukosis Complex was much lower in the crosses between Rhode Island Reds and light Sussex than in either of the parent stocks.

Chubb and Gordon (1957) did not find any significant difference in the incidence of Avian Leukosis Complex between light and heavy breeds or btween breeds in either group.

The occurrence of leukosis in birds of almost all breeds have been reported (Sharma et al.,1967) but the majority of these cases have been encountered in White leghorns (Viraraghavan and Chandrasekharan Nair, 1965; Sharma et al., 1967).

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(b) AGE INCIDENCE:

Pappenheimer et al. (1926) notedthat when the pullets of one year age from a fowl-paralysis-free flock were kept in contact continuously for 9 months with the fowls from the paralysis-affected flock, the former were highly refractory to fowl paralysis. They also noted that the males of 8 weeks age from the affected flock when removed to a clean farm, several of them developed paralysis when they were 12 weeks of age.

Johnston and Wilson (1937) observed a marked reduction in the incidence of fowl paralysis when day-old chickens from a flock affected with fowl paralysis were kept isolated from the parent stock.

Olson Jr. and Bullis (1942) reported the average age of birds with leukosis (Erythroblastosis and Granuloblastosis) 47 weeks. Waters (1951) recorded that the visceral form and neural form of lymphomatosis prevailed among birds of age group 300-600 days. He also reported that the visceral form was much more prevalent after 180 days. Das (1951) reported that the incidence of Avian Leukosis Complex was significantly higher in the younger than in older birds. Darcel et al.(1952) noticed that the losses due to visceral lymphomatosis were more shortly before and shortly after 5 months of age. Coles (1955) observed mortality due to leukosis being more during the adult period (20-78 weeks) than during the rearing period

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(0-20 weeks). Jordon (1956) recorded that neurolymphomatosis prevailed chiefly at 4-6 months of age and that over 60% of the birds with visceral lymphomatosis died at 6-12 months of age.

Viraraghavan and Chandrasekharan Nair (1965) reported that the youngest chick found affected due to Avian Leukosis Complex was 15 weeks of age and that the oldest was found to be 208 weeks of age. According to them the average age of birds affected with erythroblastosis was 35.8 weeks and for visceral and ocular laymphomatosis was 48.8 weeks.

(c) SEX INCIDENCE:

Pappenheimer et al. (1926, 1929) found that fowl paralysis occurred equally in both sexes.

Das (1951) reported that the incidence of Avian Leukosis Complex did not vary significantly in males and females.

Viraraghavan and Chandrasekharan Nair (1965) did not find any influence of sex on Avian Leukosis Complex. However, incidence of visceral lymphomatosis was more in females and that of osteopetrosis more in males. Previously too, the incidence of osteopetrosis in males in England had been reported by Pugh (1927) and in India by Venkatraman (1936).

(d) SEASONAL INCIDENCE:

Doberstein (1935) observed autumn and spring to be

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the usual season for prevalence of fowl paralysis.

Smith and Long (1959) reported that the date of hatch was one of the factors affecting the incidence of visceral lymphomatosis. A higher incidence was found in birds hatched in January, February and March than in birds hatched in April, May and June.

Viraraghavan and Chandrasekharan Nair (1965) reported that the incidence of Avian Leukosis Complex was highest in north-east monsoon period. The incidence in the cold season and south-west monsoon period were nearly equal but it was lowest in the hot season.

(d) ORGAN INCIDENCE:

So far as the involvement of organs and distribution of lesions is concerned, Viraraghavan and Chandrasekharan Nair (1966), Sharma et al. (1967) have reported that liver, spleen, Kidney and ovary were the organs most frequently involved in visceral lymphomatosis and also in other form of leukosis.

PRESENT OBSERVATIONS:

In the present investigation, the incidence of Avian Leukosis Complex at the poultry Extension Centre, Bihar Sharif, Patna for a period from 1965 to 1967 is given in the following tables :-

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T A B L E - I.

Incidence of Avian Leukosis Complex at Poultry Extension Centre, Bihar Sharif, Patna.

Year.	Total no.of birds examined at autopsy	Total no.of positive cases of A.L.C.	Percentage.
1965	221	33	14.9%
1966	386	50	12.9%
1967	128	34	26.5%
TOTAL:	735	117	18.1%

NOTE: The data has been analysed on the basis of total mortality as the total strength of the flock at all times remained the same viz. 500.

Total incidence observed is 18.1% of the mortality.

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TABLE - II.

Breed-wise incidence of Avian Leukosis Complex at Poultry Extension Centre, Bihar Sharif, Patna.

Year	Total no. of positive cases of A.L.C. in the flock.	No. of positive cases of A.L.C. in White leghorns.	Percentage incidence in relation to the no. of total positive cases.	No. of positive cases of A.L.C. in Rhode island reds.	Percentage of incidence in relation to the no. of total positive cases.
1965	33	18	54.54%	15	44.46%
1966	50	13	26.0 %	37	74.0 %
1967	34	16	47.05%	18	52.95%
TOTAL:	117	47	40.7 %	70	59.3 %

NOTE: The number of W.L.H. and R.I.R. birds maintained at the centre was more or less same throughout the present study. Incidence observed in the White leghorn breed is 40.7% of the total no. of positive cases.

Incidence observed in the Rhode island red breed is 59.3% of the total no. of positive cases.

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T A B L E - III.

Age-wise incidence of Avian Leukosis Complex at Poultry Extension Centre, Bihar Sharif, Patna.

Age group	White Leghorn breed.		Rhode island red breed.	
	No. of positive cases of A.L.C.	Percentage (out of a total no. of positive cases i.e. 117)	No. of positive cases of A.L.C.	Percentage (out of a total no. of positive cases i.e. 117)
Upto 10 months.	12	10.2%	13	11.1%
10-15 months.	26	22.2%	49	41.8%
15-20 months.	9	7.6%	8	6.8%

T A B L E - IV.

Seasonal incidence of Avian Leukosis Complex at Poultry Extension Centre, Bihar Sharif, Patna.

Year.	No. of positive cases of Avian Leukosis Complex			Total.
	Winter	Summer	Monsoon	
1965	8	14	11	33
1966	14	26	10	50
1967	8	18	8	34
TOTAL:-	30	58	29	117
Percentage-	25.6%	49.6%	24.7%	

CAPTION: Winter = November to February. Summer = March to June. Monsoon = July to October.

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TABLE - V.

Incidence of Avian Leukosis Complex at Veterinary College Poultry Farms, Mathura for a period from 1960-1967.

Year.	No. of birds examined at autopsy.	Total no. of positive cases of A.L.C.	Percentage.
1960	776	28	3.6 %
1961	2621	57	2.18%
1962	2799	27	0.96%
1963	2530	52	2.05%
1964	5302	95	1.87%
1965	6175	159	2.5%
1966	7130	193	2.7%
1967	3210	50	1.55%
TOTAL:-	30,543	661	2.1 %

Total incidence observed is 2.1% of the total mortality.

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T A B L E - VI.

Incidence of Avian Leukosis Complex on the basis of autopsies conducted by the author at different centres.

Name of the centre	Total no. of birds examined at autopsy.	Total no. of positive cases	Percentage.
1. Poultry Extension Centre, Bihar Sharif, Patna.	201	17	8.4%
2. Central Poultry Farm, Patna.	758	12	1.5%
3. Veterinary College, Mathura.	559	-	-

N O T E :- The autopsies at Veterinary College, Mathura mentioned in the above TABLE were conducted in the month of May only while those at the other two centres throughout the period of study.

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The frequency of involvement of various organs on the basis of histopathological studies carried out by the author on the materials collected during the period of this study is tabulated below:-

TABLE - VII.

Sl. No.	Organs involved.	Number of cases in which the organ was involved out of 32 cases.
1.	Liver.	24
2.	Spleen.	24
3.	Kidney.	9
4.	Ovary.	8
5.	Heart.	4
6.	Intestine.	2
7.	Pancreas.	2
8.	Oviduct.	2
9.	Proventriculus.	1

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It can be seen from the foregoing tables that there was a fairly high incidence of Avian Leukosis Complex at the Poultry Extension Centre, Bihar Sharif, Patna. The total incidence of 18.1% out of the total mortality has been worked out during the period from 1965-67. Similarly, the incidence at Veterinary College Farms, Mathura has been worked out to 2.1% of the total mortality during the period from 1960-67.

On working out the incidence on the basis of autopsies conducted by the author during the tenure of the present study, an incidence of 8.4% at the Poultry Extension Centre, Bihar Sharif, Patna and 1.5% at the Central Poultry Farm, Patna have been noted. No case of Avian Leukosis Complex was encountered by the author at Veterinary College Poultry Farms, Mathura in the month of May during which period he attended the ~~peutri~~ poultry post-mortems at the above institutions

More cases of Avian Leukosis Complex have been observed in the Rhode island red breed than White leghorn breed. Out of 117 positive cases of Avian Leukosis Complex, 70 cases (59.3%) occurred in Rhode island reds and 47 cases (40.7%) in White leghorns.

Maximum number of cases were observed in the age group of 10-15 months and minimum number of cases in birds over 15 months of age.

It was also seen that more of the deaths due to

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Avian Leukosis Complex occurred during the summer season i.e. from March to June, lesser in the winter season i.e. November to February and least deaths were noted in the monsoon season i.e. July to October.

The involvement of different organs in Avian Leukosis Complex was observed to occur in the following order of priority - Liver, spleen, kidney, ovary, heart, pancreas, intestine and proventriculus.

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REVIEW OF LITERATURE AND PRESENT OBSERVATIONS ON AVIAN HAEMATOLOGY AND HAEMATOLOGY OF AVIAN LEUKOSIS COMPLEX

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REVIEW OF LITERATURE AND PRESENT OBSERVATIONS ON AVIAN HAEMATOLOGY AND HAEMATOLOGY OF AVIAN LEUKOSIS COMPLEX.

INTRODUCTION:

Studies on the avian blood have long been hampered to a great extent due to lack of a proper and quick quantitative method for the enumeration of the leukocytes. The differentiation of the leukocytes from the erythrocytes is rather difficult in the counting chamber as the latter are nucleated in birds. One of the most controversial aspects of avian haematology centres around the morphology of its polymorphonuclear leukocytes (Heterophils), especially when Giemsa's or Wright's staining techniques are employed. The heterophils and eosinophils of the birds take the acid stain unlike the human neutrophils. As such, the difference in the shape of their cytoplasmic granules was utilised as the chief criterion for distinguishing the heterophils from the eosinophils. Studies made by Olson (1937) and Natt and Herrick (1954) on the chicken leukocytes revealed that contrary to the generally accepted description of the heterophils, the shape of their cytoplasmic granules showed marked variations from that of a rod or a spindle to definite spherical bodies. Because of this variation, the identification of the heterophils and eosinophils on the basis of the morphology of their cytoplasmic granules alone is somewhat difficult in the chicken.

Another difficulty in the study of avian blood is the identification of the "Lymphoidal" cells with certainty. In some pathological conditions, it is not unusual to come across cells morphologically similar to that of the lymphocyte and the lymphoblast. Some of the latter cells may actually be the monocytes or their precursors or even proerythrocytes.

REVIEW OF LITERATURE:

1. NORMAL BLOOD VALUES:

Normal blood values for chickens have been reported by a large number of workers. Biely and Palmer (1935), Forkner (1929), Olson (1937), Devilliers (1938), Twisselman (1939) and Hamre and McHenry (1942) have reviewed and summarised the literature on the normal number of erythrocytes and leukocytes of chickens while Devilliers has also reviewed the literature on haemoglobin values and the number of thrombocytes of chickens. Mean values presented in the literature as normal values for haemoglobin, erythrocytes, leukocytes and thrombocytes have ranged from 8.5 to 12.6 gms. per 100 c.c. of blood, 2.1 to 4.7 millions per c.m.m. of blood, 14.7 to 55.2 thousand per c.m.m. of blood and 17.9 to 130.0 thousands per c.m.m. of blood respectively. Devilliers (1938) in his review of the literature on blood values of fowls stated that no values for the volume of packed red blood cells had been reported for chickens. Heller and Paul (1934), as quoted by Hamre and

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McHenry (1942), in a study of the effectiveness of various anti-coagulants in the determination of cell volume, recorded the value of 27%. Hamre and McHenry (1942) obtained an average value of 29% of packed cell volume.

2. DIFFERENTIAL COUNT:

Devilliers (1938) and Twisselman (1939) reviewed the values for the differential leukocyte counts of birds. Hamre and McHenry (1942) presented the average values of 30.8% for heterophils, 4.6% for eosinophils and 2.7% for basophils. The mean values presented in the literature for lymphocytes vary greatly. Devilliers (1938) and Twisselman (1939) had presented the average values of lymphocyte count. Only very few investigators have differentiated between the large and small lymphocytes. Hamre et al (1942) experienced great difficulty in clearly distinguishing between large lymphocytes and monocytes in the study of avian blood smears. According to Devilliers (1938) a number of investigators had reported that monocytes do not occur in the blood smears of birds while others have reported that they occur very infrequently. Hamre et al. (1942) found that the large lymphocytes and monocytes could be separated only arbitrarily since a line of leukocytes showing all degrees of transition between recognised monocytes and large lymphocytes occurred in the blood smears. They designated those leukocytes as monocytes

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which were usually slightly larger than the common types of large lymphocytes. Twisselman (1939) reported 13.7% and 16.5% for monocytes for chickens obtained from blood smears stained with wright's stain and by the supravital technic. Hamre and McHenry (1942) observed a range of 0 to 3 percent and they designated as monocytes only those cells which could be clearly identified as that type. Thus the great variation in the normal values reported for lymphocytes and monocytes of birds was due to a lack of agreement as to the characteristics of these two types of leukocytes. Hayden and Fish (1928) reported 81.35% lymphocytes, 1.54% mononuclear leukocytes, 10.285% polymorphs, 5.173% eosinophils and 1.623% basophils. Cook (1937) reported 73.3% lymphocytes, 15.2% polynuclear rods and granules, 2.7% basophils and 6.3% monocytes. Olson (1937) noted marked variation in the shape of granules of heterophils and eosinophils specially by wright's and Giemsa's staining procedures.

3. TECHNIQUES:

The literature on procedures for counting avian blood cells was reviewed by Natt and Herrick (1952). The first method for counting leucocytes in avian blood was proposed by Warthin (1907) who suggested an indirect method for counting these cells. He used a diluent which was quite capable of preserving all the blood cells. The calculation

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of the total count of leukocytes was made by determining the total number of cells (erythrocytes and leukocytes) in a cubic m.m. of blood and subsequently ascertaining the ratio between these cells in a stained smear. Blain (1928) was the first to describe a direct method for counting the leukocytes of avian blood. His method involved the use of two solutions consisting of neutral red as the differentiating dye. Forkner (1929) stated that Blain's method did not give a correct leucocytic count as the dye stained polymorphonuclear leukocytes and monocytes only. So he used only one diluting fluid which contained 25 m.gms. of neutral red in 100 ml. of normal saline solution, and he found it possible to count monocytes and polymorphonuclear leukocytes in a haemocytometer. The total leukocyte number was calculated from the relative percentage of these cells, as determined from a differential count and haemocytometer count. The use of brilliant cresyl blue was suggested by Coates (1929) quoted by Olson (1935) for differentiating the blood cells of the fowl. This method involved mixing of the dye solution with a solution of potassium cyanide, filtration and use of subdued light to differentiate the leucocytes. Olson (1935) in a comparative study found it unsatisfactory. Wiseman (1929) quoted by Olson (1935) evolved a new blood diluent which included the dye, phloxine. This dye stained the polymorphonuclear leukocytes and eosinophils satisfactorily and permitted the count to be made in a haemocytometer. The total leukocyte count was

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calculated with the aid of a differential smear count as in the Forkner's (1929) technique. But there was a drawback in the use of this method since it took several hours to obtain maximum staining reaction, thus consuming time and making rapid counting impossible. Another direct method of counting the leukocytes was introduced by Shaw (1930) who used two solutions containing the dyesx neutral red and crystal violet respectively. This method no doubt gave a satisfactory differential stain but the preparation of the solution daily made the process cumbersome. This was further limited as the solutions were required to be filtered before use and thus consuming lot of time while series of observations were to be made. Wetmore (1940) suggested the use of brilliant cresyl blue and pyronin and claimed to have obtained equally good results when checked against Shaw's method. This diluent was found unsatisfactory as it was not stable. A further modification of the procedures adopted by Wetmore (1940) and Blain (1928) was suggested by Yokoyama (1947) who was using a diluent for counting the blood cells of fish. Her diluent consisted of two solutions, one of which contained the dyes, pyronin and methyl violet 2B. This dye solution was mixed with a modified formalin-Locke's solution and filtered. The stock solutions were quite stable. Darcel (1950) introduced a new blood diluent consisting of two solutions one contained Giemsa stain and the other sodium chloride, sodium citrate

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and neutral formalin in distilled water. The two solutions were mixed and filtered daily. Natt and Herrick (1952) described a new diluent which permitted ready, differentiation of the several types of chicken blood cells and the procedure was simple since it was a mere direct count to be made from the blood sample. The diluent consisted of methyl violet 2B as the differentiating dye. This method had the advantage of using a single solution and was quite satisfactory for routine work.

Verma (1960) in his early studies used Shaw's (1930) method but later on adopted Dixon and Torbert's (1959) method. Srinivasan (1965) in his studies on the haematology of avian spirochaetosis used Natt and Herrick's (1952) method for total red and white cell counts.

There is wide divergence of opinion regarding the normal cellular elements of fowl blood. As reported by various workers, the range of total cell counts and differential leukocyte counts vary to such an extent that it is difficult to judge between the normal and the abnormal. The figures given by some workers are tabulated below:-

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TABLE - VIII.

Normal values of total and differential cell counts as reported by various workers

Author.	Total R.B.C. in m/c. m.m. of blood	Total W.B.C. in thousands per c.m.m. of blood	Differential count in percent				
			Neutrophils	Eosinophils	Lymphocytes	Monocytes	Basophils
Mack *	3.0	37.7	32.7	2.7	54.9	6.2	3.3
Burnet*	3.3	17.9	28.8	3.3	58.0	5.5	4.3
Ellermann and Bang.*	3.0	30.0	-	37	-	-63-	-
Good Wall.*	3.2	19.0	37	6	-56-		1
Hedfield*	4.2	24.0	30	12	43	12	3
Schmeisser*	3.5	50.0	29.6	4.3	42.3	19.4	2.2
Warthin.*	2.5	20.5	21.5	10.0	35.5	14.5	2.0
Seagar (1933)	2.9	27.0	34.0	5.0	54.0	5.0	2.0
Blakemore (1934)	2.7	25.1	-21.5-		69.4	7.5	-
Gaiger and Davies (1946)	3.0	25.0	33.0	3.0	45.0	15.0	4.0

* Quoted by Johnson and Conner (1933).

4. HAEMOGLOBIN ESTIMATION:

Accurate determination of the amount of haemoglobin in avian blood has always been more difficult than in the mammalian blood because the acid haematin method produces a turbidity in the sample which gives a false light transmission

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value. Dukes and Schwarte (1931) were the first to try to apply a compensating correction factor. Schultze and Elvehjem (1934) estimated the haemoglobin in fowl's blood by using the standard alkali haematin method and stated that it gave consistently good results. Olson (1935) made a comparative study of the amount of haemoglobin in the fowl blood by acid haematin method as read in different standard haemoglobinometers used for the purpose for human blood, against photoelectric haemoglobinometer. Due to the turbidity of fowl's blood he found that the standard haemoglobinometers gave a higher value than was found by the photoelectric haemoglobinometer. This variation was due to the presence of nucleus in the avian erythrocytes and according to him is constant in case of New Comer Haemoglobinometer, which could be corrected easily. The correction factor given by him is as follows:

$$\text{Gms. of haemoglobin per 100 c.c.} = \frac{0.16365 \times \text{New Comer percent}}{0.437}$$

or New Commer (Percent) = 6.1106 x photoelectric (gms.) + 2.67.

Blackemore (1945) estimated the haemoglobin in fowls' blood by using standard alkali haematin method and stated that it gave consistently good results. Dennington and Lucas (1955) suggested a modification of the alkaline acid haemoglobin technique, by which the turbidity of avian blood was eliminated. Varma (1960) used alkaline haematin method for estimation of haemoglobin satisfactorily. Srinivasan (1965) in his studies estimated haemoglobin by acid haematin method using Sahli's haemoglobinometer and found no difficulty.

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5. PACKED CELL VOLUME:

Sturkie et al. (1951) recorded a value of 28.5% in laying adult chickens. Johnston (1955) found that the cell volume values varied from 19% to 33% while studying the blood of chicks from 5-24 days. A value ranging from 27.5% to 31.1% in growing white Leghorns was recorded by Medway and Kare (1959). Srinivasan (1965) recorded a mean value of 28.0% in fowls from 10-12 weeks age.

6. CLOTTING TIME:

Johnson et al.(1933) reported a range varying from 1-14 minutes with an average of 6 minutes. Cook (1937) recorded an average value of 4.6 minutes while his range was varying from 1.3 minutes to 14.5 minutes. Dukes (1947) reported coagulation time to be 4.5 minutes. Varma (1960) obtained a value of clotting time in chickens ranging from 23.66 records to 52.25 seconds. Lucas and Jamroz (1961) had presented the normal avian blood morphology in their monograph, "Atlas of avian Haematology". Srinivasan (1965) recorded an average clotting time of 262 seconds.

HAEMATOLOGY OF AVIAN LEUKOSIS COMPLEX:

REVIEW OF LITERATURE:

Ellermann and Bang (1908) and Schmeisser (1915) have described the blood changes in the anaemic form of leukosis in which a large number of erythroblasts appeared in the peripheral blood. Bedson and Knight (1924) described a similar condition in fowls in England but in addition demonstrated

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the presence of a lipochrome in the serum and tissues of the affected birds accompanied by an increase in the ether extracts of the serum. Bayon (1929, 1930) described the haemopathies of the fowl in which increasing number of various immature blood cells appeared in the peripheral blood. These conditions described by him as Erythromyelosis, Leucomyelosis and Myelo-lymphocytosis (akin to Erythroleucosis, Myeloid leukosis and lymphoid leukosis of Ellermann respectively) were specific and when the immature cell types appeared in blood stream in increasing number it indicated bone narrow involvement.

Macfie (1914) in Nigeria and Adler (1925) in Palestine reported to have detected intracytoplasmic annular inclusion bodies entirely composed of chromatin material lodged in the mononuclear leukocytes of fowls suffering from an acute infectious disease. The former worker also reported their presence in the cytoplasms of the erythrocytes. One of Adler's fowls had leukaemia and none had pronounced anaemia. Stubbs and Furth (1932) described the presence of this type of bodies in the cytoplasm of undifferentiated mononuclear cells in fowls suffering from spontaneous erythroleukosis.

Furth (1931) stated that in myeloid leukaemia the total leukocyte count was unusually high, the ratio between red cells and white cells being 3:1 frequently, and sometimes the number of white cells out numbered red cells. Stubbs (1934) stated that in myeloid leukosis the ratio between red cells and white cells instead of being 100:1 in normal chickens

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very often became 2:1 and in some cases the number of white cells exceeded the number of red cells.

Furth and Strumia (1931) stated that leukaemia produced by transmission was preceded by an aleukaemic stage, in which the lymph nodes and spleen were uniformly enlarged, and the white cell count and the percentage of lymphocytes remained within normal range, but immature lymphocytes were numerous in the circulating blood. Stubbs and Furth (1931) stated that in theirstrain two types of leukaemia were observed in experimental chickens viz. one in which the blood picture showed large number of erythroblasts and the other showed large number of large mononuclears and myelocytes. Fenestermacher (1932) reported a total erythrocyte count of 1,090,000 to 2,300,000 per cu.m.m; a total leukocyte count of 1,850,000 to 3,690,000 per cu.m.m. with a marked eosinophilia or lymphocytosis sometimes reaching upto 64% and 93% respectively in cases of fowl leukaemia. Non-specific azurophilic granules and vacuoles were demonstrated by the same author in the cytoplasms of lymphocytes of some of the affected birds.

Blood changes were not found significant in cases of fowl paralysis by a number of workers e.g. Pappenheimer et al. (1926), Blount (1934), Dobson (1934), Hamilton (1934), Blakemore (1934) and Jungherr (1934). Similarly Johnson and Conner (1933) reported no significant alteration in blood picture from the birds suffering from lymphoid leukosis. Bayon (1931) stated that differential counts in paralytic birds showed a definite

increase of lymphocytes. Seagar (1933) claimed that cases of fowl paralysis showed an increase in total leukocyte count by 20 to 30% and in differential count the large mononuclear cells were found to be higher than the established normal count. In cases of fowl paralysis Johnson and Conner (1933) found that they lymphocytes showed vacuolation of cytoplasm and budding. Furth (1933) reported subleukaemia or leukaemic alteration in the blood in some cases. Blakemore (1934) also observed a slight increase in both total white cell count and lymphocyte count in fowls suffering from neurolymphomatosis but he stated that it was of doubtful diagnostic value, as the increase was not consistent. Similarly no significance could be attributed to the differential leukocytic count without reference to the total leukocytic count in cases of fowl paralysis. Emmel (1936) stated that haemocytoblastosis is a condition in chickens where the blood cells showed various types of degeneration and could be induced by oral exposure and intravenous injection of microorganisms of the paratyphoid group and by intraperitoneal injection of tissue emulsion and filtrates of the organs of birds affected with fowl paralysis. Such an induced haemocytoblastosis lacked in the necessary impetus for the development of paralysis or leukaemia. Emmel definitely confused between salmonellosis in fowls and leukosis as can also be seen from his work (1936) where he claimed that fowl paralysis was produced by the members of paratyphoid group of organisms. Beach and Twisselmann (1939) proved the falacy of

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Emmel's findings. They attributed doubtful diagnostic value to the blood changes in advanced cases of neurolymphomatosis and visceral lymphomatosis as in their observations the changes in the blood cells did not conform to any particular trend as to their nature or to the type of lymphomatosis. Emmel (1940) used the term haemocytoblastosis for the condition characterised by an increase or decrease of leukocytes and the presence of immature and degenerated blood cells in the peripheral circulation. Further he stated that this condition in fowls leads to the development of fowl paralysis and allied diseases.

Blakemore (1945) observed that in fowl paralysis the total erythrocytes and haemoglobin index of the affected fowls remained within normal range. Leukocytosis with an increasing number of monocytes was recorded by him but he did not observe any basophilia as was detected by Johnson and Conner (1933).

Patterson et al. (1932) stated that a positive blood finding in erythroleukosis and myeloid leukosis was diagnostic, but a negative blood picture did not mean that the bird was not affected with certain expression of the disease.

Furth (1931a) noticed Plasma, Leukocyte and Erythrocyte ratio of about 88:1:11 in erythroleukosis and 16:69:15 in granuloblastosis. This worker while studying blood of erythroleukotic birds, reported an intense thrombocytopenia.

Marked rise in leukocyte column of centrifugalised blood in the myeloblastosis had been reported by Furth (1931a).

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Feldman and Olson (1933) described in detail the blood changes in leukaemia. They stated that in erythroleukosis polychrome erythrocytes and erythroblasts with two nuclei and mitotic bodies were common. In myeloid leukosis presence of large numbers of promyelocytes in a stained film coupled with a very low total erythrocytic count ranging from 500,000 to 1,000,000 per cu. m.m., a low thrombocyte and a high leukocytic count were characteristic features. Correspondingly the haemoglobin percentage was lowered. Oberling and Guerin (1934) and others reported the presence of primitive cells of the myeloblastic series especially myeloblasts and promyelocytes in the blood picture of myeloblastic birds. Beard (1956) reported immature cells in large number up to 2 millions per cu. m.m. indicative of leukaemic or subleukaemic state.

Schmitt (1936) reported fall in red blood count and haemoglobin, presence of erythroblasts and erythrogonia in stained blood smear, decrease in blood viscosity and increase in sedimentation in erythroleukosis. The cytoplasm of these immature bl-ood cells appeared to be narrow basophilic and occasionally vacuolated (Furth, 1931 b; Oberling and Guerin, 1934).

Johnson and Conner (1933) reported no alteration in the blood picture in uncomplicated cases of ocular form of leukosis.

Jungherr (1959) concluded that blood picture may be characterised by the presence of immature lymphocytes in the circulating blood along with budded lymphocytes and

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lympholeukaemic alterations may be accompanied by anaemic changes such as the presence of basophilic erythrocytes, erythroblasts and erythrogonia suggestive of erythroleukosis.

Jungherr (1959) also stated that birds suffering from myelocytomatosis were found usually aleukaemic when uncomplicated, although heterophil leukocytosis might be observed but blood picture was dominated by the presence of polychromic myelocytes and acidophilic metamyelocytes in leukaemic conditions.

Sato (1960) reported no difference in packed red cell volume of chickens suffering from lymphoid leukosis.

Campbell (1961) reported anaemia in the osteopetrosis due to obliteration of marrow and no changes in blood picture in respect of total red cell count although there was a decrease in number of white cells characteristic of a myelophthisic anaemia.

Bell and Campbell (1961) reported the presence of some proerythroblasts in the peripheral blood in the birds suffering from osteopetrosis but not in sufficient numbers to resemble erythroleukosis.

PRESENT OBSERVATIONS ON HAEMATOLOGY:

In the present investigation the different haematological values of the sixty birds examined are given in the following table:-

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TABLE - IX.

Details of the observations on Haematology.

Sl.No. of the Bird.	Total Erythrocyte count in millions /c.m.m.	Total Leukocyte count in thousands /c.m.m.	Haemoglobin in gms. per 100 c.c. of blood.	Clotting time in seconds	Packed cell volume in percentage	S.E.R. at 2 hrs. in m.m.	Differential leukocyte count in percentage					Buffy layer in %
							Lymphocytes.	Heterophils	Monocytes.	Eosinophils.	Basophils.	
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
1.	2.3	24	11.4	220	31	5	63	25	8	3	1	1.2
2.	2.4	20	11.0	240	28	7	65	28	6	1	-	1.0
3.	2.3	25	10.4	260	28	5	62	29	6	2	1	1.0
4.	2.1	18	11.0	180	31	6	60	35	4	1	-	1.0
5.	2.0	20	9.8	260	29	3	59	38	3	-	-	1.0
6.	1.9	27	10.0	180	31	5	60	29	8	2	1	1.0
7.	2.2	24	10.4	260	30	5	62	30	7	1	-	1.0
8.	2.1	25	9.0	240	26	5	63	30	4	2	1	1.0
9.	1.8	21	9.8	240	25	7	60	35	4	1	-	1.2
10.	1.9	18	9.0	220	30	5	59	35	5	1	-	1.2
11.	1.8	18	8.2	260	28	5	58	33	7	2	-	1.0
12.	2.1	20	8.8	280	30	6	55	39	6	-	-	1.0
13.	2.8	27	8.0	300	27	7	54	35	8	2	1	1.0
14.	2.2	18	8.6	320	25	6	60	30	7	2	1	1.0
15.	2.8	29	8.8	240	27	6	62	33	4	1	-	1.0

Contd.....

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
16.	1.9	28	8.6	220	28	5	63	25	8	3	1	1.0
17.	2.9	46	10.2	280	32	5	60	30	7	2	1	2.0*
18.	1.9	25	9.8	320	25	6	64	30	4	1	1	1.0
19.	2.6	24	7.4	360	29	7	54	39	5	1	1	1.0
20.	1.9	30	7.2	280	28	7	55	35	9	1	-	1.2
21	2.4	17	7.6	260	29	6	56	38	4	1	1	1.1.
22.	2.6	18	9.0	280	30	5	54	39	5	1	1	1.0
23.	1.9	24	10.8	260	32	5	54	39	7	-	-	1.0
24.	2.3	25	10.0	280	28	6	62	30	7	1	-	1.0
25.	2.1	22	9.8	320	28	5	65	25	8	1	1	1.2
26.	1.9	18	8.2	210	30	5	62	30	5	2	1	1.2
27.	1.8	29	9.0	280	28	6	53	36	9	1	1	1.0
28.	2.1	42	9.8	320	29	6	61	30	8	1	-	3.2
29.	2.1	24	8.8	280	29	5	60	35	4	1	-	1.0
30.	2.2	25	8.2	360	28	7	80	18	2	-	-	1.5
31.	1.9	23	8.8	300	32	7	55	35	8	1	1	1.0
32.	2.0	25	8.8	280	32	7	54	38	7	1	-	1.1
33.	2.3	28	8.6	300	29	5	63	30	6	1	-	1.0
34.	2.5	24	9.4	280	30	5	60	36	3	1	-	1.0
35.	2.1	18	8.0	320	31	5	58	35	6	1	-	1.0

Contd.....

Contd.... Table - VIII.

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
36.	2.4	25	12.0	240	31	4	60	35	4	1	-	1.2
37.	2.9	26	10.6	240	33	5	60	32	5	2	1	1.5
38.	2.3	20	9.6	180	25	6	72	26	2	-	-	1.0
39.	2.4	25	9.2	260	29	6	55	39	5	1	-	1.2
40.	2.9	23	9.8	280	27	7	54	39	7	-	-	1.2
41.	2.5	30	10.0	300	30	7	60	28	9	2	1	1.0
42.	2.6	27	9.6	220	30	5	60	30	8	1	1	1.0
43.	1.8	24	8.4	200	25	5	58	32	8	1	1	1.0
44.	2.1	29	8.8	240	25	5	58	35	6	1	-	1.0
45.	2.1	27	9.2	240	28	7	58	38	4	-	-	1.2
46.	2.1	28	9.6	180	27	6	62	29	8	1	-	1.0
47.	1.8	23	8.2	220	30	5	59	30	9	1.	1	1.0
48.	2.2	18	9.2	320	28	6	55	35	8	1	1	1.0
49.	2.1	23	8.4	300	31	5	54	35	9	1	1	1.2
50.	2.1	22	9.8	360	28	7	58	38	4	-	-	1.0
51.	1.9	24	8.2	320	29	6	58	35	4	2	1	1.0
52.	2.5	24	9.0	120	30	5	54	39	5	1	1	1.0

Contd.....

Contd..... Table - VIII.

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
53.	2.2	23	9.8	120	31	6	55	38	6	1	-	1.1
54.	2.1	26	8.4	180	25	6	58	35	6	1	-	1.00
55.	2.4	24	8.4	300	31	6	60	34	5	1	-	1.0
56.	2.4	59	8.9	260	30	5	62	29	8	1	-	4.0*
57.	2.1	18	8.8	240	31	5	62	28	9	1	-	1.0
58.	2.5	22	8.0	250	27	7	62	35	3	-	-	1.0
59.	2.2	24	9.4	280	25	5	60	28	9	2	1	1.0
60.	2.4	20	8.0	260	30	5	60	30	9	1	1	1.0

In the present observations, as can be observed from table - IX, fowls No.17, 28 and 56 had a very high total leukocyte count whereas fowls No.30 and 38 had an unusually high lymphocyte percentage. Unusually thick buffy layer was seen in case of fowls No.28 and 56. On histopathological examination of the different organs of these five birds only fowl No.56 proved to be positive for lymphoid leukosis.

In the case of this fowl positive for lymphoid leukosis, the total leukocyte count was 59 thousands/c.m.m. of blood and the buffy layer was 4.0%.

The haematological values of all other fowls did not show any deviation from the normal values.

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REVIEW OF LITERATURE & PRESENT OBSERVATIONS ON GROSS LESIONS & HISTOPATHOLOGICAL CHANGES.

REVIEW OF LITERATURE AND PRESENT OBSERVATIONS ON GROSS LESIONS AND HISTOPATHOLOGICAL CHANGES

REVIEW OF LITERATURE:

LYMPHOID LEUKOSIS

SYNONYMS:

Big liver disease, Lymphatic leukosis (Ellermann, 1922), Visceral lymphomatosis (Pappenheimer et al.,1926), hemocytoblastic myelosis (Battaglia and Leinati, 1929), Lymphadenoma (Mathews and Walkey, 1929), Lymphocytoma (Fieldman,1932), Lymphomatosis (Johnson, 1932), Lymphosarcomatosis (Burmester, 1967), lymphoid leukosis-lymphocytoma and lymphoblastoma (Campbell, 1964).

GROSS LESIONS:

Furth (1935) described the lesions in the organs produced by lymphomatosis virus strain - 2. In most instances the liver was enlarged upto 4 times its normal size. In many cases numerous grey lymphomas varying in size from 0.1 cm. to 1.0 cm. in diameter were produced.

Campbell (1961) recorded the presence of creamy white nodules in liver, spleen, kidney, heart, lung and intestine and enlargement or atrophy of spleen in lymphocytoma. In the case of lymphoblastoma he recorded the enlargement of liver which turned from creamish to grey with red streaks. He reported that affection of the central and peripheral nervous system was rare.

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HISTOPATHOLOGICAL CHANGES:

According to Lee et al. (1937) the infiltrating cells were described as variable sized, undifferentiated mononuclear, basic staining nongranular cells. The infiltration in the liver varied from mild periportal accumulation to massive infiltration destroying almost all the normal liver tissue. In the kidney the cellular infiltrations were between the tubules and in extreme cases separated apart the parenchymal elements or actually replaced them. In the spleen the infiltrations were found both in the pulp and the follicle. Ovary was the frequent site of infiltration. Dalling (1938) described that the cells seen in the lesions of lymphomatosis were young lymphoblasts, cells resembling the mammalian plasma cells; less commonly polymorphonuclear leukocytes.

Waters and Pricket (1944) described that the condition called lymphomatosis was characterised by abnormal extravascular accumulation of mononuclear cells having some characteristics of the lymphoid series, in any system or part of the body of the chicken. In the liver the lymphoid accumulations were usually placed perivascularly around the hepatic vessels and appeared to be well circumscribed or encapsulated, the capsule being apparently formed by a portion of the vessel wall. No evidence of compression of the liver cells was noted, although the lymphoid nodule caused impingement of the lumen of the vessel. In another type of perivascular lymphoid aggregation, the mononuclear cells were present around all visible branches of hepatic vessels but without compression of the

parenchymal elements.

Iyer and Hashmi (1945) described the tumours of visceral organs to be composed of small and large basic - staining, nongranular, mononuclear cells (lymphocytes and lymphoblasts) closely packed together. The normal elements showed no degenerative changes, remained intact until entirely replaced by the infiltrating cells. The infiltration of cells in the liver varied from mild periportal aggregation to massive infiltration.

Davis and Doyle (1947a) indicated that lymphoblasts were predominantly the typical cells of lymphomatous lesions, but cases were noted in which a more mature lymphocyte was the predominating cell. They 1947(b) described the appearance of eosinophilic leukocytes in many cases in both the normal as well as the parivascular lymphoid nodules in the liver.

Das (1951) recorded that the histopathological examination of the lesions in lymphoid leukosis comprised of a generalised infiltration of the liver, spleen and kidneys with large mononuclear nongranular cells; focal necrosis of the liver; reduction of stroma of spleen; proliferative changes of vascular endothelium and fibroblastic changes of the vessel walls in a few cases; mild parenchymatous degeneration to necrosis in renal cells; cellular infiltration in the epicardium, in the myocardium in more advanced cases; cellular infiltration throughout the lung tissue; accumulation of large round cells

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with little stroma and very rare blood vessels in the tumours on the peritoneal surface of the intestine.

Arvy and Gabe (1961) indicated an increase in the cortical tissue of the adrenal glands and the percentage of acidophile cells of the pituitary glands, but no change in the structure of pancreas and parathyroid.

Campbell (1961) described diffuse or focal infiltration of lymphocytes and lymphoblasts in visceral organs of birds affected with this disease on the basis of histopathological examination.

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OCULAR FORM OF AVIAN LEUKOSIS COMPLEX

SYNONYMS:

Blindness, grey eye, pearly eye, glass eye, fish eye, iritis (Pappenheiner et al.,1926), epidemic blindness (Findley and Wright, 1933) and Uveitis (Doyle, 1928).

GROSS LESIONS:

Pappenheimer et al. (1926, 1929) observed discolouration of iris from yellow brown to slate grey in several of the paralysed birds. They (1926) also reported a granular or amorphous material in the anterior chamber of eye.

Doyle (1928) reported that in advanced stages the iris presented a diffuse greyish opacity with pin point pupil. Gibbs and Johnson (1936) observed lesions in the cornea near schlemm's canal, bulbar conjunctiva, pecten, retina and optic nerve, in a comparatively small number of cases.

Jungherr (1934) reported concentric annular or spotty depigmentation or diffuse bluish grey fading of the iris in one or both eyes, with gradual loss of light accomodation.

Iyer and Hashmi (1945) reported the thickened, oedematous and pigmented iris and the distortion of pupil in the ocular form of A.L.C.

Rigdon (1959) reported the occurrence of Cataracts in chickens affected with ocular lymphomatosis.

HISTO PATHOLOGICAL CHANGES:

Jungherr (1934) by microscopic study, described that iris was most frequently affected and showed mononuclear

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spleen were greatly enlarged and became firm, granular and often dryish in appearance.

HISTOPATHOLOGICAL CHANGES:

Johnson (1934, 1941) observed extravascular accumulations of all stages of immature granulocytes including haemocytoblasts in many of the visceral organs.

Olson and Bullis (1942) reported that in the liver affection infiltration with myelocytes was moderate and usually confined to the periportal areas. The spleen showed diffuse infiltration of the pulp with neoplastic myelocytes. The degree of involvement of the kidneys varied from a slight inter-tubular infiltration to almost complete replacement of parenchyma with masses of myelocytes. Blood vessels of the tissues contained a variable number of myelocytes.

Campbell (1961) observed massive accumulations of large myeloblasts and promyelocytes in the parenchymatous tissue in which cells might overflow in to circulation shortly before death, to produce a true leukemia.

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MYELOCYTOMATOSIS.

SYNONYMS:

Myelocytoma (Pentimalli, 1915; Feldmann, 1932), myeloid leukosis (Kilermann, 1920), myelocytomatosis (Furth, 1933), aleukemic myeloblastosis (Nyfeldt, 1934), myeloid leukosis-myelocytoma (Campbell, 1954).

GROSS LESIONS:

Campbell (1961) observed friable masses of tumour tissue resembling bakers yeast in the internal aspect of the sternum, along the costal borders and specially at the costo-chondral junctions. The ovary was usually involved but was not much enlarged. The kidneys were not always affected.

HISTOPATHOLOGICAL CHANGES:

According to Campbell (1961) the liver frequently showed periportal accumulation of myelocytes to a considerable degree. He described that the organs were infiltrated with myelocytes with a vesicular chromophobe nucleus and prominent eosinophilic granular cytoplasm. The bone narrow was composed of myelocytes, occasionally accompanied by a terminal leukermia.

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ERYTHROLEUKOSIS

SYNONYMS:

Severe anaemia, "light" intravascular lymphoid leukosis (Ellermann, 1921), erythroleukocis (Ellermann, 1923), erythromyelosis (Bayon, 1930), erythroblastosis (Engelbreth - Holm and Rothe Meyer, 1932).

GROSS LESIONS:

Furth (1931) described that in erythroleukosis the bone marrow on gross examination was reddish or pale greyish in colour and showed increase in consistency, becoming semi-solid and also showed an absence of fat.

Lee et al. (1937) reported that the gross pathology of the lesions in erythroleukosis comprised swollen liver with rounded edges, enlarged spleen with more grey colour than normal, bone marrow being swollen, pale grey or deep red in colour ~~cour~~ and more solid in consistency. Tumour like nodules occurred in liver and in spleen in many cases.

Campbell (1961) described the gross lesions as diffusely enlarged liver, spleen and kidney with cherry red discolouration while in anaemic cases he observed absence of hyperplasia of visceral organs and atrophied spleen.

HISTOPATHOLOGICAL CHANGES:

Furth (1931, 1931b) observed that liver, spleen

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and bone narrow showed accumulations of the precursors of erythrocytes in their blood sinusoids and capillaries. This condition was termed leukostasis.

Stenszky (1934) reported that in 21 experimental fowls which died of erythroleukosis, severe leukostasis in the blood vessels of the liver, spleen and less regularly in the kidney was present. A slight leukostasis was present in the spleen of 4 of the aneamic fowls.

Johnson (1934, 1941) recognised erythroleukosis by the accumulation of a large number of haemocytoblasts and immature erythrocytes in the spleen pulp, capillaries of liver and bone narrow.

Lee et al.(1937) reported that the histophatology revealed accumulation of haemocytoblastic cells in the capillaries, usually intravascularly but sometimes with tumour like proportions in the liver, accumulation of similar cells in the pulp and follicles of spleen; lymphocytic and myelocytic foci in the bone narrow which in advanced cases appeared as a mass of lymphoid-like cells.

Hall and Pollard (1943) reported extensive perivascular infiltrations in addition to the presence of numerous haemocytoblasts in the blood vessels and sinusoids of the liver, occasional focal necrosis in liver and spleen and extensive extra and intravascular haemocytoblastic infiltrations in the kidney and spleen.

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Das (1951) reported intensive intralobular infiltration of the liver with mononuclear round cells (Leukostasis) in erythroleukosis.

Ishitani (1956) studied 75 experimental and 9 natural cases of erythroleukosis and reported hyperplasia of the cells of erythrocytic series in the liver sinusoids, and activation of reticuloendothelial cells in the spleen, liver and bone narrow.

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FOWL PARALYSIS

SYNONYMS:

Fowl paralysis, polyneuritis (Marek, 1907), neuritis (Doyle, 1926), neurolyphomatosis gallinarum (Pappenheimer *et al.*, 1926), neurogranulomatosis (Lerche and Fritzsche, 1934), Fowl paralysis granuloma (Campbell, 1961) and Marek's disease (Biggs, 1961).

GROSS LESIONS:

Pappenheimar *et al.* (1926, 1929) described grey iris with blindness in fowl paralysis.

McLennan (1935) described that the sciatic nerve and dorsal root ganglion were enlarged in advanced cases of fowl paralysis.

Jungherr (1959) described localized or occasionally diffuse greyish soft swelling of the peripheral nerves especially the femoral portion of sciatic trunk.

Campbell (1961) described pink or cream coloured ovary and thickened and granular mesentery in pullets suffering from fowl paralysis.

HISTOPATHOLOGICAL CHANGES:

Pappenheimer *et al.* (1926, 1929) described infiltrations of lymphoid cells, plasma cells and large

mononuclear cells in the peripheral nerves and central nervous system as the prominent lesions. Infiltrations of such cells were also observed in the lungs, myocardium, liver, Pancreas, kidneys, ovary and iris.

Patterson et al. (1932) described lymphoid infiltrations forming tumours in the nerves and practically all the organs of the body.

Campbell (1961) proposed its separation from the leukosis on the basis of different histopathological findings. He also reported in central lesions a form of perivascular cuffing with lymphoid cells.

Biggs (1961) observed some oedema and variable proliferation of Schwann cells with nuclear swelling in the nerves in early stage of fowl paralysis. There was a light scattering of mononuclear cells (small and medium lymphocytes, Blast cells and Plasma cells) which appeared to be derived from small perivascular foci. This stage of infiltrating cells was followed by diffuse scattering of the cells, which appeared to spread out from these foci eventually involving a large part of the nerve. In advanced stages, lymphocytes dominated among the infiltrating cells replacing the nerve ultimately with solid mass of lymphoid cells with no or few plasma cells. In the liver lesions he observed an- increase in size and number of lymphoid foci. By electron microscopic studies a marked endoneural fibrosis was observed by Deutsch and Siller (1961).

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Biggs (1961) and Campbell (1961) both described that the most common site of proliferative lesions was in and around the ovary. The lesions were infiltrative and spread through the mesentary and sometimes to other nearby organs like kidney and even lungs.

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OSTEOPETROSIS.

SYNONYS:

Diffuse osteoperiostitis (Pugh, 1927), thick leg disease, marble bone and hyperplastic osteitis (Reinhardt, 1930), osteo-dystrophia fibrosa cystica (Gohs, 1934), Paget's disease (Venkatraman, 1936) and osteopetrosis gallinarum (Jungherrand Landauer, 1938).

GROSS LESIONS:

Jungherr and Landauer (1938) reported abnormal thickening or convexities in the diaphyses or metaphyses of the leg bones. In advanced cases a characteristic "boot-like" thickening of the shanks was seen.

Iyer and Hashmi (1945) reported thickening of the long bones particularly in the Shanks in birds which showed other forms of Avian Leukosis Complex as well.

Das (1951) reported that the bones of a bird affected with osteopetrosis were very brittle.

HISTOPATHOLOGICAL CHANGES:

Jungherr (1952) described 3 phases of histo-pathological structure according to maturation of the process. 1. The initial phase was characterised by sequestration and granular degeneration of old trabeculae, fibrosis

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of marrow, increased osteoclasia accompanied by the development of new large-celled vascular fibrous bone tissue failing to undergo osteochondral ossification. 2. Florid phase characterised by gradual replacement of original sponngiosa and compacta by new bone tissue and regression of osteoclastic activity. 3. Arrested phase characterised by condensation and hypercalcification of new bone lamellae and their subdivision by a number of thick irregular cement lines.

Campbell (1961) described that swollen fusiform bones showed a thickened periosteum and were highly vascular. The medullary cavity was obliterated by new bone with an abnormal architecture due to enlarged and elongated Haversian canals.

PRESENT OBSERVATIONS:

During the present investigation tissues from various organs of 90 fowls were examined histopathologically and 32 cases of Avian Leukosis Complex were recorded. The details of gross and histopathological examination has been given in the following description.

A. LYMPHOID LEUKOSIS.

The following cases were categorised as Lymphoid leukosis. The histopathological examination of the lesions were characterised by diffuse or focal accumulations of

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lymphoblasts and lymphocytes in extravascular tissue areas in various organs.

1 (P).

HISTORY:

WLH hen, aged about 11 months, general body condition-poor and emaciated, autopsied on 27.3.1967.

GROSS PATHOLOGY:

LIVER :- Enlarged about 2½ times the normal size, studded with grey tumorous nodules projecting out of the surface of the organ (figure-1).

SPLEEN:- Enlarged about twice the normal size, minute grey dots all over its surface.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER :- Parenchyma has been extensively infiltrated by lymphocytic cells predominantly the lymphocytes and lymphoblasts so much so that hardly any recognisable tissue could be left. The normal lobular pattern of the liver was also disorganised and only thin strands of connective tissue elements particularly those of reticulin could be seen in between the neoplastic lymphoidal cells. Few

periportal areas could also be visualized (figure - 12).

SPLEEN:- The grey pulp of the organ appeared to be considerably increased and assumed the form of a number of follicles of variable size. However, a diffused marked involvement of the organ could not be recognised (figure - 13).

OTHER TISSUES:- Non-leukotic.

2 (P).

HISTORY:

WLH hen, aged about 11 months, general body condition poor and emaciated, died in the night of 29.3.1967, autopsied on 30.3.1967.

GROSS PATHOLOGY:

LIVER:- Enlarged to about twice the normal size, bands and patches of grey coloured areas.

SPLEEN:- Enlarged to about 1½ the normal size, grey foci all over its surface.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER :- The hepatic epithelial cells appeared degenerated and disorganised. The sinusoids appeared dilated and there appeared hyperplasia of the

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reticular cells and fibres situated in the walls of the sinusoids leading to the neoplastic character. The differentiation of various types of cells particularly the lymphoblasts and the lymphocytes from these hyperplastic elements could also be recognised (figure - 14).

SPLEEN:- Similar to 1 (P).

OTHER TISSUES:- Non-leukotic.

3 (P).

HISTORY:

WLH hen, aged about 12 months general body condition poor and emaciated, autopsied on 31.3.1967.

GROSS PATHOLOGY:

LIVER:- Enlarged moderately, patches of grey areas.

SPLEEN:- Slightly enlarged.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- The hepatic epithelial cells appeared to be disorganised and there occurred a number of various sized areas distributed irregularly in the hepatic parenchyma. Majority of these foci consisted of cells of lymphocytic and lymphoblastic

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variety. A number of immature cells have been a constant feature of these foci. Cells showing mitotic figures were also been distributed irregularly in these focal areas (figure - 15).

SPLEEN:- Diffuse reticular hyperplasia of the organ making red and white pulp in distinguishable.

OTHER TISSUES:- Non-leukotic.

4 (P).

HISTORY:

WLH hen, aged about 22 months general body condition good, autopsied on 31.3.1967.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged, red lines on its surface.

SPLEEN:- Slightly enlarged, patches of greyish colour.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- A number of focal areas of cellular infiltrations particularly in the viscinity of blood vessels and arterioles. The majority of the infiltrating cells appeared to be the immature heterophils alongwith some plasma cells, lymphoblasts and lymphocytes. The hepatic epithelial cells and chords appeared to be disorganised (figure-16).

SPLEEN:- Similar to 3 (P).

OTHER TISSUES:- Non-leukotic.

5 (P).

HISTORY:

WLH hen, aged about 22 months, general body condition - poor and emaciated, autopsied on 5.4. 1967.

GROSS PATHOLOGY:

LIVER :- Enlarged slightly, patches of congestition (figure - 2).

SPLEEN:- Slightly enlarged, areas of congestion.

OVIDUCT:- Congested.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Diffuse infiltration of the liver parenchyma by the immature lymphocytes. The hepatic epithelial cells being reduced to thin rims surrounding the inflitrating neoplastic cells.

SPLEEN:- Diffuse proliferation and infiltration of lymphoidal cells throughout the pulp.

OTHER TISSUES:- Non-leukotic.

8 (SP).

HISTORY:

RIR cock aged about 20 months.

GROSS PATHOLOGY:

LIVER:- Enlarged slightly.

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HEART:- Red spots.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER :- Non-leukotic.

HEART :- The myocardium revealed focal accumulation of laymphocytes.

PROVENTRICULUS:- Focal accumulation of lymphocytes and lymphoblasts.

OTHER ORGANS:- No lesions.

42 (P).

HISTORY:

WLH hen, aged about 12 months, general body condition - good, autopsied on 22.4. 1967.

GROSS PATHOLOGY:

LIVER:- Enlarged slightly, discrete greyish small nodules.

SPLEEN:- Enlarged about twice the normal size, grey spots.

KIDNEY:- Slightly enlarged, greyish nodules.

OVARY:- Greyish nodules.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER :- Infiltrated by neoplastic lymphoidal cells replacing the normal hepatic parenchyma. A few blast cells were also recognised (figure - 20).

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SPLEEN:- The pulp of the spleen showed lymphoidal follicles of various sizes. The distribution of lymphocytes was focal (figure - 21).

KIDNEY:- The intertubular spaces of the kidney were infiltrated by immature lymphocytes at various foci.

OVARY:- The stroma was infiltrated with foci of lymphocytes.

OTHER TISSUES:- Non-leukotic.

43 (P).

HISTORY:

WLH hen, aged about 12 months, general body condition-poor and emaciated, autopsied on 23.4.67.

GROSS PATHOLOGY:

LIVER:- Enlarged about twice the normal size, studded uniformly with minute nodules of grey colour.

SPLEEN:- Enlarged about twice the normal size, grey mottling.

KIDNEY:- Enlarged about thrice the normal size, grey nodules.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Similar to 5 (P) (figure - 22).

SPLEEN:- A few foci of lymphocytes and blast cells.

KIDNEY:- In the cortex region were found a few foci

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of lymphoidal cells.

OTHER TISSUES:- Non-leukotic.

44 (P).

HISTORY:

RIR hen, aged about 12 months general body condition - weak, necropsied on 27.5.67.

GROSS PATHOLOGY:

LIVER:- Enlarged to about twice the normal size, studded with minute grey foci.

SPLEEN:- Enlarged to about twice the normal size, minute grey foci.

KIDNEY:- Enlarged to more than twice the normal size, greyish in colour.

OVARY:- Enlarged and tumorous.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Parenchyma infiltrated diffusely by a large number of neoplastic lymphoidal cells of the blast series. There was perivascular arrangement of these cells in portal triads.

SPLEEN:- Small focal infiltration of mononuclear cells of the lymphoid series.

KIDNEY:- The intertubular spaces were massively infiltrated by lymphocytes and lymphoblasts both in the cortex and in the medulla (Figure - 23).

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OVARY:- The stroma of the ovary was diffusely infiltrated by mature and immature lymphoidal cells obliterating the normal architecture of the organ (figures - 24 & 25).

OTHER TISSUES:- Non-leukotic.

45 (P).

HISTORY:

RIR hen, aged about 12 months general body condition - good, autopsied on 25.4.67.

GROSS PATHOLOGY:

LIVER:- Enlarged slightly.

SPLEEN:- Slight enlargement.

KIDNEY:- Slightly enlarged.

OVARY:- Enlarged slightly.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Lymphoidal cells were mostly found proliferating around the periportal sheath, some infiltrating deep into the sinusoids (figure - 26).

SPLEEN:- Similar to 5 (P).

KIDNEY:- Similar to 44 (P).

OVARY:- Diffuse infiltration of blast cells and lymphocytes.

OTHER TISSUES:- Non-leukotic.

46 (P)

HISTORY:

WLH hen, aged about 18 months general body

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condition-poor and emaciated, necropsied on 28.4.67.

GROSS PATHOLOGY:

LIVER:- Enlargement with greyish discoloration.

SPLEEN:- Enlarged with greyish discoloration.

KIDNEY:- Slightly enlarged.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Similar to 44 (P).

SPLEEN:- Similar to 5 (P).

OVARY:- The stroma is mildly infiltrated with round mononuclear cells.

OTHER TISSUES:- Non-leukotic.

47 (P).

HISTORY:

WLH hen, aged about 12 months general body condition - good, autopsied on 30.4.67.

GROSS PATHOLOGY:

LIVER:- White nodules of a hard consistency.

SPLEEN:- Enlarged slightly, discoloured grey and had hard white nodules.

OVARY:- Slightly enlarged and tumorous.

KIDNEY:- White hard nodules.

OTHER ORGANS :- No lesions.

HISTOPATHOLOGY:

LIVER:- Similar to 4 (P).

SPLEEN:- Neoplastic growth consisting mostly of lymphoblasts was found attached to the capsule

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of the organ. The pulp of the spleen was also infiltrated with similar type of cells disrupting the normal architecture of the organ. There was lymphoreticular hyperplasia in the adenoid sheath (figures - 27 & 28).

KIDNEY:- The cortex and medulla were infiltrated with lymphoidal cells separating the tubules wide apart, some of which showed degenerative changes.

OVARY:- Similar to 44 (P) (figure - 29).

OTHER TISSUES:- Non-leukotic.

48 (P).

HISTORY:

RIR hen, aged about 10 months general body condition - good necropsied on 31.4.67.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged.

SPLEEN:- Studded with minute grey nodules (figure -30).

KIDNEY:- Studded with minute grey nodules and enlarged slightly.

HEART:- Neoplastic nodular growths on the epicardium (figures 31 & 32).

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Non-leukotic.

SPLEEN:- Similar to 43 (P).

KIDNEY:- Similar to 47 (P).

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HEART:- The myocardium was diffusely infiltrated with lymphoidal cells disrupting the cardiac muscle fibres.

OTHER TISSUES:- Non-leukotic.

49 (P).

HISTORY:

RIR hen, aged about 12 months general body condition - poor and emaciated, necropsied on 11.5.67.

GROSS PATHOLOGY:

LIVER:- Enlarged about twice the normal size, greyish discolouration.

SPLEEN:- Enlarged slightly, greyish yellow discolouration.

KIDNEY:- Diffuse grey discolouration.

OVARY:- Greyish discolouration, moderately enlarged.

OTHER ORGANS:- Non- lesions.

HISTOPATHOLOGY:

LIVER:- Large foci of lymphoid cell proliferation merging with each other and forming confluent growth. The nodular growths were found to be separated by thin connective tissue septa particularly those of reticulin.

SPLEEN:- Isolated lymphoidal follicles were found in the white pulp. Nodular growths under the capsule as well as deep into the organ.

KIDNEY:- The medulla was found to be infiltrated

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with lymphoidal cells replacing the normal pattern of the organ. Large lymphoidal follicles were also noted (figure - 33).

OVARY:- Large foci of lymphoidal cell proliferation merging with each other were found (figure - 34).

OTHER TISSUES:- Non-leukotic.

50 (P).

HISTORY:

RIR hen, aged about 10 months, general body condition-good, necropsied on 16.5.67.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged and discoloured grey.

SPLEEN:- Slightly enlarged.

OVARY:- Greatly enlarged and tumomous.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Similar to 44 (P).

SPLEEN:- Similar to 5 (P).

OVARY:- Diffuse proliferation of mature and immature lymphocytes in the stroma of the ovary replacing the normal stromal elements and even inside of the follicle (figure - 35).

OTHER TISSUES:- Non-leukotic.

64 (P).

HISTORY:

RIR hen, aged about 16 months, general body condition - poor and emaciated necropsied on 21.4.67.

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GROSS PATHOLOGY:

LIVER:- Enlarged slightly, grey discolouration(fig.3).

SPLEEN:- Slightly enlarged,grey discolouration(fig.3).

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Mild proliferation of lymphoidal cells mostly around the periportal tracts, preportal sheath and blood vessels.

OTHER TISSUES:-

66 (P).

HISTORY:

RIR hen, aged about 12 months, general body condition - good, necropsied on 21.4.67.

GROSS PATHOLOGY:

LIVER:- Enlarged to about twice the normal size, greyish discolouration (figure - 36).

SPLEEN:- Slightly enlarged.

OVARY:- Slightly enlarged.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Similar to 5 (P).

SPLEEN:- Similar to 5 (P).

OTHER TISSUES:- Non-leukotic.

67 (P).

HISTORY:

RIR hen, aged about 12 months, general body

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condition - poor and emaciated, necropsied on 23.4.67.

GROSS PATHOLOGY:

LIVER:- Enlarged slightly, with grey nodules uniformly (figure - 5).

SPLEEN:- Enlarged slightly, grey discolouration (figure - 5).

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER :- Similar to 66 (P).

SPLEEN:- Large foci of lymphoid cells proliferation. A few necrotic foci were also visualised.

OTHER TISSUES:- Non-leukotic.

68 (P).

HISTORY:

WLH hen, aged about 10 months, general body condition - good, necropsied on 28.4.67.

GROSS PATHOLOGY:

KIDNEY:- Greatly enlarged and discoloured.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:-

KIDNEY:- Similar to 47 (P) (figure - 37).

OTHER TISSUES:- Non-leukotic.

72 (P).

HISTORY:

WLH hen, aged about 14 months, necropsied on 24.6.67. General body condition - weak.

GROSS PATHOLOGY:

LIVER:- Enlarged 1½ times normal, grey colour(fig.10).

SPLEEN:- Slightly enlarged, patches of greyish colour (figure - 10).

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

SPLEEN: Similar to 5 (P) (figure - 49).

OTHER TISSUES:- Non-leukotic.

76 (P).

HISTORY:

WLH hen, aged about 6½ months, general body condition - weak, necropsied on 2.8.67.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged, studded with minute grey and red foci uniformly (figure - 11).

SPLEEN:- Minute grey and red foci (figure - 11).

OTHER ORGANS:- No change.

HISTOPATHOLOGY:

LIVER:- Similar to 1 (P).

SPLEEN:- Only parenchyma is involved with proliferation of lymphoidal cells.

OTHER TISSUES:- Non-leukotic.

78 (P).

HISTORY:

RIR hen, aged about 18 months, general body condition - weak, autopsied on 4.6.67.

GROSS PATHOLOGY:

LIVER:- Red stripes of congestion.

SPLEEN:- Greyish spots.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

SPLEEN:- Slight lymphoreticular hyperplasia and lymphoid cell infiltration.

OTHER TISSUES:- Non-leukotic.

80 (P).

HISTORY:

RIR hen, aged about 15 months, general body condition - good, necropsied on 8.6.67.

GROSS PATHOLOGY:

LIVER:- Greatly enlarged, small greyish red foci.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Mild lymphoidal cells infiltration in the parenchyma of the organ.

OVIDUCT:- Diffuse infiltration by lymphoidal cells in the serous surface of the oviduct.

OTHER TISSUES:- Non-leukotic.

82 (P).

HISTORY:

WLH hen, aged about 18 months, general body condition - weak, necropsied on 12.6.67.

GROSS PATHOLOGY:

LIVER:- Enlarged to some extent, grey discolouration.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

HEART:- Slight infiltration of lymphoid cells at a

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few foci disrupting the cardiac muscle fibres.

OTHER TISSUES:- Non-leukotic.

84 (P).

HISTORY:

RIR hen, aged about 14 months, general body condition good, necropsied on 8.8.67.

GROSS PATHOLOGY:

LIVER:- Enlarged, minute grey foci all over its surface.

SPLEEN:- Slightly enlarged, grey discolouration.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- The parenchyma was infiltrated with mature and immature lymphocytes. Among the lymphoidal elements were some macrophages also.

SPLEEN:- Diffuse infiltration of mature and immature lymphoidal cells. Degenerative changes were also discernible in the mesh of the pulp.

OTHER TISSUES:- Non-leukotic.

85 (P).

HISTORY:

RIR hen, aged about 18 months, general body condition - weak, necropsied on 13.8.67.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged.

KIDNEY:- Slightly enlarged.

OTHER ORGANS:- No lesions.

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HISTOPATHOLOGY:

KIDNEY:- Similar to 68 (P).

OVIDUCT:- Serous surface and muscular layer of the oviduct was infiltrated diffusely by lymphoidal cells (figure - 54).

88 (H).

HISTORY:

WLH hen, aged about 18 months, general body condition - good. On haematological examination the total leukocyte count was found to be 59 thousands per c.m.m. of blood and the buffy layer was 4%. It was destroyed on 29.8.67.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged, grey spots all over its surface.

OVARY:- Underdeveloped.

OVIDUCT:- Underdeveloped.

OTHER ORGANS:- Normal.

HISTOPATHOLOGY:

LIVER:- A few foci of lymphoid cell infiltration particularly perivascularly arranged were observed.

OTHER TISSUES:- Normal.

B. ERYTHROBLASTOSIS

The following case was categorised as erythroblastosis. The histopathological examination of the lesions in the organs was characterised by engorgements of blood sinusoids and capillaries with primitive blood cells particularly of the erythroblastic series.

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77 (P).

HISTORY:

RIR hen, aged about 18 months, general body condition - weak, necropsied on 4.6.67.

GROSS PATHOLOGY:

LIVER:- Slightly enlarged, cherry red discolouration.

SPLEEN:- Slightly enlarged, cherry red discolouration.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- Focal perivascular areas of infiltration with mononuclear round cells taking basophilic stain associated with degenerative changes were seen. Intravascular accumulation of polychromatic erythrocytes with slightly eosinophilic cytoplasm and relatively small nucleus was also observed. Blood sinusoids and capillaries appeared to be dilated and some of these were filled to capacity with immature erythrocytes and erythroblasts having elongated nucleus and mononuclear cells. There was accumulation of immature erythrocytes and erythroblasts in the parenchymatous tissue also.

SPLEEN:- Diffuse infiltration of mononuclear cells. There was hyperplasia of the reticular cells of the adenoid sheath of the follicle. Several foci of infiltration with immature erythrocytes with elongated nucleus were seen.

PANCREAS:- Infiltration of mononuclear cells mild and

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focal only. Blood vessels were filled with immature erythrocytes having elongated nucleus (figs.52& 53).

OTHER ORGANS:- Non-leukotic.

C. MYELOBLASTOSIS

The following case was categorised as myeloblastosis. The histopathological examination of the lesions in the organs was characterised by extravascular accumulation of lymphoid cells of myeloid series i.e. myeloblasts and myelocytes particularly in the liver and also in other organs.

79 (P).

HISTORY:

RIR hen, aged about 15 months, general body condition - weak, necropsied on 18.6.67.

GROSS PATHOLOGY:

LIVER:- Enlarged and spotted with greyish yellow areas.

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

LIVER:- The parenchyma was heavily infiltrated by a large number of lymphoidal cells of myeloid series i.e. myeloblasts and myelocytes which were essentially extravascular. Vacuolization of the cytoplasm of these cells characteristic of primitive stages of myelocytes could be seen under oil immersion objective of the microscope. Some of the blood vessels showed moderate leukostasis.

SPLEEN:- Diffuse lympho-reticular hyperplasis and infiltration of lymphoid cells.

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OTHER TISSUES:- Non-leukotic.

D. LYMPHOID LEUKOSIS MIXED WITH OTHER NON-LEUKOTIC CONDITIONS

The following cases were categorised under this sub-head as some of the organs revealed other tumorous conditions while most of the other organs revealed lymphoid leukosis.

9 (SP).

HISTORY:

The specimen was received from a field case and the history supplied mentioned it to be from a WLH hen, aged about 22 months. Pieces from Liver, lung, heart, kidney and a tumour from the intestine were received in 10% formalin.

HISTOPATHOLOGY:

LIVER :- Focal infiltration of lymphoid cells.

HEART:- There was infiltration of cells of lymphoid series predominantly lymphocytes and lymphoblasts in the myocardium separating the cardiac muscle fibres (figure - 17).

TUMOUR FROM THE INTESTINE:- The neoplastic growth consisting of mature and immature lymphoidal cells was found to infiltrate the serosa of the intestine. The growth also penetrated into the muscular coat causing disruption of the normal architecture of the organ (figure - 18).

LUNG:- It was diagnosed as metastatic adenocarcinoma replacing the normal parenchyma; made up of a large number of immature epithelial cells having an occasional acinar appearance. Due to the supervening post mortem changes the morphology of the

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cells could not be made out. Van Gieson's staining gave negative reaction for connective tissue (figure - 19).

69 (P).

HISTORY:

RIR hen, aged about 12 months, general body condition - weak, necropsied on 25.4.67.

GROSS PATHOLOGY:

SPLEEN:- Enlarged about twice the normal size, tumorous (figure - 6).

OVARY:- Greatly enlarged and tumorous, studded with grey coloured nodules (figure - 7).

INTESTINE, CAECA, PANCREAS & MESENTERY:- All tumorous with grey coloured nodules (figure - 8).

OTHER ORGANS:- No lesions.

HISTOPATHOLOGY:

SPLEEN:- Hyperplasia of the lymphoid follicles. Red pulp was infiltrated by a number of lymphoid cells. The capsule showed growth consisting of lymphoid cells. Substance of spleen showed atrophy (fig.38).

OVARY:- The granulosa cells having an alveolar arrangement were seen. At places large amount of interstitial connective tissue was present. The granulosa cells were typically arranged around open spaces which is characteristic of granulosa cell tumours (figures - 39 & 40).

SMALL INTESTINE & PANCREAS:- Neoplastic growths consisting

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of granulosa cell like structures were found attached to the serosa of the intestine. The neoplastic growths had a very scanty stroma. Some acini like structures were also seen(fig. 41 & 42).

MESENTERY:- The granulosa cells were having an alveolar arrangement. At places large quantities of interstitial connective tissue was also seen. This was diagnosed as a granulosa cell tumour which has produced metastases in the mesentery (fig.43 & 44).

70 (P).

HISTORY:

RIR he, aged about 18 months, general body condition weak, necropsied on 11.5.67.

GROSS PATHOLOGY:

LIVER:- Enlarged to about twice the normal size and grey discolouration (fig. 9).

SPLEEN:- Enlarged to about three times the normal size and tumefied with nodular growths on its surface. Its diameter was about 5 cms.

OVARY:- Enlarged and tumorous, grey coloured (fig.9).

SMALL INTESTINE & PANCREAS:- Studded with tumorous nodules (figure - 9).

OTHER ORGANS :- No lesions.

HISTOPATHOLOGY:

LIVER:- Discrete and confluent foci of lymphoidal cells throughout the parenchyma of the organ and around the blood vessels.

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SPLEEN:- The neoplastic growth consisting of lymphoidal cells was found attached to the capsule of the organ. The red pulp also showed lymphoidal hyperplasia. Diffuse degenerative changes were also seen (figures 47 & 48).

SMALL INTESTINE & PANCREAS:- The neoplastic growths consisting of immature lymphocytes were found attached to the serosa of the duodenum and acinar structure of the pancreas. In neither of these organs the growth penetrated ~~my~~ beyond the serous lining. Lymphoidal foci consisting of mature and immature lymphocytes were also present separated by thin connective tissue stroma. The mesentrian serosa was infiltrated with round cells (figures 45 & 46).

OVARY:- Diffuse proliferation of lymphoidal cells in the stroma interspersed with the granulosa cells having an alveolar arrangement. Large quantities of fibrous connective tissue forming trabeculae like structures were also seen. It was diagnosed as a case of a granulosa cell tumour.

OTHER MISCELLANEOUS CONDITIONS

This head includes tumorous materials which were suspected for Avian Leukosis Complex on gross examination

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but histopathological examination revealed a different picture.

41 (S.P)

HISTORY:

Tumor of the oviduct, from a WLH hen, aged about 24 months, preserved in 10% formalin.

HISTOPATHOLOGY:

The section of tumor revealed bundles of neoplastic smooth muscle fibres arranged harrum-scarrum with very little connective tissue in between the bundles. The nuclei of muscle cells were elongated. It was diagnosed as leiomyoma.

74 (SP).

Diagnosed as leiomyoma.

75 (SP).

Diagnosed as leiomyoma.

During the present investigation, 32 natural cases of the various forms of A.L.C. were recorded. These cases were classified as follows:-

Sl.No.	Forms of A. L. C.	No.of cases.
1	Lymphoid leukosis	30
2	Myeloblastosis.	1
3	Myelocytomatosis.	-
4.	Erythroblastosis.	1
5	Fibrosarcoma, endothelioma and nephroblastoma.	-
6.	Osteopetrosis.	-
7.	Marek's Disease (Fowl paralysis).	-

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During the present study the liver was involved in 24 cases. The gross lesions encountered in lymphoid leukosis were greyish discolouration of the liver interspersed in some cases with red spots or stripes. The organ was greatly enlarged and sometimes various sized greyish white nodules were observed raised above the surface of the liver, while in some other cases greyish white areas or minute grey foci were seen.

Similar discolouration was also observed in other organs such as spleen, kidney and ovary. Enlargement of all the above organs was observed. The spleen was involved in 24 cases and was found enlarged in a number of cases. Sometimes it was studded with greyish foci.

Tumour formation was observed in the ovary and small neoplastic foci were observed in the liver and spleen. Small nodules were sometimes observed in liver, spleen and ovary. Minute grey foci were noted in liver, spleen, kidney and ovary. The heart sometimes showed characteristic neoplastic nodular growths on the epicardium.

In most of the cases of lymphoid leukosis grey discolouration of the visceral organs particularly liver was observed. In one case of erythroblastosis cherry red discolouration of the liver and spleen was seen.

In the present studies, a careful and thorough search was made during the histopathological examination of sections of various organs, for the presence of cytoplasmic and intranuclear inclusion bodies, if any. Even special staining techniques did not reveal the presence of inclusion bodies.

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During the course of present investigation some interesting cases were observed. On the basis of examination of gross lesions no differentiation could be made between these conditions and A.L.C. In one of these cases lymphoid leukosis was found to be associated with metastatic adenocarcinoma in the lung replacing the normal parenchyma of the organ while in two other cases it was associated with granulosa cell tumour of the ovary having metastases in different organs. Three cases of leiomyoma were also recorded.

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Figure No. 1

Figure No. 2.

Figure No.3

Figure No.4

Figure No.5

Figure No.6

Figure No.7

Figure No.8

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Figure No. 9

Figure No. 10

Figure No. 11

Figure No. 12

Figure No. 13

Figure No. 14

Figure No.15

Figure No.16

Figure No.49

Figure No.50.

Figure No.51

FigureUNo.52.

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DISCUSSION AND CONCLUSIONS.

DISCUSSION AND CONCLUSIONS

(A) INCIDENCE:

The present investigations on incidence of A. L. C. revealed that there was a high incidence of this disease at the poultry Extension Centre, Bihar Sharif, Patna. A total incidence of 18.1% out of the total mortality has been worked out during the period from 1965-67. On the other hand similar studies carried out at Veterinary College Poultry Farm, Mathura for the period from 1960-67 revealed the total incidence of A. L. C. to be only 2.1% of the total mortility.

Further, incidence was also worked out on the basis of autopsies conducted by the author during the period of the present study and an incidence of 8.4% has been recorded at the Poultry Extension Centre, Bihar Sharif, Patna; 1.5% at the Central Poultry Farm, Patna and no case was recorded from Veterinary College Poultry Farm, Mathura in the month of May during which period the author attended poultry post-mortems at the above institution.

The highest incidence of A. L. C. at Poultry Extension Centre, Bihar Sharif, Patna can be exaplained on the basis of the observations of Smith and Long (1959) who reported that a higher incidence was found in birds hatched in January, February and March than in birds hatched in April, May and June. In the above farm also more chicks are hatched during the months of January to April. Therefore, it is concluded

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that the date of hatch is one of the factors affecting the incidence of A. L. C.

The lower percentage of incidence of this disease at the Central Poultry Farm, Patna can be explained on the basis of the fact that most of the birds examined were from the stock that was destroyed at the Poultry Dressing Plant, Patna on a large scale for table purpose. These birds came from healthy broiler flocks maintained separately for the purpose. There is a possibility that the broiler flock might have been comparatively resistant to A. L. C. Therefore, an overall incidence of 1.5% should not be considered as low.

The incidence recorded for Veterinary College Poultry Farm, Mathura is not very high (2.1%) as worked out for the period from 1960-67. For the year 1967, only the data of post-mortem records upto April were included. On the basis of autopsies conducted no positive case of A. L. C. was recorded on histopathological examination because of the fact that the author attended the autopsies at the above institution only in the month of May. The incidence of A. L. C. is very low in this period of the year according to Krishnan (1960) who did not record any case of A. L. C. in the months of May during his three years of study from 1956-58 in Madras State.

On critical perusal of the observations, it is evident that Rhode island red breed is comparatively more susceptible to A. L. C. than White leghorn as out of 117 positive cases of A. L. C. during 1965-67 at the Poultry

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Extension Centre, Bihar Sharif, Patna, 59.3% cases were recorded in the R. I. R. breed and 40.7% in the W.L.H. breed. This observation is in agreement with the observations recorded by Das (1951) and Krishnan (1960).

On consideration of the effect of age on the incidence of A. L. C. it has been the observation that out of a total number of 117 positive cases of A. L. C. maximum number of cases (41.8%) were recorded in the age group of 10-15 months. Lowest number of cases was found in birds more than 15 months of age. This observation is in close conformity with the observations of Krishnan (1960) and Chandrasekharan Nair (1965). In older birds, although there is a decline in incidence, cases do occur. The fact that only young adults die in large numbers due to this disease may be due to the reason that in any flock young adults are in larger numbers than older birds (Isaakson, 1950).

On considering the seasonal incidence on the basis of present results it is revealed that maximum number of cases of A. L. C. were encountered during the summer i.e. from March to June during the period from 1965-67. This observation agrees with the findings of Schaff (1936) who considered low incidence during June to October but differs from that of Krishnan (1960) who observed few deaths in March to July. Soman (1967) recorded cases of this disease in all the seasons of the year.

In the light of the fact that in the Poultry Farm at Bihar Sharif more chicks are hatched during the months of

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January to April and deaths due to this disease are mostly observed at about an year of age, it is well expected that maximum deaths will occur from March to June. From these observations the conclusion drawn is that the date of hatch is one of the factors responsible for seasonal incidence of A. L. C.

However, the results of various authors indicate that season does not play any significant role in the incidence of A. L. C. Ellermann (1922) observed most of the cases from January to April; Jarmai (1930-31) from November to February; Kitt (1931) to November; Luttschwager (1930-31) in the winter and spring months. Viraraghavan and Chandrasekharan Nair (1965) reported that the incidence was greatest in north-east monsoon period (October to December). The incidence in the cold season (January to February) and south-west monsoon (June to September) was observed to be nearly equal.

From the foregoing discussion it appears that the date of hatch has never been taken into consideration while studying the seasonal incidence of this disease except by Smith and Long (1959) who reported a higher incidence in birds hatched in January, February and March than in birds hatched in April, May and June. The author, on the basis of these studies concludes that further study on the seasonal incidence in respect to the date of hatch should be undertaken to elucidate this observation.

While considering the involvement of different

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organs and distribution of lesions, the present histopathological studies have revealed that liver, spleen, kidney, ovary, heart, pancreas, intestine and proventriculus were the organs involved in order of priority. These observations are akin to those observed by Viraraghavan and Chandrasekharan Nair (1965), Sharma et al. (1967) and Soman (1967). However, in the present investigation liver and spleen were involved in equal number of cases.

(B) HAEMATOLOGY:

On perusal of the results of haematological study it is evident that in the fowl positive for lymphoid leukosis the packed cell volume was normal. Sato (1960) also got similar results while studying the cases of lymphoid leukosis. Soman (1967) also reported no deviation from the normal values of packed cell volume in lymphoid leukosis.

The buffylayer in this case was found to be 4.0% whereas the normal value is about 1.0%. This increase in the thickness of the buffy layer in leukosis is in complete agreement with the observations recorded by Sato (1960). It is very difficult to say if this observation is useful in the diagnosis of lymphoid leukosis because on the hand the study is limited to only one case and on the other hand another bird having a buffy layer 3.2 mm. % proved to be non-leukotic histopathologically.

Two birds showing abnormally high values of lympho-

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lymphocytes in the differential leukocyte count also proved to be normal on histopathological examination. This is contrary to the observations of Bayon (1931) who stated that differential counts in paralytic birds showed a definite increase of lymphocytes. It is, therefore, concluded that high lymphocyte count alone can be of no help in the diagnosis of this disease.

The high values of total leukocyte count were found in two apparently healthy birds and only one of these birds proved to be a case of lymphoid leukosis whereas the other proved to be normal on histopathological studies. On the contrary, high total leukocyte count was observed in myeloid leukemia by Furth (1931). The present observation indicates its in-applicability in diagnosing clinical cases of this disease as this value must have increased due to some other unknown infection.

From the above discussion it is concluded that haematological studies particularly total leukocyte count and differential lymphocyte percentage, do not reveal anything significant regarding the diagnosis of clinical cases of A.L.C. However, increase in thickness of the buffy layer, which is a constant feature of lymphoid leukosis according to Soman (1967), needs further study to prove its usefulness as a diagnostic aid in this disease, because Soman (1967) also observed this only in two cases of lymphoid leukosis.

(C) GROSS LESIONS AND HISTOPATHOLOGY:

It is well known that A. L. C. is a disease of

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varied manifestations in the forms of lymphoid leukosis; myeloblastosis (Leukemic); Myelocytomatosis (aleukemic); erythroblastosis (Proliferative and anemic); fibrosarcoma, endo thelioma and nephroblastoma; osteopetrosis and Marek's disease. Difference of opinion has always existed in the terminology of the various manifestations of A. L. C. Hungerford (1951) quoted by Campbell (1958) stated that 69 different synonyms prevailed in the English language alone to designate the various forms of A. L. C.

During the present investigation, on examination of gross lesions of the various organs of 1430 birds, 90 cases could be suspected for A. L. C. Detailed histopathological examination of the various organs of these cases was carried out and 32 postive cases of various forms of A.L.C. were recorded. Out of 32 cases of A. L. C. 30 were categorised under lymphoid leukosis, one under erythroblastosis and one under myeloblastosis.

During the course of present investigation, the author did not come across any case of myelocytomatosis, osteopetrosis and fowl paralysis. This observation is in agreement with the observations of Soman (1967) who could not get any case of the above 3 forms. Similarly, Waters and Pricket (1948) did not meet with any osteopetrotic forms out of 3257 autopsies. From Utter Pradesh, Awadhiya (1960) reported only 2 cases of osteopetrosis out of 47 positive cases of A. L. C. studied by him. It is, thus, evident that the above forms are

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of rare occurrence in this country.

In contradiction to the observations made above, Krishnan (1960) reported that the Osteopetrotic form was much more common and in this observation he differed from the findings of other workers. Burmester and Gentry (1956) stated that males were mostly susceptible to osteopetrotic form and females to visceral form. Probably this is true as Krishnan (1960) described 9 osteopatrotic cases out of which 6 were found in females. During the present study most of the birds examined were females and this may be one of the reasons of not encountering cases of osteopetrosis. There is a possibility that this form may be common in Madras State but rare in Bihar State.

No case of neural form of the disease was encountered during the present study. This is in distinct contrast to the observations made by other workers out side India. On the other hand Krishnan (1960) reported that the neural form of the disease which was supposed to be common in foreign countries was very rare in this country. Awadhiya (1960) did not record any case of neurolymphomatosis in Uttar Pradesh. Soman (1967) made similar observations. It is, therefore, concluded that the neural form of A. L. C. is extremely rare in India.

No ocular form of the disease has been recorded during the period of present investigation and it appears that this form is un-common in Bihar.

Under the present study out of a total of 32 cases

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of lymphoid leukosis none showed either peripheral or central nervous system involvement. This observation is akin to those made by Campbell (1945); Burmester, Prickett and Belding (1946); Davis et al. (1947). Burmester et al. (1959) and Soman (1967).

The observations of the author on gross lesions are in agreement with those of other previous workers viz. Furth (1935), Campbell (1954), Krishnan (1960) and Soman (1967).

Observations of cherry red discolouration grossly and leukostasis in the blood vessels of liver in erythroleukosis are in agreement with those described by Campbell (1961) and Stenszky (1934) respectively.

One case of myeloblastosis was recorded. In this case there was an infiltration of myelocytes and myeloblasts in the parenchymatous tissue of the liver as described by Campbell (1961).

The present studies have shown that grey discolouration of the visceral organs or greyish foci are mostly found in cases of lymphoid leukosis whereas a cherry red discolouration of the liver is found in erythroblastosis.

As described by all the workers histopathological studies revealed that the changes are essentially similar in almost all cases of lymphoid leukosis. In most instances the infiltrating cells had replaced the parenchyma of the affected organs. Both diffuse and nodular forms were noted in the liver as described by Feldman (1932). In nodular forms

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focal aggregates of lymphocytes and blast cells were noticed.

Sections of spleen revealed lymphoid hyperplasia in many cases as described by Jungherr (1943) giving rise to a reduction in the red pulp. The infiltrations were found both in the pulp and the follicle, which is in agreement with Lee et al. (1937).

In the kidney the changes were in the nature of interstitial accumulation of lymphoid cells in between the tubules causing great damage to them. In some extreme cases the tubules were separated wide apart and the space between them was occupied by the infiltrating cells of lymphoid series. In some cases the parenchymal elements were replaced by lymphoidal cells. This is in agreement with Lee et al. (1937). In some cases the kidney tubules were so much atrophied by the infiltrating cells that identification of the organ was made difficult.

Sections of the ovary revealed follicular infiltration in one case where it was transferred in to a tumorlike mass and in other cases only infiltrating lymphoid cells were found encroaching upon few of the follicles.

In the heart the infiltrating lymphoidal elements involved the myocardium with disruption of the muscle fibres in advanced cases. This observation is in close agreement with Das (1951).

The tumours of the visceral organs in leukosis were composed of lymphocytes and lymphoblasts packed together

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closely as stated by Iyer and Hashmi (1945).

The infiltrating cells were either small mature lymphocytes or earlier cells in the lymphoid series. Many of these cells had a vesiculated nucleus as described by Mac Gaughey and Dawnie (1930).

From the histopathological studies it is concluded that during present studies the frequency of involvement of different organs was in the following order - liver, spleen, kidney, ovary, heart, pancreas, oviduct, intestine and proventriculus. Thus, it is noticed that liver, spleen, kidney and ovary are the organs mostly affected with lymphoid leukosis. This is in agreement with the observations of Viraraghavan and Chandrasekharan Nair (1965), Sharma et al. (1967) and Soman (1967).

A careful and thorough search was made for the presence of inclusion bodies in different organs but no distinct inclusion bodies could be observed in the sections stationed with Mann's eosin methyl blue or with Lendrum's stain.

It is further concluded on the basis of histopathological observations that some of the tumorous conditions are grossly so similar to leukosis tumours that their diagnosis can be made only after a thorough histopathological examination of the affected tissues. This inference is in agreement with the observations of Das (1951) who described that this disease may, at times, be mistaken for a few other conditions viz. Round cell sarcoma, Lymphosarcoma, nerves from post new castle disease vaccination reactors, spirochaetosis and fowl typhoid. This

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During the present study, 1530 birds were examined and 90 cases were suspected for Avian Leukosis Complex on the basis of gross examination of the lesions, out of which 32 cases were confirmed as positive for the disease on histopathological examination.

The incidence of Avian Leukosis Complex has been worked out by a study of the post-mortem records and also on the basis of autopsies conducted by the author at three poultry farms, naemly - Poultry Extension Centre, Bihar Sharif, Patna; Central Poultry Farm, Patna and Veterinary College Poultry Farm, Mathura. The incidence was maximum in the poultry Extension Centre, Bihar Sharif, Patna; being 18.1% of the total mortality during the period from 1965-67. An incidence of 2.1% was revealed at Veterinary College Poultry Farm, Mathura during 1960-67. At the Central Poultry Farm, Patna the incidence was studied on the basis of autopsies performed together with the post-mortem observations made on the birds killed at the Poultry Dressing Plant and it worked out to be 1.5%.

The disease occurred more commonly in the Rhode Island Red breed than in the White Leghorn breed. The broiler flocks might be comparatively resistant to Avian Leukosis Complex.

Maximum deaths occurred between 10-15 months of age. Although there is a decline in incidence in older birds, cases do occur in them.

Seasonal incidence of Avian Leukosis Complex was worked out and it was found that most cases were encountered during summer i.e. from March to June. It was concluded that it is the date of hatch and not the season that affects the incidence of Avian Leukosis Complex at different periods of the year.

Attempts were made to correlate the haematological observations of some of the previous workers with the present histopathological as well as haematological studies. Only lymphoid form of leukosis was studied. Values for the buffy layer and total leukocyte count were found to be unusually high in a positive case of lymphoid leukosis, but it was concluded that haematological studies do not reveal anything significant regarding the diagnosis of clinical cases of Avian Leukosis Complex. However, the increase in the thickness of buffy layer in lymphoid leukosis needs further study to prove the use fulness of this observation.

In lymphoid leukosis the organs of predilection were liver, spleen, kidney, ovary and heart in order of priority. Other organs were involved in less number of cases. Grossly, enlargement of the affected organs was seen in most of the cases. In the liver both discrete and diffuse forms were seen. Greyish discolouration interspersed in some cases

with red spots or stripes was found mostly in liver and sometimes in other organs such as spleen, kidney and ovary. Tumour formation was found in the ovary and small neoplastic foci were observed in the liver and spleen. Small nodules were sometimes seen in liver, spleen and ovary. The heart sometimes showed neoplastic growths on the epicardium. Cherry red discolouration of the liver was found in one case of erythroblastosis.

During the period of present study only cases of lymphoid leukosis, erythroblastosis and myeloblastosis were encountered. Out of all these cases none showed involvement of the nervous system.

No case of myelocytomatosis, osteopetrosis and fowl paralysis (including neural and ocular forms) was recorded.

Inclusion bodies could not be demonstrated in the sections stained with Mann's eosin - methyl blue stain or with lendrum's stain.

The histopathological picture was one of extensive focal or diffuse infiltration with cells of the lymphoid series. Many of these cells had a vesiculated nucleus. Cases of Metastatic "adenocarcinoma" of the lung and "Granulosa cell tumour of the ovary were found associated with lymphoid leukosis. Three cases of tumours from the oviduct were diagnosed as "Leiomyoma" on histopathological examination.

54 photographs & 9 TABLES have been included to illustrate the results achieved.

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