with a normal buff color at the base following the change in diets. This growth of normal colored feathers was noted at about five days after the diets were changed. Fig. 1 shows the banding effect produced in the feathers.

Summary. In an experiment with Buff Orpington chicks, a breed normally having no black pigment in the feathers, it was found that a deficiency of vitamin D caused a widespread deposition of black pigment in the feathers. This abnormal blackening was prevented by supplementing the diet with vitamin D.

The feeding of thyroactive iodinated casein failed to increase the deposition of black pigment in chicks fed either a vitamin D deficient or vitamin D supplemental diet.

The iodinated casein was supplied by Cerophyl Laboratories, Inc., Kansas City, Mo.

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Response of Spontaneous Lymphoid Leukemias in Mice to Injection of Adrenal Cortical Extracts.*†

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Striking alterations in blood elements have been observed to result from injections of pituitary adrenotropic hormone in various species of animals.[1,2] The blood picture observed within a few hours after injection is as follows: decrease in leukocyte count, an absolute lymphopenia and a corresponding absolute polymorphonuclear leukocytosis. Statistically significant decreases in the weights of lymphoid tissues, excepting the spleen, following injections of adrenotropic hormone, indicate a profound influence on normal maintenance of lymphoid tissue by this hormone.[3] Histological studies indicate specific degenerative changes of normal lymphocytes in all lymphatic structures with repair and recovery after definite time intervals.[4] These changes are mediated through the adrenal cortex and similar alterations, although at possibly different time intervals, occur following injections of adrenal cortical extracts.[2]

In view of these profound effects on normal lymphocytic tissues, we have undertaken a study of the effects of adrenal cortical extract on the immature elements in spontaneous lymphoid leukemias in mice. Earlier studies have indicated some relationship between the adrenals and the growth of transmitted animal leukemias. Sturm and Murphy[5] showed that adrenalectomy reduced the natural resistance of rats to a transplantable lymphoid leukemia. The rate of growth of the leukemia was also apparently increased following adrenalectomy. It has been also[6] shown that desoxycorticosterone acetate and two adrenal cortical extracts decreased the susceptibility of rats to a transplanted leukemia. Complete disappear-

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ance of a transplantable lymphoid tumor in mice followed by recurrence but definite retardation in growth after administration of 11-dehydro-17 hydroxycorticosterone (Compound E) has been reported.[7]

In the C58 strain of mice approximately 90% of mice of both sexes develop leukemia, the majority of which are lymphoid in origin. Early symptoms of leukemia can be detected within the strain by periodic palpation of lymph nodes. Usually a single node is initially involved[8] followed by a progressive systemic course of the disease. An incidence of approximately 80% leukemia, involving a significantly greater number of females than males, has been observed in the inbred RIL strain. The majority of these leukemias involve initially and principally the thymus. The first symptom observed is usually that of dyspnea and the animals are at this time in the terminal stage of the disease.

The leukocyte count is definitely elevated in leukemias of both strains of mice, although not severely, and immature lymphoid forms have been found in the peripheral blood of all leukemias observed within these strains. There occurs a progressive leukocytosis and a definite increase in circulating immature forms as the disease runs its course.

In the following experiment we are reporting 13 cases of spontaneous lymphoid leukemia arising in the inbred leukemic strains of mice, C58 and RIL (including 2 hybrids between these strains) which were injected with adrenal cortical extracts. Most of these leukemias were in the terminal stages of the disease (from 5 to 16 days following discovery of symptoms) when administration of adrenal cortical extract was begun. Mice of the C58 strain had moderate to severe lymphadenopathy of the axillary, inguinal and cervical lymph-nodes, moderate to severe splenomegaly and numerous immature leukocytes in the peripheral circulation. Mice of the RIL strain showed dyspnea and thoracic enlargement and in some cases had subcutaneous lymph-node and splenic involvement. In 5 of the 13 cases of spontaneous leukemia, biopsy tissue (axillary or inguinal lymph node) was taken prior to treatment, part of which was saved for histological section and the other part inoculated into mice of the strain of origin of the leukemia. This was done to confirm further the diagnosis of leukemia.

Leukemic mice were inoculated initially either intraperitoneally or subcutaneously with from 0.1 to 0.5 cc of adrenal cortex extract (aqueous) or lipoadrenal cortex.‡ Since the lipoadrenal cortical extract gave the most favorable effects, subsequent inoculations were continued with this at usually 0.1 cc inoculation every 24 hours. Several animals received this dose every 12 hours for a considerable period of time and optimum response as determined by blood analyses was obtained. Blood analyses were made at 0, 3, 6, 9, 24, 72, and 168 hours and at various times thereafter throughout the life of the animal.

The blood picture obtained is characterized by: (a) decrease in total white blood-cell count, (b) decrease in absolute number of lymphocytes with a corresponding polymorphonuclear leukocytosis and decrease in absolute number of immature forms (lymphocytes and lymphoblasts). Following initial administration of adrenal cortical extract, the maximum blood alterations were noted at 6 hours. After continued hormone administration these effects were enhanced so that in the majority of mice given daily injections for 168 hours or longer there resulted a severe lymphopenia and decrease in absolute number of circulating immature blood cells. Two cases, however, both in the C58 strain proved refractory after 72 hours and showed a progressive increase in circulating leukocytes and immature forms. Recovery to a leukemic blood picture tended to occur after 9 hours following injection of adrenal cortical extract. However, after continuous daily injections over a relatively long period of time an effect of the extract has been observed in animals 24 hours after injection

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‡ Obtained from the Upjohn Co., Kalamazoo, Mich.

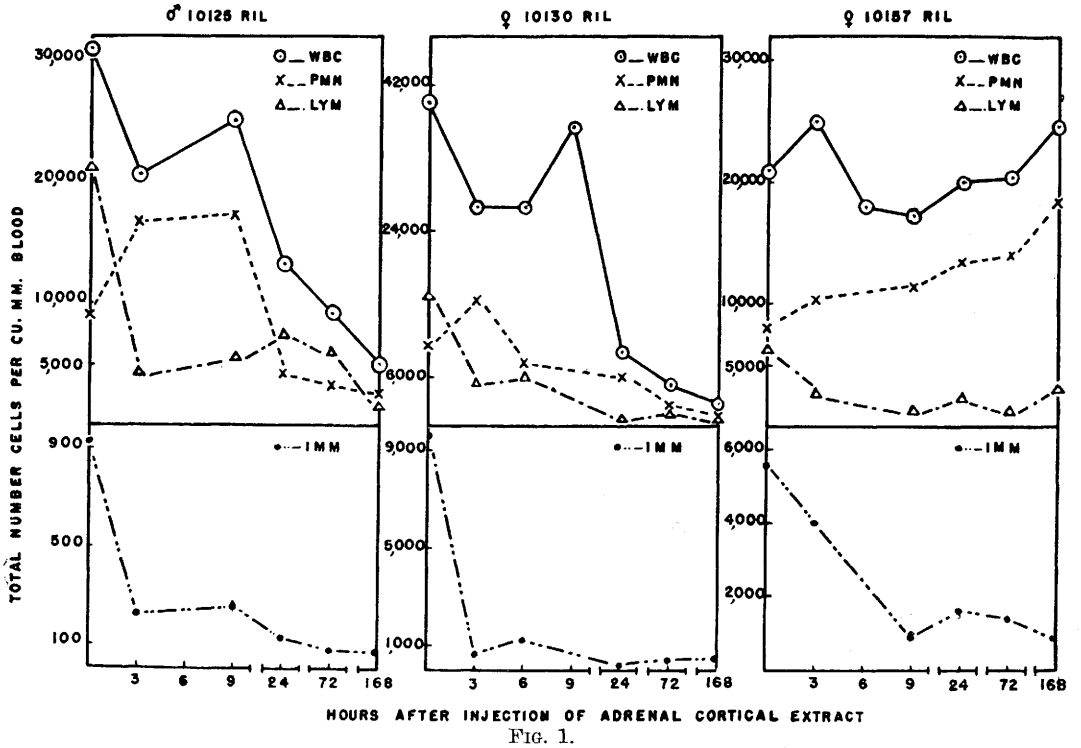

FIG. 1.

Effect of injections of adrenal cortical extract on the white blood cell counts of three mice of the RIL strain with lymphoid leukemia. Injections were given every 12 hours and blood analyses were made usually at 6 hours following injection. Blood analyses for these mice were continued throughout life and are explained further in the text.

(Fig. 1 and 2).

Degenerating lymphocytes both mature and immature were observed in peripheral blood 6 hours after initial injection of adrenal cortical hormone and constituted as much as 20% of the differential count in several leukemic animals which had received injections over a period of 10 days to 2 weeks or longer. Nuclear degenerative changes were prominent in these cells. Dougherty and White[2] have not described a peripheral removal of lymphocytes in their experimental observations in normal mice but suggest this as a possible mechanism in normal lymphocyte dissolution. Whether the phenomenon observed here is characteristic of the lymphocytes of leukemic mice or is related to a strain difference in the mice employed must await further experimentation.

Favorable effects of adrenal cortical extract on hemoglobin levels in leukemic mice have not been observed, although it is possible that the decline in hemoglobin levels is not as precipitous in treated as in control leukemic animals.

Profound palliative effects were noted within 24 to 48 hours in the majority of leukemic mice. These effects were: complete disappearance of the symptoms of dyspnea and thoracic enlargement and marked regression in infiltrated inguinal, axillary, cervical and mesenteric lymph-nodes. Definite regression of the spleen was noted in some leukemic animals but this was not constant throughout the treated series. It has been reported in normal mice receiving injections of adrenotropic hormone that the spleen did not show the characteristic weight decrease observed for other lymphoid tissues.[3] The characteristic response to adrenal cortical extract in a typical case, ♀ 10130 RIL, was as follows: Sixteen days after appearance of symptoms of leukemia this animal was in the terminal stages of leukemia. There was extreme dyspnea, moderate involvement of all subcutaneous lymph nodes, each measuring approximately 3 x 3

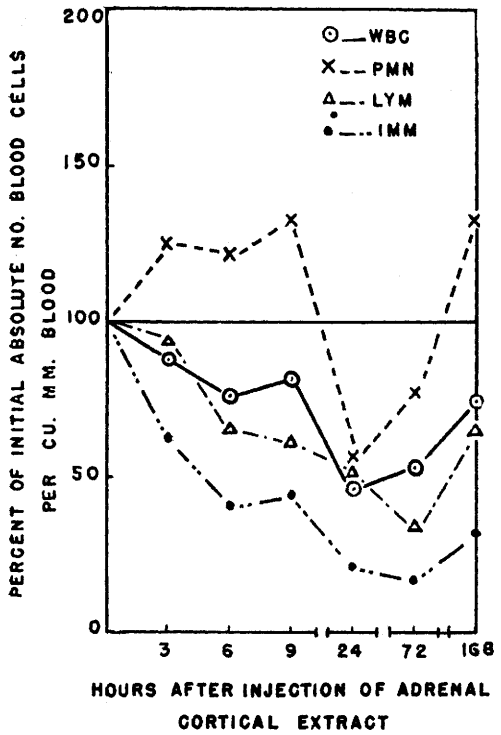

FIG. 2.

Mean percentage increase or decrease of initial number of blood cells in leukemic mice receiving daily injections of adrenal cortical extract. Each point on curve represents the mean of data obtained from at least 10 mice. Mean percentage increase at 168 hours is the result of including data of 2 mice which became refractory at this time.

mm, a moderately involved spleen and palpable mesenteric nodes. Biopsy tissue from an inguinal lymph node showed severe infiltration of immature forms and mice of the RIL strain inoculated with the remaining tissue of the inguinal node developed leukemia within 3 to 4 weeks. The leukocyte count was elevated to 40,500 and 24% of the circulating cells were lymphoblasts. Lipoadrenal cortex was injected subcutaneously 0.1 cc twice daily. Within 48 hours all subcutaneous nodes had completely regressed, there was no evidence of dyspnea and the spleen and mesenteric nodes showed definite regression. This animal continued to show regression with daily inoculation of extract and died 30 days after appearance of symptoms at which time the subcutaneous lymph nodes were normal in size, the thymus and spleen showed slight to moderate infiltration and the liver and kidneys were greatly infiltrated. (See Fig. 1 for blood response in this animal.)

Lymph nodes, spleen, thymuses, liver and other organs were removed following death of the animal for histological study. In addition several inguinal nodes were removed at 2 and 3 days following initial injection. Severe alterations in infiltrated lymph-nodes and thymuses were observed in leukemic mice which had received numerous injections of adrenal cortical extract. Extensive degenerative changes of the immature lymphocytes were evident. Nuclei were pyknotic and of various sizes and bizarre shapes. Hyperplasia of the cytoplasm was marked in numerous immature forms. Nuclear debris was scattered throughout the organ with very little evidence of phagocytosis. There was a severe depletion of immature forms and a "washed-out" appearance of nodes and thymuses. Recovery and repair processes were not noticeable. Mitotic figures were not observed. In contrast, the alterations in the spleen of leukemic mice receiving long continued injections were more localized. Numerous macrophages were present, filled with nuclear debris and there were many areas of histiocytic infiltration wherein the histiocyte-like cells had abundant cytoplasm but did not exhibit phagocytosis.

In inguinal lymph nodes examined at 2 and 3 days there were in evidence areas of recovery with many macrophages present, filled with nuclear debris. In addition in scattered areas there were present large reticular cells lying free in the sinuses but not actively phagocytic.

Degenerative changes of the immature lymphocytes were not found in the non-hemopoietic tissues studied.

Definite weight decreases of spleen, thymus and subcutaneous lymph-nodes of treated leukemic mice were obtained. The effect on thymic mass was most severe resulting in a mean weight of 270 $\pm$ 90 mg in the experimental series compared with 663 $\pm$ 66.5 mg in leukemic controls.

From the small number of mice observed in this preliminary series, it is impossible to state the effect on life expectancy. Two leukemic mice of the RIL strain have lived 30

and 42 days respectively with continued daily intraperitoneal inoculations. We have observed the course of the disease in more than 50 leukemic animals of this strain and none has survived for this period of time.

Summary. Injections of adrenal cortical extract into 13 mice of the inbred C58 and RIL strains in the terminal stages of spontaneous lymphoid leukemia resulted in the following responses: (a) An acute response (maximum at 6 hours following initial injection) resulting in a decrease of circulating leukocytes of the blood, a lymphopenia with a corresponding absolute polymorphonuclear leukocytosis and a decrease in the number of circulating immature lymphocytes. (b) These blood alterations become more pronounced following continuous daily injections and tend not to return to the leukemic blood picture within 24 hours after injection of the extract. (c) Regression of infiltrated thymuses and subcutaneous lymph nodes was observed. Regression of the spleen, although definite, was not so pronounced as that observed in other lymphoid tissues. (d) Extensive degenerative changes in immature lymphocytes in thymuses and lymph nodes resulting in pyknosis, dissolution and depletion of these cells. In the spleen similar changes occurred but were not so generalized. Pronounced degenerative changes in immature lymphocytes was observed in thymuses and lymph nodes of mice receiving daily injections over a relatively long period of time. Recovery and repair in these lymphoid organs was slight.

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Intertransformability of *Salmonella simsbury* and *Salmonella senftenberg.**

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It is recognized[1-7] that profound changes may be induced in H antigens of *Salmonella* by cultivating the organisms in serums containing appropriate H agglutinins. Such changes seem to occur particularly in monophasic cultures. Thus, in the stools of a person infected with *S. cholerae-suis* var. *kunzendorf* (VI,VII:1,5) Kristensen and Bojlen[8] found an organism with the biochemical properties of Kunzendorf whose antigens were VI,VII:c. Such variants are easily produced *in vitro* by cultivating Kunzendorf cultures in 1,5 serum. The writers have encountered 12 of these variants occurring naturally among cultures from man and swine. Likewise, Edwards and Moran[7] found that monophasic strains of *S. minnesota* (XXI,XXVI:b) were easily changed to a form with new H antigens (XXI,XXVI:z_{33}) by cultivation in b serum. Cultures with the latter formula occurred naturally among strains isolated from sewage by A. A. Hajna and it seems very

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. It was supported in part by a research grant from the U. S. Public Health Service.

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