agated in this medium for varying lengths of time.

Summary. The nutritional requirements of a strain of *Polytomella agilis* were investigated. Ethanol, butanol, acetate, propionate or butyrate were utilized as carbon sources. Glutamine was adequate as a sole source of nitrogen. Thiamine and vit. B_{12} were stimulatory. A simplified medium, adequate for continuous subculture of this organism, is presented.

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Effects of Adrenalectomy on Blood Cells of Mice with Special Reference to Eosinophiles.* (19123)

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Numerous references to the effects of adrenalectomy on the numbers of blood cells occur in the literature. The various investigators tend to agree that total removal of both adrenals results in an anemia which is manifested by decreases in total red cell count and in Hb values, provided hemoconcentration of the blood is prevented by supplying the salt factor(1-5). The situation is less clear with regard to the leucocytes. The results reported range from a decrease through little or no change to a significant increase, in total white count, in lymphocytes, and/or in neutrophils(1-13). Either there has been no consideration of eosinophilic leucocytes apart from neutrophilic or the statement has been made that the former seem unchanged(2,3,10).

In the course of a series of experiments with adrenalectomized animals some data were accumulated with regard to the white blood cell picture before and at various intervals after the operation(14) and later additional counts were made. Special attention has been given to variations in the numbers of eosinophils and bone marrow has been examined to determine the effects of adrenal insufficiency upon the myeloid-erythroid ratio and upon mitotic division.

Material and methods. All counts included in this report were made on 56 young male adult mice, hybrids of mixed ancestry, raised and maintained in wire cages in a warm animal room (72-78°F) and fed Purina dog chow supplemented with occasional milk and lettuce. Following adrenalectomy they were kept in wooden boxes and given 0.9% NaCl in tap water for drinking. Bilateral adrenalectomy was performed under light ether anesthesia following an intraperitoneal injection of 0.1 cc of 1 part Nembutal to 20 parts 0.9% NaCl solution. A subcutaneous injection of 5 cc of salt solution was given postoperatively.

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The method of Speirs and Meyer(15) was used to test for completeness of the operation, and only data from those mice which were completely adrenalectomized, as evidenced by no change or a rise in the number of eosinophiles after an injection of epinephrine, have been included. Total white counts were done in the usual manner. Differential counts of at least 200 cells were made from smears stained in May Grünwald Giemsa solutions for 31 of the mice after adrenalectomy and for all except 6 prior to adrenalectomy. Data for the latter, and for 25 of the adrenalectomized animals, are from direct differential counts. The number of eosinophils was determined according to the direct method of Speirs and Meyer(15); polymorphonuclear were distinguished from mononuclear cells by use of a 15x ocular in the course of making the total count in the Neubauer counting chamber, and the figures for the neutrophils were then obtained by subtracting the number of eosinophils from the number of polymorphonuclear cells per cu mm. A comparison of test counts on 5 mice by both methods indicated very little variance for neutrophils or mononuclear cells but a wider range and higher values for eosinophils by the smear technic. Intervals of 1 to 30 days elapsed between adrenalectomy and time of counting and data have been arranged in four series on this basis: 1-2, 4-7, 9-13, and 17-30 days. Counts were done on some of the mice at only one interval, on others at 2 or 3; but at least 3 days elapsed between successive counts on individual mice. Bone marrow smears were made directly, with the use of a hemostat, from the longitudinally split femur or sternum. Femoral smears were treated by the Feulgen method(16), those from the sternum were stained in May Grünwald Giemsa solutions. One thousand cells per smear of femoral marrow were differentiated as belonging to the myeloid or erythroid series according to the method recommended by Polli(17), and the M E ratio computed. The number of mitotic figures (chromosome stages only) per thousand cells was estimated on the same smears in most cases; for a few mice in each category, operated and intact, sternal smears were used. Comparative counts on smears from the same animals by both methods showed little or no variation. Reticulocytes were counted by the method of Friedlander and Wiedemer(18) with slight modifications.

Observations and results. Since the mice were of different generations from mixed ancestry a good deal of variation was expected between blood counts of individual animals. The effect of adrenalectomy on the leucocyte count showed the same trend in most cases, however, whether the count before operation was high or low. The picture, except at the 1-2 day interval, was characterized by a leucocytosis (Table I) for which the increased numbers of lymphocytes were mainly responsible. Absolute numbers of eosinophils were also higher. The behavior of the neutrophils was variable. The average numbers of the various types of cells at each interval after adrenalectomy, with the exception of 1-2 days, illustrate these trends and also show the increase in degree of effect with increase in length of interval after operation (Table I). The differences in numbers of mononuclear cells and eosinophils are significant within each of the 3 later series. Computations were made by Student's t test(19) and t values were higher than those expected at the 1% level. This was not true for the neutrophils; even the greatest difference, at the 4-7 day interval, was one which might be expected more than 5 times in 100 on the basis of chance alone. For all computations a comparison was made between counts of the same individuals before and after operation within each series; some of the mice are represented by counts in 2 or 3 different series. Lymphocytosis occurred after adrenalectomy in each one of the latter and only one failed to exhibit an eosinophilia. Of the 28 individuals counted at only one interval, 6 had fewer lymphocytes and 2 showed a slight eosinopenia after

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TABLE I. Average No. of White Blood Cells Before and After Adrenalectomy.

Type of count	No. of mice	Count before adrenalectomy	Interval, days	Count after adrenalectomy	Increase in No.	Stand. error	Increase, %
Total	10	11920	1-2	11340	−580		−5
	35	9280	4-7	12394	3114		33
	25	9808	9-13	13673	3865		39
	12	9758	17-30	17140	7382		76
Mononuclear	10	10350	1-2	10185	−165		−2
	35	7708	4-7	10334	2626	697	34
	25	7635	9-13	10840	3205	734	42
	12	7642	17-30	14739	7097	1950	93
Eosinophil	10	165	1-2	103	−62		−37
	35	94	4-7	160	66	24	70
	25	138	9-13	275	137	33	99
	12	129	17-30	312	183	42	141
Neutrophil	10	1405	1-2	1053	−352		−25
	35	1478	4-7	1900	422	233	29
	25	2035	9-13	2558	523	478	26
	12	1988	17-30	2089	101		5
Total	56	9600					
Mononuclear		7940					
Eosinophil		110					
Neutrophil		1550					

operation. These were from the 4-7 day series with the exception of one mouse from the 9-13 day group.

Consideration of the 1-2 day series (Table I) illustrates the importance of counting the same individuals before and after operation if accurate results are to be obtained. If a comparison of the average counts for the 12 mice in this series is made with those for all 56 mice before adrenalectomy there appears to be an increase in total count and in each type of cell except eosinophils. Actually for this particular group average decreases in all 3 types of leucocytes occurred at this interval after operation. These are obviously not significant with the possible exception of that for the eosinophils. As noted previously there is great variation in the neutrophil counts but none of the increases noted in this type of cell after adrenalectomy is significant. There is certainly no evidence of a neutropenia resulting from the operation, at least in the average counts. This is true irrespective of the method of counting and of whether the mice had had previous injections of epinephrine or not. A comparison of the percentage increases for the total count and for each type of leucocyte indicates that those for the mononuclear cells closely parallel those for the total count at the various intervals (Fig. 1). The eosinophils show by far the greatest average per cent increases.

An attempt was made to trace the origin of the increased numbers of leucocytes by a study of bone marrow and lymph node tissue. No real difference was observed from examination of stained imprints of the latter but some variations were noted in the former. The average number of mitotic figures, including late prophase (chromosome), metaphase, anaphase and telophase stages prior to reconstruction, was 5.5 ± 0.4 per thousand cells in the femoral marrow of 18 intact mice while for 25 animals adrenalectomized for from 8 to 30 days this was reduced to 3 ± 0.24 per thousand. This was a 45% decrease and the difference was found to be significant (5 X S.E.). The myeloid/erythroid ratio was also altered significantly after an 8-18 day absence of the adrenal glands. The variation in 10 intact mice was from 0.5/1 to 0.8/1, average 0.7/1; and in 14 adrenalectomized from 0.7/1 to 1.8/1, average 1.2/1. This average increase of 0.5/1 could indicate either a relative increase in myeloid or a relative reduction in erythroid cells. Since the number of reticulocytes is considered an index of bone marrow activity, counts were made on 18 adrenalectomized mice (from the 4-5 and 9-13 day series) and compared with data from the same number of controls. The average number of reticulocytes per 1000 red cells was

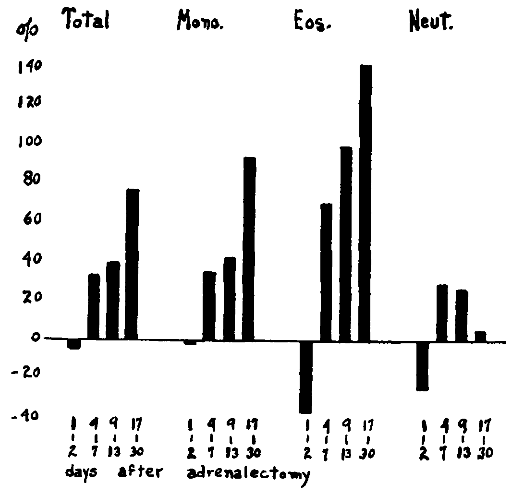

FIG. 1. % changes in absolute numbers of leucocytes at various intervals after adrenalectomy.

53 ± 4 for the former and 31 ± 2 for the latter, a 66% increase after adrenalectomy and a significant difference (4.8 X S.E.). No consistent variation was observed between degree of reticulocytosis and length of interval.

Discussion. The reasons for the difference in results obtained in investigations of the effect of adrenalectomy on circulating white blood cell values(1-13) are not apparent. Infection(8), individual reaction(12), and feline agranulocytosis(13) have been suggested. Other factors which might be involved in different instances are incompleteness of operation, length of the interval between operation and counts, small numbers of animals, and comparison of one group of adrenalectomized animals with a different group of controls rather than of counts from the same subjects pre and postoperatively. The results of the present experiment are in agreement with those of White and Dougherty(4) in showing increases in the total number of leucocytes and in the absolute number of mononuclear cells. As in their data, increases were progressively greater with length of interval after 2 days. However, counts were made at more intervals and over a longer period (2-30 versus 2-6 days) in the present study. White and Dougherty (4) reported an increase in polymorphonuclear leucocytes in their adrenalectomized mice which received salt treatment and a decrease of these cells in untreated animals. Neither a neutropenia nor a significant neutrophilia was observed in our mice but there was a significant and progressive increase in the absolute number of eosinophils after adrenalectomy. Reports in the literature either do not give data on eosinophils or state that no significant change occurred.

Examination of the bone marrow would be expected to aid in the interpretation of the mechanism of action of the adrenal hormones on the processes of erythropoiesis and myelopoiesis. On the basis of the decreased number of polymorphonuclear cells in the blood of adrenalectomized mice which were not salt treated, White and Dougherty(4) suggested the likelihood of an actual suppression in the production or delivery of these cells. Hypoplasia of the marrow of adrenalectomized cats was noted by Lewis(1). An increased myeloid/erythroid ratio was observed in this investigation in mice, and by Piliero *et al.*(5) in rats, after adrenalectomy. In conjunction with a hypoplastic marrow such an increased ratio might represent only a relatively greater inhibition of erythroid than of myeloid elements rather than a stimulation of the latter. A recent investigation(20) points to the stimulating effects of cortisone on myeloid tissue and thus lends support to the suggestion of inhibition resulting from lack of the hormone. Pilero *et al.*(5) comment on the increased level of *young* neutrophilic forms in the marrow of adrenalectomized rats and the reduction of this immaturity by Compound E. Increase in the number of young versus older myeloid cells was not observed in our mice but there was a significant decrease in the number of mitotic figures in the bone marrow of the adrenalectomized animals, indicating a reduction in hemopoietic activity.

As regards erythropoiesis the percentage of reticulocytes in the circulating blood is considered an index of bone marrow activity. The increased proportion of these cells observed by us and by previous investigators(2,5,12) after adrenalectomy might thus be interpreted as indicating a more than normally active marrow. This is not in agreement with the observations noted above. An increased rate

20. Quittner, H., Wald, N., Sussman, L. N., and Antopol, W., *Blood*, 1951, v6, 513.

of maturation and release of reticulocytes would also account for the data but it seems questionable to assume that adrenalectomy would increase the degree of immaturity in leucocytes and hasten maturation of erythrocytes. Another, and seemingly a more likely, possibility would be a decreased rate of maturation of the circulating reticulocytes thus allowing for their accumulation in the blood.

Summary. Adrenalectomy resulted in an increase in total count and increased absolute numbers of mononuclear and eosinophilic cells in the circulating blood of adrenalectomized mice. The increases were progressively greater with increases in the length of the interval after adrenalectomy from 4-5 days to 17-30 days. There was no consistent effect on neutrophiles. Reticulocyte percentages increased in the circulating blood. The number of mitotic figures in the bone marrow was reduced, while the ratio of myeloid to erythroid elements was increased. All the differences noted were found to be significant. The results suggest a decreased rate of activity and of maturation of cells in the bone marrow and an increase in the length of period of maturation and longevity in the circulating blood.

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Toxicity Studies on Subtilin. (19124)

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The possibility that it may become desirable to use antibiotics in the normal diet either for growth promotion or as food preservatives requires that adequate knowledge be available on their long-term effects. The present study dealing with the continued feeding of subtilin-containing diets to rats was prompted by the observation that subtilin appeared to have possibilities as a food-preserving substance(1).

Wilson, Humphreys, Reynolds and Lewis (2) have reported on the absorption of subtilin in acute experiments and commented on accompanying toxic reactions. Consideration of that data and the *in vitro* studies of others (mentioned below) leads to the presumption that chronic oral ingestion would be harmless. Subtilin is a polypeptide(3) inactivated and presumably hydrolyzed by proteolytic enzymes(4). Destruction in the gastrointestinal tract could be expected. In the acute toxicity studies(2) it was mentioned that a single rabbit received 1 g of subtilin/kg by stomach tube. After 5 hours only 2 μg were found in the urine, 15 mg in the intestine and 0.5 g in the stomach, or a total of 13% of the administered 4 g. The highest blood level was 0.1 ppm. This would indicate rapid inactivation or destruction of the subtilin entering the intestine. Subtilin is but sparingly soluble in physiological saline solutions and in body fluids(4). This insolubility accounted for all observed acute toxic effects* and might limit chronic oral toxicity. Andersen and Michener(1) suggested that "although *B. subtilis* is often found in foods, where it may well have produced appreciable quantities of subtilin, no cases of food poisoning have ever been attributed to it." They suggested further that the predominant intestinal microflora would not be adversely affected since subtilin is not active against Gram-negative bacteria:

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