animals in which leukopenia has been produced by administration of leukocytes or their products, and in no animal in which leukopenia was induced with sodium citrate solution, argues against the subsequent leukocytosis being a response of the bone marrow stimulated in some manner by the leukopenia. The possibility of the existence of differences in the reaction of the recipient is indicated by the production of leukocytosis in one rabbit but not in another with the same cell suspension.

The chemical nature of the active substances present in solution of lysed leukocytes has not as yet been determined. Preliminary experiments indicate that removal of lipids, including thromboplastin, by ether extraction does not reduce its activity. Considerable loss of activity results from exposure to heating at 66°C for 1 hour.

Summary and conclusions. 1. Leukocytes were obtained in large numbers by introduction of large amounts of physiologic salt solution into the peritoneal cavity of rabbits.

2. Intravenous administration of these cells to the same or different animals was followed by very rapidly developing and severe leukopenia. A subsequent leukocytosis developed in the majority of rabbits after several hours.

3. Disintegrated leukocytes, or aqueous extract of disintegrated leukocytes produced similar results.

4. Preliminary data indicate that the leukocytes stick in the capillaries of the lung. Ether extraction of disintegrated leukocytes does not cause loss of activity but heating reduces activity.

5. These experiments indicate that unsatisfactory preservation of leukocytes in stored blood is not the reason for failure to raise leukocyte counts with transfusions, but that white blood cells contain a substance (or substances) which is capable of producing leukopenia.

We are indebted to Dr. William Holden for the assay of the thromboplastic content of leukocytes.

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Effect of Adrenocorticotrophic Hormone Upon Liver Fat and Urinary Phosphorus in Normal Force-Fed Rat.

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It was shown by Baker, Ingle, Li, and Evans[1] that the administration of pure adrenocorticotrophic hormone (ACTH) to force-fed male rats caused fatty infiltration of the liver as was demonstrated histologically by Sudan stains. This observation was confirmed by chemical analysis of the liver in the present study. In addition, during the administration of ACTH there was noted a significant increase in the urinary excretion of inorganic phosphorus which accompanied a rise in urinary nitrogen and that there was excretion of glucose and loss in body weight. Changes in the weights of certain organs was noted.

Methods. Male rats of the Sprague-Dawley strain were maintained on a diet of Archer Dog Pellets until they reached a weight of approximately 300 g. They were then placed in metabolism cages and maintained on a fluid diet administered by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technic of force-feeding and the diets used were modi-

[1] Baker, B. L., Ingle, D. J., Li, C. H., and Evans, H. M., *Am. J. Anat.*, 1948, **82**, 75.

TABLE I.
Medium Carbohydrate Diet.

Constituent	g
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Diet yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vit. K (2-methyl-1,4-naphthoquinone	100 mg
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc

fications of those described by Reinecke, Ball, and Samuels.[2] The diet was made according to Table I. During the period of adaptation to force-feeding the amount of diet was increased gradually to prevent the development of "food-shock." The animals were brought to a full feeding of 26 cc per day on the 5th day.

The animals were housed in an air-conditioned room in which the temperature was maintained at 74 to 78°F and the humidity at 30 to 35% of saturation. Twenty-four-hour samples of urine were collected at the same hour each day and were preserved with thymol and 1 g of citric acid per sample to insure the acidity of the urines for nitrogen analysis. The following methods of analysis were used: urinary inorganic phosphorus, Müller;[3] urinary non-protein nitrogen by the micro-Kjeldahl procedure; tissue fat and protein, Li, Simpson, and Evans.[4]

The ACTH was prepared by the method of Li, Simpson, and Evans.[5] Following control periods of 14 days, 6 experimental animals were each given 3 mg of ACTH in 7 divided injections every 2 hours during the day for a period of 10 days. One animal was killed by a feeding accident so that the final averages are based upon 5 animals. Six control animals were given injections of physiological saline. At the end of the experiment the animals were anesthetized with ether and exsanguinated. The liver was subjected to lyophilization at low temperature and was then analyzed for fat and protein. Weights were obtained on a number of organs.

Results. The data on body weight, urinary non-protein nitrogen, inorganic phosphorus and glucose are in Fig. 1, the data on organ weights in Table II, and the data on liver composition in Table III. The administration of ACTH caused a significant increase in liver fat so that all of the individual values were higher than any of the control values. The water content of the ACTH livers was correspondingly decreased. The difference between the average protein content of ACTH and control livers was too small to be considered significant.

The injections of ACTH caused a loss of weight associated with a rise in urinary nitrogen, inorganic phosphorus and glycosuria. ACTH caused an increase in the weight of the liver, hypertrophy of the adrenal glands and atrophy of the thymus. The average weights of the organs of the gastrointestinal tract and of the testes were somewhat less in the ACTH series but the kidneys and hearts were heavier. Since the numbers of animals were small the group differences cannot be

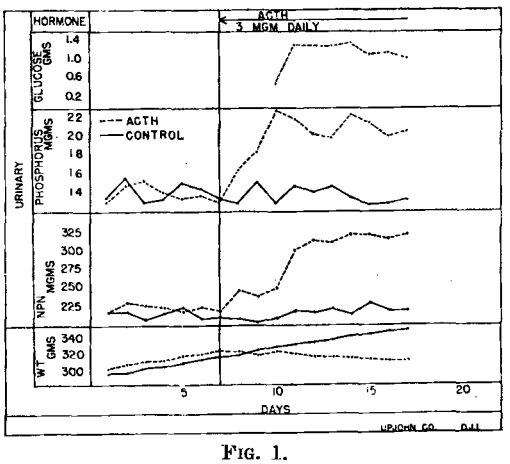

FIG. 1.
The effect of adrenocorticotrophic hormone upon body weight and some constituents of urine in the force-fed normal rat. Averages.

[2] Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 44.

[3] Müller, E., *Hoppe-Seyler's "Zeitsch. fur. Physiol. Chemie.,"* 1935, **237**, 35.

[4] Li, C. H., Simpson, M. E., and Evans, H. M., *Growth*, 1948, **12**, 39.

[5] Li, C. H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1942, **146**, 627.

TABLE II.
Organ Weight. Grams.

Organ	Adrenocorticotrophic hormone		Controls	
	Avg	Range	Avg	Range
Liver	12.17	10.11-16.21	10.27	9.50-10.80
Stomach	1.33	1.30- 1.40	1.47	1.35- 1.61
Small intestine	5.51	5.11- 5.95	6.04	5.90- 6.40
Cecum and colon	1.74	1.65- 1.80	1.94	1.70- 2.20
Kidneys	2.27	2.10- 2.49	2.01	1.86- 2.01
Testes	3.14	2.65- 3.35	3.32	3.15- 3.45
Heart	1.03	0.97- 1.05	0.94	0.93- 0.97

TABLE III.
Liver Composition of Rats Treated with Adrenocorticotrophic Hormone. Averages and standard deviations of averages.

	Body wt, g	Liver wt, g	Composition in g per 100 g wet liver		
			Water	Fat	Protein
ACTH	315	11.97	61.1	11.96	20.4
	±5.7	±1.1	±1.2	±1.4	±0.6
Control	347	10.27	67.5	5.83	21.73
	±3.1	±0.4	±0.4	±0.6	±0.6

regarded as highly reliable but there was no overlapping in individual values of the two groups in the case of the heart and kidneys and it is probable that ACTH caused these organs to gain weight.

Discussion. There are a number of experimental conditions which cause fatty livers in non-adrenalectomized animals. This response has not been described in the adrenalectomized animal,[6] although Chaikoff *et al.*[7] have reported on the production of fatty livers in hypophysectomized - thyroidectomized dogs. Hartman *et al.*[8] have prepared a fraction of adrenal cortex extracts which sustains the ability of the adrenalectomized rat to deposit fat in the liver. The relationship of adrenal cortical function to the accumulation of liver fat and to other aspects of fat metabolism is not well understood.

One of the objectives of this experiment was to study carcass fat in animals treated with ACTH without the development of glycosuria. It was not anticipated that these animals fed a medium carbohydrate diet would develop glycosuria when given ACTH. The administration of ACTH or of certain adrenal extracts and steroids either inhibits the protein anabolism or stimulates protein catabolism. If the energy represented by the missing protein is not wasted by incomplete metabolism, it must either be stored as fat (the amount of energy which can be stored as extra carbohydrate is very limited) or dissipated by a higher energy output. The effect of ACTH and of cortical hormone overdosage on energy output has not been fully studied. There are two reports[9,10] that 11-dehydrocorticosterone can cause an increase in carcass fat in the mouse. In the present study it was calculated from the extent of rise in urinary nitrogen that the administration of ACTH inhibited the accumulation of approximately 4 g of protein per rat during the 10-day period. During the

[6] Ingle, D. J., *J. Clin. Endocrinol.*, 1943, **3**, 603.

[7] Chaikoff, I. L., Entenman, C., Rinehart, J. F., and Reichert, F. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 170.

[8] Hartman, F. A., Brownell, K. A., and Thatcher, J. S., *Endocrinology*, 1947, **40**, 450.

[9] Kendall, E. C., Josiah Macy, Jr. Foundation, Conference on Metabolic Aspects of Convalescence, Transactions of 10th Meeting, 1945, 81.

[10] Kochakian, C. D., Josiah Macy, Jr., Foundation, Conference on Metabolic Aspects of Convalescence, Transactions of 6th Meeting, 1944, 13.

same period each rat excreted an average of more than 9 g of glucose. Under these conditions an accumulation of carcass fat cannot be expected and this aspect of the problem remains for further study.

The effect of ACTH in causing an increased loss of urinary phosphorus is similar to the effect of 11-dehydro-17-hydroxycorticosterone noted by Ingle and Thorn.[11]

Summary. Normal male rats were forcefed a medium carbohydrate diet. The administration of adrenocorticotrophic hormone in amounts of 3 mg per rat per day for 10 days caused an increase in liver fat, glycosuria, a rise in urinary nitrogen and inorganic phosphorus, suppression of weight gains and some changes in organ weights.

[11] Ingle, D. J., and Thorn, G. W., *Am. J. Physiol.*, 1941, **132**, 670.