TABLE II. Results of Carcass Analysis.

Salt mixture	Strain	No. of mice	Final body wt, g	Dry wt, g	Dry, fat-free wt, g	Fat, g	Ash, g
Hubbell, Mendel and Wakeman	C_{57}	6	31	13.4	6.2	7.2	.93 ± .016*
	I	5	29.6	10.7	6.5	4.3	.95 ± .015
Fenton and Carr	C_{57}	8	28.9	11.7	5.8	5.8	.86 ± .009
	I	5	26.4	8.3	5.8	2.5	.86 ± .015
Wesson	C_{57}	6	28	11	5.8	5.2	.85 ± .011
	I	5	26.9	9.1	5.8	3.5	.88 ± .021
U.S.P. XII #2	C_{57}	6	32.2	13.9	6.2	7.7	.84
	I	5	25.7	8.7	5.8	3	.86 ± .014
McCollum-Simmonds #185	C_{57}	6	29.8	12.4	6.1	6.3	.83 ± .007
	I	5	26.3	9.1	5.8	3.2	.84 ± .014

* Stand. error.

fat and mineral deposition in the carcass. Feeding the mixture described by Hubbell, Mendel and Wakeman(1) resulted in significantly higher ash values than when the other four mixtures were employed. Increasing the Ca:P ratio of the Fenton-Carr mixture(4) significantly improved mineral deposition.

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Received December 1, 1952. P.S.E.B.M., 1953, v82.

Cytoplasmic Budding of Human Lymphocytes Produced by Cortisone and Hydrocortisone in *in vitro* Preparations.* (20009)

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The destructive changes that lead to lymphocytic dissolution *in vivo* under conditions of increased adrenocortical secretions begin with cytoplasmic budding and result in pycnosis and karyorrhexis of the cells(1-3). Shedding of the cytoplasm of lymphocytes was described as a normal process in the older literature by Downey and Weidenreich and the cytoplasmic fragments were termed hyaline bodies(4). The observation of cytoplasmic budding of lymphocytes in this laboratory in blood films of patients treated with ACTH and cortisone led to an attempt to produce this phenomenon by treating lymphocytes isolated from buffy coat of normal human blood with cortisone and hydrocortisone *in vitro.*

Materials and Methods. Crystalline hydrocortisone (free alcohol) and cortisone (free alcohol) were used in these experiments. Ethylene bisiminodiacetic acid (Sequestrene Na_2) was used as an anti-coagulant according to the technique of Proescher(5). Fifteen ml samples of venous blood from normal human subjects were centrifuged in sterile tubes containing 0.1 ml of a 10% solution of Sequestrene Na_2. The hormone to be tested was added to a portion of plasma so that 1 ml of plasma contained 1 mg of hormone. Non-hormone treated plasma from the same sample was used for control studies. A drop of

* These studies were aided by a grant from the Committee on Growth, National Research Council acting for the American Cancer Society.

† The authors wish to thank Dr. Augustus Gibson of Merck and Co. for the supply of hormone used and Dr. H. W. Zussman of the Alrose Chemical Co. for Sequestrene Na_2.

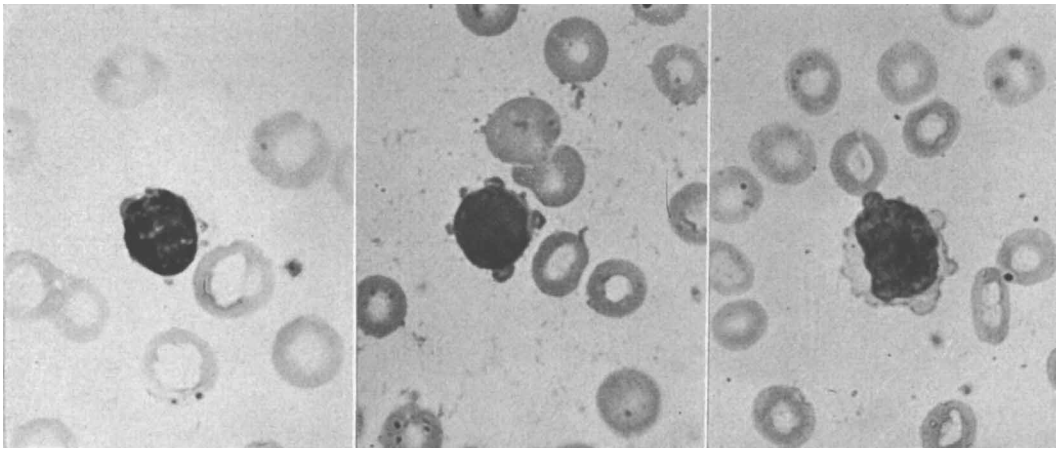

FIG. 1. Cytoplasmic budding in buffy coat lymphocytes obtained from normal human subjects and treated with hydrocortisone.

plasma was mixed gently with a drop of buffy coat from the same blood specimen on a clean cover slip and smeared. The mixing and smearing process occupied approximately 30 seconds. Cover slips were air dried and stained with May Grünwald Giemsa. A minimum of 200 lymphocytes per cover slip were counted.

Results. A comparison of the percent of budding lymphocytes in normal and treated buffy coat lymphocytes is seen in Table I. Cytoplasmic budding was found in 11.63% of the lymphocytes of the hydrocortisone treated buffy coat and in 1.2% of the controls. Cortisone also produced cytoplasmic budding but to a lesser degree than hydrocortisone. Buffy coat lymphocytes demonstrating cytoplasmic budding after treatment with hydrocortisone are presented in Fig. 1.

TABLE I. Percent of Budding Lymphocytes in Normal and Treated Buffy Coat Lymphocytes.

No. of exp.	Control	Experimental
	Buffy coat and plasma	Buffy coat, plasma and hydrocortisone
1	.25	10.5
2	0	10
3	1.25	9.75
4	0	7.5
5	0	8
6	2.5	20
7	4	18.25
8	1.5	8.75
9	0	15.75
10	2.5	7.75
$\bar{X}$	1.2	11.63
σm	.4	1.25

Discussion. Williamson recently confirmed the findings of Downey and Weidenreich(4) that lymphocytes from lymph and lymph nodes of rabbits, rats and guinea pigs shed their cytoplasm in *in vitro* preparations. He found that the medium sized lymphocytes were the most actively budding cells(6). Several authors concluded that lymphocytes contributed their cytoplasmic constituents to the body fluids by the apocrine type of secretion (2,4,6). Dougherty and White suggested that the phenomenon of lymphocytic cytoplasmic shedding is enhanced by increased concentrations of C-11-oxy adrenocortical steroids(2).

The production of cytoplasmic budding in circulating lymphocytes of hydrocortisone has been demonstrated. The free alcohol of hydrocortisone is soluble in plasma to the extent of 0.7 mg per milliliter(7). Therefore, it can be assumed that an excess of 0.3 mg per milliliter was present in suspension. No attempt was made to demonstrate the lymphocytolytic effects of adrenocortical hormones which are known to occur *in vivo*(2).

The problem as to whether or not the purified adrenocortical hormones exert lymphocytolytic effects *in vitro* is still not settled. Adrenal cortical extract (Lipo Adrenal-Upjohn) produced "bubbling" of lymphocyte cytoplasm and disintegration of lymphocytes (8). Hechter and Johnson however, found that lymphocytolysis occurred *in vitro* only

when lymphatic tissue homogenates were added to the adrenal cortical extract and suggested that a co-factor present in the tissue was essential for lymphocytolysis(9). Other investigators were unable to confirm the lymphocytolytic action of adrenal cortical extracts using *in vitro* techniques(10,11).

Cortisone and hydrocortisone produce degeneration of lymphocytes *in vitro* as measured by degeneration and inhibition of cell migration in tissue culture(12) and eosin staining in lymphocyte suspensions(13). Purified adrenocortical steroid hormones which were not lymphocytolytic *in vivo* also were without effect *in vitro*(13).

It is apparent from the data presented here that cytoplasmic budding reflects an excess of the C-11-oxy, 17-hydroxy adrenocortical hormones at the lymphocyte level and is similar to the "bubbling" phenomenon described by Feldman following treatment with adrenal cortical extracts(8). It is suggested that lymphocytes that have undergone cytoplasmic budding may revert to normal lymphocytes. On the other hand, lymphocytes may undergo karyorrhexis and pycnosis. It is not known whether these various phenomena are due to variations in the concentration of adrenocortical hormones or whether there is a difference in susceptibility of lymphocytes. This problem is being investigated at the present time.

Summary. 1. Significant numbers of lymphocytes exhibiting cytoplasmic budding were found within 30 seconds following addition of cortisone or hydrocortisone to plasma and then to buffy coat of the blood of normal human subjects. It is suggested that cytoplasmic budding of lymphocytes may be a reversible process and that the occurrence of cytoplasmic budding, pycnosis or karyorrhexis may depend upon differential susceptibility of lymphocytes.

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Received December 1, 1952. P.S.E.B.M., 1953, v82.

Effects of Age, Food Intake, and Stress on Plasma Hexosamine Levels in the Rat. (20010)

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Non-specific body injury or stress has been shown to produce an increase in the level of plasma hexosamine in many acute and chronic clinical diseases(1-6) and under a variety of experimental conditions(7-10). In order to study the physiological mechanisms involved in this response it was first necessary to examine the effects of age, and food intake in the normal and stressed animal. In the rat these are shown to be critical variables calling for carefully controlled experimental conditions.

Methods. Male Sprague-Dawley rats were fed a specially prepared diet,* unless otherwise indicated, and drank water *ad libitum.* The rats were stabilized 3-7 days on this diet