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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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

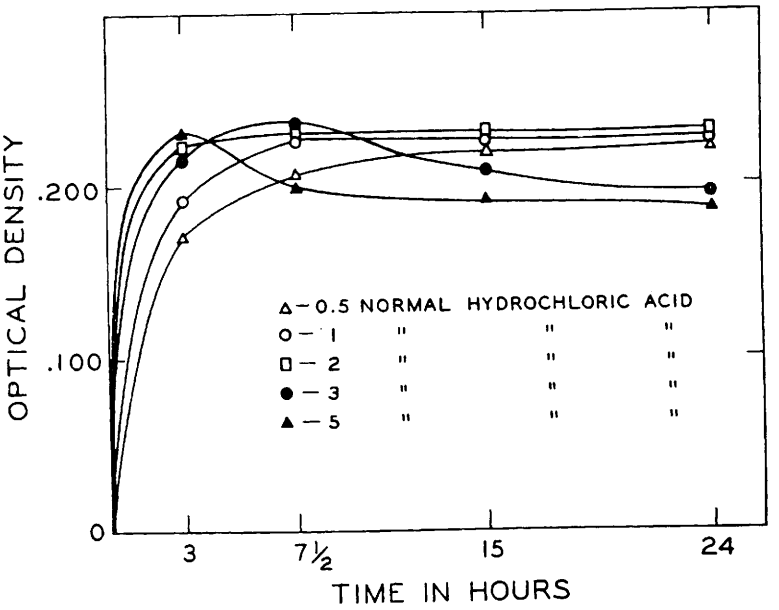

FIG. 1. Effect of acid hydrolysis (100°C) on rate of liberation and destruction of rat plasma hexosamine.

before experiments were started. Pair-fed controls, when used, were started one day later than the experimental groups. Food intake and body weights were measured daily. Tail blood samples (0.35 ml) were collected for the hexosamine determinations and external jugular vein blood for the plasma volume studies. All samples were treated with dry oxalate, transferred to Wintrobe hematocrit tubes and centrifuged at 3400 rpm for 30 minutes. Hematocrit readings were made and the plasma separated. For the hexosamine determinations 1.1 ml of water were added to 0.1 ml of each plasma sample. These diluted samples were stored in a refrigerator at 4°C until the end of an experiment,† when 1 ml aliquots were transferred to 10 ml glass-stoppered volumetric flasks. One ml of 4 N hydrochloric acid was added to each sample, producing a 2 N solution, the flasks were stoppered and placed in a boiling water bath for 15 hours. After cooling, they were taken to volume with water and filtered. The conditions for maximal liberation of hexosamine were established by hydrolyzing replicate plasma samples as indicated in Fig. 1. Two ml aliquots of the filtrates were used in a modified Elson and Morgan hexosamine method(12). Plasma chromogens interfering in the hexosamine method amount to less than 3-4% so no purification of plasma is necessary(12). For the 90-140 gram rats which were used, the hexosamine range varied generally from 120-140 mg/100 ml. In serial bleeding experiments the values are expressed as mg/100 ml change from an initial control value.

In experiments where 2 or more blood samples were obtained, the hematocrit values fell due to hemo-dilution resulting from blood loss. With fasting the hematocrit values increased as a result of dehydration. Both of these hematocrit changes were primarily the result of plasma volume changes. Since hexosamine is expressed as concentration in plasma, the hexosamine values in the fasting and serial bleeding experiments were therefore

* Diet No. 4164(11): 67.5% ground whole wheat; 15% casein; 10% whole milk powder; 5.25% cotton seed oil; 1.5% $CaCO_3$; 0.75% NaCl; Vit. A 12,000 U.S.P. units, Vit. D 2,150 U.S.P. units and alpha-tocopherol 50 mg per kilo of diet respectively. (Protein 24.3%; carbohydrate 54.8%; fat 11.0%).

† Plasma hexosamine is stable at 4° for 2 weeks. For longer preservation the samples may be stored in a deep freeze.

TABLE I. Effect of Age on Hematocrit and Plasma Hexosamine Levels in the Rat.

Age (wk)	4	7	11	16
Body wt (g)	40 ± .9*	81 ± 1.5	159 ± 4.7	271 ±10.6
Hematocrit (%)	44.8 ± .7	46.9 ± .8	51.7 ± .5	52.3 ± .2
Hexosamine (mg/100 ml)	94 ± 3.7	136 ± 6	162 ± 3.5	146 ± 1.3

* Stand. error of the mean.

TABLE II. Effect of Fasting on Blood Volume and Plasma Hexosamine.

	Control	Starved	t	P
No. rats	5	5		
Initial body wt (g)	132 ± 3.5 *	137 ± 4		
Final " " (g)	134 ± 4.9	107 ± 2.6		
Hematocrit (%)	43.7 ± 1.1	51.2 ± 1.7	7.42	<.01
Plasma Hexosamine (mg/100 ml)	119 ± 3.1	122 ± 2.9	1.41	<.50
Plasma volume (ml)	5.86 ± .24	4.47 ± .14	10.01	<.01
Red cell volume (ml)	5.02 ± .16	4.58 ± .16	3.89	<.01
Total blood volume (ml)	10.88 ± .19	9.05 ± .17	14	<.01
Total circulating hexosamine (mg)	6.96 ± .24	5.43 ± .16	10.60	<.01

* Stand. error of the mean.

TABLE III. Effect of Time of Sampling and Fasting on Plasma Hexosamine Levels in the Rat.

		1st day		2nd day	
		9 A.M.	5 P.M.	10 A.M.	5 P.M.
Control	Body wt (g)	130 ± 4.4*	126 ± 5.3	136 ± 5	129 ± 6
	Hexosamine (mg/100 ml)	131 ± 7.2	120 ± 3.5	134 ± 6.8	136 ± 4.4
Fasted	Body wt (g)	114 ± 4.3	106 ± 5.9	102 ± 2.2	91 ± 6.4
	Hexosamine (mg/100 ml)	126 ± 7	132 ± 6.2	113 ± 4.9	108 ± 2.5

* Stand. error of the mean.

corrected for these hematocrit changes as follows: (Corrected Hexosamine mg/100 ml) = $\frac{(100 - H_2)\,(\text{observed hexosamine mg/100 ml})}{(100 - H_1)}$

where H_1 = control hematocrit and H_2 = experimental hematocrit. *Plasma volumes* were measured with the dye T-1824(13) following the withdrawal of blood for a hexosamine determination. The final blood volumes were corrected for the prior removal of this sample.

Results. Age. Plasma hexosamine determinations were performed on 4 different age groups of 3 rats each, which had been raised on Purina Chow and kale. The results in Table I show that the hexosamine concentration increased from a low value in the 40 g group (4 weeks) to a high in the 160 g group (11 weeks), and then fell slightly in the 270 g group (16 weeks). These observations demonstrated the need for adequate control rats when studying plasma hexosamine levels in the rapidly growing period.

Fasting. Ten rats were used to determine the effect of fasting on the total plasma hexosamine (mg) (Table II). Five rats were permitted to eat *ad libitum* and food was withheld from the other group of 5. After 2 days, determinations were made of their hematocrits, blood volumes and plasma hexosamine levels. The total circulating hexosamine (mg) was calculated on the basis of observed values for plasma volume (P V) and plasma hexosamine concentration in mg/100 ml (Hex), *i.e.*, $\frac{(P\,V)\,(Hex)}{100}$. There were no significant differences in hexosamine expressed as concentration (mg/100 ml), however, the hematocrit values in the fasted rats increased significantly. The hematocrit changes are shown to be primarily due to a decreased plasma volume (76.0% of total blood volume decrease) and to a lesser extent to a decrease in the red cell volume (24.0% of total blood volume decrease). The total circulating hexosamine (mg), as calculated on the basis of

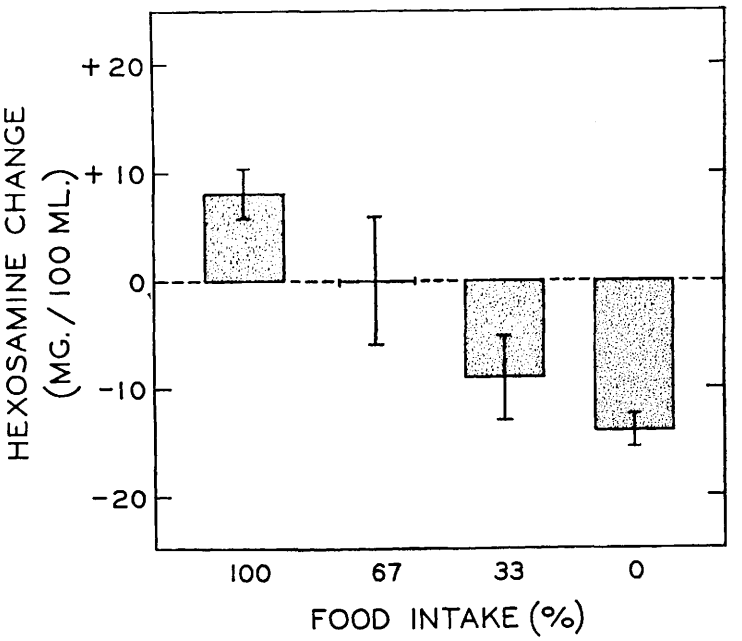

FIG. 2. The effect of different amounts of food intake over a 3 day period on plasma hexosamine levels in the rat. Vertical lines refer to S.E. of the mean.

observed plasma volume and the concentration of plasma hexosamine, were significantly reduced. Frequent plasma samples were obtained over a 2-day period to determine how soon the effects of fasting on hexosamine became manifest and to see if there were any diurnal changes in plasma hexosamine levels. A control group eating *ad libitum* (Purina Chow), and a group fasted from the start of the experiment were used. There were 16 rats in each group, with 4 being used at each time interval indicated in Table III. Each rat was bled only one time. There were no significant diurnal changes in the control group. Fasting did not significantly lower the hexosamine level until 24 hours after the start of the experiment. In another experiment in which rats had access to food and in which plasma was obtained at 9 A.M., 1, 5, and 9 P.M. of the same day, no changes were noted. The time of sampling during the day does not appear to be an important factor.

Food intake. Since fasting lowered the total plasma hexosamine, it became important to know what the critical food intake level was for this effect. Accordingly, 4 groups with 5 rats in each group (95-118 g) were permitted to eat *ad libitum* for a 3-day control period (Fig. 2). The daily average intake (g) for each rat was calculated. The first group continued to eat *ad libitum* (10.0 $\pm$ 0.3 g/day); each rat of the second group was fed daily with 66.7% of its calculated daily average of the control period (6.6 $\pm$ 0.2 g/day); each rat of the third group was fed daily with 33.3% of its calculated daily control average (3.3 $\pm$ 0.1 g/day); the fourth group was fasted. Plasma samples were obtained at the end of the control period and at the termination of the experiment which ran 3 days. The control group (100% intake) developed a slight increase in plasma hexosamine, probably due to the stress of bleeding. The group getting a 66.7% intake had lower hexosamine values some of which were well below the control range. The fasted group and those rats getting 33.3% intake had significantly reduced values. This experiment demonstrated the critical effect of only slight reductions of food intake, and stressed the need for careful dietary controls in plasma hexosamine studies.

Stress. The general plasma hexosamine response to stress is demonstrated in Fig. 3.

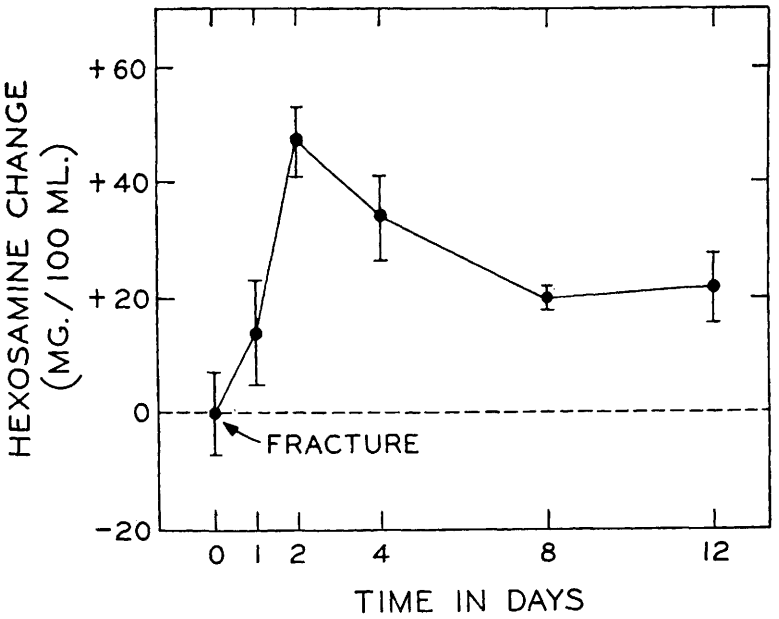

FIG. 3. Effect of fracture on plasma hexosamine levels in the rat. Vertical lines refer to S.E. of the mean.

Six groups of 4 rats weighing 100-130 g were fed Purina Chow *ad libitum*. The right femur and tibia were fractured in each rat under light ether anesthesia. Single bleedings were performed at the indicated time intervals. The plasma hexosamine level increased temporarily reaching a peak at 2 days. Repeated experiments with this and other types of stress (turpentine injections, sham operations, epinephrine, blood-letting) have all shown hexosamine increases, with maximum responses between 2 and 4 days.

Fasting and stress. To study the effect of fasting on stress-induced plasma hexosamine changes, 4 groups of rats, with 5 rats in each group were used. Two groups received 0.4 ml subcutaneous injections of Venice Turpentine in their backs. Of these, one group was permitted to eat *ad libitum* and the other group was fasted. Two control groups were used, one was pair-fed with turpentine-injected rats eating *ad libitum* and the other group was fasted (Fig. 4). The animals were bled serially, and the hexosamine values corrected for hematocrit changes and expressed as mg/100 ml change. The turpentine-injected rats which had access to food, despite a reduced intake following the stress, developed a significant rise in plasma hexosamine. Their pair-fed controls, however, developed an initial fall in hexosamine, probably due to the impaired intake. The rise in hexosamine was not only prevented by fasting the other turpentine-injected group, but was lowered to a level similar to that of the fasted controls.

Discussion. The bound plasma polysaccharides in man consist essentially of hexosamine, mannose and galactose, occurring in approximately equimolar proportions, with a combined plasma concentration greater than 3 times the normal concentration of blood glucose. Measures of these polysaccharides have been obtained in several ways—as "serum polysaccharide"(14), non-glucosamine polysaccharides(15), total mucoprotein(16), hexosamine(1), and others. Regardless of the methods used, it has been repeatedly demonstrated that following many types of stress, clinical or experimental, these substances increase in the plasma(1,17-19). Little is understood concerning the mechanism of these changes. ACTH or cortisone will lower elevated serum hexosamine(6) and serum mucoprotein(20) levels. A thyrotropic hormone fraction produced striking increases in the serum of 2 patients(21). The specificity of

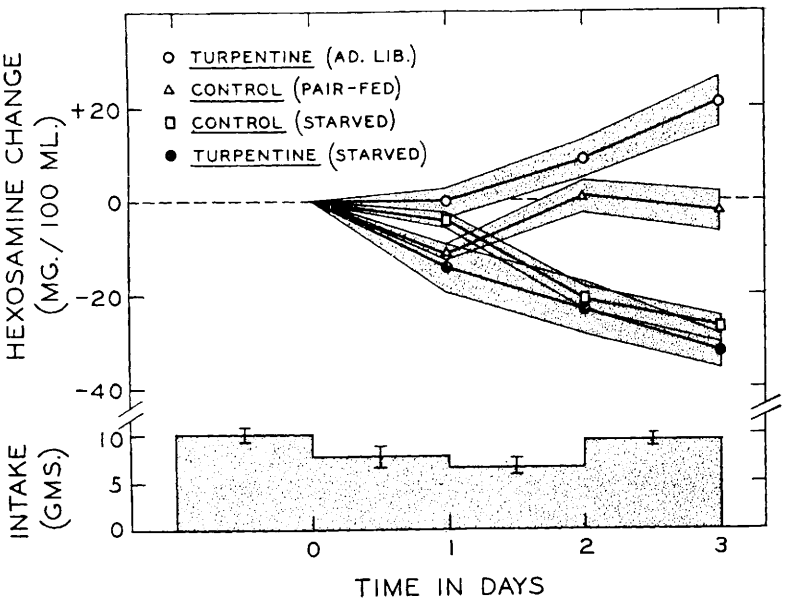

FIG. 4. Effect of fasting on stress-induced (turpentine injections) plasma hexosamine changes. Shaded areas on curves and vertical lines refer to S.E. of the mean.

this response has not been established. It has been suggested, however, that the serum hexosamine response is a stress-induced change mediated by a pituitary hormone, probably related to the thyrotropic hormone fraction(21).

Summary. The following observations were made on plasma hexosamine levels in the rat: 1) there is an increase during the period of rapid growth, 2) no significant diurnal changes could be demonstrated, 3) the amount of food eaten is important in the maintenance of a normal level, 4) stress increases and fasting lowers the plasma hexosamine level in the normal rat, 5) fasting prevents the expected increase following stress.

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