Effects of Growth, Fasting, and Trauma on the Concentrations of Connective Tissue Hexosamine and Water. (21203)

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Hexosamines* are present in the ground substance of connective tissue as components of mucopolysaccharides(1) and mucoproteins (2). Since the ground substance is altered in the collagen diseases, it has been suggested by many that changes in mucopolysaccharides may be related to these observed lesions. Schlamowitz *et al.*(3) have demonstrated that "nodules", experimentally induced by injections of trypsin and other enzymes, contain increased concentrations of hexosamine. This has also been noted in nodules obtained from patients with rheumatic fever(2) and in the corium and subcutaneous connective tissue of patients with scleroderma(4). In order to evaluate further the factors influencing connective tissue hexosamine levels, the effects of growth, fasting and various traumatic agents have been studied.

Methods. Male Sprague-Dawley rats were individually caged, fed a stock diet(5) and were permitted to drink water *ad libitum.* Rats of similar weight were used in each experiment. At the end of an experiment the rats were killed with ether. In those instances in which orbital connective tissue was obtained the rats were killed by exsanguination under ether anesthesia. Subcutaneous connective tissue samples were obtained from an area of the back, caudal to the scapular adipose tissue. The skin was cut along the midline of the back, retracted and the sample (50-200 mg) removed by scraping the loose connective tissue from the underside of the corium with a pair of wing-tipped forceps. Orbital connective tissue was obtained following removal of the eyeballs by dissection from the Harderian glands. After weighing, all the samples were placed in an oven at 101-104°C for 18 hours, reweighed, then extracted in a Soxhlet for 3 hours with redistilled petroleum ether (B.P. 60-70°C). The dry, fat-free samples were weighed and the water and fat contents calculated. The amount of fat in a wet sample of subcutaneous connective tissue may vary from 2-40%, causing a wide variation in calculated water and hexosamine values from sample to sample. The concentrations of tissue hexosamine and water are independent of the amount of fat present, therefore, all expressions of concentration for water and hexosamine are presented relative to fat-free tissue. Water is expressed as per cent (wet wt—dry wt) x 100/(wet wt—fat), and hexosamine as mg/100 g of fat-free dry tissue. To determine the hexosamine content, the dry defatted samples were each placed in 10-ml glass-stoppered volumetric flasks. After adding one ml of 4 N hydrochloric acid, they were hydrolyzed 15 hours at 100°C, taken to volume with water, and filtered. Hexosamines were determined by a modification of the Elson and Morgan method after separation from the hydrolysates on columns of Dowex-50(6).

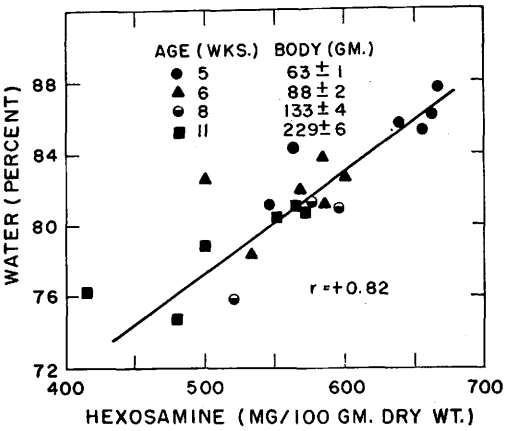

FIG. 1. Correlation between water and hexosamine concentrations in subcutaneous connective tissue of rats of varying ages.

* The 2 amino sugars normally encountered in connective tissue are galactosamine and glucosamine. The method of Elson and Morgan used here does not distinguish between them.

Results. Growth. Four groups of rats, 5, 6, 8, and 11 weeks of age, with 4-6 rats in each group, were used. Subcutaneous connective tissue samples were obtained and analyzed (Fig. 1). The water and hexosamine concentrations, high in the youngest group, decreased with increasing age. Water and hexosamine values obtained on the same samples in each animal were found to have a high degree of correlation ($r = +0.82$), which was significant at a probability level of <0.01.

The effect of body weight on the concentrations of water and hexosamine in orbital connective tissue was similarly studied by analyzing the data from 93 rats which had served as controls in several different experiments. Correlation studies were made between body weight (range was 60-235 g) and hexosamine concentration (range was 357-493 mg/100 g dry wt), body weight and water concentration (range was 76.6-79.8%), and hexosamine and water concentrations. There was a significant negative correlation between hexosamine concentration and body weight ($r = -.81$), and also between water concentration and body weight ($r = -.77$). Thus, with increasing body size there was a progressive decrease in the concentrations of hexosamine and water. The hexosamine and water changes were tested and found to have a significant positive correlation ($r = +.69$). In each instance the values for r were significant at a probability level of $<.01$.

Fasting. A reduced food intake decreases the amount of plasma hexosamine(5). It was therefore considered of interest to assess the effect of fasting on the concentrations of hexosamine in normal connective tissue. Sixteen rats were used, and sacrificed in groups of 4. One group was killed at the start of the experiment and the remaining groups following one, 2, and 3 days of total fasting. The rats had drinking water available. Fasting had no significant effect on the concentrations of hexosamine which were 581 ± 20, 599 ± 20, 577 ± 6, and 584 ± 11 mg/100 g dry wt on 0, 1, 2, and 3 days, respectively. There was a decrease in connective tissue water, most striking on the second day (77.0 ± .8% as compared with 81.6 ± .2% at the start of the experiment). The normal relationship between water and hexosamine was thus altered by the second day of fasting (compare with Fig. 1). During this fast the mean body weight fell from 109 ± 7 g at the start of the experiment to 80 ± 4 g on the third day. Since fasting did not affect hexosamine levels in connective tissue, food intake was not measured in subsequent experiments.

Local trauma. Following a preliminary observation that epinephrine injections increased the hexosamine concentration at an injection site, an experiment was designed to determine the rate of this change. In the first experiment, 45 rats weighing 110-130 g were used; 5 were used as an initial control group; a group of 20 rats was injected subcutaneously twice daily in the same site (right dorsum) with 0.1 ml of physiological saline; a group of 20 rats was injected similarly with 0.1 ml of epinephrine (1:3000). Five rats in each injected group were sacrificed at 6, 24, 48, and 72 hours and connective tissue samples obtained (Fig. 2). In both groups the concentrations of water and hexosamine increased, reaching highest observed values at 24 hours (epinephrine) and 48 hours (saline). The increases in the hexosamine levels in response to saline and epinephrine were not significantly different. At 24 hours, the increase in water concentration produced by epinephrine was greater than that produced by saline. In subsequent studies, a response time of 48

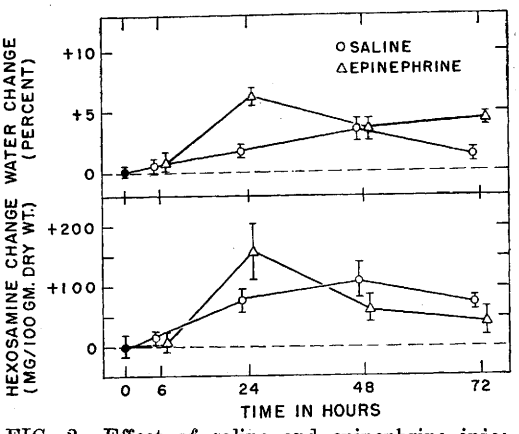

FIG. 2. Effect of saline and epinephrine injections on concentrations of hexosamine and water in subcutaneous connective tissue. Vertical lines refer to S. E. of mean.

TABLE I. Effect of Local Traumatizing Agents on Concentrations of Connective Tissue Water and Hexosamine.*

Traumatizing agent	No. rats	Body wt (g)	Water (%)	Hexosamine (mg/100 g dry wt)
Control	5	122 ± 5	81.8 ± 1.0	561 ± 12
Formalin (0.25 ml of 2%)	4	112 ± 4	89.6 ± .4	580 ± 16
Ammonia (0.25 ml of 1%)	5	117 ± 6	81.4 ± 1.3	601 ± 27
Ethyl alcohol (0.25 ml of 50%)	4	120 ± 6	87.3 ± .6	616 ± 26
Plier pinch	4	110 ± 2	84.1 ± .7	632 ± 28
Incision	4	109 ± 2	85.0 ± .6	640 ± 12
Venice turpentine (0.3 ml)	4	104 ± 1	84.4 ± .3	666 ± 23
Tannic acid (0.25 ml of 4%)	4	111 ± 2	85.0 ± .4	736 ± 26

* Mean ± S. E.

hours was used for testing other noxious agents.

In the next experiment several traumatizing agents were tested for their effect on connective tissue hexosamine levels. All rats received a single stressing stimulus to the subcutaneous connective tissue of the lower right dorsum. Various groups received subcutaneous injections of either formalin, ammonia, ethyl alcohol, turpentine, or tannic acid. In one group the skin was squeezed firmly with a pair of pliers. In another group the skin of each animal was incised (2-3 cm) and sewn with silk sutures. The latter 2 injuries were produced under ether anesthesia. Samples were obtained 48 hours following the tissue trauma (Table I). In almost all instances the concentrations of water and hexosamine increased, but in varying degrees and not necessarily paralleling one another. Following formalin, for instance, there was a marked edema (89.6% water) with only minimal hexosamine changes (580 mg/100 g dry wt), whereas with tannic acid the edema was less pronounced (85%) but the hexosamine strikingly increased (736 mg/100 g dry wt). The response was generally characteristic for each agent. For example, repeated experiments demonstrated that an incision or tannic acid injection consistently induced significant increases in hexosamine concentration, whereas formalin usually failed to stimulate such a response despite its edema-inducing properties.

Fasting and local trauma. Since fasting prevents the expected increase in the plasma hexosamine level following body injury(5), the effect of fasting on the response of connective tissue to local trauma was studied. Three groups of rats were used, 1) a control group eating *ad libitum,* 2) a group injected with tannic acid (.25 ml of 4% solution) eating *ad libitum,* and 3) a group injected with tannic acid after 24 hours of a 72-hour fast. All rats were sacrificed 48 hours following the time of the injection. The fasted group weighed 92 ± 2 g, whereas the injected group eating *ad libitum* weighed 140 ± 4 g. Although the fasted group had a reduction in water content (81.5 ± 1.1% as compared with 85.3 ± .8 in the injected group eating *ad libitum*), the hexosamine concentration increased the same amount in both injected groups (761 ± 22 mg/100 g dry wt in the injected group eating *ad libitum* and 776 ± 21 in the injected group which was fasted) as compared with the controls (561 ± 10 mg/100 g dry wt).

Systemic stress. Since many types of body injury will produce a rise in the plasma hexosamine level(4), a systemic stress was induced to determine whether or not the hexosamine in non-traumatized connective tissue participates in this response. As a stress the right femur and tibia were fractured under ether anesthesia. Connective tissue samples from the dorsum were obtained from a control group at the start of the experiment and from groups 2, 4, and 8 days following the day of fracture. No significant changes were noted in the concentrations of hexosamine and water.

Discussion. The concentrations of connective tissue hexosamine and water decrease with growth. Similar observations on age changes have been made in the skin of the rat(7), where the decrease in the concentration of hexosamine was shown to result primarily from a relative increase in collagen. It is

likely that mucopolysaccharides, which account for approximately 50% of the total connective tissue hexosamine(8) and behave as hydrophilic colloids, would largely account for the parallelism between the tissue water and hexosamine concentrations. The close correlation between hexosamine and water has been observed in experimental myxedema(9), and in human skin(4). This relationship is altered in tissue from fasted rats and in traumatized tissue.

It is well recognized that following many types of body injury the plasma hexosamine level increases(3,5,10). The site of production or source of this hexosamine has not been defined. The synthesis of hexosamine by isolated connective tissue cells has recently been demonstrated(11) and it therefore is possible that the increased amount of plasma hexosamine following trauma may be produced by connective tissue. Although it was not possible to detect changes in hexosamine concentration in non-traumatized connective tissue following trypsin injections(3) or fracture, as reported here, the possibility that a concurrent increase in plasma hexosamine(3,5) may result from its synthesis by connective tissue is not excluded.

Summary. 1. The concentrations of hexosamine and water in connective tissue decrease with growth in the rat. There is a high degree of correlation between the concentrations of water and hexosamine in normal connective tissue. 2. Most traumatic agents (physical and chemical) effect a local non-specific increase in hexosamine and water concentrations. The degree of change of each of these constituents varies with the agent administered. Fasting does not influence this increase in hexosamine in response to local trauma, or the hexosamine concentration in untraumatized connective tissue. 3. Changes in the concentration of connective tissue hexosamine were not detectable following systemic stress (fracture).

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Pantothenic Acid Deficiency Induced in Human Subjects.* (21204)

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Shortly after the classic demonstration by Woods(1) of the molecular antagonism of sulfanilamide by para-amino benzoic acid, one of us (WBB) became interested in seeking similar relationships between individual B-complex vitamins and their chemical analogues. A series of pyridine compounds was studied for vasodilating effects and therapeutic potency against pellagra(2). Observations at that time suggested that some of the compounds might have toxic properties. This suspicion had been expressed by Woolley(3) as a result of his work with canine 'blacktongue'. McIlwain(4) suggested that pyridine-3-sul-

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