

10^{-4} g/lit with a standard deviation of less than 10^{-5} g/lit.

Shallow pyrex dishes containing samples are placed in an enclosed space at a distance of approximately 4 in. from a battery of six Sylvania or G.E. 15-w germicidal lamps. The area of the light source is approximately 324 in.², and the area used for exposure of samples to this light is approximately 34 in.² The germicidal lamps were chosen because 90 percent of the radiant energy is at the 2537 Å line (4) where the quantum efficiency is about 0.60.

Five standards were prepared in 500-ml volumetric flasks as follows: 10 ml of 0.1M H₂C₂O₄ was placed in each flask, followed by the addition of 70, 50, 30, 10, and 0 ml of a standard uranyl nitrate solution (0.0117 mg U/ml), respectively. Each was diluted to the mark with demineralized water and thoroughly mixed.

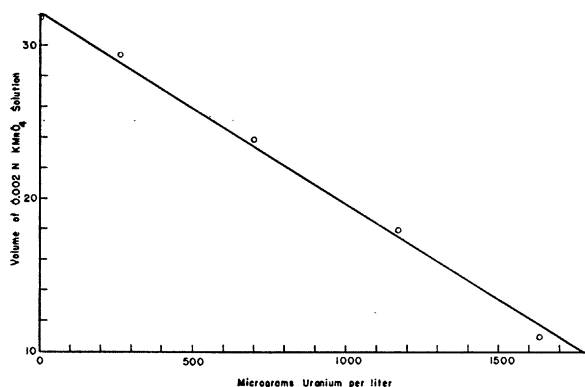


Fig. 1. Photodecomposition of oxalic acid as a function of uranium content of the solution (2-hr exposure; 2537 Å radiation).

Eight 15-ml aliquots of each sample were exposed to the ultraviolet light for a period of 2 hr. After rinsing the samples into titration flasks, adding 5 to 10 ml 18N H₂SO₄, and diluting to 100 ml, the samples were titrated with a 0.002N solution of KMnO₄. The graph of Fig. 1 was made to serve as a standard curve. A new series of samples was prepared and treated as the others were, and the amount of uranium present was determined from this standard curve. Table 1 shows these data.

Although the accuracy and precision are good and

Table 1. Uranium concentration as determined by the photodecomposition of oxalic acid.

Uranium taken (μg/lit)	Uranium found (μg/lit)	Std. dev. in μg of uranium/lit
117	85	43
351	333	43
936	952	36
1287	1303	64
1521	1526	26

the time required for a determination compares favorably with that of other methods, there are many variables yet to be studied, some of which are other light sources, time of exposure, ratios of uranium to oxalic acid, and interferences.

References and Notes

1. Leighton and Forbes, *J. Am. Chem. Soc.* **52**, 3146, 3148 (1930).
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Synthesis of Hexosamine by Connective Tissue (*in Vitro*)

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Although labeled hexosamine has been found in streptococcal cultures (1-3), ovomucoid (4), and rat serum (5) following administration of C¹⁴ glucose, the synthesis of hexosamine has not been previously demonstrated in isolated animal tissue.

The method devised by Parker (6) for the study of antibody production *in vitro* was adapted to this problem. Using aseptic methods, dorsal subcutaneous connective tissue was obtained from rabbits that were sacrificed with ether. No attempt was made to obtain fat-free connective tissue, since fat did not interfere with determinations and the control samples used were of similar composition. Control samples were used to estimate the hexosamine content of the test samples before incubation (7). Tissue samples were cut into 1- to 2-mm fragments and incubated in 25-ml Erlenmeyer flasks after the medium was adjusted with 5 percent CO₂ in air to pH 7.8. Parker's ratio of 100 mg of tissue per 3 ml of medium was observed. Three-

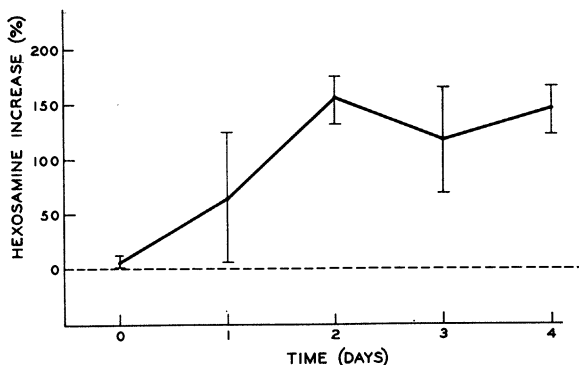


Fig. 1. Production of hexosamine by rabbit subcutaneous connective tissue cultured in Hanks' solution containing 25-percent ox serum ultrafiltrate. Vertical lines refer to standard error of the mean.

Table 1. Production of hexosamine by cultures of rabbit subcutaneous tissue.

Tissue	Medium	Hexosamine contents (mg)								
		3 days					4 days			
		Initial	Final			Change	Initial	Final		Change
		(tissue)	Tissue	Medium	Total	(%)	(tissue)	Tissue	Medium	Total
Living	Hanks' solution with 25 per- cent ox serum ultrafiltrate	.0374	.0215	.0440	.0655	+ 75	.0263	.0166	.0450	.0616
		.492	.362	.393	.755	+ 54	.254	.253	.465	.718
		.366	.189	.171	.360	- 2	.215	.284	.312	.596
		.286	.344	.445	.789	+ 175	.234	.250	.380	.630
		.251	.415	.497	.912	+ 263	.418	.217	.459	.676
Heat in- activated	Hanks' solution with 25 per- cent ox serum ultrafiltrate	.450	.348	.084	.432	- 4	.400	.218	.119	.337
		.398	.230	.087	.317	- 20				
Living	Hanks' solution (with glucose)	.545	.565	.431	.996	+ 83	.193	.199	.370	.569
		.300	.232	.259	.491	+ 64				
Living	Hanks' solution without glucose	.355	.237	.175	.412	+ 16	.452	.239	.087	.326
		.391	.273	.151	.424	+ 9				

hundred milligram tissue samples were divided equally into three flasks and were maintained without significant proliferation. All flasks contained 50 units of penicillin and 50 μ g of streptomycin (7a) per milliliter of medium. At the end of the period of incubation, the sterility of each culture was established by subcultures on blood agar and Sabouraud's mediums. The tissue fragments were separated from the medium by filtration. The tissue residue and an aliquot of the medium were hydrolyzed in 2N HCl for 15 hr, and the total amounts of hexosamine were determined following their isolation on Dowex-50 (7).

Whole serum or embryo extracts could not be used as nutrients, since they contain relatively large amounts of hexosamine. Hanks' balanced salt solution (8), with or without ox serum ultrafiltrate, was used as the suspending medium, since both were found to be free of hexosamine; hence, the only hexosamine in the culture was the amount initially present in the tissue and that synthesized during incubation. The combined hexosamine content of tissue and medium was significantly increased after 48 hr or more of incubation in either medium (Fig. 1).

When the glucose was omitted from Hanks' solution, no increase in total hexosamine occurred (Table 1). When the tissue was inactivated by heating at 100°C for about 15 min prior to incubation, no increase in the total hexosamine occurred. In control cultures in which no synthesis occurred, hexosamine diffused out of the tissue into the medium, as was evidenced by the fact that the total hexosamine content of tissue and medium corresponded with that estimated to be present in the original tissue sample.

Histological studies made on tissues cultivated under the specified experimental conditions but not used for hexosamine determinations revealed that some necrosis occurred in the tissue fragments early and increased progressively during incubation. The progressive tis-

sue damage may explain why most of the hexosamine production occurred in the first 48 hr.

Synthesis of hexosamine by rabbit synovial tissue was also demonstrated by incubating infrapatellar fat pads that are lined by synovial cells. No increase in hexosamine was found when muscle tissue was used.

References and Notes

1. Y. J. Topper and M. M. Lipton, *J. Biol. Chem.* **203**, 135 (1953).
2. S. Roseman *et al.*, *J. Biol. Chem.* **203**, 213 (1953).
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- 7a. Note added in proof: The methylglucosamine in the streptomycin does not produce an appreciable color in the hexosamine determination, probably because of incomplete hydrolysis of the methyl group.
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Toxicity to Pineapple Plants of Biuret Found in Urea Fertilizers from Different Sources*

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During investigations involving the spraying of urea on pineapple plants, it has been observed that in some cases injury to the plants occurred. This injury consists of leaf-tip dieback, which is separated from the normal tissue by a zone of yellow tissue. Dieback varying from 1 to 12 cm has been observed. In extreme cases there has been yellowing of the edges of the lower leaves. In the past, the assumption has been made that under