thinning of the hair especially in the posterior portion of the dorsum.

Discussion. It is of interest that both cadmium and fluorine caused a loss of incisor enamel pigmentation and a drop in blood hemoglobin indicating in each instance an effect on normal iron metabolism. Although the exact mechanism of the interference is in need of further clarification, the ability of cadmium to precipitate iron-containing proteins has been noted[5] and it has been observed that the iron content of fluorosed enamel is markedly diminished.[6]

The failure of dietary cadmium to limit experimental caries in the same manner as fluorine suggests that the caries limiting property of the latter is not related to the inhibition of lactic acid fermentation by oral organisms. Likewise, it does not appear to be dependent on an inhibitory action on bone phosphatase since it has been shown that both cadmium[1] and fluorine[7] limit the activity of this enzyme.

Summary. The ability of cadmium to diminish the degree of pigmentation of rat incisor enamel and to effect a reduction in blood hemoglobin has been confirmed. The ability of fluorine to bring about a comparable reduction in blood hemoglobin has also been observed. It is suggested that the pigmentary changes in the enamel and the fall in hemoglobin that results in the ingestion of both cadmium and fluorine is related to the ability of these elements to interact with iron-containing proteins. Unlike fluorine, cadmium in the concentration studied is ineffective in reducing experimental rat caries.

5 Granick, S., and Michaelis, L., *Science*, 1942, **95**, 439.

6 Bowes, J. ., and Murray, M. M., *Brit. Dent. J.*, 1936, **60**, 556.

7 DeEds, F., *J. A. D. A.*, 1936, **23**, 568.

14750

Glycogen in Alloxan-Treated Rats.

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Fisher and Lackey[1] found the glycogen content of the heart of the dog to be increased following pancreatectomy. Evans *et al.* found a similar increase in cardiac glycogen in 3 depancreatized cats.[2] Since, in these experiments, both exocrine and endocrine secretions of the pancreas were eliminated, it seemed desirable to study the glycogen changes in the heart when the endocrine secretion alone is disturbed. The demonstration by Dunn and McLetchie[3] and Gomori and Goldner[4] that diabetes mellitus can be produced in rats by a single injection of alloxan, and that this chemical destroys specifically the islet tissue of the pancreas, makes it possible to carry out such a study. Also, alloxan makes it possible to study diabetes mellitus in the rat, an animal in which surgical removal of the pancreas is very difficult.

Method and Results. The glycogen content of liver, heart, and skeletal muscle was determined in 20 control animals (Table I) and 19 animals with alloxan diabetes (Table II). The animals used were full grown rats of approximately the same weight. Three rats in each series were females and the remainder were males. Each animal was kept in a separate metabolism cage with ample food and water.

1 Fisher, N. F., and Lackey, R. W., *Am. J. Physiol.*, 1925, **72**, 43.

2 Evans, Gerald, and Bowie, Morris A., PROC. SOC. EXP. BIOL. AND MED., 1936-37, **35**, 68.

3 Dunn, J. Shaw and McLetchie, G. B., *Lancet*, 1943, **245**, 384.

4 Gomori, G., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 287.

Alloxan was injected as a freshly prepared 5% solution. 200 mg of alloxan monohydrate per kilogram of body weight was given as a single dose intraperitoneally. Not all animals injected with alloxan developed diabetes as determined by 2 daily urine tests for ketones and sugar. Those animals which showed neither glycosuria nor ketonuria were not used for glycogen analyses. Some alloxan-injected rats died or were in a moribund state before tissues were obtained for analysis. These, too, were discarded. Therefore, the 19 animals in Table II represent all alloxan-injected animals which survived three or more days and developed moderate to severe diabetes, without becoming moribund.

At autopsy rats were injected with sodium pentobarbital, which has been shown to have no effect on the glycogen content of tissues.[5] While anesthetized, a lobe of the liver, the entire heart, and a portion of thigh muscle were quickly removed and cut into small pieces with scissors and immersed in hot potassium hydroxide. The entire procedure for all 3 tissues was completed in less than one minute. Blood was also obtained for ketone and glucose determinations in animals C6 to C21 and A19 to A33. Glycogen analyses were done according to the method of Good, Kramer, and Somogyi.[6] The determination of glucose obtained from hydrolysis of the glycogen was made by the method of Shaffer and Somogyi.[7] The blood ketones were determined by the method described by Weichelbaum and Somogyi,[8] and the blood sugars by the method of Hagedorn and Jensen.[9]

After removal of tissues for glycogen analysis, the pancreas, one kidney, and portions of the liver and heart were removed and prepared for histological study. Tissues were fixed immediately in 10% formalin. After fixation, the tissues were imbedded in paraffin and sectioned at 7 microns. All sections were stained with Harris' hematoxylin and eosin.

In Tables I and II are summarized the

TABLE I.
Glycogen Content of Liver, Heart, and Skeletal Muscle in Untreated Adult Rats.

Rat	% Liver glycogen	% Heart glycogen	% Muscle glycogen
C 5	4.2	.34	.52
1	3.48	.29	.57
C 2	3.42	.43	.57
5	1.88	.40	.396
6	2.88	.31	.568
7	4.41	.68	1.02
8	3.7	.43	.39
3	1.63	.24	.47
9	4.15	.47	.54
10	3.71	.44	.54
11	3.83	.56	.59
12	3.18	.42	.51
13	3.10	.41	.45
14	1.24	.53	.476
15	1.58	.60	.42
16	4.24	.518	.58
17	6.39	.49	.51
18	1.164	.462	.405
19	1.13	.408	.354
21	1.819	.410	.434
Mean	3.056 ± .317*	.442 ± .024*	.515 ± .032*

* Standard error.

TABLE II.
Glycogen Content of Liver, Heart, and Skeletal Muscle in Adult Rats Having Alloxan-Produced Diabetes Mellitus.

Rat	% Liver glycogen	% Heart glycogen	% Muscle glycogen
A 1	.091	.582	.138
3	1.01	.827	.309
8	.79	1.24	.36
9	.68	.88	.43
10	.25	.73	.37
12	.05	.63	.06
13	.14	.82	.092
17	.248	1.0	.028
19	1.39	.95	.56
20	1.37	.32	.49
21	1.28	1.19	.31
22	1.23	1.21	.37
23	.72	.77	.32
25	.95	1.22	.59
27	.98	.87	.28
29	.65	.735	.066
30	1.76	.604	.543
32	.0795	.814	.391
33	.833	.834	.473
Mean	.833 ± .122*	.854 ± .056*	.325 ± .040*

* Standard error.

5 Shelley, W. B., Code, C. F., and Visscher, M. B., *Am. J. Physiol.*, 1943, **138**, 652.

6 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

7 Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

8 Weichelbaum, T. E., and Somogyi, M., *J. Biol. Chem.*, 1941, **140**, 5.

9 Hawk and Bergeim, *Practical Physiological Chemistry*, P. Blakiston's Son and Co., Inc., Philadelphia, Pa., 1937, p. 439.

analytical data on tissues of normal rats and alloxan-treated rats respectively. A comparison of these tables shows that alloxan-injected rats have an elevated heart glycogen and a decreased glycogen content of both liver and skeletal muscle. The mean value for heart glycogen in control animals was .442%, and for alloxan-treated animals was .854%. The mean value for liver glycogen of control animals was 3.056%, and for alloxan-treated animals was .806%. The mean value for muscle glycogen of control animals was .515%, and for alloxan-treated animals was .325%. These data were analyzed for significance by the T method of Fischer, and for each tissue a P value of less than .01 was obtained.

The alloxan-injected animals on which blood sugar and ketone determinations were made showed a marked elevation of both. The ketones averaged 29.5 mg % as compared to 3.2 mg % for the controls. Blood sugars averaged 443 mg % in the alloxan group and 136 mg % in the controls.

Discussion. Our experience with alloxan confirmed that of others[3,4,10] in that the animals showed considerable individual variation in susceptibility. Approximately 60% of the animals developed moderate to severe diabetes mellitus. The remaining 40% either died in the first 72 hours or failed to develop any apparent diabetes. In the limited number of females used, no sex difference was observed either in susceptibility to alloxan or in glycogen distribution. Contrary to Gomori and Goldner,[4] we observed no difference in reaction to alloxan between albino and hooded strains of rats. Approximately one-half of the rats used by us were albino and one-half hooded. A most striking polyuria was observed in some of the diabetic animals. In some instances rats weighing 175 to 200 g excreted as much as 60 ml of urine in 24 hours.

The fact that the glycogen content of the heart increases in diabetes, while that of skeletal muscle decreases, suggests some fundamental difference in the metabolism of these two types of muscle tissue. Other conditions such as reduced barometric pressure,[11] and fasting,[12] are reported to alter the glycogen content of these 2 tissues differentially, but no satisfactory explanation has been offered. The most striking metabolic changes between normal and diabetic animals are the great increases in blood sugar and blood ketone levels. Whether or not these changes bear any real relation to the increased heart glycogen in diabetes mellitus, we cannot say on the basis of the present work.

A study of microscopic sections from the alloxan-injected animals sacrificed between the third and seventh days after injection revealed fairly uniform histological changes. In the pancreas under low magnification islets appear fewer in number, more cellular, and smaller than in those of control animals. Higher magnification revealed marked reduction in the amount of cytoplasm of the cells which make up the bulk of each islet. The majority of the cells present are transformed from the usual large beta cells with abundant pale-staining cytoplasm into cells with shrunken, granular, somewhat eosinophilic cytoplasm or with extensive vacuolation of the cytoplasm. Loss or reduction of the amount of cytoplasm is the most outstanding change and causes what we believe is a real collapse of islets so that they appear smaller and more cellular than normal. In some cases this collapse of islets may simulate cellular proliferation. The nuclei tend to be smaller and more pycnotic than those of the original cells, but it is often difficult or impossible to be certain of a real nuclear change even in the cells with markedly depleted or vacuolated cytoplasm.

At this stage (*i.e.*, between third and seventh days) very few of the islet cells in our animals are actually necrotic, although necrosis has been described as occurring within 7 hours of poisoning.[13] Our study in this series does not include the very early stages, and we are not prepared to comment at this time on the origin or the nature of the cellular proliferation which must occur to provide the cells which have replaced the normal parenchyma of islets in our animals studied from 3 to 7

[10] Bailey, C. C., Bailey, O. T., and Leech, R. S., *New England J. Med.*, 1944, **230**, 533.

[11] Kreienberg, W., and Wisehutter, E., *Pfluger's Arch. f. d. Ges. Physiol.*, 1943, **247**, 11.

[12] Evans, G., *J. Physiol.*, 1934, **82**, 468.

[13] Ridout, J. H., Ham, A. W., and Wrenshall, G. A., *Science*, 1944, **100**, 57.

days after alloxan injection. From examination of the tissues in this series it might be suspected that the majority of the cells present are degenerate forms of the original beta cells. There is little difference in the appearance of islets between the third and seventh days. In several alloxan-treated animals (30A, 6A, 26A), the pancreatic islets retain a normal appearance. In each instance where this occurred the animals failed to develop diabetes.

Almost all the sections of kidney in this series of animals with alloxan diabetes showed varying grades of parenchymatous degeneration most notable in epithelium of the convoluted tubules. In several cases there was marked swelling and hydropic degeneration of individual and small groups of epithelial cells. Two animals (29A, 10A) showed marked degeneration of epithelium of both limbs of loops of Henle. In these cases the changes appeared most marked in the ascending limbs where much of the epithelium was necrotic and the lumina were often filled with blue-stained granular debris.

Liver sections revealed frequent widespread parenchymatous degeneration and early stages of hydropic degeneration. No consistent changes were observed in the myocardium.

Summary. Glycogen determinations were made on the heart, liver, and skeletal muscle of rats with alloxan-produced diabetes mellitus and on the same tissues of untreated controls. The alloxan-treated animals showed a statistically significant increase in glycogen content of heart muscle and a statistically significant decrease in liver and skeletal muscle glycogen.

Rats which developed diabetes mellitus had a high blood sugar and high blood ketones at the time of autopsy.

On histological examination of the tissues, the islet cells of alloxan-treated rats which developed diabetes showed a marked reduction in cytoplasm, pycnosis and decrease in size of the nuclei with resultant collapse of islet structure. Almost all kidney and liver sections showed some degenerative changes.

14751

Effect of Trypan Blue upon Cardiac Explants in Tissue Culture.

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During some previous work with trypan blue in tissue cultures it was observed that the growth of explants in the dye-containing medium appeared to be more luxurious than in the control cultures. When buffy coat[1] of chicken blood was cultivated in a trypan blue medium, monocytes markedly increased in numbers as they became transformed into macrophages. Attempts were made to estimate this increase in numbers of phagocytic cells by making surface counts of macrophages in so many contiguous microscopic fields and comparing them with similar counts made upon control cultures stained by neutral red at the time of counting. There was a noticeable difference, but since cultures of buffy coat did not make a compact or membranous growth on the under surface of the cover-slip, comparative areal measurements could not be made and the matter was dropped.

The following experiment was designed to test the presence or absence of a stimulating effect upon growth of tissue cultures in a medium containing trypan blue.

At any one planting, control and experimental cultures were made on cover-slips from fragments of the same heart removed from a 10-day chick embryo. Lyophiled embryo juice (20%) and plasma diluted to volume with

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1 Hetherington, Duncan C., *Arch. f. exp. Zellforsch.*, 1931, **11**, 520.