Effect of Alloxan Administration on Liver Nucleoproteins.* (18922)

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Evidence has been accumulating that the desoxyribose nucleic acid (DNA) content of cells is approximately constant in all nuclei of any one species(1,2). It has been reported by Cunningham, Griffin, and Luck(3) that, although an increase in content based on whole tissue occurs in many tumors and in certain tissues following carcinogen administration, nuclei isolated by the Dounce method show no such increase. In the present study liver tissue of alloxan diabetic rats showed not only a significant increase per unit of wet or dry tissue, but also an absolute increase per cell nucleus.

Methods. Rockland Farms male white rats, (180 to 250 g) maintained on Purina Dog Chow and water *ad libitum* were used in these experiments. The animals were fasted 48 hours prior to subcutaneous administration of 175 mg/kg of alloxan-monohydrate (Eastman Kodak Co.) and were sacrificed at intervals up to 6 days following treatment. Liver samples (350-500 mg) taken for nucleoprotein determinations were rapidly weighed and frozen. The nucleic acids were extracted and determined as described by Schmidt and Tannhauser(4). The data obtained using normal rat liver correlated closely with those reported by various authors employing this method(5). Glycogen determinations were made according to the method of Good, Kramer and Somogyi(6). A modification of Payne's method(7) was employed for fat analysis. Tissues for cytochemical study were placed in Carnoy's acetic alcohol (1:3) and cytochemical analyses were performed, utilizing microspectrophotometric technics(1,8-13).

Sections to be stained by the Feulgen nucleal method were cut at 10 μ. All preparations were hydrolyzed simultaneously in 1 N HCl at 60°C for 12 minutes(10,11), then stained for 1 hour in the Schiff reagent(14). Unhydrolyzed control sections, one for each animal, were stained simultaneously in the same reagent. For studying the effect of treatment on the histone and non-histone protein fractions of the cell, 10 μ sections were stained by means of the Millon reaction(8). Ultraviolet absorption measurements of nuclei and cores of cytoplasm (4 μ in diameter) were made on sections 3 μ thick. All absorption measurements were made with a Photovolt electronic multiplier photometer (Mod. 512). For measurement of the amount of Feulgen dye bound by the nuclei, the desired wavelength of light was isolated from a G. E. AH4 mercury lamp and a Farrand interference filter with a transmission peak of 560 mμ; for measuring the intensity of the Millon reaction in nuclei and cores of cytoplasm a Corning

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TABLE I. Nucleoprotein Changes Occurring in Alloxan Treated Diabetic Rats.

Exp	Treatment	No. of rats	No. of observations	Days after treatment	Per 100 g wet wt DNA ± SE*	Per 100 g wet wt RNA ± SE	Liver nucleic acids, mg P Per 100 g dry wt DNA ± SE	Per 100 g dry wt RNA ± SE	Per 100 g protein dry wt DNA ± SE	Per 100 g protein dry wt RNA ± SE
1	Controls (saline)	28	56		20.15 ± .51	102.9 ± 1.18	68.17 ± 4.13	349.4 ± 2.40	100.3 ± 3.9	513.63 ± 6.74
Nov. 1950	Alloxan†	14	28	4	27.4 ± .69	108.9 ± 2.00	95.27 ± 2.41	344.2 ± 5.15	133.23 ± 4.33	482.6 ± 12.17
2	Controls (saline)	4	8		17.2 ± .55	106.6 ± 4.30				
Apr. 1951	Alloxan	3	6	1	21.2 ± .84	123.4 ± 2.30				
	"	9	18	3	21.1 ± .90	112.8 ± 2.36				
	"	7	14	5-6	22.8 ± 1.15	108.6 ± 2.88				

* $SE = \frac{SD}{\sqrt{n}}$.

† Alloxan diabetic rats had blood sugar values ranging from 150 to 885 with a mean of approximately 400 mg %.

glass filter having a maximum transmission at 365 mμ was used. For the ultraviolet absorption measurements, the 2537 Å line was isolated from a Hanovia mercury lamp, by means of a Cooke double prism quartz monochromator. Throughout this series of measurements, care was taken to choose only round nuclei having approximately the same diameter (about 7 μ).

Results. DNA per unit of whole liver was increased in the alloxan treated animals (Table I). When calculated on the basis of dry weight or total protein this difference was still apparent. The liver lipid and glycogen content of the diabetic rats did not differ significantly from that of the control animals. Liver lipid in the untreated rats was 5.59 ± 0.15%, in the alloxan diabetic rats 5.13 ± 0.145%; corresponding values for liver glycogen were 6.36 ± 0.23% and 5.9 ± 0.25%, respectively. Each figure represents a mean with its standard error. Their P values are > .05 and > .1, respectively. The increase in DNA was confirmed by cytochemical analysis (Table II).

Tissue taken 4 days after alloxan administration showed no deviation in total RNA from that of the controls. Ultraviolet absorption measurements on the cytological preparations indicated no change in cytoplasmic RNA. Though no estimations of nuclear RNA have been made, indirect evidence that it, as well as DNA, is increased after alloxan is given in Table II. Since the percentage increase in total nucleic acid is greater than that of DNA, the difference is presumably due to RNA.

An increase in total protein content of the nucleus was indicated by the increase in intensity of the trichloracetic acid Millon reaction. The measured extinction value for a control animal (No. 211) was 0.422 ± 0.006, the corresponding value for a treated animal (No. 204) 0.482 ± 0.006. In the cytoplasm, however, no such increase was observed. Since the ratio of trichloractic acid Millon extinction to Feulgen extinction remained constant for both control and experimental tissues, the protein increased in proportion to the DNA. That this protein in-

TABLE II. DNA and Total Nucleoprotein Changes Occurring in Alloxan Treated Diabetic Rats.

Treatment	Animal No.	DNA‡	Feulgen nucleal† reaction for DNA, $\overline{E}_{560} \pm SE$	Total nucleic acid† ultraviolet absorption (nucleus), $\overline{E}_{2537} \pm SE$
Alloxan*	200	27.2	.336 ± .004	.339 ± .006
	204	30.5	.342 ± .005	.323 ± .008
	198	28.6	.335 ± .005	.329 ± .006
Saline	211	18.9	.288 ± .003	.230 ± .006
	212	19.6	.290 ± .003	.243 ± .005
	213	22.7	.295 ± .004	.248 ± .004

* Animals were sacrificed 4 days after alloxan administration.
† Each figure represents an average extinction value of 15 to 25 nuclei.
‡ mg P/100 g of liver (wet wt).

crease was due primarily to an increase in the histone fraction was indicated by the observation that removal of histone from both treated and control sections eliminated the differences in the intensity of color produced by the standard Millon reaction.

The increase in DNA could have resulted from either an increase in the number of nuclei present per unit of tissue or from a change in nuclear volume. The cytochemical studies exclude the first possibility; the second was excluded by measurements which showed that no change in the nuclear volume resulted from the alloxan administration. It should be emphasized that the determination of DNA is expressed as mg/100 g of wet liver weight, whereas the Feulgen and ultraviolet extinction data are expressed as extinction values/nucleus. The data therefore, are not directly comparable, but results obtained with both methods show an increase in DNA.

Discussion. High DNA values were found uniformly on the 4th, 5th and 6th days following alloxan administration. The increases observed were small during the first 3 days. This is consistent with the reports of Brues *et al.*(15) that the DNA turnover, as measured by isotope technics, is relatively slow. Rerabek(16) reported that alloxan administration to rats produced an increase in liver RNA but no change in DNA. It is not possible to compare Rerabek's results with ours since he gives no evidence to indicate that his animals were diabetic, nor does he state what interval elapsed between alloxan administration and removal of the tissue for analysis.

The relationship of the DNA increase to the diabetic state is being studied. The data presented do not indicate whether the effect noted is specifically due to the action of alloxan on hepatic tissue or arises from the destruction of beta cells in the islands of Langerhans. Possibly this will be clarified by experiments in progress on rats made diabetic by partial pancreatectomy. Since the DNA increase cannot be correlated directly with blood sugar levels, it seems reasonable to assume that it is a direct effect of alloxan.

Summary. Administration of alloxan to white rats causes an increase in the DNA and the histone of liver nuclei. Direct measurements indicate no change in cytoplasmic RNA, but, indirect evidence suggests an increase in nuclear RNA.

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