Responses of Liver Lipid Fractions, Liver and Plasma Electrolytes Shortly After Alloxan Administration in Rat. (21190)

ABRAHAM DURY.

From the Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pa.

The response of the blood sugar to alloxan administration is well documented(1,2). Recently, Nichols and Sheehan(2) clearly demonstrated that the primary and permanent hyperglycemic phases after alloxan are eliminated in dogs with "chemical ablation" of the zona reticularis and fasciculata of the adrenal glands. However, little is known of the nature of the alterations of other chemical constituents of tissues or the factors responsible shortly following alloxan injection which might be relevant to its action on the organism as a whole and the subsequent development of "alloxan diabetes." This paper reports the changes in liver lipids fractions, and liver and plasma electrolytes in groups of rats sacrificed at 4, 24, and 72 hours after intravenous administration of alloxan. Determinations also were made of adrenal ascorbic acid and total cholesterol concentration as indices of adrenocortical activation in these animals.

Materials and methods. Male Wistar rats weighing 250-300 g were used. A 5% solution of Alloxan (Eastman No. 1722) was injected directly into an exposed femoral vein at a dose level of 40 mg/kg body weight in all experimental animals. The control rats received an injection of an equal amount of physiological saline (0.1 ml/100 g body weight) and killed 4 hours later. The rats killed 72 hours after alloxan were fasted approximately 20 hours before the administration of the drug but food and water was available *ad libitum* from then to the time they were sacrificed. The animals of the other groups were not fasted before alloxan; solid food and water were available *ad libitum* until they were sacrificed. The rats were anesthetized with EVIPAL (n-methyl-cyclo-hexenyl-methyl barbituric acid) for all surgical procedures, injections, and the removal of tissues for chemical analyses. The following tissue constituents were determined: Sodium and potassium of liver and plasma with aid of an internal lithium standard flame photometer; liver glycogen(3,4) as glucose equivalents; plasma glucose(5); adrenal ascorbic acid(6), and adrenal total cholesterol concentration(7). The details of the methods employed at this laboratory for the determination of the liver lipids fractions have been previously reported(8). Total lipids were calculated from weight of residue after distillation of petroleum ether and drying to constant weight; lipid phosphorus by the method of Youngsburg and Youngsburg(9), and Fiske and SubbaRow(10); phospho-lipids derived from lipid-P values x 26; cholesterol fractions by method of Schoenheimer and Sperry (7); and neutral fat content was derived by calculation of difference. Heart blood was obtained by direct puncture in a syringe moistened with one drop of heparin. The blood was centrifuged immediately and the plasma separated off for the chemical determinations. Specimens of liver for glycogen and lipids determination were obtained immediately after the blood sample was secured.

Results. The chemical constitution of liver and plasma secured from rats at 4, 24, and 72 hours after alloxan administration is presented in Table I. Inspection shows that at 4 hours after alloxan there was a significant increase in the liver total lipids content mainly due to an increase in the liver neutral fat fraction, whereas potassium and glycogen content of the liver were significantly decreased. The hyperglycemic condition of these rats was marked and associated with a significantly elevated plasma potassium concentration. Analyses of tissues obtained from the group 24 hours after alloxan revealed that the liver total lipids content was significantly greater than the respective value of the controls. This change in lipids content was due mainly to the marked increase in the liver neutral fat content and to a significant increase in the phospho-lipids fraction. The changes in the liver

TABLE I.
Changes in Chemical Constituents of Tissues following Intravenous Alloxan Administration.

Tissue constituents	Controls (10)	Exp. groups—Time after alloxan 4 hr (6)	24 hr (4)	72 hr (6)
	Part A. Liver lipids fractions (g %)			
Total lipids	4.96 ± .11	*5.32 ± .10**	*7.10 ± .57‡*	*6.27 ± .16 ‡*
Phospho-lipids	4.11 ± .08	4.07 ± .09	*4.47 ± .07†*	*4.66 ± .11 †*
Total cholesterol	.20 ± .004	.21 ± .007	.20 ± .006	*.23 ± .009†*
Free "	.17 ± .003	.18 ± .001	.17 ± .01	*.21 ± .01 ‡*
Ester "	.02 ± .003	.03 ± .003	.03 ± .004	.03 ± .005
Neutral fat	.63 ± .09	*1.03 ± .09**	*2.30 ± .46‡*	*1.34 ± .26 ‡*
	Part B. Liver and plasma electrolytes,§ water, glucose			
Liver:				
% water	69.2 ± .13	69.8 ± .2	69.6 ± .6	69.1 ± .12
K	93.5 ±1.1	*88.1 ±1.5**	*81.1 ±3.9†*	*82.7 ±2.0 ‡*
Na	23.4 ±1.0	25.6 ± .5	*30.8 ±3.0**	*30.8 ±1.5 ‡*
Glycogen (g %)	3.15 ± .22	*1.97 ± .29†*	*1.54 ± .15‡*	*1.62 ± .45 ‡*
Plasma:				
% water	92.9 ± .09	92.1 ± .17	91.7 ± .2	92.2 ± .2
K	4.60 ± .17	*5.60 ± .27†*	*7.01 ± .82‡*	*5.30 ± .06 ‡*
Na	165.4 ±3.9	167.7 ±2.4	*153.2 ±6.3†*	*155.6 ±4.3 †*
Glucose (mg %)	119 ±4	*500‡‖*	*500‡*	*500‡*

All values are mean ± S.E. Italicized values are significantly different from respective control values. "P" values of significant differences are indicated as follows: * ~.05 to .01; † ~.01 to .001; ‡ ~.001 and less.

§ Liver and plasma electrolytes are expressed as mEq/kg wet tissue and mEq/kg plasma water, respectively.

‖ Glucose values of experimental groups were at least that shown.

No. of rats in each group are figures in parentheses.

and plasma of the other constituents were similar to that found at 4 hours after alloxan-decreased potassium and glycogen content of liver, hyperglycemia, elevated plasma potassium level, and in addition a significant increased sodium content of liver and decrease in sodium concentration of the plasma.

It is generally agreed that by 72 hours following administration of the drug that an "alloxan diabetes" is established. Reference to Table I shows that the alterations in the chemical constitution of the liver and plasma at this time following alloxan injection were of the same general nature and the changes were comparable to those found in the groups at 4 and 24 hours after administration of the drug. The only additional changes found in the former group and different from the controls and the other 2 alloxan-injected groups were the significant increases in the free and total cholesterol content of the liver. Polyuria and glycosuria were marked in this group, but also were considerably evident in the 24-hour group.

The adrenal ascorbic acid and total cholesterol concentrations determined in the control and alloxan-injected groups are presented in Table II. Evidence of depletion of these adrenal chemical constituents is believed to be a reliable index of acute adrenocortical activation. These data therefore indicate a condition of increased adrenocortical activity had been induced in the group killed 4 hours after alloxan injection. The mean ascorbic acid concentration in the group 24 hours after alloxan was not different from the control value but the adrenal cholesterol concentration still was significantly lower in the former group. This type of time-response relationship between adrenal ascorbic acid and cholesterol changes are believed typical of a sudden temporary increased demand for cortical hormones when animals are exposed to a temporary period of stress(11).

Long(12) had expressed the view that increased secretion of the adrenal medulla was a common element in the various forms of stress. Consideration of this proposition and the evidence presented in Table II prompted a study of the chemical constitution of liver

TABLE II. Adrenal Ascorbic Acid and Cholesterol Concentrations in Groups of Rats at Indicated Time-Intervals following Alloxan Administration.

Groups	Right adrenal: Ascorbic acid (mg/100 g adrenal)	"P"*	Left adrenal: Total cholesterol (mg/g adrenal)	"P"
Controls (10)	419 ± 10		35.1 ± 3.2	
Alloxan inj.				
After 4 hr (6)	259 ± 8	<.0001	21.4 ± 3.4	.02
24 (4)	408 ± 22	ND	14.0 ± 2.5	.003
72 (6)	397 ± 27	ND	30.6 ± 2.3	ND

All values are mean ± S.E. Figures in parentheses are No. of rats in each group.
* Difference between means occurring by chance compared with controls.

and plasma in adrenal demedullated rats 72 hours after alloxan administration. Adrenal enucleation was accomplished in 20 rats. They were given 1% NaCl solution to drink for the first 10 days and then given tap water for the next 15 days. After an overnight fast 10 rats were injected with alloxan and the remainder with physiological saline. Tissues were secured for chemical analyses 72 hours later. The data are presented in Table III. All the liver lipid fractions, except phospholipids, and the liver and plasma electrolytes were found to be significantly different in the alloxan-injected group from the respective values of the controls. It is notable that the absence of the adrenal medulla and its secretory products did not measurably affect the response of the tissue constituents to alloxan administration. The changes in tissue chemistries were essentially the same in the demedullated and intact groups 72 hours after alloxan.

Discussion. The above results show that at the 3 periods of time chosen to determine the responses of certain tissue constituents to alloxan administration there were certain alterations in the chemistries common to all groups—particularly the increased neutral fat content and decreased potassium content of the liver, and the increased plasma potassium level. The alterations in the liver lipid fractions and the electrolytes of liver and plasma 72 hours after alloxan are those manifestations of the changed metabolism expected with insulin insufficiency. On the other hand, the altered chemistries found in the 4- and 24-hour groups cannot be presumed to reflect solely the change in metabolism in consequence of alloxan's action on the islet beta cells although it is known that alloxan has a toxic action on these cells within 5 minutes of its administration. In regard to these alterations in tissue constitution found in the latter 2 groups the following explanations can be proposed: 1) these changes in tissue chemistries may be manifestations of alloxan poisoning, particularly a hepatotoxic action of alloxan. It is of interest, therefore, that Beach *et al.*(13) expressed the belief that alloxan may damage mechanisms or factors other than insulin as explanation of their results. Recently, Goldner and Jauregui(14) reported evidence from perfusion experiments demonstrating an alloxan effect on the liver. They showed that alloxan inhibits the spontaneous glycogenolysis as well as the adrenalin induced glycogenolysis. The effect upon the liver appeared to be an immediate one. 2) The alterations in liver and plasma chemical constitution reported here might be adrenocortical conditioned "stress" type of responses to alloxan injection at least during the first 24 hours. Indeed, the data presented in Table II show that adrenocortical activation had been induced following alloxan injection. It is evident that further studies are necessary to elucidate the nature of these chemical responses and the factor(s) which may be responsible.

Summary. The responses of the lipid fractions of the liver, and liver and plasma electrolytes to alloxan administration were determined at 4, 24, and 72 hours. The adrenal ascorbic and cholesterol concentrations were also measured as indices of adrenocortical activation in these animals. Besides the known

TABLE III. Response of Tissue Constituents in Adrenal Demedullated Rats 72 Hours after Intravenous Alloxan.

Tissue constituents	Controls (10)	Alloxan inj. (10)	"P" value*
Part A. Liver lipids fractions (g %)			
Total lipids	5.22 ± .11	6.42 ± .22	.0002
Phospho-lipids	4.20 ± .10	4.42 ± .08	ND
Total cholest.	.20 ± .004	.24 ± .01	.002
Free "	.19 ± .003	.21 ± .008	.055
Ester "	.01 ± .004	.03 ± .002	.015
Neutral fat	.75 ± .05	1.74 ± .14	<.0001
Part B. Liver and plasma electrolytes,† water, glucose			
Liver:			
% water	69.3 ± .3	69.6 ± .4	ND
K	86.2 ±1.0	77.7 ±1.4	.0002
Na	25.0 ± .8	30.7 ± .9	.0005
Glycogen (g %)	3.60 ± .22	1.56 ± .28	<.0001
Plasma:			
% water	92.8 ± .09	91.9 ± .23	.02
K	5.26 ± .05	5.87 ± .08	<.0001
Na	169.9 ±2.0	155.6 ±2.2	.0004
Glucose (mg %)	126 ±6	500‡	<.0001

All values are mean ± S.E. No. of animals in each group is figure in parentheses.

* Difference between means occurring by chance compared with controls.

† Liver and plasma electrolytes are expressed as mEq/kg wet tissue and mEq/kg plasma water, respectively.

‡ All glucose values were at least 500 mg %.

responses of hyperglycemia and decreased liver glycogen content, there were similar changes in other chemical constituents in all groups—an increased neutral fat and decreased liver potassium content, and an increased plasma potassium level. The changes in the tissue chemistries of the 4- and 24-hour groups after alloxan were discussed as signs of an hepatotoxic action of alloxan, or responses of a stress type which were adrenocortically conditioned. It was also shown that the nature of the altered chemistries 72 hours after alloxan were essentially the same in intact and adrenal-demedullated rats.

The technical assistance of Frank Szematowicz is gratefully acknowledged.

1. Lukens, F. D. W., *Physiol. Rev.*, 1948, v28, 304.

2. Nichols, J., and Sheehan, H. L., *Endocrinol.*, 1952, v51, 362.

3. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

4. Somogyi, M., *ibid.*, 1945, v160, 61.

5. Kingsley, G. R., and Reinhold, J. G., *J. Lab. and Clin. Med.*, 1949, v34, 713.

6. Roe, J. H., and Keuther, C. A., *J. Biol. Chem*, 1943, v147, 399.

7. Schoenheimer, R., and Sperry, W. M., *ibid.*, 1934, v106, 745.

8. Dury, A., and Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 719.

9. Youngsburg, G. E., and Youngsburg, M. V., *J. Lab. and Clin. Med.*, 1930, v16, 158.

10. Fiske, C. H., and SubbaRow, Y., *J. Biol. Chem.*, 1925, v66, 375.

11. Sayers, G., and Sayers, M. A., *Recent Progr. Hormone Research*, 1948, v2, 81, Academic Press, New York.

12. Long, C. N. H., *Bull. N. Y. Acad. Med.*, 1947, v23, 260.

13. Beach, E. F., Bradshaw, P. J., and Blatherwick, N. R., *Endocrinol.*, 1952, v50, 212.

14. Goldner, M. G., and Jauregui, R. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 116.

Received June 15, 1954. P.S.E.B.M., 1954, v86.

Virulence of Strains of Herpes Simplex Virus for Mice. (21191)

ISAAC RUCHMAN.

From the Department of Microbiology, College of Medicine, University of Cincinnati and Cincinnati General Hospital.

It has been our experience that in the use of neutralization tests the HF strain(1) of herpes simplex virus usually yields low and at times inconclusive neutralization indexes (2-4). In spite of the many factors involved, apparently good indexes can be obtained with some of the recently isolated strains of herpes(2-5). In subsequent reports it will be shown that the strain of virus and the method of preservation are of importance. This paper will report on the virulence of various strains of virus for mice and the ultimate selection of