Alloxan in Blood Plasma before and after Ingestion of Glucose. (20925)

HARRY SOBOTKA AND ANITA V. LUISADA-OPPER.

From the Department of Chemistry, Mount Sinai Hospital, N. Y. City.

The parenteral injection of alloxan to animals produces a syndrome, which resembles in many respects diabetes mellitus. Typical lesions have been observed in the pancreas. The question remains open, whether some of the collateral symptoms are manifestations of diabetes, or primary signs of alloxan poisoning. Studies of the reverse effect, namely of carbohydrate intake upon alloxan metabolism, may throw light on the mechanism underlying "alloxan diabetes." The quantitative analysis of alloxan in body fluids is a capricious problem, because of the great reactivity of this compound with numerous oxidizable constituents of the blood serum. Archibald(1) has described 6 methods for the quantitative determination of alloxan and recommends the sixth method(1, p. 634) as the most sensitive one for the small amounts present in blood plasma. This photofluorometric method, based on the fluorescent condensation product of phenylenediamine with alloxan, gives according to Archibald values of less than 0.02 mg per 100 ml of plasma of dog or man. Shiøler(2) using this method, finds 0.015-0.030 mg in 100 ml of plasma in fasting rats and similar values in horse and man. Shiøler, in his preliminary report, describes what he calls "provoked" blood alloxan upon feeding glucose, pyruvic acid, succinic acid or fumaric acid in quantities of one g kg of body weight to rats. The blood alloxan in his experiment rises to values from 0.55 to 1.10 mg/100 ml. Huisman, Van Hulten, and Stel(3) could not reproduce these findings in rats. Their values for blood alloxan in fasting rats average 0.115 mg/100 ml and, after ingestion of one g glucose/onekg body weight, 0.110 mg/100 ml. Thus, their fasting values are about 5 times higher than those of Archibald and Shiøler and no provoked alloxan was observed.

Results. In experiments on dogs we find normal fasting alloxan values of 0.125-0.175 mg/100 ml. Upon administration of 5 g glucose/kg of body weight the alloxan values showed but minor fluctuations from 0.090-0.235 mg/100 ml during the 3 ensuing hours.

TABLE I. Plasma Alloxan in the Normal Dog (mg/100 ml).

Exp.	Fasting	30 min.	1 hr	1.5 hr	2 hr	3 hr
1	.135	.100	—	.165	—	.090
2	.175	.235	.155	—	.170	.145

Fasting alloxan in 2 other dogs was: .125 mg/100 ml
.160 "

All analytical results are averages of duplicates. Blood filtrates were prepared by the Folin-Wu method, which yielded the same results as the method of Herbert, Bourne and Greene(5) used by Archibald.

In 9 patients we obtained fasting values from 0.075-0.240 mg alloxan/100 ml. Six other patients were given glucose at the rate of 1.75 g/kg of body weight and both their blood alloxan and glucose were determined before and 30 minutes, one, 2 and 3 hours after administration. Here again the fasting alloxan values varied from 0.115 to 0.270 mg/100 ml and the irregular postprandial fluctuations were within the same range. Fasting and non-fasting values above 0.200 mg/100 ml were confined to cases 4, 5, and 6, suffering from diabetes.

As regards the higher fasting values found by Huisman *et al.* and ourselves, we have confirmed that the fluorescence of the standards develops rather slowly, reaching a maximum between 16 and 24 hours(4). The plasma alloxan develops maximum fluorescence within one hour. Thus, the immediate comparison would yield even higher levels. Our results extend the observation made by Huisman *et al.* in rats on the absence of alloxan provocation by glucose ingestion to dog and man.

Summary. Using Archibald's photofluorometric orthophenylene diamine method for plasma alloxan, we find plasma alloxan values of 0.1-0.2 mg in 100 ml of plasma for normal dogs and man. Slightly higher fasting values were observed in 3 cases of diabetes. No

TABLE II. Plasma Alloxan and Glucose in 6 Patients.

Initials	G.M.	S.M.	S.S.	I.G.	S.W.	E.S.
Diagnosis	Necr. of pancreas	Adrenal hypotens.	Glom. nephr.	Diab. & nephr.	Diab.	Cirrhosis & Diab.
Alloxan (hr)						
Fasting	.150	.200	.120	.235	.277	.235
½	.210	.150	.105	.265	.240	.090
1	.210	.110	.125	.330	.280	.195
2	.165	.135	.110	.285	.225	.200
3	—	.140	.130	.240	.240	.200
Glucose (Folin-Wu) (hr)						
Fasting	90	103	107	93	140	190
½	146	172	250	198	218	310
1	94	216	294	240	316	300
2	94	162	280	183	254	382
3	94	165	193	128	250	398

significant increase in plasma alloxan was provoked by a glucose meal in man and dog.

1. Archibald, R., *J. Biol. Chem.*, 1945, v158, 347.
2. Shiøler, P., *Biochim. et Biophys. Acta*, 1948, v2, 260.
3. Huisman, B. H., Van Hulten, A., and Stel, H., *Biochim. et Biophys. Acta*, 1950-51, v6, 290.
4. Karrer, P., Koller, F., and Sturzinger, H., *Helv. Chim. Acta*, 1945, v28, 1529.
5. Herbert, F. K., Bourne, M. C., and Greene, J., *Biochem. J.*, 1930, v24, 291.

Received March 2, 1954. P.S.E.B.M., 1954, v85.

Method for Separation of Human Epidermis into Cellular and Keratinous Components.* (20926)

PETER FLESCH AND ABRAHAM SATANOVE. (Introduced by P. Gyorgy)
From the Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia.

One of the major difficulties which has hampered experimental work on human epidermis is its heterogeneous composition. Experiments done on whole epidermis do not differentiate between the cellular components and the inert keratinous fibers. This paper describes a simple method for the separation of pulverized, dry human epidermis into its keratinous and cellular layers.

Methods. Human epidermis from autopsy or surgical material was separated from the corium with the stretch method(1), dried, pulverized in a mortar and passed through a 200-mesh sieve. The resulting powder was defatted by washing 3 times with ether in a centrifuge tube. Before the last washing the epidermal powder was allowed to settle and then centrifuged at moderate speed, whereupon 2 layers developed in the precipitate. After drying, the 2 layers could be separated easily. The upper layer was lightly pigmented and of chalk-like appearance, while the lower layer was darker and granular (Fig. 1). Powder from each of the 2 layers was embedded in paraffin, sectioned and stained with various histochemical reagents, such as Schiff's reagent and the Barrnett-Seligman stain for sulfhydryl groups(2,3), and with Giemsa stain. Sulfhydryl groups and disulfide bonds in each layer of the dry powder were determined with Bennett's reagent(4,5) quantitatively.

Results. Microscopic studies showed that the upper layer consisted mainly of Schiff-

* This investigation was supported by Research Grant, U. S. Public Health Service.