15133

Alloxan Diabetes in Rabbits.* Production of Hypercholesterolemia, Hyperlipemia and Adrenal Cortical Lesions.

FORREST E. KENDALL, WALTER MEYER, LIESE LEWIS, AND JOSEPH VICTOR.

From the Research Service, First Division, Goldwater Memorial Hospital, Welfare Island, N.Y., and the Department of Medicine, Columbia University, N.Y.

Dunn and his coworkers[1] reported that a single intravenous injection of alloxan into rabbits caused extensive damage to pancreatic islet cells and suggested that it should lead to the production of sustained diabetes mellitus. Alloxan diabetes has since been produced in the rabbit, rat, dog, cat, rhesus monkey, pigeon, and guinea pig.[2,3,4] Hyperlipemia and hypercholesterolemia are commonly found in patients with diabetes mellitus. However, up to the present, few reports have been made of the effect of alloxan upon the serum lipids of experimental animals. Bailey and Bailey[5] reported that one rabbit with uncontrolled diabetes following an intravenous injection of 200 mg of alloxan per kilo of body weight, developed pronounced hyperlipemia with total blood lipids of 8412 mg per 100 ml. The hyperlipemia was not observed in other rabbits in which the diabetes was controlled with insulin. Goldner and Gomori[6] found that alloxan diabetes in dogs was characterized by hyperlipemia and fatty liver which developed within 10 to 12 days. A more extensive study of the effect of alloxan upon the blood lipids of rabbits is reported in this paper.

Experimental. Thirty-eight rabbits varying in age between 5 months and 3 years were used. The rabbits were kept in individual cages and fed a diet of Purina Rabbit Chow and oats. Fresh greens were given twice a week. Fresh drinking water was supplied daily. Without a preliminary fasting period each animal was given a single intravenous injection of a freshly prepared 5% solution of alloxan monohydrate (Eastman Kodak Co.). Three rabbits were given a dosage of 50 mg of alloxan per kilo of body weight, 3 a dosage of 100 mg/kg, and the remaining 32 received a dosage of 125 mg/kg. The hypoglycemic shock which frequently follows alloxan injection was controlled in most cases by subsequent intravenous injection of 1 ml of 50% glucose per kilo of body weight after 6 hours, and by substituting a 5% glucose solution for the drinking water during the first 24 hours. Blood samples were taken for analysis at various intervals. Blood sugar was determined by the colorimetric copper method of Benedict,[7] and total cholesterol by the Sperry-Brand method.[8] The total lipids were determined gravimetrically by a method shortly to be described. Autopsies were done on all animals dying during the experiment and histological examination was made of all tissues.

Results. Alloxan Dosage 50 mg/kg: The 3 rabbits of this group all developed hyperglycemia within 3 hours of the injection. No hypoglycemic phase was observed in samples taken at 6 and 24 hours. The blood sugar of 2 of the rabbits returned to normal within 4 days; that of the third remained elevated above 200 mg % for 60 days and then gradually fell to 130 mg % 6 months after the injection of alloxan. At no time were serum lipid or cholesterol values found above basal

* This investigation has been aided by a grant from the Albert and Mary Lasker Foundation.

1 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., *Lancet*, 1943, **1**, 484.

2 Review: Goldner, M. G., *Bull. N. Y. Acad. Med.*, 1945, **21**, 44.

3 Review: Joslin, E. P., *New Eng. J. Med.*, 1944, **230**, 425.

4 Review: Kennedy, W. B., and Lukens, F. D. W., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 143.

5 Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1165.

6 Goldner, M. G., and Gomori, G., *Endocrinol.*, 1943, **33**, 297.

7 Benedict, S. R., *J. B. C.*, 1929, **83**, 165.

8 Sperry, W., and Brand, F., *J. B. C.*, 1943, **150**, 315.

levels. One rabbit with normal blood sugar died 112 days after alloxan injection. No lesions referable to alloxan were found. The other 2 animals were still living 176 days after injection.

Alloxan Dosage 100 mg/kg: All 3 rabbits of this group developed diabetes. Two of the 3 have survived 176 days since the injection. The blood sugar of one of these rabbits has remained above 300 mg % during the whole period; that of the other was above 300 mg % for 20 days and then gradually fell to normal values. The serum cholesterol and lipid values of both animals have remained within normal limits during the entire period. The third rabbit died on the 4th day after injection. At that time the blood sugar was 490 mg % and the serum cholesterol 520 mg %. The serum was extremely milky. At autopsy, in addition to the expected damage to the pancreas, the animal showed extensive fatty infiltration of the liver and kidneys and marked necrosis and leucocytic infiltration of the cortex of both adrenal glands. No alterations were visible in the adrenal medulla.

Alloxan Dosage 125 mg/kg. The triphasic nature of the blood sugar response was studied in 3 rabbits to which no glucose was given after the injection of alloxan. Samples of blood were taken hourly for 10 hours following injection and at daily intervals thereafter. Blood sugar levels rose from normal (98, 92, 90 mg %) to hyperglycemic levels (416, 296, 436 mg %) in from 3 to 4 hours and then fell to a low level (68, 22, 17 mg %) 8 to 10 hours after injection. At 24 hours the values were above normal (122, 234, 166 mg %) and one day later hyperglycemic levels were found (372, 400, 340 mg %). One rabbit died on the 4th day after injection, one on the 5th, and one has survived 60 days without a decrease in the blood sugar. All 3 animals had developed hypercholesterolemia (200, 333, 410 mg %) and hyperlipemia (1.5, 4.6, 6.3 g %) 4 days after injection. Morphological examination of the tissues of the rabbit dying on the fourth day revealed no lesions except loss of the beta islet cells of the pancreas. The rabbit dying on the 5th day showed necrosis of the renal convoluted tubules in addition to the pancreatic lesion.

Of the remaining 29 rabbits of this group, all of which received glucose, two-thirds died within 5 days, and all but 7 died within 60 days following the injection.

Determinations of blood sugar and serum cholesterol were made on all (25) animals surviving 4 days or longer. Total serum lipids were determined on 11 animals. Of the 7 rabbits surviving more than 60 days 2 were resistant to alloxan and showed normal values for blood sugar, cholesterol, and total lipid throughout the entire period. One developed hyperglycemia (blood sugar values in excess of 300 mg % throughout the entire period) without any elevation in cholesterol or total lipids, while 4 showed elevation of all 3 substances. Details of the data on 3 of the surviving rabbits are shown in Fig. 1. Two other survivors with hyperglycemia gave a pattern similar to that of the second.

All of the animals that died later than 4 days following the injection developed hyperglycemia (blood sugar >300 mg %), hypercholesterolemia (values ranging between 200 and 780 mg %) and hyperlipemia (values ranging between 2.8 and 18.5 g %). Fig. 2 shows the data on 8 of the 13 animals in this group. Since but one analysis was obtained for each of the other five animals, they are not included in the figure. They all showed cholesterol values above 200 mg % and the sera were visibly lipemic.

Post Mortem Findings. Rabbits dying after 3 days had a weight loss of from 10 to 30%, associated with considerable decrease in fat in subcutaneous, omental, and intraperitoneal tissues. After the 4th day livers were decreased in size, were dark brown and had sharp edges. In several instances the adrenal glands were congested and some showed greenish yellow mottling in the cortex. The kidneys of many of the rabbits were pale, swollen, and tan colored. Their capsules were easily stripped and their widened cortices revealed gray and yellow streaks running parallel to the tubular striations.

Microscopically, aberrations from normal similar to those described by other investiga-

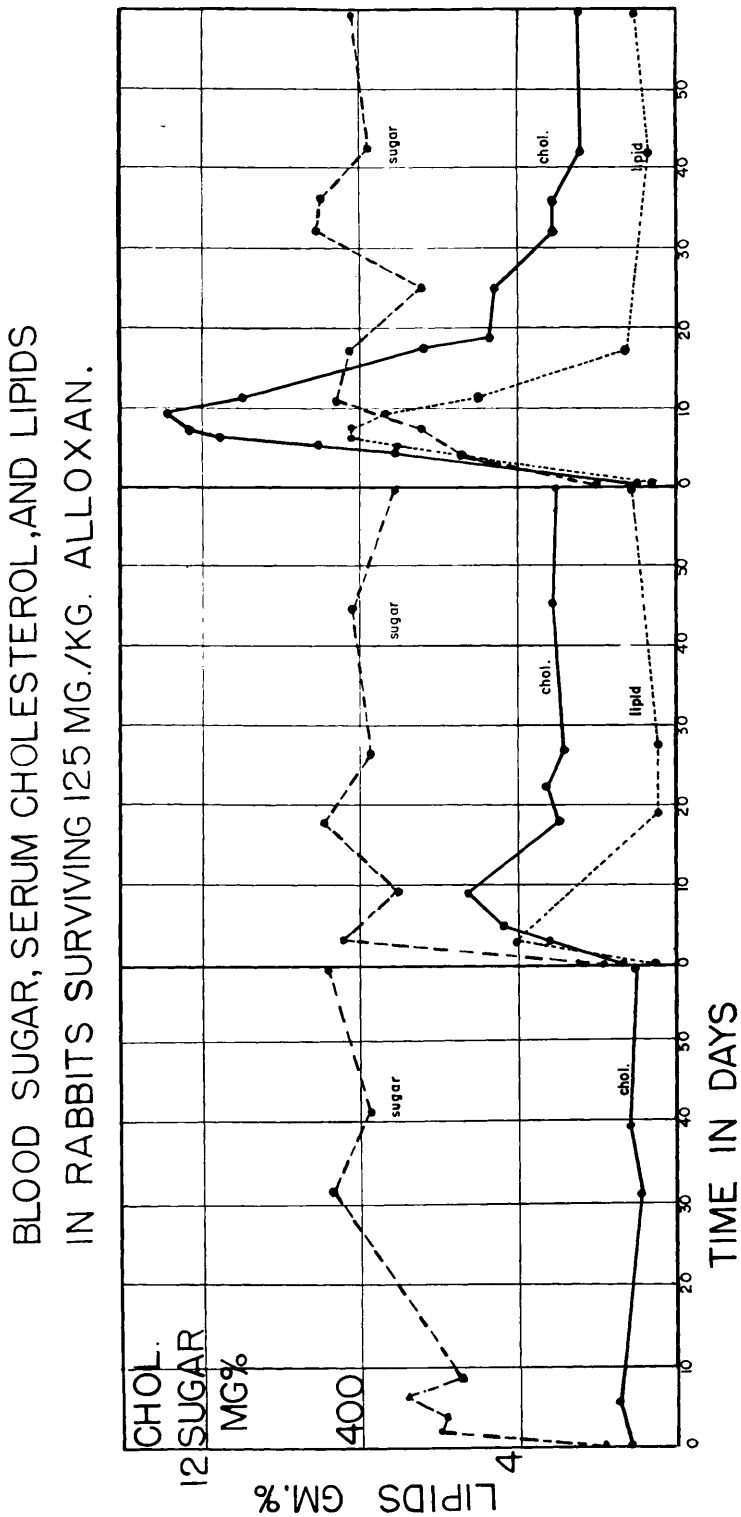
BLOOD SUGAR, SERUM CHOLESTEROL, AND LIPIDS
IN RABBITS SURVIVING 125 MG./KG. ALLOXAN.
CHOL.
SUGAR
MG%
400
12
4
LIPIDS GM.%
sugar
chol.
lipid
TIME IN DAYS
0
10
20
30
40
50

FIG. 1.

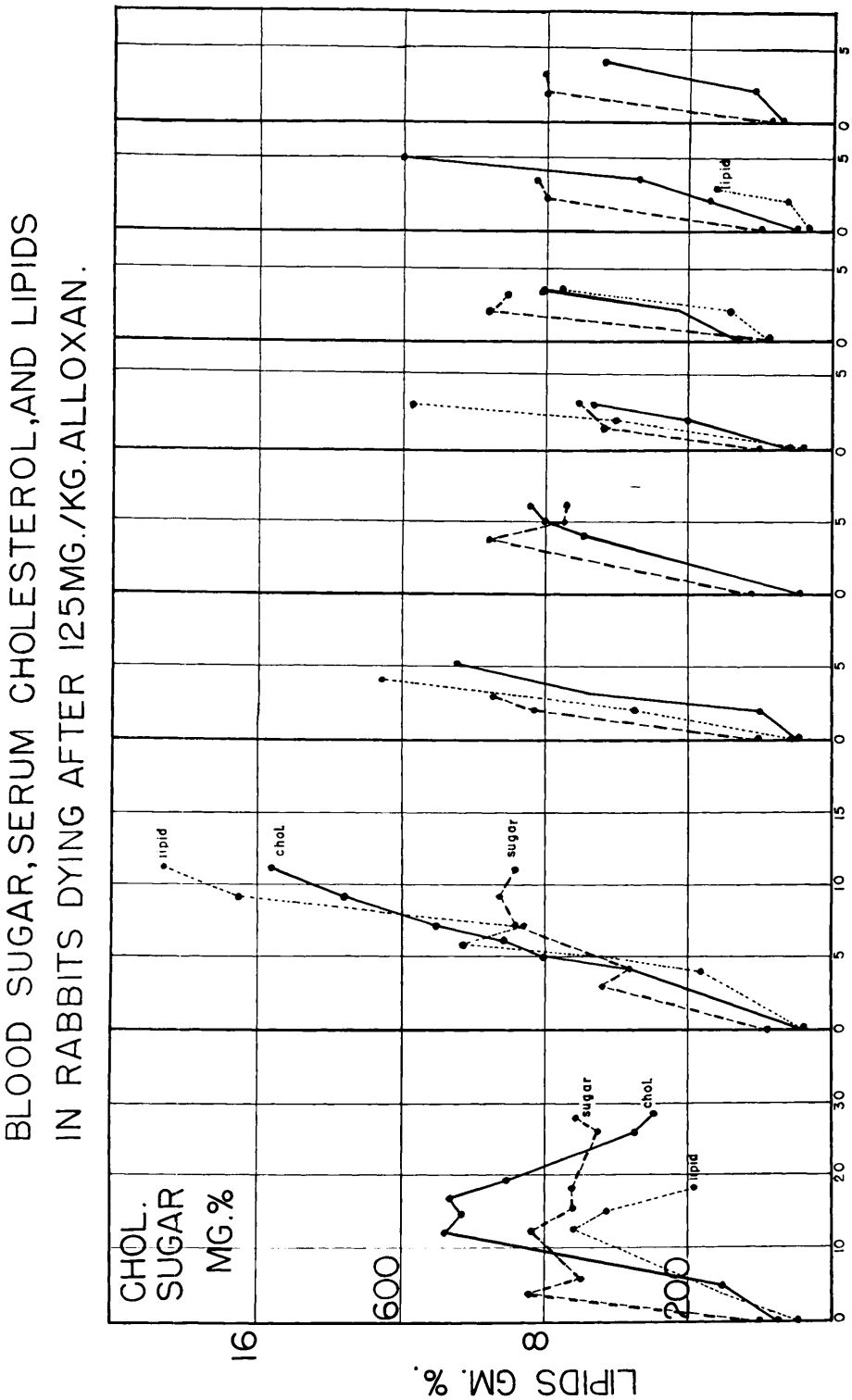
BLOOD SUGAR, SERUM CHOLESTEROL, AND LIPIDS
IN RABBITS DYING AFTER 125MG./KG. ALLOXAN.
CHOL.
SUGAR
MG.%
600
200
16
8
LIPIDS GM. %.
lipid
chol.
sugar
TIME IN DAYS

FIG. 2.

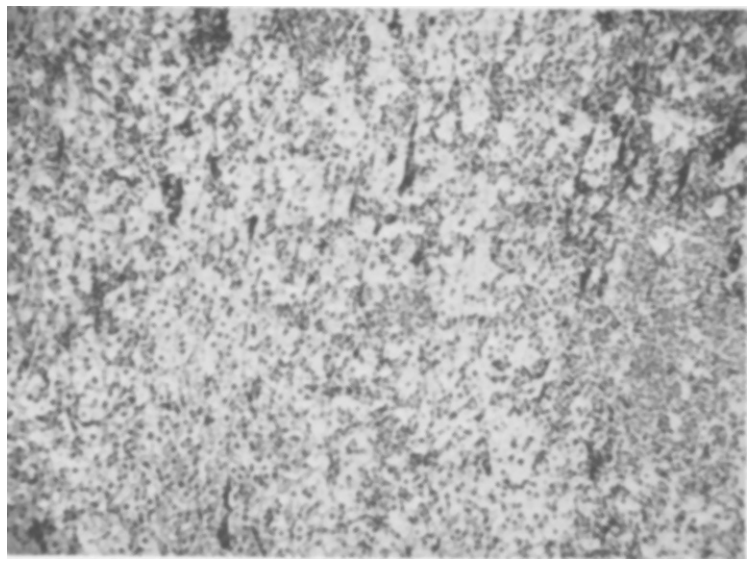

FIG. 3.
Adrenal Cortex rabbit No. 354, four days after i.v. injection of 125 mg/kg of alloxan. Necrosis of cortical cells and infiltration with polymorphonuclear, neutrophilic, and eosinophilic leucocytes. Magnification 100 ×. Giemsa stain.

tors were found in the pancreas,[9,10] liver,[6] and kidneys.[9] Lysis of beta cells of the islets of Langerhans was observed in all rabbits dying within 3 days after alloxan administration. Thereafter the islets consisted of eosinophilic alpha cells surrounding the collapsed stroma which previously had supported beta cells. Liver cells were well vacuolated until the 4th day. After that time they lost their vacuoles and their cytoplasm became more granular and denser than normal. Necrosis and regeneration of epithelium of renal convoluted tubules occurred bilaterally in 9 of 23 rabbits. Hyalin casts and, in one rabbit, calcium containing casts were seen in the damaged tubules.

Lesions of a type not previously described in alloxan poisoning were found in the cortices of both adrenal glands of 12 of 25 animals. Diffuse and focal necrosis was encountered bilaterally in the adrenal cortices of 7 of these animals. The necrosis was accompanied by infiltration with many polymorphonuclear leucocytes together with a few mononuclear and eosinophilic cells (Fig. 3). Bacterial stains, such as Giemsa or Brown, revealed no organisms in these areas. In 4 cases loss of lipoid and decrease in size of cortical cells was seen. One rabbit lived 32 days after alloxan treatment and showed considerable focal lymphocytic infiltration in addition to cell atrophy in the adrenal cortex. In contrast with the findings of Hard and Carr[10] no adrenal medullary changes were observed.

Discussion. It is evident that uncontrolled diabetes mellitus produced in rabbits by the intravenous injection of alloxan may be accompanied by a great increase in serum cholesterol and lipids. Hypercholesterolemia and hyperlipemia always occurred if the diabetes was severe enough to cause the death of the animal. Death after the fourth day is attributed to the diabetic condition rather than to the acute effects of alloxan. If the animal survived, great variations occurred in the degree of hyperlipemia and hypercholesterolemia, as shown in Fig. 1 for 3 rabbits all of which had diabetes of comparable severity as meas-

[9] Dunn, J. S., Kirkpatrick, J., McLetchie, N. G. B., and Telfer, S. V., *J. Path. and Bact.*, 1943, **55**, 245.

[10] Hard, W. L., and Carr, C. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 214.

ured by blood sugar levels. In the animals that died, no correlation could be found between the occurrence of lesions in the adrenal cortex or the kidneys and the development of high serum levels of cholesterol and lipid. In view of the results reported by Bailey, Bailey, and Leech[11] on rabbits and by Goldner and Gomori[6] on dogs, the hyperlipemia is probably caused by the diabetes itself. They found that it did not occur if the hyperglycemia was controlled with insulin. The type of curve found for these values in surviving animals together with the observation made upon the animals that died, suggests that the high levels were caused by a flood of fat being mobilized from the body depots. The mechanism of this fat mobilization will be studied further.

Age seemed to play some role in the reaction of the rabbits to alloxan. Of 32 rabbits given 125 mg/kg of alloxan, 17 were 5 months old and 15 of ages varying between 9 months and 3 years. Six of the 17 young rabbits, one of which was resistant to alloxan, survived, whereas only one old rabbit out of 15 survived and that one was resistant to alloxan. Only 2 of the 17 young rabbits died before the 5th day as compared with 9 of the 15 older ones. The younger group was remarkably uniform in that 7 of the 11 fatalities occurred on the 5th day. Only 4 of the 11 young rabbits that died showed extrapancreatic lesions, whereas only 2 of the 14 in the older group failed to show such lesions. Necrosis of the adrenal cortex was found only in the older animals. Loss of vacuolization of the cells of the adrenal cortex occurred in both age groups. An adequate number of control animals in the old age group were not available for study at this time. However, during the past five years the adrenals of 279 rabbits in this age group have been examined without disclosing any comparable lesions. Moreover, the acute nature of the lesion relates it definitely to the alloxan injection rather than to an unknown factor referable to the age of the animal. This demonstration of damage to the adrenal cortex is of particular interest in view of the findings of Kirschbaum, Wells, and Molander[12] that the adrenal cortex is involved in the initial hyper- and hypoglycemic reactions to alloxan.

Summary. A transient period of hypercholesterolemia and hyperlipemia may accompany the early stage of severe diabetes mellitus in rabbits following the injection of alloxan. It occurred in varying degrees in many rabbits that survived with persistent diabetes and always occurred if the diabetes was severe enough to cause death. A dosage of 50 mg/kg of alloxan failed to cause hyperlipemia or hypercholesterolemia, 100 mg/kg produced it in one of 3 rabbits, while 125 mg/kg was effective in 29 of 32 animals. Lesions were found in the adrenal cortices of 12 of 25 animals dying after receiving 125 mg/kg of alloxan.

11 Bailey, C. C., Bailey, O. T., and Leech, R. S., *New Eng. J. Med.*, 1944, **230**, 533.

12 Kirschbaum, A., Wells, L. J., and Molander, D., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 294.