acetate was consistently found to lower the GSH content, the fall in GSH being greater as the dose of acetoacetate increased.

2. The GSH level could be reduced to zero after repeated injection of acetoacetate.

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Studies on Alloxan Diabetes

1. INCREASE OF SUSCEPTIBILITY CAUSED BY ACETOACETATE

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Acetoacetate, which has been found in this laboratory to cause hyperglycaemia (Nath & Brahmachari, 1944), to reduce glucose tolerance (Nath & Chakrabarti, 1950), to raise blood lactic acid (Nath & Chakrabarti, 1951) and to inactivate insulin both *in vitro* and *in vivo* (Nath & Brahmachari, 1946), has recently been reported to cause decrease in the reduced glutathione (GSH) content of blood by Nath & Hatwalne (1950) and Nath, Hatwalne & Gadgil (1953). Griffiths (1948, 1950), in his studies on the production of diabetes by the administration of uric acid, noticed that diabetes could be produced

in the animals only when the blood GSH level could be lowered by a diet deficient in cysteine and methionine and by feeding a large amount of ascorbic acid, which was shown by Prunty & Vass (1943) to lower the glutathione content of the blood, and to increase the susceptibility of rats to alloxan diabetes (cf. Lazarow, 1946). The increased sensitivity of starved animals to the diabetogenic action of alloxan, as noticed by Kass & Waisbren (1945), might be due to the fall of blood GSH which takes place during starvation (Hirano, 1934). It is also worth noting that animals depleted in

Table 1. *Effect of acetoacetate injection on the lowering of blood GSH and increase of blood sugar*

(Average values with the calculated mean deviations.)

Group no.	No. of animals in the group	Blood GSH (mg./100 ml.)		Blood sugar of the treated animals (mg./100 ml.)
		Initial	After injection of acetoacetate for 5 weeks (150 mg./kg.)	
I	6	28.8±3.6	2.7±0.1	131.8±4.9
II	6	27.8±3.8	2.5±0.2	131.0±3.1
III	6	31.3±3.9	2.7±0.2	134.0±2.7
IV	6	30.7±2.9	2.5±0.7	133.0±3.6
V	6	28.8±3.4	2.7±0.5	133.0±4.4

blood GSH by a sodium-deficient diet have also been shown to have increased susceptibility to alloxan (Grunert & Phillips, 1949).

Archibald (1945) and Lazarow, Patterson & Levy (1948) have shown that when alloxan is administered it undergoes destruction partly by the glutathione of the blood and partly by other mechanisms. When the dose is high, some of it reaches the β cells of the pancreas and reacts with the glutathione of these β cells and even causes cell death by reacting with the sulphhydryl groups of the protein and the enzymes. It is, therefore, expected that any substance which would decrease GSH in the body would also increase the susceptibility to alloxan.

This work was undertaken to see whether acetoacetate, which can produce various diabetic symptoms and can cause reduction of blood GSH, can cause an increase in the susceptibility to alloxan.

EXPERIMENTAL

Thirty male rabbits weighing 1.5–2 kg. were selected and divided into five groups of six and their normal blood GSH was determined by the method of Woodward & Fry (1932). They were then injected subcutaneously with 150 mg./kg. of sodium acetoacetate daily for 5 weeks, when usually all the animals had turned over from their initial hypoglycaemic to the hyperglycaemic state (Nath & Chakrabarti, 1950). Blood sugar was estimated by the method of Hagedorn & Jensen (1923). After 5 weeks, when the animals in all the five groups were found to have very low blood GSH, those in one group were kept as control while those in other groups were injected intravenously with different doses of alloxan after allowing them to fast for about 18 hr. Twenty-four normal rabbits were also used to ascertain the diabetogenic and lethal doses of alloxan on the normal animals in our laboratory.

RESULTS AND DISCUSSION

Table 1 shows that animals in all the groups show a slight rise in the fasting blood-sugar level and a sharp decline in the blood GSH content on injection of acetoacetate for 5 weeks. Urine of all the animals was analysed at this time and none was found to contain glucose, ketone bodies or albumin.

It will be evident from Table 2 that although a dose of 25 mg./kg. of alloxan is absolutely without any effect, those having 50 mg./kg. showed a definite tendency to have hyperglycaemia without glycosuria. A dose of 75 mg./kg., however, produced marked increase in the blood sugar with glycosuria and acetoneuria and also albuminuria in some cases. A dose of 100 mg./kg. was found fatal to about 80 % of the animals who died within 48 hr., the only one surviving showing blood sugar as 410 mg./100 ml. and 4.5 % sugar in urine. The test for acetone in the urine was strongly positive and the albumin content high.

Table 2. *Effect of different doses of alloxan on normal animals and on animals whose blood GSH was depleted by acetoacetate injections*
(Average values with the calculated mean deviations.)

Group no.	Nature of animals	No. of animals in the group	Dose of alloxan (mg./kg.)	Pathological constituents in urine			Remarks
				Blood sugar 48 hr. after alloxan injection (mg./100 ml.)	Sugar (%)	Acetone bodies	
I	GSH depleted	6	0	131.7 $\pm$ 3.5	0	-	No effect of alloxan
II	GSH depleted	6	25	131.0 $\pm$ 3.1	0	-	Hyperglycaemia without glycosuria
III	GSH depleted	6	50	152.0 $\pm$ 6.8	0	-	One animal died within 48 hr. The remaining animals were diabetic. Glycosuria and hyperglycaemia continued for 5 weeks in all the animals
IV	GSH depleted	6	75	212.0 $\pm$ 13.6	2.0 $\pm$ 0.8	+	High glycosuria and hyperglycaemia in the only animal surviving for 5 days. All other animals died within 48 hr.
V	GSH depleted	6	100	410.00	4.5	++	Hyperglycaemia without glycosuria
VI	Control	4	125	163.5 $\pm$ 2.5	0	-	Only two animals became diabetic
VII	Control	4	130	186.25 $\pm$ 12.8	0.775 $\pm$ 1.1	+	Only three animals became diabetic
VIII	Control	4	135	206.75 $\pm$ 23.5	2.13 $\pm$ 0.25	+	All the animals became diabetic
IX	Control	4	140	259.00 $\pm$ 5.3	2.7 $\pm$ 0.13	+	Severe diabetes
X	Control	4	200	335.00 $\pm$ 2.6	3.4 $\pm$ 0.3	+	All animals died within 48 hr.
XI	Control	4	225			.	

From Table 2 it will also be seen that the diabetogenic dose of alloxan in normal animals, as found in this laboratory, ranges between 130 and 140 mg./kg. and the lethal dose is observed to be in the neighbourhood of 225 mg./kg. It is interesting to note that the diabetogenic and lethal doses of alloxan in acetoacetate-treated animals, having blood GSH in the neighbourhood of 3 mg./100 ml., are only 75 and 100 mg./kg., respectively. Continued injection of acetoacetate for a few weeks has thus a definite effect in making the animals susceptible to alloxan.

It may be mentioned here that the diabetogenic and lethal doses of alloxan in normal animals, as quoted by Lukens (1948) and others, are 150–200 and 300 mg./kg., respectively, which are somewhat higher than the values observed by us. The dis-

parity may be explained by the difference in diet, climate and breed of the animals.

SUMMARY

1. Rabbits injected subcutaneously with 150 mg./kg. sodium acetoacetate daily for 5 weeks show a slight rise in blood sugar, and an enormous fall in reduced glutathione in blood.

2. The susceptibility of these treated animals to alloxan is increased to a great extent, 75 mg./kg. being the diabetogenic dose and 100 mg./kg. the lethal dose, as compared with 130–150 and 225 mg./kg., respectively, for controls.

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The Assimilation of Amino-acids by Bacteria

14. NUCLEIC ACID AND PROTEIN SYNTHESIS IN *STAPHYLOCOCCUS AUREUS*

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If washed suspensions of *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) are incubated in buffered salt solution containing glucose and glutamic acid, free glutamic acid accumulates to a high concentration within the cell (Gale, 1947*a*, *b*). If certain other amino-acids, such as cysteine or alanine, are added singly to the incubation mixture, the accumulation of free glutamic acid within the cells is reduced while peptides of glutamic acid appear in the external medium (Gale & Van Halteren, 1951). If a complete mixture of amino-

acids and glucose is present, then the cells no longer accumulate free glutamic acid but the combined glutamate of the cellular substance increases, glutamic acid from inside and outside the cell becoming directly incorporated into this combined glutamate fraction (Gale, 1951*b*).

In the investigations so far described in this series, no account has been taken of the nucleic acid content of the cells or of its variation. In the present work, the nucleic acid content has been studied and the effect of the addition of purines and