Progressive Depletion in the Reduced Glutathione Content of the Blood following Acetoacetate Injections

By M. C. NATH, V. G. HATWALNE AND J. S. GADGIL
The University Department of Biochemistry, Nagpur, India

(Received 16 April 1952)

Glutathione has long been known to be an essential constituent of cells and to be indispensable for their normal activity since it serves to protect the cell enzymes. Several diabetogenic substances have been found to affect the reduced glutathione (GSH) content of various tissues. The injection of anterior pituitary preparations has been found to lower GSH content of tissues (Goss & Gregory, 1935; Gregory & Goss, 1939) and it has recently been shown by Conn, Louis & Wheeler (1948) that there is a direct correlation between the production of a transitory diabetes in man produced by injecting a purified adrenocorticotrophic hormone and the blood GSH level. It has also been reported that the GSH of blood, tissues and, in particular, pancreas is decreased in diabetes (Campanacci, 1930). Leech & Bailey (1945) have investigated the effect of alloxan, and they found that the blood GSH may fall to near zero values on alloxan injection.

On the basis of these findings Lazarow (1949) attempted to correlate the diabetogenic action of a chemical compound with its ability to lower the GSH content of blood. He states that diabetes is usually developed in later stages of life, and hence it may not be unreasonable to suppose that gradual accumulation of some metabolic factors may be held responsible for a progressive degeneration of the β cells of the pancreas. Attempts have been made in this laboratory to show that the intermediary fat metabolites, namely 'ketone bodies', might play the role of such a factor. Acetoacetate and β -hydroxybutyrate have already been shown to produce hyperglycaemia and to lower the potency of pancreatic insulin (Nath & Brahmachari, 1944, 1949) and to cause lowering of glucose tolerance and a rise of blood lactic acid (Nath & Chakrabarti, 1950, 1951).

In some preliminary observations it has already been shown from this laboratory (Nath & Hatwalne, 1950) that acetoacetate has some effect in lowering the GSH content of blood in rabbits. It is interesting to note that after the publication of our letter, Illing, Gray & Lawrence (1951) found that there was a significant reduction in glutathione in the diabetics with severe ketosis.

This paper gives an account of experiments on rabbits under various conditions.

EXPERIMENTAL

Thirty male rabbits weighing from 1.5 to 2 kg. were chosen, and the normal GSH content of their blood was determined by the method of Woodward & Fry (1932). For this purpose 1.5 ml. of blood were withdrawn from the marginal ear vein of the rabbit by means of a syringe and immediately mixed with water and only reduced glutathione determined. The animals were fed not more than 2 hr. prior to taking a sample of blood for analysis to ensure that they were not starved. After the initial determination of GSH the rabbits were injected subcutaneously with the different doses of acetoacetate and GSH was estimated periodically.

Sodium acetoacetate was prepared in the usual way in the laboratory, other chemicals required being of the B.D.H. Analar quality.

Blood sugar was determined by the method of Hagedorn & Jensen (1923).

RESULTS

First, the effect of a single injection of acetoacetate in doses of 300 mg./kg., 200 mg./kg. and 100 mg./kg. was studied on three groups of six rabbits each for a period of 3 hr., and a GSH determination was also made at the end of 24 hr. Blood sugar was determined simultaneously in the case of the first group alone (300 mg./kg.). It will be noted from Fig. 1 that the maximum drop in GSH is observed at the end of 1.5 hr. after the injection and that it returns to almost normal values after 3 hr. The blood-sugar curve shows that the fall in GSH is accompanied by a rise in blood sugar.

If the injection of the same doses of acetoacetate were repeated on consecutive days results as depicted in Fig. 2 were obtained. Every day GSH was determined 1.5 hr. after the injection of acetoacetate since it was known from the previous experiment that the maximum effect could be observed only after that time. It can be seen from Fig. 2 that the GSH level falls to zero on the third, fourth and fifth days, respectively, with successively decreasing doses. Injections were discontinued from the sixth day and it was found that it took several days more for the GSH to return to normal.

The effect of smaller but gradually increasing doses of acetoacetate was also studied. For this purpose twelve rabbits were selected and injected with 50 mg./kg. of sodium acetoacetate for the

initial 10 days; 100 mg./kg. for the next 10 days and then 150 mg./kg. for a further 10 days. The GSH was determined after ten, twenty and thirty injections, i.e. on the eleventh, twenty-first and thirty-first day, before the next injection of sodium acetoacetate, and after making sure that the rabbits were fed within 2 hr. of the withdrawal of the blood.

Fig. 1. Effect of single massive subcutaneous dose of acetoacetate on blood GSH content. ■—■, 100 mg./kg.; ●—●, 200 mg./kg.; ▲—▲, 300 mg./kg.; ○—○, blood-sugar curve.

Fig. 2. Effect of repeated daily injection of acetoacetate on blood GSH content. ■—■, 100 mg./kg.; ●—●, 200 mg./kg.; ▲—▲, 300 mg./kg.

Results are represented in Fig. 3. There is a steep fall in GSH at the end of the first 10 days, and subsequently a further gradual fall can be noted. At the end of the experiment the values have fallen to slightly less than 30 % of the original.

To ascertain whether bleeding has any effect on the GSH content, 1.5 ml. of blood were withdrawn twice daily for a period of 5 days from six rabbits and GSH determined. It was found that there was no effect on the GSH content for 5 days.

DISCUSSION

The experimental data presented indicate that acetoacetate causes a fall in the blood GSH content, although it is not as sharp and rapid as is reported in the case of alloxan (Leech & Bailey, 1945). It is only natural that this should be so since aceto-

acetate is an intermediary fat metabolite which normally occurs in very minute proportions in the system. Repeated daily injection brings the blood GSH value to zero in 3–5 days, less time being required with higher dose. Though alloxan causes an immediate fall of the GSH content to zero, nearly 1.5 hr. are required to obtain the maximum effect of an acetoacetate injection. The mode of injection may also account for this difference since alloxan is given intravenously and acetoacetate subcutaneously.

Fig. 3. Effect of smaller but gradually increasing doses of acetoacetate for a number of days on blood GSH.

The fall in the blood GSH caused by the injection of a 300 mg./kg. dose of acetoacetate is accompanied by a simultaneous transitory hyperglycaemia. It appears that blood sugar may have some relation with GSH and the presence of a substance like acetoacetate, which becomes toxic at higher concentrations. This also supports the hypothesis put forward by Nath & Brahmachari (1944) that accumulation of acetoacetate may be a predisposing cause of diabetes.

The decrease in GSH content in the blood, as a result of acetoacetate accumulation, could be due to many factors. It has been suggested that sodium depletion may be a contributory factor (Grunert & Phillips, 1949; Illing *et al.* 1951). Since we have used a sodium salt of acetoacetic acid, sodium depletion is unlikely to be the contributory factor in our experiments. It is probable that the fall is due to increased requirement as also suggested by Illing *et al.* (1951).

SUMMARY

1. The effect of acetoacetate injections (single and repeated) on the reduced glutathione (GSH) content of the blood of rabbits was studied. Aceto-

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.

The authors record their grateful thanks to the Indian Council of Medical Research for the award of a Research Fellowship to one of them (V.G.H.).

They are also thankful to Shri V. K. Sahu for his help in determining the blood-sugar values recorded in Fig. 1.

REFERENCES

- Campanacci, D. (1930). *Klin. Wschr.* **9**, 1212.
 Conn, J. W., Louis, L. H. & Wheeler, C. E. (1948). *J. lab. clin. Med.* **33**, 651.
 Goss, H. & Gregory, P. W. (1935). *Proc. Soc. exp. Biol., N.Y.*, **32**, 681.
 Gregory, P. W. & Goss, H. (1939). *Growth*, **3**, 159.
 Grunert, R. R. & Phillips, P. H. (1949). *J. biol. Chem.* **181**, 821.
 Hagedorn, H. C. & Jensen, B. N. (1923). *Biochem. Z.* **135**, 46.
 Illing, E. K. B., Gray, C. H. & Lawrence, R. D. (1951). *Biochem. J.* **48**, 637.
 Lazarow, A. (1949). *Physiol. Rev.* **29**, 48.
 Leech, R. S. & Bailey, C. C. (1945). *J. biol. Chem.* **157**, 525.
 Nath, M. C. & Brahmachari, H. D. (1944). *Nature, Lond.*, **154**, 487.
 Nath, M. C. & Brahmachari, H. D. (1949). *Ind. J. med. Res.* **37**, 71.
 Nath, M. C. & Chakrabarti, C. H. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 326.
 Nath, M. C. & Chakrabarti, C. H. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 369.
 Nath, M. C. & Hatwalne, V. G. (1950). *Nature, Lond.*, **166**, 692.
 Woodward, G. E. & Fry, E. G. (1932). *J. biol. Chem.* **97**, 465.

Studies on Alloxan Diabetes

1. INCREASE OF SUSCEPTIBILITY CAUSED BY ACETOACETATE

BY M. C. NATH, J. S. GADGIL AND V. G. HATWALNE

The University Department of Biochemistry, Nagpur, India

(Received 5 August 1952)

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