The rate of hydrolysis of esters in solution is highly dependent on the nature of the alcohols, e.g. hydrolysis of methyl acetate is twice as fast as that of ethyl, *n*-propyl and *n*-butyl acetate and six times as fast as that of isopropyl acetate (Smith & Olsson, 1925). The fact that the different wool esters hydrolyse at approximately the same rate suggests that the rate-controlling step in the heterogeneous reaction may not be the actual hydrolysis but a diffusion process.

SUMMARY

1. The effect of concentration of acid catalyst, temperature and water on the esterification of the carboxyl groups in wool with alcohols has been studied.

2. The number of carboxyl groups capable of being esterified decreases with increasing molecular weight of the alcohol.

3. The esters formed are readily saponified by alkali and the rate of this reaction is independent of the alcohol used.

4. Of a number of 1:2-epoxides examined only epichlorhydrin was found to esterify more than 10 % of the carboxyl groups of wool. The ester of monochlorhydrin formed reacts with dilute alkali at room temperature to form an epoxide which is combined in the wool.

5. The effect of esterifying the carboxyl groups on the titration curve, dyeing behaviour and elastic properties of wool have been investigated.

6. It is shown that the combination of acids with wool can be greatly speeded up by efficient agitation and that equilibrium can be established in a few minutes.

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Blood Glutathione and Non-glucose Reducing Substances in Diabetes

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A relation between glutathione and experimental diabetes was first suspected when Leech & Bailey (1945) observed a simultaneous fall in blood alloxan and glutathione following the injection of diabetogenic doses of alloxan into rabbits. Lazarow (1946, 1947) subsequently demonstrated that glutathione and other sulphhydryl compounds such as cysteine and BAL (2:3-dimercaptopropanol) were capable of

protecting the rat against the diabetogenic activity of alloxan. More recently Conn, Louis & Johnston (1949) have shown that the intravenous administration of reduced glutathione inhibited the temporary diabetes produced by the administration of adrenocorticotrophic hormone (ACTH).

It is possible that the great variation observed by Mosenenthal & Barry (1946) in the non-glucose reducing

substances in the blood from diabetics might be explained by variations in the concentration of glutathione in the blood and that in turn these might be related to the type or severity of diabetes. Previous investigations concerning the concentration of glutathione in the blood of diabetic patients have given conflicting results. Thus, while Campanacci (1930) and Varela, Apolo & Vilar (1930) found low levels, Platt (1931) and Dogliotti & Meloni (1935) found the blood levels in diabetic patients to be the same as in normal individuals. More recently, Stock & Currence (1950) have determined the concentration of reduced glutathione in the blood of 14 fasting diabetic patients and observed no significant difference from the normal range. This paper reports similar investigations on 39 diabetic patients, under somewhat different conditions from those studied by Stock & Currence, by a method which determines both reduced and oxidized glutathione. At the same time, we have compared the concentration of glutathione in the blood with the difference between the 'true' and 'apparent' blood-sugar concentrations.

CLINICAL MATERIAL

The 39 subjects studied were all ambulatory diabetic patients attending the out-patient diabetic clinic at King's College Hospital. Some attending for routine checks had relatively normal blood sugars; others,

some of whom have not received insulin treatment, had high blood sugars and showed ketosis. Blood samples were taken by venepuncture into a mixture of fluoride and oxalate at 10 a.m., in most cases about 2 hr. after breakfast. A series of 20 normal healthy medical students and laboratory staff were investigated under similar conditions.

METHODS

All estimations were performed in duplicate. 'True' blood sugar was determined by the Harding & Downs (1933) modification of the Schaffer-Hartmann (1921) method performed on CdSO_4 -NaOH filtrates (Fujita & Iwatake, 1931). 'Apparent' blood sugar was determined by the same method using Folin & Wu tungstic acid filtrates. The nitroprusside reaction for glutathione was always negative when performed on the CdSO_4 filtrates, but was strongly positive in all the tungstic acid filtrates. The concentrations of reduced and total glutathione in the blood were determined by the method of Woodward & Fry (1932) on sulphosalicylic acid filtrates of whole blood prepared within 10 min. of venepuncture.

RESULTS

Table 1 presents the glutathione levels observed in the 20 normal individuals, in 18 diabetics without ketosis, and in 21 diabetics who had heavy ketosis and whose urine gave positive tests for acetoacetic acid with ferric chloride.

Table 1. *Glutathione levels in blood of normal and diabetic patients*

Group	No. of cases	Reduced glutathione (mg./100 ml.)		Total glutathione (mg./100 ml.)	
		Mean $\pm$ S.E.M.	Range	Mean $\pm$ S.E.M.	Range
Normal	20	33.5 $\pm$ 0.82	25.6-40.5	43.0 $\pm$ 0.88	36.8-50.5
All diabetics	39	30.1 $\pm$ 0.74	20.4-43	39.1 $\pm$ 0.81	27.3-53
Diabetics without ketosis	18	32.1 $\pm$ 1.18	23.6-43	41.1 $\pm$ 1.22	32.2-53
Diabetics with ketosis	21	28.5 $\pm$ 0.81	20.4-36.8	37.5 $\pm$ 0.93	27.3-45.4
Comparison of groups					
				<i>t</i>	<i>P</i>
Reduced glutathione	Normals/diabetics			3.1	<0.01
	Normals/ketosed diabetics			4.1	<0.01
	Normals/non-ketosed diabetics			0.99	0.4-0.3
	Ketosed diabetics/non-ketosed diabetics			2.5	0.02-0.01
Total glutathione	Normals/diabetics			3.26	<0.01
	Normals/ketosed diabetics			4.3	<0.01
	Normals/non-ketosed diabetics			1.26	0.3-0.2
	Ketosed diabetics/non-ketosed diabetics			2.4	0.02-0.01

Table 2. *Correlation of blood-sugar differences with total glutathione levels*

(Difference = 'Apparent' - 'True' blood sugar.)

Group	No. of cases	Mean blood sugar difference (mg./100 ml.)		Mean blood glutathione (mg./100 ml.)	<i>r</i> *	<i>P</i>
			Range			
Normals	7	19.0	15-23.5	43.1	+0.422	0.1
Ketosed diabetics	21	26.9	16-52	37.5	+0.072	0.1
Non-ketosed diabetics	18	24.6	2-39	41.1	+0.217	0.1

* *r* is the correlation coefficient.

Total glutathione. The values for total glutathione in the whole group of diabetics were significantly below normal. While the concentration of total glutathione in the diabetics without ketosis was within the normal range, a significantly lowered concentration was observed in those with ketosis, and accounted for the significant difference found between our normal values and those of the whole group of diabetic cases.

Reduced glutathione. Table 1 also shows that the changes in reduced glutathione paralleled those of total glutathione.

Non-glucose reducing substances. Table 2 presents the mean values of the difference between the 'true' and 'apparent' blood-sugar concentration in the same diabetic patients. Statistical analysis shows that there is no correlation between this difference and the total glutathione concentration in the blood either for normal or diabetic subjects.

DISCUSSION

The reduction in the blood glutathione concentration in diabetes is in agreement with a recent report of Binkley, Fujii & Kimmel (1950), whose normal values, however, differ from those we have observed. This difference may be because Binkley *et al.* used a method for the determination of glutathione depending on hydrolysis to cysteine which was then estimated by the Sullivan & Hess (1936) method. Our results for normal subjects, which agree with those of previous workers (Platt, 1931; Stock & Currence, 1950), were obtained by the iodometric method. Binkley *et al.* found in diabetes that the lowered value of glutathione was compensated by an increase in the concentration of γ -glutamylcysteine. Since this substance would be estimated as glutathione by the iodometric method, a fall in blood glutathione due to its conversion to γ -glutamylcysteine would therefore not be detected by this method. The question of the specificity of the methods of estimation of glutathione clearly requires further investigation in the light of the results of Binkley *et al.*

The complete lack of correlation between total blood glutathione and the non-glucose reducing substances demonstrates clearly that in addition to glutathione other non-glucose reducing substances must be present in variable quantities in the blood. One case showed this very clearly because the blood glutathione was 33 mg./100 ml., equivalent to 8.6 mg. glucose/100 ml. by the Schaffer-Hartmann method, and yet the difference between the 'true' and 'apparent' blood sugar was only 2 mg./100 ml. The discrepancy is beyond any experimental error

and shows that the blood must have contained a non-glucose reducing substance other than glutathione which passed into the cadmium filtrate to be estimated with the 'true' glucose. Recently Fraser (1950) has shown that the ergothioneine concentration is significantly higher in diabetics than in normals, and it is therefore possible that the above results may be due to an increase in the ergothioneine concentration or some other unknown substance in this particular patient.

The decrease in glutathione concentration in the blood could be due to one or more of several factors. There is no published evidence of an increased glutathione excretion in diabetes and it has not been possible for us to investigate this point. It is possible that the fall in glutathione in diabetic ketosis is due either to decreased production or to increased requirements for sulphhydryl compounds. Blood glutathione, however, is contained almost entirely within the red cells and it is therefore more probable that the fall in glutathione content of the blood is associated with some change occurring within the red cells. Grunert & Phillips (1949) have shown that sodium deficiency is accompanied by a low level of blood glutathione in the rat. Binet & Pautonet (1943) have reported that the low serum sodium found in patients with Addison's disease is accompanied by low levels of blood glutathione which are restored to normal by the administration of deoxycorticosterone acetate. Diabetic ketosis is known to be associated with an increased elimination of sodium and it is possible, therefore, that the concomitant fall in blood glutathione is brought about directly by sodium deficiency. This clearly requires further investigation.

SUMMARY

1. Total and reduced blood glutathione have been determined in 20 normal and 39 diabetic persons. A significant reduction in glutathione was found only in the diabetics with severe ketosis. This reduction may be related to the reduction of base in diabetic ketosis.

2. The glutathione content of blood has been compared with the difference between the concentration of 'true' glucose and total reducing substances. Glutathione does not always account for this difference, which suggests that other reducing substances not yet identified must be present in the blood.

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