Acetylation as a means of detoxication is not very common. So far it has been observed in the case of amino compounds only (1). \( p \)-Aminobenzoic acid, for example, is partially acetylated by the rabbit and eliminated as \( p \)-acetylaminobenzoic acid (2). It is conceivable that the acetyl group may be derived from carbohydrates, fats, or even proteins. In order to study this mechanism somewhat more in detail, the effect of the injection of insulin upon the acetylation process was examined. The observation of du Vigneaud (3) that insulin is inactivated by glutathione led us to extend our observations to the effect of the simultaneous injections of these substances upon the acetylation process.¹

EXPERIMENTAL

Female rabbits weighing 1 to 2 kilos were maintained on diets of 75 gm. of oats and 100 gm. of cabbage daily. They were injected subcutaneously with the \( p \)-aminobenzoic acid (in the form of the sodium salt), the 48 hour sample of urine collected, and the acetylated compound isolated. Insulin, together with the \( p \)-aminobenzoic acid, was next injected, then the glutathione plus the acid, and finally the insulin, glutathione, and the acid. For the sake of economy of space, the results are compressed into one table (Table I). The intervals between injections were approxi-

¹ We have for some time past been of the opinion that one of the functions of glutathione may be its detoxicating property, inasmuch as all three of its amino acids are typical detoxicating agents of the body.
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approximately 1 week. During the course of the experiment, the weights of the rabbits varied from 1.3 to 2.2 kilos. The total nitrogen in urine (in gm. per 48 hours) varied as follows: Rabbit 1, 1.10 to 2.00; Rabbit 2, 1.00 to 1.25; Rabbit 3, 1.46 to 2.21; Rabbit 4, 1.03 to 1.91; Rabbit 5, 1.52 to 2.00. To make clear certain points in Table I, we call attention to the second column. When 1 gm. of the $p$-aminobenzoic acid was injected, 0.30 gm. of the acetylated compound was excreted. This was followed by a 2 gm. injection

| TABLE I |
| Effect of Injection of $p$-Aminobenzoic Acid, Insulin, and Reduced Glutathione |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| $p$-Aminobenzoic acid injected | 
| gm. | 
| 1.0 | 2.0 | 1.0 + insulin* | 2.0 + " | 1.0 + reduced glutathione† | 2.0 + reduced glutathione† | 1.0 + insulin and glutathione | 2.0 + insulin and glutathione |
| 0.30, 0.28 | 0.65, 0.50 | 0.62 | 1.02 | 0.29 | 0.55 | 0.44 | 0.55 |
| 0.15, 0.16 | 0.20, 0.21 | 0.28 | Died | Died | 0.41 | 0.30 | 0.57 |
| 0.32, 0.29 | 0.40, 0.42 | 0.57 | 1.18 | 0.30 | 0.41 | 0.57 | 0.27 |
| 0.10 | 0.15 | 0.16 | Died | Died | 0.23 | 0.19 | 0.27 |

* 0.5 unit per kilo of body weight.
† 5.0 mg. per kilo of body weight.

of $p$-aminobenzoic acid and an excretion of 0.65 gm. of the acetylated compound. Again 1 gm. of $p$-aminobenzoic acid was injected and 0.28 gm. of the acetylated compound was eliminated. This was followed by an injection of 2 gm. of $p$-aminobenzoic acid and an elimination of 0.50 gm. of the acetylated compound. The first two lines in the third and fourth columns are to be viewed similarly.

For the isolation of the acetylated compound, the 48 hour sample of urine, preserved with toluene, was neutralized, evaporated on a steam bath to a thick syrup, cooled, acidified to Congo
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red with sulfuric acid, and finally extracted with ether for 10 hours in a continuous extractor.\textsuperscript{2} The ether extract was evaporated to dryness on a steam bath and the residue taken up with 10 cc. of water. Enough 3\textsubscript{N} hydrochloric acid was now added to convert any unchanged \(p\)-aminobenzoic acid into its water-soluble hydrochloride. The insoluble acetylated compound was filtered and recrystallized from boiling water and norit. The product was filtered, dried at 60\textdegree, and its melting point determined. A mixed melting point (with some of the acetylated compound obtained from the Eastman Kodak Company) was also determined. The melting point was found to be 250\textdegree. The nitrogen determined was 7.78 per cent and 7.80 per cent; calculated 7.82 per cent.

\textbf{DISCUSSION}

It is quite obvious that insulin markedly increases the output of the acetylated compound. That this is the result of a stimulating effect upon carbohydrate metabolism seems probable. It further emphasizes the probable importance of acetaldehyde as an intermediate product in carbohydrate (and fat?) metabolism. Reduced glutathione\textsuperscript{3} alone, on the other hand, has no effect upon the acetylation process. But, in striking confirmation of du Vigneaud's \textit{in vitro} experiments, the simultaneous injections of insulin and glutathione very definitely inhibit the output of \(p\)-acetylaminobenzoic acid. Such a result, as du Vigneaud indicates, is probably due to the inactivation of insulin by glutathione.

Approximately 75 per cent of the original \(p\)-aminobenzoic acid has still to be accounted for. On an average, for 1 gm. of the acid injected an amount of acetylated product is recovered in the urine which, calculated as \(p\)-aminobenzoic acid, amounts to about 0.25 gm. Undoubtedly some of the acid is eliminated in the form of the glucuronate. Indeed, after removing the acetylated product with ether, the residue gives a very striking color test with naph-

\textsuperscript{2} This continuous extractor is a modification of one designed by Professor H. T. Clarke. It was built by Mr. R. Rosenthal of the College of the City of New York, to whom our thanks are due.

\textsuperscript{3} The glutathione was neutralized to litmus in an atmosphere of nitrogen. We wish to thank Professor du Vigneaud for details of the procedure. We also wish to thank Professor E. C. Kendall for some of the glutathione.
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thoresorcinol. This is in accord with the views of Quick (4), who, however, overlooks the possibility of acetylation.

SUMMARY

1. p-Aminobenzoic acid is acetylated by the rabbit to the extent of about 25 per cent.
2. The injection of insulin (0.5 unit per kilo of body weight) markedly increases the output of the acetylated compound.
3. Reduced glutathione (5 mg. per kilo of body weight) does not affect the acetylation process.
4. The simultaneous (but separate) injections of insulin and reduced glutathione produce a decrease in the amount of acetylated product as compared to the injection of insulin alone, thus indicating an inhibition of insulin activity by reduced glutathione in vivo.
5. The probable bearing of these results on intermediate metabolism is stressed.

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STUDIES IN ACETYLATION. THE FATE OF p-AMINOBENZOIC ACID IN THE RABBIT
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