

Acetylation of Sulfanilamide as Influenced by the Thyroid.*

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The acetylation of sulfanilamide has proved to be a useful reaction for the determination of the acetylating capacity of the animal organism. The reaction has been shown to take place in the liver^{1,2} in most animal species and to be irreversible.³⁻⁵ An increase in the degree of acetylation was observed following the administration of various metabolites, such as acetate,⁶⁻⁹ pyruvate,^{7,10} lactate,⁷ alcohol⁶ and glucose.⁷ The *in vitro* experiments of Klein and Harris² and of Lipmann¹¹ demonstrated an increased reactivity if acetate, pyruvate, lactate, acetoacetate, or acetoin were added to the reaction vessels. Lipmann, using pigeon liver as the most adaptable tissue for these studies, found the conjugation to take place not only in the slice but also in homogenates and even in extracts of the liver.

Inasmuch as the thyroid greatly affects metabolism and products of intermediary metabolism have been shown to influence the degree of acetylation of administered aromatic

amines, a study of the effect of the thyroid on the *in vivo* acetylation of sulfanilamide was undertaken.

Methods and Results. The experiments here reported were carried out on female rats weighing approximately 200 g. The series consisted of untreated controls, rats which had received a diet consisting of 1% desiccated thyroid for 2 to 3 weeks, rats which had been thyroidectomized 2 to 3 months prior to the experiment, and rats which had received 0.1% thiouracil in their drinking water for 3 weeks. The animals were fasted overnight, injected with 5 ml of approximately 1% sulfanilamide solution, put in metabolism cages with free access to food and water for 24 hours, and the urine collected and analyzed according to the method of Bratton and Marshall.¹² The total amount of sulfanilamide excreted was approximately the same in all instances. The per cent of this amount which was acetylated varied with the experimental conditions; these data are recorded in Table I.

Statistical analyses¹³ of the data were carried out by the standard procedure for small samples of determining the standard error of the difference between the 2 means. In the experiments using hyperthyroid rats, the difference between the means of the control and experimental groups was more than 3 times the standard error, the difference between the means of the controls and thiouracil-treated group was 2.5 and between the controls and thyroidectomized group it was 2.2. Taken in conjunction with the probability of the occurrence of the deviations derived from Fisher's "t" table, which is shown in Table I, the difference found in the hyperthyroid group appears to be definitely significant; the differences in the thyroid-

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¹ Stewart, J. D., Rourke, G. M., and Allen, J. G., *Surgery*, 1939, **5**, 232.

² Klein, J. R., and Harris, J. S., *J. Biol. Chem.*, 1938, **124**, 613.

³ Fishman, W., and Cohn, M., *J. Biol. Chem.*, 1943, **148**, 619.

⁴ van Winkle, W., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1940, **69**, 40.

⁵ Robinson, E. J., and Crossley, M. L., *Arch. Biochem.*, 1943, **1**, 415.

⁶ Bernhard K., *Z. physiol. Chem.*, 1940, **267**, 91, 99; 1941, **271**, 208; 1942, **273**, 31.

⁷ Harrow, B., Mazur, A., Borek, E., and Sherwin, C. P., *J. Biol. Chem.*, 1934, **105**, xxxiv.

⁸ Harrow, B., Mazur, A., and Sherwin, C. P., *Biochem. Z.*, 1937, **293**, 302.

⁹ James, G. O., *Biochem. J.*, 1939, **33**, 1688; 1940, **34**, 633.

¹⁰ Martin, G. J., and Rennebaum, E. H., *J. Biol. Chem.*, 1943, **151**, 417.

¹¹ Lipmann, F., *J. Biol. Chem.*, 1945, **160**, 173.

¹² Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

¹³ Arkin, H., and Colton, R. R., *An Outline of Statistical Methods*, 4th edition, 1939, pp. 126-127.

TABLE I.
Acetylation of Sulfanilamide.

Exp. condition	% acetylated		Diff.	P†
	Exp.	Control*		
Hyperthyroidism	30 ± 1.8 (11)†	39 ± 1.6 (11)	—23%	.01
Thyroidectomy	33 ± 2.2 (7)	39 ± 1.2 (6)	—15%	.05
Thiouracil treatment	44 ± 1.5 (11)	39 ± 1.4 (11)	—13%	.05

* For each experimental animal a control animal was run at the same time to minimize differences due to temperature, humidity, diet, etc.

† Figures represent mean values ± the standard error. Numbers in parentheses refer to number of rats per group.

‡ Probability of occurrence of deviation, obtain from Fisher "t" table.

ectomized- and thiouracil-treated groups just barely significant.

The results show that both hyperthyroidism and thyroidectomy produced a decrease in the degree of acetylation of sulfanilamide, whereas thiouracil treatment caused an increase. It is difficult to interpret the variance in the effects of thyroidectomy and thiouracil. A possible explanation may be that thiouracil exerts other effects than those depressing the activity of the thyroid.

It may be noteworthy to compare these results with the changes observed in an entirely different experiment, in which the high energy containing phosphate compounds of

the liver were determined under similar conditions;¹⁴ hyperthyroidism was associated with a decrease in the specific activity of the labile phosphorus, thyroidectomy with no change in this value, and thiouracil treatment with an increased specific activity of the labile phosphorus.

Summary. The influence of the thyroid on the acetylation of sulfanilamide was studied. Hyperthyroidism and thyroidectomy caused a decrease in the amount of sulfanilamide acetylated; thiouracil treatment led to an increase.

¹⁴ Greenberg, D. M., Fraenkel-Conrat, J., and Glendening, M. B., unpublished observations.

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Effect of Penicillin on Blood Coagulation.*

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Moldavsky, Hasselbrook and Caterno¹ have recently reported that both oral and intramuscular penicillin consistently shorten the coagulation time of normal blood and produce

a nonretractile clot. These authors also observed decreased bleeding times in patients receiving penicillin. Hines and Kessler² demonstrated increased sensitivity to heparin in 2 of 10 cases receiving penicillin but found no changes in the platelet counts or prothrombin times.

The widespread use of penicillin makes this an important clinical problem. Therefore, it was decided to investigate more thor-

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¹ Moldavsky, L. F., Hasselbrook, W. B., and Caterno, C., *Science*, 1945, **102**, 38.

² Hines, L. E., and Kessler, D. L., *J. A. M. A.*, 1945, **128**, 794.