THE FUNCTION OF VITAMIN A IN ADRENAL STEROID PRODUCTION

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Vitamin A deficiency has been found to cause a decrease in total steroid production by quartered rat adrenal glands (Van Dyke and Wolf, 1958), and using adrenal homogenates it has been found that vitamin A deficiency causes a decrease in the incorporation of cholesterol-4-C^{14} into corticosterone (Van Dyke, Johnson and Wolf, 1960). In this paper we would like to report the effect of the in vitro addition of vitamin A on the incorporation of radioactivity from cholesterol into corticosterone.

To minimize general morbidity effects, rats were used while still in the relatively early stages of deficiency. In Experiment 1 and 3 (Tables I and III) the rats showed no gross symptoms but had plateaued in weight, although no loss had been demonstrated. These rats had been consuming the vitamin A-deficient diet for 2 weeks (Wolf, Lane and Johnson, 1957). In Experiment 2 (Table II) the rats had been on the deficient diet for 5 weeks and were losing weight.

Table I
Conversion of Cholesterol to Corticosterone in Adrenal Tissue

Incubation Addition	C^{14} Activity in Corticosterone in Deficient Tissue	C^{14} Activity in Corticosterone in Normal Tissue
	dpm	dpm
Homogenate	2650	7548
Homogenate + glucose-6-phosphate*	3104	9196
Homogenate + glucose-6-phosphate* + glucose-6-phosphate dehydrogenase**	2412	8552

DPN and TPN, 0.5 mM; ATP, 1 mM. 0.65 μc cholesterol-4-C^{14} was added to each incubation. Incubated for 2 hours under 95% O_2, 5% CO_2 at 37° C. Total volume was 3 ml.

*Glucose-6-phosphate, 4 mM.

**Glucose-6-phosphate dehydrogenase, activity to produce 3 μmoles TPNH per minute.

Table II
Effect of In Vitro Addition of Vitamin A Acid on the Conversion of Cholesterol to Corticosterone

Incubation Addition	C^{14} Activity in Corticosterone in Deficient Tissue	C^{14} Activity in Corticosterone in Normal Tissue
	dpm	dpm
Homogenate	2862	14,086
Homogenate + vitamin A acid*	7641	13,975

Incubation conditions were the same as in Table I.

The adrenals were removed from the animals and homogenized in the medium of Eichhorn and Hechter (1957), an equal weight of deficient and normal adrenal tissue being used in all cases. In all incubations 3 ml of the whole homogenate, which contained 50 mg wet weight of adrenal tissue, were used. The incubations with cholesterol-4-C^{14} were carried out as given in the tables.

The incubations were stopped by the addition of acetone and the precipitate was removed. The acetone was then removed under nitrogen and the aqueous solution was extracted with benzene-chloroform (6:1) three times to remove the steroids. The benzene-chloroform mixture containing the steroids were separated by chromatography in the propylene-glycol-toluene system of Burton, Zafforoni and Keutman (1951). Following chromatography the radioactive compounds were eluted and the eluates were counted in a Packard Tri-Carb liquid scintillation counter. The different steroids were identified by chromatography and comparison with standards as well as by preparation of their acetates and rechromatographing.

The data in Table I show a marked depressing effect of vitamin A deficiency on the incorporation of cholesterol-4-C^{14} into corticosterone. Added TPNH regenerating systems were tried and found to have essentially no effect on corticosterone synthesis.

The data in the second experiment show a definite increase in corticosterone production when vitamin A acid is added to the deficient adrenal homogenates in vitro. The experiment was repeated in order to examine the effect of in vitro addition of vitamin A alcohol as well as of the acid, and as can be seen in Table III both vitamin A alcohol and vitamin A acid increased corticosterone production.

*1.3 μmoles of vitamin A acid added per incubation.

Table III
Effect of the In Vitro Addition of Vitamin A Acid on the Conversion of Cholesterol to Corticosterone

Incubation Addition	C^{14} Activity in Corticosterone in Deficient Tissue	C^{14} Activity in Corticosterone in Normal Tissue
	dpm	dpm
Whole homogenate	9630	13,706
Whole homogenate + vitamin A alcohol*	14,998	-**
Whole homogenate + vitamin A acid*	16,576	-**

DPN and TPN, 0.5 mM; ATP, 1 mM. 1 μc cholesterol-4-C^{14} was added to each incubation. Incubated for 1 hour under 95% O_2, 5% CO_2 at 37° C. Total volume was 3 ml.

It must be pointed out that the aberration in adrenal glucocorticoid biosynthesis occurs very early in the development of the deficiency and before the block in cholesterol biosynthesis shown by Gloor and Wiss (1959) can be demonstrated in an in vitro system (Wright, Wolf and Johnson, 1960). In the rats used in these experiments it was not possible to demonstrate a decrease in cholesterol biosynthesis by liver homogenates (Wright, Wolf and Johnson, 1960), but as noted in the tables, a marked decrease in the ability of the homogenates to synthesis corticosterone from cholesterol was shown.

The ability to restore, at least partially, the synthesis of corticosterone in an in vitro system by the addition of vitamin A alcohol and acid may indicate the possibility that some form of vitamin A may be functioning as a cofactor or coenzyme for one of the enzymes necessary for the transformation of cholesterol to corticosterone.

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*Vitamin A alcohol and vitamin A acid added at levels of 1.3 μmoles per incubation.
**Not determined.

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