TABLE II. Fixation of C'1 from Various Hemolysins.

	Hemolysin A	Hemolysin B	Hemolysin C	Hemolysin D
	O.D.			
EA	1.315	.260	.063	.015
EA (EDTA)	.007	.017	.009	.007
EA (extracted)*	.010	.008	.000	—

* EA incubated at 37°C for 15 min. in presence of .008 M Na_3HEDTA, centrifuged, washed twice and restandardized.

TABLE III. Stability of Rabbit C'1 at 56°C.

	Incubation time			
	0 min.	30 min.	45 min.	60 min.
	O.D.			
EA	1.170, 1.180	.890, .850	.790, .752	.541, .535
EA (EDTA)	.039, .030	.026, .024	.028, .031	.021, .031

ponent of C' reacts prior to C'1. even in the absence of cations. the further precaution of using purified rabbit antibody(8,9) may be necessary.

Summary. Commercial rabbit antisheep cell hemolysins contain rabbit C'1 which combines with the sheep erythrocyte-rabbit antibody complex. Heating at 56°C for one hour does not completely destroy the activity of the C'1. EDTA blocks the reaction of rabbit C'1 and can be used to elute rabbit C'1 combined with the erythrocyte-antibody complex.

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Received November 13, 1961. P.S.E.B.M., 1962, v109.

Effect of Diets and Hormones on Two Urea Cycle Enzymes.* (27215)

R. A. FREEDLAND AND C. H. SODIKOFF

University of California School of Veterinary Medicine, Agricultural Experiment Station, Davis, Calif.

Recently developed methods have made it possible to assay quantitatively all the enzymes in the urea cycle(1). It has further been shown that in most species, including the rat, the arginine synthetase system had the lowest activity of enzymes participating in the urea cycle(2). Arginine synthetase consists of 2 reactions, the conversion of citrulline and aspartate to argininosuccinate followed by the cleavage of this compound to arginine and fumarate. In all cases studied (2,3), the first of these reactions was shown to be the rate limiting reaction. Since there have been numerous reports(4,5,6) on the effect of high protein diets in raising liver arginase values and on the effect of adrenalectomy, which decreases protein catabolism, and causes a decrease in liver arginase and

* This work was supported in part by U.S.P.H.S. National Institute of Health Research Grant A-4732.

TABLE I. Effect of Conditions Causing Excess Protein Catabolism on Several Rat Liver Enzymes.

Group	No. of animals	% of body wt as liver	% liver protein	% of control values* Arginase	Arginine synthetase	Lactic acid dehydrogenase
Control	50	4.62 ± .04†	100	100 ± 5	100 ± 4	100 ± 5
Hydrocortisone	12	6.21 ± .11	100	251 ±21	285 ± 17	143 ±16
Cortisone	12	6.24 ± .15	100	278 ±19	276 ± 17	128 ± 9
Hyperthyroid	8	4.58 ± .11	110	118 ± 8	172 ± 12	94 ± 6
Diabetic	11	3.86 ± .09	98	116 ± 9	184 ± 15	72 ± 7
Fasted	8	3.41 ± .08	117	58 ± 5	140 ± 7	50 ± 4
High protein	26	4.92 ± .07	115	145 ± 8	266 ± 13	111 ±12

* See text for actual values. † Stand. error of mean.

arginine synthetase(3), it was thought to be important to study arginine synthetase levels under conditions of excessive protein catabolism.

Materials and methods. Male albino rats weighing between 100 and 200 g were used. The animals were offered food *ad libitum*. The basic diets have been described(7). All animals except the high protein, fasting, and hyperthyroid groups received the dextrin diet. The high protein group received the high protein diet and the hyperthyroid group received the dextrin diet fortified with 4% iodinated casein. All animals received the special diets or treatments from 3-5 days before sacrifice. Hydrocortisone and cortisone were administered as a suspension of the acetate at the rate of 5 mg per animal per day subcutaneously. The rats were made diabetic by alloxan injection. Arginase and arginine synthetase were performed by the method of Brown and Cohen(1), lactic acid dehydrogenase by DPNH oxidation(8), blood glucose by Somogyi's(9) modification of Nelson's (10) method, carbon-dioxide by titration, and protein by the method of Lowry, *et al.*(11).

Results and discussion. The results have been calculated as units per liver, per g of liver protein or per 100 g of body weight, since this method more closely reflects the animal's total ability to produce a given product in relation to its need. Since many individual experiments were performed and control values varied between experiments, all values are reported as percent of control value. Average control values and standard errors are as follows: Percent liver protein 15.2 ± 0.3, arginase activity/100 g body weight (μM urea formed/minute) 1,427 ± 64, arginine synthetase activity/100 g body weight (μM urea formed/minute) 1.83 ± 0.08, lactic acid dehydrogenase activity/100 g of body weight (μM DPNH oxidized min) 1,505 ± 69. Diabetes was confirmed by blood glucose levels of 300 mg % or higher in all animals and the hyperthyroidism was confirmed by an increase in CO_2 production from .370 mM CO_2/min/$Kg^{3/4}$ for the normals to 1.318 mM CO_2/min/$Kg^{3/4}$ for the hyperthyroid group. It is apparent from the data in Table I that all treatments which would be expected to increase protein catabolism, regardless of whether the animal has gained or lost weight, also produce a significant increase in arginine synthetase. The results with arginase are not as clear cut as those with the arginine synthetase as only in 3 of the 6 groups did this enzyme show a significant increase. Moreover, in all groups which had an increase in protein catabolism, and maintained or gained weight, the arginase increased, and in those groups in which the animals were losing weight arginase did not appreciably change or decrease. It also appears that the arginase activity pattern somewhat parallels that of lactic acid dehydrogenase, which was run as a control enzyme since, like arginase, it occurs in great excess of theoretical demands. The activity of at least one of the urea cycle enzymes, arginase, may be controlled by two factors: First, degree of protein catabolism, and second, nitrogen balance of the animal, if one correlates weight loss to negative nitrogen balance and a gain to positive nitrogen balance. Unfortunately, nitrogen balance was not measured

in these experiments, since the observed results with arginase were unexpected. Apparently the change in body weight, which may be related to nitrogen balance, affects both arginase and lactic acid dehydrogenase similarly, but shows less of the effects on arginine synthetase activity. This may be of importance to the overall protein economy of the animal as both arginase and lactic acid dehydrogenase do not appear to be limiting or pace setting enzymes in either the urea cycle or the glycolytic scheme. In contrast, arginine synthetase, which apparently is the limiting enzyme in the urea cycle and therefore probably the "pacemaker enzyme", increases during excessive protein breakdown regardless of the nutritional state of the animal, even when the other 2 enzymes show significant decreases in activity as during fasting. The synthetase also shows a greater percentage increase than arginase in all cases of increased protein catabolism, except after administration of glucocorticosteroids. Thus, arginine synthetase may be a better indicator of nitrogen catabolism than arginase. Further studies on the importance of nitrogen balance and hormonal dietary interrelationships are being conducted using more than one treatment per animal and using surgically altered animals.

Summary. All treatments increasing protein catabolism produced an increase in arginine synthetase activity. Arginase activity was increased only in cases where the increased protein breakdown was accompanied by an increase or maintenance of body weight. The changes in lactic acid dehydrogenase, used as a control enzyme, more nearly parallel those in arginase and show very little relationship to changes in arginine synthetase. It has been suggested that arginine synthetase would be a better indicator enzyme of nitrogen catabolism than arginase.

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Received November 13, 1961. P.S.E.B.M., 1962, v109.

Hemagglutinin Loss by Vaccinia Virus and Its Relationship to Virus Adaptation in Tumor and L Cells. (27216)

WILLIAM A. CASSEL,* R. EARL GARRETT, AND WARNER L. BLAIR
Department of Microbiology, Division of Basic Health Sciences, Emory University, Atlanta, Ga.

An earlier report described how vaccinia virus strain IHD-E lost its hemagglutinin-producing ability on serial passage in the Ehrlich ascites tumor(1). This loss occurred along with a marked increase in ability of the virus to multiply in the tumor, and to cause lysis of the tumor cells. At the time, there was no way of determining how closely integrated the hemagglutinin loss was with the other concomitant changes in the virus. It became possible to examine this relationship when a hemagglutinin-negative virus was derived in an L cell culture system containing a hemagglutinin inhibitor from the Ehrlich tumor(2). The present report describes this examination.

Materials and methods. Viruses TF-1, TF-32, and TF-33 are respectively the first, thirty-second, and thirty-third passages of

* This investigation was supported by a research grant and by a Senior Research Fellowship from U. S. Public Health Service.