ture showed a classical diploid karyotype but with some increase in frequency of tetraploids (6.9%). The spontaneous chromosome alterations that characterize the terminal phase of human fibroblasts in culture(20,21) are not yet present in these cells at their 95th generation. The same cell strain, however, demonstrated the typical terminal aneuploid degeneration after 50-70 generations on the standard medium(22). It is concluded therefore that these chromosomal alterations need not occur after any fixed number of cell generations in culture.

Summary. Serially transferred hamster or human fibroblasts maintained on synthetic medium containing 10% calf serum could be carried through about 10 and about 50-70 cell generations respectively, before growth ceased. Addition of crystallized serum albumin to the medium to a concentration of 20 mg/ml affected the growth potential so that in both cases over 100 cell generations could be obtained. The human fibroblasts after 95 cell generations showed no chromosomal abnormalities while in the hamster fibroblasts some deviation from the diploid number did occur. In neither case was there evidence of the evolution of improved growth properties that characterize established cell lines.

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Urea Cycle Adaptations in Intact and Adrenalectomized Rats.* (29347)

R. A. FREEDLAND

Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis

In studies on protein catabolism, especially the final stages as urea synthesis, the work of Ratner(1-3), and that of Cohen's group (4-6) in elucidating the individual steps of the urea cycle and in providing relatively simple methods for assaying these enzymes

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has made it possible to study changes in individual urea cycle enzymes in detail. Since the formation of urea is a vital segment of protein catabolism in the mammal, this process provides a natural place to look for "adaptations" or "metabolic controls" where an adaptation may be described as "a change in the nature or capacity of a biological system in response to a change in the internal or external environment."

Arginase, the terminal enzyme of the urea cycle, is easily measured due to its relatively high activity in the liver. Therefore, this enzyme was the first of the urea cycle enzymes to be examined for a possible adaptation. Early reports(7-11) indicated that increased ingestion of protein caused increases in liver arginase activity. In the latter of these reports(11), it appears that all of the urea cycle enzymes exhibit a behavior pattern similar to arginase in respect to amount of dietary protein intake. It further appears that in the case of the 2 of these enzymes, arginase and ornithine transcarbamylase, the normal and "induced" enzymes have identical properties by several criteria(11). Thus, it appears there is an increase in amount of enzyme present and not an activation of existing enzyme. During our present study on metabolic adaptations, it was observed that both high protein diets and glucocorticosteroid injections increased 2 urea cycle enzymes, arginase and arginine synthetase (12). Similar findings have been reported for other enzymes involved in protein catabolism(13). Other differences were observed between the behavior of arginase and arginine synthetase activities with various dietary and hormonal treatments(12). It was also reported that adrenalectomy decreased the activity of these two enzymes(14). It has also recently been shown that cortisone can restore the activity of these enzymes to normal in adrenalectomized rats(15,16) and that adaptation to high protein diets occurs in these adrenalectomized animals(16). Therefore, it appeared important to delineate the role of the adrenal hormones, if any, in the adaptation of these 2 enzymes, especially in response to diets which are not exceptionally high in protein content and non-adrenal hormones. This report concerns a closer study of this problem and of factors affecting the activities of arginase and arginine synthetase.

Materials and methods. Animals and diets. Male Sprague-Dawley rats weighing between 100-200 g obtained from Diablo Animal Laboratories, Berkeley, Calif., were used. All rats were kept on identical diets for 3 days before being transferred to experimental diets. This diet was either the control (dextrin diet) or commercial laboratory rat chow. During the experiment, all animals were housed in screened bottom cages and provided with food and water *ad libitum*. In the case of the adrenalectomized rats, a 1.5% NaCl solution was used in place of water. Adrenalectomies were performed by Diablo Animal Laboratories and the effectiveness of the operation was examined by randomly placing animals on salt free diets. Ten out of 12 adrenalectomized animals succumbed to this treatment within 3 weeks, whereas only one out of 12 normal animals died from this treatment within this period. Thus, the operation appeared to be successful in a high percentage of cases and the chances of having more normal than adrenalectomized animals in a group thought to be adrenalectomized is highly improbable. The adrenalectomized rats were used for experimental purposes at varying times after the operation, ranging from one to 4 weeks. No differences were observed in the results with length of time after operation, nor were any noticeable differences observed with the time of the treatment period between 3 and 6 days.

In some cases the adrenalectomized rats would not accept the control diet. In these groups, the animals were offered ground laboratory rat chow with progressively more dextrin diet (control) mixed into the ground chow, until they accepted the experimental diets. At this time, food consumption and weight gains were approximately the same as that of intact animals. The diets are described in Table I. The dextrin (obtained from Nutritional Biochemicals Co.) diet also served as the control.

The animals were killed from 3-6 days after being offered the experimental diets

TABLE I. Composition of Diets.

	Control	Sucrose	Fructose	Margarine	High protein	Hyper-thyroid
Salts*	4	4	4	4	4	4
Vitamins*	1	1	1	1	1	1
Corn-oil	5	5	5	5	5	5
Casein	25	25	25	25	90	25
Dextrin	65					61
Sucrose		65				
Fructose			65			
Margarine				65		
Iodinated casein						4

Composition are given as % of diet.
* Salt mixture P-H and vitamin diet fortification mixture obtained from Nutritional Biochemicals Co., Cleveland, Ohio.

or initial injections. After exsanguination by decapitation, the livers were carefully and completely removed, weighed and placed in ice. A portion of the liver was homogenized in exactly 9.0 volumes of 0.1% solution of hexadecyltrimethyl-ammonium bromide. The homogenate was centrifuged at 4,000 $\times$ *g* for 30 minutes and the supernatant solutions were used for all enzyme assays. Assays for arginase and arginine synthetase were performed by the method of Brown and Cohen (6), except that only 0.2 ml of supernatant was used in the arginine synthetase assay, since results using 0.5 ml were at the far end of the linear response. The resulting urea was measured by Ratner's(17) modification of Archibald's(18) method. Lactate dehydrogenase was measured by the rate of DPNH oxidation in presence of pyruvic acid(19), and proteins by the method of Lowry *et al* (20). Carbon dioxide in the respiration trials was trapped in standardized NaOH, precipitated with $BaCl_2$, and the remaining NaOH was titrated. Amount of NaOH consumed prior to titration is directly proportional to amount of CO_2 produced. Hyperthyroidism was confirmed in the iodinated casein group by distinctly higher CO_2 production, 3.70 mM CO_2/min/$Kg^{3/4}$ for control animals and 1.318 mm CO_2/min/$Kg^{3/4}$ for hyperthyroid rats. The livers of the hyperthyroid rats were practically devoid of glycogen compared to levels of from 4-5% glycogen in control animals. All animals except those receiving iodinated casein in the diet gained weight; the iodinated casein fed animals lost weight.

Results. Enzyme units, as suggested by the International Union of Biochemistry Commission on Enzymes(21) on the reporting of enzyme results, are defined as the amount of enzyme that will convert 1 μmole of substrate to 1 μmole of product in 1 minute. Since the final catabolism of nitrogen to urea takes place primarily in the liver, a true "metabolic adaptation" of a system that provides a detoxification for all body tissues should result in a change in activity of the system on the basis of total body requirements, which may be related to body weight. Therefore, the emphasis is placed on the activity per 100 g of body weight and standard errors are reported for these values. Although both the kidney and brain have functional urea cycles(22), it is most probable that their quantitative aspects are very small compared to that of the liver. Lactate dehydrogenase was followed as a control enzyme. Since it occurs in a large excess of theoretical demand it might be thought to give a picture of the overall change in non-limiting enzyme systems.

Adrenalectomy caused decreases in both arginase and arginine synthetase activity (Table II). No differences in liver weight to body weight ratio, liver protein, or lactate dehydrogenase activity were observed after adrenalectomy. In intact rats only the high protein diet and fructose diet caused significant increases in liver arginase activity. Both these diets and the iodinated casein diet caused increases in liver arginine synthetase activity. Apparently, conditions which increase protein catabolism but cause a loss of weight result in increases in liver arginine synthetase activity but not in arginase activity, as is also true of diabetic rats(12). The

TABLE II. Effect of Diets and Hormones on Several Liver Constituents in Intact and Adrenalectomized Rats.

	Intact					
	Control	Iodinated casein	High protein	Sucrose	Fructose	Margarine
No. of animals	70	8	26	32	15	12
Liver wt/body wt × 100	4.66 ± .05‡	99	117	116	130	102
Liver protein %	17.7 ± .04	100	115	89	91	106
Arginase	1805 ± 59	118 ± 8	145 ± 8†	110 ± 5	152 ± 8†	109 ± 13
Arginine synthetase	2.06 ± .08	172 ± 12†	266 ± 13†	112 ± 9	142 ± 8†	92 ± 6
Lactate dehydrogenase	1670 ± 102	94 ± 6	111 ± 12	126 ± 11	111 ± 7	184 ± 11†
	Adrenalectomized					
	Control	Iodinated casein	High protein	Sucrose	Fructose	Margarine
No. of animals	40	8	10	12	16	6
Liver wt/body wt × 100	4.56 ± .10	88	97	115	117	105
Liver protein %	17.2 ± .8	100	116	90	97	100
Arginase	1322 ± 79	95 ± 8	158 ± 10†	95 ± 6	158 ± 18†	92 ± 12
Arginine synthetase	1.49 ± .08	148 ± 18†	258 ± 18†	140 ± 20	163 ± 14†	87 ± 9
Lactate dehydrogenase	1556 ± 183	100 ± 13	106 ± 9	140 ± 14*	158 ± 10†	142 ± 8*

Control values are absolute values. Enzyme values are reported in units. All other values are reported as % of corresponding control value.

* and † differs from control value at * $p < .05$ and † $p < .01$, respectively. Calculated only for enzyme activity per 100 g body weight.

‡ Standard error of mean.

margarine diet which would be expected to increase protein catabolism, in order to maintain a normal blood glucose level, did not cause an increase in either of the measured urea cycle systems. However, the diet did cause an increase in liver lactate dehydrogenase activity. This does not rule out the possibility of excess protein catabolism during this dietary regimen. It is also of interest that although the fructose diet increased the activity of the urea cycle enzymes the sucrose diet did not produce a similar effect. The sucrose diet has previously been shown to have a negligible effect on nitrogen balance (23).

The role of the adrenals in the mediation of these changes of enzyme activity is particularly important since administration of glucocortical steroids increases the activity of these enzymes in the liver(12). This is of further interest since it has been reported that increases of liver glucose-6-phosphatase in response to various diets were not observable in adrenalectomized rats(24). This observation has been questioned by the finding that the glucose-6-phosphatase adaptation was not affected by adrenalectomy(25). The results after adrenalectomy in response to the various diets were similar to those observed with intact rats. Although the absolute increase in activity after removal of the adrenal glands was less than in intact animals, due to lower control values, the relative increases were comparable. It was interesting to note that both the sucrose and fructose diets caused significant increases in lactate dehydrogenase activity in adrenalectomized but not intact animals. It was particularly hopeful that the increase observed after feeding the fructose diet, which does not contain excess protein above the level found in the control diet, would be eliminated by adrenalectomy. However, this was not the case. Since this response to ingestion of fructose is not hormonally mediated it is difficult to explain.

It is apparent that if these "adaptations" are hormonally mediated, the adrenal is not the sole controlling gland. A hormone-diet interrelationship in these "adaptations" is being investigated.

Summary. Adrenalectomy causes significant decreases in arginase and arginine synthetase activity, but has no effect on lactic acid dehydrogenase activity. All treatments known to increase protein catabolism in-

creased the arginine synthetase activity. The results with arginase were not as clear cut as they also appeared to be affected by losses in weight. All changes in activity of the 3 enzymes, arginine synthetase, arginase, and lactic acid dehydrogenase, observed with various treatments in intact rats were also observed in adrenalectomized animals.

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Biologic Relationship of Endotoxin and Other Toxic Proteins. IV. Effect of Heparin on Endotoxin-Induced Hypersusceptibility to Snake Venoms.* (29348)

RICHARD M. CONDIE, EDWARD V. STAAB AND ROBERT A. GOOD

Pediatric Research Laboratories, Variety Club Heart Hospital, University of Minnesota, Minneapolis

Parenteral administration of sublethal doses of endotoxin is known to alter the nonspecific resistance of mammals to bacteria and viruses, and has recently been shown to affect susceptibility of rabbits to some snake venoms(1), including those of *Agkistrodon piscivorus* (water moccasin), *Vipera russellii* (Russell's viper), and *Notechis scutatus* (tiger snake)(2). Although these are very complex toxins and their multiple activities are not well defined, it is known that Russell's viper and tiger snake venoms are powerful coagulants and that water moccasin venom has important hemorrhagic activity(3). Many venoms combine anticoagulant and coagulant activity, and this is true of all three of these venoms: *Vipera russellii* and *Notechis scutatus* also have anticoagu-

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