terized by marked degeneration and necrosis of monolayer cultures. Multiple small foci of degeneration and necrosis were observed as early as 16 hours post inoculation. These focal areas enlarged until most cells were affected by 72 hours after exposure to the virus.

Early cellular changes consisted of rounding of cells with condensation of the cytoplasm. These changes were often accompanied by vacuolization or ballooning of the cytoplasm and shrinkage of the nucleus. Next, necrosis, as evidenced by pyknosis of the nucleus and lysis of the cell, occurred rapidly. Many of the pyknotic nuclei assumed a rod or horseshoe shape. Neither intranuclear nor intracytoplasmic inclusions were observed.

Discussion. Duck hepatitis virus had been propagated in cell cultures derived from chicken embryos but cytopathic changes were not observed(2-4). CPE was first noticed in our experiments during the 8th passage of DHV in DEK cell cultures. Cytopathic effects occurred consistently from the 16th through the 26th passage in this system and usually appeared 24 to 36 hours after inoculation with virus.

Cell culture fluid containing infectious virus produced gross lesions of DHV in chicken embryos(5). Duck serum, known to contain antibodies against DHV, inhibited the production of CPE in DEK cell cultures and prevented occurrence of lesions in chicken embryos.

In other experiments, ducks inoculated with 2nd egg passage origin DHV exhibited gross and microscopic lesions of duck hepatitis(6). A liver suspension obtained from one of the affected birds was inoculated into DEK cell cultures. Focal areas of cytopathic changes similar to those described above have been sporadically observed during 5 passages of the liver material in DEK cell cultures. Fluid from the 25th DEK cell passage containing infectious virus and showing CPE failed to produce cytopathic changes in 2 passages in chicken embryo kidney cell cultures prepared in the same manner and with the same nutrient medium used for preparation of DEK cultures. These findings would suggest that the host cell is of prime importance in determining the nature and degree of cytopathic changes resulting from infection by DHV.

Summary. The cytopathic effect of duck hepatitis virus on duck embryo kidney cell cultures was characterized by marked degeneration and necrosis of the cells of the monolayers. Cytopathic effects occurred consistently through several viral passages and were usually apparent within 24 to 36 hours after inoculation. Inclusion bodies were not observed in the infected cells.

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Immediate and Prolonged Effects of Hydrocortisone on the Free Amino Acids of Rat Skeletal Muscle.* (28808)

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The facilitation of the transport of glucose from the extracellular to the intracellular space by insulin may be typical of the manner by which hormones control cellular me-

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tabolism(1). Endocrine control of the transport and availability of amino acids for metabolism and protein synthesis may also be an example of such control, and several studies have described increased transport and increased amino acid pools in cells of target organs following administration of hormones (2,3).

Similarly, it would be expected that hormones producing catabolic effects on tissues would decrease amino acid transport and concentration of amino acids in these tissues. The catabolic effects of hydrocortisone on tissues other than the liver are well known and it would be expected that hydrocortisone would decrease the free amino acid concentrations in these tissues. However, Kaplan (4) recently reported an increase in free amino acids of the skeletal muscle of adrenalectomized mice, 24 hours after injection of hydrocortisone. Because an increase in the free amino acids of skeletal muscle did not appear to be consistent with other evidence, the following study of both acute and chronic effects of hydrocortisone on skeletal muscle was initiated.

Experimental. For the acute experiments, white male rats (Sprague-Dawley) weighing 90-120 g were injected subcutaneously with 5.0 mg hydrocortisone acetate. Twenty-four hours after injection, the animals which had been fasted for 12 hours were sacrificed by decapitation and the blood collected in beakers containing heparin. To obtain adequate plasma for analyses, the blood from 5 or more animals was pooled and the protein removed with picric acid. Skeletal muscles from the thighs of these animals were also pooled and frozen immediately. All samples were prepared for chromatography according to Spackman *et al*(5).

The chronic experiments were carried out in the same manner except that 5 mg hydrocortisone was given every other day for 10 days.

Some of the samples of muscle were placed in an oven at 110° and dried to a constant weight to determine the dry weight of the tissues. The amount of blood in the tissues was determined by homogenizing a sample of tissue with distilled water, centrifuging and then measuring the amount of hemoglobin in the supernatant. The amount of blood found was so small that it was not necessary to perfuse the tissues or correct the muscle amino acids values for the presence of blood amino acids.

Results. The data of Table I show that hydrocortisone produced an elevation of the plasma amino acids during the 24 hours following first injection of hydrocortisone. However, a significant depression of the free amino acids of plasma occurs after repeated administration of hydrocortisone for 10 days. A catabolic response was obviously produced in rats since the average weight gain of the control rats was 64 g as compared to an average weight gain of 14 g in the hydrocortisone treated rats.

An elevation of the plasma amino acids by hydrocortisone has been reported by Kaplan(4), Bondy(6), Friedberg(7) and Lotspeich(8). Recently, however, Soupart(9), in studies on patients being treated with hydrocortisone, reported a decrease in the plasma amino acids. This difference appears to be resolved by the data of Table I in which it can be seen that hydrocortisone causes an initial elevation with a subsequent depression of the free amino acids. The investigators reporting an increase in free amino acids studied the effects of hydrocortisone during a period of 24 hours or less, whereas the latter study was the result of continued use of hydrocortisone.

The free amino acids of the muscles of the acute and chronically treated rats show the same general trend as does the plasma. An increase in free amino acids occurs at 24 hours with a decrease after prolonged treatment with hydrocortisone. An exception to this generalization is: a significant increase in aspartic acid in the muscle of chronically treated rats. There is also a large increase in muscle/plasma ratio of aspartic acid. The elevation of the aspartic acid concentration in muscle would suggest either formation in the muscle or increased transport from the plasma. Because of the high concentration of glutamic-oxaloacetic transaminase in muscle(10) stimulation of this transaminase would appear to be a plausible mechanism

TABLE I. Effect of Hydrocortisone on Ninhydrin Reactive Substances of Rat Muscle.

	Plasma			Muscle			Muscle/plasma		
	Control	Acute	Chronic	Control	Acute	Chronic	Control	Acute	Chronic
Phosphoethanolamine	—	.036	—	.172	.224	.130	—	6.22	—
Taurine	.130	.178*	.177*	2.29	8.27 *	8.80 *	56.08	46.46	49.72
Urea	2.95	3.21	3.83 *	3.57	4.77	6.14 *	1.21	1.49	1.60
Hydroxyproline	.038	.043	— *	.272	.209*	—	7.16	4.86	—
Aspartic acid	.012	.018*	.007*	.204	.254	.319*	17.00	14.11	45.57
Threonine	.167	.267*	.123*	.440	.544*	.285*	2.63	2.04	2.32
Serine	.192	.274*	.120*	.641	.812	.366*	3.34	2.96	3.05
Glutamine	.521	.672	.474*	2.16	2.56	1.32 *	4.15	3.81	2.78
Proline	.117	.149*	.071*	.195	.315*	.171	1.67	2.11	2.41
Glutamic acid	.090	.070*	.060*	1.46	1.46	.937*	16.22	16.22	15.62
Citrulline	.066	.90 *	.033*	.190	.186	.066*	2.88	2.66	2.00
Glycine	.330	.466*	.137*	4.51	5.08	1.10 *	13.67	10.90	8.03
Alanine	.296	.468*	.267	1.73	2.46	1.64	5.85	5.26	6.14
α-Amino-n-butyric acid	—	.012	—	—	—	—	—	—	—
Valine	.123	.154*	.126	.170	.220	.193	1.38	1.43	1.53
Half cystine	.014	.017	.015	—	—	—	—	—	—
Methionine	.037	.945	.029	.054	.074	.067	1.46	1.64	2.31
Isoleucine	.067	.088*	.069	.107	.130	.112	1.60	1.48	1.62
Leucine	.096	.131*	.104	.144	.193	.166	1.50	1.47	1.54
Tyrosine	.049	.062*	.038*	.075	.091	.076	1.53	1.47	2.00
Phenylalanine	.049	.068*	.052	.067	.091	.082	1.37	1.34	1.58
β-Alanine	.005	—	—	.086	.073	.133*	17.20	—	—
Ornithine	.030	.032	.039	.035	.064	.030	1.17	2.00	.76
Ethanolamine	.007	.009	.007	.048	.044	.063	6.86	4.89	9.00
Ammonia	.261	.453*	.338	4.40	5.01	3.59	16.85	11.06	10.62
Lysine	.266	.341*	.250	.681	.893	.319*	2.56	2.62	1.28
l-Methylhistidine	—	.005	—	—	—	—	—	—	—
Histidine	.045	.053	.046	.156	.147	.100*	3.47	2.77	2.17
Anserine	—	—	—	1.00	.794	1.26 *	—	—	—
Tryptophan	.004	—	—	—	—	—	—	—	—
Creatinine	—	—	—	6.47	10.74	9.82	—	—	—
Carnosine	—	—	—	2.35	1.51	1.63	—	—	—
Arginine	.075	.059	.048	.179	.203	.131	2.39	3.44	2.73
Total free amino acids	2.69	3.58 *	2.11 *	16.91	18.36 *	10.50 *	6.29	5.13	4.98
Total ninhydrin reactive	6.04	7.47 *	6.46	38.86	47.42 *	39.04	6.43	6.35	6.04

Plasma value = μmoles/ml. Tissue values = μmoles/g wet wt.
Five determinations each of 5 pooled samples.
* Determinations significant to $p < .05$ by Mann-Whitney U test(18).

for the increased aspartic acid. To our knowledge, no study of the effect of hydrocortisone on muscle transaminase has been reported; however, the effect of hydrocortisone on glutamic-pyruvic and glutamic-oxaloacetic transaminase in liver has been reported and only glutamic-pyruvic transaminase was increased (11).

The only significant increase in muscle/plasma ratio was found in the case of proline. The hydroxyproline ratio for chronically treated rats could not be calculated because of the complete disappearance of hydroxyproline from the plasma and muscle (< 0.010 μmole).

All the remaining significant changes in muscle/plasma ratios were decreases (lysine, glycine, glutamine and histidine). This suggests that with these exceptions the free amino acids of the muscle increase and decrease with the plasma concentrations so that the same concentration gradient is maintained. Therefore, the catabolic activity of hydrocortisone may be a result of lowering the free amino acids of the plasma rather than decreasing the rate of transport into the muscle.

A comparison of the amino acid concentrations found in rat skeletal muscle in these experiments with those found in mice by

Kaplan(4) indicated two notable differences. Tryptophan values of 0.64 μmole/g wet weight were found in mice whereas the values found in rat muscle were too small to measure accurately (> 0.05 μmole). The results described here are similar to those found in cat muscle by Tallan, Moore and Stein (0.07 μmole/g wet weight)(12). In addition, no anserine was reported for mouse muscle although 1.00 μmole/g wet weight was found in rat control muscles and Tallan *et al* reported 8.0 μmoles/g wet weight in cat muscle. Because of the similar chromatographic mobility of anserine and tryptophan, the rat muscle samples were rechromatographed after addition of 0.5 μmole of anserine to the sample. This procedure confirmed that the peak was anserine and not tryptophan.

The discrepancies previously reported in the action of hydrocortisone on the plasma amino acids appear to be resolved by this study. The general picture that emerges suggests that hydrocortisone initially produces an elevation of the plasma amino acids. This would be expected to produce the amino aciduria that has been described by Roberts (13). The cause of the immediate effect of hydrocortisone may be the inhibition of transport of amino acids into the cells of organs other than the liver. The decreased levels of amino acids in tissues in which a catabolic response is produced by hydrocortisone would seem to be consistent with most of the literature, that is, increased protein synthesis requires a high intracellular level of amino acids(14,15,16). Recently, Riggs has related the intracellular level of amino acids to rate of incorporation of amino acids into protein and further confirmed the relation of intracellular amino acid concentration to protein synthesis(17). Therefore, the decreased amino acid levels in rat muscle following chronic administration of hydrocortisone would seem to be the immediate cause of the catabolic effect of hydrocortisone on skeletal muscle. The lowered amino acid pool of muscle appears to result from a decreased level of amino acids in the plasma.

Summary. 1. Twenty-four hours after injection hydrocortisone was found to increase the free amino acids of the plasma and muscle of the rat. These acids are decreased after 10 days of treatment with hydrocortisone. 2. Hydrocortisone produced increases in aspartic acid in the muscles of rats treated for 24 hours and for 10 days. This increase after 10 days occurred in spite of a 40% decrease in aspartic acid concentration in the plasma. 3. The muscle/plasma ratios of glycine, glutamine and glycine were found to decrease at 24 hours and at 10 days. This suggests that hydrocortisone inhibits the transport of these amino acids.

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