thrombin yields were increased essentially to normal.

Discussion. Owren's(4) nomenclature impresses us with the advantages of clarity and adaptability to differentiation of precursor (pro) and active forms. Thus, we have designated these 2 cases as hypoproconvertinemia, probably familial in type as suggested by their histories. In severity, frequency and types of bleeding episodes, these cases are indistinguishable from other kinds of congenital hemorrhagic disorders. Hence, the laboratory tests are essential for the differential diagnosis. Quick tests on both patients were markedly prolonged. Our initial examination(9) showed that this was not due to abnormality of fibrinogen, antithrombin or proaccelerin and, therefore, a diagnosis of hypoprothrombinemia, unresponsive to Vit. K, seemed justified. Re-examination of R.S. and restudy of M.T.'s frozen specimens refutes this diagnosis and establishes hypoproconvertinemia. Normal plasma prothrombin levels in both patients are clearly demonstrated by the 3 test methods, but it should be noted that the addition of proconvertin, either bovine (Table II) or human (Fig. 1) was required. The very low proconvertin index denotes a severe deficiency and its small positive value, rather than the completely negative Owren's test, is in keeping with the facts that clotting did occur, albeit slowly, in whole blood and the Quick tests. The finding of a moderate deficiency in prothrombin consumption in our cases is new. This may depend upon the severity of the proconvertin deficiency.

Summary. Two unrelated patients had a congenital, probably familial, hemorrhagic disorder associated with a prolonged "prothrombin time." Laboratory examination showed that this prolongation was not due to deficiency of prothrombin, proaccelerin, or fibrinogen, nor to excess of inhibitors. The diagnosis of hypoproconvertinemia was established by the findings of a low "proconvertin index," by Owren's test, and by the ability of added proconvertin to restore normal prothrombin conversion *in vitro*. Prothrombin consumption tests in both cases showed a moderate but significant decrease.

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Metabolic Patterns in Experimentally Induced Muscular Dystrophy.* (20742)

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Lesions in striated and cardiac muscle have been described in animals receiving large amounts of desoxycorticosterone(1), and in animals maintained on diets deficient in vit. E, or in potassium(2). Recently Ellis noted degenerative changes in the striated muscles of rabbits treated with cortisone(3) and main-

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tained on the Rockland pellet diet which we had previously found to be inadequate in vit. E content. We have made a biochemical study of the muscular dystrophy which cortisone administration produces in the rabbit receiving adequate amounts of vit. E, and have compared these results with those observed in muscular dystrophy of vit. E-deficiency. Selye reported that many of the effects of overdosage of cortisone in rats can be prevented by simultaneous admininstration of approximately equal amounts of pituitary growth hormone(4). It appeared of interest to determine whether growth hormone would modify or prevent the dystrophy producing effects of cortisone in the rabbit.

Most of the data heretofore published on vit. E-deficiency were obtained on animals during the advanced stages of deficiency. As a result, these data are difficult to interpret since many of the observed changes could have been the non-specific result of anorexia, malnutrition and loss of muscle substance, and may give little indication of the primary metabolic changes. Therefore, in the present investigations, observations on animals during the early period following the withdrawal of tocopherol were made.

Methods and materials. Animals. Young growing chinchilla rabbits of both sexes weighing about 800 g were used. All animals were kept in individual metabolism cages during the experiments. Urinary total nitrogen, creatine, creatinine, calcium, sodium and potassium were determined daily. Urinary glucose was determined when indicated. In the cortisone experiments, each animal was observed for a control period of 7 days. Hormone injection periods lasted from 18 to 21 days. A second control period was often studied after the cessation of hormone administration. In these experiments, the amounts of urinary constituent "retained" or "lost" means the difference between the amounts excreted during a control period and during a subsequent period of treatment. In the experiments on vit. E-deficiency, the metabolic periods were separated on the basis of the level of creatine excretion. The first metabolic period was the one during which the animal was maintained on the deficient diet without obvious metabolic or clinical abnormality and lasted from 9 to 15 days. The second period was that in which creatinuria was pronounced but in which there was no change in food intake. Data from the last period preceding death, and characterized by anorexia, weight loss and paralysis, were not included.

Diets. Each day the animals were fed measured amounts of food which were constant for each animal during all periods. *Vit. E-deficient animals* were fed diet 11 of Goettsch and Pappenheimer(5) which had been treated with 1% ferric chloride. This treated diet is designated diet 13. The controls received 50 g of this diet plus 4 mg of α-tocopherol per day. This amount previously had been found to be the quantity of food which a dystrophic rabbit would eat in the early stage of the disease, and therefore, this regimen yielded a period in which the intake of the dystrophic animal was comparable to that of the non-dystrophic animal. *Cortisone-treated animals*: Group A was maintained on 70 g of the Rockland pellet diet plus 4 mg α-tocopherol per day. Groups B and C were given 60 g of diet 11(5) plus 4 mg α-tocopherol per day. These amounts of food supported growth but were slightly less than the animals would have eaten if they had been fed *ad lib* but this assured constancy of food intake throughout the experiments. Both the *Rockland pellet diet* and diet 11 were found to contain so little vit. E as to produce dystrophy and early death in young growing animals. That this was nutritional muscular dystrophy was demonstrated by the fact that the administration of vit. E reduced creatinuria and induced recovery. Histological sections of tissues of animals not receiving supplements of tocopherol revealed the characteristic lesions found in vit. E-deficiency. It will be noted in the discussion that these diets contained different amounts of sodium and potassium and different ratios of these ions. These are listed below:

	Rockland pellet diet	Diet 11
	g %	
Sodium	.06	.55
Potassium	1.52	.89
Calcium	1.04	1.14
Protein	24.0	24.8

Hormone Administration. Cortisone acetate (Merck) was injected intramuscularly into the right hind leg once daily. The dose was 10 mg per kg per day. Growth hormone (Armour) was injected intramuscularly in the left hind leg once daily. Group C-1 received 10 mg per day; Group C-2 received 20 mg per day. Since these animals all weighed about one kg, these doses were approximately equal to the amount of cortisone administered in Group C-1 and twice as large in Group C-2 respectively. *Chemical Methods.* Urinary creatine and creatinine were determined directly on undiluted urine by the semi-micro autoclave adaptation of the Folin method(6). Calcium was determined volumetrically by titration with potassium permanganate of the acid-dissolved calcium oxalate precipitate. A semi-micro Kjeldahl method was used for urinary nitrogen. Glucose excretion was estimated colorimetrically by the anthrone method(7). Sodium and potassium in the urine and in ashed samples of the diets were determined by flame photometry(8).

Results. The effect of deprivation of vit. E on the urinary excretion of creatine, creatinine, calcium, sodium and potassium is shown in Table I. The first column, showing the averages of data of seven control animals, is compared with the second column of data obtained from vit. E-deficient animals during that period after the withdrawal of tocopherol when the rabbits were growing at a normal rate and had not yet developed excessive creatinuria. The third column contains data on the same animals after creatinuria had increased and muscular performance had become impaired but food intake had not yet diminished. It may be seen that there were changes in composition of urine before the level of creatinuria was increased. The level of calcium excretion was elevated from a normal average of 23 mg per day to 70 mg, whereas the output of sodium was significantly lower than that of the control group. Although the intake of food was unchanged, the average daily rate of gain in body weight was curtailed from 25 g to 8.5 g. During the following stage, when dystrophy was well established, the amount of sodium in the urine was reduced even further; the average output at this time was only about 1/3 that of the normal rabbits. Potassium excretion increased, and although the excretion of calcium decreased, it still remained somewhat higher than that of the control animals. Other investigators have noted that the muscles of dystrophic animals contain abnormally large amounts of sodium and calcium(9,10). Our findings are consistent with these observations insofar as sodium is concerned. Our data do not account for the increased amounts of calcium found in the muscles and urine. The source of this extra calcium might be the skeleton, in view of our preliminary investigations that indicate a lower concentration of calcium in the bones of vit. E-deficient animals. The decrease in the rate of sodium excretion either coincided with, or immediately preceded, the increase in creatinuria. Fig. 1 illustrates these time relationships.

TABLE I. Effect of Deprivation of Vitamin E. Seven rabbits in each series.

	Control animals	Deficient animals: Before onset of dystrophy	Deficient animals: During dystrophy
Diet (50 g per day)	13 + vit. E	13	13
Avg daily body wt gain in g	28 ± 2.4	25 ± 3.0	4.4 ± 4.5
	Avg daily urinary excretion (mg)		
Creatine	13 ± 3.1	15 ± 2.5	72 ± 4.1
Creatinine	36 ± 3.2	36 ± 3.6	38 ± 3.6
Ca	23 ± 4.9	70 ± 5.3	37 ± 6.0
Na	313 ± 12.	241 ± 19.	101 ± 14.
K	222 ± 13.	216 ± 17.	260 ± 16.

All the vit. E-deficient rabbits developed a stiffness in the extremities and back resembling that described in guinea pigs(11). Movement at the wrist and knee joints became greatly restricted. This did not seem to be due to structural changes in these joints, but rather to a decrease in the length of the muscle-tendon unit. Subsequent treatment with vit. E failed to restore full freedom of movement at these joints. The condition once produced may be irreversible or its reversibility may depend upon passive stretching or exercise as well as upon vit. E-treatment. The microscopic appearance of the skeletal muscles of these animals was characterized by coagu-

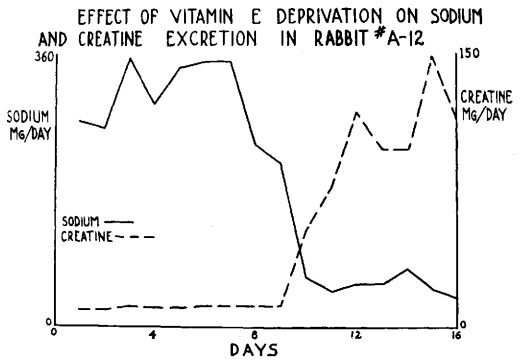

FIG. 1.

lative necrosis of the fibers, increase in nuclei of the fibers, and infiltration by adipose tissue; in short, by lesions identical to those first described by Goettsch and Pappenheimer(5).

The first two columns of Table II illustrate the effect of prolonged administration of cortisone to rabbits maintained on the Rockland pellet diet supplemented with vit. E. The daily level of urinary creatine was increased and there appeared to be an increase in urinary creatinine also. Unlike the vit. E-deficient animals, these rabbits retained little if any of the ingested sodium, and lost potassium at a much greater rate (*i.e.* about 100 mg per day). The total urinary nitrogen was increased by an average of 865 mg per day. The food intake was restricted to that of the control period; the growth rate was considerably reduced. Hyperglycemia, lipemia and glycosuria developed soon after the injections of cortisone were begun and persisted during the entire period of administration and for a time beyond the injection period.

Microscopic examination of the striated muscles of these animals showed the same types of structural change which Ellis has described(3); namely, swelling of the fibers, rupture of the sarcolemmal sheath, loss of cross-striations, and Zenker's degeneration.

The effect of identical doses of cortisone was then studied in rabbits maintained on the purified diet which, without added vit. E, induces muscular dystrophy. The animals were given adequate supplements of α-tocopherol daily, and this regimen thus permitted a comparison of the two types of muscular dystrophy, one induced by vit. E-deficiency and the other by cortisone, in 2 groups of rabbits maintained on the same basic diet. The results of these experiments appear in the third and fourth columns of Table II. The changes in the amounts of urinary constituents on this diet, due to cortisone administration, were analogous to those exhibited by animals on the Rockland pellet diet. Total nitrogen, creatine, creatinine, calcium and potassium in the urine increased and there was only a slight change in sodium metabolism. The rate of growth of the animals was very much reduced.

The muscle shortening noted in the vit. E-deficient animals did not occur in the cortisone-treated animals. Both groups of animals showed muscular weakness, and slowness in getting up after being placed on their backs. Many of the cortisone injected animals developed tremors in all 4 extremities. These

TABLE II. Effect of Prolonged Cortisone Administration on the Rabbit (10 mg/day for 3 wk).

	Group A		Group B	
	Control period	Inj. period	Control period	Inj. period
Diet	R* + vit. E	R + vit. E	11† + vit. E	11† + vit. E
No. of animals	6	4	6	3
Avg daily change body wt in g	+29 ± 3.5	−6 ± 1.7	+30 ± 2.5	+5 ± 6.5
	Avg daily urinary excretion (mg)			
Total N	685 ± 49.	1550 ± 32.	871 ± 5.8	2088 ±200.
Creatine	20 ± 1.7	67 ± 2.1	19 ± 2.5	81 ± 11.
Creatinine	35 ± 2.7	43 ± 1.7	47 ± 2.1	51 ± 3.9
Ca	22 ± 1.5	90 ± 6.0	23 ± 2.4	71 ± 22.
Na	23 ± 9.2	27 ± 3.8	294 ± 13.	280 ± 26.
K	779 ± 5.6	875 ± 16.	335 ± 8.5	455 ± 26.

* 70 g daily. † 60 g daily. R = Rockland pellets.

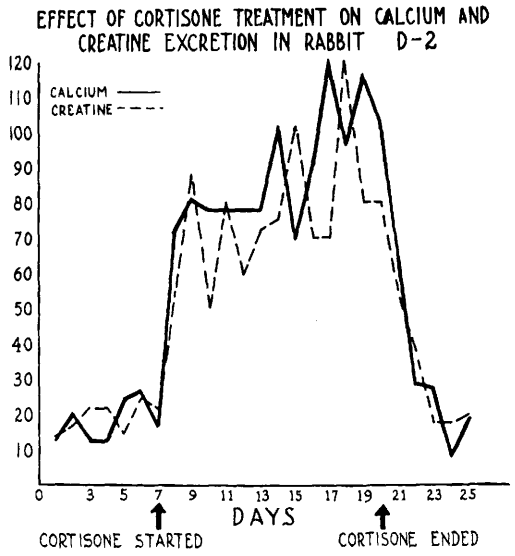

FIG. 2.

tremors were most pronounced in the group given the Rockland pellet diet. The reason for this difference is not known, but it might be the difference in K:Na ratio of the diets. In the Rockland pellet diet the K:Na ratio is 25; that of the purified diet is 1.6.

The alterations in metabolism due to cortisone administration, as reflected in urinary excretion, were prompt and were maximal by the second or third day after the first injection. Withdrawal of cortisone was followed by an equally rapid return to normal levels of urinary calcium and creatine, but the excessive excretion of nitrogen diminished gradually over a period of several days. Fig. 2 illustrates these time relationships.

It seemed possible that the pituitary growth hormone might counteract at least one of the effects of cortisone, namely, the acceleration of the rate of protein catabolism. Therefore, simultaneous treatment with both hormones might reasonably be expected to reveal the significance of this increased nitrogen loss in the genesis of the degenerative changes in striated muscle. Accordingly, cortisone-injected animals were treated with growth hormone at two different dosage levels, and biochemical studies similar to those made in the other group of animals were made in these doubly injected animals. The dose of cortisone and the food consumption remained constant. These data are presented in Table III. The simultaneous injections of growth hormone did not diminish the effect of cortisone either on creatine excretion or on histological degeneration.

When growth hormone was given in doses of 10 mg per day, the loss of nitrogen in the doubly injected animals was about 30% less than in those given cortisone alone. However, treatment with a larger amount of growth hormone (20 mg per day) not only did not enhance this partial reversal of the catabolic action of cortisone, but actually accentuated the effect of cortisone. Moreover, at this dosage level, growth hormone treatment alone, produced an increase in daily nitrogen excretion. The increased nitrogen excretion of the doubly injected animals was close to the arithmetical sum of the increases obtained with each hormone separately. Glycosuria appeared on the second day of injection of either growth hormone or cortisone and disappeared gradually after cessation of treatment. The effect of growth hormone on nitrogen excretion in vitamin E-deficient animals was also studied, and is recorded in Table IV. The food intake was included with these data because of the variability of appetite in the vit. E-deficient animals which must be taken into account in evaluating the data. The daily nitrogen excretion in vit. E-deficient animals after the onset of dystrophy was found to be the same as, or somewhat greater than, the amount excreted during the period immediately preceding other evidence of dystrophy (creatinuria and weakness). Since food consumption was decreased in all these animals, it is evident that less nitrogen was retained by the dystrophic animals. In three vitamin E-deficient animals given growth hormone, the food consumption before and during the injection periods was practically unchanged. In two of these animals, growth hormone appears to have induced a small retention of nitrogen, as well as a slight decrease in creatinuria. However, the clinical condition was not improved and the life span of either animal was not prolonged. At these lower dosage levels of growth hormone (3 to 5 mg per day), glycosuria appeared as rapidly as it did when

TABLE III.
Effect of Pituitary Growth Hormone on Urinary Nitrogen Excretion in Cortisone Dystrophy.

Group	Cortisone, mg/kg/day	Growth hormone, mg/day	Change in urinary N from control level, mg/day	Urinary glucose, mg/day
B	10	0	+1128	+ 795
	10	10	+ 841	+1680
C	0	20	+ 185	+ 810
C-1	10	0	+1217	+2420
C-2	10	20	+1363	+4150

TABLE IV. Effect of Growth Hormone on Nitrogen Retention in Nutritional Muscular Dystrophy (4 Day Periods).

	Control period			Growth hormone, 3-5 mg/day			
Animal No.	Avg daily food eaten, g	Avg daily N intake, g	Avg N excreted daily, g	Avg daily food eaten, g	Avg daily N intake, g	Avg N excreted daily, g	Difference, g
M-65	57	2.26	1.55	53	2.10	1.09	−.46
-78	34	1.35	1.00	37	1.42	1.00	0
-49	70	2.78	1.32	69	2.74	1.27	−.05

larger doses were given but was not so pronounced.

Discussion. Tocopherol deprivation and cortisone overdosage afford two different methods of studying metabolic patterns which are present in animals with progressive muscular degeneration.

Cortisone overdosage did not impair the appetite, and could be tolerated for much longer periods of time, at the dosage levels used in these investigations, than could total tocopherol deficiency. The animals with nutritional muscular dystrophy usually developed inanition in from 4 to 9 days after onset of creatinuria and all growing animals died in from 3 to 14 days after the onset of elevated creatinuria. This variation in survival time is partly related to age; as a general rule, the younger the animal, the shorter the survival time is apt to be. Older mature animals may actually develop a chronic form of nutritional muscular dystrophy and live for a considerable period. On the other hand, even young animals have survived a month of cortisone treatment, but definitive studies on the ability of rabbits to survive cortisone administration were not made.

During the period of dystrophy, the vit. E-deficient animals developed muscle shortening and paralysis, neither of which were symptoms of cortisone overdosage.

The two types of experimentally induced dystrophy were characterized by differences in the blood constituents. Cortisone treatment induced a severe lipemia that was not seen in vit. E-deficiency. On the other hand, preliminary experiments indicate that the ratios of the various blood proteins are altered in muscular dystrophy of vit. E-deprivation, with considerable increase in the third globulin component. These observations will be reported in detail later.

Disturbances in carbohydrate metabolism were observed in both forms of muscular dystrophy. Cortisone treatment produced hyperglycemia and glycosuria. In vit. E-deficiency the concentrations of glycogen in liver and muscle are decreased(12), as we have confirmed in some of our animals.

In a qualitative sense, the daily urinary excretions of creatine, nitrogen, potassium, calcium and sodium were similar in the two types of muscular dystrophy, but there were significant quantitative differences. The maximal creatinuria due to cortisone overdosage was less than that seen in vit. E-deficiency, but loss of nitrogen and potassium was greater. A significant retention of sodium was an early symptom of vit. E-deficiency, in contrast to the slight fall in sodium excretion observed in

cortisone treatment. However, chemical analyses of the muscles of animals given cortisone, revealed sodium concentrations that were significantly greater than in the muscles of untreated controls(3).

Small doses of growth hormone induced nitrogen retention in cortisone treated and in vit. E-deficient rabbits. The fact that larger amounts of growth hormone have an opposite effect and result in an increased amount of urinary nitrogen, may be due to the diabetogenic action of the hormone to which the rabbit appears to have a sensitivity similar to that exhibited by the cat(13). Glycosuria developed within from 1 to 3 days days after administration of growth hormone had been started.

It has been demonstrated that cortisone administration induces an acceleration in the rate of protein catabolic processes resulting in increased nitrogen excretion and in decreased body substance(14). The question of the genesis of the muscular wasting in vit. E-deficiency still is unsettled. It has generally been supposed that the loss of muscle is due to an increased breakdown of muscle protein, but whether protein breakdown actually is increased is not known and cannot be decided by the methods employed in these studies. The possibility, however, that reduction in the rate of synthesis of normal muscle protein may be a primary defect in nutritional muscular dystrophy is suggested by our studies on the liberation of free amino nitrogen by the tissues of vit. E-deficient hamsters(15), and possibly by the alterations in the electrophoretic pattern of the blood proteins, to be reported later.

Summary. 1. A study has been made of the metabolic patterns of rabbits with muscular dystrophy due to vit. E-deficiency and to chronic overdosage with cortisone. Both conditions are characterized by an increased urinary excretion of creatine, nitrogen, potassium and calcium. These urinary losses are quantitatively greater in cortisone induced dystrophy, although the clinical symptoms of muscular weakness and diminished life span were most marked in vit. E-deficiency. 2. The effect of pituitary growth hormone administration on both types of experimental muscular dystrophy was studied. Combined with cortisone in large doses, it exacerbated the metabolic abnormalities. Smaller doses partially curtailed the increase in nitrogen excretion. Superimposed on vit. E-deficiency, growth hormone was without effect clinically and mildly active with respect to nitrogen storage for a few days. 3. Both cortisone and growth hormone were diabetogenic in the rabbit.

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