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Numerous investigators have encountered deficiency symptoms variously referred to as muscular dystrophy, paralysis, or merely stiffness. These symptoms have been encountered in vitamin E deficiency and another nutritional deficiency described by Wulzen and Bahrs.

Muscular dystrophy accompanying vitamin E deficiency is characterized by a high urinary excretion of creatine and a very low level of muscle creatine. The "pl" deficiency of Wulzen and Bahrs studied in this paper appears to be characterized by essentially normal levels of muscle and urine creatine.

THE METABOLISM OF CREATINE IN GUINEA PIGS

by

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THE METABOLISM OF CREATINE IN GUINEA PIGS

INTRODUCTION

Numerous investigators have encountered deficiency symptoms variously referred to as muscular dystrophy, paralysis, or merely stiffness. These symptoms have been encountered in vitamin E deficiency and another nutritional deficiency described by Wulzen and Bahrs. Since it has been demonstrated that the muscular dystrophy accompanying vitamin E deficiency is characterized by a high urinary excretion of creatine and a very low level of muscle creatine, it was thought desirable to make a comparative study of vitamin E deficiency and the Wulzen deficiency through creatine metabolism.

LITERATURE REVIEW

"Factor pl" deficiency. Bahrs and Wulzen (31) found that when planarian worms were fed tissues from guinea pigs maintained on supposedly adequate diets, the worms developed a dietary disease. The disease appeared when all of the recognized vitamins were supplied in the diet of the guinea pigs (1). Only heart and liver tissues of pigs which had been fed fresh green kale supported normal growth and health of the worms. This protective factor, present in the kale, was given the name "factor pl."

Throughout these experiments the guinea pigs killed to supply the tissue were quite normal in appearance and no overt signs of disease were exhibited. Wulzen and Bahrs then carried out extended feeding experiments (32) with guinea pigs to bring out possible latent nutritional disturbances. They used three basal diets, differing only in minor points. One of these rations appears below.

Diet 10

	parts
Baked milk	25
Ground barley	42
Bran	23
Yeast	5
Cod-liver oil	3
Ferric ammonium citrate	
NaCl	
$CaCO_3$	
KI	0.5
Viosterol	Two drops twice weekly

Groups of animals were given this diet with various supplements, including kale, tomato juice, and orange juice. Those animals receiving kale increased 100 per cent in weight in the course of twenty weeks, while those animals fed orange or tomato juice as a supplement gained only from 30 to 65 per cent in weight.

With progress of the disease a characteristic syndrome developed in each animal after about four months on the diet. The first symptom was a loss in muscle tone, followed by definite indications that the voluntary muscles were losing their normal contractability. The guinea pigs finally lost the power of locomotion and death resulted from inanition. Some animals were maintained by pipette feeding for an additional period. The severity of the paralysis continued to increase until just before death there was strong adduction of the muscles of the limbs and neck.

Autopsy revealed a hypertrophied and liquefied liver in some cases. The most striking abnormalities were evident in the skeletal muscles. They were hard, rigid, and unrelaxed after death. Their appearance was grayish or yellowish in color. Fibrous tissue predominated and probably accounted for the paralysis of the limbs. There were also abnormalities of the skeleton indicating loss of bone minerals.

Wulzen made further investigations (30) of the "pl factor" in which guinea pigs were fed a basal milk ration.

This ration is described in the words of the author, "To each 100 cc. of the type of milk being used were added 10 grams skim milk powder, one cc. copper sulfate solution (.078%), and one cc. of ferric chloride solution (.482%). The milk was fed in as large quantities as could be consumed. All groups were bedded in straw and were provided with iodized salt. Orange juice at the level of one cc. per 100 grams of body weight and carotene were added to the milk once a day. Once a week two drops of viosterol were administered to each animal."

When guinea pigs were fed whole raw milk, treated as described above, they grew at an excellent rate while the guinea pigs in whose ration the whole milk was replaced by pasteurized whole milk or raw skim milk showed decided and persistent muscular stiffness as shown by a wrist test. The technique of applying the wrist test was explained by the author in detail. "The fore leg of the guinea pig in the opposite side of the body wall, from the experimenter is extended posteriorly, close to the body wall, by pressing down with the thumb on the olecronon process and at the same time supporting the proximal and distal portions of the leg with the fingers. The leg should be as straight as possible. The disengaged hand of the operator is then used to gently flex the foot by pressing upward on its medial aspect. The foot of a normal animal will flex easily until it forms a right angle with the leg. If the

animal has become quite stiff it is not possible to flex the foot at all. No force is used at any time; pressure ceases and the estimation of the position of the foot is taken as soon as the leg offers resistance to the movement."

Vitamin E avitaminosis. Evans and Bishop (5) demonstrated, with the aid of special diets, that rats require vitamin E for successful reproduction. Then followed the discovery, by Ringsted (22) that the further depletion of the body stores of this vitamin gave rise to a muscular dystrophy. The disease was difficult to induce in rats unless they were fed a very high fat diet. Goettsch (8) was able to demonstrate that a muscular dystrophy developed in guinea pigs maintained on a specially treated diet for approximately two months. The diet is listed below.

Goettsch ration

	parts
Rolled oats	355
Wheat bran	180
Casein	75
Lard	80
Cod-liver oil	10
Skim milk	275
NaCl	10
$CaCO_3$	15
$FeCl_3$	1%
Orange juice supp.	3 cc. per day

The destructive power of ferric chloride upon vitamin E has been shown by Waddell and Steenbock (28). The ferric chloride is added as an ether solution to the dry parts of the mixture and then after mixing, the ether is allowed to

evaporate. The vitamin A requirement in this diet is supplied by cod-live oil. Madsen (16) observed that vitamin E is very readily destroyed by this oil. When included in a diet it hastens the onset of muscular dystrophy. Weber, Irwin, and Steenbock (29) found that any rancid fat accomplishes the same purpose, although the general opinion of various workers (10) (14) (17) is the cod-liver oil has some special influence in aggravating the severity of the dystrophy.

The Goettsch diet was not readily eaten by the guinea pigs and it was therefore necessary to supplement it with greens during the first ten days of the experiment. Thereafter the feeding of greens was discontinued. Madsen and co-workers (18) experimented with various diets upon guinea pigs, goats, and sheep, using a diet similar to the Goettsch ration. When the cod-liver oil supplement was omitted from their ration the onset of the disease was delayed for a considerable period of time. Morgulis and Spencer (20) found that the Goettsch and Brown diet (9) produced vitamin E deficiency in rabbits.

During the period of the last five years a group of papers has been published centering around the production of muscular dystrophy in guinea pigs and rabbits with a subsequent cure by a-tocopherol. The diets used by these investigators were quite similar. A typical diet is that of Shimotori and co-workers (24).

Diet III (Shimotori)

	parts
Casein	15
Regenerated cellulose	15
Cornstarch	40
Sucrose	14
Agar	5
Yeast	5
Salt mixture	2
Lard	2
Cod-liver oil (fed separately)	2

This is an entirely synthetic diet and its use with guinea pigs is accompanied by feeding difficulties as was the case with the Goettsch ration (8). If the food does not appeal to the guinea pig, the animal prefers to starve rather than eat it. Shimotori accustomed the animals to the synthetic food mixture by feeding it in conjunction with the stock diet for a period of weeks. Cannon and Emerson (4) obtained satisfactory feeding by mixing agar and water with their ration to form a slab which was broken up into small hard fragments. Another diet reported by Kohler, Elvehjem, and Hart (13) was also quite successful with guinea pigs, although it was used for the assay of the grass juice factor and was not vitamin E free. They had used whole milk as the basal ration. In spite of the fact that the digestive tract of the guinea pig is designed for bulky foods, the animals prospered on this diet. The milk ration used by Wulzen (30) and discussed previously, was also quite satisfactory and it should be feasible to adopt a milk ration for vitamin E studies.

Vitamin E deficiency has been produced and described in rabbits, guinea pigs, dogs, and rats. The clinical symptoms are similar in all species, although there are slight variations. The complete picture of the condition includes sterility as well as muscular dystrophy, but only the latter phase of the disease has a bearing upon the subject of this thesis.

Goettsch (8), as previously mentioned, produced dystrophy of the voluntary muscles by dietary means. The guinea pigs grew normally for the first two months on the ration. During the third month growth stopped, muscles became flabby, and death terminated the experiment. Autopsy revealed muscle lesions like those found in rats by other workers (6) (21). The metabolism of the voluntary muscles is greatly disarranged in vitamin E deficiency. Evans and co-workers (7) have made histological examinations of a large number of rat muscle tissues. They found a marked infiltration of connective tissue and fat, multiplication of cell nuclei, and degeneration of the muscle cells. Knowlton and Hines (12) measured the functional capacity of the gastrocnemius of the rat. The normal rat muscle showed a great superiority, in maximal contractile effort, over the muscle of dystrophic animals.

Goettsch and Brown (9), realizing the importance of creatine in muscle metabolism, made a quantitative study of the muscle creatine, fat, and water content of

dystrophic rabbits. For normal rabbit muscle (gastrocnemius) the mean level of creatine was 463 milligrams per 100 grams of fresh tissue. The mean level in tissues exhibiting extreme necrosis or fibrous replacement was 171.4 milligrams. Intermediate creatine levels corresponded to less acute lesions. Accompanying the low creatine level, Goettsch found the moisture content to be 79.5 per cent in dystrophic tissue as compared with 75.7 per cent in normal tissues. Fat analysis showed extreme variations in the dystrophic tissues. One rabbit with severe lesions had five times the normal level of fat in the muscle. The normal fat content of fresh muscle tissue was always near one per cent.

Telford, Emerson, and Evans (25) have reported that dystrophic rats also have low muscle creatine. The muscle creatine level was 37 per cent below normal in rats which had been raised in a vitamin E-free ration.

The most complete muscle creatine data in guinea pigs are found in a report by Shimotori, Emerson, and Evans (24). Their vitamin E-free diet was typical of the synthetic diets discussed elsewhere in this thesis. The muscle creatine of paralyzed guinea pigs varied from 117 mg. to 288 mg. per 100 grams of fresh tissue. In contrast, those animals which received a wheat germ oil supplement had from 390 mg. to 471 mg. of creatine per 100 grams of tissue.

Accompanying the low creatine content of muscle in

vitamin E deficiency is the excretion of creatine in the urine by these dystrophic animals. Creatine is either entirely absent or present in very small quantities in the urine of normal animals. Mackenzie and McCollum (15) found that the dystrophic rabbit had an extremely high level of creatine in the urine. A record of creatine excretion in animals maintained on the special vitamin E-free ration revealed that creatinuria was a primary symptom of impending dystrophy. The creatine excretion mounted as the severity of the disease increased. There was a maximum excretion of 80 mg. per day. A daily supplement of a-tocopherol resulted in an immediate 50 per cent drop in creatine excretion. In a later paper (14) Mackenzie and McCollum reported that a 50 mg. per day level of creatine was completely erased by feeding a-tocopherol. Verar (27) studied the influence of a-tocopherol acetate on the creatinuria of rats with vitamin E deficiency, and found that the creatine excretion quickly dropped to zero after injestion of the ester. The work of these investigators which is summarized above is generally regarded as proof that the muscular dystrophy accompanying sterility in vitamin E avitaminosis can be completely cured by a-tocopherol.

A preliminary experiment on the creatine excretion of guinea pigs suffering from the lack of the "pl factor" was made by Gouley (11). The results indicated that these

animals had a continuous creatinuria. The ratio of creatine to creatinine varied from 0.30 to 0.98 in the urine of diseased animals, but no data were available to show the creatine excretion of normal animals.

The description of the syndrome developed in guinea pigs by Wulzen (32) and Gouley's experiment with urine from these guinea pigs indicate that a comparison of the creatine metabolism in "pl" deficiency and vitamin E deficiency would be of interest. Such a comparison has been the object of the experimental work reported in this thesis.

EXPERIMENTAL PROCEDURE

Preparation and feeding of diets. A vitamin E-free diet similar to the one used by Shimotori (24) was prepared for use in a preliminary experiment with seven guinea pigs. The animals ate the ration readily at first but lost weight steadily. Within a month five of the animals had died from disorders which probably arose from malnutrition and the experiment was discontinued. A second experiment was begun, using the same diet, but the animals were allowed more than a week to become accustomed to the ration before the stock diet was removed from the cages. These animals also lost weight steadily and their food consumption was relatively small. The ration was then slightly modified by adding enough agar-agar (2%) and water to form a hard cake after the mixture had dried. The guinea pigs ate this material in small quantities but continued to lose weight. In both of the above dietary experiments the animals received a daily supplement of 3 cc. of orange juice, and 0.5 cc. of cod-liver oil fed by pipette.

Only the basal milk ration proved to be successful as a vitamin E-free diet for guinea pigs. It was prepared as follows. A mixture of 850 grams of raw skim milk, 50 grams of cod-liver oil, and 100 grams of powdered skim milk were shaken together until a suspension was formed. Fresh portions of this liquid diet were placed before the animals

twice daily. The supplements were 3 cc. of orange juice, 1 mg. of iron as the citrate, 0.1 mg. of manganese as the sulfate, and 0.1 mg. of copper as the sulfate. These supplements were fed by syringe each day. The animals in the experiment were divided into two groups of six animals each. One group was given a supplement of 0.5 cc. of wheat germ oil and the other group was given 0.05 cc. of crude molasses, which was sufficient to protect the animal from the "pl" deficiency. The results of this experiment are summarized in Table I.

There were two diets used in studying the urinary excretion of creatine and creatinine. The normal animals were given a stock diet consisting of rolled oats, alfalfa meal, lawn clippings or fresh kale, and iodized salt. This diet was always before the animals except during the short periods when they were on the metabolism cages. The dystrophic animals were given Wulzen's basal milk ration described on page three of this thesis.

Methods of analysis. Urine samples were collected every twenty-four hours from animals placed in metabolism cages. Each twenty-four hour total sample was diluted to 500 cc. in a volumetric flask. Since this solution was quite turbid, an excess (1.5 g. per 100 cc. of solution) of solid basic lead acetate was added. A flocculant precipitate formed immediately, carrying down suspended particles and colloidal material. Basic lead acetate does

not adsorb creatinine or creatine (26). The clarified solution was then suitable for colorimetric analysis.

The determination of creatine and creatinine in urine were made by the colorimetric method of Folin (7). A number of substances which interfere in the creatinine picrate color reaction have been discovered and the accuracy of the method was held in doubt until Baker and Miller (19) reported the isolation of a microorganism which specifically decomposed creatinine. They compared their method with the Folin procedure and found that less than one per cent of the chromogenic material in urine was other than creatine or creatinine.

A Klett photoelectric colorimeter, with a light filter transmitting a band at 540 mu. was used for the measurement of color intensity. After the scale of the instrument had been adjusted to zero with a blank solution containing reagents only, readings were taken on standard solutions containing from 0.1 to 1.5 mg. of creatinine per 100 cc. A straight line response was obtained over this range and a standard curve was constructed for the calculation of analyses.

For the determination of creatinine, 10 cc. aliquots of the clarified and filtered sample solutions were then transferred to 100 cc. volumetric flasks. To each flask were added 20 cc. of a saturated aqueous solution of picric acid and 1.5 cc. of a solution containing 10 per cent

each of sodium hydrozide and Rochelle salt. The color was allowed to develop for twenty minutes before the sample was diluted to volume for the colorimetric comparison.

The determination of total creatinine-creatine was made by the autoclave modification of the original Folin method to avoid the charring that occurred when samples were evaporated to dryness. Aliquots (10 cc.) of the clarified urine samples were autoclaved in flasks with 2.5 cc. of concentrated HCl for two hours at 15 lb. pressure. This acid was then neutralized and the same procedure followed as in the creatinine determination.

The method of Rose, Helmer, and Hamutin (23) was used for the determination of tissue creatine. Some modifications introduced by Baker and Miller (2) were also used. The specific enzyme method of tissue creatine developed by these workers has shown that 96 per cent of the chromogenic substance in the gastrocnemius muscle is creatine.

Guinea pigs selected for analysis were killed by decapitation. The gastrocnemius was excised from each hind leg and quickly transferred to a stoppered weighing bottle. Approximately 0.2 g. samples were then removed to 50 cc. flasks containing 20 cc. of 2N sulfuric acid. After autoclaving for one hour the segments were disentigrated with a stirring rod while still in the flasks and the autoclave treatment repeated for one additional hour. The samples

were then transferred to 100 cc. volumetric flasks containing 5 cc. of a 10 per cent sodium tungstate solution. Sodium hydroxide (2N) was then added from a burette to make the samples just acid to congo red. The samples were then diluted to volume and filtered. From this filtrate 10 cc. aliquots were taken and placed in test tubes. The following reagents were then added to each test tube; 0.38 cc. of 0.1 N NaOH, 0.25 cc. of molar phosphate buffer (pH7), 6.25 cc. of alkaline picrate solution (1 part 2.5N NaOH and 5 parts sat. picric acid), and 2 cc. of water. The contents of the tubes were mixed and the color allowed to develop. Baker and Miller (2) allowed only twelve minutes to elapse before measuring the color. It was found in this laboratory that the stable level of color development was reached only after thirty minutes.

The moisture content of fresh tissue was determined by drying one gram samples at 86 degrees in an electric oven for 15 hours.

The analysis of fat was made by the microsoxhlet extraction of dried tissues. Six hours of extraction gave a constant weight of extracted lipids.

EXPERIMENTAL RESULTS

Urine analysis. Twenty-five guinea pigs from the breeding stock of the colony were placed upon metabolism cages. The total excretion for twenty-four hours was collected for analysis. All animals were normal and had been raised on the stock diet of grain and greens. The summary of the urine creatine analysis of ten of these animals appears in Table I. Creatinuria occurred in all individuals during at least some portion of the time during which they were under observation.

It was thought desirable to obtain a record of the urine excretion over a prolonged period and three normal animals were placed in metabolism cages for twelve consecutive days. The data from this experiment are shown in Table II. These young animals had a low creatine level at the beginning of the experiment, but between the days 7 and 12 animal 1060 had a continuous and high creatinuria.

Table III shows the results of an experiment with sixteen guinea pigs which had developed the "pl" deficiency symptom of stiffness. All animals had a creatinuria although two of them had been changed to the deficient diet only ten days preceding the collection of the samples.

Tissue analysis. Urinary creatine determinations were attempted on samples from the experiment with the vitamin E-free milk ration but charring of the samples

made the results unreliable. The experiment was then terminated by sacrificing the animals for tissue analysis. In Table IV there are data from two animals which received a wheat germ oil supplement and therefore were protected from vitamin E deficiency. The other three animals listed in this table received a supplement of livestock molasses which protected them from "pl" deficiency. The creatine level of the animals receiving no vitamin E was 100 mg. lower than those which were fed wheat germ oil.

In Table V are compared tissue creatine determinations of "pl" deficient animals with those of normal animals. The "pl" deficient guinea pigs were taken from a group maintained on the Wulzen (30) basal skim milk diet. Three of the normal animals had been given a whole milk ration with the necessary supplements and the other three normal pigs had been supplied with the stock ration. The tissue creatines of these individuals are quite uniform throughout both groups.

TABLE I

Urinary excretion of creatine and creatinine in milligrams per 100 g. of body weight per 24 hr. Creatine excretion calculated as milligrams of creatinine.

Animal Number	Body Weight	Day 1 C.*	Day 1 Cr.*	Day 2 C.	Day 2 Cr.	Day 3 C.	Day 3 Cr.	Day 4 C.	Day 4 Cr.	Day 5 C.	Day 5 Cr.	Day 6 C.	Day 6 Cr.
702	860 g.	.29	3.3	.46	2.8	.00	2.4	.00	3.3	.09	2.4	.17	3.5
887	905	.00	2.6	.00	3.3	.00	2.5	.51	3.3	.38	3.4	.33	2.7
903	929	.34	3.1	1.3	3.3	.00	3.3	.64	3.1	.20	3.1	.32	3.3
952	800	2.5	3.4	.12	2.9	.00	3.6	.30	3.9	.00	3.1	.32	3.4
981	1039	.37	2.8	.36	2.6	.00	1.9	.10	3.2	.14	2.6	.32	2.4
1065	300	.71	2.5	.83	1.9	.91	2.2	1.8	2.1	.88	2.3		
1061	330	.27	2.6	.30	2.2	.92	2.4	1.4	2.2	.68	2.5		
1048	280	1.6	2.7	.94	2.4	1.6	2.7	2.4	1.8	.81	2.4		
1057	370	.00	2.8	.3	2.1	1.3	1.9	.30	2.4	.00	2.3		

* The abreviations C. and Cr. indicate Creatine and Creatinine respectively.

TABLE II

Urinary excretion of creatine and creatinine of three guinea pigs (given stock diet) over a period of 12 days. Data are calculated as milligrams per 100 g. per 24 hr.

Day	Animal 1060 W.	C.	Cr.	Animal 1052 W.	C.	Cr.	Animal 1056 W.	C.	Cr.
1	385	.00	.8	340	.07	1.4	385	.00	1.8
2	383	.00	.8	340	.00	2.6	370	.06	2.2
3	382	.06	.7	328	.12	1.3	382	.23	.65
4	381	.20	1.9	324	.20	1.9	374	.18	1.8
5	390	---	---	340	.56	1.8	385	---	3.4
6	391	.34	1.6	341	.85	2.1	384	.40	1.7
7	400	1.4	1.7	334	.75	.75	380	.46	1.9
8	397	2.0	2.0	350	.29	.96	375	.00	2.0
9	402	3.0	2.1	345	.32	1.8	370	.34	2.4
10	403	2.5	1.8	344	.11	1.1	379	.20	2.0
11	404	1.9	1.9	348	.19	2.3	388	.38	2.1
12	403	2.3	2.3	342	.11	1.6	388	1.1	2.3

TABLE III

Urinary creatine and creatinine of "p1" deficient guinea pigs. Wulzen skim milk diet. Calculated excretion per 100 g. of body weight per 24 hr. Creatine expressed as mg. creatinine.

Animal Number	Body Weight	Wrist Stiffness	Day 1		Day 2	
			C.	Cr.	C.	Cr.
815	966	moderate	.87	2.1	.91	2.6
828	820	moderate	.73	2.8	.88	2.4
867	771	severe	.54	1.7	.60	2.2
1034	530	moderate	.17	3.0	.81	3.5
1031	590	moderate	.76	4.0	2.4	3.4
1036	443	moderate	.52	2.9	.65	2.4
1043	350	moderate	.14	3.2	.49	3.0
972	797	severe	.33	3.1	.13	3.2
966	728	severe	.51	2.9	.00	3.3
963	666	severe	.41	4.0	.99	2.5
952	789	moderate	.16	2.9	.00	2.9
960	678	moderate	.44	3.1	.15	3.1
986	801	moderate	.40	3.7	.10	3.9
980	718	moderate	.49	3.3	.00	3.6
973	760	severe	.57	3.3	.00	3.6
967	755	severe	.46	2.9	.46	3.8

TABLE IV

Muscle creatine in milligrams per 100 g. of fresh tissue. Animals from vitamin E-free skim milk diet with supplements.

Animal Number	Supplement	Wrist Stiffness	Mg. creatine Left leg	Right leg
1086	wheat germ oil	extreme	334	---
1061	wheat germ oil	extreme	312	320
1048	molasses	moderate	252	240
1047	molasses	moderate	209	227
1068	molasses	moderate	272	277

TABLE V

Muscle creatine per 100 g. of fresh tissue. Normal and "pl" deficient animals from Wulzen skim milk diet. Moisture and fat were calculated to the fresh tissue weight.

Animal Number	Body Weight	Diet	mg. creatine Left leg	Right leg	Wrist Stiffness	% HO	% Fat
1070	827	whole milk	403	357	none	73.2	1.9
1082	824	whole milk	378	383	none	76.0	1.4
1080	829	whole milk	356	356	none	75.9	2.0
1055	570	stock	399	435	none	75.7	2.0
1053	630	stock	418	418	none	76.3	1.8
956	820	skim milk	401	397	severe	75.3	1.7
971	680	skim milk	435	468	severe	76.0	2.1
924		skim milk	365	370	moderate	76.4	1.9
904		skim milk	387	382	moderate	76.3	1.6
1024		skim milk	366	366	moderate	75.4	2.0

DISCUSSION

The data from tables I and II show that the normal guinea pigs had a creatinine excretion between 2mg. and 4mg. of creatinine per 100 grams of body weight. These same animals had an extremely variable level of creatine excretion. At some time when under observation each animal had a high creatinuria, although the same animal perhaps would excrete no creatine for the period preceding or following the creatinuria.

When the creatine excretion of "pl" deficient animals was investigated, similar variability was noted. Table III deals with a typical group of dystrophic animals and creatinuria was present in all of the animals on at least one day of the experiment, but four animals from the group had no creatinuria on the second day. There is no continuous high creatinuria present in these animals such as described by Mackenzie and McCollum in vitamin E deficient rabbits (15). The results of the vitamin E-free milk ration dietary experiment were not conclusive because a number of the animals showed symptoms of malnutrition. However, two weeks after changing to the diet those animals receiving wheat germ oil as a supplement were extremely stiff and yet their muscle creatine levels were normal. In contrast, those animals fed a supplement of molasses were moderately stiff and their muscle creatine levels

30 per cent below those of the wheat germ fed animals, indicating vitamin E depletion. If this experiment were repeated, using less cod-liver oil, more decisive results might be anticipated.

Table V deals with a comparison of five normal guinea pigs with five pigs showing the "pl" deficiency stiffness. There is little difference in creatine, moisture, and fat contents of the gastrocnemius between the two groups. This observation is in marked constrast to the picture presented by vitamin E deficiency and discussed elsewhere in this thesis.

SUMMARY AND CONCLUSIONS

I. Normal guinea pigs show a creatinuria which varies from zero to 15 per cent of the creatinine excretion.

II. "P1" deficient guinea pigs have a creatinuria which is comparable to the creatinuria of normal guinea pigs.

III. The muscle creatine level of "p1" deficient guinea pigs is the same as the muscle creatine level of normal stock guinea pigs.

IV. The disturbance in creatine metabolism of guinea pigs caused by vitamin E deficiency is not exhibited by guinea pigs suffering from the "p1" deficiency.

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