AN ABSTRACT OF THE THESIS OF

Victor Schocken for the M.S. in Chemistry
(Name) (Degree) (Major)

Date Thesis presented August 1943

Title A COMPARATIVE STUDY OF THE EFFECTS OF AN ANTI-STIFFNESS FACTOR AND ALPHA TOCOPHEROL UPON THE CREATINE EXCRETION OF GUINEA PIGS RAISED ON A N E-FREE DIET

Abstract Approved [redacted]
(Major Professor)

Since the muscular dystrophy produced in guinea pigs by a deficiency of vitamin E suggested a possible relationship to the impaired flexion of the limbs resulting from a dificiency of the anti-stiffness factor, we undertook the investigation of simple and combined deficiencies of the two factors with the dual purpose of distinctly differentiating between the syndromes of each and investigating the possibility of synergy between the two.

Avitaminosis E has been studied in such varying animals as sheep, goats, ducks, the tree kangaroo, mice and hamsters, but most particularly for rats, rabbits, and guinea pigs. For all these species it has been shown that vitamin E plays an essential part in the metabolism of skeletal muscle. The development of the disease in the guinea pig is as follows. The animal gradually stops gaining weight and becomes flabby. This is accompanied by a shrinking and degeneration of the voluntary muscles and the development of paresis, particularly in the hind quarters. There also develops a lack of coordination, and in most cases the animal is considered dystrophic if it proves to be unable to

right itself when placed on its back.

A deficiency of the anti-stiffness factor was first observed when Bahrs and W ulzen found that planarian worms, fed on tissue from guinea pigs maintained on certain stock diets, developed a dietary disease. The disease appeared when all of the recognized vitamins were supplied in the diet of the guinea pigs, and the worms thrived only on heart and liver tissues of animals which had been fed fresh green kale. From this observation it was assumed that there existed in the kale a new essental dietary factor. With the progress of the deficiency 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 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.

In order to study and compare simple and combined deficiencies of vitamin E and the anti-stiffness factor, five groups of guinea pigs were used, each of which was on a special diet. The distribution was as follows.

Group I: Four animals on stock diet without supplements

Group II: Six animals on E-free diet unsupplemented

Group III: Six animals on E-free diet supplemented by daily portions of the anti-stiffness factor

Group IV: Six animals on E-free diet suppleneted by daily portions of alpha tocopherol

Group V: Six animals on E-free diet supplemented by daily portions of alpha tocopherol and the anti-stiffness factor.

The animals were kept in metal cages, bedded on sawdust and segregated as to group and sex. Each morning a fresh portion of the diet in the form of a dry powder was put into the food dishes, and the animals were given orange juice and supplements by pipette. After receiving their supplements, each day a third of the animals were weighed and put on metabolism cages where twenty-four hour urine samples were collected. In this way a urine sample was collected from each guinea pig every three days.

A deficiency of the anti-stiffness factor produces in guinea pigs a characteristic wrist-stiffness but has no effect upon the creatine excretion. Avitaminosis E produces a muscular dystrophy accompanied by creatinuria. On a combined deficiency these symptoms develop simultaneously and apparently independently of each other, resulting in a greater degree of paresis than that produced by a deficiency of either of the factors individually.

A COMPARATIVE STUDY OF THE EFFECTS OF AN ANTI-STIFFNESS FACTOR AND ALPHA TOCOPHEROL UPON THE CREATINE EXCRETION OF GUINEA PIGS RAISED ON AN E-FREE DIET

by

VICTOR SCHOCKEN

A THESIS

submitted to the

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

August 1943

APPROVED: [redacted]

Assistant Professor of Chemistry

In Charge of Major

[redacted]

Head of Department of Chemistry

[redacted]

Chairman of School Graduate Committee

[redacted]

Chairman of State College Graduate Council

ACKNOWLEDGEMENT

I wish to express my sincere gratitude and appreciation to Dr. Willem J. van Wagtendonk and Dr. Rosalind Wulzen, under whose constant supervision this study was carried out, for their many helpful suggestions and complete cooperation.

A COMPARATIVE STUDY OF THE EFFECTS OF AN ANTI-STIFFNESS FACTOR AND ALPHA TOCOPHEROL UPON THE CREATINE EXCRETION OF GUINEA PIGS RAISED ON AN E-FREE DIET

PREFACE

Vitamin deficiencies are observed prior to the discovery of vitamins, and therefore their physiological applications are frequently known rudimentarily even before their isolation and identification. However, there are usually subtler manifestations associated with a deficiency than those which beckon observers to the discovery of the vitamin involved, and these must be subsequently detected and systematically studied. The detection of these secondary effects of a deficiency of a new vitamin is particularly difficult since they have in many cases been incorporated into the syndromes of previously investigated vitamins, and therefore, as in the classic example of the B complex, the specific symptoms caused by each new member of the group must be reclaimed from the syndrome of the group as a whole.

Since the muscular dystrophy produced in guinea pigs by a deficiency of vitamin E suggested a possible relationship to the impaired flexion of the limbs resulting from a deficiency of the anti-stiffness factor, we undertook the investigation of simple and combined deficiencies of the two factors with the dual purpose of distinctly differentiating between the syndromes of each,

and investigating the possibility of synergy between the two.

REVIEW OF THE LITERATURE

The history of vitamin E begins in 1922 when Evans and Bishop (1) announced the discovery of an anti-sterility factor for rats. In the course of investigating this anti-sterility factor, Evans and Burr (2) observed that young rats from vitamin E deficient mothers were paralyzed in part of the musculature of the body wall and posterior extremities. With this discovery that the effects of avitaminosis E are not restricted to the reproductive sphere, the research on the vitamin branched in two directions: the investigation of the reproductive disorders and the investigation of the muscular dystrophy.

The next significant step in the pursuit of the latter was reported by Goetsch and Pappenheimer (3) in which they describe a diet which leads to "progressive, highly selective, and ultimately fatal dystrophy of the voluntary muscles". The diet which they used is typical of those used in the early investigations of avitaminosis E, and consisted of the following:

Rolled oats	355 parts
Wheat bran	180
Casein	75
Lard	80
Cod liver oil	10
Sodium chloride	10
Calcium carbonate	15

The entire diet was freed of vitamin E by treatment with ferric chloride dissolved in ether, according to the meth-

od of Waddell and Steenbock (4). Most of the animals so fed died in a dystrophic condition after about three months.

Using a similar diet, Ringsted (5) was able to produce a paresis or muscular dystrophy in adult rats. Since both Goetsch and Pappenheimer, and Ringsted used cod liver oil in their diets, and since neither was able to cure the dystrophy by the administration of vitamin E, the question arose as to the part played by the cod liver oil, and the possibility of other factors being involved in the deficiency. Although various workers (3, 6, 7) indicated their belief that the cod liver oil played a toxic, aggravating role in the production of the dystrophy, it was finally shown (8,9) that the cod liver oil merely hastened the onset of the disease. The explanation offered for its action is that it oxidizes traces of vitamin E in the gut (10). The question of the complexity of the deficiency has also been clarified since Shimotori, Emerson, and Evans, and others (11, 12, 13, 14) have shown that the muscular deficiency developed on E-free diets can be prevented and cured by the addition of alpha tocopherol.

Avitaminosis E has been studied in such varying animals as sheep, goats (15), ducks (16), the tree kangaroo (17), mice (18), and hamsters (19), but most particularly for rats, rabbits, and guinea pigs, as in the work pre-

viously cited. For all these species it has been shown that vitamin E plays an essential part in the metabolism of skeletal muscle. The development of the disease in the guinea pig is as follows. The animal gradually stops gaining weight and becomes flabby. This is accompanied by a shrinking and degeneration of the voluntary muscles and the development of paresis, particularly in the hind quarters. There also develops a lack of coordination, and in most tests the animal is considered dystrophic if it proves to be unable to right itself when placed on its back.

Goettsch and Pappenheimer (3) describe the necrotic muscular condition of the animals which had been on their diet and sacrificed when moribund in the following way. "The muscles of the thigh and abdomen were particularly abnormal, although the muscles of the back and the extremities, the diaphragm, and the intercostal regions were involved. They were atrophied, pale, with a yellowish or yellowish brown color and less translucent than those of the controls."

Frequently lesions are observed on the degenerating muscles. The lesion is a rapidly produced necrosis of muscle fibers characterized by conversion of a part of the whole of individual fibers into hyaline structureless necrotic material, which breaks up into globular and irre-

gularly fragmented masses. The fiber lesions in E deficient animals as described by Pappenheimer are essentially the same as those described in muscle from persons who died in consequence of epidemic influenza with pneumonia (20).

Evans and coworkers (21), on the basis of histological examination of a large number of rat muscle tissues report that they found a marked infiltration of connective tissue and fat, multiplication of cell nuclei, and degeneration of the muscle cells. In addition to these effects on the muscles, the complete syndrome of avitaminosis E includes complex reproductive disorders.

Early in the work it was recognized that the dystrophy of the muscles was accompanied by a diminished creatine content. Goettsch and Brown (22) summarize their extensive quantitative investigations as follows. "In nutritional muscular dystrophy of the rabbit there is an absolute as well as relative loss of creatine in the skeletal muscle without any demonstrable loss in either heart or brain. Both white muscle (which normally contains 420 to 500 milligrams of creatine per hundred gram, fresh tissue) and red muscle (260 to 360 mg.) contain, when in the last stages of degeneration, 110 to 250 mg. This is the level at which creatine is normally present in the heart."

It was further found that the loss of creatine from the muscles was effected through its excretion in the urine. Therefore the progress of the dystrophy could be followed by tracing the increase in creatine in the urine. Morgulis and Spencer (23) who developed this criterion for the appraisal of dystrophy, using rabbits, divide the creatinuria into the following stages: 1. During the period of approaching dystrophy the creatinine excretion remains practically constant while that of creatine increases markedly.

2. At the "critical point" an enormous increase in creatine elimination occurs.

3. From the "critical point" until death the creatinine and total nitrogen decrease from the high level reached at the "critical point", while the creatine excretion continues to rise till the time of death.

4. During the period of recovery following a change of diet at the "critical point" the creatine elimination approaches the normal level.

Verzar (24) cites Morgulis (25) as having followed dystrophy in guinea pigs by urine analyses for creatine, and we have found the method successful.

A deficiency of the anti-stiffness factor was first observed when Bahrs and Wulzen (26) found that planarian worms, fed on tissues from guinea pigs maintained on certain stock diets, developed a dietary disease. The dis-

ease appeared when all of the recognized vitamins were supplied in the diet of the guinea pigs (27), and the worms thrived only on heart and liver tissues of animals which had been fed fresh green kale. From this observation it was assumed that there existed in the kale a new essential dietary factor.

Throughout these experiments the guinea pigs which were sacrificed to supply the tissue for the feeding of the planarian worms were quite normal in appearance and exhibited no overt signs of disease. However, believing that the factor must also be essential for the well-being of guinea pigs, Wulzen and Bahrs then carried out extended feeding experiments (28) on these animals to bring out possible latent nutritional disturbances. They used three basal diets of which the following is a typical example.

Diet 10

Baked milk	25 parts
Ground barley	42
Bran	23
Yeast	5
Cod liver oil	3
Ferric ammonium citrate	0.5
Sodium chloride	1
Calcium carbonate	0.5
Potassium iodide	0.002
Viosterol	2 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 the 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 smooth 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 possibly accounted for the paralysis of the limbs. There were also abnormalities of the skeleton including loss of bone minerals.

Wulzen made further investigations (29) of the 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 sulfate solution

(0.078%), and one cc. of ferric chloride solution (0.482%). 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 the wrist test. The technique of applying the wrist test was explained by the author in detail. "The foreleg of the guinea pig in the opposite side of the body 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."

EXPERIMENTAL

In order to study and compare simple and combined deficiencies of vitamin E and the anti-stiffness factor, five groups of guinea pigs were used, each of which was on a special diet. The distribution was as follows.

Group I: Four animals on stock diet without supplements.

Group II: Six animals on E-free diet without supplements.

Group III: Six animals on E-free diet supplemented by daily portions of the anti-stiffness factor.

Group IV: Six animals on E-free diet supplemented by daily portions of alpha tocopherol.

Group V: Six animals on E-free diet supplemented by daily portions of alpha tocopherol and the anti-stiffness factor.

The E-free diet was based on that used by Mackenzie, Mackenzie, and McCollum (30) and consisted of the following:

Casein (Smaco vitamin free)	1050 grams
Dextrin (commercial corn starch)	4830
Yeast (Fleischman's irradiated brewers')	700
Salt mixture	420
Orange juice (given separately)	1 cc/100g. wt.

The salt mixture consisted of the following:

$CaCO_3$	135 grams
KCl	90
NaCl	45
$NaHCO_3$	63
MgO	18
KH_2PO_4	153
Fe citrate	45

The stock diet consisted of rolled oats, straw, and greens, the latter in the form of lawn clippings or fresh kale. The supplements were prepared and administered as follows:

Anti-stiffness Factor: Forty milliliter portions were made up weekly by dissolving 20 mg. of the crystalline material in 5 ml. of ethyl laurate and diluting with Wesson oil. Daily doses of 0.25 ml. (125 gammas of n.e.d.f.) were fed by pipette to animals in groups III and V.

Alpha Tocopherol: Forty milliliter portions were made up weekly by dissolving 240 mg. of the pure material in 40 ml. of Wesson oil. Daily doses of 0.5 ml. (3 mg. alpha toc.) were fed by pipette to animals in groups II and V.

The animals were kept in metal cages, bedded on sawdust, and segregated as to group and sex. Each morning a fresh portion of the diet in the form of a dry powder was put into the food dishes, and the animals were given orange juice and supplements by pipette. After receiving their supplements, each day a third of the animals were weighed, and put on metabolism cages where twenty-four hour urine samples were collected. In this way a urine sample was collected from each guinea pig every three days.

The determination of the creatine and creatinine in the urine was carried out by the method of Folin (31). The twenty-four hour samples were diluted to 500 cc. in

volumetric flasks, three grams of basic lead acetate was added to each to clarify, and then a portion of each was filtered. Two ten ml. aliquots were taken from the filtrate, the first to be treated for the determination of creatinine, the second for total creatine and creatinine.

For the determination of the creatinine the aliquot was treated with twenty ml. of saturated picric acid solution and one and one-half ml. of a ten per-cent sodium hydroxide, ten per-cent Rochelle salt solution. After the addition of these reagents, the mixture was allowed to stand for twenty minutes for the color to develop and then diluted to one-hundred ml. The amount of creatinine present was then determined by measuring the color intensity of each sample with a Klett-Summerson colorimeter.

For the determination of the total creatine and creatinine the second aliquot was treated with two-and-one-half ml. of thirty-six normal, chemically pure hydrochloric acid and autoclaved for two hours at fifteen pounds per square inch pressure to convert the creatine to creatinine. After the autoclaving, the solution was made just alkaline to litmus with six normal sodium hydroxide, and then picric acid and basic Rochelle salt solution were added and the determination was carried out as in the previous case.

The wrist and elbow stiffness of the guinea pigs was

measured and recorded each week by Dr. Wulzen.

The experiment was carried out in this way for four months, but, as the results of the urine analyses during this time as shown in Table I indicated that the dystrophy was developing very slowly, it was decided to expedite the experiment by the addition of cod liver oil to the diet. One milliliter of cod liver oil was therefore given to each animal on alternate days, while the regular supplements in double doses were given on the intervening days. This procedure was continued to the end of the experiment.

DISCUSSION OF RESULTS

Table one shows that muscular dystrophy, as observed by the characteristic creatinuria accompanying it, is slow to develop in animals on an E-free diet not including cod liver oil. The succeeding charts, figures two to six, depict the extent of dystrophy, degree of stiffness, and changes in weight of a typical animal from each of the groups.

The control animals in group one survived the experiment unimpaired. By the end they had doubled their weights and were in prime physical condition. In striking contrast to these were the animals in group two which had been on the E-free diet without supplements. These animals developed wrist-stiffness as well as the creatinuria indicating muscular dystrophy. As the experiment progressed, these animals lost weight, and their muscles, particularly in the hindquarters, became weak, diminished in size, and seemed to lose coordination. When placed upon their backs they could regain their footing only with difficulty and in some cases not at all, and when they moved about on the sawdust in the cages they did so laboriously and tended to drag their hind legs. When the experiment was terminated, one of these was dead, two were in an emaciated, moribund condition, and the others of the group were gradually becoming stiff and paralyzed.

The animals in group three which had been on the E-free diet with only the anti-stiffness factor supplemented fared somewhat better than those in the previous group. Although they too developed muscular dystrophy, their joints remained limber throughout, and, as a result, their paresis was of a lesser extent than that of the animals suffering from the combined deficiencies.

In group four, the animals which had been on the E-free diet supplemented with alpha tocopherol, there appeared no signs of dystrophy through the creatinuria criterion, but the joints of the animals became stiff and their muscles became weak. The disturbances to these animals seemed to be of a more chronic nature, for although their appearance and behavior seemed to suggest some sort of illness, the characteristic severe wrist stiffness was the only outward symptom which could be clearly defined.

The animals which had received both alpha tocopherol and the new essential dietary factor as supplements to the E-free diet, group five, thrived well, and, although they did not increase in weight as rapidly as the animals on the stock diet, they remained in good health throughout the experiment.

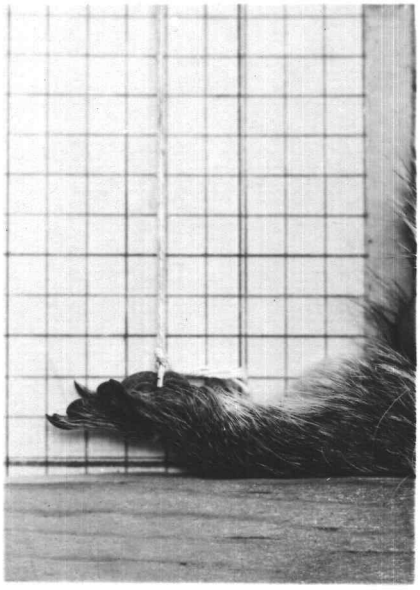
The growth trends lend themselves to qualitative considerations only, since there are inconsistencies within the groups due to variation of individual adapta-

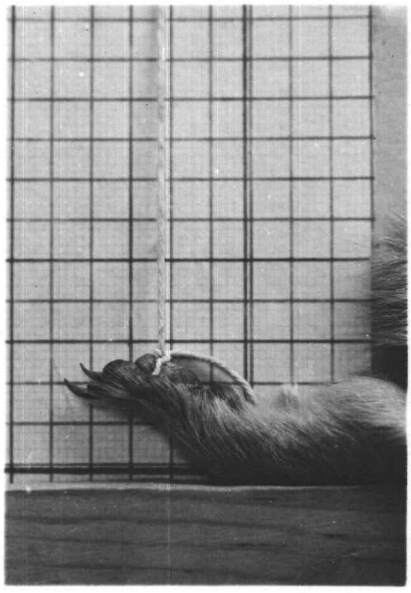
tion to the diet. It can be said however that each of the deficiencies as well as their combination hampers growth.

DEFICIENCY INDICES PRIOR TO ADMINISTRATION OF COD LIVER OIL

	1st day				10th day				20th day				53rd day				69th day				79th day			
	Creatine				Creatine				Creatine				Creatine				Creatine				Creatine			
Animal	Creatinine	Weight	Stiffness		Creatinine	Weight	Stiffness		Creatinine	Weight	Stiffness		Creatinine	Weight	Stiffness		Creatinine	Weight	Stiffness		Creatinine	Weight	Stiffness	
5	1.14	328	4	4	1.10	367	4	4	0.52	433	4	4	0.11	510	4	4	0.28	573	4	4	0.10	597	4	4
7	0.58	419	4	4	0.11	434	4	4	-	516	4	4	0.25	592	4	4	1.00	650	4	4	0.07	683	4	4
1284	0.47	444	4	4	0.07	481	4	4	0.30	570	4	4	0.71	647	4	4	0.47	673	4	4	0.48	654	4	4
1288	0.28	484	4	4	0.02	463	4	4	0.17	516	4	4	0.44	566	4	4	0.47	603	4	4	0.02	587	4	4
4	0.25	484	4	1.5E	0.50	474	3	1.5E	0.69	529	4	1.5E	0.26	520	4	1.5E	2.27	517	4	1.5E	0.30	520	4	1.5
6	0.13	370	4	3	0.16	375	4	2	0.47	415	4	2	1.94	445	2	1.5	1.98	471	2	1	0.33	450	2	1-
1W	-	440	3	3	0.31	361	4	4	0.06	503	4	4-	1.20	519	4-	4-	2.52	541	3	3	0.22	519	3	3
3	0.12	340	4	2	0.71	372	4	2	0.60	381	4	2-	1.08	395	4P	1.5	9.15	435	4-	1.5	1.08	398	3	1.5
10	0.81	365	E	E	1.90	334	E	E	1.29	425	E	E	1.00	466	E	E	0.71	507	E	E	0.72	459	E	E
11	0.22	359	4P	3	0.63	374	4P	4P	0.21	453	4	4-	0.14	502	4P	3	0.50	462	4P	2	1.14	431	4P	2
8	0.27	507	4	4P	0.37	484	4	4P	1.47	479	4	4	1.21	487	4	4E	2.51	378	4	4E	-		-	
1271	0.81	489	4	4	0.73	424	4	4	2.61	505	4	4	1.01	576	4	4	0.89	581	4	4	1.34	588	4	4
12	0.17	386	4	4	0.08	430	4	4	0.58	479	4	4	1.26	504	4	4	1.23	471	4	4	4.18	418	4	4
1270	0.49	444	4	4E	0.31	438	3	4E	1.43	474	4-	1.5E	0.88	553	4-	1E	0.50	580	4-	1E	1.08	624	4-	E
1272	0.66	536	4	4E	0.21	524	4	4	0.13	481	4	4	0.74	493	4	4	1.83	471	4	4	1.20	433	4	4
1273	0.42	477	4	4	0.04	477	4	4	0.41	487	4	4	0.80	494	4	4	0.52	475	4	4	2.15	377	4	4
1274	0.29	429	4-	2	0.32	459	4P	1.5	1.04	501	2E	1.5	0.42	555	1.5E	1.5E	0.49	524	1.5E	1.5E	0.76	569	1.5E	1E
1278	0.90	502	3	2	0.17	515	3	2	0.60	522	3	3	0.04	549	2	1.5E	0.36	572	2	1.5E	1.06	565	2	1.5E
1275	0.70	443	3	2-	-	464	3	1.5E	0.79	525	2+	1.5E	0.32	556	2+	1.5E	2.09	587	2+	1.5E	0.27	609	2+	1.5E
1276	0.38	448	4	3	0.45	426	4	3	1.70	396	4	4P	0.57	424	4	3	0.62	438	4	4-	1.08	438	4	4
1277	0.38	360	4	4P	0.46	349	4	4	0.35	394	4	4	0.51	383	4	4	0.78	375	4	4P	1.03	360	4	4P
1280	-	502	4	3	0.22	498	4	3	0.11	554	4	3	0.45	498	4	3	1.47	455	4	2	0.87	437	4	2+
1285	-	486	4	4P	0.25	527	4	4P	0.05	600	4	4	0.24	573	4	4	0.79	582	4	4P	2.12	597	4	4
1287	5.10	453	4	4	0.13	485	4	4P	0.02	551	4	4	0.25	543	4	4	0.26	562	4	4	0.26	580	4	4
1	0.13	388	4	4P	0.19	420	4	4	-	457	4	4	0.21	450	4	4	1.53	476	4	4	1.34	456	4	4
1282	0.26	396	4	4	0.42	411	4	4	0.10	402	4	4	0.64	453	4	4	0.50	495	4	4	0.83	472	4	4
1283	0.45	449	4	4	0.92	448	4	4	0.13	515	4	4	0.17	557	4	4	0.24	607	4	4	0.62	619	4	4
1286	-	449	4	4-	0.34	463	4	4P	0.17	476	4	4	0.12	498	4	4P	0.61	503	4	4P	0.40	470	4	4

Figure 1. Varying degrees of stiffness. A vertical force of two hundred grams is applied to the toes while the fore arm of the guinea pig is held palm upward in a horizontal position. Upper left, a typical 1 joint; upper right, a typical 2 joint; lower left, a typical 3 joint; lower right, a typical 4 joint.





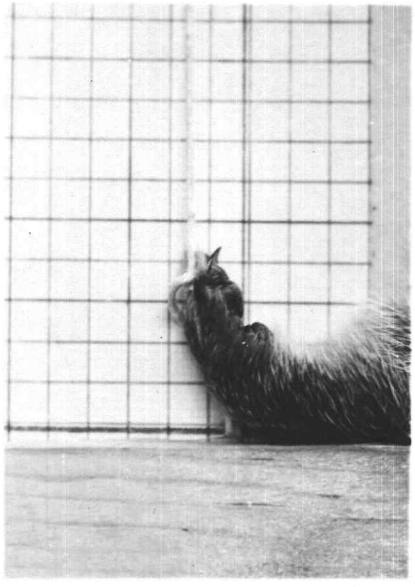


Figure 2. Creatine/creatinine ratio (A), wrist-stiffness (B), and weight in grams (C) of guinea pig number five, a typical representative of group I (stock).

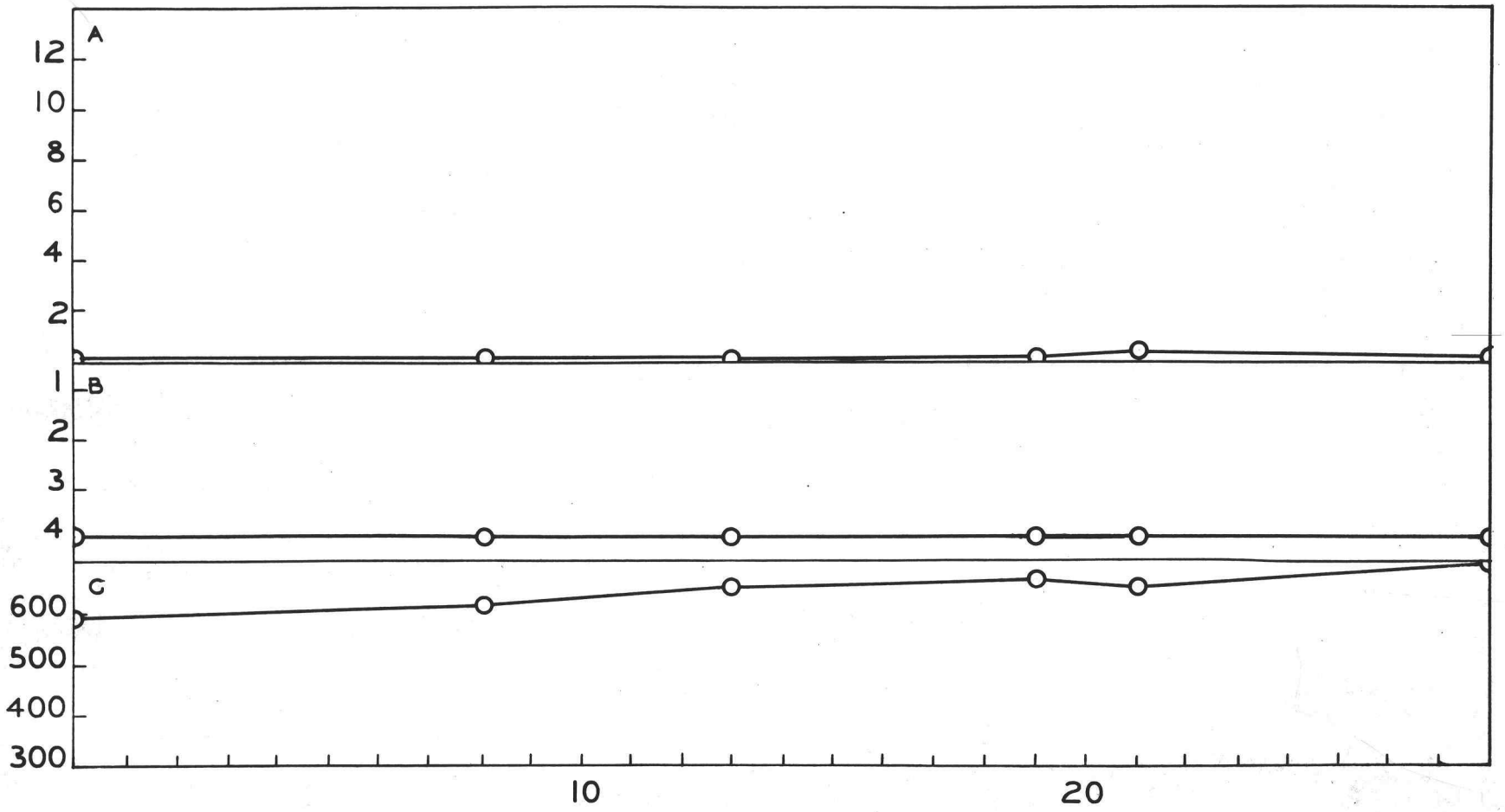

A
12
10
8
6
4
2
1
B
2
3
4
C
600
500
400
300
10
20

Figure 3. Creatine/creatinine ratio (A), wrist-stiffness (B), and weight in grams (C) of guinea pig number thirteen, a typical representative of group II (E-free diet unsupplemented).

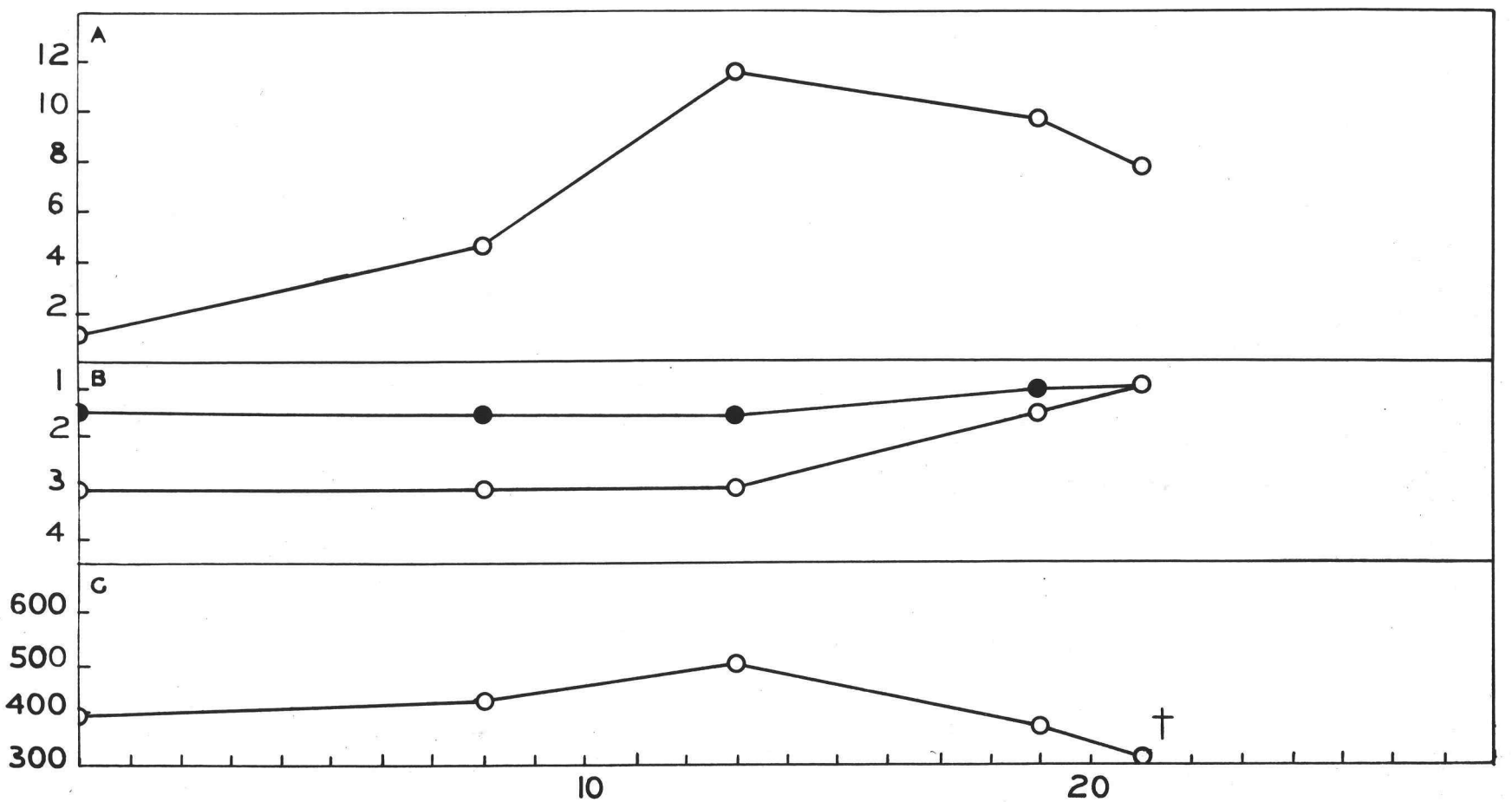

A
12
10
8
6
4
2
B
1
2
3
4
C
600
500
400
300
†
10
20

Figure 4. Creatine/creatinine ratio (A), wrist-stiffness (B), and weight in grams (C) of guinea pig number twelve, a typical representative of group III, (E-free diet supplemented with the anti stiffness dietary factor).

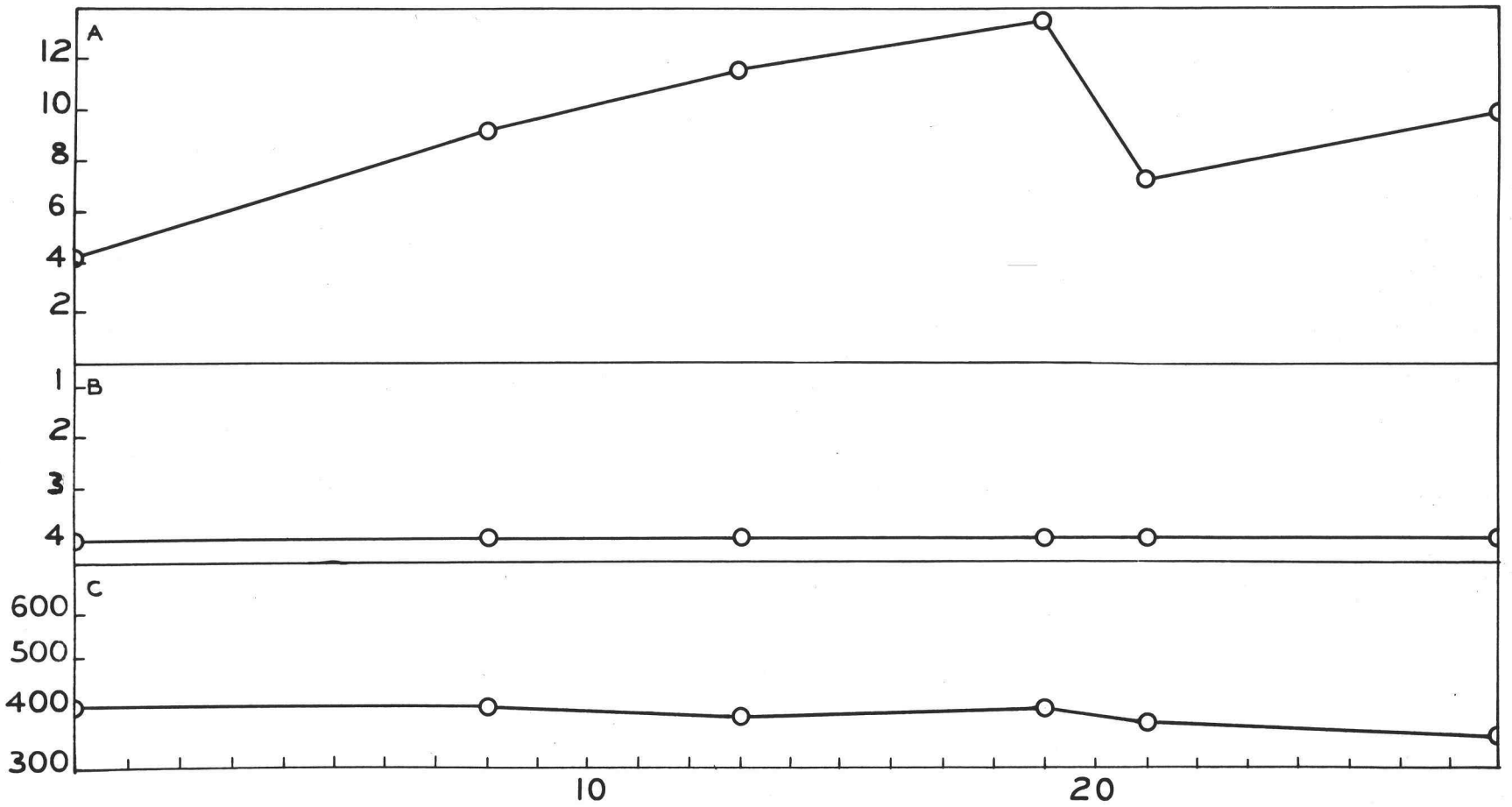

A
12
10
8
6
4
2
1
B
2
3
4
C
600
500
400
300
10
20

Figure 5. Creatine/creatinine ratio (A), wrist-stiffness (B), and weight in grams (C) of guinea pig number twelve-hundred seventy-eight, a typical representative of group IV, (E-free diet supplemented with alpha tocopherol).

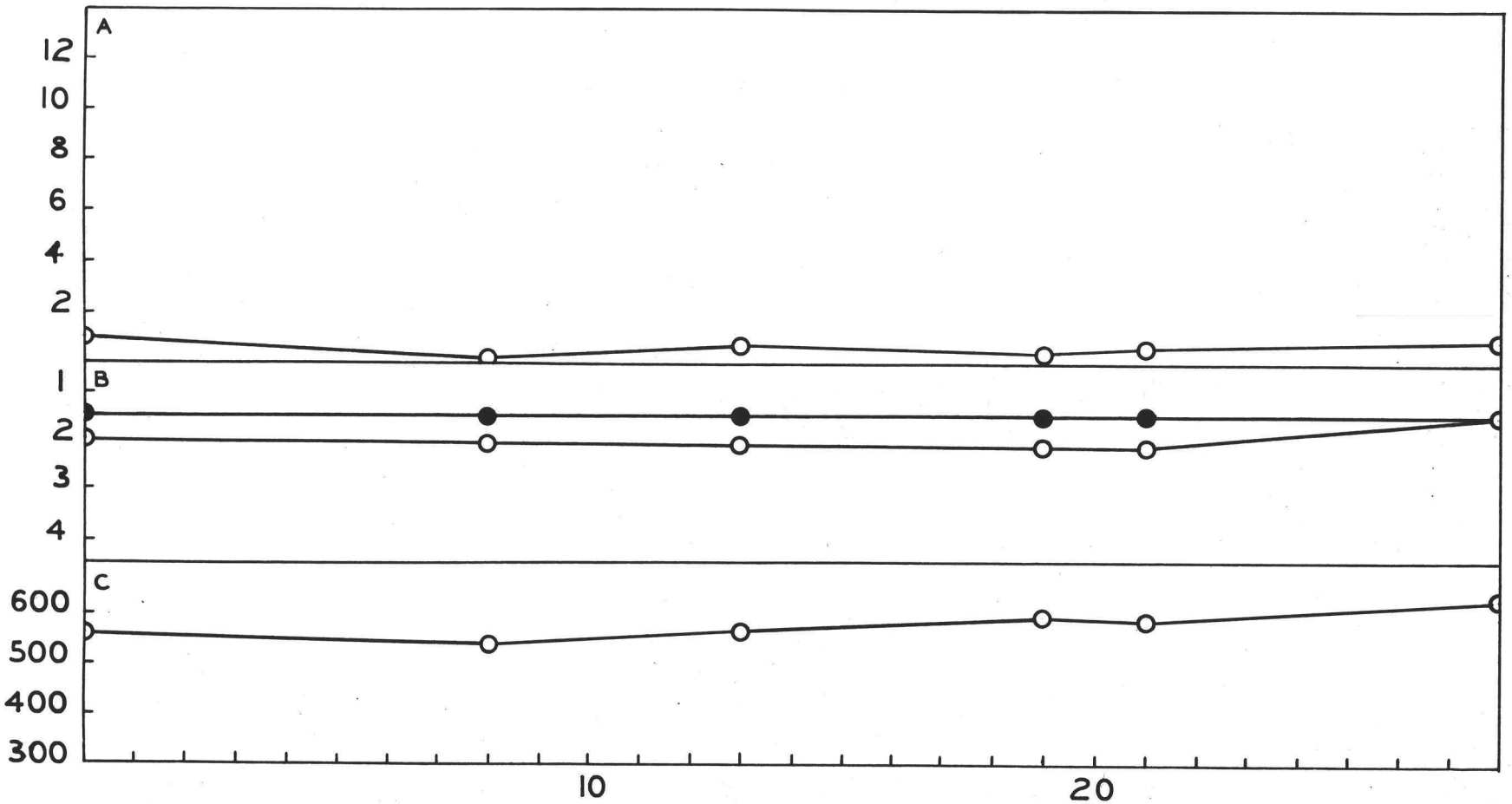
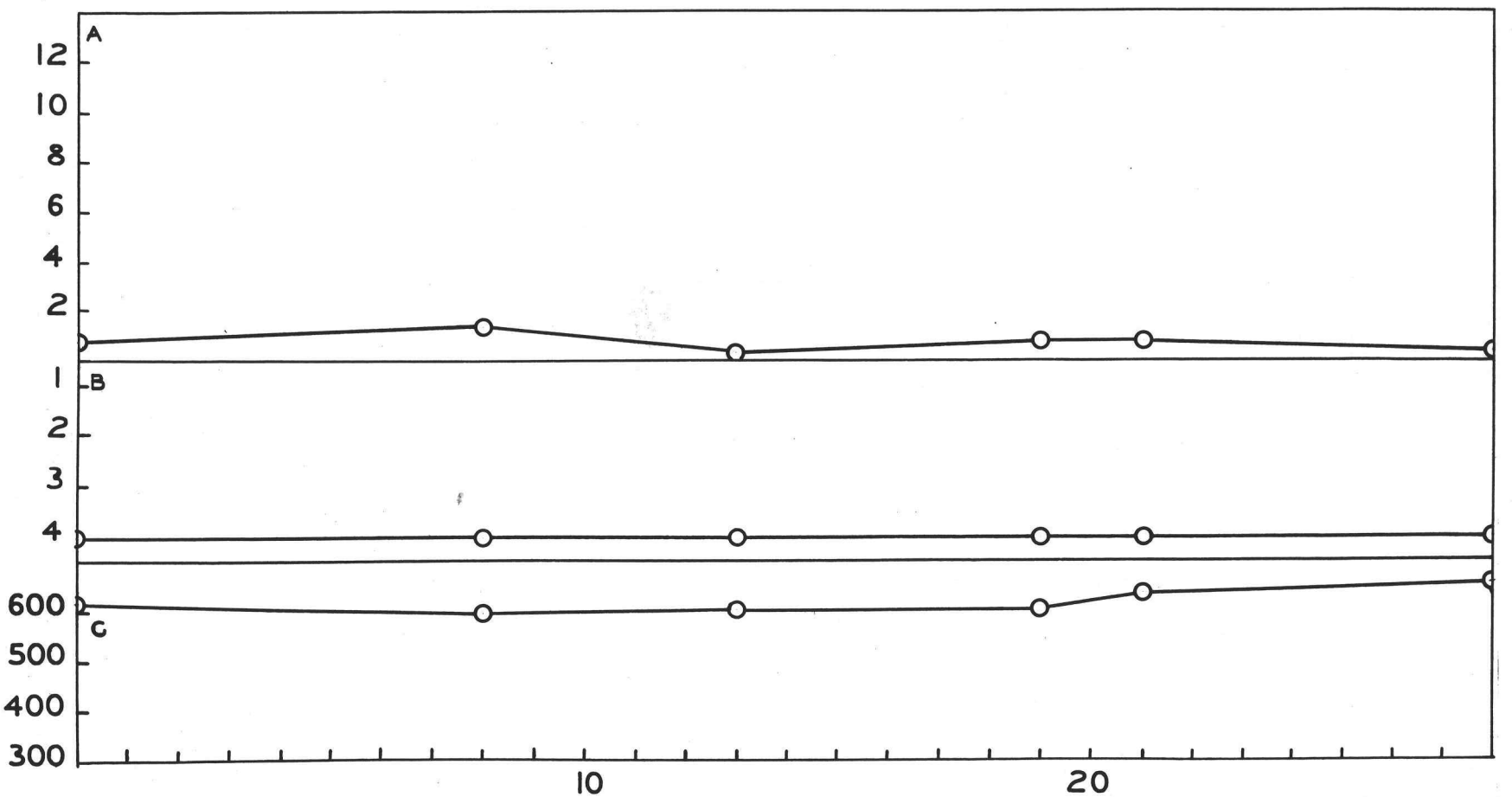

A
12
10
8
6
4
2
1
B
2
3
4
C
600
500
400
300
10
20

Figure 6. Creatine/creatinine ratio (A), wrist-stiffness (B), and weight in grams (C) of guinea pig number twelve-hundred eighty-five, a typical representative of group V, (E-free diet supplemented with the anti-stiffness dietary factor and alpha tocopherol).


A
12
10
8
6
4
2
1
B
2
3
4
600
C
500
400
300
10
20

SUMMARY

A deficiency of this anti-stiffness dietary factor produces in guinea pigs a characteristic wrist-stiffness but has no effect upon the creatine excretion. Avitaminosis E produces a muscular dystrophy accompanied by creatinuria. On a combined deficiency these symptoms develop simultaneously and apparently independently of each other, resulting in a greater degree of paresis than that produced by a deficiency of either of the factors individually.

BIBLIOGRAPHY

(1) Evans and Bishop, Science, 56, 650 (1922)

(2) Evans and Burr, J. Biol. Chem., 76, 273 (1928)

(3) Goettsch and Pappenheimer, J. Exp. Med., 54, 145 (1931)

(4) Waddell and Steenbock, J. Biol. Chem., 80, 431 (1928)

(5) Ringsted, Biochem. J., 29, 788 (1935)

(6) Mackenzie, Levine, and McCollum, J. Nutrition, 20, 398 (1940)

(7) Madsen, McCay, and Maynard, Proc. Soc. Exp. Biol. Med., 30, 1434 (1933)

(8) Madsen, J. Nutrition, 11, 471 (1936)

(9) Mackenzie, Mackenzie, and McCollum, Science, 94, 216 (1941)

(10) Mattil and Golumbic, J. Nutrition, 23, 625 (1942)

(11) Shimotori, Emerson, and Evans, Science, 90, 89 (1939)

(12) Barrie, Nature, 142, 799 (1938)

(13) Goettsch and Ritzman, J. Nutrition, 17, 371 (1939)

(14) Mackenzie and McCollum, Science, 89, 370 (1939)

(15) Madsen, McCay, and Maynard, Cornell Univ. Agric. Exp. Station Memoir 178 (1935)

(16) Pappenheimer and Goettsch, J. Exp. Med. 57, 35 (1934)

(17) Goss, Zoologica, 25 (part 4), 523 (1940)

(18) Pappenheimer, Am. J. Path., 18, 169 (1942)

(19) Houchin, Federation Proc., 1, 117 (1942)

(20) Wolbach and Howe, Arch. Int. Med., 32, 571 (1923)

(21) Evans, Emerson, and Telford, Proc. Soc. Exp. Biol. Med., 36, 625 (1938)

(22) Goetsch and Brown, J. Biol. Chem., 97, 549 (1932)

(23) Morgulis and Spencer, J. Nutrition, 12, 191 (1936)

(24) Verzar, Ztsch. f. Vitaminforsch. 9, 242 (1939)

(25) Morgulis, Nutritional Muscular Dystrophy, Act. Sc. et Indust. 752, Paris, Hermann & Cie (1938)

(26) Wulzen and Bahrs, Physiological Zoology, 3, 457 (1933)

(27) Bahrs and Wulzen, Proc. Exp. Biol. Med., 33, 528 (1936)

(28) Wulzen and Bahrs, Physiological Zoology, 9, 508 (1936)

(29) Wulzen, Rural New Yorker, vol. CI (January 10, 1942)

(30) Mackenzie, Mackenzie, and McCollum, J. Nutrition, 21, 225 (1941)

(31) Folin, J. Biol. Chem. 17, 469 (1914)