THE SYNTHESIS, STORAGE, AND EXCRETION OF CREATINE, CREATININE, AND GLYCOCYAMINE IN PROGRESSIVE MUSCULAR DYSTROPHY AND THE EFFECTS OF CERTAIN HORMONES ON THESE PROCESSES

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Progressive muscular dystrophy is a disease characterized by degeneration, weakness, and atrophy of various groups of voluntary muscles, notably those of the lower extremities, pelvis, and shoulder girdles. Several clinical varieties have been described, based on the age of onset and location of the affected muscles. It is becoming increasingly clear, however, that no sharp division can be made among many of the clinical types, and that some fundamental defect common to the various syndromes may be responsible for the continuity and sequence of phenomena observed in this group of muscular disorders. Although isolated cases are observed frequently, there is excellent evidence that the disease is hereditary and familial. Males are more frequently affected than females, and in a ratio of about 6:1. In the male the disease also tends to be of greater severity and more rapidly progressive. In females the disease may progress with only slight symptoms, and in some cases might pass unnoticed were it not for the occurrence of the syndrome in other members of the family.

Notwithstanding the fact that progressive muscular dystrophy has engaged the attention of the clinician, the pathologist, and the neurologist since the original description of this syndrome in the middle of the Nineteenth Century, biochemists and physiologists, for the most part, had failed to exhibit any sustained interest in this disorder until the work of Levene and Kristeller (1) in 1909. These workers showed that the feeding of protein resulted in an an excessive excretion of creatine in patients with progressive muscular dystrophy. Subsequently, many studies have revealed that marked derangement in the metabolism of creatine exists in this disease, and that endogenous creatine, formed from protein and amino acids, is not retained nearly so effectively as in the normal subject (2). When creatine is ingested by patients

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with progressive muscular dystrophy, much of it may be excreted in the urine, the amount retained depending to some extent on the severity of the disease. This observation has given rise to the concept that there is in progressive muscular dystrophy a "diabetic-like state" with respect to the ability of the patient to retain either ingested creatine, or creatine which is formed endogenously from proteins and amino acids. Whether or not this is a true concept, the recognition of the biochemical aberration in creatine metabolism is perhaps the only truly significant contribution to have been made toward an understanding of the essential nature of this disease within the last thirty years.

Considerable speculation has arisen in recent years over the significance which may be attached to the aberrant metabolism of creatine in diseases of muscle, with particular reference to progressive muscular dystrophy. There has been much controversy over factors which influence the excretion of this substance, not only in patients with diseased muscles, but in normal subjects as well. An examination of the results of these studies reveals that in many instances little or no dietary control was exercised, while in others almost no cognizance was taken of the factor of variation in physiological creatinuria with age and sex.

The development of methods of greater specificity for the determination of creatine and creatinine in biological material, and recent studies in which isotopes of nitrogen and hydrogen have been employed, have given us new and important information on the origin and metabolism of creatine and creatinine. As a consequence, many of the early statements concerning the metabolism of creatine and creatinine in diseases of muscle need to be revaluated, and in some instances rejected entirely.

In line with the appearance of this information, it was decided to perform anew certain studies on the excretion of these substances, both in normal subjects and in patients with diseases of muscle, and to secure additional data on some of the many factors which are known to influence this process.

Studies on the excretion of creatine in patients with progressive muscular dystrophy have been made by numerous investigators. In general the level of creatine excretion has been found to be roughly proportional to the degree of severity of the disease, and is, therefore, of importance in clinical appraisal and prognosis. Increased excretion of creatine in the urine, however, is by no means confined to diseases of muscle, but is observed in a variety of physiological conditions, *e.g.* starvation (3, 4), carbohydrate privation (5), acidosis (6), and as a result of feeding diets high in protein (7, 8). Increased excretion of creatine is also observed in a number of entirely unrelated diseases, notably malignancies, fever, diphtheria, thyroid disease, and parathyroid disease (9). In children, utilization of data on the excretion of creatine as a guide to the state of progression of muscle disease is difficult, owing to the excretion, under physiological conditions, of considerable quantities of creatine.

Of equal importance with the increased excretion of creatine in progressive muscular dystrophy, is the diminished output of creatinine. Due to the specific association of

creatinine formation and excretion with the integrity of muscle processes, the urinary concentration of this material may, in certain cases, give a more reliable indication of the severity of the disease than the level of urinary creatine. Moreover, the relatively great constancy in the excretion of creatinine, even under widely differing conditions of diet and health of the subject, permits the attachment of greater significance to small changes in the urinary concentration of this material than is the case with creatine, which may show wide fluctuations from day to day.

The constancy with which creatinine is excreted, and its apparently complete lack of relation to the total metabolism of protein were cited by Folin (15) as evidence that creatinine is the product of a special type of metabolism which proceeds at a uniform velocity in all living cells of the body independent of the many events which influence the excretion of creatine. Later Shaffer (10) suggested that creatinine is not derived from all tissues, but that it arises as a result of a special process of tissue catabolism taking place "largely, if not wholly in the muscles." Upon this hypothesis was based the belief that the amount of creatinine excreted in the urine "bears a direct relation to the potential efficiency of the muscles and is a reliable index of the muscular development of an individual." The belief, originally stated by Folin, that creatinine formation represents a type of endogenous metabolism distinct from the exogenous metabolism of food protein is no longer tenable, as a result of the studies performed by Schoenheimer and his associates (11) with isotopic nitrogen. The constant daily excretion of creatinine, in contrast to the highly inconstant excretion of other nitrogenous constituents of the urine, however, indicates that the formation of creatinine is an orderly and well regulated process, the biological significance of which is not entirely apparent. That it arises directly from creatine is abundantly clear from the isotope experiments of Bloch, Schoenheimer, and Rittenberg (12). Moreover, it is also clear from the results obtained by numerous investigators that its origin from creatine is somehow associated with the utilization of creatine by the muscles, and that the quantity of creatinine in the urine is a fairly adequate measure of the extent to which creatine is being metabolized.

Materials and Methods

Data reported in this communication have been obtained from six male children with progressive muscular dystrophy, and from five normal male children, within the same age group, in whom no evidence of muscle disease could be elicited. The subjects reported herein were selected from a larger group of 40 patients with progressive muscular dystrophy as being most representative of the disease in its principal stages, from moderate to severe involvement. An account of certain clinical studies performed on the larger group of patients has appeared in an earlier communication (13). A preliminary statement regarding the nature of the specific changes in the muscles of some of the cases reported in this paper, as revealed by ultraviolet light photomicrography, has also appeared (14). Throughout the period of observation, patients and normal subjects were maintained on a diet of constant composition, high in protein and moderately low in carbohydrate and fat. By careful control of the diet, fluctuations in the excretion of creatine due to this factor were held to a minimum. During the control period, and for several days after initiation of a given experiment, urine specimens were collected quantitatively over periods of 24 hours. These were kept at 5°C. until ready for analysis, a period of time which rarely exceeded 48 hours. When the trend of the analytical data was established, the interval of collection was lengthened and the urine stored at low temperature until the collection for a given period was complete. Determinations of pre-

formed creatine and total creatinine were made by the method of Folin (15), with certain modifications of the classical method in order to adapt it to the photoelectric cell colorimeter. The determination of glycocyamine was carried out by the technique of Dubnoff and Borsook (16), with slight modifications.

EXPERIMENTAL

In this study, the creatine excretion of a series of normal preadolescent males was found to be approximately one-half to two-thirds that observed in a group of children with progressive muscular dystrophy. Although stabilization of the excretion of creatine was enhanced by feeding a diet of constant composition from day to day, there was, nevertheless, considerable fluctuation in creatine excretion for a variable interval following the ingestion of food. A certain daily rhythm in the excretion of creatine in both the normal children and the children with muscular dystrophy was observed, with its peak occurring in the morning and its lowest point occurring during the night. This so called rhythm of excretion of creatine in normal children was first recorded by Powis and Raper (17) in 1916. As a consequence of the physiological creatinuria and its marked fluctuation in the subjects under study, it was considered unwise to attempt an evaluation of any but the major changes in creatine excretion observed during the study of each case.

Relation between the Level of Excretion of Urinary Creatinine and Residual Mass of the Muscles in Progressive Muscular Dystrophy

The marked decrease in the output of creatinine observed consistently in patients with progressive muscular dystrophy has been attributed frequently to the reduction in the total mass and efficiency of the muscles which occurs in the disease (2). In Table I are listed the results of an attempt which was made to give quantitative expression to the degree of loss of mass and efficiency in the muscles in this disease, by a consideration of the ratio of creatinine excretion in patients with progressive muscular dystrophy to that observed in a series of normal subjects within a comparable age group. The calculations were made on the assumption that in the normal subject 40 per cent of the body weight is muscle. Normal creatinine values used in these calculations were obtained from the five control subjects. The values, expressed as milligrams of creatinine excreted per kilogram of body weight in 24 hours, agree closely with those given by other workers for normal male children in this age group (9). On the basis of the results given in Table I, it can be seen that in advanced progressive muscular dystrophy the mass of functioning muscle, as inferred from the creatinine findings, may be as little as 17 per cent of the body weight, or less than half of that of normal subjects. That the concentration of creatinine in the urine bears more than a casual relationship to the mass and efficiency of the muscles in children with progressive muscular dystrophy is seen clearly in the results obtained on the case material reported above. The

values recorded in Table I are typical of those found for a larger number of patients exhibiting this disease in whom studies have been less complete. In all cases in which the creatinine excretion has been carefully studied, there has been found a marked correlation between the amount of muscle as per cent of body weight, inferred from a consideration of the data on creatinine excretion, and the degree of physical performance of the patients, in so far as such performance could be appraised in a quantitative fashion. In our experience the calculation of the muscle mass on the basis of the creatinine excretion has been a fairly reliable index of the extent of involvement of the muscular system in patients with this disease as determined clinically. Moreover, by performing this type of calculation at frequent intervals on a given subject with progressive muscular dystrophy, a fairly quantitative appraisal of the clinical condition of the patient and the rate of progression of the disease can be ascertained.

TABLE I

Calculation of Residual Muscle Mass of Patients with Progressive Muscular Dystrophy from Data on Creatinine Excretion

Case No.	Excretion of creatinine Mg./kg. body weight/24 hrs.	Muscle mass Per cent of body weight	Degree of clinical involvement
Average values on 5 normal subjects.....	21.6	Estimated at 40 per cent	None
Patients with progressive muscular dystrophy			
1	9.5	17.6	++++
2	10.0	18.5	+++
3	10.3	19.0	+++
4	11.6	21.5	++
5	13.1	24.2	++
6	18.7	34.6	+

Methyl Group Reserves in Progressive Muscular Dystrophy

Following the elucidation by Borsook and Dubnoff (18), and du Vigneaud and associates (19), of the rôle of methionine in the synthesis of creatine and creatinine, speculation arose in this laboratory over the possibility that a deprivation of methyl groups may occur in certain diseases characterized by excessive creatinuria. In progressive muscular dystrophy, for example, does there develop a state of methyl group deprivation as a result of the excessive and prolonged creatinuria which is characteristic of this disease? The idea that an exhaustion of methyl groups may occur when the demand for

methyl groups is artificially raised by feeding an excess of methyl acceptor is implied in the results obtained by du Vigneaud and associates (20), who found that growth in rats, which was inhibited by feeding of excess glycocyamine, was resumed following addition to the diet of the methyl donors, choline and methionine. It has also been reported that the addition of glycocyamine to an otherwise adequate diet resulted in the production of fatty livers and in the reduction of liver choline to values below that observed when animals were

TABLE II

Total Quantities of "Methyl" Excreted as Creatine and Creatinine in Normal Subjects and Patients with Progressive Muscular Dystrophy

	Age	"CH_3" output as		Total
		Creatine	Creatinine	
		Mg./kg. body weight/24 hrs.		
	yrs.			
Normal subjects				
1	10	2.10	2.51	4.61
2	11	1.29	2.61	3.90
3	11	0.74	3.01	3.95
4	9	1.87	3.01	4.88
5	10	1.22	3.22	4.44
Average		1.44	2.87	4.39
Patients with progressive muscular dystrophy				
1	8	2.27	1.37	3.64
2	7	2.91	1.54	4.46
3	4	2.22	2.49	4.71
4	8	2.55	1.33	3.88
5	11	3.06	1.26	4.32
Average		2.60	1.60	4.20

placed on a choline-free diet (21). Handler and Dann (22), moreover, have recently reported that inhibition of growth produced by an excessive intake of nicotinamide was prevented by the administration of methionine or by choline and homocystine. Although alternative hypotheses must be entertained, the results of these experiments indicate that in the presence of an excess of substances which act as methyl acceptors, a deficiency of methyl groups may occur, and that this deprivation may be prevented by an adequate intake of methylating agents such as methionine and choline. In order to learn whether deficiency of methyl groups occurs in progressive muscular dystrophy, the total quantity of methyl groups excreted as creatine and creatinine was compared

with that of control subjects without evidence of muscle disease. The results are given in Table II. It will beseen at a glance that no evidence of exhaustion of methyl group reserves can be obtained from a consideration of the levels of urinary excretion of creatine and creatinine. It will be observed that any loss in methyl groups, greater than that seen in normal children, occurring as a result of excessive creatine output, is fully compensated for by a diminution in the output of creatinine in the diseased patients. It may be observed also that there is a surprising agreement between the total quantity of "methyl" excreted by the group of normal subjects and that excreted by patients with progressive muscular dystrophy. These data suggest that there is no increase in the output of total creatine compounds in progressive muscular dystrophy over that of normal subjects with a comparable age range, and that the difference in children between the normal subjects and the patients with dystrophy resides rather in the partition of the substance excreted in the urine. In view of the fact that creatinine has been shown by means of isotopes to be derived from creatine, it would appear from a consideration of these results, that in progressive muscular dystrophy the excessive creatinuria does not arise as a result of an increase in the synthesis of creatine, but rather as a result of the incomplete metabolism of this material in the muscle with a consequent decrease in the amount converted to creatinine.

Effects of the Administration of Certain Hormones on Creatinuria in Progressive Muscular Dystrophy

During a period of 3 years in which the problem of progressive muscular dystrophy has been under study at the Rockefeller Hospital, only five females with unequivocal signs of progressive muscular dystrophy have been encountered in a group of 46 patients who have been admitted to the clinic with this disease. Moreover, as pointed out previously, the disease generally runs a more acute course in male children than in the occasional female child in whom the disease is encountered. These observations tend to lead one to the hypothesis that the essential aberration in progressive muscular dystrophy is one connected closely with the biological phenomenon of maleness on the one hand, and perhaps with those systems concerned with growth and development on the other. Certain of these phenomena are mediated through the hormones of the anterior pituitary, the adrenal cortex, and the gonads. It was believed, therefore, that an investigation of the effects of certain of these agents on the metabolism and clinical course of patients with this disease should be carried out.

Several papers have appeared recently, including a preliminary report from this laboratory, dealing with the effect of the steroid hormones, particularly testosterone propionate, on the growth and development of the muscular system, and more specifically on the metabolism of creatine (23–29). Since creatinuria is considered to be the most consistent abnormality in the metabolism of pa-

tients with progressive muscular dystrophy, any agent which may affect the excretion of this substance seemed to us to possess particular interest in connection with a study of this syndrome. Accordingly a study has been made of the effect of certain of the steroid hormones, including testosterone propionate and methyl testosterone,[1] on the metabolism of creatine, creatinine, and glycocyamine in a group of male children with progressive muscular dystrophy, and, for purposes of comparison, in a group of normal male children as controls. The determination of glycocyamine was included in these studies because of its known rôle in the biological synthesis of creatine, and because its excretion has been shown to be altered in patients with progressive muscular dystrophy (30). Less complete studies were also carried out on the effects of desoxycorticosterone acetate, a purified preparation of chorionic gonadotropin, and a concentrate containing both the gonadotropic and thyrotropic hormones of the hypophysis. As in the foregoing studies on creatine excretion, patients and control subjects were placed on diets of constant composition and base lines obtained in each case for the excretion of creatine, creatinine, and glycocyamine. After a control period, during which the excretion of these substances was found to be constant, the hormone selected for study was administered over a period of time adequate to test its effect. The hormone was then discontinued and determinations of creatine, creatinine, and glycocyamine made until approximately the former levels of excretion of these substances were reached.

Effects of the Administration of Testosterone Propionate.—Muscular development is promoted by the male sex hormones in animals (31) and in man (32–34). Various tests, objective in character, have shown a measurable increase in muscular performance in human subjects, following the administration of male hormone. The male hormone has been shown to foster the deposition of creatine in muscle, resulting in added weight and strength of this organ system (27). After a suitable control period, testosterone propionate was administered intramuscularly to male children with progressive muscular dystrophy in doses of 20 mg. a day over a period of 10 to 14 days. In five patients the administration of the hormone was continued in doses of 10 to 20 mg., 2 to 3 times weekly, for periods of 2 to 3 months with determinations of creatine and creatinine performed at regular, but less frequent intervals. Certain differences between the effect of testosterone propionate on the excretion of creatine and glycocyamine in normal subjects and in patients with progressive muscular dystrophy were immediately apparent. In the normal controls there was a marked drop in the excretion of creatine within 72 hours following the administration of testosterone propionate, which persisted as

[1] We are greatly indebted to Dr. L. A. Pirk, of Roche-Organon, Inc., Nutley, New Jersey, for the testosterone propionate (neo-hombreol), methyl testosterone (neo-hombreol M), ambinon, pregnyl, and desoxycorticosterone acetate (doca) used in these studies.

long as the hormone was administered. Immediately following the withdrawal of the hormone there ensued a marked creatinuria for several days, at which time the creatine excretion reached a level greater than that of the control period. Results on a typical normal subject are given in Fig. 1. In patients

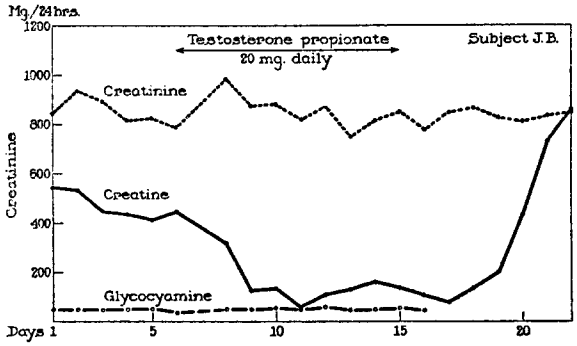

FIG. 1. Curves showing the influence of testosterone propionate on the urinary excretion of creatine, creatinine, and glycocyamine in a normal child of 8 years.

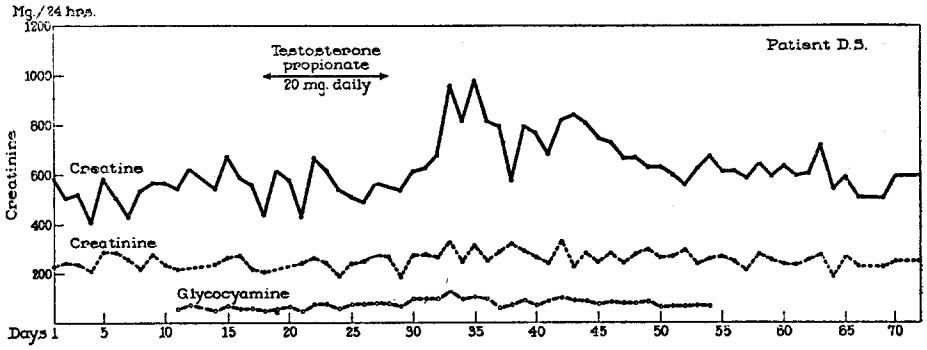

FIG. 2. Curves showing the influence of testosterone propionate on the urinary excretion of creatine, creatinine, and glycocyamine in a child with moderately advanced progressive muscular dystrophy.

with progressive muscular dystrophy, no significant change occurred in the excretion of creatine during the interval in which the hormone was administered. However, as in the normal subjects, a marked increase in the excretion of creatine was observed for several days following the withdrawal of testosterone propionate. Data on a typical case with progressive muscular dystrophy are represented in graphic form in Fig. 2. No significant change in the excretion of creatinine was apparent either in patients with progressive muscular dystrophy or in normal subjects following the administration of testosterone pro-

pionate. Clinical response to the hormone, manifested by marked changes in the secondary sex characters, was manifested in each case receiving testosterone propionate within 10 days to 2 weeks. These changes, which were evidenced in lowered pitch of the voice, acceleration of the growth of pubic and axillary hair, and enlargement of the genitalia, persisted in some cases for several weeks following withdrawal of the hormone. Certain psychological changes similar to those which occur normally at the onset of adolescence, were also observed. No final statement regarding the effect of testosterone propionate on the clinical course of the disease can be made at this time. Although marked clinical improvement in coordination and strength of the muscles followed the administration of the hormone in one case of early progressive muscular dystrophy, it is not certain at this time that the effect can be directly attributed to treatment with testosterone propionate. Certainly no dramatic or unequivocal signs of improvement were observed in other cases receiving the hormone. It should be remarked in this connection, however, that the rate of progression of the disease is extremely variable from case to case, and adequate controls are so difficult to set up that many cases will have to be studied before it can be stated with certainty whether or not improvement is obtained.

Effects of the Administration of Methyl Testosterone.—Methyl testosterone is said to be a completely potent androgen and to be similar in its clinical effects to testosterone propionate. The methyl derivative differs from testosterone propionate, however, in that it can be administered by mouth. It differs from testosterone propionate, also in that it has been shown to produce marked creatinuria (35). Creatine storage, however, is said to occur with this agent, and the accompanying creatinuria has been explained as being due to an accelerated synthesis of creatine (36).

Three patients with progressive muscular dystrophy were given 20 mg. of methyl testosterone orally for a period of 10 days, and an analysis of the urine for creatine, creatinine, and glycocyamine performed as before. The results obtained with a typical patient are given in Fig. 3. Since the effects of methyl testosterone on the excretion of creatine in normal children have been thoroughly explored by others (35, 36), only one normal male child was deemed necessary as a control subject. In contradistinction to the effects observed with testosterone propionate, however, there was a marked increase in creatine excretion in the normal subject immediately following the administration of methyl testosterone, which was maintained as long as the hormone was continued. A marked increase in urinary glycocyamine likewise occurred. Upon withdrawal of methyl testosterone, no further increase occurred in the output of either creatine or glycocyamine. On the contrary, there was a rapid drop in the excretion of these substances until the previous levels of output were attained. No significant alteration in the excretion of creatinine occurred

in either the normal control subject or in the patients with progressive muscular dystrophy. Various explanations of the effect of methyl testosterone in provoking creatinuria have been offered. Most workers assume that creatinuria following the administration of this substance is due to an actual increase in the rate of creatine synthesis, with consequent excretion of the excess creatine (36). The marked increase in excretion of glycocyamine, a known biological intermediary in the synthesis of creatine, is in keeping with this idea. In this connection it is interesting to speculate on the possibility that the creatinuria following the administration of methyl testosterone results primarily from an increase in the rate of formation of glycocyamine, and that

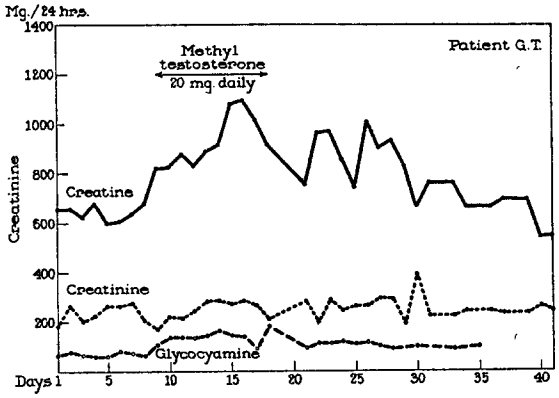

FIG. 3. Curves showing the influence of methyl testosterone on the urinary excretion of creatine, creatinine, and glycocyamine in a child with moderately advanced progressive muscular dystrophy.

methylation of the glycocyamine occurs secondarily, resulting in an excessive output of creatine.

The phenomenon of creatinuria following the administration of methyl testosterone has been cited by Tager (37) as evidence that the hormone acts in large doses as a methylating substance. The idea that methyl testosterone provokes creatinuria in this manner may be attractive but, unfortunately, is not supported by the facts. Indeed, there is considerable evidence to the contrary inherent in Tager's own data. A comparison between the amount of "methyl" supplied in the form of methyl testosterone and the amount of methyl appearing in the excess urinary creatine in his cases, shows that on the consideration of mass alone, the hormone could account at the most for only a small part of the total methyl excreted, assuming that all of the "methyl" contained in the hormone were available for methylation.

The Effects of Concentrates of Hormones of the Hypophysis on the Excretion of Creatine and Creatinine in Progressive Muscular Dystrophy.—Because of

the current concept that the formation of creatinine is intimately bound up with the normal metabolism of creatine, it was considered logical to test the effects of those hormones which are believed to accelerate the production of creatine and promote the formation of creatinine in animals and in normal human subjects. According to some workers the excretion of urinary creatinine is increased in animals and man by the growth hormone of the anterior pituitary (38), the gonadotropic hormone of the hypophysis (39), and by certain extracts of the anterior pituitary (40). Two hormone concentrates were made available for this study. The first, a concentrate of the gonadotropic and thyrotropic principles of the hypophysis (ambinon[1]), containing 50 rat units of gonadotropic principle and 200 guinea pig units of the thyrotropic principle per cc., was administered in doses of 1 cc. three times weekly for 20 and 40 days, respectively, to two male children with progressive muscular dystrophy. No detectable changes in the excretion of creatine or creatinine were observed in either case. Moreover, no detectable change in the clinical status of the patients was evident. The second preparation, a chorionic gonadotropic concentrate with pituitary-like properties (pregnyl[1]), containing 500 I.U. per cc., was administered intramuscularly to another patient with muscular dystrophy, in doses of 1 cc. 2 times a week for 1 month. No changes in the levels of excretion of creatine or creatinine were observed, and no changes in the clinical status of the patient were detected.

The Effect of Desoxycorticosterone Acetate on the Excretion of Creatine and Creatinine.—Two patients with moderately severe progressive muscular dystrophy were selected for study, and placed on 5 and 10 mg. respectively of desoxycorticosterone acetate (doca[1]), by intramuscular injection, 3 times weekly. No changes in the levels of excretion of creatine or creatinine, or in the clinical status of the patients were detected after 40 days of this regimen.

DISCUSSION

Although much speculation has accompanied the published results of investigation on progressive muscular dystrophy, little has been added to our concept of the essential nature of this syndrome since the discovery of an aberrant metabolism of creatine in this disease by Levene and Kristeller, in 1909 (1). After thirty years of investigation, we must continue to regard creatinuria as the most striking manifestation exhibited by patients with this disease. Evidence has been presented which leads us to believe, however, that the essential defect is not to be found in the mechanisms which control the synthesis of creatine or in the mechanisms responsible for transport of this substance, but rather in those systems which in normal muscle are linked with the production of creatinine. Although creatinuria may arise in many diseases, only in those diseases in which there is extensive atrophy and degeneration

of muscle does there occur at the same time a marked diminution in the urinary excretion of creatinine. Of the diseases of muscle which are characterized by creatinuria and diminution in excretion of creatinine, progressive muscular dystrophy is the most outstanding example. Notwithstanding the invariable association of diminution of creatinine excretion with this syndrome, however, we have no evidence that the disease arises as a result of a defect in the synthesis of this substance. It is perhaps best to regard the phenomenon of diminished creatinine excretion as evidence of a defect which is far more subtle, and which results among other things in the lessened production of this substance. In fact there has been no adequate evidence presented thus far to show that genuine qualitative changes occur in the metabolism of muscle in this disease. Until such changes are demonstrated the disease will continue to escape classification with the rare inborn diseases of metabolism, *e.g.* alkaptonuria, cystinuria, etc., in which changes of a qualitative character in the metabolism are evident. It is even possible that the inherent defect in progressive muscular dystrophy lies entirely outside the musculature, and that the chief symptoms of the disease are expressed in degenerative changes in the muscles.

Of the factors which are believed to influence the synthesis and utilization of creatine, the hormones of the adrenal cortex, the gonads, and the anterior pituitary are the best known. It was in an effort to learn something of the extent to which these factors operate in the production of creatinuria in progressive muscular dystrophy that the foregoing study was initiated.

In general, it may be said that the effects of testosterone propionate and methyl testosterone in patients with progressive muscular dystrophy, are qualitatively like those in normal male children of comparable ages, except that the storage of creatine in the normal child receiving testosterone propionate is more readily demonstrated than in patients with marked muscle disease. This may be explained in part by a diminution in the capacity of the muscle in progressive muscular dystrophy to store creatine, and also by the fact that in the presence of an excessive creatinuria the storage of creatine, in so far as it is reflected by a diminution in output, is harder to demonstrate. The possibility must be kept in mind, however, that the male hormones may be unable to influence the storage and utilization of creatine to the same extent in patients with muscle disease as in normal subjects, owing to a possible defect inherent in the muscles *per se*.

If creatine retention, which occurred as a result of the administration of testosterone propionate, is to be regarded as a true storage phenomenon, it would suggest that in progressive muscular dystrophy the capacity to store creatine is not irreversibly altered. It seemed, therefore, that we were justified in administering the hormone over long intervals of time in order to learn

whether improvement in muscle function could be accomplished thereby. In this connection, results of the present study have been disappointing. In one case only did it appear that favorable clinical results had been attained. In this instance the disease was in its earliest stages, and although there was unequivocal evidence of marked improvement in muscle function and in the clinical status of the patient following the administration of testosterone propionate, it cannot be ascribed with certainty to a specific effect of the male hormone. It should be pointed out that in progressive muscular dystrophy effective controls are nearly impossible, because of the extreme variation in severity and rate of progression of the disease from case to case. Therefore, the effect of any agent on the rate of progression of the disease, unless truly dramatic, would be difficult to evaluate clinically.

SUMMARY

The diminished excretion of creatinine in progressive muscular dystrophy is a more striking and specific phenomenon than the excess excretion of creatine, marked though this is. While creatinuria is invariably encountered in all cases of long-standing dystrophy, the extent to which the excretion of creatinine is decreased provides a more reliable indication of the severity of the disease since an excess output of creatine may occur physiologically in normal human subjects and in many pathological conditions not known to be associated with muscle disease.

In progressive muscular dystrophy the residual muscle mass, as inferred from the excretion of creatinine, provides a useful index of the state of the disease at any given time.

Although there is excessive creatinuria in progressive muscular dystrophy, there is no evidence that a deprivation of methyl stores occurs through a loss of urinary creatine. The loss of methyl groups contained in the excess creatine is, under ordinary conditions of diet, almost exactly compensated for by a drop in the excretion of methyl groups in the urinary creatinine.

Testosterone propionate, administered over variable periods of time, resulted in the retention of creatine both in normal male children and in male children with progressive muscular dystrophy, as shown in the normal subjects by a diminution in creatine output, and in both by an excess creatinuria for variable periods of time following withdrawal of the hormone. An increase in the excretion of creatine in progressive muscular dystrophy occurred following the administration of methyl testosterone. Neither testosterone propionate nor methyl testosterone appeared to effect any consistent change in the output of urinary creatinine.

No effects on the excretion of creatine and creatinine were observed following the prolonged administration of a concentrate of gonadotropic and thyrotropic

principles of the hypophysis, or from the administration of desoxycorticosterone acetate to patients with progressive muscular dystrophy.

Except in one case, in which marked improvement was observed following the administration of testosterone propionate, no effects on the clinical course of the patients with progressive muscular dystrophy were observed as a result of treatment by any of the various hormones employed in this study.

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