EFFECTS OF PARABIOSING DYSTROPHIC MICE TO NORMAL MICE ON THE DAY OF WEANING

By

Richard S. Pope, B.S.

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APPROVED:

[redacted]

Leonard H. Elwell, M.S., Ph.D.
Professor of Physiology

[redacted]

[redacted] B. Bump, D.M.D., Ph.D.,
Chairman, Graduate Education Committee

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TABLE OF CONTENTS

LIST OF FIGURES

Figure | Page

Figure | Page

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

dydy	Dystrophic (homozygous genotype)
DyDy	Homozygous Normal
Dydy	Heterozygous Normal
Dy+	Normal phenotype, includes both DyDy and Dydy normals
P	Parabiont
S	Single
O.T.	Ovary transplanted female A normal female with an ovary transplanted from a dydy female, used for breeding purposes
(DyDy+dydy)	Parabiotic pair; homozygous normal and dystrophic
(Dydy+dydy)	Parabiotic pair; heterozygous normal and dystrophic
(DyDy,dydy)	Single control pair; homozygous normal and dystrophic
(Dydy,dydy)	Single control pair; heterozygous normal and dystrophic

INTRODUCTION

Dystrophia Muscularis (gene symbol dydy) occurred in the mouse inbred strain 129 as a spontaneous mutation in 1955 (1). The clinical, histological, and biochemical manifestations of this disease have been shown by Coleman, West, and others to be comparable to the Duchenne type of human Progressive Muscular Dystrophy (2,3). As such, mice with muscular dystrophy are the only available good research animals with which to study muscular dystrophy.

Much work has been done on the dystrophic 129 strain mouse since the occurrence of the mutation for this defect in early 1955 was first reported by Michelson, Russell and Harman (1). Many reports have been concerned with finding differences in concentrations of various enzymes in serum. If and when a difference is found, the question of causality must be examined. A physiological means of determining whether blood is etiologically involved, either because of the absence or the presence of some substance, would be a significant aid in the study of muscular dystrophy.

One hypothesis as to the cause of this disease would invoke the presence or absence of some substance in the blood or serum. Such a substance might be a hormone or an enzyme, the absence of which results in the development of progressive muscular dystrophy. Conversely, one could conceive of the presence of a faulty enzyme or toxic by-product which would be a causative factor, at least secondarily. This hypothesis can be tested by providing a common circulation between a dystrophic mouse and a normal mouse.

A technique approximating these requirements is that of parabiosis, whereby two animals are united surgically in such a way that there is a shared circulation of blood.

Previous work (4) has shown that if dystrophic mice are parabiosed to normal mice there is no difference in life span between parabiosed and single dystrophics. It would appear that parabiosis does not provide the means for a cure of dystrophy. Because of severe limitation of life span parameters, study of organ weights and histopathology of various tissues could not be adequately done. When the dystrophic parabiont dies, it soon becomes severely congested with blood transfused from the live partner. Autolysis of tissue and congestion of vessels and organs as a result of the terminal state, preclude any reliable search for possible subtle effects, either in the normal parabiont or the dystrophic partner, arising as a result of the common blood circulation. The purpose of this study was to determine whether effects of parabiosis between normal and dystrophic mice could be found if serial sacrifice of pairs was done at varying times following union, as well as to further evaluate the effects on life span. Since diet has been shown to have important effects on lifespan of dystrophic mice (5), a further evaluation of lifespan of parabiosed dystrophics is considered important for this study.

REVIEW OF LITERATURE: PARABIOSIS

Finerty (6) states that Paul Bert first described the technique of parabiosis in 1862. The technique used today remains essentially the same, although some workers modify the technique slightly. Bunster and Meyer reported on an improved technique in 1933 (7). An excellent review of the subject was published in 1952 by Finerty (6). Some of the areas of investigation which have found parabiosis a useful tool are:

Immunology and Genetics (8 - 15),

Transplantation and Tumor Studies (16 - 21),

Endocrinology (22 - 32),

Blood Pressure (33 - 35), and

Irradiation Effects (36, 37).

As can be seen the parabiotic technique has been used in many different areas of study. Two of the problems associated with use of this technique, particularly when used on small animals such as rats or mice, are the establishment of a common circulation and the avoidance of "parabiotic intoxication".

Establishment of a common circulation between parabionts has been demonstrated in many ways. Radioactive red blood cells have been injected into one partner and found to circulate in the other (38,39). Hill and others injected vital dyes and noted the movement of the dye from one partner to the other (24,40). Fluorescein injections also have demonstrated the presence of a common blood circulation (41). These and many other studies show that a common circulation exists between parabiosed mice after about five to seven days. This circulation is via anastomotic vessels across the healed skin incisions.

As a test of this investigator's technique, parabiotic pairs were injected with methylene blue and the dye was seen to appear in the tissues about the mouth, ears, anus and feet of the non-injected animal. The appearance of the dye in these areas was seen prior to diffusion of the dye through the joined skin. A more definitive test is shown in Figure 1, where one animal had a latex solution injected into the ascending aorta, while its partner had the right ventricle incised. The latex injection technique used was a modification of that described by West and Gorham (42). Figure 1 shows the vascular pattern outlined by latex in skin of both parabionts. It is thus demonstrated that an anastomotic network of vessels occurs across the sutured skin between two parabiotic partners.

"Parabiotic intoxication" is not a problem when inbred strains of mice are used. This has been shown by Eichwald et al (10) and by the absence of this complication in this investigator's previous studies (4), and in the present studies (43).

Fig. 1 Latex Injection of Parabionts

The parabiont on the right had a latex solution injected directly into the ascending aorta. Latex can be seen to be present in vasculature of the left parabiont, demonstrating blood exchange between parabionts.

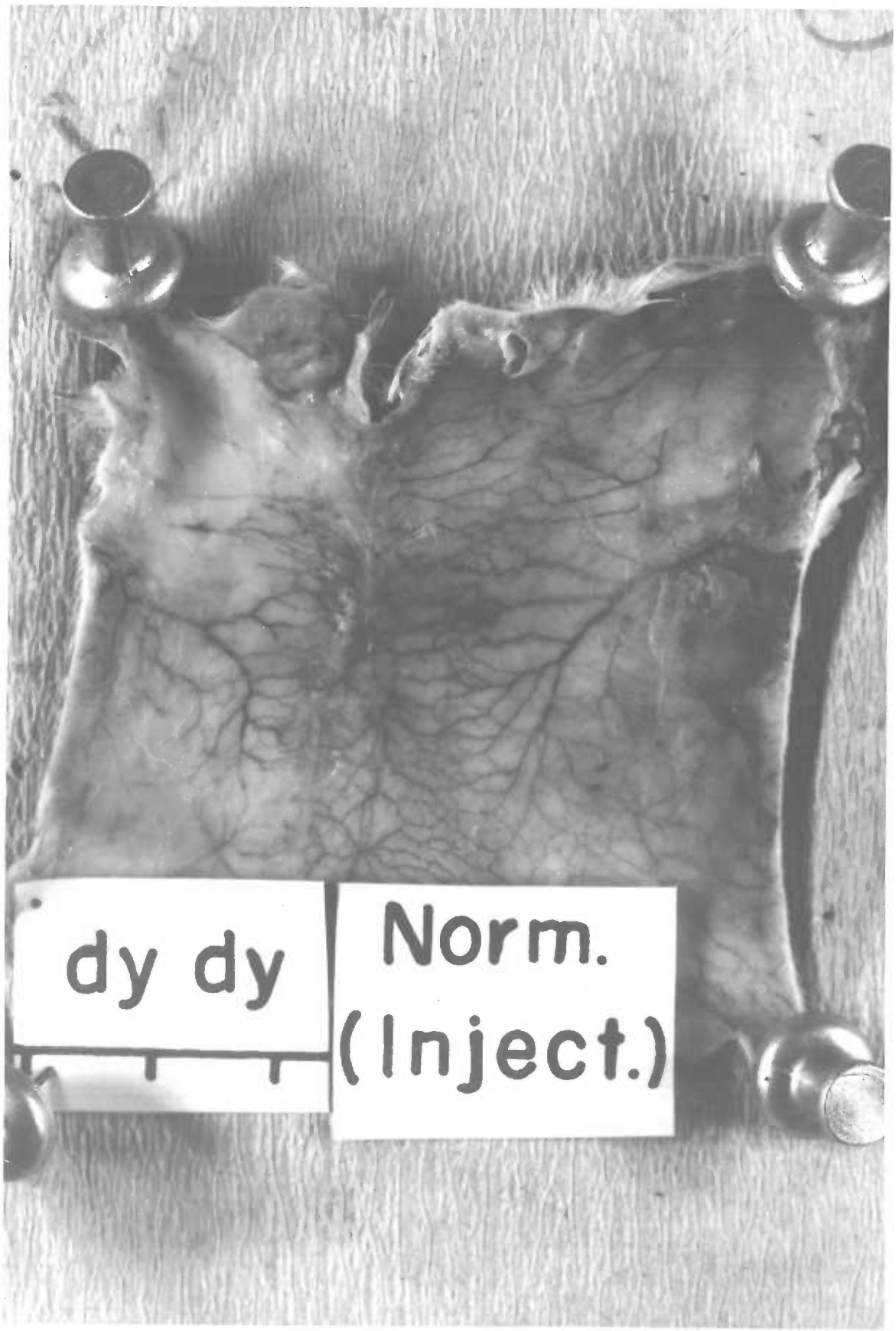

FIG. 1.

REVIEW OF PERTINENT LITERATURE, MUSCULAR DYSTROPHY

Hall, Hall & Nevis (44) reported that when dystrophic mice were parabiosed to normal mice, "the usual brief survival of dystrophic mice was much prolonged by parabiosis and the characteristic signs were often much reduced in severity". These authors also claimed an "apparent complete recovery in one" dystrophic parabiont. The age at the time of parabiosis was not stated and no mention of histologic examination of muscle tissue was made. A life expectancy for single dystrophic mice of 8-10 weeks with an increase to 24 weeks for males and 25 weeks for females, was reported. No figures were given for means, S.E. or S.D. The diet was Purina Laboratory Chow. The number of animals was "15 pairs and their controls".

At variance with Hall et al is the report by Pope and Murphy (4). In their study dystrophic mice were parabiosed to normal mice at seven weeks of age. Diet consisted of Purina Laboratory Chow. Muscle samples from all animals were prepared for microscopic study.

Their results showed a mean attained age for single dystrophic control mice of 18.7 $\pm$ S.E. 1.5 weeks and a mean attained age of 17.0 $\pm$ S.E. 1.4 weeks for dystrophic parabionts. The conclusion was that no significant difference was present between the two groups in attained age. No improvements in the physical condition of dystrophics was found and no improvements were present in muscle examined histologically.

Any improvement which might be found in lifespan of parabiosed dystrophic mice might be attributed to nutritional factors provided by the normal partner via the common blood circulation. This possibility was mentioned by Hall et al (44), but little else could be said about

it. Coleman and West (5) studied the effect of different diets on growth, lifespan and histopathology of dystrophic mice. These authors reported an increased mean lifespan for dystrophic mice; for males from 9.1 weeks to 21.8 weeks and for females from 13.1 weeks to 22.3 weeks, when the mice were reared on Morris formula diet[1,2] instead of Purina Laboratory Chow.[3] An improvement in physical condition and less severe muscle lesions were attributed to the improved diet.

Considering the fact that there was disagreement concerning the effects of parabiosing dystrophic mice to normal mice and that diet alone can result in significant changes in lifespan of dystrophic mice, it was felt that further studies of parabiosed dystrophic mice would be worth while. Parabiosis should be done at an early age, the mice should be reared on the improved diet, and attempts to study lifespan and histopathologic changes should be made separately.

The present studies were planned so as to take into account an early age of parabiosing, nutritional factors of improved diet, and separation of lifespan and histological parameters.

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1. Old Guilford Mouse and Rat Breeder Pellets, the Emory Morse Co., Guilford, Connecticut. This diet is manufactured essentially according to the Morris formulation.

2. Purina Mouse Breeders Chow, Ralson Purina Company, St. Louis 2, Missouri. This diet is manufactured essentially according to the Morris formulation.

3. Purina Laboratory Chow, Ralston Purina Company, St. Louis 2, Missouri.

PARABIOTIC TECHNIQUE

Parabiosis was performed under sodium pentobarbital anesthesia (0.06 mg per gram, I.P.). Veterinary Nembutal[1] 60 mg per cc was diluted ten times to a concentration of 6 mg per cc for injection. The dose was 0.01 ml per gram body weight injected intraperitoneally. The hair was then clipped with electric clippers from opposing sides of two mice from the lumbar region to the ear and extending from side to side, from the mid dorsal line to the mid lateral line. Shaving and the use of depilatory preparations were found to be unnecessary. After clipping the hair from opposite sides of two anesthetized animals an incision was made along the sides from just below the ear to the pelvic region. (Fig. 2).

Figure 2 shows the extent of hair clipping and the location of the skin incision. No particular attention was made regarding weight of partners nor was there any attempt to pair only litter-mates. Dystrophic mice are significantly lighter (12 grams mean weight) at weaning than normal mice (17 grams mean weight). Thus, any attempt to parabiose partners having similar weights would lead to unwarranted and unnecessary selection. Since all mice used in this series were from the inbred strain 129 (see appendix 1), it was felt unnecessary to restrict partners to litter-mates (10). Sexes were never mixed as partners, however.

After making skin incisions along opposing sides of two mice, a running mattress suture (6-0 black braided silk) was started at one

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1. Pentobarbital Sodium, NEMBUTAL, Veterinary, 60 mg per/ml. Abbott Laboratories, North Chicago, Illinois

Fig. 2 Technique of Parabiosis, Hair Removed, Incision Made.

Showing the area of clipped skin and the position and extent of the skin incision.

Fig. 3 Technique of Parabiosis, Stay Sutures in Place.

Showing completion of anastomosis of ventral skin edges, the placement of stay sutures, and the peritoneal incisions.

Fig. 4 Technique of Parabiosis, Completion.

Showing the completed parabiosis.

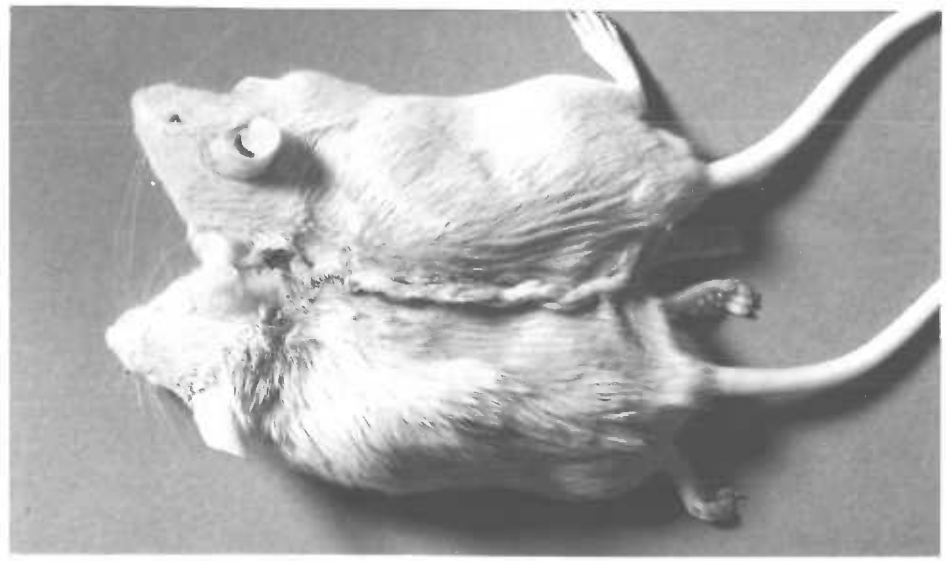

FIG. 4.

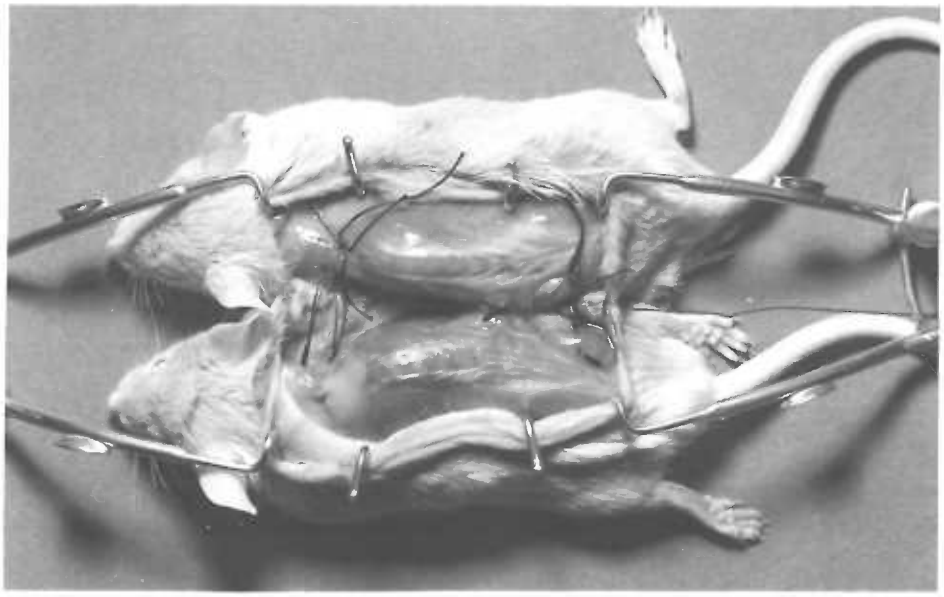

FIG. 3.

FIG. 2.

end, joining the skin edges of both mice and continuing along the ventral skin edges. Suturing was done from the outside. After reaching the opposite end the pair was placed belly down and stay sutures (4-0 black silk or nylon) were placed between the scapulae and between the ilia or the pelvic girdle region. An anastomosis of the peritoneum between the partners can be made at this stage or just prior to the placement of the caudal stay suture. Figure 3 shows completion of ventral suturing and the placement of stay sutures. The dorsal skin edges are next approximated, as were the ventral edges. Figure 4 shows the completed preparation.

No further attention is required unless there is undue strain with resulting tearing of skin along the incision. This occurs infrequently and is usually a result of placing the skin incisions too high or as a result of inadequate extension of the skin incisions. Usually a suture or two is sufficient to close a gap resulting from tearing but an extension of the incision may be necessary in order to relieve the pulling. If initial skin incisions are made too short there also is a tendency for the partners to become twisted, with resultant tearing apart of the parabionts and/or ischemia of the anastomosed skin. Initial incisions which are sufficiently extended prevent this difficulty. When peritoneal anastomoses are made there is the possibility for loops of intestine to slip through the connection and to become twisted, ischemic and necrotic due to stoppage of circulation. This difficulty does not occur if the peritoneal incision is made large and is located high on the body wall. (see Figure 3).

LIFESPAN STUDY, MATERIALS AND METHODS

The strain 129 mice (see appendix 1.) used in these experiments were of three genotypes: dystrophic (dydy), heterozygous or carrier "normal" (Dydy), and homozygous normal (DyDy). The first two genotypes were obtained from ovarian transplant (O.T.) litters, subline 129/Re-dy (45,46). The normals were obtained from a reference colony of subline 129/J. Phenotypic normals (Dy+) include both DyDy and Dydy. All animals were weanlings (mean age, 30.9 ± S.E. of mean 0.5 days). Initial mean weight for normals (Dy+'s) was 16.5 ± 0.2 gm, for dydy's 11.4 ± 0.1 gm.

One hundred twenty dystrophic weanlings were studied (76 female and 44 male): (A) 30 parabiosed with normal DyDy partners, (B) 30 parabiosed with carrier "normal" Dydy partners, (C) 30 sham-operated and caged with DyDy sham-operated partners, and (D) 30 sham-operated and caged with Dydy sham-operated partners.

The following designations will be used when referring to the groups: for parabionts (P), (DyDy+dydy) and (Dydy+dydy): for sham-operated controls (S), (DyDy, dydy) and (Dydy, dydy). Dystrophic weanlings from ovarian transplant litters, and non-dystrophic weanlings from either ovarian transplant litters or from a reference colony of strain 129 were randomly paired, recorded as dydy and DyDy or dydy and Dydy and then alternately parabiosed or sham-operated. Each pair was housed separately and received a modification of the Morris formula (see footnotes 1 and 2, page 17), and tap water ad libitum.

The technique of parabiosis used was the same as described above. Animals sham-operated had the skin incised from the shoulder to the pelvic girdle, the peritoneum incised 3-5 mm and stay sutures placed through the scapula and pelvis but were not connected together in pairs.

Skin and peritoneum were then closed separately. Parabiosed and sham-operated pairs were subsequently treated the same. All operative procedures were done under sodium pentobarbital anesthesia (0.06 mg per gram). TERRAMYCIN[1] was administered in the drinking water (0.2 gm/L) for one week post-operatively. Sexes were not intermixed as partners.

No attempt was made to select the healthier-appearing dystrophics. Deaths which occurred during the first 24 hours post-surgically were arbitrarily attributed to surgery, although it was recognized that dystrophic mice could be expected to have a high incidence of pre-weaning and early post-weaning deaths. The incidence of early deaths was lowered when dystrophic mice were reared on the diet used herein(5).

All animals were checked at least daily. When one member of a pair died the partner was killed with ether and an autopsy performed immediately in order to mimimize autolysis. At autopsy, parabiosed pairs were separated along the suture line. Muscle from the brachium and thigh was fixed in either Bouin's or Zenker's solution for 24 hours, washed in water for 12 hours, imbedded in paraffin, sectioned at 6 to 7 u and stained with hematoxylin and eosin (H and E).

Weights of single and separated animals were recorded. It was recognized that weight data for the separated mice was not completely reliable, since the animal dying first was generally congested with blood from the surviving parabiont.

The period of survival (and attained age) was recorded for all dystrophic mice. (Attained age is defined as the time from birth to death.) Survival data were analyzed for each type of pair. Two pairs were eliminated from the (DyDy,dydy) sham-operated group as a result of animal care accidents, which occurred after 144 to 161 days.

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1. Oxytetracycline HCl, TERRAMYCIN (Pfizer).

LIFESPAN STUDY, RESULTS

Dystrophic mice in parabiotic union with normal mice showed no sign of clinical improvement. All muscle of dydy's whether parabiosed or single, showed evidence of advanced degrees of muscular dystrophy. The final mean body weight of dydy parabionts was less than that of single dydy controls. In no case was death attributable to the surgical procedure. The earliest death occurred on the third post-operative day with only four deaths in less than one week: one (DyDy+dydy) pair at three days: one (Dydy+dydy) pair at four days: one (DyDy+dydy) pair and one (Dydy+dydy) at six days. There was no tearing of skin anastomosis, no intestinal strangulation and no "parabiotic intoxication". Rarely did a pair become twisted.

In two pairs, the normal partner died first and the dystrophic was sacrificed. These occurred as follows: a (DyDy+dydy) pair at thirteen days and a (DyDy+dydy) pair at thirty-three days after surgery. A third pair (Dydy+dydy) was found with both partners dead at 117 days. The first dying partner was considered to have been the Dydy parabiont because of the degree of congestion and autolysis present. These three pairs were eliminated from attained age calculations. Autopsy and histological study did not reveal the cause of death of these pairs but muscle from the normals showed no significant pathology. Of the remaining 115 pairs, the dystrophic always died prior to its partner, whether parabiont or single. In 95 pairs (83%) the normal member of the pairs was found alive. In the remaining 20 pairs (all parabionts) both members were found dead, but there was no question concerning the sequence of death between the two partners since autolysis was more advanced and congestion of blood vessels was found in the dystrophic.

Weight. Final mean body weights for dystrophics showed no significant difference between parabionts and controls or between sexes. Mean body weight of parabiosed dystrophics and single dystrophics was 13.0 ± 0.6 gm and 12.1 ± 0.4 gm respectively. Final mean body weights for normals in parabiotic union were significantly less than for normal counterpart controls, 17.9 ± 0.7 gm and 30.9 ± 1.0 gm respectively, $P < 0.01$.

Histology. Microscopic study of muscle from dystrophic mice, whether single or parabiosed, confirmed the diagnosis of muscular dystrophy in all cases. No normal mouse (parabiont or single) showed significant muscle pathology. Mild inflammatory lesions were found in heart muscles of one normal parabiont, two dystrophic parabionts and one single dystrophic.

Due to the presence of autolysis and severe blood congestion of first-dying parabionts, the value of microscopic study in this series of experiments is limited to determining the diagnosis of muscular dystrophy.

Attained Age. The mean attained age for single dystrophic mice was 212.0 ± 9.6 days. The mean attained age for parabiosed dystrophic mice was 110.0 ± 11.6 days. There was a significantly decreased life-span for parabiosed dystrophic mice of 52% as compared to single dydy controls. ($P < 0.01$). These results are shown in Figure 5.

Fig. 5 Attained Age Following Parabiosis.

The curves show the attained age of dystrophic mice in parabiotic union, and of single control dystrophic mice. A significantly decreased lifespan for parabiosed dydy's is apparent.

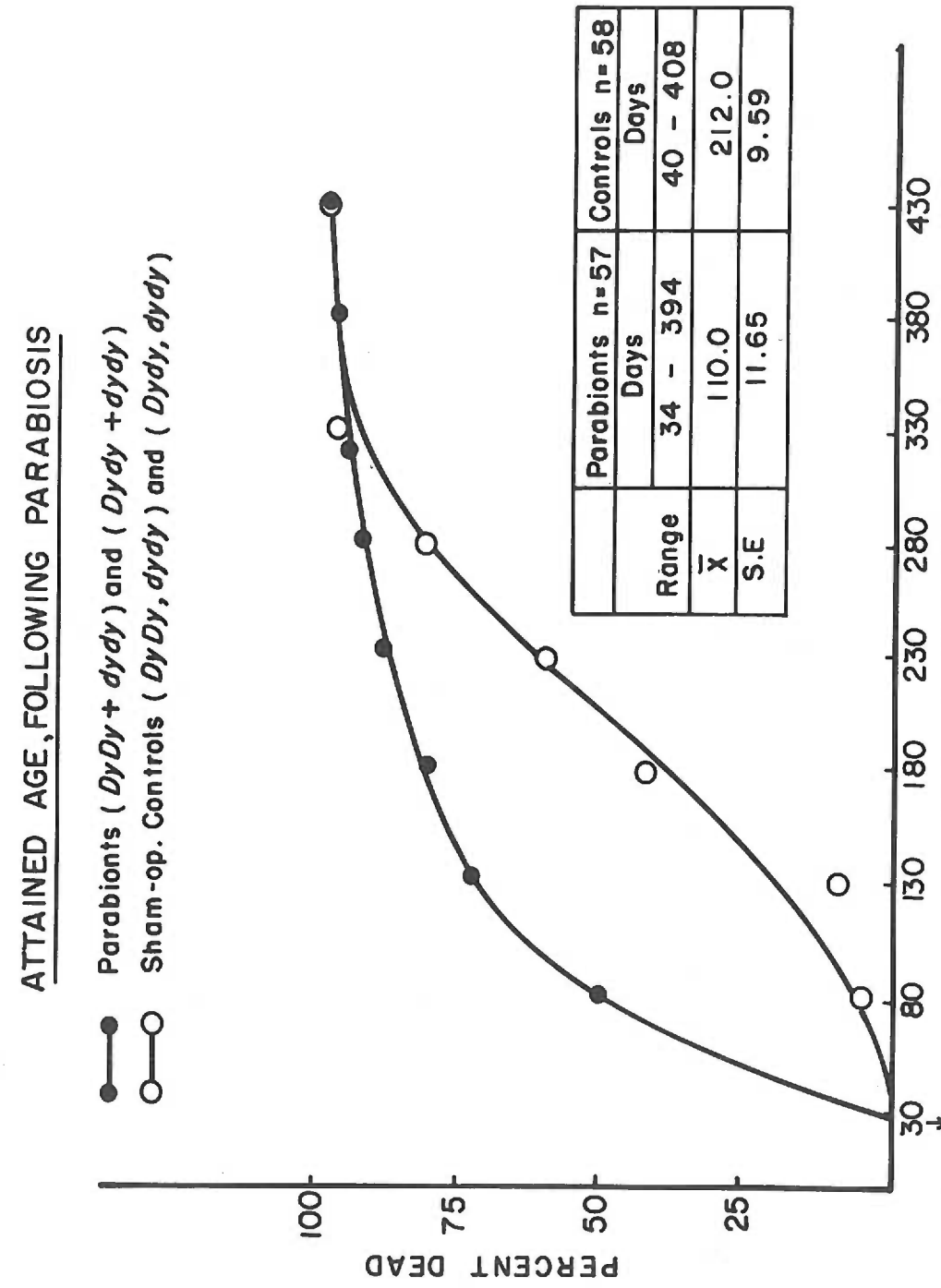

ATTAINED AGE, FOLLOWING PARABIOSIS
Parabionts (DyDy + dydy) and (Dydy + dydy)
Sham-op. Controls (DyDy, dydy) and (Dydy, dydy)
PERCENT DEAD
100
75
50
25
30
80
130
180
230
280
330
380
430
AGE , 50 DAY INTERVALS
Parabiosis or sham-op, weaning.
Parabionts n=57
Controls n=58
Range
Days
34 - 394
40 - 408
X̄
110.0
212.0
S.E
11.65
9.59

FIG. 5.

HISTOPATHOLOGY AND ORGAN WEIGHT STUDIES, MATERIALS AND METHODS

The strain 129 mice used in these experiments were of the same three genotypes as used for the lifespan study. The different genotypes (DyDy , Dydy, and dydy) were obtained from the same sources as for the lifespan study. All animals were weanlings, mean age 31.1 ± 0.6 days. Initial mean weight for normals was 16.9 ± 0.3 gm, for dydy, 12.2 ± 0.2 gms.

One hundred twenty dystrophic weanling mice were used (64 females, 56 males). Parabiosis or sham-operations were performed on the day of weaning. Dystrophic weanlings (dydy), and non-dystrophic weanlings were randomly paired, recorded as dydy and Dydy or dydy and DyDy and then alternately parabiosed or sham-operated.

Four types of pairs were prepared and designated as follows: homozygous normal parabiosed to dystrophic (DyDy+dydy): heterozygous normal parabiosed to dystrophic (Dydy+dydy): homozygous normal paired with sham-operated dystrophic (DyDy,dydy): and heterozygous normal paired with dystrophic (Dydy,dydy). Each pair was housed separately and received a modification of the Morris formula (see footnotes 1 & 2, page 17) and tap water ad libitum. There was no attempt to select healthier or stronger-appearing dydy's, and no attempt to pair according to weight. Sexes were not intermixed as pairs.

The technique of parabiosis used was that described previously. Sham-operations were performed the same as those in the lifespan study. At the time of operation, pairs were designated for sacrifice at 4, 8, 12 or 16 weeks. Additional pairs were set up in reserve for the longer intervals due to the high death rate of dystrophic mice in parabiosis (4). Pairs were considered suitable for sacrifice only if both partners

were alive on the predetermined date. Sacrifice times were not changed after being assigned, except for the reserves which were used to fill in for pairs which died prior to sacrifice date.

Sacrifices were performed by exposure to ether. Autopsies were done and parabionts were separated along the line of anastomosis. Body weights were recorded. Samples of muscle (thigh and brachium), as well as heart, lung, liver, spleen, adrenals and kidneys were taken for further study. Muscle samples were fixed in a solution of two parts 10% formalin and one part 10% trichloracetic acid.

Samples of muscle for cross and longitudinal sections were embedded in paraffin, sectioned at 6 to 7 u, and stained with hematoxylin and eosin. Other organs were fixed in 10% formalin (24 hours) and washed in running tap water (24 hours). Heart, liver, spleen, kidneys and adrenals were weighed from the wash water after fat and connective tissue were trimmed and excess water blotted. All organ weights were performed on a Roller-Smith scales to the nearest 0.01 mg. Samples of organs were then embedded in paraffin and treated the same as muscle samples.

The examination of all histological slides was done independently by two different individuals, (the author and W. T. West). There was no communication between these two individuals concerning the slides before study, nor was any attempt made to set up a uniform scale for evaluation. Instead, each individual interpreted every slide and recorded his evaluation. Only after both examiners had completed the study of slides were interpretations compared.

HISTOPATHOLOGY AND ORGAN WEIGHT STUDIES, RESULTS HISTOPATHOLOGY

A total of fifty-nine pairs were collected, distributed over the four time intervals as follows: eight pairs of parabionts and eight pairs of singles were sacrificed at four and eight weeks after being parabiosed: six pairs of parabionts and eight pairs of singles at twelve weeks: and five pairs of parabionts and eight pairs of singles at sixteen weeks (Table 1). No difference in any respect between groups containing either homozygous or heterozygous normals was apparent. Therefore, the (DyDy+dydy) and (Dydy+dydy) groups will be considered together as the parabionts (P), and the (DyDy,dydy) and (Dydy,dydy) groups will be considered together as the singles (S).

There was nearly perfect agreement between the two separate histopathological interpretations. Out of over 800 responses required there was agreement for 92%. The few incidences of disagreement (8% of all slides) were always concerned with minor details, or slight differences in the interpretation of degree of severity.

The diagnosis of amyloidosis as used in these studies indicates deposition of material between cells, which appears like amyloid in H. and E. stained slides where the material was proved to be amyloid by crystal violet metachromasia on adjacent sections. None of the slides from these experiments were subjected to specific staining for the proof of amyloid.

LUNG: Lesions of lung tissue were limited to mild degrees of edema and/or effusion, and slight septal or alveolar thickening. The diagnosis and incidence are listed in Table 2. The over-all incidence was 18.6% (22/118, twenty-two out of 118 animals) with some degree of pathologic process. More parabionts than singles had lesions, but this

TABLE 1. Number and Distribution of Pairs Sacrificed.

TIME	PARABIONTS (P)	SINGLES (S)
4 wks.	8 pairs, N = 16 (8 female)	8 pairs, N = 16 (8 female)
8 wks.	8 pairs, N = 16 (8 female)	8 pairs, N = 16 (8 female)
12 wks.	6 pairs, N = 12 (6 female)	8 pairs, N = 16 (8 female)
16 wks.	5 pairs, N = 10 (4 female)	8 pairs, N = 16 (8 female)
TOTALS	27 pairs, N = 54 (26 female)	32 pairs, N = 64 (32 female)

TABLE 2. LUNGS: List of Pathologic Lesions, with Incidences.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	1 edema 1/8	1 edema 1/8	1 edema ± 1/8	1 alveolar thickening 1/8
8 wks.	1 alveolar thickening 1 edema 2/8	2 edema, effusion 2/8	2 edema, spotty 1 edema 3/8	1 septal thickening 1/8
12 wks.	1 edema 1 septal thickening 2/6	1 septal thickening 1/6	1 septal thickening 1 edema, effusion 2/8	None
16 wks	None	3 edema 1 septal thickening 4/5	1 septal thickening 1/8	1 edema 1 edema, effusion 2/8
TOTALS	5/27	8/27	7/32	4/32

was due to the increased number of lesions among parabiosed dydy's, since there was no difference between parabiosed normals and single normals, 5/27 (18%) and 7/32 (22%) respectively. The increased incidence for parabiosed dydy's over single dydy's (8/27, 30% and 4/32, 12%) became apparent after eight weeks, and became greater with the longer periods in parabiotic union.

ADRENALS: (Table 3) Essentially all animals, whether parabiosed or single, or Dy+ or dydy, were free of pathologic lesions in adrenal tissue. Only two adrenals with lesions were found, both from animals with generalized amyloidosis, and both in the twelve-week group. One Dydy parabiont showed junctional fibrosis and possible moderate amyloidosis; one single dydy showed moderate amyloidosis.

MYOCARDIUM: (Table 4) Those lesions found in myocardium were of a minor nature and generally consisted of focal areas of fibrosis, acute inflammation, or periarteritis of one or a few vessels. Most of the lesions occurred in the parabionts rather than in singles, 13/54 (24%) and 3/64 (5%) respectively. In both groups more dydy's were affected than were Dy+'s. For the P dydy's and P Dy+'s the respective incidences were 10/27 (37%) and 3/27 (11%).

SPLEEN: (Table 5) Lesions of spleen were limited to amyloidosis of varying degrees, and, except for one isolated case, were limited to parabionts. No lesions were found in the four-week groups. Incidences between sexes were essentially the same. The peak incidences were at 12 weeks. The incidences for P Dy+'s and P dydy's were 5/27 (18%) and 7/27 (26%) respectively. In most cases, when one parabiont had amyloidosis, the partner was similarly affected. In all cases of amyloid of the spleen there was generalized amyloidosis in the animal. Figures

TABLE 3. ADRENALS: List of Pathologic Lesions, with Incidences.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	None	None	None	None
8 wks.	None	None	None	None
12 wks.	1 junctional fibrosis (amyloid) 1/6	None	None	1 amyloidosis++ 1/8
16 wks.	None	None	None	None
TOTAL	1/27	0/27	0/32	1/32

TABLE 4. MYOCARDIUM: List of Pathologic Lesions, with Incidences.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	None	1 sm. area periarteritis 1 fibrosis 2/8	None	None
8 wks.	1 focal necrosis 1/8	1 periarteritis 1 periarteritis, one vessel 2/8	None	None
12 wks.	1 sl. fatty infiltration 1 myocarditis with thrombi 2/6	1 sl. fatty infiltration 1 periarteritis, myocarditis 1 inflammation L. ventricle valve 1 sm. area fibrosis 4/6	None	1 focal degeneration, fibrosis 1 sl. fibrosis 2/8
16 wks.	None	2 focal fibrosis 2/5	1 sl. fatty infiltration 1/32	None
TOTAL	3/27	10/27	1/32	2/32

TABLE 5. SPLEEN: List of Pathologic Lesions, with Incidences.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	None	None	None	None
8 wks.	1 amyloidosis+ 1/8	3 amyloidosis+ 3/8	None	None
12 wks.	2 amyloidosis+ 1 amyloidosis++ 3/6	2 amyloidosis++ 1 amyloidosis+ 3/6	None	1 amyloidosis++
16 wks.	1 amyloidosis+ 1/5	1 amyloidosis+ 1/5	None	None
TOTAL	5/27	7/27	0/32	1/32

Fig. 6 Photomicrograph Spleen 8 week P Dy+.

Spleen showing mild (+) amyloidosis is shown. This was from a parabiosed eight week normal female. Stain is H. and E. Magnification 100x.

Fig. 7 Photomicrograph Spleen 8 week P dydy.

Spleen showing mild (+) amyloidosis is shown. This was from a parabiosed eight week dystrophic female (parabiosed to the above). Stain is H. and E. Magnification 100x.

Fig. 8 Photomicrograph Spleen 12 week P Dy+.

Spleen showing moderate (++) amyloidosis, from a parabiosed twelve week normal male, H. and E. stain, 100x.

Fig. 9 Photomicrograph Spleen 12 week P dydy.

Spleen showing moderate (++) amyloidosis, from a parabiosed twelve week dystrophic male parabiosed to male above. H. and E. stain, 100x.

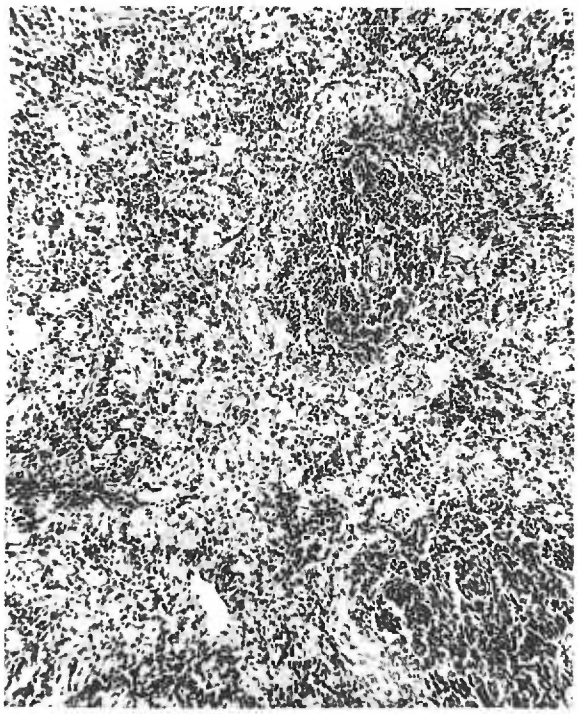


Fig. 6

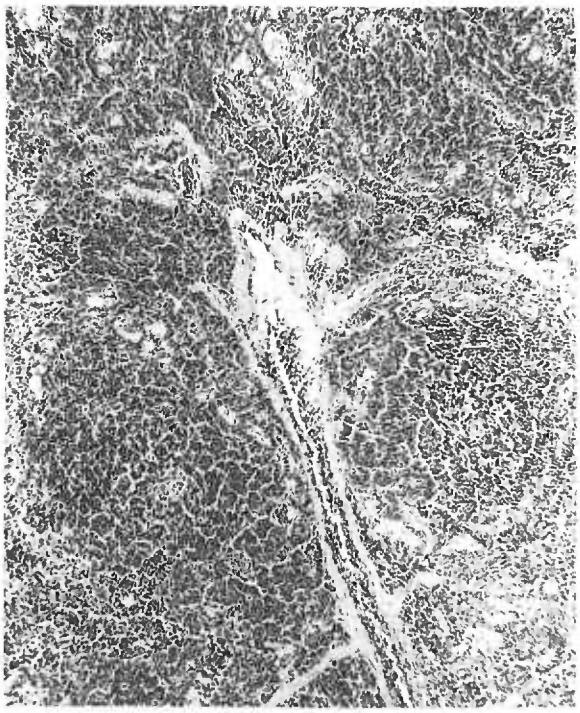


Fig. 7

Fig. 8

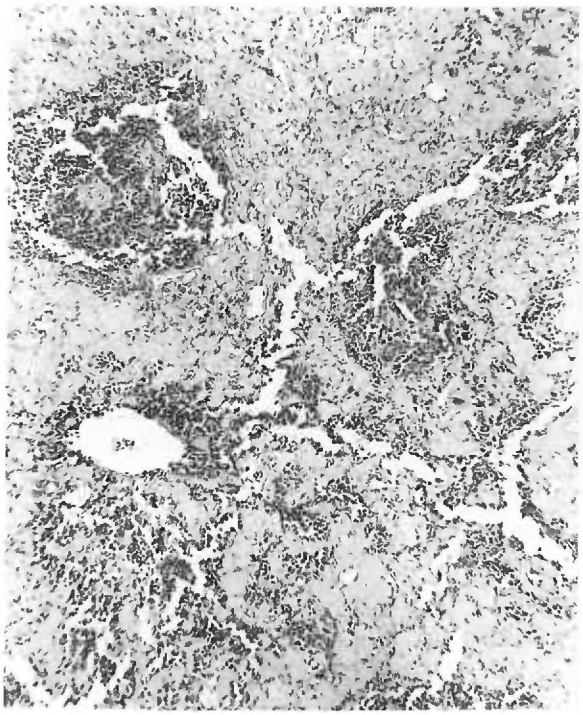
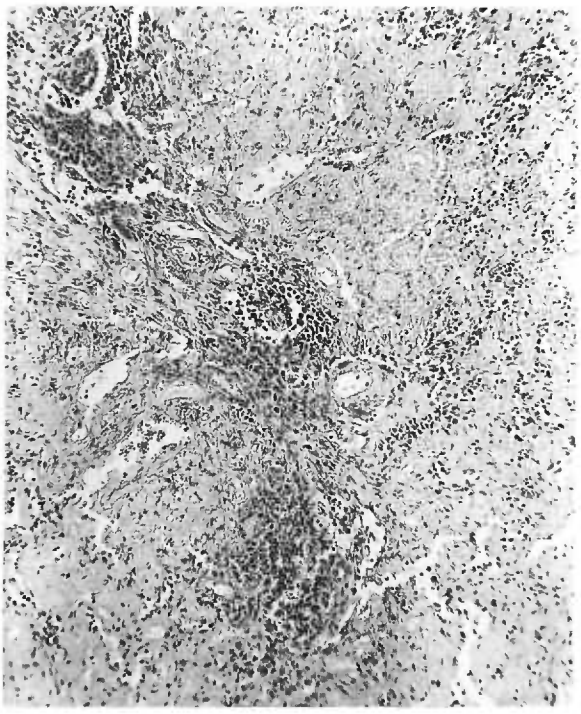


Fig. 9



6, 7, 8 and 9 are photomicrographs of spleen, showing varying degrees of amyloidosis at eight and twelve weeks.

LIVER: (Table 6) Pathologic processes of liver were generally mild to moderate in severity and consisted predominately of varying degrees of fatty changes. The diagnosis of amyloidosis was next in frequency followed by a few cases of focal necrosis, focal inflammation, periarteritis, and two parabionts with bile duct hyperplasia. Usually only a single diagnosis was present in one animal. There was a peak incidence of all conditions at twelve weeks. There was no difference in overall incidence between parabionts, 14/54 (26%) and singles, 16/64 (25%) but there was a definite difference in the types of lesions present. Fourteen of the 16 singles with pathologic processes showed fatty changes, one had amyloidosis and the last one slight focal necrosis. In contrast only one parabiont had fatty changes present, six of the 14 had amyloidosis and the remaining seven had various diagnoses including two with bile duct hyperplasia. Thus 14/64 (22%) singles had fatty changes while only 1/54 (2%) parabionts had a similar lesion. Figures 10, 11, 12 and 13 are photomicrographs of liver showing typical fatty change findings, and the bile duct hyperplasia.

KIDNEY: (Table 7) Lesions present in kidney were varying degrees of tubular amorphous casts, amyloidosis and, in the older groups, chronic pelvic inflammation. Also found were pyelonephritis, nephrosis, and focal areas of necrosis. Parabionts were more often affected than singles, and P dydy's more often than P Dy+'s. In the four and eight week groups the only lesions found were 10/16 dydy parabionts with amorphous casts, and a single eight week DyDy parabiont with pyelonephritis. There was a peak incidence at 12 weeks for all categories, (P and S).

TABLE 6. LIVER: List of Pathologic Lesions, with Incidences.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	None	None	1 fatty changes+ 1/8	None
8 wks.	1 amyloidosis+ 1/8	1 sl. focal inflammation 1 sl. focal necrosis 1 focal periarteritis 3/8	1 fatty changes+ 1/8	1 fatty changes+ 1/8
12 wks.	1 amyloidosis+ 1 bile duct hyperplasia 1 focal necrosis amyloidosis 1 amyloid+, bile duct proliferation 4/6	2 amyloid+ 1 giant cells, round cell infiltration 1 bile duct hyperplasia 4/6	4 fatty changes+ 4/8	1 fatty changes+ 1 few fat cells 1 sl. focal necrosis 1 amyloidosis+ 4/8
16 wks.	1 fatty changes 1 focal necrosis 2/5	None	3 fatty changes+ 1 fatty changes++ 4/8	1 fatty changes+ 1/8
TOTAL	7/27	7/27	10/32	6/32

TABLE 7. KIDNEYS: List of Pathologic Lesions, with Incidences.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	None	5 few amorphous casts 5/8	1 few casts 1/8	None
8 wks.	1 pyelonephritis 1/8	4 amorphous casts 1 pyelonephritis 5/8	None	None
12 wks.	1 pyelonephritis(?) 2 pyelonephritis 2 chronic pelvic inflammation 5/6	1 pyelonephritis and casts 2 casts and nephrosis 1 pyelonephritis 1 chronic pelvic inflammation 1 amyloidosis++ 6/6	1 focal inflammation, necrosis 1/8	2 chronic pelvic inflammation 1 casts 3/8
16 wks.	2 chronic pelvic inflammation 2/5	2 nephrosis and casts 1 few casts 3/5	4 chronic pelvic inflammation 4/8	4 chronic pelvic inflammation 1 perivascular cuffing 5/8
TOTAL	8/27	19/27	6/32	8/32

Fig. 10 and 11 Photomicrographs liver, 12 week S Dy+.

Liver from a female and male respectively. The diagnosis was fatty changes. Sacrifice was at twelve weeks after parabiosis. H. and E. stain, 450x.

Fig. 12 and 13 Photomicrographs 12 week P Dy+ and P dydy.

Two parabiotic partners at twelve weeks with bile duct hyperplasia present in liver. The liver is shown at 450x, stained with H. and E. The normal parabiont is Fig. 13, and the dydy parabiont is Fig. 14.

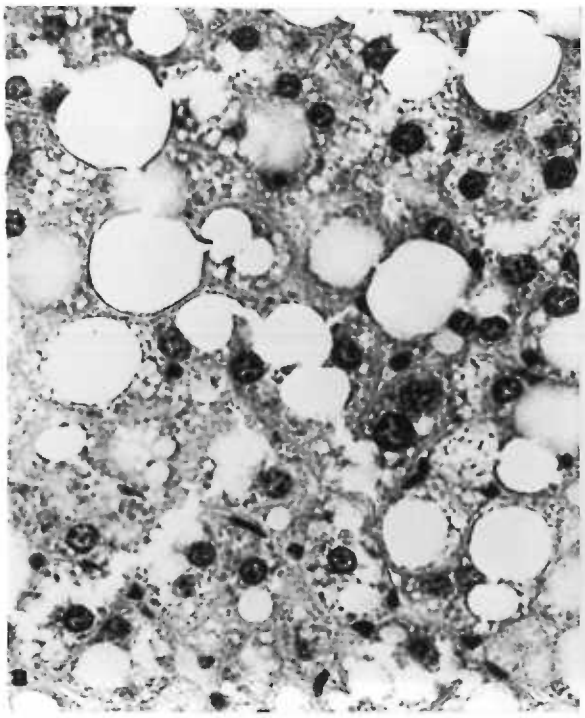


Fig. 10

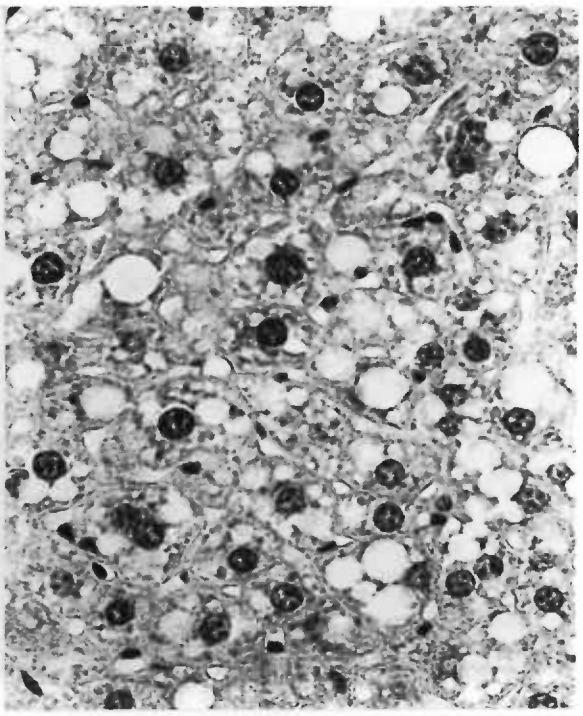


Fig. 11

Fig. 12

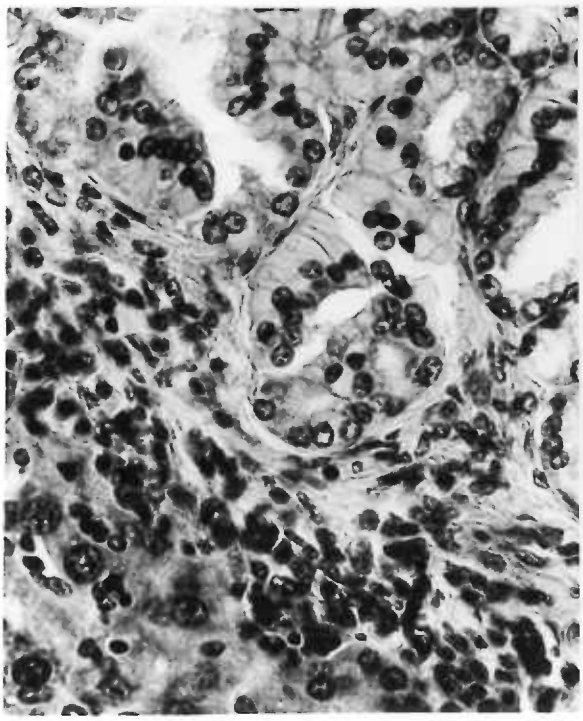
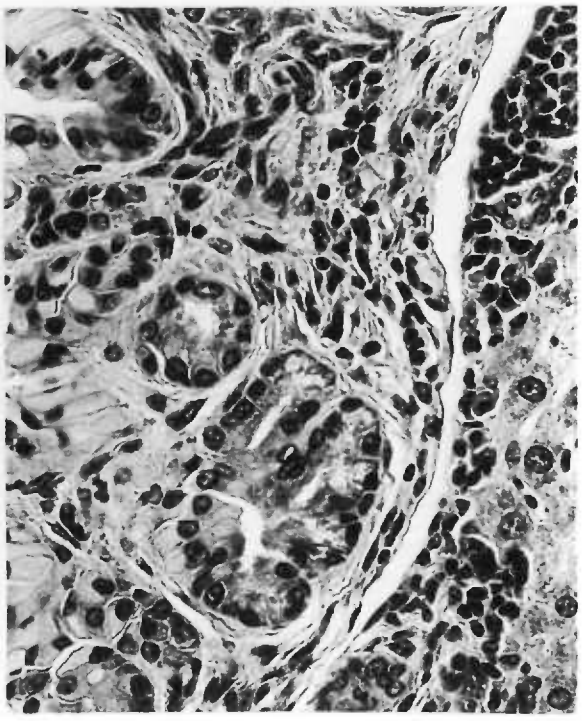


Fig. 13



MUSCLE: (Table 8) All dystrophics had the usual histologic signs of muscular dystrophy. The severity increased with time for single dystrophics, less so for parabiosed dystrophics. (All normals had normal appearing muscle). In each age group male dydy's tended to be somewhat more severely affected than were female dydy's. Parabionts were less affected as a group than were single controls. This difference was present at each age group, but was more pronounced at older ages. Figure 14 shows the severity score for dydy muscle. The severity was rated 1+, 2+ or 3+, and the degree of severity was equated to one, two or three. The severity ratings were summed for the group and divided by the number of mice in the group. This was then multiplied by 100 to give the "severity score". The formula was as follows: $\frac{\text{Sum ratings x 100}}{N}$

Figures 15 through 30 are photomicrographs of dydy muscle from parabionts and from singles, showing varying degrees of muscle lesions at different time intervals.

In muscle of 6 normal mice (one parabiont and six singles) small areas of pathology were noted suggestive of dystrophic changes. Two of these were listed as areas of necrosis. In each case the lesion was limited to a single small area in an animal. The two with necrotic patches were males, the remaining four were females. In two cases remains of suture material were found in the same muscle. Not all cases were on the operated sides of animals.

TABLE 8. MUSCLE: List of Pathologic Lesions, with Severity.

TIME	P DY+	P dydy	S DY+	S dydy
4 wks.	None	6+ 2++ 8/8	None	2+ 6++ 8/8
8 wks.	None	5+ 3++ 8/8	1 changes 1/8	8++ 8/8
12 wks.	1 changes 1/6	2+ 4++ 6/6	2 patch of necrosis 2 changes 4/6	6++ 2++ with fat 8/8
16 wks.	None	3+ 2++ 5/5	None	1+ 6++ 1+++ 8/8
TOTAL	1/27	27/27	5/32	32/32

Fig. 14 Severity Scores for Parabiosed and Single Dystrophics.

A bar graph showing severity as calculated according to the formula: $\frac{\Sigma \text{ severity ratings} \times 100}{N}$ is shown for each sex, parabiont and single, at each time interval.

The frequency of lesions was compared between parabiosed and single dystrophics using the chi square statistic.

$\chi^2 = 144.84.$ $(P < 0.001)$ This value indicates a highly significant difference between S dydy's and P dydy's in severity of muscle lesions.

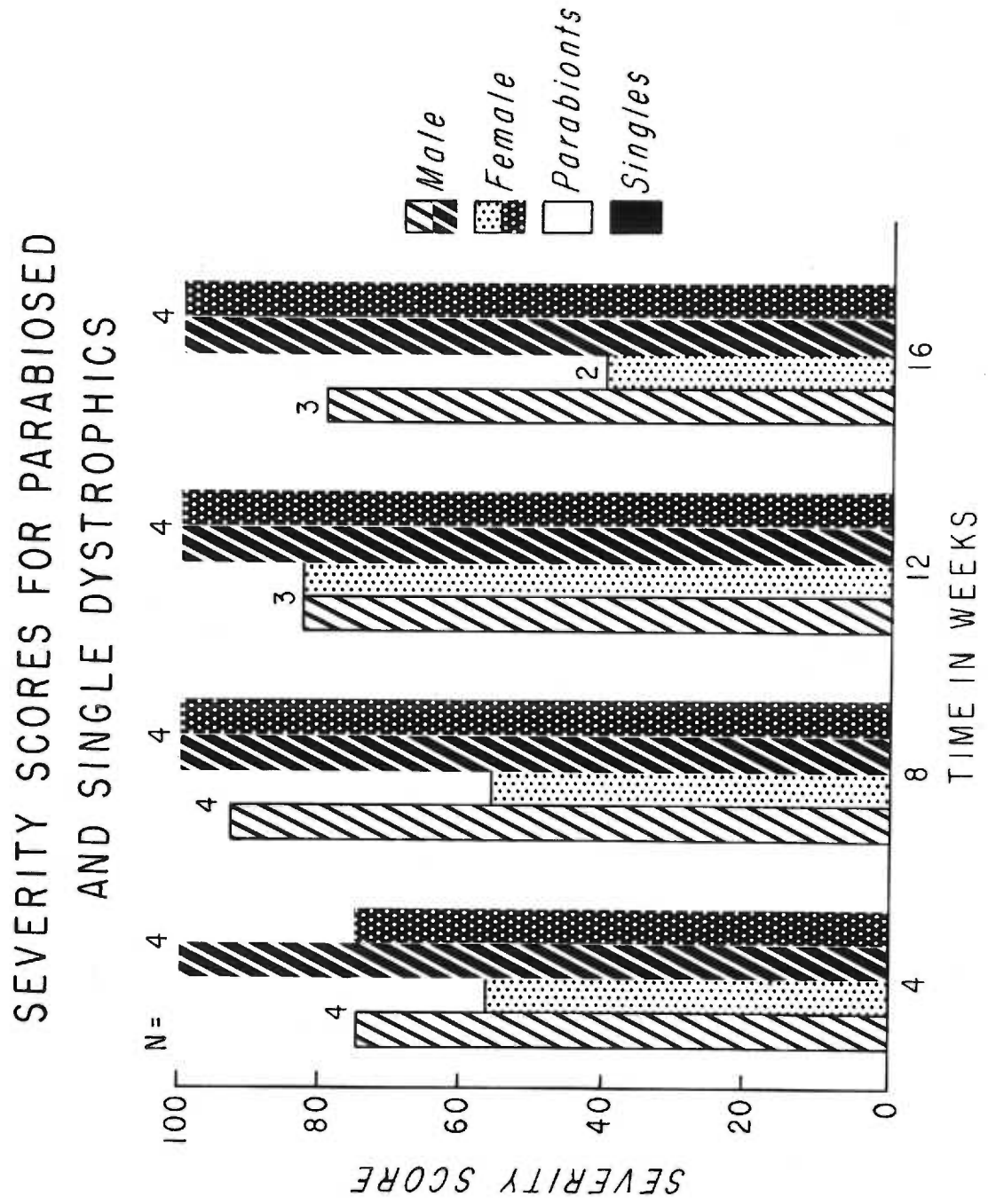

SEVERITY SCORES FOR PARABIOSED
AND SINGLE DYSTROPHICS
Male
Female
Parabionts
Singles
N=
4
4
4
4
4
3
4
3
4
2
SEVERITY SCORE
100
80
60
40
20
0
4
8
12
16
TIME IN WEEKS

FIG. 14.

Fig. 15 and 16 Photomicrographs S dydy muscle 4 weeks.

Muscle of single dydy's at 4 weeks showing moderate (++) condition of dystrophy in cross-section and longitudinal section. H. and E. stain, 100x.

Fig. 17 and 18 Photomicrographs P dydy muscle 4 weeks.

Muscle of parabiosed dydy's at 4 weeks showing mild (+) condition of dystrophy in cross section and longitudinal section. H. and E. stain, 100x.

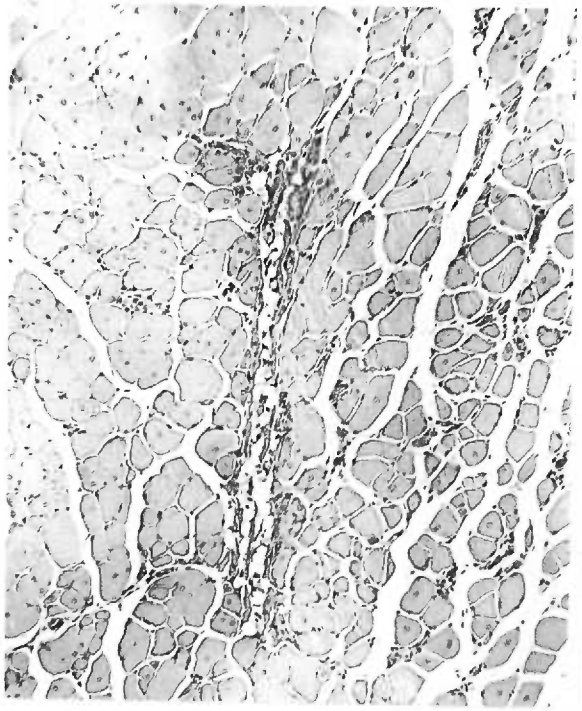


Fig.15

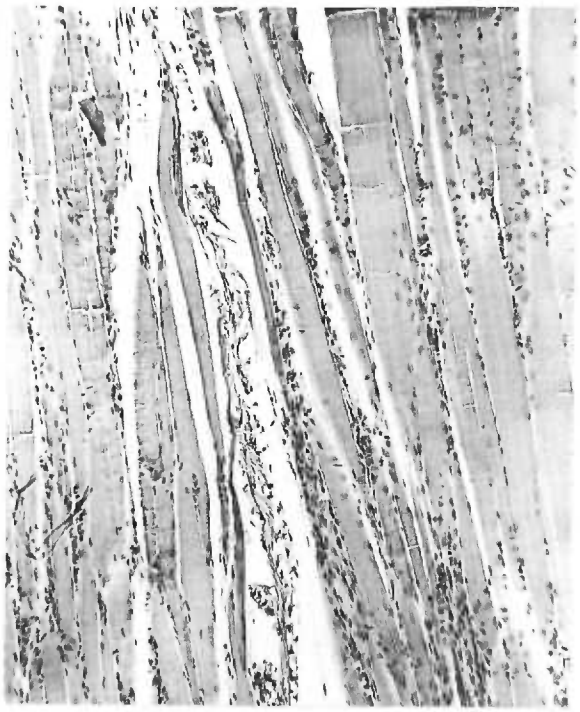


Fig.16

Fig.17

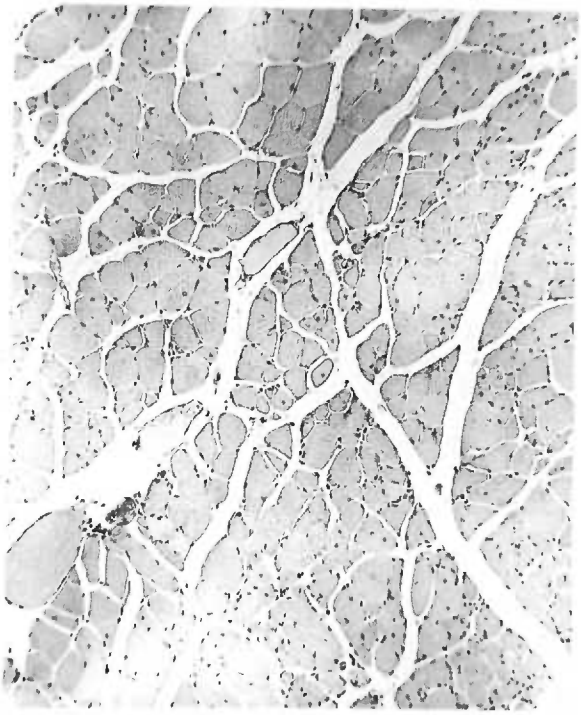


Fig.18

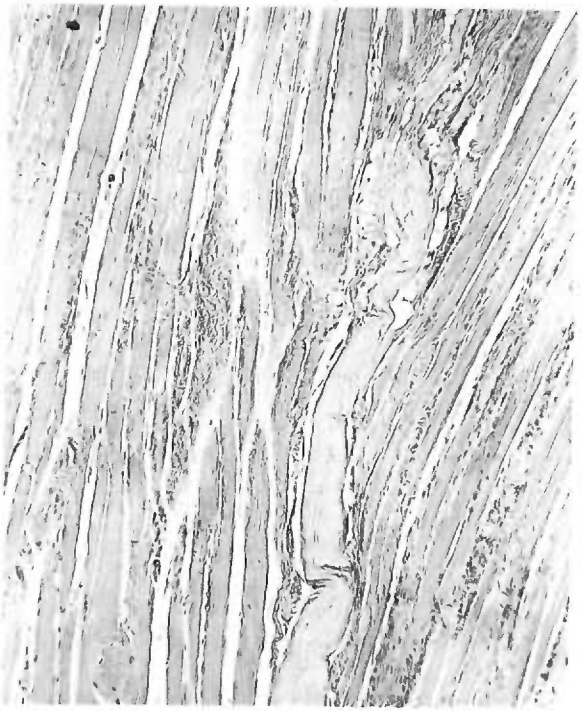


Fig. 19 and 20 Photomicrographs S dydy muscle 8 weeks.

Muscle from eight week single dystrophics showing moderate (++) degrees of dystrophy. (female and a male) H. and E. stain, 100x.

Fig. 21 and 22 Photomicrographs P dydy muscle 8 weeks.

Muscle from eight week parabiosed dystrophics showing mild (+ and +/++) degrees of dystrophy. H. and E. stain, 100x.

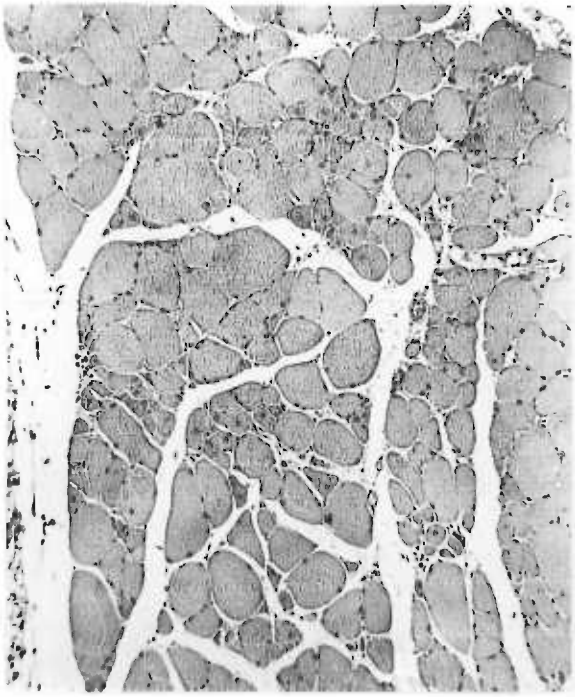


Fig. 19

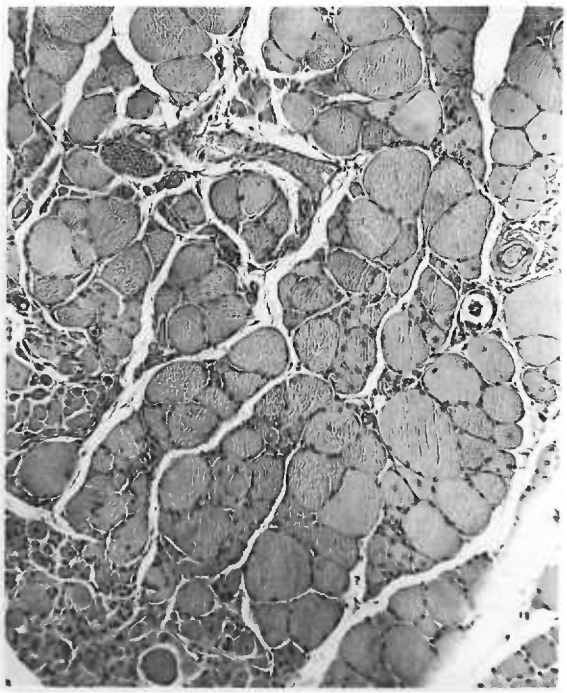


Fig. 20

Fig. 21

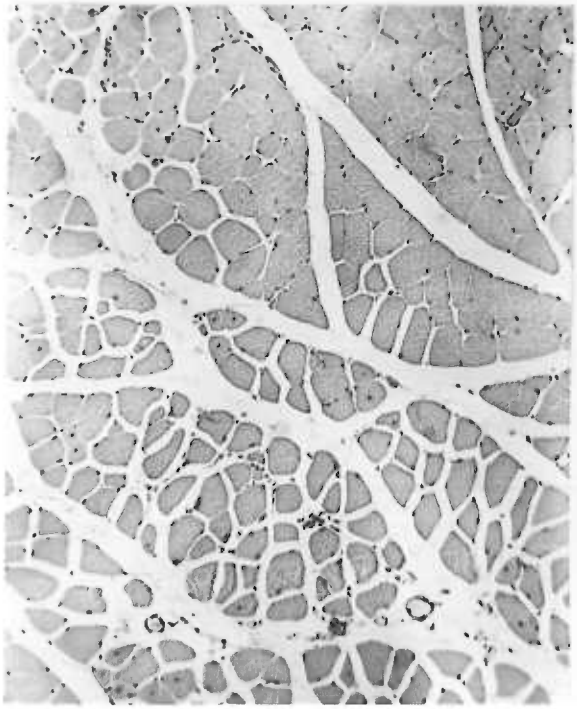


Fig. 22

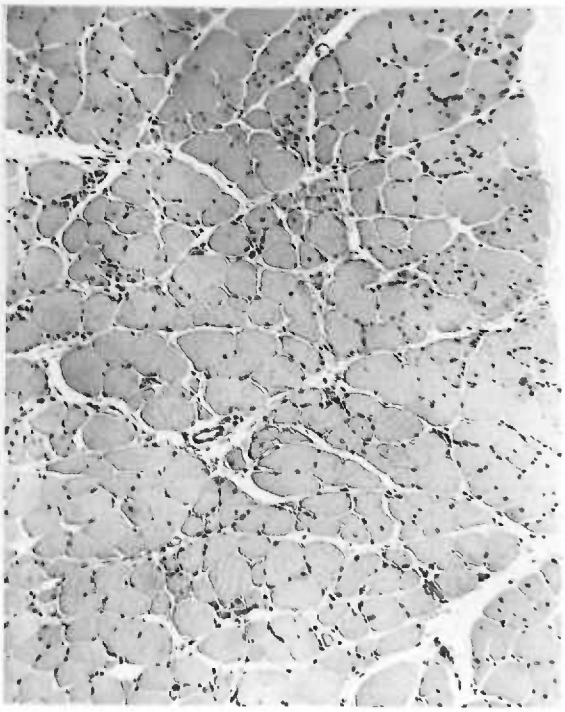


Fig. 23 and 24 Photomicrographs S dydy muscle 12 weeks.

Muscle from twelve week single dystrophics showing moderate (++) degrees of dystrophy. H. and E. stain, 100x.

Fig. 25 and 26 Photomicrographs P dydy muscle 12 weeks.

Muscle from twelve week parabiosed dystrophics showing mild (+) and moderate (++) degrees of dystrophy. H. and E. stain, 100x.

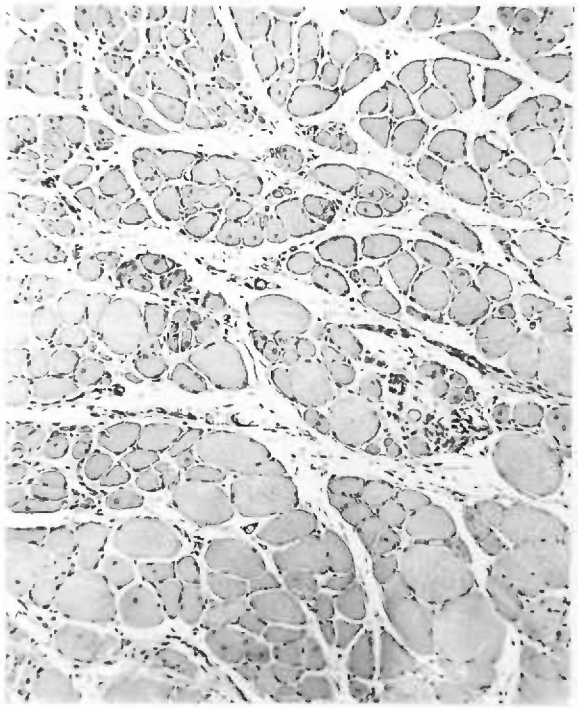


Fig. 23

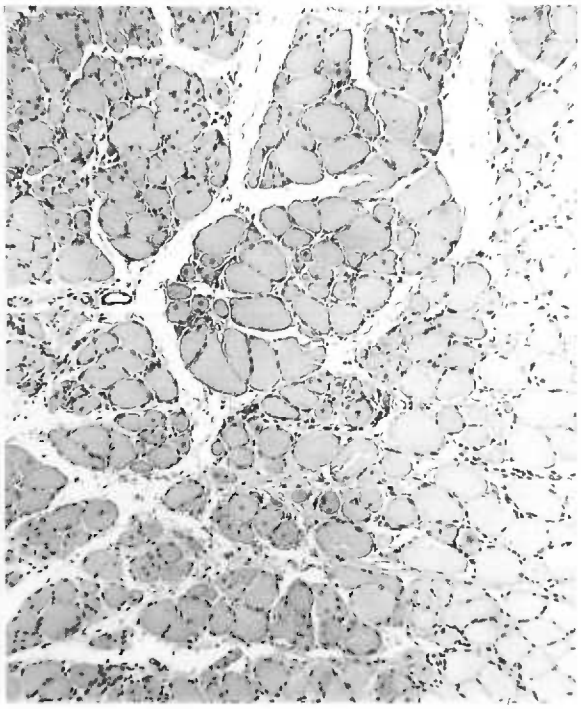


Fig. 24

Fig. 25

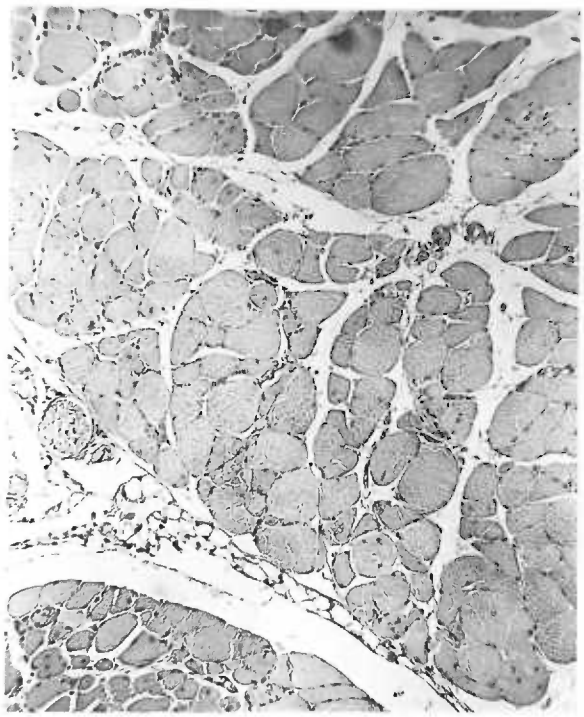


Fig. 26

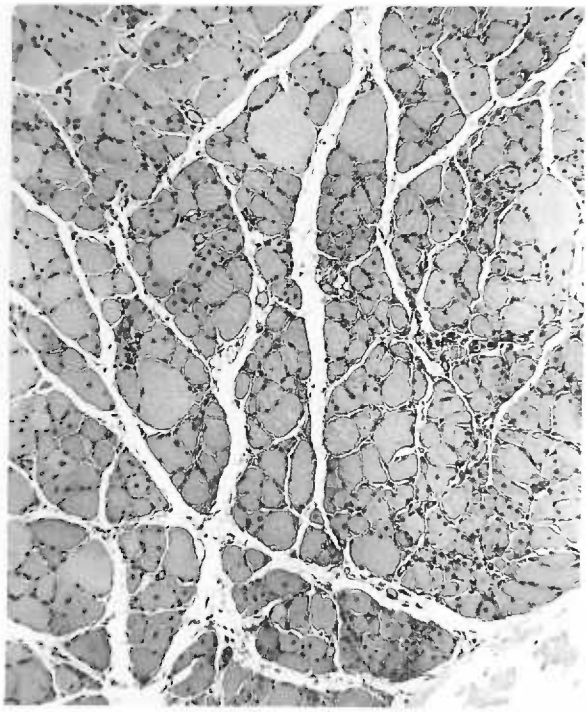


Fig. 27 and 28 Photomicrographs S dydy muscle 16 weeks.

Muscle from sixteen week single dystrophics showing moderate (++) and severe (+++) degrees of dystrophy. H. and E. stain, 100x.

Fig. 29 and 30 Photomicrographs P dydy muscle 16 weeks.

Muscle from sixteen week parabiosed dystrophics showing mild (+) degrees of dystrophy. H. and E. stain, 100x.

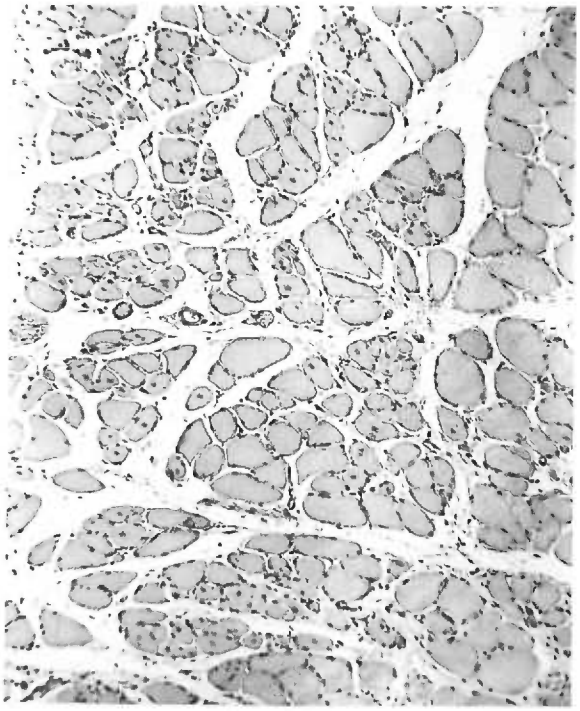


Fig. 27

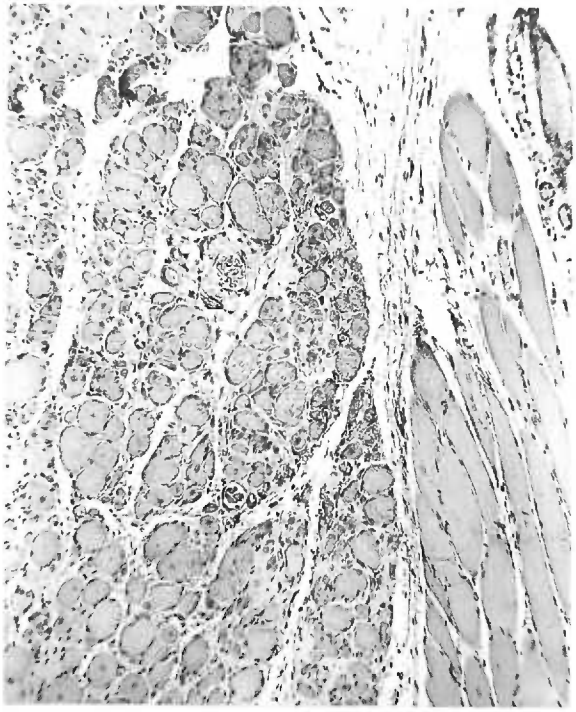


Fig. 28

Fig. 29

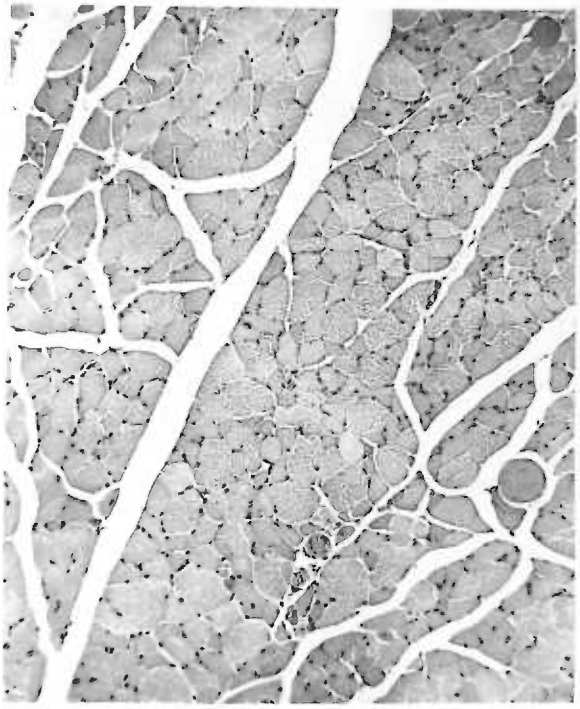
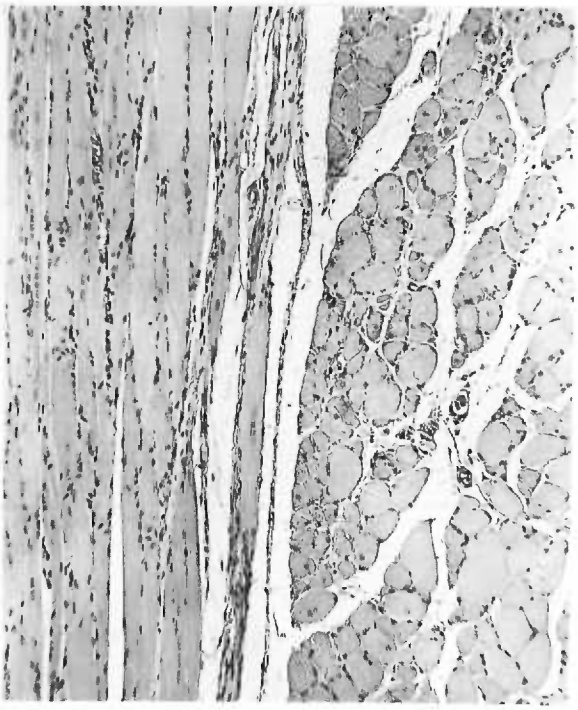


Fig. 30



HISTOPATHOLOGY AND ORGAN WEIGHT STUDIES,

RESULTS BODY AND ORGAN WEIGHTS

Initial Body Weight. The mean initial weight for DyDy mice was 16.6 gm ± S.E. 0.3, with a range of 12.0 - 19.0 gm and S.D. 1.7. There was no significant difference between sexes ($P > 0.40$). The mean initial weight for Dydy mice was 17.1 ± 1.4 gm. The range was 13.5 - 24.0 gm, S.D. 2.4. No significant difference was present between homozygous and heterozygous normals ($P > 0.30$). The combined mean initial weight for all normals (Dy+) was 16.9 ± 0.3 gm with a range of 12.0 to 24.0 gm and S.D. 2.1.

The mean initial weight for dydy mice was 12.2 ± 0.2 gm with a range of 9.0 - 17.0 gm and S.D. 1.6. No significant difference was present between sexes ($P > 0.50$). The mean weight difference between normal (Dy+) and dydy mice was highly significant ($P < 0.001$).

Weight Gain. Weight gain following parabiosis is shown in Figures 31a. and 31b. as percent of initial weight. It can be seen that parabiosis significantly decreases the amount of weight gained at each time interval for normals as well as dystrophics. (P values were all less than 0.05 when comparing P Dy+ with S Dy+, and P dydy with S dydy). In no case was there a significant difference between sexes for normal mice, whether parabiosed or single. At the four week interval dydy males gained more weight than did dystrophic females, either in parabiosis or as singles ($P < 0.05$). At no other time was there a sex difference in weight gain for dydy's. Figure 32 shows mean whole weight after parabiosis, for normal parabionts and singles, and for dydy parabionts and singles. This figure also shows a lighter body weight for parabionts, compared with singles and a lighter body weight of dydy's compared with normals.

Fig. 31a. and 31b. Weight Change After Parabiosis, Percentage.

The percentage of the mean initial weight is plotted at each time period after parabiosis for P Dy+'s, S Dy+'s, P dydy's, and S dydy's.

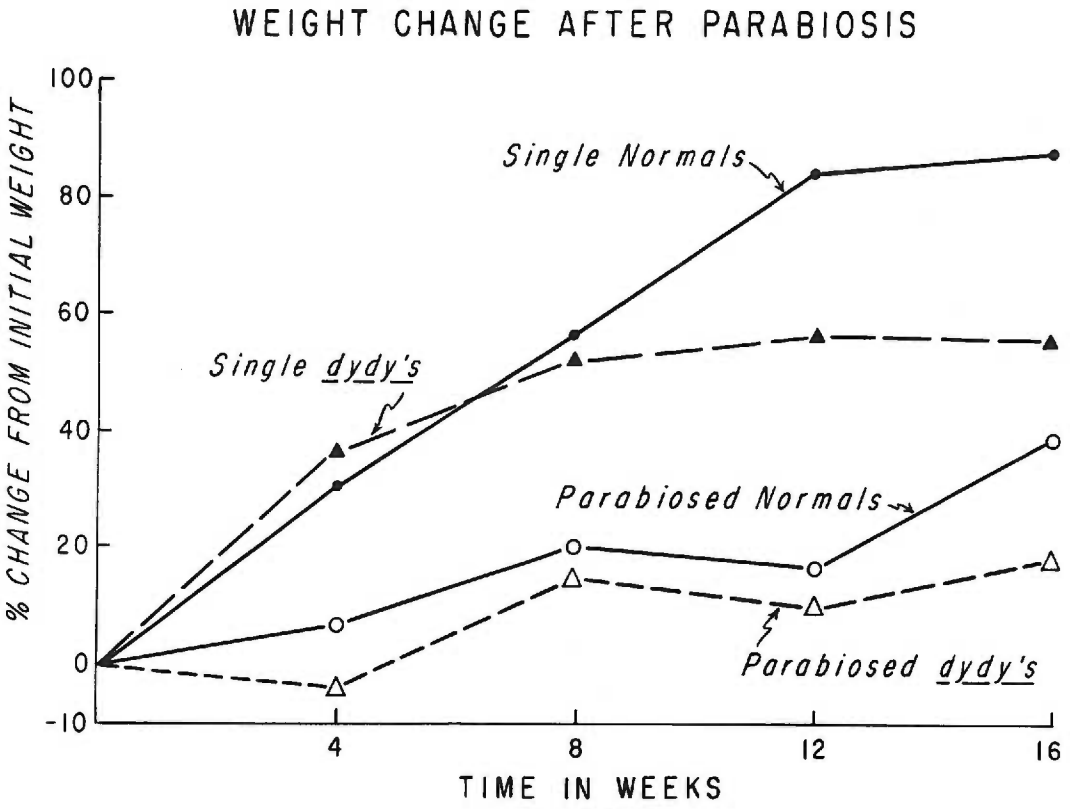


FIG.31a.

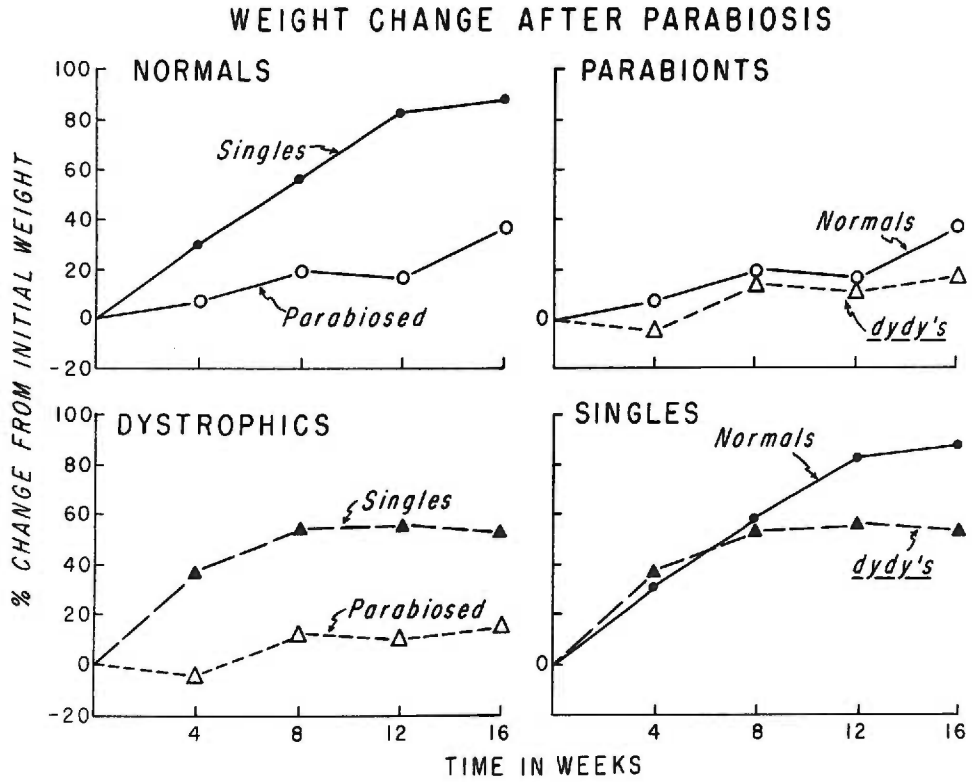


FIG.31b.

Fig. 32 Mean Whole Body Weights, Following Parabiosis.

The mean weights of single and parabiosed Dy+'s, and single and parabiosed dydy's, are plotted for each time period after parabiosis. The initial mean weights of Dy+'s and dydy's are shown at the time of parabiosis.

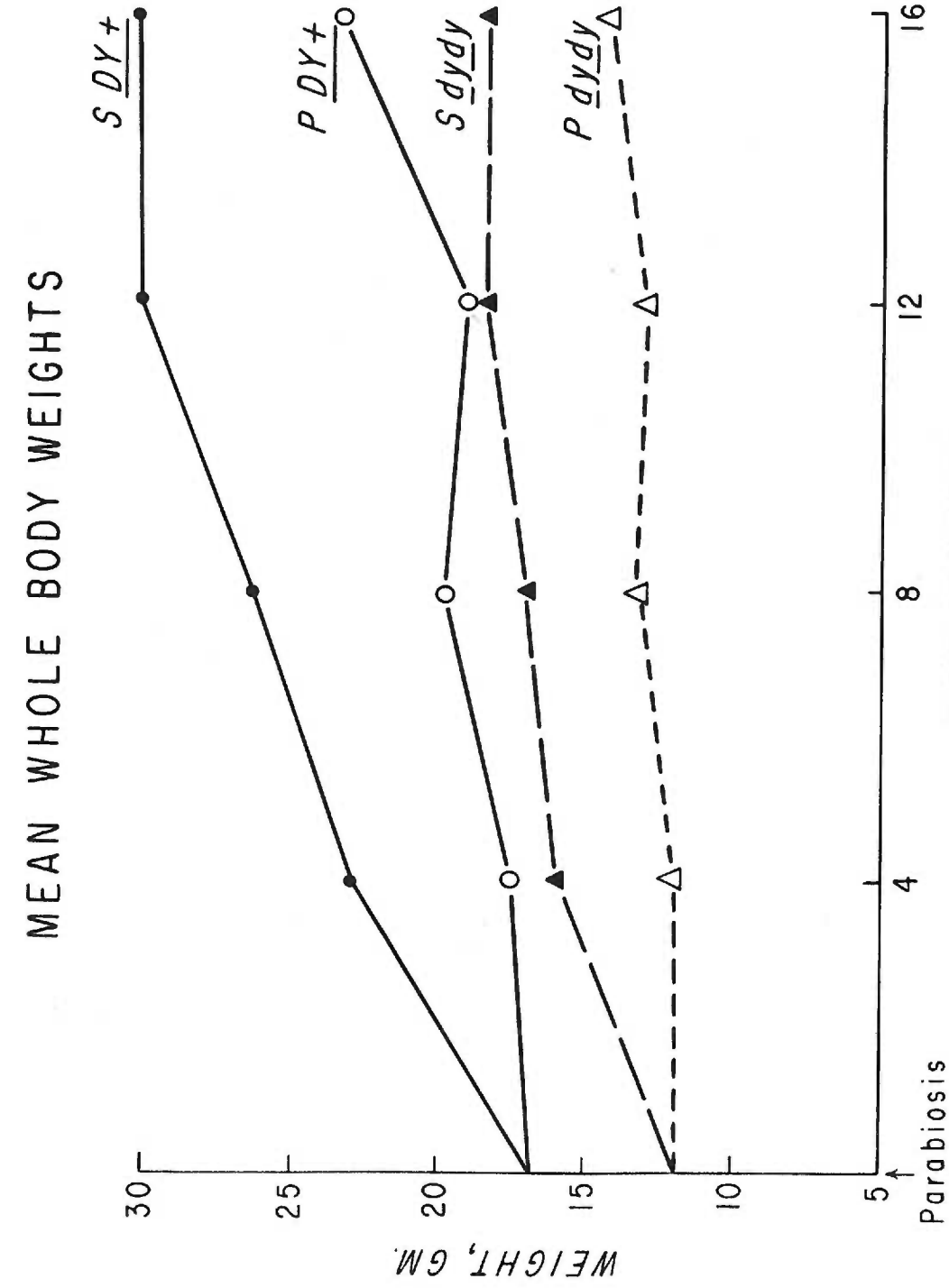

MEAN WHOLE BODY WEIGHTS
S DY+
P DY+
S dydy
P dydy
WEIGHT, GM.
30
25
20
15
10
5
4
8
12
16
Parabiosis
TIME IN WEEKS

FIG.32.

Organ Weights. Organ weight data for adrenal, heart, spleen, liver and kidney are presented in four different ways. For each organ, mean whole weights, and the mg organ weight/gm body weight ratios, are plotted for each time group. Because body weight varies between dystrophics and normals as well as for different time groups, neither of these techniques is completely adequate to separate different effects on body and organ. In order to obviate confusion resulting from changes in body weight without comparable changes in organ weight, two additional plots are presented in which ratios of organ weights are charted against ratios of body weights. In one case the ratios of parabiont to single are used, where each animal is of the same genotype and sex. For the other case the ratio of dystrophic to normal is used where the animal is of the same sex and where either both are parabionts or both are singles - in other words, where they are partners.

Adrenals. Mean whole weights for adrenals (Fig. 33) are less for parabionts for both Dy+ and dydy mice than for singles. Dystrophic mice have smaller adrenals than do normal mice. The change with time, a gradual increase through 16 weeks, is not present in the parabiosed dystrophics.

Figure 34 shows the organ weight/body weight ratios, and shows that the proportion of body weight due to adrenals is greater for dystrophics than for normals. It is also greater for parabionts than for singles, but this difference is significant only at 12 weeks ($P < 0.05$ and < 0.025 for normals and dystrophics respectively).

When adrenal weight ratios of parabionts to comparable singles are plotted against body weight ratios for the same animals, the plot for normals and for dystrophics is as shown in Figure 35. Generally the

Fig. 33 Adrenal Mean Weight, Following Parabiosis.

The mean weights of adrenals for P and S Dy+'s, amd P and S dydy's are shown at each time interval after parabiosis.

Fig. 34 Adrenal Weight mg/gm Body Weight.

The ratio of organ in mg to body weight in grams is plotted for each group, for each interval after parabiosis.

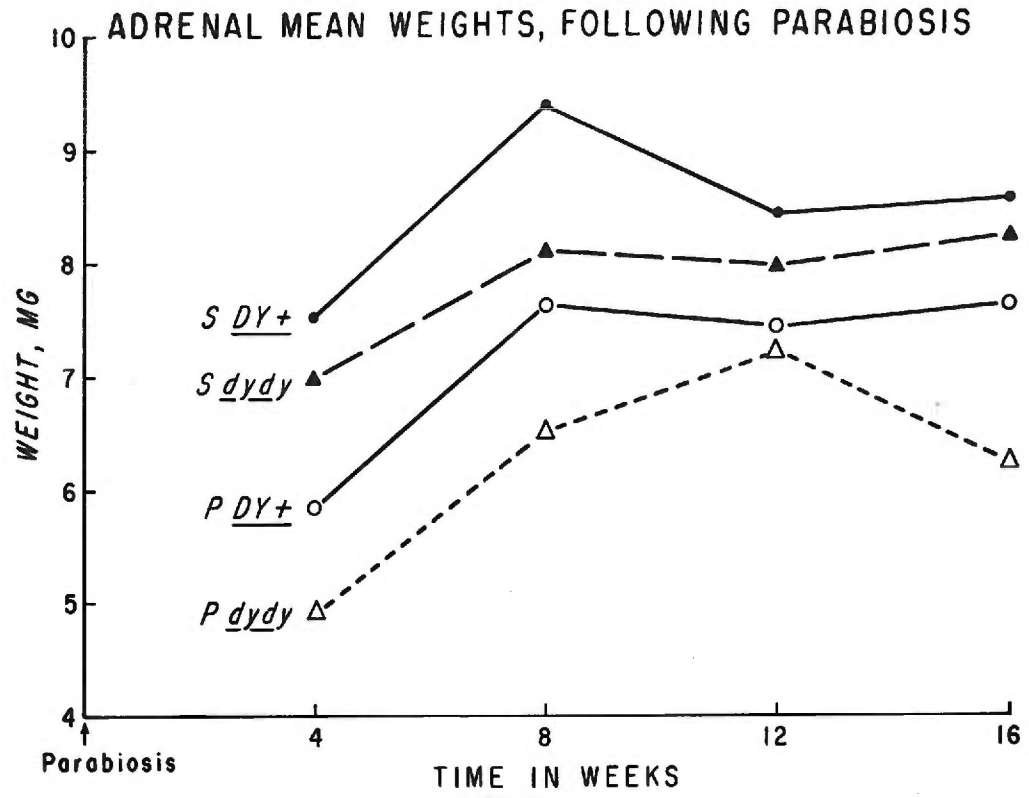

ADRENAL MEAN WEIGHTS, FOLLOWING PARABIOSIS
WEIGHT, MG
10
9
8
7
6
5
4
S DY+
S dydy
P DY+
P dydy
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG.33.

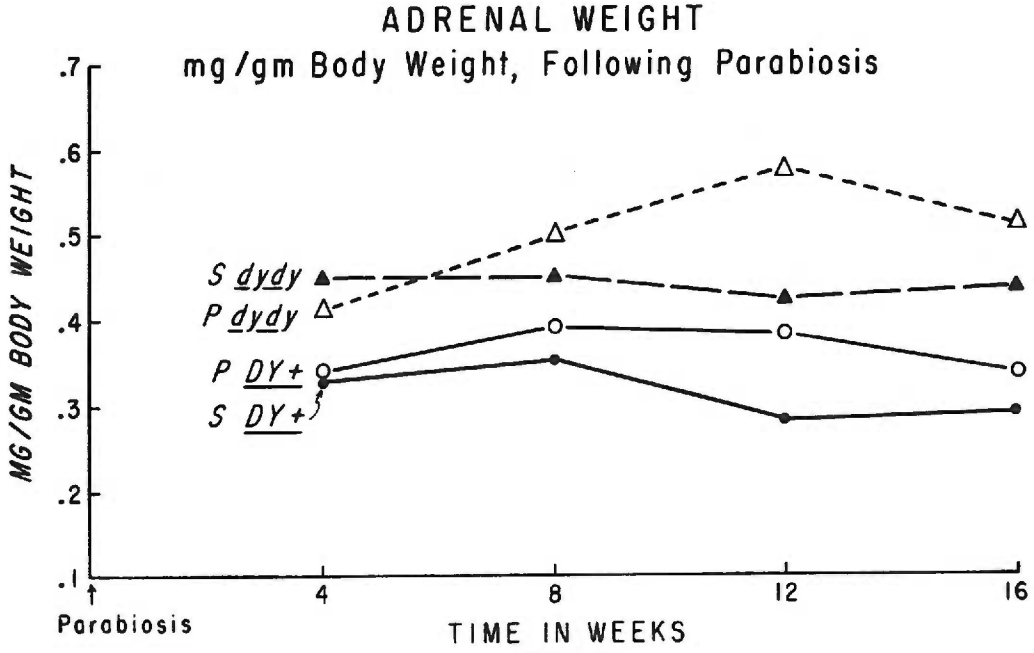

ADRENAL WEIGHT
mg/gm Body Weight, Following Parabiosis
MG/GM BODY WEIGHT
.7
.6
.5
.4
.3
.2
.1
S dydy
P dydy
P DY+
S DY+
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG. 34.

Fig. 35 Adrenal Weight Ratio ($\frac{P}{S}$) Plotted Against Body Weight Ratio ($\frac{P}{S}$).

The ratio of adrenal weight, parabiosed to single, is plotted against the ratio of body weight, parabiosed to single. The 45° line indicates similar values for the two ratios. Open symbols represent means for like sex at each time interval. Closed symbols represent a single value.

Fig. 36 Adrenal Weight Ratio ($\frac{dydy}{Dy+}$) Plotted Against Body Weight Ratio ($\frac{dydy}{Dy+}$).

This figure is similar to that above, but compares dydy to Dy+ instead of P to S.

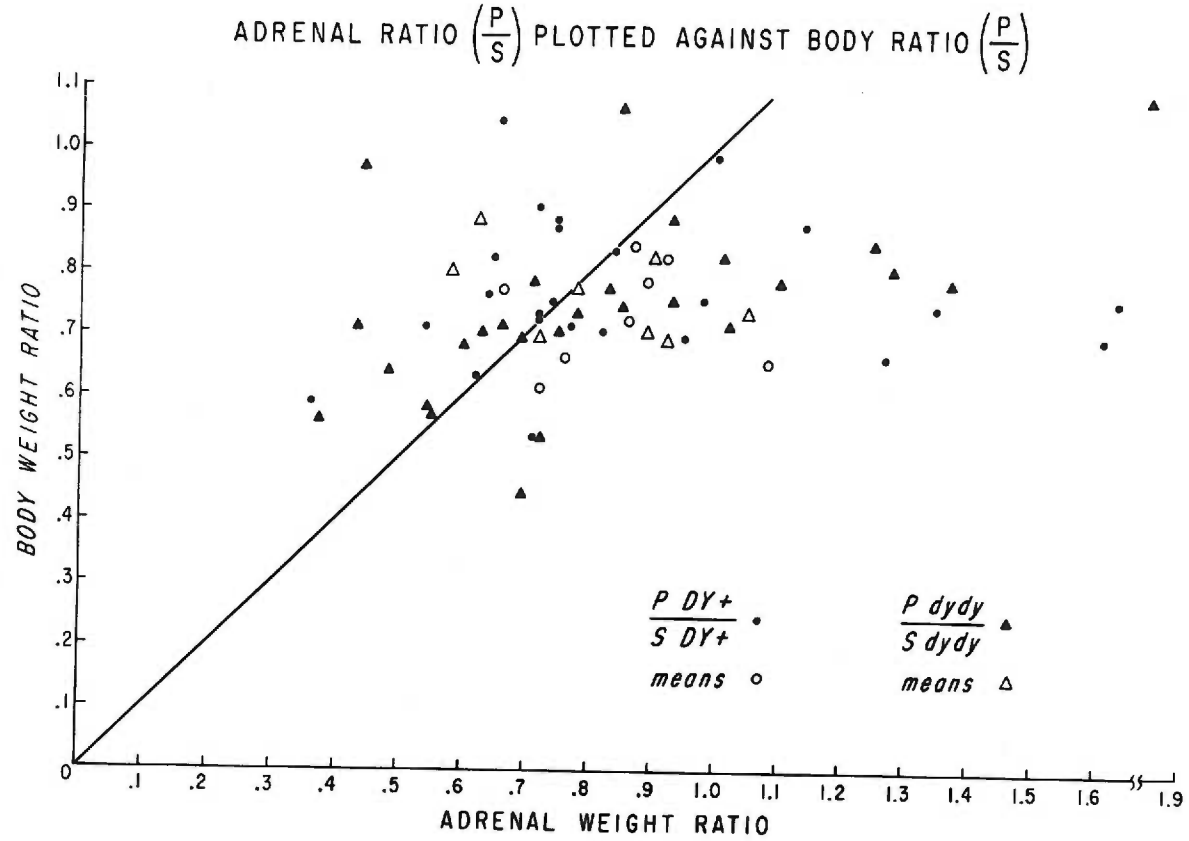

ADRENAL RATIO (P/S) PLOTTED AGAINST BODY RATIO (P/S)
BODY WEIGHT RATIO
ADRENAL WEIGHT RATIO
P DY+ / S DY+
means
P dydy / S dydy
means

FIG.35.

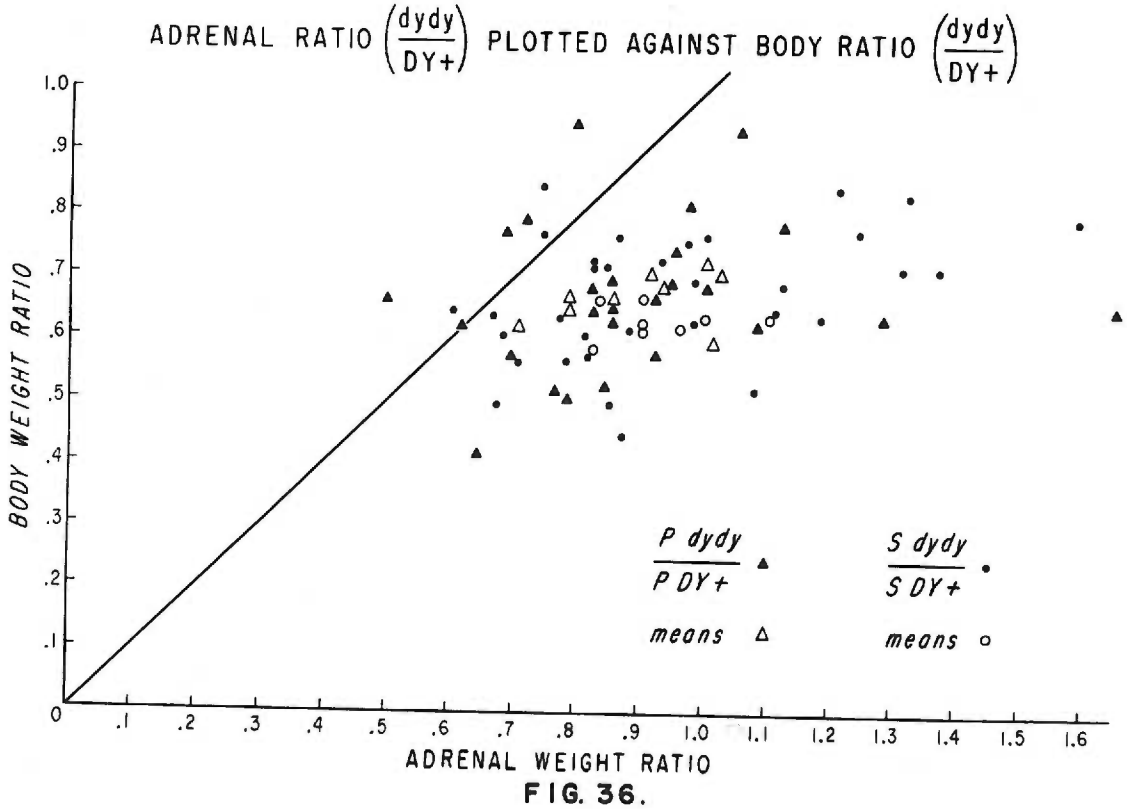

ADRENAL RATIO (dydy/DY+) PLOTTED AGAINST BODY RATIO (dydy/DY+)
BODY WEIGHT RATIO
ADRENAL WEIGHT RATIO
P dydy / P DY+
means
S dydy / S DY+
means

FIG. 36.

points are not far from the forty-five degree line, indicating that the weights of adrenals are proportionally the same as the body weights, for both parabionts and singles.

Figure 36 is the adrenal weight ratio plotted against body weight ratio, for parabionts and for singles. Here the ratios are of dystrophic and normal animals of a single pair, either parabionts or singles. Generally, as shown by Figure 36, dystrophics have a greater adrenal weight proportionate to body weight than do normals. This difference is the same for both parabionts and singles.

Heart. The heart weighs more in single mice than in either P normals or P dystrophics, until about 16 weeks. At that time a larger increase in heart weight is seen in both P Dy+'s and P dydy's (Fig. 37). The mean weight of parabiosed dystrophic hearts actually becomes greater than that of dydy singles, and is only slightly less than that of either parabiosed or single normals. The mean weight of the parabiosed normal heart is seen to be less than that of either single or parabiosed dystrophics from before eight weeks until after twelve weeks. A larger proportion of body weight is due to heart in the dystrophics than in the normals (Fig. 38) at all time periods. Parabionts, as compared to singles, also have a larger proportion of weight present in heart. The difference between parabionts and singles is significant for dystrophics at 12 weeks and at 16 weeks ($P < 0.05$), and for normals at 16 weeks ($P < 0.05$).

Figure 39 shows a clustering of points close to the 45 degree diagonal but tending toward the right, which indicates an increase in heart weight proportionate to body weight, for parabionts as compared with normals. Here the greater heart weight in proportion to body weight is more pronounced when parabionts are compared with singles. No difference is apparent here between single pairs and parabiosed pairs (Fig. 40).

Fig. 37 Heart Mean Weights, Following Parabiosis.

The mean weights of heart of P Dy+'s, P dydy's, S Dy+'s and S dydy's are shown at each time interval after parabiosis.

Fig. 38 Heart Weight mg/gm Body Weight.

The ratio of heart weight to body weight (mg/gm) is shown for each group at successive intervals after parabiosis.

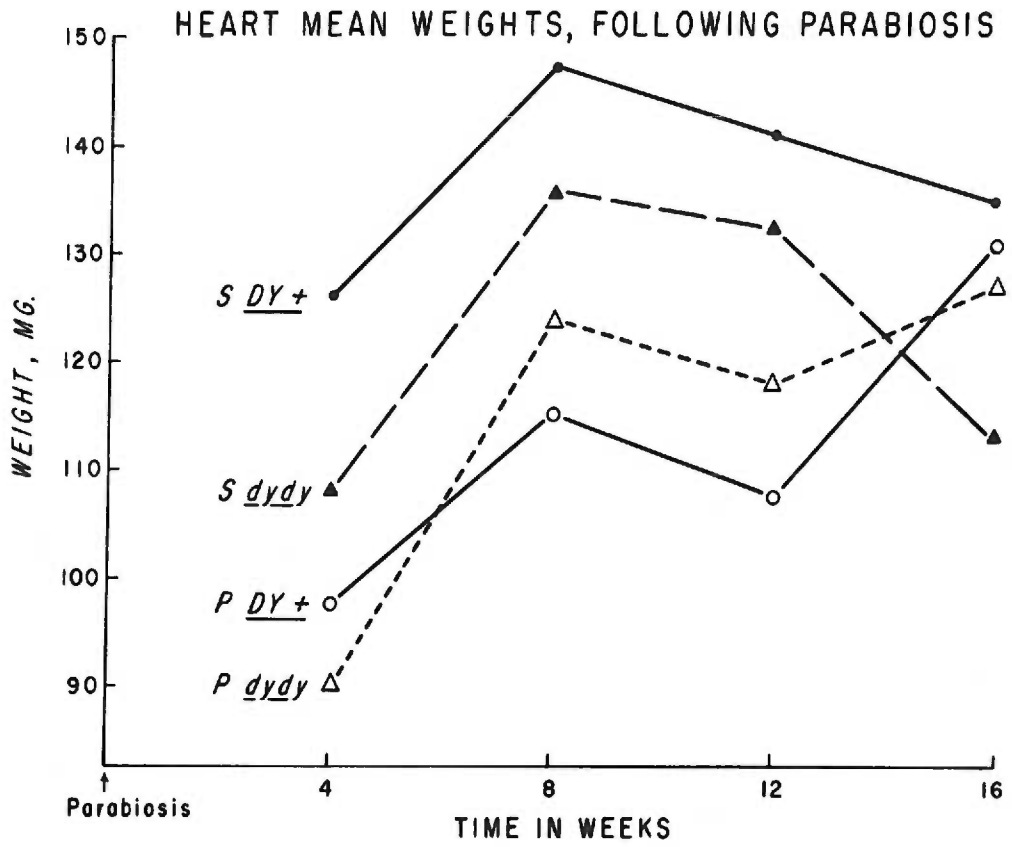

HEART MEAN WEIGHTS, FOLLOWING PARABIOSIS
WEIGHT, MG.
150
140
130
120
110
100
90
S DY+
S dydy
P DY+
P dydy
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG. 37.

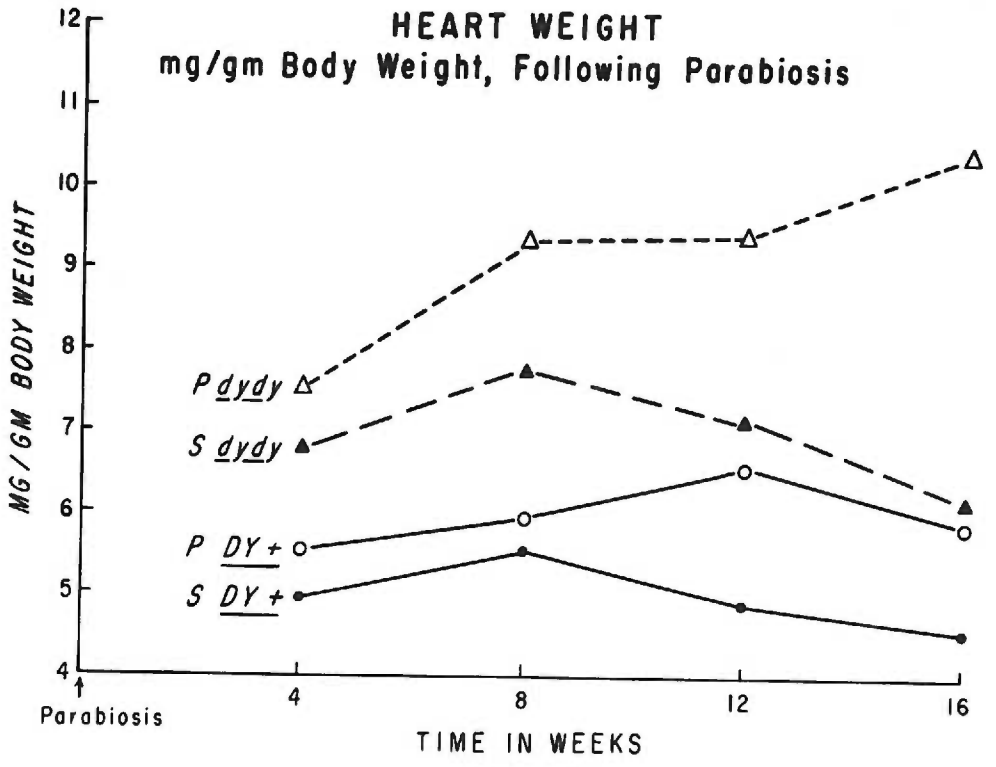

HEART WEIGHT
mg/gm Body Weight, Following Parabiosis
MG/GM BODY WEIGHT
12
11
10
9
8
7
6
5
4
P dydy
S dydy
P DY+
S DY+
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG. 38.

Fig. 39 Heart Weight Ratio $(\frac{P}{S})$ Plotted Against Body Weight Ratio $(\frac{P}{S})$.

The ratio of heart weight, parabiont to single, is plotted against the ratio of body weight, parabiont to single. The 45° line indicates similar values for the two ratios. Open symbols represent means for like sex at each time interval. Closed symbols represent a single value.

Fig. 40 Heart Weight Ratio $(\frac{dydy}{Dy+})$ Plotted Against Body Weight Ratio $(\frac{dydy}{Dy+})$.

This figure is similar to that above, but compares dydy to Dy+ instead of P to S.

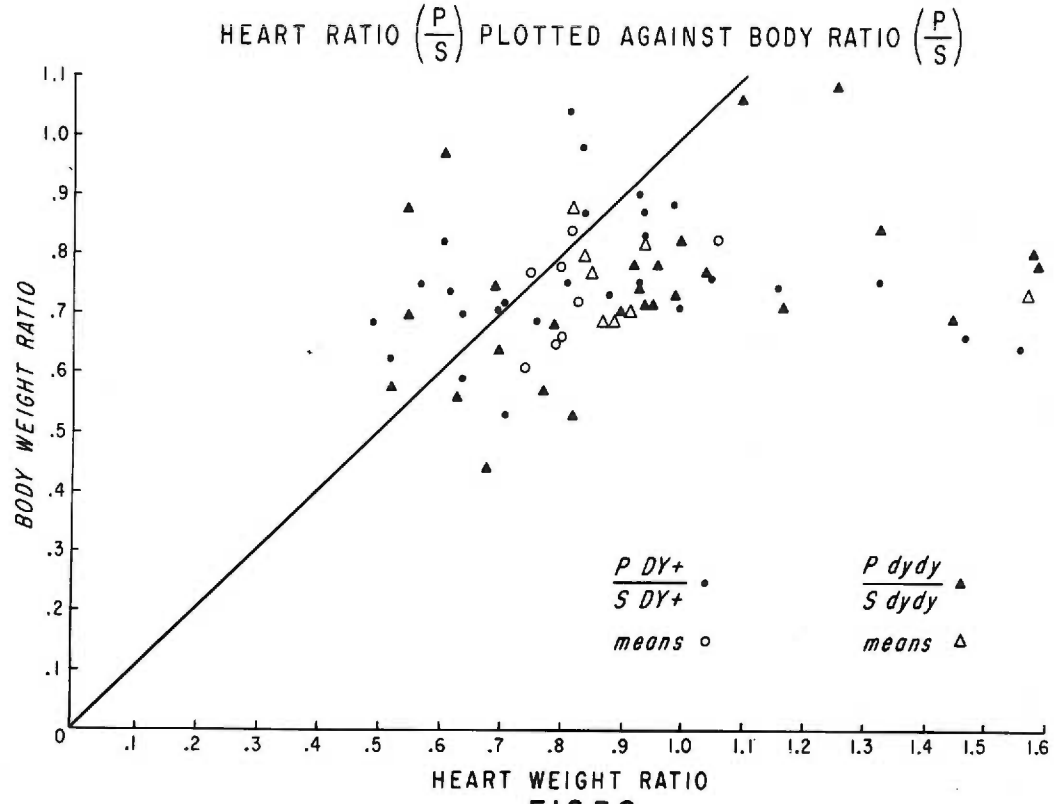

HEART RATIO (P/S) PLOTTED AGAINST BODY RATIO (P/S)
BODY WEIGHT RATIO
HEART WEIGHT RATIO
P DY+ / S DY+
means
P dydy / S dydy
means

FIG.39.

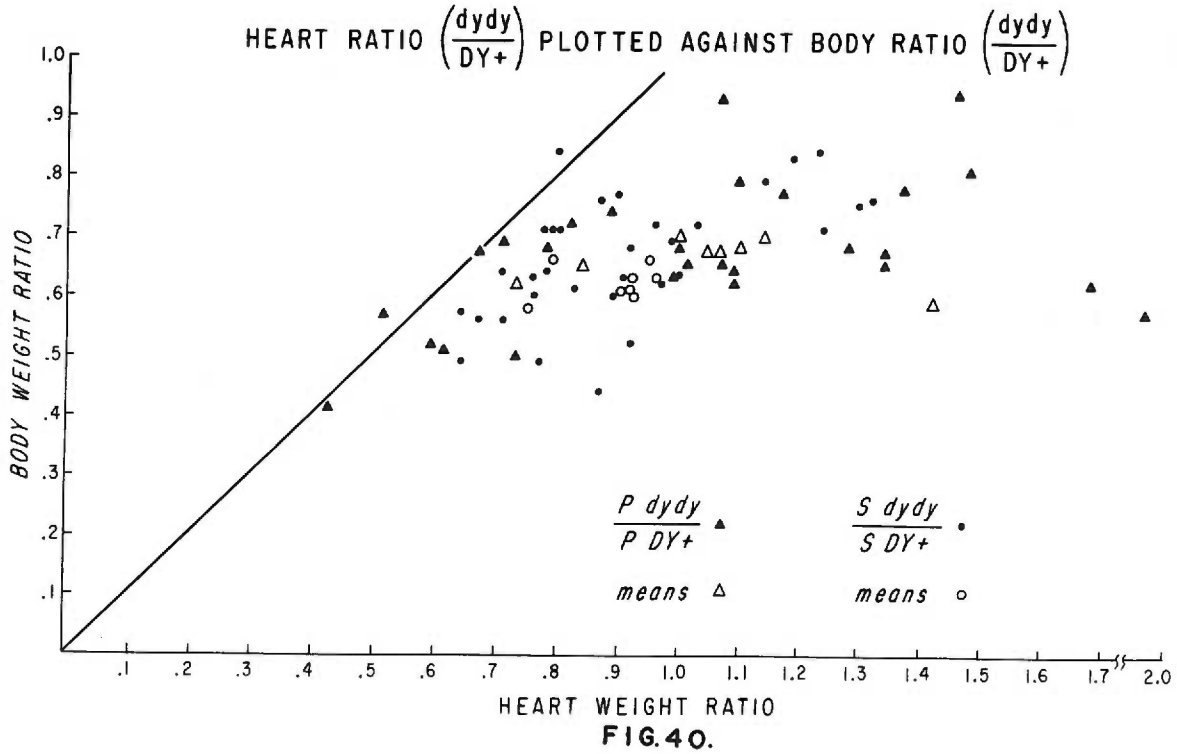

HEART RATIO (dydy/DY+) PLOTTED AGAINST BODY RATIO (dydy/DY+)
BODY WEIGHT RATIO
HEART WEIGHT RATIO
P dydy / P DY+
means
S dydy / S DY+
means

FIG.40.

Spleen. Weights of spleen after parabiosis are shown in Figure 41. No difference is apparent between single Dy+'s and single dydy's at any time interval, nor is there any difference between parabiosed Dy+'s and parabiosed dydy's. At twelve weeks after parabiosis, parabionts (both Dy+'s and dydy's) have heavier spleens than singles. This increase is still present at sixteen weeks but is less pronounced. Plotted as a ratio to body weight (Fig. 42), spleen weight is greater for dystrophics than for normals and is greater for parabionts than for singles. The difference between parabionts and singles is significant only at twelve weeks, ($P < 0.01$). When spleen weight ratios of P Dy+'s to S Dy+'s and P dydy's to S dydy's are plotted against respective body weight ratios (Fig. 43), it is seen that there is a tendency toward greater spleen weight in proportion to body weight for parabionts. Figure 44 similarly shows that dystrophic spleen comprises a larger proportion of body weight than does spleen of normals, whether single or parabiosed.

Liver. Liver weight is greatly less in parabiosed dystrophics as shown in Figure 45. No change is apparent in whole weights between single and parabiosed normals. The lighter liver weight for P dydy's compared with S dydy's and P or S Dy+'s is apparent at each time period following parabiosis. The proportion of body weight represented by liver is greater for P Dy+'s than for S Dy+'s (Fig. 46), but for dystrophics this relation is reversed. The dydy parabionts not only have a lighter liver than dydy singles, but the organ-body weight ratio is less. This difference was significant at four and at eight weeks following parabiosis, ($P < 0.05$), but not at later periods. Figure 47 indicates that, whereas P Dy+'s have greater liver weights in proportion to body weights than S Dy+'s, P dydy's have less liver weight in proportion to body weights than S dydy's. Figure 48 shows that P dydy's

Fig. 41 Spleen Mean Weights, Following Parabiosis.

The mean weights of spleen of each group are plotted at successive intervals after parabiosis.

Fig. 42 Spleen Weight mg/gm Body Weight.

The ratio of spleen weight to body weight (mg/gm) is shown for each group at successive intervals after parabiosis.

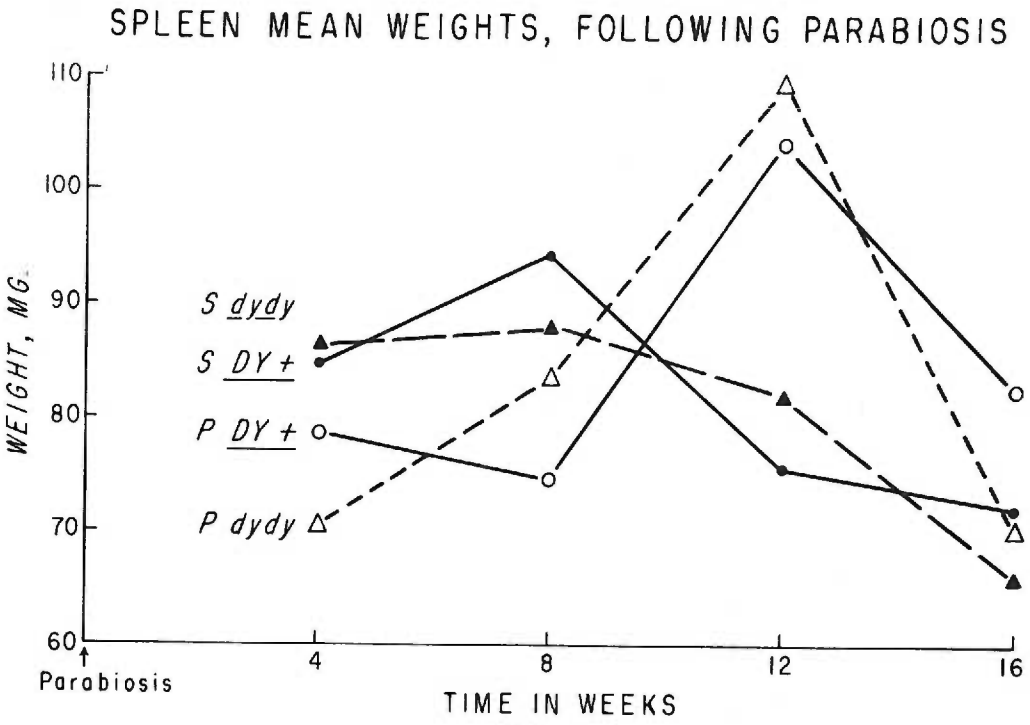

SPLEEN MEAN WEIGHTS, FOLLOWING PARABIOSIS
WEIGHT, MG.
110
100
90
80
70
60
S dydy
S DY+
P DY+
P dydy
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG.41.

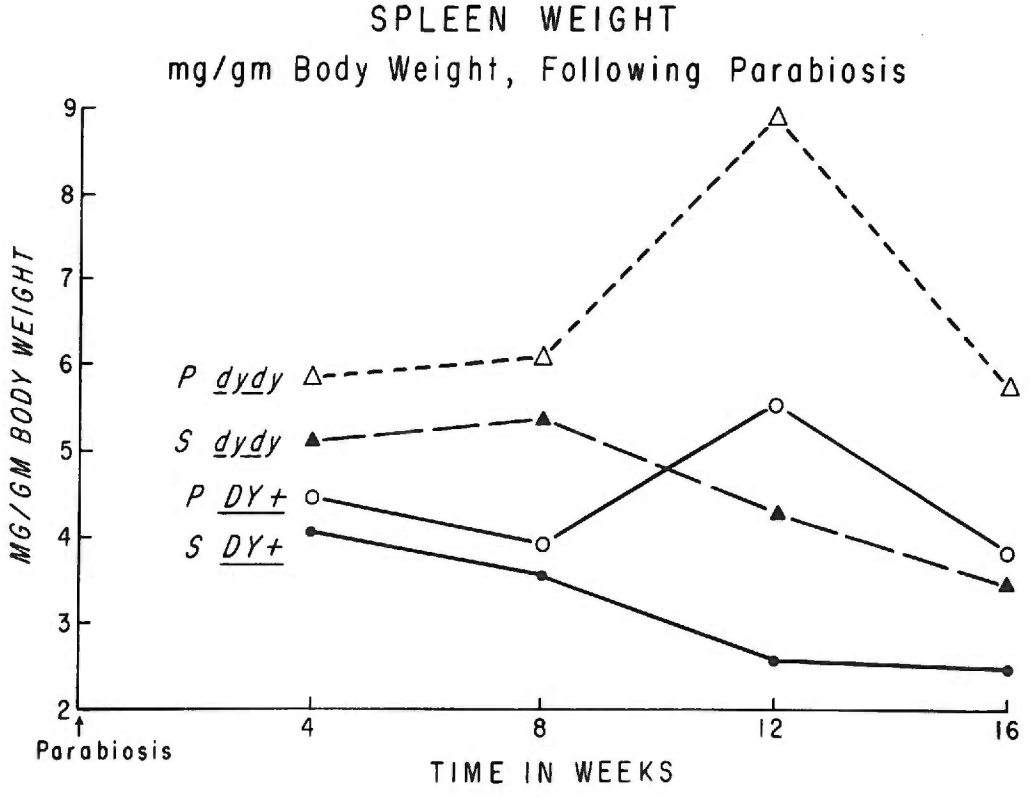

SPLEEN WEIGHT
mg/gm Body Weight, Following Parabiosis
MG/GM BODY WEIGHT
9
8
7
6
5
4
3
2
P dydy
S dydy
P DY+
S DY+
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG.42.

Fig. 43 Spleen Weight Ratio $(\frac{P}{S})$ Plotted Against Body Weight Ratio $(\frac{P}{S})$.

The ratio of spleen weight, parabiont to single, is plotted against the ratio of body weight, parabiont to single. The 45^{o} line indicates similar values for the two ratios. Open symbols represent means of like sex at each time interval. Closed symbols represent a single value.

Fig. 44 Spleen Weight Ratio $(\frac{dydy}{Dy+})$ Plotted Against Body Weight Ratio $(\frac{dydy}{Dy+})$.

This figure is similar to that above, but compares dydy to Dy+ instead of P to S.

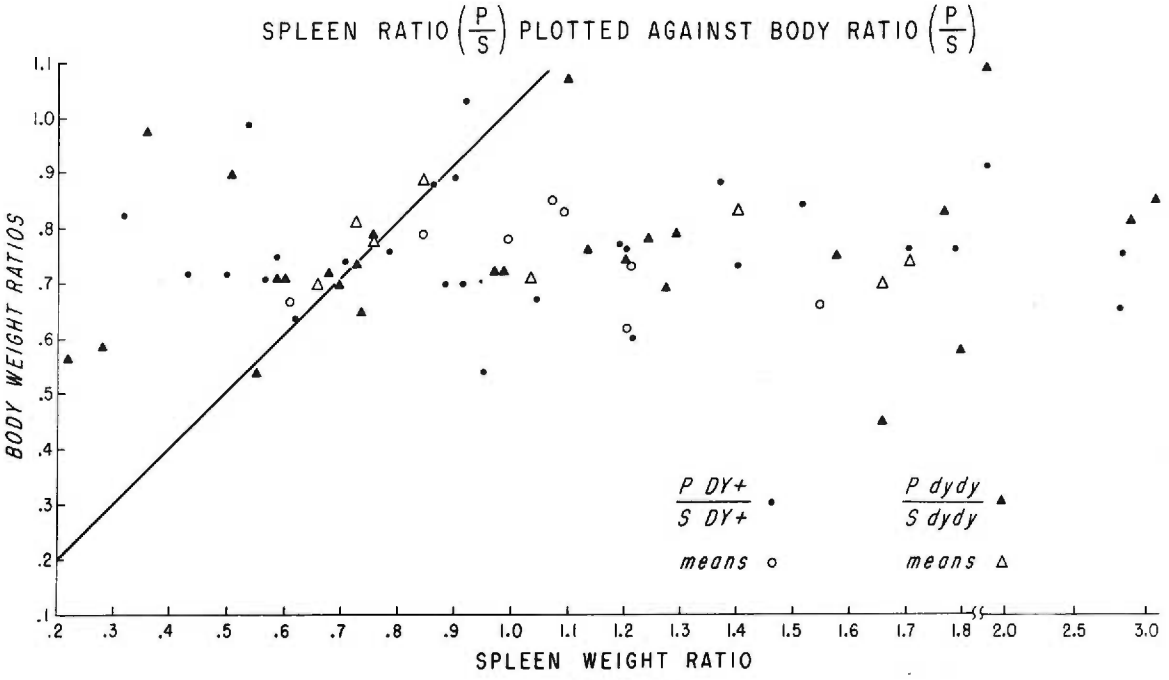

SPLEEN RATIO (P/S) PLOTTED AGAINST BODY RATIO (P/S)
BODY WEIGHT RATIOS
SPLEEN WEIGHT RATIO
P DY+ / S DY+
P dydy / S dydy
means
means

FIG.43.

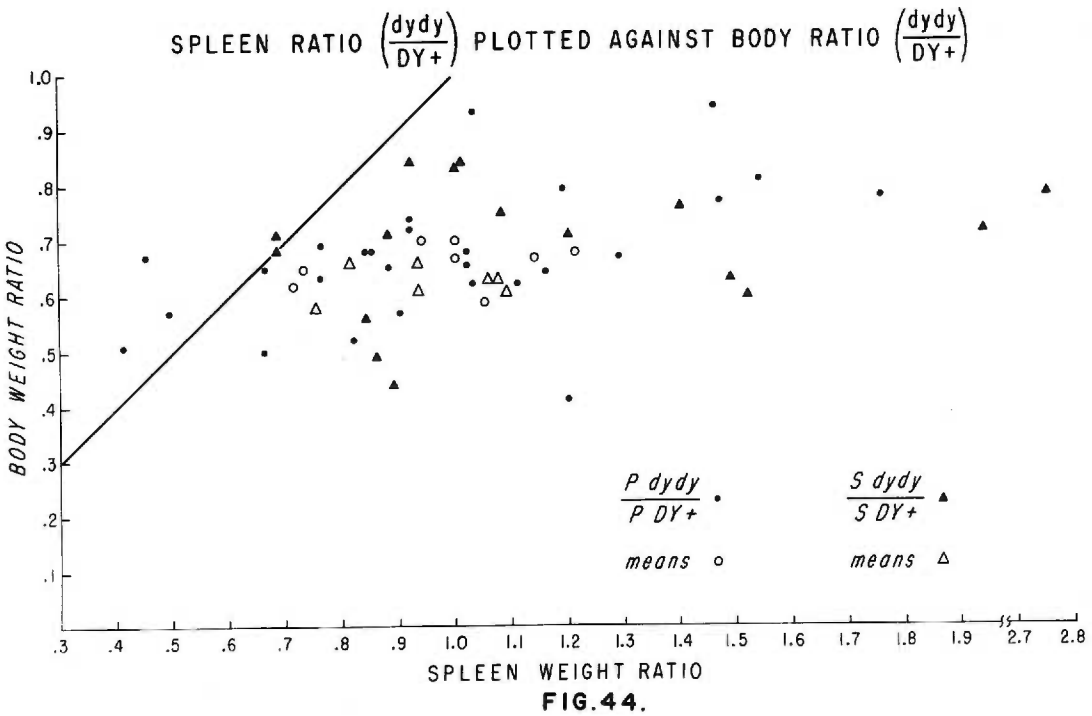

SPLEEN RATIO (dydy/DY+) PLOTTED AGAINST BODY RATIO (dydy/DY+)
BODY WEIGHT RATIO
SPLEEN WEIGHT RATIO
P dydy / P DY+
S dydy / S DY+
means
means

FIG.44.

Fig. 45 Liver Mean Weight, Following Parabiosis.

The mean weights of liver of each group are plotted at successive intervals after parabiosis.

Fig. 46 Liver Weight mg/gm Body Weight.

The ratio of liver weight to body weight (mg/gm) is shown for each group at successive intervals after parabiosis.

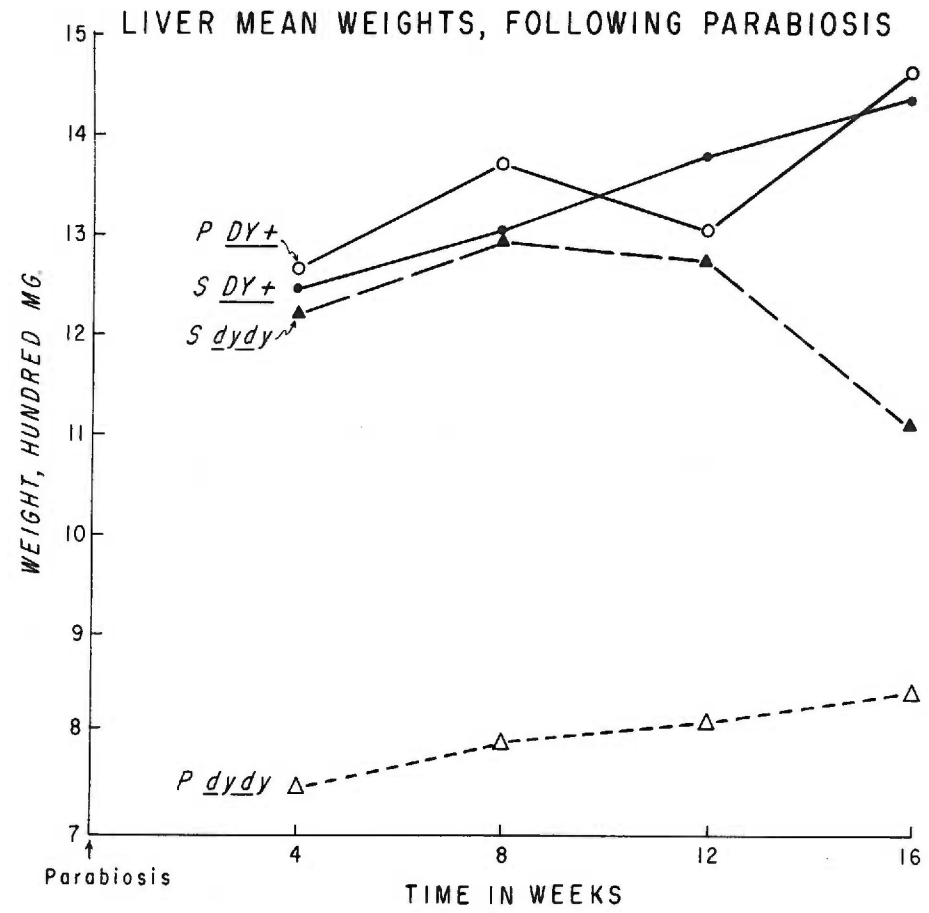

LIVER MEAN WEIGHTS, FOLLOWING PARABIOSIS
WEIGHT, HUNDRED MG.
15
14
13
12
11
10
9
8
7
P DY+
S DY+
S dydy
P dydy
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG. 45.

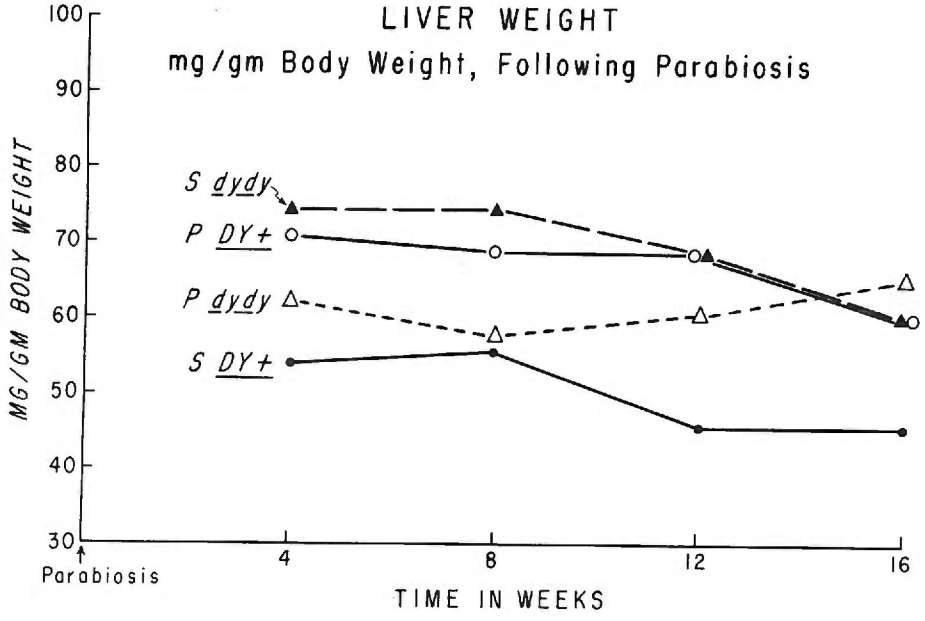

LIVER WEIGHT
mg/gm Body Weight, Following Parabiosis
MG/GM BODY WEIGHT
100
90
80
70
60
50
40
30
S dydy
P DY+
P dydy
S DY+
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG. 46.

Fig. 47 Liver Weight Ratio $(\frac{P}{S})$ Plotted Against Body Weight Ratio $(\frac{P}{S})$.

The ratio of liver weight, parabiont to single, is plotted against the ratio of body weight, parabiont to single. The 45^{o} line indicates similar values. Open symbols represent means of like sex at each time interval. Closed symbols represent a single value.

Fig. 48 Liver Weight Ratio $(\frac{dydy}{Dy+})$ Plotted Against Body Weight Ratio $(\frac{dydy}{Dy+})$.

This figure is similar to that above, but compares dydy to Dy+, instead of P to S.

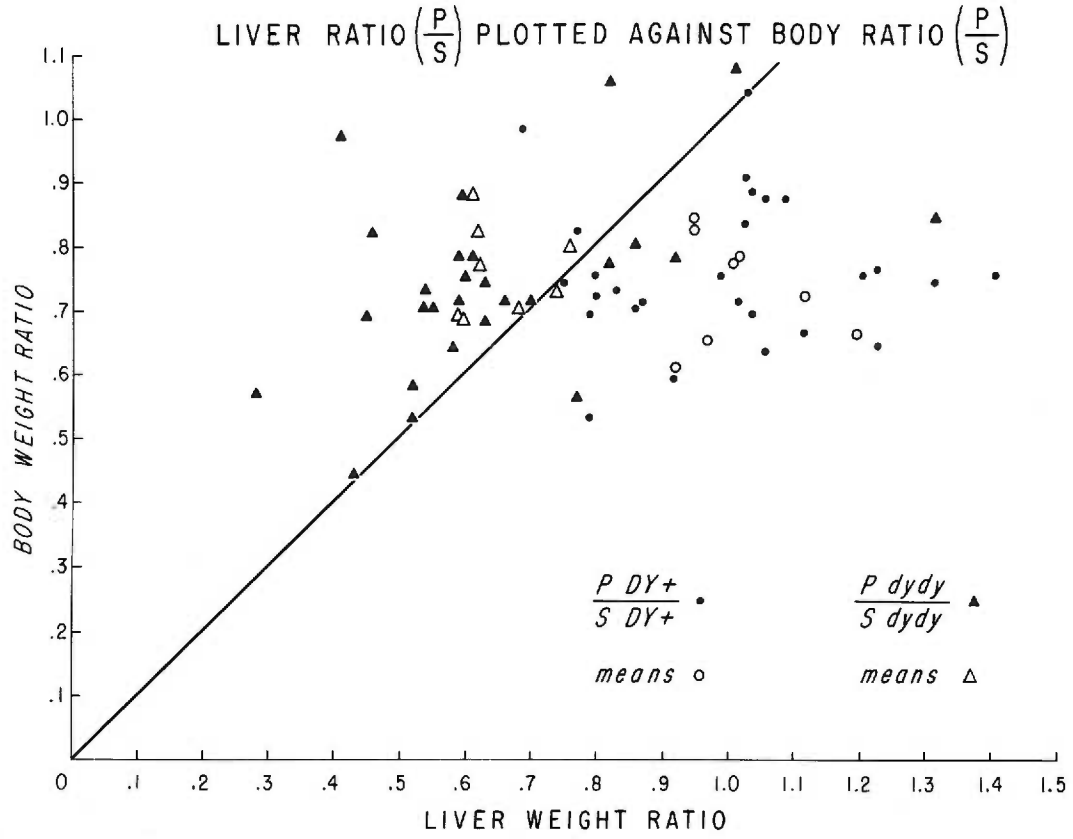

LIVER RATIO (P/S) PLOTTED AGAINST BODY RATIO (P/S)
BODY WEIGHT RATIO
LIVER WEIGHT RATIO
P DY+ / S DY+
means
P dydy / S dydy
means
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
1.5

FIG. 47.

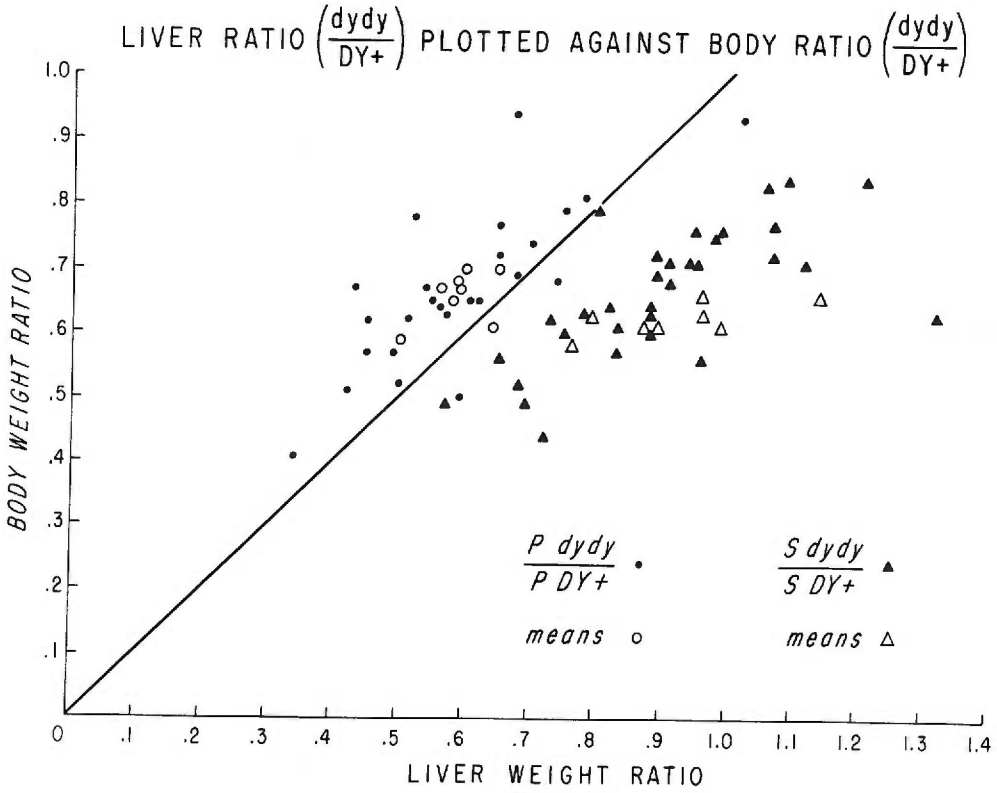

LIVER RATIO (dydy/DY+) PLOTTED AGAINST BODY RATIO (dydy/DY+)
BODY WEIGHT RATIO
LIVER WEIGHT RATIO
P dydy / P DY+
means
S dydy / S DY+
means
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4

FIG. 48.

have less liver weight proportionate to body weight than do P Dy+'s and S dydy's have more liver weight proportionate to body than S Dy+'s.

Kidney. Mean whole weights of kidneys are shown in Figure 49. After parabiosis the kidney weight of parabiosed dydy's is less than that of any other group at all time intervals. Pronounced differences between the other groups are not apparent at any time period. At four and eight weeks, P Dy+'s have lighter kidneys than S Dy+'s. This relationship is not present at twelve and sixteen weeks. The ratios of kidney weight to body weight (Fig. 50) show that dystrophics have a larger kidney weight proportionate to body weight than do normals, and that for both Dy+'s and dydy's the parabionts have a larger proportion of body weight contributed by kidney than do the singles. The difference between P Dy+'s and S Dy+'s are significant only at twelve and at sixteen weeks, ($P < 0.05$). Kidney weight of parabionts compared to that of singles, for both Dy+'s and dydy's (Fig. 51), shows that the loss in weight of parabionts is nearly proportional for kidney and whole body, the kidney probably weighing slightly more in proportion to the body as a whole. Figure 52 shows that dystrophics have heavier kidneys in parabiosis than do normals, proportionate to body weight, and that a similar relationship holds for S dydy's relative to S Dy+'s.

Fig. 49 Kidney Mean Weight, Following Parabiosis.

The mean weights of liver of each group are plotted at successive intervals after parabiosis.

Fig. 50 Kidney Weight mg/gm Body Weight.

The ratio of liver weight to body weight (mg/gm) is shown for each group at successive intervals after parabiosis.

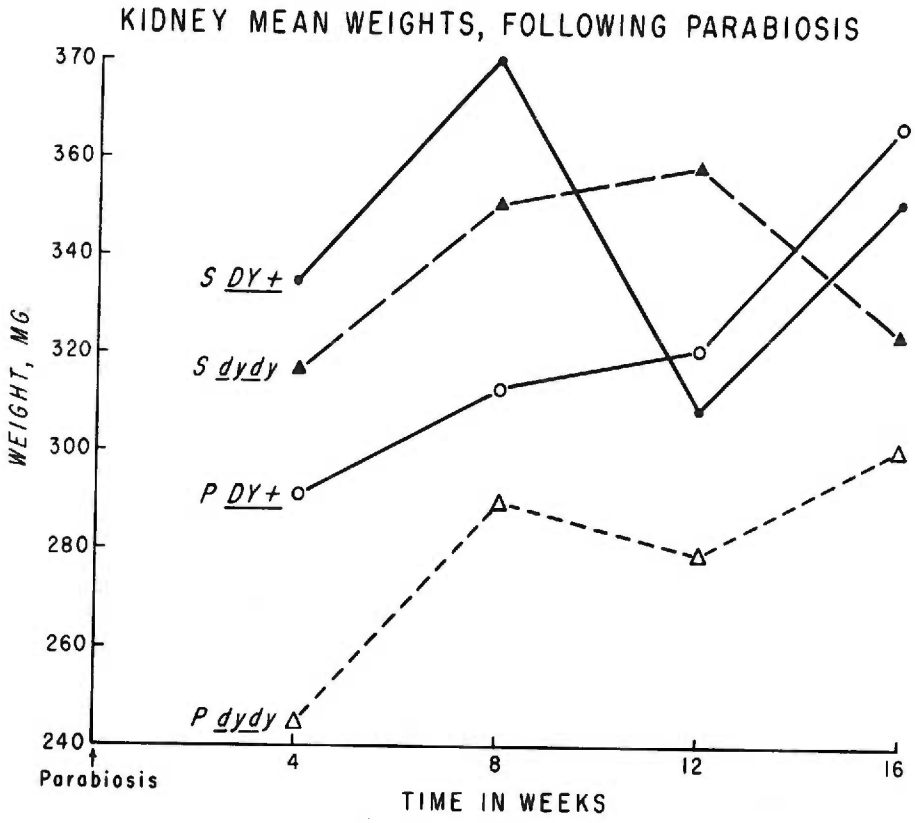

KIDNEY MEAN WEIGHTS, FOLLOWING PARABIOSIS
WEIGHT, MG
370
360
340
320
300
280
260
240
S DY+
S dydy
P DY+
P dydy
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG.49.

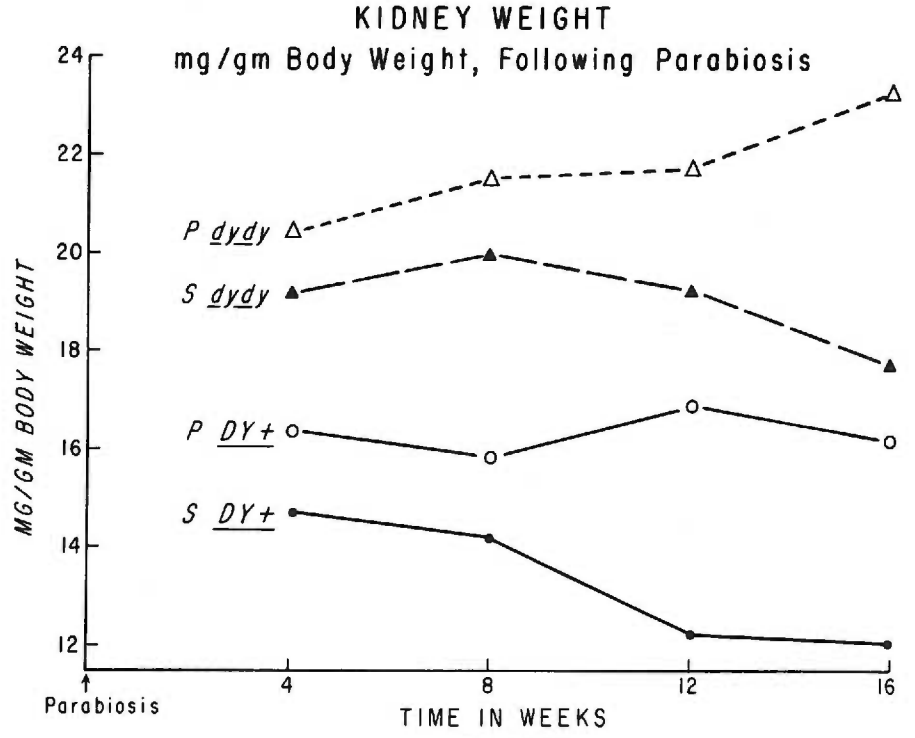

KIDNEY WEIGHT
mg/gm Body Weight, Following Parabiosis
MG/GM BODY WEIGHT
24
22
20
18
16
14
12
P dydy
S dydy
P DY+
S DY+
Parabiosis
4
8
12
16
TIME IN WEEKS

FIG.50.

Fig. 51 Kidney Weight Ratio $(\frac{P}{S})$ Plotted Against Body Weight Ratio $(\frac{P}{S})$.

The ratio of kidney weight, parabiont to single, is plotted against the ratio of body weight, parabiont to single. The 45^{o} line indicates similar values. Open symbols represent means of like sex at each time interval. Closed symbols represent a single value.

Fig. 52 Kidney Weight Ratio $(\frac{dydy}{Dy+})$ Plotted Against Body Weight Ratio $(\frac{dydy}{Dy+})$.

This figure is similar to that above, but compares dydy to Dy+, instead of P to S.

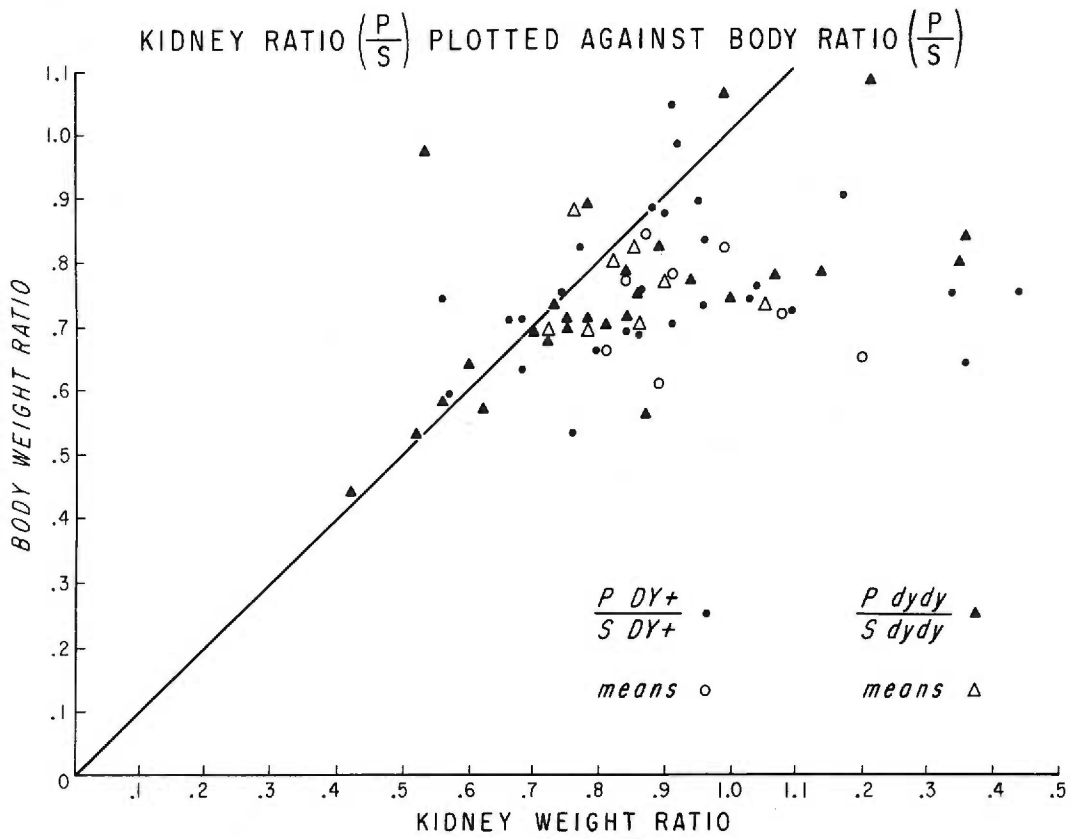

KIDNEY RATIO (P/S) PLOTTED AGAINST BODY RATIO (P/S)
BODY WEIGHT RATIO
KIDNEY WEIGHT RATIO
P DY+ / S DY+
means
P dydy / S dydy
means

FIG.51.

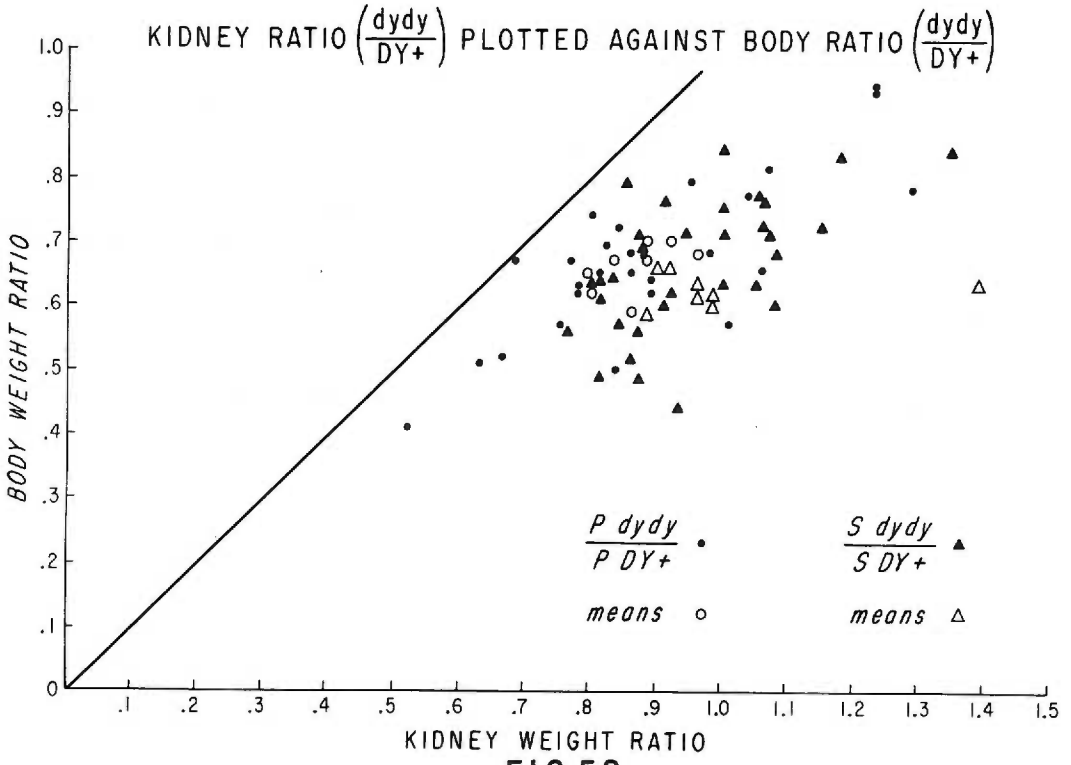

KIDNEY RATIO (dydy/DY+) PLOTTED AGAINST BODY RATIO (dydy/DY+)
BODY WEIGHT RATIO
KIDNEY WEIGHT RATIO
P dydy / P DY+
means
S dydy / S DY+
means

FIG.52.

DISCUSSION AND CONCLUSIONS

Dystrophic mice parabiosed to normal mice have a highly significant decrease in lifespan of 52% compared to that of single dystrophic mice. There is no clinical improvement in the parabiosed dydy's, but histological evidence shows less severe dystrophic lesions in muscle of P dydy's compared to S dydy's.

No decrease in lifespan was apparent in earlier work (4) because the dystrophic mice were not provided with an adequate diet (5). On the old diet single dydy's had an attained age of 130 ± 10 days, and parabiosed dydy's reached 119 ± 10 days. Improved diet increased mean attained age for single dydy's to 212 ± days, but parabiosed dydy's failed to benefit from the improved diet and had an attained age very close to that previously found for parabionts; 110 ± 12 days. It appears then that parabiosis exacts a penalty from the attained age of dydy mice nearly equivalent to the improvement provided by an adequate diet.

Histological improvement of dydy muscle was apparent only when muscle of sacrificed parabionts was examined. If the dystrophic mouse is permitted to reach a terminal condition and die, the presence of autolysis of tissues and congestion of blood vessels does not permit suitable histological evaluation. During the last stages of life the severity of the dystrophic lesions may increase rapidly, so that an improvement in parabiosed dystrophics would no longer be apparent.

Parabiosis results in a lighter body weight compared with singles for both Dy+'s and dydy's. The effect of parabiosis on body weight is not remarkable, but rather what would be expected. Parabiosis by itself can be assumed to exert a stress on the partners such that they would

not be expected to grow as well as single mice. Organ weights are less affected than body weights except for the liver. In the dydy, the liver weighs less in proportion to the body weight when parabiosed than when single. In the Dy+ just the opposite occurs; that is, the P Dy+ has a heavier liver proportionate to body weight than does the S Dy+. The effects on the liver are different from that on other organs and are different between dydy's and Dy+'s. The liver, then, shows an effect of parabiosing dystrophics to normals, that is not merely a result of parabiosis per se. It is likely that this effect on liver weight is related to some metabolic difference between the dydy and the Dy+.

Parabiosis did not result in different histological effects between Dy+'s and dydy's in heart, lungs, adrenals, kidneys or spleen. Most of the pathologic conditions found were not different, except in incidence from those in singles. Parabiosis appears to cause amyloidosis, but this is considered to be an incidental finding of no particular relevance to the study of dystrophy because it was found in both Dy+'s and dydy's. Another incidental finding histologically was the presence of bile duct hyperplasia in two parabiotic partners. Whatever caused this condition in one parabiont apparently passed to the other partner via the common circulation to cause the same condition there. Other than amyloidosis and bile duct hyperplasia, pathologic lesions found were of the kind which would be expected (48,50) in single mice; the only difference was in the incidence and age of onset.

Parabiosis affects the muscle of the dystrophic to result in a less severe dystrophic lesion but does not result in any noticeable clinical improvement. Perhaps a greater exchange of blood between the

normal and dystrophic partners could exaggerate the muscle findings and result in clinical improvement. If dystrophy is the result of a deficiency in some blood-borne substance, this could be provided by the normal parabiont. If dystrophy is the result of a deficient metabolic process, this also might be provided by the normal parabiont. Dystrophy also could be the result of the presence of a substance which the dystrophic is unable to handle metabolically. Under any of these hypotheses an increased blood exchange between parabionts would result in an increased effect on the dystrophic.

The decreased liver weight in the P dydy with a concomitant increase in liver weight of the P Dy+ points to a metabolic deficiency in the dystrophic. Thus, if the liver of the dystrophic is unable to detoxify or metabolize some substance this process might be handled by the P Dy+ liver. The P Dy+ liver then would increase in size since it is handling an increased load, while the P dydy liver would decrease in size since it is no longer providing or attempting to provide the function in question. Histological changes in liver between parabionts and singles were apparent. Single mice had a high incidence of fatty livers, a finding which was practically absent from the parabiosed group.

The weight difference between P dydy and P Dy+ liver is apparent prior to the development of histological differences so that the large number of fatty livers present in only single dydy's and S Dy+'s cannot account for the weight difference. Gould (51,52) has shown that, in contrast to normal mice, dystrophic mice do not utilize acetoacetate in muscle homogenates. Acetoacetate accumulates in the muscle homogenates of the dystrophic. Apparently the dystrophic muscle lacks an (or contains a defective) enzyme required for fat metabolism in muscle.

Since the liver is a major site of fat metabolism, it can be speculated that the normal parabiont is metabolizing fat in the liver to compensate for this lack in the muscle of the dydy parabiont. This could account for the noted decrease in size of P dydy liver, increase in size of P Dy+ liver, and decreased severity in muscle of the P dydy.

The decreased lifespan of dydy's in parabiosis is probably a reflection of the weakened condition of the dystrophic in general, resulting in a greater effect from the stress of parabiosis per se. No adequate information is available regarding the effect on lifespan of parabiosis of normal mice. Likewise no information is available on the effects of parabiosis of two dystrophic mice. It would be of some significance to measure the effect on lifespan of normal parabionts. At present it is felt that the effect may be negligible. The cost of measuring the effect of parabiosing dystrophic mice together is prohibitive relative to the small amount of information likely to result.

A massive blood exchange might permit essential cure of parabiosed dystrophics and at the same time prevent the decreased lifespan of P dydy's if this decrease is due to the general weakness of dystrophic mice. On the other hand, a massive blood exchange between normal and dystrophic parabionts could possibly result in such great mechanical effects on vasomotor tone, heart load, and blood pressure that the dystrophic could not handle the exchange.

These studies indicate that some factor in blood may be related to the cause of muscular dystrophy or at least to the improvement of the muscle lesions. This should be investigated further, realizing that the search may be basically biochemical and may be aimed at finding (1) the absence of something in the dystrophic, (2) the presence of

something in the normal, (3) a faulty or deficient metabolic process in the dystrophic, (4) the production of a toxic or deficient substance by the dystrophic, or some combination of these.

Parabiosis with a massive blood exchange could be a step toward answering which of the above possibilities is operative, particularly if the blood exchange could be selectively directed between parabionts; that is, if parabionts could be prepared where the arterial blood from the Dy+ is directed to the dystrophic or away from the dystrophic. A technique toward perfecting such a system is presently being developed.

SUMMARY

Dystrophic (dydy) mice were surgically united in parabiotic union with normal (Dy+) mice. One hundred and twenty pairs, half of which were parabiotic pairs and half single control pairs, were used to measure the lifespan of dydy mice in parabiosis. Two hundred and eight dydy mice were similarly paired as parabiotics, or as controls, and sacrificed at predetermined times. Intervals of four, eight, twelve and sixteen weeks after parabiosis were selected. The number of pairs sacrificed was 16, 16, 14 and 13 for the time intervals respectively. Histopathological studies and weight studies of body, heart, liver, spleen, kidney and adrenal were done on these mice.

It was found that dydy mice in parabiotic union with normal mice show no clinical improvement and have a lifespan decreased fifty percent of that of single dydy mice. Histologically the lungs, adrenals, heart and kidneys showed little or no effect as a result of parabiosis. The spleen showed a high incidence of apparent amyloidosis present in both dydy and Dy+ parabionts. The liver of single dydy's and single Dy+'s showed a high incidence of fatty changes, which was not present in dydy or Dy+ parabionts. Muscle of dydy parabionts showed a marked decrease in severity of dystrophic lesions as compared to muscle of single dydy's.

Body weight is decreased about the same for both dydy and Dy+ parabionts. Adrenal, heart, spleen and kidney show little or no effect on weight, as a result of parabiosis for either dydy's or Dy+'s. The liver of parabiosed dydy's is decreased in weight, while the liver of parabiosed Dy+'s is increased.

Thus, parabiosis of dydy mice to Dy+ mice, while decreasing life-span by 50%, also decreases the severity of dystrophic lesions and decreases liver weight of dystrophic mice. These effects could be explained on the basis of the liver of parabiosed Dy+'s carrying out certain metabolic functions which the dydy muscle and liver are unable to accomplish. The Dy+ liver easily could increase in size while carrying on increased function, and the dydy liver could decrease in size since it is doing less work.

A technique which would increase the blood exchange between parabionts might increase the beneficial effects for dydy mice, perhaps even to the point of cure or clinical improvement.

The results reported here indicate that there is a reasonable possibility that some substance, either present in or absent from blood of dydy mice, may be involved in the progression of the dystrophic condition. If such a substance could be found and isolated it would be of considerable importance in understanding the causation of the disease and possibly providing the means for treatment or cure.

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APPENDIX 1.

The following revised classification of strain 129 applies to all such mice used in the present studies as well as in all previous communications by other authors concerning a hereditary progressive muscular dystrophy in mice derived from the Jackson Memorial Laboratory dystrophy colony. This classification is in accordance with the rules and standard symbols given in "Standardized Nomenclature for Inbred Strains of mice, second listing" prepared by the Committee on Standardized Genetic Nomenclature for mice, published in Cancer Research 20: February, 1960.

I. 129/Re-+dy designates the subline of the 129 strain in which the dy (dystrophic) mutation arose. The following classes of mice stem from non-sibling matings in the 129/Re-+dy subline:

A. all dydy dystrophic individuals.

B. all known Dydy heterozygous carrier individuals.

C. some mice of normal phenotype, which may be wither Dydy carriers or DyDy homozygous normals, derived from matings between known heterozygous carriers.

II. 129/J designates a subline of the 129 inbred strain maintained in the Jackson Memorial Laboratory Foundation Stocks. All guaranteed DyDy homozygous normals are offspring of full-sibling matings between animals descended from this 129/J Foundation Stock colony.

(Reference: West, W.T., and E.D. Murphy. Histopathology of Hereditary, progressive Muscular Dystrophy in Inbred strain 129 mice. The Anatomical Record 137: 279-295, 1960.)