GENETIC AND PHYSIOLOGICAL CONTROL OF ESTERASES IN EXPERIMENTAL SMALL ANIMALS V. CONGENITAL OBESITY AND ESTERASE VARIATION IN MICE

GENETIC AND PHYSIOLOGICAL CONTROL OF ESTERASES IN EXPERIMENTAL SMALL ANIMALS

V. CONGENITAL OBESITY AND ESTERASE VARIATION IN MICE

By

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Introduction

Several forms of hereditary and nonhereditary obesities in mice have been reported (1–8). Mayer has grouped the obesities into two categories, regulatory and metabolic (9). In the regulatory type, the primary impairment is of the hypothalamic mechanism regulating food intake, which causes habitual hyperphagia. In the metabolic type, the primary disturbance is an inborn or acquired error in the metabolism of tissues. Some genetic obesities of mice such as "Hereditary hyperglycemic syndrome" and "NZO" obesity have an inborn metabolic dysfunction (10, 11).

A congenital obesity of a mouse was spontaneously produced in AA inbred strain at this laboratory. The obese mice were very plump and sterile as the other known hereditary obese mice which have the gene obese "ob" and the gene adipose "ad", respectively (2, 4).

In this report, some characterisitics hormone treatment and esterase variation of the congenital obese mice in AA strain will be dealt with.

Materials and Methods

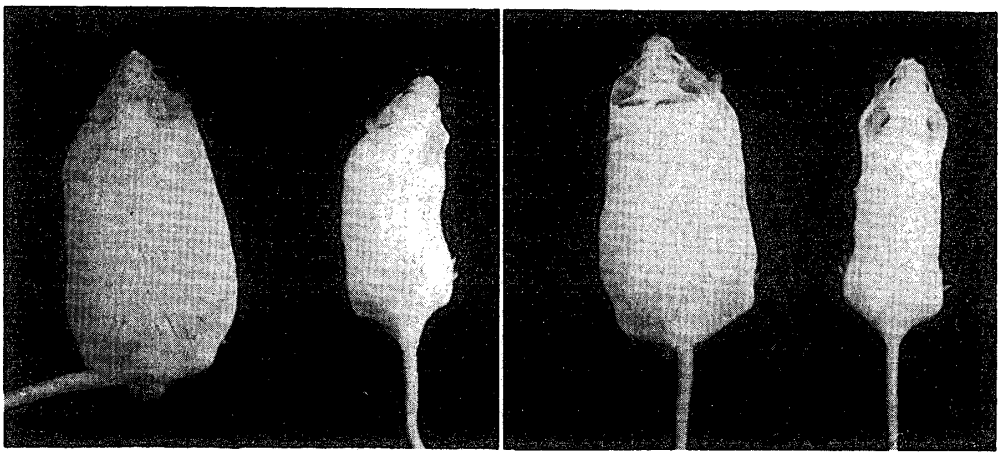
Two obese male mice were produced with a non-obese female mouse in AA inbred strain of tenth selected generation at the breeding laboratory in this Department in June, 1965. One of the obese mice was treated with testosterone heptanoate and propionate (4:1) (Shering A.G., Germany), 0.5 mg daily for 7 days. Sera and organs were obtained from the original obese mice, and some obese offspring from first generation cross between the testosterone treated obese male and non-obese female sibs.

The activities of the serum cholinesterase and kidney esterase isozymes in the obese mice were compared with those in the normal mice by starch-gel electrophoresis, according to the method stated in the previous reports (12, 13).

Results

1. Description of character of the congenital obese mice

Body weight: Obese male and female mice were distinguishable from normals at about six weeks of age. From this time they increased in weight rapidly, and by the time they were three months of age they weighed average 45 grams or about 1.5 times as much as normal mice of AA strain. After three months they still increased in weight until they weighed 65 to 75 grams at seven months as pictured in Fig. 1. Growth curves of the obese and normal mice are shown in Fig. 2. The obese mice showed moderate hyperphagia, that is, 40 per cent overeat as compared with normal mice.



A B

Fig. 1. Comparison of growth of the obese and normal mice.
A: shows a male obese mouse and normal control of AA strain at seven months of age, when the obese mouse weighed 75 grams.
B: shows a female obese mouse and normal control of AA strain at seven months of age, when the obese mouse weighed 65 grams.

Organ weights: Organ weights of the obese and normal male mice are compared in Table 1. The weights of the liver, kidney, spleen, heart and pancreas from the obese were 2 to 3 times as great as those from the normal in proportion to the body weight. However, there was no significant increase in weights of the testicles and epididymis in the obese group. On the other hands, there was a significant atrophy of the uterus and ovaries in the old obese female, which were about half as small as those in the normal, although the other organ weights abnormally increased as those in the obese male.

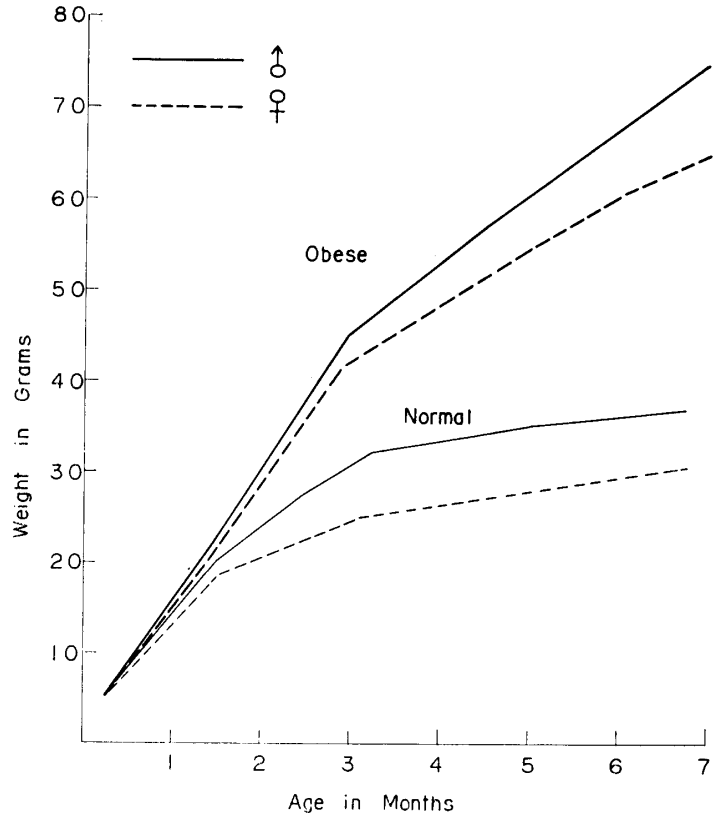


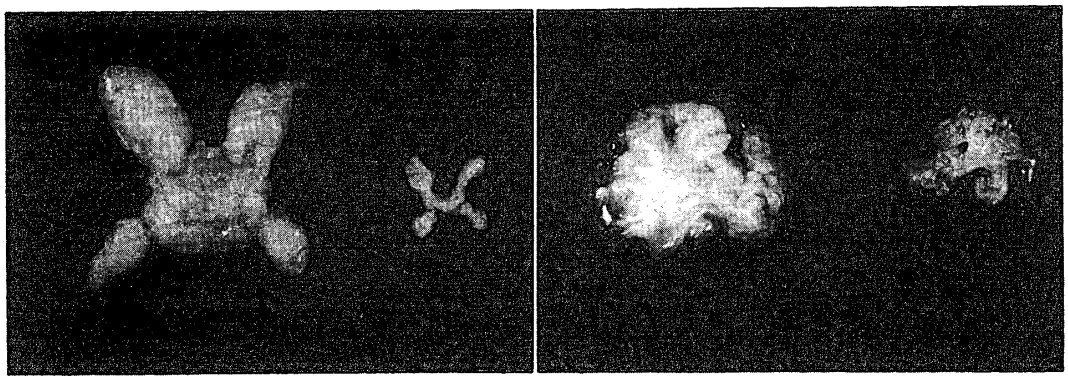
Fig. 2. Growth curves of normal and obese mice.
The mice of AA strain were weihged monthly up to seven months. The weights of the normal and obese mice up to at least one month could not be distinguished.

Table 1. Organ weights of the obese and normal mice

Adult males*	Young adults (3-4 months)		Adults (7-8 months)	
Organs (gm.)	2 Obese	3 Normal	1 Obese	3 Normal
Body wt.	49.0	25.3	76.0	32.0
Liver	2.373	1.230	4.300	1.325
Kidneys	0.485	0.370	0.860	0.422
Spleen	0.105	0.080	0.180	0.088
Heart	—	—	0.250	0.148
Pancreas	—	—	0.530	0.203
Brain	—	—	0.400	0.415
Adrenals	—	—	0.010	0.008
Testicles	0.195	0.210	0.200	0.214
Epididymis	0.060	0.074	0.080	0.078
Epididymal adipose	—	—	6.100	1.037
Mesenteric adipose	—	—	2.800	0.446

Note: Values are means. * AA strain.

Body adipose: The obese mice showed considerable high fat contents in body composition and they were associated with serious diabetes containing more than 2



A B

Fig. 3. Epididymal and mesenteric adipose tissues of the obese and normal mice
A: shows epididymal adipose tissues of the obese and normal mice.
B: shows mesenteric adipose tisses of the obese and normal mice.

grams per cent of glucose in urine. Epididymal and mesenteric adipose tissues prepared from the old obese and normal mice are shown in Fig. 3. Weights of fat in the adipose tissues from the obese were much greater than those from the normal.

2. Hormonal treatment and reproduction of the obese mice.

The obese male mice which were found with a non-obese female mouse in AA strain were sterile. One of the obese males was treated with testosterone heptanoate and propionate (4:1). In matings between the testosterone treated obese male and the non-obese female sibs, the non-boese female was delivered of two male and two female mice, about 40 days after the hormone adminsitration to the obese male. However, all the offspring died before weaning period. About 50 days after the first parturition, the non-obese female was successfully delivered of five male and one female offspring in mating with the same male. Thereafter, all the F1 offspring were demonstrated to be obese and sterile as the parental obese male, except for one male animal which died before the weaning period.

3. Variation of esterase activities in the obese mice.

The activity-levels of serum cholinesterase isozymes on the starch-gels were compared among the normal, castrated, testosterone treated castrated males and the obese males as shown in Fig. 4. The activity of serum cholinesterase, zone C_3, of the normal male was very low (Fig. 4a). The activity of the zone C_3 increased by castration and were depressed to normal level by testosterone treatment (Fig. 4b and c). The activitylevels of the zone C_3 in the parental obese male mice were greater than that in the normal male and similar to that in the non-obese castrated male, although the obese males were not castrated (Fig. 4 d and e). Testosterone administration to the obese male depressed the enzyme activity to a level as low as that in the normal male.

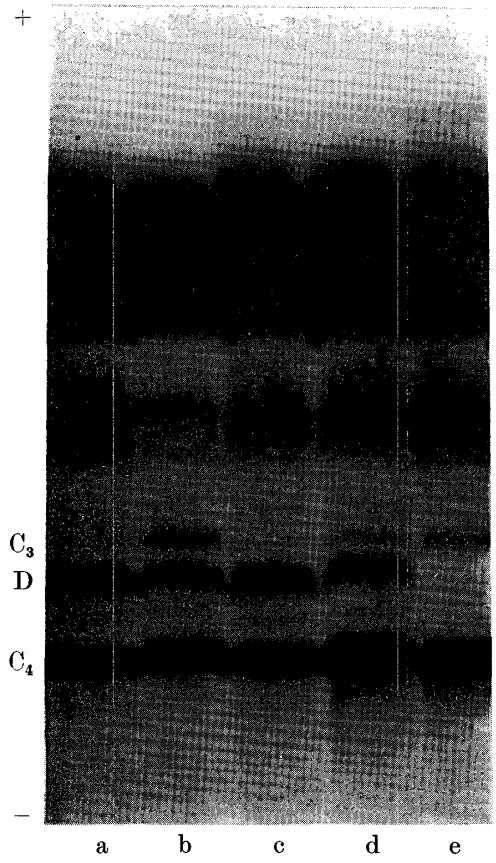


Fig. 4.

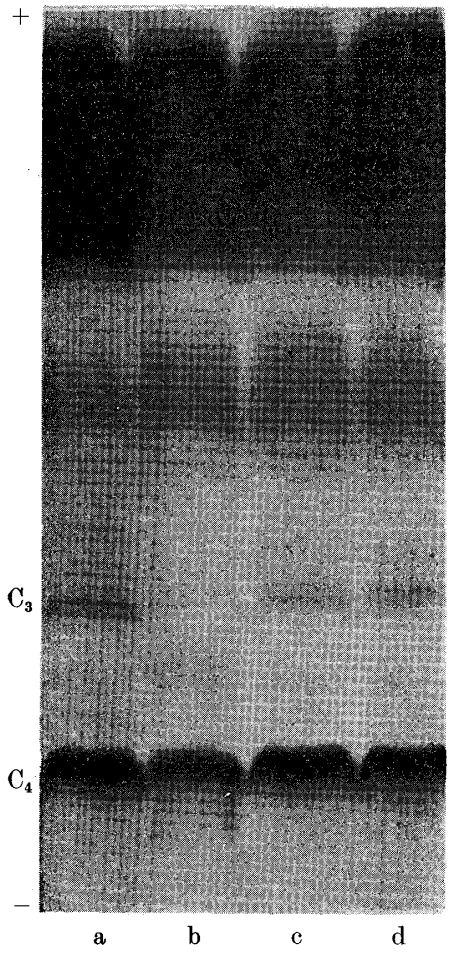


Fig. 5.

Fig. 4. Comparison of the activity-levels of serum cholinesterase isozyme, zone C_3, among the normal, castrated, testosterone treated castrated and obese male mice. (a) Normal male, (b) castrated male, (c) castrated male treated with testosterone, and (d) and (e) obese males.

Fig. 5. Activity-level of the serum cholinesterase isozyme, zone C_3, of the obese offspring produced from a mating of the testosterone treated obese male and the non-obese female sibs. (a) Normal female, (b) normal male, and (c) and (d) obese males.

The activity-levels of serum cholinesterase isozymes of the offspring from a mating of the testosterone treated obese male and the non-obese female are shown in Fig. 5. The activities of the cholinesterase, zone C_3, in the male offspring which were all obese and sterile were greater than that in the normal male and similar to that in the normal female (Fig. 5 a, b, c and d). The activities of cholinesterase isozymes of serum and uterus (12) in the female offspring which was also sterile-obese was very low as those in the ovariectomized female.

On the other hand, the activity-levels of the "sex hormone-dependent esterases" in normal male mouse kidney (13, 14) were always much greater than those in the normal female kidney as shown in Fig. 6 (a and b). While, the activities of the

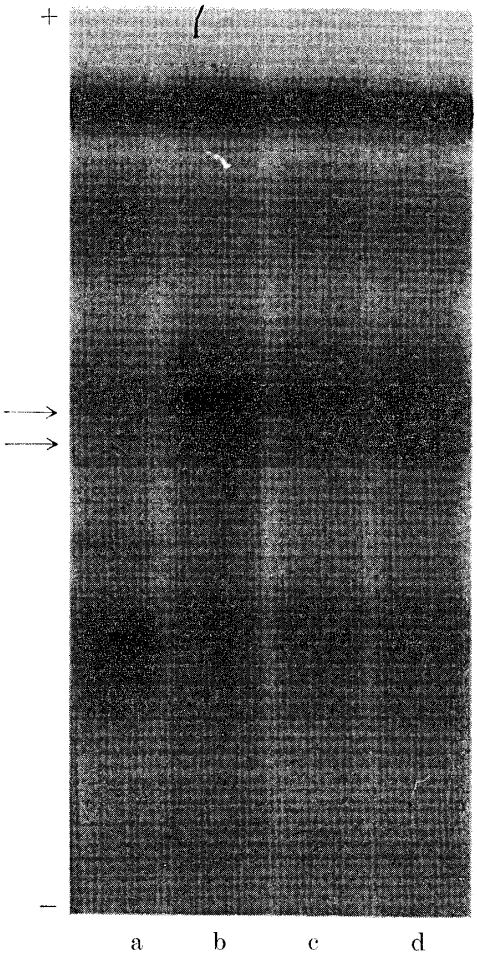


Fig. 6. Comparison of zymograms of the kidney esterases in the normal and obese mice. Arrows indicate loci of the kidney esterases. (a) Normal female, (b) normal male, and (c) and (d) obese males.

kidney esterases in the obese male indicated the intermediate activities between the normal male and female (Fig. 6 c and d).

Discussion

The obese male mice which were first produced spontaneously in AA inbred strain were sterile. The activity-level of serum cholinesterase isozyme, zone C_3, in the obese male was much greater than that in the normal male and was in the high level as that of the castrated male. In the previous report (12) the activity of the enzyme in normal male mouse has been demonstrated to be elevated by castration and depressed by testosterone administration. From the results, it was considered that the secretion of testosterone in the obese male was low. Thus, one of the obese male mice was treated by testosterone hepatonate and propionate (4:1) which was continuously effective agent. After the treatment, the obese male recovered to the normal low level in the enzyme activity and gradually decreased in the body weight. An non-obese female mouse was successfully delivered of the six offspring, one being female, in mating with the testosterone treated obese male. However, all the offspring were demonstrated to be sterile and obese in adult

ages. The activities of serum cholinesterase isozyme in the obese male offspring were abnormally in high level as that in the parental obese male before the hormonal treatment. From the results, it was demonstrated that the abnormally high-level of the activity of cholinesterase isozyme was inherited the obese male offspring, by the parental obese male, although the activity-level of the enzyme in the parental obese male was able to be quantitatively regulated and depressed with testosterone. Thus, it was considered that the obese male offspring were also accompanied with a deficiency of the secretion of the male hormone like that of the parental obese male. The comparatively low activity of the "male kidney esterase" (13, 14) in the obese male also supports this hypothesis.

On the other hand, the activities of pseudo-cholinesterase of serum and uterus (12) in the obese female were lower than those in the normal female and were similar to those in the ovariectomized female. This probably means the deficiency of the secretion of female hormone in the obese female. Such a hypothesis is supported by the facts that the obese female indicated the atrophy of uterus, although the other organs, i.e., the liver, heart, kidney and spleen etc. were much greater in weights than those in the normal.

A sterile-obese syndrome in the V stock of mice has been reported by Ingalls et al. (2). Offspring have been obtained from the sterile-obese female mice, which were homozygous for the recessive gene ob, following hormonally induced ovulation and transplantation of resulting eggs to fertile recipients (15). It has been suggested that the sterile-obese syndrome was associated with reproductive organs arrested in a prepuberal condition due to insufficiency of pituitary gonadotropins (16).

On the other hand, the obese mice in AA strain had enlarged pancreas and were associated with a serious diabet, which was similar to the "Hereditary obese hyperglycemic syndrome" in mice (obob) associated with a pancreatic dysfunction (10, 17).

From the results, it was concidered that the congential sterile-obese syndrome occured in AA strain at this laboratory was a hereditary metabolic disease, associating with deficiency of the secretion of the sex hormones, and probably a pancreatic dysfunction.

Summary

Obese male mice were found in the AA inbred strain at this laboratory. The congenital obese were sterile and increased rapidly in weights. One of the obese males was treated by testosterone. Six offspring were obtained from a mating between the hormone treated obese male and the non-obese female sibs, but they were demonstrated to be sterile and obese in adult ages. The obese female and male mice increased in weights up to 65 and 75 grams at seven months, respectively. The weights of the liver, heart, kidney, spleen and pancreas from the obese were much greater than those from the normal, but not the weights of the reproductive

organs, i.e., testicles, epididymis, ovaries, and uterus.

The activity-levels of the serum cholinesterase isozyme in the obese males were greater than those in the normal males and similar to those in the castrated males. Testosterone treatment to the obese male depressed the enzyme activity to the normal level. The activity of the enzyme in the obese female was as low as those in the normal male and the ovariectomized female. The activity of the "male kidney esterase" in the obese male was lower than that in the normal male. On the other hand, the obese mice were associated with serious diabetes.

From the results, it was concluded that the congenital sterile-obese syndrome in AA strain was a kind of hereditary metabolic disease, associating with deficiency of secretion of the sex hormones and probably a pancreatic dysfunction.

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