EVIDENCE OF PROLACTIN CELL DEFICIENCY IN CONNECTION WITH LOW REPRODUCTIVE EFFICIENCY OF FEMALE ‘TORPID’ MICE

H. C. DUNG

Department of Anatomy, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284, U.S.A.

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Summary. The pituitary of female ‘torpid’ mice at the age of sexual maturity was deficient in prolactin cells. This deficiency is thought to contribute to the infertility of the affected mice.

INTRODUCTION

The ‘torpid’ mouse was first described in 1965 by Dickie. It resulted from a single recessive gene mutation which occurred in the DBA/2J strain. Homozygous affected animals were known to have abnormal behaviour and were classified as neurological mutants (Sidman *et al.*, 1965). These mutants were also shown to have low reproductive efficiency, runting, and other clinical symptoms. The pathology underlying the clinical symptoms was unknown. It is important that investigations be undertaken to determine the pathogenesis of the clinical symptoms found in the mutants. Only then can these mutants be considered as useful experimental models for similar diseases in man. Without such knowledge, the value of the mutants for experimental studies remains limited.

Since the female mutants were known to show low reproductive efficiency and poor growth, a number of studies were carried out in our laboratory to determine if there were pathological changes in the pituitary and ovaries.

MATERIALS AND METHODS

Sources of the mutant stock and body weights

The animals used were all bred in this laboratory. The original stock were obtained from the Jackson Laboratory, Bar Harbor, Maine, in 1967. The mutants were identified by their abnormal behaviour and smaller body weight than that of normal littermates after 18 days of age. The curves for body weight at different ages were obtained following the method previously described by Dung & Swigart (1971).

Vaginal smears

Six normal and six ‘torpid’ female mice were randomly chosen for study of the oestrous cycle. The age of the animals was between 3 and 4 months. The

vagina was washed with a small quantity of distilled water with a clean pipette and vaginal smears were made daily for 30 days. The mouse was considered to be in oestrus if the smear contained predominantly cornified epithelial cells.

Organ weights

Mice were killed with an overdose of sodium pentobarbital and the pituitary, ovaries, uterus, brain, heart, kidney, and spleen were removed. After cleaning from surrounding fat and connective tissue under a dissecting microscope, the tissues were frozen in wells drilled in $\frac{3}{8}$ -in. aluminium plate packed in powdered dry ice. The tissues were freeze-dried and their weights were determined as described previously (Dung & Swigart, 1971).

Histological and pathological studies

Four normal and nine 'torpid' females between 3 and 4 months of age were prepared by cardiac perfusion under appropriate anaesthesia with mammalian physiological saline followed by 10% neutral formol saline or Bouin's fixative. After perfusion, the pituitary, ovaries, thyroid, adrenal glands, brain, thymus, spleen, submaxillary glands with lymph nodes, and pancreas were removed and allowed to remain in the fixative overnight. The tissues were washed, dehydrated, and embedded in paraffin wax ($53 \pm 1^\circ\text{C}$). Blocks of all tissues except brain were cut into $8\text{-}\mu\text{m}$ sections and stained with haematoxylin and eosin or with periodic acid-Schiff (PAS)-haematoxylin. The brain tissues were sectioned between 12 and $25\text{ }\mu\text{m}$ and stained with one of three methods: (1) the combined methods of Klüver & Barrera (1953) for nerve cells and fibres, (2) thionine stain for Nissl substance (Fletcher, 1947), or (3) Weil-Weigert stain for myelin (Lillie, 1954).

Electron microscopic study

The pituitaries of four normal and four 'torpid' female mice between 3 and 4 months of age were exposed by removal of the calvarium and brain immediately after decapitation, and were fixed in a mixture of equal parts of ice-cold 2% paraformaldehyde buffered in cacodylate buffer solution (0.2 M, pH 7.2) and 2% osmic acid mixture. After fixation for 6 to 8 hr, the tissues were kept in 0.5% ice-cold uranyl acetate solution overnight, and were then transferred to cold absolute alcohol for 5 to 12 hr. Two hr after the tissues were returned to room temperature, they were transferred to fresh absolute alcohol for a further 2 hr and then placed in propylene oxide for 5 hr. All tissues were embedded in the plastic mixture recommended by Spurr (1969). Tissue blocks were cut into serial sections. Thin sections were mounted on copper grids with special 100 mesh (Ernest F. Fullam, Inc., Schenectady, New York). After staining with lead citrate, thin gold sections on grids were examined with the electron microscope. One good section was selected from every fifteen to twenty grids. A total of ten such sections were studied from each pituitary under a Siemens 1A or 101 electron microscope. Under a low magnification ($\times 2500$), twelve negatives were taken from a grid, one from each mesh space. Each negative was printed to make a photograph with a final enlargement of $\times 10,000$ that of the original structures: Pl. 2, Fig. 8 is typical of such prints.

All cells showing profiles of their nuclei in the print were counted. These cells were then classified into (1) prolactin, (2) growth hormone, or (3) other cells. Growth hormone cells were the most prevalent and easy to identify (Rinehart & Farquhar, 1953; Yamada & Sano, 1960; Barnes, 1962).

RESULTS

Sources of the mutant stock and body weights

Text-figure 1 shows growth curves for normal and 'torpid' mice between 20 and 95 days of age.

TEXT-FIG. 1. Growth curves for phenotypically normal (—) and 'torpid' (---) mice. Each dot or circle represents at least twelve and no more than fifteen animals. ●, Males; ○, females.

Breeding records and low reproductivity

When 55 pairs of normal mice (TD/+ × TD/+) bred, 54 litters were produced (96.3% fertility). Breeding of normal males with mutant females (TD/+ × tdfd) resulted in only five litters from 36 pairs (13.8% fertility). Mutant males crossed with normal females (tdtd × TD/+) resulted in nine litters from 28 pairs (32.2% fertility).

Vaginal smears

All the normal females studied had regular oestrous cycles (Text-fig. 2). Similar regularity was observed in only 1/3 'torpid' females examined. Three of the mutant females studied exhibited no oestrous periods. Vaginal smears of these mice throughout the 30-day period contained only leucocytes.

Organ weights

No differences between the normal and 'torpid' mice were found for the weights of the brain, heart, kidney or spleen. The mean weight ($\pm$ S.E.M.) of the uterus (mg/100 g body wt) was lower in 'torpid' (55.54 ± 9.58 , N = 9) than

TEXT-FIG. 2. Periodicity of oestrus in (a) normal and (b) 'torpid' female mice. Filled-in squares represent periods of oestrus.

in normal (79.72 ± 6.58 , $N = 9$) mice, but the difference was not significant. The pituitary (1.70 ± 0.10 , $N = 9$) and ovary (7.05 ± 0.79 , $N = 9$) weights of 'torpid' mice were significantly different ($P < 0.01$ and $P < 0.001$) from those of normal mice (pituitary: 2.13 ± 0.20 , $N = 9$; ovary: 11.50 ± 0.84 , $N = 9$).

Histological and pathological studies

The pituitary and ovaries showed consistent differences between 'torpid' and normal mice. Pathological changes characterized by degenerative involution were frequently observed in the thymus of the mutant.

In the ovaries of 'torpid' mice, corpora lutea were absent (Pl. 1, Fig. 1), although always present in the normal animals (Pl. 1, Fig. 2). Growing follicles of all sizes were found in the ovaries of 'torpid' mice, although the interstitial cells were dystrophic (Pl. 1, Fig. 3) when compared to those of normal animals (Pl. 1, Fig. 4).

Histological differences between pituitaries of 'torpid' and normal mice were characterized by alterations in (1) the density of the cell population and (2) the nuclear/cytoplasmic ratio. The cells were smaller and more closely packed in the pituitary of 'torpid' (Pl. 1, Fig. 5) than in normal animals (Pl. 1, Fig. 6). This increase in the population density was associated with a scanty amount of cytoplasm in the pituitary cells. The pituitary cells were definitely larger in

EXPLANATION OF PLATE 1

FIG. 1. The ovary of a 'torpid' mouse. No corpora lutea are seen. H & E, $\times 87$.

FIG. 2. The ovary of a normal littermate of a 'torpid' mouse showing a corpus luteum (CL). H & E, $\times 87$.

FIG. 3. Interstitial cells taken from the central portion of the ovary shown in Fig. 1. H & E, $\times 400$.

FIG. 4. Interstitial cells taken from the area between two corpora lutea shown in Fig. 2. H & E, $\times 400$.

FIG. 5. A section of the pituitary of a female 'torpid' mouse. PAS, $\times 320$.

FIG. 6. A section of the pituitary of a female normal littermate of a 'torpid' mouse. PAS, $\times 320$.

PLATE 1

(Facing p. 94)

PLATE 2

(Facing p. 95)

normal mice than in the 'torpid' mice. The increase in the nuclear/cytoplasmic ratio reduced the distance between nuclei of pituitary cells and the cells became more closely packed.

Electron microscope study

Growth hormone cells were readily identified by their medium size, oval shape, and by the presence of many large round secretory granules. Their nuclei were round and were usually eccentrically placed within the cell.

Table 1. Numbers and percentages of the prolactin, growth hormone, and other cells in the pituitary of 'torpid' and littermate control mice at the age of 3 to 4 months

Phenotype of mice	Total no. of cells counted	Prolactin cells		Growth hormone cells		Others	
		No.	%	No.	%	No.	%
Control	783	241	30.7	252	32.2	290	37.1
	1106	520	46.8	348	31.6	238	21.6
	805	383	47.8	307	37.8	115	14.4
	1046	460	44.0	320	30.6	266	25.4
'Torpid'	996	85	7.8	407	38.3	504	53.9
	846	118	14.0	369	43.6	359	42.4
	1260	107	8.5	505	40.1	648	51.4
	1179	90	7.6	438	37.2	651	55.2

Prolactin cells were also easy to identify. They had pleomorphic granules which varied considerably in size. The endoplasmic reticulum of prolactin cells was always well developed in normal female mice. In the electron micrographs, the nuclei of prolactin cells often showed an indentation. After all of the cells had been classified and marked as prolactin, growth hormone, or other, the number of cells in the three categories was counted and tabulated. Table 1 contains the summary of the calculations derived from this study.

There were few prolactin cells in the pituitaries of 'torpid' female mice. In three of the four animals, they constituted less than 10% of the entire cell population in the gland. By comparison, prolactin cells in control animals accounted for more than 40% of the total cell population. No significant difference occurred in the growth hormone cells. A greater percentage of 'other' cell types was present in the pituitaries of 'torpid' mice compared to those of normal control mice. The pituitaries of 'torpid' mice had more cells which contained no granules (Pl. 2, Fig. 7) than the glands of normal controls (Pl. 2, Fig. 8).

The prolactin cells in the pituitary of 'torpid' mice usually contained fewer granules than those of control mice, although the size and shape of the granules

EXPLANATION OF PLATE 2

FIG. 7. A representative micrograph of the pituitary of a female 'torpid' mouse. Several growth hormone (GH) cells are seen. There are only two cells which are identified as possible prolactin (PR) cells. $\times 6000$.

FIG. 8. A representative micrograph of the pituitary of a normal female littermate of 'torpid' mouse. A number of prolactin (PR) and growth hormone (GH) cells are seen. $\times 6000$.

were unaffected. The lamellated endoplasmic reticulum was poorly developed and Golgi complexes were inconspicuous.

DISCUSSION

Approximately 100 mutant genes occurring in the mouse are known to produce behavioural abnormalities (tremor, paralysis, convulsions, seizures, instability of gait) in the affected animals. Because of these abnormalities, the mice are classified as neurological mutants (Sidman *et al.*, 1965). The mutant 'torpid' mouse is one of them. Neurological mutant mice are considered to be important research animals for investigation of the nervous system, but histological examination of the nervous system of many mutants has failed to reveal pathological changes which can be taken as the cause of behavioural abnormalities. Typical examples are 'jittery', 'agitans', 'trembler', 'spastic', 'lethargic', and 'torpid' mutants (DeOme, 1945; Braverman, 1953; Martinez & Sirlin, 1955; Chai, 1961; Dung & Swigart, 1970). In 'tumbler', 'lethargic', and 'torpid' mutant mice, pathological changes are consistently found in some of the endocrine glands, reproductive organs, and lymphoid tissues. It seems that the usefulness of neurological mutant mice as research animals may not be in the nervous system, but rather in relation to endocrine malfunction, infertility, and immunological disorders. The literature reveals that, in addition to behavioural abnormalities, many mutant mice have three aspects of their conditions in common: growth retardation, low fertility, and frequent mortality around weaning age or at the 4th week after birth. Examples of such mouse mutations are 'varitint-waddler' (Cloudman & Bunker, 1945), 'jittery' (DeOme, 1945), 'trembler' (Falconer, 1951), 'reeler' (Falconer, 1951), 'ducky' (Snell, 1955), 'ataxia' (Lyon, 1955), 'quivering' (Yoon & Les, 1957), 'waddler' (Yoon, 1959), 'tremulous' (Yoon, 1959), 'staggerer' (Sidman, Lane & Dickie, 1962), 'spastic' (Chai, 1961), 'tumbler', 'lethargic' (Dung & Swigart, 1970), and now 'torpid' mice.

Very little is known of the pathogenesis of the growth retardation, infertility, and high mortality of these different types of mutations. It was thought that difficulty in obtaining food and water due to the behavioural abnormalities probably resulted in the growth retardation and death of the affected animals (Falconer, 1951; Searle, 1952; Snell, 1955; Chai, 1961), but autopsies we have performed on 'torpid', 'lethargic', 'quaking', and 'tumbler' mice have shown that the stomachs and intestines contained food. A similar observation was made by DeOme (1945) in 'jittery' mutants. It is probable, therefore, that the growth retardation, infertility, and high mortality are due to other factors in addition to the difficulty in obtaining food.

The results of our experimental studies on a number of mouse mutations as well as those reported by other investigators seem to indicate that endocrine disturbances, particularly in the pituitary, may be involved in causing growth retardation and infertility, and that high mortality may be related to an immunological deficiency.

Two types of 'dwarf' mice (Snell, 1929; Schaible & Gowen, 1961) are known to have growth retardation and infertility caused by pituitary deficiencies

(Smith & MacDowell, 1930, 1931; Kemp, 1938; Osborn, 1938; Marshak, 1938; Francis, 1944, 1945; Ortman, 1956; Rennels & McNutt, 1958; Elftman & Wegelius, 1959; Carsner & Rennels, 1960; Cavallero *et al.*, 1963; Bartke, 1964, 1965a, 1965b, 1965c, 1966a, 1966b). There is evidence to indicate that the high mortality of 'dwarf' mice is related to a selective deficiency in the cell-mediated type of immunity which is thymus-dependent (Baroni, 1967; Duquesnoy *et al.*, 1970). Other neurological mutations of the mouse which are known to have pathological changes in the pituitary include 'jittery' (DeOme, 1945), 'tumbler' (Dung & Swigart, 1967), and 'lethargic'. The histology of the pituitary of these mutants is very similar to that of 'dwarf' mice. Their pituitary is congested and considerably more cellular than those of normal mice. Cell cytoplasm is scanty and nuclei are hyperchromatic. The pituitary from these three mutants as well as the other mouse mutations, except 'dwarf', has not been investigated thoroughly either by light or electron microscopy. In regard to a high mortality rate among affected mice, more types of mutations have been studied. All of those studies, including those for 'dwarf' mice, appear to indicate that a deficiency in immunity mechanisms involving a thymic involution and a severe lymphocytopenia may play an important role in causing early death in many mutant mice. DeOme (1945) reported that the thymus in 'jittery' mice is much smaller than that of the normal siblings and shows involutionary changes. He suggested that perhaps thymic involution was related to the cause of the malady of the 'jittery' mutant. Mufson & Starr (1958) reported that 'wabblers-lethal' mice have lymphocytopenia, and therefore the mutants may be more susceptible to certain environmental factors which might play a role in initiating their disease. Mice carrying a new hairless gene described by Flanagan (1966) and studied by Pantelouris (1972) were found to show thymic involution and a deficiency in cell-mediated immunity. We reported that thymic involution occurred in 'tumbler' and 'lethargic' (Dung & Swigart, 1970, 1971, 1972; Dung, 1973). It is possible that the pathological changes in the thymus are directly related to the high mortality of the mutant mice.

In the present study, 'torpid' mutant mice were found to have definite growth retardation between 20 and 95 days of age. Most of the mutants, male and female, were incapable of reproducing. Those female 'torpids' which were incapable of becoming pregnant were found to have either irregular oestrus cycles or none. Their pituitaries and ovaries were atrophic as shown by weight determination and histological examination. It is reasonable to suggest that 'torpid' mice have an endocrine deficiency, and their growth retardation and infertility may be caused by this rather than by behavioural difficulties in obtaining food and in copulation.

The nature and degree of specific pituitary hormone deficiencies which may be responsible for growth retardation and infertility in 'torpid' mice cannot be known from the present studies. Our electron microscopical studies on the pituitary suggest that a growth hormone deficiency may not be the main factor in causing the growth retardation and that further studies should be focused on the prolactin activity in 'torpid' mice. Prolactin is known to act synergistically with growth hormone in promotion of growth in animals, and it seems possible that the growth retardation in 'torpid' mice might be a result of

prolactin deficiency in the animal. Infertility associated with a deficiency in prolactin activity is known in 'dwarf' mice (Bartke, 1965b, c, 1966a, b). Such a deficiency may also account for the low reproductive efficiency in female and male 'torpid' mice. It is, of course, quite possible that other hormones such as FSH and LH may be deficient in the mutant mice.

'Torpid' mice have a high mortality rate between 20 and 40 days of age, a period when the thymus often showed involutionary changes. More studies will be undertaken to determine if 'torpid' mice have any immunological deficit which may account for the increased mortality in these mutants.

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