Immunohistochemical detection of changes in growth hormone cells in rat pituitaries in protein deficiency

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1. An immunohistochemical method was used to study the effect of a low-protein diet on growth hormone (GH) cells in the pituitaries of developing rats. The deficient diet (80 g protein/kg) was administered during gestation and lactation, or during the time after weaning until 90 d of age, or during both periods.

2. GH-cell changes were much more striking in males than in females.

3. In males, GH-producing cells were usually reduced in size and number in all treatments. The effect was most intense when protein deprivation occurred throughout gestation and sucking, and continued until 90 d of age, but it was also evident in animals given the low-protein diet only after weaning. Recuperation appeared to be almost complete when offspring of deprived dams were fed on a normal diet after weaning.

4. It is concluded that a low-protein diet reduces the amount of GH in the rat pituitary in a way similar to that with a protein-free diet.

Several studies indicate that the fasting plasma growth hormone (GH) levels are increased in undernourished infants (Pimstone, Wittmann, Hansen & Murray, 1966; Hadden, 1967; Milner, 1970; Becker, Pimstone, Hansen & Hendricks, 1971) and the extent of the increase appears to be directly correlated with the severity of protein depletion (Pimstone, Barbezat, Hansen & Murray, 1968). Beas and co-workers (Beas, Contreras, Maccioni & Arenas, 1971) report that in kwashiorkor the process of malnutrition is acute, with high values of plasma GH, whereas in extreme, chronic-marasmus, there is a progressive adaptation to undernutrition with low GH secretion. However, in rats, starvation or feeding with a diet completely free of protein results only in a decrease of plasma GH (Srebnik, Nelson & Simpson, 1959; Srebnik, 1965; Trenkle, 1970). A lack of any indication that a rise in GH levels, similar to that found in humans, occurs in rats suggests that the GH response to protein deprivation should be investigated further in lower animals. Since most of the work with rats has been done with diets totally free of protein and since even undernourished children normally have some small amino acid intake, a diet containing a minimal amount (80 g/kg) of protein was used in the study to be described.

Within the last 10 years there has been much concern about the possible irreversibility of effects of nutrition during gestation and studies have been done using both children with a history of foetal undernutrition (Hillman & Colle, 1969; Winnick, 1970), and experimental animals (Chow & Lee, 1964; Stephan, Chow, Frohman & Chow, 1971). My work has been designed to investigate the possibility of reversing the effects on GH-producing cells after early deprivation, and to study the differences in the intensity of the effects when the deprivation occurs at different periods of development.

In rats, the reduction in plasma-GH or pituitary-GH content resulting from severe food restriction was accompanied by a parallel decrease in size and number of pituitary acidophils (Scott, 1956; Srebnik & Nelson, 1962). These studies were done using traditional histological techniques in which the differentiation of GH-producing cells from other cell types such as prolactin cells is difficult, especially when a study of cellular changes is involved, and highly specific methods may be expected to yield more exact information. In the present investigation, therefore, an immunochemical method (Baker, 1969) for the intracellular localization of GH was used to observe structural changes in GH-producing cells of rats resulting from a reduction in dietary protein levels in early life.

METHODS AND MATERIALS

Two diets were used. The control diet (180 g protein/kg) was a modification of the balanced diet used for rats at the University of São Paulo, Brazil (de Moura-Campos, 1949), providing (g/kg): protein 180, carbohydrate 660, fat 100 and fibre 20, supplied by casein, maize starch, cottonseed oil and cellulose, together with a complete supplement of fat- and water-soluble vitamins. The experimental, low-protein diet was isoenergetic with the control diet and the quantity of casein reduced to provide a total of 77 g protein/kg diet. Both diets were given *ad lib.*

Female albino rats, of a strain maintained for many years at the Federal University of Pernambuco, were mated and placed on one of the two diets as soon as vaginal smears revealed the presence of spermatozoa. On the 3rd day after birth the litters were reduced to six, to provide controlled feeding conditions. Any litter in which two or more animals died was discarded. Following weaning the offspring were placed on either the control or the low-protein diet and they were killed at 90 d of age. There were four experimental groups (1–4) in which the diets during pregnancy and lactation and from weaning to 90 d of age were respectively: 1, control–control; 2, low-protein–low-protein; 3, control–low-protein; 4, low-protein–control.

At 90 d of age the rats were decapitated and the hypophyses excised, fixed in Bouin's solution, embedded in paraffin and sectioned at 3 μm. Immunochemical staining was carried out with the peroxidase-labelled antibody technique of Nakane & Pierce (1967); 3,3′-diaminobenzidine was used as a substrate for the peroxidase reaction. The antiserum was prepared at the Department of Anatomy of the University of Michigan Medical School, using human growth hormone (HGH) provided by the (US) National Institute of Arthritis and Metabolic Diseases. The HGH was administered to rabbits, which were bled, using the procedure described by Midgley, Niswender, Gay & Reichert (1971).

When histological sections showed a break in the membrane surrounding the pituitary which might have influenced fixation and staining of the hormone contained in the cells, the gland was discarded. Intact, well-stained sections were obtained from the following number of animals in the different groups: group 1, six males and six females; group 2, five males and five females; group 3, six males and five females; group 4, five males and five females.

Sections were examined with a light microscope using magnifications of 100 and 440. Relative number of cells and intensity of stain were noted by two independent observers. All slides in a group were first examined for consistency within the group. The slides were then re-examined in pairs; one control and one unidentified experimental.

RESULTS

Representative sections from each group of males are shown in the microphotographs of Plate I. When compared to the 90-d-old male controls (Plate 1 *a*), the animals of group 2 (subjected to protein deficiency throughout the experiment) showed a dramatic reduction in size and number of GH-producing cells (Plate 1 *b*). The female rats of the control group had, as expected, fewer GH-producing cells than the males of the same group, and when compared to the females of the protein-deprived group 2, no consistent changes could be observed.

The male animals of group 3 (fed on a protein-depleted diet only after weaning) showed GH-producing cells reduced in size and number (Plate 1 *c*) but with granules of hormone tightly packed, resulting in a sharply outlined dark mass which indicates a cell which is neither in a secretory state, nor in the exhausted state of the longer-term deprived group 2. In the females, no significant change was observed.

Both male and female animals of group 4 (offspring of dams given the low-protein diet during pregnancy and lactation, which after weaning were placed on a normal diet) showed GH-producing cells similar in size and number to those of the control group in almost all instances (Plate 1 *d*).

DISCUSSION

The immunohistochemical method employed proved to be helpful in revealing changes in the GH-producing cells of rats subjected to a restriction of dietary protein during the time of development. Highly specific labelling of hormone within certain cells leaves no doubt that the changes observed were in the cells which I set out to study. The striking differences among the groups of male animals indicate that a moderate protein deprivation in a chronic situation also brings about a reduction in number and size of GH-producing cells in the pituitary of the male rat, just as do starvation or absence of protein in the diet, as reported by other workers. Therefore some element other than the severity of the protein deprivation must be the cause of the differences of the findings in rats compared to those in malnourished humans.

Fasting and malnourished adults (Adibi & Drash, 1970; Alvarez, Dimas, Castro, Rossman, Vanderlaan & Vanderlaan, 1972) were found to have increased levels of plasma GH, but the results of Godard & Zahnd (1971) support the hypothesis that under certain conditions, GH deficiency can exist in severe infantile malnutrition. Post-mortem studies of severely malnourished children revealed that acidophils are usually, but not always, decreased (Tejada & Russfield, 1957). The findings of Lunn, Whitehead, Hay & Baker (1973) in children with progressively severe hypoalbuminaemia culminating in kwashiorkor, indicate that rapid increases in plasma GH concentration may be of comparatively short duration.

It has been shown that after 1–6 d of food deprivation in hamsters, there is a very great increase in the number of GH granules in the process of secretion (Dubois & Girod, 1968). However, in the adult rat, starvation for 24–96 h brought about a progressive decrease in the content of GH in the anterior pituitary gland (Friedman & Reichlin, 1965). Perhaps the hamster would be a better choice of experimental animal for investigations of this type, if this species exhibits a set of metabolic responses to dietary deprivation which are more nearly parallel to those observed in humans.

The fact that GH cells of male animals showed such definite changes, whereas those in the female animals were inconsistent, may be due to the influence of other hormonal factors or to the smaller amounts of somatotrophin often found in female rat pituitaries. Other authors (Srebnik *et al.* 1959; Srebnik, 1965; Trenkle, 1970) also found slighter differences in females than in males after protein deprivation or starvation.

The reduction in GH content of the pituitaries from progeny of dams fed on a low-protein diet is in accord with the results of Stephan *et al.* (1971). However, although nutritional deprivation during gestation and lactation results in more drastic cellular changes than a later deprivation, these changes were reversible, at least with respect to histological observations. When studies of protein-energy malnutrition were carried out in piglets a short time after birth (Platt & Stewart, 1967) there were indications of reversible changes, and the cellular changes were similar to those observed in the present work.

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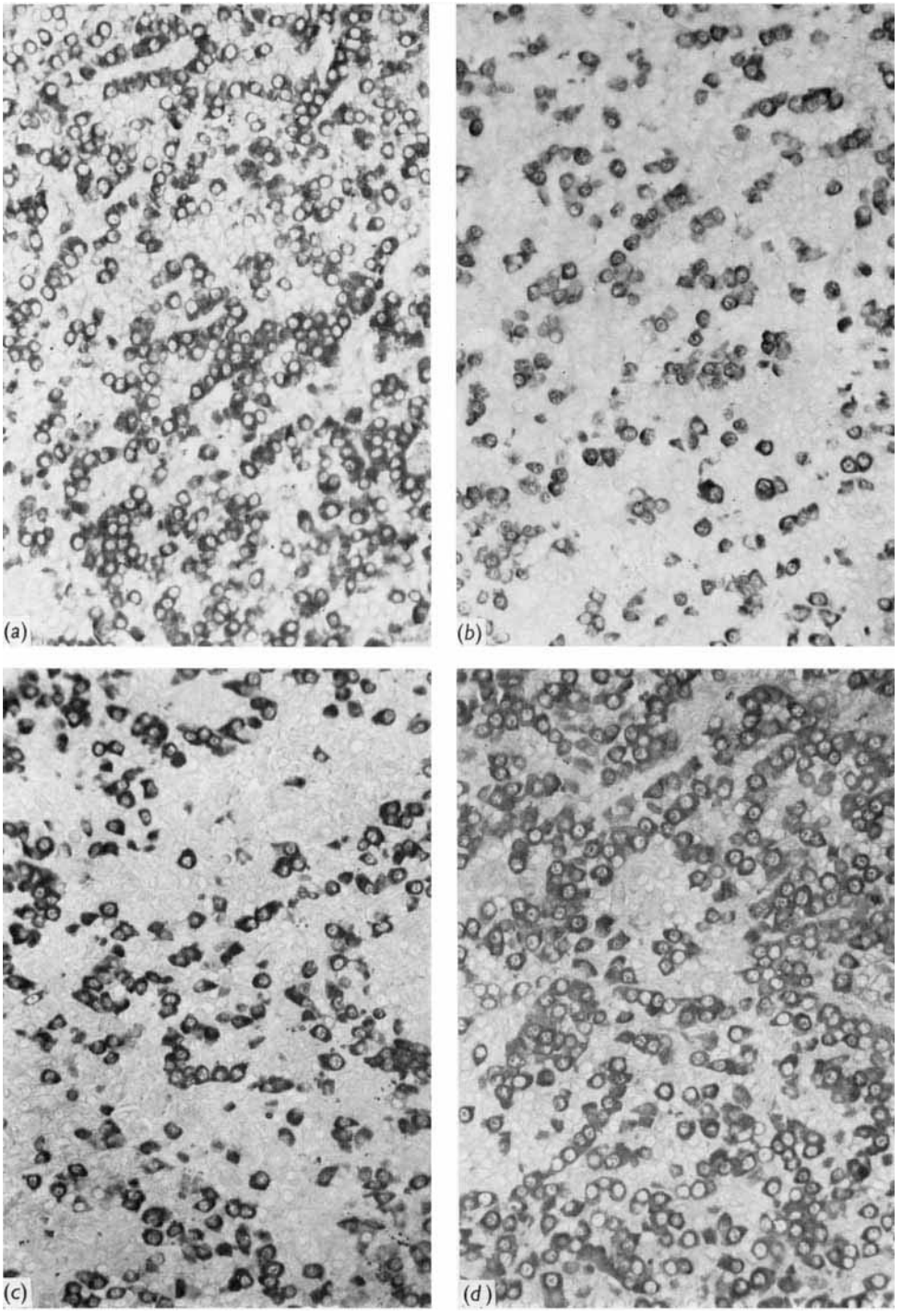
REFERENCES

Adibi, S. A. & Drash, A. L. (1970). *J. Lab. clin. Med.* **76**, 722.
Alvarez, L. C., Dimas, C. O., Castro, A., Rossman, L. G., Vanderlaan, E. F. & Vanderlaan, W. P. (1972). *J. clin. Endocr. Metab.* **34**, 400.
Baker, B. L. (1969). *J. Histochem. Cytochem.* **18**, 1.
Beas, F., Contreras, I., Maccioni, A. & Arenas, S. (1971). *Br. J. Nutr.* **26**, 169.
Becker, J., Pimstone, B. L., Hansen, J. D. & Hendricks, S. (1971). *J. Lab. clin. Med.* **78**, 865.
Chow, B. F. & Lee, C. J. (1964). *J. Nutr.* **82**, 10.
de Moura-Campos, F. A. (1949). *Problemas Brasileiros de Alimentacão* p. 232. Rio de Janeiro: Serviço de Alimentação da Previdência Social.
Dubois, P. & Girod, C. (1968). *C. r. Séanc. Soc. Biol.* **162**, 2116.
Friedman, R. C. & Reichlin, S. (1965). *Endocrinology* **76**, 787.
Godard, C. & Zahnd, G. R. (1971). *Helv. paediat. Acta* **26**, 266.
Hadden, D. R. (1967). *Lancet* ii, 589.
Hillman, D. A. & Colle, E. (1969). *Am. J. Dis. Child.* **117**, 636.
Lunn, P. G., Whitehead, R. G., Hay, R. W. & Baker, B. A. (1973). *Br. J. Nutr.* **29**, 399.
Midgley, A. R., Niswender, G. D., Gay, V. L. & Reichert, L. E. (1971). *Recent Prog. Horm. Res.* **27** 235.



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Milner, R. D. (1970). *Archs Dis. Childh.* **45**, 276.
Nakane, P. K. & Pierce, G. B. Jr (1967). *J. Cell Biol.* **33**, 307.
Pimstone, B. L., Barbezat, G., Hansen, J. D. L. & Murray, P. (1968). *Am. J. clin. Nutr.* **21**, 482.
Pimstone, B. L., Wittmann, W., Hansen, J. D. L. & Murray, P. (1966). *Lancet* ii, 779.
Platt, B. S. & Stewart, R. J. C. (1967). *J. Endocr.* **38**, 121.
Scott, E. B. (1956). *Proc. Soc. exp. Biol. Med.* **92**, 134.
Srebnik, H. H. (1965). *Anat. Rec.* **151**, 476.
Srebnik, H. H. & Nelson, M. M. (1962). *Endocrinology* **70**, 723.
Srebnik, H. H., Nelson, M. M. & Simpson, M. E. (1959). *Proc. Soc. exp. Biol. Med.* **101**, 97.
Stephan, J. K., Chow, B., Frohman, L. A. & Chow, B. F. (1971). *J. Nutr.* **101**, 1453.
Tejada, C. & Russfield, A. B. (1957). *Archs Dis. Childh.* **32**, 343.
Trenkle, A. (1970). *Proc. Soc. exp. Biol. Med.* **135**, 77.
Winnick, M. (1970). *Am. J. Dis. Child.* **120**, 416.

EXPLANATION OF PLATE

The Plate shows sections of the hypophyses of 90-d-old male rats, in which the growth hormone (GH) granules are darkly stained by a peroxidase-labelled antibody technique (× 440).

(*a*) Group 1, fed on the control diet throughout life; GH-cells are abundant. (*b*) group 2, in which a low-protein diet was given to the dams during pregnancy and lactation and to the progeny during the post-weaning period; there is a definite reduction in size and number of GH-cells; (*c*) group 3, in which the dam was fed on the control diet but, beginning at 21 d of age, the offspring were given a low-protein diet; there is a reduction in size and number of the GH-cells but the dark masses of densely packed granules do not indicate an exhausted state such as is found in *b*; (*d*) group 4, in which the dam was given a low-protein diet during pregnancy and lactation and the offspring received the control diet during the post-weaning period. Similarities between *a* and *d* indicate recuperation in the amount of GH produced in the latter animals.

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