

## Growth hormone in infant malnutrition: the arginine test in marasmus and kwashiorkor

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1. Experiments in children and in animals seem to demonstrate that in marasmic malnutrition there is a reduction in the secretion of growth hormone. To study this problem the fasting, resting plasma concentrations of growth hormone were determined, before, and 45 and 60 min after, stimulation with an intravenous dose of arginine, in six infants with marasmus, in six infants with kwashiorkor and in five normal infants.

2. The values of plasma growth hormone ( $4.1 \pm 0.97$  ng/ml) in marasmic infants were significantly ( $P < 0.001$ ) lower than those of the controls ( $7.8 \pm 2.6$  ng/ml), and responded little to stimulation with arginine ( $5.1$  and  $5.8$  ng/ml at 45 and 60 min respectively), in contrast to those in the control group, which rose to  $16.4$  and  $14.6$  ng/ml. In children with kwashiorkor the values were very variable, but generally high, and showed little response ( $25.6 \pm 13.3$  before and  $25.0 \pm 17.6$  and  $14.2 \pm 5.3$  ng/ml at 45 and 60 min after stimulation respectively).

3. These results demonstrate that the responses of the hypophysis to deprivation of calories and protein (marasmus) and to protein deprivation (kwashiorkor) are different: in marasmus there is a progressive adaptation, with low secretion and poor reaction to stimulation, whereas in kwashiorkor the process is acute with high basal values of plasma growth hormone.

The relationship between growth hormone and severe malnutrition has been the subject of controversial results published in the literature. The existence of high concentrations of growth hormone in plasma has been reported in children suffering from kwashiorkor (Pimstone, Wittmann, Hansen & Murray, 1966). Similar findings have been reported in adults with anorexia nervosa (Landen, Greenwood, Stamp & Wynn, 1966) and chronic starvation (Marks, Howarth & Greenwood, 1965; Conte, Kaplan, Abrams & Grumbach, 1966). In contrast, on the basis of indirect data a growth hormone deficiency has been suggested in malnutrition. A decreased number of anterohypophyseal beta cells has been described on necropsy of severely malnourished children (Tejada & Russfield, 1957). Furthermore, in infants with malnutrition, a weight gain accompanied by nitrogen, phosphorus and potassium retention during the administration of exogenous human somatotrophic hormone has been demonstrated (Monckeberg, Donoso, Oxman, Pak & Meneghello, 1963). It has also been shown in our laboratory that human growth hormone produces 'catch-up growth' in rats with experimental marasmus (Muzzo & Beas, 1968).

It is a well-established fact that a single determination of growth hormone while the subject is fasting is frequently of limited value, because the level in subjects with normal pituitary function varies greatly (Landen & Greenwood, 1968). It has been recently shown that increased secretion of growth hormone is caused by a wide variety of stimuli, such as exercise (Hunter, Fonseka & Passmore, 1965), hypoglycaemia (Roth, Glick, Yalow & Berson, 1963*a, b*), amino acids (Knopf, Conn,

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Fajans, Floyd, Guntzsch & Rull, 1965), in particular arginine (Landen & Greenwood, 1968). The response of the plasma concentration to an intravenous dose of arginine ('arginine provocative test') is believed by some to be a satisfactory test for the evaluation of human growth hormone reserve (Merimee, Riggs, Rimoin, Rabinowitz, Burgess & McKusic, 1967).

The purpose of the work now described was to investigate further the function of the adenohypophysis in relation to growth hormone in infants with malnutrition. To study this problem, plasma growth hormone concentrations were measured in infants with calorie and protein malnutrition (marasmus) and protein malnutrition (kwashiorkor), before and after the administration of arginine.

#### EXPERIMENTAL

Six infants suffering from severe marasmus, six infants with typical kwashiorkor and five normal control infants were studied (Table 1). Marasmic patients (aged 6–13 months) had a weight deficit of at least 50% for their age and sex, as estimated by Chilean standards (Donoso, Undurraga, Weshler, Barja & Saez, 1962), and were not premature, and had birth weights within the normal range. The aetiology of marasmus was a deficiency in the calorie and protein intake, determined by social and economic factors. Thus all the patients had been breast-fed for a very short period of time and for this reason had been severely undernourished from a very early age. None of the patients showed evidence of oedema, hypoproteinaemia or fatty infiltration of the liver, or any of the changes in the skin and hair typical of kwashiorkor. No organic causes, such as parasitism or metabolic disease, were found in any of the infants studied. Furthermore, none of them gained weight during the period of study (at least 10 d before and after the test) notwithstanding an adequate diet for their weights (120 cal/kg per d of whole milk).

Patients with kwashiorkor (aged 8–33 months) presented a typical acute disease with oedema, low plasma serum albumin, evident enlargement of the liver and typical changes in the skin and hair produced by vitamin deficiency. In these patients, in contrast to all the others, the arginine test was performed on the 1st or 2nd day of hospitalization, while these clinical conditions prevailed.

The ages of the normal control infants ranged from 6 to 15 months and their weights and heights were in the normal percentiles, judged by Chilean standards (Donoso *et al.* 1962).

All subjects were admitted to the Metabolic Unit of the Laboratorio de Investigaciones Pediátricas, Hospital Manuel Arriarán, Santiago, Chile. The tests were made after an overnight fast of 10 h. During each study the children remained in bed; furthermore, except for those with kwashiorkor, the patients were in bed for at least 10 d before and after the test in the same unit under the same environmental conditions (temperature, food, personnel). An indwelling scalp vein needle was inserted into a forearm vein and was used for the injection of the test materials and collections of blood sample. Plasma was separated and stored at  $-20^{\circ}$  until samples were assayed.

At the time of the injection 1 ml/kg body-weight (0.5 g/kg body-weight) of stock

solution of arginine hydrochloride was mixed with saline (2 ml/kg body-weight) and administered over a 30 min period.

Plasma growth hormone was measured at Bio-Science Laboratories, Los Angeles, California by radioimmunological assay using growth hormone I-125 and a double antibody precipitation method (Hunter & Greenwood, 1964; Morgan, 1966). The growth hormone used as a standard was obtained from the National Pituitary Agency (Lot. No. NIH-GH-HS 1216 C). The potency of the growth hormone was 1.45 i.u./mg and it had a prolactin activity of 6.5 i.u./mg. The sensitivity of the procedure was down to 2 ng/ml.

## RESULTS

*Plasma growth hormone values in the fasting, resting state*

Table 1 shows the values found in normal infants and patients while fasting. The mean concentration in fasting normal infants was  $7.8 \pm 2.6$  ng/ml, significantly higher ( $P < 0.001$ ) than the mean value for the fasting marasmic infants, which was  $4.1 \pm 0.97$  ng/ml. The mean value for fasting infants with kwashiorkor was  $25.6 \pm 13.3$  ng/ml, significantly higher ( $P < 0.001$ ) than the mean value for the normal infants (Table 1).

Table 1. *Clinical information and basal values for plasma concentration of growth hormone during fasting and after arginine infusion in undernourished infants suffering from marasmus or kwashiorkor and in normal controls*

Patient	Age (months)	Sex	Birth wt (kg)	Wt		Ht		Plasma growth hormone		
				kg	As % of ideal	cm	As % of ideal	Fasting	After 45 min	After 60 min
Marasmus	6	♀	3.2	3.9	50	54	83	5	6	9
Marasmus	7	♀	2.5	3.4	51	54	80	3	5	5
Marasmus	6	♀	3.4	4.5	46	55	84	4	3	7
Marasmus	7	♂	3.2	4.3	49	57	83	5	8	6
Marasmus	8	♂	3.2	4.8	41	60	85	5	5	5
Marasmus	13	♂	3.3	4.2	42	63	81	3	5	3
Mean with SE								$4.1 \pm 0.97$	$5.1 \pm 1.72$	$5.8 \pm 2.03$
Kwashiorkor	17	♀	2.5	6.7	61	72	89	11	58	—
Kwashiorkor	16	♂	3.6	7.7	68	70	88	20	16	11
Kwashiorkor	8	♂	3.8	5.1	61	64	95	42	15	14
Kwashiorkor	14	♀	3.2	7.8	76	68	88	9	12	9
Kwashiorkor	16	♂	3.0	7.3	68	75	95	40	17	23
Kwashiorkor	33	♂	3.5	10.5	75	88	95	32	32	14
Mean with SE								$25.6 \pm 13.3$	$25.0 \pm 17.6$	$14.2 \pm 5.38$
Normal	9	♀	2.9	8.5	100	70	98	12	20	18
Normal	6	♂	3.1	7.8	100	65	100	8	16	16
Normal	15	♂	3.4	10.3	100	78	101	8	15	12
Normal	8	♂	3.2	8.2	100	69	99	6	20	15
Normal	12	♀	3.4	9.0	100	72	97	5	11	12
Mean with SE								$7.8 \pm 2.6$	$16.4 \pm 3.7$	$14.6 \pm 2.6$

Difference between fasting levels in controls and marasmus ( $P < 0.001$ ).

Difference between fasting levels in controls and kwashiorkor ( $P < 0.001$ ).

Difference between fasting levels in marasmus and kwashiorkor ( $P < 0.001$ ).

*Plasma growth hormone values after arginine stimulation*

A significant growth hormone response ( $P < 0.001$ ) was observed in normal infants after stimulation with arginine. The basal value of 7.8 ng/ml increased to  $16.4 \pm 3.7$  ng/ml after 60 min (Table 1). No significant response was observed in the marasmic patients after the administration of arginine. The values were 4.1 ng/ml during fasting,  $5.1 \pm 1.72$  at 45 min and  $5.8 \pm 2.03$  at 60 min after stimulation with arginine (Table 1). The growth hormone response to stimulation with arginine was variable in patients with kwashiorkor (Table 1): the mean values for plasma growth hormone 45 and 60 min after the test were  $25 \pm 17.6$  and  $14.2 \pm 5.38$  ng/ml respectively, not significantly different from the values found for the same group in fasting condition ( $25.6 \pm 13.3$  ng/ml) (Table 1).

## DISCUSSION

Different authors have studied the somatotrophin plasma levels in patients with severe malnutrition and have obtained conflicting results. This may be because the patients present different clinical conditions with a different aetiology. We deliberately selected two types of patients with severe malnutrition, marasmus and kwashiorkor. In our patients marasmus malnutrition had begun during the first months of life, because of decreased breast feeding, so at the time of study they had made little or no increase in height or weight (Table 1). These patients showed no hypoproteinaemia, no clinical signs of oedema, no signs of fatty liver and few or no changes in the skin and mucus membranes. This situation is different from that found in other developing countries in which undernutrition more frequently begins after the first or second semester of life. On the other hand, the patients selected suffering from kwashiorkor had a relatively short history (established by questioning the mothers) of protein malnutrition with an adequate, or more than adequate calorie intake. They all showed hypoproteinaemia, oedema, evident enlargement of the liver and severe changes in the skin and mucus membranes. It must be pointed out that these two extremes are exceptional and that most patients with severe malnutrition have mixed symptoms and aetiology. The reason why we selected patients of these types was precisely in order to differentiate clearly between these two syndromes.

It has been established that the radioimmunological technique is a sensitive and accurate method for the determination of growth hormone in serum plasma (Glick, Roth, Yalow & Berson, 1965; Roth *et al.* 1963 *a, b*). With this technique it has been possible to appraise the ability of the anterior pituitary gland to secrete growth hormone, in response to certain stimuli such as hypoglycaemia, exercise, fever, amino acids and others (Hunter *et al.* 1965; Roth *et al.* 1963 *a, b*; Landen & Greenwood, 1968). The arginine provocative test has been described as suitable for this purpose (Knopf *et al.* 1965; Merimee *et al.* 1967; Parker, Hammond & Daughaday, 1967); it was chosen for this study because it is harmless. The use of insulin to induce hypoglycaemia and subsequent secretion of growth hormone was considered dangerous in patients in whom disturbances of carbohydrate metabolism have been described (Biag & Edozien, 1965; Oxman, Maccioni, Zúñiga, Spada & Monckeberg, 1968). A group of

normal infants was studied simultaneously under the same experimental conditions, because plasma growth hormone has been rarely studied in normal children under 2 years of age.

The fasting concentrations for plasma growth hormone in our normal infants were similar to those reported by others. Kaplan, Abrams, Bell, Conte & Grumbach (1968) consider that the response to a test after hypoglycaemia has been induced with insulin is normal when an increase of 7 ng/ml or more is observed; a border-line rise of 3–5 ng/ml may be the first evidence of growth hormone deficiency.

Plasma growth hormone concentrations of the marasmic patients were significantly low when fasting, with no response after the arginine test; the lack of response to one stimulus (Raiti, Davis & Blizzard, 1967), in this instance arginine, does not necessarily imply growth hormone deficiency, but it is significant that all our marasmic patients behaved in a similar way (Table 1). Furthermore, C. Godard & G. Zahnd (1970, personal communication) have reported a low plasma growth hormone concentration in fasting infants with severe marasmic malnutrition ( $3.6 \pm 1.34$  ng/ml) and no significant increase during acute hypoglycaemia produced by insulin ( $5.2 \pm 3.22$  ng/ml).

These results seem to show that in marasmic infants chronic low intakes of calories and protein are accompanied by low concentration of plasma growth hormone and absence of response to different stimuli. This finding is significant in relation to the fact that for the same type of patient it has been shown that the administration of human growth hormone to marasmic infants causes positive nitrogen, phosphorus and potassium balances and gain in weight (Monckeberg *et al.* 1963.) Furthermore, Muzzo & Beas (1967) have produced experimental marasmus in rats by varying the number of animals suckled by the same mother, and have shown that, whereas under normal conditions growth rate and final size of these animals are permanently reduced, 'catch-up growth' can be obtained by administration of human growth hormone (Muzzo & Beas, 1968).

Studies of severe marasmus have shown that a low calorie intake also provokes a thyroid mechanism of adaptation, characterized by a decrease in iodine uptake and of thyroid hormone production and also a diminished oxygen consumption by the peripheral tissues (Beas, Monckeberg, Horwitz & Figueroa, 1966). The last finding has been corroborated by Muzzo, Egaña & Beas (1970) in rats with experimental marasmus in which the 'in vitro' oxygen consumption by the brain mitochondria was significantly lower than in the normal animals. The administration of thyroid-stimulating hormone to marasmic infants corrects the low values of thyroid iodine uptake and protein butanol-extractable iodine (Beas *et al.* 1966). We have also studied adrenal function in marasmic infants and have shown a low urinary sodium conserving capacity, which suggests a deficit in the secretion of aldosterone, low secretion of urinary 11-oxysteroids, 17-ketogenic and 17-ketosteroids in basal conditions and after stimulation with ACTH (Beas, 1970). All these results seem to indicate that a progressive and severe low calorie intake produces an adaptive pituitary hypofunction (Beas, 1970). In our laboratory, Brunser, Reid, Monckeberg, Maccioni & Contreras (1968) have shown a low duodenal mitotic index in marasmic infants, and Salinas & Colombo (1968) showed a decrease in DNA synthesis in the brain of rats with experimental marasmus.

The same low mitotic index has been described in hypophysectomized and thyroidectomized rats (Leblond & Carriere, 1955). It is therefore an attractive hypothesis to postulate that the endocrine mechanism of adaptation, in which pituitary hypofunction is very important, brings into operation an equilibrium between the calorie intake, the rate of growth and the synthesis of protein. It will be necessary to perform several investigations in order to test this hypothesis.

The high concentrations of plasma growth hormone presented by our kwashiorkor patients are in agreement with the values published by Pimstone *et al.* (1966). It seems as if protein deprivation can cause secretion of pituitary growth hormone similar to that produced by hypoglycaemia, exercise, or fever.

Consistently high plasma cortisol levels have been found in children with kwashiorkor (Alleyne & Young, 1966). Moreover, we have found no alteration in oxygen consumption in infants with kwashiorkor, and cell division measured in duodenal biopsy material is maintained at a rate not significantly different from that in material from normal controls (Brunser *et al.* 1968).

We were not surprised to find that our patients with kwashiorkor presented a variable response to the arginine test, because Kaplan *et al.* (1968) have shown that the response of the plasma concentration of growth hormone to a given stimulus depends on the initial concentration. In the presence of a moderate elevation of fasting concentration, normal children tend to have either a less pronounced rise or a fall in plasma concentrations after hypoglycaemia induced by insulin. These observations suggest that temporary depletion of the growth hormone content of the pituitary following an initial surge of secretion may modify the response to a second stimulus, e.g. arginine. An alternative explanation is that the high fasting growth hormone concentration in itself can suppress further secretion by the pituitary through a feed-back effect on the hypothalamic growth hormone releasing centre.

To summarize, the above results seem to indicate that the response of the pituitary to the secretion of growth hormone depends mainly on the type of malnutrition. Marasmus is characterized by a deficient secretion of growth hormone in response to chronic deprivation of calories and protein, in addition to a global pituitary mechanism of adaptation involving the thyroid and adrenal functions. Conversely, kwashiorkor shows an acute and high plasma concentration of growth hormone as a result of a deficit, mainly of proteins, with high production of corticoids, but without involvement of the thyroid function and cell division. These results show a very good correlation with the clinical characteristics that marasmus and kwashiorkor (not mixed forms) present in our environment. Marasmus is a chronic illness, produced by very early weaning, with a torpid evolution, difficult recovery and, when severe, high mortality rate; kwashiorkor in typical cases is an acute disease which usually responds well after a few days of treatment, and shows a significantly lower mortality rate.

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