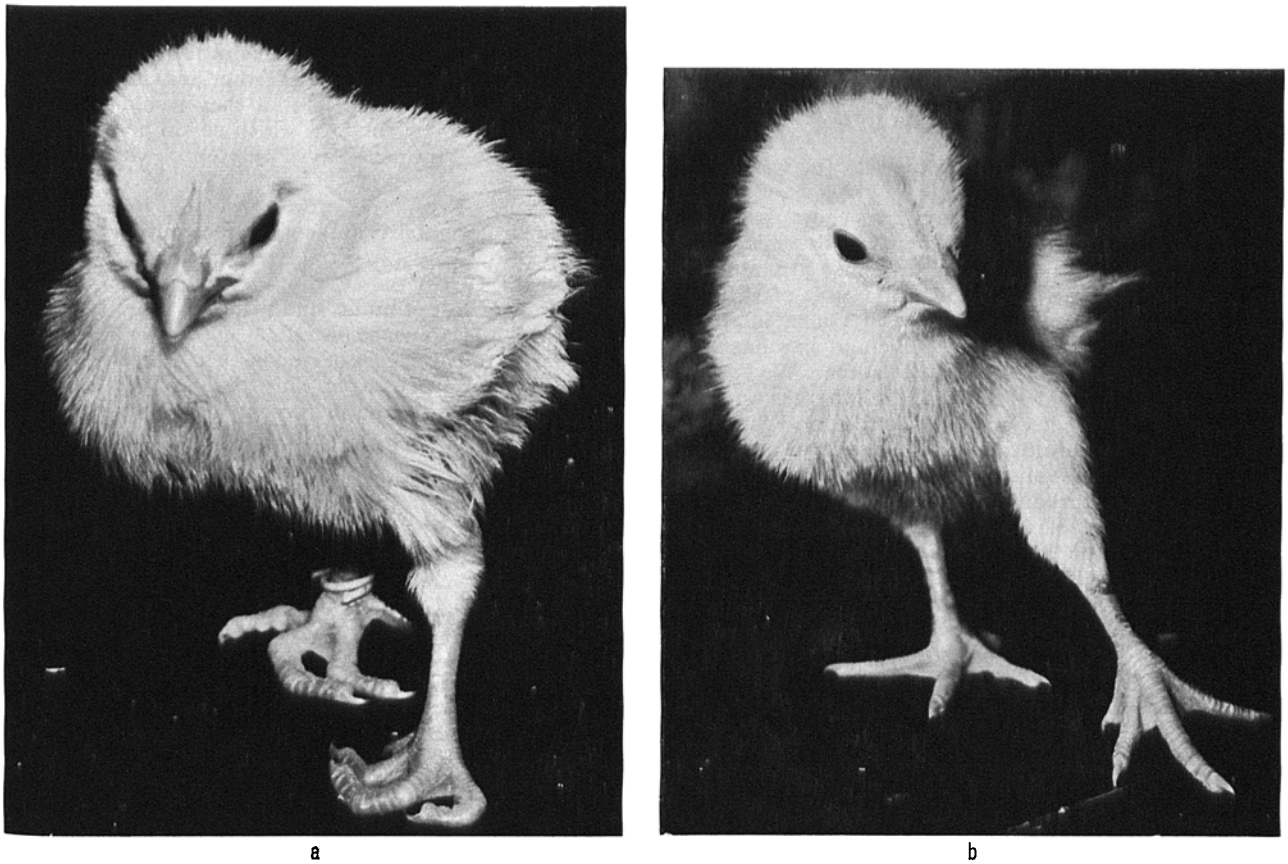
Teratogenic Effects of the Amino Acid Leucine in the Chicken

In conjunction with an earlier study of the depressive effects of thalidomide on the electrical activity of the brain in the guinea-pig foetus[1], it was suggested that this substance, or some metabolite of it, might exercise a neurotoxic effect on the immature brain. On examining a number of other agents with effects which might be teratogenic, only glutethimide (α-phenyl-α-ethyl-glutarimide), which like thalidomide is a derivative of glutaminic acid, displayed similar neurotoxic effects judging from the influence exerted on the electrical activity of the brain[2]. As a result, and in view of the fact that a change in the amino acid feeding pool may affect the code of the morphological development in certain green plants[3], a study has been made of the effects of amino acid on the development of animals. Because of its relative stability in the metabolism and its possible significance in the maturation of the brain leucine was chosen as the first agent[4,5]. The chicken was the test animal, because of the ease of application of the agent, and the possibility of comparing this material with the well-known thalidomide effects[6].

The material comprised 263 chicken (White leghorn) embryos. 167 of these were subjected to a single injection of 0.2 ml of a 1% solution of leucine into the amnionic sac of a nine-day-old embryo. The rest of the material served as control; thalidomide in a dose of 0.2 ml of 2% solution was applied to 44 of them in the same manner and at the same age. The remaining 52 were left in a normal state during the development. Moreover, sodium chloride, in a physiological concentration and equal in volume to leucine, was injected into 20 eggs of the normal material, in the same manner and at the same age. All the eggs were stored in ordinary breeding boxes (moisture 63–70%, temperature 38.5–39.5 °C). A minor proportion of the material in each group was tested before hatching by opening eggs, and examining the condition of the foetus.

In the leucine group (Table) the % of morphologically abnormal cases of fully developed chickens is 86, the corresponding numbers in the thalidomide group being 33, and that in the normal control material 13. As regards the eggs opened, the respective numbers were 41, 22 and 2. The most pronounced morphological changes in the abnormal cases of the leucine group were anomalies of the legs (91.7%). Visual examination showed them to be of normal appearance in the proximal part, but with an inward flexion of the toes, including the back toe (Figure, a). In some of the cases, one of the legs was either wholly

[1] R. M. Bergström, Lea Bergström and P. Putkonen, Medna exp. *11*, 119 (1963).
[2] R. M. Bergström, K. Sainio and P. Pitkänen, Acta physiol. scand. *68*, 25. Suppl. 277 (1966).
[3] H. Waris, Physiologia Pl. Part I: *12*, 753 (1959); Part II: *15*, 736 (1962).
[4] S. Piha, personal communication.
[5] S. Oja, Suomal. Tiedeakat. Toim. Ser. A.V. *125*, 1 (1966).
[6] C. A. Bock and A. Peters, Nature *199*, 1204 (1963).



a b

Effect of the amino acid leucine (0.2 ml of 1% solution injected into the amnion sac of the 9 days old egg) on the development of the chicken showing (a) an inward flexion of the toes, (b) extension of the left leg.

extended (Figure, b) or flexed at the hipjoint so that the chicken could not stand on it. In a small portion of the leucine anomalies, defects were apparent in the ossification of the skull: in 1 of them a part of the brain was extruded from the defect. In some cases, torsion of the neck appeared to occur. X-ray pictures of the toes mostly evidenced remarkable positional anomalies of the joints, the same being the case of joints of the neck vertebrae. The electroencephalographic changes mostly consisted of depression of the brain waves. The thalidomide material mostly evidenced the same changes as reported by BOCK and PETERS 1963[6]. The anomalies of the normal group consisted of functional inability to stand and to move.

The observation of disturbances in development of the electrical maturation of the brain, present at the same time as severe morphological and behavioural anomalies, mostly in the extremities, is in accord with the assumption made that teratogenic effects might reflect a disturbance in the interaction of the central and peripheral organ development. A normal interaction might depend on a normal biochemical maturation of the brain stem, possibly on a normal code function for the amino acid spectrum, at a sensitive developmental stage, with the brain stem showing intense electrical maturation (at a stage of 7–8 weeks prenatally in the human[7,8]).

Percentage and number of the morphologically normal and abnormal cases in the leucine, thalidomide and control material of chickens

	No. of cases	Hatched				Opened			
		Normal		Abnormal		Normal		Abnormal	
		No.	%	No.	%	No.	%	No.	%
Leucine	167	2	14	14	86	89	59	62	41
Thalidomide	44	5	67	2	33	29	78	8	22
Control	52	14	87	2	13	35	98	1	2

Zusammenfassung. Leucin wurde in einer Dosis von 0,2 ml einer 1%igen Lösung im Amnion von 9 Tage bebrüteten Hühnereiern injiziert. Morphologische Anomalien mit abnormen Funktionen, meistens in den Beinen und der Nackenregion, wurden beobachtet, während Röntgen-Aufnahmen Stellungsanomalien in Gelenken der Extremitäten und der Halswirbel zeigten.

R. M. BERGSTRÖM, T. ERILÄ and R. PIRSKANEN

Institute of Physiology, University of Helsinki, (Finland), 23rd March 1967.

[7] R. M. BERGSTRÖM and LEA BERGSTRÖM, Annls Chir. Gynaec. Fenn. *52*, 1 (1963).

[8] This work was aided by grants from Sigrid Juselius Foundation.

The Ability of Human Mothers to Identify the Hunger Cry Signals of their Own New-Born Infants During the Lying-In Period

From ethological studies we know that the vocal utterings of young offspring of many animals have specific meaning to their mothers and can cause behaviour directed towards satisfying the infant's needs. This process is based on innate release mechanisms[1]. In studying spectrographic and auditive identification of different types of cry signals of new-born and young infants[2–6] we have often wondered how specific and significant the cry signals of a new-born are to its mother. We are concerned here with the determination of whether a mother can, in fact, identify her own new-born's cry. Whether or not the mother's identification is the essential part of her interpretation of the infant's needs, initiating her actions to remove the disturbance causing the cry, has not been determined[7].

Material. All the 35 mothers in this study were II-paras, normal and healthy and had had an uncomplicated delivery after an uneventful, full-term pregnancy. The age of the mothers ranged between 18 and 35 years. The babies used were all healthy and normal and their age is shown in Table II.

Testing technique. The cries were recorded 5–10 min before a meal; about 3–4 h after the previous one. All possible external disturbance was carefully avoided. The cries were played for the mothers on the same day before the next meal.

The test tape consisted of 10 separate hunger cry signals from 9 different babies mixed at random; 2 cries from the baby of the testee and 8 from other babies. Every cry signal was repeated 3 times with an interval of 3 sec; the pause between different samples being 10 sec. Conscious subjective selection was avoided when making the tape. The mothers were instructed that they would hear 1 or more hunger cries on the tape which would be from their own baby. The tape was played only once, without stopping.

Two types of responses were considered in measuring the identification ability: (1) correct recognition of signals of one's own baby; (2) correct recognition of signals of other's babies.

In terms of test stimulus and response these types are designated as (1) *own-own* and (2) *other-other* responses. The number of both types of responses have been recorded and totalled for every testee. This total number of correct answers, along with the chance probability value, was used as the measure of identification. This chance probability is the statement of obtaining at least x correct answers by chance. This was calculated by ordinary bi-

[1] N. TINBERGEN, *The Study of Instinct* (Oxford University Press, Oxford 1951).

[2] O. WASZ-HÖCKERT, T. J. PARTANEN, K. MICHELSSON and E. VALANNE, Experientia *20*, 154 (1964).

[3] O. WASZ-HÖCKERT, T. J. PARTANEN, V. VUORENKOSKI, E. VALANNE and K. MICHELSSON, *6*, 393 (1964).

[4] J. LIND, O. WASZ-HÖCKERT, V. VUORENKOSKI and E. VALANNE, Annls Paediat. Fenn. *11*, 32 (1965).

[5] V. VUORENKOSKI, J. LIND, O. WASZ-HÖCKERT, T. J. PARTANEN, J. LEJEUNE and J. LAFOURCADE, Annls Paediat. Fenn. *12*, 174 (1966).

[6] T. J. PARTANEN, O. WASZ-HÖCKERT, E. VALANNE, K. THEORELL, V. VUORENKOSKI and J. LIND, Annls Paediat. Fenn., in press.

[7] H. S. SULLIVAN, *The Interpersonal Theory of Psychiatry* (W. W. Norton and Co., New York 1953).