neurales. Mentionnons encore que les structures neurales induites dans nos expériences ne construisent jamais de cerveaux aussi bien conformés que ceux obtenus à la suite du contact permanent entre le greffon et l'ectoblaste réagissant.

L'examen des coupes de certains de nos blastodermes, qui ont été fixés à des stades de plus en plus avancés du développement, montre que déjà après 2 h d'application du greffon contre l'ectoblaste, ce dernier change d'aspect. Il s'épaissit et devient un épithélium cubique de même caractère que celui que l'on trouve à la périphérie de l'aire pellucide. Durant les 8 h suivantes, cet ectoblaste ne se modifie plus de façon appréciable, même si le greffon n'a pas été détaché. Ce n'est que beaucoup plus tard, au moment de la neurulation de l'embryon hôte, que l'ectoblaste soumis à l'action inductrice interrompue au moment opportun, commence à se transformer en plaque neurale. Il appert donc que les effets de l'action inductrice sur l'ectoblaste demeurent latents pendant une longue période. Soulignons que l'épaississement de l'ectoblaste que l'on observe déjà après 2 h de contact avec le greffon ne signifie nullement que l'ectoblaste ainsi transformé soit capable de fournir des structures neurales. Néanmoins, ce changement structural semble nécessaire pour que le feuillet externe puisse réagir au stimulus inducteur.

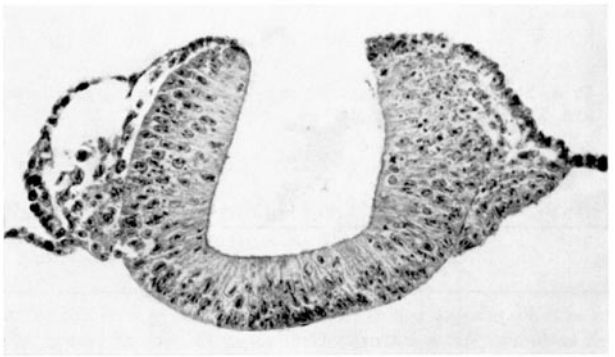

Fig. 2. Coupe transversale d'une gouttière cérébrale induite par le greffon laissé en place pendant 9 h et $^1/_2$. Sur les deux côtés de cette gouttière se sont accumulés des éléments de la crête neurale. Le feuillet interne ne s'est pas reformé sous la structure induite. Même grossissement que sur la Figure précédente.

Conclusions. Contrairement à ce qu'on pourrait supposer, une longue durée de contact entre l'inducteur et l'ectoblaste est indispensable pour produire des structures neurales chez les Oiseaux. Après 6 h de contact, nous obtenons dans certains cas une différenciation neuroïdale de l'ectoblaste. Par contre, dans tous les cas où le contact a été maintenu au minimum pendant 8 h 30, le feuillet externe a fourni des structures cérébrales.

Summary. The anterior region of the full primitive streak was transplanted onto the ectoblast in the area opaca of the young blastoderms (generally of medium primitive streak stage). After a lapse of fixed time, the graft was removed. At least 6 h of contact between the inductor and the ectoblast are necessary to obtain neuroidal response in the reactive ectoblast. On the other hand, contact of $8^1/_2$ h is sufficient to provoke the formation of cerebral structures.

J. GALLERA

Laboratoire d'Embryologie expérimentale, Institut d'Anatomie de l'Université de Genève (Suisse), le 25 novembre 1964.

The Changes of Gastric Histidine Decarboxylase Activity During Fasting and Feeding

In preliminary experiments on the histidine decarboxylase activity (HDA) in different organs of the rat, we observed that the enzymatic activity of the stomach was extremely variable. This fact seemed related to the fasting and feeding. The possible connection between histamine production and digestive rhythm had already been noted by SCHAYER, who reported that the histamine urinary excretion decreases during fasting and increases after feeding[1]. Recently KAHLSON, ROSENGREN, and THUNBERG reported that 'feeding stimulates gastric secretory activity, which is accompanied by a substantial increase in the histidine decarboxylase activity in the gastric mucosa'[2].

In this report we describe the changes of rats' gastric HDA during starvation and refeeding. Enzymic activity was determined in vitro, using the method of TELFORD and WEST slightly modified[3].

The amount of histamine produced by 1 g of fresh tissue during 3 h incubation was chosen as a measure of HDA. Each enzymic preparation was obtained from male albino rats of Wistar stock. The pooled pyloric tissue of 3–4 animals was carefully cleaned, homogenized in H_2O 1:2–1:5 and then centrifuged.

1 ml of the supernatant (S_1) was incubated for 3 h at 37°C in a medium containing phosphate buffer (pH 7.2 0.098 M), $2.93 \cdot 10^{-2} M$ histidine, $6 \cdot 10^{-5} M$ aminoguanidine, and $1.39 \cdot 10^{-4} M$ pyridoxal phosphate. After incubation, the mixture was acidified with HCl N, boiled for 1 min at 100°C and centrifuged. The supernatant (S_2) was neutralized with NaOH 14% and its histamine content bioassayed on the guinea-pig ileum. The standards were prepared by adding known histamine amounts and all the other components of the medium to the supernatant (S_1) previously inactivated by boiling for 1 min at pH 5. The changes in HDA, with respect to feeding, were studied in three types of experiments.

(1) Animals, fed for several days either with Rockland diet or with meat, were made to fast and then sacrificed at different times: 3, 6, 12, 24 and 48 h later. The average production of histamine at the 3rd, 6th, 12th, 24th and 48th h of fasting was 12.392 μg/g, 11.228 μg/g, 4.395 μg/g, 3.55 μg/g and 2.869 μg/g respectively. No differences in activity of animals fed either on the Rockland diet or on

[1] R. W. SCHAYER, Am. J. Physiol. *189*, 369 (1957); *195*, 400 (1958).
[2] G. KAHLSON, E. ROSENGREN, and R. THUNBERG, Am. J. Physiol. *169*, 467 (1963).
[3] J. M. TELFORD and G. B. WEST, J. Pharm., Lond. *13*, 75 (1961).

meat were noted, and therefore general averages are reported. The decrease in histamine production appears to be progressive from the 6th h onwards. Yet the considerable variability of results does not permit definitive conclusions to be reached. In the 12 experiments (56 rats) carried out on animals in fasting conditions varying from 12 to 48 h, the average production of histamine was 3.745 ± 2.308 μg/g fresh tissue.

(2) Animals kept in fasting conditions for 48 h were refed either with the Rockland diet or with meat and then sacrificed in groups of 4 to 6 at periods of 1, 2, and 3 h following feeding. For results see Table I and II.

The differences between the meat and Rockland diet were not significant. The increase in HDA after refeeding was considerable and rapid in both cases, inasmuch as, even in the first hour, very high histamine production was observed. After the first hour, enzymic activity underwent a slight increase, reaching a maximum at the 3rd h of refeeding. The first hour values were very variable and considerably higher than the corresponding values in the following experiment. The results of fasting were also not very homogeneous and clearly higher than those of the previous experiment.

(3) After fasting for 24 h, rats were fed for 1 h on the Rockland diet, and then food was withdrawn. The animals were sacrificed in groups of three, either immediately after food had been withdrawn or else 1, 2, and 5 h later. For results see Table III.

Enzymic activity seems to increase progressively, reaching a maximum at the 5th h following withdrawal of food. The above values are not very homogeneous, and in several experiments (3 out of 5) very low production was observed.

Parallel tests carried out on histamine present in the pyloric part of the stomach gave the following results, using the fluorimetric method of SHORE, BURKHALTER, and COHN[4]. For results see Table IV.

[4] P. A. SHORE, A. BURKHALTER, and V. H. COHN JR., J. Pharm. exp. Therap. *127*, 182 (1959).

Table I. Meat

	Fasting	1 h after feeding	2 h after feeding	3 h after feeding
No. of rats	27	26	28	29
No. of experiments	6	6	6	6
HDA	6.243 ± 3.542	18.845 ± 12.003	18.082 ± 6.845	24.729 ± 10.859
%	100	291.7 ± 167.6	316.2 ± 224.5	373.6 ± 177.1

Table II. Rockland diet

	Fasting	1 h after feeding	2 h after feeding	3 h after feeding
No. of rats	24	24	22	24
No. of experiments	5	5	5	5
Activity	7.495 ± 6.23	24.222 ± 16.943	27.807 ± 2.967	29.035 ± 4.787
%	100	395.76 ± 198.3	573.26 ± 402.41	619.72 ± 476.09

Histamine values are calculated as μg/g fresh tissue/3 h.

Table III

	Fasting	0 h after feeding	1 h after feeding	2 h after feeding	5 h after feeding
No. of rats	14	15	14	15	14
No. of experiments	5	5	5	5	5
Activity	4.395 ± 2.99	10.875 ± 4.97	15.701 ± 3.927	14.483 ± 5.514	17.740 ± 8.62
%	100	309.62 ± 153.8	366.68 ± 98.74	390.16 ± 117.59	496.32 ± 298.472

Histamine values are calculated as μg/g tissue/3 h.

Table IV

	Fasting	0 h after feeding	1 h after feeding	2 h after feeding	5 h after feeding
No. of rats	16	17	16	17	17
No. of experiments	6	6	6	6	6
Histamine μg/g	15.852 ± 3.191	12.250 ± 2.917	12.322 ± 2.968	11.985 ± 3.192	16.524 ± 3.543

It is evident that histamine slightly decreases after feeding for 1 to 2 h, and rises again, at the 5th h, towards fasting values.

The conclusions drawn from the above results are that HDA of the stomach undergoes rapid increases in response to refeeding, and that the return to basal values requires several hours.

The mechanism determining the rapid enzymic induction is unknown. It may be supposed that the increase of free histamine or else reduction of its bound fraction are the triggers of induction. In fact, the concentration of total gastric histamine is reduced 1 h after refeeding. Probably the distinction of gastric histamine pool in free and bound amine at different times of fasting and feeding may furnish an answer to the above hypothesis.

Riassunto. L'autore descrive le variazioni dell'attività istidino decarbossilasica nello stomaco di ratto in funzione del ritmo digestivo. L'attività enzimatica già alla 1ª–2ª ora di rialimentazione è molto superiore a quella dello stomaco in condizioni di digiuno. Contemporaneamente all'aumento dell'attività enzimatica si manifesta riduzione della quantità della istamina gastrica. Si prospetta l'ipotesi che la liberazione di tale amina sia responsabile dell'incremento dell'attività enzimatica osservata.

A. Castellucci

Istituto Farmacobiologico Malesci, Firenze (Italy), December 4, 1964.

Nervous Activity of the Frog's Epiphysis Cerebri in Relation to Illumination

Maintained activity in the absence of an external stimulus is a common feature of sense organs. While in the invertebrate lateral eye the impulse frequency is reported to rise with the level of illumination[1], the discharge rate of retinal ganglion cells of the cat changes with illumination, depending on whether the on-region or the off-region of the receptive field is dominant[2,3]. In the frog's optic nerve there are, among many others, units continuously active, even under bright light; their activity is inversely proportional to the light intensity and increases to a maximum in darkness[4]. Light sensitivity of individual neurons of the epiphyseal stalk (pineal organ) of the frog's diencephalon has recently been studied. In contrast to what has been found in the vertebrate lateral eye, the action of light on the frog's diencephalon is only of one type, namely causing inhibition of the spontaneous discharge[5]. At cessation of the light stimulus, this is followed either by a return of the spontaneous discharge (low stimulus intensities), by an off-discharge (moderate stimuli), or by a persisting inhibition of the spontaneous discharge (strong stimuli) which may last for many seconds[6].

In the present experiments, the impulse activity of single elements of the frog's epiphyseal stalk has been recorded by means of microelectrodes under steady lighting conditions. The experiment started with the measurement of the absolute threshold in preparations which had been dark-adapted for at least 2 h[7]. Then the diencephalon was exposed to constant lights of several intensities from zero to the highest obtainable. After some minutes (usually 5) of light adaptation to each intensity, the spontaneous discharge was recorded and its frequency was measured. Figure 1 illustrates the electrical activity of a single unit of the frog's pineal stalk during darkness and in response to steady illumination of several intensities. During darkness and at a low level of illumination, the frequency of impulses is high. It decreases to about 50% if the illumination is increased to $3.2 \cdot 10^{-2}$ lm/m². At 7.9 lm/m² no impulses can be seen. At the end of illumination of this intensity, impulse activity suddenly reappears (Figure 1, lowermost record). For several units of the epiphyseal stalk investigated in this way, there are individual differences of the absolute frequency of the maintained activity in the dark-adapted state, ranging from 4 to 10 per sec. However, the relative change of frequency with rising illumination is about the same for all units investigated (Figure 2). At low levels of light adaptation, 10^{-5} to 10^{-4} lm/m², a small (4 to 12%) rise of frequency, as compared to the steady discharge in the dark, can be

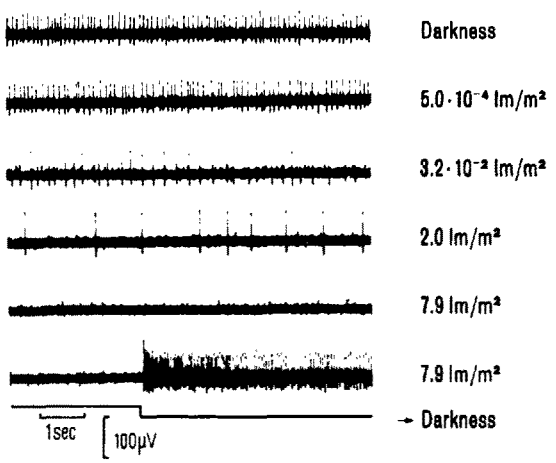

Fig. 1. Microelectrode recording, by means of a steel needle electrolytically sharpened and insulated except at the tip, of a single excitable unit, probably ganglion cell, of the epiphyseal stalk of *Rana esculenta* in the dark (upper record) and at different levels of illumination (intensities as given). The effect of sudden termination of the light stimulus is shown in the lowermost record. Note the strong change of size of the individual spike at different frequencies of discharge[6].

[1] H. K. Hartline and C. H. Graham, J. cell. comp. Physiol. *1*, 277 (1932).
[2] S. W. Kuffler, R. Fitzhugh, and H. B. Barlow, J. gen. Physiol. *40*, 683 (1957).
[3] A. Arduini and L. R. Pinnea, Arch. ital. biol. *100*, 425 (1962).
[4] H. R. Maturana, J. Y. Lettvin, W. S. McCulloch, and W. H. Pitts, J. gen. Physiol. *43*, Suppl. 2, 129 (1960).
[5] E. Dodt and M. Jacobson, J. Neurophysiol. *26*, 752 (1963).
[6] Y. Morita, Pflügers Arch. ges. Physiol. *279*, R2 (1964).
[7] E. Dodt and Y. Morita, Vision Res. *4*, 413 (1964).