groupées dans le nœud de Hensen en recul, ce qui signifie qu'au stade 4, il n'y a plus d'invagination active dans le nœud de Hensen.

Du greffon B dérivent des cellules qui ne participent qu'à la formation du mésoblaste paraxial: nous les retrouvons dans le mésenchyme de la tête, le cœur, les lames latérales de la partie antérieure du corps embryonnaire et quelques-unes dans les somites.

Le greffon C correspond à la région médiane de la ligne primitive achevée. Il est destiné à donner du mésoblaste paraxial, mais beaucoup plus latéral que dans le cas du greffon B, puisque nous observons le marquage surtout dans les cellules du péricarde, du myo-épicarde et derrière le cœur, dans celles de la somatopleure et de la splanchnopleure.

Quant au greffon D, situé dans le tiers postérieur de la ligne primitive, il ne donne que du mésoblaste extra-embryonnaire.

Si la région post-nodale est échangée au stade 5, plus précisément au stade du prolongement céphalique moyen, ses cellules se groupent exclusivement dans les somites, sauf les tous premiers.

Conclusions. Ces résultats préliminaires sont suffisamment précis pour que nous puissions considérer certains points comme définitivement acquis.

Quoique l'origine gastruléenne de l'endoblaste ait été déjà démontrée (VAKAET[3], MODAK[4], NICOLET[1]), nous sommes maintenant en mesure d'en préciser les modalités. L'endoblaste commence à s'invaginer de façon prépondérante, sinon exclusive, dans la région antérieure de la ligne primitive dès le stade 2. Cette invagination doit se terminer au stade 4. D'après les dispositions des cellules marquées dans ce feuillet observées dans nos différents types d'échange, il paraît indubitable que la totalité de l'endoblaste jusqu'aux limites de l'aire transparente provient de cellules invaginées. L'endoblaste vitellin au contraire doit provenir du bord interne de l'aire opaque.

Quant à la partie postérieure de la ligne primitive, elle ne fournit dès le début que du mésoblaste extra-embryonnaire. Elle est par conséquent comparable au nœud postérieur de la ligne primitive des Mammifères qui a la même fonction.

Nous tenons également à faire remarquer que la totalité du matériel chordal présomptif est rassemblé dans le nœud de Hensen de la ligne primitive achevée et qu'aucune cellule de la région post-nodale ni au stade 4, ni au stade 5 ne participe à la formation de la chorde.

Notons enfin que la région post-nodale au stade 4 (greffon B) fournit un nombre très restreint de cellules destinées à former les somites. L'invagination massive du mésoblaste somitique débute bien dans cette région, mais un peu plus tard, c'est à dire au moment de l'apparition du prolongement céphalique. Elle doit en tout cas se poursuivre jusqu'au début de la neurulation[5].

Summary. The exchange of various regions of the primitive streak by similar parts labelled by the tritiated thymidine shows that all the endoblast of the area pellucida comes from invaginated cells, and that this invagination starts very early in the development. On the contrary, the posterior part of the primitive streak produces only extra-embryonic mesoblast. As for the somite material, its massive invagination begins in the post-nodal region, as soon as the head process appears.

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The Effect of Central Nervous System Stimulation on Plasma Erythropoietin Levels in Rhesus Monkeys

The participation of the central nervous system in the regulation of erythropoiesis has been reported by many investigators[1–13]. It is the purpose of this communication to present our findings primarily on the effect of electrical stimulation of the hypothalamus on the presence of plasma erythropoietin in rhesus monkeys.

Rhesus monkeys in normal health and nutrition were used in these studies. The monkeys were non-uremic and non-anemic. The animals were treated in the following manner. Group I: electrical stimulation was applied to intact monkeys in the area of (1) the supraoptic nucleus, (2) the preoptic nucleus, (3) the posterior median eminence, (4) the mammillary nucleus, and (5) the anterior commissure; group II: hypophysectomized male monkeys received electrical stimulation in the area of (1) the supraoptic nucleus and (2) the posterior median eminence; group III: gonadectomized male monkeys received electrical stimulation in the area of (1) the supraoptic nucleus and (2) the posterior median eminence.

Nickel chromium wire electrodes were stereotaxically inserted into the brains of rhesus monkeys under general anesthesia (pentobarbital sodium) using the stereotaxic localization coordinates of SNYDER and LEE[14] and a correction factor for the skull cap based on animal body-weight charts. The electrodes were fully insulated except at the tip and were 0.09 mm in diameter. 48 h following electrode insertion, stimulation was applied with a BRS (Behavior Research Systems) constant current stimulator. The following currents were individually used on each electrode: a current varying from 0.6–0.7 mA was administered for a 300 msec duration every 30 sec for $^1/_2$–8 h periods on 4 consecutive days unless otherwise stated.

Blood was collected before and during the period of stimulation; care was taken, however, to prevent monkeys from becoming anemic. In some instances, following completion of stimulation of the brain, a lesion was burned in each area to assist in the later anatomical

localization of the position of the electrode. The brain was fixed in buffered formalin solution, and serial sections and multiple staining procedures were used to locate the lesion.

Hypertransfused-polycythemic Ha/ICR Swiss male mice were used to assay plasma for erythropoietin (ESF)[15].

The results in Table I show that stimulation of the supraoptic nucleus in 4 monkeys, 1–8 h on 4 consecutive days, induced the release of high levels of plasma erythropoietin. The levels of iron^{-59} uptake by the blood were 3–13 times greater than the pre-stimulatory levels in these animals and levels in sham control animals.

Significant levels of plasma erythropoietin were obtained following stimulation in the area of the preoptic nucleus for $^1/_2$–8 h on 4 consecutive days (Table II). The stimulation of the posterior median eminence, for 1–3 h on 4 consecutive days, produced the highest levels of plasma ESF (Table III). Stimulation of other areas of the hypothalamus, i.e. the mammillary nucleus and the anterior commissure, for 4–8 h on 4 consecutive days, did not produce significant levels of plasma ESF (Table IV). Table IV also shows that although stimulation of some areas produced high levels of plasma ESF in some monkeys, in many more instances there was no increase in plasma ESF levels. This is perhaps due to different functional states in various animals. Also, we may not have stimulated similar areas in all animals due to the variation in the localization of hypothalamic nuclei and fiber tracts.

Since it is known that specific areas of the hypothalamus regulate pituitary function[16,17], it was deemed advisable to determine if ESF is released via a hypothalamic-pituitary-kidney pathway. 8 monkeys were hypophysectomized and 2 areas, the supraoptic nucleus and posterior median eminence, were stimulated. Only 1 hypophysectomized monkey, stimulated in the posterior

Table I. Plasma erythropoietin levels in rhesus monkeys subjected to hypothalamic stimulation in the area of supraoptic nucleus

Animal	Duration of stimulation (h)	% 24 h blood radio-iron uptake in polycythemic mice		
		Pre-stimulatory plasma S.D.[a]	Stimulatory plasma S.D.	Sham control S.D.
(A) Experimental				
(1) Male	1	0.46 ± 0.09	1.59 ± 0.30	
(2) Male	2	0.38 ± 0.03	5.08 ± 0.66	
(3) Male	4	0.53 ± 0.07	3.29 ± 0.50	
(4) Male	8	0.65 ± 0.02	3.86 ± 0.90	
(B) Sham control (8 h)				
(1) Male	–			0.55 ± 0.06

[a] S.D., standard deviation.

Table II. Plasma erythropoietin levels in rhesus monkeys subjected to hypothalamic stimulation in the area of preoptic nucleus

Animal	Duration of stimulation (h)	% 24 h blood radio-iron uptake in polycythemic mice		
		Pre-stimulatory plasma S.D.[a]	Stimulatory plasma S.D.	Sham control S.D.
(A) Experimental				
(1) Female	$^1/_2$	0.39 ± 0.11	0.90 ± 0.10	
(2) Female	1	0.52 ± 0.11	1.14 ± 0.16	
(3) Male	$1^1/_2$	0.38 ± 0.13	2.05 ± 0.48	
(4) Male	8	0.43 ± 0.15	2.66 ± 0.41	
(B) Sham control (8 h)				
(1) Female				0.38 ± 0.07
(2) Male				0.54 ± 0.14

[a] S.D., standard deviation.

Table III. Plasma erythropoietin levels in rhesus monkeys subjected to hypothalamic stimulation in the area of posterior median eminence

Animal	Duration of stimulation (h)	% 24 h blood radio-iron uptake in polycythemic mice		
		Pre-stimulatory plasma S.D.[a]	Stimulatory plasma S.D.	Sham control S.D.
(A) Experimental				
(1) Male	1	0.40 ± 0.09	5.44 ± 0.98	
(2) Female	2	0.62 ± 0.12	6.87 ± 1.34	
(3) Male	3	0.43 ± 0.07	9.40 ± 2.19	
(B) Sham control (3 h)				
(1) Male				0.60 ± 0.07
(2) Female				0.51 ± 0.05

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median eminence, gave us a high level of plasma ESF (4.85% ± 0.58 iron-^{59} uptake vs. sham control 0.54% ± 0.04) (Table V). It appears from this finding that the hypothalamus may directly affect the target organ producing erythropoietin.

Testosterone[18] has been shown to influence the production of ESF. For this reason, 8 male monkeys were gonadectomized and the areas of the supraoptic nucleus and posterior median eminence were stimulated. No significant increase in ESF levels was detected. It must be pointed out, however, that stimulation was carried out for 4–8 h on only 1 day, instead of on 4 successive days (Table VI).

The question remains as to how the hypothalamus exerts its influence on erythropoiesis. There are 3 possible pathways by which the hypothalamus may exert its control on erythropoiesis: (1) through specific nervous circulation affecting erythropoietin-producing target organ(s) directly[19]; (2) through the release of pituitary secretions which have been reported to have either a primary or secondary influence on erythropoiesis: thyroxin[20], androgens[15,18,21–23], growth hormone[24–26], and prolactin[27] have been found to stimulate erythropoiesis and/or erythropoietin production, or to interact with erythropoietin in an additive manner[18,28]; and (3) through the autonomic nervous system: hypothalamic stimulation or lesions interfere with the function of the autonomic nervous system; both may influence the cardio-pulmonary function and induce hypoxia. Both also may affect renal circulation and, therefore, affect erythropoietin production.

FELDMAN[29] demonstrated the effect of the central nervous system stimulation on erythropoiesis in rats with chronically implanted electrodes. However, unlike HALVORSEN'S[7] findings in the rabbit and ours in the monkey, stimulation of the hypothalamus did not result in the increase of plasma erythropoietin. One must keep in mind the nature of stimulation, species and age difference in relating the involvement of the hypothalamus to the presence of plasma erythropoietin[30].

Table IV. Plasma erythropoietin (ESF) levels in rhesus monkeys subjected to stimulation in various areas of hypothalamus

Duration of stimulation (h)	Area stimulated	No. of animals	No. positive for ESF	No. negative for ESF
1/2–8	Preoptic nucleus	14	4	10
1/2–8	Supraoptic nucleus	15	4	11
1–3	Posterior median eminence	7	3	4
4	Mammillary nucleus	1	–	1
8	Anterior commissure	1	–	1
		38	11	27

Table V. Plasma erythropoietin (ESF) levels in hypophysectomized male rhesus monkeys subjected to stimulation in various areas of the hypothalamus

Duration of stimulation (h)	Area stimulated	No. of animals	No. positive for ESF	No. negative for ESF
2	Supraoptic nucleus	1	–	1
4		2	–	2
8		1	–	1
2	Posterior median eminence	1	–	1
4		1	–	1
8		2	1[a]	1

[a] 4.85% blood radio-iron uptake (S.D. ± 0.58).

Table VI. Plasma erythropoietin (ESF) levels in gonadectomized male rhesus monkeys subjected to hypothalamic stimulation

Duration of stimulation (h)[a]	Area stimulated	No. of animals	No. positive for ESF	No. negative for ESF
4	Supraoptic nucleus	2	–	2
8		2	–	2
4	Posterior median eminence	2	–	2
8		2	–	2

[a] Stimulation administered only once for 4 or 8 h.

Zusammenfassung. Elektrische Reizung der Nuclei supraoptici und preoptici hypothalami, ebenso der eminentia mediana hatte eine signifikante Erhöhung des Plasma Erythropoietins zur Folge, nicht aber bei Reizung der Nuclei mammillaria hypothalami und commissura anterior. Es wird der hypothalamische Einfluss auf die Erythropoiese kurz erörtert.

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