normal development[5]. Plasticity of the forebrain region was also evident in the donors of the explanted neural tissue, as normally proportioned paired eyes were found in all seven *Xenopus* larvae examined histologically after earlier removal of large portions of the anterior neural plate. Except for two explants in which a pair of eyes developed, eye formation in vitro always resulted in a single, compact structure.

Discussion. It is clear from the above results that in an anuran as well as in urodeles the forebrain area of the future central nervous system behaves initially like a 'morphogenetic field'. The final developmental instructions, although limited to forebrain types, are not yet fixed in the individual cells. Although the kinetics of 'crystallization' into the definitive pattern are unknown, field properties are in evidence at least through the end of neurulation. This is not necessarily to say, however, that the presumptive forebrain area is uniform ('equipotential') at the time of testing. Recent quantitative experiments have in fact established, by measuring the *differentiation tendencies* of different areas, that regional differences exist at least as early as the open neural plate stage[6]. Apparently the postulated initial *equi*potentiality of the field is quickly lost. The way in which this occurs is not known, but it is noteworthy that a qualitatively normal structural pattern develops even in tissue activated in vitro[4]. This implies that slight differences in the 'micro-environment' at different points within the cell mass are sufficient to select different developmental pathways. Such differences could in turn consist partly of gradients resulting from metabolic or secretory activity[7].

The determination of the forebrain structural pattern in situ is strongly influenced by regional factors which are non-existent under in vitro conditions. The best established of these is an *eye-depressing* influence from the underlying medial mesoderm[8]. This effect could also explain why no eye developed in a percentage of cases in the *Xenopus* forebrain material reported here[9]. The relatively small size of the neural fragments used was possibly also a contributing factor since it is known that eye formation frequently fails to occur if the mass of forebrain cells is small[4,6]. Taking in perspective the known facts about the histogenesis of the eye, it becomes clear that the developmental fate of individual eye cells is specified in the following step-wise sequence. During 'primary induction' all potentialities ('genes'?) become blocked for cellular differentiation other than to one of the *forebrain* types. The potentialities become further restricted in the future eye-forming region by a secondary process of *segregation* during neurulation, so that ultimately only cell types of the eye can develop. Eye morphogenesis begins shortly afterwards by cellular migration and finally, at about the optic cup stage, the definitive development of each cell is determined according to its location within the 'eye field'[10].

Résumé. Une analyse du développement des yeux faite sur de petits fragments de l'ébauche neurale («neural plate») a montré que la région du futur prosencéphale se comporte comme un *champ* morphogénétique. Bien que cette région ne soit plus «*équi*potentielle» même dans la neurula jeune, les propriétés du champ persistent pendant toute la neurulation.

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Central Institute for Brain Research, Amsterdam (The Netherlands), September 6, 1965.

[5] M. A. Corner, J. exp. Zool. *153*, 301 (1963) and [1] for an approximate fate map of the major brain divisions in the *Xenopus* neurula. – C. O. Jacobson, J. Embr. exp. Morph. *7*, 1 (1959). – C. von Woellwarth, Roux Arch. Entw. Mech. *152*, 602 (1960). (For precise maps of the forebrain area in the early neurulae of *Amblystoma mex.* and *Triturus alp.* respectively.)

[6] P. D. Nieuwkoop, J. Anim. Morph. Physiol. *11*, 21 (1964) using *Triturus alp.*; also from unpublished experiments of the author with *Amblystoma mex.*: the presumptive eye region of the early neurula formed almost exclusively eye in vitro while relatively less eye and more neural material developed from the other forebrain areas.

[7] W. F. Loomis, in *Biological Structure and Function* (Academic Press, New York 1961), p. 509, suggests a differential gene activation according to the pCO_2 built up at each point in the tissue, while S. M. Rose, Biol. Rev. Cambr. Philos. Soc. *32*, 351 (1957) has proposed that a hierarchy of specific self-inhibitory cell products initiates the spatial pattern. Boterenbrood[2] has suggested that telencephalic differentiation is favoured in cells located towards the *surface* of the neural mass, which in itself is consistent with either of the mechanisms mentioned above.

[8] See *Discussion* in Nieuwkoop et al.[3], this factor may in fact be identical with the 'transforming principle', which blocks all forebrain differentiation tendencies in the more caudal neural regions. It appears also to favour diencephalic differentiation within the forebrain area at the expense of telencephalic.

[9] M. A. Corner, unpublished (*Amblystoma mex.*): the inclusion even of ventral mesoderm in an explant led to the formation of extensive mesenchyme and absence of eye formation in presumptive eye tissue. A neural structure formed in its place.

[10] V. Lopashov and O. E. Stroeva, in *Advances in Morphogenesis* (Academic Press, New York 1961), p. 331: differentiation can still be guided into either tapetum or retina, by properly choosing the environmental conditions. The experiments of L. S. Stone, J. exp. Zool. *145*, 85 (1960) and of G. Székely, Acta biol. hungar. *5*, 157 (1954) have demonstrated, moreover, that at about this time individual retinal cells become specified to make synaptic connections at appropriate points in the optic tectum.

Relationships Between Cerebral Transit Time of Non-Diffusible Indicators and Cerebral Blood Flow. A Comparative Study with Krypton^{85} and Radioalbumin

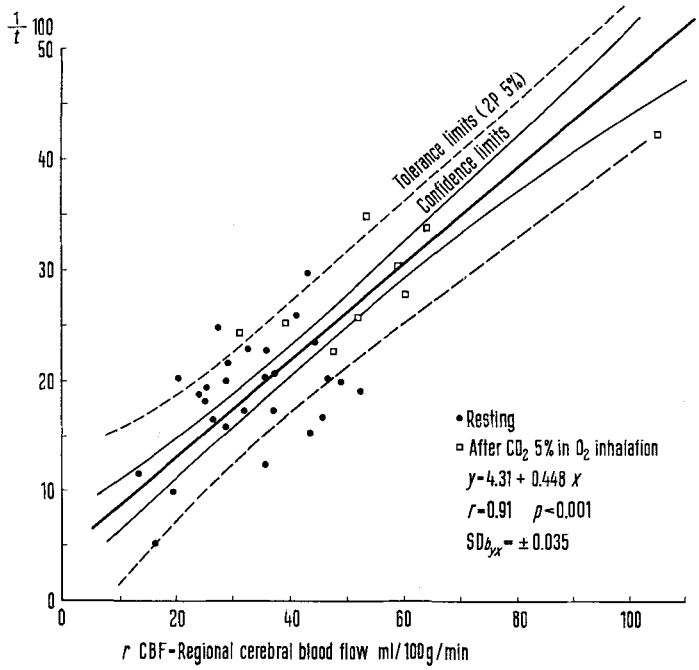
Methods available at present have not yet yielded reliable measurements of the parameters of the cerebral circulation suitable for clinical use. In recent years a consistent effort has been directed towards estimation of indices of cerebral blood flow (CBF) by externally recording thepassage through cerebral vessels of a bolus of a γ-emitting, non-diffusable tracer, such as radiohyppurane or radioalbumin[1,2].

The reliability of such methods is based essentially on two assumptions: (1) the recorded curve of radioactivity versus time secures reliable information about the mean transit time ($\bar{t}$) of the blood circulating in the explored region of the head; (2) the cerebral blood volume is a constant. Under these conditions, $\bar{t}$ would behave as a linear index of CBF: $1/\bar{t} = F/V$, where F is the blood flow and V the blood volume in the explored region[3].

[1] W. H. Oldendorf, J. nucl. Med. *3*, 382 (1962).

[2] C. Fazio, C. Fieschi, and A. Agnoli, Neurology *13*, 561 (1963).

[3] M. Zierler, in *Dynamic Clinical Studies with Radioisotopes* (Ed., R. Kniseley, Atomic Energy Commission, 1964), p. 55.



Inverse of mean transit time of RISA ($1/\bar{t} \cdot 100$) on the ordinate, and rCBF calculated from the regional clearance of Kr^{85} on the abscissa. Radioactivity was recorded over the temporo-parietal area, ipsilateral to the injected internal carotid artery. The following values are given in the figure: the equation of the regression line, the standard deviation of the coefficient b (SDb_{yx}), and the coefficient of correlation r. The confidence limits of the regression line of y on x and tolerance limits of y are shown in graphical form.

Both assumptions are faced with serious limitations, as has been extensively discussed in previous papers[4,5]. Therefore, in the absence of convincing evidence, parameters of the curve of transit of radioalbumin externally recorded on the head have only been used in the past as *approximate and non-linear indices of CBF*[4,5].

The present study aims at a better definition of the relationships between the indices of CBF as measured with radioalbumin, and the quantitative value of CBF in the same region measured by the regional inert gas clearance technique of LASSEN et al.[6].

Following incannulation of an internal carotid artery with a 0.9 mm polyethylene catheter, 3 ml of Kr^{85} solution (specific activity $\sim$ 1 mc/ml) were injected, and the build-up and subsequent clearance of radioactivity in the brain recorded by an external detector. The clearance curve was analysed into two compartments, and the mean cerebral blood flow in the explored region (rCBF) was calculated according to LASSEN et al.[6].

After 15 min, a bolus of radioiodized serum albumin (I^{131} RISA) was rapidly injected through the same catheter (40 μc diluted in 0.5 ml), and the passage of the radioactive molecules recorded by means of the same detector. Due to a similar energy of γ-emission (I^{131} = 0.364 MeV, Kr^{85} = 0.562 MeV), both tracers are seen with a rather similar counting efficiency in the same brain area. The mean transit time of RISA was calculated according to FIESCHI et al.[5].

27 patients were studied. Among them there were 8 normal subjects and 19 patients with cerebral vascular lesions. In 9 patients, two injections of Kr^{85} and RISA were made, the second one during administration of a gas mixture containing 5% CO_2 in O_2. As the majority of pathological cases had a reduced CBF, and – on the other hand – 5% CO_2 brings about a consistent increase of CBF, a wide range of CBF values (from 14–104 ml/100 g/min) and of $\bar{t}$ (from 1.2–18 sec) is represented in our sample.

The results are given in graphical form in the Figure, where a dot indicates each couple of measurements of rCBF and $\bar{t}$ of RISA in basal conditions, and measurements taken during CO_2 inhalation are denoted by a square.

The analysis of the results, and the conclusions deriving therefrom, can be summarized as follows: (1) The regression equation has been calculated, and its linearity tested, showing that there is a linear regression between rCBF and $\bar{t}$ of RISA; the coefficient of correlation is 0.91. (2) Confidence limits ($p < 0.05$) of the regression line and tolerance limits ($p < 0.05$) of expected CBF values, for any $\bar{t}$ observed, have also been calculated, and are graphically represented in the Figure. A wide range of values of CBF can be expected for any observed values of $\bar{t}$. To give an example, a $\bar{t}$ of 5 sec ($1/\bar{t} \cdot 100 = 20$ on the ordinate of the Figure) tolerates CBF values ranging from 24–46 ml/100 g/min.

These results prove that the mean transit time in cerebral vessels of a bolus of non-diffusible indicator is a linear, though approximate, index of regional cerebral blood flow.

Riassunto. Il flusso sanguigno cerebrale è stato misurato con il metodo della clearance regionale del Kr^{85}. I valori del flusso sono stati confrontati con le misure del tempo medio di transito di radioalbumina nella stessa regione cerebrale. È stata dimostrata una correlazione significativa tra le due serie di misure, e sono stati calcolati i limiti fiduciali e di tolleranza per la regressione.

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