During 'habituation' in man[8] 'the main visual evoked response changes' are 'reduction of amplitude or disappearance of all its components' but particularly of the 'last' waves caused by unspecific polysynaptic pathways[7]. On the contrary, our results are exclusively concerned with the 'primary evoked response' directly recorded from the optic pathways. It is likely that, in man, non-specific responses caused by unspecific afferent systems may undergo 'habituation' phenomena which, according to our results, are not observed in the specific primary responses.

Zusammenfassung. Unter Atropinmydriase verschwinden beim Menschen die primären visuellen Antwortpotentiale auch bei wiederholter photischer Stimulation nicht, d.h. es tritt in bezug auf diese Potentiale keine Habituation auf.

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RNA-Induced Biosynthesis of Adult Haemoglobin in Cooley's Disease

An abnormal disposition of amino acids has been demonstrated for various pathological haemoglobins[1,2]. It is well known, for example, that sickle cell haemoglobin differs from normal haemoglobin by the replacement of one amino acid (valine for glutamic acid) in a specific peptide of the β-polypeptide chain[3,4]. This demonstrates that abnormal information for protein synthesis exists in hereditary haemoglobinopathies, due, very probably, to a defect in the DNA and therefore of the messenger-RNA.

In recent research in the field of molecular biology, it has been observed that the information required for the synthesis of haemoglobin can be transferred from one cell to another by means of RNA. In fact, the synthesis in *E. coli* of a protein resembling normal rabbit haemoglobin has been observed when ribosomal-RNA of rabbit reticulocytes and *E. coli* have been reacted[5]. On the other hand, RNA extracted from the erythropoietic cells of subjects suffering from sickle cell anaemia has been shown to be capable of causing *in vitro* the synthesis of pathological haemoglobin in the megaloblasts and the normal reticulocytes[6].

The possession of this valuable information induced us to study the possibility of producing *in vivo* the synthesis of adult haemoglobin in the erythropoietic cells of subjects affected with Cooley's disease, by means of RNA extracted from normal haemopoietic cells. Obviously, by extracting RNA from human bone marrow it is impossible to obtain only RNA of the erythropoietic cell line. In the experiment here described, therefore, the RNA used was that produced in all haemopoietic cells; only a certain part of it therefore possessed the information necessary for the normal haemoglobin formation.

Methods. The haemopoietic tissue was obtained, by a sternal puncture, from twenty normal adult volunteers. After isolation of the cells, the RNA was extracted by the method described by GEORGIEV and MANTIEVA[7]. In the experiment the nuclear RNA II, corresponding in base ratio and turnover rate to the type of messenger-RNA, was used. 2 mg of this RNA, dissolved in phosphate-buffered saline, were injected into the medullary cavity of the sternum of two children affected with Cooley's disease. Before the injection and 48 h afterwards the physico-chemical characteristics of the haemoglobin present in the erythropoietic cells of the sternum were determined. The second medullary biopsy was effected in the same region where the RNA was injected. The haemoglobin was analysed by means of starch-block electrophoresis[8,9] and by the method of alkali denaturation[10].

Results. In the haemoglobin of the two patients, the percentage of HbF (foetal haemoglobin) was predominant. Because of this the separation of HbA (rapid adult haemoglobin) from HbF was impossible using starch-block electrophoresis: in fact only one component occupying the position of HbF was present (Figure 1). The fraction of slow adult haemoglobin (HbA β) was absent. Only by measuring the sensitivity to alkalis was it possible to determine, by the difference, the percentage of HbA present in the specimens under observation. The percentages observed were as follows: *Patient 1* – HbF 86.51, HbA 13.49; *Patient 2* – HbF 95.30, HbA 4.70. Both cases were, therefore, typical for Cooley's disease.

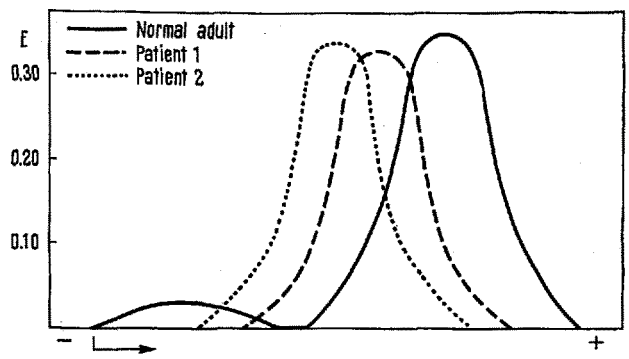

Fig. 1. Electrophoretic diagrams of the Hb extracted from the erythropoietic cells of two patients affected with Cooley's disease. For purposes of comparison, an electrophoretic diagram of Hb of a normal adult is also reproduced.

[1] V. M. INGRAM, Brit. Med. Bull. *15*, 27 (1959).
[2] H. LEHMANN and J. A. M. AGAR, in *The Metabolic Basis of Inherited Disease* (McGraw-Hill Book Co., New York 1960), p. 1086.
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[9] H. G. KUNKEL and G. WALLENIUS, Science *122*, 288 (1955).
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Following the injection of RNA into the marrow cavity of the sternum, the electrophoretic diagrams revealed an asymmetrical main component (Figure 2). The base of this component was substantially HbA, but the upper part tended towards the region of slow Hb. This was because the percentage of HbF in the solution was still high, and on account of its slow movement caused a trail phenomenon on the starch-block. In the second examination a component of HbA β similar to that of the normal adult was also present. The divided quantitative determination of Hb gave the following results: *Patient 1* – HbF 16.90, HbA β 1.50, HbA 81.60; *Patient 2* – HbF 22.80, HbA β 2.30, HbA 74.90.

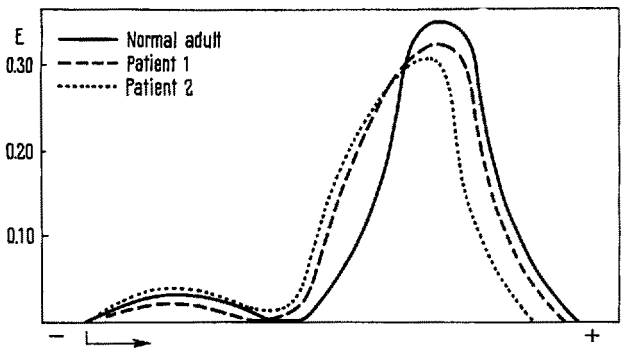

Fig. 2. Electrophoretic diagrams of the Hb extracted from the erythropoietic cells of two patients suffering from Cooley's disease after the introduction of RNA into the marrow cavity of the sternum.

The experiment therefore demonstrated that by injecting into the marrow cavity RNA possessing the information necessary for the normal haemoglobin formation, it is possible to obtain a notable increase in the quota of HbA in the erythropoietic cells of patients affected with Cooley's disease.

Riassunto. L'introduzione nella cavità midollare dello sterno di soggetti affetti da morbo di Cooley di ARN II° estratto dalle cellule emopoietiche normali, provoca un netto aumento della quota di HbA nelle cellule della serie eritroblastica.

S. ESPOSITO

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PRO EXPERIMENTIS

Eine Anlage zur Perfusion isolierter Organe

Die Perfusion isolierter Organe[1–5] hat sich als wertvolle Methodik erwiesen, um bestimmte Leistungen dieser Organe in einem möglichst physiologischen Zustand zu erfassen. Die Funktionen laufen während einiger Zeit mit befriedigender Geschwindigkeit oder Intensität ab, ohne von anderen Organsystemen beeinflusst zu werden. Dabei ist die Struktur im Gegensatz zu Gewebsschnitten oder Homogenaten intakt.

Trotzdem sind die klassischen Perfusionssysteme mit *einem Kreislauf* mit Nachteilen behaftet. Da die zu Beginn des Experimentes verwendete Perfusionslösung nicht ohne weiteres quantitativ durch eine andere ersetzt werden kann, ergeben sich rasch Grenzen in der experimentellen Anwendbarkeit.

(1) So ist zum Beispiel die *Streubreite* der Resultate recht gross[6] und spiegelt die Streubreite der Resultate wieder, die mit gleicher Bestimmungsmethodik an intakten Ganztieren erhoben worden sind. Es wäre deshalb vorteilhaft, *Vergleichs*werte von *einem* Organ aus *einem Experiment* zu erhalten. Das ist theoretisch möglich, da die Geschwindigkeit von Stoffwechselvorgängen zu Beginn der Experimente konstant ist.

(2) Perfusionsanlagen mit *einem* Kreislauf gestatten nicht, die *Reversibilität* verschiedenster Einflüsse am perfundierten Organ zu studieren.

(3) «*Short pulse labelling*»-Experimente können am perfundierten Organ nur unter unphysiologischen Bedingungen durchgeführt werden. Die Methodik des «short pulse labelling» besteht darin, biologische Materialien *kurze Zeit* einer bestimmten markierten Verbindung auszusetzen. Diese markierte Verbindung oder ihre Umwandlungsprodukte finden sich dann dort, wo die Stoffwechselaktivität in Bezug auf die Gruppe dieser Verbindungen am höchsten ist, zum Beispiel in einer Fraktion *z*. Wird die markierte Verbindung nach kurzer Zeit aus dem System entfernt, so erscheint nur die Fraktion *z* markiert, während die markierte Verbindung in der kurzen Zeit nur geringgradig in weniger aktive Stoffwechselwege eingeschleust wird. Damit kann das Schicksal der allein markierten Fraktion *z* über verschiedene Zeiten nach Wegnahme des Vorläufers verfolgt werden.

In der Praxis lässt sich indessen eine markierte Verbindung nicht mehr ohne weiteres aus einem biologischen System entfernen. Man hat sich deshalb mit Isotopenverdünnungsmethoden beholfen. Dieses Vorgehen ist selbstverständlich nicht physiologisch, da das biologische Objekt plötzlich mit grossen Mengen der nicht markierten Verbindung überschwemmt werden muss.

Beschreibung der Apparatur. Bei dem hier zu beschreibenden Perfusionssystem handelt es sich um eine Weiterentwicklung des von JEUNET und QUITT[5] beschriebenen Apparates. Es soll die eben erwähnten Nachteile älterer

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