Within the workers' group itself, the lower the social rank, the smaller the cholesterol concentration ($P < 0.001$). However, the higher value for laboratory technicians might be partly due to their greater age. Their mean age is equal to 30 years, while in the other groups the averages vary from 22 for the students to 28 for the sedentary workers.

Although the students' food is abundant and well balanced, their diet varies following their family traditions. We have classified them roughly into vegetarians and non-vegetarians. It is also important to note that some of them regularly take ghee, a kind of melted clarified butter, with their meals while others never use it, the food being cooked in oil, mostly gingely oil (from *Sesamum indicum*) and groundnut oil (from *Arachis hypogea*). The presence of ghee in the diet seems to affect considerably the cholesterol level among vegetarian people but not among the others (Table II). Vegetarians who do not eat ghee have a much lower cholesterol level than vegetarians who do ($P < 0.001$) and a slightly lower value than non-vegetarians ($P < 0.05$). These data do not permit us to conclude that ghee, when taken with a mainly carbohydrate diet, is directly involved in these variations. In some families, an accidental association between certain food habits and some genetic factors which regulate cholesterol metabolism should not be excluded although, in the present survey, each subgroup is composed of individuals taken at random and originating from numerous different endogamic groups of the former Hindu system of caste.

Table I. M = mean, $\sigma$ = standard deviation, and N = number of subjects

| Groups | Cholesterol, mg/100 cm³ | | |
|---|---|---|---|
| | N | M | $\sigma$ |
| Workers: | | | |
| Technicians | 12 | 196.92 | 47.94 |
| Servants | 20 | 164.38 | 33.40 |
| Coolies | 40 | 153.31 | 27.73 |
| Sedentaries | 30 | 152.62 | 35.06 |
| Total | 102 | 160.41 | 36.18 |
| Students: | 91 | 204.83 | 39.65 |

Table II. Means and standard deviations of plasma cholesterol values among students, classified after the type of their diet; N = number of subjects

| | No ghee | Ghee |
|---|---|---|
| Vegetarians | N = 11 $179.8 \pm 28.8$ | N = 16 $225.3 \pm 31.9$ |
| Non-vegetarians | N = 49 $208.2 \pm 42.9$ | N = 14 $190.0 \pm 30.4$ |

Contrary to our expectation in a previous study[1], a familial diabetic background does not seem to be associated with hypercholesterolaemia: 23 healthy students who reveal one or several diabetics in their families have an average of $207.9 \pm 25.2$ mg of plasma cholesterol, while 66 students with no familial diabetics have almost an identical value equal to $202.7 \pm 43.6$ mg of cholesterol per 100 ml of plasma. However, the variances of the two groups are vastly different ($P < 0.01$). Further analysis will be published later.

*Résumé.* Le cholestérol plasmatique a été dosé chez 193 jeunes Indiens de Madras. Plus leur niveau économique est bas, plus leur cholestérolémie est faible. Chez les sujets végétariens de niveau économique élevé, la présence de ghee, une sorte de beurre clarifié, dans la nourriture est associée à une cholestérolémie élevée. Les variations quantitatives et qualitatives de la nourriture semblent modifier profondément la cholestérolémie des Indiens.

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## Primary Visual Potentials in Man During Repetitive Photic Stimulation

The reduction of amplitude and the disappearance of VEP (visual evoked potentials) during prolonged repetitive photic stimulation, observed in animal experiments[1] and referred to as 'habituation', was held probably to be caused by central inhibitory mechanisms[1]. VEP have been recorded from the scalp in man, by means of integrative methods or superposition of sweeps, and the phenomenon of visual 'habituation' was held to be present also in the human[2]. However, successive observations[3] suggested that, in animals at least, visual 'habituation' was caused by a very marked pupillary constriction. In fact, if pupillary constriction is prevented by atropine and darkened contact lenses with artificial fissurated pupils, neither reduction nor disappearance of VEP are observed[4].

The aim of this investigation is to study the modifications and the variability of primary VEP in man during repetitive photic stimulation, and its relationship to the pupillary diameter. The recordings have been performed

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[3] A. Fernández-Guardiola, E. Roldan, M. L. Fanjul, and C. Castells, EEG clin. Neurophysiol. *13*, 564 (1961).

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in patients who underwent operation for Parkinson's disease. At the end of the operation a hollow screw was applied to the occipital bone[5]; in case of early recurrence an electrode can be reintroduced to hit and coagulate again the target area (nucleus ventralis lateralis thalami). The recordings discussed in this paper were carried out on patients who underwent further coagulations with this technique. The tip of the electrode (which was inserted through the occipital bone, 15 to 17 mm laterally from the midline and 10 to 20 mm above the protuberance) before reaching the deep target area, was placed in the optic radiations and/or in the visual cortex, so as to record primary VEP. The recordings were performed in a dark, quiet room. The pupils were dilated with atropine. The patient was instructed to keep his head steady, his eyelids closed and his gaze towards the stimulus. The photic stimulation consisted of flashes of yellow light by a Xenon-filled tube (Lumitron photostimulator) placed at 30 cm from the face of the patient. Intensity and frequency of flashes (70 to 90 per min) were kept constant throughout the period of the experiment, which lasted 60 min. The VEP were recorded with a Wyss monopolar recording-stimulating electrode[6] on a cathode-ray oscilloscope (Polyscope-Horstfehr) and continuously checked on the oscilloscope screen: every 3 to 5 min, 5 to 15 series of 10 responses each were photographed by superposition technique.

The primary VEP were clearly visible during the experiments. Neither marked reduction nor disappearance of the response was observed. The latency of VEP underwent no meaningful changes (Figure 1). With our technique we could observe, on some patients only, a very slight reduction (less than 10%) in amplitude of some series of evoked responses; this seems to be independent from the attention or distraction of the patient (Figure 1).

The recording of long series of successive identical visual responses in man is difficult; this fact must be remembered before discussing our results. The response in man varies continuously in amplitude; this may be caused by very slight movements of the head and eyes (Figure 2).

In conclusion we have been able to demonstrate that in atropinized men, during repetitive photic stimulation, disappearance of primary VEP never occurs.

Our results do not confirm other reported observations in man[2]. Two points may perhaps explain this discrepancy:

(1) Experiments in man were performed in non-atropinized patients[2], while in our cases photic stimulation was started only after a maximal mydriasis had been provoked in both eyes with atropine.

(2) At the scalp, a very complex response of great latency and of extensive topographic distribution was recorded[2]. These 'facts make it unlikely that the recorded response be in every case made up of the primary response'[7].

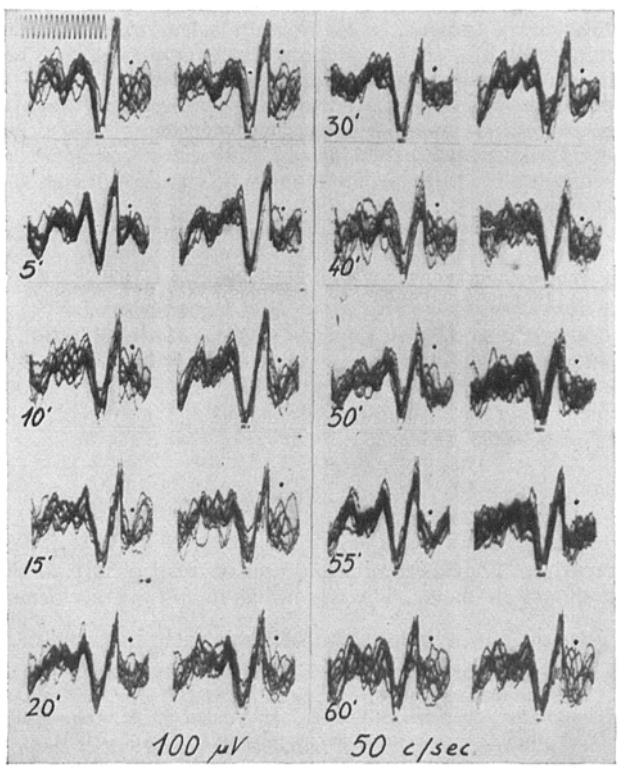


Fig. 1. Evoked responses recorded from the primary visual area, during photic repetitive stimulation, lasted 60 min. The responses were photographed by superposition technique. They were recorded at the very beginning of the experiment (upper left) and later after 5, 10, 15, 20, 30, 40, 50, 55, 60 min of repetitive photic stimulation. Two series of 10 responses each are reported. During the whole experiment the primary visual evoked response was clearly identifiable and never disappeared. Only a very slight reduction in voltage of the primary visual evoked response was noted. There were no modifications in the latency (stimulus artifact is marked with a point). The recordings must be read from right to left.

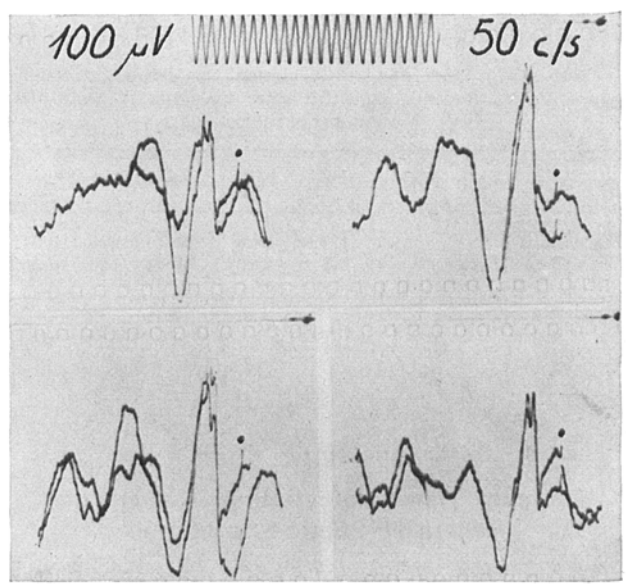


Fig. 2. 8 successive primary visual evoked responses obtained with rhythmical stimulation at 1 per sec recorded in the same patient as Figure 1 before 'habituation' experiment. The evoked responses have been photographed, superposing two at a time. Note the great variability in amplitude and morphology in the various evoked responses.

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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 $\beta$-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 $\beta$) 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.

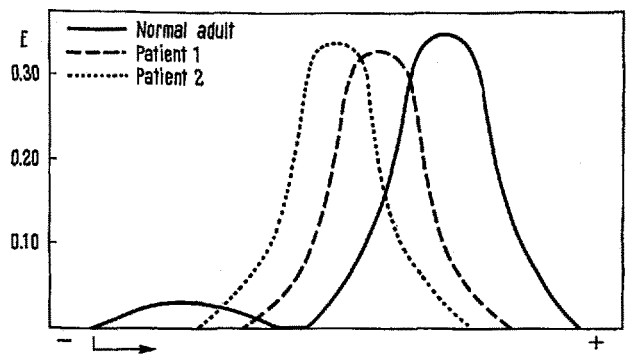


Fig. 1. Electrophoretic diagrams of the Hb extracted from the erythropoietic cells of two patients affected with Cooley's disease. For purposes of comparison, an electrophoretic diagram of Hb of a normal adult is also reproduced.

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