The rats were then killed by decapitation and the livers removed and stored at −15°C. The plasma cholesterol was determined according to the method of PEARSON et al.[13] and liver cholesterol, according to that of SPERRY and WEBB[14]. Fats were determined by the method outlined by HANANHAN et al.[15].

Results. A comparison of the results obtained using diets with the same nitrogen content, with and without lysine and threonine supplementation, appears in Table II. As shown, the rice diet deficient in lysine (first limiting amino acid) and threonine (second physiologically limiting amino acid) induces, in growing rats, in addition to a lower growth rate, fatty liver of the portal type as seen in kwashiorkor and an increase of total liver cholesterol. In these conditions plasma cholesterol is low. Lysine and threonine supplements are effective in promoting growth and in preventing the accumulation of fats in the liver, and, while the liver cholesterol is lowered, the plasma level is increased.

Our results on plasma cholesterol are in agreement with those found by SCHENDEL and HANSEN in kwashiorkor[9] and KEMPNER[6] and OLSON et al. in man[7].

In regard to SINGAL's[10] result with amino acid ration, our data are in agreement with his findings on liver cholesterol. The failure to find variations in serum cholesterol under those conditions might be ascribed to the use of different diets.

Discussion. Since plasma cholesterol is lower in deficient rats than in those with lysine and threonine, while the opposite is observed regarding liver cholesterol, the phenomenon, as a whole, supports the view that serum lipoproteins transporting cholesterol are not elaborated at normal rates in amino-acid-deficient rats as in children with kwashiorkor. In addition to choline[12], methionine[16], and EFA[17]; the amino acids lysine and threonine also seem to be able to control the cholesterol transport from liver to blood in the rat.

If we also consider that the protein level acts on the biosynthesis and catabolism of liver cholesterol[2,3], it seems possible that the variations we observed in the liver may even depend on these mechanisms.

In view of the fact that our results are in conflict with those of JOHNSON et al.[4] and KOKATNUR et al.[5], who found that the growth failure in amino acid deficient chickens is associated with an increase in serum cholesterol, it would appear that uricotelic animals react differently to ureotelic animals in essential amino acid deficiency to cholesterolemic response.

Therefore, the rat seems to be a suitable animal for studies of deficiency of, balance of, and imbalance of essential amino acids in cholesterol metabolism, if the results are to be referred to man.

Riassunto. È stato studiato l'effetto di diete ipoproteiche e isoazotate carenti e supplementate in lisina e treonina sul contenuto in colesterolo del fegato e del plasma.

La carenza di aminoacidi essenziali induce negli animali più elevati livelli di colesterolo nel fegato, mentre diminuisce il colesterolo del plasma.

In queste condizioni il ratto dimostra un comportamento simile all'uomo.

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Department of Biological Chemistry, University of Bologna (Italy), December 3, 1962.

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Table II. Effect of supplementation of rice diet with lysine and threonine on growth, total lipid content of the liver, liver and blood plasma cholesterol content (Mean values ± Standard Deviation)

Diet	Average weight in g	Total liver lipids g/100 g liver	Total plasma cholesterol mg/100 ml	Total liver cholesterol mg/100 g tissue
No. 1	74.5 ± 12.3	9.49 ± 1.5	111.4 ± 14.3	367 ± 55.8
No. 2	144.8 ± 27.9	5.93 ± 0.72	130.3 ± 12.8[a]	203 ± 46.5[b]

[a] Significantly different from values of diet No. 1. $P = < 0.01$.

[b] Significantly different from values of diet No. 1. $P = < 0.01$.

Somatic Evoked Responses in Cats during Natural Sleep[1]

The aim of the present research is to study the patterns of somatic evoked responses in some central nervous structures of the cat during natural sleep. Such an experimental approach seems justified in view of some recent studies[2] which have shown that sleep can be subdivided into two fundamental phases: (i) light sleep, which is characterized by EEG slow waves (2–4/sec) intermingled with spindles (8–15/sec), moderate muscular tonus and basal blood pressure level; (ii) deep sleep, which is characterized by EEG low voltage fast activity, complete muscular relaxation and a marked decrease in blood pressure.

Material and Methods. The experiments were carried out on 30 adult, unanaesthetized, freely moving cats, carrying stimulating and recording electrodes previously introduced aseptically during barbiturate anaesthesia. It

[1] Part of these results has been presented at a meeting of the Società Italiana di Biologia Sperimentale held in Genova on July 25th 1962.
[2] O. CANDIA, E. FAVALE, A. GIUSSANI, and G. F. ROSSI, Arch. Ital. Biol. *100*, 216 (1962).

should be noted that: (a) the electrodes for bipolar recording (epidural) of the cortical responses consisted of two metal screws, isolated except for their tips, which were fixed into the floor of the frontal sinuses, just above the somatic sensory cortex; (b) recording of the EMG activity was made by means of two thin steel wires passing through the posterior muscles of the neck; (c) the peripheral stimulation, consisting of square pulses (0.1 msec in duration, 0.4–0.8 V in amplitude, frequency 0.2/sec), was given through bipolar electrodes inserted into the subcutaneous tissue of one foreleg. The stimulation was given for periods lasting from 40 to 80 sec at 5 to 10 min intervals, in order to prevent habituation; (d) central stimulation was effected by means of bipolar electrodes inserted stereotaxically into the medial lemniscus and the ventropostero-lateral nucleus of the thalamus, and consisted of square pulses (0.1–0.5 msec, 1–8 V, 0.2–5/sec), which were usually given for periods lasting from 40 to 80 sec, at 2 to 5 min intervals. It should be pointed out that the results did not vary substantially when the stimulation lasted for uninterrupted periods of 5 to 10 min; (e) the responses evoked by peripheral stimulation were recorded simultaneously from the medial lemniscus, the ventro-postero-lateral nucleus of the thalamus, the somatesthetic radiation and the cortex. Simultaneous recordings of the responses evoked in the thalamus, the somatesthetic radiation and the cortex by stimulation of the medial lemniscus were made during the same experimental session; (f) the records were begun not less than 48 h after the operation and were carried on for an average of one week. During the experiment the animal was kept in a small sound-proof cage where it was able to move freely. The animal's behaviour was controlled both directly and by means of simultaneous recording of the EEG and EMG activity; (g) the precise anatomical location of the electrodes was verified *post mortem* on sections of the brain stained according to Weil and Nissl methods; (h) the evoked responses were recorded photographically from a dual-beam Cossor oscilloscope driven by a Galileo a-c amplifier. The EEG and EMG activity was recorded by a Galileo 10-channel electroencephalograph. Two Tektronix stimulators were used.

Results. These will be divided into two parts: (A) Description of the changes occurring in the cortical responses evoked by peripheral and central stimulation during the different stages of sleep; (B) Description of the data yielded by the comparative evaluation of both cortical and subcortical evoked responses. (A, 1) Cortical responses evoked by peripheral stimulation have a latency varying from 7.3 to 8.6 msec. Both shape and amplitude of the responses appeared to vary greatly according to the depth of the sleep. In particular, it was noticed that: (a) during light sleep the amplitude of the responses, although varying greatly, could on the whole be defined as moderate. Morphologically, they consisted of a positive–negative deflection, the negative component being always smaller than the positive one and sometimes scarcely discernible (Figure I, A); (b) during deep sleep the responses were always facilitated, their increase in amplitude ranging from 30 to 300% above the mean amplitude values of light sleep. The increase in amplitude was almost the same for both positive and negative phases (Figure I, B). This facilitation lasted throughout the deep sleep stage, being occasionally quite remarkable. (A, 2) The stimulation of the medial lemniscus and the ventro-postero-lateral nucleus of the thalamus evoked on the somatic sensory cortex responses having a latency varying from 3 to 4 and from 2 to 3.5 msec respectively. Amplitude and shape of the responses appeared to vary considerably according to the stage of sleep. In particular, it was noticed that: (a) during light sleep both lemnisco-cortical and thalamo-cortical responses had a very low amplitude, being sometimes scarcely visible. They consisted of a positive–negative deflection, their negative component being smaller than the positive one, occasionally even completely absent (Figure II, A and III, A); (b) during deep sleep the responses increased greatly in amplitude (from 200 to 400%), such increase being almost equally divided between the two components of the response (Figure II, B and III, B). This facilitation lasted throughout the deep sleep stage, being sometimes very prominent.

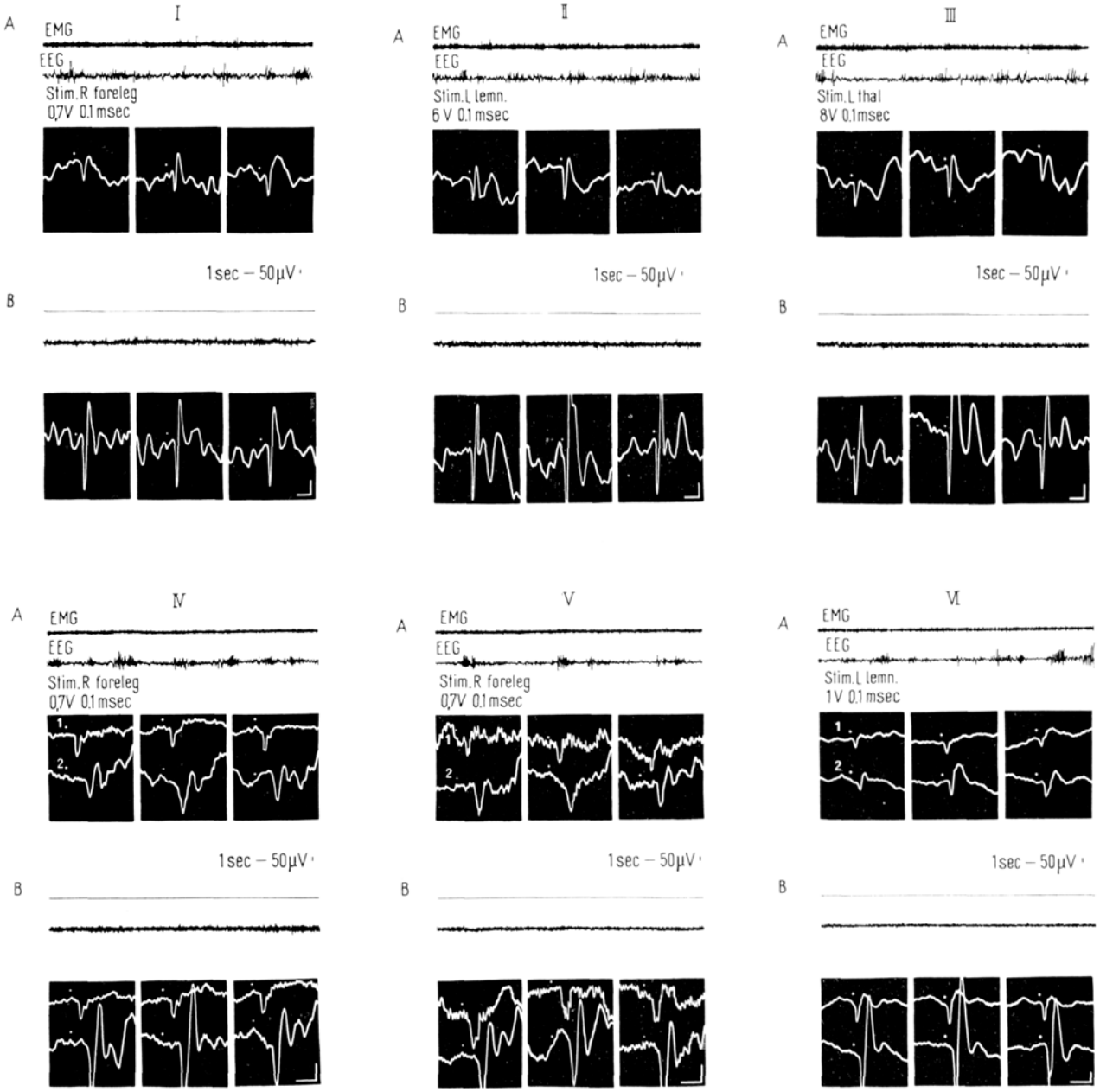
(B) With the aim of assessing the respective roles of the different stations of the afferent pathway (bulbar, thalamic, cortical) in determining such a facilitation, we recorded simultaneously the responses from the ventro-postero-lateral nucleus of the thalamus, the somatesthetic radiation and the somatic sensory cortex when the medial lemniscus was stimulated. During peripheral stimulation responses from the medial lemniscus were recorded as well. Our results can be summarized as follows: (B, 1) the peripheral stimulation evoked in the lemniscus, thalamus, radiation and cortex responses having latencies of 3–3.7, 3.7–4.5, 5–6.5 and 7.3–8.6 msec respectively. It was noticed that: (a) during light sleep lemniscal (Figure IV, A), thalamic (Figure V, A) and radiation responses consisted of a fast positive deflection of small amplitude, sometimes followed by a slower negative deflection, usually hardly measurable; the cortical responses have been described above; (b) during deep sleep, lemniscal responses remained practically unchanged, without paralleling in any way the marked facilitation of the cortical responses (Figure IV, B). On the contrary, both thalamic and radiation responses were constantly facilitated (from 20 to 50%), their shape being unchanged (Figure V, B). The facilitation of the thalamic responses ensued simultaneously with that of the cortical ones, lasting throughout the deep sleep. It should be pointed out, however, that there seemed to be a certain independence between the facilitation of the thalamic responses and that of the cortical ones. In fact, sometimes a marked facilitation of the cortical responses was accompanied by a slight facilitation of the thalamic ones, and *vice versa*, both these possibilities occurring not only in the same animal but also during one single spell of deep sleep. Apart from these slight occasional discrepancies, it might justifiably be said that during deep sleep there exists a constant parallelism between the facilitation of the thalamic responses and that of the cortical ones. The same considerations hold true as to the responses evoked in the somatesthetic radiation. (B, 2) The stimulation of the medial lemniscus evoked in the ventro-postero-lateral nucleus of the thalamus, the somatesthetic radiation and the somatic sensory cortex responses having latencies varying from 1 to 1.5, 1.5 to 2.5 and 3 to 4 sec respectively. With regard to the different phases of sleep, it has been observed that: (a) during light sleep, both lemnisco-thalamic and lemnisco-radiation responses (Figure VI, A) were constituted by a fast deflection very low in amplitude, often hardly detectable. The lemnisco-cortical responses have been described above; (b) during deep sleep, both lemnisco-thalamic and lemnisco-radiation responses (Figure VI, B) showed constantly a marked facilitation which persisted throughout the deep sleep phase; in particular, with respect to its course and entity, the facilitation of these responses closely resembled the cortical one.

Summing up, during deep sleep a marked facilitation of the responses evoked by central stimuli was observed both in the cortex and in the thalamus. On the other hand, during the peripheral stimulation in the deep sleep phase

the evoked responses appeared to be facilitated both in the cortex and in the thalamus, but remained unchanged in the medial lemniscus. In particular, a statistical evaluation (carried out by the student 't' test) of the mean amplitude values of the responses obtained during light sleep and deep sleep showed that: (a) the increase in amplitude of the responses evoked in the thalamus both by central and by peripheral stimulation proved constantly highly significant ($P < 0.001$); (b) in more than 90% of the cases the responses evoked upon peripheral stimulation in the medial lemniscus did not show statistically significant variations in amplitude.

Such results enable us to state that during deep sleep the transmission of somatic centripetal impulses, either during peripheral or central stimulation, was constantly facilitated at the level of the ventro-postero-lateral thalamic nucleus. The first synaptical relay—i.e. nuclei gracilis and cuneatus—on the contrary, did not seem to play any relevant role in the facilitated transmission of the ascending volley during deep sleep.

While the importance of the thalamus in determining the cortical facilitation of the evoked response seems to be well established, no hint is provided by the present data as to the possible role played by the cortex in such a mechanism. The latter could be verified in further researches by means of direct stimulation of the somatesthetic radiation. In such a case, in fact, a possible facilitation of the responses would certainly not be due to a



EMG = electromyogram. EEG = electroencephalogram. In I, II, and III CRO recording from the left somatic sensory cortex; in IV from the left medial lemniscus (1) and the left somatic sensory cortex (2); in V from the left ventro-posterolateral nucleus of the thalamus (1) and the left somatic sensory cortex (2); in VI from the left somatesthetic radiation (1) and the left somatic sensory cortex (2). (See text for explanations.) CRO calibration: 50 μV. Time base: upper half of the figure, 20 msec; lower half of the figure, 10 msec.

thalamic mechanism and could therefore be attributed only to the cortex.

The facilitated transmission of the ascending impulses in the ventro-postero-lateral nucleus of the thalamus could be accounted for either by a functional depression of structures controlling the transmission of the centripetal volley during light sleep or by the intervention of mechanisms actively facilitating the transmission of the impulses in the thalamus during deep sleep.

Riassunto. Le risposte corticali evocate da stimoli somatici, sia periferici che centrali, aumentano di ampiezza durante il sonno profondo. Tale amplificazione è dovuta, almeno in parte, ad una facilitata trasmissione degli impulsi ascendenti a livello del nucleo ventro-postero-laterale del talamo.

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b-Nucleic Acid, and the Initial Step of Deoxyribonucleic Acid-Degradation by Deoxyribonuclease I

In 1935, FEULGEN[1] observed that the solution of DNA[2] prepared according to his own method[3] was strongly liquefied by the action of pancreatin without the liberation of purines and phosphates, and the reaction product, which was obtained in high yield, yet maintained nearly the same acid-precipitability as that of the parent DNA. He called this substance b-nucleic acid, and the enzyme concerned nucleogelase.

For some time we have also studied the nucleogelase reaction and obtained the following results. To 0.4 ml of 1% solution of Feulgen's DNA in $M/10$ acetate buffer (pH 6.0) was added 0.1 ml of enzyme solutions of various activities by extracting NBC's pancreatin with water, and the viscosity change of the reaction mixture was observed. The relative viscosity which was at first ca. 4, dropped to the final value of ca. 1.2 after various time passages, according to the concentrations of nucleogelase. An aliquot of the reaction mixture was poured into 30 vol of cold $0.25N$ HCl and the resultant precipitate was dissolved in $M/15$ Na_2HPO_4; then an aliquot of this solution was subjected to paper electrophoresis (200 V, 4 mA, the current flow lasting 2 h, the electrolyte solution used being $M/50$ acetate buffer of pH 5.0). After the finish the paper was observed with mineralight Model SL 2537. The original DNA of FEULGEN did not move from the starting line, while b-acid showed a circumscribed sharp shadow having a fairly large migration rate. In accordance with the drop in the viscosity of the reaction mixture the spot of the original DNA detectable by paper electrophoresis diminished rapidly, while that of b-acid increased quickly. When crystalline DNase 1 was used instead of pancreatin without Mg^{++}, the same electrophoretic pattern as above was observed, suggesting that FEULGEN's nucleogelase might be the same enzyme with DNase 1. Therefore, more detailed comparisons of the two enzymes were carried out as follows. A solution of DNase 1 and the nucleogelase solution prepared as described above were fractionated with ammonium sulphate, and every fraction of both enzymes was compared in DNA-liquefying activity. As indicated in Figure 1 (a), the enzyme activity in both cases was found to be concentrated on the fraction between 57 and 71% ammonium sulphate saturation. As the next step, the b-acid-decomposing activity of nucleogelase was compared with that of DNase.

To 1% solution of FEULGEN's DNA dissolved in $M/10$ acetate buffer of pH 6.0 was added each of the following solutions containing, in liquefying activity order, DNase 1(10 γ/ml) > pancreatin extract > DNase 1(5 γ/ml) respectively. After incubation, an aliquot was taken from each reaction mixture and poured into 30 vol of $0.25N$ HCl and centrifuged. The supernatant solution was investigated for its light absorbency at 260 mμ in order to estimate the degradation of b-acid. As indicated in Figure 1 (b), no difference was seen between the b-acid decomposing activity of nucleogelase and that of DNase 1. Furthermore, the pH-activation curve of liquefying activity (production of b-acid) and that of b-acid decomposing were compared between these two enzymes. For this purpose, DNA or b-nucleic acid was dissolved to 1% in phosphate buffer of various pH's as indicated in Figure 1 (c), and to this solution was added nucleogelase solution

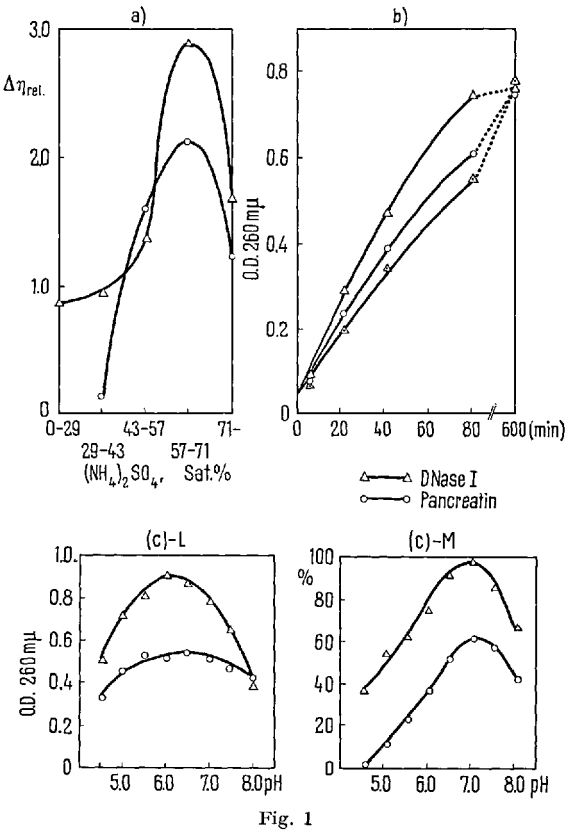

Fig. 1

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[2] Abbreviations: DNA, deoxyribonucleic acid; DNase 1, deoxyribonuclease 1; NBC, Nutritional Biochemicals Corporation, Cleveland, Ohio; OD, optical density; P, phosphate.

[3] R. FEULGEN, Z. physiol. Chem. *90*, 261 (1914).