range both on the non-effected characteristic (point B) and for point C on the characteristic for baropacing.

For intermittent stimulation of a CSN, however, apart from the blood pressure reduction, also the measuring range was displaced towards lower pressure values, as compared to the non-effected characteristics. Results of such a test are plotted in Figure 3. When with this animal the blood pressure is decreased from 160 mm Hg (point A) to 120 mm Hg, the control range is almost completely exhausted for the noneffected characteristic (point B). However, for a stimulation of the CSN with pulse-synchronous impulse trains, a mid-position of the operating point is maintained with regard to the static characteristic, as is indicated by point C. Therefore, further reduction in blood pressure can be compensated with intermittent stimulation of the CSN, in contrast to the continuous stimulation, Even for a continuation of the intermittent stimulation over a period of 40 min, no trend of back-displacement of the characteristic towards the non-effected characteristics was observed. The stimulation was effected with impulse trains of 100 msec duration, which started 30 msec after the R-wave of the E.C.G.; the impulse frequency was 40 impulses/sec; the amplitude was 2 volts and the duration of single impulse 0.5 msec. The selection of the parameters of stimulation, the time interval between stimulation and R-wave of the E.C.G. (WARZEL and BRATTSTRÖM[14]) and the impulse train duration had been dealt with in preceding studies.

According to the results presented, the intermittent stimulation of the CSN appears to be superior to baropacing with continuous impulse frequency. With pulse-synchronous triggering of the impulse trains, the static characteristic of the blood pressure control system is displaced towards lower ISP values (input pressure), without any adaptation being perceptible. The arterial pressure reduced by baropacing keeps its mid-position with regard to the characteristic. In contrast to the findings for continuous stimulation, sufficient buffering capacity exists both in case of reduction and increase in blood pressure.

Zusammenfassung. Bei narkotisierten Hunden wurde der Karotissinusnerv sowohl mit Dauerimpulsen als auch mit R-Zacken-gesteuerten Impulszügen elektrisch gereizt. Die Blutdruckcharakteristik zeigt, dass der Messbereich des Kreislaufreglers unter kontinuierlicher und ohne Reizung bei etwa gleichen Druckwerten liegt. Die Reizung mittels Impulszügen führt dagegen zu einer Verlagerung des Messbereiches zu niederen endosinualen Drücken.

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DNA Synthesis and Distribution of First and Second Divisions in 72-h-Cultures of Human Leukocytes

Cytological observations have demonstrated that in whole, unmanipulated blood, the level of DNA synthesis is very low and mitoses are undetectable[1]. Based on studies of in vitro incorporation of tritiated thymidine, it has been estimated that only 0.06% of unstimulated lymphocytes of human peripheral blood can synthesize DNA[2]. A significant DNA synthesis has been recorded in phytohemagglutinin (PHA) stimulated cultures about 30 h after initiation[3].

The purpose of this work has been to collect information on DNA synthesis in human 3-day leukocyte cultures. Cytological observations on mitoses and interphases at 48 and 72 h have been used in order to study the dynamics of this system. An estimate has been made of the distribution of first and second divisions at 72 h.

Materials and methods. The experiments have been carried out with blood cells from a normal 24-year-old male. The cells were grown as described by BRØGGER[4]. Tritiated thymidine (spec. activity 2.0 Ci/m*M*) was obtained from the Radiochemical Centre (Amersham, England). For the study of DNA synthesis, measurements of incorporated tritiated thymidine were made in a Beckman LS-150 scintillation counter. For the study of mitoses and interphases, 31-h-old cultures were exposed to tritiated thymidine (1 μCi/ml) for 2 h. Autoradiographs were made from cultures harvested at 48 and 72 h (colcemid treatment for the last 2 h).

Mitoses were classified by counting silver grains over metaphase chromosomes. A metaphase was scored as labelled if it contained 5 or more grains over the 2 longest chromosomes (No. 1) and unlabelled in all other cases. The labelled metaphases were scored as first or second divisions depending on the distribution of grains over one or both chromatids. Interphase nuclei were scored as labelled or unlabelled according to the relative number of grains over the nuclei compared to the background.

Results. Two similar experiments were made, and the rate of the DNA synthesis at different times is shown in the Figure. A shoulder on the curves can be seen at about 40 h, after which the incorporation increases steadily.

The distribution of metaphases from cultures harvested at 48 and 72 h are shown in Table I. Provided that not any of the unlabelled cells have finished their DNA synthesis before 31 h, the S- and G2-period of unlabelled cells at 48 h is 15 h or shorter.

Cells showing labelling at 48 h would do the same at 72 h. The distribution of first and second divisions among these cells can easily be decided. However, it is impossible directly to differentiate among the unlabelled mitoses at 72 h. Their distribution has been estimated in the following way.

At 48 h $0.68/(84.8 + 0.68)$ of the unlabelled cells appear as unlabelled mitoses. Presuming that only cells which are in mitosis at 48 h could appear as second divisions 24 h

[1] M. A. BENDER and D. M. PRESCOTT, Expl. Cell Res. *27*, 221 (1962).
[2] V. P. BOND, E. P. CRONKITE, T. M. FLIENDER and P. SCHORK, Science *128*, 202 (1958).
[3] A. MICHALOWSKI, Expl. Cell Res. *32*, 609 (1963).
[4] A. BRØGGER, *Translocation in Human Chromosomes* (Universitetsforlaget, Oslo 1967).

later, while interphases at 48 h could appear as first divisions only, and that each unlabelled cell has the same probability of having entered mitosis at 72 h, the frequency of unlabelled second divisions after 3 days cultivation can be estimated as $(2\times0.68/(84.8 + 2\times0.68))\times 2.55\% \simeq 0.04\%$.

One interphase at 48 h can give one first division at 72 h, while one mitosis at 48 h can give two second divisions or interphases at 72 h. Therefore, the unlabelled mitoses at 48 h amount to approximately as large a part of the unlabelled cells as half of the unlabelled second divisions amount to of the unlabelled mitoses at 72 h. After 72 h cultivation $(0.34 + 0.04)/(2.55 + 0.51 + 0.34)$ or 1 mitosis out of 9 is estimated to be a second division, whereas the others will be first divisions.

Table I. Distribution of metaphases in PHA stimulated human leukocyte cultures harvested at 48 and 72 h (colcemid treatment for the last 2 h)

Harvesting (h)	Metaphases analyzed	Metaphase number and percentage		
		Unlabelled	Labelled	
			1st division	2nd division
48	235	93 (40)	142 (60)	0 (0)
72	408[a]	304 (75)	62 (15)	40 (10)

The cultures were exposed to tritiated thymidine for 2 h from 31 h. Autoradiographs were developed and examined in the microscope. [a] Two cells were scored as unclassified.

Table II. Percentage distribution of interphases and mitoses in PHA stimulated human leukocyte cultures harvested at 48 and 72 h (colcemid treatment for the last 2 h)

Harvesting	Interphases		Mitoses		
(h)	Unlab.	Lab.	Unlab.	Lab.	
				1st division	2nd division
48	84.8	13.5	0.68	1.02	0
72	87.3	9.3	2.55	0.51	0.34

The cultures were exposed to tritiated thymidine for 2 h from 31 h. Autoradiographs were developed and 1000 cells at each harvesting time were examined under the microscope. The cells were scored as unlabelled and labelled interphases and mitoses. The distributions of the mitoses are based on the distributions of metaphases shown in Table I.

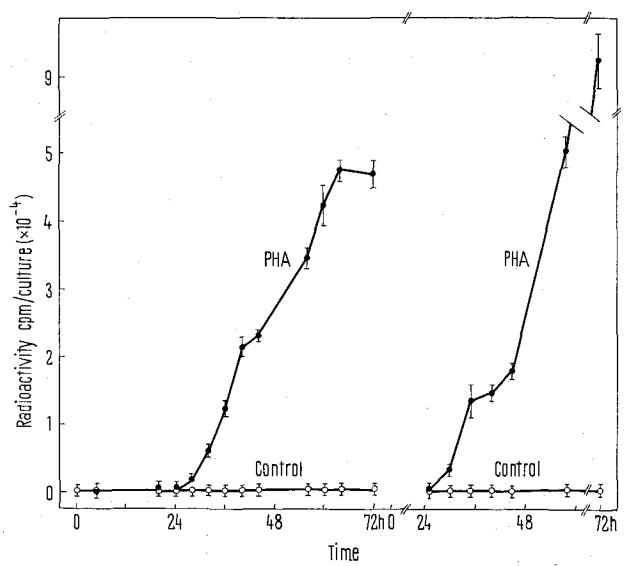
Discussion. The unstimulated cultures incorporate ^{3}H-thymidine to a minimal extent from the beginning up to 72 h, and the sample counts of these cultures do not exceed those of the blanks appreciably. This is consistent with the results of BENDER and PRESCOTT[1] and BOND et al.[2].

In accordance with data obtained by MICHALOWSKI[3], it is found that cells in PHA stimulated cultures start a considerable DNA synthesis about 24 h after initiation of the culture. As mitoses are frequent at 48 h, the significance of the shoulder can be disregarded. If the S-period lasts for 12–30 h and the G2-period for a maximum of 6 h[1], most of the cells which have entered mitosis at 48 h must have finished their DNA synthesis at about the time the shoulder appears. The mitosis is presumed to last for not more than about half an hour[5].

Figure 1 in the report of MICHALOWSKI[3] shows a shoulder on the synthesis rate curve between 40 and 48 h similar to what I have found. This result, together with my own data, seems to indicate the presence of 2 or more PHA stimulated cell populations in the cultures.

BENDER and PRESCOTT[1] found that the first mitoses appeared about 42 h after PHA stimulation and incubation. Addition of tritiated thymidine at 31 h and washing 2 h later revealed labelled as well as unlabelled mitoses at 48 h. It is not likely that any of the unlabelled mitoses would have finished their S-period before the radioactivity was administered. These cells must have started S-period after 33 h. Choosing a later period for radioactive administration, this would perhaps cause labelling of all mitoses appearing at 48 h. Second divisions at 72 h would then presumably be easier to determine.

At least two PHA stimulated cell populations seem to be present in human leukocyte cultures. One of these is composed of early dividing cells (48 h) while cells from another population show first divisions approximately 24 h later.



DNA synthesis in PHA stimulated and unstimulated (control) human leukocyte cultures. The synthesis rate curves of 2 similar experiments are shown. At each point 6 cultures (3 stimulated and 3 unstimulated) were given ^{3}H-thymidine (1 μCi/ml) and harvested 2 h later. After dissolving DNA, 2 samples from each culture were counted, the average estimated, and the average counts of the background (blanks) subtracted. The radioactivity (cpm/culture) was then calculated and plotted in the figure together with the standard deviation.

Zusammenfassung. Lymphocyten eines gesunden Erwachsenen lassen ohne Stimulation keine messbare DNS-Synthese erkennen. Nach Stimulation durch Phytohaemagglutinin finden am 2. und 3. Tag je eine Phase intensiver DNS-Synthese statt, was auf die Anwesenheit von mindestens zwei verschiedenen Zellpopulationen hinweist.

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[6] Grateful acknowledgment is made to Dr. A. BRØGGER and Dr. P. EKER for their kind advice and criticism.