Tab. II. Vergleich der Acetoin-, 2,3-Butylenglykol- und Brenztraubensäurewerte in Blut und Liquor von Nierenpatienten (R), Leberpatienten (H), Patienten mit *Diabetes mellitus* (D) und einer gemischten Krankheitsgruppe (M). C(Liquor): Konzentration im Liquor; C(Blut): Konzentration im Blut. N: Anzahl der Bestimmungen

	Bestimmung	N	C(Liquor) = C(Blut) N	C(Liquor) > C(Blut) N	C(Liquor) < C(Blut) N
Total (R, H, D, M)	Acetoin	41	10	18	13
	2,3-Butylenglykol	38	3	5	30
	Brenztraubensäure	39	5	19	15
Nierenpatienten (R)	Acetoin	12	5	4	3
	2,3-Butylenglykol	11	1	2	8
	Brenztraubensäure	11	0	10	1
Leberpatienten (H)	Acetoin	9	3	5	1
	2,3-Butylenglykol	9	1	2	6
	Brenztraubensäure	9	1	2	6

in 4 Stadien eingeteilt: I. Keine Bewusstseinsstörung, IV. Stärkste Bewusstseinsstörung.

Resultate. BTS, AMC und BD in Blut von Nierenpatienten (Tabelle I): Patienten mit Bewusstseinsstörungen haben durchschnittlich erhöhte BTS-, AMC- und BD-Werte. Die höchsten Konzentrationen von AMC wurden bei Patienten mit mittelschweren Bewusstseinsstörungen (Stadium III), die höchsten BD-Konzentrationen bei Patienten mit leichten Bewusstseinsstörungen (Stadium II) beobachtet.

BTS, AMC und BD in Blut von Leberpatienten (Tabelle I): BTS-Werte sind bei Patienten mit Bewusstseinsstörungen erhöht. Die durchschnittlichen Acetoin-Konzentrationen nehmen parallel zum Grad der Bewusstseinsstörungen zu. Patienten mit mittelschweren und schweren Bewusstseinsstörungen haben im Durchschnitt sehr hohe BD-Konzentrationen.

Vergleich der BTS-, AMC- und BD-Konzentrationen in Blut und Liquor von Nierenpatienten, Leberpatienten und Patienten mit verschiedenen Affektionen (Tabelle II). Von 39 BTS-Bestimmungen zeigten 19 im Liquor höhere Werte als im Blut und 15 im Blut höhere Werte als im Liquor. 10 von 11 urämischen Patienten hatten im Liquor höhere BTS-Konzentrationen als im Blut. Die AMC-Werte waren im Liquor in 18 Fällen höher, in 13 Fällen niedriger als im Blut. 10mal wurden zwischen Blut und Liquor keine Unterschiede festgestellt. 30 von 38 Patienten wiesen im Liquor höhere BD-Werte auf. 3mal wurden keine Unterschiede gefunden.

Diskussion. Nieren- und Leberpatienten haben erhöhte AMC- und BD-Werte im Blut, wenn Bewusstseinsstörungen vorhanden sind. Diese Befunde können bei Leber- und Nierenpatienten auf die Intensivierung des anoxydativen BTS-Abbaus zurückgeführt werden. Bei der Erhöhung der AMC- und BD-Blutwerte bei Urämikern kommt ausserdem der Retention dieser beiden Substanzen eine gewisse Bedeutung zu. Ob die Intensivierung der AMC-Synthese die Folge einer vermehrten intermediären BTS-Bildung darstellt oder ob sie durch eine Störung des oxydativen BTS-Abbaus verursacht wird, bleibt abzuklären.

Aus unseren Befunden geht nicht hervor, ob AMC und BD in der Pathogenese hepatischer und urämischer Bewusstseinsstörungen eine Rolle spielen, da zwischen der Höhe der Blutkonzentrationen von AMC und BD einerseits und dem Grad der Bewusstseinsstörungen andererseits keine Korrelation besteht. Es ist zwar sehr fraglich, ob sich Störungen des Gehirnstoffwechsels auch im peripheren Blut manifestieren. Man muss wohl eher annehmen, dass in ihrer Wirkungsweise unbekannte Schrankenmechanismen zwischen Blut und Gehirn, Blut und Liquor, Liquor und Gehirn existieren, die die Erkennung metabolischer Veränderungen des Gehirns im peripheren Blut verunmöglichen. Von den erwähnten Schrankenmechanismen des Zentralnervensystems können bei Menschen nur die Beziehungen zwischen Blut- und Liquorraum untersucht werden. Unsere Untersuchungen ergaben in 85% Konzentrationsunterschiede für BTS, AMC und BD zwischen Blut und Liquor. Diese Befunde deuten möglicherweise auf ein Barrierenphänomen für die erwähnten Substanzen an der Blut-Liquorgrenze hin. D.h., Bildung und Abbau der BTS in Blut- und Liquorraum sind unter den untersuchten pathologischen Bedingungen verschieden. Obwohl Untersuchungen des Liquorraumes wahrscheinlich keine Aussage über den Hirnstoffwechsel gestatten, so lassen die vorliegenden Ergebnisse doch vermuten, dass Störungen des zerebralen BTS-Stoffwechsels nicht im peripheren Blut erkannt werden können.

Summary. Acetoin and 2,3-butylene glycol in blood of renal and hepatic patients are raised, when consciousness is disturbed. There is no correlation between blood levels of acetoin and 2,3-butylene glycol and the degree of impairment of consciousness. Simultaneous determinations of acetoin and 2,3-butylene glycol in blood and cerebrospinal fluid show that alterations of the cerebral pyruvic acid metabolism are difficult to detect in circulating blood.

H. Thölen, F. Bigler, A. Heusler, W. Stauffacher und H. Staub

Medizinische Universitätsklinik, Basel (Schweiz), 5. Juli 1962.

Interferometric and Refractometric Determinations of the Dry Matter Concentration of the Mitochondria in a Cell

The use of interference microscope for a wide variety of special quantitative cytological studies on living cells, dependent on immersion refractometry, was first reported by Barer et al.[1,2] at Oxford. These studies were applied by Barer et al. (Barer and Ross[3]; Barer, Ross, and Tkaczyk[4]; Barer and Joseph[5]) to determine the refractive index of the cytoplasm of living cells by immersion

[1] R. Barer, Nature *169*, 366 (1952).
[2] R. Barer, J. Roy. Microscop. Soc. *72*, 10 (1952).
[3] R. Barer and K. F. A. Ross, J. Physiol. *118*, 38 (1952).
[4] R. Barer, K. F. A. Ross, and S. Tkaczyk, Nature *171*, 720 (1953).
[5] R. Barer and S. Joseph, Quart. J. Microscop. Sci. *95*, 399 (1954).

refractometry, by using isotonic solutions of bovine plasma albumin as immersion medium. DAVIES and WILKINS[6,7], and BARER[1,2] had independently pointed out that such measurements, when applied to living cytoplasm and many other cell constituents, could give approximate dry mass concentration of the solids present. In 1954 Ross[8] extended this method to determine the refractive index of the mitochondrial nebenkern of the male germ cells of locust. STENRAM[9] reported the dry matter concentration of nuclei and nucleoli by interferometric and refractometric methods.

In my present investigations I have made an attempt to determine the dry mass concentration of mitochondria in the spermatids of the cricket, *Gryllus domesticus* Linnaeus, by refractometry and interferometry, so as to correlate the results obtained by these two methods. Only those cells were selected, for the determination of dry matter concentration, in which all the mitochondria had coalesced completely. The refractometric studies were completed with Baker Interference Microscope, while interferometric determinations were undertaken with the New Leitz Interference Microscope.

Refractometry. The double refracting interference microscope of 'A. O. Baker' was used for refractometric determinations of the dry mass of the nebenkern. The cells were suspended in an isotonic insect saline medium consisting of enough bovine plasma albumin so that its refractive index matches exactly with that of the cytoplasm. All the measurements were taken with the shearing objective and condenser system. The phase-change retardation was measured with light from ribbon filament lamp, filtered through a green filter giving a narrow spectrum of maximum intensity at about 546 mμ. This optical retardation of light wave produced by the nebenkern was measured by an extinction method, i.e. the analyzer was first turned so as to darken the suspension medium and cytoplasm as much as possible, and then the extinction is transferred over to the nebenkern by further turning the analyzer. The phase shift was recorded from the scale of the analyzer which is supposed to give phase difference values up to fiftieth of a wavelength.

The measurements were taken on the nebenkerns of three different sizes commonly found in the material. The nebenkern is approximately spherical, therefore its diameter along the optical axis of the microscope can be taken as being the same as that at right angles to the optical axis. It is probable that in no case the error of this measurement could be more than half a micron.

The dry weight mass (M) of the nebenkern was calculated from the formula:

$$M = \frac{\Phi A}{100\alpha}$$

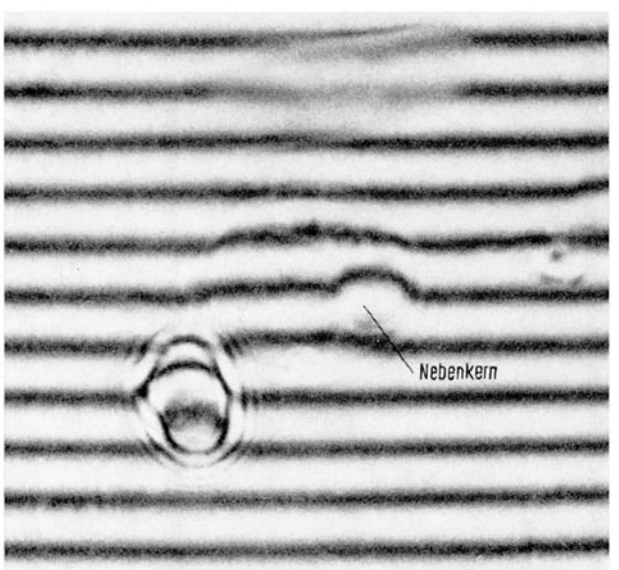
where Φ is the phase shift due to the nebenkern; A is area calculated from the diameter of the nebenkern, α is refractive increment (assumed 0.0018 for mitochondria, chiefly consisting of protein and lipoprotein).

As shown in the Table the dry weight mass of the nebenkern was derived from measurements of this body in twenty four different cells, and the mean figure obtained is 11.24×10^{-12} g%.

Interferometry. The new Leitz Interference Microscope was used for the interferometric determinations of the dry weight mass of the mitochondrial nebenkern. This microscope permits exact phase measurements, as no correction is required for the oblique rays due to the small numerical aperture of the illuminating system. The light source was a mercury arc lamp with an interference filter transmitting maximally at 546 mμ. A pair of oil immersion objectives 85X/N.A.I.36 were used. In this monochromatic

Nebenkern diameter in microns (μ) t	Phase-change retardation in wavelengths (λ) Φ	Dry mass of the nebenkern in picograms ($g \times 10^{-12}$) M
3.0	0.288	11.29
3.0	0.295	11.57
3.0	0.265	10.38
3.0	0.288	11.29
3.0	0.278	10.90
3.0	0.276	10.82
3.0	0.263	10.31
3.0	0.273	10.70
3.2	0.257	11.48
3.2	0.273	12.19
3.2	0.273	10.58
3.2	0.248	11.07
3.2	0.248	11.07
3.2	0.263	11.74
3.2	0.263	11.74
3.2	0.265	11.83
3.5	0.231	12.48
3.5	0.209	11.14
3.5	0.218	11.62
3.5	0.221	11.12
3.5	0.221	11.12
3.5	0.209	11.14
3.5	0.212	11.30
3.5	0.212	11.30

Data tabulated above shows the phase-retardation of light, of wavelength 546 mμ, passing through the living spermatids of *Gryllus domesticus* Linnaeus mounted in an isotonic insect saline medium, consisting of bovine plasma albumin, with a refractive index equal to that of their cytoplasm; the dry mass is calculated from these measurements obtained by the use of 'A. O. Baker Interference Microscope' (shearing system).



Photomicrograph taken under the New Leitz interference microscope, with monochromatic light and dark fringes focused in the field of view. 85 × immersion objectives with N.A.I.36. Diameter of the nebenkern is 3 μ.

[6] H. G. DAVIES and M. H. F. WILKINS, Report to the Cytochemistry Commission of the Society for Cell Biology: *Physical Aspects of Cytochemical Methods* (Stockholm 1951).
[7] H. G. DAVIES and M. H. F. WILKINS, Nature *169*, 541 (1952).
[8] K. F. A. Ross, Nature *174*, 836 (1954).
[9] U. STENRAM, Exp. Cell Res. *22*, 545 (1961).

light, a system of monochromatic light and dark fringes were focused in the whole field of view.

The cricket spermatids were mounted in an isotonic insect saline medium for interferometric investigations. The phase-change retardation of the nebenkern becomes visible as the lateral shift of the fringes in the image which was photomicrographed (Figure). The accuracy obtained by this method is expected to be $\pm$ 0.005 wavelength. The average optical path difference determined from the photomicrographs is 0.58 wavelength of the green light; thus the absolute optical path difference of the nebenkern is (0.58×0.546 μ). Cell diameters were measured with a micrometer ocular.

The basic formula relating the optical path difference to the dry mass of the mitochondrial nebenkern is the same as used in refractometry. However, since in this case the cells were suspended in isotonic saline medium, a correction factor is added to the formula:

$$M = \frac{\Phi A}{100\alpha} + (\mu_m - \mu_w)\frac{At}{100\alpha}$$

where μ_m, μ_w are the refractive indices of the saline medium and water, respectively. t is thickness equivalent to the diameter.

The dry weight mass of the nebenkern is determined to be 12.56×10^{-12} g%.

The value 11.24×10^{-12} g obtained for total dry mass of mitochondrial nebenkern by refractometry agrees well with the dry mass value of 12.56×10^{-12} g obtained by interferometry. Differences in values were well within the errors of both methods[10-12].

Résumé. L'auteur a effectué des mesures réfractométriques et interférométriques sur le résidu sec, concentré, de la matière mitochondriale du «nebenkern» des spermatides du Grillon. La valeur obtenue par réfractométrie (11×10^{-12} g) en utilisant le microscope à interférence de A. O. Baker concorde avec celle qui a été obtenue par interférométrie (12.56×10^{-12} g) avec le nouveau microscope à interférence de Leitz.

H. C. Chopra[13]

Division of Cell Biology, Institute for Muscle Disease, Inc., New York (U.S.A.), June 1, 1962.

[10] H. G. Davies, *General Cytochemical Methods* (J. F. Danielli, Ed., Academic Press Inc., New York 1958), vol. 1, p. 57.

[11] K. F. A. Ross, *General Cytochemical Methods* (J. F. Danielli, Ed., Academic Press Inc., New York 1961), vol. 2.

[12] The author wishes to thank Dr. W. G. B. Casselman for teaching interference microscopy. This work was supported by a fellowship grant from the Muscular Dystrophy Association of America.

[13] Present address: The Rockefeller Institute, New York (U.S.A.).

The Perception of Vibration by the Subgenual Organ in *Zootermopsis angusticollis* Emerson and *Periplaneta americana* L.

In their investigations of the vibration-sensitivity of the insect subgenual organ, Autrum[1,2], and Autrum and Schneider[3] have concluded that there was a transformation of the stimulus (*Reiztransformation*), in which the vibration gave rise to vortices in the blood around the subgenual organ. The constant pressure of these vortices was held to be responsible for stimulating the sensory cells. During the course of investigations of the vibration-sensitivity of *Z. angusticollis* and also of *P. americana*, evidence has accumulated which suggests a slightly different interpretation.

In both insects oscillographic recordings were taken with electrodes in the ventral nerve cord, but in the cockroach mainly with electrodes in the femur of an isolated leg. The legs were rested on a surface which could be vibrated by the coil of a suitably modified moving-coil loudspeaker worked from an audiofrequency oscillator.

In both insects the response to a pulse of vibration had a latency of 10–20 msec and died out after about the first half second (Figure 1 for the cockroach preparation). However, a series of rapid pulses at the same frequency of vibration elicited a synchronous non-adapting response for up to at least 30 pulses/sec for *P. americana* (Figure 1). At between 5 and 20 pulses/sec for *Z. angusticollis*, the response, although synchronous, tended to be lost after about 10–30 pulses (Figure 2). After a break of 1/50 sec in a long pulse of vibration, an immediate response can be recorded, and a phenomenon frequently encountered with a cockroach preparation is a response originating from the end of the pulse as well as from the start of the next (Figure 1). Further, after the response to a long pulse has died away, a synchronous response can be obtained to modulation of the vibration by tapping the support with a glass rod (Figure 3).

This evidence clearly suggests that the sense-organ is responding not to steady-state conditions, but to phenomena that precede or interfere with the steady state. Before going further it is necessary to consider the structure of the subgenual organ which has been studied in *Z. angusticollis*. (Further details of this will be published elsewhere.)

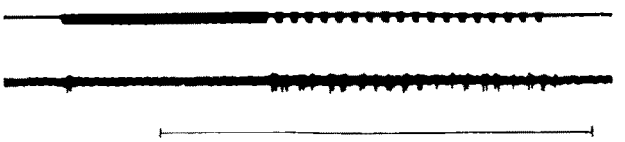


Fig. 1. Responses recorded from the cockroach femur to vibration with a frequency of 1000 c.p.s., partly in pulses of 30/sec.

Fig. 2. Responses recorded from the nerve cord of the termite to vibration with a frequency of 1250 c.p.s., in pulses of 10/sec.

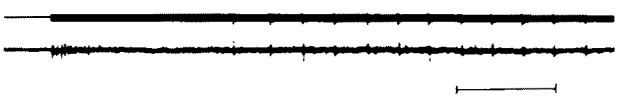


Fig. 3. Responses recorded from the nerve cord of the termite to vibration with a frequency of 1250 c.p.s., modulated by taps with a glass rod.
Scale line = 1 sec in each case.

[1] H. Autrum, Naturwiss. *30*, 69 (1942).

[2] H. Autrum, in *Handbook of Physiology*, Section 1 (Ed. Field, Washington 1959), vol. 1.

[3] H. Autrum and W. Schneider, Z. vgl. Physiol. *31*, 77 (1948).