and transferred on to microscopic slides. These pieces are pressed into thin layers with cover-slips. The particle size is determined microscopically, better microphotographically. The picture quality is adequate to allow exact measurement of the particle size, as can be seen from Figures 1 and 2. An example of the feasibility of the method is shown in the Table. The emulsion is completely characterized by the size frequency distribution. If the oil quantities added during the fermentation are known, it is possible to calculate the interfacial area and other parameters of the emulsion. Under the present conditions an interfacial area of 230 m^2 per litre of culture fluid can be calculated.

Zusammenfassung. Eine mikroskopische Methode zur Bestimmung des Emulsionsgrades von Kohlenwasserstoffen in Kultursubstraten wird beschrieben. Der jeweilige Zustand solcher Öl/Wasser-Emulsionen in bewachsenen Fermentationsmaischen wird für die Bestimmung mittels Gelatine stabilisiert. Einflussfaktoren und Bedeutung des Emulgierens werden diskutiert.

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Acetylcholine Content in Brain White Matter as Determined after Extraction with a New Solvent Mixture

There are few studies which report acetylcholine (ACh) content in the brain white matter. This is probably due to the very low levels of ACh found in this tissue. With the older conventional extraction procedures, it was especially difficult to obtain reproducible data. Recently we reported that the extraction of brain ACh using acidified acetone with formic acid yields a higher value than when other extraction procedures were used (TORU and APRISON[1]). This solvent extracts free ACh and the ACh which is bound to the lipidlike substances. With this new solvent it was not only possible to study the ACh distribution in smaller brain samples but also to study the chemical nature of bound ACh. As the first step in the re-investigation of ACh distributions in the brain employing this procedure, brain white matter, the lipid-rich tissue, was studied.

Adult, male albino rats (Wistar strain) and guinea-pigs were used. The animals were decapitated in the refrigerated room at 2 °C. The skull was opened and the dura mater was cut with a scalpel without removing the brain from the cranium. The grey matter of the cerebrum was gently removed by using a stainless steel spatula. Pieces of the white matter from the parietal and temporal areas and corpus callosum were carefully cut out and immediately frozen in liquid nitrogen. The collected brain tissue from 3 or 4 animals was pulverized in a stainless steel mortar and was divided into 2 portions. These samples were extracted by 2 extraction procedures.

One solvent system consisted of 15% *N*-formic acid plus 85% acetone (TORU and APRISON[1]), while the other was acidic-ethanol (CROSSLAND[2]). The guinea-pig ileum bioassay was used to measure the ACh content in samples; the effects of 5-hydroxytryptamine, histamine and substance P were eliminated as previously described (TORU and APRISON[1]).

ACh content in the brain white matter of rat after formic acid-acetone extraction (7.71 nmoles/g) was 23% higher than that found after acidic-ethanol extraction ($p < 0.05$, Table I). In the guinea-pig, the ACh content of the white matter (6.82 nmoles/g) was also higher when tissue samples were extracted with formic acid-acetone than with acidic-ethanol (4.73 nmoles/g). The difference in these 2 mean values is significant at the 0.01 level (Table II).

ACh concentrations in the brain white matter reported in the literature are lower when compared with the other brain parts (MACINTOSH[3], TAKAHASHI and APRISON[4]). The only exception is the cerebellum. The ACh values obtained with the new extraction solvent in the present study was higher than any of the previously reported values. However, the difference of measured ACh in the white matter after using the 2 extraction methods was comparable to that found for whole brain of the rat when duplicate samples of this tissue were also extracted with the same 2 solvent systems. Thus, the ratio of ACh values in the white matter to that in the whole brain of rat by the new extraction procedure (0.25) is close to that calculated in a recent report[4] employing the acidic-ethanol procedure (0.18).

Since higher ACh values are obtained with the 15% 1*N* formic acid-85% acetone extraction method, several additional points were checked further. It was found that the addition of chymotrypsin to standard ACh solutions as well as to brain samples did not affect nor influence the bioassay tissue in any way. Acetone sensitization also did not occur when using our procedure. Since very low choline

Table I. ACh concentrations (nmoles/g) in the brain white matter of rat[a] by 2 extraction procedures

Experiment No.	Acid-ethanol	Formic acid-acetone
1	6.05	6.49
2	6.55	8.92
3	6.44	7.49
4	6.05	7.93
Mean ± S.D.	6.27 ± 0.26	7.71 ± 0.99
	$P < 0.05$	

[a] 4 animals (weighed between 200 and 300 g) were used in each experiment.

[1] M. TORU and M. H. APRISON, J. Neurochem. *13*, 1533 (1966).
[2] J. CROSSLAND, in *Methods in Medical Research* (Ed. J. H. QUASTEL; Year Book Medical Publishers, Chicago, 1961), vol. 9, p. 125.
[3] F. C. MACINTOSH, J. Physiol. *99*, 436 (1941).
[4] R. TAKAHASHI and M. H. APRISON, J. Neurochem. *11*, 887 (1964).

acetylase activity was found in guinea-pig ileum homogenates, the possibility of ACh synthesis during the assay was also tested. No evidence of measurable ACh synthesis was found when choline and acetyl CoA (SCHUBERTH et al.[5]) were added in the bioassay bath at levels normally found in brain samples of the size we used nor after repeated additions over a normal work period. These checks plus those used in the original study (TORU and APRISON[1]) suggest strongly that the higher values of ACh obtained with the formic acid-acetone extraction solution are true values.

Table II. ACh concentrations (nmoles/g) in the brain white matter of guinea-pig[a] by 2 extraction procedures

Experiment No.	Acid-ethanol	Formic acid-acetone
1	6.11	8.97
2	6.82	6.94
3	5.17	5.50
4	4.51	6.55
5	3.25	6.99
6	4.29	5.67
7	3.08	7.32
Mean ± S.D.	4.73 ± 1.13	6.82 ± 1.15
	$P < 0.01$	

[a] 2 or 3 animals (weighed between 600 and 1000 g) were used in each experiment.

Zusammenfassung. Eine stark verbesserte Methode zur Extraktion und damit zur Bestimmung von Acetylcholin in der weissen Substanz von Ratten- und Meerschweinchengehirn, die bis zu 20–40% höhere Werte ergibt als die bisherigen Verfahren, wird beschrieben.

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DISPUTANDUM

Fluid-Drop as a Cosmological Model:

1.1. *Qualitative observations on discontinuous evaporation of certain fluid systems.*

In the course of routine biological techniques, while warming drops of certain biological stains on a glass slide, some curious patterns of their discontinuous evaporation can be observed. If these observed patterns can be extrapolated to cases of freely suspended static or rotating spheres of the fluid system, this leads to very curious iso-morphic or homomorphic analogues which seem to be repeated, apparently at various levels of cosmological hierarchies. Therefore, a possibility of examining such fluid drops as a cosmological model has been tentatively indicated. However, the author as a biologist can only restrict himself to mere qualitative observations and concepts with a hope that some physicists and cosmologists may like to re-examine this case more critically and quantitatively.

Cosmological enquiries necessarily go beyond the limits of direct objectivity or precise measurements[1]. In this sense, cosmology has to be more or less speculative with its usual reliance on models, analogies or bisociations as initial starting points for further enquiries through prediction, observation and verification or falsification. Even mathematicians like Laplace have deliberately preferred to be purely qualitative while proposing their cosmological conjectures. Modern cosmology has been trying to be more quantitative and exact within a limited[1] scope. But even in these cases, pure qualitative hypothetical concepts first precede and then motivate any such quantitative attempts. History of science presents several cases of pure concepts or qualitative observations which were quantitatively treated much later by someone else. Prout's Hypothesis or Brownian Motion may serve as examples. It was a *biologist* who first reported his simple qualitative observations on Brownian motion, later to be examined by Einstein, Perrin, Wiener and other physicists and mathematicians until it developed into the modern theory of probabilistic potential[2].

It is hoped that conspicuous absence of any quantitative approach in the following pages may be received in this perspective.

1.2. In earlier communications[3,4] it has been shown that evaporation of drops of certain liquids and consequent reduction in drop diameter does not occur as a continuum process. It proceeds in discrete discontinuous steps. If a drop of a biological stain like propiano-carmine (0.3% carmine in 45% aqueous propionic acid) is placed on a horizontal glas-slide and allowed to evaporate slowly by gentle heat, reduction in its diameter occurs in discrete steps due to discontinuous emission of the solvent phase (45% propionic acid) in sequential series of discrete fractions or serial quanta as ($Q1 \rightarrow Q2 \rightarrow Q3, \ldots QL$),

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