Estimation of Emulsification of Hydrocarbons in Culture Fluids

Many investigations are concerned with the biosynthesis of protein using hydrocarbons as raw material. In this relatively new technique, the fermentation process itself presents various difficulties, one of them being the fact that hydrocarbons (n-alkanes) are extremely insoluble in water. On the basis of the solubility figures published by BAKER[1], one can assume that the water soluble fraction of the alkanes is of minor importance for yeast nutrition during such fermentation processes. The uptake of these substrates by the cell must therefore occur through direct contact between the cell and the hydrocarbon globules[2]. To enable this type of mass transfer, it is necessary that the nutritious substrate be presented to the cell in the form of an emulsion. The size of the oil globules of the emulsion determines the interfacial area of the hydrocarbon substrate. Therefore the 'actual substrate concentration' or availability of the substrate is determined by two factors: firstly, through the amount of alkanes supplied, and secondly, by the size frequency distribution of the emulsified globules. As it is very important to examine to what extent growth rate and maximum yield of the microorganisms depend on the degree of emulsification, we endeavoured to estimate this factor as accurately as possible.

The application of the traditional determination methods in characterizing emulsions, i.e. measurement of absorbtion and light dispersion, electronic particle counting and sizing, sedimentation analysis[3-5] is greatly hindered by the exceptional conditions existing during the fermentation process. The fineness and stability of the emulsion, number of cells and air bubbles, viscosity, etc., are strongly influenced by the stage of culture development. Thus, only the microscopic method enables the direct determination of the globule size distribution in the presence of these disturbing factors. Should the microscopic investigation show a distribution of the emulsion globules in a form suited to give a picture of the actual emulsion degree of the dynamic state of equilibrium in the agitated culture medium, then it is mandatory that this condition be maintained until an evaluation can be carried out. This can be achieved by the following method: the sample is taken by inserting a syphon of small diameter into the fermenter. One opening of the syphon is submerged into the middle of the agitated culture medium. By closing the air discharge tube the fermenter is set under pressure whereby the culture medium is discharged with a relatively high speed of flow. After having rinsed the syphon with overflow, 1–2 ml of the sample is drained directly into a gelatine solution at 30 °C and cooled at once in ice water until the gelatine has hardened. For this purpose a solution of 15% microscopically pure gelatine is used. 10 ml of this solution is emptied into Erlenmeyer flasks and kept until use at 30 °C. The flask should be kept in circular motion during the entire procedure. The time needed for a fluid particle from the fermenter to reach the gelatine does not exceed 3 sec. The high viscosity and the stabilizing effect of the gelatine hinders the emulsified globules from coalescing as can be shown microscopically. Gelatine solidifies in approximately 30 sec under the above-mentioned conditions. Thus an absolutely stable emulsion is obtained. For the microscopic evaluation of particle size, pieces are cut from the gel at different places

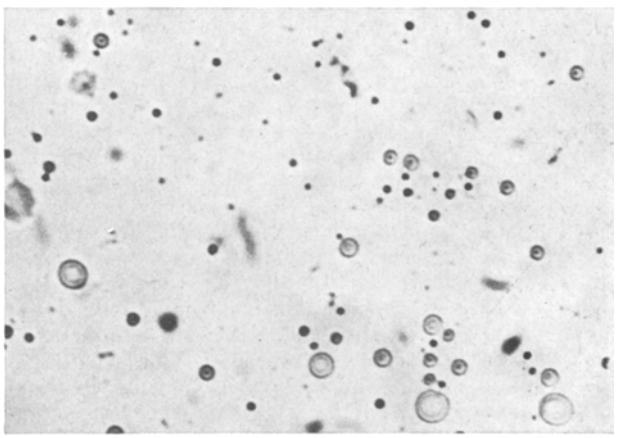

Fig. 1. Gelatine stabilized globules of a pure oil/water emulsion. × 1700.

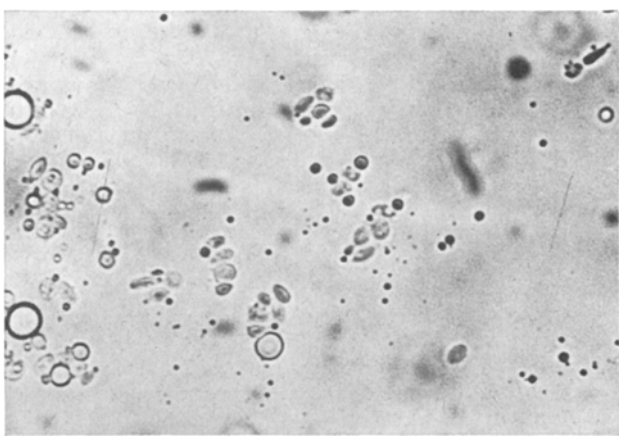

Fig. 2. Gelatine stabilized emulsion of a culture fluid. × 1700.

Size of globules μ	Average diameter μ	No. of globules measured	Volume μ^3	Interfacial area μ^2
– 1	0.5	844	55	663
1– 2	1.5	120	212	848
2– 3	2.5	68	556	1335
3– 5	4	23	771	1156
5–10	7.5	1	221	177
		1056	1815	4179

Size distribution of oil globules in a culture fluid containing 10% v/v of gas oil, turbine stirred (2200 rpm) fermenter, 1 l working volume

[1] E. G. BAKER, Chimia. *21*, 504 (1967).
[2] M. J. JOHNSON, Chemy. Ind. *36*, 1532 (1964).
[3] P. BECHER, *Emulsions: Theory and Practice* (Rheinhold Publishing Co., New York 1965).
[4] G. E. LANGLOIS, J. E. GULLBERG and T. VERMEULEN, Review scient. Instrum. *25*, 360 (1945).
[5] N. E. LLOYD, J. Colloid. Sci. *14*, 441 (1959).

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).
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