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BP PROTEINS
A SURVEY OF BP'S PROCESSES AND PRODUCTION FACILITIES WITH PRODUCT EVALUATION1/

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INTRODUCTION

Following the initial discovery made in 1959, BP have been investigating the possibility of growing high-protein containing yeast on hydrocarbons for over twelve years.

The work originated in France at the BP Research Centre at Lavera, near Marseille, under the leadership of Dr. Alfred Champagnat, who was subsequently awarded the French Legion of Honour, the French Chemical Industrial Society's 50th Anniversary Prize and the Redwood Medal by the Institute of Petroleum of Great Britain.

Work has steadily continued on the development of the form of process utilising gas oil as a substrate. A second centre was established at Grangemouth in Scotland in 1964 and the work there concentrated on the development of a process using pure normal paraffins as a substrate.

Considerable effort has also been put into the more basic research studies on all aspects of hydrocarbon microbiology at research centres in France (Epernon) and in the U.K. (Sunbury).

The considerable experience gained by the various research teams has culminated in the construction and operation of two large-scale development plants and in the design of a 4,000 t.p.a. plant at Grangemouth and a 20,000 t.p.a. plant at Lavera and in the design of a 100,000 t.p.a. plant in the process of erection in Sardinia. The improvement and development of the two processes mentioned and also in the production of more advanced processes is well in hand.

BP, therefore, now have a very strong and balanced team with a lot of experience in this relatively new field, both in the production of high-protein yeasts and also in the utilisation of these materials in various animal feeding stuffs.

I. BP PROCESSES

1. The Normal Paraffin Process

a) Feedstock

The feedstock used in this process can be normal paraffins boiling in the kerosine or gas oil range from C_{10} to C_{23}. This material can be produced in an extremely pure state by use of the molecular sieve process. BP is amongst the companies who have developed and engineered this process on a large scale. Details of the process are attached.

The purity of the feedstock is assessed by testing against the various U.S. and Western European regulations for the use of hydrocarbons in food. The most important of these regulations is the F.D.A. test 121.1146 which forms the basis of the FAO/WHO specifications.

b) Process

Besides n-paraffin, feedstock includes mineral nutrients (potassium, magnesium, sulfate and phosphate), air, nitrogen as ammonia, and water. A last component is an inoculum of the appropriate yeast strain.

Every feed stream except the yeast inoculum passes through a sterilisation "filter" before entering the fermenter. Mineral nutrients are held at a high enough temperature to kill all micro-organisms present before being charged to the fermenter. Heat economy is achieved by exchange between the raw feed and hot, sterilised feed. Minerals, water and n-paraffin all enter the fermenter via the same sterilising filter. Air, at a controlled rate, and gaseous ammonia enter via the other filter.

In the fermenter, fermentation commences under batch growth until the dry cell weight reaches a level that will support continuous operation. At

steady state approximately one ton of yeast is produced for every ton of hydrocarbon feedstock. (Some hydrocarbon feed is metabolized to form CO_2 and water; mineral nutrients make up the balance of the ton of product produced from each ton of hydrocarbon feed).

Process control is maintained by careful manipulation of pH and temperature; pH is controlled by the addition of ammonia and temperature is maintained by passing degassed broth through an external cooling circuit to exchange heat against site cooling water.

The continuous product stream from the fermenter is passed to a yeast/water centrifugal concentrator, which separates a clear aqueous phase and a yeast cream containing about 15% solids.

The concentrated cream is then dried to give a product of about 95% solids in a conventional spray drier. Alternatively, a more granulated material could be produced using a fluidised bed granulator, depending on the use envisaged for the final product.

Dried yeast is cooled while being pneumatically conveyed to storage. Product is shipped in bulk or packaged in multiwall paper bags. Quality control is continued through these last steps to check for nitrogen and moisture content. The storage stability of the product is indefinite under normal conditions.

Process equipment is in large part made of stainless steel, in line with food-industry general practice for sanitary product handling.

2. The Gas Oil Process

a) Feedstock

For this process the feedstock is raw gas oil boiling in the range

300-380°C and the yeast utilises the hydrocarbons present ranging from C_{10} to C_{30}. In this form of process only 10-20% of the hydrocarbons fed to the fermenter is utilised by the yeast, depending on the n-paraffin content of the feedstock, the remainder of the gas oil must be removed from the offtake stream during the harvesting stage. It is not a difficult process to remove the bulk of this hydrocarbon, but in order to remove the last residual traces down to the specification level of less than 0.08%, it is necessary to employ a solvent extraction stage at the end of the harvesting train. Because of this solvent extraction stage the final yeast product is extremely pure and the quality regarding aromatic levels can be assessed from the attached anlyses.

b) Process

As with the n-paraffin protein process all mineral nutrients are added to the fermenter together with the gas oil feed and the initial inoculum. The fermenters are open to the atmosphere and the operation is non-aseptic. It has been found possible to select suitable conditions of temperature, pH, dilution rate and nutrient medium composition such that the culture yeast remains dominant throughout the fermentation. Consequently, there is no need to sterilise rigorously the fermenter feed streams, with attendant cost savings.

Air-lift fermenters are used which operate on the principle of compressed air being sparged into an annulus between the fermenter wall and an internal guide-cylinder to provide both sufficient oxygen for yeast cell growth and sufficient energy to mix intimately the four phases and to circulate the broth around the fermenter.

The flow of ammonia to the fermenters serves two purposes: firstly, to provide a source of nitrogen, essential for the yeast growth, secondly to control the pH of the broth.

During steady, continuous operation, one tonne of protein biomass is produced for every tonne of hydrocarbon consumed, despite the fact that about half the consumed paraffin is converted to carbon dioxide and water: the incorporation of oxygen and mineral nutrients makes up the balance.

The broth leaving the fermenter can have a dry cell weight which varies between wide limits, depending on the conditions used and, to a certain extent, the process feedstock applied.

The separation train employed used centrifugation to remove yeast cells from the broth. Because of the use of the air-lift principle to mix and circulate the fermenter contents, broth emerging from the fermenter is considerably aerated. The broth is, therefore, passed initially to a decanter/degasser where part of the aqueous phase is removed. The resultant supernatant containing yeast cells, gas oil and water is de-aerated and passed to a first centrifugation stage where the majority of the gas oil is separated from the yeast cream. The samll amount of gas oil remaining with the yeast is later removed in the solvent purification stage to be described below and the bulk of the dewaxed gas oil is further clarified before being returned to the refinery stream.

The yeast cream passes to a second centrifuge stage where it is further concentrated prior to spray drying to provide dry material suitable for solvent purification.

All but traces of the gas oil remaining on the spray dried yeast is removed by means of a counter-current solvent leaching, which simultaneously also removes the cellular lipids. Finally, the extracted yeast is treated in specially adapted driers to remove residual solvent before passing to hopper storage and bagging.

The spent solvent passes to a recovery stage where both fresh solvent for re-use and the cellular lipids (which can be sold as a separate by-product of the process) are recovered.

II. PRODUCTION PLANTS

Pilot scale operation with both forms of process has been continuous from about 1964. Initially a half ton/day gas oil plant was constructed at Lavera. This provided material for all the initial toxicological and feeding trials for the gas oil based product. The Grangemouth research unit was provided with pilot plant fermenters of various sizes giving a range of capacities to provide a 10 times scale-up to the largest pilot plant of 2,000 litres. Both these plants provided sufficient material to test pilot and full scale equipment within the harvesting train.

The next stage was to design and construct two large units:

a) the 4,000 ton/annum at Grangemouth based on n-paraffin feedstock. This plant represented a 25 times scale-up from the pilot plant and has been successfully commissioned and operated and has demonstrated that the scaling-up factors used were entirely correct. The plant is now producing material for marketing in the U.K. and for test marketing in other European countries. The opportunity has been taken to put additional equipment onto this unit in order to test out alternative forms of drying, etc.

b) The second large plant is the 20,000 ton/annum gas oil based plant constructed and commissioned at Lavera. This is a larger scale of operation than the Grangemouth unit and will provide sufficient material for a genuine entry to be made to the French animal feed market. The plant is based on two streams and the detailed operating experience being gained will be used as the basis of a design for a larger gas oil plant. The product from this plant is now being sold into the French market.

The first very large scale plant has now been designed and engineered for the Italproteine project in Sardinia. This operation, a joint BP/ANIC venture, will be at a level of 100,000 tons/annum. This is a size of

operation which will enable a significant impact to be made on the high protein feedstuff market in Western Europe. The scale will also enable the largest and most up-to-date forms of equipment, e.g. centrifuges, spray driers, etc. to be used with the consequent economic advantages of this scale of operation.

III. CHARACTERISTICS OF BP YEASTS GROWN ON ALKANES

Yeast cultivated on a medium containing either pure n-alkanes or those in a middle distillate hydrocarbon fraction are now being produced on a commercial scale by BP processes under the registered name TOPRINA.

They are intended in the first instance for use in animal feeds, particularly in those for single stomached animals and the pre-ruminant calf and lamb.

Among other features, the yeasts are characterised by their high protein content relative to other yeasts currently in use for this purpose. This may be seen from the table below.

TABLE 1

General Characteristics of Alkane-grown Yeasts

	Yeast G	Yeast L
Moisture % wt.	<7	<8
Crude protein (N x 6.25) % wt. on dry matter	60	68-70
Lipids after acid hydrolysis % wt.	8-10	1.5-2.5
Ash % wt.	6.0	7.9
Ca % wt.	0.01	0.3
P % wt.	1.6	1.5
Pepsin digestibility %	>80	>80

Another noteworthy feature is the pattern of the amino acids in the proteins of these yeasts. It will be seen from the table which follows that they are particularly rich in lysine - an essential amino acid in which cereals tend to be deficient. The biological availability of the amino acids in TOPRINA is high, being between 92 and 100%.

TABLE 2

Amino acid Content of BP Yeasts, Fish Meal and Soya Bean Meal in Grams per 16 Grams Nitrogen

Amino acid	Yeast G	Yeast L	Fish meal	Extracted soya bean meal
Isoleucine	5.1	5.3	4.6	5.4
Leucine	7.4	7.8	7.3	7.7
Phenylalanine	4.3	4.8	4.0	5.1
Tyrosine	3.6	4.0	2.9	2.7
Threonine	4.9	5.4	4.2	4.0
Tryptophan	1.4	1.3	1.2	1.5
Valine	5.9	5.8	5.2	5.0
Arginine	5.1	5.0	5.0	7.7
Histidine	2.1	2.1	2.3	2.4
Lysine	7.4	7.8	7.0	6.5
Crystine	1.1	0.9	1.0	1.4
Methionine	1.8	1.6	2.6	1.4
Total S-acids	2.9	2.5	3.6	2.8

Note: Yeast G - grown on pure n-alkanes

Yeast L - grown on alkanes in middle distillate.

IV. TOXICITY TESTING OF BP [illegible] GROWN ON [illegible]

The subject considered in this section may be referred to as toxicity testing or safety evaluation. Both terms mean essentially the same thing though, in general, the phrase "toxicity testing" seems to be preferred by scientists.

Yeasts grown on hydrocarbons are in a situation which, whilst not unique, is different from that of most other food or feed materials. This is a direct result of their novelty combined with their appearance at a point in time when safety evaluation is not only possible but is receiving increasing attention.

BP has taken the view that these products, although used for animal as opposed to direct human feeding, should be shown to be safe by the most stringent testing procedures that can reasonably be applied.

Because, when this work was started in 1963 there were no internationally recognised testing schemes for materials of this nature, it was necessary to devise such a scheme.

A toxicity testing programme was devised in collaboration with the C.I.V.O. (Central Institute for Nutrition and Food Research) at Zeist in Holland. C.I.V.O. is part of the Dutch T.N.O. organisation, it commands State support and is completely independent of BP or any other commercial or industrial organisation. It has a high reputation internationally and does work for the United Nations agencies as well as for the Dutch and other National Governments. For reasons of the excellence of its work and its high reputation, C.I.V.O. was selected to carry out the programme of toxicity testing that it had collaborated in formulating. As a result, such testing of BP products started in 1964.

The basis of the scheme was to show that the yeasts grown on hydrocarbons according to the BP processes had no ill effects when fed at high levels in the diet of laboratory animals for short, medium or long periods of time. In addition to these tests, because of the nature of the substrates, special long term studies were carried out to prove that the yeasts had no tendency to produce cancer. Then, to complete the picture, a separate study was made to ensure that the yeasts had no bad effects upon reproduction. This involved the production of successive generations of test animals on diets containing up to one third of their total content as yeast. It involved also special tests for the absence of teratogenic effects, that is to say the absence of any effect on the number or physical characteristics of the offspring, i.e. no deformities, when the yeast comprised one third of the diet of female animals during pregnancy. As a final precaution, it was also shown that the yeast had no mutagenic effect. In other words, it was shown that even when the diet of breeding males consisted of yeast to the extent of two-thirds of its total content, the fertility of the males was not impaired nor were any deformities caused by the yeast in young stock bred from these males.

Yeast produced by both of the BP processes has been subjected to exactly the same toxicity testing programme.

It is worth noting, however, that multiple generation studies with rats and quail are still going on although international protocol only requires that three generations of rats are examined, we have gone beyond that. At the time of writing, we have reached thirteen generations of rats and twenty-three generations of quail still without any bad effects appearing.

These yeasts are probably unique at this time in the extent to which their freedom from toxicity was investigated and demonstrated prior to their commercialisation. The safety evaluation has extended over eight years and, as far as is known, no other material has ever before been tested so thoroughly before being allowed to go into general use.

V. NUTRITIONAL EVALUATION OF BP YEASTS GROWN ON HYDROCARBONS

Whilst the fundamental property of any feed ingredient is that it should be safe, it is of little practical importance unless it can make a positive nutritional contribution to the diet of the animal. Consequently, once the early results of our toxicity testing indicated that we were dealing with an inherently safe material, we were justified in going to a full scale nutritional evaluation with farm animals.

To carry out the work, we selected the I.L.O.B. (Institute for Agricultural Research in Biochemical Products). This Institute is situated in the Agricultural University Research complex at Wageningen in Holland, but is an independent foundation. As with C.I.V.O., the I.L.O.B. enjoys an international reputation for excellence and approximately 50% of its work is conducted on behalf of organisations outside Holland.

Experiments commenced here in October, 1965 and were concerned for the first few years primarily with the use of hydrocarbon-grown yeasts in the rations of pigs and poultry. This was a deliberate choice since it was realised that the single-stomached animal was confined to a narrower choice of high biological value proteins than was the ruminant which could utilise effectively low grade proteins and even simple nitrogenous compounds such as urea.

At a later stage the work was extended to cover the young calf and is, at present, being conducted with baby lambs. Both liquid and solid presentations are being examined in the case of these types of animal at the pre-ruminant stage.

The object of the work in all cases has been to use the yeasts grown on hydrocarbons as a partial or total substitute for the more orthodox high protein components of poultry and pig feeds, such as soya bean meal

and fishmeal. In the diets of the young calf and lamb we have concentrated on using the yeasts as partial replacements for dried milk and this aspect of the work is still actively being pursued and extended.

The general scheme of experiment was to feed a group of animals on a standard commercial type diet. These were the so-called "control" animals. Then other groups, as nearly similar as possible in physical characteristics, were fed on diets equivalent in nutrient content to that of the control diet but in which yeast replaced some or all of the fish and soyameal that was in the control formulation. A comparison of the performance of the various groups was then made in the appropriate parameters. For example, we compared the egg production in laying birds, fertility and hatchability of eggs from breeding birds, rate of growth and efficiency of feed conversion in broiler birds and pigs, number of young born to sows etc.

We have maintained numbers of pigs and poultry over several generations on diets containing yeast grown on hydrocarbons as well as similar animals kept on conventional diets. At this stage we are with the fifth generation of poultry and pigs without any evidence of deterioration in performance or physical characteristics.

On the basis of these test and in the light of the safety evaluation already mentioned, there can be no doubt that the hydrocarbon-grown yeasts produced by the BP processes under the trade name TOPRINA are both safe and valuable components of animal feeds.

VI. . [illegible]

Within the animal feed industry there are two categories of products. There are those which by virtue of custom or long usage are regarded as safe or, at least, acceptably so. For such products no formal approval for use is generally required.

The other category consists of feed sources that have not been in general commercial use for sufficiently long to be regarded as established in this field. Yeasts grown on hydrocarbons fall into this latter category being included in the generic description "Single-cell Proteins" (S.C.P.).

Various countries differ in their legislative attitudes towards these new protein sources. In some countries their use is prohibited unless it is specifically authorised. In other countries the reverse is the case and their use is permitted unless specifically prohibited. An example of a country adopting the former practice is France, whereas it is the latter situation that applies in the United Kingdom. However, even in the case of the "open" list countries it is necessary for the products to meet certain requirements in spite of the fact that it is not necessary to obtain formal approval for their use. In the United Kingdom, for example, any material sold for use in animal feeds must comply with the requirements of the Fertiliser and Feeding Stuffs Act and the various Statutory Instruments relating to it. Furthermore, a material can be banned from use if, in the opinion of experts in the Department of Health, it presents a hazard directly or indirectly to Public Health.

So whether control is exercised via the necessity of obtaining specific approval to use a product or by the authorities' power to apply sanctions to its use, it is ultimately and effectively in the hands of the appropriate authorities within the country concerned.

In the case of the BP products, all the toxicological and nutritional information has been made available to the appropriate Government Departments with the result that the product resulting from one or other of the processes is permitted to be used in animal feed in the following countries:-

Denmark,
France,
Germany,
Holland,
Italy,
South Africa,
United Kingdom.

It is believed that the BP products are the only yeasts grown on hydrocarbons at present which may be marketed in all of these countries.

Applications are likely to be made to several more countries in the near future depending upon the priority which will be accorded to certain marketing considerations.

There is at this time no universal standard, compulsory protocol for the toxicity testing of these new products and BP took the initiative by publishing its toxicity testing programme.

Because of the potential importances of Single-cell Proteins, the Protein Advisory Group (P.A.G.) of FAO/WHO/UNICEF set up an S.C.P. Working Group. Since 1970 this Group has published a series of recommendations or Guidelines on various aspects associated with the safety and nutritional evaluation of novel sources of food protein, available on request from U.N.O. Headquarters.

From the point of view of demonstrating the safety of such products, the definitive publication is P.A.G. Guideline No. 6. This recommends the protocol to be observed in carrying out the toxicity testing of novel

sources of protein for use in human food. The procedure followed by BP at C.I.V.O. since 1964 to demonstrate the safety of their products is in all essentials, identical with that now recommended by the P.A.G. Even though at this stage the BP products are being used only in animal feeds their safety evaluation has been to the standards recommended for human food.

This was recognised at a symposium held in Aix-en-Provence in February, 1972 which was attended by leading toxicologists and nutritionists from most countries in the world to discuss the potential role of hydrocarbon grown yeasts in human food. It was the general view that in the specific case of the BP yeasts, both the degree of toxicity testing and the satisfactory nature of the results would justify our proceeding to conduct controlled clinical trials with humans. It is impossible to believe that any such opinion would have been expressed in public by responsible experts if there had been the slightest doubt of the BP yeasts' entire suitability for animal feeding which has been its only use so far.

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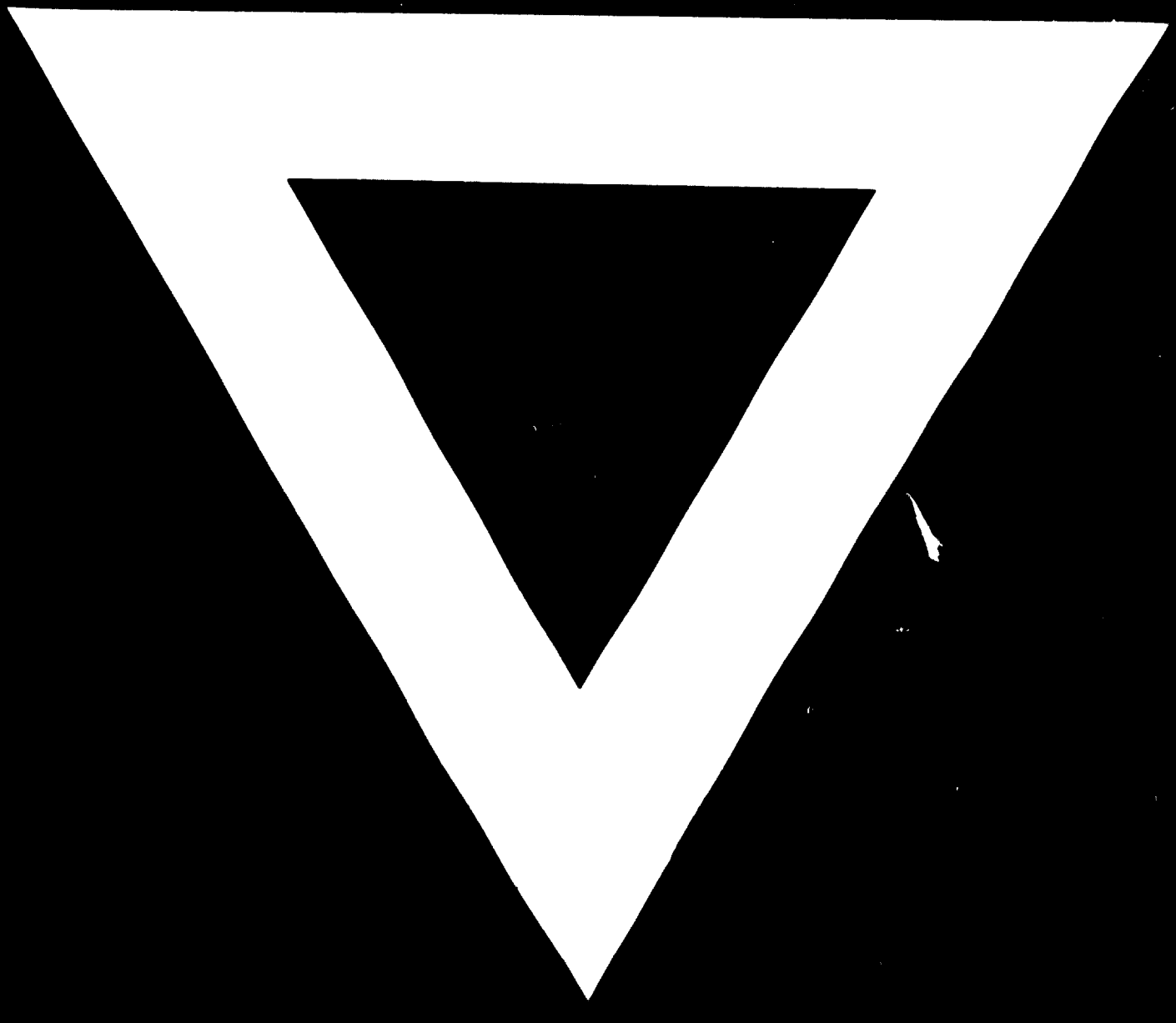

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