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HIGH LEVEL ADVISORY SERVICE FOR THE OPTIMIZATION OF THE ANTIBIOTIC COMPLEX AT MEDEA

SI/ALG/90/801

ALGERIA

Terminal report: Findings and recommendations*

Prepared for the Government of Algeria
by the United Nations Industrial Development Organization

Based on the work of Ralph Batchelor, consultant in industrial production of penicillin G and V and semi-synthetic penicillins

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* This document has not been edited.

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SUMMARY

The report deals with the major problems of penicillin fermentation, 6APA, ampicillin, amoxycillin and isoxazolyl penicillin production. It proposes solutions which could make the penicillin fermentation economic and considerably increase yields and reduce costs of production.

Potential Savings

US$ savings based on production of 10 tonnes each of 6APA, ampicillin and amoxycillin, taking into account only the content of penicillin G or 6APA.

Product	Savings
6APA	560,000
Ampicillin	331,100
Amoxycillin (includes side-chain)	191,000
Total	$1,082,400

The above savings would total $5 - 10 million per annum at expected production rates and would be obtained by a one-off cost of perhaps $500,000 to $750,000 at most.

The costs involved in improving penicillin production will be very substantial because of the need for a new culture which alone would be at least $1 - 1.5 million. With such a culture it should be possible to produce penicillin in yields comparable with the best available and make such production an economic viability as long as locally available raw materials are used. As long as materials all have to be imported then penicillin G must remain uneconomic because raw materials make up such a high proportion of manufacturing cost.

Proposals are made as to how the necessary technology could be obtained.

In addition allowance must be made for even more financial assistance for working capital without which the factory cannot produce at all.

INTRODUCTION

The report of the first visit dealt more with the types of problems, their magnitude and whether or not they were soluble. This second report is more concerned with presenting possible solutions to the problems particularly with respect to making the Medea complex economically viable within the context of the Algerian economy, so that it could make a significant contribution both economically and socially and become a production site of which the country can be proud.

Since the first visit there have been a number of organisational changes, but overall the utilisation of the factory and its production capabilities seems to have declined particularly with respect to bulk. On neither of my visits have I been able to follow the production of penicillin G or semi-synthetic penicillins first hand. I have therefore had to rely entirely on documents and discussions with individuals, from whom I have excellent co-operation, rather than observe for myself. This has naturally led to some conflicting evidence and there may thus be some minor errors of fact which I would have been able to correct if I had had-first hand sight of processes. It would also have been expected to allow identification of other potential problems for which solutions could have been proposed, but which were not apparent when the factory was not in production.

It has also been difficult to get updated raw material costs since there has been no recent purchases. In most cases current international prices have been used without taking into account any shipping costs.

General Overview

We have at the Medea Complex a generally well designed and well equipped factory for the fermentation of antibiotics, the bulk production of sterile and non-sterile semi-synthetic penicillins together with penicillin and non-penicillin formulation and packaging operations. The main part of the fermentation complex comprising 9 x 130,000 litre fermenters (5 for penicillin G and 4 for tetracycline) should be a great and possibly increasingly valuable asset over the next 10 years.

Much of the fermentation capacity of the world was constructed between 1950 and 1960 and is coming to the end of its working life. Quite apart from the high capital cost of replacement many of the factories are in environmentally sensitive areas and so will not be rebuilt there. In the last year or so both Pfizer & Rhone Poulenc have stopped making penicillin and while this report was being written the Anagni plant in Italy has been closed. While there are suggestions of increased capacity in China and also increased output for other reasons such as strain improvement this is more than counterbalanced by the

potential interest in other products produced by deep fermentation. Many chemical pesticides are environmentally damaging and there is a great interest in "friendly" fermented pesticides. At least 6 companies are known to he investigating such possibilities. It is therefore important to get the penicillin fermentations operating effectively and for the Medea factory to demonstrate that it has the skills to carry out fermentations, not just to make penicillin now but to put themselves in the market to make other and perhaps more valuable products in the future. There are after all 9 large fermenters capable to making far more penicillin and tetracycline that is required for Algeria if operated efficiently and with good processes.

There were from the start very many problems often relating directly to the initial concept as set out below.

1) No account was taken as to whether the product to be manufactured was likely to be needed or what the world market was doing. Thus, in the case of streptomycin, a very expensive plant was installed to make a product which was no longer needed, a plant which has never been operated. The demand for tetracycline has fallen and is continuing to fall.

2) The processes which were acquired with the plant were almost all old, not state-of-the-art and sometimes obsolete even when the factory was being designed and would have been uneconomic from the start even if operated at design/"guaranteed" levels. The chemical route to 6APA for example has not been operated elsewhere for many years even by the company who discovered and developed the process. The amoxycillin process provided by Cheminvest has not been used by major companies since the early 1980's. Furthermore the process provided was not the best of its type and the yields are really not comparable with what should have been obtainable. A similar situation applies to penicillin G which even at the time of acquisition was not the best, while the culture might have been the best available for purchase, the recovery yields certainly were not.

3) Another problem has been that some good processes use unstable raw materials eg the ampicillin process, so that unless the materials are used quickly they deteriorate and lead to uneconomic production. They were thus not suitable for that particular factory.

Most of the above can be corrected quite easily for 6APA and the semi-synthetic penicillins with very little modification to the equipment and a fairly modest cost for process know-how. The major problem is associated with penicillin G which is the basic raw material for many of the subsidiary processes. Here, there are two vital requirements, a new culture giving acceptable yields and most of all the production within Algeria of the greater part of the raw materials, at present some 95% are purchased overseas.

It is, in the authors view, essential to the viability of the entire bulk pharmaceutical production facility that the penicillin G production be made economic in terms of a positive contribution to the Algerian economy. While limited expenditure can correct the downstream problems the economic production of penicillin G (and tetracycline) is the key issue and requires a significant injection of funds. The longer this problem is left, the more difficult it will become, the more the management and the workers will get used to doing nothing, the more difficult it will be to start working efficiently again.

It is therefore not just a solution to the technical problems which is needed . This can be done with suitable know-how and training on injection of capital along with implementing some improvements of management . What is also needed however is a very significant level of working capital both local and hard currency so that sufficient raw materials can be purchased to operate the plant continuously on a profitable basis. Without such an injection of money for working capital as well as that needed for modification and repairs to the plant together with up-to-date technology, the factory can only continue to be a drain on the Algerian economy when it should and could be contributing to it.

PENICILLIN FERMENTATION

It was unfortunate that for neither of my visits was there any fermentation or extraction being carried out making it difficult to be sure all the major problems have been covered in this report.

It is clear the fermenters should be an important asset. These are relatively new and scarcely used and there does not appear to be any significant problem with them, nor should there be with the downstream recovery as far as crystallisation of the penicillin. The capacity of the rotary vacuum filters is such that only one needs to be used. The podbielniak extracters are accepted art and are of the right size but do not appear to be operating to the efficiency they should. Though the butanol azeotropic method is a clumsy way of crystallising penicillin there is fundamentally nothing wrong with the equipment which if operated efficiently could produce between 700 and 1,000 tonnes of penicillin from the 5 x 130m3 fermenters.

While leasing all or some of them to a third party is an interesting option which the current management are investigating, the key to a successful factory at Medea lies in producing penicillin G for downstream processing.

Accepting that there is no fundamental equipment problem, all of the difficulties are associated with other aspects of the process namely the culture, culture maintenance, fermentation raw materials, lack of pilot plant, fermentation management and know-how, and recovery of the product.

The culture

Neither of the cultures were state of the art at the time of purchase though they may well have been the best available to the particular suppliers. The highest titres obtained originally with the IB1/ISF strain were around 42,000 units (26gms/litre) and rather less with the Squibb penicillin V organism 32,000 units (21gms/litre). In both cases the yields are overstated by at least 10% because the hydroxylamine assay used is non-specific and would be including other penicillins in the total, a specific assay (HPLC) must be used. The strain has the morphology associated with the older ones. It grows as a thick filamentatous mycelium which though making it easy to filter, makes it difficult to wash and also makes aeration of the fermenter difficult by requiring a high energy consumption for the agitator. More modern strains tend to be pelletted (ie little balls of mycelium) which makes aeration and mixing easier, ideally they become "fluffy" towards the end of the fermentation cycle making filtration easier.

It would be just about possible to make penicillin G economically below the world price with a titre of 42,000 if all raw materials were available locally at a low price, such as is the case in China, but a higher yielding strain is really

essential particularly as the present strain (see below) has deteriorated through inadequate maintenance.

New strains are available but at a significant cost - it is not just a question of a new strain, a new strain must be capable of being used on the plant and this applies particularly in relation to the downstream recovery equipment.

One way of getting a new strain could be a joint venture with an existing penicillin producer. While this is unlikely to be possible with any of the current major producers there are some, particularly companies who have recently stopped fermentation themselves, who might be interested.

The best strains available can be obtained by joining the Panlabs "penicillin club". This club has had all the major producers as members at some time though at present most of the members are from the smaller producers who cannot afford strain development themselves. Present membership is around 12 companies and the annual cost of membership now is $84,000 p.a. However, because members can immediately acquire the latest strains (penicillin G and V are the same strain) there is a very substantial entry fee of $600,000. Not only does this make the Panlabs Club expensive but I would not recommend it for Medea at this stage. The latest high yielding strains are "yeast like" in their morphology and not well matched to the downstream extraction available at Medea, furthermore they require careful maintenance and some modifications to the ferementation medium and other technological assistance which Panlabs themselves are unable to provide since they have no large scale fermentation experience of their own. Even major companies such as Beecham have experienced difficulty in getting the same high yields when transferring a fermentation from one site to another despite the same organism, similar fermenter and apparently same medium. Perhaps the best way would be to acquire a strain which although not the latest would be most suitable for the Medea factory. This could be done in one of two ways. Panlabs themselves are prepared to sell a 5 year old strain which would have excellent characteristics both in its morphological form and its stability. The cost would be high $1,700,000 but this would include help to put the strain on pilot plant production and 6 weeks of personal attention which could be split between Panlabs personnel at the Medea factory and Medea staff visiting the Panlabs laboratory in Taiwan to learn in detail how to handle the strain correctly, maintain its productivity and generate the spores and innocula for the production vessels. There are also some important tricks to ensure the ideal negative form. This could be the preferred option unless a joint venture partner can be found because there will be after-sales service which there probably would not be if a slightly older strain were purchased from a Panlabs Club Member. Members of the Panlabs Club are allowed to sell on strains that are over 7 years old. Because as explained above it is not just a question of obtaining the highest yielding strain and expecting it to work in the Medea factory it is suggested that experienced assistance be employed to help

obtain a suitable culture and commission it on the factory. Such a function could be carried out by Michael Barber Associates who could not only help obtain a suitable culture but could also provide assistance to ensure it could be used satisfactorily in Medea.

Culture Maintenance

The high yielding cultures which have been produced by mutation, genetic re-combination and genetic engineering have been subject to years of selection. They are not like the original wild types and if a selective pressure is not maintained they will tend to revert and gradually lose the ability to produce high titres. Keeping a good penicillin producing culture is rather like standing on a downward moving escalator. If you simply do nothing you move down, you have to walk up steadily to maintain your position and run fast to move up. If you take no special precautions the culture will slowly and steadily produce less and less penicillin. When the culture was first acquired it gave titres of 42,000 in the large vessels. During 1990 this fell to around 35,000 and it is now about 25,000. This was very much in line with my prediction when I saw how the culture has been handled during my first visit though the situation is complicated by deterioration of the medium which may also contribute to the lower titres.

In my view the documentation provided on culture maintenance was quite inadequate and even if staff were properly trained at that time, most of those staff are no longer at the plant. Furthermore there are some very significant facilities not available which are essential to good culture maintenance.. Even the master culture will deteriorate and need regular re-selection. The culture is by no means homogenous, over time spores will die and in general it will be the less stable high penicillin producers which fail to germinate and the lower producers which will survive. This lower rate of germination is very clear From their own records the production of a seed culture from the freeze dried spores on quartz now takes 58 hours rather than 36 - 42 hours as originally.

During my earlier visit it was apparent that the then head of that section had no understanding of biological variation, or the number of samples required to get a statistically valid result. No guidance on this was given in the Italian manual. It was also clear too that the shaken flasks, which are on the small side anyway were not being properly used. It is not possible to carry out a fermentation such as for penicillin in a shaken flask in such a way that it parallels a fermenter. Even daily additions of side-chain and other materials mean that agitation has to be stopped and consequently aeration ceases resulting in substantial problems and even death and autolysis of the mould. With penicillin many parameters must be kept within very strict limits and materials such as glucose fed continuously. Too high a glucose level suppresses penicillin production. (It was understanding this fact which allowed glucose rather than lactose to be used in penicillin fermentation).

Furthermore there is considerable evaporation from the flasks, as was demonstrated to the staff, so that part of the apparent increase in titre is really due to evaporation of water. All this makes shaken-flasks really inappropriate as a means of evaluating potential yields as is obvious when one sees the maximum titre in the shaken flask from the present culture has been 13-14,000 units when the same culture gave 42,000 in the fermenter. While the fermenter titre has dropped over 40% to 25,000 units that in the flask has dropped only some 10% to 12-13,000 units.

The way in which shaken flasks should be used to test the productivity is not to look at the maximum titre since there is no way they can approach the titre obtained in fermentors, but rather to look at the rate of penicillin production over a 24 hour period when other factors are not limiting. To do this properly means using a significant number of replicates. Because the figure is obtained by the difference between two titres, the standard deviation is necessarily doubled. Even when carried out in the best way it can still give only guidance that the strain has not deteriorated, what is essential is to have a number of small mini-fermenters which are equipped exactly like the large fermenters and which can be used to mimic the condition with the same controls and additions of substrate etc. 6 - 8 of these 20 litre vessels would be desirable and at least 4 essential. They are so important to fermentation technology, maximising yield and reducing costs of medium as well culture maintenance that they are dealt with further in a later section.

Not only was the re-selection process not properly covered in the documentation but also some other important aspects could have had fuller explanation. Even the basic preservation and storage was not properly covered. Most companies consider their culture to be so important they take every care in preserving it. They do not store spores in just one way alone, but usually use a variety of methods (at least 3), which include lyophilisation and storage in liquid nitrogen. This is an area where there is clearly a need for further training and also the need for additional staff, it should be easy to teach some of the under-employed personnel to help. Training would almost certainly have to be carried out in the factory, penicillin producing companies do not normally allow any outside persons near their cultures because they are too easy to steal. They are also very jealous of their expertise. One of the best ways would be to have someone with hands on experiences of these operations to train staff on-site. MBA could certainly arrange for a recently retired expert in this field to help. It must also be mentioned that here is a good example that none of the work suggested above is of any value unless the results can be properly measured. Competent analytical assistance is essential within the department together with the right equipment such as HPLC to ensure accurate and specific assays. Certainly on my first visit (and probably this recent one) the standard deviation in the assay as being carried out was far greater than the difference in titre that was being looked for, particularly as there were insufficient or no replicate flasks or assays. Operating in such a way was bound to lead to deterioration of the culture.

Culture Medium

This is one of the most important problems for the economic operation of the Medea factory at least as important as obtaining an efficient organism. The problem has already been recognised and the present management have started taking steps to solve the problem.

One of the secrets of success of any fermentation process is to use cheap, locally available materials. Once high volume, low cost materials have to be shipped long distances, particularly if that involves unnecessarily shipping water, serious cost problems arise. The tables below highlight the problem.

Major raw materials for penicillin G production.

Data ex Medea 1986/7 Cost FOB Country of origin

Ingredient	% Fermentation Raw Materials	% Total Raw Materials
Corn Steep	9.3	7.8
Glucose	63.0	53.2
Lard Oil	11.8	9.9
Phenylacetate	10.6	8.9
	94.7	79.8

The above figures are broadly in line with experience elsewhere but hide an important problem. As stated they are FOB prices not CIF. Both cornsteep and liquid glucose are over 50% water with high shipping costs. In the case of liquid glucose which must be kept 40 - 45ºC to prevent crystallisation the shipping cost is very high. At one time when it was being shipped to Algeria in this form the transport costs were double the FOB price. Even shipping in solid glucose at FFr 3.3/Kg the situation without including shipping costs is as below.

	Cost/ Fermentation	Cost/kg Pen G 750 kg Production per fermentation	Cost/kg Pen G 3000 kg Production per fermentation
Glucose	$21,800	$29	$7.2

These should be compared wit'· the World Market price of $30/kg and probably under $20/kg for the production cost and show the cost of sugar alone makes the current production of 750Kg per fementer uneconomic. It is therefore essential to find a cheaper local source.

Taking the 4 major raw materials at todays world prices we have:

Raw Material	$ Cost/kg Pen G 750kg/Fermenter	Produced at 3000kg/ Fermenter
Corn Steep	2.0	0.5
Glucose	29.0	7.2
Lard Oil	4.5	1.1
Phenylacetate	2.5	0.5
	Total $38.0	$9.4

The above costs exclude transport.

Not only must the cost per kilo be reduced by increased yields and recovery but it is clearly important to obtain raw materials locally. At the time of my first visit the cost of glucose per kilo including the transport costs of penicillin produced was really almost $90. Put another way one could have bought 1.3Kg of 6APA or ampicillin for the cost of the glucose alone to make the G which at the Saidal yields would have given only 0.35Kg 6APA.

Saidal have already started to obtain glucose powder which although marginally more expensi has a much lower shipping cost. More to the point they are looking at producing a starch hydrolysate at Maghnia in conjuction with Novo. The Maghnia factory has in the past supplied some of their starch hydrolysate which has been produced by chemical hydrolysis rather than enzymically. It is known that acid hydrolysis of starch has a different specifity for the 1:6 and 1:4 links as compared with the enzymic route and it is not surprising that the penicillin factory found the product unsuitable for fermentation. Since Maghnia is some 300Km or more from Medea the cost of transport should be considered. It may be more economic to produce starch hydrolysate locally at Medea rather than incur high transport costs which will be the case if liquid glucose is the product to be made at Maghnia. There are a number of possible alternatives to producing glucose at Maghnia. Other sources of starch ofter than maize could be suitable and the plant could be on the Medea site. This will need detailed costing of the alternatives taking all factors including transport costs into account.

Like glucose, corn steep liquor comes from maize and should be available from the Maghnia plant. Material is in fact available and this otherwise waste product would be useable at Medea if it were within specification. At present it contains almost twice the amount of lactic acid as it should (about 20%). It obviously requires liaison between the two factories to solve the problem, it may be possible to use the material as it is if the input is altered, work that can only be carried out in small fermenters to optimise the titre from the local corn steep. One possibility the corn steep has not been kept sufficiently sterile

and has undergone a fermentation with a lactosbacillus producing not only the excess lactic acid but also using up many of the amino acids and other trace materials essential for a good penicillin fermentation.

Of the other major materials phenylacetic acid is made by only about 4 companies in the world and will have to be purchased from outside though installing an enzymic 6APA process will allow a substantial amount to be recovered and recycled into the fermentation.

Phenylacetic acid is almost 11% of the fermentation cost as it is used up either by incorporation into penicillin G or by metabolism. In view of its cost and the fact that it must be purchased outside Algeria it is important to minimise its use. This is one of the examples where rapid analytical results are important so that the correct amount can be fed. Underfeeding will give a reduced titre, overfeeding will both waste phenylacetic acid and could depress titres or even cause damage to the mycelium.

When the process was first installed the phenylacetic acid was measured by a specific assay, using gas chromatography. The instrument is apparently broken. Rather than repair it a non-specific method involving extraction into toluene and titrating using phenol red as an indicator is now used. Other substances could interfere and give high readings, more likely incomplete extraction will give low readings and cause excess phenylacetic acid to be added. This seems to be the case at present.

The amount of phenylacetic acid being added now is more than was added when gas chromatography was used despite the penicillin production being much less. This must mean too much is being added.

The Lard oil is used as an antifoam as well as a carbohydrate source (reducing the amount of sugar required). There is no reason why it should not be replaced by another oil probably a vegetable oil such as corn oil, peanut oil, sunflower oil etc, any locally available or cheaper oil should be tried but again this requires pilot-plant.

The use of locally available materials is thus clearly a key not only to reduce production costs but perhaps more important to the overall benefit of the Algerian economy, by avoiding imports.

Pilot Plant

The need for a pilot-plant has been referred to several times in the foregoing pages. It should have been included with the original factory construction. It is quite surprising that ISF did not suggest any such facility for penicillin fermentation. IBI certainly did both for chemical processes where they installed an excellent unit and for tetracycline where a small number fermenters of around 300 litres capacity were included.

It is in my view essential to have a small fermentation pilot-plant with a number of identical mini-fermenters of around 20 litres, the smallest size which can be used and operated like a production vessel. It must have all the necessary controls and the ability to make additions so that the course of the fermentation can exactly parallel what happens in a larger vessel. 6 or 8 of such vessels together with the computerised operating equipment is really the minimum useful number. The capital cost is very substantial, a group of 6 with computer and software would be around $450,000. This cost includes all the auxillary equipment such as oxygen electrodes and pH measuring equipment. There are several manufacturers of equipment of this nature but only 2 or at most 3 make good pilot-scale equipment with reliable performance suitable for fungal fermentation such as penicillin, many are no more than laboratory instruments. A suitable supplier known to be used by the major companies could be recommended, MBA could also provide a very experienced person with over thirty years experience to help the staff gain maximum benefit from the equipment. Although it is expensive it should very rapidly pay for itself, not only in helping culture maintenance but also maximising the yields and minimising the costs of running the ferementers.

In addition the tetracycline pilot plant could be modified to be used as additional penicillin G experimental equipment. If this were done it may be sufficient to purchase only four of the mini fermenters. At least one of the production fermenters should have its equipment overhauled to ensure it has working pH, dissolved oxygen and other necessary measuring equipment, to enable it to be set up to parallel results obtained in mini fermenters. Although it would be desirable to install them on all the fermenters, one would be enough to allow the parameters to be found and then all the other would be set up similarly.

A properly functioning pilot-plant with trained staff is essential to maintain an efficient and economic fermentation production unit, without it, it is very difficult to introduce any improvements particularly a new and more efficient culture.

Fermentation Management

One of the major problems throughout the whole factory but particularly important to penicillin fermentation is that there is no one at the factory who has any experience of industrial scale penicillin production. The present staff do the best they can and with the right help I am sure many of them would be very satisfactory managers and operators. There is undoubtedly a need for them to have regular acccss to fermentation expertise from outside Algeria to give them con fidence and guidance where necessary. This could be organised by having a consultant visit the factory at regular intervals.

Penicillin Recovery

The basic recovery process for penicillin G has changed very little over the years. With the exception of the introduction of whole broth extraction by a few companies the process is essentially filtration to remove mycelium, followed by solvent extraction and crystallisation. The azeotropic removal of butanol for crystallisation as used at Medea is a rather old method, most producers using continuous crystallisation with potassium acetate. The overall process at Medea should however give a very much higher recovery than the guaranteed figure of 50%. It is difficult to make any real assessment of the current recovery problem at Medea because of the non-specific assay, unless HPLC is used there is no easy way of knowing the true penicillin G content of the broth, a figure certainly lower than indicated by the present assay method. It is clear however that the whole extraction process is currently taking too long and is being carried out at too high temperature. At present, because of the infrequent runs, partial harvests have been kept 24 hours or more. I was not aware of any attempt to measure what was left after such storage but with low titres and the problems of which can be caused by autolysis it is possible that trying to recover any penicillin from this stored material was counter productive. Penicillin G is not a very stable substance particularly at extremes of pH. At pH 2.0 when being extracted into solvent the half life of penicillin G is only about 20 minutes at 20°C. It is therefore important to minimise the time and get the penicillin into solvent and back into buffer at neutral pH as quickly as possible. One part of the current recovery process which is difficult to understand is the dilution of the broth prior to the extraction with solvents. The manual provided by the Italians clearly says the broth should be diluted to a maximum of c.14,000 units. Neither I nor the manufacturers of the equipment, Podbielniak, feel this is necessary. It wastes both water and solvent. The current management of Podbielniak would be prepared to advise on the use of their specialised pieces of equipment for a nominal fee. Normally when Podbielniaks are installed the company assists in commissioning them, but for some reason they were not asked to do this at Medea. It is recommended they are requested to advise on this aspect when the plant is running. Only they have the experience of the use of their equipment over the whole world and they are in the best position to advise having seen all the problems that have occured.

With regard to an improved crystallisation proce dure, MBA would be able to provide a direct crystallisation process. Apart from the above suggestions the

only way to improve recovery further is to first find out where the major losses are occurring. To do this it is first essential to have access to dedicated HPLC equipment and then carry out material balances throughout the extraction process. When we know where the losses are we can then take action to minimise those losses. Recovery should then approach 90%, in line with the best producers.

The improvement in penicillin G yield and economics is a combination of all the above issues. A higher titre not only gives more penicillin but also helps the recovery process. All the improvments complement one another, double the titre and a 50% increase in recovery would give three times the yield and produce penicillin at one third of the cost. Every improvement helps because it is multiplied by all the others. Overall the benefits could be as follows:

Action	Potential Yield Improvement		
1) Obtain New Strain Requires money or partner but also some new equipment and training.	x 2	-	x 3
2) Reduce Costs Minimise cost of materials using local where possible. Maximise benefit by scheduling and optimising fermentation time. Requires some equipment and training.	x 1.1	-	1.5
3) Increase Recovery Training, attention to detail, materials balance and new crystallisation method.	x 1.7		
Overall improvement	3.7	-	7.6

These would reduce present raw materials cost from the present figure of around $120 per Kg down to $16 - 30. To compete with world prices it is the lower figure which must be aimed at. Certainly a target of around 60,000 units should be achievable and a recovery yield of 85% is still below the best, Gist achieve around 90% using conventional techniques. Local production of starch hydrolysate (60% of raw material cost) and corn steep should have a very significant benefit. Even the overseas purchased phenylacetic acid can be used more efficiently and with recovery and regularly from a new 6APA process would make further contributions to savings.

One of the big problems however is that there is no experienced person. Fermentation is a living process and while the training given in the past if done properly is useful it cannot replace experience. There will always be problems cropping up where the staff need help and guidance. One way to alleviate the problem would be to have a consultant who would not only visit the factory two or three times a year but would also be available to answer questions and offer advice at other times.

Optimisation of Fermentation Time

At present the penicillin G fermentations are run until there is no significant increase in the measured titre of penicillin G. The present duration is 190 hours but I was unable to obtain typical current data so the example given here is based upon the original instruction provided by the Italians when the process was installed.

The important point is that while the rate of increase of penicilin gradually slows up, some of the expensive raw materials such as sugar, lard oil and phenylacetic acid continue to be added, sometimes even in increasing quantities. In addition there is the additional energy cost to power the agitator and supply the compressed.

For the purpose of this example I have limited the data to just sugar and lard oil but a complete evaluation is really much more complex and to obtain an accurate view the data from many actual fermentations should be taken into account. On the most sophisticated plants all the relevant data is processed by computer, the existing fermentation is compared with the overall record and the computer in fact decides when the vessel should be harvested depending upon the rate of penicillin production and raw materials input.

Such sophistication is however not necessary to make use of the principle as the example below shows. Even in a simple form significant savings can be made.

The rate of increase in penicillin G can be calculated from the tangent at any point on the penicillin accumulation curve Fig I and this multiplied by the volume of liquid in the fermentor will give the number of kilos of penicillin produced per hour at that given time. The recovery yield should be taken into account and in the present case I have also allowed 10% for the over estimation of penicillin G by the analytical method used which is measuring other B lactam containing substances as well as penicillin G. At the same time the cost of the added glucose, lard oil and other substances can be calculated and their cost per kilo of extra penicillin produced at that time inferred. In this example I have taken the feed rates from the documentation provided.

In this example the figures are:

HOURS:	Increase UNITS/ hour	Production Kg/hour in 100,000 litres	Sugar Kg/hr	gms Penicillin per Kg sugar	Cost of sugar per Kg penicillin $	Allow 60% recovery of G & 90,000 litres actual volume
70 -100	280	17.5	139	125	4.8	8.8
100 - 160	240	15.0	144	104	5.8	10.7
160 - 220	160	10.0	148	67.5	8.9	16.5
220 - 250	120	7.5	148	50.5	11.8	21

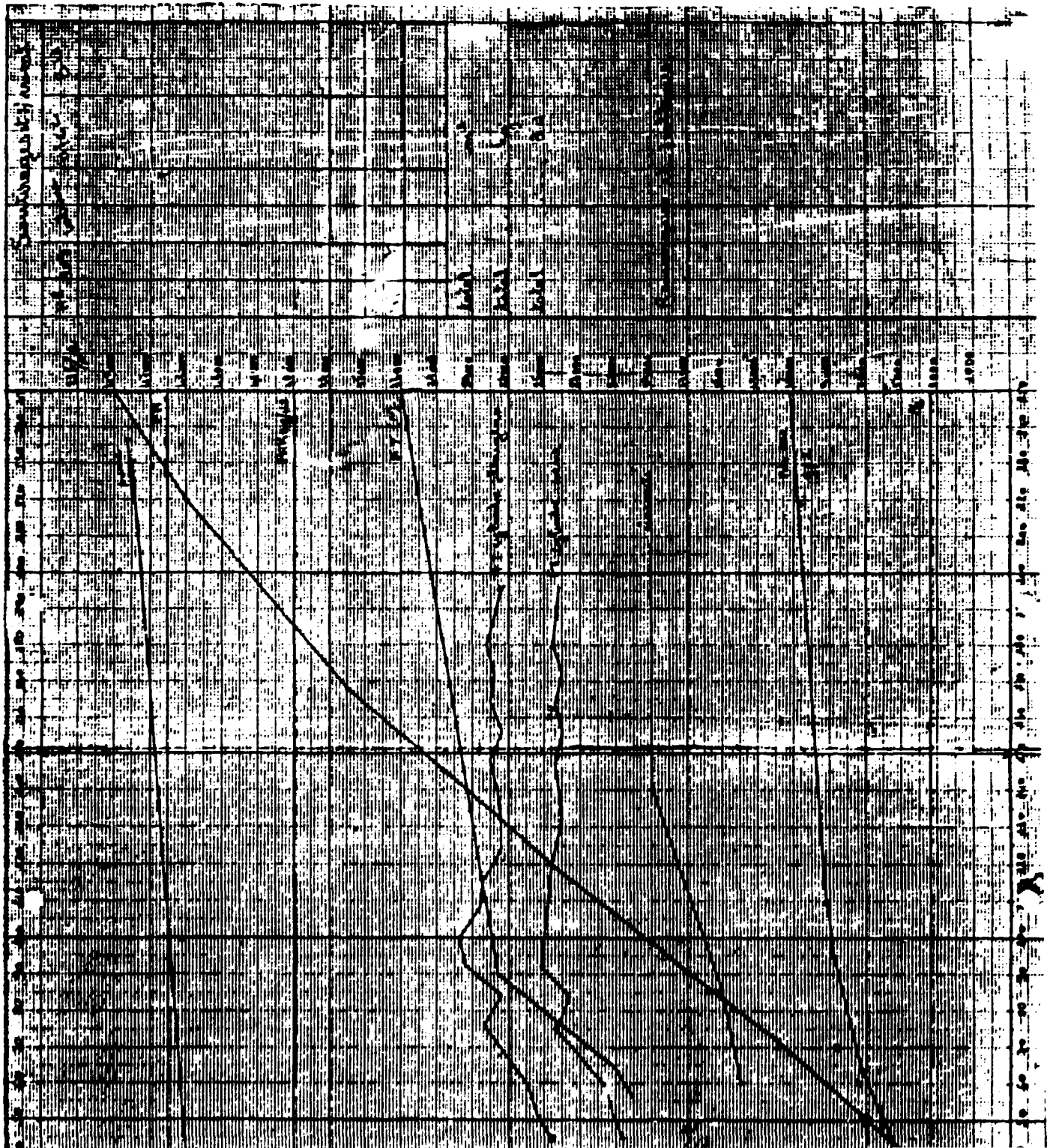
A similar calculation can be done for the lard oil and allowances can also be made for the overestimate by the assay if a non-specific assay is used.

Cost $ per Kg penicillin G Produced

HOURS	Sugar	Lard Oil	Total	Corrected for assay specifity
70 - 100	8.8	1.45	10.25	11.4
100 - 160	10.7	1.6	12.3	13.7
160 - 200	16.5	2.4	18.9	21
220 - 250	21.0	3.2	24.2	26.9

It is now easy to see that after 200 hours in this particular fermentation the cost of the sugar and lard oil alone is approximately the world selling price of penicillin ($30) and is well above what the world production cost might be (say $20) even without taking other costs into account. Around 160 hours is clear when the fermentation is no longer economic.

By keeping all fermentation data on a computer it is possible to see exactly when it becomes economically desirable to stop a fermentation and start a new one, but even simple calculations like the above and taking into account only sugar, lard oil and phenylacetic acid will improve the economics considerably.



Pencillin V

Most of what has been said about penicillin G applies equally penicillin V.

From the titres said to be obtained the culture appears to be even less efficient than the G strain. Since today the same culture is generally used for both G and V, the purchase of a new strain for G will be equally applicable to V subject to a little process development.

All the other factors applying to G fermentation are the same and what works for one product will almost certainly work for the other.

Recovery of penicillin V should be converted to precipitation of the potassium salt rather than the azeotropic system. Overall the recovery yields should be higher than with penicillin G since the compound is acid stable and much less should be destroyed during the recovery steps.

Rather than make the potassium salt and convert it to the free-acid, which is bound to be accompanied by some loss of activity though not as much as is said to occur at Medea, efforts should be made to persuade the market to accept potassium salt. The potassium salt gives higher blood levels and so is more effective. Most countries use only the salt. No free acid is available in USA, UK or Germany. There is a small quantity marketed by Farmitalia in Italy and a very small amount sold in France, though in both France and Italy the potassium salt outsells the acid. This information has already been given to Mr Mausouri. Avoidance of the need to convert to the free acid would be a financial benefit to the factory.

The penicillin V strain used at present will also produce 8 - 10% of phydroxy penicillin V by hydroxylating the side-chain. This has been a problem until the very latest strains (available from Panlabs only in the last year or so). As with penicillin G the hydroxylmine assay will have overestimated the amount in the fermentations so the recovery yield appears worse than it really is. Some of the phydroxy penicillin V will also get into the final product and unless HPLC is used will not be detected.

6APA

Comparison of yields and costs

The current production process for 6APA at the Medea plant uses the chemical de-acylation route, a route which to the best of my knowledge has no longer used by any other manufacturer in the world for many years. Even the guaranteed yield of 75% was substantially inferior to the 90% known to have been achieved by Gist Brocades the originators of the process. Not only are there yield and cost problems but the chemical method also employs dimethylaniline, a known carcinogen, a compound for which a limit test is imposed on many semi-synthetic penicillins in the EP and USP and which it is clearly best to avoid.

If one takes the standard purchase price of penicillin G at $30/Kg (The actual production cost of penicillin G will be lower and when looking at the prices at which 6APA is sold one should remember it is a way for many companies to sell their penicillin G. The profit is really taken on the penicillin G making it highly desirable that penicillin G is made efficiently within Algeria) and using the cost figures provided by Saidal the guaranteed yield of 78% of theoretical would give a cost of producing 6APA at Medea of around $90/Kg compared with the world price of around $70. The actual yields reported to be obtained at the factory were only about 60% giving a production cost as high as $117/Kg. Furthermore the total capacity of the plant when producing one batch per day 300 days per year would be as little as 51 tonnes per annum at the guaranteed yield and only 39 tonnes at the currently obtained yields.

Using the same major reaction vessel (8m3 capacity) with minimal modification involving automatic pH control with addition of either ammonia or sodium hydroxide, fitting a stainless steel sieve mesh at the bottom on which to collect the immobilised enzyme and possibly modification of the agitator blades, the total cost of which should be a few tens of thousands of dollars, it would be possible to operate the enzymic route with yields of around 85%. The inclusion of a reverse osmosis system for concentrating the 6APA solution prior to crystallization would increase the yield to around 90%. Such a reverse osmosis unit would cost $170,000 but on a 100 tonne per annum plant would give an additional yield of about 5.4 tonnes worth around $378,000 ie the pay back would be under 6 months for this additional capital investment.

The cost of purchasing the enzyme which is around $3-5per Kg of 6APA produced would be offset by there being no need for the dimethyldichlorosilane, the dimethylaniline or the phosphorus pentachloride which together come to a similar cost. The solvents and other materials would cost roughly the same and there would be a further major saving, currently 14.5% of Medea's production cost because no liquid nitrogen would be required. Because of the problems of transport and storage of liquid nitrogen

when considerable quantities are lost by evaporation the real cost of liquid nitrogen is probably higher. Using these figures and the same input cost of penicillin G the cost of 6APA produced would be reduced from the current price of around $117/Kg to $68 or $64.5 depending on whether a reverse osmosis concentration were installed and lower still if penicillin G were produced economically at Medea.

The use of the enzymic route would also give a significant increase in total capacity both batch size and annual production potential. Because of the finite life of the immobilised enzyme whether it is used or not it may be desirable to reduce the potential batch size by half and also to carry out a campaign with a batch of enzyme using it perhaps 4 times a day rather than once. There may also be a minor problem of matching centrifugation and drying capacities to production.

For ease of comparison the above data is set out in the table below:

6APA Potential Savings $

	Saving $Kg per G used	Saving $ per tonne G used	Saving $ per Kg as 6APA produced	Saving $ per tonne 6APA produced	Cost per Kg 6APA produced $30/Kg	Cost per Kg 6APA purchased	100 tonnes 6APA as G@30/Kg Loss/benefit
Existing Actual Yields	0	0	0	0	117	70	- 4.7 million
Existing Process Guaranteed Yield	19.9	19,900	27	27,000	90	70	- 2.0 million
Enzymatic process 85% yield	25.3	25,300	4.9	49,000	68	70	+0.2 million
Enzymatic process 90% yield	28.7	28,700	52.5	52,500	64.5	70	+0.55 million

Note: $30 /Kg per G is selling price not manufacturing price of G. Manufacturing price of G should be around $20/Kg. The present cost in Medea will be over $30/Kg, so if local penicillin cannot be made efficiently below that price then there is not a good case for making 6APA locally.

Potential cost and output of 6APA

	Yield % Theory	Cost 6APA	Cost G per Kg 6APA	Kg output Batch same Kg Pen G	Tonnes/ output 300 day same Kg pen G	Kg output/ batch using similar vol in vessel	Maximum tonnes/ output 300 day
Existing process Actual yield	60	$117	$86	130	39	150	39
Existing process Guaranteed yield	78	$90	$66.1	170	51	170	51
Enzymic process without concentration	85	$68	$60.7	184	55	237	284
Enzymic process with concentration	90	$64.5	$57.3	195	58.5	251	308

Assumptions: Penicillin G at $30/Kg. 1 batch per day. Same equipment used. Other costs same.

It can readily be seen cost at $117 or even $90 is well above existing price of about $70/Kg.

The table shows very clearly the effect of cost reduction when using the enzymic route as well as the potential to increase production. Used to its maximum capacity of 4 enzymations per day this process could produce over 300 tonnes of 6APA per annum from the current vessel while the current process at one batch/day could produce only 51 tonnes, a very significant difference in the capital cost per Kg of 6APA.

Installation of Enzymic Process

The immobilised enzyme is readily available from several commerical sources. There are at least three sources of penicillin G acylases as well as two companies making themselves for in house use.

None of the companies supplying commercially available penicillin G acylase has any significant experience of using it on a commercial scale in a factory and while it is easy to get their suggested yields of 6APA, and the number of enzyme cycles quoted in their literature, on a laboratory scale it is more difficult on a plant where unit process times are much greater. There are a number of important aspects related to the design and usage of such a plant in order to achieve the yields of 6APA set out in this paper. The yields quoted here are what can be achieved if the process is operated correctly under ideal conditions and are known by the author to be routinely achieved by a number of producers. To the best of the author's knowledge none of the major producers would be prepared to provide the desired know-how except in one or two cases when that company would provide such know-how only if it were

a joint venture and if the penicillin were bought from them. In the author's view the best information on plant design and recommentation on the best enzyme to use could be obtained from Michael Barber Associates (MBA) and it suggested their assistance is sought. The author has to disclose a minor personal interest in MBA but he also knows that using MBA would also gain access to real experience of production and not just paper technology as is so often provided. The yields obtained would be state of the art.

During the final debriefing with UNIDO Algiers and the Saidal Management it was suggested that they may have no need for help on a new 6APA process to be suggested by the author. During the discussions with the Danish company Novo over the glucose production at Maghnia it had been suggested a 6APA process could also be provided. This provides a good example of the need for expert advice. Novo are an excellent producer of industrial enzymes, probably one of the best in the world. Their penicillin acylase is however not one of the best available, it is mechanically somewhat fragile and needs a buffer because it cannot readily stand the vigorous agitation which is desirable to minimize loss of penicillin and 6APA. More important however is that it is a penicillin V acylase, not a penicillin G enzyme,and it would not be effective if penicillin G were the substrate. These enzymes are extremely substrate specific. Novo does not manufacture or supply a penicillin G acylase. At present the intentions of the Medea plant seem to be to concentrate on penicillin G. More penicillin G is available in the world than penicillin V and it is generally penicillin G which is used industrially to make 6APA.

The comparison of the advantages of penicillin G and penicillin V as a raw material are not part of this paper but it is significant that other than Novo the only other producer of a penicillin V acylase is Biochemie. Like Gist (a penicillin G producer) they are really only interested in producing the enzyme for themselves or for customers who purchase penicillin V from them. If Medea were to manufacture more penicillin V than penicillin G then a penicillin V acylase would be needed but that is not I understand the intention. It should also be remembered that the enzyme producers are unlikely to be too interested in maximising the use of their enzyme and will not necessarily have great experience in the maximum yield of 6APA.

AMPICILLIN

The current process used for ampicillin is based upon the so called chloride-hydrochloride route because it uses this particular form of protected side-chain. A protected side-chain is of course necessary for both the amino penicillins, ampicillin and amoxycillin because the acid chloride reaction as used for other penicillins would acylate the amino group of the side-chain just as readily as it would 6APA The amino group therefore has to be protected. Early routes to ampicillin involved protecting the amino group with a carbobenzylozy group and then removing it by hydrogenation involving expensive palladium catalyst or by making the corresponding nitro compound which was converted to an amino group again by reduction. The two major routes, one developed by Bristol and the other by Beecham, were the chloride-hydrochloride and the Dane-salt ethylchloroformate processes.

One major problem with the chloride-hydrochloride route is that this form of protected side-chain is not very stable. It slowly breaks down giving lower and lower yields of ampicillin. Furthermore there is no way of repurifying or recovering the deteriorated side-chain. Unfortunately the factory has some 40 tonnes of this side-chain which is several years old. It has now deteriorated so much that the yields from it are now so low that it is wasting good 6APA. It would be cheaper to accept the material is now worthless rather than try to make ampicillin from it. For any factory which is not scheduling regular production on a large enough scale to use the side-chain in a few months at most, this is not the right process to use. The chloride-hydrochloride process should be capable of much higher yields than the guaranteed 66%, they should be of the same order as the Dane-salt below but, in view of the stability problem, is not recommended.

A more convenient process would be to use the Dane-salt pivaloyl chloride route. Not only are the materials now stable but the process is essentially the same as for amoxycillin making it convenient to make both products on alternating campaigns on the same plant.

A comparison of the yields and costs of the ampicillin process (see table) shows the saving on 6APA alone that could be made by operating an efficient process. Overall the savings would actually be somewhat larger because of the saving in side-chain as well which represents about 30% of the cost so the total saving per tonne of ampicillin produced would be over $40,000 compared with present operations.

Information on the requirements for a 50 tonne capacity plant (combined ampicillin and amoxycillin) was set out in detail in the interior report and so is not repeated here. What is clear however is that the present equipment can be used, in fact only one of the large vessels is required together with half of the smaller vessels would be required although because the projected output is the

same all the recovery equipment, centrifuges and driers, will be used. The small glass-lined vessel will be suitable for making the mixed anhydride (ie Dane-salt - pivaloyl chloride mixture) but will need to have a system of injecting liquid nitrogen installed. Obviously with the glass-lined vessel it would not be possible to fit a coil for the nitrogen, however direct injection is very effective and although direct injection causes some loss of solvent this is more than counterbalanced by the use of less liquid nitrogen particularly if the local cost of liquid nitrogen is higher. The other vessels needing to be cooled to -50°C already have liquid nitrogen available but if small volumes are used in them direct injection may prove beneficial to costs. It is however important to get the "brine" cooling system operating, liquid nitrogen should be used only after the temperature has already been brought down to -15 - -20°C using it to cool from ambient is extremely wasteful and costly.

The pH control on the vessels will need to be repaired and upgraded, the best electrodes are those manufactured by Ingold. Unless pH is controlled accurately there will be an adverse effect on yield and greater risk of losing a batch. In the case of amoxycillin in particular there is a danger of hydrochloride salt formation. pH should be controlled to at least ±0.1unit and preferably better. Although the temperature needs to be controlled reasonably carefully it is relatively easy to do this manually. A preliminary estimate of the cost of the necessary modifications is around $5,000.

The interim report gave the equipment requirements for a 50 tonnes plant with an outline plant design. It is however quite clear that the present plant can be used with only the small modifications referred to above. Minimum operating volumes are consistent with the processes which could be installed. The cooling coils and agitators present no problems to operate with the smaller volumes which would be used. The drying equipment excellent fluid bed driers are ideal for both ampicillin and amoxycillin, other types of drier can cause problems either by not drying sufficiently well or evenly or by drying too much and taking off some of the water of crystallisation thus giving material of lower stability.

A suitable process could be provided by Michael Barber Associates which would give the yield referred to in the table, ie 86% (ie 1.6Kg of ampicillin per Kg of 6APA).

AMPICILLIN

	Yield % Theory	Kg Ampicillin Per Kg 6APA	Cost of 6APA used per Tonne Ampicillin	Savings on cost 6APA per Tonne Ampicillin
Existing Process as used with Aged Side - Chain	49	0.91	$76,860	———
Existing process to "Guaranteed" Yield. Fresh side-chain.	66	1.23	$56,910	$19,950
Dane Salt process (no problem of ageing side-chain)	86	1.60	$43,750	$33,110

ASSUMPTIONS 6APA $70/Kg
Only half existing vessels but same collection drying equipment.
Minor improvements to pH control costing perhaps $5,000

NB Same equipment exactly for Amoxycillin.

AMOXYCILLIN

Amoxycillin is an important antibiotic, it is gradually taking over the place of ampicillin although the latter is still just the greater in world tonnage. Amoxycillin has replaced ampicillin for oral use on the WHO list of Essential Drugs, and one would therefore expect its demand to continue to grow at the expence of ampicillin.

The process currently used for ampicillin at Medea can not be used for amoxycillin. The chloride-hydrochloride of the p.hydroxy phenyl glycine is not commercially available in large quantities because the cost of making it causes it to be an uneconomic route to amoxycillin, thus the Dane salt route has always been used.

The Dane-salt route as suggested by Cheminvest is a poor yielding version of the original route as used commercially until the 1980's. Even at that time a yield of around 78% (1.51Kg/Kg6APA) should have been obtainable, much higher than the 67%(1.3Kg/Kg6APA) given as the Chemeinvest process yield.

Instead of using the Dane-salt-ethylchloroformate route the Dane-salt-pivaloyl chloride route giving around 88% yield (1.75Kg/Kg6APA) should be employed. It is versions of this route which are now used routinely by all major amoxycillin producers.

The route proposed uses exactly the same process equipment as ampicillin and in many ways the process is the same though there are some small but quite significant differences. Many producers use the same plant for making both ampicillin and amoxycillin thus reducing capital costs. It also has the advantage of maintaining continuity and reducing learning period when changing products, and this would be true for the Medea plant. The very minor modification to the vessels ie adequate pH control and perhaps a new liquid nitrogen line to one of the smaller vessels as proposed for ampicillin would allow the production of both amoxycillin as well as ampicillin on the one unit in excess of the local demand.

The table sets out the cost savings compared with the proposed Cheminvest route which I understand did not perform as proposed. The overall savings including other reagents will make the total in excess of $20,000 per tonne amoxicillin produced.

As with ampicillin, a process, technical know-how and assistance could be provided by Michael Barber Associates and as agreed with Saidal and at the meeting with the Acting Minister for Industry. I will arrange for a proposal to be made by them. As stated for other processes the yields and costs when operated competently are known by the author to be amongst the best operated by anyone in the world. Furthermore MBA do not provide a "paper" process but a process which has been installed and is working efficiently giving yields of 88% or more in several factories.

AMOXYCILLIN

	Yield % Theory	Kg Amoxycillin per Kg 6APA	Cost of 6APA per Tonne Amoxycillin	Savings on 6APA per tonne Amoxycillin	Savings on Dane-Salt use per tonne amoxycillin	Total savings per tonne Amoxy-cillin
Dane-Salt Chloro-Formate process Ex Cheminvest	67	1.3	53,830	—	—	—
Dane-Salt Chloro-Formate process as used by Beecham until c.1978	c. 75	1.45	48,300	5,500	3,000	8,550
Dane-Salt Pivaloyl route as used by good producers today.	88+	1.71	40,950	12,880	6,250	19,130

Assumption 6APA $70/Kg World Price
Same plant as for Ampicillin

ISOXAZOLYL PENICILLINS

This is a related group of semi-synthetic penicillins which are particularly important for their activity against penicillin resistant Staphylococci. The family includes oxacillin, cloxacillin, dicloxacillin and flucloxacillin. The Medea factory has been making oxacillin and wishes to make dicloxacillin as well. The author does not understand why the local interest is in dicloxacillin, it is little used elsewhere and is inferior to flucloxacillin which is the best of the group. Furthermore dicloxacillin cause some necrosis at the site of injection and is generally regarded as not suitable for parenteral formulations.

Oxacillin

It is quite obvious that neither the process currently operated at Medea nor that proposed by Cheminvest compare with what should be the reasonable expected efficiency for manufacture of such products. The cost of $100,000 per process technology for each or any of the isoxazolyl penicillins as proposed by Cheminvest was also quite out of proportion to the value of such specialist products. The total tonnage usage world wide for all the isoxazolyl penicillins in around 1,000 tonnes compared with over 11,000 for ampicillin and amoxycillin together. The best available process should give a saving of over $11,000 per tonne.

As with the other semi-synthetic penicillins MBA will be asked to make a proposal for the necessary technology for oxacillin which as with the other products is comparable with the best available.

OXACILLIN COMPARISON OF PROCESSES

	Yield % Theory	Kg Oxacillin per Kg 6APA	Cost of 6APA per tonne oxacillin	Savings on 6APA/ tonne oxacillin	Cost of side-chain per tonne oxacillin	Savings on side-chain per tonne oxacillin	Total savings per tonne oxacillin
Average yield of original Medea Process	73.5	1.5	$46,900	——	$28,000	——	——
Process proposed by Cheminvest	78	1.6	$43,750	$3,150	$32,800	($4,800)	- 1650
Proposed MBA process	88	1.8	$38,500	$8,400	$25,200	$2,800	$11,200

Assumption 6APA = $70/Kg Side chain = $40/Kg.

Equipment as used at present.

Note. Cheminvest process proposes great excess 1:1.37 rather than the usual 1:1.1 of side chain hence high cost of it in their process and the negative figure under side-chain savings.

Other isoxazolyl penicillins

As with oxaxillin improved processes can also be provided for the other penicillins of this family. The yields compared with those proposed by Cheminvest are set out below. The MBA processes give yields known by the author to be comparable with the highest generally achieved and similar to those obtained by Beecham, the originator of these compounds.

Compound	Cheminvest		MBA	
	% Theory	Kg/Kg 6APA	% Theory	Kg/Kg 6APA
Cloxacillin	72	1.6	90	1.98
Dicloxacillin	76	1.8	90	2.1
Flucloxacillin	70	1.6	90	2.06

For all the above semi-synthetic the MBA processes would offer savings compared to those proposed by Cheminvest. For each one the figure would be simialr to that for oxacillin. The yields are similar and with all of them the side-chain is almost a important as costs on the 6APA.

OTHER PRODUCTION RELATED ISSUES

Besides the main product problems there are a number of other production issues which need to be addressed at some time though not with the urgency of the major ones.

While most of the solvent recovery yields are quite good there are some which require improvement. Part of the problem is undoubtedly due to too many solvents and solvent mixtures being used.

There are no standard operating procedures particularly for re-working of out-of-specification products.

Sterile production clearly has some problems which were difficult to study in any detail since no production was occuring during my visit.

All of the above issues could be dealt with in detail by MBA at the same time as the major issues.

QUALITY CONTROL & ASSURANCE & ANALYTICAL MATTERS

There are a number of reasons why there need to be separate analytical groups associated directly with, situated in or nearby and reporting to the managers of Development, Fermentation and Bulk Production. They can and perhaps should have a functional reporting line to the Senior Analyst but their work and priorities must be set by the group they are working with. Furthermore they must be properly trained in the methods they are using and be part of team with whom they are working helping to follow the progress of a fermentation or discover where losses occur in synthesis.

Quality control and quality assurance are not just there to approve or disapprove materials with regard to specification. They are there also to help solve problems, their information can be valuable, whether it be identifying an organism which has caused contamination of a sterile product (this can help identify where it came from and how to prevent recurrence).

In the case of penicillin G recovery it is essential to follow the materials balance to discover where the major losses are before any corrective action can be take.

Since the first visit management have already arranged for some analytical units to be associated with manufacturing groups. There is however a need for a functional management line to ensure these analysts understand the methods they are using and carry them out properly. From my observation it was obvious that in some cases the "analyst" was more of a liability than a help and the results obtained were totally inaccurate and misleading. There was in one section no awareness that the hydroxylamine assay could be used for other penicillins other than penicillin G and V. Attempts to get some assays carried out on oxacillin were not very successful. The attempt to show a linear relationship between optical density and concentration of oxacillin produced results that indicated a complete review of techniques was required which can only be done by a competent analyst following every step to identify all the errors. This all indicates considerable training is required. Incorrect assays are worse than no assays.

Overall co-operation with production.

There is a need for the analytical group to co-operate with others. Even the Quality Control laboratory for finished products should remember that it is not just a policeman; it should be there to help as well. It seems for example that when a batch of sterile ampoules fails all the plant manager is given a form which denotes failure and all he can do is go through the sterile cleansing procedurees for his unit, at least, that is what he believes to be the case. What should happen is that the QC laboratory identify the organism and give some idea of the level of contamination. This can be very helpful in

discovering the source. Perhaps the most likely source is from a member of staff working in the area and if the organism turns out to be a common human commensal then the cause of the problem is likely to be through someone not going through the clean up procedure properly, dressing properly or handling the material incorrectly. If it is common water borne organism or a spore former then it is a different problem. The manager of the QC department clearly understood the above and apparently carried it out. There thus seems to be a communication problem rather than anything else reinforcing the feeling I have that too many groups operate in their own little boxes rather than co-operate for the common good.

At present bulk sterile packs purchased from outside suppliers are taken to a sterile area within QC. Here they are opened and sampled. Every time the seal is broken there is a risk of contamination and this is just as likely to occur when sampling as at any other time. In fact the QC operation itself could be at times responsible for the contamination. The "sterile" pack is then re-sealed in a manner which is not in accord with GMP and transported to the production building. Bulk sterile materials should in my view be stored in a quarantine unit in the main sterile area and sampled within that area. This will minimise the risk of contaminating a bulk batch. Every time a sterile container is opened one runs a risk of contaminating it. Since the bulk batches of penicillin G and sodium ampicillin come from some of the most reputable suppliers in the world operating under the highest level of GMP and would have been thoroughly checked for sterility by them I would suggest the most likely cause of contamination if found would be the act of sampling or perhaps a false positive in the QC laboratory. Even under the best condition the sterility test can show up false positives as is clearly recognised by all the Pharmacopoeae. Retesting is allowed and is clearly set out in the protocol.

Value of analytical Group as part of production

As mentioned above there has been a move to have analytical groups within manufacturing operations and this is a step in the right direction. Major pharmaceutical companies have gone further and where possible with automated equipment use the process operators themselves to carry out the in-process analysis. While this is appropriate where labour cost are high there are other considerations in Algeria which are more important and it is clearly more desirable to employ people rather than spend money on expensive automated equipment so long as an assay result can obtained quickly and accurately within the time frame needed to make it useful.

In order to improve the recovery of penicillin G from its present low figure it is first necessary to discover where the problem is, that is at what steps do the major losses occur. When one knows that, one can then do something about solving the problem.

Taking a typical recovery as carried out at Medea the first question to be asked is. Is the titre really what it is said to be? At present it is simply assumed that the assay using the hydroxylamine method estimates the yield of penicillin G. No one in the analytical department associated with penicillin fermentation or recovery properly understands the method and how and why it works and I am sure the same situation applies to other methods. The hydroxylamine method when properly used is a very good method for measuring total B lactam. It will however give a positive figure for any penicillin or cephalosporin but will not distinguish between them. It can be used to measure total B lactam both accurately and reasonably precise but it will measure all penicillins present in the fermentations. This will include those always produced in greater or lesser amounts in a penicillin fermentation including 6APA, methyl penicillin and penicillin X, F, dihydro F and the K family. The amount of these produced will depend partly on the organism and also upon the medium particularly in the early part of the fermentation if a lot of lard or vegetable oil is used. The amounts can be quite significant upto 10% of the total penicillin content.

In the case of penicillin V all producing strains until very recently have also produced 8 - 10% of p.hydroxy penicillin V as well as those other penicillin produced in penicillin G fermentations (there will also be some G as a result of precursor substances in the corn steep).

Using only hydroxylamine as the assay thus inflates the yield and the person in charge of recovery is expected to recover something which is not actually there. There is thus a need for a more specific assay which will measure the penicillin G accurately. The best is undoubtedly HPLC and in my view it is highly desirable if not essential to have such a facility available within production.

Having the penicillin in the broth samples measured accurately it is necessary to remind everyone that the assay which everyone quotes is not really the concentration per ml of broth, it is the concentration per ml of filtered broth since the whole broth's volume also includes the mycelium and other solids from the medium. One should not multiply the titre by the number of litres in the fermenter to give the total quantity but by a somewhat lower volume or by multiplying the total volume by a lower titre obtained by preparing the sample in ways which have been discussed with the fermentation staff, a problem which at least some members were aware.

It is now necessary to measure the penicillin level in all process streams, whether penicillin rich or waste streams, in order to determine whether that part of the process is working properly. It is also important to measure the penicillin content in a solution at both the beginning and the end of any unit process that take a long time or hold the penicillin under conditions where it is likely to be unstable. Only by finding out where losses occur by preparing a material balance can corrective action be taken.

It is clearly important to remember that the samples taken are themselves often unstable and must be assayed either immediately, or stabilised if that is possible, thus it helps for the analytical facility to be as near as possible, preferably in the production unit. Similarly it must be remembered that some of the solvents used can interfere with the assay, acetone and other ketones in high concentration will for example interfere with the iodometric assay. Such problems should not occur if the analytical staff understand the need to validate assay proceedures. Copies of two papers on Assay Validation and Penicillin Assay Methods written by Mr A E Bird for the UNIDO Guangzhou project were given to Saidal. (They were part of Mr Bird's Report for that project and are thus not included again).

The details above have been put in to emphasise the importance of analytical work. No experiment will give useful information unless one is able to measure the results properly (whether this be accurately, precisely or both accurately and precisely). Appropriate and reliable assay are essential for economic production and to assist any trouble shooting.

Analytical

Analytical work is not just part of QA or QC. It is at the very heart of an efficient factory operation. Everything depends upon it being carried out properly and without it no process improvement is possible nor is the maintenance of the culture.

Thus while the individual group advocated during my first visit are desirable, they must also be competent. There is clearly a great deal of training needed here, not just among the analytical staff but also among the users of their services.

Probably the best way to start would be to hold a seminar with two speakers, an analytical expert together with someone to explain how these services should be used to improve yields and reduce costs. It would seem that no proper instruction was included at the time of factory start-up or during training in Italy. Those staff who were given some training seem simply to have been instructed in the mechanical operations of carrying out the methods without a proper understanding of how and why they worked and what were their limitation.

Such a seminar could easily be included within the scope of a proposal for assisting with upgrading the processes to be prepared by MBA Associates. This together with functional management supervision of the satellite analytical groups should make the service more useful and effective.

UTILITIES

Water supply has always been one of the problems. Fermentation needs a large quantity of water. On my first visit it appeared that the overall supply of water was the problem. The town rightly taking priority when there was a shortage. I was also informed that the main pipe from the dam could not deliver sufficient water to allow the entire factory to operate at the same time. I assume there is still a problem if everything were running but this is likely to be an infrequent occurence.. The second problem relating to water was the purification of it and work at the dam was said to be causing severe problems such that the filtration unit was producing only 30% of its design capacity. This has now improved to 60%. Certainly the company involved, Christ of Switzerland, is generally regarded as one of the leading water purification companies and there is no-one who can better overcome the problems.

The compressors for producing the cooling fluid are in disrepair, not all are working and it is essential. These and other items such as air compressors are put back in full working order with necessary back up. Even short breakdowns causing a discontinuity in supply can be very serious and back up equipment must always be ready to be switched in. The loss of compressed air for aeration of the fermenters for as little as 15 minutes can lead to lysis and death of the fermentation and loss of all fermentations in progress at that time.

While I appreciate there has been a shortage of money there have been some strange decisions. Instead of obtaining more ethylene glycol to top up the coolant (saumure) very expensive liquid nitrogen, much of which has evaporated during shipment from Algiers, has been used for the 6APA process, so much liquid nitrogen per Kg 6APA prodcued as to be equivalent to 40% of the world price of 6APA.

Because so little of the factory was actually operating I was not able to see first hand what was working and what was not or make any further judgments on this aspect during this visit.

INDUSTRIAL HEALTH

Dr Heraoui asked to meet me to discuss industrial health experience in other factories producing or handling penicillin products. The experience at Medea was similar to other factories in the types of reactions, contact dermatitis and the more serious asthma, although in relation to the amount handled the problem seemed to be numerically greater probably because of proper safety equipment/precautions were either not available or not being used.

Major companies have clearly defined safety precautions for handling each material and also clear policies in the event of personnel developing any health problems. In the case of severe life threatening conditions such as asthma staff could no longer be employed on the site which meant that following medical confirmation of the case there also had to be a defined policy of severance and compensation. The principles of the policy were common throughout all sites regardless of country although there were minor differences depending upon local laws, customs and practice etc.

The incidence of problems within Beecham varied a little between sites and probably reflected the relative succeptibilites of different populations rather than variations in standards or personnel compliance with safety. To get further information it was suggested that Dr Heraoui write to the Medical Officer of Occupational Health at the Smithkline Beecham factory at Worthing.

Dr Heraoui was provided with documents, copies attached appendix, which set out the hazards for all of the materials in making semi-synthetic penicillins including both 6APA and penicillin G together with the precautions which should be taken. For example much dermatitis can be prevented by the use of barrier creams and rubber gloves. While in the case of handling large quantities of penicillin or 6APA a full protective suit including an air hood should be used so that no dust is breathed in. Even for small amounts it is desirable a filter mask should be worn.

From my observations on the plant as when for example 100Kg of sodium ampicillin was charged into a vessel no protective clothing was worn even if it were available. I also noted that when dry penicillin was spilt on the bench no attempt was made to clear it up so everyones hands became contaminated. The handling of sterile penicillin G in the sterile area also left much to be desired. Material from the bulk container was tipped into the filling hopper with no attempt to control the dust.

It is not possible to comment in any more detail because no other work was in progress during my visits but there is no reason to believe any more care would have been taken and it is thus not surprising that there is a very high

incidence of allergy which would probably be even higher if the plant were being fully utilised.

The introduction of Good Manufacturing Practice procedures would also cover many of the requirements to minimise health problems. This is an area where there is a significant new effort being made but where help from a experienced person would be very useful. Such experience could be provided in a package which could be provided by M B A.

MANAGEMENT

The interim report raised a number of concerns about management of the Medea complex. Since that visit there have been a number of changes particularly the appointment of Mr Haddadj in overall charge of SAIDAL. This is undoubtedly leading to other changes and improvements though many of the problems discussed in the interim report must still give cause for concern.

Cost Control and Saving

As mentioned in the interim report there is a clear need for proper cost accounting to be introduced for example. Each process should have a standard operating cost so that actual costs can be compared with budget and with historical performance. Cost centres can be identified and effort then can be made to reduce these costs. It would help avoid financially detrimental decisions such as spending large sums on liquid nitrogen rather than a much smaller one-off cost of purchasing ethylene glycol (I realise this was foreign currency rather than local) to enable the refrigeration system to work. It is suggested that help may be needed in introducing these systems.

Budgetting, Production Planning and Purchasing

It is essential that an efficient system of budgetting is set up. The market/sales demand must be properly budgetted and linked in with production planning and purchasing. This will enable the right amount of materials to be purchased and to be available for production when required. It will also ensure that there are not unnecessary stocks of raw materials held, not only is expensive to have excess stock but in some cases the raw material can deteriorate as is the case with the ampicillin side-chain.

Set up properly to co-operate and work with the production staff they can help reduce costs, improve efficiency, and provide information for management to make appropriate decisions. It did not appear at the present time that any of these functions were operating effectively and in some cases not at all.

Management of Bulk Production

One of the problems associated with the Medea factory is there are so many disparate operations ranging from bulk production through pharmaceutical formulation and even printing. It does not seem that bulk production, the economic performance of which is essential to the whole operation takes its proper place in priorities. This may simply because lack of funds and poor processes has meant it has been unable to contribute properly but there is a real danger the atitude may be difficult to change even when other things are put right. It must be given its proper place in the priorities for the good of the whole plant. Management of bulk production requires understanding of

the needs of the processes and this is unlikely to be found in someone whose training is solely as a pharmacist. It is more likely to be found in a chemist or chemical engineer.

This is made more difficult because there is no-one with real experience of managing bulk production in the pharmaceutical industry. There is no tradition of a pharmaceutical industry in Algeria and it thus seems essential that some help and assistance is found by having an experienced person who could operate as a consultant on a regular basis and be available to give help and advice as required.

Overall Management

One of the great difficulties the management is going to have to face, assuming all other corrective action is taken and there is a sufficient injection of money to provide working capital as well as new processes, is to change all the staff attitudes. At present all the staff and management team have become more and more used to turning up at the factory but not working. There are presently only about 300 properly employed out of the total of over 1,500. It is not going to be easy to get people to work again effectively and some management changes may need to be made to underline the difference. It will not be easy to motivate the staff again and the difficulties should not be underestimated. It will not be just a question of introducing the new processes and everything will now be fine. Perhaps one of the best ways of improving things particularly among the more senior and technically qualified staff could be to organise training programmes and seminars on many of the subjects where there is a need as discussed in the report. Carried out properly with the aid of suitable experts this would not only provide the necessary training but help to motivate the staff again. If they are not convinced the plant can operate economically and effectively then the other corrective measures will not have a chance to succeed.

Financial

To put the factory onto full and efficient operation will require money. The sums needed to obtain suitable processes for 6APA, ampicillin, amoxycillin, isoxazolyl penicillin and improve solvent recovery and manufacture of sterile products is likely to be in the order of $500,000 or so. Penicillin G is a bigger problem because of the culture perhaps $1.5 - $2.0 million, but may be more to start with as no real maintenance has been carried out since the factory was built.

The cost of capital improvements is likely to be no more than $1 million to start with even including the mini-fermenters though money must be budgetted each year for repairs and maintenance. This should probably be between $1 - 2 million.

The other financial problem is the need for working capital. At present no raw material can be bought because there is no money, and nothing can be made to sell because there are no raw materials. At this stage I can make only a guess at what might be required, it would depend how quickly materials could be processed and sold. I would be surprised if much less than $10,000,000 were required because to get the factory working will need starting with bought-in penicillin G and 6APA rather than locally made. What does concern me is that until now the management does not seem to have addressed this problem of working capital but has simply used existing materials in stock even if their use was obviously uneconomic.

MATERIAL HAZARDS

Hazards

Several of the raw materials and intermediates used in the production of BRL 2333 are very hazardous (e.g. concentrated Hydrochloric Acid), whilst others are less obviously hazardous but must still be treated carefully, especially those that give off dust or fumes. Any spillage must be cleaned up immediately and it is essential that everyone follows the safety precautions as laid down in the Materials Hazard Manual. It is important that everyone maintains high standards of personal cleanliness to protect themselves and the quality of the product. To achieve this, regular washing of the hands and exposed body surfaces is essential, especially before working with the finished product. It is particularly important after working with these materials to see that the hands are clean before eating, to prevent the ingestion of trace amounts of chemicals. Barrier cream is provided on the plant and should be used to guard against possible skin irritation and dermatitis.

6-APA, NAP-OH and finished Product (Pen. G, Ampi, Amoxi, Clox ox, etc.)

Inhalations of these dusts may give rise to irritation of the respiratory tract, and skin contact may cause dermatitis. Any sensitivity to these materials should be reported to the Surgery. RFD airhoods/Airstream helmets and short rubber gloves must be worn during charging of 6APA and NAP-OH, and when changing kegs on the Krauss-Maffei drier. Siebe Gorman masks and short rubber gloves must be worn at all times in Drying Formulation Area (D.F.A.)

Methylene Dichloride (MDC)

This is a colourless liquid, which may cause irritation of the skin and eyes. Inhalation of the vapour can cause irritation of the respiratory system and in extremely high concentrations can cause narcosis. Goggles and short rubber gloves MUST be worn when handling MDC. In the event of skin or eye contact, flush well with water. Spillage must be reported to the Supervisor.

Triethylamine (T.E.A.)

This is a colourless liquid with a strong ammoniacal odour which causes severe irritation of the eyes and respiratory system. RFD airhood, green suit and short rubber gloves must be worn when handling. In the event of skin or eye contact, flush well with water, and seek medical attention. In the event of a spillage, dilute with large amounts of running water and ventilate area to dispel fumes.

2-Ethylhexoic Acid (2-EHA)

This is a colourless, dense, acidic liquid. Goggles and short rubber gloves must be worn when handling. In the event of skin or eye contact, wash affected skin with soap and water, wash eyes with water and obtain medical attention. In the event of a spillage, dilute with a large amount of water.

2,6-Lutidine/Pyridine

This is a straw coloured liquid with a pyridine-like odour, which can cause severe irritation to the eyes and skin. A visor, and short rubber gloves must be worn when handling. In the event of skin or eye contact, wash thoroughly with water and seek immediate medical attention. In the event of an spillage, inform Supervisor and spread suitable absorbent liberally and scoop up, placing the absorbed material in a suitable, labelled container prior to disposal. Wash down the area with detergent and water.

Trimethylacetyl Chloride (TMAC)

This is a colourless, acidic liquid which is inflammable. The liquid and vapour can cause irritation to the skin, eyes and respiratory system, if inhaled. RFD airhood, green protective suit and short rubber gloves MUST be worn when handling. In the event of skin or eye contact, wash thoroughly with water and seek immediate medical attention. In the event of a spillage, inform Supervisor, and smother the area with Sodium Bicarbonate. Flush well with water.

N,N'-Dimethylacetamide (DMA)

This is a colourless liquid which can cause severe irritation of the eyes and skin. Goggles and short rubber gloves must be worn when handling. In the event of skin or eye contact, flush well with water and obtain medical attention. In the event of inhalation or ingestion, obtain medical attention immediately. Wash away spillage with large amounts of water.

Concentrated Hydrochloric Acid

This is a fuming, greenish liquid which can cause severe burns. Goggles, corrosive resistant gloves and green protective suit must be worn when handling. In the event of skin or eye contact, flush well with water and obtain medical attention. In the event of a spillage, smother the area with sodium carbonate and wash well with water.

Sodium Hydroxide (Caustic Soda) Solution

This is a colourless liquid which can cause burns. Goggles corrosive resistant gloves and green protective suits MUST be worn when handling. In the event of skin or eye contact, flush well with water and obtain medical attention. In the event of spillage, wash away with copious amounts of water.

Methyl iso butyl ketone (MIBK) (4 methyl Pent-2-one)

This is a colourless liquid with a characteristic ketonic odour. Avoid skin contact, eye contact and inhalation. When handling use gloves and goggles and breathing apparatus if handling large amounts in poorly ventilated area. In the event of spillage, wash area thoroughly with detergent and water followed by degreasing agent.

N.B. Goggles and short rubber gloves must always be worn when sampling process streams.

UNIDO's comments on expert report
SI/ALG/90/801/11-51

Dr. Batchelor assignment to assist MEDEA complex in the field of improvement of the production of antibiotics has been completed after finalization of his second mission.

The expert evaluated the present situation the complex is facing and presented specific recommendations to both the plant managers and health authorities in the country for the improvement of the productivity of the complex and for the application of modern techniques in production and managerial activities. The presented recommendations are of extreme value for the development of the pharmaceutical sector in the country and it is advisable to follow their implementation.

The analysis of the problems the complex is facing has been done following the specific characteristics of each of the production processes the plant is managing.

The process based on fermentation techniques have been affected mainly by lack of experience in the management of the microbiological culture, low productivity of the strains; inconstancy on the production programme, etc. Factors like lack of raw materials, quality inconsistence of the available materials, etc. has also affected the rentability of the whole industrial complex.

The cost of production of Penicillin G will directly influence the economic indicators of the industrial installation. The availability of adequate raw materials from national origin will influence the above. The study of the production of glucose syrup, corn steep liquour, starting from available raw materials and placed not far from the antibiotics complex will contribute to the economical efficiency of the antibiotic production.

Through genetic engineering techniques, it could be possible to develop or acquire varieties of corn resistent to specific climates conditions. Technological know how for production of both corn steep liquour and glucose are commercially available.

Another important aspects which requires to be improved is related to the utilized strains, their genetic conservation and proper utilization. The available strains are poorly conserved and their productivity is extremely low compared those available in the market and utilized in the industry. The optimal recommendation could be to purchase some high productivity strains including the required know hows and to train the personnel on their conservation and utilization in production. For the above, microbiological laboratory must be properly equipped and a pilot installation supplied with necessary measuring facilities and computerized controls is requested.

It could be possible to train the personnel, test the different raw materials and adjust the parameters for more efficient production processes if the mentioned facilities are available.

Once the production of penicillin is properly established with the appropriate quality and yields, the enzyme production of 6APA will have assured the desired economic indicators as penicillin G is the basic material for 6APA. Also the production of semisynthetic penicillins (Amoxicillin, Cloxacilline, Ampicillin, etc.) will improve the economic aspects. For the production of the above mentioned products, it is advisable to look for more productive and modern technological processes as the presently utilized are given extremely low yields which make their production uneconomical. Organization of technology transfer procedures and training of the personnel will be necessary.

UNIDO could assist on the preparation and execution of a specific programme for the rehabilitation of the production activities of the complex.