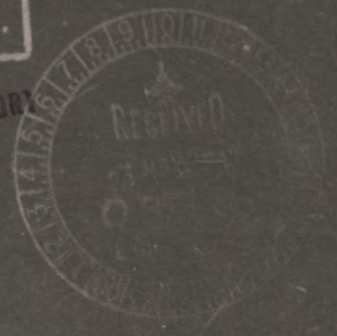


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SUB-COMMITTEE

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SECRET

DUTCH SCIENTIFIC INSTITUTIONS
IN UTRECHT AND AMSTERDAM

MOULD METABOLIC PRODUCTS:
MISCELLANEOUS SUBJECTS:

Reported by

Lt. Col. H.J. BARBER, M. of S.

CIOS Target Numbers 24/28, 24/28a,
24/28b, 24/98 & 24/199

Medical

June 6, 1945

COMBINED INTELLIGENCE OBJECTIVES SUB-COMMITTEE
G-2 Division, SHAEF (Rear) APO 413

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Investigator

Lt. Col. H.J.Barber,
Ministry of Supply

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Part I. Introductory and General Observations

The assessors' preliminary observations and the investigator's further examination of this group of University Institutions makes it clear that they cannot really be regarded as C.I.O.S. targets in the sense that they can provide useful information of immediate practical value. These Dutch University Institutions have in general remained undisturbed by the Germans and have managed to continue with some of their scientific work under considerable difficulty. Dutch University life in the teaching sense has been practically dead for the past few years and many of the research workers have disappeared for various and obvious reasons. It could not be expected therefore that any far reaching developments would arise in such circumstances and this is in fact the case.

The investigator takes the view that these Dutch Scientific men have performed a valuable service to their country (and therefore to the Allies as a whole) by maintaining some kind of continuity of work and preserving their integrity. They can and must play an important part in the full restoration of university life in every sense. The importance of this - especially in such fields as Health Services - needs no emphasis and British and American authorities must make every effort to supply their urgent and essential needs so that they can stand on their own feet as soon as possible. This question is probably dealt with in other places but it cannot be kept out of an account of these visits.

The urgent needs are:

1. Supplies of essential chemicals, biological standards, equipment, etc.
2. Close contact with allied scientific men.
3. Scientific publications (especially review journals) from 1940 onwards.

This investigator would urge that this matter be made the subject of special consideration as soon as possible by a competent body set up for the purpose.

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Part II. Interview with Dr. V.J. K nigsbergerStatus of Organisation

Dr. K nigsberger is Director and Professor of Botany at the Botanisch Laboratorium, Utrecht.

This institution is part of the Faculty of Science of the University and works in close association with the Pasteur Instituut. The Botanical gardens and buildings for research and teaching are situated at the rear of Professor K nigsberger's residence, Nieuwe Gracht, 18 f, Utrecht.

Summary and Recommendations

1. The work reported here has no immediate bearing on Penicillin production.
2. *Penicillium expansum* produces "Expansin" (Patulin).
3. These extracts have been assayed using *Pythium mammillatum* and *P. de Baryanum*.
4. The therapeutic properties have been explored tentatively in lupus, tricho and epidermophytotic infections of the skin with some success.
5. All work should be considered in relation to British work on Patulin.
6. A visit of Professor K nigsberger to London to contact Professor Raistrick and other M. of S. Scientific advisers is desirable in the near future.
7. This group of workers would be a suitable agency through which to establish Penicillin production in Holland.
8. Van Luyk is examining oral flora in relation to head colds and infections of the upper respiratory tract.

Introductory Remarks

C.I.O.S. Secretariat, C.A.F.T. and "T" Force were all emphatic that Professor K nigsberger was a leading member of the Dutch Resistance movement

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and was therefore to be regarded as a friend. The interview was conducted in the light of these directions and the investigator was convinced that Professor Köningsberger was only too anxious to communicate all his information to allied scientists, although he was fully conscious of the fact that he was much behind other workers, largely owing to the difficulties under which he has worked and is still working. Professor Köningsberger is in the course of translating a full report by Van Luyk on his work and this will be sent by him to "T" Force in the course of the next two or three weeks. This report is therefore confined to an assessment of the status of this work in relation to that of British and American Groups. It should also be born in mind that the investigator is an organic chemist whose knowledge of antibiotics is primarily chemical whereas these Dutch workers are microbiologists.

Information Available to Professor Köningsberger

Professor Köningsberger had obtained some information on Penicillin from a leaflet dropped by the R.A.F. This was not of a detailed technical kind but was nevertheless of interest to him. Further details recently reached him in a photo copy of a paper from the Schweizer Medizinische Wochenschrift June 1944, which reviews the information on antibiotics. This paper is probably available in England but part of a table in the paper is reproduced here as it illustrates the situation.

Penicillia

P. notatum Fleming	Penicillin	$C_{24}H_{32}N_2O_{10}$
P. notatum Westling		
P. glaucum	Penatin	
P. petulum	Patulin	
P. claviforme	Claviformin	$C_9H_8O_5$
P. puberulum Bainier	Penicillic acid	$C_8H_{10}O_4$
P. cyclopurin	-do-	-do-

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P. spinosum Thom.	Spinulosin	$C_8H_8O_5$ (Toluidinone derivative)
P. citrinum	Citrinin	$C_{15}H_{14}O_3$
P. resticulosum	Peptide	
P. fimbriatum	Gliotoxin	$C_{14}H_{16}N_2S_2O_4$

Aspergiller

A. flavus		
A. clavatus		
A. fumigatus Fresenius	Fumigatin	$C_8H_7O_4$
A. fumigatus Fresenius		
A. fumigatus mut. Helvoea	Hevolic acid	$C_{32}H_{42}O_8$
A. niger		
A. gigentus	Gigantic acid	

The inaccuracy and incompleteness of the data on Penicillin is readily appreciated. This investigator has had access to the secret reports of the British C.P.S. Committee and the corresponding American documents and great care was taken during the discussions not to disclose any information not published. It was possible however, to inform Professor Königsberger on many points on which there has been publication in England or the U. S. A.

Description of Professor. Königsberger's WorkCulture

The organism used was almost exclusively *Penicillium expansum*. This was very easily obtained from a piece of apple in contact with ordinary soil in a Petri dish. The apple rapidly

becomes covered with the mould and is removed to a sterile dish. The pure organism is readily obtained since, when grown in this way, it forms well marked upright conidia which resemble macroscopic stalks. The clean separation of the spore head is thus a simple matter. The culture medium is Knöps solution with 2% sucrose added. It is grown for 10 days at 25°C. If kept longer it gradually loses its power to produce the antibiotic principle. An obvious sign of this is the lightening in colour of the mould, an active one is dull green the inactive ones dirty white. Solutions of maximum antibiotic activity are obtained in 3 weeks and no culturing is done from such solutions.

Professor Königsberger stressed the need for frequent re-isolation of *P. expansum* from soil. The isolated organism grown in pure culture media loses its activity in 3 months. *P. notatum* was stated to behave similarly but not *P. claviformum*. The investigator does not know if this agrees with British experience. Königsberger said that the Germans obtained *P. notatum* from the Bureau of Cultures at Baarn but Van Luyk was confident that, as these had been grown for 25 years on pure culture media, they would have negligible power of producing penicillin. Some two or three dozen German institutes had obtained strains from Baarn.

Testing of Activity

Professor Königsberger stressed that this method was only an improvisation forced on them by the inadequacy of their resources. *Pythium mammillatum* and *Pythium de Baryanum* were used as test organisms. The technique is as follows. Small rectangular sections (about 6 x 4 mm.) are cut with a punch from a sheet of agar inoculated with pythium spores and dropped into a tube containing tap water (control) or tap water and the appropriate volume of the expansin liquor. The control rapidly develops a fibrous growth of pythium resembling very loose cotton and the height to which this extends is clearly visible by transmitted light. In the control this usually extends about

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half way up the liquid. By serial dilution it is a simple matter to establish the concentration of active liquor which just inhibits the growth of pythium.

Isolation of active principles

This has been carried out at Amsterdam and the methods employed are described in the report on the visit to Professor Jansen. It should be said here that there is every probability that the main product is Patulin but other substances may be produced. The plotting of "bacteriological spectra" would doubtless clear up many points of this kind.

Discussion

It is clear that this work has not reached a stage of immediate practical importance although future developments may well prove interesting. Progress is in the first place dependent on the restoration of the essential services - gas and electricity - needed for the running of thermostats, sterilising ovens, air conditioning, refrigerators and so on. It will then be possible for Professor Köningsberger to assess his products by standard methods developed by allied scientists. He is aware of the Oxford Unit for Penicillin but he will obviously need some standardised Penicillin of a fairly high activity in order to establish his own technique. Sufficient should be obtainable from military sources in the locality in the first place (the amount would be extremely small) but later a very small quantity of pure crystalline sodium penicillin II should be made available. Köningsberger will then be able to describe his products in known units and their true place in the scale of activities can then be seen.

The investigator felt strongly that Köningsberger and Van Luyk may be able to make very valuable contributions in the field of antibiotics from various angles. They are two experienced men who have shown great ingenuity in surmounting difficulties, they have the space in good laboratories in a fine modern building, they have the equipment and they have ideas. Van Luyk's examination of oral flora and their possible bearing on infections of the respiratory tract referred to in Anderson's assessment report is an example, as is also his early association with ideas on antibiotics.

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Ways and means should be found to integrate this work with that of allied groups especially of Raistrick, Fleming and others. It is assumed that the U. of S. will wish Penicillin production to be established in allied countries, and this group of Dutch workers in the opinion of the investigator would be a valuable nucleus on which to base a suitable set-up.

It should not be difficult to work out a scheme of collaboration on lines similar to Anglo-American Co-operation on Penicillin in its various aspects. If this view is accepted in principle the first step should be to organise a visit of these Dutch Scientists - particularly Köningsberger and Jansen to London to establish direct contact with the Ministry and its Scientific Advisers in this field.

It should be added that this work in Holland has received some support from Dutch Chemical industry. The firm of Brocades, Stheeman and Phamacia (Brocaparm) of Amsterdam is making a small annual grant believed to be equivalent to about £600. They have as yet done nothing towards Semi-Scale development of the methods of preparation. They might have done had communications been possible. Enquiries in England show that this firm is the leading house of its kind in Holland and enjoys a good reputation.

The complete paralysis of all communications in Holland has to be experienced before it can be appreciated. There are no trains, no buses, no trams, no postal service, into or in Holland (apart from B.L.A.). Professor Köningsberger in Utrecht cannot keep in touch with Professor Jansen in Amsterdam. There are many bicycles but most have to be ridden without tyres. Wood blocks have been removed from roads for fuel. Utrecht has no gas or electricity and a reasonable water supply has only just been achieved. This parenthetical note has been inserted to emphasise the difficulties facing these scientists at present. They are, however, cheerful and uncomplaining to a remarkable degree and are planning energetically for the restoration of university life in Holland.

Date of Visit: 26th May, 1945.

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Part III. Interview with Professor S.C.P. JansenStatus of Organisation

Dr. Jansen is Director of the Physiological Chemistry Laboratory at the Netherlands Institute of Nutrition.

J.D. Meyerplein, 3, Amsterdam.

This laboratory is a department of the University of Amsterdam and much of its work is on problems of nutrition including vitamin assays (B₁ and C) on much of Holland's food produce.

Summary and Recommendations

1. This report should be read in conjunction with that on Professor Königsberger's work at the Botanisch Laboratorium Utrecht.
2. The main product from the growth of *Penicillium expansum* is almost certainly Patulin. The Dutch workers have called it Expansin.
3. Growth of *P. expansum* at low temperature may produce a new antibiotic with high activity against *S. aureus*.

General Remarks

Professor Jansen took the investigator from his house to the laboratories and showed him round them. These were old and had obviously suffered from neglect during the war years. Many of the staff had perforce to retire into hiding and were now slowly returning to work in the university. The laboratories were equipped for semi-scale operations though not extensively so. Jansen had not received any culture fluid from Königsberger in Utrecht for many months owing to lack of communications and in short he had made very little progress in the last few months.

The laboratory was concerned with nutritional problems and conducted Vitamin B₁ and C assays. They had a small rat house for biological assays and they had the usual physical apparatus. The photoelectric colourimeter they used for B₁ assays they had built themselves. A Hilger Spekker had recently been

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taken out of its hiding place during German occupation.

Detail of Isolation of Antibiotic Substances

Expansin

The original method of isolation was as follows. The culture fluid was concentrated at low (sic.) temperature under reduced pressure. Jansen estimated the temperature to be about 40°C. and said the liquor was about neutral. The investigator is confident that no extra special precautions were taken to prevent the destruction of very labile substances which might be present. The acidified concentrated liquor was then continuously extracted with ether. The continuous extractors were quite normal in design and held two or three litres of aqueous solution. A battery of about 6 of these was set up in a small downstairs room devoted to this kind of operation with inflammable liquids. The ether solution was treated with concentrated hydrochloric acid and this threw out a gum. The gum was extracted with boiling benzene and the benzene solution, sometimes without further concentration, deposited the crystalline product they call "Expansin". Sometimes it was necessary to pass the benzene solution through a column of alumina in order to obtain it in crystalline condition. The m.p. quoted by Jansen was 109° and the m.p. of patulin is 112° so that there is every probability that this product is in fact patulin. Jansen had isolated a product from *Penicillium patulinum* and found it identical. What has not been done is to confirm by mixed m.p. the identity of the Dutch Expansin with the British Patulin. It is just possible that *P. patulinum* of different origin in different nutrient media might produce a modified Patulin. Jansen's Expansin has a very high activity against *Pythium mammillatum* and relatively low activity against *S. aureus*. The figures are:

<i>Pythium mammillatum</i>	1 - 5,000,000
<i>Staph. Aureus</i>	1 - 50,000

Other Antibiotics

Later Jansen improved the method of isolation on the following lines. The culture fluid was passed through a norite column, which adsorbed all the active principles. An important point to note

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is that, unless the norite column is pre-treated with some substance which partly saturates the high adsorptive power of the charcoal, the activity cannot be recovered from the charcoal. Aqueous solutions of benzoic acid were passed through the column first. The charcoal was extracted with methanol in the first place but later it was found that ether was better. The ether solution on evaporation and allowing to crystallise yielded expansin as before but the liquors contained another substance which has not been isolated in a pure condition. This has a higher activity against *S. aureus* and a lower activity against *P. mammillatum*. Jansen is confident that there is another very active substance present.

A further observation of some interest was made fortuitously. Some liquor from Königsberger at Utrecht was delayed for some months in transit and was then found to contain little or no expansin. It did however contain material which had a high activity against *S. aureus*. This hypothetical substance must be a somewhat stable one. It may be the same as that responsible for the activity referred to in the preceding paragraph.

Yet another observation has been made. If the culture is rapidly stirred or aerated there is produced a fairly high *S. aureus* activity moderately quickly but after reaching a peak this rapidly disappears. However if the *P. expansum* is grown at low temperature 5-10° then little or no expansin is produced, but a high *S. aureus* activity results.

Additional evidence for the existence of this principle was quoted by Jansen. They have studied the anti-urease activity of various fractions and have found that solutions containing expansin have low activity but solutions from which expansin has been removed or in which it has not been produced have a high activity. Clearly there are some features of interest here.

Jansen hopes to publish these results shortly in the *Archiv. der Physiologie. Hollandaise*.

Jansen was very co-operative and helpful (he has a son in the Dutch Army in England who is qualifying in medicine). Again the remarks made

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in the report on Professor Königsberger's work apply here. Means should be found to re-establish contact with the scientific world. Jensen counted himself lucky in having a copy of the Annual Review on Biochemistry for 1940. This must be remedied.

Date of Visit: 26th May, 1945.

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Part IV. Interview with Dr. TimmermanStatus of Organisation

Dr. Timmerman is Director of the Pasteur Instituut and Ryks Instituut voor de Volksgezondheid, Utrecht.

Summary and recommendations

1. The Institute has been engaged almost entirely on the production of essential sera and vaccines and research work has been very limited.
2. It is important for the Health Services of the province that this Institute be restored to full efficiency as soon as possible.
3. The needs quoted on p.1. of this report are particularly important to this Institute.

Interview

This target was visited by this investigator at the request of C.A.S.T. Group 3., as it was close to the primary target. It should be noted that the investigator is an Organic Chemist and the work of the Institute is Biological. There is little to add to the Assessors' report on the activities of this Institute and Dr. Timmerman made it quite clear that research work had been almost out of the question during the occupation period. There is therefore no information of immediate value to C.S.O.S. The Institute and its Director has a high scientific status and its work obviously has great importance to the Health Services of Utrecht and the surrounding area. Clearly every effort should be made to restore the Institute to full efficiency. Apart from essential services, such as gas and electricity, which are not the concern of this investigator certain International Biological Standards are urgently required. These are:

1. Pituitary Posterior Lobe.
 2. Insulin.
 3. Sulpharsphenamine.
 4. Neo-Arsphenamine.
 5. Aneurin.
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Dr. Timmerman has already attempted to communicate with Sir Percival Hartley of the National Institute for Medical Research for these standards but does not know the result. The investigator promised Timmerman he would expedite this matter and has been able to arrange with Sir Percival Hartley and the C.I.O.S. Secretariat for the rapid dispatch and conveyance of these urgently needed materials.

This target, being, quite distinct from an enemy one, the investigator was able to answer questions on the whereabouts and welfare of Dr. Timmerman's numerous British friends in Scientific circles. It is difficult to convey in sufficiently graphic terms the sense of isolation from outside scientific circles that these Dutch Scientists have experienced for so long. This can only be remedied, slowly, but the most effective way of doing this would be to grant facilities at an early date for leading scientific men such as Dr. Timmerman to visit London and other Allied centres to re-establish personal contact with British and American scientific thought.

Allied Scientific journals have naturally not reached Holland and the supply of these must have a high priority when the first essentials have been dealt with. Such journals as British Journal of Pharmacology and Experimental Biology, Journal of Pathology and Bacteriology, and Physiological Reviews would be of considerable help.

The investigator appreciates that this report stresses what we should do to assist Dr. Timmerman and indicates that he can supply no help to us. But it must be realised that Dr. Timmerman and many others have contributed much to the Allied cause in the past years by their resistance to German penetration and by maintaining under extraordinary difficulties the coherence and integrity of the institution.

Dr. Timmerman expressed his deep appreciation of the visit of the investigator.

Date of Visit: 25th May, 1945.

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Part V. Interview with Professor K₈glStatus of Organisation

Dr. F. K₈gl is Director of the Laboratory of Organic Chemistry. This is a part of the university and is endowed by the Rockefeller Foundation and also by the Dutch Government. It is situated in modern buildings (teaching and research) in Croësesstraat, 59, Utrecht.

Summary and Recommendations

1. K₈gl claims to have substantiated his observations (subsequently disputed by other workers) that d-glutamic acid and not the normally occurring l isomer is produced in malignant tissue.
2. This is produced by a different mechanism from that which synthesises the l isomer.
3. K₈gl has shown that his (β) biotin differs slightly from the (α) biotin of du Vigneaud.
4. None of these matters is of immediate practical application.
5. It is desirable that K₈gl should contact allied scientists in London in the fairly near future.

Interview

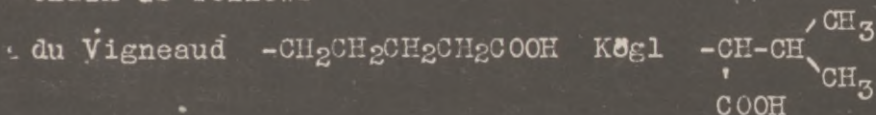
There is little of material importance to add to the outline given by the assessors. Professor K₈gl discussed his results in detail quite freely with this investigator. It was obvious that K₈gl was very unhappy about the doubt which had been cast upon his results by the failure of American workers (published) to repeat his findings. They had again confirmed them at Utrecht and the workers at Munich under Hans Fisher had also been able to confirm these findings. K₈gl said that work using heavy water fed to his animals had enabled him to prove that the d-glutamic acid was formed by a different mechanism and was not produced by racemisation followed by

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preferential elimination of the l isomer with consequent increase in the proportion of the d. Koggl thought this difference between the behaviour of normal and malignant tissue was of profound significance in cancer. The investigator is not competent to express an opinion on this point, but it is very doubtful if this factor could immediately point the way to new approaches to the problems of cancer research. In view of the fact that the work has been written up for publication the investigator pressed Koggl to send it with as little delay as possible to a British or American scientific journal. The investigator suggested Journal of Biological Chemistry as a possible medium. Later, when contact can be re-established between these Dutch scientists and their British and American colleagues, it seems highly desirable that Koggl should have the opportunity of vindicating himself by demonstrating the technique which has given these results. But this is a matter for a scientific society or academic institution.

Koggl has also done some work on biotin and demonstrated that the biotin which he has isolated differs from the one whose constitution was elucidated by du Vigneaud. The difference is in the side chain as follows



It will be noted that the total number of carbon atoms is the same but that the carboxyl group is α to the heterocyclic system and the chain is branched in Koggl's β biotin.

This work has been published (Hoppe-Seyler, 281, 65 (1944) and should be available in England by now.

Date of Visit: 25th May, 1945.

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War Dept, Combined Intelligence Objectives

Subcomm. Report # 82



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