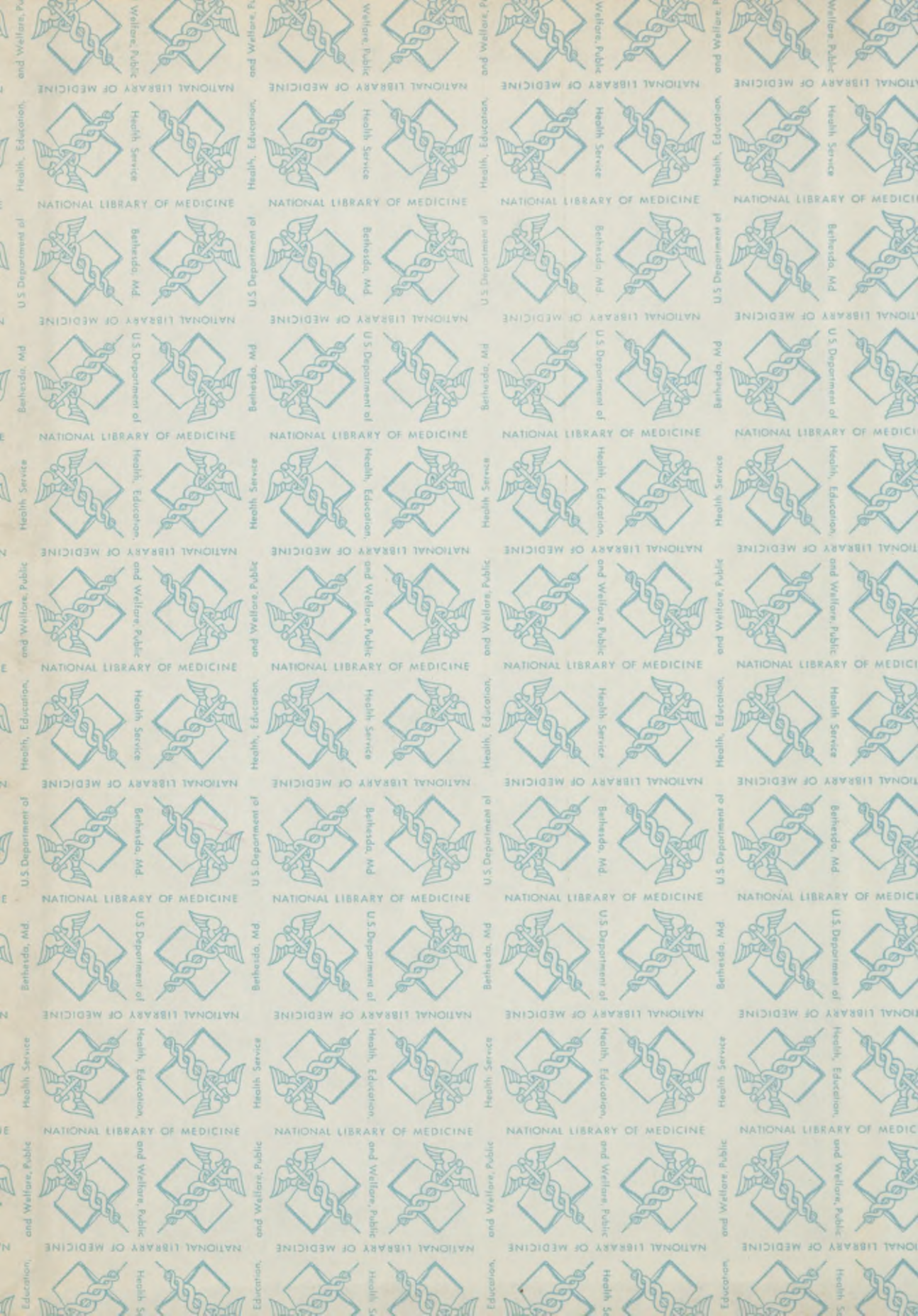
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PHARMACEUTICALS

AT THE

I.G. FARBENINDUSTRIE PLANT

ELBERFELD, GERMANY

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COMBINED INTELLIGENCE OBJECTIVES

SUB-COMMITTEE

RESTRICTED

PHARMACEUTICALS AT THE I.G. FARBENINDUSTRIE PLANT ELBERFELD, GERMANY

A SUPPLEMENTARY REPORT

Reported by:

Dr. IRVIN C. KLEIDERER, U.S. Civilian,
TIIC, Team Leader
Dr. JUSTUS B. RICE, U.S. Civilian, TIIC
Mr. VICTOR CONQUEST, U.S. Civilian, TIIC

CIOS Target Number 24/3
Medical

p. 48
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COMBINED INTELLIGENCE OBJECTIVES SUB-COMMITTEE
G-2 Division, SHAEF (Rear) APO 413

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PHARMACEUTICALS AT THE I.G. FARBENINDUSTRIE
PLANT
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COMBINED INTELLIGENCE OBJECTIVES SUB-COMMITTEE
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I. Introduction

A. General: The report of CIOS Team No. 110 contains some general comments on Elberfeld research, products and operations. In order to make the present report more complete, such information as is pertinent is repeated here. Some of the information collected at Elberfeld is not new to us; nevertheless, it is included so as to give the reader an overall picture of the status of research and development at this plant.

Our impressions of the departments at Elberfeld are as follows:

Synthetic Organic Research: This department has an excellent staff headed by Dr. Fritz Schönhöfer, who in spite of difficulties during the past years has done good work.

Development Department: This division is headed by Dr. Dörr, who has a reasonably well equipped technical room and a good staff. His reports indicate a very thorough job on the projects undertaken by this group.

Tropical Medicine: Headed by Dr. Walter Kikuth, this section does an excellent job of providing birds, mosquitoes and other test materials. Some of Kikuth's methods and conclusions, however, are subject to criticism by our experts.

Pharmacology: Headed by Dr. Weese, this department is very well set up with many new ideas on equipment and conveniences. We feel, however, that the work of this department is not of high standard, and is frequently inadequate to support conclusions drawn from it.

Points of special interest in processing were:

Barbiturates: Sodium ethylate is made in a special safety room in special reaction vessels, the alcohol being maintained under nitrogen. Sodium is cut into small pieces and introduced through a double-valve trap-door, nitrogen being flooded into the valve both from the vessel and a separate connection.

Vitamin D_2: In the vitamin D_2 process about 7 kg. per month are made. After irradiation of ergosterol the vitamin D_2 is purified via the dinitrobenzoate. Subsequently the dinitrobenzoic acid ester is hydrolyzed giving pure D_2. From 1 gm. of ergosterol about 0.25 to 0.30 gm. of D_2 is obtained.

Olive oil is used as a carrier for the commercial product.

B. Plant: The physical plant is in excellent condition, having suffered only one bomb hit in 1943 and 12 artillery shells on the last day before our occupation. Machine gun strafing was frequent but did no material damage. Several units of the plant were inspected and found to be clean, well designed and roomy; more so than most American plants. The practice at Elberfeld was to put each product manufacture in a separate building or section of a building, and to provide separate equipment for that operation alone. This results in the duplication of certain equipment that could be used for a number of products. On the other hand, it prevents contamination and the need for thorough cleanup after runs on different materials. All the equipment, while not new, was well-designed, spaced widely to give plenty of light and floor space, and maintained in excellent condition. Safety measures were up to date, and adequate provision was made for the protection of plant and employees.

Official permission was given to open the plant on 28 May 1945. Three weeks' supply of coal was on hand and arrangements for further provision were being made through the military government.

During the six weeks' shut-down the workers have been paid some money and are available for work. All of the research personnel is in Elberfeld or nearby in small communities, except Domagk, who is missing somewhere in the Hamburg area.

C. Interrogation of Dr. Hörlein, General Manager: Dr. Hörlein, general manager and director of the various institutes, was first interrogated. He had little detailed information at hand so that most of his answers were general in nature. In answer to a question regarding Japanese cooperation, he said that none existed. He alleged that even before the war the Japs were a source of trouble to the I. G. They used I. G. patents and information illegally, and some patent suits were filed against them. Hörlein gave all the appearance of intense dislike for the Japs.

Regarding the number of men employed in the laboratories, Hörlein said that research suffered during the war. At times one-third of the staff were away and the average efficiency was only 50%. When interrogated regarding any special work for the Wehrmacht or the Nazi party, Hörlein replied that no special work or contract work had been done. He said their products had been supplied in quantity to the German Army. Atebrin, Sulfonamides, Marfanil, and Periston were the large items. Periston was not supplied in the

quantities wanted because of a shortage of containers and stoppers.

Regarding the Nazi party, he stated that Julius Streicher was openly antagonistic to I. G. Soon after the Nazis came to power a series of cartoons, labelled "Isadore G. Farber", appeared in Streicher's paper. Hörlein said he joined the party only because it was necessary to carry on the work of the company. He does not know of any clinical work carried out on POW's or displaced persons.

Hörlein was also interrogated concerning his work on carcinoma. He said no results of promise had been obtained from screening tests. (Dr. Hackmann's work on cancer appears in a later section of this report.) He did say that the work of Kögl on the presence of d-glutamic acid in tumor tissue had been confirmed. The significance of this finding, even if true, is obscure. (This statement, as well as Kögl's claims in his original publication, have not been confirmed by British and American investigators.) Hörlein admitted that American and British results with diethylstilbesterol in prostate carcinoma have been confirmed in the I. G. laboratories. He also said that insulin manufacture was carried on at Höchst, but that the supply did not meet the demand. No attempts had been made to find a substitute material for insulin preparation. At present there was a big shortage of pancreas, since in normal times most glands are imported.

D. Interrogation of Dr. Lutter, General Works Superintendent:
The following points were developed:

1. All packaging of finished pharmaceutical products made at Elberfeld is done at Leverkusen. None is done at the Elberfeld plant, except large drums of rubber accelerators.

2. Those in charge at Leverkusen are Director Schellenberg, in charge of all packaging; Dr. Neubert for tablets; and Dr. Daab for ampoules.

3. Elberfeld manufactures the following vitamins:
   a. A from tuna fish liver oil.
   b. B_1, synthetic.
   c. B_2, synthetic.
   d. D_2, irradiation of ergosterol.

   The other vitamins are made at other plants, for example, vitamin C at Höchst.

4. Regarding mixtures of vitamins, Lutter said that B_1 and C were never mixed with A because of the instability of B_1 and C. Mixtures of B_2 with nicotinamide are now on

stability tests, but mixtures of B_1 with nicotinamide have never been tried.

E. Manufacturing: Dr. Lutter is in charge of all manufacturing at Elberfeld, with Dr. Rietz as his assistant. Dr. Dörr is in charge of the intermediate development of products (The "Technischer Raum" or Pilot Plant). The procedure in Elberfeld is for Dr. Schönhöfer's department to develop a product in the laboratory. Dr. Dörr's department then takes over and gets the process ready for the plant. It then becomes Dr. Lutter's responsibility to produce it for commercial sale. The research department very seldom comes into the picture again, Dörr's department being the "trouble shooters".

There is no pilot plant in the American sense. The old original laboratory plus several other laboratories constitute the entire development department. In one of these, a new laboratory, there are two 100 liter enameled stills equipped for reflux pressure or vacuum. This constitutes the only plant type equipment.

F. General Scope of the Testing of Products: There are three testing groups. They are headed by (1) Kikuth for malaria and other parasitic diseases, (2) Domagk for bacterial chemotherapy, and (3) Weese for pharmacology. Special sheets are used, and samples of these are included in this report (See Appendix 1).

II. The Organic Research Department

This Department is under the direction of Director Dr. Fritz Schönhöfer, who is a short-statured, pleasant and very cooperative subject for interrogation. He is one of the three I. G. directors at Elberfeld. Drs. Hörlein and Lutter are the other two.

A. Personnel: His staff consists of the following men:

Name	Technical School	Education Professor	Principal Researches
Dr. Schönhöfer	Munich	Wieland	Antimalarials; basic side chains for organic Hg. derivatives like ceresan, acaprin; vitamin B_1; barbituric acids.
Dr. Mietzsch	Dresden	König	Sulfonamides, sulfones, acridines such as atabrin, acrinil, antergan (RhP)

Name	:Technical School	Education :Professor	:Principal Researches
Dr. Andersag	Munich	Hans Fischer	Sontochin, Vitamin B_1, biotin constitution, desthiobiotin, benzochin, cyren.
Dr. Behnisch	Breslau	Königs Slotter	Sulfonamides and sulfones, tocopherol.
Dr. Hiltmann		Helfrich	Was with I.G. about 3/4 year, then went to Army and has only lately returned.
Dr. Breitner	Munich	Hans Fischer	Sontochin, insecticides, synthesis of oestrone-like substances.
Dr. Klarer	Munich	Hans Fischer	Sulfonamides and sulfones.
Dr. Klös			Has been in Army since 1941. Worked a little on Hg. compounds. Not a very promising chemist.
Dr. Linsert	Göttingen	Windaus	Vitamins D_2 and D_3.
Dr. Fritz Leuchs		Adolph Bayer	Zephirol and other quart. salts. Age 64.
Dr. Mauss			Atabrin (discoverer according to Dr. Schönhöfer) Zanthones, analogues of known antimalarials.
Dr. Meiser	Munich	Wieland	DDT, compounds in adaline field, laxative compounds.
Dr. Pöhls	Marburg	Meerwein	Sulfones, diisopropyl-sulfone and isopropyl sulfonamid for cancer.
Dr. Salzer	Darmstadt	Schöpf	Cyren and analogues, sulfonamides and azides and endochin.
Dr. Schrader			Worked a little on Phosphorus compounds, but worked chiefly for Prof. Hörlein.
Prof. Schmidt	Strassburg	Thiele	Antimonials, amidines.
Dr. Schütz	Jena	Knorr	Pharmacist. Worked for about a year and went to Army as meteorologist.
Dr. Westphal	Göttingen	Windaus	Vitamin B_1, Castrix, Esmodil, and pterines.
Dr. Wieder			Discharged.

B. Consultants: The I. G. consultants receive no retainer fees, but are provided with apparatus, chemicals and funds for assistants. When a consultant produces a worthwhile product which is eventually put on the market, he receives a sum of money. This sum varies, depending on two factors. If a patent has been issued, it may be as high as fifty percent of the profits, but it also depends on the proportion of the total work performed by the consultant; and this percentage is paid during the life of the patent (15 years). On the other hand, if no patent has been obtained, a smaller sum, about five percent, is paid for fifteen years. Consultants are free to carry on research of their own choice.

Among the I. G. consultants in chemistry are:
Windaus in Göttingen;
Kuhn in Heidelberg;
Brockmann in Posen;
Kögl in Utrecht;
Grewe in Strassburg, then Göttingen;
Konigs in Breslau;
Schöpf in Darmstadt;
Pfeiffer in Bonn;
Kröhnke in Berlin;
Hans Fischer in Munich (to Höchst plant);
Meerwein in Marburg.

C. Bonus Plan: The type of bonus plan for I. G. employees was much the same as that for consultants. It frequently happened that men working for Schönhöfer received larger total payments than he did. For example, as a result of his work on the sulfa-drugs, Mietzsch received a total of about 50,000 RM, while Schönhöfer was paid 48,000 RM.

Salaries paid new chemists varied from 3000 to 4000 marks per year. On the basis of the pre-war mark, valued at forty cents, this would be about 1200 to 1600 dollars. Schönhöfer stated that at least five years were necessary before a new chemist produced anything worthwhile.

D. Patent Scope: An example of the scope of the patent department of the I. G. may be gained from the following list of countries in which two representative products have been patented. This was necessary because they had sales offices in these countries.

Vitamin B_1: England, France, Italy, Switzerland, Czechoslovakia, South Africa, Japan, Poland, Austria, Hungary, Sweden, British India, Australia, Holland, Jugoslavia,

Norwey, Denmark, Spain, Rumania, Belgium, Portugal, Mexico, Brazil, Argentina, Palestine, Greece, Ireland, Canada.

Sontochin: England, France, Egypt, Czechoslovakia, Switzerland, Japan, Holland, South Africa, Argentina, Austria, Hungary, Spain, Poland, U. S. A., Canada, Brazil, Greece, Iraq, Jugoslavia, Mexico, Palestine, Rumania, India.

E. Sulfa Drugs:

1. Products: I. G. Elberfeld makes the following sulfa drugs:

Prontalbin (Sulfanilamide) $H_2N-C_6H_4-SO_2NH_2$

Prontosil rubrum $H_2NO_2S-C_6H_4-N=N-C_6H_3(NH_2)-NH_2$

Prontosil soluble $H_2NO_2S-C_6H_4-N=N-C_{10}H_5(OH)(NHCOCH_3)(SO_3Na)$ (NaO_3S)

Uliron $H_2N-C_6H_4-SO_2NH-C_6H_4-SO_2N(CH_3)_2$

Neo-Uliron $H_2N-C_6H_4-SO_2NH-C_6H_4-SO_2NHCH_3$

Uliron C $H_2N-C_6H_4-SO_2NH-C_6H_4-SO_2NH_2$

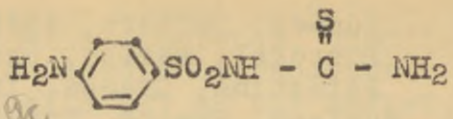
Sulfapyridine $H_2N-C_6H_4-SO_2NH-C_5H_4N$

Eleudron (Sulfathizaol) $H_2N-C_6H_4-SO_2NH-C_3H_2NS$

Debenal (Sulfapyrazine) $H_2N-C_6H_4-SO_2-C_4H_3N_2$

Marfenil $H_2NCH_2-C_6H_4-SO_2NH_2$

Tibatin Galactose $NH-C_6H_4-SO_2-C_6H_4-NH$ Galactose

Badional (Sulfathiourea) 

2. Marfanil: No information on the chemotherapy of marfanil could be obtained from Domagk's assistants. Dr. Hackmann said that since no chemical test method was available for marfanil in body fluids, a biologic method had been developed. A series of test tubes is set up, each containing 1 cc. of blood, 1 cc. of saline, 1 drop of a pararauschbrand bacillus culture, and different dilutions of marfanil. (The pararauschbrand bacillus is a member of the B. Welchii series.) The unknown fluid is put into another tube containing the above solutions, but no marfanil. By comparing the activity of the unknown on the inhibition of the pararauschbrand bacillus with the series of knowns, an approximation of the concentration of marfanil in the unknown is obtained.

A mixture of 10% marfanil and 90% sulfanilamide is used for dusting on wounds. It is not sterilized but is considered by the I. G. workers to be self-sterilizing.

Marfanil (without sulfanilamide) is sold by the I. G. in tablets of 0.5 g., and, according to Hackmann, is widely used by the Wehrmacht. No clear evidence of its activity, however, on oral administration was forthcoming. A motion picture in color showing the beneficial effects of marfanil applied to grossly contaminated wounds of rabbits, is available at Elberfeld. It was difficult to judge the activity of the drug since debridement of the wound was also carried out. The drug was administered locally as well as orally to the animals, with emphasis, however, on topical use.

Dr. Hecht, the pharmacologist, gave the following information. The maximum tolerated intravenous dose of marfanil hydrochloride for mice was 0.1 to 0.5 g/Kg. Doses of 1-1.3 g/Kg produce mild symptoms such as dyspnea in all animals. A dose of 1.4 g/Kg killed one of these mice and 1.5 g/Kg killed 2 to 3 animals.

Doses of 1.0 g/Kg administered to Rabbits caused a transitory fall of temperature of 0.5°C which lasted less than 4 hours. A dose of 2 g/Kg caused a drop of 0.8°C, 3 g/Kg a drop of 5°C lasting for 7-8 hours. The effects on the temperature were even more pronounced when the preparation was given intramuscularly.

Studies on temperature after oral and intramuscular administration were also made on cats.

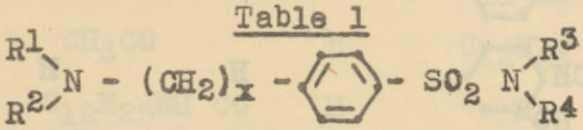
Rabbits showed no evidence of toxicity, other than local effects, after repeated doses of as much as 0.5 g/Kg daily for seven days administered intramuscularly. Toxic effects were observed on cats after intramuscular administration.

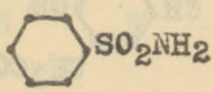
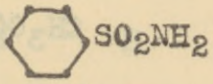
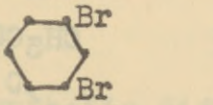
Hecht regards marfanil as relatively non-toxic, and has seen no evidence of cumulative toxicity. He believes that daily doses of 2-3 g. in divided doses can be given safely to human beings.

All in all, marfanil remains to be proved as a really effective substance in vivo. Applied locally it probably has a certain effectiveness. Information regarding attempts to find antagonistic substances to marfanil analogous to p-aminobenzoic acid for sulfanilamide, is given in the section on anti-vitamines and bacterial metabolism.

3. Compounds Made in the Search for Marfanil: Marfanil analogues were made synthetically. These were of the following general structure:

Table 1



No.	R^1	R^2	R^3	R^4	x
Kl 1877	H	H	H	H	1
1880	H	H	H		1
1881	H	H	H	H	2
1885	H	H	H		2
1888	H	H	H		2
1897	CH_3	CH_3CO	H	H	1
1914	CH_3	H	H	H	1
1992	H	H	H	CH_3	1
1979	H	H	CH_3	CH_3	1

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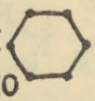
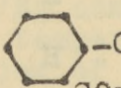
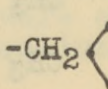
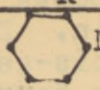
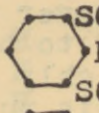
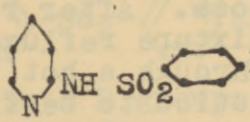
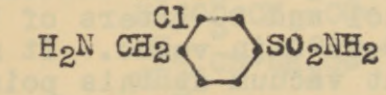
No.	R^1	R^2	R^3	R^4	x
2063	H	H	H		1
2061	H	CH_3CO	H	CH_2 	1
2062	H	H	H	CH_2 	1
2058	= CH 		H	H	1
2057	= CH 		H	H	1
2082	H 		H	H	1
2084	= CH-CH = CH 		H	H	1
2085	= CH 		H	H	1
2086	= CH 		H	H	1
2087	H	CH_3CO	H		1
2088	= CH 		H	H	1
2089	H	CH_3CO	H		1
2091	H	CH_3CO	H		1
2092	H	CH_3CO	H		1
2117	H		H	H	1
2109	H		H	H	1
2102	CH_3CO		H	H	1

No.	R^1	R^2	R^3	R^4	x
2097	H	H	H		1
2094	H	$C_{11}H_{23}CO$	H	H	1
2093	H	H	H		1
2139	H	$C_{12}H_{39}$	H	H	1
2138	H	CH_3 CH_3 CH CH_2	H	H	1
2124	CH_3	CH_3	H	H	1
2171	CH_3CO	H	H		1
2148	H	H	H		1
2174	H	$C_{12}H_{25}$	H	$C_{12}H_{25}$	1
2232	H	H_2N CO CH_2CH_2CO	H	H	1
2259	H	$C_{11}H_{23}CO$	H	$C_{11}H_{23}CO$	1
2283	H	CH_3CO	H	$C_{12}H_{25}$	1
2280	H	$C_{12}H_{25}$NH CO	H	H	1
2279	H	H	H	CH_2COOH	1
2299	H		H	H	1
2295	H	$C_{17}H_{35}COO$ CH_2CO-	H	H	1
2420	H	HOOC CH_2CH_2CO-	H	H	1
2463					

When x becomes 3 or higher, the synthetic difficulties are very great. The best compounds are those in which R equals H, and x is 1 or 2. In other cases markedly lessened activity results. It was stated that marfanil has some activity against aerobic bacteria but is specific against anaerobic bacteria. In vitro this seems to be true, but it is doubtful that it is true in vivo.

4. Tibatin: Domagk's department had nothing of interest regarding Tibatin. Dr. Hecht supplied the following. Tibatin is the bis-galactoside of 4,4' diaminodiphenylsulfone, and was developed for the purpose of supplying a well-tolerated sulfone preparation which would be highly soluble and suitable for parenteral use.

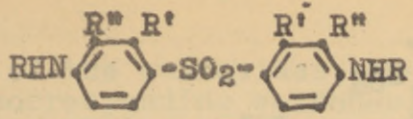
Clinically the use of Tibatin is frequently followed by fever. Hecht believes this to be due to pyrogens present in manufacturing the compounds, but not in the distilled water in which it is dissolved. Each batch produced by the I. G. is tested for pyrogens in rabbits before release. The preparation is always administered intravenously, never orally or intramuscularly. The toxicity is said to be higher after oral administration. (Hydrolysis to highly toxic 4,4' diaminodiphenylsulfone). The preparation is put up in 5 c.c. ampoules containing 2 g. of Tibatin. The solution when manufactured is almost colorless but it darkens on standing. Some three year old ampoules were dark brown. This discoloration is said not to affect toxicity or activity.

Tibatin is made as follows:

400 L Methanol (not over 0.5% H_2O)
25 Kg 4,4'-diamino-diphenylsulfone
40 Kg Galactose
150 g. Salicylic acid.

The mixture is refluxed for 24 hours. After 10-12 hours the galactose has dissolved to a greenish or yellowish solution, which is slightly cloudy due to the presence of $CaSO_4$ in the galactose. After refluxing, 2K of Kieselgur is added and the mixture refluxed for 2 hours. The solution is then filtered through a hot filter into 400 liters of butanol. The solution to be filtered is kept at 66-70°C, but the filtrate is not allowed to heat above 30°C after it has been added to the butanol, otherwise a slimy mess results. After cooling to 15°C, the product which separates out is collected in a centrifuge, washed with 20 liters of methanol and 20 liters of butanol. It is dried overnight at 60-65°C in vacuo. It must not be heated above 35°C without vacuum at this point lest it liquify due to the small amount of water present. The yield is 48-51 Kg. The butanol-methyl alcohol mother-liquors are separated by azeotropic distillation. (Details of this procedure for separation appear in Folder 7 of CIOS Team No. 110). The monthly production aimed at is 800 Kg.

5. Compounds made in the Tibatin Series:

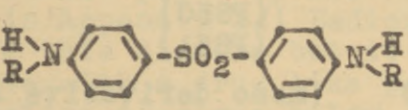


No.	R	R^1	R^{11}
859	Maltose	H	H
865	Galactose	H	H
866	Mannose	H	H
867	Glucose	H	CH_3
868	Maltose	H	OCH_3
871	Maltose	H	CH_3
919	Ch_3CO- Maltose	H	H
921	Annamaldehyde bisulfite	H	H
1105	Acetaldehyde bisulfite	H	H
1106	Propionaldehyde bisulfite	H	H
1134	Glucose bisulfite	H	H
1301	Crotonaldehyde bisulfite	H	H

6. Sulfone Derivatives for Chemotherapy other than Tibatin:

Many other derivatives of 4,4' diamino-diphenyl sulfone besides sugar derivatives have been made. Various men have worked on this problem, but Dr. Pöhls has done most of this work.

Among the types of compounds made are:

(A) 

Where both R groups are the same

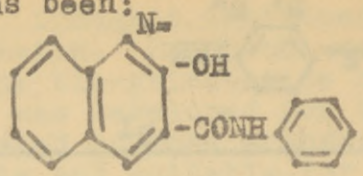
R has been: $C_6H_5-CH_2-CO-$ (P641)
$C_4H_9O-CH_2CO-$ (P672)
$C_6H_5O-CH_2CO-$ (P674)
$NASO_3-CH_2-CO-$ (P713)
$(CH_3)_2CH-CH_2CO-$ (P714)

etc.

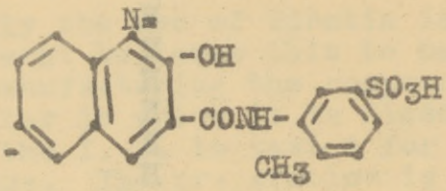
(B) Azo compounds:

Where both R groups are the same

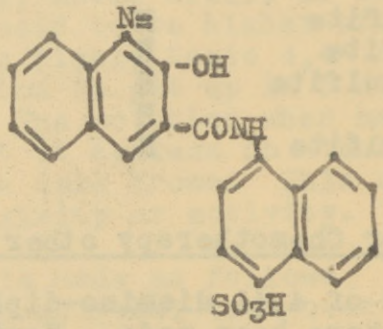
R has been:



(P754)



(P755)



(P756)

(C) Anils of 4,4' diamino-diphenyl sulfone using the following aldehydes:

propionaldehyde	(P849)
butyraldehyde	(P850)
isobutyraldehyde	(P851)
$CH_3 > CH-(CH_2)_4\ CHO$	(mono derivative P852)
crotonaldehyde	(P853)

(D) Miscellaneous compounds:

Among the many other variations made were:

1. Nitro sulfones.
2. Amino nitro sulfones
3. Derivatives of (2) such as sugar, acyl, anils, diazo-coupling compounds.
4. Corresponding sulfoxides of all the above.
5. Corresponding sulfides of all the above.

Most of these represent excellent chemical syntheses, but the toxicity and/or the activity is unfavorable. The only compound marked is Tibatin.

In the case of the sulfoxides, these are much less active than the corresponding sulfones, but the toxicity is the same. Consequently these were dropped. No work is being done on the sulfone problem at present.

7. Badional: This drug is sulfathiourea. It is being tested in bronchopneumonia by inhalation thereapy. There are reports in the literature on this compound, e.g.

Harris, T. N., Sommer, H. E., and Shapple, C. C.: Amer. J. Med. Sciences (1943), 1.

Mooser, H., and Leemann, A.: Schweiz. Med. Wchschr. (1943), 1545.

Kikuth has summarized his findings in the following statement: "Badional (sulfathiourea) is a well-tolerated substance which has been found to be active against various human infections, especially pneumonia. In our test experiments with the broncho-pneumonia virus of white mice, which produces a specific pneumonia, inhalation therapy with badional was found to be superior to subcutaneous administration, since, as we were able to show, it permitted a deposition of the substance in the pulmonary tissue and thus an intensive action upon the disease focus. Consequently, it appears promising to us to try the inhalation therapy on human pneumonia patients." (an unpublished paper by Kikuth and Bock on this subject is given in Appendix 3.) Badional probably has some usefulness in this disease but it is doubtful whether it can compete with a drug like penicillin. The method of preparation is given below.

a. Preparation of "Sulfocalcium" (Intermediate in preparation of Badional)

900 liters of water are placed in a 2000 liter iron reactor with a stirrer and 150 Kg. of crude calcium cyanamide are added. The mixture is allowed to stir at 25°C for 3-4 hours and then filtered. The solution is analyzed and adjusted so that it contains 60-80 g./liter.

The adjusted solution is mixed with 120 Kg. of 30% sodium hydroxide solution. To this is added the calculated quantity of prontyl chloride (p-acetamidobenzene sulfonchloride).

68.6 Kg. of calcium cyanamide = 200 Kg. of 100% prontyl chloride.

Upon this addition, the temperature rises to 28-30°C. After

3/4 of the prontyl chloride is added, the solution is tested to be sure it remains alkaline, and if necessary more sodium hydroxide is added. This amount of alkali is e to 25 liters. After 12 hours stirring, 300 Kg of NaCl are added. After addition of most of the salt, crystallization begins and is complete in about 5 hours. Stirring is discontinued during the addition of the salt, so as to get larger particles. Too rapid stirring during the salting out procedure results in a fine product, difficult to filter. After filtration of the product, the mother liquor is tested with salt to be sure that precipitation is complete.

The product is dried in air for 2 days at 45-50°C.

b. Acetyl Badional

200 Kg of glacial acetic acid
208 Kg of acetic anhydride
200 Kg of "Sulfocalcium"
44 Kg of sodium carbonate
100 c.c. of hydrogen peroxide

This mixture is placed in an enamel reactor with a water jacket.

320 Kg of 16.6% sodium hydrosulfide solution (53 Kg - 100%) and a 50% solution of sulfuric acid (made from 41.9 Kg of sulfuric acid) are added slowly. This generates H_2S which is passed into the first mixture over a period of 6-7 hours at 38-40°C.

When all the H_2S is added, the lot is diluted with 700 liters of water. The mixture is allowed to stir at 12-14° overnight. It is then filtered off.

The yield is 170-190 Kg (100%).

c. Resolution of Acetyl Badional

1200-1400 liters of water are placed in a reactor with 40 Kg of sodium carbonate. The acetyl badional is now added. (Care must be observed due to foam.) The mixture is heated to 35-40° and 5 Kg of charcoal added. After 1/4 hour stirring, it is filtered and acidified with hydrochloric acid until precipitation is complete (about 120-140 Kg of 30% of HCl). The precipitate is filtered and washed until the washings are neutral.

Yield is 150-175 Kg (100%).

d. Hydrolysis

The damp acetyl badional is used. For one batch, 133.5 Kg sodium hydroxide in 301 liters of water is used for the hydrolysis. The batch is heated with the alkali for 1½ hours at 75-80°C. To this are added 500 liters of water and 3.6 Kg charcoal, and after ½ hour it is filtered. The calculated quantity of acetic acid (160 Kg 50% acetic acid) is added, the mixture cooled to 10°C and filtered.

Yield: 100-130 Kg crude.

e. Purification of Badional

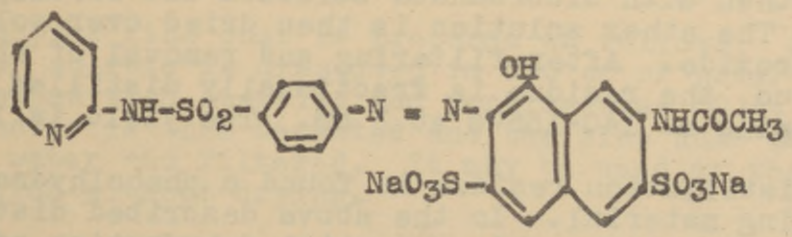
The crude product is placed in 700 liters of water and warmed to 30-35°C. To this is added 20-25 liters of 25% ammonium hydroxide, to cause complete solution. To this is added 2 Kg of medicinal charcoal and after ¼ hour it is filtered.

Addition of 70-80 Kg of 50% acetic acid causes the precipitation of pure Badional. The mixture is cooled to 15°C and filtered. The product is washed 10-12 times with distilled water and well dried on the filter. It is then dried at 30-40° for 24 hours and then at 50-60° at 24 hours.

Yield: 60-90 Kg.

8. Sulfonamides in Trachoma: Kikuth has found sulfonamides to be effective in trachoma. The German Army had considerable trouble with this disease in Poland. Sulfapyridine was used at first, but when it was supplied to the civilian population, the Poles soon began to trade the white tablets for cigarettes. The preparation was changed to a red derivative of sulfanilamide to stop this practice.

The new drug is No. B1034 and has the structure:

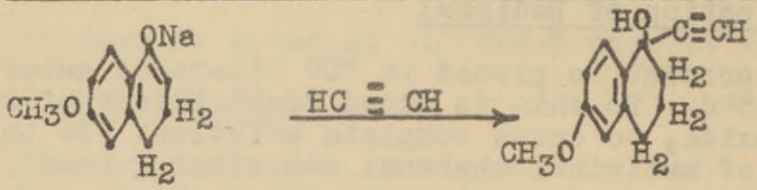


This material is much less effective, but was used in higher dosage with good results. The compound B1034 was prepared by Dr. Behnisch.

The method of preparation is described in D.R.P. 745365 to Dr. Behnisch, March 21, 1944. It is made by coupling diazotized sulfapyridine with 2-acetylamino-8-hydroxynaphthalene-3,6-disulfonic acid.

F. The Preparation of Synthetic Oestrone: Dr. Breitner has worked out a very interesting synthesis. It is given below in detail. The yields, however, are too low for commercial use. Nevertheless, it represents a possible starting point for other synthesis.

1. The Preparation of 1-Acetylenyl-6-Methoxytetralol-1

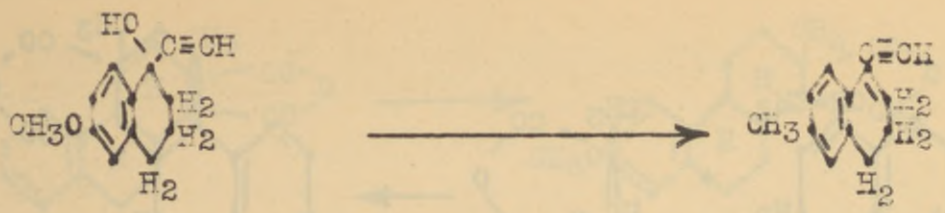


30 g. of 6-methoxy-alpha-tetralon are dissolved in 150 cc of pure dioxane; to this are added 12 g. of mono-acetylene-sodium in a pressure vessel and the mixture is saturated at room temperature with acetylene. The vessel is then closed and shaken for 48 hours at about 30-40°. After cooling to room temperature, the reaction mixture is diluted with 700 cc of ether and filtered. The filtrate is acidified with 2 per cent acetic acid, and washed with water. The etherwwas removed by distillation without drying. The residue thus obtained contains the desired product together with a certain amount of starting material. This starting material is removed in the following manner:

The crude mixture freed from ether is reacted with a cold solution of 13 g. phenylhydrazine in 30 cc of 35% acetic acid. After a short while the phenylhydrazone of 6-methoxy-alpha-tetrolone precipitates. After filtering, the filtrate is refluxed with ether and is washed twice with water, then with bicarbonate solution and finally with water. The ether solution is then dried over solid potassium hydroxide. After filtering and removal of the ether in vacuo, the residue is fractionally distilled in vacuo. It boils at 140-155°C at 3 mm. The yield is 12 g.

In the distillation residue is found a phenolhydrozone of the starting material. In the above described distillation one finds that there is a partial dehydration of 1-acetylenyl-6-methoxy-tetralol-1.

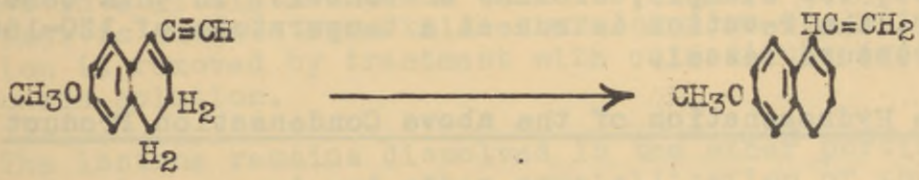
2. The Preparation of 1-Ethinyl-3,4-Dihydro-6-Methoxy-naphthalene.



24 g. of 1-acetylenyl-6-methoxy-tetralol-1 together with a small amount of hydroquinone is refluxed in 75 cc. of acetic anhydride for 20 minutes. The acetic anhydride is removed in vacuo at water bath temperature. The residue is dissolved in ether, washed in sodium bicarbonate solution, then washed with water, and dried over solid potassium hydroxide. The ether is removed by distillation, and to the residue is added a small amount of hydroquinone and fractionally distilled. It boils at 140-145° at 4 mm.

The compound yields a white precipitate ammoniacal silver nitrate solution.

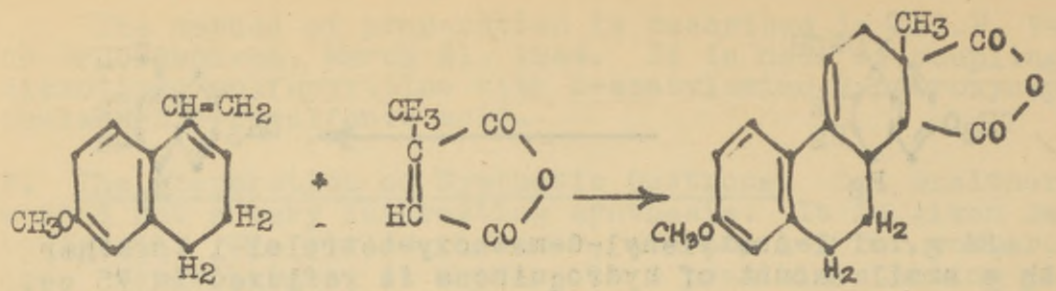
3. The Preparation of 1-Vinyl-3,4-Dihydro-6-Methoxy-naphthalene.



14 g. of this 1-vinyl-3,4-dihydro-6-methoxynaphthalene are dissolved in 150 cc of 80% alcohol and the solution is refluxed for some hours with 30 g. of freshly prepared copper zinc dust. After disappearance of silver reaction the solution is cooled, diluted with ether, filtered, and the filtrate washed with water three times. The ether is dried over sodium sulfate and removed in vacuo. The residue is then vacuum distilled and it boils at 120-125°C at 3 mm. The yield is 11 g.

Copper zinc dust is prepared as follows: 25 g. of copper sulfate is dissolved in 500 cc of water and to this are added with starting and portion-wise 30 g. of zinc dust. The water is then decanted and the zinc dust well washed with water and filtered. It may be used in the above reaction without drying.

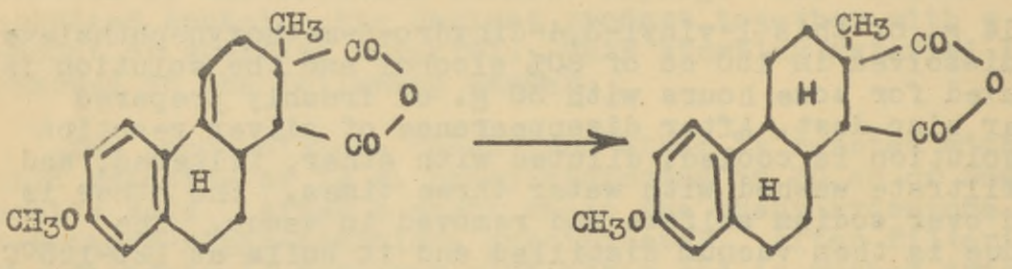
4. The Reaction of the 1-Vinyl-4,4-Dihydro-6-Methoxy-naphthalene with Citraconic Acid Anhydride



18.6 g. of the 1-vinyl-3,4-dihydro-6-methoxynaphthalene with citraconic acid anhydride are reacted with 11.2g. of citraconic acid anhydride and warmed to 50-60° on the water bath. The temperature of the reaction mixture rises spontaneously to 160°-190° and then falls. After this happens the reaction product is crystallized from methylene chloride-ether solution. After one recrystallization the product melts at about 125°. The yield is about 20 g. After several recrystallizations the melting point rises to 160°C.

The above condensation can also be carried out in other solvents, for example, dioxane or benzol. In this case, however, the reaction is run at a temperature of 120-160° in a pressure vessel.

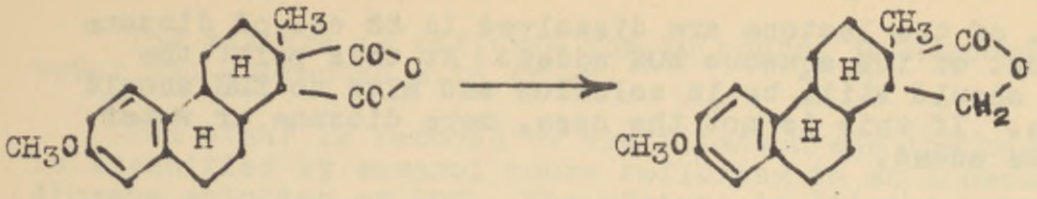
5. The Hydrogenation of the above Condensation Product



The hydrogenation of the condensation product is carried out with hydrogen and palladium catalyst in dioxane as a solvent.

14.9 g. of the substance are dissolved in 150 cc. of dioxane, 100 mg. of palladium black are added and the mixture is placed in the hydrogenation machine. After about 2 hours one mole of hydrogen is taken up. The catalyst is removed by filtration and the dioxane is removed in vacuo. The residue is crystallized from methylene chloride,-ether and melts at 125°. The yield is quantitative.

6. The Reduction of the Anhydride to the Lactone



The reduction of the anhydride to the lactone is accomplished with sodium and alcohol. 30 g. of substance from the above palladium reduction are added to 200 cc. of boiling absolute alcohol. After a short time 23 g. of sodium slices are added all at one time. After the completion of the reaction the remaining bits of sodium are dissolved by the addition of more alcohol, the alcohol is then removed by steam distillation and the alkaline solution is acidified with hydrochloric acid; the resulting precipitate contains a mixture of dicarboxylic acid and hydroxy acid. The mixture is dissolved in glacial acetic acid and treated with concentrated hydrochloric acid. The mixture is warmed to 50°C for several hours to bring about lactonization of the hydroxy acid. The solvents including the hydrochloric acid are removed in vacuo and the residue dissolved in a little methylene chloride; then a large excess of ether is added. From this ether solution the acid portion is removed by treatment with cold dilute sodium carbonate solution.

The lactone remains dissolved in the ether portion. The ether is removed and after crystallization of the residue from methylene chloride - ether melts at 214°C and distills at 216°C at 1 m.m.

The lactone may also be prepared from the unreduced anhydride, since a sodium and alcohol reduction of this also reduces the double bond.

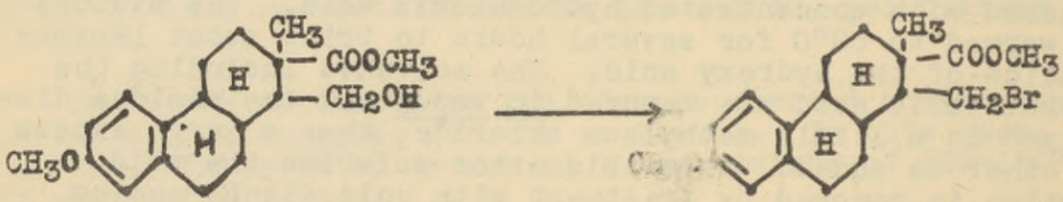
7. Preparation of the Methyl Ester of the Hydroxy-Carboxylic Acid.

37525-1

3 g. of the lactone are dissolved in 25 cc. of dioxane and 25 cc. of 10% aqueous KOH added. At this point the lactone should still be in solution and also no KOH should separate. If this is not the case, more dioxane or water should be added.

The mixture is refluxed until a small sample no longer gives a cloudiness when added to water (about 10-15 minutes). The mixture is then cooled and weakly acidified with acetic acid (with cooling). The precipitated hydroxy-acid is immediately dissolved in ether (it reverts to the lactone very easily), and treatment with diazomethane yields the methyl ester. The hydroxy ester is a thick yellow oil, which by distillation reverts to the lactone by splitting out of methyl alcohol.

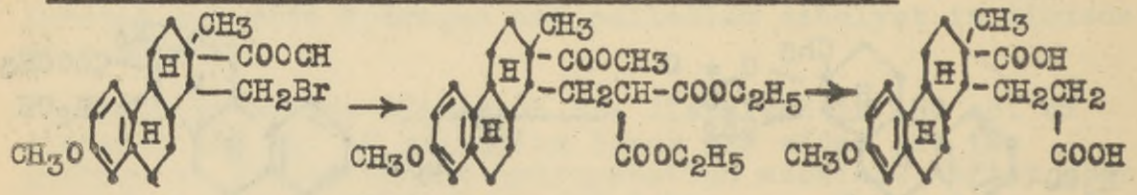
8. <u>The Preparation of the Brom-ester</u>



3 g. of hydroxy-ester were dissolved in 10 cc. of chloroform, and the solution brought to the boiling point. To this was added portionwise 4.3 g. of PBr_5 and the solution refluxed an hour longer. The mixture was then added to alcohol, diluted with water and the insoluble brom compound taken up in ether. The ether portion was washed with cold sodium carbonate solution.

The product resulting from the removal of the solvents was a thick bright yellow oil. This crude product was used without further purification.

9. <u>Reaction of the Brom-ester with Malonic Ester</u>

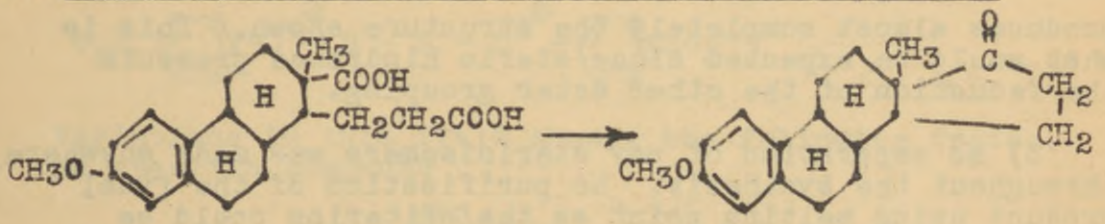


8.5 g. of the brom-ester are added to sodiomalonic ester prepared from 0.5 g. of sodium and 6 cc. of malonic ester in 30 cc. of benzol.

The mixture is refluxed for 10 hours, then poured into water and extracted with ether.

The ether is removed by distillation and the residue is saponified by several hours refluxing in an aqueous dioxane solution of KOH. The mixture is diluted with water and acidified with dilute sulfuric acid. The precipitated acid is taken up in ether and the ether washed with water. The ether is removed in vacuo. The tricarboxylic acid is decarboxylated by heating to 200°C in a flask. After all of the CO_2 has been liberated, the reaction product is warmed for a short time with glacial acetic acid and concentrated hydrochloric acid causing lactonization of any hydroxy-acid still present (of Section 7 above). The dicarboxylic acid is separated from this lactone by treatment with dilute sodium carbonate solution. The dicarboxylic acid is obtained by acidification of the carbonate solution. The product is an oil.

10. Ring Closure of the Dicarboxylic Acid to Oestrone



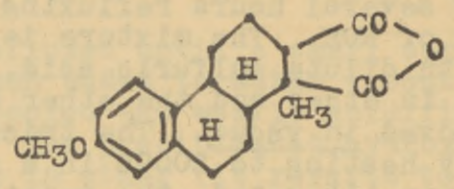
a) 0.4 g. of acid is refluxed two hours with 10 cc. of acetic anhydride. After removal of the acetic acid in vacuo, the residue is taken up in ether and washed with sodium carbonate solution and then with water.

The ether contains oestrone methyl ether. Since no crystallization took place, the entire solution was evaporated and the residue demethylated by refluxing it in a mixture of 5 cc. of glacial acetic acid and 10 cc. of hydrobromic acid (1.7) for ½ hour. The solvents are removed in vacuo and the phenolic portion of the residue dissolved by treatment with sodium hydroxide solution. This alkaline extract yields a product upon acidification, which after one high vacuum sublimation at 150-250° has a melting point of 100-110°C.

b) 1.2 g. of acid was intimately mixed with 1.2 g. of lead carbonate. This mixture was cautiously heated in small portions in a flask over an open flame. The reaction masses were mixed and extracted with ether. The ether solution was treated as described above. The final product melted at 145°C.

11. Notes on the above Synthesis

1) In step No. 4 it appears that very little if any of the isomeric compound



is produced.

2) The reduction of the anhydride

produces almost completely the structure shown. This is what would be expected since steric hindrance prevents the reduction of the other ester grouping.

3) No separation of any sterioisomers was made anywhere throughout the synthesis. No purification of the final product using melting point as the criterion could be effected using ketone reagents.

4) The oestrogenic activity on animals of the final product in doses of 1 gamma was identical with that shown by oestrone.

5) Yields were not available in all steps but it is obvious that they were quite low in the later steps.

6) This type of synthesis making use as it does of the Diels-Alder synthesis presents interesting possibilities for other syntheses in this field.

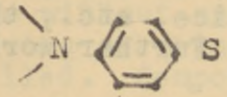
G. Research on a New Series of Antipneumococcal Compounds By Schönhöfer and Meiser

After the work on the various sulfonamides had been in progress for several years, it appeared that the following sulfa drugs were useful in the following diseases:

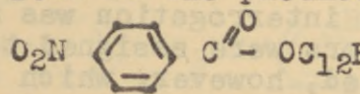
Sulfonilamide -	Streptococcal infections.
Uliron) -	Gonococcal infections.
Albucid)	

Sulfapyridine) – Gonococcal infections
Sulfathiazole) Pneumococcal infections

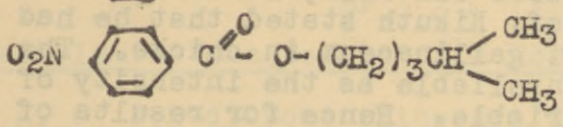
Schönhöfer began work on variations of the basic structure of sulfanilamide



and found that the esters of p-nitrobenzoic acid provided a possible lead in the pneumococcal field. For example:



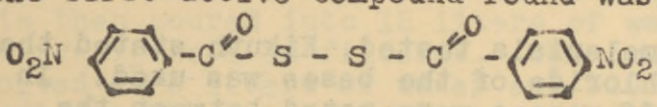
(amonal)



(amonal A)

are the best compounds found in experimental animals.

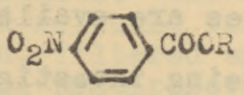
The first active compound found was:



Variations in this field showed the following trends. In the specific compound

if the nitro group is changed to -NO, -NHOH,  , NH_2 the activity decreases progressively.

In the specific compound



if the carbalkoxy group is changed to $-CONH_2$, $-NH-COCC_2H_3$ $-SO_3R$, the activity disappears.

Instead of -COOR, the group -  and  are active and equivalent.

In the case of -COOR,  and  , R may be aliphatic, aromatic, cycloaliphatic, heterocyclic or mixed; the activity of the resulting compound depends upon the character of R.

Most of these compounds show an activity against all

types of pneumococci and also frequently show weak activity against streptococci. The toxicity of these substances is low and not at all like that of the aminophenols. Hecht has found that these substances are very rapidly reduced to the non-toxic amino compounds in the animal body. While these substances are active in mice, etc., they show no promise when tested clinically. Further work has been discontinued.

H. Antimalarial Research

1. Introduction: No exhaustive interrogation was made on this subject as other investigators were assigned to do this. Several facts were elicited, however, which are of general interest. Prof. Kikuth stated that he had done some work in the past on P. gallinaceum in chicks. The test in Kikuth's opinion is unreliable as the intensity of the infection is quite variable. Hence for results of value, great numbers of chicks would be required and even then conclusions would be very difficult. This is not in agreement with findings in the U. S.

Regarding the antimalarials tested, Kikuth stated that in general, the hydrochloride of the bases was used. In canaries only minor differences were noted between the hydrochloride and an insoluble salt such as the methane bishydroxynaphthoate. He felt that greater differences would be found in humans.

All of Kikuth's screening tests are run either in canaries or in rice finches. He has no strains of lophurae and even if he had them, ducklings would be hard to get.

Clinical testing is another difficulty. In the past, many cases of malaria were obtained from incoming ships, but during the war no such cases are available.

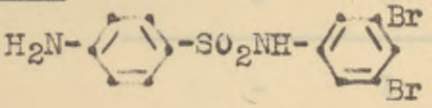
The classes of compounds being investigated at Elberfeld are:

1.	Phenyl substituted dialkylaminoalkyls	Dimeplasmin
2.	Sulfonamides	Bemural
3.	8-Aminoquinolines - - - - - -	-> Certuna
4.	4-Aminoquinolines	Resochin Sontochin Brachysan
5.	3-Alkyl quinolines	Endochin
6.	Acridines	Atabrin

The methods of preparation are given below:

2. Dimeplasmin: The directions are given in D.R.P. 499826 (June 23, 1930). In general, compounds of this type are prepared by reacting the diamine with the correct dialkylaminoalkyl chloride without any solvent. The base is taken up in ether following alkalinization of the reaction product and distilled. Compounds of this type boil around 200°C at 1-2 m.m.

3. Bemural



a. 4-Acetylaminobenzenesulphonic Acid-3',5'-dibromanilide

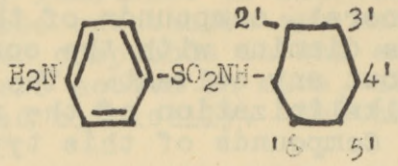
500 g. of 3,5-dibromaniline are dissolved in 2 liters of pyridine and to this are added, with stirring, 560 g. of dried 4-Acetylaminobenzenesulphonyl chloride. The mixture is warmed for 30 minutes on a boiling water bath, and is then poured into 12 liters of water. The resulting solid precipitate is filtered. The crude product is dissolved in 3 liters of water and 300 cc. of 32% sodium hydroxide. The solution is stirred with decolorizing carbon, filtered, and the filtrate treated with 500 cc. of glacial acetic acid. The resulting precipitate is filtered off, washed with water until wash water is neutral and can be used without drying for the hydrolysis step.

b. 4-Aminobenzenesulphonic Acid-3',5'-dibromanilide

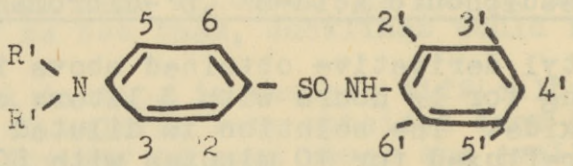
The crude acetyl derivative obtained above is refluxed with stirring for 1½ hours with 3 liters of 16 per cent of sodium hydroxide. The solution is diluted with 4 liters of water and refluxed for 10 minutes with 50 g. of decolorizing carbon, filtered and acidified with acetic acid. The resulting precipitate is filtered, washed with water and crystallized twice from diluted methanol. The crude product is then dissolved at the boiling point in about 2 liters of methanol, refluxed for 10 minutes with decolorizing carbon, filtered, and the hot filtrate is added to 1.4 liters of hot water. The mixture is then allowed to crystallize. After cooling the mixture, the crystals are filtered and put through the crystallization procedure again. In this way there is obtained a pure product in the form of short colorless crystals having a melting point of 152-153°C, with softening at 150°; the yield is 700 g. which is 86% of theory calculated on the amount of 3,5-dibromaniline used.

c. Other compounds prepared by Breitner in the Bemural Series

Table II

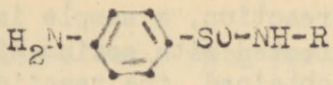


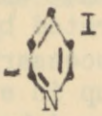
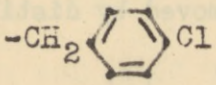
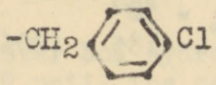
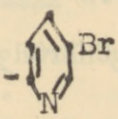
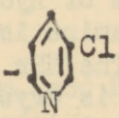
#	2'	3'	4'	5'	6'
Be 704	-	I	-	I	-
706	-	-	I	-	-
745	-	Cl	-	Cl	-
746	-	CH_3	-	CH_3	-
768	-	Br	-	Br	-
774	-	OCH_3	-	OCH_3	-
784	-	Cl	-	-	-
785	-	CH_3	-	-	-
787	-	OCH_3	-	-	-
789	-	Br	-	-	-
802	-	CF_3	-	CF_3	-
814	Cl	-	Cl	Cl	-
815	-	CH_3	-	CH_3	CH_3
817	-	Cl	Cl	-	-
843	-	Br	Br	Br	-



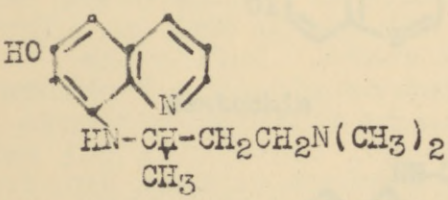
#	R'	R''	2	3	5	6	2'	3'	4'	5'	6'
Be 766	H	CH_3CO	-	-	-	-	-	NO_2	-	NO_2	-
777	O	O	-	-	-	-	-	CH_3	-	CH_3	-
778	O	O	-	-	-	-	-	OCH_3	-	OCH_3	-
779	O	O	-	-	-	-	-	Cl	-	Cl	-
828	CH_3	CH_3	-	-	-	-	-	Cl	-	Cl	-
832	-	-	-	OCH_3	-	OCH_3	-	CH_3	-	CH_3	-
833	-	-	-	OCH_3	-	CH_3	-	Cl	-	Cl	-
834	-	-	-	OCH_3	-	CH_3	-	Br	-	Br	-

Table II, continued



No.	R
Be 938	
957	
958	
963	
964	

4. Certuna (Oprochin, Cilional)



a. Preparation of Dimethylaminobutanol

$CH_3CHOHCH_2CH_2N(CH_3)_2$

230 g. dimethylaminoisobutanone are dissolved in 800 c.c. of water and 120 cc. of glacial acetic acid. The solution is cooled externally to 15-25°C and to it are added over a 5-hour period 2.6 liters of dilute acetic acid (made from 1.3 liters of glacial acetic acid diluted to 2.7 liters and 5.2 kg. of 4% sodium amalgam.

The rate of addition is so regulated that every ten minutes 100 cc. of the dilute acetic acid and 200 g. of the amalgam are added. At the end of the reaction, a sample is made alkaline, extracted with ether, and tested with sodium nitroprusside and N. NaOH. If no red color is obtained, the reaction is complete.

The aqueous portion of the reaction mixture is removed from the mercury and made alkaline with 32% sodium hydroxide solution and the product separated by steam distillation. About 5 liters of distillate are necessary. The base is salted out of the distillate with KOH, taken up in ether and the other solution dried over KOH. The ether is removed by distillation at 50-60° and the residue distilled.

$B.P._{14} = 58°$
Yield = 185 g (80%)

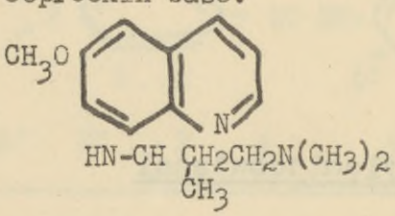
b. Dimethylaminoisobutylbromide-hydrobromide

$CH_3CHBrCH_3CH_3N(CH_3)_2$, HBr.

To 2 liters of hydrobromic acid (1.7) is added, with cooling, 530 g. dimethylamine isobutanol. The solution is heated to 80-90° for 15 hours. The HBr is removed <u>in vacuo</u> at this temperature and the residue is crystallized from 1 liter of alcohol. Colorless crystals are obtained.

M.P. = 150-151°C
Yield= 800g. (65%)

c. Ceprochin base.



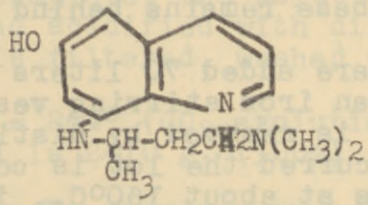
844 g. dimethylaminoisobutylbromide-hydrobromide are dissolved in 840 cc. of water and treated with 1124 g. of 6-methoxy-8-amino-quinoline (Amichin). The mixture is heated with stirring under a reflux at 45-55° external temperature for 5 hours. Within 3 hours, the temperature has risen to 100° and it is finally heated for 6 hrs. at 95-100°. The melt is dissolved in hot water and dilute HCl and upon cooling the precipitated unreacted Amichin salt is removed. The filtrate is treated with sodium acetate until Congo red no longer gives a blue color, and is extracted twice with ether. The aqueous portion is then made strongly alkaline with 32% sodium hydroxide and the insoluble base extracted with ether. The base is distilled.

$B.P._{3}$ 181-185° C.
$B.P._{0.4}$ 168° C.

The product solidifies after a long period.

Yield = 615 g. (69%).

d. Cilional hydrobromide (certuna hydrobromide)



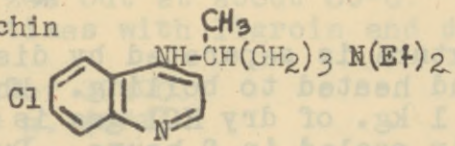
300 g. Ceprochin Base is added to 1 liter of hydrobromic acid (1.5) and the mixture refluxed at about 120° until the methyl group is removed (2½ hrs.). The HBr is removed <u>in vacuo</u> at 70-80°. The solid residue is crystallized from alcohol.

M.P. of the dihydrobromide 135-155° C.

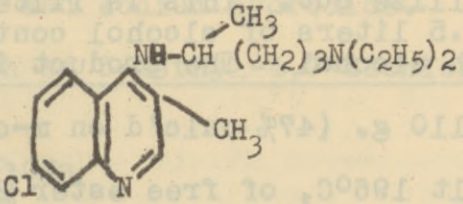
Yield 350 g.

5. Preparation of Sontochin, Resochin, and Brachysan. These products have the following structures:

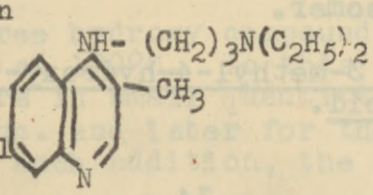
Resochin



Sontochin



Brachysan

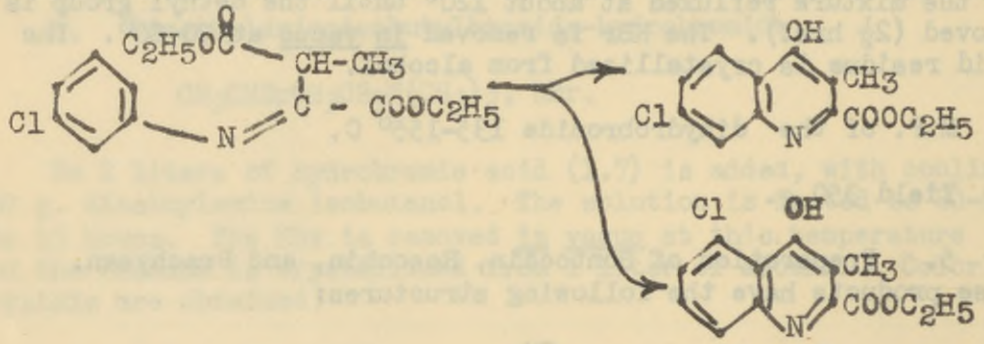


All of these compounds are made in a similar manner. A description of the preparation of Sontochin will suffice for this class of compounds. Resochin and Sontochin are patented in D.R.P. 683692 (Nov. 13, 1939).

a. Preparation of Sontochin: Preparation of 3-methyl-4-hydroxy-7(5) chlorquinoline-carboxyl acid ester.

In an apparatus for continuous azeotropic distillation, 4450 g. of oxalylpropionic acid ester, 2800 g. of m-chloraniline, 4 liters of chloroform, 7 cc. of concentrated hydrochloric acid are refluxed until the calculated amount of water is distilled off. (22 moles or 396 cc.) This requires about 8-10 hours. The chloroform is then removed by distillation in vacuo. The Schiffs base remains behind as a red oil.

To this raw product are added 70 liters of paraffin oil and heated to 255°C. in an iron stirring vessel with direct heating. The alcohol is removed by distillation as formed and after this has occurred the lot is cooled. The reaction product crystallizes at about 160°C., is filtered and washed twice with ligroin and dried at 100°C. In this reaction a mixture of isomers results and melts at 190°C.

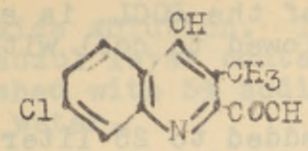


The isomeric mixture is separated by dissolving in 18 liters of alcohol and heated to boiling. While being held at this temperature 1 kg. of dry HCl gas is led in. The solution is gradually cooled in 2 hours. During the cooling, ½ kg. more of HCl is added causing the 7-chloro compound to crystallize out. This is filtered off and washed once with 2.5 liters of alcohol containing HCl and finally washed with alcohol. The product is then dried.

The yield is 3110 g. (47% calc'd on m-chloraniline)

M.P. of HCl salt 196°C, of free ester 226°C. The alcoholic mother-liquor contains the 5-chlorisomer and a little of the 7-chlorisomer.

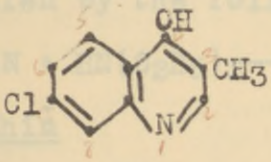
b. Preparation of 3-methyl-4-hydroxy-7-chlorquinoline-2-carboxylic acid.



3110 g. of the ester-HCl is heated for 2 hours under a reflux with 15.5 liters of 2N sodium hydroxide. After the reaction is complete, the solution is diluted to 40 liters with water and acidified with dilute hydrochloric acid. The product is filtered, washed with water and dried at 100°C.

The M.P. = 264° (CO_2 evolution)
The yield is 2410 g. (100%)

c. Preparation of 3-methyl-4-hydroxy-7-chlorquinoline

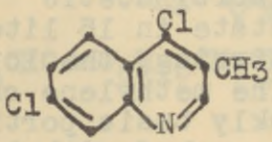


10 liters of paraffin oil are heated 275°. To this is added 2.5 kg. of the acid in small portions during 20-30 minutes. Carbon dioxide is split out of the molecule and is led away with the help of a water pump. After the evolution of the CO_2 has ceased, the mixture is cooled and the product crystallizes out at about 80°C. It is filtered off and washed three times with ligroin and dried at 100°C.

It has a m.p. of 334°C.
Yield is 1970 g. (97%)

The oil may be used for 4 or 5 runs and then purified with sulfuric acid and sodium hydroxide.

d. Preparation of 3-methyl-4,7-dichlorquinoline



5 kg. of the free hydroxy compound are added to 15 liters of dry chlorbenzol at 170°C. To this 3.4 kg. of $PCCl_3$ are added during 2 hours in small quantities, beginning with portions of about 50 cc. and later for the last 800 cc., 100 cc. at a time. After each addition, the course of the reaction

is watched (lively boiling). After all of the $POCl_3$ is added, the mixture is heated for 3 hours and allowed to cool with stirring.

The cooled chlorbenzene solution is added to 25 liters of water and stirred for ½ hour. Then about 10 kg. of ice and 5 liters of chlorbenzol are added and when the temperature has reached 20°C, ammonium hydroxide is added to alkalinize the solution (about 6 liters). The lot is stirred 15 minutes and allowed to stand 1 hour for the product to separate into layers. The lower layer contains the dichlor compound dissolved in chlorbenzol. This layer is drawn off and washed with an equal volume of water.

To determine the m.p. of the product dissolved in the chlorbenzol, a small amount of the solution is distilled and the m.p. of the product determined.

BP_2 = 140°C

M.P. = 90°C

Yield = 92%

e. Sontochin Base

The solution of the dichlor compound from (d) above is heated to distill off enough chlorbenzol to remove the water.

Then 2 kg. of pyridine, 2.5 kg of phenol, 2.5 kg. of sodium iodide are added and the temperature raised to 150-160°. To this are added, over a period of 3 hours, 5.6 kg (1.5 moles) of novoldiamine. The temperature is then raised to 160-170° and held at this point for 15 hours.

After cooling, the melt is diluted with 15 liters of methylene chloride and 6.5 liters of glacial acetic acid, and 3.0 kg crystalline sodium acetate in 15 liters of water are added. After 5 minutes stirring, the lot is allowed to stand ½ hour to separate. The methylene chloride layer which contains phenol and the weakly basic portions is filtered. The methylene chloride is washed with 1 liter of 10% acetic acid which is added to the chief portion of the lot.

The combined acetic acid contains sontochin and excess novoldiamine. To it is added 10 liters of benzol, cooled with ice and made strongly alkaline with 150 moles of sodium

hydroxide solution. The layers are allowed to separate and the benzol layer containing the sontochin removed. This is washed with 5% sodium hydroxide solution and twice with water.

The benzol is removed in vacuo and the residue distilled in vacuo.

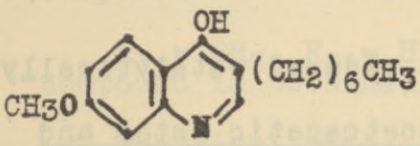
B.P.$_2$ = 210-220°C

Yield = 6.1 Kg (77.6%)

In Kikuth's opinion, sontochin is a promising antimalarial, but is rather difficult to prepare. Brachysan, on the other hand, is just as effective according to Sioli, but is much easier to prepare. The basic side chain is prepared at Ludwigshafen by the following reaction:

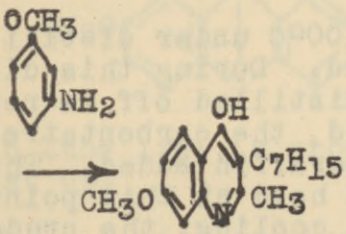
$$CH_2{=}CH{-}C{\equiv}N + HN(C_2H_5)_2 \rightarrow N{\equiv}C{-}CH_2CH_2{-}N(C_2H_5)_2 \rightarrow H_2N(CH_2)_3N(C_2H_5)_2$$

6. Endochin

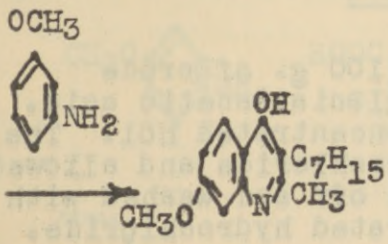


The preparation of this compound is made by the following series of reactions:

(a) $C_6H_{13}CHO + CH_3COCH_2COOC_2H_5 \rightarrow CH_3COC(CHC_6H_{13})COOC_2H_5 \rightarrow CH_3C(=O)CH(C_7H_{15})COOC_2H_5$



(b) $C_6H_{13}CHO \rightarrow C_6H_{13}CH_2OH \rightarrow C_6H_{13}CH_2Br \rightarrow CH_3{-}C(=O){-}CH(C_7H_{17}){-}COOC_2H_5$



The two syntheses do not yield the same quality of heptylacetoacetic ester. (a) Yields a quantity of highly hydrogenated acetoacetic ester. (b) Yields considerable amounts of O-alkylated acetoacetic ester.

Directions for synthesis (a) are:

a. Heptylidene - acetoacetic ester

120 g. of heptaldehyde are added to a solution of 130 g. of acetoacetic ester and 3 g. of piperidine cooled to -10 to -15°C over a period of 3 hours. The mixture is allowed to stand at -3° for 24 hours. The mixture is then added to water and made neutral with sulfuric acid. The solution is extracted with ether and after removal of the ether, the product is distilled.

B.P. 125° at 4 mm.
Yield is 190 g.

b. Heptylacetoacetic ester

The heptylidine product is reduced catalytically.

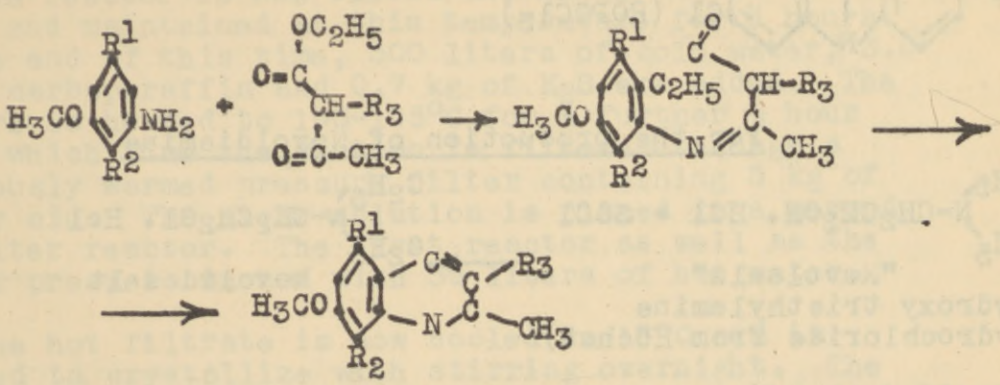
c. Schiff's base from heptylacetoacetic ester and m-Anisidine followed by ring closure

400 g. heptylacetoacetic ester
215 g. m-Anisidine
1.5 liters carbontetrachloride

This mixture is heated at 100°C under distillation until all water has been removed. During this distillation, the carbontetrachloride distilled off is replaced. After all water has been removed, the carbontetrachloride is removed, and 1.5 liters of paraffin added. The temperature is raised to 250°C and held at this point until the reaction is finished. Upon cooling, the crude product crystallizes. It is filtered off and washed with ligroin. This crude product contains a mixture of isomers (5-methoxy and 7-methoxy).

These are separated as follows: 100 g. of crude product are dissolved in 200 cc. of glacial acetic acid. To this are added about 360 cc. of concentrated HCl. The solution is seeded with Endochin-hydrochloride and allowed to crystallize. The salt is filtered off and washed with a mixture of acetic acid and concentrated hydrochloride. The salt is treated with sodium carbonate and the base crystallized from methanol. M.P. 207-212°C.

Dr. Salzer has been investigating the endochin field. The general method of preparation is as follows:



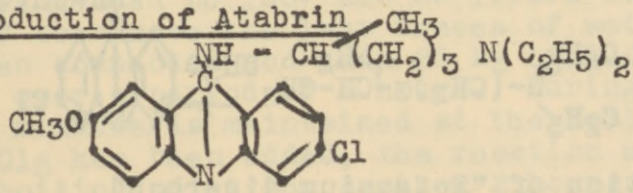
When R_1 and R_2 = H and R_3 = C_7H_{15} the compound is Endochin. Its therapeutic index in canaries is 1:120 but is inactive on clinical trial.

R_1 = H, R_2 = CH_3 and R_3 = C_7H_{15} an avian active compound is obtained.

R_1 = CH_3, R_2 = H and R_3 = C_7H_{15} an avian inactive compound is obtained.

The final ring closure of the Schiff's base is an example of the Conrad-Limpach reaction.

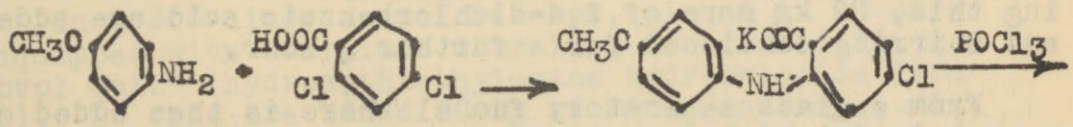
7. Production of Atabrin



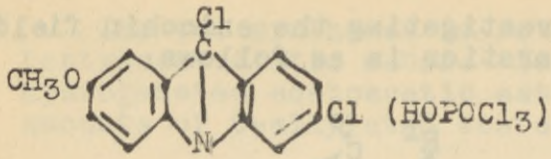
In June 1941, a production schedule of 1900 kg/month was set up.

The reactions involved are:

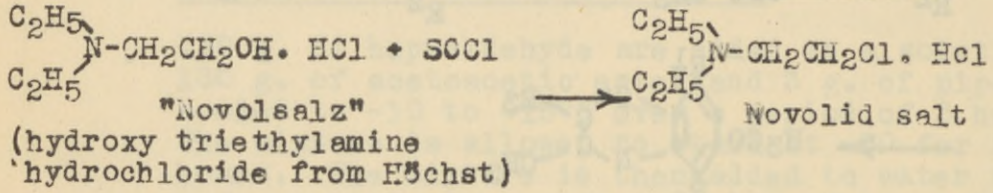
For the production of Halochrine

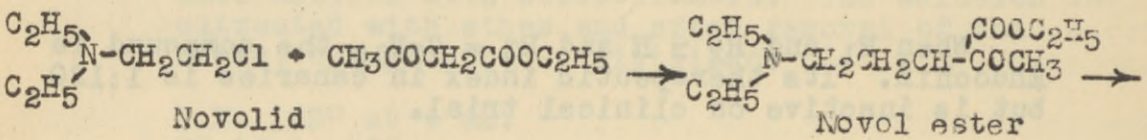


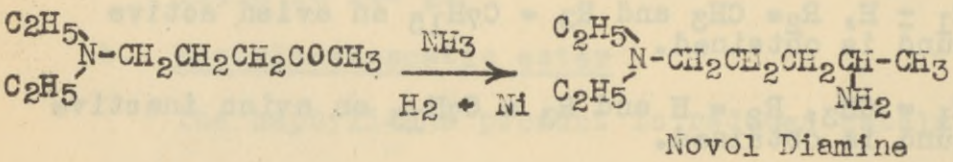
Potassium Dicarbon



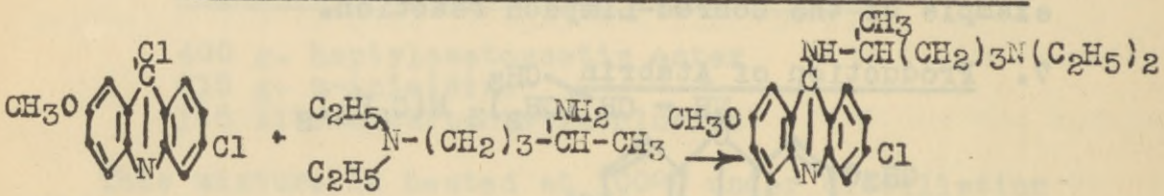
For the production of Novoldiamine







Condensation of Halochrine and Novol Diamine



a. Preparation of "Potassium dicarbon"

In a 500 liter reactor, equipped with a steam jacket and a reflux condensor and a stirrer, are placed 30 liters of water and 41 kg of p-anisidine. After warming the mixture to 60-70°C, it is stirred for ½ hour, then 27 kg of 2,4-dichlorbenzoic acid are added, stirring continued for 10 minutes and 0.36 kg of copper powder added. Following this, 27 kg more of 2,4-dichlorbenzoic acid are added and stirring continued for a further ½ hour.

From a glass separatory funnel there is then added over a period of 1-1½ hours, a solution of 40 kg of KOH in 40 liters of water. Care must be exercised in this addition

because of foaming.

The reactor is now closed and slowly heated to 103-105°C and maintained at this temperature for 3 hours. At the end of this time, 300 liters of cold water, 3.0 kg of carboparaffin and 0.7 kg of K_2S are added. The mixture is heated to 103-105°C for a further ½ hour after which time the solution is filtered through a previously warmed pressure filter containing 3 kg of filter aid. The clear solution is placed in a second 500 liter reactor. The first reactor as well as the filter press is washed with 50 liters of hot water.

The hot filtrate is now cooled to 15°C and is allowed to crystallize with stirring overnight. The crystals are collected on a Buchner funnel. The dark blue mother liquor is collected and two 20 liters of water used to wash the crystals are added to it. The light grey crystals are dried for 4 days in a drier at 80-90°C.

The yield is 70 kg which is 78.5% of theory calculated on the 2,4-dichlorbenzoic acid used.

b. Preparation of Halochrine Phosphoric Salt

In a 300 liter reactor equipped with a stirrer and a reflux condensor are placed 180 liters of chlorbenzol and to this are added 33 kg of "Potassium dicarbon". The mixture is heated to 130° and 20 liters of chlorbenzol distilled off to remove the last traces of water. The reflux is then connected and then 25 kg of $POCl_3$ are slowly added over a period of 2 hours. During this time, the reaction mixture is maintained at the boiling point. After the $POCl_3$ has been added, the reaction mixture is kept at the boiling point for another 2 hours. At this time 30 liters more of chlorbenzol are distilled off and the reaction mixture cooled with stirring to 30°C. The reaction mixture is then transferred to a 500 liter reactor for the condensation with novoldiamine. (See below)

c. Preparation of Novolid Salt

In a 300 liter enamel reactor, placed in a water bath equipped with efficient stirring, are placed 70 kg of "Novol Salz" (hydroxythiethylamine hydrochloride from Höchst). To this are added 60 Kg of $SOCl_2$ in two equal portions. The first portion is added rapidly at room temperature causing the liberation of HCl and SO_2. The

temperature rises during this addition to 35-40°C. The mass has now become liquid. The mixture is allowed to stir for ½ hour following the addition of the first portion of $SOCl_2$. The water bath is heated to 55-60°C and the temperature of the mixture reaches 50°C. Now the second portion of $SOCl_2$ is added over a period of 1-1½ hours. This causes the temperature of the mixture to rise to 55-57°C together with a vigorous evolution of HCl and SO_2 gas. Both bases are absorbed in a wash tower. Finally (after several hours) the mixture is heated (mixture temperature) to 70-75°C. In a short time, the entire mixture solidifies to a thick crystalline mass of the novolid salt. The salt is removed and dried.

The yield is 11 Kg of novolid salt which is 99% of theory.

d. Preparation of Novolketone

(1) Novolid

33 Kg of Novolid salt is dissolved in 33 liters of water. 10 Kg of ice are added and to this mixture is added with stirring a solution made from 33 Kg of solid KOH, 5.4 kg of 30% NaOH solution and 22 liters of water.

The resulting mixture is extracted for ½ hour with 16 kg of benzol. The benzol portion is separated and the alkaline water is re-extracted with 10 kg of benzol. The combined benzol extracts (about 48 kg) are dried over $CaCl_2$ (about ½-1 Kg) for about 20 hours and filtered.

Four such portions of novolid are combined and reacted with acetoacetor ester.

(2) Novolester

360 Kg of benzol, 150 kg of acetoacetic ester and 22 kg of sodium (cut up) are placed in an iron reactor equipped with a stirrer and a reflux condensor. The contents are cooled to about 50°C, and the novolid-benzol solution warmed to 50-60°C, is slowly added. After all is added, the mixture is refluxed for 3 hours. The mixture is cooled and separated from the precipitated NaCl by filtration.

(3) Novolketone

The benzol solution from the preparation of Novol Ester is divided into 4 parts. These are placed in

300 liter enamel reactors equipped with reflux condensors and steam jackets. In each are placed 75 kg of ice, 25 kg of water and 35 kg of 96% sulfuric acid. The temperature rises to about 35°C. The mixture is then heated and the benzol removed by distillation after which the solution is refluxed for twelve hours. After cooling to 30°C the solution is placed in a 300 liter iron reactor with a stirrer. To this are added 188 kg of 30% NaOH solution and 50 kg of ice, causing separation of novolketone. This is separated and the alkaline solution is extracted with 35 kg of benzol. The benzol solution and the first crude novlketone are mixed. After filtering and drying the benzol is removed and the residue distilled.

1. Fraction (novol fraction) 60-90°@ 30 mm ca 2-3 Kg
2. Middle fraction 80-88°@ 19 mm ca 3-4 kg
3. Novolketone (pure) 74-76°@ 4 mm

The novol fractions are combined with the middle fractions and refractionated.

Yield 85-85.4 kg = 71% of theory

Since 1942 novolester is prepared by the condensation of dry novolid-benzol solution with dry solid sodioaceto-acetic ester (from Höchst).

For example: 123 kg 95% sodioacetoacetic ester
290 kg dry benzol
ca.200 kg Novolid-benzol solution from 132 kg Novolid salt+104 kg benzol.

Yield is ca. 80 kg novolketone.

e. Novoldiamine

50 kg of Novolketone and 100 liters of methanol are reduced in a 250 liter iron hydrogenation vessel using 2-5 kg of nickel contact catalyst. The air is displaced with hydrogen and then with 16.7 kg of ammonia gas and 1.5 kg (15 cbm) of hydrogen. A pressure of 30 Atm. results. The mixture is heated to 95-96° raising the pressure to 50 Atm. During the hydrogenation when the pressure drops to 20 Atm. more hydrogen is added to bring it back to 50 Atm. This is continued until no more hydrogen is taken up, and requires about 6-6½ hours. The first atmosphere of hydrogen is used in about 10 minutes but this soon slows down. The mixture is cooled, filtered, and distilled.

Novoldiamine boils 70-71° @ 5 mm. The residue will be about ½ - 1 kg.

Yield is 45-46 kg of Novoldiamine which is 90-92% of theory.

f. Preparation of Atabrin Base

The chlorbenzene solution of Halochrin phosphate is used without isolation.

In a 500 liter reactor are placed 80 kg of Phenol (warmed to 80°) and 26 kg of pyridine. The mixture is stirred and allowed to cool to 70°C. To this is added the chlorbenzene solution of Halochrin phosphate and the mixture warmed to 40-50°C. After 1/2 hour of stirring, the mixture is heated slowly to 110-120°C and held at this temperature for 1 hour. To this are added over a half hour, 17 kg of novoldiamine. After stirring for 3/4 hour at 110-120°C, the chlorbenzene is distilled off under a vacuum of 50 mm. After the distillation is over, the temperature is again raised to 120°C.

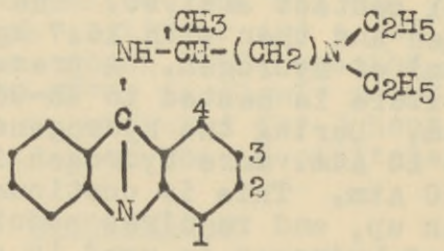
The melt is cooled to 90° and transferred into a 1000 liter enamel reactor containing 150 liters of 30% sodium hydroxide. This mixture is stirred for 4 hours at room temperature, preferably over night to produce a satisfactory product. The base is filtered off and washed with water.

This is crystallized from acetone in the form of the hydrochloride.

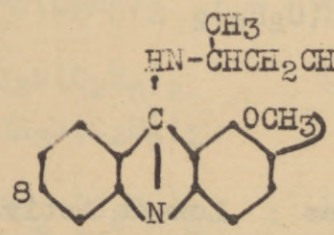
The yield of pure atabrin hydrochloride is 40 kg (75% on the basis of the Potassium dicarbon used)

g. Other Acridines prepared

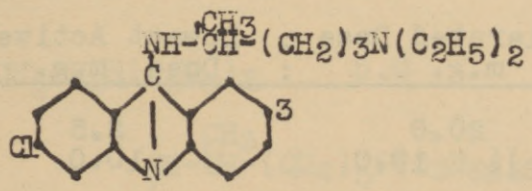
TABLE III



Substituent Position	Group	Tolerated Dose m.g.	Lowest Active Dose m.g.	Ratio
3	H	5.0	5.0	1
3	$-OCH_3$	5.0	2.5	2
3	$-C_2H_5$	10.0	2.5	4
3	$-CH_3$	5.0	1.25	4
3	-Cl	10.0	1.25	8
2	$-CH_3$	5.0	0.67	8
2	-Cl	5.0	0.33	15

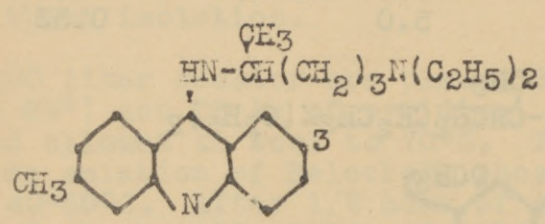


Substituent in 8 position	Tolerated Dose m.g.	Lowest Active Dose m.g.	Ratio
H	5.0	2.5	2
NO_2	10.0	10.0	1
NH_2	10.0	0.w	-
F	10.0	0.67	15
Cl	10.0	0.33	30
Br	10.0	0.67	15
I	5.0	1.25	4
CH_3	10.0	0.67	15

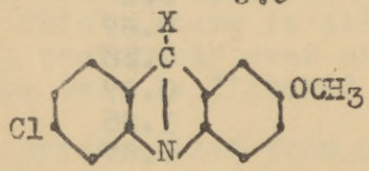


Substituent in position 3	Tolerated Dose	Lowest Active Dose m.g.	Ratio
-H	5.0	0.33	15
$-OCH_3$	10.0	0.33	30
$-OC_2H_5$	5.0	0.67	8
$-OCH(CH_3)_2$	5.0	0.67	8
$-OC_4H_9$	5.0	0.33	8-15
$-OC_6H_{13}$	5.0	0.33	15

Substituent in position 3	Tolerated Dose	Lowest Active Dose m.g.	Ratio
$-OC_{12}H_{25}$	10.0	1.25	4-8
$-SCH_3$	5.0	0.33	15
$-SC_2H_5$	2.5	0.67	4
$-CH_3$	5.0	0.33	15
$-C_2H_5$	2.5	0.33	8
-Cl	10.0	0.67	15

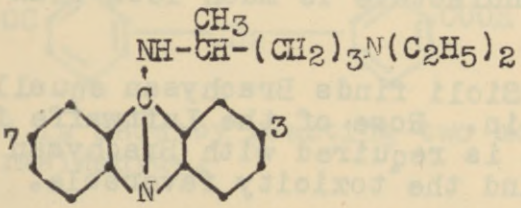


Substituent in position 3	Tolerated Dose m.g.	Lowest Active Dose m.g.	Ratio
-H	5.0	0.67	8
$-CH_3$	5.0	0.67	8
$-SCH_3$	10.0	1.25	8
$-OCH_3$	10.0	0.67	15
-Cl	5.0	1.25	4



X	Tolerated Dose m.g.	Lowest Active Dose m.g.	Ratio
$-NH-CH_2CH_2N(C_2H_5)_2$	20.0	2.5	8
$-NH-CH_2CH_2N(CH_2CH-CH_2)_2$	10.0	10.0	1
$-NH-CH(CH_3)-CH_2N(C_2H_5)_2$	10.0	2.5	4
$-NH\ CH(CH_2N(C_2H_5)_2)(CH_2N(C_2H_5)_2)$	10.0	5.0	2
$-NH\ CH_2CH_2N(C_2H_5)-CH_2CH_2N(C_2H_5)_2$	2.5	1.25	2
$-NH\ CH_2CH_2SCH_2CH_2N(C_2H_5)_2$	5.0	0.67	8
$-NH\ CH_2CH_2CH_2N(CH_3)_2$	5.0	0.67	8
$-NH\ CH_2CH_2CH_2N(C_2H_5)_2$	10.0	0.67	15
$-NH\ CH(CH_3)\ CH_2CH_2N(CH_3)_2$	10.0	0.67	15

X	Tolerated Dose m.g.	Lowest Active Dose m.g.	Ratio
-NH-CH(CH3)-CH2CH2(C2H5)2	20.0	0.67	30
-NH-CH(CH3)-CH2CH2N(CH2CH=CH2)2	5.0	0.67	8
-NH-CH(CH3)-CH(CH3)-CH2N(CH3)2	10.0	1.25	8
-NH-CH2-C(CH3)2-CH2-N(C2H5)2	20.0	2.5	8
-NH-CH2CHOH-CH2N(C2H5)2	10.0	0.67	15
-NH-CH2CH2CH2CH2N(C2H5)2	5.0	0.67	8
-NH-CH(CH3)-CH2CH2CH2N(C2H5)2	10.0	0.33	30
-NH-CH2-CH(CH2CH2N(C2H5)2)(CH2CH2N(C2H5)2)	5.0	0.67	8
-NH-(CH2)5-N(C2H5)2	10.0	0.67	15
-NH-CH(CH3)-(CH2)4-N(C2H5)2	5.0	0.67	8
-NH-C6H4-N(C2H5)-CH2CH2N(C2H5)2	2.5	1.25	2
-NH-C6H4-CH2CH2CH2N(C2H5)2	10.0	5.0	2
-NH-C6H4-O-CH2CH2N(C2H5)2	5.0	1.25	4



Substituent in 3	Substituent in 7	Tolerated Dose m.g.	Lowest Active Dose m.g.	Ratio
CH3O-	CH3O-	5.0	0.67	8
CH3O-	CH3-	10.0	2.5	4
CH3O-	Cl	10.0	2.5	4
CH3O-	-SCH3	10.0	5.0	2
C2H5O-	C2H5O-	5.0	1.25	4
CH3-	CH3-	5.0	1.25	4
Cl-	Cl-	5.0	-	-

8. Summary: The usefulness of these types was summarized by Kikuth as follows:

Dimeplasmin and Bemural: Kikuth thinks these compounds may provide a lead in prophylaxis in humans. Dimeplasmin is active in avian malaria on E forms. He thinks it may also be prophylactive for E forms in avian malaria. Clinical tests show this particular compound to be inactive in human malaria.

Bemural is of interest because it is a member of the only class of sulfonamides which shows activity in avian malaria. It was sent to Hamburg but no results have been received. Kikuth believes that a member of this class might be useful in prophylaxis in humens.

Certuna: This type has been used in Italy only. Certuna is less toxic than plasmochin, but like the latter is a good gametocide. It was developed as a substitute for plasmochin. Although a good gametocide, it does not prevent relapses, which plasmochin does. It can be used with atabrin. Kikuth feels that because of this fact the compound is promising.

Resochin. Sontochin, and Brachysan: Resochin has been proved by Sioli to be of no value in human malaria.

In Kikuth's opinion Sontochin and Brachysan are the most promising leads the I. G. has in the field of antimalarials. The toxicity is low and less than that of atabrin which they are designed to replace. Another advantage is the fact that since they are white, no coloring of the patient results. Of the two, Brachysan is now preferred since the cost of manufacture is much less than that of Sontochin.

Clinically, Sioli finds Brachysan equally as effective as Sontochin. Rose of the Luftwaffe finds a slightly higher dose is required with Brachysan over Sontochin. Both found the toxicity favorable.

Endochin is the first quinoline compound to show activity on the E forms in avian malaria. Hence it acts as a prophylactic in this type. Only one dose is necessary to protect birds. Clinically in humans, no prophylactic action can be found. Kikuth feels that somewhere in this series there will be found a substance which will be useful as a prophylactic in human malaria, and this work is being continued by the I. G.

Summary of Work to be done in the Antimalarial Field:

Dimeplasmin, Endochin, and Bemural: Some member of the series of which these compounds are examples may provide a good prophylactic for human malaria.

Prof. Schulemann at Bad Oeynhausen is working in the dimeplasmin field (See Dr. Southworth's report on his visit to Schulemann).

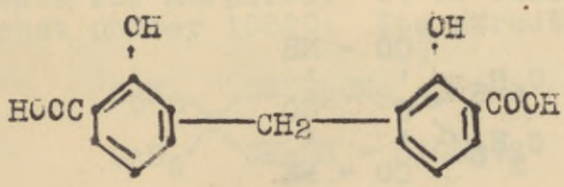
Sontochin and Brachysan: These substances or other similar types may give the answer to a search for a white, less toxic substitute for atabrin.

Certuna and other closely related compounds should be looked into as a less toxic plasmochin substitute.

I. Insoluble Salts of Quinolines and Other Basic Substances:
The I. G. workers made many of these insoluble salts in order to slow down absorption, and in this way hoped either to reduce the toxicity or prolong the action. The only good acids which were found were described a number of years ago in D.R.P. 489726.

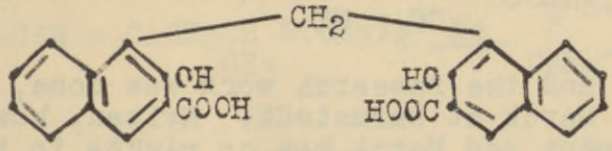
The general procedure for preparing the salt is to mix an aqueous solution of the hydrochloride of the base with an aqueous solution of the sodium salt of the acid. These are used in stoichometric proportions. The salt precipitates out of the solution. It is filtered and washed free of NaCl and dried. The acids used were:

1. Methylene disalicylic acid



This acid is made by reacting two moles of Salicylic acid with formaldehyde.

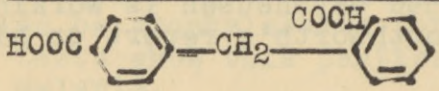
2. 2,2' Dioxydinaphthylmethane - 3,3' dicarboxylic acid



37525-I

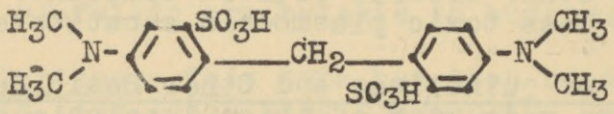
This is the acid called "methane säure" by I. G. and is used to prepare salts of plasmochin. It is prepared by reacting 2 moles of 2-hydroxynaphthalene-3-carboxylic acid with formaldehyde in glacial acetic acid.

3. Diphenylmethane-2,4'-dicarboxylic acid



This acid is made according to Ann. 309, 115.

4. 4,4'-tetramethyldiamino-diphenylmethane-2,2'-disulfonic acid

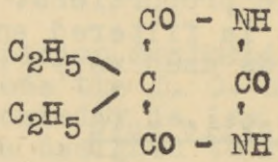


This substance is made according to D.R.P. 65017.

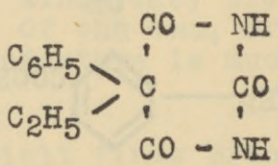
J. Barbituric Acids: The I. G. workers are no longer investigating this field. They feel that evipan is as good a drug as can be made in the barbituric acid field.

Other substances in the series which they market are:

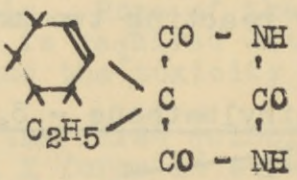
Veronal



Luminal

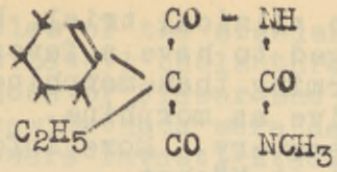


Phanodorm



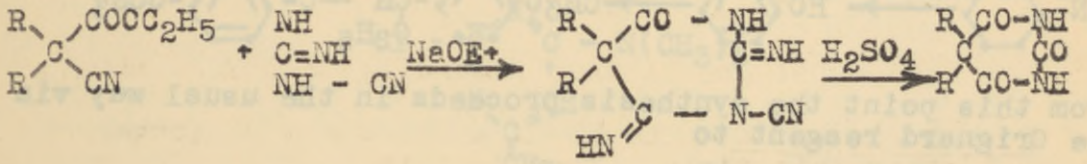
These are marketed, and the research work was done, in cooperation with E. Merck at Darmstadt. Evipan, however, is an I. G. development and Merck has no rights in this compound.

Evipan is



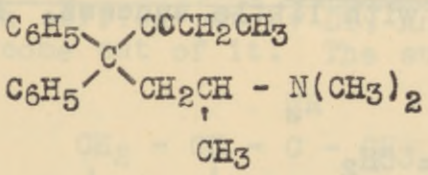
A number of thiobarbituric acids have been made, but none have been found which the I. G. thinks good enough to market. This subject is likewise not being investigated at present.

Due to the shortage of urea and other derivatives a new commercial synthesis is being used at the Elberfeld plant. Instead of Urea, guanidine or dicyandiamide is used for condensation. Most of the time, the dialkyl or aryl-alkyl cyanacetic ester is used.

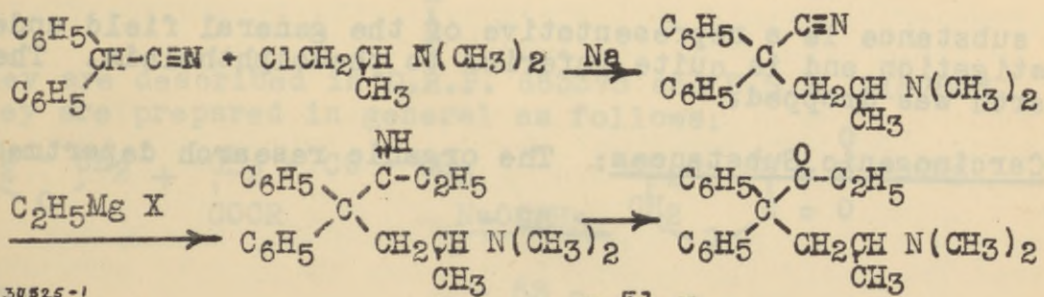


K. Miscellaneous Information Obtained as a Result of Discussions with Members of the Organic Chemical Research Staff

1. New Analgesic 10820 (Höchst): Dr. Schönhöfer stated that Dr. Eisleb at Höchst had synthesized a compound which is better than Demerol (called Dolantin in Germany) as a substitute for morphine. It is called Amidone, and has a Höchst number 10820. Its structure is

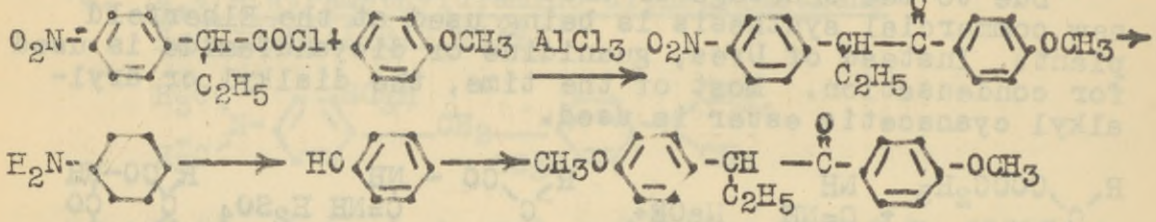


According to Schönhöfer, the method of synthesis is

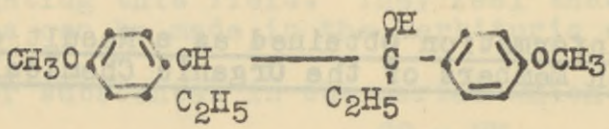


This substance is now on clinical trial, but on the basis of animal tests is alleged to have a favorable toxicity, and to be less habit-forming than morphine. It is said to be four times as active as morphine. If this is true, this is an important discovery. More information on this substance will be sought at Höchst.

2. Preparation of Diethyl Stilboestrol (war-time method): Modifications in the synthesis of diethyl stilboestrol were made at Elberfeld due to the war-time lack of anisaldehyde. These changes are shown below:

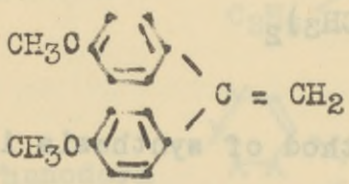


From this point the synthesis proceeds in the usual way via the Grignard reagent to



followed by dehydration and hydrolysis of the methoxy groups. This method is uneconomical and illustrates the methods used by the I. G. when products were needed but the usual starting material were not available.

3. New Laxatives (Dr. Meiser): Dr. Meiser has been investigating new laxatives with little success. His best compound was



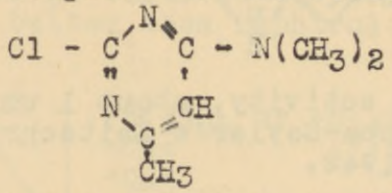
This substance is a representative of the general field under investigation and is quite inferior to phenolphthalein. The research was dropped.

4. Carcinogenic Substances: The organic research department

has prepared quantities of the standard carcinogens such as dibenzanthracene and methyl cholanthrene. The synthetic methods used were those of Fieser and Neumann in the United States. No new methods were developed, and no new series of compounds were investigated.

Schönhöfer made certain statements regarding x-ray work and carcinogenic agents. Summarizing the discussion, x-ray treatment in huge doses brings about a cancerous condition while low dosage has a curative effect. It was hoped that a similar action would be brought about by the synthetic carcinogenic substances like dibenzanthracene or methyl-cholanthrene. With these, large doses produced cancer in experimental animals, but low dosage had no curative effect.

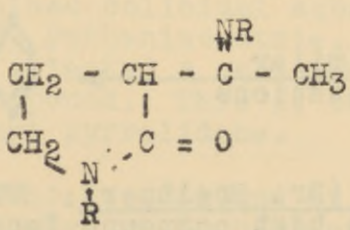
5. Rodenticides: Dr. Westphal has investigated this subject and the best compound of the series was found to be



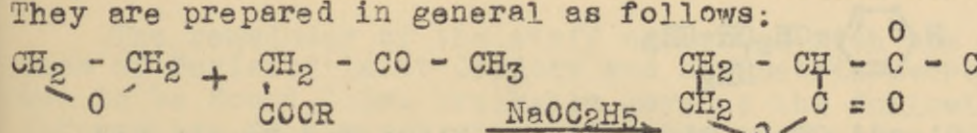
This substance is marketed under the trade name of Castrix. It is very effective but causes a certain ferociousness in the rats after ingestion.

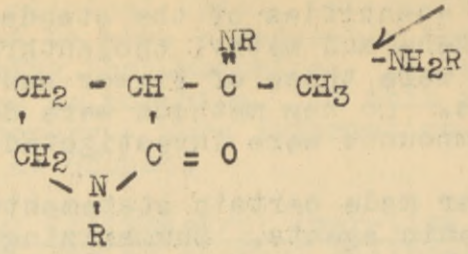
Variations in the molecule, particularly in the groups attached to the nitrogen, cause marked changes in activity. For example, if the methyl groups are replaced by ethyl groups, a marked lessening of activity results.

6. Compounds having a Cocaine-like Action: This is an old piece of research carried out by Dr. Andersag. No useful substances have come out of it. The substances are members of the class

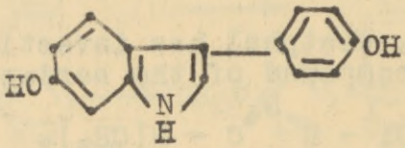


They are described in D.R.P. 663375 and U.S.P. 2187847. They are prepared in general as follows:



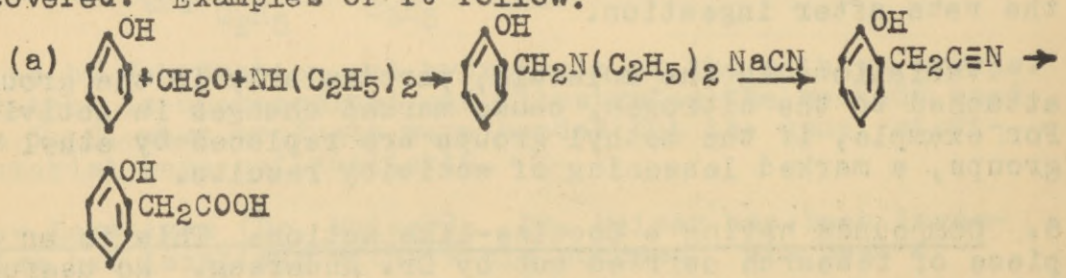


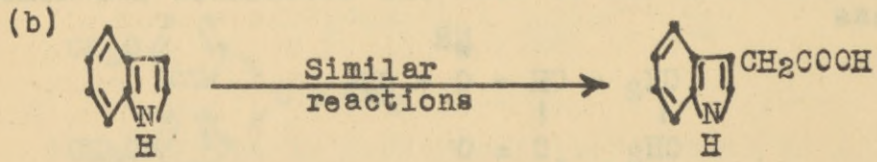
7. Other Synthetic Oestrogens (Dr. Salzer): Some work has been done in this field attempting to find other useful synthetic oestrogens. The best substance found was still very much inferior to diethylstilboestrol. It was



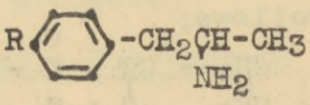
and had only a low activity, about 1 unit/mg. This work is published in Hoppe-Seyler's Zeitschrift für physiologische Chemie for 1942.

In the course of the synthetic work a new reaction was discovered. Examples of it follow:



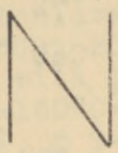


8. Vasoconstrictors (Dr. Breitner): The benzedrine field was investigated. The best compound found was

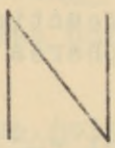


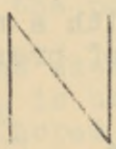
where R = Cl and CH_3. Since Smith, Kline and French had covered the field thoroughly before the I. G. did, work was discontinued on this line.

9. Zephirol Type Compounds (Dr. F. Leuchs): Dr. Leuchs is the discoverer of Zephirol and works entirely in the field of quaternary compounds. Zephirol is



This is the best of the series as a general disinfecting agent. It is of no value against tuberculosis, and at present Leuchs is attempting to find a substance in the quaternary field effective against the disease. While Leuchs is old and not very active, he has made a number of new quaternary salts, the following of which are examples. They are of no value in the treatment of tuberculosis, and no better than Zephirol as general disinfecting agents.



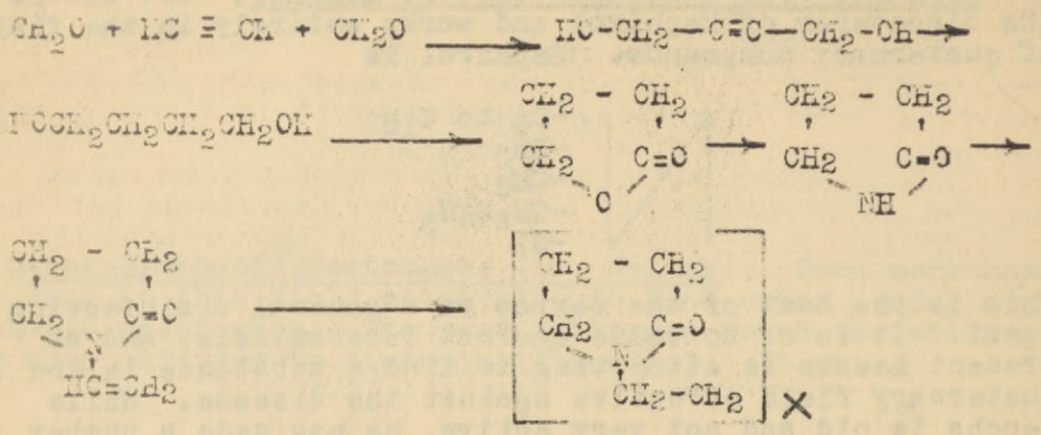


III. Periston

When a blood substitute was needed by the Wehrmacht, Professor Weese obtained colloidal substances from all sections of the I. G. Farbenindustrie. On the basis of animal tests, Weese selected a colloid made at Ludwigshafen as the best compound. This is called Kollidon, and is a polymerized vinyl pyrrolidone.

Dr. Krzikalla, who is employed at Ludwigshafen and is now living in Heidelberg, was consulted regarding the synthetic methods used to prepare this substance.

The remainder of the staff concerned with the production of Periston is at Gendorf and no "Betriebsvorschrift" was to be found. Dr. Krzikalla gave us the following information from memory regarding the production of Periston:



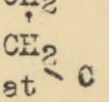
1. a. Preparation of 1,4 butinediol $HOCH_2-C\equiv C-CH_2OH$

Formaldehyde (2 mols) in water is treated with acetylene in the presence of about 1% copper acetylide, as a catalyst. The acetylene is pumped through the aqueous solution of the formaldehyde. The reaction is carried out at about 80-100°C under 20-30 atmospheres of pressure. The yield is 90-100%.

b. Preparation of 1,4 butanediol $HOCH_2CH_2CH_2CH_2OH$

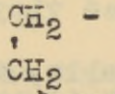
1,4 butinediol is reduced with a nickel catalyst at about 200°C and 100 atmospheres of pressure. The yield is about 90-100%.

c. Preparation of Butyrolactone



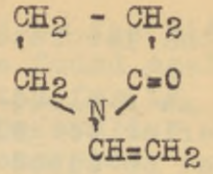
1,4 butanediol is treated with a dehydrogenating catalyst such as CrNi at 200-210°C under 170-180 atmospheres of pressure. In the course of the reaction one mole of hydrogen is produced for each mole of butyrolactone. This is removed as formed. The yield is 90-100%.

d. Preparation of 2-pyrrolidone



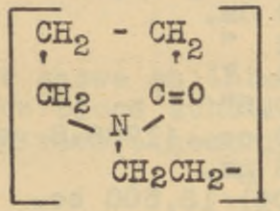
Butyrolactone is treated at 230°C with a large excess of liquid ammonia in an autoclave. About 100-200 atmospheres of pressure is obtained. The reaction is completed in a short time, about 2-3 hours. The product is purified by distillation, B.P. ca 90-100°C @ 8-9 mm. It is essential for subsequent steps that the pyrrolidone be as pure as possible. The yield is quantitative.

e. Preparation of 1-vinyl-2-pyrrolidone



2-pyrrolidone is reacted with anhydrous KOH (or NaOH) and then reacted with acetylene in an autoclave. For safety it is necessary to use about 20 atmospheres of acetylene together with 10 atmospheres of nitrogen. A temperature of 140-160°C is used. The product is purified by distillation B P, @ 8-9 mm. somewhat higher than the pyrrolidone. The yield is about 80-90% but depends upon the purity of the pyrrolidone used.

f. Polymerization of 1-vinyl-2-pyrrolidone to Periston



This work was done by Dr. Fikentscher who is also at Gendorf.

In general these polymerizations go as follows: at low temperature a higher polymer is obtained while at a temperature of about 40°C the polymer size is correct for Periston.

At first SO_2 was used as the polymerization catalyst but now 30-40% H_2O_2 is used. Exact details could not be obtained but it is hoped that teams visiting Gendorf will obtain these details.

The total product is used after dissolving to remove impurities. This is done at Elberfeld and the packaging is done at Leverkusen.

Dr. Krzikalla stated that about 6000 Kg/mo of Periston was made and about 30,000 kg/mo of the 1-vinyl-2-pyrrolidone was made, the remainder of this going into various other products.

2. Pharmacology and Clinical Use of Periston (Dr. Weese and Dr. Hecht)

The development of Periston represents the only important work done in this laboratory during the past few years. The urgency of the work on Periston for the Wehrmacht and the absence of Dr. Weese from his laboratory for two years with the army, accounts for the relative unproductiveness

of the laboratory.

As a blood substitute, Weese regards Periston as superior to gelatin, and from the practical standpoint equal to blood plasma. 400,000 ampules of 500 and 250 cc. were furnished to the Wehrmacht of which half, or 200,000, perhaps were used. The active principle of Periston is Kollidon. The army preferred a solution of Periston containing 2.5% of Kollidon. Weese, however, thinks a solution of 3.5% is admissable.

The solution is made up as follows:

NaCl	800 Gm.
KCL	42 "
$CaCl_2$ $6H_2O$	50 "
$MgCl_2$ $6H_2O$	0.5"
N/L HCl	1710 cc. (1728.8 gm)
$NaHCO_3$	168 gm.
Kollidon sol. (20%)	12,500 cc.
Distilled water	q.s. 100 liter = 101.3 Kg.

Kollidon sol. can be sterilized at 120°C.

Weese considers the preparation entirely inactive in the animal body, and says that it exhibits no toxicity in the ordinary sense of the word. Mice tolerate up to 8 gm/Kg. Very large doses (completely outside the therapeutic range) have produced a transient albuminuria in rabbits. Weese considers this a mechanical effect, and not due to any toxicity. In all animals studied, including mice, rats, rabbits, cats, monkeys and man, only dogs show any sensitivity. Weese claims this is a natural sensitivity, and is an idiosyncrasy of this species. No animal can be sensitized by repeated injections.

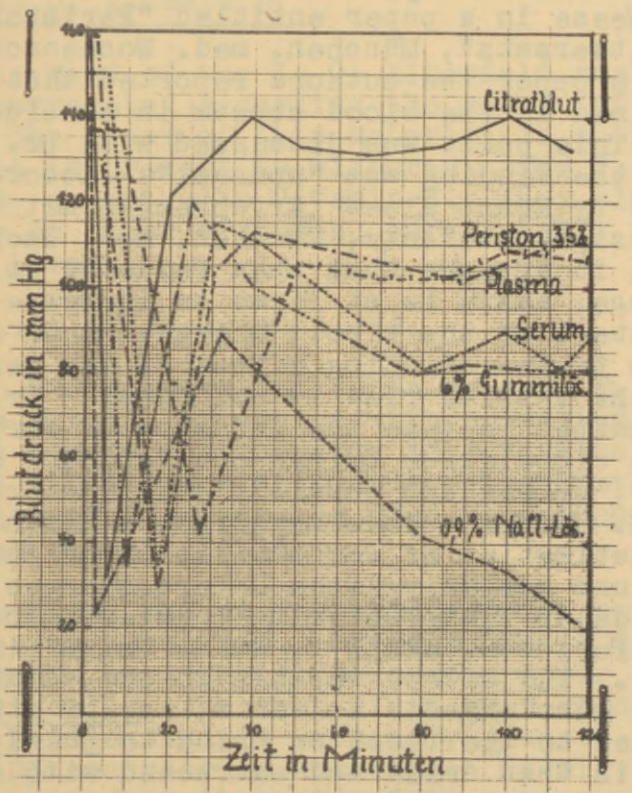
In the 100,000 to 200,000 cases of the army there was only one case of "drug fever" reported. Weese thinks this was probably due to some cause other than the administration of Periston.

When administered to animals shocked by hemorrhage, capillary permeability is reduced, the colloidal pressure and the viscosity of the blood increased, the water content of the blood raised, and the erythrocyte and hemoglobin content lowered by dilution. The fibrin, albumen and globulin content is also lowered by dilution. The preparation remains in the circulation in significant quantity for 2 or 3 days after injection, and is lost at a rate of

about 50% per day. The preparation is excreted by the kidney for two or three days, but thereafter cannot be found again in the urine. Not more than 50% can be accounted for by urinary excretion. Hecht believes that only the smaller molecules, possibly up to mol. wt. 10,000 are excreted, the rest being changed in some unknown manner in the body. The doses recommended are up to 500 cc. for adults and 25 cc/kg. body weight for infants.

Infants do not tolerate the drug as well as older patients. Of the thousands of babies treated, Weese says that there have been two or three instances of hematuria; however, the urine cleared promptly and there were no signs of permanent injury.

The attached chart shows an interesting comparison of the effect of different blood substitutes on the blood pressure of cats after severe hemorrhage.



According to the chart, citrated whole blood gave the quickest response; the blood pressure rose higher and remained high for the duration of observation.

Next in order of effectiveness is Periston. The blood pressure did not rise as high as it did after the use of citrated whole blood but it was superior to plasma in height of level and in promptness of response. The blood pressure remained high in both instances for the period of observation.

The blood pressure level after serum equaled that of plasma and Periston in rapidity of response and height of level but was less well sustained.

Saline solution was inferior to all the others in promptness of response and height of level. The blood pressure rapidly fell to shock level during the time of observation (120 min.).

For a discussion of the physiology of blood substitution, and of the chemistry and pharmacology of Periston by Dr. Hecht see Appendix 3. The pharmacology and clinical aspects of periston therapy have also been discussed by G. Hecht and H. Weese in a paper entitled "Periston, ein neuer Blutflüssigkeitsersatz", München. med. Wochenschr. 1943,Nr.1, p. 11. In this paper the authors reported that in one case Periston remained in the blood stream in considerable amounts for 15 days. This point was discussed with Dr. Hecht, who admitted that the finding was "unusual". According to another report by Ernst Klees (Erfahrungen mit "Periston", einem Blutflüssigkeitsersatz, München. med. Wochenschr. 1943, Nr. 2, p. 29), it appears that when Periston is administered after hemorrhage, there is at first an increase in the hemoglobin content of the blood, which is then followed by a fall, due to the diluting effect of the periston. The cause of the initial rise is not clear. Dr. Hecht's view on this matter is that the infusion of Periston by increasing the circulation brings out "hidden" erythrocytes from the liver, spleen and possibly other internal organs. J. Korth and H. Heinlein have described a method for the quantitative estimation of Periston in blood and urine (Funktionelle und morphologische Untersuchungen über die Wirkung kolloidaler Blutersatzmittel unter besonderer Beachtung des Periston, Arch. f. klin. Chirurgie 205: 230-286, 1943). The method depends on the solubility of kollidon in most organic solvents, except ether. Alcohol is added to the blood or urine to precipitate the proteins. It is then dried and extracted with ether to remove the fat lipids and foreign nitrogenous substances. The insoluble part is extracted with chloroform. The kollidon is dissolved in the chloroform, and a nitrogen

determination by means of the Kjeldahl method gives the amount of kollidon nitrogen. Hecht considers the method accurate within ±10%. Other reports on Periston are:

H. Weese: Blutersatzprobleme, Med. Zeitschr. 1944, pp. 19-23.

G. Düttman: Klinische Untersuchungen und Erfahrungen mit Periston, Der Deutsche Militärarzt 9:320-323, 1944.

Comments:

1. The claim that Periston is equal or superior to blood plasma is probably unfounded. However, it does appear to be superior to gum acacia and simple saline.

2. No comparisons have been made with degraded gelatin. This should be done, and if Periston is superior to gelatin, there is probably a place for it in therapeutics.

IV. The Assay of Vitamin B_1

Dr. Loth, head of the I. G. Elberfeld analytical laboratory, was interrogated regarding the method of assay of Vitamin B_1. The assay of this material is a troublesome matter when it is present in mixtures. The I. G. makes no mixtures of B_1 except with Vitamin C. They claim that B_1 is not stable in other mixtures, particularly those with Vitamin D or liver.

Two methods of assay are used:

1. When the preparation contains only B_1, and no other organic matter, oxidation with potassium ferricyanide is used. The excess ferricyanide is determined by iodine titration and titration with sodium thiosulfate. From the actual quantity of ferricyanide used, the amount of Vitamin B_1 may be calculated.

2. Thiochrome method. Vitamin B_1 is converted to thiochrome in the usual way. After suitable dilution, usually 0.5 gamma/cc., the fluorescence under a quartz lamp is compared visually with that given by a standard preparation of B_1. This method is extremely crude. Dr. Loth stated that 10% accuracy is attained. No microbiological assay is made, in fact it appeared that none was known to them. No stabilizing substances are added to ampoule solutions.

V. Virus and Rickettsial Infections

The I. G. Farben scientists, and in fact German investigators in general, are behind the rest of the world in research on these diseases because of the lack of expensive equipment such as ultra-microscopes and high speed centrifuges, and since the war they have been hampered by the impossibility of obtaining eggs (chick embryos) and larger laboratory animals for scientific purposes.

From the chemotherapeutic standpoint, Kikuth divides the viruses into two arbitrary groups, the large and the small viruses. In the group of large viruses, he includes those that cause bronchopneumonia of mice, lymphogranuloma venereum, trachoma, and psittacosis, if the latter is considered a virus disease. Kikuth believes that all the viruses in this group are affected by the sulfonamide compounds; the others, such as the viruses of influenza A, lymphocytic chorio-meningitis, avian pox, ectromelia and infectious myxomatosis, are unaffected by the sulfonamides or any other form of chemotherapy. Kikuth considers his major contribution to the study of virus infection to be his method of inhalation treatment. He has used this method in experiments with the viruses of influenza, lymphogranuloma venereum, bronchopneumonia of mice, and avian pox. See Appendix 4 for a table of sulfonamides active against viruses.

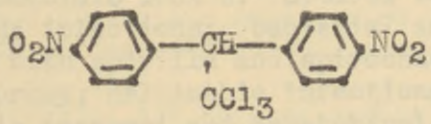
The mice are infected in the usual way by placing a few drops of virulent culture in the nostril. An aqueous solution of the medicament to be used is then made with a concentration of 1.5 or 10 mg. per liter depending upon the dose desired. A box of 65 liters capacity is then sprayed with the solution, using compressed air so that a very fine fog is produced. The fog remains in the air in practical concentration for about half an hour. Kikuth does not test samples of air for the drug, but calculates the concentration per liter of air. For example, if he uses 6.5 cc. of solution in the 65 liter box, he will have 6.1 cc. per liter. In general, he treats his animals 30 minutes a day for 3 days. Through the use of Badional he is able to cure bronchopneumonia in mice with regularity. This is the only way in which he has tested Badional, but he thinks well of the drug. He has written a paper on the subject and sent it in for publication. See Appendix 3.

A. Infectious Myxomatosis: This is a disease occurring naturally in rabbits. In the laboratory the disease is transmitted readily from one animal to another by subcutaneous injection of macerated organs or subcutaneous tissue of

infected animals. Several years ago Kikuth tested a few sulfonamide compounds in this condition, but without effect. He then gave up the work because he considers the disease of little interest as it has no counterpart in human beings.

Preparation B1034 was tested on the viruses of bronchopneumonia and lymphogranuloma venereum. Infected mice were given 1 cc. of solution per 20 gm. of body weight. The concentration of the solutions varied. In bronchopneumonia a concentration of 1:100 was effective when given orally or subcutaneously; a concentration of 1:200 was ineffective by both routes of administration. In lymphogranuloma venereum 1:800 was effective both orally and subcutaneously, whereas 1:1500 was inactive or nearly so. Sulfadiazine was far superior in its effect on bronchopneumonia. A concentration of 1:50,000 was effective subcutaneously, and 1:12,000 orally. In the case of lymphogranuloma venereum the figures were respectively 1:50,000 and 1:100,000. In view of these results Kikuth lost interest in B1034.

B. Rickettsial Infections: Kikuth has worked with rickettsial diseases from time to time, but has shown no sustained interest in them. He has tested several immune sera (not made by I. G. Farben), but was not impressed by them. Most of the animals eventually died despite serum treatment, although death seemed to be delayed. Of more interest to Kikuth was chemotherapy with methyleneblue and nitro gesarol.



A full report of his work with methylene blue appears in Z. f. Bakt., Parasitenkunde and Infektionskrankheiten 151, 293 (1944).

As a result of the world-wide interest aroused by gesarol (D.D.T.), the I. G. Farben chemists made a number of similar compounds in the hope of improving the original substance. One of these preparations, nitro-gesarol, was tested by Kikuth in a routine manner on murine typhus in mice. To his great surprise he found the preparation highly active (activity much greater than methylene blue) and with very low toxicity. This work was done shortly before the surrender of Germany, and due to the subsequent confusion no further work has been attempted.

General Remarks:

1. No extensive or very important work has been carried

out at the Elberfeld plant, I. G. Farbenindustrie on virus or rickettsial diseases in recent years.

2. Kikuth's belief that sulfonamide drugs are directly active on certain large viruses (i.e. beneficial effects not due to action on secondary bacterial invaders) is unproven and open to question.

3. Kikuth's method of treating virus infections by means of inhalation of an air suspension of the drug certainly has clinical as well as scientific application, but the technique is not new. Penicillin and other drugs are used in this way in America.

4. Kikuth's results taken alone do not justify further interest in preparation B1034.

5. Nitro-gesarol may be a preparation of considerable interest and should be studied further.

VI TROPICAL MEDICINE

A. Introduction:

Inasmuch as Professor Walter Kikuth is a recognized authority on laboratory tropical medicine, and has made many outstanding contributions to this field, he was questioned intensively for 4½ days. He was most cooperative and seemed anxious to impart all the knowledge at his command. He has an excellently equipped laboratory, and is ably assisted by Drs. Bock and Mudrow who were also interrogated.

Dr. Kikuth has kept his laboratory open throughout the war, but since the occupation his sole work has been to keep his cultures, strains of organisms and laboratory animals intact. During the war his work was greatly hampered by an inability to obtain or keep trained help, and the shortage of laboratory animals as well as feed for them. His most recent work has been confined to cultures and small animals, such as mice, rats, guinea pigs, and a few rabbits. He has not been able to work with larger animals, such as cats, dogs, and monkeys. In normal times he was equipped to carry out toxicity studies on mice, rabbits birds and cats. Chemotherapeutic studies were regularly carried out on such parasitic infections as trypanosomiasis (congolense and brucei), bird malaria (using canaries and rice finches, amoebiasis (in cultures and kittens) and coccidiosis. Studies were also made on leishmania and hemoproteus infections; bacterial and related infections such as those with spirilla and spirochaetes (recurrens, rabbit syphilis); leprosy; helminthic infections such as cat ascariasis, schistosomiasis (mansoni and hematobium) bothriocephalus in dogs, oxyuriasis of mice, tapeworm of mice and sheep (studied in cats); and virus infections such as mouse herpes, canary virus, choriomeningitis, lymphogranuloma venereum, and influenza.

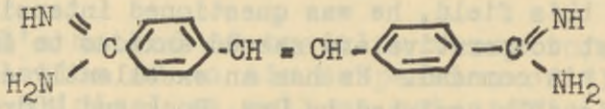
Professor Kikuth is in charge of all testing for tropical diseases. Below are presented the diseases being investigated, Kikuth's present recommendations as to the best remedies, his test methods, and the methods for the preparation of new substances.

B. Trypanosomiasis

Kikuth feels that Bayer 205 (Germanin) is the drug of first choice, with Fuadin second. He has done very little in this field because he considers Bayer 205 highly specific and almost ideal for the treatment of African Sleeping Sickness. When Lwoff, Bovet and Funke reported on the activity of N- (p-isopropylbenzyl)-ethylenediamine dihydrochloride, he made some preliminary studies on this compound, but dropped it when he was unable to confirm the reported results. His latest publication on African Sleeping Sickness appears in the Deutsche medizinische Wochenschrift (1940),

page 1363. In this paper combined treatment with Bayer 205 and Fuadin, or Bayer 205 and Tryparsamide are also recommended.

Dr. Schmidt has prepared the most active members of the York series for test purposes on trypanosomiasis. He made 4,4[1] diamidinostilbene.



Kikuth tested these and found them no better than the antimonials. Since the field had been thoroughly covered by York and Kikuth's test indicated no superiority, the work was dropped.

C. Leishmaniasis

The drug of choice in Kikuth's opinion is solustibosan. He studies Leishmaniasis in German hamsters, a considerably larger animal than the chinese or golden hamster. He believes the German variety far superior for test purposes in leishmaniasis. His technique is as follows: Remove the liver and spleen of infected animals under sterile precautions. Grind the organs in a sterile mortar with a little sterile saline and inject about 0.1 cc. into the peritoneum of a second animal. After 4 to 6 weeks a liver puncture is made by means of an ordinary syringe, and the tissue fluid is dried on a slide and stained with Giemsa stain. If the inoculation has been successful, the Leishman bodies are readily observable.

Kikuth prefers German hamsters because the infection produced is very constant whereas with Chinese hamsters the infection results are quite erratic.

His exact assay technique is published in a paper with Professor Hans Schmidt in Zeitschrift für Immunitätsforschung" 100:157-178 (1941). In this paper the authors state:

"Daily doses of concentrated substance (500 mg Sb/Kg) for 10 successive days with either Solustibosan or Neostibosan resulted after 2 weeks in a complete clinical and parasitological cure of leishmania infected hamsters (Cricetus frumentarius)".

To judge the effects of treatment recourse must be had to cultures of leishmania, or to many smears made over an extended period of time. Neostibosan is used as a base line with which new preparations are compared (Kikuth uses N.N. medium for culturing leishmania). The only new preparations that Kikuth has tested are solustibosan-concentrated (containing 100 mg antimony per cc.;

old solustibosan contained 20 mg. antimony per cc.) and solustibosan suspended in a mixture of olive oil and 1 or 2 % lanolin. The antimony content of this preparation is about 54 mg. per cc. Kikuth is convinced of the great superiority of solustibosan over neostibosan.

The advantages which he claims for solustibosan are as follows:

(1) It is easier and cheaper to manufacture (this is confirmed by others).
(2) It is better tolerated locally when given intramuscularly (confirmed by others)
(3) It is less toxic generally (confirmed by others).
(4) It can be furnished in solution in ampules ready for administration (amply confirmed).
(5) Although it contains less antimony than neostibosan, it is at least as active in fact, weight for weight; he thinks it is more active (not entirely confirmed by others - Schmidt is apparently not in entire agreement on this point).

In addition Kikuth says that the stability in solution is quite satisfactory. Storage in the tropics for 3 years caused no increase in toxicity or loss of potency. Incubation studies apparently were not available. (Schmidt agrees that stability was satisfactory, but this has not been confirmed by others).

Kikuth believes that the concentrated solution is superior to the older preparation. The intravenous and intramuscular tolerance is excellent. The only real advantage appears to be that larger doses can be given intramuscularly with less pain because the smaller amounts of fluid produce less pressure pain.

The advantage of solustibosan in oil and lanolin consists in the depot action. Kikuth tested the activity of the oil proportion and found it satisfactory, but no studies on duration of the effect are available.

Summary of information gained

(1) There is nothing new in Kikuth's method of studying leishmaniasis in the laboratory or in his method of testing new drugs in this condition.

(2) If not already available, European hamsters should be imported into the United States for laboratory use.

(3) Solustibosan is almost certainly superior to neostibosan, and is easier to prepare and more convenient to use. It is less toxic.

(4) Solustibosan-concentrated solution has minor advantages over the older preparation. It might be quite superior in the treatment of filariasis where large doses are required.

(5) The advantages of solustibosan in oil and lanolin are questionable, but this preparation should be studied further.

D. Schistosomiasis

Kikuth and his collaborating chemists, Mauss and Schmidt, have exhibited an active and sustained interest in the chemotherapy of schistosomiasis for a number of years and several hundred compounds have been tested systematically. Kikuth has developed an excellent technique for laboratory study of this disease. Strains of S. mansoni and S. hematobium are maintained in the laboratory together with strains of Egyptian and Venezuelan snails. Egyptian snails are used for transmission of S. mansoni and Venezuelan snails for S. hematobium. The snails are propagated in glass acquaria of about 2 gal. capacity in a room at a constant temperature of 80°F. Kikuth has found that lighting is the most important factor in his successful propagation of these snails. (He is the only one, so far as is known to raise Venezuelan snails successfully). Natural light is afforded by a window facing south, and in addition, in winter 1 or 2 hours exposure to a sun lamp is added. Altogether enough light is present to produce an abundant growth of algae in the aquaria. The water is of course aged.

The technique for culturing the schistosomes and reinfecting animals is as follows: Schistosome ova from human urine or stools are left in water in the refrigerator for about 12 to 16 hours. Then the container with eggs is placed in the warm room (80°F) and exposed to light for 24 hours. In the case of eggs isolated from stools, the eggs are separated from the feces by repeated washings with ice water before being placed in the refrigerator. As soon as the miracidia emerge, snails are placed in the water for a few hours. The snails are then returned to the acquarium; in about 2 to 3 weeks cercaria develop, and the snails are ready to infect animals. (The snails are fed on fresh lettuce placed in the aquarium). The snails are then placed in small containers and allowed to discharge their cercaria into a few cc. of water. The water is examined under the microscope and a sufficient amount taken to contain from 40 to 60 cercaria. This water which contains cercaria is injected subcutaneously into a mouse. After 3 weeks the adult worms have developed in the mouse and ova are being passed in the urine or feces.

The technique for infecting rabbits and rhesus monkeys is the same. Drugs are screened on mice and promising ones are then tested on rabbits and monkeys, if the latter are available. The drug under test is administered once daily for 6 days, urine and stools are examined twice weekly for ova. If ova disappear under treatment the

test is rated as an apparent cure. If eggs reappear or if living worms are found on autopsy, the drug is rated as of temporary benefit. If on autopsy all worms have disappeared or are all found dead, the drug under test is given the rating of a permanent cure.

In recent years the investigations have followed two main lines:- (1) to find a drug which might prove to be superior to Fuadin, and (2) to find an antimony preparation which might be administered orally. This latter aim is of considerable importance from the standpoint of the mass treatment of native populations where the disease is highly endemic.

The specific method of test as used by Kikuth using Schistosomum mansoni is as follows:

1. Infection of the snails (Planorbis guadalupensis). Freshly collected stools from monkeys or mice are washed three times (for 20-30 minutes) with 7.5% sodium chloride solution and then twice with ice water. The stools are allowed to stand in the refrigerator for 16 hours in the second ice wash water. The following morning the ice water is carefully poured off, and the number of eggs are counted in a 0.2 cc. sample of stool residue. About 40-50 eggs are required for each snail. The correct amount of stool residue is placed in warm water (45°C) and the snails are bathed in this water for 24 hours. After about 1-2 hours treatment with Vita Lux (light) the miracidia emerge from the eggs and infect the snails. The length of time of infection of the snails until they discharge the cercaria is about 14 days to three weeks.

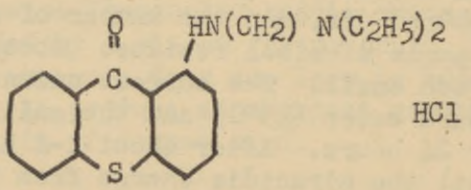
2. Infection of Mice: When the snails are ready to infect animals, they are placed in small glass containers together with a small amount of water. If the weather is cloudy, they are treated intermittently with the sun lamp for 1-3 hours. By 2 p.m. the high point of the excretion of the cercaria is reached, and the water containing these is removed. The number of cercaria in a given volume of water is determined under the microscope. Since the cercaria move very rapidly, careful stirring is essential for a uniform infection. Mice are infected by subcutaneous injection. One attempts to use 40-60 cercaria per mouse. The infection time in mice requires about 4-6 weeks. On the 48th day following the infection the mice are examined to determine if they have been positively infected. If they are, treatment is started. They treated six times per os and examined twice a week for miracidia. If none are found the stools are examined microscopically for eggs. Mice found to be negative after 4-6 weeks are killed. The liver, portal veins and mesentery are examined for worms and eggs. If none are found, the mouse is assumed to have been cured.

With the first object in view 72 compounds of the Miracil series were screened on mice and the more promising ones were also tested on rabbits. For a general description of the undertaking see Appendices 5 and 6. Appendix 5 was written up primarily for the German government with a view to saving some of Kikuth's laboratory workers from the army. Its tone is therefore somewhat more optimistic than the results really justify. Appendix 6 is a more sober evaluation by Hecht and may be too conservative.

Of all the compounds in this series, Miracil (Pa 7669) seems the most promising. The toxicity in laboratory animals is low and the activity high. The dosage figures in appendix 7 are in terms of the amount of drug per 20 gm. of body weight:

1/100 - 10 mg. of drug in 1 cc. of water per 20 gm. body wt.
1/1000 - 1 mg. drug dissolved in 1 cc. sal. per 20 gm. body wt.
1/6000 - 0.16 mg. drug dissolved in 1 cc. sal. per 20 gm. body wt.

1. Preparation of Miracil (1-Diethylaminoethylamino - 4 methylthioxanthone)



In a 1000 cc. autoclave are placed -

260.5 g. 1-Chlor-4-methylthioxanthon/ 1-methyl-4-Chlorthioxanthon mixture, prepared according to Ullmann and V. Glenck, Ber. 42, 2491.

130 g. Diethylaminoethylamine (1,1 mole)

130 g. Pyridine

and the mixture heated on an oil bath at 180-190°C under a pressure of 2-3 atmospheres. After cooling, the autoclave, the reaction mixture is treated with 500 cc. 2N- NaOH and the excess volatile reagents and solvents removed by steam distillation. The crude base which still contains in addition to the desired 1-diethylaminoethylamine 4-methylthioxanthone, unreacted 1-methyl-4-chlorthioxanthone, separates upon cooling from the alkaline solution as a yellow product. This product is purified by refluxing twice with 300 cc. of 10% acetic acid. In this solvent (m.p.138 9°C;120g) the 1-methyl-4-chlorthioxanthone is

insoluble while the desired product dissolves. The acetic acid filtrate is refluxed with decolorizing charcoal, filtered and then added to 250 cc. of ice. This causes the 1-diethylamino-ethylamino-4-methylthioxanthone to precipitate as a yellow powder which is filtered, washed with water and dried in vacuo at 30-40°C. The yield is about 165 g.

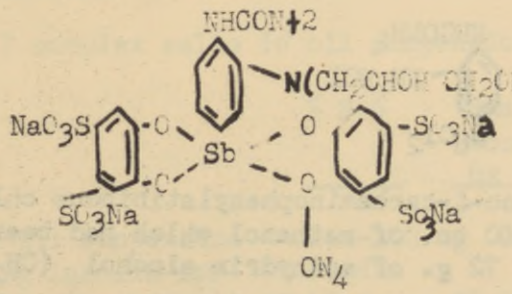
In order to prepared the hydrochloride, 165 g. of the base are placed in 415 cc. of alcohol together with charcoal and the mixture heated to boiling, filtered, and is added an alcoholic solution of HCl to the hot filtrate until the solution gives a blue color with congo red. Upon cooling, the salt crystallizes out as yellow crystals. These are filtered off, washed with cold alcohol, and dried in vacuo. The yield is 192 g., m.p. 195-6°C. For purification the salt is recrystallized from 400 cc. of alcohol, again using charcoal. Yield 147 g. - 39% of theory calculated on the entire isomeric mixture used. The melting point is 195-6°C.

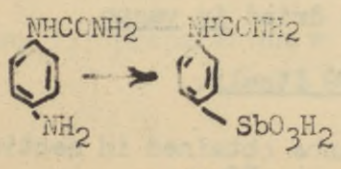
The second line of investigation was an endeavor to obtain an antimony preparation suitable for perceral administration and covered a large series of trivalent antimony compounds.

Representative compounds of different chemical groups are described in the summary given at the end of this section.

While several of the preparations intended for oral use were sufficiently active and well tolerated by mice, the doses required were so great as to be impracticable for human beings from the standpoint of gastro-intestinal tolerance. The compound most worthy of note is Fuadin in oil, which was intended to give a depot action on parenteral administration.

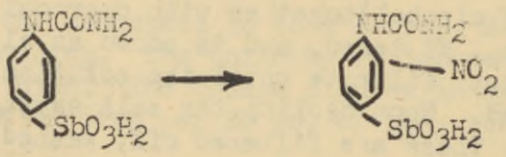
3. Preparation of Schmidt # 779



a. 

37526-I

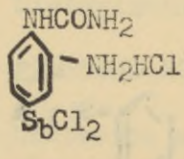
150 g. of 4-carbamido-1-aminobenzol are diazotized in hydrochloric acid solution with a solution containing 70 g. of sodium nitrite. To this is added a solution of 170 g. antimony oxide in 700 cc. of concentrated HCl, and if necessary also a little glycerine. To this is then added, with vigorous stirring and cooling, excess sodium hydroxide solution. After the reaction in finished, the solution is filtered and the 4-carbamidophenylstibinic acid is purified in the usual manner by treatment with NH_4Cl-HCl and dried.

b. 

100 g. of 4-carbamidophenylstibinic acid are added with stirring to 700 cc. of concentrated sulfuric acid, keeping the temperature below 15°C. To this is added dropwise a mixture of 22.8 cc. of nitric acid (Sp.G 1.405) and 22.8 cc. of H_2SO_4, keeping the temperature below 10°C. The temperature is then allowed to rise to 20°C. Ice is added causing precipitation of the product, which is wwashed with water and dried.

c. 

100 g. 3-nitro-4-carbamidophenylstibinic acid are placed in solution with 175 cc. of glacial acetic acid and 100 cc. of concetrated HCl. To this is added, the solution being well cooled, a solution of 250 g. of stannous chloride in 200 cc. of concentrated HCl. The temperature is allowed to rise to 30-40°C and allowed to stand. The powder which precipitates is filtered, washed free of tin and dried.

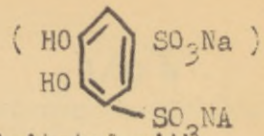
d. 

48 g. of 3-amino-4-carbaminophenylstibinous chloride hydrochloride is placed in 240 cc. of methanol which had been cooled to 10°C. To this are added 72 g. of epihydrin alcohol (CH_2  $CH\text{-}CH_2OH$).
The mixture is stirred at 10°C for 24 hours. The product is filtered off, washed with methanol and dried <u>in vacuo</u>.

e. <u>Preparation of # 779 Itself</u>.

44 g. of the substance obtained in section d are treated

with an aqueous solution of 68 g. of "Brenznatrium" and a neutral solution obtained by the addition of 2 N sodium hydroxide solution. The solution is filtered, diluted with methanol, and immediately precipitated with ethyl alcohol. The product is filtered and dried in vacuo.



There is thus obtained a light, colored powder which is very soluble in water. A 6.6% solution is isotonic and neutral with an antimony content of 12%. The aqueous solution decolorizes iodine and this may be used for the assay of Sb III. (0.5 g. in about 200 cc of water plus 10.5 of N/10 iodine solution and after 5 minutes back titrate with thiosulfate solution).

SUMMARY OF ANTIMONY COMPOUNDS USED IN THE CHEMOTHERAPY OF SCHISTOSOMUM MANSONI INFECTION OF MICE.

I. Antimony III complex salts - parenteral:-

		% S6 III	Dose to 1/20g Mouse 6x subcutan.	Smallest active dose 20g. Mouse 6X subcutan.
Pa 2011	Tarter emetic	36.5	1/2000	1/2000 H 1/4000 W
Pa 6505	Fuadin	13.5 as solid product	1/400	1/1500 W

Fuadin-concentrated; cf. Schmidt-Peter, Recent advances in the therapeutics of antimony, p. 247 ff.

II. Antimony III complex salts in oil suspension:-

		% S 6	Smallest active dose 20g. Mouse 6x subcutan
Std 735 Pa 6451	Fuadin in oil suspension (containing lanolin for permanent suspension)	1cc-100 mg Fuadin - 13.5 mg. S6	0.05 cc H 0.02 cc W

III. Antimony III preparation with aromatically bound S 6:-

		% S6 III	Dose tol/20g Mouse 6x subcutan.	Smallest active dose 20g Mouse 6x subcutan.
Std 779) Pa 7029)	For structure see above.	36.5	1/10	1/100 W

IV. Antimony III preparation peroral

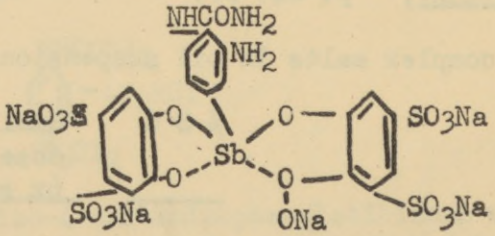
		% S6 III	Dose tol/20g Mouse 6x peroral	Smallest active dose 20g Mouse 6x peroral
Pa 2011	Tartar emetic	36.5	1/400	1/400 W., H 1/800 H
Pa 6505	Fuadin	13.5	1/25	1/50 W

		% S6 III	Dose tol/20g. Mouse 6x peroral	Smallest active dose 20g. Mouse 6x peroral
St 187) Pa 8819)	Antimony III Saccharic acid complex (Structure unknown).	27.5	1/100	1/400 W.

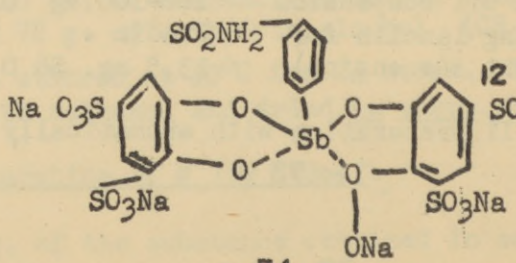
These substances have such a low emetic dose that they are not useable.

B.

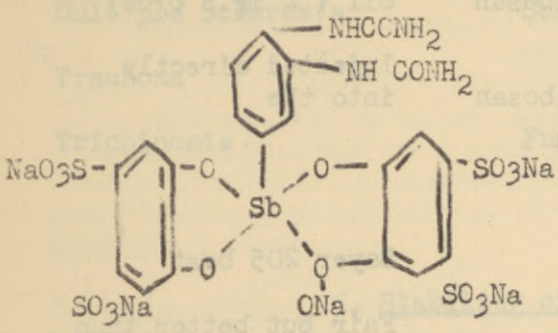
	%S6 III	Dose tol/ 20g Mouse 6x peroral	Smallest active Dose/20g. Mouse
SD 397) Pa 8816)	12	1/15	1/50 W



SD + 614) Pa 3528)	12	1/5	1/5 H 1/20 W



Sd + 779) Pa 7029)	See above	12	1/5	1/20 W



SD+ 884) Pa 8243)		10.5	1/5	1/40 W

Doses are fractions of a gram /20g. mouse.

These show good action on mice but have such a low emetic dose on dogs etc. as to be useless.

V. Antimony V Preparation

None found to be of value.

General Comments.

1. Kikuth's method for the study of human schistosomiasis is of great importance, and if successful in other laboratories should lead to much new knowledge of this disease.

2. Kikuth's method for screening and testing new drugs for schistonomasis is simple and efficient and the results can probably be regarded as a fairly reliable index of what may be expected clinically, inasmuch as the parasites used are actually those which are parasitic for human beings.

3. The toxicity of Miracil should be re-examined together with other active members of the series, and the drug should be tested cautiously in the treatment of human cases of schistosomiasis.

4. Fuadin in oil should be given clinical trial, especially in mass treatment.

4. ANTIMONY COMPOUNDS USED IN TROPICAL DISEASES

This summary is repetitive in some respects, but it is given here, as it is believed to present a more complete picture of the ideas now held by investigators at Elberfeld.

Disease	Antimonial	Remarks
Intestinal leishmaniasis	Neo-stibosan Solustibosan	conc. (100 mg Sb/cc) oil (54 mg.Sb/cc)
Cutaneous leishmaniasis (oriental sore)	Fuadin Solustibosan	Injected directly into the
South American Mucocutaneous Leishmaniasis)	Fuadin	
Trypanosomiasis	-	Bayer 205 best
	Fuadin	Fair but better than York diamidine stilbes.
Malaria	Solustibosan	Useful in cases which are resistant to atebrin, plasmochin or quinine.
Spirochetal Diseases	-	No antimony compound useful. Salvarsan is best.
Schistosomiasis (Bilharziasis)	Fuadin 779 -	 Mircacil also useful
Filiariasis	779	Possibly useful. Sent to Calcutta for clinical trial.
	Fuadin	Possibly
	187	Possibly; this is an antimony oxide complex with saccharic acid.
Lympho-Granuloma venereum (Granuloma inguinale)	Fuadin	Specific for this.
Leprosy	-	Chalmoogra oil best, Fuadin and Solustibosan, sometimes used but results not good.

Disease	Antimonial	Remarks
Lymphogranuloma	Solustibosan	--
Multiple Sclerosis	Solustibosan	--
Trachoma	--	Sulfonamides best,
Trichinosis	Fuadin	Used by Germans quite extensively in last five years.

5, Stability of Antimonials.

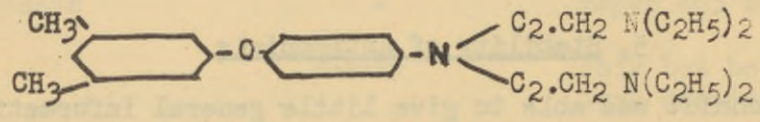
Dr. Schmidt was able to give little general information on this subject. In general, each preparation is a special case. It is well known that organic antimonials are much less stable than the corresponding arsenicals. Furthermore, the preparation of these antimonials requires as much art as science. Most of the preparations are amorphous, and the structure of these is assumed on the basis of analysis rather than as a result of rigorous proof of structure. Pentavalent antimonials, such as solustibosan, are stable in aqueous solution. There are exceptions, however, since neo-stibosan is sensitive to moisture and must be packed dry in ampoules. Trivalent antimonials, such as fuadin, are stable in solution, but must be protected against air and light. In this case the solution contains bisulfite but only as a bleach and not as an antioxidant. Compound #779 is as stable as fuadin. The stability of compound #386B is similar to that of salvarsan. It is unstable towards oxygen, and is packed dry in ampoules after evacuating in the presence of nitrogen. When antimonials decompose, Sb_2O_3 or Sb_2O_5 or the sodium salts of these are precipitated.

6. AMEBIASIS

Kikuth has been working sporadically with amoebiasis for many years. His culture of Entameba histolytica has been going in his laboratory for 12 years and has had more than 2000 culture passages. In the past few years he has tried to inoculate animals with it only once and this was a failure, either because of loss of virulence or because the infecting dose was too small. His method of culturing E. histolytica does not differ from that in common use. The method for inoculating animals is as follows: Young kittens of 3 or 4 weeks are used. The bowel is first washed 3 or 4 times with saline and then a stiff brush is inserted into the rectum and the mucosa scarified so that bleeding is produced (animals are anesthetized with ether). A culture of amoebae or an infected stool is then placed into the rectum and the anus closed for 2 hours with a clamp. It is important to select the kittens with great care,

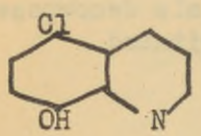
excluding all those contaminated with agranulocytosis virus or other viruses and other parasitic or bacterial diseases. The mortality from this procedure is high. Of 10 kittens so treated, only 3 or 4 survive and are suitable for drug assay. When amoebae appear in the stools, treatment must be started at once, or the animals succumb to the infection in a few days.

Kikuth has never been able to save infected kittens with emetine but he has been able to cure them with "Gavano". Gavano is a preparation made by Dr. Bruno Pütter (now in U.S.A. - Vick Chemical Co.) about 15 years ago. It has the following structure:



The intravenous toxicity for mice, rabbits, cats, and dogs is said to be about the same as that of emetine, i.e., 25 to 30 mg/kg. for mice and 4 to 7 mg/kg. for rabbits, cats and dogs. The subcutaneous toxicity for mice is somewhat superior to emetine (M & D Gavano 100 mg/kg. emetine 65 mg/kg. For rabbits the subcutaneous toxicity is about half that of emetine and in cats about 1/8. The intramuscular toxicity for rabbits, cats and dogs is from 1/3 to 1/10 that of emetine. Further details on the pharmacology of Gavano may be found in Appendix 8.

Before the war nearly all German iodine came from Japan. When this source of supply was cut off, an attempt was made to substitute other atoms for iodine in Yatren. A number of compounds were made, the best of which appeared to the following:



This preparation was tested by Kikuth in amoeba cultures and found to have some activity, but it was not tested in kittens.

Kikuth believes the preparation is certainly inferior to Yatren and has no interest in it now that the war is over.

Gavano was not considered by the research workers at Elberfeld to have sufficiant advantage over emetine to warrant commercial introduction.

The following reference on the use of Gavano were obtained.

1. Fitzgerald: Ind.Med. Gaz. 68, 458, 1933.
2. Akashi: Jam. Med. Assn. Formosa XXXIV, 2, 1935.
3. Chopa, N; Sen,S., and Sen, B: Ind. Med. Gaz.

vol, 119, p.130, 1934 (In this reference the preparation is said to be an ipecac-like preparation; this is an error)

4. Ghosh, N.N.: Antiseptic (English), 1934 No. 31, No.5, P 281.

5. Waldorp, C P.: Prensa Med.(Argentine)No. 46,p.2215, 1935.

Kikuth's method of test is as follows:

Culture of Amoebae

For propagation of Amoeba hystolytica, use is made of two kinds of cultures:

1. Feeding base of serum

100 cc of sterilized horse-serum (nothing added)
25 ccm of buffered Ringer solution (gepufferte Ringerlösung) (pH 7,4 -7,5) to be well mixed in Erlenmeyer flasks and poured off into tubes. After setting, it should be sterilized in the serum sterilizing apparatus for two successive days. On the first day it is kept at a temperature of 75-80°C for two hours, and likewise for a similar period on the second day at a temperature of 85-90°C.

Overlay fluid for above.

Albumen thinning solution.

The white of a fresh hen's egg is sterilized as.follows:- it is rubbed with soap or a solution of sublimate,- dried,- and then cleansed with alcohol, which is allowed to evaporate. The sterilized egg-white is then lifted with glass into 200 cc. "Erlenmeyer flasks and gently shaken. During this process, 100 cc. of thinning solution is added a little at a time.

The white of an egg is about 20 cc. in volume. The solution of albumen in which the amoebae thrive is obtained by augmenting the above solution with 492,5 cc. of pH 7.5-7,4.

2. Agar solution for amoebae.

1,000 cc buffered thinning solution (pH 7,4-7,5)
20 g of Agar.

The finely sliced agar is left to soften in the thinning solution for 12 hours. Thereafter, it is kept at a temperature of 100°C in a steamer, until dissolved, and passed through cottonwool until perfectly clear, after which it is filled into tubes, replaced in the steamer, and left for a further two hours to sterilize and set.

Overlay fluid for above.

Dextrin-Albumen solution.

1 g. Dextrin and 90 cc of thinning solution (Ringerlösung).
10 cc of physiological saline.

The Dextrin is dissolved by cooking in the salt solution and allowed to cool well, then it is added to 90 cc of the Ringerlosung and with a similar amount (100 cc) of the above albumen solution to 'top up'.

The following is also required:-

1. Rice. (for the feeding base of serum and for the Ringer Agar.) The rice is rubbed fine in a grater, poured into small tubes, and sterilized in the hot chamber for one hour at 100°C.

2. Blood Agar. (For feeding base)
225 cc distilled water.
3,5 g. of Agar Agar.
1,5 g. of salt.

The finely sliced agar agar is left for 12 hours in 225 cc of distilled water, after which the fluid is poured off and measured. Fresh distilled water is added to the same amount as has been poured off and the salt added. It should then be placed in the steamer for 2 hours to dissolve and finally passed through a filter of cottonwool until the Agar is perfectly clear, and poured into small tubes. These should then be placed in the steamer for sterilization. After cooling the tubes to a temperature of about 45°C, the same quantity of human blood (undisturbed) is added, well-mixed by shaking, and left to set. The tubes should be placed in a cool place for keeping.

Buffered Ringer solution for the production of feeding bases and albumen solutions.

2,000 cc Distilled Water.
12 g Salt.
0,2 g. Calcium Chloride.
0,2 g. Potassium chloride
0,2 g. Sodium bicarbonate
10 g. Calcium Biphosphate.
Approximately 52 cc. n/1 sodium hydroxide.

2000 cc. distilled water should be sterilized. From 50 to 70 cc. thereof should be poured into 5-10 cc. Erlenmeyer flasks. The

additional chemicals, after being weighed out, should be placed in one of the glasses and put into the steamer to be sterilized and, at the same moment, the larger glass should go into the steamer to be sterilized as well for a period of two hours. After cooling off, the contents of each smaller glass should be poured in turn into the larger one, being well shaken each time, and the whole brought up to pH 7,4-7,5 through the addition of sodium hydroxide and lactic acid.

For cultivation of Amoeba hystolytica, it has been found advisable to interchange the feeding bases, i.e. to transfer them from the serum to the Ringer Agar and vice-versa.

Shortly before using, 4,5 cc. of the solution is poured over the bottom of the vessel in which the amoebae are to be bred, and the latter placed in the incubation chamber to be pre-heated.

This preliminary warming process should last for about an hour, as the amoebae are extremely sensitive to changes of temperature. The culture (which exists in the form of small flakes of slimy substance) should be lifted from the bottom of the tubes with a pre-warmed capillary and transferred to one or more culture tubes, according to the amount of growth which has taken place - the tubes have already been made ready for their reception. A small quantity of agar prepared from human blood is then added to the feeding base with a glass spoon sterilized in a flame (rabbit's blood was found unsuitable for the purpose) - growth of the amoebae was greatly retarded when rabbit's blood was used, but they immediately sprang into vigorous life again as soon as agar from human blood was added. Both serum and the feeding base of Ringer agar should receive a small addition of rice thickening (it must be finely powdered) Incubation of the amoebae takes place at a temperature of 37-38°C. They should be changed over into fresh tubes every second day. We tried adding Trypaflavin in the proportion of one to one hundred thousandths to prevent the growth of bacteria, but we found this was superfluous and that it didnot lead to any increase in the rate of growth of the culture.

Checking the Preparations by Testing.

The serum feeding base is covered with a layer of Ringer solution (pH 7.2) and 0.5 ccm of the dilutant is then added. If, for instance, a concentration of one thousandth is desired, then one hundredth of the diluent should be added, since the substance has already been made ten times weaker by the addition of the Ringer solution. Thereafter, the tubes are placed in the incubation chamber, and left there for three hours. At the end of this period 0.5 cc. of a suspension of amoebae (Aufschwemmung) is added. This should consist of amoebae culture (abpippettierte Amöbenkultur) - human blood agar, and the ricethickening; it should then be of a raspberry color. The temperature should be carefully watched. Microspopic and other testing of the experiment takes place after an interval of 48 hours.

General Comments.

1. Kikuth's laboratory methods for the study of amebiasis are those in common use.

2. Gavano is probably of no interest initself, but since it appears to be highly active and is not similar in structure to emetine, possibly related substances might be produced which would be less toxic.

3. The chlorine substituted Yatren preparation has little if any interest.

7. Piroplasmosis

Inasmuch as piroplasmosis is unknown in Germany, it has been a subject of little interest to Kikuth until the German invasion of Russia at which time circumstances forced him to give some attention to the disease.

There are two types of piroplasmosis, both naturally transmitted by ticks. One type is caused by various species of Babesidae which attack man, dogs, horses, cattle, sheep and hogs. At least ten species of Babesidae are known. They are all characterized by fact that their whole life cycle in vertebrates is spent in the red blood corpuscle.

The other type of piroplasmosis is caused by Theileridae, of which four species are known and all of which parasitize cattle. The Theileridae inhibit not only red blood cells but also the endothelial cells, lymphocytes and monocytes of the internal organs especially of the liver and spleen. In the laboratory Kikuth was able to work only on Babesidae infections of dogs. However, several trips to Poland and the East afforded him an opportunity to test drugs on other forms of the parasite that attack other animals. Of the many drugs tested on dogs in the laboratory such as antimalarials (atebrin and plasmochin), sulfonamides, antimony and arsenic compounds, and various dyestuffs, none were found to possess even a trace of activity. Trypflavin, trypan blue, and a new preparation, Acaprin, showed varying degrees of activity in dogs, and were also tested in other animals.

Of the three preparations that were tested, trypan blue was found to be somewhat active in dogs infected with B. canis, in cattle infected with B. bigemina, B. argentina, B. bovis, and in B. caballi infection of horses. It was inactive against the other species of Babesidae and in all the Theileridae infections of cattle.

Trypaflavin was satisfactorily active only in B. canis infections of dogs and B.bovis infections of cattle. It was slightly active against B.bigemina, B. argentina and B.berbera in cattle; B. caballi, and B. equi in horses, and B. ovis infections of sheep. It was entirely inactive in all infections of cattle with Theileridae. The new preparation Acaprin was by far the most active. It is considered by Kikuth as providing a completely satisfactory treatment against B. canis infections of dogs and B. bigemina, B.argentina, B. Bovis and B. berbera infections of cattle. It is somewhat less active in cattle infected with B.divergens.

It is entirely satisfactory in the treatment of infections with the horse parasites B.caballi and B. equi, and is about equal to trypaflavin in B. ovis infections of sheep. It is also satisfactory in B.trautmanni infections of hogs. In addition it is slightly active against two of the Theileridae (T.dispar and Th.annulata) that infect cattle.

According to Kikuth the results of Acraprin treatments are most dramatic. In less than twenty-four hours after the beginning of treatment by subcutaneous or intramuscular injection, the animal's temperature falls to normal, and in a few days the animal appears quite well and normal.

A description of the use of Acraprin in animals is given in Kikuth's paper in the Deutsche Tierarztliche Wochenschrift 49, 190 (1941).

The following table indicates the activity of Acaprin in various veterinary infections, as comp red with Trypaflavin and Trypan blue.

Organism	Animal	Acaprin	Trypa-flavin	Trypan blue
Family: Babesidae du Toit				
B.canis	Dog	± ±	± ±	±
B. Bigemina (Texas fever)		± ±	±	±
B. argentina		± ±	±	(±)
B.bovis (Bovine haemoglobinuria)	Cattle	± ±	± ±	± ±

B. divergens (British bovine Redwater)		±	--	0
B. (Babesiella) berbera				
B. caballi		± ±	±	±
B.equi (Nuttallia)	Horse	± ±	±	0
B. (Babesiella) ovis	Sheep	±	±	0
B.trautmanni (B. suis?)	Swine	± ±	-	-

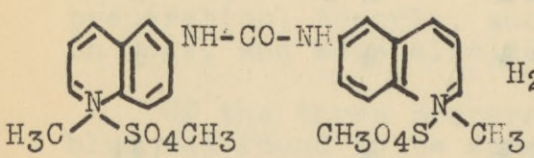
Family Theileridae du Toit

Thparva (African coastal fever)		0	0	0
Th. dispar		±	0	0
Th. annulata	Swine	±	0	0
Th. mutans (Gonderia, pseudo-coastal fever)		0	0	0

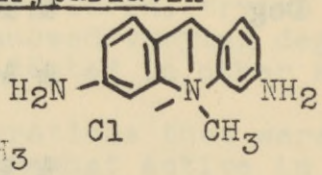
The designation of activity is as follows ± = active, ± ± = very active, 0 = inactive, - = not tried.

The structural formula of the compounds listed above are:

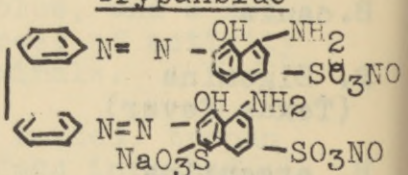
Acaprin



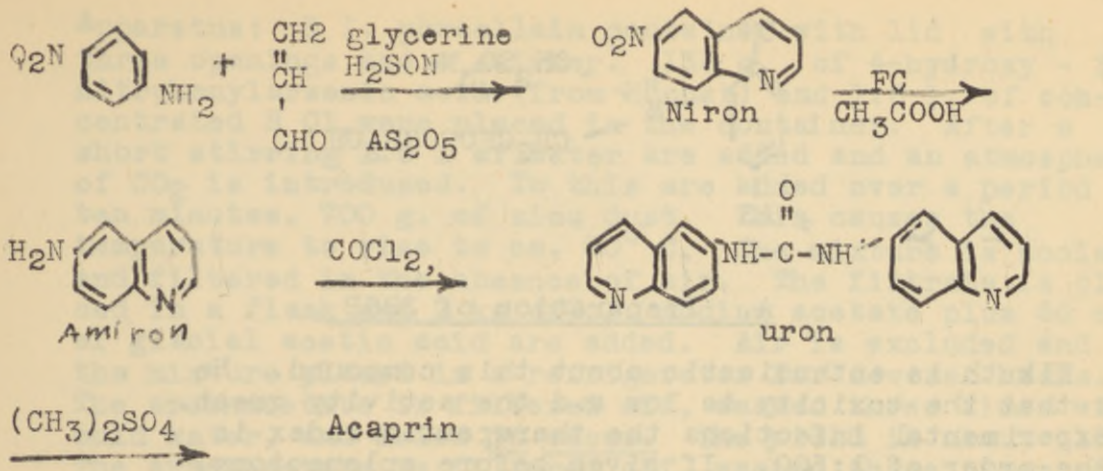
Trypaflavin



Trypanblue



The method of synthesis of Acraprin (Akiron) is as follows:

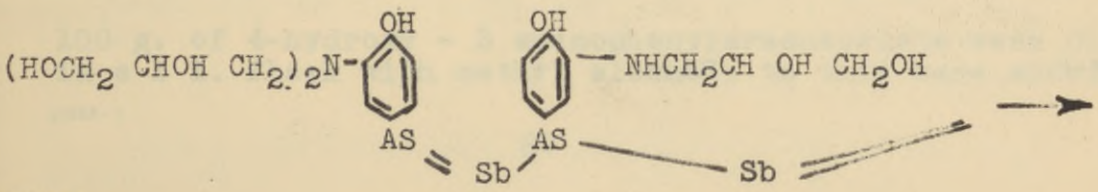


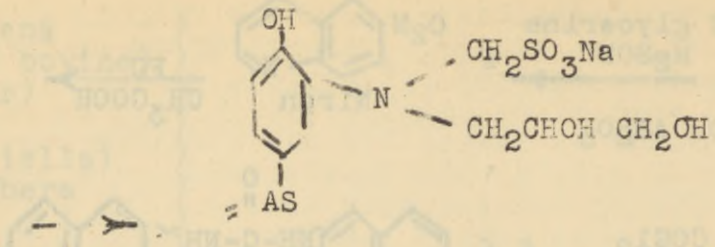
K. Conclusion.

Because of its ability to cure many of the more common types of piroplasmosis in domestic animals Acaprin is likely to be of great economic importance in veterinary medicine in the tropics.

8. BARTONELLOSIS

Nearly all laboratory rats available in Germany are naturally infected with Bartonella muris. Kikuth has found only two or three exceptions to this rule. The infection is latent and only becomes evident upon splenectomy. Upon removal of the spleen the organisms appear in the red blood cells, usually on the third day, and a profound anemia develops which is fatal in a large number of un treated animals. Drugs are tested in two ways. They are administered for two or three weeks before the spleen is removed to show presence or absence of a prophylactic effect, or treatment is started three or four days after splenectomy to determine the effect of the drug on the course of the disease. Many drugs have been tested in rat bartonellosis, including Acaprin and the sulfonamides, but all were found inactive except preparation 386 B. This compound is similar to neoarsphenamine, except that two of the arsenic atoms are replaced by antimony. The structure is not known with certainty but the preparation analyzes for the following structure.





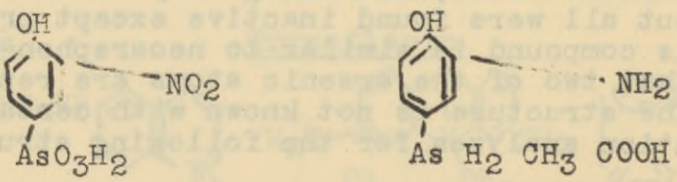
A. Preparation of 386B

Kikuth is enthusiastic about this compound. He says that the toxicity is low and the activity great. In experimental infections the therapeutic index is of the order of 1:500. If given before splenectomy, 386B willregularly prevent the appearance of bartonella in the blood, and if given after the appearance of the disease the anemia responds rapidly, the bartonella disappear from the erythrocytes and all animals not moribund before treatment begin to recover. The disease does not recur in treated animals. Clinical studies have been carried out in South America in human bartonellosis, i.e. Oroya fever and Verruga peruana. The doses used were 0.1 to 0.3 g. intravenously in courses with the total dosage 5 to 7 g.

The favorable results in clinical bartenellosis are reported in Archiv für Schriffs - und Tropen-Hygiene 41 729 (1937), but these have not been confirmed by American observers. A discussion of Kikuth's methods for studying bartonellosis in the laboratory and a review of the pharmacology of the drug als appears in the aforementioned paper. There is no noteworthy toxicity according to Kikuth until large doses are reached when symptoms of acute arsenic or antimony poisoning occurs. The product contains 18% arsenic and 20% antimony.

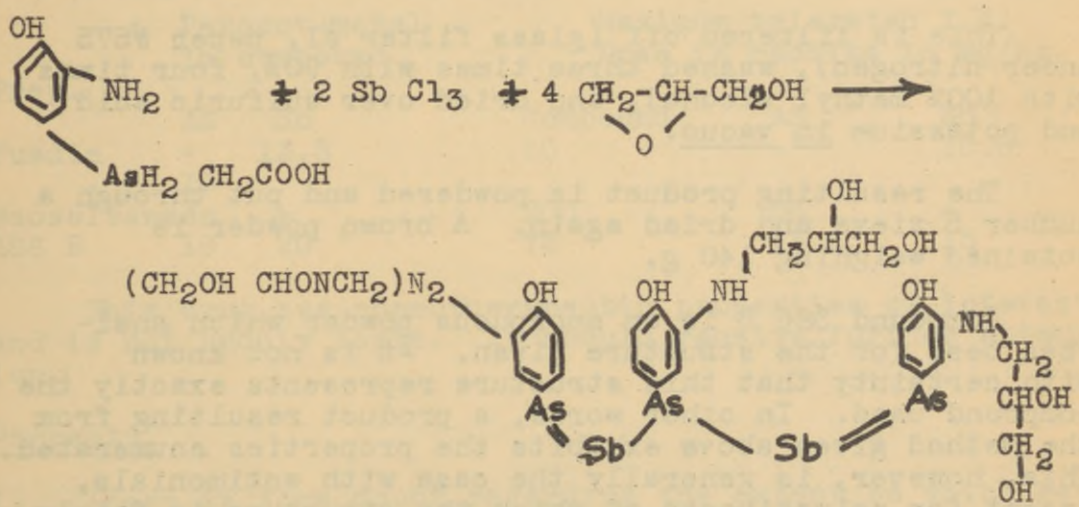
Schmidt Compound #386 B

1 4 hydroxy - 3 - aminophenylarsenate.



Apparatus: 8 l. porcellain container with lid with three openings and a stirrer. 135 g. of 4-hydroxy - 3-nitrphenylarsenic acid (from Höchst) and 1.5 l. of concentrated H Cl were placed in the container. After a short stirring 1.5 l of water are added and an atmosphere of CO_2 is introduced. To this are added over a period of ten minutes. 700 g. of zinc dust. This causes the temperature to rise to ca. 80° C. The mixture is cooled and filtered in the absence of air. The filtrate is placed in a flask and 2 kg ofC.P. sodium acetate plus 50 cc, of glacial acetic acid are added. Air is excluded and the mixture placed in a refrigerator for several hours. The arsenacetate is filtered off, washed three times with cold water, and dried in vacuo. The yield is about 110 g. The arsenacetate is filtered off, washed three times with cold water, and dried in vacuo. The yield is about 110 g. of colorless product. This should be kept in ampules under nitrogen. Its m.p. is 140°C and 0.1 g requires ca. 24 cc. N/10 iodine solution. 2. Glycid (Epihydrin alcohol $CH_2{-}CH - CH_2OH$)1065 g. obmonochlorhydrin ($CH_2Ce\ CHON\ CH_2OH$) dissolved in 1 l. of absolute alcohol were added to a cold solution of 538 g. of KOH in 2.50 cc. of absolute alcohol. The cold mixture was allowed to stand for several hours and evaporate. It was fractionally distilled. B.P. 62° @ 15 mm. Yield 400 g.

3. 4-hydroxy - 3 - dihydroxypropylamino-aminibenzene arsenantimony hydrochloride.



100 g. of 4-hydroxy - 3 aminophenylarsenacetate were placed in a 1 l. flask with methyl alcohol; to this were added

90 cc. of glycid and the flask filled to the top with methyl alcohol. Agitation was accomplished by the use of glass balls. The mixture was allowed to stand for 24 hours.

At the end of this time the mixture was cooled to about 15°C and to it was added a solution of 58 g. of antimony trichloride in 600 cc. of methanol to which had been added 30 cc. of glycid. A yellowish red precipitate is obtained which redissolves in a few minutes. The temperature is allowed to rise to 1°C and the yellow red color disappears. The solution is stirred into 8 l. of acetone. The resulting precipitate is filtered off and washed with acetone (under CO_2). The precipitate is then dried with ether and dried over sulfuric acid and potassium in vacuo. The yield is 160 g. of a yellowish brown powder.

4. Reaction with formaldehyde - bisulfite.

The hydrochloride from step 3 above is dissolved in water and ice is added. To this is added 2 N sodium hydroxide to obtain a neutral solution (ca.200 cc). A brown suspension is obtained to which is added 200 g. of formaldeyhde-bisulfite. The flask (2 l.) is filled completely with distilled water, and shaken for several hours to effect solution (glass balls are used to agitate).

After twenty-four hours the dark brown solution is filtered through a glass filter and the filtrate added to 5.6 l. of methanol which causes precipitation of the product.

This is filtered off (glass filter #1, paper #575 under nitrogen), washed three times with 90%, four times with 100% methyl alcohol, and dried over sulfuric acid and potassium in vacuo.

The resulting product is powdered and put through a number 5 sieve and dried again. A brown powder is obtained weighing 140 g.

Compound 386 B is an amorphous powder which analyzes best for the structure given. It is not known with certainty that this structure represents exactly the compound used. In other words, a product resulting from the method given above exhibits the properties enumerated. This, however, is generally the case with antimonials, except for solustibosan of which the structure is fairly well defined.

5. Glucose mixture.

Equal mixtures of the sieved compound from 4 and pure glucose are mixed under nitrogen through a #5 sieve.

Filtration with iodine, 0.2 g. require 16 cc. N/10 iodine.

b. Pharmacology of 386 B

Single Doses. The subcutaneous M.L.D. for mice is 0.15 g/kg. With an intravenous dose of 100 mg/kg. rabbits die in three days, with 120 mg/kg. in two days, and with 200 mg/kg. in twenty-four hours. The autopsy findings were massive hemorrhage of intestines, hyperemia of the kidneys, fatty degeneration of the liver and albuminuria but no glycosuria. Rabbits receiving 75 mg/kg. intravenously survived.

When cats were given 0.2 g/kg. subcutaneously, acceleration of respiration was noted within two hours; in three hours the animals were down and could not rise; fifty minutes later death occurred with pulmonary edema and evidence of insufficiency of the left heart. Symptoms of acute antimony or arsenic intoxication were not produced.

Repeated Doses. One rabbit died after five daily subcutaneous injections of 50 mg/kg. Another rabbit tolerated six such injections with only a small decrease in haemoglobin and erythrocyte content of the blood. There were no urinary changes or loss of body weight.

Comparisons of the tolerance of Fuadin, neosalversan, and #386 B are shown in the following table:

Prep.	Percent metal in compound		Maximum tolerated I.V. Dose in rabbits in mg/kg.		
	AS	Sb	Compound	AS	Sb
Fuadin	- 19	13.5	80	-	10.8
Neosalvarsan	-		200	38	-
386 B	18	20	75	13.5	15

This drug has chemotherapeutic properties of interest and is not unduly toxic. It should receive further attention.

Comments.

1. There is some doubt regarding the extent to which results in rat bartohelloses can be interpreted in terms of human Oroya Fever or Verruga peruana, nevertheless the technique should be useful for screening drugs.

2. Prep. 386 B deserves further clinical study.

9. Leprosy

Kikuth's interest in leprosy has tended to follow along the line of the development of a good laboratory technique for studying the disease, rather than along the line of testing new chemotherapeutic agents for the disease although some chemotherapy experiments have been done. He uses Mycobacterium stefanski infections in rats. He obtained his strain of organisms from Paris and is able to infect 100% of animals. His technique is briefly as follows:

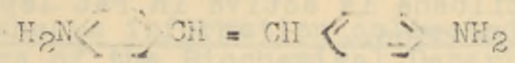
A leproma is removed from an infected animal under as nearly sterile conditions as possible and washed several times in normal saline. The leproma is divided into pieces about $\frac{1}{2}$ the size of a pea and these are transplanted under the skin of new rats in the inguinal region. If the animals are in a good nutritional state lepromata invariably appear. Kikuth made the interesting observation that in one batch of animals which were in poor condition (poor state of nutrition, infested with lice and fungi) lepromata did not appear. On more careful examination, however, M. stefanski could be demonstrated in the internal organs. This observation led to more careful study, and it was found that B1 avitaminosis could produce the same effect. Whereas animals on a diet normal with respect to B_1 content developed lepromata, animals on a diet deficient in B_1 did not develop lepromata but acquired a generalized infection with M. stefanski. Kikuth believes that this observation opens up a new way which more nearly approaches the human disease for studying leprosy in the laboratory. It is his opinion that in testing chemotherapeutic agents in the laboratory, the systemic disease should be studied rather than the local lesions. Furthermore, he believes that further studies on vitamin deficiencies and nutrition of native populations where leprosy is endemic may shed light on some of the baffling problems of the transmission of leprosy.

Papers dealing with the methods of test and the effect of vitamin deficiency are to be found in Z.f. Bakt., Parasit, und Infectionskrankheiten 151, 50 (1943), and Medizin und Chemie V.4.

The only studies on the treatment of rat leprosy were made with 4,4^1-diaminostilbene. In large doses this compound seems to have a beneficial effect upon the systemic disease of rats but the results are statistical.

Activity of 4,4[1] - Diaminostilbene in Rat leprosy

This product has the structure



It is used as an aqueous suspension and is injected subcutaneously. The toxicity is roughly 200 mg/20 g mouse. In the first experiment, 1500 and 1200 mg/kg was used three times a week, until the animals had received 5 doses. A retarding effect was definitely noticeable on the development of the disease; however, the drug was badly tolerated.

In the second experiment, much smaller doses were used, namely, a dose of 30 mg/kg once a week for six weeks. In this case, no activity was to be seen.

A third series, using 70 mg/kg. once a week showed definite activity.

The question to be answered is whether this substance exerts a general influence on rat leprosy. A longer period of observation is also necessary as at least 15 weeks are required before untreated rats show sloughing of organs. Doses of 1000 and 700 mg/kg once a week are not tolerated. The rats die at the latest in 9-13 weeks, but the organs are free of leprosy bacilli.

In a new experiment, 20 rats were given 100 mg/kg once a week. Eight survived the full 18 weeks, and a ninth died in the 16th week. The results on these nine mice are given in the table.

	Positive culture in organs	Negative culture in organs
Diaminostilbene treated	2	7
Controls	9	11

From these results, it appears that this substance actually shows a slight activity in rat leprosy, but owing to its toxicity higher doses can not be used.

Comments.

1. Kilduth's suggestion that the systemic disease of rats be studied further should be followed.

2. The opinion that the systemic disease offers a better criterion for judging chemotherapeutic agents appears to be correct, although the method is much more laborious and time consuming than the use of lepromata as criteria.

3. Further study of the systemic disease may throw light on the transmission of human leprosy.

4. Diaminostilbene is active in rat leprosy and deserves further attention.

5. In order to lessen the toxicity of diaminostilbene, it might be possible to detoxify this drug with galactose, as is done with diamino-diphenyl sulfone in Tibatin.

VII PENICILLIN

Very little research work has been under way on penicillin at Elberfeld. Dr. Auhagen haw made experimental runs in 10-20 l. flasks. No trouble with contamination was experienced in these cases, but considerable trouble was had in obtaining good culturues of Penicillium notatum. Captured Burroughs Wellcome material of about 200 u/mg. was used as a standard. The highest purity obtained by Auhagen was also 200 u/mg. Sodium and calcium salts were prepared in minute amounts. War scarcities of materials prevented him from proceeding to work on a larger scale. No preparations of the salts from the frozen state were made. In fact the impression was gained, that Dr. Auhagen knew nothing about such drying procedures.

In these case of surface cultures, the broth obtained had an activity of about 50 u/cc while submerged cultures yielded only about 5u/cc. The fermentation was carried out at a temperature of about 24-27°C following the British published results of 1942-3 and the fermentation was allowed to proceed for 10-14 days.

Purification of the broth was carried out by acidifying it to pH 2.0 with phosphoric acid and extracting the acidified broth with butyl acetate. Further purification was effected by extraction of the butyl acetate with sodium bicarbonate solution, reextraction of this with butyl acetate and finally extraction with calcium hydroxide or sodium bicarbonate. The penicillin salt was obtained by evaporation of this aqueous solution.

Due to Domagk's influence, the feeling at Elberfeld was that commercially penicillin was not a serious competitor with the sulfa drugs.

It is obvious that no structural work could be done on penicillin preparations of the low order of purity in the hands of the Elberfeld investigators. The only empirical formula known to these chemists was that published by the British workers in 1942-3, which did not provide a starting point for synthetic work. No work on any

syntheses aimed at penicillin are under way or are contemplated by Elberfeld. No other antibiotics are being investigated there.

VIII Miscellaneous information collectedcby interrogation of the members of the Biochemical Department.

1. Prof. Weyland is the head of this Department. He has done practically no research in the last three years because he was unable to get any starting material, such as glands from slaughter houses. He was-interested in liver and in the P.A. principle but knew nothing of interest and in fact attempted to interrogate us on the new developments, if any, during the war.

He had done some work on penicillin; thus is described under section VII.

2. Very little work has been done on protein hydrosates. No progress has been made on the isolation or purification of any new growth factors.

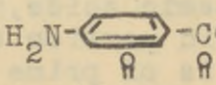
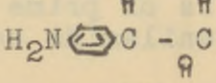
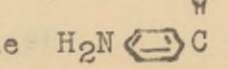
3. Folic acid in very crude form had been made several years ago from liver. No ideas regarding structure could be obtained. Doubtless none could be ascertained from such a crude mixture.

4. Auhagen brought up the question of the Vitamin D_2 story of Dimroth and his assistant. For the sake of the record, Dimroth and a junior author published a series of papers five years ago in the Berichte in which it was claimed that a synthesis of Vitamin D had been accomplished. It was subsequently learned by Dimroth that the student had falsified the reports. Consequently in Dec. 1943, he published a note in the Berichte repudiating the entire series of papers. Certain preparations provided by Dimroth were tested by Auhagen; natural Vitamins D_2 had been added to these so that positive results were obtained.

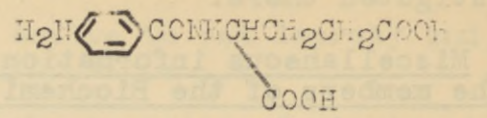
IX. Antivitamins and bacterial metabolism. (Dr. Auhagen).

Work has been done at Elberfeld on this subject with particular emphasis on sulfanilamide antagonists. Allcompounds were tested in vitro using the lactobacillus as the test organism.

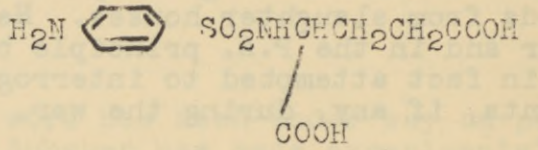
Auhagen finds that the following compounds inhibit sulfamilamide in the test.

1. p-Aminobenzoic Acid 
2. p.p. - Diaminobenzil 
3. p,p.-diaminobenzophenone 

4. N-(p-aminobenzoyl) - l - glutamic acid.

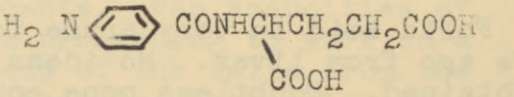


5. N-(p-aminobenzenesulfonyl) - d - glutamic acid.

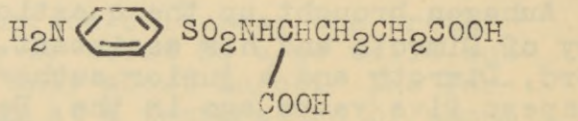


He finds that the following do not inhibit sulfamilamide under identical conditions:

1. N-(p-aminobenzoyl) - d-glutamic Acid.



2. N-(p-aminobenzenesulfonyl) - d - glutamic acid.



The order of activity of compounds exhibiting sulfanilamide activity is:

p-aminobenzoic acid	1.0
p,p' - diaminobenzil	0.2
p,p' - diaminobenzophenone	0.1

N - (p-aminobenzoyl) - l - glutamic acid 5.0

An interesting property of N-(p-aminobenzoyl) - l - glutamic acid is that while p - aminobenzoic acid in high concentrations has a slight toxicity towards the lactobacillus, the N-acyl-l-glutamic acid exhibits no demonstable toxicity.

It is also significant that in the series of N-acyl-glutamic acids, the stereo configuration of the glutamic acid portion as well as the character of the acyl group is of prime importance so far as the inhibition of the sulfanilamide action on the lactobacillus is concerned.

Acyl Group	d-glutamic	l-glutamic
H_2N-⟨⟩-CO-	No inhibition	Inhibition
H_2N-⟨⟩-SO_2	inhibition	in inhibition

Compounds have also prepared with the idea of finding inhibitors for manfanil. To date none have been found. Types of compounds tried include.

Domagk still does not think the p-aminobenzoic acid theory of Fildes is the explanation of the activity of the sulfa drugs. This theory states that sulfanilamide is active by virtue of its interference with the action of p-aminobenzoic acid as a vitamin or essential metabolite of the bacteria. Aughagen is in disagreement with Domagk on this point.

Very little work has been done on the metabolism of parent and sulfa-resistant strains of bacteria. The only results of importance learned were that the sulfa-resistant streptococcus produces thirty times more p-aminbenzoic acid as a metabolic product than the parent strain. No such result is observed with the genococcus.

No substances has been found by Auhagen which antagonizes Vitamin B_2. This indicates that he at least is behind such American workers as Woolley.

Pantoyltaurine has been found to be an anti-pantothenic acid. This is in agreement with Kuhn and Woolley.

An anti-p-aminobenzoic acid has been found.

This is p-aminoacetophenone.

No active anti-nicotinic acid has been found. It is interesting to note that while p-aminoacetphenone antagonizes p-aminobenzoic acid, p-acetopryidine is not an antagonist for nicotinic acid.

X. Dibromsalicyl and Analogs.

Following Prof. Kuhn's published reports on the salicyl derivatives work was started at Elberfeld along these lines.

Dibromsalicyl and tetrachlorsalicyl were prepared by Dr. Meitzsch and submitted to Domagk for testing. He was unable to confirm Kuhn's reported activity against tubercle bacilli in vitro tests.

Concerning this point a memorandum from Domagk to Hörlein dated June 23, 1944 is of interest: "5,5' - dibrom - 2,2' dioxybenzil obtained from Prof. Kuhn was tested by us under number Q 335 and Prof. Kuhn has already been informed that the preparation shows a good inhibitive action against staphylococcus in vitro. Dr. Kuhn's observation concerning the activity against tubercle bacilli was not confirmed; even in a concentration of 1:1000, in contrast to Eleudron (sulfathiazol), ét showed no activity against the tubercle bacillus. Prof. Kuhn was given a complete report of the technique of the test and results of the test on March 7, 1944. With Tbc infected animals (Type bovine as well as Type human) the substance "showed no activity, administered subcutaneously, orally or locally. For further staphylococcus tests new material was required from Prof. Kuhn, since all of the material had been used in the Tbc test. On June2, 1944 we were supplied with 5,5'-dibromsalicyl by our Dr. Linsert. This showed, when used as a suspension on agar plates, total inhibition against staphylococcus in concentrations of 1:50,000. Eleudron showed Under identical conditions, inhibition up to 1:250,000 (Test of June 21), with weaker strains the inhibition may be demonstrated up to 1:100,000. An aqueous solution prepared by Dr. Linsert, (called by him LST 1125 and by us R314) showed in a test on June 21, complete inhibition of staphylococcus up to 1:30,000, The inhibitive activity of 5,5' - dibrom - 2.2' - dioxybenzil against staphylococcus in plate tests is exceeded by the following sulfonamides: Sulfathiazol (Eleudron); Globucid (sulfaethylthiadiazol) is equally effective.

Domagk"

The March 7, 1944 memorandum from Domagk to Kuhn is as follows:

		1/1000	1/5000	1/10,000	1/25,000	1/50,000	1/100.000
	a						
Q335 (Kuhn)	b	~~+++~~	~~+++~~	~~+++~~	~~+++~~	~~+++~~	~~+++~~
	a	~~+++~~	~~+++~~	~~+++~~	~~+++~~	~~+++~~	~~+++~~
Q336 (Kuhn)	b	~~+++~~	~~+++~~	~~+++~~	~~+++~~	~~+++~~	~~+++~~
	a	0	0	0	0	0	0
Sulfathiazol	b	0	0	0	0	0	0
Q 372		~~+++~~					

The control shows a ~~+++~~ growth
Q 335 is 5,5'-dibrom - 2,2' - dioxybenzil.
Q 336 is 3,5 - diiodosalicylic acid.
(a) The culture on egg substrate plus magnesium sulfate.
(b) The culture on egg substrate minus magnesium sulfate.
Q 372 is tetrabromistizin using Tubercle bacillus, (typus humanus).

The following protocol was written by Dr. Brömmelhüs on May 29, 1945.

"Bacteriostatic and fungicidal activity.

1. R318 = pure dibromsalycylic acid.
2. R 85 = dibromsalicylic acid containing some tetrabrom.
3. S122 = Tetrachlorsalicylic acid.

The investigation was carried out on

(a) Pathogenic Molds.
   1. Sporotrichon sp. (Prof. Grütz, Bonn)
   2. Aspergillus Fischeri, Wehmer
   3. Epidermophyton, Kaufmann - Wolf.
   4. Trichophyton disecides.
   5. Actinomyces buccalis or
   6. Actinomyces innominatus.

(The culture medium consisted of 2% biomalt agar and these were tested in Petri dishes)

(b)
   7. Staph. haemolyticus aureus, in Bouillon
   8. Strept. haemolyticus Waaker, in Serum bouillon.
   9. Pneum. Typ. I in Serum bouillon
   10. B. dysenteriae, Shiga Kruse (Rob. Koch) in the modification substrate from Koser.

The results of the tests are given below. The tables give the number of the preparation and the lowest concentration in which no growth results.

	R 318	R 85	S 122
1. Sporotrichon	1:50,000	1:10,000	1:10,000
2. Aspergillus	1:3000	1:1000	1:1000
3. Epidermophyton	1:5000	1:2000	1:5000
4. Trichophyton	1:20,000	1:20,000	1:5000

5.))Act. 6.)	buccalis or innominatus	1:30,000	1:20,000	--
7.	Staphylococcus	1:100,000	1:100,000	1:100,000
8.	Streptococcus	1:10,000	--	1:10,000
9.	Pneumococcus	1:100,000	1:100,000	--
10.	B. dys., Shiga Kruse	1:1000	1:1000	1:1000

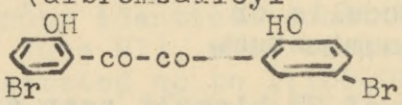
The pronounced fungicidal and bacteriocidal activity is shown by thepreparations in the greatest dilution only if the preparation completely dissolves.

The good results in vitro are not borne out in in vivo tests. In this case the preparations are inactive.

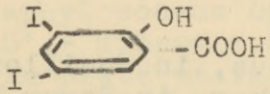
Mietsch has prepared tetrabromistizin. This is an old compound. In concentrations of 1:1000 it has no activity against tuberculosis as shown above, and also shows no in vivo activity.

For comparative purpose the compounds mentioned in the various memos and protocols above are given below.

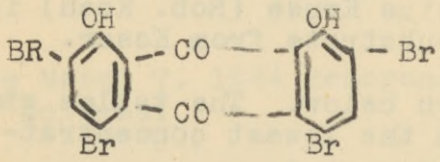
Q335-5,5' dibrom-2,2' dioxybenzil (dibromsalicyl



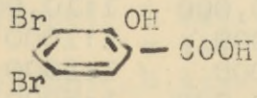
Q336 3,5 diiodosalicylic acid



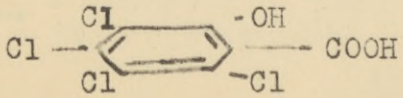
Q372 Tetrabromistizin



R318 Dibromsalicylic acid



S122 Tetrachlorsalicylic Acid



XI Ectromelia

Some preliminary work done several years ago was dropped because the infection once introduced into a laboratory is likely to kill all mice. Kikuth considers ectromelia too much of a nuisance with which to work.

XII Cancer Research (Dr. Hackmann)

One of Dr. Hackmann's chief interests is the study of neoplasms in animals and a search for an agent which will have a beneficial effect in this disease. For his study he uses Maud Slye spontaneous tumors in mice, Walker carcinoma in rats, Jensen sarcoma in rats, Brown-Pierce carcinoma in rabbits and Erlich implanted carcinoma in mice. Hackmann has no rational plan for this study. Every new compound made by the chemical department is divided into several parts. Some is sent to Domagk for bacterial studies, some to Kikuth for tests on protozoal infections and virus and rickettsial diseases and some to Hackmann for study in cancer. The compounds are tested blindly in routine fashion. They are either injected subcutaneously or given per os in repeated doeses of from 1/10 to 1.30 of the lethal dose. Oral doses are given daily and subcutaneous doses semi-weekly for periods ranging from 2 weeks to 1 month. Transplanted tumors are used to screen the compounds. Any that look promising are then tried on spontaneous tumors. The technique for the former is as follows: A tumor is removed and ground up, mixed with a little saline and injected into a healthy animal. For spontaneous tumors, young mice of cancer heredity having tumors of 1 or 2 mm. diameter are chosen. At the end of the treatment, tumors are measured again, removed, weighed, and compared with controls -- not litter mates but from the same strain. He has worked with a large number of compounds, none of which appeared very promising. Included are the organic silver compounds and organic and inorganic fluorides. This work was abandoned years ago as hopeless. The most promising preparation which Hackmann has found so far is A 1888/3 prepared by Dr. Auhagen. It is dl-alanylglycyl-glycine.

The following are typical of the results obtained with transplanted tumors.

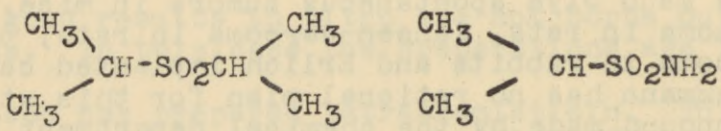
In 20 treated animals, the average weight of the tumor after treatment was 4.2 g. In 20 untreated controls it weig ed 7.9 g.

In another experiment with the same compound, the tumors of 25 treated animals averaged 11.0 g. and the control tumors averaged 12.4 g.

In a third experiment, the average weight of the tumors in treated animals was 7.5 g. and the untreated controls averaged 12.0 g.

This preparation was placed on clinical test on the basis of such results as these. To date the only report is that the preparation is well tolerated.

Another series of compounds were thought at one time to show promise. These were made by Dr. Pöhls but since have been shown to be entirely inactive. These were the aliphatic sulfones and sulfonamides.



Hackmann also studied cancer immunity by injecting rabbits with suspensions of cancer cells and tumor extracts. He says that some immunity is produced. He has also studied the carcinogenic properties of diethyl stilboestrol, and has found nothing which antagonizes the carcinogenic action of such compounds. Tissue cultures were not used in this investigation as Hackmann feels that his method of study has no relation to human cancer.

All in all, the approach to the problem at Elberfeld is entirely hit or miss, illogical, and unlikely to produce results in the near future.

APPENDIX 1

D. Nr. .....

.....

i. v. .....

s. c. .....

per os .....

.....

Streptoc. .....

Staphyloc. .....

Pneumoc. Typ I .....

Typ II .....

Typ III .....

Typ X .....

Coli .....

Paratyphus .....

Rotlauf .....

.....

.....

.....

Carcinom. .....

.....

.....

.....

.....

37525-I

Nr. ..... des untersuchenden Laboratoriums:

Dr. Kikuth
Chemo.-Therap. Lab.

Nr. ..... der Abteilung: .....

Am ..... 19 ..... erhalten mit der Signatur: .....

Untersuchungsbefund: .....

.....

Maus-Gift: .....

Tryp. congolense: .....

Tryp. brucei: .....

Spirillen: .....

Recurrens-Spirochäten: .....

Mäuse-Herpes: .....

Kaninchen-Gift und -Lues: .....

Vogel-Gift: .....

Vogel-Malaria: .....

Malaria-Prophylaxe: .....

Haemoproteus: .....

Kanarien-Virus: .....

Mäuse-Oxyuren: .....

Mäuse-Bandwurm: .....

Katzen-Gift: .....

Katzen-Leberegel: .....

B. w.

37525 - I

Herrn Dr. ..... W-Nr.: .....

Pharmakolog. Untersuchung Ihres Präparates Nr.: .....

Giftigkeit:	Maus: intrav. Ratte: subcut. Kanin.: per os
Blutdruck:	Kanin.: Katze:
Herz:	Frosch: Katze:
Atmung:	Kanin.: Katze:
Darm:	Kanin.: Katze:
Uterus:	Kanin.: i. situ. Meerschw.: isol.
Antipyrese:	Norm. Katze: Fiebernd. Kanin.:
Analgesie:	
Sonstiges:	

Ergebnis:

Untersucher: ..... W.-Elberfeld, den .....

37525-1

APPENDIX 2

Zur Inhalationstherapie der experimentellen Bronchopneumonie mit Sulfathioharnstoff (Badional).

von
Walter Kikuth u. Marianne Bock

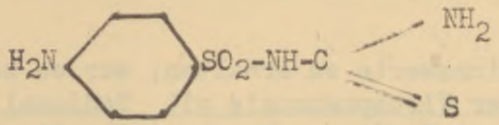
Aus dem Chemotherapeutischen Laboratorium der J.G.,
Werk Wuppertal-Elberfeld.
(Leiter: Prof.Dr. Walter Kikuth)

Vor kurzem konnte Bock zeigen, dass die von Gönnert beschriebene Virusbronchopneumonie der Maus und die durch das Lymphogranuloma inguinale-Virus experimentell hervorgerufene spezifische Pneumonie, deren Erreger nahe miteinander verwandt sind und zu der Gruppe der grossen Virusarten gehören, durch Inhalation von sulfonamidhaltigen Substanzen chemotherapeutisch zu heilen sind, wobei das Debenal (Sulfapyrimidin) und seine Derivate sich als besonders wirksam erwiesen. An Hand zahlreicher Inhalationsund Injektionsversuche und mit Hilfe zu verschiedenen Zeiten vorgenommener Debenalbestimmungen im Blut und Lungengewebe konnte Bock den Nachweis erbringen, dass die Inhalations-behandlung der parenteralen überlegen ist; sie fand, dass die aufgenommenen Substanzmengen dabei geringer sind als diejenigen, die auf peroralem oder parenteralem Wege zum gleichen Ziele, nämlich zur Ausheilung der Infektion, führen. Das war um so bemerkenswerter, als die Blutkonzentrationen der Medikamente nach Inhalation hinter denen nach parenteraler Verabreichung zurückblieben. Durch die Inhalation gelingt es offenbar, eine lokale Anreicherung des Debenal im Lungengewebe zu bewirken und den Krankheitsprozess auf diese Weise nachhaltiger zu beeinflussen.

So interessant diese experimentellen Ergebnisse auch sind, so lassen sie für die Praxis nur vorsichtige Rückschlüsse zu. Da die Sulfonamide im allgemeinen schlecht wasserlöslich sind und insbesonders das Debenal sich nur durch Zusatz von Natronlauge in genügender Menge löst, ist der Einwand nicht von der Hand zu weisen, dass solche alkalischen Lösungen, namentlich wenn sie in grossen Mengen und wiederholt inhaliert werden, Schädigungen im gesunden und krankhaft veränderten Lungengewebe erzeugen und somit dem Heilungsprozess in nachteiliger Weise beeinflussen könnten.

Wir haben uns deshalb nach einer anderen Substanz umgesehen, die diesen Nachteil nicht besitzt und von der es möglich ist, neutrale wässrige Lösungen in höherer Konzentration herzustellen. Unsere Wahl fiel auf den Sulfathioharnstoff, der unter dem Namen Badional z.Zt. klinisch geprüft wird und bei verschiedenen bakteriellen Infektionen, insbesonders bei Lungenentzündung, sich

therapeutisch als gut wirksam erweist und sich gleichzeitig durch eine besonders gute Verträglichkeit auszeichnet. Das Badional ist ein Sulfanilylthiocarbamid (Sulfathiocarbamid) und hat folgende chemische Konstitution:



Es ist ein weisses Pulver. Sein Molekulargewicht beträgt 231.29, sein Schmelzpunkt 174-175°C. Besonders hervorzuheben ist die gute Löslichkeit in Wasser mit neutraler Reaktion (pH 6,8-7,2). Sie betragt bei 20°C 547 mg/Ltr. Die Salze mit Alkalien in organischen Aminen sind besonders gut löslich und zeigen praktisch neutrale Reaktion. In den zur Verfügung gestellten Ampullen liegt das Badional-Diäthanolaminsalz vor. 1 ccm von dieser Lösung entspricht 0,5 g Badional.

Pharmakologisch wird die Substanz von Gewebe und Organismus auszerordentlich gut vertragen. Nach bisher unveröffentlichen Feststellungen von Hecht liegen die akut toxischen Dosen von Badional in derselben Grössenordnung wie die der bestverträglichen Sulfonamidpräparate, z.B. vertragen Kaninchen oral 2 g/kg ohne Störung im Befinden. Bemerkenswert ist aber die besonders gute Verträglichkeit bei intrvenöser Injektion; auch bei dieser Verabreichung zeigen Kaninchen nach einer Gabe von 2 g/kg, innerhalb von 2 Min. in Form der 50%igen Lösung injiziert, keinerlei Symptome. Diese Lösung führt bei intramuskulärer Injektion nicht zu lokalen Schädigungen. Badional wird bei innerlicher Verabreichung beim Menschen rasch resorbiert. Bei Tagesdosen von 6 g werden Blutkonzentrationen von etwa 5 mg % erreicht. Dabei befindet sich die grössere Menge im Plasma und ganz überwiegend in freier Form. Die Ausscheidung mit dem Harn beginnt ebenfalls sehr bald nach der Zufuhr, such im Harn liegt die grössere Menge des Präparates in freier Form vor (2/3-4/5).

Als Modellversuch benutzten wir diesmal nur die von. Gönnert beschriebene Bronchopneumonie der Maus. Wir halten Virusinfektion für unseren Zweck für besonders geeignet, weil es sich bei ihr um eine vorwiegend lokale, spezifische Pneumonie handelt, ohne dass die anderen Organe in Mitleidenschaft gezogen werden. Deshalb ist sie auch einer experimentellen Pneumokokkeninfektion gegenüber als geeigneter anzusehen, bei der es zu einer akuten, tödlich verlaufenden Septikaemie kommt, die ohne makroskopisch wahrnehmbare lokale Erscheinungen abläuft.

Auf die Technik der Versuchsanordnung braucht hier nur ganz kurz eingegangen zu werden, da sie bereits von Bock ausführlich beschrieben wurde. Stark virushaltige Mäuselungen werden mit physiologischer Kochsalzlösung verrieben und von dieser Aufschwemmung, deren Konzentration je nach dem Virusgehalt der Lunge gewahlt

wird und die im allgemeinen 1:5 000 beträgt, erhalten die Tiere in Athernarkose 0,05 ccm intranasal instilliert. 2-3 Tage später erkranken die Mäuse an der spezifischen Bronchopneumonie, der sie gewöhnlich am 4.-5. Tage erliegen. Als Stichtag zur Beurteilung des therapeutischen Effekts haben wir den 10. Tag nach der Infektion herangezogen.

Um entsprechende Vergleichswerte zu erhalten, war es zunächst notwendig, die Therapie dieser Viruspneumonie mit Badional auf subcutanem Wege durchzuführen. Zu diesem Zwack wurden die erkrankten Mäuse an 5 aufeinanderfolgenden Tagen 1 x täglich mit Badional behandelt. Die erste Verabreichung fand 3 Stunden nach der Infektion statt, die 4 anderen folgten im Abstand von je 24 Stunden. Für jede Dosis wurden 8 Tiere eingesetzt. Im Vergleich zu einer Reihe früher von uns geprüfter Sulfonamide erwies sich der Sulfathioharnstoff subcutan gegeben als wenig wirksam, denn selbst eben noch verträgliche Dozen von 1/25g/20g Maus liessen nur eine im allgemeinen geringe Beeinflussung des Infektionsverlaufes erkennen, und die Hälfte dieser Dosis, nämlich 1/50g/20g Maus, war fast vollkommen wirkungslos.

Im Gegensatz hierzu gelang es, durch Inhalation leicht Heilungen zu erzielen. Es wurden Dosen angewandt von 10 mg, 5 mg und 1 mg/ltr., welche in einem 65 Liter fassenden Emaillekessel versprüht wurden. Die Versprühung geschah, wie auch in früheren Versuchen, mittels Druckluft aus einer Flury-Pistole, auf welche Weise eine besonders feine Vernebelung erzielt werden konnte. Die Substanzkonzentration wurde stets so gewählt, dass die angewandten Dosen pro Liter Raum in 0,1 ccm untergebracht waren. So gelangten also in dem 65 Literkessel 6,5 ccm zur Versprühung. Die Tiere wurden 30 Min. im vernebelten Milieu belassen. Wie aus der Tabelle hervorgeht, war eine einmalige Inhalation selbst in der grossen Dosis von 10 mg/ltr. wirkungslos. Dagegen gelang es mit 3x5 mg/ltr. und 3x10 mg/ltr. deutliche Einwirkungen und mit 5x10 mg/ltr. sichere Heilungen zu erzielen.

Tabelle

Substanzmenge pro 20 g Maus	Inhalation	subcutane Injektion
1/1500	1x1mg/ltr./30 min.Ø	
1/1500	3x1mg/ltr./30 min.Ø	
1/1500	5x1mg/ltr./30 min.Sp.W.-Ø	
1/300	1x5mg/ltr./30 min.Ø	
1/300	3x5mg/ltr./30 min.Sp.W.-W.H.	
1/300	5x5mg/ltr./30 min.Sp.W.	

1/150	1x10mg/ltr./30 min.	Ø
1/150	3x10mg/ltr./30 min.	Sp,W.
1/150	5/10mg/ltr./30 min.	W.H.
1/100		5xØ
1/50		5xØ-Sp.W.
1/25		5xSp.W.-W.H.
1/10		1xtot

Ø-ohne Wirkung
Sp.W.--Spur Wirkung
W.H.-Wirkung Heilung

Um eine Erklärung für das gute Ergebnis der Inhalationsbehandlung zu finden, erschien es uns notwendig, zuerst einmal zu berechnen, wieviel von der versprühten Substanz durch Einatmen in den Organismus der Mäuse gelangt, und diese Menge mit der nach Injektion aufgenommenen zu vergleichen. Diesen Wert einigermassen genau zu bestimmen, ist schwer, da sich bei der Berechnung eine Reihe von Fehlerquellen nicht ganze ausschliessen lassen. Es ist selbstverständlich, dass der Substanzverbrauch in der Luft von der Anzahl der Versuchstiere abhängig ist. Wir haben deshalb immer die gleiche Zahl von Mäusen, nämlich 14, eingesetzt. Ferner ist es nicht gleichgültig, ob die Tiere sich während des Versuches in Ruhe oder Bewegung befinden, da anzunehmen ist, dass bei lebhafter körperlicher Betätigung auch grössere Substanzmengen aufgenommen werden. In der Regel laufen die Tiere in den ersten 5 bis 10 Minuten ziemlich lebhaft umher, beruhigen sich aber allmählich und kauern dann meistens in einer Ecke des Käfigs zusammen. Hierbei wird es vorkommen, dass die unten sitzenden Tiere eine Luft einatmen, die entweder stärker oder schwächer mit der Substanz gesättigt ist als im übrigen Raum. Auch ist nicht zu verhindern, dass die Tiere durch Ablecken der auf dem Fell niedergeschlagenen Substanz zusätzlich noch eine geringe Menge aufnimmt. Zu ähnlichen Schlussfolgerungen sind auch Harris, Sommer und Shapple bei der Berschnung ihrer Ergebnisse gekommen. Wir glauben, im allgemeinen doch zu recht brauchbaren Resultaten gelangt zu sein.

Die bei der Inhalation aufgenommene Substanzmenge haben wir auch diesmal in folgender Weise aus dem Atemvolumen der Mäuse während der Versuchszeit und aus der Menge des versprühten Präparates errechnet. Unter Zugrundelegung des von Blume für die weisse Maus angegebenen durchschnittlichen Atemvolumens von 0.3 ccm setzten wir als Atemfrequenz den Wert von 150/min. ein, was einem Volumen von 45 ccm/min. und von 1/35 ltr./30min. entspricht. Bei einer versprühten Substanzmenge von 1 mg/ltr. und bei gleichbleibender Konzentration während der Versuchszeit

würden also etwa. 1,35 mg von dem Präparat aufgenommen werden. Da aber die in den Käfig eingebrachte Konzentration mit vorrückender Zeit abnimmt, weil sich die Substanz an den Wänden und am Boden des Gefässes sowie am Fell der Tiere absetzt und ausserden mit der Atemluft verbraucht wird, haben wir angenommen, dasse die von den Tieren eingeatmetet Substanzmenge etwa nur die Hälfte, nämlich 0,65 mg, entspricht. Nach Umrechnung kamen wir deshalb zu folgenden Werten:

Eine Inhalation von 1 mg/ltr./30 min. entspricht einer subcutanen Injektion von etwa 1/1500 g/20 g Maus, eine Inhalation von 5 mg/ltr/30 min. einer von 1/300 g/20 g Maus und eine von 10 mg/ltr/30 min. der einer von 1/150 g/20 g Maus. Auf der Tabelle lassen diese errechneten Werte, verglichen mit den Injektionsdosen, erkennen, dass die therapeutischen Dosen nach Inhalation sehr viel niedriger liegen als die nach subcutaner Verabreichung. Selbet wenn wir auf Grund nicht genügender Berücksichtigung der Fehlerquellen zu falschen, in diesen Falle viel zu niedrigen Werten gekommen wären, so wäre sogar bei einer Verdoppelung der errechneten Werte ein offensichtlicher Verteil der Inhalationsbehandlung herauszulesen.

Zu ähnlichen Feststellungen gelangten auch Mooser und Leemann (1943). Wenn zwar wegen der verschiedenen Methodik die Ergebnisse ihrer Versuche sich nicht direkt mit den unsrigen vergleichen lassen, so sind doch die Schlussfolgerungen dieselben, dass nämlich bei Inhalationsbehandlung der Bronchopneumonie der Maus durch direkte Einwirkung der Präparate auf das im Lungengewebe angesiedelte Virus bessere Resultate zu erzielen sind als auf dem Umwege über die Bluthahn nach peroraler bzw. parenteraler Verabreichung.

Eine Erklärung für diese Überlegenheit der Inhalationsbehandlung lässt sich nur geben, wenn wir annehmen, dass es in den Mäuselungen nach Einstmung des Sulfathioharnstoffs zu einer Anreicherung des Medikaments im erkrankten Lungengewebe und somit zu einer lokalen Einwirkung auf den Erkrankungsherd kommt. Um diese Annahne zu erhärten, führten wir eine Reihe von Badionalbestimmungen im Blut und Lungengewebe der Mäuse durch.

Die Bestimmungen wurden nach der Methode von Druey und Oesterheld angesetzt und die Resultate am Pulfrich'schen Stufenphotometer abgelesen. Für die Ermittlung eines jeden einzelnen Wertes wurde das Blut bzw. der Lungenbrei von 3-4 Mäusen benutzt. Da es bei der Substanzbestimmung im Gewebe auch bei völliger Entblutung der Tiere praktisch nicht möglich ist, die Organe blutfrei zu erhalten, haben wir die jeweils vorhandene Hämoglobin- bzw. Blutmenge festgestellt und diesen Wert in Anrechnung gebracht. Wie aus der Kurve 1 hervergeht, erreichen die Blutwerte nach subcutaner Injektion bereits nach 1/2 Stunde

ihren Höhepunkt, um dann im Verlauf von etwa 1 Stunde bis auf einen Rest stark abzufallen, der anschliessend sehr langsam völlig abgebaut bzw. ausgeschieden wird. Offenbar wird bei hoher Blutkonzentration ein Teil des Badional von dem Gewebe der Organe aufgenommen, der dann langsam wieder in den Kreislauf abgegeben wird und so den flacheren Abfall der Kurve bedingt.

Wird Sulfathioharnstoff dagegen von den Mäusen inhaliert, so ist der Verlauf der Blutkonzentrationskurve zu Beginn ein völlig anderer (Kurve 2). Hier erreicht die Badionalkonzentration ihr Maximum 1 Stunde nach Ende der Inhalation, also 1 1/2 Stunden nach Beginn der Substanzaufnahme. Die absoluten Werte sind hier bei annähernd gleichen Mengen der sube. aufgenommenen und der inhalierten Substanz wasentlich niedriger. Ähnlich wie nach subcutaner Injektion sinkt die Blutkonzentration in den nächsten 5 Stunden weiter ab. Sie hält sich dann aber längere Zeit auf einer gewissen Höhe, so dass sich noch nach 24 Stunden ein Rest, der aber verhältnismässig gering ist, nachweisen lässt. Gleichzeitig vorgenommene Badionalbestimmungen im Lungengewebe ergaben, dass hier die Werte zu Beginn sehr viel höher liegen als die im Blut - in der Regel etwa 2 mal so hoch - um dann abzusinken und sich nach 1 Stunde ungefähr in gleicher Höhe mit diesen zu befinden. Beide Kurven laufen dann parallel zueinander, wobei die Lungengewebswerte ständig ein wenig höher als die Blutwerte liegen.

Wir ziehen aus diesen Ergebnissen den Schluss, dass das Badional vom Lungengewebe bei der Inhalation zunächst in grossen Mengen aufgenommen und im Laufe der folgenden Stunden ständig ins Blut abgegeben wird. Wichtig erscheint uns, <u>dass die Konzentrationen in der Lunge stets höher sind als die im Blut</u>. Es ist wahrscheinlich, dass das Lungengewebe im besonderen Masse befähigt ist, das Badional aufzunehman und im gewissen Sinne zu speichern. Wir gehen hier wohl nicht fehl, wenn wir glauben, dass die von uns erzielten guten Heilwirkungen auf eine direkte, lokale Beeinflussung des Krankheitsprozesses zürückzuführen sind, zumal der Erreger im vorliegenden Falle sich im Lungengewebe ansiedelt und vermehrt.

Nach subcutaner Injektion von Badional bei der Maus lag, wie nicht anders zu erwarten war, die Blutkonzentration wesentlich höher als diejenige im Lungengewebe. Bei gleichzeitig durchgeführten Bestimmungen in Leber, Milz und Gehirn zeigte sich, dass hier relativ wenig Substanz vorhanden war und dass die Konznetrationen noch unter denen in der Lunge blieben. Es hat also offenbar das Lungengewebe überhaupt die Fähigkeit in erhöhtem Masse Badional aufzunehmen.

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Die von Bock bei der Inhalationsbehandlung mit Debenal erhobenen Befunde konnten somit nicht nur bestätigt, sondern in gewisser Weise sogar noch übertroffen werden. Bei einer parenteralen Applikation bewirkt der Sulfathioharnstoff zwar eine vorübergehende höhere Konzentration im Blut, die anscheinend jedoch nicht ausreicht, auch im erkrankten Gewebe eine für die Beeinflussung des Krankheitsprozesses notwendige hohe Gewebskonzentration zu erzielen. Wird dagegen das Badional in genügender Menge mit der Atemluft in die Lunge gebracht, so kommt es, wie wir gezeigt haben, zu einer gewissen Anreicherung in der Lunge, wodurch die Lungekonzentration eine wesentlich grössere ist als die Blutkonzentration. Es ist sogar anzunehmen, dass diesses Verhältnis für die Lungenkonzentration vorübergehend noch wesentlich günstiger liegst. Wie oben beschrieben, wurden unsere therapeutischen Inhalationsversuche stets für die Dauer von 1/2 Stunde durchgeführt und dementsprechend auch die Versprühungen, die den Substanzbestimmungen im Blut und Gewebe vorausgingen, eingerichtet. Sicherlich ist aber die Substanzkonzentration im Käfig direkt nach der Vernebelung wesentlich höher als nach der Versuchszeit von 1/2 Stunde, in der ein Teil des Therapeuticums mit der Atem-luft verbraucht wurde oder nicht niedergeschlagen hat. Man müsste daher auch erwarten, dass die Substanzkonzentration in den Lungen dann schon ihrer Höhepunkt überschritten hat, weil bereits ein Teil der Substanz an das Blut abgegeben wurde. Eine Bestätigung dieser Annahme erbrachten Versuche, in denen Mäuse nur 10 oder 15 Minuten in dem vernebelten Käfig belassen und sofort nach Ablauf dieser Zeit getötet wurden, Bei den folgenden Substanzbestimmungen übertraf die Lungenkonzentration die Blutkonzentration etwa um das 3 fache und lag höher als in den Versuchen, in denen die Inhalation auf 30 Minuten ausgedehnt war.

Makroskopische Veränderungen im Lungengewebe der Mäuse, die auf Badionalinhalation zurückgeführt werden könnten, wurden von uns in keinem Fall beobachtet. obwohl wir eine gross Anzahl von Tieren sezierten, die entweder 3 mal in kürzeren Abständen oder je 1 mal an 5 aufeinanderfolgenden Tagen im Versuch waren und zu verschiedenen Zeiten bis zu 24 Stunden danach abgetötet wurden.

Diese experimentellen Ergebnisse lassen die Schlussfolgerung zu, dass die Inhalationsbehandlung such bei Pneumonien des Menschen Aussicht auf Erfolg hat. Da beim kranken Menschen die Verhältnisse ähnlich liegen wie in unserem Testversuch, so wäre auch hier bei der Inhalation mit der gleichen Substanzmenge eine etwas intensivere Behandlung möglich als bei der sonst üblichen Therapie bzw. würde eine Reduzierung der Dosen ebenfalls zum gleichen Erfolg führen, was den Vorteil hätte, einer etwaigen toxischen Schädigung durch das Medikament wirkungsvoll ant-

gegenzutren. Hierfür scheint uns das Badional auf Grund seiner Löslichkeit, seiner guten Verträglichkeit und seiner bakteriziden Wirkung insbesondere gegen Pneumococcen hervorragend geeignet zu sein, (nach den klinischen Erfahrungen besitzt Badional, sofern es in massive Dosierung intravenös angewandt wird, gegenüber der Pneumonie zumindest den gleichen Effekt wie Eleudron) um so mehr, als Inhalationsversuche mit Sulfonamiden von anderer Seite bereits zu guten Ergebnissen geführt haben (Bosse,Franke,Bass,Castex u. Mitarbeiter).

Zusammenfassung:

Badional (Sulfathioharnstoff) ist eine gut verträgliche Substanz, die sich bei verschiedenen Infektionen des Menschen, vor allem bei der Pneumonie, als gut wirksam erwies. In unserem Modellversuch mit dem Virus der Bronchopneumonie der weissen Maus, das vorwiegend eine spezifische Pneumonie verursacht, erwiess sich die Inhalationstherapie mit Badional der subcutanen Verabreichung überlegen, da es, wie wir nachweisen konnten, zu einer vorübergehenden Substanzanreicherung im Lungengewebe und somit zu einer intensiven Einwirkung der Substanz auf den Erkrankungsherd kommt. Es erscheint uns daher aussichtsreich, auch bei an einer Pneumonie erkrankten Menschen die Inhalationsbehandlung anzuwenden.

Schrifttum

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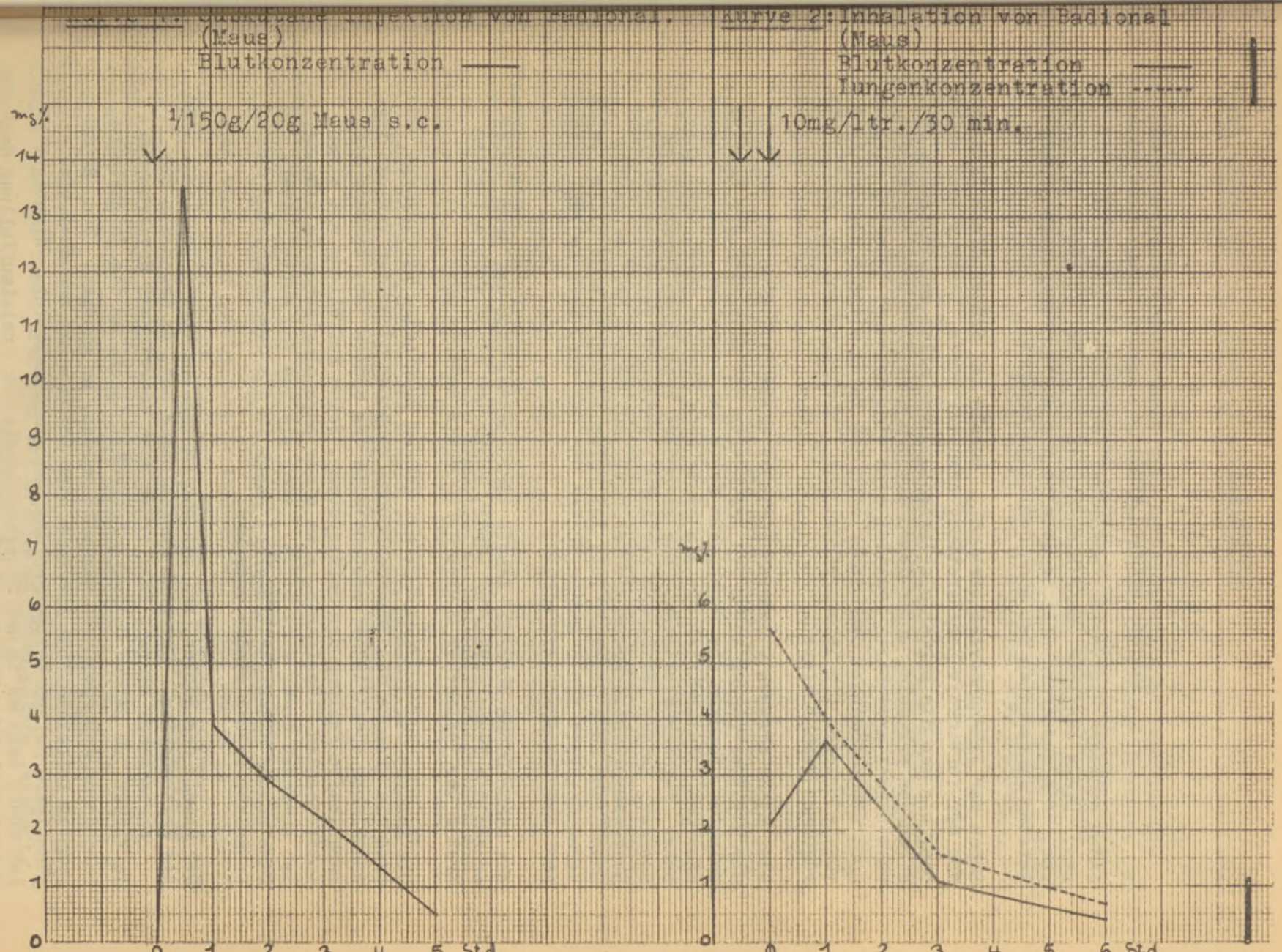
Weitere Literaturangaben in der Arbeit von Bock


Kurve 1: Subkutane Injektion von Barbital.
(Maus)
Blutkonzentration
mg%
1/150g/20g Maus s.c.
0 1 2 3 4 5 Std.
Kurve 2: Inhalation von Radional
(Maus)
Blutkonzentration
Lungenkonzentration
10mg/1 tr./30 min.
mg%
0 1 2 3 4 5 6 Std.

APPENDIX 3

P E R I S T O N .

Von Dr. Gerhard Hecht.

Jeder grossere Blutverlust erfordert gebieterisch Ersatz des Verlorenen. Diese einfache und einleuchtende Tatsache ist heute Allgemeingut. Die ausserordentliche Zunahme der Blutübertragungen in den letzten Jahrzehnten ist auch dem Laien bekannt.

Schon vor Jahrhunderten versucht, musste man früher diese Massnahme, von der instinktiv Lebensrettung erwartet wurde, immer wieder aufgeben, bis man die bakteriologischen und ver allem serologischen Bedingungen erkannte, unter denen übertragenes Blut beim Empfänger kein Unheil anrichtet. Restlos sind sie, nebenbei bemerkt, offenbar noch nicht bekannt, denn immer noch ist keine sichere Ausschaltung von leichteren bis lebensgefährlichen Nebenwirkungen bei Blutübertragungen gewährleistet.

Aber nicht diesses - dauernd geringer werdende-Risiko ist es, was die Blutübertragung unter den besonderen Verhaltnissen des Krieges belastet, sondern die technische Seite der Operation. Im friedensmässigen Klinikbetrieb ist sie heute kein Problem mehr, wohl aber unter Feldverhältnissen. Die erfordert ärztliches Personal und Spender, beides ist gerade bei Anfall grösserer Verwundetenzahlen überbeansprucht. Konserviertes Blut, auf dessen Haltbarmachung unendliche Mühe verwandt ist, ist keine durchgreifende Hilfe, seine Verwendbarkeit zählt wenige Wochen und das nur, wenn as kühl gelagert und transportiert wird.

Wer diese Dinge übersah, konnte voraussehen, dass der neue Krieg erneut die Fruge aufwerfen würde, komat man nicht auch mit einem Ersatz des verlorenen Blutes durch etwas anderes aus?

Die Analyse der Vorgänge bei der akuten Verblutung hat ergeben, dass die Urssche des Zusammenbruchs zunächst nicht der Verlust irgendwelcher spezifischer Bestandteile das Blutes ist, sondern die mangelhafte Fullung des Gefässystems. Seine Auffüllung mit einer indifferenten Flüssigkeit, etwa einer physiologischen Salzlösung, ist zunächst immer eine Hilfe. Aber sie ist von kurzer Dauer. Eine solche Flüssigkeit sickert rasch in die Gewebe ab, und der Kreislauf bricht wieder zusammen.

Der Anteil des Blutes, auf den man am ehesten verzichten kann, sind die Erythrozyten. Der akut Ausgeblutete hat, im Gegensatz zum chronischen Bluter, fast immer noch ausreichend Erythrozyten im Vorrat, um seinen O_2-Transportbedürfnissen. an die ja garkeine besonderen Anforderungen gestellt werden, zu genügen. Von Erythrozyten befreites Blut - Plasma oder Serum - hat nun aber viel günstigere Haltbarkeitseigenschaften als Gesamtblut. Wichtiger ist aber, dass es in der Dauerhaftigkeit der Kreislauffullung dem Vollblut gleichkomnt und nicht, wie die erwähnten Salzlösungen,

rasch wieder aus der Blutbahn schwindet.

Starling lehrte, dass das Verweilen des Lösungswassers, der Bestand an Flüssigkeit in der Blutbahn eine kolloidosmotische Funktion des Plasmaeiweiss ist. Sicherlich ist das nicht die einzige Funktion der 6-8 % Eiweiss, die in Blutwasser gelöst sind, aber im Rahmen derBetrachtung des akuten Blutverlustes steht sie an erster Stelle. Es muss nicht nur die verlorene Flüssigkeit ersetzt werden, sondern auch die Vorbedingung für ihr Verbleiben in der Blutbahn mit erfüllt werden. Und das gelingt mit der Transfusion von Blutplasma oder Serum.

Die Literatur der angelsächsischen Länder liess bereits vor 1939 erkennen, dass dort dieses Problem in lebhafter Entwicklung war. Bei uns aber fehlte damals eine ausreichende Vorarbeit auf diesem Gebiete. Inzwischen ist durch die Initiative von Lang auch beim deutschen Heer die Bereitstellung konservierten Serums in grossem Masstabe in die Wege geleitet worden. Im Anfang des jetzigen Krieges aber war noch nicht zu übersehen, wieweit diese Möglichkeit den praktischen Erfordernissen gerecht werden könnte. Dieser Stand der Dinge erforderte gebieterisch, auch jeden anderen denkbaren Weg zur Schaffung eines durchgreifend wirksamen Blutersatzes einzuschlagen. Das veranlasste Weese nach einem Kolloid Umschau zu halten, das geeignet sein sollte, in einer physiologischen Salzlösung die wasserbindende Fuktion der Plasmaeiweisskörper zu übernehmen, um dieser einen dauerhaften therapeutischen Effekt zu verleihen. Das damit eine Aussicht auf Erfolg bestand, war nicht mehr zweifelhaft, nachdem im vorigen Kriege Bayliss, vor dem gleichen Problem stehend, im Gummi arabicum ein Kolloid gefunden hatte, das den erwarteten Effekt durchaus besass. Das Problem bestand also jetzt darin, ein Kolloid zu finden, das in Deutschland unter Kriegsverhältnissen in ausreichender Menge und Beschaffenheit zur Verfügung gestellt werden konnte. Das Gummi arabicum musste als subtropischer Rohstoff also schon ausscheiden, ganz abgesehen davon, dass die Frage seiner Unschädlichkeit noch der völligen Klärung harrte. Das Problem war auch von anderen Seiten in Angriff genommen, es wurde erneut versucht, Tierblut zu präparieren, dass es für menschlichen Blutersatz geeignet würde, ja man kam auf den seltsamen Gedanken, Haemoglobin als das benötigte Kolloid zu verwenden.

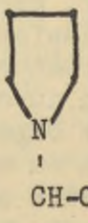
Weeses Idee war, die synthetische Chemie einzuschalten, unter den zahlreichen synthetischen hochpolymeren Verbindungen, die bei den Arbeiten über Kautschuk, Lack, Kunstseide und Kunststoffverbindungen gewonnen waren, ein geeignetes herauszufinden. Sie werden mir zugeben, dass das eine recht kühne Idee war, gerade dem Eiweisschemiker ist die einzigartige Sonderstellung der Eiweisskörper in ihrer spezifischen chemischen und physiologischen Struktur so geläufig, dass ihm die Möglichkeit, sie in ihrer Funktion durch ein synthetisches Kolloid vertreten zu Lassen, zunächst eine Ungeheuerlichkeit zu zein scheint. Demgegenüber ist aber gleich daran zu erinnern, dass ihre Vertretbarkeit durch Gummi arabicum bereits als erwiesen angesehen werden konnte, und das Gummi arabicum hat um Eiweiss keine nähere Verwandt-

schaft, als ein synthetischer Körper, ich werde noch zeigen, dass das Umgekehrte gilt.

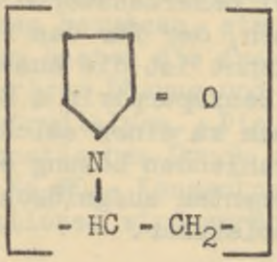
Ich möchte nun übergehen, welche Verbindungen wir damals alle auf ihre Eignung geprüft haben und nur kurz andeuten, wie die Prüfung der zur Auswahl gestandenen Körper durchgeführt wurde. Natürlich mussten sie wasserlöslich sein. Da es im allgemeinen nicht das Ziel der auf einschlägigen Gebieten tätigen Synthetiker war, wasserlösliche Verbindungen zu gewinnen, war die Auswahl unter den bereits hergestellten Verbindungen nicht übermässwig gross, und engte sich noch weiter ein, als sie auf ihre Wechselwirkungen mit den in physiologischen Lösungen notwendig enthaltenen Salzen geprüft wurden: viele von ihnen bildeten u.B. unlösliche Kalksalze. Dann kam die wichtigste Forderung in Sinne des primus non nocere: ihre anstandslos Verträglichkeit bei intravenöser Injektion grösster Dosen. Dann erst kam die kolloidchemische Seite: Der kolloidosmotische Druck musste grössenordnungsmässig zu dem des Plassweiweissen passen und schliesslich der Tierversuch mit einem grösseren Blutverlust des Leben retten, als es die kolloidfreie Salzlösung es tut.

In dieser Weise kamen wir zur Auswahl eines bestimaten Kolloides, des "Kollidons", das in geeigneter Salzlösung gelöst unter der Bezeichnung "Periston" dann klinisch geprüft und seit 3 Jahren in nunmehr hunderttausenden von Fällen beim Heer praktisch benutzt worden ist. Über den klinischen Erfolg nur einige ganz kurze Angaben: Zunächst ist die Verträglichkeit über allem Zweifel erhaben, ausser ganz vereinzeltn Fallen von Fieberreäktion - unendliche viel seltener als nach Blut- oder Seruntransfusionen - sind uns keine für das Präparat spezifischen Nebenwirkungen gemeldet worden. Und über den Effekt liegen neben kühlen und reservierten, in der Mehrzahl absolut positive Berichte von kriegschirurgischer Seite vor. Daneben wird in heimischen Kliniken, selbst dort also, wo die Bluttransfusion möglich wäre, viel Periston benutzt, sicherlich wäre es noch viel mehr, wenn die Produktion nicht von der Seite der Glasampullen her hinter den Anforderungen zurückbliebe.

An dieser Stelle möchte ich es als meine Aufgabe ansehen, Sie mit einigen Eigenachaften dieses Kolloides bekannt zu machen. Es ist in dem von Herrn Dr. Reppe geleiteten I.G. Laboratorium in Ludwigshafen erfunden worden. Nach einer von Reppe entwickelten Synthese wird N-Vinylpyrrolidon



hergestellt und dieses der Polymerisation unterworfen. So entsteht das "Polyvinylpyrrolidon", das also eine kettenförmige Verknüpfung von Einheiten der Struktur



darstellt. Wie Sie sehen, enthält es Säureamidgruppen. Wir vermuten, dass dieser Punkt für einige seiner Eigenschaften bedeutsam ist. Dieses Kolliden fällt fabrikatorisch als glasiges Harz an, das sich zu einem weissen Pulver mahlen lässt. Es löst sich, ausser in Wasser, auch in vielen organischen Lösungssmitteln, besonders in Chloroform, unlöslich ist es in Aether. Durch Zugabe von Aether zu einer Lösung in Chloroform wird es zur Ausfällung gebracht. Bemerkenswert ist seine Verteilung zwischen Wasser und Chloroform. Durch Ausschüttelungsversuche lässt sich zwar hier das Gleichgewicht nicht ermitteln, weil sich beim Schütteln hoffnungslose Emulsionen bilden. Überschichtet man aber eine Lösung von Kollidon in Chloroform vorsichtig mit Wasser, so tritt das Kolloid in erztaunlich kurzer Zeit und vollständig in die wässrige Phase über, seine hydrophilen Eigenschaften überwiegen also, es ist unmöglich, es aus wässriger Lösung mit Lösungsmitteln zu extrahieren, was für die Analysenverfahren sehr bedauerlich ist.

Das Kollidon ist in wässriger Lösung recht beständig, z.B. verändert es sich durch langeres Kochen nicht. Das ist eine wichtige Voraussetzung für die einwandfreie Sterilisierbarkeit der damit hergestellten Blutersatzflüssigkeit Periston. Aber selbst Kochen in mineralsaurer Lösung ergibt keine erkennbare Veränderung. Etwas anders ist es bei der Einwirkung von Alkali. Kochen in schwachem Alkali führt bereits zu einer Viskositätszunahme, starkes Alkali führt zur schleimigen Ausfällung eines nicht mehr wasserlöslichen Produktes. Chemisch sind diese Veranderungen noch nicht aufgeklärt.

Das Kollidon ist eine elektroneutrale, undissozierte Verbindung. Das ergibt nicht nur die Konstitutionsformel, sondern auch die elektrometrische Titration. Letztere fördert die gleichen Werte wie Wasser zutage, es ist keinerlei Pufferwirkung vorhanden. Hierin liegt physiko-chemisch wohl die stärkste Abweichung vom Eiweiss.

Kollidon ist gegen eine ganze Anzahl von Eiweissfällungsmitteln indifferent, so z.B. gegen Mineralsäuren, Sulfosalicylsäure, p-Toluolsulfosäure, Uranylacetat, Alkohol. Mit anderen lässt es sich ausfällen, in erster Linie mit Trichloressigsäure, auch mit einigen Mineralsalzen, z.B. Natriumsulfat (Bennhold), von dem Konzentrationen von 9 % an fällend wirken. Hierbei ist eigentümlich, dass sich

die zunächst auftretende Trübung nicht als feste Flockung abscheidet, sondern als konzentrierte flüssige Phase. Beide Fällungen sind vollständig reversibel.

Das Kollidon zeigt mancherlei bemerkenswerte Schutzkolloideffekte. Ich berichte nur einen Versuch, der für das folgende wichtig und sehr eindrucksvell ist. Bekannt ist die ausserordentlich intensive Strömungsdoppelbrechung des Benzopurpirin 4 B in wässriger Lösung von geringem Salzgehalt. Fügt man zu einer solchen Mischung gekreuzten Nicols bewegten und hell strahlenden Lösung ein wenig Kollidon hinzu, so ist die Doppelbrechung momentan ausgelöscht, die Kristallstäbchen zerfallen, vermutlich molekular.

Natürlich ist noch eine ganze Reihe von Eigenschaften des Kolldions von Polymerisationsgrad bezw. Molekulargewicht abhängig. Die Polymerisation lässt eich so leiten, dass Produkte von gewünschter, verschiedener Kettenlänge erhalten werden, aber, wie stets bei synthetischer Kettenlänge. Es fällit stets als Gemisch Versohiedener, um einen veränderlichen Mittelwert schwankender Molekülgrössen an. Das praktisch benutzte Produkt hat nach Messungen mit Ultrazentrifuge und nach seines osmotischen Druck ein mittleres Molekulargewicht von 25000-500000. Da alle diese Messungen bestimmte, aber etwas unsichere Annahmen über die Teilchengestalt und ähnliches zur Voraussetzung haben, lassen sich genaue Werte noch nicht angeben. Dagegen ist es einfach, die Gleichmässigkeit des Produktes zu Überwachen. Das Norm dafür ist die Viskositat. Eine etwa 3 %ige Kollidonlösung hat dieselbe Viskosität wie das Blutplasma.

Zur Messung des kolloidosmotischen Druckes von Kollidonlösungen benutzen wir das sog. Onkometer nach Hepp. Diese Messungen zeigen gegenüber der einfachen und raschen Messbarkeit des Druckes der Bluteiweisskörper bemerkenswerte Schwierigkeiten: Die Einstellung eines konstanten Druckniveaus bei Verwendung eiweissdichter Membranen ist verzögert, zudem istdie Konstanz nicht immer für lange Beobachtungszeiten gewährleistet, meist zeigt sich nach Stunden wieder eine sinkende Tendenz. Wir möchtendies auf die Polydispersität zurückführen. Eben eiweissdichte Membranen sind für die kleineren Moleküle eben noch durchlässig, aber doch schon sehr langsam, sodass die Gleichgewichtseinstellung sehr verzögert ist. Man kann auch daran denken, dass die Fadenmoleküle sich langsam durch die Poren hindurchschlängeln. Diese Dinge machen die Druckmessung etwas unerfreulich, und die Werte sind nicht innerhalb der engen Grenzen reproduzierbar wie diejenigen des Serums, sodern nur etwa innerhalb ± 10 %.

Im Durchschnitt unserer sehr zahlreichen Messungen ergibt eine 2.5 % ige Kollidonlösung einen Druck von 300, die 3.5 %ige einen solchen von 400 mm H_2O. Wir können also sagen, dass die 3 %ige Lösung ebenso wie in der Viskosität auch im osmotischen Druck mit dem Blutplasma übereinstimmt. Übrigens wird dieser Druck, in Gegensatz

zu den Verhältnissen bei Gummi arabicum, von geringen Salzgehalten der Lösungen garnicht beeinflusst.

In Laboratorium ist es möglich, das Kolliden in Fraktionen verschiedener Molekulargrösse zu zerlegen, man kann dazu verschiedene der genannten Fällungsreaktionen benutzen, etwa die mit Aether aus Lösungen in Chloroform oder am besten die fraktionierte Ausfällung mit Natriumsalfat aus wässriger Lösung und Befreiung der Fraktionen von Natriumsulfat mit Elektrodialyse. Die Abb. zeigt die Abhängigkeit von Viskosität und osmotischen Druck so gewonnener Fraktionen von der jeweils fällenden Na_2SO_4- Konzentration. Auf die biologischen Unterschiede der Fraktionen wird zurückzukommen sein.

Wir kommen nun zu dem Verhalten dieses Körpers in der Blutbahn. Dass er bei intravenöser Zufuhr sehr verträglich ist, war, wie gesagt, eine der Voraussetzungen für seine Auswahl. Die üblichen Versuchstiere vertragen bis zu 8 g/kg. Was sich ereignet, wenn man mehr gibt, kann man zuden weniger als eine Folge einer spezifischen Toxicität, als vielmehr als solche der notwendig damit verbundenen physikalischen Störungen des Wasserhaushaltes ansehen: es kommt zu einer gewaltigen hydraemischen Plethora und in deren Gefolge zu Lungenoedem.

Es muss aber erwähnt werden, dass der weitgehenden Unempfindlichkeit der meisten Versuchstiere eine eigenartige Empfindlichkeit einer spezielen Tierart gegenübersteht, die des Hundes. Dieses Tier zeigt schon bei kleinen Dosen eine Kollapsneigung mit Blutdruckabfall, deren Wesen recht unklar ist und noch rätselhafter wird durch die gesetzmässige Erscheinung, das dies Tier nach einmaligem Überstehen dieser Reaktion dann für nachfolgende, selbst viel grössere Dosen, genau so unempfindlich ist, wie andere Tiere. Das erinnert an die Desensibilisierung bei der Anaphylaxie. Übrigens ist aus der Literatur ähnliches Verhalten des Hundes gegenüber anderen Kolloiden, selbst gegenüber arteigenen Serum bekannt. Dieses abweichende Verhalten des Hundes hat aber für die Anwendung beim Menschen keinerlei Bedeutung: Unter Hunderttausenden von Infusionen ist hier eine ähnliche Reaktion nie beobachtet worden.

Die erwartete Leistung des Kollidon sollte in erster Linie die Wasserbindung in der Blutbahn sein. Zum Nachweis dieser Leistung eignet sich in einfachster und übersichtlichster Weise der Durchströmungsversuch am Läwen-Trendelenburg'schen Froschpräparat. Werden die Gewebe des Frosches von der Bluthahn aus mit eiweissfreier Ringerlösung durchströmt, so quellen sie langsam auf, es ergibt sich ein Oedem, messbar an der Gewichtszunahme des Präparates. Enthält die durchströmende Lösung aber Kollidon, so kann die Gewichtszunahme abgestoppt warden, bei hohen Konzentrationen kann sogar eine Gewichtsabnahme erreicht werden, weil das Kolloid das Quellungswasser aus den Geweben in die Blutbahn zurücksaugt.

Bei der Verblutung geht es um Tod und Leben. Daraus ergibt sich als letzten Endes entscheidender Masstab für die Leistungsfähigkeit eines Blutersatzmittels der Rettungsversuch des stark ausgebluteten Versuchstieres.

Die Leistungen des Periston in solchen Versuchen im Vergleich zu anderen Blutersatzflüssigkeiten gehen aus den Abbildungen hervor.

Mit diesen kurzen Hinweisen auf die therapeutische Seite des Peristons möchte ich mich begnügen und sich nun der Frage nach dem Schicksal des Kollidons im Organismus zuwenden. Es ist uns gelungen, Verfahren zur quantitativen Bestimmung des Kollidons im Blut, in den Geweben und im Harn auszuarbeiten, mit deren Hilfe sich folgendes Bild ergeben hat; ein grosser Teil des Kolloides verlässt die Blutbahn sehr rasch wieder in den ersten Stunden nach der Infusion. Parallel mit diesem Abfall der Blutkonzentration geht eine Ausscheidung beachtlicher Mengen mit dem Harn. Wir müssen annehmen, dass diese Vorgänge eine Art Fraktionierung im Organismus vorstellen: Am ersten werden die niederpolymeren Anteile die Blutbahn verlassen und ausgeschieden werden. Diese Auffassung wird durch folgenden Versuch belegt: Stellt man sich, wie vorher besohrieben, Fraktionen her und verfolgt deren Harnausscheidung beim Kaninchen nach intravenöser Infusion, so zeigt sich, dass von den niedersten Fraktionen etwa 50% im Harn der ersten 24-Stunden wiedererscheinen, von den höchsten aber praktisch garnichts mehr. Von dem praktisch benutzten unfraktionierten Kolloid werden etwa 20% wiedergefunden, das trifft auch beim Menschen zu. Am 2. Tage findet sich meist noch eine ganz kleine Menge im Harn, damit ist aber die Ausscheidung des unveränderten Kolloids dann beendet. Der Abfall der Blutkonzentration ist aber stärker, als allein durch die Ausscheidung des Kolloides in Harn zu deuten wäre, sie ist nach 24 Stunden stets schon auf weniger als die Hälfte gesunken. Der weitere Abfall geht dann immer langsamer, noch nach 3 Wochen konnten wir eben noch quantitativ fassbare Mengen ermitteln, letzte Spuren mögen sich noch wesentlich länger halten. - Was ist mit dem aus dem Blute geschwundenen, aber nich im Harm wiedererschienenen Kolloid geschehen? Zwei Möglichkeiten sind zu erörtern: Speicherung in Organen und chemischer Abbau. Unsere Organanalysen haben ergeben, dass sich das Kollidon ziemlich gleichmässig über die Organe verteilt, spezifische Speicherungen haben sich nicht nachweisen lassen. Auch mit histologischer Methodik sind keine Anzeichen für eine Dauer speicherung des Kolloides zu fassen (Heimlein). Nach 3-4 Wochen ist, wie in Blut, auch in den Organen kein Kollidon mehr vorhanden. Es bleibt also nichts anderes übrig, als anzunehmen, dass es chemisch abgebaut worden ist. Ein direkter Nachweis von Abbauprodukten ist aber bislang nicht moglich gewesen.

Ich hoffe, deutlich gemacht zu haben, dass das Kollidon in einer praktisch sehr brauchbaren Weise die wasserbindende Funktion der Serumeiweisse zu vertreten vermag. Das war das Ziel bei der Entwicklung des Periston. Darüberhinaus aber haben Bennhold und Schubert Versuche mitgeteilt, diees noch weiter rechtfertigen, das Interesse der Eiweisschemiker für neue Kolloid in Anspruch zu nehmen.

W. - Elberfeld. den 23.6.44. - 118 -

APPENDIX 4

Sulfonamides against Viruses.

Substance	Mouse Toxicity Subc.	Mouse Toxicity per os	Bronchopneumonia subc.	Bronchopneumonia per os	Lymphogranuloma inguinale subc.	Lymphogranuloma inguinale per os.
Prontalbin	40 D 30 L	67 D 40 L	5 W 2.5 Ø	10 W 5 Sp.W	10 W 5 Sp.W	10 W 5 Sp.W
Prontosil	67 D 40 L	40 D 30 L	10 W 5 Sp.W	2.5 W 1.3 Sp.W	10 W 5 W-Sp.W 2.5 Sp.W.	5 W 2.5 Sp.W.
Uliron	40 D 30 L	125 D 100 L	2.5 W 1.3.W-Sp.W 0.67 Sp.W.	5 W 2.5-1.3 W-Sp.W 0.67 Sp.W	2.5 W 1.3 W-Sp.W 0.67 Ø	5 W 2.5 Sp.W.
Sulfapyridine	20 D 13 L	30 D 20 L	5 W 2.5 Sp.W	2.5 W 1.3 W-Sp.W 0.67 Ø	2.5 W 1.3-0.67 W-Sp.W. 0.33 Sp.W.	2.5 W 1.3 Sp.W.
B 1034	67 D 40 L	-- 200 L	10 W 5 Sp.W	10 W 5 W-Sp.W 2.5 Sp.W	1.3. W. 0.67 Ø	1.3 W. 0.67 W-Sp W
Sulfathizol	40 D 30 L	100 D 67 L	5 W 2.5 Ø	5 W 2.5 Ø	2.5 W 1.3 Sp.W	5 W 2.5 Ø
Dibenal (Sulfapyrimidine)	10 D 6.7 L	30 D 20 L	0.08 W 0.02-.01W-Sp.W 0.005 W-Sp	0.16 W 0.08-.04 Sp.W .02 Ø	0.02 W 0.005 Ø	0.01 W 0.005 Ø

4-methyl Debenal	30 D 20 L	30 D 20 L	0.02-.01 Sp.W 0.005 Ø	0.08 W 0.02 W-Sp.W. 0.01 Ø	0.04 W.H. 0.02 W 0.01 Ø	0.04 W.H.-W. 0.02 W 0.01 Ø
4,6-dimethyl debenal	67 D 40 L	67 D 40 L	0.67 W 0.33 Ø	0.67 W 0.33 Ø	1.3 W.H. 0.33 W 0.16 W-Ø	0.67 W.H. 0.16 W-Sp.W. 0.08 Sp.W. 0.04 Ø
	Azo compounds with 2- Amino - 5 - naphthol - 7 - sulphonic acid.					
Debenal	12.5 D 10 L	-- 200 L	0.08 W-Sp.W. 0.04 Sp.W 0.02 Ø	0.67 W-Sp.W 0.33 Sp.W 0.16 Sp.W - Ø	0.33 W.H. 0.04 W 0.02 Sp.W.-Ø	0.33 W.H -W. 0.16 W.H - Ø
4- Methyl Debenal	40 D 30 L	-- 200 L	0.08 W 0.04 Sp.W	0.04 W 0.02 Sp W	0.04 W.H.-Sp.W 0.02 Ø	0.16 W.H. 0.02 Sp.W. 0.01 Ø
4,6 - dimethyl Debenal	12.5 D 10 L	-- 200 L	2 W 0.33 W - Ø	0.67 W-Sp.W 0.04 Sp.W 0.02 Ø	1.3 W.H. 0.67 Sp.W. 0.33 Ø	2.5 W.H -W 0.67 Sp.W. 0.33 Ø
	Azo compounds with 2 - Acetylamino - 8 - maphthol - 3,6 - disulfonic acid.					
Debenal	125 D 100 L	-- 200 L	0.08 W-Sp.W 0.02 Sp.W.-Ø	0.16 W-Sp.W 0.08 Sp.W.-Ø	0.67 W.H. 0.14 Sp.W 0.08 Ø	0.33 W.H. 0.04 W -Sp.W. 0.02 Ø
4. Methyl Debenal	100 D 67 L	-- 100 L	0.67 W 0.08 Sp.W	0.08 W 0.04 Sp.W	0.33 W.H.-W 0.04 W-Sp.W 0.02 Ø	0.33 W.H. 0.08 W. 0.04 Ø

4,6 dimethyl Debenal	67 D 40 L	-- 200 L	0.16 W 0.08 Sp.W 0.04 Ø	0.67 W 0.16 Sp.W 0.08 Ø	1.3 W.H. 0.33 W 0.16 W-Ø	2.5 W.H. 0.33 W 0.16 Sp,W-Ø

The doses given are in mg./ 20g. mouse

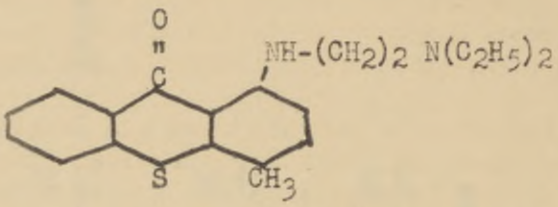
D - died W.H. - cure Sp.W - slightly active

L - living W - active Ø - no activity

APPENDIX 5

Miracil bei Bilharziose.

Xanthonderivat



Durch jahrelange Arbeiten an einem besonders konstruierten Testmodell ist es nun gelungen, ein Präparat (Miracil) von besonderer chemischer Konstitution aufzufinden, das sich experimentell bei der Bilharziose als "ausserst wirksam erwies und sich in Wirkung und Verträglichkeit sowohl dem Brechweinstein als auch dem Fuadin einwandfrei überlegen zeigte.

Die Versuchsanordnung ist kurz folgende. Aus dem Kot bilharziainfizierter Affen aus Westafrika werden die Eier (B.mansoni) gewaschen und isoliert und für 12 Stunden im Eisschrank bei einer Temperatur von ca. + 6° aufbewahrt. Mit Hilfe physikalischer, insbesondere thermischer Reize bringt man die Hülle der Eier zum Platzen, so dass die Larven (Mirazidien) frei werden und in Schnecken der Gattung Planorbis eindringen. Die für diesen Zweck benutzten Planorbenarten stammen aus Venezuela. Es handelt sich um Planorbis guadalupensis, die seit mehreren Jahren in besonders eingerichteten, ihren Lebensbedingungen entsprechenden Aquarien gezüchtet werden. In diesen Schnecken machen nun die Parasiten bei künstlich geschaffenen geeigneten Umweltbedingungen eine komplizierte Entwicklung und morphologische Umwandlung durch. Nach etwa 3-4 Wochen finden sich in der Mitteldarmdrüse der Schnecken die infektiösen Endformen, die unter bestimmten biologisch-physikalischen Bedingungen mit dem Kot ins Freie gesetzt werden und sich aktiv mit Hilfe eines Ruderschwanzes forthewegen. Bringt man sie auf geeignete Weise in Berührung mit einem tierischen Organismus, so dringen sie aktiv durch die Haut in den Blutkreislauf ein. Auf dem Blutwege erreichen sie schliesslich die für sie geeignetste Stelle ihres neuen Wirtsorganismus, wo sie in dem Venen zu geschlechtsreifen und geschlechtlich differenzierten Würmern heranwachsen.

Mit der hier geschilderten Versuchsanordnung gelingt es, experimentell Mäuse, Kaninchen, aber auch Affen zu infizieren und bei diesen Tieren ein der menschlichen Krankheit analoges Krankheitsbild hervorzurufen. Es konnte auf diese Weise gezeigt werden, dass die bei der menschlichen Bilharziose bisher als wirksam anerkannten Präparate, sowohl der Brechweinstein als auch das Fuadin, eine spezifische Wirkung auf die Wurmer entfalten, wobei allerdings erwähnt werden muss, dass ein Behandlungserfolg

erst durch eine mehrmalige Verabreichung der betreffenden Mittel zu erzielen ist. Im Vergleich zu diesen beiden Präparaten zeigt nun das Miracil, das auf diese Weise aufgefunden worden ist, eine den beiden anderen Präparaten stark überlegene Wirkung, die bei Mäusen und Affen mit Sicherheit und Regelmässigkeit zu erreichen ist. Diese Überlegenheit äussert sich nicht nur in einer wesentlichen Verkürzung der Behandlungszeit, sondern sie kommt auch in bezug auf den sog. chemotherapeutischen Index zum Ausdruck.

Während beim Fuadin bei der Maus ein Index von nur 1:2 festzustellen ist, haben wir beim Miracil einen Index von 1:30, mit anderen Worten, es ist im Vergleich zum Fuadin etwa 15 mal stärker wirksam. Besonders hervorzuheben ist aber noch, dass mit Miracil in einem sehr viel grösseren Prozentsatz Radikalheilungen erzielt werden können und dass das Miracil sich durch eine besonders gute Verträglichkeit auszeichnet. Die Radikalheilungen treten ganz eindeutig bei der sonst viel schwerer zu beeinflussenden Bilharziainfektion der Affen zutage. Die Wirkung des Miracils ist allerdings nur bei B. haematobia und B. mansoni festzustellen, während nach den bisherigen experimentellen Ergebnissen eine Wirkung auf die Bilharzia japonica nicht vorhanden ist.

Da es sich bei dieser Versuchsanordnung um einen menschenpathogenen Erreger handelt, der bei einem höher organisierten Tier wie dem Affen ein dem des Menschen ähnliches Krankheitsbild auslöst, welches prompt auf die Behandlung mit Miracil anspricht, so ist die Hoffnung berechtigt, dass eine Behandlung der Menschenbilharziose ebenso erfolgreich sein wird wie die des Affen.

Da, wie anfangs gesagt, die Krankheit nur in aussereuropäischen Gebieten, in tropischen und subtropischen Zonen zuhause, ist, ist es z.Zt. infolge des Krieges nicht möglich, klinisch-therapeutische Versuche beim Menschen durchzuführen, um sich von der Wirksamkeit des Präparates zu überzeugen.

Wuppertal-Elberfeld, den 4.2.1943

/s/ Kikuth
gez. Prof. Kikuth.
I.G. FARBENINDUSTRIE
WERK ELBERFELD.

Appendix 6.

A b s c h r i f t

Abteilung: Pharmakologisches Labor

an Herrn Prof. K i k u t h, H i e r

Unsere Zeichen Dr. Hcht/Hi. Tag 14. 4. 1943

Betreff: Mauss 752 (Miracil) = W. 2093.

Wir haben die Arbeiten uber die Toxikologie dieser Substanz zu einem gewissen Abschluss gebracht. Das wesentliche Ergebnis ist, dass sie bei geringer akuter Giftigkeit zu sehr charakteristischen und noch nach langer Zeit nachweisbaren fettigen Organdegenerationen führt, die ihre therapeutische Verwendbarkeit in Frage stellen oder wenigstens die Nennung einer vertraglichen Dosierung sehr erschweren. Im einzelnen hatten wir folgende Ergebnisse:

1.) Lokale Wirkung:
M. 752 fuhrt am Orte der Applikation zu starken Gewebsschadigungen. Noch 1%ige Losungen ergeben in der Ohrquaddel am Kaninchen Nekrosen, am Kaninchenauge Konjunktivalodeme mit eitriger Sekretion. Selbst intramuskuläre Gaben (Rattenschenkel, o,1 ccm 2%ige Losung) haben starke Ödeme und Zerfall der Muskelfasern zur Folge. Nach wiederholten intravenösen Injektionen bei Kaninchen kommt es langsam zu weit uber die Venen selbst hinausgreifenden Zerstorungen. Eine parenterale Anwendung von M. 752 demnach kaum durchfuhrbar sein.

Bei Katzen äussert sich die Reizwirkung auch bei oralen Gaben durch Auslosung von Erbrechen, das selbst noch bei der kleinen Dosis von 0,025 g/kg eintritt und erst bei 0,01 g/kg nicht mehr erfolgt.

2.) Toxische Dosen:
a) Bei oraler Gabe: Mäuse vertrugen bis zu o,3 g/kg, nach, 0,5 g/kg ging die Hälfte der Tiere zugrunde. Kaninchen vertrugen 0,2-0,6 g/kg und zeigten innerhalb einer einwöchigen Beobachtungszeit keine erkennbaren Schäden. 0,7 g/kg führten nach 3 Tagen zu zunehmender Albuminurie, später trat Durchfall hinzu und am 8. Tag trat der Tod ein. Die Sektion ergab makroskopisch neben Hyperämie des Darmkanals vor allem ein auffallend grosses und schlaffes Herz mit graubrauner Farbe des Herzmuskels. Übrige Organe makroskopisch ohne Befund. Mikroskopisch zeight das Herz trüb-körnige Andeutungen von Verfettungen. Die Leber zeigt schmale, glykogenarme Zellbalken

37525-1

mit geringer fein tropfiger Verfettung, daneben auffallende Verfettungen endothelialer und bindegewebiger Elemente, die Niere verstreute nephrotische Degeneration mit Zylinderbildung und Verfettungen.

Ein anderes Tier ging 10 Tage nach der Gabe von 1,0 g/kg zugrunde. Hier bestand ein ausgedehnter Lungenabscess, sodass der pathologische Befund nicht zu werten ist.

Bei Katzen konnte die oral-toxische Dosis night ermittelt werden, da das Praparat ausgebrochen wurde.

b) bei intravenöser Gabe: Hier war bei Katzen und Kaninchen das Bild nicht unterschiedlich, beide Tierarten vertrugen bis zu 15 mg/kg ohne schwere Erscheinungen, 20-25 mg/kg führten sofort zu Krämpfen und Lähmungszuständen, von denen sich die Tiere wieder erholten. 40 mg/kg waren sofort tödlich.

3.) Versuche mit wiederholter Verabreichung:

a) Kaninchen:
2 Kaninchen erhielten an 8 aufeinanderfolgenden Tagen je 50 mg/kg per os. Beide Tiere zeigten keine auffälligen Störungen, der Blutstatus ergab keine einheitlichen Veränderungen. Eines ging 13 Tage nach Abschluss der Behandlung zugrunde, die Sektion ergab eitrig-fibrinöse Pleuritis. Dieses Tier scheidet also fur die Beurteilung aus. Das andere Tier zeigte 14 Tage nach Abschluss der Behandlung auffällige Gewichtszunahme, dann Durchfall und verendete am 18. Tage. Die Sektion ergab hier makroskopisch keinen besonderen Befund, mikroskopisch dagegen Verfettungen in Leber, Niere und Herz, ähnlich wie oben beschrieben.

Im Gegensatz dazu vertrugen 2 Tiere die gleiche Behandlung mit der doppelten Dosis, also taglich 0,1 g/kg, sie zeigten wahrend der Behandlung keine Storungen, doch ergab das Blutbild bei beiden beachtliche Anamie, die ihr Maximum ca 14 Tage nach Abschluss der Behandlung erreichte. Einen Monat nach der letzten Gabe wurde die Beobachtung abgabrochen, die Blutwerte waren fast wieder normal, das Körpergewicht angestiegen.

Ferner wurden einige Kaninchen mit wiederholten intravenösen Gaben behandelt, jedoch infolge der starken Nekrotisierung der Ohren nur bis zu 5 Einzeldosen. 2 Tiere erhielten jedes Mal je 10 mg/kg. Das eine zeigte nach der 4. Gabe Albuminurie und wurde 4 Tage spater getötet. Die Sektion ergab neben Nierenveränderungen im Sinne einer Lipoidnephrose auffällige fettig-körnige und wachsartige Degeration

des Herzmuskels. Das andere Tier erhielt nach 10 tägiger Unterbrechung noch eine 5. Dosis. Es bot keine Störung, auffallend war die starke Gewichtszunahme.

2 weitere Kaninchen erhielten 3 bzw. 4 intravenöse Einzeldosen von 20 mg/kg, die jeder Mal von leichten Krämpfzuständen gefolgt waren. Das erste Tier mit der Gesamtdosis von 60 mg/kg entwickelte eine dauernde Albuminurie. Das Körpergewicht nahm bis zu 14 Tagen nach Abschluss der Behandlung zu, dann wieder ab. Das Tier wurde am 24. Tag getötet. Es zeigte einen Pericarderguss, der für Herzschäden spricht, ferner Hyperämie der inneren Rindenschicht der Niere. Histologisch stehen schwere degenerative Veranderungen der Herzmuskelfasern in Vordergrund, daneben fettige Degenerationen in Niere und Nebenniere. Das andere Tier - Gesamtdosis 80 mg/kg - zeigte ebenfalls Albuminurie und wurde 4 Tage nach der letzten Gabe getötet. Im Gegensatz zu dem unauffälligen makroskopischen Befund enthüllte hier die mikroskopische Untersuchung neben Fettinfiltration der Leber wieder nephritischnephrotische Nierenveranderungen leichteren Grades und vor allem sehr charakteristische eigenartige wachsartigstreifige und körnige Degenerationen der Herzmuskelfasern.

Im Ganzen ergibt sich also bei Kaninchen bei relativ guter Verträglichkeit quo ad vitam eine sehr schleichende Vergiftung mit Organdegenerationen, die insbesondere Herz und Nieren zu betreffen scheinen.

b Bei Katzen: ist die Vergiftung nun schon bei viel kleineren Dosen von tödlichem Erfolgt.

2 Tiere wurden mit je 9 täglichen oralen Dosen von je 10 mg/kgbehandelt. Im Gegensatz zu den Kaninchen zeigten beide Tiere bereits under der Behandlung, Gewichtsabnahme, vielleicht infolge der durch Magenreizung verringerten Fresslust. Ferner trat hier keine Anämie auf, sondern Im Gegenteil beachtliche Bluteindickung. Das eine Tier wurde 5 Wochen nachbeobachtet und war am Schluss wieder vollig munter. Das andere Tier zeigte weiter starke Gewichtsabnahme, und wurde 14 Tage nach Abschluss der Behandlung getötet und seziert. Auffallend war eine gelbe Verfärbung der Organe, insbesondere war die Aortenintima zitronengelb, vermutlich infolge Speicherung des Farbstoffes. Die schon makroskopisch als verfettet kennliche Leber erwies sich histologisch als nahezu maximal fettinfiltriert. Daneben wieder eine starke fettige Degeneration des Herzens, Nierenveranderungen und auch Verfettung des Markes der Nebenniere.

2 weitere Tiere erhielten 8 mal je 25 mg/kg per os. Beide zeigten wieder unter der Behandlung Gewichtsabnahme - sie gaben durch häufiges Erbrechen die Folgen des Praparates fur den Magen zu erkennensowie Bluteindickung. Beide Tiere verendeten, das eine 2, das andere

7 Tage nach Schluss der Behandlung. Bei beiden ergab die Sektion übereinstimmend maximale Fettleber - bei einem Tier waren die Leberzellen kernlose Fettropfen bei gut erhaltenem Endothel und Stützgewebe -, starke fettige Degeneration der Herzmuskelfasern, nephritisch-nephrotische Nierenveränderungen.

Je 2 weitere Katzen waren mit täglichen Dosen von 0,05 bzw. 0,1 g/kg behandelt worden. Alle 4 Tiere zeigten analoges Verhalten-Gewichtsabnahme, Bluteindickung. Sie gingen nicht früher (einige Tage nach Abschluss der 8-maligen Behandlung) zugrunde als bei kleineren Dosen, ein Zeichen dafür, dass die Vergiftung schleichend verläuft und Zeit zu ihrer Auswirkung benötigt. Die pathologischen Veränderungen der Organe waren hier neben den fettig-degenerativen Vorgägen auch durch entzündlich infiltrative Prozesse charakteristisch. Vielleicht steht damit die beobachtete stärkere Leukozytose in Zusammenhang, die übrigens auch bei dem kleineren Dosen und auch bei den Kaninchen deutlich war.

4.) Das Schicksal des Giftes:

Bei den Katzen sprach die starke Gelbfärbung der Leichen für eine starke und dauerhafte Speicherung der Substanz. Im Gegensatz dazu konnte bei den Kaninchen schon kurze Zeit nach der Gabe keine Gelbfärbung mehr beobachtet werden. Das spricht dafür, dass diese Tiere die Substanz abbauen, denn eine Ausscheidung konnte auch bei ihnen selbst nach intravenöser Gabe subletaler Dosen nicht festgestellt werden. Zom Zweck des Nachweises wurde der Harn schwach angesäuert und im Extraktionsapparat mit Chloroform erschöpfend extrahiert. (M.752 ist auch aus sauer wässriger Lösung mit Chloroform extrahiertbar). Das Chloroform wurde verjagt, der Rückstand wieder in Chloroform gelöst, mit der zehnfachen Äthermenge verdünnt, mit Sodalösung gereinigt und wiederholt mit kleinen Portionen verdünnter Salzsäure ausgeschüttelt. Mit diesem Verfahren war 1mg M. 752 in 10 ccm Harn bequem nachweisbar. Dagegen war der Nachweis bei allen behandelten Tieren negativ.

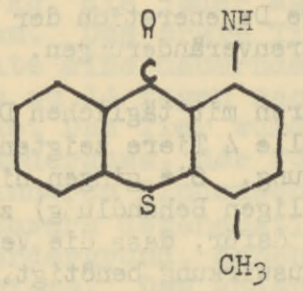
Es muss also angenommen werden, dass Kaninchen das Gift viel schneller zerstören als Katzen, und damit hängt wohl zusammen, dass sie etwa 10 mal weniger empfindlich sind. Die Annahme, dass sie die Substanz schlechter resorbieren, ist nicht gerechtfertigt, sie vertragen offenbar intravenös eine chronische Behandlung mit grösseren Dosen als Katzen per os.

Zusammenfassend muss also festgestellt werden, bass M. 752 zu sehr schleichend verlaufenden Vergiftungen führt, die auch, wenn sie nicht tödlich enden, zu schweren Organveränderungen führen. Dosen, die auch bei chronischer Gabe hermlos bleiben, können noch nicht genannt werden.

gez. Dr. Hecht

APPENDIX 7

Ba 7669 = Mauss 752 = Miracil 1.11.1939



Giftigkeit : per os 1/50 tot 1/75 lebt)
subcutan 1/100 tot 1/150 lebt) 1 dose

Bilharziose: Per os 1/200 tot 1/400 tot 1/400 Wirkung-Heilung 1/800 Wirkung-Heilung, Wirkung 1/1500 Wirkung 1/3000 ohne Wirkung

" " jugendliche Würmer 1/400 Wirkung 1/800 ohne Wirkung

" " " " 1 x 1/150 Wirkung-Heilung 1/300 Wirkung 1/400 Spur Wirkung 1/800 ohne Wirkung

" " " " Prophylaxe: 1 x 1/200 ohne Wirkung

subcutan 1/400 Wirkung 1/800 Wirkung 1/1500 ohne Wirkung
" jugendliche Würmer 1/400 Wirkung 1/800 Spur Wirkung

Affen-Bilharziose: per os 2 x 800 mg leichtes Erbrechen 400 mg vertragen.
100 mg Wirkung-Heilung, 35 mg Wirkung Heilung, 10 mg Wirkung-Heilung, 5 mg Wirkung-Heilung, 2,5 mg ohne Wirkung.

subcutan: 2 x 20 mg Wirkung-Heilung, 2 x 10 mg Wirkung-Heilung, 2 x 5 mg ohne Wirkung. Judendliche Würmer: 25. Tag 100 mg Wirkung Rezidiv, 15. und 5. Tag ohne Wirkung.

Kaninchen-Giftigkeit: intravenös 30 mg tot, 20 mg lebt.

I.G. FARBENINDUSTRIE AKTIENGESELLSCHAFT
WERK ELBERFELD.

ppa. /s/ KIKUTH

Appendix 8

E x p o s é .-

G a v a n o .-

Chemie:

Gavano ist eine synthetisch dargestellte Base, die in Wasser leicht löslichen Hydrochlorid bildet (Ampullen). In den Tabletten leigt es in Form eines in wasser schwer löslichen Salzes vor, das vollkommen geschmacklos ist.

Pharmakologie:

Allgemeinwirkungen:

In Anbetracht der spezifischen Wirkung des Gavano gegen Amoeba histolytica war es von besonderem Interesse, seine pharmakologischen Eigenschaften im Vergleich zum bisher zur Behandlung der Amoebendysenterie verwendeten Emetin zu erkennen.

Wirkung des Gavano und des Emetin auf den Magendarmkanal:

Das Gavano hemmt die spontanen Bewegungen des isolierten Kaninchendarms noch in der starken Verdünnung von 1:100 000. Mit dieser Konzentration erreicht man einen atonischen Stillstand, der sich nur schwer durch Auswaschen beseitigen lässt. Im Gegensatz dazu hemmt Emetin sogar in 10 mal höherer Konzentration (1:10 000) die Darmmotilität nur teilweise. Der Effekt ist durch einmalige Waschung quantitativ zu beseitigen, Da Pilocarpin den Gavanostillstand des Darmes nicht beseitigt, liegt der Angriffspunkt des Gavano wie des Emetin in der glatten Muskulatur selbst.

In situ lässt sich die spasmolytische Wirkung des Gavano nicht nachweisen, weil bei der intravenösen Injektion gleichzeitig mit der spasmolytischen Wirkung auf den Darm eine Blutdrucksenkung erfolgt. Letzteres zeigt, dass die Strömungsgeschwindigkeit des Blutes verringert ist und damit der Kohlensäuretransport aus dem Gewebe verzögert wird. Im Gewebe entsteht also eine Kohlensäurestauung. Auf dieseble istdas Vaguszentrum ebenso wei Ganglienzellen des Darmes besonders empfindlich. Die beiden nervösen Apparate, die die Darmmotilität fördern, werden durch die Kohlensäurestauung erregt. Der Erfolg ist, dass am gesunden (narkotisierten) Tier die spasmolytische Wirkung des Gavano durch die Kohlensäureerregung überdeckt wird.

Anders liegen die Verhältnisse beim Menschen, insbesondere bei Amoebendysenteriekranken. Der durch die Amoebeninfektion bereits schwer geschädigte Darm am dem sich Ulcerationen ausbilden, ist auf Spasmolytica ganz besonders empfindlich (s.die Arbeiten von Foci). Deshalb isttzu erwer-

ten, dass die spasmolytische Wirkung des Gavano sich sicherlich vorteilhaft auswirken wird.

Am isolierten Uterus sieht man, dass auch hier das Gavano eine, allerdings nur geringe, erschlaffende Wirkung hervorruft. Mit Emetin lässt sich dieselbe kaum nachweisen.

Am Kreislauf findet eine Erschlaffung der kleinen Arterien durch Gavano statt. Sie äussert sich am narkotisierten Tier (Kaninchen, Katze) in einer geringen aber nachhaltigen Senkung des arteriellen Blutdrucks. Eine Pulsoveränderung findet während dieser Senkung nicht statt.

Bis zu einer Vergiftung mit der halben tödlichen Dosis sind die an sich unbedeutsamen Kreislaufwirkungen von Gavano und Emetin ungefähr dieselben. Nach höheren Dosen treten Hemmungen der Reizbildung und Reizleitung auf, die beim Gavano lediglich zu Pulsverlangsamung führen, während nach Emetin bedrohliche Irregularitäten (partieller Block, totaler Block, ventrikuläre Tachycardie usw.) erscheinen. Die Rhytmusänderungen nach Emetin bleiben entsprechend seiner kumulierenden Wirkung ausserordentlich lange erhalten.

Von den Funktionen der Medulla oblongata lässt sich zwischen Gavano und Emetin nur bezgl. der Atemtätigkeit eine Parallele findem. Beide Substanzen steigern die Atemtätigkeit nach Injektion von 1/4 der tödlichen Grenzdosis ganz erheblich. Das Respirationsvolumen kann dabei bis um das Freifache zunehmen. Nach höheren Dosen erfolgt eine Hemmung der Atemfrequenz und -tiefe.

Die bekannte Brechwirkung des Emetin, die nach parenteraler Applikation therapeutischer Dosen auch am Menschen beobachtet wird, fehlt beim Gavano parenteral im Tierexperiment auch nach höchsten Dosen völlig.

Lokale Reizwirkung:

Gavano übt einen Reiz auf das Gewebe aus. In 1-2%iger Lösung stellen sich im Quaddelversuch wie nach intramusculärer Injektion starke Infiltrate und Nekrosem eim. Am Kaninchenohr führt auch die intravenöse Injektionen zu Schwellungen und Entzündungen des Ohres. Gelegentlich stellen sich Thrombosen ein. Wird anschliessend an eine intravenöse Gavanoinjektion etwa physiologische Kochsalzlösung nachgespritzt, so lassen sich die gesamten entzündlichen Reaktionen dadurch vermeiden. Die lokalen Reizerscheinungen waren in allen Versuchen geringer als nach denselben Mengen Emetin.

Toxikologisches:

Bei der akuten Vergiftung durch intravenöse Injektion der tödlichen Grenzdosis tritt erst die eingangs beschriebene Tachypnoe auf. Es folgen Atmungsirregularitäten. Etwa 3 Minuten nach der Injektion gehen die Kaninchen an Krämpfen, begleitet von Laufbewegungen und ausgeprägten Opistotonus an primärem Herztod ein. Nach chronischer Vergiftung durch wiederholte subcutane Injektion verenden die Tiere unter langsamen Verfall. Die Sektion ergab Verfettung in der Nierenrinde und in den Übergangabschnitten der Harnkanälchen. Im Harn treten Cylinder und Eisweiss sowie Gallenfarbstoffe, Urobilibin und Urobilinogen auf. In der Milz fand sich eine hochgradige Atrophie der Follikel. Es liegen also Parenchymschädigungen vor.

Die tödliche Grenzdosis (D.l.m.) beträgt pro kg Tier für Gavano und vergleichsweise Emetin:

		Gavano	Emetin
1.Maus	subcutan	100 mg	65 mg
	i.v.(in 2 Minuten)	25 mg	30 mg
2. Kaninchen	subcutan	75 mg	30 mg
	intramuskulär	75 mg	20 mg
	i.v.(in 2 Minuten)	4-5 mg	3 mg
3. Katze	subcutan	150 mg	20 mg
	intramusculär	100 mg	15 mg
	i.v.(in 2 Minuten)	6 mg	6 mg
4. Hund	subcutan	-	-
	intramusculär	50 mg	5 mg
	i.v.(in 2 Minuten)	7 mg	5 mg

Kumulation:

Um die Kumulation, d.h. die Speicherungsfähigkeit einer Substanz exakt zu erfassen, muss die Resorption ausgeschaltet werden. Daher geben nur Kumulationsversuche, in denen die zu prüfenden Substanzen intravenös gespritzt werden, einwandfreie Ergebnisse. Die Kumulationsfähigkeit der einzelnen Tierspezies ist wechselnd. Nach allgemeiner Erfahrung ist die Kumulationsfähigkeit des Kaninchens geringer als die der Katz, die in dieser Beziehung meist mit dem Menschen parallel geht.

Versuche:

1. Kaninchen:

Tier a erhielt in 10 Tagen 10 x 1,5 mg Gavano pro kg Tier i.v.
also in 10 Tagen 3 3/4 tödliche Einzeldosen (4 mg)
Tier b erhielt in 6 Tagen 6 x3 mg Gavano pro kg Tier i.v.
also in 6 Tagen 4 1/2 tödliche Einzeldosen.

Da beide Tiere diese Injektionsserie symptomlos vertrugen, scheidet das Kaninchen täglich nindestens 80% der D.l.m. aus.

2. Katz:

Tier c erhielt in 7 Tagen 1 x 4 mg und 6 x 3 mg Gavano pro kg Tier i.v. Nach dem vierten Tag (13 mg) trat eiweiss im Harn auf. Erst 7Tage nach Abschluss der Injektionsserie ging das Tier anscheinend an Nierenschädigung ein. Es hatte während 7 Tagen 22 mg Gavano oder 5 1 2 tödliche Einzeldosen erhalten. Die Katze schied also rund 50% Gavano pro Tag aus. Gavano wird also bei intravenöser Injektion sehr schwach gespeichert.

Nach intramuskulärer und subcutaner Injektion des Gavano tritt also eine "scheinbare Kumulation" auf, die lediglich die Folge der vom Gewebe aus verhältnmässig langsamen Resoption ist.

Die ausserordentlich starke Kumulationsfähigkeit des Emetin ist lange bekannt. Mattei und Ribon fanden 1917 noch 60 Tage nach einer Emetininjektion Alkaloidspuren im Harn. Diese hohe Speicherungsfähigkeit ist ein sicheres Zeichen, dass der Organismus das Emetin weder abbauen nochentgiften kann. Die folgenden Experimente bestätigen die Kumulationsfähigkeit des Emetin: Die subcutane D.l.m. an der Katze beträgt 20 mg. Wird sie einem Tier in 2 Einzeldosen zu je 10 mg 10 mg am 1- und 4.Versuchstag appliziert, so geht es am 6. Tage ein. Erst 6 Einzelinjektionen zu je 4 mg in Verlauf von 6 Tagen, also 1 1/5 D.l.m. werden überlebt.

Sowohl nach dem akuten, wie nach dem Gewöhnungsexperiment ergab die histologische Untersuchung der Leber entweder gar keine Veränderung oder gutartige Verfettung nebst Stauungshyperämie; also uncharakteristische, sekundäre Symptome.

Ein Nachweis der Ausscheidung von Gavano ist nach den bisherigen Bemühungen noch nicht gelungen.

Zusammenfassung:

Der pharmakologische Vergleich zwischen Gavano und Emetin legte klar, dass beide Substanzen die glatte Muskulatur des Magendarmtraktus des Uterus und der Gefässe zur Erschlaffung bringen. Die Wirkung des Gevano auf die Darmmuskulatur ist intensiver und nachhaltiger. Beide Substanzen erregen das Atemzentrum anfänglich, um es schliesslich zu hemmen. Nur Emetin löst den Brechakt aus. Emetin ist bei einmaliger tödlicher Vergiftung wie bei partieller Vergiftung, nicht nur spezifisch erheblich giftiger, sondern auch in Folge seiner starken Kumulations-

fähigkeit Gavano kumuliert kaum. Es wird lokal besser vertragen als Emetin.

Chemotherapeutische Prüfung.-

Die spezifische Wirkung von Gavano auf die Amoeba hist., dem Erreger der Amoebenruhr, konnte zuerst in Vitroversuchen festgestellt werden. Gavano wirkt auf eine Amoeba histolyt.- Kultur in einer ganz analogen Weise, wie Emetin hydr. Eine besondere Versuchsanordnung zeigte, dass die Wirkung sowohl von Gavano wie von Emetin als spezifische angesehen werden musste, denn sie war im Prinzip eine andere als die von Yatren und Rivanol.

Die noch wirksame Konzentration beider Lösungen war in zahlreich wiederholten Vitroversuchen nicht immer konstant, was auf die besonderen sich ändernden biologischen Eigenschaften der Kulturamoeben zurückgeführt werden kann. Diese Schwankungen waren allerdings beim Emetin etwas weniger stark ausgesprochen als beim Gavano. Im grossen und ganzen kann man aber doch sagen, dass beide Substanzen in annähernd gleicher Konzentration noch wirksam waren, deren durchschnittlicher Wert mit 1/1 000 000 festgelegt werden kann.

Nachdem die spezifische emetinähnliche Wirkung von Gavano auf Kulturamoeben festgestellt worden war, wurden Versuche angestellt, um mit Amoeba hist. inficierte Katzen chemotherapeutisch zu beeinflussen.

Es ist bekannt, dass sich Katzen nur in einem kleinen Prozentsatz inficieren lassen. Ist aber einmal die Infektion erfolgt, so verläuft sie in der Regel sehr viel stürmischer und akuter als beim Menschen. Spontanheilungen werden im allgemeinen nur selten beobachtet, bei unseren Versuchen sind sie überhaupt nicht geschen worden.

Was die therapeutische Beeinflussung der Katzenamoebenruhr mit Emetin anbelangt, so verliefen alle diese Versuche, die von deutschen, englischen und amerikanischen Autoren angestellt worden waren, und von uns bestätigt werden konnten, negativ, d.h. eine Heilung war nicht möglich.

Es ist nicht ganz klar warum das Emetin bei der Katzenruhr versagt. Höchstwahrscheinlich liegt es daran, dass das Emetin auf Grund seiner stark ausgesprochenen kumulierenden Eigenschaften zu toxisch ist, die Wirkung aber längere Zeit in Anspruch nimmt, sodass eine Beeinflussung des akuten Infektionsverlaufs bei den Katzen nicht möglich ist.

Umso überraschender waren die Versuche mit Gavano und Präpara-

ten aus derselben Reihe. Nach 2 resp. 3 subcutanen Injektionen von bestimmten Mengen Gavano konnten in der überwiegenden Mahrzahl der Fälle im Darm der inficierten Katzen keine Amoeben mehr nachgewiesen werden.

In der Regel gelingt es mit 15 mg pro kg Katze an drei Tagen hintereinander subcutan (also in ganzen 45 mg) gegeben, die Amoebenruhr der Katzen zur Ausheilung zu bringen, nur in einzelnen Fällen sind Rezidive beobachtet worden, die mit ziemlicher Sicherheit vermieden werden können, wenn man den Katzen 15mg/kg an 4 Tagen oder 20 mg/kg an 3 Tagen hinteeinander gibt.

Man darf allerdings mit dem Beginn der Behandlung nicht allzu lange warten. Am besten ist, wenn man mit der Behandlung sofort oder am nächsten Tag beginnt, nachdem lebende Amoeben in Darm nachgewiesen werden konnen. Es konnten aber auch noch Heilungen erzielt werden, wenn die Behandlung erst am dritten Krankheitstage einsetze.

Wenn man in Betracht zieht, dass Katzen 120 mg/kg Gavano subc. an 6 Tagen hintereinander (20mg/kg täglich) vertragen, ohne Schädigungen irgendwelcher Art zu zeigen und dass es auf der anderen Seite gelingt, mit 45 mg/kg verteilt auf drei aufeinanderfolgende Tagesdosen von 15 mg/kg Heilungen zu erzielen, so hätten wir einen Index von 1:3.

Die chemotherapeutischen Erfolge sind deshalb besonders beachtenswert weil zum ersten Mal den Beweis erbrancht haben, dass die Amoebenruhr der Katze auf parenteralen Wege zu heilen ist.

Die gleichen günstigen Heilungsresultate erhält man (Versuch von Höchst), wenn man die rektale Applikation anwendet. Es genügt hierfür 1 ccm einer 5%igen Losung, die auf 10 ccm verdünnt und den Katzen per clysma eingeführt wird.

Schliesslich gelingt es auch, die amoebenkranken Katzen per os mit Erfolg zu behandlen. Die tödliche Grenzdosis lasst sich oral bei Katzen wegen Erbrechens nicht feststellen. 400 mg/kg sind aber mit Sicherheit symptomlos vertragen worden. Bei 600 mg/kg brechen die Katzen regelmässig.

Mit einer täglichen Dosis von 100 mg/kg Gavano an vier bis fünf aufeinanderfolgenden Tagen gegeben, gelingt es in der Regel die Katze rezidivfrei zu heilen. Unter Umständen genügen schon 75 mg/kg.

Diese Heilungserfolge bei der akuten Amoebeninfektion der Katzen, namentlich bei der parenteralen Therapie, geben uns die Berechtigung das Gavano auch bei der Amoenbenruhr der Menschen therapeutisch zu prüfen.

Da das Gavano emetinähnliche Wirkung aufweist, kommen für die klinische Prüfung in erster Linie solche Fälle in Betracht, bei denem Emetin dem Yatren und Rivanol vorgezogen wird, nämlich akute Fälle von Amoebenruhr, oder solche, die mit Hepatitis oder Leberabszess kompliziert sind.

Zweckmässig wäre es gewesen, die Behandlung am Menschen mit der intramuskulären Injektion zu beginnen. Zu diesem Zwecke wurde das Gavano in 5% Lösung in Ampullen zu 1 ccm geliefert, so dass jede Ampulle 50 mg Gavano enthielt. Als Tagesdosis wurden 100 mg vorgeschlagen, also vor-und nachmittags je eine Ampulle. Die Behandlung hätte man voraussichtlich über 6 Tage ausdehnen müssen.

Die intramuskuläre Behandlung kommt aber nicht in Betracht. weil das Gavano bei klinischen Vorversuchen bei intramuskulärer Injektion von den Patienten als äusserst schmerzauslösend empfunden wurde.

Aus diesem Grunde ist die perorale Applikation zu empfehlen. Die Behandlung per os ist nach Manson-Bahr bei der Amoebendysenterie jeglicher Injektionsbehandlung vorzuziehen. Sie lässt sich mit Emetin nur nicht durchführen, weil das Emetin vom Menschen per os nicht vertragen wird.

Bei Katzen braucht man, um mit der Behandlung per os denselben therapeutischen Effekt zu erzilen, etwa 4 die 5 bis 6fache Dosis der intramuskulären Injektion. Es ist in Betracht zu ziehen, dass Katzen peroral etwa 4-5 mal mehr vertragen als intramuskulär.

Beim Menschen wird man auf der Basis der intramuskulären Injektion von 100 mg täglich per os etwa 300-500 mg täglich geben müssen. Da die gelieferten Gevanotabletten 250 mg enthalten, ist zu empfehlen die Behandlung mit einer Tablette täglich zu beginnen.

Man wird aber diese Dosis bei guter - Verträglichkeit ohne Gefahr auf zwei Tabletten täglich steigern können. Evtl. wird man auch drei Tabletten täglich geben können. Die Behandlung wird man voraussichtlich auf 6 Tage ausdehnen müssen.

Was die intravenöse Behandlung anbetrifft, so gelten hierfür dieselben Bedenken wie beim Emetin.

Es muss mit besonderer Vorsicht vorgegangen werden, da bei zu grossen Dosen die Gefahr einer ernstlichen Herzschädigung nicht auszuschliessen ist. Im Tierversuch konnte gezeigt werden, dass Gavano intravenös etwas weniger giftiger ist als Emetin. Emetin wird vom Menschen intravenös 100 mg täglich vertragen. Für die intravenöse Injektion wird es deshalb zweckmässig sein, mit einem 1/2 ccm der 5% Gavanolösung, also mit 25 mg Gavano zu beginnen. Diese Injektion wird man 2 oder 3 mal täglich wiederholen können. Auch bei der intravenösen Injektion wird man voraussichtlich die Behandlung über mehrere Tage ausdehnen müssen. Eine kumulative Schädigung ist bei der intravenösen Applikation weniger zu fürchten als bei der intramuskulären. Bei der intravenösen Injektion ist besonders darauf zu achten, dass langsam injiciert wird. Eventuell soll etwas physiologische Kochsalzlösung nachgespritzt worden.

Schliesslich sei noch erwähnt, dass auch die Behandlung per clysma versucht werden kann.

Elberfeld, den 25. November 1931.

37526-1

Appendix 9

Production Methods and Reports from I.G. Elberfeld and Leverkusen.

First 12 Folders indexed by E. Kleiderer and listed in Team 110 Report.

Folder 13: Production methods of some Leverkusen products.

1. Study of the constitution of 2 methyl, 2 formyloxy 3 chlor, tetrahydrofuran.

2. A thru P. Miscellaneous library notes and comments on chemotherapy-inter company notes on subjects of cancer.

Folder 14: Chinolin Acrolin Synthesis (Prof. Tschitschibabine)

1. Intercompany correspondence on financial arrangements with Tschitschibabine on his patent.

2. Patent reference on T. process.

3. Photostat in French of T. Patent application to French patent office - gives full details of methods.

Folder 15: Report for year 1944 by Dr. Bauer (Elberfeld) assistant to Dr.Dörr, Development Department on his development work on Badional, and Adolin, with log of plant runs.

Folder 16: Report for year 1937 thru 1944 of develop. work done in Develop Dept by Dr. Deichsel assistant to Dr. Dörr, Elberfeld.

1937-
1. Report on Zephirol mfg. and purification
2. Report on Dontolol mfg.
3. Report on "Vermillion" a homologue of Rotylon.
4. Report on Ethylenbromide from Ethanol,
5. Bromine and Sulphurous acid.
5. Report on work to increase Italian Atebrin production.
6. Report on work on Clinical production.
7. Report on production of Diethanalamine
8. Report on work on Barbituric acid.

1938-
1. Formula for Mittigal solution for Spain
2. Saponified cresol for Veterinary use.
3. Report on mfg of Isobutylbromide from sodium bromide, isabutylalcohol and sulphurous acid
4. Manufacture of L-Dimethylamino - 3 bro.-butanes,
5. Calculations on plant run of Isobutylbromide
6. Italian "Iodazone M" and "Iodazone T".

7. Method and equipment for making L - Dimethylamino -3-methoxybutane (M.B. Amin) M.W. 131.
8. Method and equipment for making L - dimethyl- amino-3-butanehydrobromide (Bromaminobromide) M.W. 261.

1939-
1. D-179 for toothpaste
2. Report of visit of Dr. Hecht to Prof. Killian at Freiburg on testing of narcotics (alkaloids).
3. New method for making phenylacetic acid ethylester and methylester.
4. "Desil" detergent compared in pH to NaOH and sodium metasilicate.
5. Explanation of difference in laboratory and plant yields in making "Phenester M". (Phenylacetic acid ethylester)
6. Note on cost of Lonester M". (Phenylmalonic acid dimethylester)
7. Note on solvents in manufacturing of aspirin
8. Soap from C_5 to C_{10} acids for cattle grub control.

1940-
1. Galactose for use with Eupatin
2. Making of galactose from milk sugar.
3. Short monthly statements on work - no detail.

1941-
1. Discussion on Tibatin process and possibility of increasing production.
2. Improvement in 4,4- diaminodyphenylsulfon.
3. Tibatin + galactose experiments.
4. Detailed method for making galactose from lactose
5. Discussion on method for "E-Sulfon".
6. Detailed method for making Tibatin
7. Method for making Eupatin

1942-
1 Summary of years work, mentions purification of wheat germ oil, sublimation of aminodichloride oxidation of 8-oxychinoline, a substitute for Zephirol, work on Atebrine and Tibatin.
2. Discussion on B-Picolin and Degenal
3. Report on wheat germ oil
4. Report on wheat germ oil
5. Fractionation of Kollidon with acetone
6. Study on preparation of Nicotinic acid.

1943-
1. Report on wheat germ oil concentrate
2. Status of production of Tibatin
3. Purification of Amylehydrate

4. Report of work on 2-aminopymidin and 2-amino-4-methylpyrimidin.
5. Report on Propional
6. Report of Acetone vs petroleum ether to purify aspirin

1944-
1. Very short reports on nicotinic acid, galactose, 2-aminophyrimidin, prospinaldehyde, methyldebenal.

Folder 17: Report of Dr. Haensel, Develop. Dept. for 1944.
1. Method for isolating B-picolin from Pyridine III Fraction, and making nicetonic acid.
2. Removal of contamination from Prontosil.

Folder 18: Reports of Dr. Wiegand, Develop. Dept.

1937-
1. Expose on Bedoran (E 8977)
2. List of indazol derivates (phenyl, naphthyl, dihydro, methoxy, tetrahydra)
3. Literature search on 2,4, Dichlorbenoic acid.
4. Method for making septazin.
5. Method for 2-4 Dichlorotolulol.
6. Discussion on Astrocid.
7. Detailed method on analysis of Septazin.
8. Report on acetparamid and its chlorhydrate (dated Nov. 5, 1935)

1938-
1. Report on work on production of Methyl and Ethyl ethers of 2-methyl-2-oxy-3-chlortetrahydrofuren.
2. Detailed method and discussion on C Ester.
3. Method for preparation of Aricyl.
4. Preparation of Pyrazolene from Dialkylmalonicacid.
5. Preparation of Novantisol.
6. Work sheet on "W.214" (axphenyl-B-(p-methoxyphenyl)-acrylicacidnitrile)
7. Detailed method for production of Aricyl.

1939-
1. Brief discussion of sodium Prominal.
2. Discussion on patent application No.A 1316-34 on n-alkyl-methyl-barbituric acid.
3. Discussion of some oestrone syntheses.
4. Some substituted barbiturates.
5. Report on barbituric acid.
6. Patent disclosure on Molamidester (J-60-724)(IV D/120)

7. Patent disclosure - Dial and Urethane - new barbitrate.
8. Light absorption and constitution studies on Ethylene & diazomethane.
9. Details of laboratory method to make 2-methyl-2-methoxy-tetrahydrofuran from a-chlor-a-acetox-änthyl-acecit acid ester.
10. Analysis of Eupurgen.

1940-
1. Report of work on Sulphathiazol.
2. Report of work on Thiazol-pyrimidine.
3. Report of work on anthranilicacid analid.
4. Report of work on condensation of B-ketoacid-ester and Malonicacidester and their derivatives with 2-amino-thiazole.
5. Discussion of homologs of barbituric acid.
6. Analysis of sulphathiazol.
7. Patent discussion - "sun burn" salve with pyrentet-rasulfonic acid- also used in ringworm.

1941-
1. Report on sulphathiazol operations.
2. Report on Sulfapyridine.
3. List of products similar to Delial.
4. Detailed method for making sulphathiazol.
5. Work on using NaOH instead of Na_2CO_3 in the reaction of 2-aminothiazol with Prontylchloride.
6. Sulphathiazol for ampules.
7. Laboratory method for making sulphathiazol.
8. Laboratory method for 2-aminothiazol.
9. Condensation of Ethylene and azomethane.
10. Report on saponification of intermediates in production of sulphathiazol.
11. Report on lowering cost of purification of sulphapyridine.
12. Report on improvement of reacting prontyl-chloride with 2-aminothiazol.

Folder 19: Annual reports of Dr. Dörr, head of Develop. Dept. 1941 thru 1944. Gives subject index of work done by each member of department.

Folder 20: Patent application on thio-urea derivatives.

Folder 21: Report of Dr. Goth, Develop. Dept. for 1937, 1938 and part of 1939.

1937-
1. Report of work done on the stabilization of Phanodorm.
2. Short discussion on Prontosil S.
3. Short report on "Vogan" (Vit A).
4. Report on Sodium Salicylate granules for Uruguay.
5. Report on insect repellent work.

6. Detailed method for making Prontosil S.

1938-
1. Discussion of a substitute material for "Zeliokörner" (mouse bait)
2. Brief discussion on Tutocain.
3. Brief report on effervescent sodium salicylate for Uruguay.

1939-
1. Short notes on poison baits for mice.
2. Formula for effervescent aspirin tablets.
3. Short report on packaging studies on Paranoval.
4. Outline of patent application on mouse baits.
5. Short note on method of drying effervescent sodium salicylate for Uraguay.
6. Short discussion of poison wheat for mouse bait: Thallium fue; zinc phosphide strychnine

Folder 22: Reports for years 1937 and 1938 by Dr. Lautenschläger (later head at Hoechst)

1937:
1. Abstract of years report of work done on Atebrin, Vitamin B_1, Benzytol, Phäophytine.
2. Short report on Atebrine yield from various Italian pyridin samples.
3. Note on analysis of Halocrin-Phosphorous.
4. Short report on Atebrin work.
5. Note on Atebrin method
6. Note on Vitamin B_1 by Pyrimidine-Amid method.
7. Short report on Italian Pyridine
8. Detailed report on work to prepare a easily filtrable Halokrin-Phosphorus.
9. Brief description of method for Phaophytin from Blood meal.

1938-
1. Outline of work done in 1938 on Atebrin, "Dicarbon" intermediate for Halocrin, Dicarbon-K-Salt, Phäophytin from stinging nettle, nitrochinolin, aminochinolin, and Benzpyren.
2. Short note on method for Piperazine
3. Short note on 2-4 Dichlorobenzoic acid.
4. Report on work on Akiron
5. Detailed method on Atebrin
6. Detailed report on Halocrin (Dicarbon-K salt).
7. Further discussion on Atebrin - thru Halocrin
8. Detailed method for making Phäophytin.
9. Further work on Atebrin and its purification.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