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The Metabolic Fate of Thalidomide*

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In our studies on the metabolism of drugs endowed with central-sedative properties[1–4] we have also included thalidomide, as this hypnotic occupies a special position in comparison with other sedatives and hypnotics known to date. Before we describe the results of our investigations, we feel that it is important to discuss in some detail the peculiarities of thalidomide as regards its chemical structure, pharmacological activity, and toxicological properties.

Chemical Structure, Pharmacological Activity, and Toxicological Properties of Thalidomide

The peculiar nature of thalidomide, to which those who originally synthesised it have already drawn attention[5], becomes particularly evident when its structural formula is compared with that of other sedatives. Table I lists most of the known drugs exerting central-sedative effects (hypnotics, sedatives, and anticonvulsive agents) grouped according to their structural features and to the ring system they contain. A comparison of the structural formulae outlined at the top of the Table (A, B, and C) reveals that common to all the compounds listed is the grouping R_1R_2C–CO–N< (R_1 and R_2 being alkyl or aryl residues). Thus, these substances are derived from carboxylic acids substituted at the α-carbon atom with two hydrocarbon

* Generic name for the hypnotic known as Contergan® (Chemie Grünenthal AG.).

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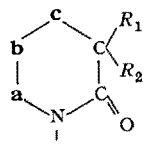
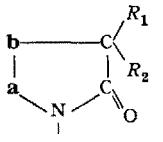
Tab. I



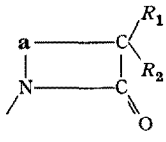
A

a	b	c	Ring system
CO	N–	CO	barbituric acids[7]
CO	CH_2	CH_2	glutarimides[2,8,9]
CO	CH	CH	glutaconimides[10,11]
CO	CH_2	N–	2,6-dioxo-piperazines[12]
CO	CH_2	O	3,5-dioxo-morpholines[13]
CO	O	CH_2	2,4-dioxo-tetrahydro-1,3-oxazines[14]
CO	N–	CH_2	dihydrouracils[15]
CO	CH_2	S	3,5-dioxo-thiomorpholines[16–19]
CH_2	CH_2	CO	2,4-dioxo-piperidines[20–23]
CH	CH	CO	2,4-dioxo-tetrahydro-pyridines[23,24]
CH_2	N–	CO	4,6-dioxo-hexahydro-1,3-diazines[25,26]
N–	CO	N–	3,6-dioxo-hexahydro-1,2,5-triazines[27]
CH_2	CH_2	CH_2	2-oxo-piperidines[28]



B

a	b	Ring system
CO	CH_2	succinimides[29,30]
CO	N–	hydantoins[24,31]
CO	O	2,4-dioxo-1,3-oxazolidines[24]
CO	S	2,4-dioxo-1,3-thiazolidines[29,32]
CH_2	CH_2	2-oxo-pyrrolidines[30]
CH_2	N–	4-oxo-imidazolines[33]

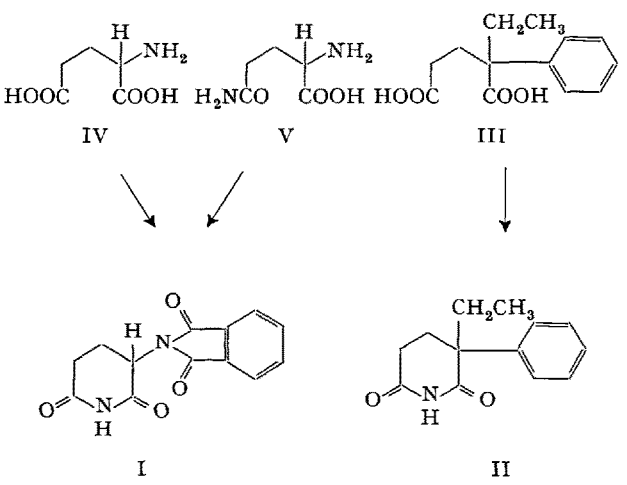


C

a	Ring system
CO	2,4-dioxo-acetidines[34]
CH_2	2-oxo-acetidines[35,36]

R_1 and R_2 in the formulae A, B, and C signify an alkyl or an aryl residue.

residues. The type of ring system into which this grouping is incorporated seems to be of minor importance. As will be seen from the structural formula of thalidomide (I), this compound cannot be assigned to any of the groups listed in Table I, because it bears, in the α-position, an acylated amino group instead of two hydrocarbon residues. Of all the sedatives known to date, thalidomide, a derivative of glutamic acid (IV) or glutamine (V), is the only preparation derived from a natural endogenous α-amino acid. Superficially, the formula of thalidomide might perhaps appear to resemble that of glutethimide[6] (II). Glutethimide, however, contains the same characteristic grouping as the other compounds listed in Table I and, like the others, bears two hydrocarbon residues in the α-position in the corresponding carboxylic acid (III).



It is not only in its chemical structure, however, but also with regard to its pharmacological properties that thalidomide displays differences as compared with the other known sedatives and hypnotics. In animal experiments[5,37–42] thalidomide was found to be a mild sedative with a rapid onset of effect. Its central-sedative action merely induces a reduction in spontaneous movement without any initial excitatory phase. Unlike preparations exerting an anaesthetic effect (barbiturates, glutethimide, ethinyl-cyclohexyl carbamate, and methylpentynol), thalidomide does not interfere with coordination of movement, even when given in very high doses[5]. Nor does it produce any anticonvulsive effect. One particularly striking feature of thalidomide is its extremely low toxicity in acute experiments. Even when the drug was administered in the highest possible dosage, it was impossible to determine the lethal dose or even to establish the dose at which an anaesthetic effect occurs—an observation which contrasts with the properties of the sedatives and hypnotics listed in Table I.

The findings emerging from animal experiments were confirmed in the course of clinical trials. Even when the drug was taken in extreme overdosage, it exerted no anaesthetic effect in man[43–45]. This cannot be ascribed solely to the fact that the compound is sparingly soluble in water, since both in animals[46] and in man[47] a considerable amount of the administered dose is absorbed.

In view of these facts it must be assumed that thalidomide constitutes a unique type of sedative[5].

During the year 1961, reports appeared in the medical press referring to neurotoxic side effects caused

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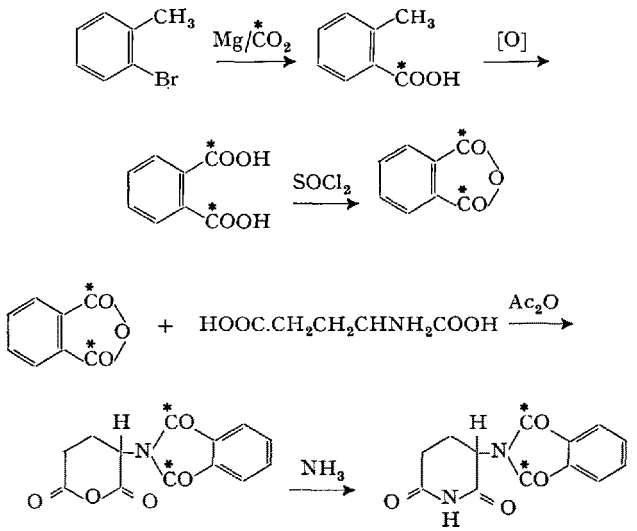
by thalidomide[48–59]. In November 1961, LENZ[60] voiced the suspicion that treatment with thalidomide during early pregnancy might be responsible for the occurrence of WIEDEMANN's syndrome[61] in Germany. With the publication of further papers on this subject[62–83], the opinion became more and more firmly established that thalidomide—if taken by pregnant women during the critical phase of limb development (4th–6th week of pregnancy)—could be the causative agent of deformities of this type. It would appear that, during this phase, even very small quantities of thalidomide are sufficient to cause congenital malformations[64 77]. Bearing in mind the fact that in adults an oral therapeutic dose of 100 mg thalidomide yields at the utmost a blood concentration of 0.9 μg/ml[47], it appears that only a few micrograms of the active substance may suffice in the embryo to produce a teratogenic effect. On the face of it, this seems hard to reconcile with the very low acute toxicity of thalidomide in man and animal, and raises the question whether it is the preparation itself or, more likely, one of its metabolites that is the factor adversely affecting the embryo.

Since nothing was known about the metabolites of thalidomide or their pharmacological and toxicological properties, investigations along these lines were undertaken to shed light on the cause of the neurotoxic damage caused by thalidomide and on the nature of the possible teratogenic factor.

Studies on the Metabolism of Thalidomide

For the animal experiments which we conducted with thalidomide, a radioactively labelled compound was prepared as outlined below:



This method of synthesis was deliberately chosen so as to ensure that the final product would be labelled in the phthalic acid residue. Thanks to this type of labelling, if thalidomide undergoes cleavage in the organism, it would still be possible to trace the exogenous phthalic acid or its derivatives. The ^{14}C-thalidomide prepared in this way had a specific radioactivity of 0.7 μC/mg; its chemical purity was demonstrated by radiochromatography, by measuring the melting point, and by its IR spectrum[84].

Tab. II. Excretion, in % of radioactivity administered, following a single oral dose of ^{14}C-thalidomide[a] in rats

	100 mg/kg (n = 6) urine %	faeces %
1st day	44.20	36.90
2nd day	4.59	7.40
3rd day	0.91	1.81
4th day	0.38	0.35
5th day	0.14	0.16
6th–10th day	0.22	0.20
	50.44	46.82
	97.26%	

[a] Suspended in 1.5% aqueous carboxymethyl cellulose.

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Tab. III. Thalidomide concentration[a] in μg/g organ after acute (single dose) and chronic treatment with ^{14}C-thalidomide in rats

Time	Gastro-intestinal tract	Liver	Lungs	Kidney	Adrenals	Fat	Muscle	Heart	Brain	Spleen	Skin[c]	Nerve[d]	Salivary glands	Plasma	Formed elements of blood[e]	Ratio of formed elements/plasma
1. Single dose[b]																
15 min	1066	21	12	10	1	12	6.7	4.5	5	4.3	5.9	5.7	6.7	4.7	1.5	0.31
30 min	1089	30	—	23	23	32	19	11	11	12	7.6	17	22	10	1.6	0.16
1 h	1184	31	15	32	29	4	15	14	14	17	14	17	18	15	2.3	0.15
2 h	824	45	20	57	50	—	38	22	25	28	7.3	27	28	28	4.5	0.16
4 h	753	53	26	71	56	5	28	23	30	31	25	45	32	32	10	0.31
8 h	518	38	17	60	36	9.4	31	28	21	19	14	28	25	21	10	0.48
16 h	116	11	8	19	11	8.4	5.6	2.6	4.6	5.6	7.2	6.8	5.6	4.5	3.8	0.84
1st day	242	7	4	16	5.6	2.8	9.6	3.9	4.1	4.4	2.9	6.8	4.3	3.3	1.8	0.55
2nd day	11	3.4	0.9	5.1	1.4	0.6	1.3	1.1	0.3	2.3	2	1.8	1.1	1.3	1.4	1.07
4th day	3.4	2.5	0.5	3	0.8	0.8	0.8	0.7	0.3	1.3	1.3	—	0.7	0.6	1.3	2.17
10th day	0.1	0.7	0.3	0.8		0	0.2	0.5	0.1	0.5	0.4	—	0.3	0.1	0.7	7
%[f]		1.3	1.2	1.1		0	0.5	1.8	0.3	1.6	1.6		0.9	0.3	7	
2. Chronic treatment[b]																
6th day[g]	2.2	6.4	2.2	8.8	4.6	0.6	6.1	4.0	1.9	6.4	7.1	4.4	2.0	1.4	10.6	7.57
12th day[g]	0.9	2.0	1.6	5.3	3.8	0.5	3.4	3.8	1.8	4.2	5.2	3.3	0.9	0.3	9.3	31

[a] Average values for 6 animals. The thalidomide concentration was calculated from the radioactivity measured. [b] The preparation was administered suspended in 1.5% aqueous carboxymethyl cellulose. [c] Dorsal skin. [d] N. gastrocnemius. [e] The formed elements were obtained by centrifuging whole blood to which oxalate had been added beforehand. [f] Concentration after 10 days in % of the maximum concentration measured in the organ in question. [g] Time elapsed following the last dose.

To study the metabolism of ^{14}C-thalidomide, the compound was administered orally to albino rats and to dogs under various conditions. The results obtained in rats following a single oral dose are listed in Table II. The air exhaled by the animals was also tested; its total content of radioactivity, measured throughout the entire experiment, was found to be less than 0.1% of the dose of radioactivity administered. It can therefore be concluded that no radical degradation or decarboxylation of the phthalic acid residue occurs.

In order to study the distribution and possible accumulation of thalidomide in the animal organism, we administered the ^{14}C-preparation in a single dose of 110 mg/kg to each of 6 rats, which were then sacrificed after varying intervals of time. The individual organs were removed and their radioactivity measured. Similarly, we also examined the organs of rats which had received the compound for 28 days at a dose of 50 mg/kg and which were sacrificed either 6 or 12 days after the last dose. Table III shows the results of both experiments.

The data reproduced in Table III show that thalidomide is evenly distributed throughout almost all the organs. The maximum concentration is attained after about 4 h and averages 40 ± 15 μg/g, with the exception of the higher values found in the gastrointestinal tract and the kidneys. The values for the body-fat and formed elements in the blood are somewhat lower than for the other organs; moreover, in the case of the body-fat the maximum is attained later than in the other organs.

Some idea of the rate at which the preparation is eliminated from the various organs was obtained by calculating the concentration still present after 10 days as a percentage of the maximum concentration recorded

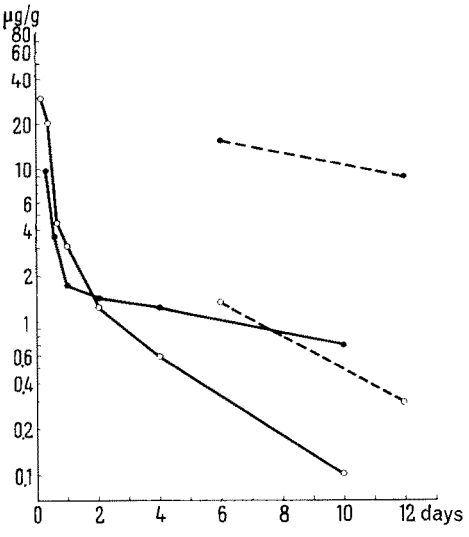


Fig. 1. Thalidomide content in μg/g plasma and formed elements in the blood, following a single dose and following chronic administration in rats (••• formed elements, ··· plasma, — single dose, - - - chronic administration).

in the organs in question (Table III). These values range from 0 to 1.8%, except in the case of the formed elements in the blood, which show a far higher value (7.0%). In Figure 1 the concentrations measured in the blood cells and in the plasma are shown separately. During the first 24 h the concentrations in both fractions decrease at the same speed, whereas later the rate of elimination from the formed elements is much slower. The same finding also emerges from the results of the 28-day experiments. It remains to be seen whether this phenomenon is due to a difference in the adsorption of thalidomide or its metabolites, or, possibly, to chemical incorporation of one of these compounds into the blood cells.

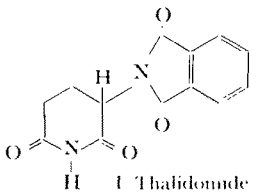
Finally, experiments in which the distribution of ^{14}C-thalidomide (100 mg/kg) was studied in pregnant rats have shown that thalidomide or its metabolites pass through the placental barrier, since the concentration measured in homogenates prepared from newborn rats was roughly the same as in the parent animal.

In a further effort to clarify the metabolism of thalidomide, the nature of its excretion products was studied in the following experiments on dogs.

^{14}C-Thalidomide was administered orally in gelatine capsules to 2 dogs for 10 days in a dosage of 100 mg/kg daily. 27.9% of the radioactivity administered was excreted in the urine; the rest was found in the faeces. Analysis based on the isotope-dilution technique revealed that all the radioactivity in the faeces was accounted for by unchanged thalidomide, i.e. by thalidomide present in the same form as had been administered; on the other hand, unchanged thalidomide accounted for only 1.8% of the radioactivity in the urine. From a sample of untreated urine we were able to extract approximately 2% of the radioactivity present. After various purification procedures, crystalline thalidomide was isolated from this extract. Hydrolysis with hydrochloric acid was necessary in order to extract the remaining radioactivity from the sample; isotope-dilution analysis showed 95% of the labelled material obtained from the hydrolysed urine to be phthalic acid. Therefore, the bulk of the thalidomide metabolites must contain an intact phthalyl residue.

Various methods for concentrating the metabolites present in the urine invariably yielded highly radio-

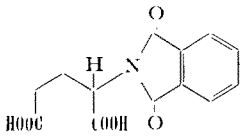
Tab. IV. Theoretically possible hydrolytic breakdown products of thalidomide



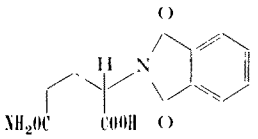
I Thalidomide

Group A

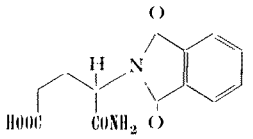
Single ring opening



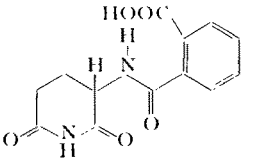
VI N-phthalyl-*D,L*-glutamic acid



VII N-phthalyl-*D,L*-glutamine



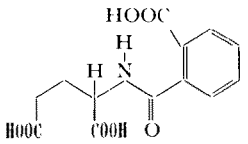
VIII N-phthalyl-*D,L*-isoglutamine



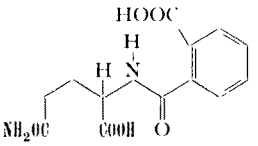
IX N-[o-carboxybenzoyl]-*D,L*-glutamic acid imide

Group B

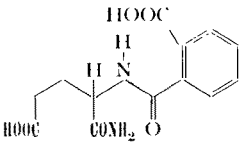
Double ring opening



X N-[carboxybenzoyl]-*D,L*-glutamic acid



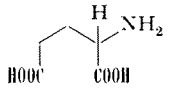
XI N-[o-carboxybenzoyl]-*D,L*-glutamine



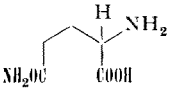
XII N-[o-carboxybenzoyl]-*D,L*-isoglutamine

Group C

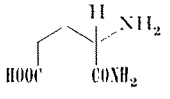
Cleavage of the molecule



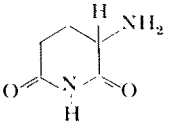
XIII *D,L*-glutamic acid



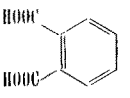
XIV *D,L*-glutamine



XV *D,L*-isoglutamine



XVI *D,L*-glutamic acid imide



XVII phthalic acid

active fractions containing substances of an acidic nature which, following total hydrolysis with hydrochloric acid, gave rise to phthalic and glutamic acids which were identified by qualitative paper chromatography. After methylation of the concentrated metabolites and separation of the resultant esters by thin-layer chromatography, it was found that the radioactivity excreted in the urine was divided among several different acids. The same finding was obtained when concentrates of the free acids were subjected to high-voltage electrophoresis in suitable buffer systems.

Since in the hydrolysis experiments it had so far only been possible to trace unsubstituted phthalic acid and glutamic acid in the concentrated fractions, it was deduced that the major portion of the acids excreted in the urine must be compounds derived from thalidomide by mere hydrolysis. Theoretically, these could be any of the compounds listed in Table IV. In order to determine the properties of these compounds and to demonstrate their presence in the urine both qualitatively and quantitatively by means of isotope-dilution analysis, an authentic sample of each was prepared synthetically.

Compounds VI, VII, and VIII were synthesised from N-phthalyl-*D,L*-glutamic acid anhydride by known methods[85]. Acid IX, which has not been described before, was obtained by condensation of *D,L*-glutamic acid imide[86] with phthalic acid anhydride. The acids of Group B were synthesised by partial hydrolysis from the corresponding compounds of Group A. The identity and purity of the compounds were established with the aid of IR and UV spectroscopy, chromatography, and electrophoresis. On the basis of the partition coefficients, which were determined in several solvent systems, it was to be expected that a mixture of the various hydrolysis products could be separated by countercurrent distribution.

The method adopted was as follows: 100 ml of the dog's urine was first concentrated by partial evaporation at low temperature and then brought to pH 2, after which it was distributed between 1/100 *N* hydrochloric acid and butyl alcohol in a 100-step Craig apparatus. To measure the radioactivity, an aliquot portion of each fraction was used after mixing the upper and lower phases by addition of methanol. The distribution pattern (Figure 2) clearly reveals that several metabolites occur in the urine. Of the theoretically possible products of hydrolysis outlined in Table IV, the amidocarboxylic acid IX was reliably identified in Fraction C, and the amidocarboxylic acids VII and VIII in Fraction E.

Now that the presence of some of the possible hydrolysis products had been confirmed by the method described, the concentration of these compounds present in the urine was determined quantitatively by separate isotope-dilution analyses. As shown in Table V, we were able in this way to account for 60% of the radioactivity excreted in the urine.

It is not yet possible to offer quantitative data on the presence of derivatives XI and XII from Group B, as these compounds were only recently obtained in crystalline form. Their partition coefficients would seem to indicate that they, too, are likely to be found within the main peaks of the Craig distribution curve. It therefore appears probable that these substances are present in the urine and that they will account for a large proportion of the urinary excretion products not yet identified. The cleavage products XIII to XVI, listed in Group C, cannot be traced radiochemically, as they no longer bear the labelled phthalyl residue. From

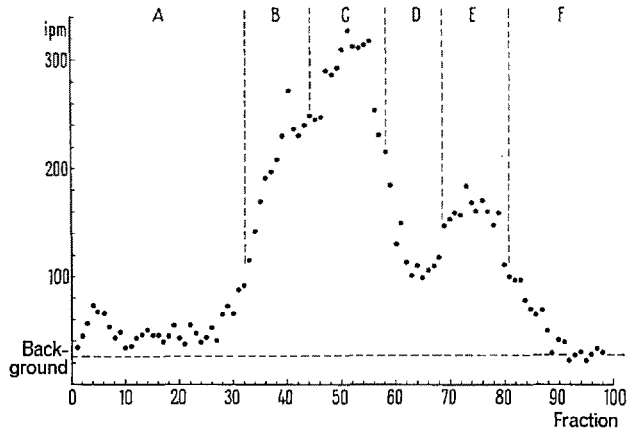


Fig. 2. 100-step Craig distribution of substances contained in the urine (dog), following administration of ^{14}C-thalidomide.

Tab. V. Excretion of various compounds in the urine and faeces of dogs following oral treatment with ^{14}C-thalidomide (100 mg/kg for 10 days)

	In % of radioactivity administered[a]	
	urine	faeces
Total accounted for	27.9	64.1
Identified by isotope-dilution analysis:		
Thalidomide I	0.5 (1.8)	62.0 (96.7)
N-phthalyl-glutamic acid VI	2.5 (9.0)	
N-phthalyl-glutamine VII	3.8 (13.6)	
N-phthalyl-isoglutamine VIII	0.6 (2.2)	
N-[o-carboxybenzoyl]-glutamic acid imide IX	6.4 (22.9)	
N-[o-carboxybenzoyl]-glutamic acid X	1.3 (4.7)	
Phthalic acid XVII	1.7 (6.1)	
	16.8 (60.3)	

[a] The figures in brackets refer to the radioactivity excreted in the urine or faeces.

[85] F. E. KING, B. S. JACKSON, and D. A. A. KIDD, J. chem. Soc. (London) *1951*, 243.

[86] E. SONDHEIMER and R. W. HOLLEY, J. Amer. chem. Soc. *79*, 3767 (1957); *76*, 2467 (1954).

the calculated content of phthalic acid in the total urine (Table V), however, it can be deduced that, at the most, only 6.1% of the thalidomide absorbed is broken down into such compounds.

In Table V the excretion products identified in the urine and faeces are expressed as a percentage of the dose administered. From this it will be seen that approximately 80% of the dose administered has been accounted for by compounds of known constitution.

Discussion of Results

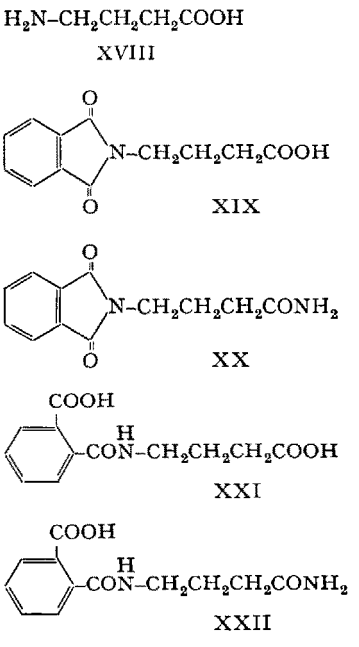
When thalidomide is administered in an oral dose of 100 mg/kg in the rat, roughly 50% is absorbed, and in the dog roughly 30%. The non-absorbed portion is excreted unchanged in the faeces. Similar findings have recently been published by MACKENZIE and MCGRATH[46] in rats. According to these authors, when single oral doses of 10, 100, and 1000 mg/kg were given, the portion excreted in the urine amounted to 40.8%, 39.4%, and 28.8%, respectively. Thus, over a wide range of dosage, the percentage of substance absorbed is independent of the size of the dose administered. Absorption takes place fairly rapidly, maximum concentrations being attained in the blood and organs after only 4 h (Table III). The substance circulating in the blood at this time—as shown by MACKENZIE and MCGRATH—consists mainly of unchanged thalidomide.

Our results indicate that thalidomide or its metabolites become evenly distributed throughout the various tissues and organs. The fact that higher concentrations are encountered in the gastro-intestinal tract and the kidneys is not surprising, as these organs are directly involved in the processes of absorption and excretion. One remarkable feature is the strikingly low affinity of thalidomide for the fat depots. By way of contrast, it is a well-known fact that the short-acting barbiturates, as well as non-barbiturates such as glutethimide, show a marked tendency to become rapidly but transiently concentrated in the adipose tissues[1].

Once absorbed, thalidomide is relatively quickly excreted in the urine, almost entirely in the form of metabolites. The metabolites in the dog are products of thalidomide hydrolysis and consist chiefly of derivatives of glutamic acid. In this respect, the metabolism of thalidomide differs markedly from that of the other known hypnotics and sedatives. As a rule, the latter are inactivated by oxidation—in other words, by the introduction of an hydroxyl group or by the formation of carbonyl or carboxyl groups, usually in the lipophilic portion of the molecule, i.e. the hydrocarbon moiety. Some of the resultant metabolites are excreted in the urine in the form of conjugated products[87]. Studies on the metabolism of glutethimide[1], for example, have shown that this hypnotic is inactivated by oxidation, i.e. by the introduction of hydroxyl groups, and by subsequent conjugation with glucuronic acid.

If the hydrolytic cleavage of the thalidomide molecule in the organism is due to the intervention of enzymes, then one would expect to obtain optically active excretion products. As thalidomide is a racemate and can therefore be regarded as being derived in equal portions from the natural *L*-glutamic acid, and from the unnatural *D*-glutamic acid, one would also expect to find in the urine optically active metabolites of both the *D* as well as the *L* series. As far as isotope-dilution analysis is concerned, the optical activity of the substance being measured is unimportant, provided a sufficiently large excess of racemic material is added. On the other hand, this method can provide no information as to the absolute configuration of the substance analysed. To determine whether the metabolites are optically active, they would have to be isolated in pure form—a task presenting considerable difficulties.

Table IV lists all the possible cleavage products of thalidomide resulting only from hydrolysis. The possibility that secondary products might be formed from these substances by other enzymatic reactions has been ignored; that such products may occur, however, is an eventuality which cannot be excluded. In particular, it is conceivable that γ-aminobutyric acid derivatives may be produced by decarboxylation of the C_1-carbon in the glutamic acid chain. In this connection, it is intended to undertake analytical studies in order to determine whether compounds XVIII to XXII occur in the urine.



Possible Reasons for the Side Effects of Thalidomide

The fact that thalidomide is the only known sedative whose metabolites are glutamic acid derivatives, and that it possesses neurotoxic and possibly teratogenic properties, gives rise to a number of considerations and

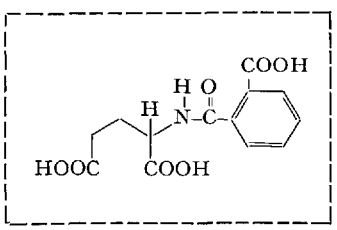
[87] W. BLOCK and J. EBIGT, Arzneimittelforsch. *10*, 709 (1960).

speculations which might serve as a guide to further experiments.

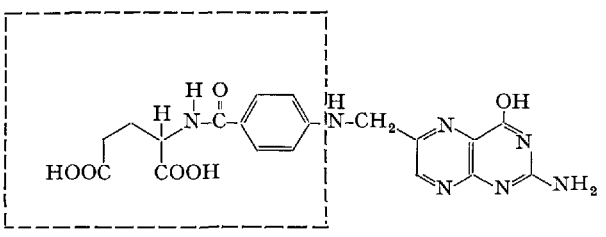
Glutamic acid is known to be involved in a wide variety of biochemical processes[88]. Apart from its importance as a component of proteins, it occupies a key position at many points within the network of intermediary metabolism. *Via* α-ketoglutaric acid it is concerned in the citric acid cycle, and thereby with carbohydrate and fat metabolism. In amino-acid and protein metabolism it plays a major role as an $-NH_2$ donor during transamination. The fact that it is maintained in equilibrium with glutamine means that it is directly concerned with the synthesis of urea. The important function it fulfils in connection with the physiology of nervous tissue is evidenced by the fact that it is the precursor of γ-aminobutyric acid[89]. Finally, it should be mentioned that biological substances closely related to glutamic acid, such as glutamine, glutathione, carbamyl-glutamine, and folic acid, likewise play important physiological roles. Glutamic acid has also proved of limited clinical interest in certain neurological disorders[90–93].

The thalidomide metabolites containing a phthalic acid residue or derived from the *D* series are unnatural glutamic acid derivatives, i. e. they do not occur in nature. It is therefore possible that they may in some way interfere with the biochemical and physiological functions of natural glutamic acid or its derivatives, i.e. by taking the place of the latter or by blocking enzymes or coenzymes (e.g. oxidases, dehydrogenases, acylases, transaminases, glutaminase, etc.) which are involved in the metabolism of these compounds. Some evidence pointing in this direction is already emerging:

KEMPNER[94] has demonstrated that thalidomide interferes with the growth and sexual development of cockerels and has drawn attention to the possibility that thalidomide or its metabolites may be folic-acid antagonists. The biological importance of folic acid is well known[95]. Folic acid has the structure XXIII shown below:



X



XXIII

A comparison between this formula and that of metabolite X reveals a similarity between the two structures:

ROBERTSON[57] and TEWES[96] recently reported independently that they had successfully treated thalidomide neuropathies with vitamin B; MURPHY. DAGG, and KARNOFSKY[97] have investigated the effect of various teratogenic compounds in chicken and rat embryos. They found that the teratogenic effect of the nicotinamide antagonist, 6-amino-nicotinamide, could be counteracted by the simultaneous administration of nicotinamide. LECK and MILLAR[98] have suggested that thalidomide may interfere with the metabolism of riboflavin, as riboflavin deficiency is capable of producing malformations in rats. Finally, the findings reported a number of years ago by ABDERHALDEN[99] and FISHMAN and ARTOM[100] should be mentioned. It was found that vitamins of the B group reduced the toxicity of *D,L*-amino acids. A causal connection between the findings mentioned above can be sought in the fact that the vitamins of the B group constitute important co-factors for numerous enzymes such as the oxidases, the transaminases, and glutamic acid-γ-aminobutyric acid decarboxylase.

In recent years, several review articles dealing with the problem of congenital malformations have appeared[101–109]. So far, some 70 different methods are known by which malformations can be produced in animals; most of them are based on the induction of a metabolic disorder in the pregnant animal during the sensitive phase of embryonic development. These metabolic disorders can be produced experimentally by a wide variety of methods, e.g. by supplying a diet deficient in vitamins or by administering specific

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[93] H. WEIL-MALHERBE, Naturwiss. *40*, 541 (1953); *41*, 124 (1954).
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[96] H. TEWES, Münch. med. Wschr. *104*, 269 (1962).
[97] M. L. MURPHY, C. P. DAGG, and D. A. KARNOFSKY, Pediatrics *19*, 701 (1957).
[98] J. M. LECK and E. L. M. MILLAR, Brit. Med. J. *1962*, II, 16.
[99] E. ABDERHALDEN and E. TETZNER, Z. physiol. Chem. *232*, 79 (1935).
[100] W. H. FISHMAN and C. ARTOM, Proc. Soc. exp. Biol. Med. *57*, 241 (1944).
[101] J. G. WILSON, J. chron. Dis. *10*, 111 (1959).
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[104] R. MCINTOSH, J. chron. Dis. *10*, 139 (1959).
[105] H. JACKSON, Pharmacol. Rev. *11*, 135 (1959).
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[109] G. E. W. WOLSTENHOLME and C. M. O'CONNOR (Editors), Ciba Foundation Symposium on Congenital Malformations (J. and A. Churchill Ltd., London 1960). – Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity (J. and A. Churchill Ltd., London 1958).

vitamin antagonists or antimetabolites. It is significant that, in most instances where a vitamin deficiency has a teratogenic effect, the vitamins in question belong to the B group, including particularly riboflavin and folic acid. A deficiency of the latter, or their blockade by antagonists such as galactoflavin, x-methyl-folic acid, or amethopterin, leads to severe malformations affecting the limbs and various organs. Among the antimetabolites, the glutamine antagonists azaserine (o-diazoacetyl-*L*-serine) and DON (6-diazo-5-oxo-*L*-norleucine) have attracted particular interest as potent teratogenic factors[109].

Although proof is still lacking that the neurotoxic and possible embryotoxic effects of thalidomide or its metabolites are due to faulty glutamic acid metabolism, this assumption would appear to offer an attractive working hypothesis.

Zusammenfassung. In einer vergleichenden Betrachtung werden die chemische Struktur, die pharmakologischen und toxikologischen Eigenschaften von Thalidomid diskutiert. Dabei zeigt sich, dass Thalidomid unter den heute bekannten Sedativa und Hypnotica eine besondere Stellung einnimmt.

Für die beschriebenen Tierversuche wurde ein mit ^{14}C markiertes Präparat verwendet. Bei Ratten wurde die Resorption, die Verteilung in den einzelnen Organen und die Ausscheidungsgeschwindigkeit nach einmaliger wie auch nach chronischer oraler Verabreichung studiert. Von der verabreichten Radioaktivität werden ca. 40% resorbiert. Die resorbierte Menge verteilt sich rasch in allen Organen und wird in verhältnismässig kurzer Zeit ausgeschieden. Eine Ausnahme bildet die Elimination aus den Blutkörperchen, die auffallend langsam vor sich geht.

Die chemischen Veränderungen, die Thalidomid im tierischen Organismus erfährt, konnten weitgehend aufgeklärt werden. Beim Hund werden ca. $^2/_3$ des verabreichten Präparates unverändert mit den Faeces eliminiert. Die im Urin ausgeschiedene Radioaktivität liegt zur Hauptsache in Form von Metaboliten vor. Von diesen konnten bisher 6 Substanzen, entsprechend 60% der im Urin vorhandenen Radioaktivität, quantitativ erfasst und identifiziert werden. Alle diese Verbindungen sind Glutaminsäurederivate, die aus Thalidomid durch hydrolytische Aufspaltung entstanden sind. Der Abbau von Thalidomid im Organismus führt daher zu einer Anzahl von Stoffwechselprodukten, die Derivate einer biogenen Aminosäure sind. Auf Grund dieser Feststellung werden verschiedene Hypothesen über die möglichen Ursachen der Nebenwirkungen von Thalidomid diskutiert.

Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Alkaloid Studies[1]. The Structure of Aspidofiline

Three alkaloids–pyrifolidine (I)[2], pyrifoline (II)[2], and aspidofiline[3]–have been isolated from the Brazilian tree *Aspidosperma pyrifolium* Mart. and the structures of the first two (I, II) have recently been elucidated[4,5]. We should now like to report evidence which leads to the assignment of expression III to aspidofiline.

Earlier studies[3] attributed the empirical formula $C_{20}H_{22}N_2O_2$ to aspidofiline and also indicated the presence of an N-acyldihydroindole moiety and of a strongly hydrogen-bonded phenolic group. The presence of these two structural features was confirmed by the n.m.r. spectrum[6], which exhibited a signal at 2.30 ppm due to the N-acetyl grouping and one at 10.13 ppm associated with a hydrogen-bonded C-17 phenolic grouping (see aspidocarpine[7] and spegazzinidine[8]). Furthermore, the n.m.r. spectrum established the absence of an ethyl group or of the C-2 hydrogen (quartet in the 3.8–4.5 ppm region[9]) typical of alkaloids based on the aspidospermine skeleton (e.g. I), but it did show signals in the 6.70–7.35 ppm region for the three aromatic protons. Chemical confirmation for the presence of the phenolic grouping was adduced by acetylation (20 h refluxing with acetic anhydride in benzene) to 0-acetylaspidofiline (IV) (m.p. 179–181°, $[\alpha]_D^{30} + 53°$ (all rotations in chloroform)) or methylation (diazomethane in methanol; 24 days at 0°) to 0-methylaspidofiline (V) (colorless glass distilled at

[1] This paper represents part XXXVII. For preceding paper see H. Vorbrueggen and C. Djerassi, J. Amer. chem. Soc. *84*, in press (1962).

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[3] L. D. Antonaccio, J. org. Chem. *25*, 1262 (1960).

[4] C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert, and L. D. Antonaccio, Tetrahedron *16*, 212 (1961).

[5] B. Gilbert, J. M. Ferreira, R. J. Owellen, C. E. Swanholm, H. Budzikiewicz, L. J. Durham, and C. Djerassi, Tetrahedron Letters 59 (1962).

[6] Measured with a Varian A-60 spectrometer in $CDCl_3$ solution with tetramethylsilane as internal standard ($\delta = 0.0$ ppm). All signals are reported in ppm as δ units (δ = cps/60).

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