determinations give values of 425 ± 21. There are three primary amino groups (Van Slyke), one N-methyl, and one C-methyl (Kuhn–Roth) in both III and IV.[7] C_1 and C_2 give positive Elson–Morgan and ninhydrin tests, and negative maltol, furfural, and Sakaguchi reactions. The two bases are very similar in all of their properties and are undoubtedly closely related in structure. Upon N-acetylation of III and IV, the parent triacetyl derivatives, I and II, are regenerated, respectively. That III and IV are not artifacts of the vigorous basic hydrolysis is shown by the identities of their paper chromatographic mobilities, rotations, infrared spectra, and elemental analyses with the same constants of gentamicin (the parent mixture). Acid hydrolyses of gentamicin, obtained either by reconstitution from III and IV or directly from the fermentation, affords mixtures with identical paper chromatographic patterns. From the acid hydrolyses of III, IV, and gentamicin, 2-deoxystreptamine is formed. The nature of the co-produced degradation products is under study, as are the total structures of III and IV.

The *in vitro* activity of gentamicin was determined by the twofold serial tube dilution method and results show this new antibiotic to be highly active against Gram positive and Gram negative bacteria. The acute toxicity (LD_{50}) of gentamicin in mice is 72 mg./kg. intravenously, 484 mg./kg. subcutaneously, 433 mg./kg. intraperitoneally, and greater than 9050 mg./kg. by oral administration. The therapeutic activity of gentamicin has been demonstrated by subcutaneous administration in mice infected intraperitoneally with *Klebsiella pneumoniae, Salmonella schottmuelleri, Pseudomonas aeruginosa, Diplococcus pneumoniae, and Staphylococcus aureus.*

TABLE II

ANTIBACTERIAL SPECTRUM OF GENTAMICIN

Microorganism	Minimal inhibitory concentrations.[a] (γ-base/ml.)
Aerobacter aerogenes	0.6
Alcaligenes fecalis	.6
Bacillus subtilis	.012
Escherichia coli	1.2
Klebsiella pneumoniae	0.14
Proteus mirabilis (5 strains)	.8–2.0
Proteus rettgeri	.8
Proteus vulgaris	4.8
Pseudomonas aeruginosa (4 strains)	0.08–0.20
Staphylococcus aureus (8 strains)	.028–0.30
Staphylococcus aureus (10 penicillin resistant strains)	.5
Salmonella schottmuelleri	1.2
Salmonella typhimurium	2.4
Streptococcus fecalis	9.6

[a] Gentamicin sulfate potency: 647 γ base/mg., Difco Antibiotic Medium No. 3 (Penassay Broth), Difco Laboratories, Detroit 1, Michigan.

Extensive toxicity studies with gentamicin demonstrate that the following doses can be administered chronically intramuscularly without demonstrable toxicity: 5.6 mg./kg. for at least 50 days in dogs; 12 mg./kg. for at least 40 days in cats; 40 mg./kg. for at least 40 days in rats. Considerably higher doses, 40 mg./kg. in dogs and 100 mg./kg. in rats, regularly produce renal tubular necrosis and vestibular function damage in 30 days. Pharmacological studies show that the antibiotic is almost completely excreted by glomerular filtration with some biliary excretion. Intramuscular administration in dogs gives blood levels for approximately 6 to 8 hr., and peak titers at 1 hr. are approximately 2.0 γ/ml. with a 1.0 mg./kg. dose. Fractionated urine samples show maximal antibiotic levels 50 times the peak blood levels, and 50 to almost 100% of the administered dose is excreted in 24 hr.

Intramuscular administration of single 0.2–3.2 mg./kg. doses to 40 normal volunteers showed onset and duration of blood levels and excretion patterns similar to the dog, though human blood levels, *e.g.* 4.0 γ/ml. at 1 mg./kg. dose, were double canine levels. The peak blood levels in volunteers given single doses of 0.4 mg./kg. intramuscularly averaged 1.6 γ/ml. and were above those levels necessary to inhibit the growth of most *Proteus* and all *Pseudomonas* strains tested as well as most other Gram negative bacteria and for penicillin sensitive and resistant *Staphylococcus*. The peak average antibiotic level in the urine in fractionated specimens at the 0.4 mg./kg. dose was 70 γ/ml. Single oral doses up to 1500 mg. of gentamicin in man result in approximately 0.2% absorption as detected in 24 hr. urines, with no measurable blood levels.

Gentamicin is being studied extensively in the clinic for the treatment of infections caused by Gram negative bacteria.

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(7) Nuclear magnetic resonance measurements in D_2O and deuteriopyridine solutions confirm the presence of both C-methyl and N-methyl in C_1, C_2, and their respective amides.

Effects of *d,l*-3-Phthalimidoglutarimide and N-Phthalyl-*d,l*-aspartimide on Rat Pregnancy

Sir:

Thalidomide-induced malformations have been reported in mice,[1,2] rats,[3–5] and rabbits.[1,2,6–9] It also

(1) A. Giroud, H. Tuchmann-Duplessis, and L. Mercier-Parot, *Lancet*, 298 (1962-II).

(2) A. Giroud, H. Tuchmann-Duplessis, and L. Mercier-Parot, *Compt. Rend. Soc. Biol.*, **156**, 705 (1962).

(3) C. T. G. King and F. J. Kendrycek, *Lancet*, 1116 (1962-II).

(4) G. Bignami, D. Bovet, F. Bovet-Nitti, and V. Rosnati, *ibid.*, 1333 (1962-II).

(5) G. Bignami, F. Bovet-Nitti, and V. Rosnati, IIIrd International Meeting in Forensic Immunology, Medicine, Pathology & Toxicology, London, April, 1963.

(6) C. F. Somers, *Lancet*, 912 (1962-I).

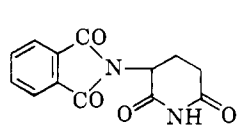
(7) K. E. V. Spencer, *ibid.*, 100 (1962-II).

(8) M. J. Seller, *ibid.*, 249 (1962-II).

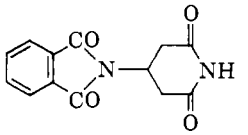
(9) D. Felisati, *ibid.*, 724 (1962-II).

has been shown that a few N-alkylphthalimides may have adverse effects on rat pregnancy, although no malformations have been produced.[4,5]

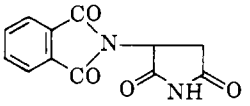
We now wish to report preliminary results obtained with two new substances closely related to thalidomide (I), namely the *d,l*-3-phthalimidoglutarimide (II) and the N-phthalyl-*d,l*-aspartimide (III).



I



II



III

While the imide II is a racemic derivative of an unnatural amino acid, and is isomeric with thalidomide, III is a racemic derivative of a naturally occurring amino acid, as is thalidomide itself.

The imide (II) has been synthesized through the following reaction sequences: phthaloylation of *d,l*-3-aminoglutaric acid to *d,l*-3-phthalimidoglutaric acid (m.p. 174–176° dec.); dehydration of the acid to *d,l*-3-phthalimidoglutaric anhydride (m.p. 212–220° dec.); conversion of the latter to *d,l*-3-phthalimidoglutaramic acid (m.p. 190–193° dec.) by anhydride ring opening with ammonia; finally, ring closure of the glutaramic acid to the six-membered cyclic imide (II) by treatment with acetic anhydride. The imide (II) is a crystalline substance, sparingly soluble in water, melting with decomposition in a wide range of temperature (217–270°). N-Phthalyl-*d,l*-aspartimide (III) has been synthesized by treating urea with N-phthalyl-*d,l*-aspartic anhydride at high temperature. Compound III is a crystalline substance with a very low water solubility, m.p. 213–215°.

The toxicity of II and III, as compared with that of thalidomide, is reported in Table I. The main effect observed for the three drugs is sedation; however, compounds II and III appear to exert in the rodent a stronger depressant action than thalidomide. The latter caused only a mild sedation at doses up to 10,000 mg./kg. p.o., while 2000 mg./kg. p.o. of compound III induced a deep sleep of long duration (6 hr. in rats and more than 24 hr. in mice). A similar effect of shorter duration was observed with 10,000 mg./kg. p.o. of II.

TABLE I

ACUTE TOXICITY OF THALIDOMIDE, II AND III

Compound	LD_{50} mg./kg. orally Mouse	Rat
I (Thalidomide)	>10,000	>10,000
II	>10,000	>10,000
III	3,500	5,000

The compounds were administered by stomach tube, in suspension with gum tragacanth. The animals were observed for one week after treatment. With compound III most animals died after more than 24 hr.

The effects observed in pregnant rats (Table II) have been similar for the three drugs. Administration of thalidomide and III in the first days of pregnancy resulted in a failure of implantation in a high proportion of the treated animals. An increased incidence of resorption and a reduction of the mean fetal weight followed later administration of the three drugs. However, no malformations have been so far observed after treatment with II and III.

Preliminary experiments have shown that II and III, like thalidomide, are teratogenic for the chick embryo.

It has been suggested that the adverse effects of thalidomide on the nervous system and the mammalian embryo might be due to an interference with the physiological functions of natural glutamic ac'd.[10] Our results seem to indicate that similar adverse effects on rat pregnancy can be produced by N-phthaloyl derivatives of glutamic acid (thalidomide), aspartic acid (III), and 3-aminoglutaric acid (II). In view of the fact that the latter is a derivative of an unnatural amino acid, it seems doubtful that such adverse effects on rat pregnancy may be due solely to a faulty metabolism of a natural amino acid.

TABLE II

EFFECTS OF THALIDOMIDE, II AND III, ON RAT PREGNANCY

Administration of drug 500 mg./kg. p.o. daily		Number of rats	% Full term pregnancies	Fetuses Mean number	Fetuses Mean weight	Resorptions mean number
Controls		72	86.1	9.5	4.83	0.82
I (Thalidomide)						
Days	0– 2[a]	12	66.7	9.7	4.88	1.25
	3– 6[b]	12	83.3	9.0	4.56	1.89
	7–10	12	75.0	10.5	4.74	1.25
	11–15	7[b]	...	10.9	4.53	0.86
II						
Days	0– 2	10	70.0	11.4	4.49	0.57
	3– 6	10	80.0	10.7	4.92	1.00
	7–10	11	72.7	8.2	4.54	1.00
	11–15	8[b]	...	10.9	4.68	0.75
III						
Days	0– 2	10	60.0	11.2	4.91	0.83
	3– 6	11	45.5	9.0	4.84	2.40
	7–10	11	81.8	9.6	4.45	0.89
	11–15	8[b]	...	11.7	4.34	0.37

The drugs were adminstered once a day in suspension with gum tragacanth. The days indicate the time of the pregnancy at which the treatment was given, day 0 being the day after mating. The rats were killed on day 21 of pregnancy, *i.e.* a few hr. before the expected parturition.

[a] Data from G. Bignami, D. Bovet, F. Bovet-Nitti, and V. Rosnati[2] and G. Bignami, F. Bovet-Nitti, and V. Rosnati.[3] [b] In the groups treated after mid-pregnancy, non-pregnant animals without signs of resorption were not included in the results.

(10) J. W. Faigle, H. Keberle, W. Ries, and K. Schmid, *Experientia* **18**, 389 (1962).

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