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Walter Modell, M.D.

cement on December 12 that
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his job must have been. We

The Editor

Commentary

The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man

The usefulness of dogs and monkeys in predicting potential qualitative drug toxicity in man was examined retrospectively for twenty-five anticancer compounds of diverse chemical and functional classification. It was found that the large animal screen served to alert the physician to a significant proportion of the total spectrum of drug effects, which were encountered during the clinical use of a new toxic compound. The dog and monkey correctly predicted bone marrow depression, gastrointestinal disturbance, and hepatotoxicity for each drug producing these effects in the clinic; in the case of renal, cardiovascular, and neuromuscular toxicity, however, the large animal screen failed in each instance to predict one drug that produced these toxicities in man. The correct predictions were accomplished at the expense of a high percentage of false positives, which resulted from the necessity of using severely toxic dose levels in order to demonstrate all potential toxicities inherent in any compound. While organ system toxicity observed during an animal study can never be disregarded, it should be viewed with an understanding of certain limitations of animal toxicologic data. While toxicity may develop in man in an organ system predicted susceptible by an animal, a different specific clinical or chemical parameter may be involved. The adverse reaction may appear in man at a greater or lesser dose level or may follow a different order of appearance in relationship to the total spectrum of qualitative toxicity inherent in the compound.

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One of the most controversial areas in the development of new drugs for use in man involves the efficacy of animal toxicology studies as a predictive system for qualitative toxicity. The general approach of evaluating a new compound for safety in several animal species is performed in an attempt to generate information that may alert the clinical pharmacologist to potential hazards. The process requires that toxicity data be extrapolated from one species to another, and implicit is the assumption that particular animal species have significant predictive value for toxicity in man and that important toxicity will not go unpredicted. While this topic has

Table I

Drug	*Chemical classification*	*Mechanism of action*	*Route*	*Clinical MTD*	*Principal clinical toxicities*	*Dog*	*Monkey*
NSC-13875 Melamine, hexamethyl Ref. 13, 64, 111	Triazene	Alkylating agent	PO	12 mg./Kg./day × 21 (480 mg./M.²) 8 mg./Kg./day × 90 (320 mg./M.²)	Leukopenia Thrombocytopenia Vomiting	+ + +	
NSC-17256E Pregn-4-ene-3, 11, 20-trione, 6α-methyl- Ref. 55	Steroid	Steroid hormone	PO	9 mg./Kg./day × 56 (360 mg./M.²)	Diarrhea Mild leukopenia	+ –	– –
NSC-19893 Uracil, 5-fluoro- Ref. 22, 94, 102	Pyrimidine	Antimetabolite	IV	15 mg./Kg./day × 5 (600 mg./M.²) 7.5 mg./Kg. q.o.d.) (300 mg./M.²)	Diarrhea Bone marrow depression Stomatitis	+ + –	+ + –
NSC-24559 Mithramycin (USAN) Ref. 21, 59, 84, 88, 97	Tricyclic glycoside	Antibiotic, complexes with DNA, inhibits RNA synthesis	IV	0.025 mg./Kg./day × 8-10 (1 mg./M.²)	Bleeding with or without thrombocytopenia Bone marrow depression Hypocalcemia Vomiting	– + + +	– + – +
NSC-2698-0 Mitomycin C Ref. 3, 31, 32, 54, 71, 103, 105	Indolequinone	Antibiotic, alkylating agent	IV	0.05 mg./Kg./day × 5-6 (2 mg./M.²)	Bone marrow depression Vomiting	+ +	+ +
NSC-40774 9*H*-Purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate Ref. 7, 49, 68	Purine nucleoside	Antimetabolite	IV	5 mg./Kg./day × 5 (200 mg./M.²)	Stomatitis Mucositis Leukopenia	– – –	– – +
NSC-45388 Imidazole-4 (or 5)-carboxamide, 5 (or 4)-(3, 3-dimethyl-1-triazeno)- Ref. 64, 67, 95	Triazeno imidazole	Unknown	IV	5 mg./Kg./day × 10 (200 mg./M.²)	Vomiting Bone marrow depression Hepatotoxicity	– + +	– + +

					Vomiting		
NSC-2698-0 Mitomycin C Ref. 3, 31, 32, 54, 71, 103, 105	Indolequinone	Antibiotic, alkylating agent	IV	0.05 mg./Kg./day × 5-6 (2 mg./M.²)	Bone marrow depression Vomiting	+ +	+ +
NSC-40774 9*H*-Purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate Ref. 7, 49, 68	Purine nucleoside	Antimetabolite	IV	5 mg./Kg./day × 5 (200 mg./M.²)	Stomatitis Mucositis Leukopenia	- - -	- - +
NSC-45388 Imidazole-4 (or 5)-carboxamide, 5 (or 4)-(3, 3-dimethyl-1-triazeno)- Ref. 64, 67, 95	Triazeno imidazole	Unknown	IV	5 mg./Kg./day × 10 (200 mg./M.²)	Vomiting Bone marrow depression Hepatotoxicity	- + +	- + +
NSC-51095 Ammonium, trimethyl purine-6-yl-chloride Ref. 50, 101	Purine	Antimetabolite	IV	30 mg./Kg./day × 7 (1,200 mg./M.²)	Cholinergic stimulation Bone marrow depression Neurological	+ + -	
NSC-52947 Pactamycin Ref. 5, 110	Unknown antibiotic	Antibiotic	IV	0.083 mg./Kg./day × 7 (3.32 mg./M.²)	Cardiopulmonary Vomiting Bone marrow depression	- + +	- - +
NSC-53398 Restrictocin	Polypeptide	Antibiotic	IV	0.008 mg./Kg./day × 5 (0.32 mg./M.²)	Hypotension Neurologic	- +	- -
NSC-56408 7 *H*-Pyrrolo [2, 3-*d*] pyrimidine, 4-amino-7-β-D-ribofuranosyl-(Tubercidin) Ref. 1, 6	Deazapurine nucleoside	Antibiotic, antimetabolite	IV	1.5 mg./Kg./week × 2 then 0.75 mg./Kg./month (60 mg./M.²/→30 mg./M.²)	Injection-site Vomiting Bone marrow depression Hepatotoxicity	+ - - +	+ - - +
NSC-56410 Porfiromycin Ref. 24, 33, 40, 66, 106	Indole quinone	Antibiotic, alkylating agent	IV	0.6 mg./Kg./week × 4-6 (24.0 mg./M.²) 0.5 mg./Kg. twice a week × 3-4 (20.0 mg./M.²) 0.2 mg./Kg./day × 21 (8.0 mg./M.²)	Bone marrow depression Vomiting	+ +	

Continued on next page.

Table I. *Cont'd*

Drug	*Chemical classification*	*Mechanism of action*	*Route*	*Clinical MTD*	*Principal clinical toxicities*	*Dog*	*Monkey*
NSC-56654 Azotomycin (USAN) Ref. 2, 105	Diazonium alkylating agent	Antibiotic, glutamine antagonist	IV	2 mg./Kg./day × 8-10 (80 mg./M.²)	Bone marrow depression Vomiting	+ +	– No data
NSC-58404 Glycine, *N*-(diazoacetyl), hydrazide Ref. 38	Diazohydrazide	Unknown	IV	37 mg./Kg./day × 21 (1.48 Gm./M.²)	Vomiting	+	–
NSC-60339 Terephthalanilide, 2-chloro-4', 4''-di-2-imidazolin-2-yl- Ref. 8, 48, 78, 93	Terephthalanilide	Unknown	IV	6 mg./Kg./day × 10 (240 mg./M.²) 6 mg./Kg. twice a week × 8 (240 mg./M.²)	Ophthalmoplegia Abdominal cramps	– –	+ –
NSC-60387 Tylocrebrine	Polycyclic alkaloid	Antibiotic	IV	0.40 mg./Kg./day × 24 (16 mg./M.²)	Neurologic Vomiting Bone marrow depression	– + +	– + +
NSC-62512 Acetophenone, 2-(dimethylamino) -3', 4'-dihydroxy-, hydrochloride	Acetophenone	Unknown	IV	275 mg./Kg./day × 10 (10.0 Gm./M.²)	Bone marrow depression Vomiting Hepatotoxicity	– + +	– + +
NSC-63878 Cytosine, 1-β-*D* arabinofuranosyl-, monohydrochloride Ref. 16, 20, 46, 58, 65, 81, 98	Pyrimidine nucleoside	Antimetabolite	IV	3.5 mg./Kg./day × 10 (IV push) (140 mg./M.²) 1-3 mg./Kg./day (24 hr. infus. to toxicity) (40-120 mg./M.²)	Bone marrow depression Vomiting Hepatotoxicity Stomatitis	+ + + –	+ – – –
NSC-65346 Sangivamycin Ref. 19	Deazapurine nucleoside	Antibiotic, antimetabolite	IV	Not fully established	Cardiac Bone marrow depression	– +	– –
NSC-68626 Hydrazine, 1-acetyl-2-picolinoyl-	Hydrazine	Chelating agent, peroxide former	IV	12-15 mg./Kg./day × 7-10 (480-600 mg./M.²)	Neurologic	+	+

NSC-63878 Cytosine, 1-β-*D* arabinofuranosyl-, monohydrochloride Ref. 16, 20, 46, 58, 65, 81, 98	Pyrimidine nucleoside	Antimetabolite	IV	3.5 mg./Kg./day × 10 (IV push) (140 mg./M.²) 1-3 mg./Kg./day (24 hr. infus. to toxicity) (40-120 mg./M.²)	Bone marrow depression Vomiting Hepatotoxicity Stomatitis	+ + + –	– – – –
NSC-65346 Sangivamycin Ref. 19	Deazapurine nucleoside	Antibiotic, antimetabolite	IV	Not fully established	Cardiac Bone marrow depression	– +	– –

NSC-68626 Hydrazine, 1-acetyl-2-picolinoyl-	Hydrazine	Chelating agent, peroxide former	IV	12-15 mg./Kg./day × 7-10 (480-600 mg./M.²)	Neurologic	+	+
NSC-69945 Phosphorodiamidic acid, *N,N*,-bis (2-chloroethyl)-, compd. with cyclohexylamine (1:1) Ref. 70, 76	Phosphorus-containing alkylating agent	Alkylating agent	IV	1.5 mg./Kg./day × 9 (60 mg./M.²) 2.5 mg./Kg. twice a week × 2 (100 mg./M.²) 5.0 mg./Kg./week × 19 (200 mg./M.²)	Bone marrow depression Vomiting Hemorrhagic cystitis	+ + +	+ – +
NSC-82151 Daunomycin Ref. 18, 29, 99, 100	Tetracyclic glycoside, polyhydroxyanthraquinone glycoside	Antibiotic, complexes with DNA, inhibits RNA synthesis	IV	~1 mg./Kg./day × 3-5 (45 mg./M.²)	Bone marrow depression Cardiac	+ –	+ –
NSC-85998 Streptozotocin Ref. 86, 92	Nitrosourea amino sugar	Antibiotic, depression of NAD	IV	25-75 mg./Kg./week (1-3 Gm./M.²) >40 mg./Kg./day × 5 (>1,600 mg./M.²)	Injection site Vomiting	– +	– –
NSC-94100 Mannitol, 1,6-dibromo-1,6-dideoxy-, D- Ref. 17, 30, 52	Halogenated reduced sugar	Alkylating agent	PO	6 mg./Kg. to toxicity or therapeutic effect (240 mg./M.²)	Bone marrow depression	+	+
NSC-409, 962 Urea, 1,3-bis(2-chloroethyl)-1-nitroso- Ref. 26, 27, 28, 36, 37, 53, 77, 85, 87, 89, 108	Nitrosourea	Alkylating agent		2.5 mg./Kg./day × 3 (100 mg./M.²) 7.5 mg./Kg./day × 1 (300 mg./M.²)	Bone marrow depression Hepatotoxicity Vomiting	+ + +	+ + +

Table II. *Number of animals and patients receiving each drug*

Drug	*Route*	*Dog*	*Monkey*	*Man*
NSC-13875 Melamine, hexamethyl-	PO	Beagle—10	*Macaca mulatta*—3	100
NSC-17256E Pregn-4-ene-3,11,20-trione,6α-methyl-	PO	Beagle—4	*Macaca mulatta*—2	93
NSC-19893 Uracil, 5-fluoro-	IV	Beagle—15	*Macaca mulatta*—3	> 100
NSC-24559 Mithramycin (USAN)	IV	Beagle—14	*Macaca irus*—6	900
NSC-26980 Mitomycin C	IV	Mongrel—28 Beagle—9	*Macaca mulatta*—6	1,004
NSC-40774 9*H*-Purine,6-(methylthio)-9-β-*D*-ribofuranosyl-, dihydrate	IV	Beagle—9	*Macaca mulatta*—7	70
NSC-45388 Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-	IV	Beagle—14	*Macaca mulatta*—7	250
NSC-51095 Ammonium, trimethylpurin-6-yl-chloride	IV	Beagle—13	*Macaca mulatta*—3	36
NSC-52947 Pactamycin	IV	Beagle—18	*Macaca mulatta*—6	81
NSC-53398 Restrictocin	IV	Beagle—12	*Macaca mulatta*—7 *Macaca speciosa*—4	12
NSC-56408 Tubercidin	IV	Beagle—20	*Macaca mulatta*—3	132
NSC-56410 Porfiromycin	IV	Beagle—9	——	93
NSC-56654 Azotomycin (USAN)	IV	Beagle—15	——	105
NSC-58404 Glycine, *N*-(diazoacetyl)-, hydrazide	IV	Mongrel—10 Beagle—6	*Macaca irus*—7	22
NSC-60339 Terephthalanilide, 2-chloro-4′,4″-di-2-imidazolin-2-yl	IV	Beagle—4	*Macaca mulatta*—6	35
NSC-60387 Tylocrebrine	IV	Mongrel—5 Beagle—7	*Macaca irus*—7	10
NSC-62512 Acetophenone, 2-(dimethylamino)-3′,4′-dihydroxy-, hydrochloride	IV	Beagle—18	*Macaca mulatta*—4	59
NSC-63878 Cytosine, 1-β-*D*-arabinofuranosyl-, monohydrochloride	IV	Mongrel—10	*Macaca mulatta*—20	> 100
NSC-65346 Sangivamycin	IV	Mongrel—16	*Macaca mulatta*—4	45
NSC-68626 Hydrazine, 1-acetyl-2-picolinoyl-	IV	Beagle—17	*Macaca irus*—8	40

onkey	Man
atta—3	100
latta—2	93
latta—3	> 100
—6	900
latta—6	1,004
latta—7	70
latta—7	250
latta—3	36
latta—6	81
latta—7 eciosa—4	12
latta—3	132
——	93
——	105
us—7	22
ulatta—6	35
us—7	10
ulatta—4	59
ulatta—20	> 100
ulatta—4	45
rus—8	40

Table II. *Cont'd*

Drug	Route	Dog	Monkey	Man
NSC-69945 Phosphorodiamidic acid, *N,N,*-bis (2-chloroethyl)-, compd. with cyclohexylamine (1:1)	IV	Beagle—14	*Macaca mulatta*—7	55
NSC-82151 Daunomycin	IV	Beagle—18	*Cercopithecus aethiops*—5	> 100
NSC-85998 Streptozotocin	IV	Beagle—12	*Macaca mulatta*—6	5
NSC-94100 Mannitol, 1,6-dibromo-1,6-dideoxy-, *D*-	PO	Beagle—9	*Macaca mulatta*—7	50
NSC-409,962 Urea, 1,3-bis(2-chloroethyl)-1-nitroso-	IV	Beagle—47	*Macaca mulatta*—15	> 200

been the subject of numerous editorials and discussions, only two comparative studies with the use of relatively few drugs are found in the pharmacologic literature. In this communication, we present a detailed study of the qualitative toxicity of twenty-five anticancer drugs in dog, monkey, and man.

Methods

Twenty-five compounds of diverse chemical and functional classification are examined. For a compound to be included, there had to be detailed toxicologic data available for at least one large animal species and for man (Table I). The animal toxicologic evaluations were performed under the guidelines of The Cancer Chemotherapy National Service Center Protocol[15] at laboratories under contract to the National Cancer Institute. Dogs and monkeys (for species used see Table II) were used exclusively. The dogs weighed between 7 to 9 kilograms and were immunized against distemper and hepatitis and purged of parasites. The monkeys weighed between 2 to 5 kilograms and were screened for tuberculosis and purged of parasites prior to being placed on test. Acute lethal toxicity was elucidated in dogs and subacute repeated-dose toxicology was carried out in both dogs and monkeys. The numbers and types of animals used for each of the twenty-five drugs are listed in Table II. In all cases, doses administered to the animals were in excess of the maximum tolerated dose level (MTD) (that dose which produced only minimal reversible toxicity) as summarized in Tables III and IV. When treatment ended, half of the animals on each dose level were randomly selected for immediate killing, while the remaining animals were observed for delayed toxicity and reversibility of toxicity. The clinical studies were carried out at the Clinical Center Program of the National Cancer Institute, one or several of the Cancer Chemotherapy Cooperative Study groups, or at an independent institute. The patient data were cumulated from individual patient records, literature publications, and personal communications. Approximately 170 parameters were examined, and a set of criteria of toxicity in animals was established (Table V).

The criteria for hematologic and chemical toxicity in man were based on the range of normal values outlined by the Clinical Pathology Department of the Clinical Center, National Institutes of Health. In patients in whom base-line values were abnormal as a result of primary disease, metastasis, or prior therapy, decisions re-

Table III. *Maximum tolerated dose—mg./Kg. (mg./M.²)**

Drug	*Route*	*Dog*	*Monkey*	*Man*
NSC-13875 Melamine, hexamethyl	PO	<50 mg./Kg./day × 10 (<1,000 mg./M.²)	21 mg./Kg./day × 15 (252 mg./M.²)—IV	12 mg./Kg./day × 21 (480 mg./M.²) 8 mg./Kg./day × 90 (320 mg./M.²)
NSC-17256E Pregn-4-ene-3,11,20-trione, 6α-methyl-	PO	<30 mg./Kg./day × 28 (<600 mg./M.²)	<30 mg./Kg./day × 28 (<360 mg./M.²)	9 mg./Kg./day × 56 (360 mg./M.²)
NSC-19893 Uracil, 5-fluoro-	IV	5 mg./Kg./day × 5 (100 mg./M.²) 10 mg./Kg./week × 6 (200 mg./M.²)	7.5 mg./Kg./day × 6 (90 mg./M.²)	15 mg./Kg./day × 5 (600 mg./M.²) 7.5 mg./Kg. q.o.d. (300 mg./M.²)
NSC-24559 Mithramycin (USAN)	IV	0.025 mg./Kg./day × 24 (0.5 mg./M.²)	0.025 mg./Kg./day × 24 (0.3 mg./M.²)	0.025 mg./Kg./day × 5 (1 mg./M.²)
NSC-26980 Mitomycin C	IV	<0.063 mg./Kg./day × 10 (<1.26 mg./M.²)	0.2 mg./Kg./day × 10 (2.4 mg./M.²)	0.05 mg./Kg./day × 5-6 (2 mg./M.²)
NSC-40774 9*H*-Purine, 6-(methylthio)-9-β-*D*-ribofuranosyl-, dihydrate	IV	4 mg./Kg./day × 10 (80 mg./M.²)	4 mg./Kg./day × 14 (48 mg./M.²)	5 mg./Kg./day × 5 (200 mg./M.²)
NSC-45388 Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-	IV	2.5 mg./Kg./day × 28 (50 mg./M.²)	15-30 mg./Kg./day × 28 (185-370 mg./M.²)	5 mg./Kg./day × 10 (200 mg./M.²)
NSC-51095 Ammonium, trimethylpurin-6-yl-chloride	IV	8 mg./Kg./day × 28 (160 mg./M.²)	30 mg./Kg./day × 28 (360 mg./M.²)—PO	30 mg./Kg./day × 7 (1,200 mg./M.²)
NSC-52947 Pactamycin	IV	0.02 mg./Kg./day × 28 (0.4 mg./M.²)	0.02 mg./Kg./day × 23 (0.24 mg./M.²)	0.083 mg./Kg./day × 27 (3.32 mg./M.²)
NSC-53398 Restrictocin	IV	<0.01 mg./Kg./day × 28 (<0.2 mg./M.²)	0.02 mg./Kg./day × 28 (<0.24 mg./M.²)	0.008 mg./Kg./day × 5 (0.32 mg./M.²)
NSC-56408 Tubercidin	IV	<0.25 mg./Kg./day × 28 (<0.50 mg./M.²)	<1 mg./Kg./day × 24 (<12 mg./M.²)	1.5 mg./Kg./week × 2 then 0.75 mg./Kg./month (60 mg./M.² → 30 mg./M.²)

NSC-51095 Ammonium, trimethylpurin-6-yl-chloride	IV	8 mg./Kg./day × 28 (160 mg./M.²)	30 mg./Kg./day × 28 (360 mg./M.²)—PO	30 mg./Kg./day × 7 (1,200 mg./M.²)
NSC-52947 Pactamycin	IV	0.02 mg./Kg./day × 28 (0.4 mg./M.²)	0.02 mg./Kg./day × 23 (0.24 mg./M.²)	0.083 mg./Kg./day × 27 (3.32 mg./M.²)
NSC-53398 Restrictocin	IV	<0.01 mg./Kg./day × 28 (<0.2 mg./M.²)	0.02 mg./Kg./day × 28 (<0.24 mg./M.²)	0.008 mg./Kg./day × 5 (0.32 mg./M.²)

NSC-56408 Tubercidin	IV	<0.25 mg./Kg./day × 28 (<0.50 mg./M.²)	<1 mg./Kg./day × 24 (<12 mg./M.²)	1.5 mg./Kg./week × 2 then 0.75 mg./Kg./month (60 mg./M.² → 30 mg./M.²)
NSC-56410 Porfiromycin	IV	<0.310 mg./Kg./day × 10 (<6.2 mg./M.²)	—	0.6 mg./Kg./week × 4-6 (24.0 mg./M.²) 0.5 mg./Kg. twice a week × 3-4 (20.0 mg./M.²) 0.2 mg./Kg./day × 21 (8.0 mg./M.²)
NSC-56654 Azotomycin (USAN)	IV	0.1 mg./Kg./day × 14 (2.0 mg./M.²)	—	2 mg./Kg./day × 8-10 (80 mg./M.²)
NSC-58404 Glycine, *N*-(diazoacetyl)-, hydrazide	IV	10 mg./Kg./day × 24 (200 mg./M.²)	20 mg./Kg./day × 24 (240 mg./M.²)	37 mg./Kg./day × 21 (1.48 Gm./M.²)
NSC-60339 Terephthalanilide, 2-chloro-4′,4″-di-2-imidazolin-2-yl-	IV	0.5 mg./Kg./day × 28 (10 mg./M.²)	<1 mg./Kg./day × 28 (<12 mg./M.²)	6 mg./Kg./day × 10 (240 mg./M.²) 6 mg./Kg. twice a week × 8 (240 mg./M.²)
NSC-60387 Tylocrebrine	IV	0.125 mg./Kg./day × 24 (2.50 mg./M.²)	0.25 mg./Kg./day × 24 (3.0 mg./M.²)	0.40 mg./Kg./day × 24 (16 mg./M.²)
NSC-62512 Acetophenone, 2-(dimethylamino)-3′, 4′-dihydroxy-, hydrochloride	IV	87 mg./Kg./day × 28 (1.74 Gm./M.²)	260 mg./Kg./day × 28 (3.12 Gm./M.²)	275 mg./Kg./day × 10 (10.0 Gm./M.²)
NSC-63878 Cytosine, 1-β-*D*-arabinofuranosyl-, monohydrochloride	IV	6 mg./Kg./day × 15 (120 mg./M.²)	12 mg./Kg./day × 15 (144 mg./M.²)	3.5 mg./Kg./day × 10 (IV push) (140 mg./M.²) 1-3 mg./Kg./day (24 hr. infus. to toxicity) (40-120 mg./M.²)

[a]Maximum tolerated dose—the highest dose that either produced no toxicity or that produced toxicity that was in all cases rapidly and completely reversible.

Continued on next page.

Table III. *Cont'd*

Drug	*Route*	*Dog*	*Monkey*	*Man*
NSC-65346 Sangivamycin	IV	0.050 mg./Kg./day × 24 (1 mg./M.²)	<0.4 mg./Kg./day × 28 (<4.8 mg./M.²)	Not fully established
NSC-68626 Hydrazine, 1-acetyl-2-picolinoyl-	IV	12 mg./Kg./day × 24 (240 mg./M.²)	24 mg./Kg./day × 24 (288 mg./M.²)	12-15 mg./Kg./day × 7-10 (480-600 mg./M.²)
NSC-69945 Phosphorodiamidic acid, *N,N,*-bis (2-chloroethyl)-cpd. with cyclohexyl-amine (1:1)	IV	4 mg./Kg./day × 14 (80 mg./M.²)	2 mg./Kg./day × 14 (24 mg./M.²)	1.5 mg./Kg./day × 9 (60 mg./M.²) 2.5 mg./Kg. twice a week × 2 (100 mg./M.²) 5.0 mg./Kg./week × 19 (200 mg./M.²)
NSC-82151 Daunomycin	IV	<0.5 mg./Kg./day × 12 (<10 mg./M.²) 2.5 mg./Kg./day × 1 (50 mg./M.²) <1 mg./Kg./week × 30 (<20 mg./M.²)	0.25 mg./Kg./day × 14 (3.0 mg./M.²)	1 mg./Kg./day × 3-5 (45 mg./M.²)
NSC-85998 Streptozotocin	IV	1 mg./Kg./day × 14 (20 mg./M.²)	3 mg./Kg./day × 14 (36 mg./M.²)	>40 mg./Kg./day × 5 (>1,600 mg./M.²)
NSC-94100 Mannitol, 1,6-dibromo-1,6-dideoxy-,*D*	PO	13 mg./Kg./day × 90 (260 mg./M.²) 25 mg./Kg./day × 14 (500 mg./M.²)	10 mg./Kg./day × 200 (120 mg./M.²)	6 mg./Kg. to toxicity or therapeutic effect (240 mg./M.²)
NSC-409,962 Urea, 1,3-bis(2-chloroethyl)-1-nitroso-	IV	1.25 mg./Kg./day × 15 (25 mg./M.²)	1.25 mg./Kg./day × 15 (15 mg./M.²)	2.5 mg./Kg./day × 3 (100 mg./M.²) 7.5 mg./Kg./day × 1 (300 mg./M.²)

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Mannitol, 1,6-dibromo-1,6-dideoxy-D		(260 mg./M.²) 25 mg./Kg./day × 14 (500 mg./M.²)	(120 mg./M.²)		therapeutic effect (240 mg./M.²)
NSC-409,962	IV	1.25 mg./Kg./day × 15 (25 mg./M.²)	1.25 mg./Kg./day × 15 (15 mg./M.²)	2.5 mg./Kg./day × 3 (100 mg./M.²) 7.5 mg./Kg./day × 1 (300 mg./M.²)	
Urea, 1,3-bis(2-chloroethyl)-1-nitroso-					

garding specificity of toxicity were based on the results of serial testing and consistency with findings in similarly treated patients.

The incidence of a given toxicity for each drug was not given special weight in the evaluation of a specific parameter. If an abnormal symptom or sign was observed in more than one animal or patient and considered drug related, it was designated as positive for toxicity.

The data were transferred to IBM cards from IBM mark sense sheets by an IBM 1231 Optical Mark Sense Reader and then computer processed.

The relative frequency of toxicity for each parameter in the individual species was determined (Table VI), and the ability of the animal system to predict for man was defined with the use of the following system: true positive, toxicity was noted in both animal and man; false positive, animal was positive for toxicity but man was negative (an index of overprediction); true negative, neither animal nor man demonstrated toxicity; false negative, man was positive for toxicity but animal was negative (an index of underprediction); false negative/true positive plus false negative, an analysis of the number of false negative predictions as a per cent of the incidence of toxicity in man. The latter category has been designated the "corrected false negative." Groups of parameters whose composite served to represent different specific organ systems, as listed in Tables XI, XII, and XIII, were computed as follows: If for a given animal and a given drug any individual parameter within the group was found to be positive, the organ system was designated positive for toxicity; otherwise, the organ system was called negative. The same process was followed for man. The results for the animal species were then compared and tabulated as true positive, false positive, true negative, false negative, or false negative/true positive plus false negative (corrected false negative) in regard to predicting for toxicity in man.

Results

Prediction of organ system toxicity

Bone marrow. The combined animal screen predicted for all drugs that produced anemia, but at the expense of 44 per cent overprediction (Table IX). It can be noted that neither species predicted for all instances of lowered red blood cell parameters. The dogs were more accurate and efficient, having predicted a higher percentage of true positive at the expense of less false positive while failing to predict for only one of twelve compounds that produced anemia in man (Table VII). However, it can be seen that the monkey data served to correct for this one false negative prediction.

The monkey and dog performed equally well in predicting for leukopenia, with a 60 or 61 per cent individual and 68 per cent combined true positives (Tables VII, VIII, and IX). However, false negative, or underprediction, which was not corrected by the combination was found for both species. Table X presents those compounds in which one or both animal species failed to predict for leukopenia. In addition, the individual animal data did not distinguish between general depression of the white blood cell count and specific toxicity to the granulocytic versus lymphocytic series; the distinctive granulocytopenia of nine of twenty compounds was not predicted by the animal screen.

Thrombocytopenia was correctly predicted for thirteen of the eighteen compounds that caused this toxicity in man (Table IX). The monkey showed significant underprediction, with 41 per cent false negatives and a corrected false negative index of 56 per cent (Table VIII). The specific compounds for which thrombocytopenia was not predicted are summarized in Table X.

The combination of dog and monkey as a toxicologic screen predicted for the myelosuppression for each of the twenty-two compounds that produced this toxicity in man (Table XIII). By examining Tables XI and XII, it can be seen that the lack

Table IV. *Maximum dose administered to each of the three species*

Drug	*Route*	*Dog*	*Monkey*	*Man*
NSC-13875 Melamine, hexamethyl-	PO	260 mg./Kg./day × 8 (5,200 mg./M.²)	84 mg./Kg./day × 15 (1,008 mg./M.²)—IV	12 mg./Kg./day × 21 (480 mg./M.²) 8 mg./Kg./day × 90 (320 mg./M.²)
NSC-17256E Pregn-4-ene-3, 11, 20-trione, 6α-methyl-	PO	30 mg./Kg./day × 28 (600 mg./M.²)	30 mg./Kg./day × 28 (360 mg./M.²)	9 mg./Kg./day × 56 (360 mg./M.²)
NSC-19893 Uracil, 5-fluoro-	IV	10 mg./Kg./day × 5 (200 mg./M.²)	30 mg./Kg./day × 6 (360 mg./M.²)	20 mg./Kg./day × 5 (600 mg./M.²)
NSC-24559 Mithramycin (USAN)	IV	0.1 mg./Kg./day × 8-10 (2 mg./M.²)	0.1 mg./Kg./day × 4-6 (1.2 mg./M²)	0.05 mg./Kg./day × 5-6 (2.0 mg./M.²)
NSC-26980 Mitomycin C	IV	3.2 mg./Kg./day × 10 (64 mg./M.²)	0.2 mg./Kg./day × 8 (4.8 mg./M.²)	0.007 mg./Kg./day × 5 (0.280 mg./M.²)
NSC-40774 9*H*-purine, 6-(methylthio)-9-β-*D* ribofuranosyl-, dihydrate	IV	12 mg./Kg./day × 8 (240 mg./M.²)	8 mg./Kg./day × 8 (96 mg./M.²)	—6.25 mg./Kg./day × 5 (250 mg./M.²)
NSC-45388 Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-	IV	20 mg./Kg./day × 28 (400 mg./M.²)	60 mg./Kg./day × 28 (1,200 mg./M.²)	5.5 mg./Kg./day × 10 (220 mg./M.²)
NSC-51095 Ammonium, trimethylpurin-6-yl-chloride	IV	16 mg./Kg./day × 28 (320 mg./M.²)	120 mg./Kg./day × 9 (1,440 mg./M.²)—PO	100 mg./Kg./day × 4 (infusion) (4,000 mg./M.²) —34 mg./Kg./week × 3 (push) (1,350 mg./M.²)
NSC-52947 Pactamycin	IV	0.375 mg./Kg./day × 6 (7.5 mg./M.²)	0.4 mg./Kg./day × 3 (4.8 mg./M.²)	0.083 mg./Kg./day × 7 (3.3 mg./M.²)
NSC-53398 Restrictocin	IV	0.08 mg./Kg./day × 6 (1.6 mg./M.²)	0.32 mg./Kg./day × 8 (3.84 mg./M.²)	0.032 mg./Kg./day × 3 (1.28 mg./M.²)
NSC-56408 Tubercidin	IV	8 mg./Kg./day × 6 (160 mg./M.²)	8 mg./Kg./day × 4 (96 mg./M.²)	0.3 mg./Kg./day × 7-10 (12 mg./M.²)
NSC-56410 Porfiromycin	IV	2.5 mg./Kg./day × 21 (50 mg./M.²)	——	0.25 mg./Kg./day × 21 (10 mg./M.²)
NSC-56654 Azotomycin (USAN)	IV	0.6 mg./Kg./day × 7-8 (12 mg./M.²)	——	5.9 mg./Kg./day × 5-10 (236 mg./M.²)

Pactamycin		(7.5 mg./M.²)	(4.8 mg./M.²)	(3.3 mg./M.²)
NSC-53398 Restrictocin	IV	0.08 mg./Kg./day × 6 (1.6 mg./M.²)	0.32 mg./Kg./day × 8 (3.84 mg./M.²)	0.032 mg./Kg./day × 3 (1.28 mg./M.²)
NSC-56408 Tubercidin	IV	8 mg./Kg./day × 6 (160 mg./M.²)	8 mg./Kg./day × 4 (96 mg./M.²)	0.3 mg./Kg./day × 7-10 (12 mg./M.²)
NSC-56410 Porfiromycin	IV	2.5 mg./Kg./day × 21 (50 mg./M.²)	——	0.25 mg./Kg./day × 21 (10 mg./M.²)

NSC-56654 Azotomycin (USAN)	IV	0.6 mg./Kg./day × 7-8 (12 mg./M.²)	——	5.9 mg./Kg./day × 5-10 (236 mg./M.²)
NSC-58404 Glycine, *N*-(diazoacetyl)-,hydrazide	IV	40 mg./Kg./day × 16-24 (1,600 mg./M.²)	160 mg./Kg./day × 12 (1,920 mg./M.²)	37 mg./Kg./day × 21 (1,500 mg./M.²)
NSC-60339 Terephthalanilide, 2-chloro-4′,4″-di-2-imidazolin-2-yl-	IV	0.5 mg./Kg./day × 28 (10 mg./M.²)	2.5 mg./Kg./day × 28 (30 mg./M.²)	6 mg./Kg./day × 10 (240 mg./M.²)
NSC-60387 Tylocrebrine	IV	1 mg./Kg./day × 7-24 (20 mg./M.²)	2.0 mg./Kg./day × 4 (24 mg./M.²)	0.5 mg./Kg./day × 21 (20 mg./M.²)
NSC-62512 Acetophenone, 2-(dimethylamino)-3′,4′-dihydroxy-, hydrochloride	IV	400 mg./Kg./day × 10-28 (8,000 mg./M.²)	400 mg./Kg./day × 4-28 (4,800 mg./M.²)	375 mg./Kg./day × 10 2-8 hr. infusion (15 Gm./M.²)
NSC-63878 Cytosine, 1-β-*D*-arabinofuranosyl-, monohydrochloride	IV	100 mg./Kg./day × 6 (2,000 mg./M.²)	100 mg./Kg./day × 15 (1,200 mg./M.²)	4 mg./Kg./day × 10 (160 mg./M.²)
NSC-65346 Sangivamycin	IV	0.4 mg./Kg./day × 13 (8.0 mg./M.²)	1,6 mg./Kg./day × 16 (19.2 mg./M.²)	0.35 mg./Kg./day × 7 (114 mg./M.²)
NSC-68626 Hydrazine, 1-acetyl-2-picolinoyl-	IV	48 mg./Kg./day × 9 (960 mg./M.²)	96 mg./Kg./day × 18 (1,052 mg./M.²)	18 mg./Kg./day × 7-10 (720 mg./M.²)
NSC-69945 Phosphorodiamidic acid, *N,N*,-bis (2-chloroethyl)-, compd. with cyclohexylamine (1:1)	IV	32 mg./Kg./day × 5 (640 mg./M.²)	16 mg./Kg./day × 7 (192 mg./M.²)	4 mg./Kg./day × 9 (160 mg./M.²)
NSC-82151 Daunomycin	IV	1 mg./Kg./day × 12 (20 mg./M.²)	1 mg./Kg./day × 12 (12 mg./M.²)	1.5 mg./Kg./day × 5 (60 mg./M.²)
NSC-85998 Streptozotocin	IV	4.2 mg./Kg./day × 14 (50.4 mg./M.²)	12 mg./Kg./day × 14 (144 mg./M.²)	40 mg./Kg./day × 5 (1,600 mg./M.²)
NSC-94100 Mannitol, 1,6-dibromo-1,6-dideoxy-, *D*	PO	200 mg./Kg./day × 6 (4,000 mg./M.²)	150 mg./Kg./day × 8 (1,800 mg./M.²)	6 mg./Kg./day to toxicity or response (240 mg./M.²)
NSC-409,962 Urea, 1,3-bis(2-chloroethyl)-1-nitroso-	IV	20 mg./Kg./day × 7 (400 mg./M.²)	10 mg./Kg./day × 7 (120 mg./M.²)	10 mg./Kg./day × 3 (300 mg./M.²)

Table V

A. *Criteria employed for the definition and semiquantitation of drug toxicity*

Hematology	Dogs		Monkey	
	Mild-moderate	*Marked*	*Mild-moderate*	*Marked*
Hct. (%)	< 38 - ≥ 32	= or < 32	< 37 - > 30	= or < 30
Hgb. (Gm./%)	< 13 - ≥ 11	= or < 11	< 12.3 - ≥ 9.5	= or < 9.5
WBC ($\times 10^3$/mm.^{3})	< 6 - ≥ 4	= or < 4	< 6 - > 4	= or < 4
Platelets ($\times 10^3$/mm.^{3})	< 175 - ≥ 100	= or < 100	< 200 - > 100	= or < 100

B. *Criteria for the semiquantitation of toxicity in dogs and monkeys based on changes from normal pretreatment values*

Chemistry	*Mild-moderate*	*Marked*
FBS	< 2 × and > 120/mg./100 ml.	= or > 2 × normal pretreatment value
BSP	< 2 × and > 8%	= or > 2 × normal pretreatment value
Alk. p'tase	< 2 × and > 7 Sigma units (dog) > 30 Sigma units (monkey)	= or > 2 × normal pretreatment value
SGOT	< 3 × and > 60 Sigma-Frankel units	= or > 3 × normal pretreatment value
SGPT	< 3 × and > 60 Sigma-Frankel units	= or > 3 × normal pretreatment value
BUN	< 2 × and > 20 mg./100 ml.	= or > 2 × normal pretreatment value
Creatinine	< 2 × and > 1.5 mg./100 ml.	= or > 2 × normal pretreatment value
Bilirubin	< 2 × and > 1.0 mg./100 ml.	= or > 2 × normal pretreatment value
Prothrombin time	< 2 × and > 10 seconds	= or > 2 × normal pretreatment value

of false negatives, or underprediction, was the result of an overlap in information contributed by using the two species; neither animal species predicted for all toxicity, the dog alone missed two compounds, and the monkey failed to predict for one.

Lymphoid organs. The large animal screen overpredicted 76 per cent for lymphopenia and lymph node toxicity. This form of toxicity was noted with only one compound, daunomycin, during the clinical studies in man.

Gastrointestinal tract. The most consistent clinical gastrointestinal toxicity induced by the drugs was vomiting, which appeared with eighteen of twenty-five compounds. The combined screen gave 72 per cent true positives, but missed 12 per cent drug-related emesis (Table IX). Again these results were primarily contributed by the dog, since the monkey gave a significant 57 per cent underprediction with a corrected false negative index of 68 per cent (Table VIII). The same situation holds true for diarrhea; the dogs gave 36 per cent positives, failing to predict for only one compound, while the monkey failed for six drugs, giving a 67 per cent corrected false negative prediction for that species. The toxicities of stomatitis and gastrointestinal bleeding were observed with relatively few compounds and were generally underpredicted by the animal screen.

The combined dog and monkey screen gave 92 per cent true positives and no false negatives for gastrointestinal toxicity when compared with that of man (Table XIII). When the individual species are compared, it can be seen that the high degree of correspondence is entirely contributed by the dog; since the monkey failed to predict for four of twenty-three compounds (Tables XI and XII).

Liver. The large animal screen predicted for all instances of hepatotoxicity, but at the expense of a 48 per cent overprediction. The comparison of the dog and monkey data failed to disclose a species selectivity.

The individual parameters in the animal

Table VI. *Groups of parameters selected as representatives of organ-specific toxicity and their frequency in each of the three species*

	Dog	Monkey	Man
Bone marrow			
Hemoglobin ↓	19/25*	18/23	12/25
Hematocrit ↓	20/25	19/23	12/25
Red blood cell count ↓	19/25	18/23	10/25
Recticulocytes ↓	8/25	2/23	3/25
Normoblast ↑	4/22	1/15	0/25
White blood cell count ↓	18/25	17/23	20/25
Granulocytes ↓	12/25	8/23	20/25
Lymphocytes ↓	12/25	4/23	1/25
Thrombocytopenia	18/25	7/23	19/25
Bone marrow pathology	16/25	12/23	1/2
Lymphoid			
Lymphocytes ↓	12/25	4/23	1/25
Lymph node pathology	12/25	4/23	0/2
Splenic lymph follicle pathology	12/25	2/23	0/2
Gastrointestinal			
Weight loss	25/25	14/23	0/25
Nausea	—	—	23/25
Vomiting	24/25	9/23	21/25
Hematemesis	5/25	0/23	2/25
Salivation ↑	14/25	4/23	3/25
Abdominal pain	1/25	0/23	3/25
Diarrhea	21/25	9/23	10/25
Constipation	0/25	1/23	1/25
Melena	3/25	0/23	2/25
Hematochezia	19/25	5/23	1/25
Stomatitis	2/25	1/23	5/25
Mucositis	1/25	0/23	2/25
Ulcerations	0/25	0/23	1/25
Steatorrhea	0/25	0/23	0/25
Esophagus pathology	3/25	2/23	0/2
Stomach pathology	13/25	6/23	0/2
Small intestine pathology	17/25	10/23	0/2
Large intestine pathology	19/25	9/23	0/2
Hepatic-biliary			
BSP retention ↑	14/18	7/9	4/25
Alkaline phosphatase ↑	19/25	5/21	8/25
SGOT ↑	12/20	10/18	11/25
SGPT ↑	13/21	11/21	3/14
Bilirubin ↑	6/18	2/15	4/25
Albumin ↓	0/2	0/2	0/25
Globulin ↑	0/2	0/2	0/25
Prothrombin time ↑	4/23	2/7	3/25
Liver pathology	25/25	14/23	1/2
Gallbladder pathology	2/25	2/23	0/2
Kidney			
Polyuria	6/25	1/23	0/25
Polydypsia	8/25	2/23	0/25
Dysuria	—	—	1/25
BUN ↑	16/25	15/23	9/25
Creatinine ↑	3/14	5/11	0/13
Proteinuria	10/13	3/7	1/25
Hematuria	5/13	1/8	1/25
Pyuria	0/13	0/8	0/25
Specific gravity ↓	6/16	1/9	0/25
Kidney pathology	17/25	13/23	0/2
Urinary bladder pathology	8/25	4/23	0/2
Ureter pathology	0/25	0/23	0/2
Neuromuscular			
Stupor	0/25	1/23	2/25
Coma	0/25	0/23	1/25
Agitation	5/25	2/23	2/25
Confusion	1/2	—	4/25
Psychosis	—	—	1/25
Sensory loss	0/25	0/23	0/25
Deafness	0/25	0/23	0/25
Blindness	0/25	0/23	0/25
Ophthalmoplegia	0/25	1/23	1/25
Loss of pupillary reflex	2/25	0/23	0/25
Nystagmus	0/25	0/23	1/25
Excessive lacrimation	8/25	3/23	2/25
Eye or optic nerve pathology	2/25	1/23	0/2
Ataxia	11/25	4/23	3/25
Vertigo	0/0	0/0	2/25
Weakness	15/25	9/23	4/25
Myalgia	—	—	3/25
Tremor	7/25	5/23	0/25
Seizures	5/25	2/23	1/25
Brain pathology	2/25	1/23	0/2
Spinal cord pathology	0/25	0/23	0/2
Peripheral nerve pathology	0/25	0/23	0/2
Skeletal muscle pathology	2/25	0/23	0/2
Cardiovascular			
Flushing	0/25	1/23	4/25
Hypotension	1/2	3/3	4/25
Hypertension	0/2	0/2	1/25
Arrhythmia	5/8	1/3	2/24
Tachycardia	4/25	1/23	5/25
Edema	0/25	0/23	4/25
Bradycardia	3/25	2/23	1/25
ECG abnormalities	0/2	0/1	3/23
Cardiac pathology	7/25	7/23	0/2

* $\frac{\text{Numerator}}{\text{denominator}} = \frac{\text{the number of drugs producing toxicity}}{\text{the number of drugs tested}}$.

Continued on next page.

Table VI. *Cont'd*

	Dog	*Monkey*	*Man*
Respiratory			
Dyspnea	7/25	5/23	3/25
Tachypnea	5/25	0/23	1/25
Cough	2/25	0/23	1/25
Bronchospasm	0/25	0/23	1/25
Hemoptysis	0/25	0/23	0/25
Lung pathology	18/25	11/23	0/2
Injection site			
Pain with injection	1/23	0/20	5/23
Inflammation	9/23	8/21	3/23
Thrombosis	3/23	4/21	4/23
Injection site pathology	8/23	7/23	0/0
Integument			
Desquamation	0/25	0/23	1/25
Dermatitis	0/25	1/23	2/25
Loss of pigmentation	0/25	0/23	0/25
Petechiae	0/25	1/23	5/25
Ecchymosis	0/25	2/23	5/25
Cyanosis	2/25	1/23	1/25
Jaundice	3/25	0/23	2/25
Alopecia	1/25	0/23	2/25
Eyes			
Conjunctivitis	2/25	0/23	0/25
Corneal scarring	0/25	0/23	0/25
Cataracts	0/25	0/23	0/25

species were analyzed for abnormalities of the same individual parameters in man (Tables VII, VIII, and IX). In general, the transaminase and alkaline phosphatase proved to be the most useful parameters of liver function. The transaminases, serum glutamic oxaloacetic (SGOT) and serum glutamic pyruvic (SGPT), had the highest true positive index, 23 to 35 per cent; the highest total true prediction of 46 to 55 per cent (true positive plus true negative) and the lowest false negative prediction 0 to 5 per cent with a corrected false negative index of 12.5 per cent (Table IX). These were closely followed by the alkaline phosphatase in the dog which gave a 25 per cent true positive index, a 46 per cent total true prediction, with a 14 per cent corrected false positive prediction for abnormal alkaline phosphatase values in man (Table VII). The bromsulphalein (BSP) determination gave only an 11 and 32 per cent true positive and total true predictions, respectively, while overpredicting 63 per cent and missing one of three compounds that produced this toxicity in man. The over-all true prediction of bilirubinemia was 72 per cent with no false negatives. The three instances of prolonged prothrombin time in man were missed by the large animal screen.

Each of the individual liver parameters was then separately analyzed to determine which best predicted for liver toxicity, as represented by the entire group of hepatic function tests (i.e., BSP versus liver, alkaline phosphatase versus liver). The data for each species and the combination are presented in Table XIV. One notable feature of the data was the poor performance of the alkaline phosphatase in monkeys, having failed to predict for seven of ten hepatotoxic compounds. With the use of the monkey and dog combination, the best predictive index was presented by the transaminase determinations which gave a total true prediction of 53 to 55 per cent with a false negative prediction of 0 to 5 per cent while overpredicting for 40 to 46 per cent of compounds tested. The BSP determination gave a 44 to 50 per cent overprediction with the combination of the two species with a total false prediction (false positives plus false negatives) of 55.5 to 66.7 per cent, having failed to predict for four of nine compounds. The data were analyzed to determine whether the alkaline phosphatase and transaminase tests could substitute for all cases in which the BSP in either species gave a true positive and could correct for all false negative predictions. In Table XV it can be seen that the alkaline phosphatase in the dog could substitute for BSP in the five cases in which the latter determination gave a true positive. The addition of the SGPT and SGOT in dog and monkey to the alkaline phosphatase gives added confidence. The data were similarly analyzed to determine whether the alkaline phosphatase and transaminase tests could

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Table VII. *Dog as a predictor for selected parameter toxicity in man*

	*TP**	*FP*†	*TN*‡	*FN*§	$\frac{FN‖}{TP + FN}$
Injection site inflammation	2/22¶	7/22	12/22	1/22	1/3
	9.1%	31.8%	54.5%	4.5%	33.3%
Injection site thrombosis	2/22	1/22	17/22	2/22	2/4
	9.1%	4.5%	77.0%	9.1%	50.0%
Dermatitis	0/25	0/25	23/25	2/25	2/2
			92.0%	8.0%	100.0%
Petechiae	0/25	0/25	20/25	5/25	5/5
			80.0%	20.0%	100.0%
Jaundice	0/25	3/25	20/25	2/25	2/2
		12.0%	80.0%	8.0%	100.0%
Flushing	0/25	0/25	21/25	4/25	4/4
			84.0%	16.0%	100.0%
Tachycardia	1/25	3/25	17/25	4/25	4/5
	4.0%	12.0%	68.0%	16.0%	80.0%
Lethargy	3/25	13/25	9/25	0/25	0/3
	12.0%	52.0%	36.0%		
Depression of sensorium	3/25	7/25	14/25	1/25	1/4
	12.0%	28.0%	56.0%	4.0%	25.0%
Agitation	1/25	3/25	20/25	1/25	1/2
	4.0%	12.0%	80.0%	4.0%	50.0%
Ataxia	2/25	9/25	13/25	1/25	1/3
	8.0%	36.0%	52.0%	4.0%	33.0%
Tremor	0/25	7/25	18/25	0/25	0/0
		28.0%	72.0%		
Seizures	1/25	4/25	20/25	0/25	0/1
	4.0%	16.0%	80.0%		
Dyspnea	1/25	6/25	16/25	2/25	2/3
		24.0%	64.0%	8.0%	66.0%
Lacrimation ↑	2/25	6/25	17/25	0/25	0/2
	8.0%	24.0%	68.0%		
Ophthalmoplegia	0/25	0/25	24/25	1/25	1/1
			96.0%	4.0%	100.0%
Weight loss	0/25	23/25	2/25	0/25	0/0
		92.0%	8.0%		
Vomiting	18/25	4/25	0/25	3/25	3/21
	72.0%	16.0%		12.0%	14.4%

*True positive, toxicity was observed both in dogs and in man.

†False positive, toxicity was observed in dogs but not in man.

‡True negative, no toxicity was observed in dogs and man.

§False negative, toxicity was not observed in the dogs but was recorded in man.

‖$\frac{\text{False negatives}}{\text{True positives plus false negatives}}$ —corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

¶$\frac{\text{Numerator}}{\text{denominator}} = \frac{\text{the number of drugs producing toxicity}}{\text{the number of drugs tested}}$.

Continued on next page.

Table VII. *Cont'd*

	*TP**	*FP†*	*TN‡*	*FN§*	$\frac{FN\|}{TP + FN}$
Salivation ↑	3/25 12.0%	9/25 36.0%	13/25 52.0%	0/25	0/3
Diarrhea	9/25 36.0%	10/25 40.0%	5/25 20.0%	1/25 4.0%	1/10 100.0%
Melena	0/25	3/25 12.0%	20/25 80.0%	2/25 8.0%	2/2 100.0%
Hematochezia	1/25 4.0%	17/25 68.0%	7/25 28.0%	0/25	0/1
Stomatitis	1/25 4.0%	1/25 4.0%	19/25 76.0%	4/25 16.0%	4/5 80.0%
Hemoglobin ↓	11/25 44.0%	7/25 28.0%	6/25 24.0%	1/25 4.0%	1/12 8.3%
Hematocrit ↓	11/25 44.0%	7/25 28.0%	6/25 24.0%	1/25 4.0%	1/12 8.3%
Red blood cell count ↓	9/25 36.0%	9/25 36.0%	6/25 24.0%	1/25 4.0%	1/10 10.0%
White blood cell count ↓	15/25 60.0%	1/25 4.0%	4/25 16.0%	5/25 20.0%	5/20 25.0%
Thrombocytopenia	13/22 59.1%	3/22 13.6%	3/22 13.6%	3/22 13.6%	3/16 18.7%
Hypercalcemia	0/3	2/3 66.7%	1/3 33.3%	0/3	0/0
BSP retention ↑	2/18 11.1%	12/18 66.6%	4/18 22.2%	0/18	0/2
Alkaline phosphatase ↑	6/24 25.0%	12/24 50.0%	5/24 20.8%	1/24 4.2%	1/7 14.2%
SGOT ↑	5/18 27.8%	7/18 38.9%	4/18 22.2%	2/18 11.1%	2/7 28.4%
SGPT ↑	3/11 27.3%	5/11 45.5%	3/11 27.3%	0/11	0/3
Bilirubinemia	2/16 12.5%	4/16 25.0%	10/16 62.5%	0/16	0/2
Prothrombin time ↑	0/21	4/21 19.0%	15/21 71.4%	2/21 9.5%	2/2 100.0%
Blood urea nitrogen ↑	6/25 24.0%	9/25 36.0%	7/25 28.0%	3/25 12.0%	3/9 33.0%
Creatinine ↑	0/6	1/6 16.7%	5/6 83.3%	0/6	0/0
Hyperglycemia	0/25	4/25 16.0%	20/25 80.0%	1/25 4.0%	1/1 100.0%
Hypoglycemia	0/25	3/25 12.0%	22/25 88.0%	0/25	0/0

Table VII. *Cont'd*

	*TP**	*FP†*	*TN‡*	*FN§*	$\frac{FN\|\|}{TP + FN}$
Proteinuria	1/11 9.1%	8/11 72.7%	2/11 18.2%	0/11	0/1
Glycosuria	0/11	4/11 36.4%	7/11 63.6%	0/11	0/0
Bilirubinuria	0/11	5/11 45.5%	6/11 54.5%	0/11	0/0
Hematuria	1/11 9.1%	4/11 36.4%	6/11 54.5%	0/11	0/1
Hyposthenuria	0/14	6/14 42.9%	8/14 57.1%	0/14	0/0

FN§	$\frac{FN\|\|}{TP + FN}$
0/25	0/3
1/25 4.0%	1/10 100.0%
2/25 8.0%	2/2 100.0%
0/25	0/1
4/25 16.0%	4/5 80.0%
1/25 4.0%	1/12 8.3%
1/25 4.0%	1/12 8.3%
1/25 4.0%	1/10 10.0%
5/25 20.0%	5/20 25.0%
3/22 13.6%	3/16 18.7%
0/3	0/0
0/18	0/2
1/24 4.2%	1/7 14.2%
2/18 11.1%	2/7 28.4%
0/11	0/3
0/16	0/2
2/21 9.5%	2/2 100.0%
3/25 12.0%	3/9 33.0%
0/6	0/0
1/25 4.0%	1/1 100.0%
0/25	0/0

correct for the underprediction, or false negatives, made by the BSP determination. Table XVI presents the results for the four compounds whose liver toxicity was not predicted by BSP. It can be seen that a true positive correlation is made in all cases with the use of both the alkaline phosphatase and SGOT determination and the two laboratory animal species.

Kidney. The large animal screen gave 36 per cent true positives for renal toxicity but underpredicted for one of twenty-five drugs and gave a 56 per cent overprediction (Table XIII). In general, no specific advantage could be attributed to use of either the dog or monkey. The blood urea nitrogen determination gave 24 per cent true positives, while overpredicting 52 per cent, and a 33 per cent corrected false prediction (Table IX). Serum creatinine was measured in man for only seven compounds, and the animal data overpredicted 43 per cent for toxicity. Proteinuria in the animals gave a gross overprediction of 73 per cent for this abnormality in man, as did hematuria and hyposthenuria. The high incidence of renal function abnormalities in the animals had its counterpart in the renal histopathologic changes which were documented for 64 and 56 per cent of drugs in dogs and monkeys, respectively (Table VI).

Neuromuscular. The combination of dog and monkey predicted for the neuromuscular abnormalities of six of seven compounds which produced this form of toxicity in man. Neither species presented a distinctly greater predictive value, though the monkey demonstrated a lesser degree of overprediction. The screen predicted for two of the three instances of ataxia and the one drug that produced convulsions. The two drugs that produced excessive lacrimation and the one case of opthalmoplegia had true positive correlations with the monkey data. There was a large overprediction of specific parameters which contributed to the favorable predictive index for neuromuscular toxicity as a group.

Cardiovascular. The combined large animal screen gave a 36 per cent true positive index, with a total true prediction of 64 per cent, and failed to predict for the cardiovascular toxicity of one of ten compounds. The overlap of monkey and dog data contributed to both the higher incidence of true positive and lower index of false negative when compared with predictions of the individual species.

Respiratory. The screen predicted for the respiratory signs or pathology for four of the five compounds at the expense of a large percentage of false positives. In general, neither the dog nor monkey proved superior in predictive value.

Injection site. The injection site toxicity

Table VIII. *Monkey as a predictor for selected parameter toxicity in man*

	*TP**	*FP*†	*TN*‡	*FN*§	$\frac{FN\|}{TP + FN}$
Injection site inflammation	2/20¶	6/20	11/20	1/20	1/3
	10.0%	30.0%	55.0%	5.0%	33.0%
Injection site thrombosis	1/20	3/20	14/20	2/20	2/3
	5.0%	15.0%	70.0%	10.0%	66.0%
Dermatitis	0/23	1/23	20/23	2/23	2/2
		4.3%	87.0%	8.7%	100.0%
Petechiae	1/23	0/23	18/23	4/23	4/5
	4.3%		78.3%	17.4%	80.0%
Jaundice	0/23	0/23	22/23	1/23	1/1
			95.7%	4.3%	100.0%
Flushing	1/22	0/22	18/22	3/22	3/4
	4.5%		81.8%	13.6%	75.0%
Tachycardia	0/23	1/23	17/23	5/23	5/5
		4.3%	73.9%	21.7%	100.0%
Lethargy	1/23	7/23	13/23	2/23	2/3
	4.3%	30.4%	56.5%	8.7%	66.0%
Depression of sensorium	2/23	6/23	13/23	2/23	2/4
	8.7%	26.1%	56.5%	8.7%	50.0%
Agitation	0/23	0/23	21/23	2/23	2/2
			91.3%	8.7%	100.0%
Ataxia	1/23	3/23	17/23	2/23	2/3
	4.3%	13.0%	73.9%	8.7%	66.0%
Tremor	0/23	5/23	18/23	0/23	0/0
		21.7%	78.3%		
Seizures	1/23	1/23	21/23	0/23	0/1
	4.3%	4.3%	91.3%		
Dyspnea	0/23	5/23	15/23	3/23	3/3
		21.7%	65.0%	13.0%	100.0%
Lacrimation ↑	2/23	1/23	20/23	0/23	0/2
	8.7%	4.3%	87.0%		
Ophthalmoplegia	1/23	0/23	22/23	0/23	0/1
	4.3%		95.7%		
Weight loss	0/23	14/23	9/23	0/23	0/0
		60.9%	39.1%		
Vomiting	6/23	3/23	1/23	13/23	13/19
	26.1%	13.0%	4.3%	56.5%	68.4%

*True positive, toxicity was observed both in monkeys and in man.

†False positive, toxicity was observed in monkeys but not in man.

‡True negative, no toxicity was observed in monkeys and man.

§False negative, toxicity was not observed in the monkeys but was recorded in man.

‖$\frac{\text{False negatives}}{\text{True positives plus false negatives}}$—corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

¶$\frac{\text{Numerator}}{\text{Denominator}} = \frac{\text{number of drugs producing toxicity}}{\text{number of drugs tested}}$.

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FN§	FN‖ / TP + FN
1/20 5.0%	1/3 33.0%
2/20 10.0%	2/3 66.0%
2/23 8.7%	2/2 100.0%
4/23 17.4%	4/5 80.0%
1/23 4.3%	1/1 100.0%
3/22 13.6%	3/4 75.0%
5/23 21.7%	5/5 100.0%
2/23 8.7%	2/3 66.0%
2/23 8.7%	2/4 50.0%
2/23 8.7%	2/2 100.0%
2/23 8.7%	2/3 66.0%
0/23	0/0
0/23	0/1
3/23 13.0%	3/3 100.0%
0/23	0/2
0/23	0/1
0/23	0/0
13/23 56.5%	13/19 68.4%

prediction which analyzes for

Table VIII. *Cont'd*

	TP*	FP†	TN‡	FN§	FN‖ / TP + FN
Salivation ↑	2/23 8.7%	2/23 8.7%	18/23 78.3%	1/23 4.3%	1/3 33.0%
Diarrhea	3/23 13.0%	6/23 26.1%	8/23 34.8%	6/23 26.1%	6/9 66.0%
Melena	0/23	0/23	21/23 91.3%	2/23 8.7%	2/2 100.0%
Hematochezia	0/23	5/23 21.7%	17/23 73.9%	1/23 4.3%	1/1 100.0%
Stomatitis	0/23	1/23 4.3%	18/23 78.3%	4/23 17.4%	4/4 100.0%
Hemoglobin ↓	8/22 36.4%	9/22 40.9%	2/22 9.1%	3/22 13.6%	3/11 27.0%
Hematocrit ↓	8/23 34.8%	10/23 43.5%	2/23 8.7%	3/23 13.0%	3/11 27.3%
Red blood cell count ↓	6/22 27.3%	11/22 50.0%	2/22 9.1%	3/22 13.6%	3/9 33.0%
White blood cell count ↓	14/23 60.9%	3/23 13.0%	2/23 8.7%	4/23 17.4%	4/18 22.2%
Thrombocytopenia	7/22 31.8%	0/22	6/22 27.3%	9/22 40.9%	9/16 56.3%
Hypercalcemia	0/1	0/1	1/1 100.0%	0/1	0/0
BSP retention ↑	1/9 11.1%	6/9 66.7%	1/9 11.1%	1/9 11.1%	1/2 50.0%
Alkaline phosphatase ↑	3/20 15.0%	2/20 10.0%	11/20 55.0%	4/20 20.0%	4/7 57.0%
SGOT ↑	6/18 33.3%	4/18 22.2%	6/18 33.3%	2/18 11.1%	2/8 25.0%
SGPT ↑	2/13 15.4%	4/13 30.8%	6/13 46.2%	1/13 7.7%	1/3 33.0%
Bilirubinemia	0/14	2/14 14.3%	11/14 78.6%	1/14 7.1%	1/1 100.0%
Prothrombin time ↑	0/6	1/6 16.7%	4/6 66.7%	1/6 16.7%	1/1 100.0%
Blood urea nitrogen ↑	4/22 18.2%	10/22 45.5%	4/22 18.2%	4/22 18.2%	4/8 50.0%
Creatinine ↑	0/5	3/5 60.0%	2/5 40.0%	0/5	0/0
Hyperglycemia	0/22	7/22 31.8%	14/22 63.6%	1/22 4.5%	1/1 100.0%
Hypoglycemia	0/22	1/22 4.5%	21/22 95.5%	0/22	0/0
Proteinuria	0/7	3/7 42.9%	3/7 42.9%	1/7 14.3%	1/1 100.0%

Continued on next page.

Table VIII. *Cont'd*

	TP*	FP†	TN‡	FN§	FN‖ / TP + FN
Glycosuria	0/9	2/9 22.2%	7/9 77.8%	0/9	0/0
Bilirubinuria	0/8	1/8 12.5%	7/8 87.5%	0/8	0/0
Hematuria	0/8	1/8 12.5%	6/8 75.0%	1/8 12.5%	1/1 100.0%
Hyposthenuria	0/9	1/9 11.1%	8/9 88.9%	0/9	0/0

of four of six compounds was predicted by the screen with a 36 per cent overprediction. There was no evidence of an animal species specificity. The animal studies did not allow anticipation of pain of injection with four of five compounds that produced this difficulty.

Integument and dermal manifestations of systemic toxicity. The skin of animals failed to correlate with the dermal lesions noted with therapy in man. The screen missed all cases of dermatitis and jaundice while predicting for one of five drugs that caused cutaneous bleeding.

Eyes. There were no cases of drug-related conjunctivitis, corneal scarring, or cataracts associated with therapy in either the monkey or man, but dogs developed conjunctivitis with two of the twenty-five compounds.

Requirement for the administration of highly toxic dose levels for the prediction of specific abnormalities. Moderately to severely toxic dose levels were required in the dog and monkey to disclose the drug-produced thrombocytopenia of mithramycin, mitomycin C, and dibromomannitol and for the leukopenia caused by 5-fluorouracil, the ammonium compound, azotomycin, and sangivamycin. Toxic doses were required for uncovering the hepatotoxicity for the acetophenone compound and cytosine arabinoside and the neurologic toxicity of restrictocin and 1-acetyl-2-picolinoyl-hydrazine.

Discussion

The medical community has taken an ambivalent or generally negative view of the ability of animal toxicology studies to predict for toxic drug effects in man.[4, 12, 14, 23, 39, 60-62, 96, 113] There has been much support for the concept that prediction from animal data is perilous and that only through trial by ordeal in man can clinical toxicities be delineated. The field of chemical pharmacology has generated a wealth of data clearly demonstrating that species' differences in drug metabolism are the rule rather than the exception, while absorption, distribution, storage, and excretion tend to be similar.[9-12] However, it must be noted that to the greatest extent these studies were conducted to explain quantitative differences in principal therapeutic effect on the primary target organ (i.e., the length of barbiturate anesthesia) and have been much less concerned or correlated with accompaning qualitative toxicities in other organ systems. Another source of concern regarding the usefulness of animal toxicology stems from examples in the literature in which an animal screen failed to predict or overpredicted for a specific drug effect in man. Several of these reports have been repeatedly cited as classic illustrations of the futility of animal experimentation. Many of these examples should be re-examined in a positive sense for clues that may lead to more realistic expectations from animal data

FN§	FN‖ / TP + FN
0/9	0/0
0/8	0/0
1/8 12.5%	1/1 100.0%
0/9	0/0

mmunity has taken an rally negative view of l toxicology studies to ug effects in man.[4, 12] here has been much ncept that prediction perilous and that only deal in man can clini- lineated. The field of logy has generated a rly demonstrating that in drug metabolism are n the exception, while tion, storage, and ex- similar.[9-12] However, it to the greatest extent conducted to explain nces in principal thera- e primary target organ barbiturate anesthesia) ach less concerned or companing qualitative rgan systems. Another egarding the usefulness y stems from examples which an animal screen r overpredicted for a t in man. Several of been repeatedly cited ns of the futility of ani- n. Many of these exam- examined in a positive at may lead to more ns from animal data

Table IX. *The combination of the dog and monkey as a predictor for selected parameter toxicity in man*

	TP*	FP†	TN‡	FN§	FN‖ / TP + FN
Injection site inflammation	2/22¶ 9.1%	10/22 45.5%	9/22 40.9%	1/22 4.5%	1/3 33.0%
Injection site thrombosis	2/22 9.1%	3/22 13.6%	15/22 68.2%	2/22 9.1%	2/4 50.0%
Dermatitis	0/25	1/25 4.0%	22/25 88.0%	2/25 8.0%	2/2 100.0%
Petechiae	1/25 4.0%	0/25	20/25 80.0%	4/25 16.0%	4/5 80.0%
Jaundice	0/25	3/25 12.0%	20/25 80.0%	2/25 8.0%	2/2 100.0%
Flushing	1/25 4.0%	0/25	21/25 84.0%	3/25 12.0%	3/4 75.0%
Tachycardia	1/25 4.0%	3/25 12.0%	17/25 68.0%	4/25 16.0%	4/5 80.0%
Lethargy	3/25 12.0%	15/25 60.0%	7/25 28.0%	0/25	0/3
Depression of sensorium	4/25 16.0%	11/25 44.0%	10/25 40.0%	0/25	0/4
Agitation	1/25 4.0%	3/25 12.0%	20/25 80.0%	1/25 4.0%	1/2 50.0%
Ataxia	2/25 8.0%	11/25 44.0%	11/25 44.0%	1/25 4.0%	1/3 33.0%
Tremor	0/25	9/25 36.0%	16/25 64.0%	0/25	0/0
Seizures	1/25 4.0%	4/25 16.0%	20/25 80.0%	0/25	0/1
Dyspnea	1/25 4.0%	7/25 28.0%	15/25 60.0%	2/25 8.0%	2/3 66.0%
Lacrimation ↑	2/25 8.0%	6/25 24.0%	17/25 68.0%	0/25	0/2
Ophthalmoplegia	1/25 4.0%	0/25	24/25 96.0%	0/25	0/1
Weight loss	0/25	23/25 92.0%	2/25 8.0%	0/25	0/0

*True positive, toxicity was observed in both animals (dog or monkey) and in man.

†False positive, toxicity was observed in the animals but not in man.

‡True negative, no toxicity was observed in the animals and man.

§False negative, toxicity was not observed in the animals but was recorded in man.

‖$\frac{\text{False negatives}}{\text{True positives plus false negatives}}$ —corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

¶$\frac{\text{Numerator}}{\text{Denominator}} = \frac{\text{number of drugs producing toxicity}}{\text{number of drugs tested}}$.

Continued on next page.

Table IX. *Cont'd*

	*TP**	*FP*†	*TN*‡	*FN*§	$\frac{FN\|}{TP + FN}$
Vomiting	18/25 72.0%	4/25 16.0%	0/25	3/25 12.0%	3/21 14.0%
Salivation ↑	3/25 12.0%	9/25 36.0%	13/25 52.0%	0/25	0/3
Diarrhea	9/25 36.0%	11/25 44.0%	4/25 16.0%	1/25 4.0%	1/10 10.0%
Melena	0/25	3/25 12.0%	20/25 80.0%	2/25 8.0%	2/2 100.0%
Hematochezia	1/25 4.0%	18/25 72.0%	6/25 24.0%	0/25	0/1
Stomatitis	1/25 4.0%	2/25 8.0%	18/25 72.0%	4/25 16.0%	4/5 80.0%
Hemoglobin ↓	12/25 48.0%	10/25 40.0%	3/25 12.0%	0/25	0/12
Hematocrit ↓	12/25 48.0%	11/25 44.0%	2/25 8.0%	0/25	0/12
Red blood cell count ↓	10/25 40.0%	12/25 48.0%	3/25 12.0%	0/25	0/10
White blood cell count ↓	17/25 68.0%	3/25 12.0%	2/25 8.0%	3/25 12.0%	3/20 15.0%
Thrombocytopenia	13/24 54.2%	3/24 12.5%	3/24 12.5%	5/24 20.8%	5/18 27.8%
Hypercalcemia	0/3	2/3 66.7%	1/3 33.3%	0/3	0/0
BSP retention ↑	2/19 10.5%	12/19 63.2%	4/19 21.1%	1/19 5.3%	1/3 33.0%
Alkaline phosphatase ↑	6/25 24.0%	12/25 48.0%	5/25 20.0%	2/25 8.0%	2/8 25.0%
SGOT ↑	7/20 35.0%	8/20 40.0%	4/20 20.0%	1/20 5.0%	1/8 12.5%
SGPT ↑	3/13 23.1%	7/13 53.8%	3/13 23.1%	0/13	0/3
Bilirubinemia	2/18 11.1%	5/18 27.8%	11/18 61.1%	0/18	0/2
Prothrombin time ↑	0/21	5/21 23.8%	14/21 66.7%	2/21 9.5%	2/2 100.0%
Blood urea nitrogen ↑	6/25 24.0%	13/25 52.0%	3/25 12.0%	3/25 12.0%	3/9 33.0%
Creatinine ↑	0/7	3/7 42.9%	4/7 57.1%	0/7	0/0
Hyperglycemia	0/25	10/25 40.0%	14/25 56.0%	1/25 4.0%	1/1 100.0%

FN§	FN‖ / TP + FN
3/25 12.0%	3/21 14.0%
0/25	0/3
1/25 4.0%	1/10 10.0%
2/25 8.0%	2/2 100.0%
0/25	0/1
4/25 16.0%	4/5 80.0%
0/25	0/12
0/25	0/12
0/25	0/10
3/25 12.0%	3/20 15.0%
5/24 20.8%	5/18 27.8%
0/3	0/0
1/19 5.3%	1/3 33.0%
2/25 8.0%	2/8 25.0%
1/20 5.0%	1/8 12.5%
0/13	0/3
0/18	0/2
2/21 9.5%	2/2 100.0%
3/25 12.0%	3/9 33.0%
0/7	0/0
1/25 4.0%	1/1 100.0%

Table IX. *Cont'd*

	TP*	FP†	TN‡	FN§	FN‖ / TP + FN
Hypoglycemia	0/25	4/25 16.0%	21/25 84.0%	0/25	0/0
Proteinuria	1/11 9.1%	8/11 72.7%	2/11 18.2%	0/11	0/1
Glycosuria	0/13	5/13 38.5%	8/13 61.5%	0/13	0/0
Bilirubinuria	0/12	5/12 41.7%	7/12 58.3%	0/12	0/0
Hematuria	1/12 8.2%	5/12 41.7%	6/12 50.0%	0/12	0/1
Hyposthenuria	0/15	6/15 40.0%	9/15 60.0%	0/15	0/0

and a better understanding of their limitations. Typical is the case of 6-azauracil, an antimetabolite selected for therapeutic trial in man on the basis of its antitumor activity in a number of rodent tumor systems. During the clinical studies, it was discovered that the principal dose-limiting toxicity involved the central nervous system, as manifested by lethargy leading to a wide range of sensory, motor, and psychic disturbances associated with electroencephalographic changes.[96] These profound CNS effects occurred at doses that were considered tolerated in a wide range of animal species. However, ample indications of CNS toxicity were available in the animal data, though very high doses were required. In mice, intraperitoneal injection of doses of 3,000 mg. per kilogram produced deep hypnosis, respiratory depression, and loss of response to painful stimuli. Narcosis was produced in rats at a dose of 1,000 mg. per kilogram.[107] Other studies in rats have demonstrated electroencephalographic patterns similar to those noted in man, with subsequent production of profound ataxia.[75] Temporary paralysis of lower extremities has been reported in dogs and cats after administration of doses of 1,200 and 1,400 mg. per kilogram, respectively.[107] The quantitative differences in dose level required to achieve neurologic toxicity in animals as compared to those required in man may relate to differences in metabolism and/or species-related variance of target organ sensitivity.

The analysis of the CNS toxicity of 6-azauracil can be used to make two important points. Very large toxic doses should be administered to animals in order to elicit all the potential qualitative toxicities of a compound. If a particular toxicity is observed during the animal studies, it cannot be disregarded since it may appear in man, though at a much different dose level, or may follow a different order of appearance in relationship to the total spectrum of toxic signs. In our comparative study we have encountered a similar situation in the case of 1-acetyl-2-picolinoyl-hydrazine, NSC-68626. The principal dose-limiting toxicity in man is somnolence leading to coma. Neurologic toxicity was seen in the dog in the form of ataxia and convulsions but required high-dose treatment for elicitation.

Another point of concern is that overprediction by a sensitive animal species may prevent a potentially important drug from ever reaching therapeutic trial in

Table X

	Species that failed to predict for leukopenia in man	*Species that failed to predict for thrombocytopenia in man*
NSC-13875 Melamine, hexamethyl-	Monkey	Monkey
NSC-17256E Pregn-4-ene-3,11,20-trione,6α-methyl-	Dog, monkey	—
NSC-19893 Uracil, 5-fluoro-	—	Monkey
NSC-24559 Mithramycin (USAN)	Monkey	Dog, monkey
NSC-26980 Mitomycin C	—	Monkey
NSC-40774 9*H*-Purine, 6-(methylthio)-9-β-*D* ribofuranosyl-, dihydrate	Dog	Monkey (not measured in dog)
NSC-51095 Ammonium, trimethylpurin-6-yl-chloride	Monkey	Monkey
NSC-52947 Pactamycin	—	Monkey
NSC-53398 Restrictocin	—	Dog, monkey
NSC-56408 Tubercidin	Dog, monkey	—
NSC-62512 Acetophenone, 2-(dimethylamino)-3′,4′-dihydroxy-, hydrochloride	—	Dog, monkey
NSC-65346 Sangivamycin	Dog	—
NSC-69945 Phosphorodiamidic acid, *N,N,*-bis (2-chloroethyl)-, compd. with cyclohexylamine (1:1)	—	Dog, monkey

man. The model often used for illustration is the high degree of toxicity produced by penicillin in the guinea pig.[14, 60] The original study by Hamre and associates[42] showed that the single-dose toxicity of penicillin in guinea pigs was similar to that in mice and rabbits. A species difference arose when repeated-dose toxicity studies were conducted in which, at relatively low dosage, the guinea pigs showed a significant mortality rate. The mechanism of this mortality rate has been the subject of study for many years. The data suggest that death is not a direct drug effect but is, at least in part, related to the replacement of the normal gram-positive intestinal bacterial flora with an overgrowth or superinfection with gram-negative coliforms.[25] Thus, the guinea pig would have predicted for one of the principal clinical problems associated with the use of penicillin in man—superinfection; though in the clinic, overgrowth with *Staphylococcus aureus* is more commonly

ies that failed to predict
hrombocytopenia in man

y

—

y

nonkey

y

y (not measured in dog)

y

y

nonkey

—

nonkey

—

nonkey

many years. The data is not a direct drug st in part, related to the normal gram-posi- ial flora with an over- ction with gram-nega- ius, the guinea pig d for one of the prin- ns associated with the man—superinfection; ic, overgrowth with us is more commonly

Table XI. *Dog as a predictor for organ-specific toxicity in man*

Organ system	TP* (%)	FP† (%)	TN‡ (%)	FN§ (%)	FN‖ / TP + FN (%)	No. of compounds
Injection site	16	36	40	8	33	25
Integument	12	32	40	16	57	25
Cardiovascular	28	24	36	12	30	25
Respiratory	16	64	16	4	20	25
Bone marrow	80	12	0	8	9	25
Lymphoid	4	72	24	0	0	25
Gastrointestinal	92	8	0	0	0	25
Liver	52	44	4	0	0	25
Renal	32	56	4	8	20	25
Neuromuscular	24	60	12	4	14	25

*True positive, toxicity was observed in both the dogs and in man.

†False positive, toxicity was observed in the dogs but not in man.

‡True negative, no toxicity was observed in the dogs and man.

§False negative, toxicity was not observed in the dogs but was recorded in man.

‖$\frac{\text{False negatives}}{\text{true positives plus false negatives}}$ —corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

Table XII. *Monkey as a predictor for organ-specific toxicity in man*

Organ system	TP* (%)	FP† (%)	TN‡ (%)	FN§ (%)	FN‖ / TP + FN (%)	No. of compounds
Injection site	13	26	52	9	40	23
Integument	13	17	57	13	50	23
Cardiovascular	22	26	30	22	50	23
Respiratory	13	48	30	9	40	23
Bone marrow	83	13	0	4	5	23
Lymphoid	0	31	65	4	100	23
Gastrointestinal	74	9	0	17	19	23
Liver	52	35	13	0	0	23
Renal	35	48	13	4	11	23
Neuromuscular	22	30	39	9	28	23

*True positive, toxicity was observed in both the monkeys and in man.

†False positive, toxicity was observed in the monkeys but not in man.

‡True negative, no toxicity was observed in the monkeys and man.

§False negative, toxicity was not observed in the monkeys but was recorded in man.

‖$\frac{\text{False negatives}}{\text{true positives plus false negatives}}$ —corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

encountered.[104] In addition, it has been shown that penicillin can be used effectively to cure the guinea pig of an otherwise lethal infection of leptospirosis.[44] Similar analysis can be made of many other cases in which animals seemingly falsely predicted for toxicity. It becomes important to re-examine the animal data not only for the study of these negative predictive aspects but also for documentation of valuable positive contributions.

Although the literature contains a number of thorough reviews and discussions on the subject of animal prediction of toxicity, a considerable "data gap" exists. Three previous comparative studies have been reported and are worthy of review. Litchfield[61, 62] reported on his analysis of

Table XIII. *The combination of dog and monkey as a predictor for organ-specific toxicity in man*

Organ system	*TP** (%)	*FP†* (%)	*TN‡* (%)	*FN§* (%)	*FN‖* / *TP + FN* (%)	*No. of compounds*
Injection site	16	36	40	8	33	25
Integument	24	36	36	4	14	25
Cardiovascular	36	32	28	4	10	25
Respiratory	16	76	4	4	20	25
Bone marrow	88	12	0	0	0	25
Lymphoid	4	76	20	0	0	25
Gastrointestinal	92	8	0	0	0	25
Liver	52	48	0	0	0	25
Renal	36	56	4	4	10	25
Neuromuscular	24	60	12	4	14	25

*True positive, toxicity was observed in both the animals and in man.

†False positive, toxicity was observed in the animals but not in man.

‡True negative, no toxicity was observed in the animals and man.

§False negative, toxicity was not observed in the animals but was recorded in man.

‖$\frac{\text{False negatives}}{\text{true positives plus false negatives}}$ —corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

six unnamed drugs which included "an antibiotic, a synthetic antibacterial agent, a tranquilizer, a central nervous system depressant, a chemical which blocks oxidation of alcohol, and a glucocorticoid." The compounds were fed to dogs for at least six months and to rats for one year, and, though doses are not stated, the reader is assured that "dosage levels were used which exceeded the tolerance of the animals." Information on the response in man consisted of 500 or more case reports for each compound. Analysis was made on 39 equally weighted physical signs or hematologic parameters, giving a total of 234 possible signs for the six drugs. Analysis of the data demonstrates that the rat predicted for 34 per cent of the 53 total toxic signs observed in man, thus leaving a 66 per cent corrected false negative index. The dog predicted for 55 per cent of total positive signs, while the composite of dog and rat data predicted for only 57 per cent of the cumulative positive signs with 43 per cent corrected false negatives. When the criteria for predictive toxicity were limited to a sign seen in both dog and rat, the result was the lowest forecast for the total number of toxic signs in man, 32 per cent, leaving a significant 68 per cent corrected false negative correlation. This study further analyzes this latter group for incidence of correct prediction as a per cent of the total positive predictions made. It is stated that toxicity in dog and rat as a predictor showed ". . . 68 per cent of the positive prediction and 79 per cent of the negative prediction were right, for an over-all score of 74 per cent."[62] This was interpreted as demonstrating that a toxic parameter shown in two species would more likely predict for toxicity in a third. However, this type of analysis distorts the data and gives a false impression of high correlation with man. It represents only the efficiency of a positive prediction, the number of times it predicted correctly of those times it predicted for toxicity, but fails to take into account the number of false negatives, which in this case represents 26 signs or 68 per cent of the toxicity seen in man.

Of great interest was the remarkable duplication in the rat and dog data. Eighteen out of 234 toxic signs were seen in the rat, while 29 were detected in the

an-specific

FN‖ / TP + FN (%)	No. of compounds
33	25
14	25
10	25
20	25
0	25
0	25
0	25
0	25
10	25
14	25

prediction which analyzes for city in man.

of toxic signs in man, g a significant 68 per negative correlation. analyzes this latter of correct prediction total positive predic- ed that toxicity in dog ctor showed ". . . 68 tive prediction and 79 gative prediction were -all score of 74 per nterpreted as demon- parameter shown in nore likely predict for However, this type of data and gives a false correlation with man. ıe efficiency of a posi- number of times it of those times it pre- but fails to take into er of false negatives, represents 26 signs or icity seen in man.

was the remarkable ıt and dog data. Eigh- ic signs were seen in were detected in the

Table XIV. *The use of single liver function parameters in dogs and monkeys as a predictor for hepatotoxicity in man*

	TP*	FP†	TN‡	FN§	FN‖ / TP + FN
Dog					
BSP ↑	5/18¶ 27.8%	9/18 50.0%	1/18 5.6%	3/18 16.7%	3/8 37.0%
Alkaline phosphatase ↑	8/24 33.3%	10/24 41.7%	4/24 16.7%	2/24 8.3%	2/10 20.0%
SGOT ↑	5/18 27.8%	7/18 38.9%	4/18 22.2%	2/18 11.1%	2/7 28.0%
SGPT ↑	3/11 27.3%	5/11 45.5%	3/11 27.3%	0/11	0/3
Bilirubin ↑	3/16 18.8%	3/16 18.8%	7/16 43.8%	3/16 18.8%	3/6 50.0%
Prothrombin time ↑	3/21 14.3%	1/21 4.8%	10/21 47.6%	7/21 33.3%	7/10 70.0%
Monkey					
BSP ↑	3/9 33.3%	4/9 44.4%	1/9 11.1%	1/9 11.1%	1/4 25.0%
Alkaline phosphatase ↑	3/20 15.0%	2/20 10.0%	8/20 40.0%	7/20 35.0%	7/10 70.0%
SGOT ↑	6/18 33.3%	4/18 22.2%	6/18 33.3%	2/18 11.1%	2/8 25.0%
SGPT ↑	3/13 23.1%	3/13 23.1%	6/13 46.2%	1/13 7.7%	1/4 25.0%
Bilirubin ↑	0/14	2/14 14.3%	6/14 42.9%	6/14 42.9%	6/6 100.0%
Prothrombin time ↑	0/6	1/6 16.7%	3/6 50.3%	2/6 33.3%	2/2 100.0%
Dog and monkey					
BSP ↑	5/19 26.3%	9/19 47.4%	1/19 5.3%	4/19 21.1%	4/9 44.0%
Alkaline phosphatase ↑	8/25 32.0%	10/25 40.0%	4/25 16.0%	3/25 12.0%	3/11 28.0%
SGOT ↑	7/20 35.0%	8/20 40.0%	4/20 20.0%	1/20 5.0%	1/8 12.5%
SGPT ↑	4/13 30.1%	6/13 46.2%	3/13 23.1%	0/13	0/4
Bilirubin ↑	3/18 16.7%	4/18 22.2%	7/18 30.9%	4/18 22.2%	4/7 57.0%
Prothrombin time ↑	3/21 14.3%	2/21 9.5%	9/21 42.8%	7/21 33.3%	7/10 70.0%

*True positive, toxicity was observed in both the animals and in man.

†False positive, toxicity was observed in the animals but not in man.

‡True negative, no toxicity was observed in the animals and man.

§False negative, toxicity was not observed in the animals but was recorded in man.

‖$\frac{\text{False negatives}}{\text{true positives plus false negatives}}$ —corrected false negative, an index of false negative prediction which analyzes for only those compounds which produced the specific toxicity in man.

¶$\frac{\text{Numerator}}{\text{denominator}} = \frac{\text{number of drugs producing toxicity}}{\text{number of drugs tested}}$.

Table XV. *Reaction of the alkaline phosphatase and serum transaminase determinations when BSP retention gave a true positive correlation for liver toxicity*

	Acetophenone, 2-(dimethylamino)-3' 4'-dihydroxy-, hydrochloride	*Pactamycin*	*9H-Purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate*	*Tubercidin*	*Urea, 1,3-bis (2-chloroethyl)-1-nitroso-*
Dog					
Alkaline phosphatase	TP	TP	TP	TP	TP
SGOT	TP	FN	—	—	TP
SGPT	—	TP	—	—	TP
Monkey					
Alkaline phosphatase	FN	FN	FN	TP	FN
SGOT	TP	FN	TP	—	TP
SGPT	—	FN	TP	—	—
Dog and monkey					
Alkaline phosphatase	TP	TP	TP	TP	TP
SGOT	TP	FN	TP	—	TP
SGPT	—	TP	TP	—	—

TP = true positive; FN = false negative; — = test not performed.

Table XVI. *Reaction of the alkaline phosphatase and serum transaminase determination when BSP retention gave a false negative correlation for liver toxicity*

	Cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride	*Hydrazine, 1-acetyl-2-picolinoyl-*	*Imidazole-4(or 5)-carboxamide, 5(or 4)-(3, 3-dimethyl-1-triazeno)-*	*Tylocrebrine*
Dog				
Alkaline phosphatase	TP	—	TP	FN
SGOT	—	TP	TP	FN
SGPT	—	TP	—	—
Monkey				
Alkaline phosphatase	FN	FN	TP	FN
SGOT	—	TP	FN	TP
SGPT	—	TP	—	—
Dog and monkey				
Alkaline phosphatase	TP	FN	TP	FN
SGOT	—	TP	TP	TP
SGPT	—	TP	—	—

TP = true positive; FN = false negative; — = test not performed.

dog. When the rat and dog data were combined, the total number of signs observed was 30. Thus, the rat added very little and compared with the dog gave a significantly lower percentage of true positives and a higher incidence of false negatives for toxicity in man. The list of toxicities unpredicted by the animal screen included, in addition to symptoms, such parameters as diarrhea, gastrointesti-

ninase
liver

ercidin	*Urea, 1,3-bis (2-chloroethyl)-1-nitroso-*
TP	TP
—	TP
—	TP
TP	FN
—	TP
—	—
TP	TP
—	TP
—	—

ıse
liver

or 5)-ide, 3, l-1-)-	*Tylocrebrine*
	FN
	FN
	—
	FN
	TP
	—
	FN
	TP
	—

ıer incidence of false
ty in man. The list
licted by the animal
addition to symptoms,
diarrhea, gastrointesti-

nal inflammation, aplastic anemia, thrombocytopenic purpura, and dermatitis.

This study, while valuable as a pioneer analysis of animal predictability, has several deficiencies. Only six compounds were used, though there was an attempt to include a wide range of chemical and functional types. The lack of details on the actual compounds, dosage range employed, maximum degree of toxicity produced in the animals, and size of the animal sample make interpretation difficult. The clinical hematologic and chemical parameters monitored and the criteria used for designation of toxicity were not stated. In addition, analysis by individual parameters alone made no allowance for possible correct prediction of toxicity in an organ, which may be indicated by an abnormality in a different specific parameter.

Owens,[79] in his species comparison review, analyzed the animal predictability for bone marrow, gastrointestinal, hepatic, renal, neurologic, and skin toxicities. The compounds studied were anticancer drugs of varied chemical and functional classification, and three to thirteen were employed for the estimation of each organ system prediction. The animal species included mice, rats, and dogs, with limited data on monkeys. There was no specific information given on doses, duration of therapy, route of administration, number of animals, parameters followed, or criteria for toxicity. The analysis showed that both rodent and dog predicted satisfactorily for general bone marrow depression but failed to detect thrombocytopenia. The dog predicted well for both gastrointestinal and hepatic toxicity, demonstrating definite superiority over the rodent in the latter category. Both rodent and dog gave good correlation for renal toxicity, but neither species proved to be satisfactory indicators for either neurologic or skin and hair toxicity.

Extending the prior observations of Pinkel,[84] Freireich and associates[85] conducted a retrospective study designed to demonstrate the ability of certain mammalian species to predict the toxic dose levels of anticancer drugs for man. The analysis of the data from eighteen compounds revealed that the ratio of the animal-human dose for comparable quantitative toxicity approached unity when measured on the basis of average body surface area for the species (milligrams per square meter). A set of factors was proposed by which a dose in milligrams per kilogram for one species could be used to closely estimate a dose which would produce a similar degree of toxicity in another animal species or in man. For example, the maximum tolerated dose (MTD) in man (milligrams per kilogram) approximates $\frac{1}{12}$ the LD_{10} in mice, $\frac{1}{9}$ the LD_{10} in hamsters, $\frac{1}{7}$ the LD_{10} in rats, $\frac{1}{3}$ the MTD in rhesus monkeys, and $\frac{1}{2}$ the MTD in dogs. The study supported the concept that animal systems can be effectively used to evaluate the quantitative toxicity of anticancer drugs prior to use in man.

The present study of qualitative toxicity deals with twenty-five anticancer drugs of diverse chemical structure and biochemical function which have been developed and/or evaluated by the Chemotherapy Program of the National Cancer Institute. The toxicologic evaluation of new compounds in animals has been an essential facet of this program for several reasons. A significant proportion of the patients in whom these compounds were tested were severely debilitated from disease and thus rendered less tolerant to drug toxicity. In this situation perhaps more than in any other general disease category, the clinician must be alerted to all potential hazards to his patients. The problem is made complex by the fact that cancer chemotherapy drugs, to varying degrees, fail to discriminate efficiently between normal and target tissues. A wide range of toxicities is encountered, and with many compounds administration of toxic doses may be required in order to achieve a therapeutic response. This situation pro-

vided a unique opportunity for the study of drug toxicity per se and facilitated the evaluation of predictions made from animal data.

It is important to note the problems that were encountered in the formulation and analysis of this study. The animal data were collected in both mongrel and beagle dogs and four species of primates. While there appeared to be no differences in predictions in these subgroups, this could not be quantitatively ascertained because of the relatively small numbers in each individual animal group. Normal healthy animals were used in the toxicology studies, while the patients, as a group, were generally debilitated and some measurements were abnormal prior to therapy. In addition, many of the patients were receiving concurrent analgesics, hypnotics, and antiemetics. The animal studies were generally carried out along the format of the CCNSC protocol,[15] which involved the delineation of acute and subacute qualitative toxicities. The animals were generally not treated for longer than 28 days, but this is longer than the average duration of therapy in the clinical studies in man. The route of administration in both animal and man was comparable, but the schedule and dose on both a milligrams per kilogram and milligrams per square meter basis varied (Tables III and IV). The animal and human data were collected by many different laboratories and institutions. While the clinical observations, laboratory determinations, and pathology readings were all considered to be of high quality, they cannot be considered uniform. A set of criteria for estimation of toxicity was formulated, but flexible judgment was employed on the final decision, depending on the animal's pretreatment hematologic and blood chemistry values. No attempt was made to adjust for the incidence of a given specific toxicity observed with each individual compound. It is important that serious toxicity be predicted even though it is subsequently found that its relative incidence in clinical studies is low. The estimation and comparison of incidence are made impractical, if not impossible, by several factors. The total number of animals receiving the drug was generally significantly lower than the total patient population tested. Treatment in the clinical studies was carried out until the first dose-limiting toxicity was encountered. At this time therapy was halted with the specific intent to prevent further adverse drug reaction. This is in marked contrast to the animal studies in which very toxic and lethal dose ranges had to be administered in order to complete the spectrum of qualitative toxicities inherent with any compound. An additional factor that complicates the usefulness of incidence measurements is that the order of appearance of the qualitative toxicities observed in the different species, as a function of increasing dose, may differ markedly. Such was the case with BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea, in which the first dose-limiting toxicity noted in dogs was liver damage followed by bone marrow depression. In patients who inadvertently sustained severe drug toxicity the sequence was the reverse, with leukopenia and thrombocytopenia being the predominant toxicities at or near the maximum tolerated dose. Thus, a complete analysis of incidence could be obtained only by carrying out clinical studies in a manner analogous to that of the animal experiment.

We have attempted to establish some of the limitations and useful properties of the large animal toxicology screen by analyzing for prediction of both individual parameters and for organ system toxicity. The species were studied individually and in combination in order to determine whether there was an additive overlap in prediction. This would be represented by an over-all reduction in false negative prediction made by the individual animal species. Such a complementary effect was noted for nine of the ten organ system groups. Perhaps most important was the case of bone marrow depression. The dog

had underpredicted for two drugs, while the monkey failed to indicate the myelosuppressive properties of a third. By combining the dog and monkey data, all false negative predictions were removed. This favorable overlap was also demonstrated for the individual hematologic parameters. The dog had failed to predict for the leukopenia produced by five compounds. Two of these false negative predictions were corrected by the monkey. Though neither species proved an adequate indicator of thrombocytopenia, the dog was demonstrated to be the superior predictor, the monkey having failed to predict for nine of sixteen drugs producing platelet depression in man. However, the monkey still served to correct for two of the dog's five false negative predictions. The over-all additive effect of the monkey data to the predictions made by the dog is in contrast to the minor contribution made by the rat in Litchfield's studies.

The other category in which the dog showed superiority over the monkey was in the indication of gastrointestinal toxicity, in which the monkey had a 17 per cent underpredictive index as compared to none for the dog. The monkey was shown to be remarkably resistant to the production of vomiting, the sign that was observed in man with 84 per cent of the twenty-five compounds. The monkey also demonstrated a lower incidence of diarrhea, coupled with a 26 per cent false negative prediction. While the combined dog and monkey toxicologic screen gave an over-all 92 per cent true positive correlation for gastrointestinal toxicity in man, this high degree of correspondence was contributed by the dog data alone.

Liver toxicity was never underpredicted, but there was a 48 per cent frequency of false positives. An important source of overprediction was the BSP determination. As an initial investigation of the usefulness of this time-consuming procedure, each liver function test in the animals was analyzed for its over-all prediction of liver toxicity in man. It was found that by using both species and the alkaline phosphatase and a serum transaminase determination, all cases in which the BSP gave a true positive were similarly predicted; while all BSP false negatives were corrected to positivity. Thus, for the compounds studied, the BSP determination served only as a confirmatory test for liver toxicity and failed to augment information already obtained by using the transaminase and alkaline phosphatase. Because of the small number of drugs involved in each case, a definitive statement regarding the usefulness of the BSP determination cannot be made. The alkaline phosphatase in the monkey proved to be a particularly poor determinant of liver toxicity. The range of normal within the species and in individual animals tends to be quite broad; with the use of the present liberal criteria, a significant percentage of hepatotoxicity in man is not predicted. The data suggest that these tests, like many others currently employed, should undergo quantitative evaluation for efficacy.

While the animal toxicologic screen failed to predict for only one of ten compounds that produced renal toxicity in the clinical studies, this was accomplished at the expense of a 56 per cent false positive index. The high percentage of BUN elevation noted in the animal studies may, in part, reflect extrarenal factors such as gastrointestinal bleeding and dehydration, but a greater susceptibility of the animal kidney to drug effects was borne out by correlative increases in serum creatinine and histopathologic changes. The incidence of false prediction as a function of the total number of compounds producing specific organ toxicity in man ranged from 5 per cent for respiratory, 10 per cent for cardiovascular, to 14 per cent for neurologic and dermal-related toxicities.

In the case of neurologic toxicity, several general factors become apparent during the analysis of the data. When compared with the entire spectrum of toxicities inherent in a compound, objec-

tive neurologic toxicity required the largest dose levels for elicitation. The form of the toxicity observed generally differed greatly from that seen in man. Signs such as personality change and confusion cannot be directly predicted by the animals but may be represented by the development of stupor, ataxia, or seizures.

Cardiopulmonary toxicity represents a difficult predictive area, primarily because it is not known which parameter or parameters will be chosen by an animal to express a harmful drug effect. The present study demonstrated a heavy reliance on pathologic changes in organs of the animals in order to predict for clinical parameters in man. In an attempt to develop a better model, future protocols should be designed which include both short- and long-term monitoring of a battery of physiologic parameters including blood pressure, cardiac output, electrocardiogram, and pulmonary x-rays.

The combined large animal screen failed to predict for two of the six compounds that produced injection site toxicity in man. This is explained in part by the failure of the animals to indicate in some way that the compound might produce pain or burning with injection. This symptom was a major problem with the administration of streptozotocin in patients and required that the drug be diluted and infused. The dogs and monkeys showed apparent complete tolerance to preparations of comparable pH (4.5) and buffer molarity.

The animal screen failed to predict dermatitis or correlate well with dermal manifestations of systemic toxicity observed in patients. This deficiency in prediction has long plagued toxicologists. Fortunately, dermatitis, though an unpleasant reaction, rarely poses a life-threatening problem as is more consistently encountered with bone marrow depression.

It is clear that toxicologic data collected in a dog and monkey screen forewarn the clinician of a useful proportion of the total spectrum of organ-specific and, with certain stated limitations, specific parameter toxicities that might be encountered. With the possible exception of central nervous system and dermal toxicity, all serious organ system toxicities are well predicted. This is accomplished, as in the case of anemia and hepatotoxicity, at the expense of a high percentage of false positive predictions. This degree of inefficiency in the system is justifiable. In order to demonstrate all possible qualitative toxicities, the animals must be given a spectrum of doses including severely toxic and lethal doses. This has been shown for the prediction of bone marrow depression, hepatotoxicity, and neurologic toxicity in our studies. If in the clinic the drugs had been given at more toxic dose levels than the estimated maximum tolerated dose, it is quite possible that the frequency of false positive predictions by the animal species would be significantly lower. Some of the overprediction is likely the result of a more extensive toxicologic evaluation in the animals. It is not common practice in clinical trials to perform lymph node and pulmonary biopsies for histologic evaluation during or immediately after chemotherapy. Both of these parameters showed a high frequency of pathology in the animals but were rarely recorded in man.

In addition to large toxic doses, chronic low-dose administration to animals will serve to elicit toxicities that require repeated subacute insult. These drug effects might be overlooked if an animal is prematurely killed by an earlier appearing, severe qualitative toxicity. An extended observation period of at least two months is required in order to predict for the unusual compound whose toxicity is delayed in onset. Such was the case with BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea, in the present study. Once organ system toxicity is observed during an animal study, it can never be disregarded but should be viewed with a realistic understanding of certain limitations of animal toxicologic data: Toxicity may develop in man in an organ system predicted susceptible by the animal, but it may be

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after chemotherapy.
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whose toxicity is de-
h was the case with
chloroethyl)-1-nitroso-
ıt study. Once organ
ɔserved during an ani-
never be disregarded
ed with a realistic un-
n limitations of animal
xicity may develop in
ɔystem predicted sus-
imal, but it may be

expressed in a different specific clinical or chemical parameter. The adverse reaction may appear in man at a greater or lesser dose level or may follow a different order of appearance in relationship to the total spectrum of qualitative toxicity inherent in any compound. The effective use of animal toxicologic data, coupled with careful monitoring, considered judgment, and expectation by the physician, jointly serve to forewarn the development of critical organ system toxicities during an initial Phase I drug trial.

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