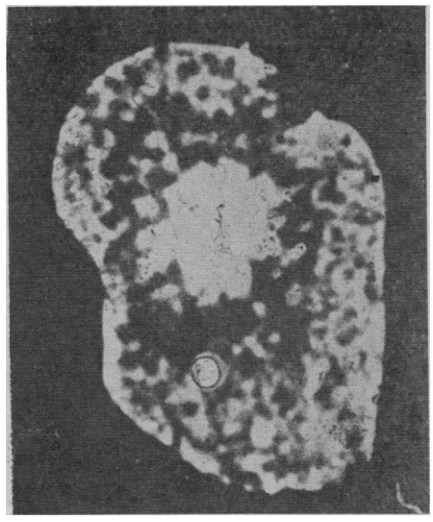

Fig. 1. Hemolysis in human blood agar covering a section of rabbit kidney, 6 hours after inoculation (rabbit No. 117) (× 6). Clear zones of hemolysis are seen against a dark background of unhemolyzed erythrocytes. The peripheral band of spotty hemolysis is over the renal cortex; the central solid zone of hemolysis is over the outer medulla.

staphylococcal lesions were produced either by infection with *Escherichia coli* or by making multiple punctures of the kidney with a red-hot needle. No hemolytic activity could be detected in any of these lesions.

Although it appeared that staphylococci could produce hemolysin when growing within the renal parenchyma of a living animal, it was necessary to exclude the possibility that the conditions for producing hemolysin were present only in the necrotic tissue and exudate of the lesions. If the hemolysin is considered to have an important pathogenic role it should be produced in the tissue before necrosis or exudation. To investigate the temporal relations among hemolysin production, bacterial growth, and tissue injury, 20 rabbits were inoculated intravenously with 10^8 cells of *Staphylococcus aureus* (Wood 46 strain) per kilogram of body weight. Two or three animals were killed at each hour from 6 to 12 hours after inoculation; five were killed 18 hours after inoculation. Half of one kidney was homogenized for quantitative culture. At least five tissue blocks from each animal were studied for the presence of hemolysin by the method already described, rabbit and human erythrocytes being used in each test. Histologic sections prepared from the same tissue blocks used for the hemolysin studies were stained conventionally and studied microscopically (Table 1).

Hemolytic activity was detected in three of the six rabbits killed 6 to 8 hours after inoculation (Fig. 1). At this time the kidneys contained an average of 3.9×10^2 bacteria per gram of tissue, but no bacteria could be found in the microscopic sections. There were no distinct histologic changes, but very subtle degeneration was present in some groups of renal tubules. By 9 to 12 hours after inoculation, hemolytic activity was present in the kidneys of seven of nine rabbits studied. The mean bacterial count was 4.3×10^5 per gram of tissue, and small colonies of bacteria were seen in the interstitial tissue of some of the specimens. Large zones of slight tubular degeneration were seen in several specimens. In one animal with microscopically visible bacterial colonies, small foci of early necrosis were seen adjacent to some of the colonies. No leukocytic infiltrates were present. Eighteen hours after inoculation, hemolytic activity was present in the kidneys of all five animals. The mean number of bacteria per gram of tissue had risen to 6.8×10^6; bacterial colonies were seen frequently in the sections and were larger than at 10 to 12 hours. Foci of renal necrosis were found in all animals. Occasionally it was possible to see necrosis in a concentric zone around a bacterial colony. Slight polymorphonuclear leukocytic infiltration was present at the periphery of some of the foci of necrosis. Six of the 15 rabbits in whose kidneys hemolysin was detected had activity against rabbit cells only; all of these were in the 6-to-12-hour group. There were no kidneys that showed hemolysis of human erythrocytes but no hemolysis of rabbit cells. In the kidneys in which both rabbit and human hemolysis was detected, the activity against rabbit cells was always somewhat greater than against human cells.

Evidence of hemolysin production during the genesis of staphylococcal lesions has been obtained in these experiments. The characterization of the hemolysin, however, was not certain. Its activity against various species of erythrocytes indicated that it could not consist entirely of α-hemolysin and suggested the presence of δ-hemolysin. Because of conflicting opinions about the heat stability of δ-hemolysin it is difficult to fully interpret the results. The appearance of detectable hemolysin before the occurrence of necrosis was consistent with the hypothesis that the hemolysin contributed to the subsequent tissue damage. The evidence at hand, however, did not exclude the possibility that other substances were responsible for at least part of the injury.

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4. Supported by research grant HD-857 from the National Institutes of Health. I thank Eleanore Sturgill for technical assistance.

25 June 1965

Melphalan Therapy and Exercise

In his refutation of the report of Bergsagel *et al.* that myeloma patients excreting type II (λ) Bence Jones protein fail to respond to melphalan therapy, Osserman [*Science* **149**, 564 (1965)] cites two such patients who did very well on this drug—a golfer ("in the 90's") and a pool swimmer ("100 to 150 yards daily"). As the pool swimmer cited, I find the underestimation of my athletic prowess annoying. The actual distance covered by my daily swim is 500 to 550 yards. This performance has been maintained regularly over almost 4 years of melphalan treatment. . . .

NAME WITHHELD

5 August 1965

Assessment of Drugs

Schneiderman, Myers, Sathe, and Koffsky [*Science* **144**, 1212 (1964)] have introduced "a substitute ranking measure for the therapeutic index . . . that would be based on minimizing the losses from the failure to cure plus the losses due to toxicity." The authors say that this new measure would allow better ranking of the net effectiveness of drugs than the therapeutic index. We do not agree. We object particularly on the following grounds:

1) Any drug-ranking measure should yield better results for drugs that provide good therapeutic properties over a wide range of dosage than for those

effective only in one specific dosage. The proposed measure takes into account only the therapeutic properties of a certain dose—the one with a minimum loss or maximum gain. The course of the loss or gain function around this minimum or maximum or in another dose range will not be taken into account. If, for instance, of two drugs one gives a great gain for a very small range of dosage but for all other doses a small gain, whereas the other offers a constantly high gain over a wide range of dosage, although this gain is slightly lower than the maximum of the first drug (Fig. 1), then according to Schneiderman *et al.* the first drug is to be preferred to the second. Thus the measure may produce highly inaccurate assessments.

2) Another drawback of this method is that it does not indicate the limits of variation from the best dose—that is, of overdosage and underdosage—beyond which the drug may give rise to serious lesions or be ineffective. In the case of drug A in Fig. 1, a slight increase of the "best" dose produces a considerably increased toxicity, whereas with drug B the "best" dose can be considerably exceeded without toxic lesions; and similarly with decrease of dosage and loss of effectiveness. For clinical practice, knowledge of the margin of safety and thus of the drug's safety in use is much more important than the knowledge of the "best" dose. Therefore the proposed measure can by no means substitute for the therapeutic index, but only give additional information.

3) Schneiderman *et al.* consider it to be a special advantage of the minimum-loss approach that a certain optimum dose is given, whereas the therapeutic index only fixes the limits of dosage. It should be remarked, however, that the results obtained in animals cannot be transferred to man without certain restrictions. Therefore the clinical pharmacologist is less interested in an optimum dose in animal experiments than in a comparative measure which permits assessment of both the therapeutic and toxic potency of a drug.

4) The authors mention various model substances which yield different ranking orders with the therapeutic index and the minimum-loss approach. This speaks neither against nor in favor of the therapeutic index. It is only due to the fact that the two methods of assessment have different bases: in one it is loss or gain, in the other degree of safety. Since these two principles are not identical, the two ranking systems may yield different rankings of substances. Which ranking order is to be considered applicable depends on the individual case. Neither is it true that the minimum-loss approach should be preferred because more data (slope information) are used in it. The therapeutic index also includes slope information [N. Brock and B. Schneider, *Arzneimittel-Forsch.* **15**, 266 (1965)].

5) The "best" dose may also lie beyond the zone between LD_{05} (dosage lethal in 5 percent of the cases) and CD_{95} (dosage curative in 95 percent of the cases). In Schneiderman's model substances this is true for an agent with a therapeutic index of less than 1. In this case the difference between LD_{05} and CD_{95} is negative, so that there is no real therapeutic range. The "best" dose is smaller and therefore less therapeutic than LD_{05}. This fact is neither an advantage of the minimum-loss approach nor a drawback of the therapeutic index; it is due to the arbitrary choice of 5 percent as the limit of toxicity for the therapeutic index and of 1 as the weighting factor for the minimum-loss approach.

These objections are directed mainly not against the minimum-loss principle, but only against the statement that it could substitute for the therapeutic index. For many other problems the principle may prove quite useful.

To demonstrate the foregoing statements we have computed the therapeutic index and the gain function according to Schneiderman *et al.* (with the weighting factor $\lambda = 1$) for some cytostatic drugs for which we have sufficient data from our own experiments. The parameters for the drugs,

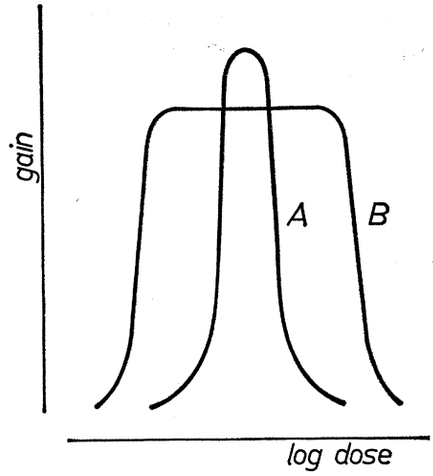

Fig. 1. Comparison of two gain functions (theoretical example).

the therapeutic index, the "best" dose, and the maximum gain are shown in Table 1. The course of the gain function can be seen from Fig. 2. When the therapeutic index is used as the ranking measure, a stepwise series with values from 0.2 to 10 is obtained. Cyclophosphamide and desmophosphamide rank first (TI = 8.5 and 10); oxy-HN2 has an intermediate position (TI = 2.3); nor-HN2 has an index value below 1. This ranking order is largely in accord with clinical experience. With the method of Schneiderman *et al.*, this ranking order is not greatly changed, but the differences between the first three drugs are hardly noticeable; cyclophosphamide, desmophosphamide, and oxy-HN2 give almost the same maximum gain. As can be seen from Fig. 2, the gain function of oxy-HN2 has a considerably narrower curve than that of the other two drugs. Therefore oxy-HN2 entails a much greater risk of toxic response on over-

Table 1. Parameters of the regression lines, therapeutic index, best dose, and gain (computed according to Schneiderman *et al.*, with weighting factor $\lambda = 1$) of some cytostatic drugs on single intravenous administration to rats suffering from Yoshida's ascitic sarcoma. (T, toxicity; A, activity.)

LD_{50} (mg/kg)	μ_T*	CD_{50} (mg/kg)	μ_A*	σ_T*	σ_A*	LD_{05} (mg/kg)	CD_{95} (mg/kg)	TI	Best dose (mg/kg)	Gain
				Cyclophosphamide						
160	2.204	4.5	0.653	0.099	0.280	110	13	8.5	60	2.000
				Desmophosphamide						
440	2.643	14.0	1.146	0.075	0.227	330	33	10.0	185	2.000
				Oxy-HN2†						
50.0	1.699	5.4	0.732	0.094	0.279	35.0	15.5	2.3	27.2	1.992
				Nor-HN2‡						
100	2.000	40	1.602	0.229	0.397	42	180	0.2	55	1.510

* μ_T = log LD_{50}, μ_A = CD_{50}, σ_T and σ_A are the reciprocal slopes of the dose-response curves. † 2,2′-Dichloro-*N*-methyldiethylamine *N*-oxide hydrochloride. ‡ 2,2′-Dichloro-*N*-diethylamine hydrochloride.

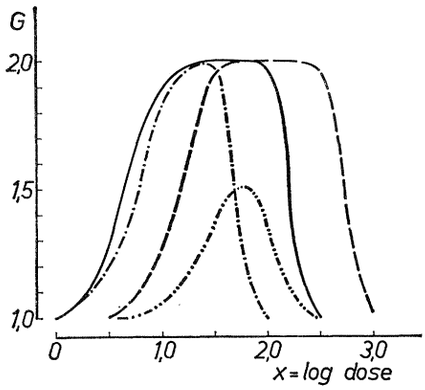

Fig. 2. Comparison of the gain functions of some cytostatic drugs under test on single intravenous administration to Yoshida's ascitic sarcoma of the rat. ——, Cyclophosphamide; -----, desmophosham-ide; -·-·-·, oxy-HN2; ·····, nor-HN2.

dosage or of inefficacy on underdosage and hence is of much smaller value for treatment. This difference is not made clearly noticeable by Schneiderman's ranking measure, whereas it is shown in a clear-cut way by the therapeutic index.

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Brock and Schneider raise a very practical issue in their Fig. 1 which has led us to consider combining some aspects of the minimum-loss approach with a procedure related to the therapeutic index. Their question is: Suppose the loss functions have clearly different shapes; should you depend on only the one value, minimum loss, to establish your ranking? Their answer is no, and they are right. Conversely, can you depend on the therapeutic index alone? The answer to that question is also no. What, then, can you do? We suggest that one might specify the maximum loss one is willing to tolerate and then find those drugs that have a dose range which produces this or a smaller loss. Choose as "best" the drug that has the widest range of doses at this maximum permitted loss. This range of doses will be related to the therapeutic index, but not on a one-to-one basis.

Brock and Schneider's example (their table and Fig. 2) serves as a good medium on which to test this suggestion. It is unfortunate that they chose to plot a gain function (one which they developed, related to but not the one given in our paper) rather than the loss function we proposed. It is difficult to see differences between some drugs by examining the gain function of Brock and Schneider. Our Fig. 1 shows the loss functions for their four materials with loss plotted on a logarithmic scale, as we originally proposed. The differences they speak of now clearly appear. Our ranking turns out to be identical with theirs (not, as they say, "not greatly changed").

Suppose we agree that 0.1-percent loss is the maximum permissible. At this level there are only two candidate drugs, desmophosphamide and cyclophosphamide. Oxy-HN2 and nor-HN2 are not in the running. Desmophosphamide has a range of doses capable of producing this loss, or less, from 1.84 to about 2.42 (logs). The range for cyclophosphamide is from 1.51 to about 1.90. We would then rank desmophosphamide ahead of cyclophosphamide, and would also know which doses would be "safe" and the dose for each drug which would produce the minimum loss.

One minor additional point, which we believe may reflect a problem in translation rather than a scientific issue: Brock and Schneider, referring to their paper in *Arzneimittel-Forschung*, remark that the therapeutic index includes slope information. They show in that paper that if one has not experimentally determined the LD_{05} and the CD_{95} from which one computes the therapeutic index, one can use slope data along with the 50-percent points to

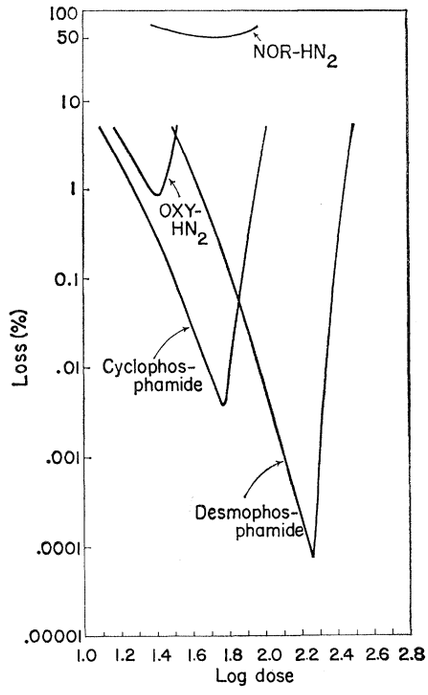

Fig. 1. Loss functions for four cytostatic drugs (data from Brock and Schneider).

estimate them. From then on, in using the TI, slopes are disregarded. It is in this latter sense that we assert that the minimum-loss approach uses slope information and the therapeutic index does not.

We are grateful to Brock and Schneider for having made so clear that the complete loss function, not merely the minimum loss, should be used in ranking drugs. We believe that adding the concept of a maximum allowable loss (which is *not* the loss at the maximum tolerated dose of Ehrlich) now permits safety considerations to enter while allowing a choice among materials of equal or unequal TI's and equal or unequal minimum loss. Use of the gain function of Brock and Schneider can be seriously misleading.

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