of the microsomes with n-butanol. Although the purification achieved is only 15 to 25 times that of the original homogenate of the tissue, the resulting preparation appears to be particularly suitable for further purification by fractional precipitation with acetone.

1. Hers, H. G., Berthet, J., Berthet, L., and de Duve, C., *Bull. Soc. Chim. Biol.*, 1951, v33, 21.

2. Kallman, F. G., *J. Cell. and Comp. Physiol.*, 1951, v38, 137.

3. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.

4. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, 1951, v35, 89.

5. Albers, H., and Albers, E., *Z. f. Physiol. Chem.*, 1935, v232, 165, 189.

6. Morton, R. K., *Nature*, 1950, v166, 1092.

7. Zittle, C. A., and Della Monica, E. S., *Arch. Biochem. and Biophys.*, 1952, v35, 321.

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Duration of Drug Action. (19953)

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A drug effect has two major features, 1) *intensity* and 2) *duration*. Intensity of drug effect is unquestionably a variable magnitude, but its quantitation meets considerable obstacles because its dimension is difficult to define. The assumption, followed in some cases, that intensity of effect can adequately be measured in such simple dimensions as length (muscle shortening), volume (glandular excretion), weight, etc., is certainly fallacious; in other cases, *e.g.*, drug-produced convulsions, one is at a loss to quantitate in any physical dimension. Yet the need to analyze the relationship between intensity of effect and its independent variable, *drug dose*, and to compare intensities of effect of different drugs has led to the elaboration of a workable yardstick for intensity of effect. This was possible by circumventing the problem of the physical dimension and focusing attention to the biological dimension. The biological yardstick is "notched" in terms of *endpoints, i.e.*, selected effects of well specified intensity, such as: *threshold effect; peak effect* = greatest intensity attained in the course of the action of a dose; *maximal effect* = greatest intensity obtainable with any dose; and numerous intermediate endpoints of appropriate specification. This instrument, refined by an abundant armamentarium of biostatistical technics to overcome variability, has placed evaluation of intensity of effect on a satisfactory conceptual and procedural basis.

The concepts and principles for quantitation of the other feature of drug effect, namely, the *duration*, are as yet on an incomparably lower level of development. This may seem paradoxical because, at first thought, the problem of dimension does not appear to offer any difficulties; by definition, the temporal features of drug effect present themselves in the physical dimension of time. Nevertheless the concept of *duration of effect* has remained rather vague and hence of little constructive value. The reasons become obvious when the intensity and time characteristics of the effect of a drug dose are represented graphically (Fig. 1). Such a diagram points out that, quite similar to the intensity axis, the temporal axis of effect is also subdivided into biological "endpoints". Since evidently the temporal features are correlated with intensity, the temporal endpoints are connotatively coupled to those of intensity. As exemplified in the diagram for action III, the temporal endpoints are time intervals measured from the time of drug administration, T_o, to that of an endpoint of intensity of effect. Major temporal endpoints are: *latency time* = time of onset of threshold effect (T_a); *peak time* = time of (onset of) peak effect (T_1); *persistence time* = time of disappearance of threshold

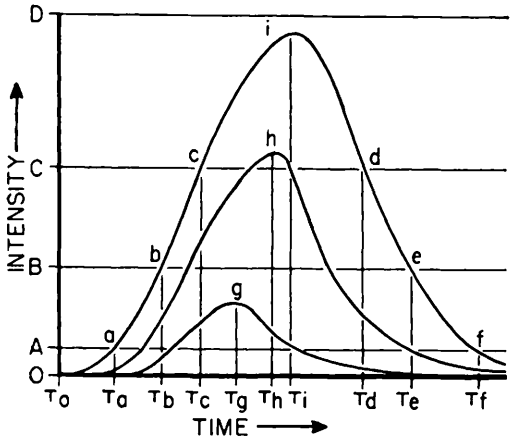

FIG. 1. Time-intensity curves of effect of 3 different drug doses, I, II and III. A: threshold effect; B: therapeutic effect; C: toxic effect; D: maximal effect. g, h, i: peak effects of the 3 doses (time sequence g<h<i selected as one out of 3 possibilities). T_g, T_h, T_i: peak times of the 3 effects. For other explanations see text.

effect (T_f). Other endpoints are the times of onset and disappearance of other intensities of effect, such as T_b, T_c, T_d, etc. It is obvious that an important use of the temporal endpoints is to measure differential periods of time, such as (T_f-T_a), (T_e-T_b), (T_d-T_c), etc., and that only these periods are "durations" (total duration of effect, duration of therapeutic or toxic effect, etc.).

These contemplations are not submitted for their academic significance; very practical necessities,—experimental, therapeutic, and toxicologic,—call for their consideration. Need and remedy may be illustrated by two examples.

1) *Duration of effect* is an important connotation of the action of drugs. Particularly in the field of short-acting antibiotics, *e.g.*, penicillin, a vast amount of work has been done to determine duration of effect and its prolongation by numerous so-called "repository" measures. Since, with secondary drawbacks, blood level of antibiotic activity can be a yardstick of intensity of effect, the time of persistence of a certain blood level of activity is usually employed to indicate duration of effect. However, the results derived from the host of investigations on repository agents are presented in surprisingly indefinite terms; duration of effect is merely expressed either by the latest time at which a therapeutic level of antibiotic activity was still found in the blood of an occasional individual or by the percentage of individuals still exhibiting such a blood level of antibiotic activity at a certain hour. It was in order to avoid such inadequacies that this writer has recently suggested the concept of *median persistence time* (PT_{50}) and the means of applying it for obtaining biostatistically defined values for duration of effect(1). Quantal (yes-or-no) statements on persistence of an appropriate endpoint (of intensity of effect or of antibiotic activity in the blood) are procured from a population at various, adequately narrowly spaced intervals around the expected persistence time; these statements are subjected to probit analysis, the median value (PT_{50} of the decremental endpoint effect) is established by interpolation, and the variation of sensitivity in the population is determined by an appropriate statistical procedure. (Further details will be found elsewhere)(2).

2) *Comparisons of the potency* of drugs are the pharmacologist's daily bread and the main approach to new and improved drugs. All such comparisons repose on the concept of ED_{50}. This is the dose whose *peak effect* reaches a certain intensity in 50% of experiments, and (relative) potency is the ratio: ED_{50}standard/ED_{50}test drug. Modern estimates of ED_{50} are so highly elaborate that an ED_{50} can hardly appear in public without an ornate biostatistical attire. Unfortunately, however, this attire may adorn a value which lacks the main criterion of an ED_{50}, especially in many of those cases in which the assay procedure does not automatically record the entire rise and fall of the individual effect of the test doses. The intensity is then often measured at a uniform, rigidly pre-set time interval after drug administration and no precautions are taken to insure that this time interval is the *peak time*. The intensity measured may then not be the *peak* effect, but rather some other intensity of effect reached anywhere during the rise to, or the fall from, the peak effect of the dose examined. One can well imagine the errors in potency values obtained from such spurious "ED_{50}s",—for instance, if the standard drug has a small and

the test drug a large peak time, and the potency ratio between the two drugs is formed by use of doses which are equieffective at the same rigid time interval from administration. According to the preceding discussion, potency evaluations by way of ED_{50} comparisons are, to say the least, highly problematic unless they include determination of peak time as the indispensable criterion of ED_{50} and define this value with the same biometrical elaborateness as the values presented as ED_{50}s. How Median Peak Time can be determined is indicated in the preceding example.

Summary. The intertwining of temporal and intensity features of drug effects and the necessity of its consideration in their quantitation are demonstrated by two examples: 1. "Duration of effect" requires reference to an endpoint of intensity of effect; it is then appropriately determined by "median duration" and suitable confidence limits. 2. The customary "median effective dose" is meaningless as a measure of potency unless determined at the time of peak effect; thus, determination of "median peak time" and its confidence limits is the prerequisite of ED_{50} determinations.

1. Greaves, Carol, Loewe, S., and Marcus, S., paper presented at 8th Semi-ann. Meet., Intermount. Branch, Soc. Am. Bact., Ogden, Utah, April 4, 1951.
2. Loewe, S. and Hogan, D., in press.

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Increased Excretion of Dehydroascorbic and Diketogulonic Acids by Rats After X-Ray Irradiation.* (19954)

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Recent work has shown that rats excrete increased amounts of dehydroascorbic acid (DHA) and diketogulonic acid (DKA) in the urine when they are exposed to environmental temperatures of 0°C(1). In order to establish whether this increased excretion of oxidized ascorbic acid (AsA) is a specific response to cold stress or a more general one obtained from stress of other kinds, adult albino rats were exposed to a second type of stress differing from the cold environment, namely, X-ray irradiation.

Experimental. Eight rats of the same inbred strain as those used in the cold experiments(1) with starting weights of about 200 g, paired as to sex, were used. The animals were placed 2 at a time in a flat wooden box and carried to the X-ray wing of the hospital, and then returned without treatment to the constant temperature animal room and placed in individual metabolism cages. The floors and funnel portions of the cages were silicone-coated, and the 24-hour urine was collected and analyzed for AsA, DHA and DKA(2). Any possible effect of the trip in the irradiation box was thus reproduced in the controls. The control excretion was determined for 10-14 days, and then the same rats were taken back to the X-ray wing and exposed, 2 at a time, to a single, total body irradiation of 800 r, in air. (200 KV, 15 ma., 0.5 mm Cu and 3.0 mm aluminum filters, 50 cm target distance, 15 x 15 cm cone, and 25 r per minute dose rate). This dose represents an LD_{50} in 30 days in rats, and has been used by Smith and coworkers in metabolic studies(3). At each time of exposure, 2 new rats were carried along to start as controls. All irradiated animals survived the experimental period 10 days after exposure in apparent good health. The urinary excretion of AsA, DHA and DKA was determined exactly as in the control period.

Results of the analyses are summarized in Table I. As in the animals exposed to low

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