shown, it seems unlikely that either auxin destruction or inhibition of synthesis could be responsible for the observed differential. Third, when the tips were totally split, and an impermeable barrier was placed between the halves, unilateral light failed to produce any differential at all. Again, if either auxin destruction or inhibition of synthesis were responsible for the observed differential in the partially split tips, one would not expect a total barrier to make any difference in the amounts of auxin obtained. Thus, diffusions 1*g* and 1*h* should be comparable to 1*e* and 1*f*. The most reasonable explanation for these results is that unilateral light actually induces a lateral movement of auxin from the light to the dark side of the coleoptile, and in this way effects the observed auxin differential.

Further experiments are in progress to determine the effect of a range of light dosages on lateral auxin movement, and to attempt to determine in what part of the tip the lateral transport is occurring.

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7. This work was supported in part by a grant to one of us (W.R.B.) from the National Science Foundation.

24 May 1957

Method of Anesthetizing Large Sharks and Rays Safely and Rapidly

In the course of an experimental study dealing with the influence of the anterior pituitary on mating behavior and reproduction in elasmobranchs, it recently became necessary to handle sharks and rays of considerable size. Of the many tranquilizers and anesthetics that were tried, the narcotic known commercially as M.S. 222, a meta-amino-benzoic acid-ethylester in the form of a methan-sulfonate, proved to be the most useful (*1*).

For a quarter of a century, M.S. 222 has been used by the experimental embryologist as an anesthetic for amphibian embryos and small teleosts. Rothlin (*2*) notes that it is isomeric with anesthesin, 3 times less toxic than Novocain, 10 times less toxic than cocaine, and highly

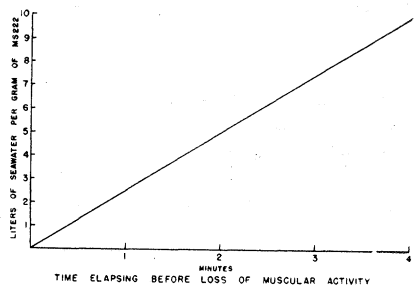

Fig. 1. Relationship between the concentration of M.S. 222 utilized and the rapidity of its action on a young female lemon shark. An initial dose of 100 ml of M.S. 222 solution was sprayed over the gills at the beginning of each experiment. When weaker concentrations were used, additional doses of 100 ml were administered every 60 seconds.

soluble in water. McGovern and Rugh (*3*), using a dilution of 1/3000 on frog eggs and sperms, found that M.S. 222 does not affect sperm motility and fertilizing power, nor does it have any effect on ciliary action; however, it does act quickly on skeletal muscle and effectively immobilizes frog embryos immersed in the solution in 30 to 80 seconds. Schotté and Butler (*4*) point out that M.S. 222 is of great value as an anesthetic in experiments with urodele larvae, since a stock solution of 1/1000 may be sterilized in the autoclave without loss of its narcotic properties and with no increase in toxicity. These investigators have "often kept animals for two consecutive days, and longer, in a 1/10,000 solution without ill effects. Moreover, no cumulative effects have been observed, inasmuch as the same larvae have frequently been submitted to a total duration of 10 days of narcosis within a single month." Many others, including Christensen (*5*), Copenhaver (*6*), Glucksohn (*7*), Rotmann (*8*), Sato (*9*), and Witschi (*10*), have successfully employed M.S. 222 as an anesthetic by simply immersing amphibian embryos or small teleosts in a solution of 1 g of M.S. 222 dissolved in 3000 ml of spring water.

Because of the large size of the elasmobranchs that were investigated, complete immersion in a solution of M.S. 222 was out of the question. An alternative method of utilizing this narcotic was therefore developed, and because of the ease with which it is applied while the fish is still vigorously straining at the hook or harpoon in the water alongside the boat, because its action is dramatically prompt, and because recovery after narcotization is invariably complete, we believe our method may be of some interest and value to others (*11*).

For the most rapid results, a concentration of 1/1000 (1 g of M.S. 222 in 1 lit of sea water) is utilized. On smaller species, 100 ml, and on larger fish, up to 1 lit, of this solution is introduced into the mouth of a shark or the spiracles of a ray and sprayed over the gill exits of the pharynx by means of a water pistol, rubber-bulb syringe, or small, pump-type hand sprayer. During the period of application, the head of a large fish should be held above the level of the water by means of a gaff or the leader that is attached to the hook or harpoon point; smaller fish may be temporarily removed from the water. The drug is quickly absorbed by the gills, and its action is rapid. Within 15 seconds the M.S. 222 solution begins to take effect and, as a rule, even a 400-pound shark is anesthetized in 1 minute or less. The shark or ray may then be readily handled, either out of or in the water, until the first stage of recovery takes place, within 5 to 30 minutes after the animal is returned to the water; this varies with the size of the elasmobranch and the dosage. Recovery may be delayed with an additional application of M.S. 222 solution at this time or may be hastened by washing the gills with fresh sea water (either by "walking" a large shark, with its mouth kept open, around the pool or by directing a stream of fresh sea water into its mouth).

Figure 1 is based on a series of tests in which concentrations ranging from 1/1000 to 1/10,000 were used on a lemon shark (*Negaprion brevirostris*) that weighed 9½ pounds. A 1/1000 concentration of M.S. 222 solution acted in approximately 20 seconds, while weaker concentrations of M.S. 222 solution acted much less rapidly. When the recommended 1/1000 concentration of M.S. 222 solution is utilized, the size of the dose needed to anesthetize a shark or ray within 1 minute is suggested in Fig. 2.

M.S. 222 has been employed effectively on four genera of sharks and two genera of rays, and, in every case, recovery has been complete and the fish appears to have been quite unharmed by

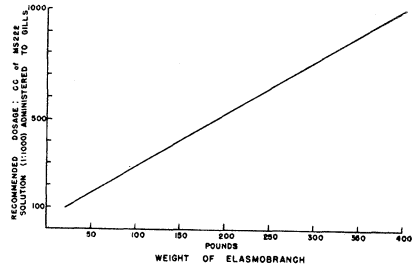

Fig. 2. Size of dose of M.S. 222 solution (1/1000 concentration) recommended to anesthetize a shark or ray in 60 seconds or less. The solution should be sprayed over the gill exits of the pharynx while the head of the elasmobranch is held above the level of the water. If the head remains under water, proportionally stronger concentrations must be utilized.

the treatment. Sharks have been kept alive for as much as 1 month after treatment and have shown no ill effects.

The advantages of this simple method for anesthetizing elasmobranchs are obvious. M.S. 222 is easily applied to the gills by way of the mouth or spiracles and acts rapidly on large or small fish (*12*), and recovery is gradual and invariably complete. It would appear that this drug, and the method of application we have outlined here, might be useful not only to the experimental zoologist but to those concerned with capturing large fish, either for exhibition purposes or for food (*13*).

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11. This study was initiated by both authors at the Marineland Research Laboratory and was subsequently carried on by the senior author at the Lerner Marine Laboratory, Bimini, Bahamas. It was supported, in part, by a grant from the National Science Foundation.
12. M.S. 222, applied in this manner, also works effectively on teleosts of various sizes.
13. M.S. 222 does not make the flesh unpalatable, and fish that have been anesthetized with M.S. 222 have been consumed with no deleterious effects.

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6 June 1957

Fundamental Limit to Certainty in Scientific Generalizations

It is of theoretical interest, at least, to consider the degree of certainty that can be attained for a scientific generalization. In this report, the derivation of a maximum confidence limit for a generalization is described (*1*).

Let U and X designate two combinations of one or more variables each, which may be divided into d and e mutually exclusive, observed classes, respectively. Class widths need not be equal. Values d and e may be as large as desired, subject to limits of observation, but let $d \geqq e$. Now, an apparently perfect relationship between U and X would be indicated if it were observed that for each of n_i occurrences of U which fall into a given class U_i, the concurrent (or subsequent) occurrence of X falls into just one class X_j, and this were true of every U_i and X_j. Here, U_i can be regarded as a hypothetical infinite population from which a random sample n_i has been obtained. According to the statistical method of confidence limits (*2*), then, a lower confidence limit P_i to the "true" (population) probability for X_j to be observed when (or after) U_i is observed, is given by

$$P_i = \left[\frac{1-S}{2}\right]^{\frac{1}{n_i}} \qquad (1)$$

where S is defined as the confidence level. Limits for P_i and S are 0 and 1. A value of S is chosen subjectively; 0.95 and 0.99 are frequently used.

A lower confidence limit $\overline{P}_i$ for the apparently perfect relationship between U and X can be defined as the arithmetic average of all d of the P_i's. By means of first and second derivatives of $\overline{P}_i$ with respect to n_i (*3*), with S, d, and N (the total number of observations) held constant, it is found that $\overline{P}_i$ is a maximum when all n_i's are equal to each other (*4*). It follows that

$$P = \left[\frac{1-S}{2}\right]^{\frac{d}{N}} \qquad (2)$$

where P is the maximum value of $\overline{P}_i$. P increases as N increases or as d decreases. (In general, now, d may be regarded as the maximum number of observed classes of any factor or combination of factors in the relationship.)

A simple example will illustrate the use of the last equation. Consider an idealized situation in which it is observed that barometric pressure gives perfect predictions of weather at a given location. Let the weather (X) there at noon be classified as *rain, changeable,* and *fair* ($e = 3$); and the pressure (U) there 24 hours earlier be classified as *low, intermediate,* and *high* ($d = 3$). A total of 750 independent forecasts (N) are available to show that low, intermediate, and high pressure invariably preceded rain, changeable, and fair weather, respectively. With just this information and a value of 0.95 for the confidence level S, Eq. 2 can be used to find a maximum confidence limit $P = 0.985$ for pressure as a predictor. In effect, this means the chances are 95 out of 100 that the seeming perfection of the observed relationship between noon pressure and subsequent weather at the given location is at least 98.5 percent certain, provided that the forecasts are distributed equally among the three pressure classes. No stronger statement of confidence can be made in this case; if the distribution is unequal, there is less certainty.

Note that when $d = e = 1$, P is the maximum confidence limit for an event observed to happen the same way each time, under the same circumstances.

As is indicated by Wilson (*5*), "All sciences start with a process of selection or classification. . . . Furthermore, all scientific laws are based on classifications." And Caldin (*6*) states that "The elementary generalisations on which science, considered as a system of beliefs about nature, is dependent, are derived from observations by generalisation . . . a process . . . treating observations not as bare data but as signs of a regularity; leading not to certainty, but to likelihood. . . ." However closely a scientific generalization describes observed regularity, and however perfect the regularity appears to be, absolute certainty for the generalization cannot be claimed. At best, the degree of uncertainty is as small as the uncertainty in the regularity. The latter uncertainty is given by Eq. 2 in terms of a maximum confidence limit. This leads to the interesting conclusion that, in the final analysis, this expression furnishes a maximum confidence limit for the generalization—law, hypothesis, or theory.

It is apparent that such an analysis could be quite complicated, particularly in the more advanced sciences, chemistry and physics (*7*). But this complication does not invalidate the conclusion.

Finally, it can be noted that Eq. 2 is relevant too for a regularity wherein assumptions of random, independent samples (required by statistical theory) and objective, rather than subjective, classifications, are not fully satisfied. In that event, the computed value of P still is a maximum confidence limit not to be exceeded, for it is the closest possible approach to certainty when S, d, and N are specified.

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1. The work in this report was supported, in part, by the Geophysics Research Directorate of the Air Force Cambridge Research Center under contract No. AF19(604)-753. A more detailed derivation of Eq. 2 in this article has been given, with application to weather prediction [T. A. Gleeson, *J. Meteorol.* **14** (Aug. 1957)].
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3. It is assumed that the n_i scale is continuous rather than being composed of whole numbers. This assumption is deemed sufficient for the present purpose.
4. Furthermore, each $n_i > -\frac{1}{2}\log_e\left[\frac{1-S}{2}\right]$. This condition is easily satisfied in practice. For example, if each n_i were as small as 4, S could be chosen as high as 0.999.
5. E. B. Wilson, *An Introduction to Scientific Research* (McGraw-Hill, New York, 1952), p. 151.
6. E. F. Caldin, *The Power and Limits of Science* (Chapman and Hall, London, 1949), p. 69.
7. For discussions of the complexities involved here, see E. B. Wilson (*5*, section 7.5) and M. R. Cohen and E. Nagel, *An Introduction to Logic and Scientific Method* (Harcourt, Brace, New York, 1934), chap. 14.

3 May 1957