cleaving eggs or embryos were found *in corpore*.

Eggs and sperm from each of the ovotestes were isolated in dishes of sea water. Fertilization *inter se* gave entirely normal larvae. Normal eggs fertilized with sperm from the ovotestes gave normal larvae, as did also the eggs of the ovotestes fertilized with normal sperm.

Subsequently all five gonads were preserved and sectioned. There were thus three types of section: ovarian, testicular, and ovotesticular (from the median zone). Both ovarian and testicular lobes showed normal structure, with masses of ripe and immature eggs or sperm, as the case might be. In the median section, where the ovarian and testicular structures lie side by side, the acini were intermingled. Ripe ova occurred among the sperm. A few eggs showed fertilization membranes, which must have been the result of the recent handling, since no division stages or embryos were found *in corpore*.

It is worthy of note that in a similar hermaphroditic specimen of *Strongylocentrotus pulcherrimus,* Okada and Shimoizumi (*3*) found that, when selfed, the eggs and sperm resulted in imperfect larvae, while larvae resulting from outcrossing were normal. Thus, their findings differed from those in the experiments described above (*4*).

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4. This study was aided by a University of California research grant.

25 August 1958

"Shutoff Pulse Illusion"

Abstract. Visual signals produced by sharp illumination decrements are commonly misinterpreted because of the presence of an illusory sharp increment at the moment of switching. Conditions for occurrence of the illusion are outlined, as well as conditions under which it is not reported.

During evaluation of a device in which information was tranmitted by means of a step-modulated light beam, the visual appearance of certain light signals was found to disagree consistently with these same signals as electronically received and recorded. The discrepant element

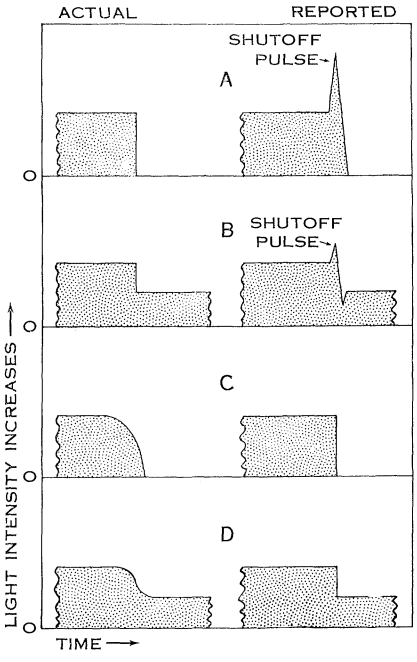

Fig. 1. Conditions associated with "shutoff pulse" illusion.

in all instances was found to be a positive pulse of short duration, reported when the illumination ceased or declined sharply. This illusory signal, here called the "shutoff pulse," is shown diagrammatically in Figs. 1*A* and 1*B*. It was noted regardless of whether the light source was a high-temperature filamentary lamp or a glow lamp, provided the lamp supply was direct-current; it was noted at all supply frequencies above about 40 cycles/sec with high-temperature filamentary lamps and at all supply frequencies above about 250 cycles/sec with glow lamps. It was not noted with low-temperature filamentary lamps, and it was masked by flicker and stroboscopic effects at low supply frequencies.

The illusion could be lessened or removed entirely by "fading" the light source in place of "stepping" it, as shown in Figs. 1*C* and 1*D*, but the amount of "rounding" seemed to be different for different observers, and some inconsistencies suggested hour-to-hour changes for the same observer.

No simple relation could be observed between the rate of change of illumination and the observed "iris overshoot" of the observer. This well-known "hunting" phenomenon is an oscillatory change in the iris aperture in response to a rapid change in illumination. When illumination changes by a factor of 2, the iris attains a new equilibrium aperture in from 50 to 250 msec with most subjects. When the rate of change of illumination was slow enough for the iris overshoot to be undetectable, the "shutoff pulse" was never reported.

The conditions for maximum illusory effect appear to be moderate illumination, light intensity change by a factor of 2 or more, and illumination filling at least several degrees of the observer's visual field. With very strong illumination, the illusion, if present, is masked by afterimages. At very weak illumination, it is not reported. If the change in light intensity is very small, the illusion is not reported, and, in some instances, the step modulation is not perceived.

When the key light source is not only weak but also fills only a small portion of the observer's visual field, such as 30 min of arc, the shutoff pulse is not reported. If the light is near the edge of the observer's visual field and is relatively weak, he is likely to report that "it moved" when the intensity is keyed.

This illusion is possibly related to some phenomena recently reported by Baker (*1*) and Bouman (*2*). It is roughly analogous to the subjective portions of the "key click" problem, which has plagued the wire and radio-communications industries since their inception, and the "shutoff pulse" illusion is one of the reasons why keyed or step-modulated light beams, with visual reception, are not a satisfactory means of rapid communication.

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11 August 1958

Venom of the Stonefish *Synanceja verrucosa*

Abstract. Moderate doses in rabbits produced hypotension, increased respiratory rate, and myocardial injury. Respiratory arrest occurred with fatal doses when the blood pressure had declined to very low values. The active substance (or substances) was nondialyzable, and the potency of the protein-containing lyophilized or glycerol-treated extracts was maintained well on prolonged storage.

Stonefishes of the genus *Synanceja* have caused a number of deaths in human beings through stings by the venomous spines (*1, 2*). Two large venom sacs are present on each of the 13 dorsal spines, and much smaller ones on two pelvic and on three anal spines (*1*). Wounds have occurred commonly on the hand or foot as a result of punctures by the dorsal spines of the fish, which inhabits shallow water over wide areas of the tropical Indian and Pacific oceans. Extreme pain ensues within a few minutes after the sting and then spreads from the wound over the entire extrem-

ity. Ischemia and edema soon appear, and complete recovery from the local effects may be delayed for days or weeks. Systemic effects include weakness, sweating, respiratory distress, and convulsions; death may occur within a few hours (*1–3*). Duhig and Jones (*4*) and Gail and Rageau (*5*) have described the behavior of a few animals injected with stonefish venom; in addition, a hemolytic action has been reported (*6*). However, no data are available on the nature of the systemic effects of the venom or on the properties of the active substance (or substances).

Specimens of *Synanceja verrucosa* Bloch and Schneider were collected at depths of 25 feet or less at Parry Island, Eniwetok Atoll, Marshall Islands, and were maintained alive in the aquarium until used (*7*). The mean weight of the fish was approximately 2 kg; the mean standard length, 30 cm. The 13 dorsal spines were removed, and the thick outer integumentary sheath of each spine was peeled away, revealing two large, attached venom sacs. Extracts were prepared at 3°C, either by slitting open the sacs under 0.9-percent NaCl solution or by aspirating the fluid venom from the sacs and adding it to 0.9-percent saline. The extract was centrifuged at approximately 500 *g* for 5 minutes and the clear, colorless supernatant (*p*H approximately 6.8) was decanted from a small amount of precipitate. Suitable dilutions of the extracts were injected intravenously into tail veins of albino mice (mean weight 25 g). The animals died in about ½ to 30 minutes, depending upon the dose; animals which did not die within ½ hour usually survived indefinitely. Injected venom in amounts fatal within 2 to 5 min produced any or all of the following symptoms initially: ataxia, circling movements, and partial or complete paralysis of the limbs. A period of inactivity usually followed, after which violent rolling or pedaling movements occurred for about 15 seconds prior to respiratory arrest and death.

The undiluted venom aspirated from the sacs (0.03 to 0.07 ml per spine) was a clear, colorless fluid with an average nitrogen content of approximately 2 percent and a protein content (biuret determination) of approximately 13 percent. The total solids amounted to about 14 percent. The mean LD_{50} of freshly prepared extracts in mice was approximately 200 μg of protein per kilogram (range, 148 to 276 μg/kg) or 30 μg of nitrogen per kilogram; 10,000 to 25,000 LD_{50} doses for mice were present in extracts prepared from individual fish. Dialysis of extracts for 24 hours at 2°C in Visking cellulose casing against 0.9-percent NaCl solution indicated that the material lethal to mice was nondialyzable.

Extracts prepared from freshly killed specimens were adjusted to *p*H 7.5 and preserved by lyophilization or by the addition of glycerol to a final concentration of 40 percent. The samples (in sealed glass vials) were maintained for about 1 week in Dry Ice and subsequently were maintained at −20°C. Bioassays of representative glycerol-treated and lyophilized samples after 1 year of storage have indicated that 50 to 100 percent of the original activity was retained. The symptoms produced in mice with these stored samples appeared identical with those evoked by freshly prepared extracts. Spines were also removed from fish and stored at the afore-mentioned temperatures in sealed polyethylene bags for 10 months; extracts prepared after this period were of comparable potency and produced in mice the same symptoms as did extracts prepared from freshly killed fish.

Experiments were performed to determine the nature of the systemic effects of the venom. Twelve rabbits (average weight about 2 kg) were anesthetized with urethane (1 to 1.5 g/kg) intraperitoneally. Blood pressure was recorded from a carotid artery; the respiration was recorded, and electrocardiographic tracings were obtained simultaneously. Venom extracts were injected into a jugular vein. Lyophilized and glycerol-treated samples produced the same responses. Injection of small doses produced a slight fall in blood pressure accompanied by an increase in respiratory rate, with no electrocardiographic changes; these effects disappeared within about 5 min. Larger doses caused a more marked fall in blood pressure and an increase in respiratory rate which was sometimes accompanied by a period of decreased depth of respiration. There was evidence of myocardial ischemia or injury (inversion of the T wave in some animals, and marked displacement of the S-T segment in all animals), despite the absence of significant changes in the heart rate. All effects had disappeared after about 10 minutes (Fig. 1, *A*).

Injection of fatal doses produced similar effects initially; additional electrocardiographic changes (for example, occasional premature auricular and ventricular impulses, first-degree atrioventricular block, ventricular tachycardia, ventricular fibrillation) occurred as the blood pressure continued to decrease. The respiration then slowed and often became gasping for ½ minute or less and finally ceased in inspiration (in approximately 1.5 minutes in the typical experiment illustrated in Fig. 1, *B*); cardiac standstill followed within a few minutes. The auricles (and sometimes the ventricles) were usually still beating when the chest was opened, approximately 1 minute after respiratory arrest. The mean lethal dose in these experiments was approximately 10 μg of protein per kilogram.

The above results indicate that the primary action of the venom upon the cardiovascular system is the production of a marked hypotension, which is associated in the case of larger doses with

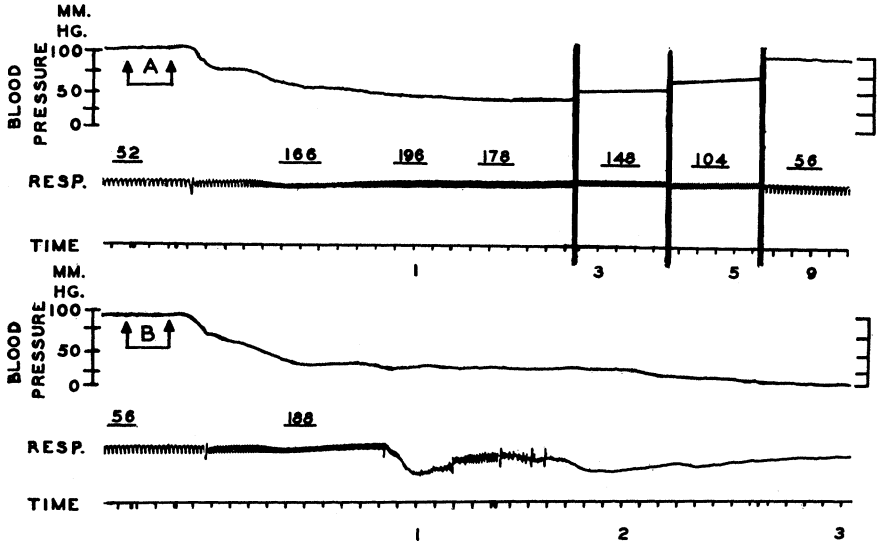

Fig. 1. Effect of venom of *Synanceja verrucosa* upon the blood pressure and respiration of a rabbit. The space between the arrows at *A* (top) indicates the interval during which 7.7 μg of venom protein per kilogram was injected; at *B* (bottom), the interval during which 11 μg/kg was injected 25 min later. The heavy vertical lines in the upper record indicate interruption of the recording. The numbers above the record of respiration indicate the respiratory rate per minute during the time interval denoted by the horizontal lines beneath these numbers. Signal marks at 5-second intervals and the numbers below the signal marks indicate time (minutes) after injection of venom. Inspiration is indicated by the downward stroke on the respiration record.

injury to the myocardium; whether these cardiac effects are due to a direct action of the venom upon the myocardium or coronary vessels or are secondary to the hypotension remains to be determined (*8*).

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7. This paper represents results of research carried out by the University of Southern California under contract with the Office of Naval Research. I am grateful to the Scripps Institution of Oceanography, University of California, and in particular to Prof. D. L. Fox of that institution, for the use of facilities during this investigation, and to the Atomic Energy Commission for general assistance and the use of facilities of the Eniwetok Marine Biological Laboratory, Marshall Islands. I also wish to thank Dr. D. W. Strasburg, Pacific Oceanic Fishery Investigations, U.S. Fish and Wildlife Service, Honolulu, T.H., for identification of the specimens used, and Mr. Peter B. Taylor for technical assistance in portions of this work.
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Purification of Folic Acid

Abstract. Various purification procedures for folic acid were investigated. The criteria of purity were a negative Bratton-Marshall test and the absence of fluorescent spots on paper chromatograms. Since no method provided a pure product, a procedure consisting of cellulose column chromatography followed by filtration through charcoal was developed.

Commercial folic acid contains a number of impurities, principally photochemical decomposition products. At least one of these substances, 2-amino-4-hydroxypteridine-6-aldehyde, has an intense effect on certain enzymes. It strongly inhibits the enzyme (or enzymes) of milk that oxidizes xanthine (*1–3*), xanthopterin (*1–3*), and 2-amino-4-hydroxypteridine, (*3*), as well as liver xanthopterin (*1*) and quinine oxidase (*1*). This report (*4*) describes the purification of folic acid by chromatography on cellulose powder followed by filtration through charcoal.

Various other purification procedures have been investigated—washing with dilute HCl solution (*5*), charcoal treatment and recrystallization (*6*), and crystallization of calcium foliate followed by charcoal treatment (*7*). The purity of the products was studied by paper chromatography and by determination of *p*-aminobenzoylglutamic acid by the Bratton-Marshall procedure (*8*). Since folic acid undergoes photochemical decomposition (*7*), all operations were carried out under dim illumination.

Descending paper chromatograms, 30 cm long, or more, were prepared; 2-cm² spots of neutral sodium foliate solution on Whatman No. 1 paper and two solvent systems—0.2*M* sodium phosphate buffer (*p*H 7.0) saturated with isoamyl alcohol (*9*) and *n*-butanol:acetic-acid:water (4:1:5) (*5*)—were used. When the phosphate buffer was used, 0.1-mg samples of folic acid were chromatographed. Three ultraviolet-fluorescing spots (R_f 0.48, 0.33, and 0.13, respectively) and a single ultraviolet-absorbing spot [R_f 0.40 (sodium foliate)] were present on chromatograms of the commercial product.

When the *n*-butanol:acetic-acid:water system was employed, denser (0.25 mg) spots were used, since sodium foliate does not move in this solvent. Three fluorescent spots (R_f 0.38, 0.26, and 0.11, respectively), and a single ultraviolet-absorbing spot (R_f 0) were visually detectable on chromatograms of commercial folic acid. The first two spots were decomposition products of the pteridine portion of the folic acid molecule (*5*).

The absence of fluorescent spots on the chromatograms and a negative test for diazotizable amine (*p*-aminobenzoic acid and *p*-aminobenzoylglutamic acid) were employed as the criteria of folic acid purity.

Since none of the purification procedures that were studied yielded a pure product, the problem was investigated, and the method described below was developed. The procedure involves a combination of cellulose chromatography and filtration through charcoal.

The cellulose column is prepared in the following manner. Two hundred grams of Whatman standard-grade cellulose powder are mixed with 0.1*M* phosphate buffer (*p*H 7) saturated with isoamyl alcohol. After it has stood for ½ hour, the mixture is poured into a tube (7.5 by 55 cm) plugged with cotton. The cellulose is packed to a height of 40 cm by suction, covered with a circle of heavy filter paper (Eaton-Dikeman No. 627-030), and compressed with a plunger to a final height of 37 cm. The column is then washed with 500 ml of buffer and placed in a dark room. The remaining operations should be carried out under dim illumination.

Commercial folic acid (550 mg) is suspended in 30 ml of water, and sufficient 1*N* NaOH is added to dissolve the folic acid. The solution [*p*H 7 (universal indicator paper)] is introduced into the column, and sodium foliate is moved down from the top with three 2-ml portions of the isoamyl-alcohol–saturated buffer. The column is then eluted with buffer at the rate of about 75 ml/hr. The yellow foliate band, which is clearly visible, is collected between approximately 550 and 730 ml of effluent. Since *p*-aminobenzoylglutamic acid is eluted just before folic acid, collection of the folic acid fraction should not be started until the effluent is distinctly yellow. If the folic acid band is so uneven that the folic acid fraction exceeds 200 ml, it probably will be contaminated with *p*-aminobenzoylglutamic acid.

Cellulose chromatography reduces the content of *p*-aminobenzoylglutamic acid (from 1 to 2 percent in commercial folic acid) to less than 0.02 percent. It also removes most, but not all, of the fluorescent material present in the folic acid. To remove the remaining trace, the folic acid is filtered through charcoal, as described below.

The eluate from the cellulose column is placed in a 250-ml polyethylene bottle, acidified to *p*H 2 (universal indicator paper) with 3*N* HCl, and centrifuged at 0°C in an International PR-1 refrigerated centrifuge. The sediment is then suspended in water and dissolved with sodium hydroxide, as previously described. It is important that the solution be completely clear, otherwise filtration will be extremely slow.

A charcoal column is prepared, an 8- by 100-mm chromatography tube being fused at the top to a 25- by 200-mm section of tubing. The tube is plugged with cotton and filled to a depth of 1 cm with cellulose powder, which serves to trap charcoal particles. A mixture of 0.5 g of Darco G-60 decolorizing charcoal and 1 g of cellulose powder is then slurried in water, poured into the tube, and held in place with a cotton plug. After the column has been washed with 25 ml of 6*N* HCl and 100 ml of water, the folic acid solution is filtered through the charcoal by suction. The column is washed until the effluent is colorless. Filtration through charcoal removes all of the fluorescent material remaining after cellulose chromatography.

Folic acid is recovered from the charcoal filtrate as follows. The solution is acidified to *p*H 2 with 3*N* HCl and centrifuged at 0°. The sediment is washed four times by stirring with 50-ml portions of 1-percent acetic acid and by centrifugation at 0°; it is then suspended in water and lyophilized. A pale yellow powder is obtained in a 70- to 75-percent over-all yield.

The product, which is completely pure according to the criteria that have been described, should be stored in the dark.

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