readings the electrophoretograms were all oriented alike (with the cathode end at the left) and the precipitin-band count of the lower run was subtracted from that of the upper run. In every case straight-line graphs resulted from the comparison of serum samples from the same individual. Comparisons between sera of different individuals never resulted in straight lines. However, the particular shapes and slopes of the intercomparative graphs have no known significance.

This study, designed to reveal inherent differences in the protein fractions of serum from different individuals, does not necessarily promise direct applicability to the practical determination of an individual source of a blood sample. It does not, for example, provide a classification of individuals based upon immunoelectrophoretic patterns, since it is applicable only to pairs of individuals whose serum is examined under identical conditions.

In the field of criminal investigation blood—often contaminated and usually dry—is frequently important evidence. However, before this method can be adapted to the practical problem of blood identification, it is essential to establish that the serum proteins in the fresh blood of different persons respond differently to immunoelectrophoretic treatment, and that this differentiation is reproducible. Preliminary work with sera extracted from dry blood indicates that, although the system operates at a somewhat reduced sensitivity, the results are essentially comparable with those obtained from fresh serum (*4*).

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Gas Spitting by Alarmed Fish Disturbs Their Hydrostatic Equilibrium

Abstract. Besides maintaining hydrostatic equilibrium, the physostomatous gas bladder of fish has the broader function of adapting the density of the fish to momentary needs.

Physostomatous teleosts can decrease the amount of gas in the gas bladder by "spitting" bubbles. This "gas spitting reflex" is generally assumed to be controlled by proprio- or exteroreception of ascent to higher levels in the water, for instance, during the ascent phase of vertical migration (*1*, *2*). With the release of gas the volume of the gas bladder is kept constant during ascent, and the hydrostatic equilibrium of the fish is maintained by keeping the density of the fish constant. In order to maintain equilibrium during descent, the amount of gas in the gas bladder must be increased. The means by which this is accomplished is not quite clear. A possible, but rather peculiar, method might be by swallowing air at the surface before descending. Another method, secretion of gas from the blood into the gas bladder, is very slow (*2*).

During experiments into the occurrence of an alarm substance in some North American fish (*3*) gas spitting related to downward movements was observed in the hornyhead chub (*Hybobsis biguttata* Kirtland, Cyprinidae) and in the white sucker (*Catostomus commersoni* Lacépède, Catostomidae). Release of gas bubbles was first observed when the fish were disturbed optically or mechanically. Both species are rather shy. Other cyprinids such as the creek chub (*Semotilus atromaculatus* Mitchell) and the bluntnose minnow (*Pimephalus notatus* Rafinesque) were tame within 1 week after collection by seining. But even 3 weeks after collection the hornyhead chub and the white sucker became very frightened if noise or visible movement occurred near their aquaria.

Ten hornyhead chubs (total length 8 to 12 cm) were kept in a 200-liter tank; ten white suckers (total length about 15 cm) were kept in a similar tank.

If undisturbed the fish swam quietly in the aquarium, and searched sporadically for food along the bottom. Sometimes one swam in its own direction, at other times all fish swam together in a polarized group [aggregating or schooling according to the definitions of Breder (*4*)]. In an undisturbed state the fish seemed to be in perfect hydrostatic equilibrium and were able to swim at any level with minimal correcting fin movements. When a noise occurred, such as the banging of a door, or when I approached their aquarium, they immediately dashed along the bottom, concentrated in a corner, and formed a tight "pod" (*4*). At one time they lined up nose-by-nose, at another time they spaced themselves more randomly. Gas spitting started almost immediately after the disturbance, or after some tens of seconds. Without further disturbance the fish would stay in a stationary pod and continue gas spitting for up to 20 minutes. After spitting, the fish were completely out of hydrostatic balance, and were too heavy to ascend from the bottom without great effort.

While studying whether a similar behavior pattern is elicited by the administration of alarm substance (aqueous skin extract), I took care to avoid frightening the fish by other means. The fish were observed through a peep-hole in a screen. The skin extract was put in the aquarium through a tube. Control fluid (aquarium water) was introduced in the same way in order to check whether the fish reacted to other stimuli accompanying this procedure. In view of the extreme variability of the response of the suckers, a definite decision with respect to their reactivity to skin extract does not seem justified. In the chubs, however, the responses to skin extract—forming of a pod and gas spitting as described previously—were convincing.

Another indication of alertness after disturbance in both species was the erectness of the fins while the fish were lying on the bottom of the aquarium. I noticed that frequently the pectorals were spread wide, with the caudal margin turned upward in a position so that the surface of the fins made an angle of about 45° with the bottom. The results of some improvised experiments in flowing water tentatively suggest that the fish were pressed down toward the bottom by the force of flowing water on the pectoral fins. Together with the increased density of the body, a result of excessive gas spitting, this attitude of the fins would enable the fish to remain motionless in a current. By this method they would avoid the production of key stimuli (movements) which would release attack behavior in several predators.

This type of gas spitting may be more widespread. Dijkgraaf (*5*) observed gas spitting resulting in sound production in the European minnow (*Phoxinus*

laevis Agassiz) when the fish were alarmed. He doubted (*6*) whether sound production is a functional aspect of the gas emission and suggested that gas spitting might help the fish in diving downward.

The release of gas bubbles in alarm reactions is not the result of an ascending movement of the fish. Gas spitting initiates, or is at least closely connected to, a downward movement of the fish, and it purposefully disturbs the hydrostatic equilibrium of the fish instead of maintaining this situation.

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Neutron Activation Analysis for Phosphorus in a Study of Development in a Beetle Wing

Abstract. Neutron activation analysis was used to measure phosphorus in individual beetle wings during pupal and early adult stages. By counting neutron-induced P^{32} radioactivity it was possible to measure 0.005 ± 0.001 μg of phosphorus. The phosphorus content of the wings rises to maximum at eclosion and subsequently decreases with loss of cells.

While investigating an effect of radiation upon wing development in the confused flour beetle (*Tribolium confusum*), we found it desirable to quantitate in some manner differences between normal and affected wings at different stages of development. A method was developed for reproducibly isolating the minute (≈20 μg) membranous wings which, during the pupal stage, contain a population of more than ½ million hypodermal cells (*1*). We wished to make measurements upon individual wings in order to assess the variation from one animal to another, while sampling enough wings to detect small differences among different groups. Neutron activation analysis is a method of unparalleled sensitivity for many elements of biological importance (*2*), and preliminary experiments showed that we could easily measure phosphorus in these wings by this method. The occurrence of phosphorus in both nucleic acids and lipoproteins makes it a reasonable choice as an indicator of changes in cellular state and number.

When samples of nearly any material are exposed, under identical conditions, to the same flux of neutrons, a constant fraction of the various nuclides in each sample becomes radioactive. For each element the intensity of this induced activity is proportional to the amount of the element present. The radioactivity induced in each element is unique and can often be distinguished from other radioactivities by physical or chemical means. Therefore, by activating a standard sample containing a known amount of the element of interest, together with the sample to be analyzed, one can determine the amount of a given element in the specimen.

Several samples of wings and a separate, dry, weighed sample of KH_2PO_4 were exposed simultaneously for 8 hours in the Livermore Pool-Type Reactor (LPTR). The flux was about 5×10^{12} thermal neutrons per square centimeter per second. For the first experiment the wings were packaged in two groups in polyethylene vials, which in turn were sealed in a standard LPTR aluminum container. These wings suffered appreciable radiation damage and became somewhat brittle, making them difficult to handle. We had previously found that radiation damage to thin polyethylene was reduced by maintaining good thermal contact with the coolant of the reactor. In the subsequent experiments, therefore, good thermal contact was established by packaging each group of wings in a tiny polyethylene envelope, sandwiching these envelopes between aluminum foils, and stacking them in a standard LPTR container.

For counting, each wing was attached with double-faced tape to a polyethylene sample holder. Background counts were obtained from similar preparations without wings. Standards containing 0.28 μg of phosphorus were prepared in duplicate by dissolving the activated KH_2PO_4 in H_2O and evaporating 2 μl of this solution onto a holder. These standards were placed in an automatic sample changer and successively counted with an end-window Geiger-Mueller tube. A Sr^{90} source was also counted to monitor the long-term stability of the counting system. Counts and counting times were recorded by a printer, making the entire counting operation automatic.

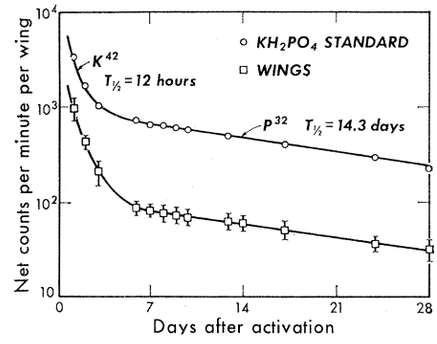

Fig. 1. Radioactive decay of activated wings and standards.

In the first experiment eight wings from adults approximately 24 hours past eclosion were analyzed. The radioactivities of the individual wings were averaged; the averages are plotted semilogarithmically against time after activation in Fig. 1. The vertical line at each point represents twice the observed standard deviation among the wings. The early counts reflect the presence of short-lived radionuclides, primarily Na^{24} (half-life, 15 hours). From the 6th day to the 28th day (the time of the last count) the activity decayed with the same half-life as that of the standard, which corresponds to the 14-day half-life of P^{32}. Gamma-ray scintillation spectroscopy of a pooled sample failed to detect the presence of long-lived gamma-emitting nuclides. Comparisons with the KH_2PO_4 standard indicated 0.03 μg of phosphorus per wing.

In another experiment we measured the amount of phosphorus in imaginal (final) wings removed from the animal

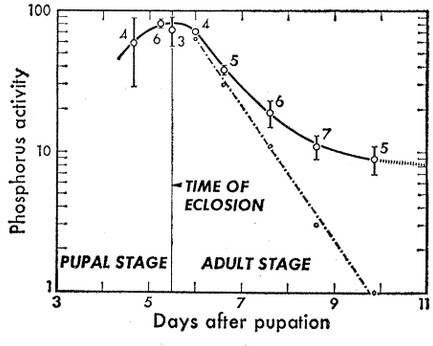

Fig. 2. Phosphorus activity (arbitrary units) as a function of stage of development. Numbers of wings in each sample are shown on the graph.