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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References and Notes

1. F. R. H. Jones and N. B. Marshall, *Biol. Rev. Cambridge Phil. Soc.* **28**, 16 (1953).
2. F. R. H. Jones, in E. Brown, *The Physiology of Fishes* (Academic Press, New York, 1957), vol. 2, chap. 4.
3. F. J. Verheijen, manuscript.
4. C. M. Breder, *Bull. Am. Museum Nat. Hist.* **117**, 399 (1959).
5. S. Dijkgraaf, *Z. Vergleich. Physiol.* **17**, 802 (1932).
6. ———, *Zool. Anz.* **136**, 103 (1941).

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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.

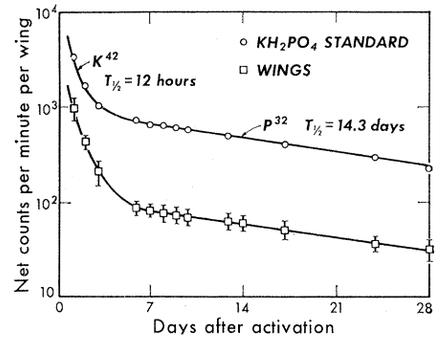

Fig. 1. Radioactive decay of activated wings and standards.

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

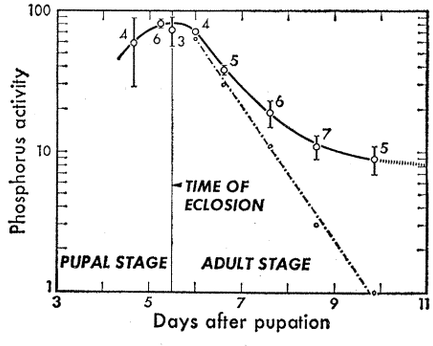

Fig. 2. Phosphorus activity (arbitrary units) as a function of stage of development. Numbers of wings in each sample are shown on the graph.

at various times during pupal and early adult life. Dissection of imaginal membranous wings from within the pupal cuticle is difficult at earlier stages, and larger sampling errors result. However, useful data were obtained, even with small numbers of wings. Counting was begun 7 days after the activation and continued until each wing had been counted twice. The interval between the two counts of any given sample was 5 days. The expected 14-day half-life was verified for each sample, and the counts were corrected for decay to an arbitrary time to make comparison possible. In Fig. 2 these values are plotted semilogarithmically as phosphorus activity per wing against the time of development at which the wing was sampled (solid curve). If we assume that the amount of phosphorus in wings 1 day after eclosion was the same as in the first experiment, the latest sample, taken 10 days after pupation, contained 0.005 μg of phosphorus per wing. The standard deviation for the five wings of this sample is 0.001 μg. This includes both the variation among the wings and the variation of the counts. The standard deviation of the counting of this smallest quantity (2×10^3 net counts in 1 hour) is only about 2 percent.

Observations by light and electron microscopy have revealed that after eclosion the hypodermal cells are lost from the wing. Failure to observe cell fragments suggests that this process is a transfer of whole cells from the wing. Accordingly, the observed decline in the phosphorus would reflect this loss. The curve seems to approach a constant value which would represent the amount of phosphorus in the wing structure exclusive of that in the hypodermal cells. When our estimate of this value, obtained by extrapolation (dotted line of Fig. 2), is subtracted from the data, an exponential curve results (broken line of Fig. 2). Our interpretation of this result is that the cells leave the wing at a rate proportional to the number in the wing—that is, randomly. This is in striking contrast with the pre-eclosion state of the hypodermal cell population (*1*). The cells were then a fixed population precisely synchronized in a regulated sequence of steps in differentiation and morphogenesis (*3*).

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References and Notes

1. J. S. Beck, Semiannual Report, Donner Laboratory, *UCRL-10211* (June 1962).
2. R. C. Koch, *Activation Analysis Handbook* (Academic Press, New York, 1960); K. Hsu, *Advan. Biol. Med. Phys.*, in press.
3. This study is based on work performed under contracts with the U.S. Atomic Energy Commission. We are grateful to Dr. A. Kirschbaum and E. Hill for the use of the reactor and counting facilities and to R. Padden for technical assistance.
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Secretion of Iodide by the Nasal Gland of Birds

Abstract. The nasal gland of the gull can secrete a solution of sodium chloride about 5 to 6 times more concentrated than that found in blood plasma. The gland can also concentrate iodide to several times the plasma concentration, but chloride seems to be preferred in the secretory process. These experiments were undertaken in the hope that a particularly high iodide clearance could form the basis of a method for determining the blood flow to the gland. The results made this approach to blood flow measurements unfeasible.

The main route of salt elimination in marine birds is through the nasal salt gland (*1*). The activity of this gland enables the birds to eliminate excess salt which is ingested with invertebrate food or sea water. In the herring gull, *Larus argentatus,* the secreted fluid may have a concentration of 0.8*M* with a chloride concentration 6 times greater than the concentration in the blood plasma. The fluid has a rather constant composition, with the principal ions of the blood plasma, sodium and chloride, making up most of its solutes. We therefore examined the ability of the gland to secrete other ions.

Iodide secretion was studied after either tracer amounts of radioactive iodide (I^{131}) or a massive dose of unlabeled iodide (I^{127}) had been injected. Since the nasal gland normally is active only during an osmotic load, secretion was stimulated by injection of 10 ml of a 10-percent NaCl solution into birds weighing 800 to 1000 g. All solutions were injected into leg veins, usually through a polyethylene catheter. To avoid contamination, all blood samples were withdrawn from a catheter in the opposite leg. After each withdrawal the catheter was flushed with saline containing heparin. The bird's upper beak was inserted into a vial to catch the nasal gland secretion. Urine samples were collected as they were eliminated. Chloride determinations were made by titration with silver nitrate and thiocyanate (*2*). The I^{131} was counted in a well scintillation counter, and the I^{127} was titrated with silver nitrate to a rose bengal end point (*3*). For the iodide titration the plasma and urine samples were deproteinized with $Ba(OH)_2$ and $ZnSO_4$ (*4*).

In one experiment on a 1-kg bird, 80 μc of I^{131} was injected and allowed to equilibrate for 25 minutes. The nasal gland was then stimulated with NaCl, and produced a copious secretion that continued in diminishing amounts for about an hour. The chloride content of the secreted fluid was high and relatively constant (Fig. 1). After a brief initial decrease in concentration paralleling that in the plasma, the iodide

Table 1. Excretion of toxic quantities of iodide in the herring gull with 10.3 m*M* NaI injected at time zero, and 17.2 m*M* NaCl injected at 48 minutes. *S*/*P* ratio, secretion concentration to plasma concentration ratio.

Time (min)	Volume (ml)	Concentration (m*M*)		*S*/*P* ratio		Cl^- preferment as % of I^-
		Cl^-	I^-	Cl^-	I^-	
			Plasma			
− 10		119				
35		98	33			
45		100	31			
53		152	22			
68		139	21			
			Gland secretion			
4	2.23	526	117	4.56	3.55*	128
10	1.66	543	110	4.85	3.34*	145
15	0.12	568	107	5.16	3.24*	159
49	1.65	770	76	4.90	3.42	143
55	1.23	755	76	5.02	3.46	145
60	0.33	795	78	5.49	3.58	153
65	0.18	796	82	5.69	3.84	148
			Urine			
3		122	75	1.03	3.20	32
54		145	85	0.96	3.86	24
63		159	67	1.06	3.11	34

* These ratios are based on iodide concentrations of plasma at 35 minutes.