weights, are unlikely to induce an immunological process like proteins; and thirdly, rats and mice, devoid of any digitalis-like principles in their body secretions, are comparable to toads in their tolerance to cymarin, ouabain, and coumingine HCl.

In the assay of digitalis-like principles, cats and frogs are most frequently used. When the results in cats are compared with those of frogs published in a previous communication,[3] cats appear to be more susceptible. However, part of the difference may be due to temperature variations, for the frogs were studied in a water bath maintained at 20°C, while the cats were investigated at a room temperature of 24.5°C.

Summary. 1. The acute toxicity of ouabain, cymarin, and coumingine hydrochloride has been compared by subcutaneous injection in cats, rabbits, guinea pigs, rats, and mice. 2. Both mice and rats are much more tolerant to ouabain and cymarin than cats in the ratios of 62:1 to 671:1. Regarding the alkaloid, coumingine HCl, which also has a digitalis-like action, their resistance is not so marked, being in the ratios of 13:1 to 29:1. 3. Guinea pigs and rabbits, like cats, are highly susceptible to ouabain, cymarin, and coumingine HCl.

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Preparation of Living Nuclei from Hen Erythrocytes.

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The early attempts to separate the nuclei from avian erythrocytes resulted in obtaining an amorphous mass of nuclear material. Warburg[1] was the first to isolate the separate nuclei. He used a freezing-melting technic. The same method was used by Miyake.[2] The disadvantages of this method are: partial agglutination and damage of nuclei, incomplete hemolysis and the necessity of further separation.

The method to be described leads to a stable suspension of free

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1 Warburg, O., *Z. physiol. Chemie*, 1910, **70**, 413.

2 Miyake, M., *Keijo J. Med.*, 1933, **4**, 247.

nuclei which probably have been less damaged than in the freezing technic. The method is based on the hemolysis of hen erythrocytes with lysolecithin in neutral saline solution, washing the nuclei with saline and resuspension in saline.

Lysolecithin was prepared as follows: Lecithin emulsion (0.5 g per 20 ml) was made in phosphate buffer pH 7.0-7.1 (crude lecithin recovered from cadmium salt of student preparation was used). The lecithin emulsion was ground with poison glands of 100 bees (lecithinase A), incubated during 24 hours at 37°C and filtered through a Berkefeld filter.

Chicken blood was obtained by cardiac puncture, according to the method of Sloan and Wilgus.[3] Citrate was used to prevent clotting. The blood was centrifuged at a speed just sufficient to sediment the erythrocytes and leave the bulk of the leucocytes in suspension. The plasma with suspended leucocytes and the top layer of erythrocytes were pipetted off. Isotonic saline solution was added to the rest of the erythrocytes and the procedure was repeated several times (5-6). Finally, the erythrocytes were centrifuged to close packing, the pipette introduced to the bottom of the centrifuge tube, and the lower layer of erythrocytes pipetted into a second centrifuge tube and suspended in saline. Microscopic examination of this suspension showed only occasional leucocytes.

To 30-40 ml of the erythrocytes suspension, corresponding approximately to the same volume of original blood, about 5-8 ml of hemolysing solution were added. The hemolysis was followed by observation of color in the liquid and controlled by microscopic examination. After the hemolysis was completed, usually 20 to 40 minutes at room temperature, the nuclei suspension was centrifuged, washed several times (5-6) with saline, and resuspended in the volume of saline approximately corresponding to the volume of the original blood.

The nuclei suspension thus obtained had a very faint yellow color. Examined under the microscope the free nuclei appeared smaller and more compact than the nuclei within the intact erythrocytes. The free nuclei stained readily with the aqueous methylene blue.

The nuclei suspension was stable when stored during 2 weeks in the refrigerator. When a few drops of nuclei suspension were poured into 3-4 ml of water almost immediate agglutination occurred. The nuclei suspension was stable in 0.1 M KH_2PO_4, but not in 0.1 M Na_2HPO_4. Microscopic examination in Na_2HPO_4 solution showed the nuclei with partially broken membranes and with the nuclei con-

[3] Sloan, H. J., and Wilgus, H. S., Jr., *Poultry Science*, 1930, **10**, 10.

tent streaming out from the punctured membranes (aqueous methylene blue staining). In saturated NaCl solution the nuclei formed a transparent gel.

A few preliminary experiments showed that the nuclei thus obtained respire. Warburg's direct method was used. In saline buffered with phosphate to pH 7.3 the Q_{O_2} value was around 0.2. It did not change appreciably after 2 days' storage in the refrigerator. When glucose was added to the medium the value of Q_{O_2} rose to about 0.3. The respiration varied at different pH values (phosphate buffers) as follows:

pH	Vol.	$cmm O_{2/h}$	Q_{O_2}
6.0	1.0 ml	2.83	0.094
6.5	1.0 ml	3.87	0.13
7.0	1.0 ml	6.33	0.21
7.5	1.0 ml	5.83	0.19

Agglutination of the nuclei with water did not significantly influence the respiration. In saturated sodium chloride the respiration first increased then diminished and stopped. The temporary increase could be regarded as bringing into contact the enzymes and substrates of nuclei after the internal structure was disrupted.

The respiration of the original erythrocyte suspension in the identical conditions gave Q_{O_2} values between 0.15 and 0.22, which is about one-third of the values found in Warburg's laboratory[4] for goose erythrocytes. Addition of glucose to the medium did not increase the respiration materially.

When erythrocytes were hemolysed within the Warburg apparatus either with lysolecithin or with water a significant decrease of respiration occurred. Judgment is withheld, until more evidence can be collected, as to whether this decrease is due to the partial damage of nuclei or only to the disruption of the connection between the nuclei and the cytoplasm.

The same method of preparation can be applied to the erythrocytes of mammalian embryos.

[4] Negelein, E., *Biochem. Z.*, 1925, **158**, 121.