TABLE IV.
Influence of Procaine upon the Spreading of India Ink in the Presence and Absence of Hyaluronidase. 10 rats tested in each experiment.

Exp.	Dosage (mg/kg)	Average size of spreading		Reduction of spreading area	
		Without hyaluronidase	With hyaluronidase	Without hyaluronidase	With hyaluronidase
				%	%
1	0	5.92	8.9		
	75	5.8	8.4	3.8 ± 6.4	5.3 ± 4.5
2	0	6.1	8.8		
	225	5.3	7.0	11.1 ± 3.4	18.1 ± 3.3

the epidermal type and (b) that antihistaminic substances such as Pyribenzamine and antistine counteract the spreading effect of India ink and hyaluronidase, as well as the latter's effect upon the allergic skin inflammation. The significance of these findings for the activity of antihistaminics in epidermal sensitizations is discussed.

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Isolation and Properties of Snake Erythrocyte Nuclei.

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The separation of nuclei from avian erythrocytes has been attempted by Warburg and by Miyake using the freezing and thawing technique.[1,2] However, the nuclei obtained by this method are not well separated and usually agglutinate in a mass. Laskowski was able to prepare a stable suspension of free nuclei of chicken blood using lysolecithin in neutral phosphate buffer solution for the hemolysis with subsequent washing of the nuclei with isotonic saline.[3] The poison glands of bees were ground with the lecithin emulsion which was then incubated for 24 hours at 37°C and finally filtered through a Berkefeld filter. The lysolecithin so prepared was added to the blood and after the hemolysis the nuclei were separated by centrifugation. This technique yields good preparations but the lysolecithin is not easily obtained under standard conditions. The procurement of the bee poison and the time required are disadvantageous conditions when large amounts of blood are used for chemical analysis.

The well known hemolytic action of tyrothricin, an antibiotic obtained from *Bacillus brevis* and commercially available,* has been used in our experiments with very satisfactory results. Snake erythrocytes after rapid hemolysis liberate the nuclei which are then washed several times in 0.9% saline and 5% citric acid solution. Microscopic examination of the nuclei suspension shows that they are not agglutinated and are almost free from the cytoplasm (Fig. 1). They are easily stained with methylene blue and Giemsa. The nuclei in suspension appear microscopically smaller and more compact than in the intact erythrocytes. This observation was also reported by Laskowski for chicken blood preparations.

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

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

3 Laskowski, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 354.

* Tyrothricin 2% solution, Parke, Davis & Co.

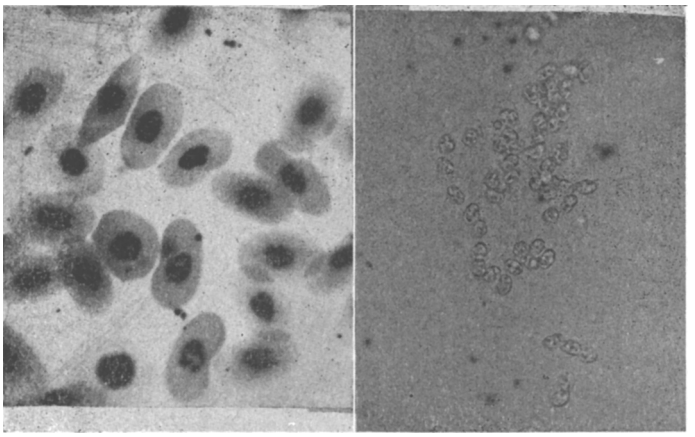

FIG. 1.
Left: Nuclei of the erythrocytes of the snake *Eudryas bifossatus* obtained with the tyrothricin hemolytic technique. Right: Blood of the same snake stained with Giemsa. × 810.

Fig. 1 shows a microphotograph of the nuclei of erythrocytes of snake blood suspended in 5% citric acid in comparison with the nuclei in the intact erythrocytes stained with Giemsa.

Method. The blood of 2 poisonous and 4 non-poisonous snakes was studied (Table I). It was collected from decapitated snakes directly into large test tubes containing 0.3% potassium oxalate. The amount obtainable (usually 25 to 40 ml) depended upon the size of the reptile. After centrifugation the plasma and leucocytes were pipetted off and discarded. The erythrocytes were washed several times (5 to 7) with 0.9% NaCl solution, until the washing was water clear. The densely packed cell layer was mixed with saline in a stoppered flask to obtain a 5% suspension. To 40 ml of the erythrocyte suspension 1 ml of the tyrothricin solution (20 mg/ml)† was added. The suspension was mixed well immediately by inversion of the flask. After 10 minutes the hemolyzed blood was centrifuged and the whitish mass of nuclei washed twice with saline, once with 5% citric acid, and finally suspended in the citric acid solution. For chemical analysis the citric acid treatment was omitted. The suspension of the nuclei was dehydrated with 95% alcohol in a centrifuge tube and the alcohol was evaporated under low pressure until a fine white powder was obtained. The powder was left over night in a vacuum desiccator and stored in the refrigerator. By this technique 74 mg could be prepared from 5 ml of washed snake erythrocytes. The same technique was applied successfully to the blood of fowls.

Results. Applying the saponin method of Dounce and Lan[4] to the erythrocytes of some snakes we obtained good preparations, but the nuclei still showed a layer of stroma. The authors cited demonstrated that the nuclei from chicken erythrocytes, which are somewhat yellow in color, contain a xanthophyll pigment and probably a flavin. Since the plasma of some snakes *(Bothrops, Eudryas)* is very rich in riboflavin, as formerly reported by us[5] it would be of interest to know if the nuclei of the erythrocytes also contain flavins. Using both tyrothricin and saponin techniques, we were unable to detect any flavin or carotenoid pigment in any sample exam-

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4 Dounce, A. L., and Lan, T. H., *Science*, 1943, **97**, 584.

5 Villela, G. G., and Prado, J. L., *J. Biol. Chem.*, 1945, **157**, 693.

TABLE I.
Desoxyribonucleic Acid Content of Nuclei of the Snake Erythrocytes.

Common names	Scientific names	No. of samples	Mg of desoxyribonuclei acid in 100 mg dry nuclei
Non poisonous snakes:			
Limpa-campo	*Eudryas bifossatus*	3	64
Giboia	Boa sp.	1	62
Caninana	*Spillotis pullatus*	2	31
Boipeva	*Xenodon merrimii*	1	44
Poisonous snakes:			
Rattlesnake (Cascavel)	*Crotalus terrificus*	3	59
Jararaca	*Brothrops jararaca*	4	56

ined. The ether extract and the remaining nuclei mass showed only a slight white fluorescence. Thiamin and nicotinamide assays on the dry nuclei showed values averaging 80 and 122 γ/g respectively (4 samples analyzed). The microbiological methods of Sarett and Cheldelin[6] for thiamin and of Krehl, Strong and Elvehjem[7] for nicotinamide were employed. The nuclei assayed were only saline-washed. Citric acid-treated nuclei are not suitable for analysis by these methods.

The desoxyribonucleic acid content of the nuclei was determined by use of the Dische reaction, as adapted by Dounce to permit spectrophotometric measurement of the blue color.[8] In a test tube 2 mg of the alcohol-dryed nuclei were suspended in 0.5 ml of distilled water and 1.0 ml of the Dische reagent. The tubes were immersed in a boiling water bath and 8.5 ml of the blank reagent (sulfuric-acetic acid mixture) was added. The slight turbidity appearing 1 to 2 minutes later was easily removed by the addition of 0.1 to 0.2 g of celite (Hyflo-supercel) and subsequent filtration or centrifugation. The clear blue color was measured in a Lumetron photocolorimeter using a 530 mμ filter. The blank value was subtracted from the unknown. To the tube containing the blank were added 1.0 ml of the Dische reagent and 7.5 ml of the blank reagent (sulfuric-acetic mixture) so that the total volume in the blank and unknown tubes was 10 ml. The color was stable for only 20 minutes and, therefore, all the operations had to be run in a short time. The values, referred to as desoxyribonucleic acid, were calculated from a calibrated curve made with pure sodium desoxyribonucleate prepared from normal liver nuclei according to a modified Hammarsten method and tested both spectrophotometrically and chemically (N : P ratio = 2.34%). The averages obtained for the nuclei of the different snake erythrocytes are shown in Table I.†

The percentage of desoxyribonucleic acid found in the nuclei of snakes showed higher values than those reported by Dounce for the nuclei of chicken erythrocytes and of rat liver (about 45%).[4,8] The total lipid content was determined only for the nuclei of the snakes *Spillotis pullatus* and *Eudryas bifossatus* and averaged 12.7%. Dounce and Lan reported for the chicken the total lipid content of about 14%.[4]

Summary. A new technique for the isolation of the snake erythrocyte nuclei using tyrothricin as an hemolytic agent is described. Some properties and chemical data are reported. The desoxyribonucleic acid content of the erythrocyte nuclei for the 6 kinds of snakes studied was higher than that reported for chicken erythrocytes and liver rat nuclei.

[6] Sarett, H. P., and Cheldelin, V. H., *J. Biol. Chem.*, 1944, **155**, 153.

[7] Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. and Eng. Chem., Anal. Ed.*, 1943, **15**, 471.

[8] Dounce, A. L., *J. Biol. Chem.*, 1943, **151**, 231.