vide a useful tool in investigations involving the examination of serums of certain species in which the *direct* complement-fixation procedures are not feasible.

Summary. 1. A simplified procedure for the *indirect* complement-fixation test is described.

2. The serums of 59 Newcastle disease immune chickens were tested by the simplified *indirect* complement-fixation and by the hemagglutination-inhibition tests.

3. Good general agreement was observed between the titers obtained by the two methods.

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Preparation and Amino Acid Composition of Salmin and Clupein.

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Salmin, on acid hydrolysis, was shown to yield arginine, proline, serine, valine, alanine, and isoleucine.[1] This observation was confirmed and extended by Tristram[2] who also identified glycine. This paper confirms the presence of glycine in salmin and also indicates that clupein, from *Clupea pallasii*, differs from salmin in that it contains threonine but no glycine.

1. *Preparation.* The procedure to be described gives approximately 10 times the yield of protamine from frozen milt than was obtained by any of the older methods.[3] One thousand grams of frozen milt are thawed and rinsed with cold tap water, mixed with an equal volume of a 1% solution of citric acid, finely ground, and 2.5 liters of 1% of citric acid are added. After standing in the refrigerator over night, the insoluble material is removed by centrifugation and the residue is washed with citric acid. This precipitate is suspended in 4 liters of 0.2 *N* HCl for 18-24 hours at room temperature with occasional mixing. The precipitate is removed and washed with 0.2 *N* HCl. The combined filtrate and washings are heated to boiling and adjusted to pH 7-8 with dilute ammonia. The resulting precipitate is removed by centrifugation and ammonia is added to the filtrate, at 30°C, to bring the pH to 9.4-9.6. The protein precipitate is removed by filtration. The contaminating proteins may also be removed by precipitation with 14% ammonia at room temperature at pH 10.4-10.6. In contrast to the first method, the histones (?) so prepared are completely soluble in 0.2 *N* HCl.

The filtrate is cooled to 12-15°C. Aliquots of the cold solution are removed and graded quantities of freshly prepared 33% of HPO_3 are added. The oily precipitates of protamine metaphosphate are thrown down by centrifuging and are washed with cold water. The quantity of HPO_3 required for optimal precipitation is thus ascertained.

The calculated quantity of 33% HPO_3 is then added slowly with stirring to the main protamine solution and the resulting oil is removed and washed with cold water until the washings are free from ammonia. The protamine metaphosphate precipitate is dissolved in a minimum quantity of hot *N* sulfuric acid. The solution is boiled until a small aliquot no longer gives a white cloud when cooled below 5°C, *i.e.*, when the metaphosphate has been completely converted to the sulfate but no longer. Protamine sulfate is precipitated and dried by the addition of alcohol or acetone. The yield is 2.0-2.5% of the weight of the original milt.

More than 30 preparations of salmin sulfate or hydrochloride and 8 preparations of clupein sulfate have been prepared from samples of

[1] Block, R. J., and Bolling, D., *Arch. Biochem.*, 1945, **6**, 419.

[2] Tristram, G. R., *Nature*, 1947, **160**, 637.

[3] Kossel, A., Protamine und Histone; Leipsig, Wien, 1929.

TABLE I.
Amino Acids in Salmin and Clupein (free base).

Amino acid	Salmin				Clupein	
	Block and Bolling[1] and Authors %	Kossel and Dakin (*cf.* [3]) %	Taylor[12] %	Tristram[2] %	Authors %	Kossel[3] %
Arginine	88.4	87.4	91.7	85.2	87.3*	88.0
"					86.8†	
Proline	7.9	11.0	10.8	5.8	8.2†	
"	8.6†					
Serine	7.0	7.8	8.7	9.1	3.4	
Valine	4.1	4.3	5.3	3.1	3.6	
Alanine	1.5			1.1	4.7	
Isoleucine	1.2			1.6	1.0	
Leucine	0†				0†	
Glycine	3.3			2.9	0	
Threonine	0				1.9	
Nitrogen	31.52	31.52			31.68	

* Kossel flavianate method.
† Microbiological method.

salmon or herring milt obtained through the kindness of Mr. Dudley Cole, Victoria, B.C., during the past 4 years. The protamine sulfate preparations were devoid of contaminating material as evidenced by negative Molisch, Millon, Pauly, ninhydrin, and phosphorus tests. (*cf.*[1,4]). The purity of each preparation was further confirmed by the use of two-dimensional paper chromatograms.[5]

2. *Amino Acid Analysis.* Arginine was determined by precipitation as the monoflavianate (*cf.*[4]) and microbiologically.[6] Proline was estimated by Guest's pyrrole method (*cf.* [4]) and by the microbiological procedures of Henderson and Snell[7] or of Dunn *et al.*[8] Serine and threonine were determined by periodate oxidation (*cf.*[4]). Glycine and alanine were determined by oxidation with ninhydrin to HCHO and CH_3CHO respectively (*cf.* [4,9,10]) Leucine, isoleucine, and valine were estimated by microbiological methods[4,11,12]. Leucine does not appear to be present in either of these protamines.

Results and Discussion. Our analytical results, as well as those of Kossel and Dakin (*cf.* [3]), Taylor,[13] and Tristram,[2] are given in Table I.

In contrast to the results of Felix and Mager,[14] there is no evidence for the presence of hydroxyproline in our samples of clupein. Because salmin contains no demonstrable amino or imino nitrogen or free carboxyl groups, Frankel-Conrat and Olcott[15] suggested that salmin lacks an end-group and that it may have a cyclic structure similar to those suggested for gramicidin and tyrocidine. Waldschmidt-Leitz *et al.*[16] reported that the

4 Block, R. J., and Bolling, D., The Amino Acid Composition of Proteins and Foods. Analytical Methods and Results. C. C. Thomas, Springfield, Ill., 1945.

5 Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 1944, **38**, 224.

6 Stokes, J. L., *et al.*, *J. Biol. Chem.*, 1945, **160**, 35.

7 Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, **172**, 15.

8 Dunn, M. S., Shankman, S., Camien, M. N., Frankl, W., and Rockland, L. B., *J. Biol. Chem.*, 1944, **156**, 703.

9 Alexander, B., Landwehr, G., and Seligman, A. M., *J. Biol. Chem.*, 1945, **160**, 51.

10 Alexander, B., and Seligman, A. M., *J. Biol. Chem.*, 1945, **159**, 9.

11 Shankman, S., *J. Biol. Chem.*, 1943, **150**, 305.

12 McMahan, J. R., and Snell, E. E., *J. Biol. Chem.*, 1944, **152**, 83.

13 Taylor, A. E., *J. Biol. Chem.*, 1908-09, **5**, 389.

14 Felix, K., and Mager, A., *Zeit. physiol. Chem.*, 1937, **249**, 112.

15 Frankel-Conrat, H., and Olcott, H. S., *Fed. Proc.*, 1947, **6**, 253.

16 Waldschmidt-Leitz, E., Ziegler, F., Schäffner, A., and Weil, L., *Z. physiol. Chem.*, 1931, **197**, 219.

imino group of proline is one terminal group of salmin and the carboxyl of arginine the other. Tristram[2] mentions unpublished results of Porter using the dinitrofluorobenzene method as strong evidence that proline is the end-group of salmin. This may account for the absence of the ninhydrin color of these protamines.

The data obtained in this study indicate that the ratio of arginine to monoamino acids in both salmin and clupein is somewhat higher (*cf.* [1]) than the 2:1 ratio postulated by Kossel.[3]

The question of homogeniety of salmin was discussed in a previous publication.[1] A similar situation holds with respect to clupein. Since that time, numerous unsuccessful attempts to fractionate salmin and clupein have been made from 90% aqueous isopropanol at 90°C, 25°C, and 0°C, and by paper chromatography using water-saturated phenol, lutidine-water-alcohol, water alone, 0.1 *N* H_2SO_4 and other solvents. The dinitrophenyl derivatives of the protamines also failed to yield more than one spot on paper chromatograms using various solvents. Although these preparations may be chemically inhomogeneous, their overall physical properties must be rather similar due to the preponderance of arginine.

Summary. A simplified procedure for the preparation of salmin and clupein is described. The amino acid content of clupein as well as salmin has been estimated.

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Effect of Electronarcosis on Level of "Adrenalin-like" Compounds in Blood.

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A chemical method for the quantitative determination of adrenalin was devised by Whitehorn.[1] This procedure was modified by Shaw[2,3] who showed that the method is not specific for this compound, and that a number of substances related to adrenalin give the same reaction. Shaw's method was applied to blood by Tietz, Dornheggen and Goldman,[4] Raab,[5] Bloor,[6] and others. It became apparent that the material thus estimated is not adrenalin alone, but that other compounds determined by Shaw's procedure are present in the blood.

Previous observations showed that the level of these "adrenaline-like" compounds is increased in the blood of patients during insulin shock.[7] It was also found to be increased during electronarcosis and electroshock treatment in humans.[8] In the present paper the effect of electronarcosis on the level of these compounds was investigated experimentally.

Methods. The technic used for the estimation of adrenalin-like compounds in blood has been reported before.[7] In order to limit the total amount of blood taken from the animal the method was adapted in the following way to the use of about 2 cc per determination. Blood was taken from the jugular vein which had been exposed previously under local anesthesia. A little more than 2 cc of blood was drawn from the vein in an iced syringe and squirted immediately into a small tube cooled in ice. Of this, 2 cc was measured

1 Whitehorn, C., *J. Biol. Chem.*, 1935, **108**, 633.

2 Shaw, F. H., *Biochem. J.*, 1938, **32**, 19.

3 Shaw, F. H., *Australian J. Exp. Biol. and Med. Sc.*, 1946, **24**, 53.

4 Tietz, E. B., Dornheggen, H., and Goldman, D., *Endocrinology*, 1940, **26**, 641.

5 Raab, W., *Endocrinology*, 1941, **28**, 325.

6 Bloor, W. R., *J. Biol. Chem.*, 1939, **128**, ix.

7 Tietz, E. B., and Birnbaum, S. M., *Am. J. Psych.*, 1942, **99**, 75.

8 Tietz, E. B., unpublished data.