TABLE I.
Precision of Urethane Standard Determinations.

Urethane, mg	0.15	0.3	0.5	0.8	1.0	1.2
Standard error, in %	5.1	2.4	0.7	1.2	0.5	0.7

TABLE II.
Recovery of Urethane Added to Blood.

Urethane added, mg %	Urethane found, mg %	% recovery
3.9	4.2	108
5.7	6.3	111
7.8	7.7	99
11.1	12.3	111
15.9	16.1	101
18.5	19.3	104
19.2	19.0	99
32.3	34.5	107
43.5	45.7	105

Such bloods gave the same urethane values when they were previously hemolyzed with one tenth volume of 1% saponin. The 1:10 tungstic acid filtrate was abandoned because the recoveries were more variable at low urethane concentrations.

Specificity of method. In 5 normal bloods we have found non-specific "urethane" values varying from 0.1 to 1.4 mg % and averaging 0.8 mg %. Five other bloods from patients receiving drugs (sulfadiazine, streptomycin, penicillin, phenobarbital and amytal) in therapeutic doses gave "urethane" values from 0 to 1.1 mg % and averaging 0.5 mg %. These "urethane" values are zero within the accuracy of the method, hence urethane values below 1.5 mg % have no significance.

Clinical blood urethane levels. Patients with wound infections receive urethane locally.[2] In 2 such cases, we have found the blood urethane to be 3.1 and 5.6 mg %, after 4 and 8 days of treatment, respectively.

Application of method for ethyl alcohol determination. The blank determination in the urethane method may be used for the determination of ethyl alcohol since the conditions of the distillation and incubation were chosen to give quantitative recoveries of ethyl alcohol in amounts yielded in the urethane determination, that is, about ½ the amount of urethane. A standard alcohol curve was constructed from dilutions of a standard alcohol solution prepared from a weighed quantity of absolute ethyl alcohol. The curve was linear up to 0.4 mg alcohol, the 0.6 mg point deviating about 5 percent from the line. The equation of the line up to the 0.4 mg point was:

Mg Ethyl Alcohol = 1.65 (0.4436 − L).

Strictly speaking the optical density at zero alcohol (0.4436 in the equation) should be that of a blank distillation with water.

Summary. A colorimetric method is described for the determination of urethane in blood, which gives 99 to 111% recoveries in the range 4 to 44 mg % urethane, and a non-specific "urethane" value of normal blood of 0.1-1.4 mg %.

A part of the method may be directly used for the determination of ethyl alcohol.

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Agglutination of Sea Urchin Eggs and Sperm by Basic Proteins.

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It is well known that basic proteins (protamines and histones) react with a wide variety of unrelated substances forming precipitates (with proteins, nucleic acids, etc.), causing cell agglutination (*i.e.* erythrocytes,[1] bacteria[1]) and exerting various physiological effects (*i.e.* parthenogenesis,[2] bacteriostasis[3]).

[1] Lajmanovich, S., and Mittelman, N., *Rev. Inst. Bact.*, "Carlos G. Malbran," 1944, **12**, 320.

It is not surprising, therefore, that such substances precipitate the jelly coat of sea urchin eggs, agglutinate sea urchin eggs, and agglutinate the spermatozoa of a variety of forms.[4,5] It is unfortunate, however, that another sea urchin egg jelly precipitin and egg agglutinin, namely antifertilizin, should be confused with basic proteins from the sperm nucleus.

In a recent study Hultin[6] attempts to show that the sea urchin egg jelly precipitins (egg agglutinins) extracted from sea urchin sperm by Frank[7] (by heating sperm) and Tyler[8,9] (by freeze-thawing or extraction at pH3) are basic proteins, that they are non-specific in their action and that they are obtained from the sperm nucleus. Hultin maintains that the extracts of Frank and Tyler do not contain the sperm surface substance, antifertilizin, that reacts with the specific sperm agglutinin, fertilizin, obtained from eggs. The last is a particularly important charge since much of our present knowledge of the role of specific substances in fertilization stems from studies on sperm extracts as prepared by Frank and Tyler.

Hultin's claims are based on the following observations: (1) basic proteins obtained by extracting sperm with acid (pH 0.9 to 1.0) agglutinated eggs of various echinoid species indiscriminately, (2) basic proteins split from sperm nucleoproteins (cytoplasm free) caused egg agglutination and jelly precipitation, (3) sperm cytoplasm prepared by citric acid extraction had no effect on eggs or egg jelly of the homologous species (*Arbacia lixula*) whereas acid extracts of the residual sperm nuclei gave strong positive reactions with eggs, (4) egg agglutinin and jelly precipitin samples prepared from sea urchin sperm by heating or extraction with weak acid (pH 3) gave positive tests for desoxyribosenucleic acid. Hultin makes no statement regarding the presence or absence of free basic protein in these last preparations.

Unfortunately Hultin reports no test for cross agglutination of sperm by heterologous fertilizin or cross agglutination of eggs by the sperm extracts of Frank and Tyler. Without such data as a standard of specificity his studies on specificities of sperm extracts have little significance since cross reactions between echinoid sperm and fertilizin are known. Hultin mentions no controls testing the effect of his basic protein extraction procedure (extraction at pH 1) or the effects of the cytoplasm extraction procedure (citric acid extraction) on antifertilizin samples as prepared by Frank and Tyler. To claim identity of the egg agglutinins of Frank and Tyler with the basic protein fraction it would appear essential to show that the former retains its activity when subjected to the extraction procedure required for preparation of the basic protein.

Some years ago the writer[4] began a study of the effects of basic proteins on eggs and sperm. It was found that basic protein prepared by acid extraction (pH 0.5 to 1.0) of sperm of 3 sea urchins (*Strongylocentrotus purpuratus, Lytechinus anemesis, Arbacia punctulata*) not only agglutinated homologous eggs, but agglutinated homologous sperm as well. The latter property is definitely not shared with sea urchin antifertilizin as prepared by Frank and Tyler. Such basic protein preparations also agglutinated sperm from a variety of unrelated species. Thus *Arbacia* basic protein agglutinated sperm of seven (three molluscs, four echinoderms) out of ten forms; *S. purpuratus* basic protein agglutinated sperm of 5 echinoderms and one mollusc. These and a variety of other basic protein sperm agglutinations will be reported in detail elsewhere.

Basic proteins give strong non-specific egg jelly precipitation and egg agglutination reactions as Hultin states. Thus basic protein

[2] Loeb, J., Artificial Parthenogenesis and Fertilization, Univ. Chicago Press, 1913.

[3] Negroni, P., and Fischer, I., *Rev. Soc. Argentina Biol.*, 1944, **20**, 487.

[4] Metz, C. B., Doctorate Thesis, California Institute of Technology, 1942.

[5] Hultin, T., *Ark. Kemi, Mineral., Geol.*, 1947, **24B**, 12.

[6] Hultin, T., *Pubbl. staz. zool. Napoli*, 1947, **21**, 2.

[7] Frank, J. A., *Biol. Bull.*, 1939, **76**, 190.

[8] Tyler, A., *Proc. Natl. Acad. Sci. U. S.*, 1939, **25**, 317.

[9] Tyler, A., and O'Melveny, K., *Biol. Bull.*, 1941, **81**, 364.

preparations from sperm of 7 forms (one annelid, 3 molluscs, 3 echinoderms) all agglutinated *Arbacia eggs*. It is of interest also that seminal fluid from the 3 molluscs agglutinated the *Arbacia* eggs. However, the basic protein egg agglutinins prepared from sea urchin sperm appear to be separate and distinct from the egg agglutinin (antifertilizin) prepared by freeze-thawing sperm. This follows from the fact that the egg agglutinating property of such antifertilizin was reduced (*S. purpuratus*) or disappeared entirely (*Arbacia punctulata*) when the antifertilizin preparations were subjected to the procedure used to extract basic protein. Thus, a sample of *Arbacia* antifertilizin (titer 16), prepared by freeze-thawing sperm, was adjusted to pH 0.8. A precipitate which formed at pH 2 to 3 was centrifuged off 4 hours later and the supernatant was neutralized. The sample was diluted to only one half the original concentration by addition of acid and base. It had no effect on the jelly coat of *Arbacia* eggs. It appears then that the antifertilizin was either inactivated by the acid or that it separated out in the precipitate (not tested further). Extracts prepared from whole sperm by this method gave powerful egg and sperm agglutination. Further evidence that the egg agglutinins in sperm extracts prepared by pH 1 extraction (basic proteins) and by freeze-thawing (antifertilizin) are separate and distinct was obtained in a neutralization experiment. A pH 1 sperm (*S. purpuratus*) extract which agglutinated both eggs and sperm was mixed with an active antifertilizin extract prepared by freeze-thawing sperm. The resulting mixture had no effect upon either eggs or sperm. Apparently the egg agglutinins in the two extracts can not coexist.

One may conclude from these experiments that at least 2 distinct jelly precipitating and egg agglutinating agents can be extracted from sea urchin sperm. The egg agglutinin(s) in pH 1 sperm extracts is probably a basic protein which acts in a highly non-specific fashion. The freeze-thaw and probably also the heat and pH 3 sperm extracts contain a different egg agglutinin(s), namely antifertilizin. For a discussion of antifertilizin specificity the reader is referred to Tyler's[10] recent review. The electrophoretic studies of Runnstrom, Tiselius and Vasseur[11] and Tyler[10] indicate that antifertilizin preparations contain a single acidic protein. In view of the foregoing the writer believes that Hultin has confused these two egg agglutinating substances obtainable from sperm. In the cases where basic protein extracts agglutinate homologous sperm as well as homologous eggs, this confusion can be rather readily avoided since antifertilizin preparations act on eggs but not on homologous sperm.

Summary. The agglutination of sea urchin eggs and sperm by basic protein extracts of homologous and unrelated sperm (reported by Hultin,[5,6]) is confirmed. Experiments are cited which show that antifertilizin, another egg agglutinin obtainable from sperm, is not present in or identical with the egg agglutinating basic protein fraction of sea urchin sperm as Hultin claims. Thus two distinct egg agglutinins are obtainable from whole sperm. One of these, antifertilizin, is obtained by heating, freeze-thawing or pH 3 extraction (Frank,[7] Tyler,[8,10]); the other, a basic protein(s), is obtained by pH 1 extraction of sperm.

[10] Tyler, A., *Phys. Rev.*, 1948, **28**, 180.

[11] Runnstrom, J., Tiselius, A., and Vasseur, E., *Ark. Kemi, Mineral., Geol.*, 1942, **15A**, 16.