PROCEEDINGS
OF THE
SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 30. DECEMBER, 1932. No. 3.

Western New York Section.

Clifton Springs Sanitarium, October 22, 1932.

6447

Combined Action of Ethyl Urethane and Sodium Thiocyanate on the Living Cell.

HENRY E. GUERLAC. (Introduced by J. B. Sumner.)

From the Physiological Laboratories, Cornell University, and the Marine Biological Laboratory, Woods Hole.

The alleged ability of sodium rhodanate (thiocyanate) to peptize heat-coagulated egg albumin led Bancroft and Rutzler[1] to elaborate a general theory, based upon an early observation of Claude Bernard,[2] that anesthesia is the result of a reversible coagulation of the colloids of the brain-cells, and that the action of narcotics in general can be antagonized by peptizing agents such as NaCNS and NaI. Their principle has been so extended in its application that now such pathological conditions as drug addiction,[3] histamine poisoning,[4] and insanity[5, 6] are held by them to result from disturbances in the colloidal state of the nervous system.

[1] Bancroft, W. D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1931, **35**, 144, 215, 1185, 3036.

[2] Bernard, Claude, *Union Med. Paris*, 1869, **8**, 109.

[3] Bancroft, W. D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1932, **36**, 1521, 2011.

[4] Bancroft, W. D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1931, **35**, 3189.

[5] Bancroft, W. D., and Richter, G. H., *J. Phys. Chem.*, 1931, **35**, 1606.

[6] Bancroft, W. D., and Richter, G. H., *Proc. Nat. Acad. Sci.*, 1931, **17**, 294.

The action of NaCNS as a narcotic antagonist was studied, to determine whether the action postulated for it could be observed in the case of a simple cell. The material was the egg of *Arbacia punctulata*, selected for its convenient size, ready availability, and because of the great amount of information amassed concerning it.[7]

Preliminary determinations were made to determine the toxic and lethal effects of different concentrations of ethyl urethane and sodium thiocyanate. In all cases freshly shed eggs of a single female were selected, using the criteria suggested by Just[8] for obtaining normal eggs. The eggs were washed twice to remove the perivisceral fluid, pipetted into sea-water solution, beakers covered, and after requisite time, washed and inseminated in sea-water. Controls were run for each determination. The solutions were made from purest reagents obtainable, with sea-water acting as natural buffer. The criterion of viability was the development of gastrulae and pluteii. When striking abnormalities were observed in the larvae the concentration was designated as toxic; when development was inhibited, as lethal.

The following table summarizes the results for the narcotic:

Concentration ethyl urethane	Effect of ½ hr. exposure	Effect of 2 hr. exposure
%		
0	None	None
1	"	
1.5	Slightly toxic	Toxic
2	Toxic	Lethal
3	Lethal	"

This indicates a value of 1.5% for the slightly toxic dose, corresponding with Heilbrunn's value[9] for the anesthetic concentration. The value of 3% for the lethal dosage is the same as his. There was no need to consider the osmotic values of the solution since the narcotic penetrates immediately into the cell.

The results for NaCNS were as follows:

All runs showed similar results. The values between 3% and 4% varied, being always toxic and sometimes lethal. The molarity values quoted in the table are for the whole solution, sea-water plus NaCNS, with the molar concentration of sea-water being taken as equivalent to 0.52M NaCl. The values of 3, 3.5, and 4% (of which the value in the table is an average result) were all made

[7] Harvey, E. Newton, *Biological Bulletin*, 1932, **62**, No. 2.

[8] Just, E. E., *Protoplasma*, 1928, **5**, 97.

[9] Heilbrunn, L. V., The Colloid Chemistry of Protoplasm, Borntraeger, 1928.

Concentration NaCNS	Effect of ½ hr. exposure	Effect of 2 hr. exposure
%		
0	None	None
2.1 (.78M)	"	Slightly toxic
3.5 (.52M)	Toxic	Lethal
5	Lethal	"

isosmotic with the eggs, the necessary amount of distilled water being added.

According to Bancroft and Rutzler the effect of the narcotic should be antagonized by some concentration of NaCNS capable of penetrating into the cell and exerting an effect upon the cytoplasmic colloids; the peptizing action of the salt should compensate for the coagulative action of the narcotic. To test this assumption concentrations of ethyl urethane were chosen which were known to coagulate the protoplasm; Heilbrunn's work[9] showed these to be our toxic or lethal values. It appeared desirable to antagonize these narcotic values with both a harmless and a toxic concentration of the salt. In results to be reported later it will be shown that NaCNS, like most strongly ionized salts, penetrates only very slowly into the cell, but that the minute quantity which does gain access is sufficient to bring about a definite change in the colloidal condition of the cytoplasm. Such a viscosity change can be demonstrated after exposure to the 2.1% or 3.5% NaCNS within one-half hour. Thus it did not appear essential to use a wide range of concentrations.

When eggs were placed in ethyl urethane plus NaCNS in seawater the following results were obtained:

Concentration ethyl urethane + NaCNS		Effect of ½ hr. exposure	Effect of 2 hr. exposure
%	%		
0	0	None	None
2	3.5	Lethal	Lethal
2	2.1	"	"

The results show no antagonism but an additive toxic effect upon treatment with the two reagents. For the half-hour period the 3.5% NaCNS and the 2% ethyl urethane were both toxic when used by themselves, but their combined effect was lethal. More convincing evidence comes from the runs with the other concentrations: 2% urethane is toxic, while 2.1% NaCNS is harmless, whereas together they are definitely lethal. It is to be remembered that the cumulative effect cannot be attributed to the increased os-

motic pressure theoretically obtainable with the 2 reagents in solution together, the narcotic being osmotically inactive due to its rapid penetration and the immediate attainment of its equilibrium.

6448

Relation of Plasma Volume to Plasma Protein Concentration.

MICHAEL J. LEPORE. (Introduced by G. H. Whipple.)

From the Department of Pathology, University of Rochester School of Medicine and Dentistry.

It was the purpose of this study to determine the relationship of plasma protein concentration to the plasma volume of non-edematous dogs. The value of plasma albumin determinations in studying fluid exchange has been amply demonstrated. It was consequently determined to perform simultaneous analyses of plasma albumin and globulin concentrations and of plasma volume in dogs that were undergoing certain experimental procedures. The method of Koch and McMeekin[1] was used for determining the plasma protein levels of the first 2 dogs and a micro-kjeldahl procedure involving distillation into N/70 H_2SO_4 and subsequent titration with N/20 alkali was used for the other 2 dogs. The circulating plasma volumes of the first 2 dogs were kindly determined for me by Mr. John Morcan, who used the brilliant vital red technique as described by Whipple and coworkers.[2] Those of the other 2 dogs were performed by the author, utilizing the spectrophotometric procedure for analyzing brilliant vital red described by Clarke and Graff.[3]

Changes in the plasma albumin concentration proved to be the most significant ones and consequently they alone will be mentioned in this report.

Two dogs in the anemia colony in this laboratory were studied weekly over a period of 2-3 months. In one of them, both the plasma volume and the plasma albumin concentration remained fairly constant. In the other, as the plasma albumin concentration decreased, the plasma volume decreased. As the plasma albumin concentration increased the plasma volume increased.

[1] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

[2] Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **51**, 205.

[3] Graff, S., and Clarke, H. T., *Arch. Int. Med.*, 1931, **48**, 808.