unchanged over a period of 4 weeks but when the positive control groups were supplemented with 3 and 10 μg respectively of synthetic vitamin B_6 the condition was first aggravated and then cured. Within 2 to 5 days of supplementation the tail lesions appeared in an additional 15% of the animals and in the more severe cases the tails became marked by a series of constrictions and intervening wet swollen areas denuded of hair. The tail lost much of its flexibility and frequently became kinked in one or more places. The stage of pronounced swelling and stiffness lasted from 3 to 7 days and was followed by a period of healing and rapid growth of the tail. The dermatitis of the face, ears and paws disappeared at the 10 μg level, and improved at the 3 μg level several days before healing of the tail lesions took place.

The same series of symptoms was noted in the groups supplemented with dried sheep or cow rumen contents. The reaction to the pure vitamin and to the rumen contents was similar also in that while the most severe swelling occurred in those groups receiving the highest levels of the supplement, complete healing occurred most quickly in these same groups.

The experimental evidence does not demonstrate whether the effect of vitamin B_6 in this condition is direct or indirect. György[4] has shown that the addition of crystalline vitamin B_6 to a choline-low diet aggravates the development of cortical necrosis of the kidneys. It is possible that a similar relationship may be involved in the present experiments.

11812

Flavin-Dinucleotide in Eggs of the Sea Urchin, *Arbacia punctulata.*

M. E. KRAHL, A. K. KELTCH, AND G. H. A. CLOWES.

From the Lilly Research Laboratories, Indianapolis, Indiana, and the Marine Biological Laboratory, Woods Hole, Mass.

In the course of an investigation of the respiratory systems of various marine eggs, the eggs of *Arbacia punctulata* have been analyzed for flavin-dinucleotide by the following adaptation of the d-amino acid oxidase method of Warburg and Christian.[1]

[4] György, Paul, and Goldblatt, H., *J. Exp. Med.*, 1940, **72**, 1.

[1] Warburg, O., and Christian, W., *Biochem. Z.*, 1938, **298**, 150.

Mature eggs were obtained and, when necessary, fertilized as previously described.[2] About 3 g of eggs, either unfertilized or taken at any desired time after fertilization and development at 20°C, were tightly packed by centrifuging at 2,000 times gravity for 10 minutes; they were then cytolyzed with 10 volumes of water, immediately put in a water bath at 80°C and held there with stirring for 10 minutes.

After cooling, the suspension was centrifuged and the cell debris and the supernatant fluid (made one-third saturated with ammonium sulfate) were each extracted 5 times, each time with 1 ml of liquid phenol at 40°C. With the initial addition of phenol to the supernatant fluid from the cytolysis, a precipitate of denatured protein appeared. This was centrifuged off and separately extracted 5 times with phenol. The combined phenol extracts were mixed with 2 volumes of ethyl ether; the flavin-dinucleotide was then extracted from the phenol-ether layer with 5 successive portions of water, the total water volume being made equal to that used in the cytolysis. Traces of phenol in this water layer were removed by repeated ether washes. The crude water solution was then concentrated and virtually freed of ether by several hours' exposure to suction in a vacuum desiccator over calcium chloride.

The flavin-dinucleotide activity was determined manometrically at 38°C, using a partially purified protein from lamb kidney[3] and d,l-alanine as substrate. The egg extracts gave zero oxygen uptake with d,l-alanine in absence of the protein. In each experiment a standard curve was run with 5 or 6 dilutions of partially purified yeast flavin-dinucleotide;[1] this material had been previously standardized against a sample of Warburg's mono barium salt of pure flavin-dinucleotide, kindly supplied by Dr. Eric G. Ball.

The results of 15 determinations by this method are given in Table I. Each determination was performed at least twice; the

TABLE I.
Flavin-dinucleotide Content, μg per g Wet Arbacia Eggs.

Exp. No.	Unfertilized	Fertilized		
		20 min	10 hr	24 hr
21 W 40	8.5	—	—	—
23 W 40	11.2	10.5	—	—
25 W 40	9.3	8.2	—	—
58 W 40	7.9	9.6	10.5	10.0
61 W 40	5.3	8.8	—	—
67 W 40	8.4	12.0	12.5	7.7

[2] Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, 1936, **20**, 145.
[3] Negelein, E., and Brömel, H., *Biochem. Z.*, 1939, **300**, 225.

results of a group of experiments on any one sample fell within ±10% of the mean value. All determinations opposite a given experimental number were made on eggs from the same batch.

The lumiflavin reaction[1] was performed with the residues of 58W, 61W, and 67W, containing, in all, approximately 0.4 mg (5×10^{-7} moles) flavin-dinucleotide, as measured by manometric test. Approximately 4×10^{-7} moles lumiflavin were obtained.

Conclusions. These experiments appear to indicate that *Arbacia* eggs contain a flavin-dinucleotide similar to, or identical with, that found in other tissues.[1,4]

11813

Measures of Respiratory Activity With Resting Cells.*

R. H. BURRIS AND P. W. WILSON. (Introduced by E. G. Hastings.)

From the College of Agriculture, University of Wisconsin, Madison.

The "resting cell" technic has gained wide acceptance and has proved extremely useful in the study of bacterial respiration. Quastel and Whetham[1] who pioneered the application of the method, defined "resting bacteria" those which had been centrifuged, washed with saline, and finally well aerated. In the absence of a nitrogen source such cells do not proliferate, and hence observed responses are not believed to be complicated by growth phenomena. They state, "Such an emulsion is always employed under such conditions that growth does not occur. The reactions brought about under these conditions we consider to be reactions produced by the resting organism. . . . The term resting organism as we have defined it is useful for comparison with resting muscle and other tissues." It should be noted that there is nothing in this definition which precludes assimilation of a part of the substrate instead of its partial or complete oxidation. Working with *Esch. coli* and a few other species, Clifton[2] has demonstrated that assimilation usually occurs; Wilson[3] has provided evidence that resting cells of the rhizobia likewise assimilate part of the glucose furnished them.

4 Ochoa, S., and Rossiter, B. J., *Biochem. J.*, 1940, **33**, 2008.

* This work was aided by a grant from the Rockefeller Foundation.

1 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1924, **18**, 519.

2 Clifton, C. E., *Enzymologia*, 1937, **4**, 246.

3 Wilson, P. W., *J. Bact.*, 1938, **35**, 601.