This slight lowering may be taken as lying within the limits of experimental error. It appears, however, that in such a long series of experiments, if there were any appreciable excretion of cholesterol into the gall bladder one surely would have expected it to become evident.

In 6 human cases who had had common duct obstruction for varying periods, the cholesterol content of the bladder bile lay within normal limits.

In the cases of cystic duct obstruction reported recently by Phemister[3] in which calcium carbonate gall stones occurred, the secretion of the gall bladder, although it contained large amounts of calcium, had but a trace of cholesterol in one case and none in the others.

We have tried to reproduce this condition in dogs and it has proven very difficult on account of the tendency of such gall bladders to form large abscesses. In another series the cystic duct was ligated and a fistula established. These were kept open for several weeks and drained a purulent material. Finally they became cleaner and drained clear mucus and were then allowed to close. Most of them became infected again and when reopened were mere bags of pus, but one such experiment succeeded. In this dog the gall bladder when opened contained 6 cc. of milky fluid which contained practically no pus cells. The calcium content was 580 mg. per 100 cc. and the cholesterol was but 21 mg. per 100 cc. This is in accord with the recent work on human cases.

Conclusion. The normal gall bladder mucosa secretes calcium in large amounts and cholesterol in negligible amounts.

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Effect of Cyanide on Respiration of the Protozoan, Colpidium Campylum.

ROBERT F. PITTS.* (Introduced by R. W. Gerard.)

From the Physiology Course of the Marine Biological Laboratory, Woods Hole.

The claim of Lund[1] that *Paramecium* is insensitive to KCN though questioned by Hyman[2] has been confirmed by Gerard and

[3] Phemister, D. B., Rewbridge, A. G., and Rudisill, H., *J. Am. Med. Asn.*, 1931, **97**, 1843.

* This work was carried out under the direction of Prof. R. W. Gerard.

Hyman.[3] A similar insensitivity of an alga, *Chlorella,* was described by Warburg and by Emerson,[4] and an even more striking resistance to cyanide by a bacterium, *Sarcina lutea,* has been reported by Gerard.[5] It seemed desirable to extend observations as to cyanide sensitivity to other protozoa. The present work is limited to the Protozoan, *Colpidium campylum.*

Methods. Pure cultures of colpidia were obtained from a single individual, and cultured on a spring water rye infusion seeded with the coccus *Achromobacter candicans* grown on agar slants for 2 days at 35° C. The colpidia were added 24 hours after the bacteria and grown for 48 hours before use. The cultures were prepared for respiration studies by gentle centrifuging, removing the bacterial layer, washing the lower sediment of protozoa and bacteria with spring water and recentrifuging, repeated 5 to 7 times. At the end the colpidia were practically bacteria free, and any of the latter still present could not have affected the respiration perceptibly. Oxygen consumption was determined by the Warburg method and the results are expressed in terms of Qo_2, the cmm. of oxygen used per million animals per hour. The colpidia were enumerated with an hæmocytometer after killing with formalin. Before mounting in the manometers, the heavy protozoan suspension was diluted with a fresh phosphate buffer at pH 7.4, made by adding 5 cc. of Sörensen's phosphate to 95 cc. of spring water. The cyanide was also made fresh every day, neutralized to phenol red and dissolved in the same buffer solution. The effect of cyanide was determined by opening the manometer chamber and adding a desired amount of cyanide in the course of a run. At the relatively low pH used here, cyanide put into a side bulb volatilized into the test solution before a control run could be made. Readings were taken at 15-minute intervals for about 4 hours, and the temperature kept constant at about 24° C. R.Q.'s were determined by comparative runs with and without alkali present in the inset.

Results. Concentrations of organisms from about 100,000 to 600,000 per cc. gave the same rates of respiration, the average Qo_2 for 35 determinations being 151.5. These values remained approximately constant over the 4-hour period of the experiment. This

[1] Lund, E. J., *Am. J. Physiol.,* 1918, **45**, 365.

[2] Hyman, L. H., *Am. J. Physiol.,* 1919, **48**, 340.

[3] Gerard, R. W., and Hyman, L. H., *Am. J. Physiol.,* 1931, **97**, 524.

[4] Emerson, R., *J. Gen. Physiol.,* 1927, **10**, 469.

[5] Gerard, R. W., *Biol. Bull.,* 1931, **60**, 227.

result is in fair accord with that of Pütter,[6] whose values, referred to the same temperature, were about twice as high. A few determinations of R.Q. gave an average value in buffer alone of 0.65, in cyanide of 0.90. The latter may be largely due, of course, to the liberation of CO_2 by stronger fixed acids under the action of the cyanide.

Due to the manner of adding the cyanide, the first 15 minutes' respiration, after its addition, were missed. At the end of this time, in concentrations between M/1000 and M/100 the respiration was regularly depressed about 20%. With the passage of time the respiration continues to increase to values as much as 25% greater than those before the addition of cyanide. In the case of M/1000, the maximum is reached in about 2 hours, and subsequently respiration falls again to about 80% of the initial value at the end of 4½ hours. In M/500 cyanide, the rise after the initial depression is slower, occupying 3 or 4 hours, and reaches only about 110% of the original value before beginning a second decrease; and in M/100 the rise from the initial inhibition is still more prolonged, requiring over 4 hours to reach 110% of the initial value. This rise, following an initial inhibition, may be due in part to the volatilization of cyanide from the suspension fluid to the alkali inset. At the pH used, such volatilization does take place, as shown by the appearance of the cyanide effect on the organisms when NaCN buffered at pH 7.4 is put in the side arm. It is extremely doubtful, however, whether a major portion of the M/100 cyanide would have left the solution in the time involved, especially since M/1000 cyanide shows a similar behavior. Likewise the increase of respiration above normal would not be so accounted for. For *Chlorella,* Warburg found a similar stimulating effect with low cyanide concentrations. It seems probable that under the conditions of these experiments—the organisms suspended in a water solution containing a very small amount of phosphate buffer and no nutrient substance—that not more than 25% of the respiration is cyanide sensitive.

[6] Pütter, A., *Z. f. allg. Physiol.*, 1905, **5**, 566.