of the height of the flask and to this 6 loopfuls of sterile rice starch were added. After having been well shaken the entire supernatant fluid from a positive 72-hour culture tube was added to a prepared 125-cc flask. After 72 hours most of the supernatant fluid from this flask was decanted off, leaving only a small amount of liquid and all of the sediment which lay on the solid egg base. Amebæ were to be found growing abundantly in this sediment, which was then well mixed and pipetted into the prepared 250-cc flask and incubated for 72 hours. This method resulted in the development of masses of amebæ which grow vigorously and abundantly.

The cultured trophozoites which have been studied had large nuclei each with a large eccentric karyosome and a nuclear membrane heavy with plaques of chromatin. The pseudopods were typically those of *Endamœba coli:* they were usually blunt, rounded, and granular, at no time hyaline. The cytoplasm was heavily granular, with many bacteria and starch inclusions, was often distinctly vacuolated and exhibited a very sluggish movement.

Encystation. Encystation in culture occurred 4 times spontaneously and was not produced at will. The medium in 3 of these instances was Locke's egg serum plus starch. In the fourth instance encystation occurred in cultures growing in a medium consisting of an egg base with a liquid overlay of a saline extract of the mucosa of the large bowel of man. This medium contained no starch. Subsequent experiments using the same media did not result in encystation. The pH value in each successful case was 6.4-6.6.

Working on the assumption that the possible cause of this encystation was the associated bacteria, the writer tried to isolate and identify the organisms present in the tubes where encystation occurred. The only ones found were *Escherichia coli communis* and an, as yet, unidentified species belonging to the *Colon-Aerogenes-Proteus group.* When these organisms, singly or together, were reintroduced into cultures containing *Endamœba coli* trophozoites, encystation did not occur.

Summary. Twenty-six strains of *Endamœba coli* were used for culture experiments, 2 strains in the trophic stage and 24 in the cystic stage. The former did not reproduce in subculture but 15 of the latter excysted and grew abundantly. Four of these are still being cultivated. A modified Locke's egg serum medium plus starch was the only one in which excystation occurred and proved to be the optimum one for continued growth of the organism. The medium was incubated at 37°, had an initial pH of 7.8-8.0 and a final pH of 6.8-7.0. Encystation occurred spontaneously but was never produced at will.

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Studies on Guinea Pigs After Repeated Administration of Paraldehyde.

EMMETT B. CARMICHAEL, F. A. KAY, AND G. W. PHILLIPS.

From the Department of Physiological Chemistry, School of Medicine, University of Alabama, University, and Bryce Hospital, Tuscaloosa.

Cervello[1] introduced paraldehyde as a hypnotic in animals and then on February 13, 1882, he ventured to try four 0.5 g doses of the drug on himself. The drug was introduced as a therapeutic agent by Morselli[2] at the Royal Asylum of Turin and within a few years, it had been employed by clinicians in both Europe and the United States. Paraldehyde gained a rather general use in most branches of medicine and especially in obstetrics and psychiatry.

Because of the wide acceptance of paraldehyde as a safe drug and because of the controversy as to whether it loses its efficacy on

[1] Cervello, V., *Arch. per le Scienze Med.*, 1882, **6**, 177.

[2] Morselli, Enrico, *Gaz. d. ospital*, 1883, **4**, 28.

continued use in patients,[3–8] we thought that some tolerance experiments on animals would be of interest.

Nineteen normal young adult male guinea pigs, weighing from 418 to 582 g, were used. One volume of the drug was diluted with water to 10 volumes. It was necessary to cool both the solution and the syringe below 18°C to keep the drug in solution. Ten cc of this solution per kilo were injected intraperitoneally, 3 times per week (Tuesday, Thursday, and Saturday). The injections were continued for 4 weeks and the average weight of the animals dropped from 499 to 469 g during this period of treatment.

To study the effects of the paraldehyde on these guinea pigs, the periods of time required for the development of 3 stages in the hypnotic state were used:[9] (1) the time from the injection until the onset of sleep, (2) the time from the injection until the end of sleep, and (3) the time from the injection until the end of hypnosis when an animal could walk about a meter with a steady gait. Each of the animals was observed by the above scheme after each of the 12 injections of the solution of paraldehyde and the data are illustrated in Fig. 1.

Summary. Nineteen adult guinea pigs received three intraperitoneal injections of a 1/10 solution of paraldehyde, 10 cc per kilo, per week for 4 weeks. The average weight of the animals dropped from 499 to 469 g during the series of injections. These animals showed a tolerance to paraldehyde, following repeated administration of large doses of the drug, as indicated by a decrease in both the sleeping time and the length of hypnosis. The average time for the onset of sleep, for each series of injections, varied from about 85 to 120 seconds. The duration of sleep dropped from 95 to 31 minutes and the length of hypnosis dropped from 231 to 167 minutes.

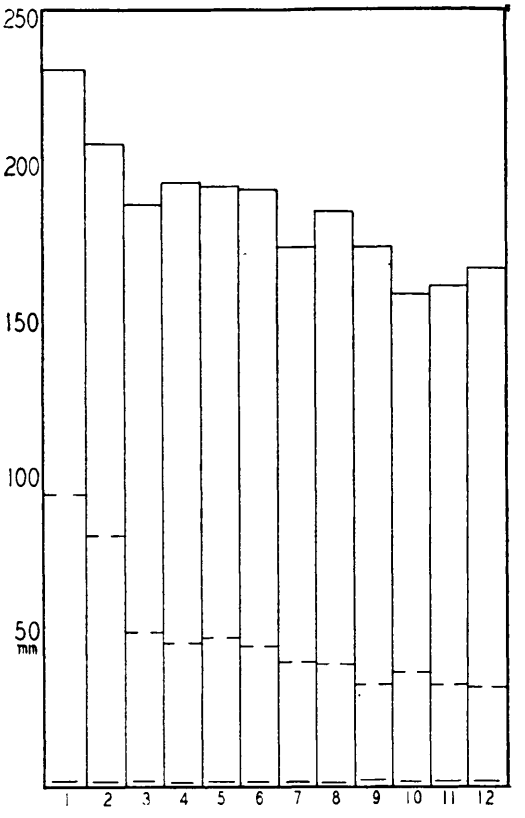

FIG. 1.
Production of Tolerance in Adult Guinea Pigs by 12 Tri-weekly Intraperitoneal Injections of Paraldehyde.

The graphs in Fig. 1 represent averages and each unit shows the results for one day. The lower line represents the time required to produce sleep and the upper broken line represents the end of sleep.

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[4] Gordon, John, *Brit. Med. J.*, 1889, **1**, 515.

[5] MacGregor, Alexander, *The Lancet*, 1899, **1**, 363.

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[7] Hoyt, F. C., *Medical Record*, 1890, **38**, 520.

[8] Mackie, W., *The Lancet*, 1899, **1**, 756.

[9] Carmichael, E. B., and Posey, L. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **33**, 1329.