osmicated nerves and roots both show the fibers inferred to correspond to the second potential associated with pain.

Both functional and histological evidence therefore support the conclusion that the impulses giving rise to the sensation of pain are mediated by myelinated fibers coursing *via* the dorsal roots, having the properties of the visceral afferent group.

6154

Observations on "Encystment Cycle" of Endamoeba histolytica in a Carrier.

H. TSUCHIYA. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology, Immunology and Public Health, Washington University School of Medicine.

The stools of a human carrier of *E. histolytica* were studied daily for 215 days to determine (1) the presence of encystment cycle, (2) the development and viability of immature cysts outside of the body, effects of changes in temperature and moisture, (3) the relative proportions of uni-nucleate cysts at various periods of encystment cycle, and (4) correlation, if any, between consistency of stools and number of discharged cysts.

The number of cysts per gram of stool was calculated according to the method previously described[1] and if cysts were very few, according to a modification of Lane's method[2] for counting hookworm eggs. All negative findings were verified by Rivas' modification[3] of ether-acetic acid concentration method, and also by the culture method recently reported.[4] Gram iodin solution was used in determining the number of nuclei present in samples of cysts examined.

Observations. 1. *Encystment Cycle.* There was a definite cycle of encystment in *E. histolytica* as has been observed in giardias by Boeck,[5] Kofoid and others[6] as well as by the author.[1] There was an apparent periodicity in appearance of the maximum number of

[1] Tsuchiya, H., *Am. J. Hyg.*, 1931, **13**, 544.

[2] Lane, C., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1924, **17**, 407.

[3] Rivas, D., *Am. J. Trop. Med.*, 1928, **8**, 63.

[4] Tsuchiya, H., PROC. SOC. EXP. BIOL. AND MED., 1931, **26**, 347.

[5] Boeck, W. C., *Uni. Col. Pub. Zool.*, 1922, **20**, 199.

[6] Kofoid, C. A., Boeck, W. C., Minnich, D. E., and Rogers, J. H., *J. Med. Res.*, 1919, **39**, 293.

cysts in each cycle, and the durations of such cycles ranged from 8 to 10 days, the average being 9.2 days. The number of cysts fluctuated from day to day with the maximum of approximately 1,250,000 per gram of stool. In most instances a peak in number was followed by a gradual diminution, and this was subsequently followed by a gradual rise until another peak was reached. However, when a negative period (the complete disappearance of cysts from stool) preceded a cycle, there was observed a sudden rise in the number of cysts.

2. *Development and Viability of Immature Cysts.* Contrary to the general belief that immature cysts were incapable of development outside of the body, it was found that under favorable environmental conditions such as an optimum temperature and a sufficient moisture, the cysts may develop to maturity *in vitro*. Thus uni- and bi-nucleate cysts developed into maturity quite readily at room temperature (22°C.) as observed by Hegner, Johnson and Stabler,[7] and in a refrigerator temperature (5°C.), while at 37°C. the development was extremely poor. The viability of such cysts was found to be greatest in a refrigerator (28-35 days for washed cysts and 9-11 days for cysts in original stools). At room temperature, washed cysts remained viable for 7-9 days, while in original stools for 3 to 6 days. The longevity of washed cysts in a refrigerator may be accounted for by the presence of fewer bacteria and the retardation of their growth.

3. *Relative Proportions of Uni-nucleate Cysts in Cycle.* The greater the total number of cysts, the greater was that of uninucleate cysts, and as the number of cysts became less and less in a cycle, there was also a gradual diminution in the proportion of uninucleate cysts. This seems to indicate that a peak in the number of cysts represents the beginning rather than the middle of an encystment cycle.

4. *Correlation Between Consistency of Stools and Number of Cysts.* The factors which influence the consistency of stools seem to have no effect upon the encystment cycle, and thus the principle governing the latter may be considered as strictly biological in nature.

Comments. The above observations emphasize the importance of frequent examinations of stools in suspected cases of the amoebic infections. Judging from the manner in which cysts were discharged from day to day, it is suggested that an optimum result may be obtained by examining stools on alternate days for a longer

[7] Hegner, R., Johnson, C. M., and Stabler, R. M., *Am. J. Hyg.*, 1932, **15**, 394.

period rather than on 6 consecutive days as advocated by Dobell.[8] The viability of immature cysts at low temperature suggests the possibility that such cysts may play an important rôle in the spread of the infection in cold season, and incidentally explains why the incidence of the carriers is relatively frequent in north temperate zones.

6155

Further Studies of the Ovaries of Monkeys.

EDGAR ALLEN, A. W. DIDDLE AND W. C. BALTZELL.

From the University of Missouri School of Medicine.

During the course of removal of ovaries from monkeys to prepare them as test animals for experiments with ovarian hormones, analyses of the ovarian follicular hormone (theelin) content have been made of individual follicles and corpora lutea. Ovariectomized mice have been used as test animals.

Solid tissues have been implanted in small pieces or simultaneous implants of solid tissues and injections of liquor folliculi have been made. Where possible ova have been recovered to check the normality or atresia of the follicles.

Similar series of experiments have been in progress with follicles of different sizes from pig ovaries, the test being made in ovariectomized rats.

Although additional tests must be added before publication of the data, enough evidence has already been obtained to justify the conclusion that theelin reaches a higher concentration in the follicles of monkeys than in the follicles of the pig. This is to be expected since the pig is a litter-bearing animal.

[8] Dobell, C., *Med. Res. C. Sp. Rep. Ser.*, 1921, **59**, 71.