fections of organs other than the intestinal tract. (3) Ingestion of serotype D433 of *E. coli* resulted in diarrhea and weight loss in a 2-months-old infant, whereas a similar exposure to the patient's own strain of *E. coli* failed to do so. Terramycin therapy was followed by clinical improvement and the disappearance of *E. coli* serotype D433. (4) Serotype D433 (4 strains received from England and 5 isolated in this laboratory) were completely or considerably inhibited on SS agar and desoxycholate citrate agar but grew well on Endo agar. Obviously, these selective culture media should not be used exclusively in examinations for the presence of this organism. (5) Serotype D433 (the above-mentioned 9 strains) was found to be highly susceptible to aureomycin, chloromycetin, terramycin, and polymyxin B (10 μg per ml), but less so to streptomycin. None of the strains were susceptible to bacitracin (100 U per ml) and penicillin (500 U per ml). (6) Treatment with aureomycin of 4 patients resulted in prompt disappearance of serotype D433 and clinical improvement; the fifth patient was treated with sulfadiazine and penicillin and continued to excrete this microorganism in the feces.

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Effects of a Water-Miscible Multiple Vitamin Preparation on Tissue Cultures.* (18247)

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(Introduced by F. J. Stare)

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The present study deals with the effects of a commercial water-miscible multiple vitamin preparation, Vi-Penta, on the morphology of mouse fibroblasts and on the beat of mouse heart fragments grown in tissue culture. This investigation was undertaken because of the possible association of the feeding of water-miscible vitamin preparations to premature infants and the development of an ocular condition, retrolental fibroplasia, leading to blindness(4).

Materials and Methods. All experiments were carried out in roller tube cultures following the technique of Gey and Gey(1). The fibroblasts studied were derived either from mouse embryo skin fragments or from heart fragments of 1- to 3-day-old mice. The tissue fragments were placed in a row in glass culture tubes and then covered with a layer of chicken plasma. After the plasma had clotted, a supernatant fluid of the following composition was added: Tyrode's or Gey's solution, 0.6 ml, heated horse serum§, 0.2 ml and chicken embryo extract, 0.2 ml. The tubes were then stoppered and incubated at 37°C in a rotor. Test materials were incorporated into the supernatant by replacing 0.1 ml of the salt solution with 0.1 ml of the material to be tested. The supernatant was changed approximately twice a week. The cultures of heart fragments were examined periodically in order to determine the number of fragments beating.

Results. Morphologic Effect. After 4 days incubation of mouse embryo skin fragments, a striking morphologic difference was observed in the unstained, living cells exposed to a 1:1,000 dilution of Vi-Penta as compared to

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1. Gey, G. O., and Gey, M. K., *Am. J. Cancer*, 1936, v27, 45.

§ The horse serum was heated at 56°C for 3 hours to decrease the lysis of the plasma clot(2).

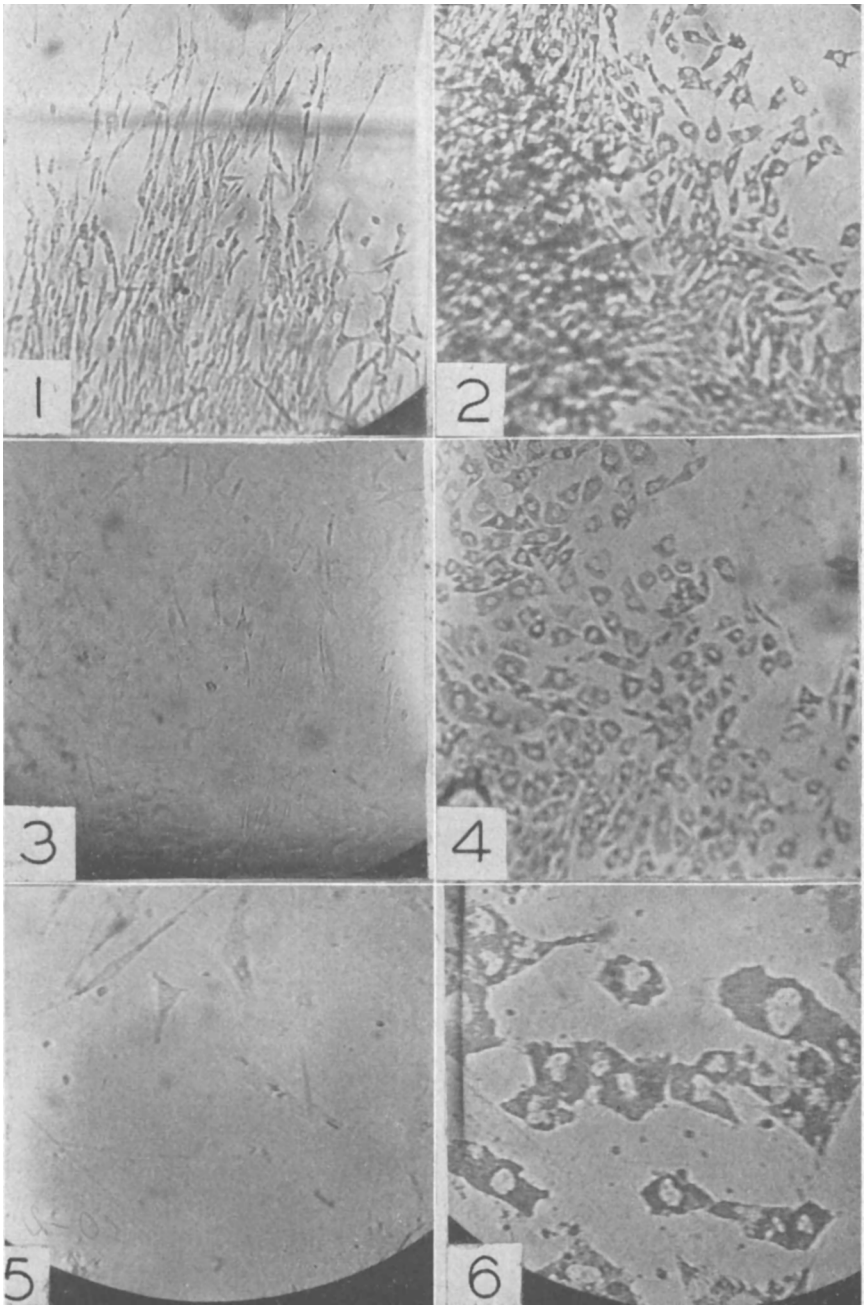

FIG. 1.

Part 1. Four-day growth of normal control cells from mouse embryo skin. (×60).

Part 2. Four-day growth of cells from mouse embryo skin which have been exposed to a 1:1,000 dilution of Vi-Penta. Note the broad, granular cells at the periphery of the outgrowth. (×60).

Part 3. Eight-day growth of normal control cells from mouse embryo skin. (×60).

Part 4. Eight-day growth of cells from mouse embryo skin which have been exposed to a 1:1,000 dilution of Vi-Penta. (×60).

Part 5. Fourteen-day growth of normal control cells from mouse embryo skin. (×120).

Part 6. Fourteen-day growth of cells from mouse embryo skin which have been exposed to a 1:1,000 dilution of Vi-Penta. Note the granularity of the cytoplasm, and the irregularity of the nuclear and cytoplasmic outlines. (×120).

TABLE I. Morphologic Effect of Various Dilutions and Fractions of Vi-Penta on Cells Derived from Mouse Embryo Skin and Heart Fragments.

Tissue	Substance tested	Dilution	No. of fragments	Presence of abnormal cells
Mouse embryo skin	Normal control	—	123	0
" " "	Vi-Penta	1:1000	93	Numerous
" " "	"	1:2000	28	Few
" " "	"	1:5000	18	0
" " "	" — vit. A & D fraction	1:1000	55	0
" " "	Vit. A & D fraction of Vi-Penta	1:1000	30	Numerous
Mouse heart	Normal control	—	115	0
" "	Vi-Penta	1:1000	110	Numerous
" "	Vi-Penta — vit. A & D fraction	1:1000	50	0

the normal control cells. Whereas the control cells maintained their normal thin, spindle-shape over the 14 days of culture, cells treated with Vi-Penta (especially those at the periphery of the outgrowth) showed an early broadening that in extreme cases amounted to a rounding with no extension of cytoplasmic processes (plate, parts 1 and 2). A more distinctive change in the treated cells was the notable increase in cytoplasmic granularity. While the control cells showed only the expected small amount of granularity in the cytoplasm near the poles of the nucleus, the treated cells appeared to have heavy granules filling their entire cytoplasm. This abnormal appearance of the treated cells was intensified after 8 days of incubation (plate, parts 3 and 4). By the fourteenth day many of the treated cells had undergone cytolysis, while most of the remaining cells had lost their processes and showed markedly irregular cytoplasmic outlines (plate, parts 5 and 6). During the early stages of the process, the nuclei of the treated cells appeared unaffected. By the fourteenth day, however, the regular, elliptical outline of the nucleus had become irregular, the nuclear material occasionally appearing to have been extruded into the cytoplasm.

The effects of various dilutions and fractions¶ of the vitamin preparation on the cell morphology are shown in the table. The data show that abnormal cells were produced by dilutions of the vitamin preparation as high as 1:2,000. In addition, the data also indicate that the responsible agent is associated with the vitamin A and D fraction of the vitamin preparation, since omission of the vitamin A and D fraction eliminated the appearance of abnormal cells and the use of this fraction alone produced abnormal cells.

Similar effects also were obtained with fibroblasts derived from newborn mouse heart fragments (table). However, tests with water-miscible solutions of either crystalline vitamin A or irradiated ergosterol (in concentrations approximating those in the Vi-Penta solutions) failed to produce the abnormal cells.

Physiologic Effect. It is well known that fragments of heart tissue grown in tissue culture will contract rhythmically for several days. As the cultures age, the number of pulsating fragments decreases progressively. Upon renewal of the supernatant, however, all the fragments usually resume their contractions. From the figure it may be seen that a marked decrease in the number of beating fragments occurred when Vi-Penta

¶ Supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.

2. Goldhaber, P., Cornman, I., and Ormsbee, R. A., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 590.

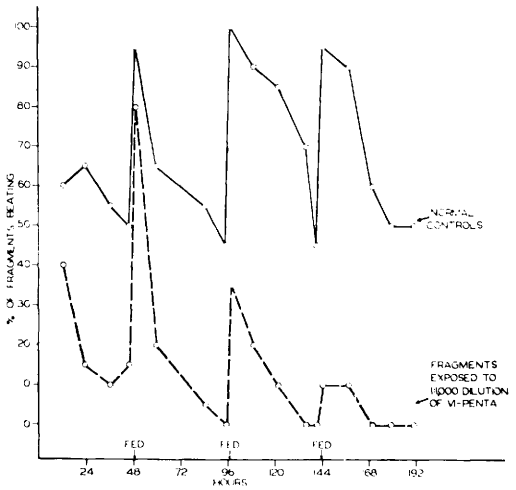

FIG. 2.

The effect of a 1:1,000 dilution of Vi-Penta on the number (in per cent) of beating heart fragments. Note the marked decrease in the number of beating fragments when Vi-Penta was present in the supernatant.

was present in the supernatant. Moreover, after renewal of the supernatant, these fragments did not completely resume their pulsations.

Discussion. The morphologic effects reported in the present experiments indicate that a 1:1,000 dilution of a commercial vitamin preparation, Vi-Penta, is toxic to mouse fibroblasts grown in tissue culture. While the responsible agent appears to be associated with the vitamin A and D fraction of the preparation, similar effects were not obtained with crystalline vitamin A or irradiated ergosterol.

Apparently, the morphologic effects observed were phenomena associated with a degenerative process. Evidence in support of this supposition was observed in the ability of the abnormal cells to stain with osmic acid, an indication of the lipoidal nature of their cytoplasmic granules, which Horning and Richardson(3) believe to be the predominant feature of the degenerating cell *in vitro*. In addition, the nuclear changes observed were similar to those found by Ludford(5) in the degenerating cell. It appears, however, that the toxic effects described are not specific for any particular agent, since similar abnormal cells have been observed in cultures not fed for a week, and also in cultures treated with high concentrations of the wetting agent[‖] used in Vi-Penta.

With regard to the pulsating heart fragments, the most likely explanation for the normal, progressive decrease in the number of beating fragments with time is the exhaustion of some necessary component of the supernatant or the increased concentration of some metabolic waste product. The action of Vi-Penta, in markedly accelerating the normal decrease in the number of beating fragments and in preventing full recovery upon the addition of fresh supernatant, is an indication of its ability, at the concentrations used, to interfere with the normal metabolic processes of this tissue as well as cells. It should be noted, however, that similar effects were obtained with another commercial water-miscible vitamin preparation from a different source, thereby indicating that Vi-Penta is not unique in its action.

Summary. 1. The effects of a commercial water-miscible multiple vitamin preparation, Vi-Penta, on mouse fibroblasts and beating heart fragments grown in tissue culture were investigated. 2. Cultures exposed to a 1:1,000 dilution of the vitamin preparation for a period of 2 weeks were markedly affected morphologically. The most distinctive changes were a notable increase in cytoplasmic granularity and a broadening of the cells. 3. The toxic agent responsible for the morphologic effects appears to be associated with the vitamin A and D fraction of the preparation. However, crystalline vitamin A and irradiated ergosterol had no abnormal effect on the cells. 4. A marked and abnormal decrease in the number of beating heart fragments occurred when Vi-Penta was present in the supernatant. 5. Vi-Penta is not unique in its morphologic and physiologic effects, since tests with a com-

3. Horning, E. S., and Richardson, K. C., *Australian J. Exp. Biol. Med. Sci.*, 1929, v VI, 229.

4. Kinsey, V. E., and Zacharias, L., *J. A. M. A.*, 1949, v139, 572.

5. Ludford, R. J., *Protoplasma*, 1935, v23, 180.

‖ The amount used was ten times the concentration of wetting agent in the 1:1,000 Vi-Penta solutions.

mercial water-miscible vitamin preparation from a different source gave similar abnormal effects.

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Comparative Bioautography of Vitamin B_{12} and Related Growth Factors Using *Euglena gracilis* and *Lactobacillus leichmannii*. (18248)

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It has been reported by Hutner *et al.*(1) that *Euglena gracilis* var. *bacillaris*, an algal flagellate, exhibits a quantitative growth response to crystalline vit. B_{12}, and that the desoxyriboside thymidine is inactive for this organism. Winsten and Eigen(2), and Cuthbertson and Smith(3,4) have used paper chromatographic and bioautographic procedures to demonstrate that certain *Lactobacillus leichmannii* strains exhibit a growth response to thymidine and other desoxyribosides in addition to vit. B_{12}. The procedures used by these authors demonstrated that the vit. B_{12} was found in the area of the slow-moving factors on the chromatograms while the desoxyribosides studied appeared as the more rapidly moving growth factors. Hutner's group(1) suggested that comparative studies using *E. gracilis* and lactobacilli would be of value in investigating the functions of vit. B_{12} and related growth factors. This communication presents a method for the use of *E. gracilis* as a bioautographic microorganism, and the application of paper chromatography and bioautography to the comparative study of the response of *E. gracilis* var. *bacillaris* and *L. leichmannii* 313 to vit. B_{12} and related growth factors.

Methods and materials. The general method employed in these comparative studies consisted of subjecting to paper chromatography selected samples of various preparations in duplicate, bioautographing one of the strips with *E. gracilis* var. *bacillaris* (ATCC 10616) and the other with *L. leichmannii* 313 (ATCC 7830) and finally comparing the positions of the areas of growth response of the two organisms. Descending chromatograms were prepared essentially as described by Winsten and Eigen(2) using *n*-butanol saturated with water as the solvent. Twenty-five μl of the material to be examined were applied to a 1 x 20 inch strip of Whatman No. 1 filter paper. After the desired distance of solvent flow had been attained, the strips were removed from the chamber and allowed to air dry in a disinfected hood.

The use of *E. gracilis* as a bioautograph organism required additional precautions against contamination because of its long incubation period. For this reason, aseptic technics, as far as practicable, were used. It appeared that *n*-butanol was in most cases an effective antiseptic agent, so that the wet chromatograms, if dried in a disinfected hood and thereafter handled aseptically, would remain essentially sterile. Large pyrex baking dishes with close fitting plate glass covers, used to contain the nutrient agar layers, were sterilized in an oven at 150°C. The medium was that of Hutner *et al.*(1) with 1.5% agar added, and was sterilized in the autoclave at

1. Hutner, S. H., Provasoli, L., Stokstad, E. L. R., Hoffmann, C. E., Belt, M., Franklin, A. L., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 118.

2. Winsten, W. A., and Eigen, E., *J. Biol. Chem.*, 1949, v181, 109.

3. Cuthbertson, W. F. J., and Smith, E. Lester, *Biochem. J.*, 1949, v44, v.

4. Smith, E. Lester, and Cuthbertson, W. F. J., *Biochem. J.*, 1949, v45, xii.