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INFLUENCE OF CARBOHYDRATES ON INTESTINAL PROTOZOA IN VITRO AND IN VIVO.*

BY

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Introduction.

That carnivorous animals are less frequently parasitized by intestinal protozoa than are herbivorous animals has been recognized for many years (Hegner, 1924). Experiments with laboratory animals have demonstrated conclusively that a diet high in protein is unfavorable and a diet high in carbohydrates favorable for the growth and reproduction of certain of these protozoa (Hegner, 1923, 1933). Soon after a method for cultivating *Endamoeba histolytica in vitro* was developed, it was found by Sautet (1926) that the addition of starch to the medium had a favorable influence on the multiplication of the amoebae. Several investigators have reported that cultures of *Balantidium coli* are likewise improved if starch is added (Rees, 1927; Schumaker, 1931a). *Balantidium coli* does not ingest starch slowly and grain by grain but gorges itself in a few minutes when given an opportunity (Nelson, 1933). Several authors claim that the number of balantidia in the intestine of pigs can be judged by the quantity of starch visible in the intestinal contents (Shegalow, 1899; Pritze, 1928; Schumaker, 1931b). Schumaker (1930) was able to infect rats with *Balantidium coli* when a diet high in carbohydrates was employed and to eliminate the ciliates when the carbohydrates in the diet were reduced. Trichomonads will ingest starch in culture (Hegner, 1932) and, as shown in the following pages, multiply more rapidly and live longer in media with starch than without starch. Thus various types of evidence involving several species of intestinal pro-

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tozoa indicate that carbohydrates exert a profound influence on these organisms both *in vitro* and *in vivo.*

At first it was suggested that changes in hydrogen-ion concentration were responsible for the differences noted, but no such changes were found to occur sufficient to account for the results obtained (Hegner and Andrews, 1925). Then the changes in the intestinal contents accompanying the changes in the nature of the intestinal bacteria from the acidophilus type, when the diet consisted largely of carbohydrates, to the putrefactive type, when the diet consisted largely of proteins, and *vice versa,* were suggested to account for the situation. This subject has been studied particularly by Ratcliffe (1928). More recently, the observations of Nelson (1933) have directed attention to the possibility that carbohydrates may serve as an essential food element and that intestinal protozoa thrive when they are present but starve when they are absent, although apparently in the midst of plenty. The experiments described in this paper were undertaken in order to test further the influence of carbohydrates on intestinal protozoa *in vitro* and *in vivo.*

Effects of starch on the multiplication of Trichomonas hominis *in culture.*

The organisms used were obtained from the same source as those employed in previous experiments (Hegner, 1929). The culture medium was also that previously found most satisfactory, consisting of 0.75 per cent NaCl solution plus 0.5 per cent of Loeffler's dehydrated blood serum Ten cc. were placed in each tube and 10,000 trichomonads were added. The tubes were incubated at about 36° C. The trichomonads were counted with a haemocytometer standardized by the Bureau of Standards. The counting method has been described in a previous publication (Hegner, 1934). Since our method of counting made it necessary to shake the tubes every time they were used for this purpose, several of the experiments were set up so as to test the effects of shaking on the multiplication of the organisms. Cultures without starch are called "normal." To other culture tubes about 2 mg. of rice starch were added daily. In certain experiments 2 mg. of soluble starch prepared by the Lintner method, were added daily. In one experiment the effects of the addition of glucose and glycogen were tested. The relative efficiency of the normal and modified media for the multiplication of *Trichomonas hominis* was judged by the comparative number of organisms present per cmm. at each examination and by the number of all counts added together.

Experiment 1. Comparison of normal and starch media. Ten tubes of normal medium and ten with 2 mg. of rice starch added daily to normal medium were inoculated with 10,000 trichomonads each from a pure-line culture and counted 2, 4, 6 and 7 days later. The average number of organisms per cmm. is given in table 1.

TABLE 1.

Number of trichomonads per cmm. in tubes containing the substances indicated.

Media	Second day	Fourth day	Sixth day	Seventh day	Total
Normal	116	132	82	55	385
Starch	64	207	168	115	554

Multiplication in the starch tubes was slower at first than in normal tubes, but after the second day was always greater. It is difficult to determine exactly what these numbers mean because we do not know in any case how many of the organisms died during the course of the experiment (See Hegner, 1934 for a discussion of this point). Only living flagellates were counted. Very few dead organisms were noted.

Experiment 2. Comparison of normal, rice starch, and soluble starch media. Ten tubes of each type inoculated with 10,000 trichomonads each from a pure-line culture were counted 2, 5 and 7 days later with the results shown in table 2.

TABLE 2.

Number of trichomonads per cmm. in tubes containing the substances indicated.

Media	Second day	Fifth day	Seventh day	Total
Normal	157	60	49	266
Rice starch	133	115	182	430
Soluble starch	30	208	234	472

The data indicate that the addition of rice starch favors increase in numbers and that soluble starch is more favorable than rice starch. Multiplication was delayed in both rice starch and soluble starch media.

Experiment 3. Comparison of normal, rice starch and soluble starch media. Fifteen tubes of each type were set up. Five were counted on the third day, 10 on the fifth day, and 15 on the tenth day. By this procedure the effects of shaking the tubes on multiplication could be tested.

TABLE 3.

Number of trichomonads per cmm. in tubes containing the substances indicated.

Media	Third day	Fifth day	Tenth day	Total
Normal (5 tubes).....	230	62	56	
(5 tubes).....	(not shaken on third day)	32	36	
(5 tubes).....	(not shaken on third and fifth days)		30	446
Rice starch (5 tubes).....	266	184	9	
(5 tubes).....	(not shaken on third day)	166	13	
(5 tubes).....	(not shaken on third and fifth days)		18	656
Soluble starch (5 tubes)...	214	184	7	
(5 tubes)...	(not shaken on third day)	114	26	
(5 tubes)...	(not shaken on third and fifth days)		17	562

Cultures containing rice starch or soluble starch were again found to be more favorable for the multiplication of trichomonads (table 3). Shaking the cultures appears to have aided rather than prevented multiplication, since in all but one of the six counts, which gave comparative numbers in cultures that had and had not been shaken, larger numbers of trichomonads were present in those that had been shaken, the total numbers being 505 and 377 respectively.

Experiment 4. Comparison of normal, raw-rice starch, cooked-rice starch, glucose and glycogen media. Five tubes of each type were prepared and inoculated with 10,000 trichomonads each from a pure-line culture. To these were added daily about 2 mg. of raw-rice starch, cooked rice starch, glucose and glycogen respectively. Counts were made on the following later days: 2, 4, 6, 8, 11 and 13. The numbers recorded per cmm. are presented in table 4.

TABLE 4.

Number of trichomonads per cmm. in cultures containing the substances indicated.

Media	Second day	Fourth day	Sixth day	Eighth day	Eleventh day	Thirteenth day	Total
Normal.....	266	126	70	38	11	+	511
Raw-rice starch.....	300	94	90	108	21	16	629
Cooked-rice starch.....	54	212	160	70	13	22	531
Glucose.....	34	162	144	82	22	21	465
Glycogen.....	72	148	184	132	44	30	604

Every medium except that containing glucose gave a better growth than the normal. Raw-rice starch was slightly superior to any other, with glycogen nearly as favorable. In both experiments 3 and 4

larger numbers were recorded on the first count in the rice starch tubes than in the normal tubes, in contrast to the situation in experiments 1 and 2.

Experiment 5. Comparison of numbers in shaken and unshaken tubes. This was a combination experiment to test the efficacy of different amounts of protein as well as the effects of shaking the tubes. Four groups of five tubes each were made up with 0.25, 0.5, 0.75, and 1.0 per cent of Loeffler's dehydrated blood serum respectively. Each tube was inoculated with 10,000 trichomonads from a pure-line culture. The organisms did not multiply well in the 1.0 per cent tubes. In the others shaking gave results such as the following: not shaken, 160; shaken once, 120; not shaken, 130; shaken once, 145; shaken twice, 250; not shaken, 40; shaken once, 60; shaken twice, 45; shaken thrice, 45. Counts were made on the following days after inoculations: 2, 5, 7, 9 and 12. So far as these results are concerned no evidence is indicated that shaking the cultures at intervals of 2 or 3 days has any appreciable effect on the numbers of flagellates present.

Experiments 1 and 2 also furnish data on the effects of shaking the tubes. In experiment 1, five of the tubes were not shaken on the second and fourth days; the later counts varied but apparently shaking had no significant effect on the numbers present. In experiment 2, five of the tubes were not shaken on the second day; here again no significant differences were noted.

It has been previously shown that trichomonad flagellates from various species of hosts will ingest red blood cells from various animals when these are added to the normal culture medium (Hegner, 1932). It was also found that several species of trichomonads will ingest starch grains from both rice and corn, although the former, which are much smaller than the corn-starch grains, were ingested more frequently. As many as 50 per cent of specimens contained grains of rice starch 2 days after they had been added to the culture medium. *Trichomonas hominis* does not seem to be so voracious, but specimens placed in a drop of culture medium in a hollow-ground slide to which rice starch was added were found to have engulfed one or several grains 15 minutes later.

Summary. The objects of these five experiments were to determine the effects of the addition of rice starch and other allied substances on the multiplication of the human intestinal flagellate, *Trichomonas hominis,* in cultures, and the effects of shaking the cultures at intervals of 2 or 3 days on the number of flagellates present.

Four separate experiments involving the counting of the tricho-

monads on three or four occasions in each of 120 tubes gave uniform results. In every case a considerably larger number of trichomonads was present in the tubes containing rice starch than in those without. The comparative numbers are as follows:

Without starch, 522, 375, 446 and 511 trichomonads per cmm.;
With added starch, 837, 727, 656 and 629 trichomonads per cmm.

Soluble starch was shown to be about as favorable for the multiplication of these flagellates as rice starch. Cooked rice starch gave a slightly higher count than normal. Glucose seemed to interfere slightly with multiplication. Glycogen proved to be almost as favorable as raw rice starch.

Shaking the culture tubes at intervals of 2 or 3 days in order to distribute the flagellates for counting purposes had no appreciable effect upon the numbers present.

Trichomonad flagellates will ingest starch grains, especially the small grains of rice starch, if these are added to the medium. The ingested starch grains are probably used as food and their addition to the diet may account for the greater numbers of trichomonads in rice starch media. Starch may, of course, be responsible directly or indirectly for various changes in the medium which may render the latter more favorable for the growth and reproduction of the trichomonads.

Five months after the experiments just described were completed it was decided to carry out two more experiments so as to determine with greater certainty the effect of the addition of rice starch to cultures of *Trichomonas hominis*.

Experiment 6. In this experiment a sample was obtained from the same human host that furnished the trichomonads used in the first five experiments but the flagellates employed did not represent a pure line. Two sets of 25 tubes were prepared containing 10 cc. each of 0.5 per cent Loeffler's dehydrated blood serum in 0.7 per cent NaCl solution and were inoculated with 10,000 trichomonads each. To one set about 2 mgm. of rice starch were added daily. The tubes were examined approximately every other day by shaking thoroughly and then counting the number of trichomonads present with a haemocytometer. The results obtained, as shown in the accompanying curves (chart 1), sustain those of the other five experiments. There was in every case a more rapid initial increase in the number of trichomonads in the normal tubes but this was soon overcome. The peak number in the normal cultures reached 101 trichomonads per cmm. on the

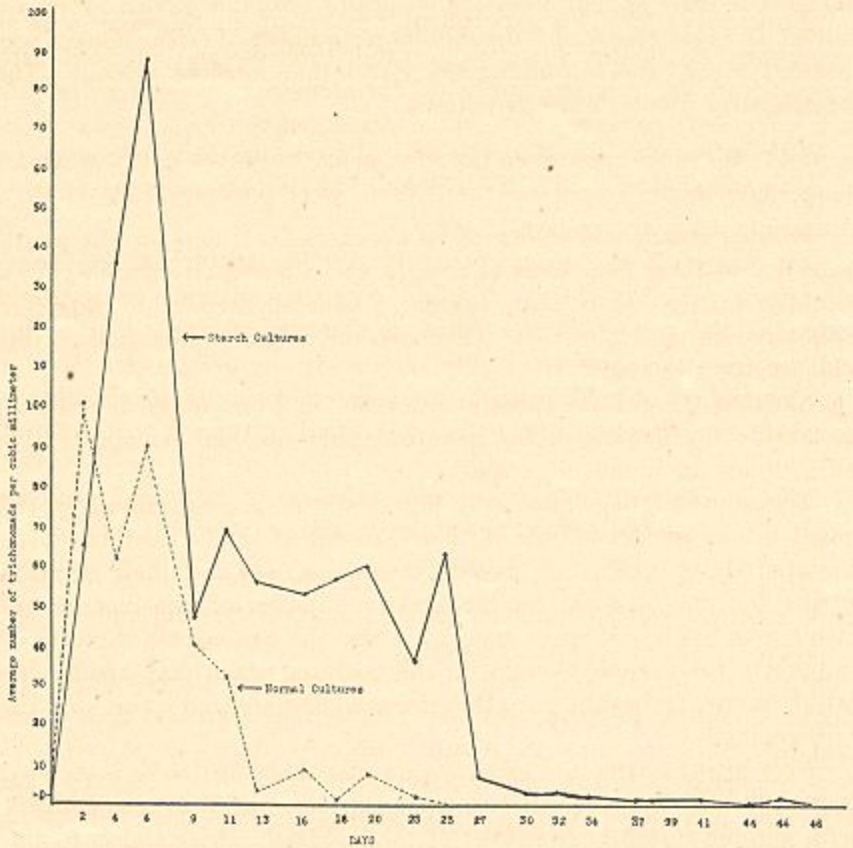

CHART 1. Curves representing the average number of trichomonads per cmm. in 25 tubes containing normal culture and in 25 tubes containing normal culture to which 2 mgm. of rice starch were added daily during the life of the cultures.

second day, whereas that in the starch cultures reached 188 per cmm. on the sixth day. The trichomonads rapidly decreased in the normal cultures, and in most tubes were too few to count after the ninth day; none of the normal tubes was positive after the twenty-third day. The number of trichomonads in the starch tubes decreased considerably after having reached the peak on the sixth day, but from the ninth day on the number decreased very slowly; counts could be made of some of the tubes up to the forty-sixth day and 17 of the 25 tubes were still positive on the forty-eighth day when the experiment was terminated. In both normal and starch tubes the number per cmm. in the different tubes within each lot of 25 tubes did not vary widely; these numbers are not included here so as to save space.

Experiment 7. This experiment was set up in the same way as experiment 6. Unfortunately the starch tubes became infected with an unfavorable organism, probably *Pseudomonas pyocyanea* and life was curtailed; however, these tubes presented the typical picture of a rapid rise in the normal cultures, a lag in the starch cultures, and a longer life in the starch cultures, which were positive 25 days while normal tubes were positive 18 days.

Summary. Two experiments with a "wild" culture of *T. hominis* covering a period of 48 days and involving 100 cultures confirmed and extended the results of five previous experiments with a pure-line culture from the same host. The data leave no doubt regarding the larger numbers of trichomonads and greater length of life in cultures to which rice starch is added than in normal cultures without starch.

Relations between the presence and number of intestinal protozoa and the quantity of starch in human feces.

This section of our report deals with methods and results obtained with material collected in Guatemala during June and July, 1933. Guatemala was selected as a collecting field because the natives, mostly mixtures of Indian and Spanish, live largely on starchy food and might, therefore, be expected to have undigested starch in their feces, and because large numbers of infections with *Balantidium coli* have been reported from this region (Aguilar, 1926). Unfortunately for this work infections with *B. coli* now appear to be rare in Guatemala and none were encountered among several hundred persons examined. As noted above it was at first believed that the effects of diets high in carbohydrates and proteins respectively were due to the changes in the bacterial flora, but the fact that starch grains are ingested in culture and seem to make up a large part of the food of such species as *B. coli* in the intestine of the pig suggested that a diet high in protein may deprive these organisms of one of their important food elements (starch), and that when carbohydrates are eliminated the protozoa undergo starvation and hence are unable to grow and reproduce.

Material and methods. One hundred fecal samples were obtained at the hospital of the United Fruit Co., in Quirigua, at the Friends Mission in Chiquimula and at the American Hospital and Public Health Laboratory in Guatemala City. A record of the presence and comparative numbers of intestinal protozoa was made from one smear from each sample within a few minutes to several hours after the

stools were passed. The number of protozoa per field was estimated and 1, 2, 3, and 4 pluses were used to designate the relative number present. Then 20 cc. of the stool were added to a 60 cc. bottle containing 40 cc. of 95 per cent alcohol. These samples were brought to our laboratory in Baltimore and their starch content determined. This was done by two methods.

(*A*) The alcohol was poured off; the sample taken from the bottle and thoroughly mixed, and 2 cc. of this were added to 56 cc. of water and heated in a double boiler for 15 minutes. Two cc. of this were added to 10 cc. of water in a test tube and 5 drops of Lugol's solution added. The appearance of a blue color was accepted as an indication of the presence of starch and the intensity of the blue color as an indication of the comparative quantity present. One, 2, 3 and 4 pluses were used to designate the quantity of starch.

(*B*) An adaptation of the Rask technique and Bidwell's modified Rask method which have been used for assaying the starch in cereal products was developed for our purpose. The method depends on the insolubility of starch in 70 per cent alcohol and its solubility in dilute hydrochloric acid. In dealing with feces preliminary treatment is indicated in order to remove matter that might interfere with the analysis. The procedure is as follows:

1. 20 gm. of fecal material are dispersed with 30 cc. of 98 per cent alcohol in a 50 cc. centrifuge tube. The moisture in the feces brings the alcohol concentration close to 70 per cent when the starch is insoluble and the alcohol-soluble products are washed out. The tube is centrifuged and the supernatant fluid aspirated.

2. The tube is filled with 70 per cent alcohol, centrifuged and aspirated. This step is repeated twice more.

3. The final alcoholic washing is made with 95 per cent alcohol to dehydrate the material.

4. Next the material is dispersed with anhydrous ethyl ether and washed as above to remove fats, etc. This step is repeated twice more and the material is finally filtered, or, after the last aspiration, the residue is broken up in the bottom of the tube and allowed to dry. It may remain in this state until convenient to finish.

The object, in the last part of the method is to precipitate pure starch and weigh it. The procedure starts by putting the starch into solution in half strength hydrochloric acid. The concentrated acid is mixed with equal parts of distilled water and adjusted by titration so that 100 cc. of the solution contains 20.5–21.0 grams of HCl. The

acid must be kept cool and is maintained below 18° C. The technique given must be followed closely to prevent hydrolysis of the starch.

1. The filtered material and paper or dried residue are put in a mortar and moistened with the hydrochloric acid and ground with a pestle. From time to time small amounts of acid are added until a complete suspension is accomplished. This step should take 10 minutes and a thick smooth paste should result.

2. The paste is transferred to a 100 cc. wide mouth volumetric flask and the mortar is washed out with hydrochloric acid and the washings are added to the flask. The level is brought to the 100 cc. mark with the acid. (If filter paper is used the flask must be recalibrated to allow for the extra volume.)

3. The flask is shaken vigorously for several minutes, allowed to settle and shaken again several times. The liquid is filtered immediately into a 250 cc. suction flask through a small Büchner filter fitted with a thin pad of asbestos and half full of fluffy dry asbestos.

4. 50 cc. of the filtrate are drawn off in a fast-delivering pipette and placed in a beaker containing 115 cc. of 95 per cent alcohol. This brings the alcohol concentration to 70 per cent thus precipitating the starch soluble in the acid. The contents of the beaker are stirred vigorously to aid the precipitation, and the starch is allowed to settle. The elapsed time from the addition of hydrochloric acid until the filtrate is placed in alcohol must not exceed 30 minutes.

5. The supernatant fluid, after settling is complete, is decanted into a weighed Gooch crucible fitted with a thin pad of asbestos and half full of fluffy asbestos. The precipitate is washed in the beaker with small portions of 70 per cent alcohol and then 95 per cent alcohol and transferred to the crucible. The sides of the beaker are washed to remove all starch adhering to the glass. Washing with ether completes the filtration. It is necessary to remove all the hydrochloric acid due to the fact that any remaining acid will char in drying.

The crucible is brought to a constant weight in a 100° C. oven at atmospheric pressure, and cooled in a desiccator and weighed. The difference of the weight of the crucible before and after filtration represents one half the starch present in the original 20 grams of feces. This method is described at some length because it proved to be a difficult problem in itself and may be useful to others who may wish to determine the quantity of starch in feces or other materials. Thus far it has been of very little service to us because the starch content of the feces we examined was for the most part too limited to weigh successfully.

Incidence of intestinal protozoa. Table 5 shows the number of single, double, triple and quadruple infections recorded for the 74 positive specimens of the 100 used in this study. The totals in the last column give the percentage incidence for each species. The incidence observed is about what one would expect from this group of people. The number of cases of *Iodamoeba* seems a little high; also with

TABLE 5.

Number of single, double, triple and quadruple infections with intestinal protozoa found in a single examination of fresh fecal samples from 100 natives of Guatemala of mixed Indian and Spanish descent.

Species	Number of species of protozoa present				
	1	2	3	4	*Total
Endamoeba histolytica	4	6	5	1	16
Endamoeba coli	17	20	10	2	49
Endolimax nana	4	11	3	1	19
Iodamoeba williamsi	5	5	10	2	22
Giardia lamblia	1	3	3	1	8
Trichomonas hominis	2	1	4	0	7
Chilomastix mesnili	0	2	3	1	6
Retortamonas intestinalis	0	4	1	0	5
Total no. of positive samples	33	26	13	2	
					132

* In every case the total represents percentage incidence since 100 specimens were examined. These data involve 74 specimens since 26 were negative for protozoa.

Giardia, since previous surveys indicate that the incidence of *Giardia* is not as high in the tropics as it is in temperate regions. This result, however, may have been due to the fact that a considerable number of children were examined at Chiquimula among whom five of the eight cases were found. As is well known, children are more highly infected with *Giardia* than are adults. The incidence of *Trichomonas hominis* seems low, compared with previous reports from Tropical America, e.g., Hegner (1925), when 20 per cent was recorded.

Multiple infections. We are accustomed to state that no antagonism exists among intestinal protozoa and that multiple infections depend on the infectibility of the host and on his exposure to infection. This is indicated by our results. The number of combinations are recorded in table 6.

It will be noted that the protozoa with the highest incidences, namely, *E. coli, I. williamsi, E. nana,* and *E. histolytica* are present in

TABLE 6.

Number of double, triple, and quadruple infections with intestinal protozoa in 100 samples.

Double infections		Triple infections	
E. coli + *E. nana*	9	*E. hist.* + *E. coli* + *I. will*	3
E. hist. + *E. coli*	5	*E. coli* + *I. will.* + *T. hom*	2
E. coli + *Iod. will*	3	*E. hist.* + *E. coli* + *E. nana*	1
E. hist. + *Iod. will*	1	*E. coli* + *E. nana* + *I. will*	1
E. coli + *G. lamblia*	1	*E. hist.* + *E. nana* + *T. hom*	1
E. coli + *C. mesnili*	1	*E. coli* + *I. will.* + *G. lamblia*	1
E. coli + *Retort. int*	1	*E. coli* + *I. will.* + *C. mesnili*	1
E. nana + *Iod. will*	1	*E. coli* + *C. mes.* + *Retort. int*	1
E. nana + *Retort. int*	1	*I. will.* + *G. lamblia* + *T. hom*	1
G. lamblia + *C. mesnili*	1	*I. will.* + *G. lamblia* + *C. mes*	1
G. lamblia + *Retort. int*	1		
T. hominis + *Retort. int*	1	Total	13
Total	26		

Quadruple infections	
E. hist. + *E. coli* + *I. will.* + *G. lamblia*	1
E. coli + *E. nana* + *I. will.* + *C. mesnili*	1
Total	2

combinations, in general, according to the order of their frequency. For example, 18 of the 26 double infections and 5 of the 13 triple infections are amoebic only and 3 of the 4 species in each of the 2 quadruple infections are amoebae. *E. coli*, which is most frequent in single infections, is also most frequent in multiple infections, being present in 20 of the 26 double infections, 10 of the 13 triple infections and both of the quadruple infections.

Relation between the presence and number of intestinal protozoa and the quantity of starch in the feces. Ninety-four of the 100 fecal specimens were successfully examined for starch. The quantity of starch present was so small that it could not be extracted and weighed satisfactorily; hence we had to depend entirely on color reactions. Twenty-seven specimens gave a color reaction and 67 did not. Of 24 samples negative for protozoa, 17 gave no color reaction and 7, or 34 per cent, gave a color reaction. Of 70 samples positive for protozoa, 50 gave no color reaction and 20, or 35 per cent, gave a color reaction. The 67 which gave no color reaction were distributed as follows: negative for protozoa, 17; single infections, 24; double infections, 16; triple infections, 8; quadruple infections, 2. The 27 that gave a color reaction were distributed as follows: negative for protozoa, 8; single infections, 5; double infections, 9; triple infections, 5. Efforts have been

made to obtain a more definite result by taking into consideration the number of pluses recorded for the various samples, both as regards the number of individuals present in the samples and the intensity of the color reactions, but the data obtained were no more conclusive than those noted above and hence are not included here.

Conclusion. Apparently the only conclusion possible is that the methods used do not reveal any definite relation between the presence and number of intestinal protozoa and the quantity of starch in human feces.

The summary of results obtained in experiments to determine the effects of starch on the multiplication of *Trichomonas hominis* in culture media will be found on pages 125 and 128.

Bibliography.

AGUILAR, R.
1926. The treatment of *Balantidium coli*. 14th Ann. Rept. Med. Dept. United Fruit Co., 1925, 246–248.

HEGNER, R.
1923. The effects of changes in diet on the incidence, distribution and numbers of certain intestinal protozoa of rats. Amer. Jour. Hyg., *3*, 180–200.
1924. The relations between a carnivorous diet and mammalian infections with intestinal protozoa. Amer. Jour. Hyg., *4*, 393–400.
1925. Intestinal flagellates in Tropical America. Amer. Jour. Trop. Med., *5*, 239–245.

HEGNER, R., AND ANDREWS, J.
1925. Effects of a carnivorous diet on the intestinal pH of rats with reference to flagellates. Amer. Jour. Hyg., *5*, 557–558.

HEGNER, R.
1929. The viability of trichomonad flagellates in milk. Jour. Parasit., *16*, 47–48.
1932. Differential reactions of species and strains of trichomonad flagellates to changes in the environment. Amer. Jour. Hyg., *16*, 513–522.
1933. The effects of a high vegetable protein diet on the trichomonad flagellates of rats. Amer. Jour. Trop. Med., *13*, 535–538.
1934. Effects of environmental changes and disinfectants and antiseptics on *Trichomonas hominis* in culture and in feces. Amer. Jour. Hyg., *19*, 22–37.

NELSON, E. C.
1933. The feeding reactions of *Balantidium coli* from the chimpanzee and pig. Amer. Jour. Hyg., *18*, 185–201.

PRITZE, F.
1928. Beiträge zur Kenntnis des *Balantidium coli*. Ztschr. Parasit., *1*, 345–415.