MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

XII. The Effect of Diet on Host Resistance.

Further Studies.

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In a previous paper we described experiments in which it was shown that mice of the Rockefeller Institute strain, when fed on a McCollum "complete" diet, were far more resistant to *per os* infection with a mouse paratyphoid bacillus than were mice of the same strain fed on the routine bread and milk diet.[1] Since this paper was published, we have had it in mind to analyze the results in order to find out, if possible, which constituents of the McCollum diet were chiefly responsible for the increased resistance to infection. This work has been done during the past year, and we have sought to ascertain (1) the constituents of the McCollum diet which promote resistance to the infection, and (2) whether the observed seasonal fluctuations in resistance to the infection[2,3] are correlated with seasonal changes in diet.

The first experiments were planned to determine (*a*) the effect of the omission of the butter fat from the McCollum diet, and (*b*) the effect of the constituents of that diet, other than butter fat, when added singly to the ordinary bread and milk ration.

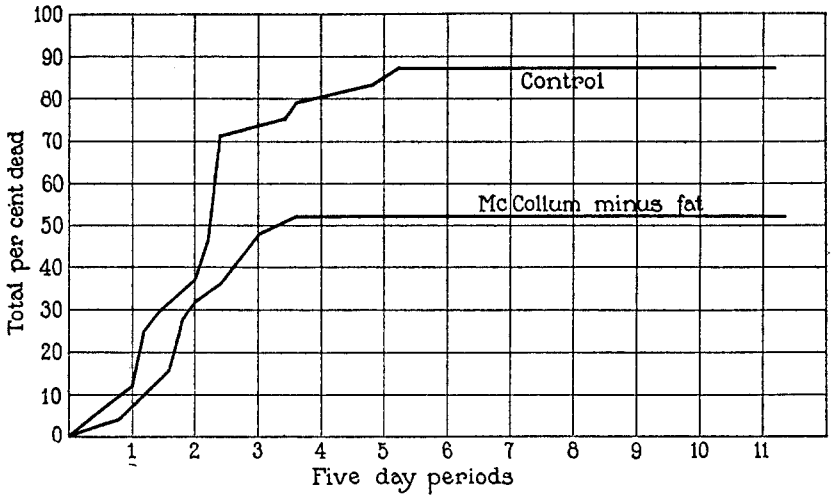
The mice employed were obtained from the Rockefeller Institute breeding room and had been reared on a diet of bread and milk, grain mixture, and dog biscuit,

[1] Webster, L. T., and Pritchett, I. W., *J. Exp. Med.*, 1924, xl, 397.

[2] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 209.

[3] Pritchett, I. W., *J. Exp. Med.*, 1926, xliii, 173.

from the time of weaning until they were 6 to 8 weeks of age. They were then removed to another building and placed in metal cages in groups of 12 and 13. At this time the various modified diet groups were arranged, a control group being maintained on the ordinary bread and milk diet. The mice were given the same care that they received in the breedng room; the cages were kept as clean as possible, uneaten portions of the ration being removed at the end of the day. On the day of inoculation each mouse was placed in a separate battery jar containing clean shavings and received by means of a stomach tube about 5,000,000 bacilli from an 18 hour broth culture of mouse typhoid (*B. pestis caviæ*). Each test was allowed to run 8 weeks.



TEXT-FIG. 1.

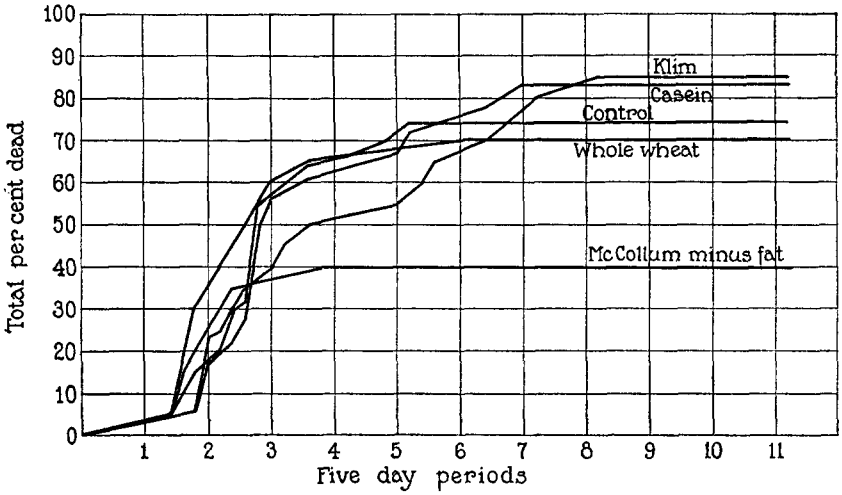
EXPERIMENTAL.

Experiment 1.—Two groups of mice of the Institute strain, one lot of 24 and another of 25, were caged in groups of 12 and 13. One lot, consisting of 25 mice, received, in addition to a constant supply of fresh water, a McCollum diet from which the 5 per cent of butter fat had been omitted, the rest of the formula being the same as that previously employed:

Whole wheat flour	67.5 per cent.
Casein (commercial)	15.0 " "
Milk powder (Klim)	10.0 " "
Sodium chloride	1.0 " "
Calcium carbonate	1.5 " "

The second lot, consisting of 24 mice, was fed on the bread and milk diet and served as controls. After having been on these diets for a period of 10 days, each mouse was given by means of a stomach tube the standard dose of about 5,000,000 mouse typhoid bacilli.

The mortality curves for these tests are given in Text-fig. 1. From them it will be seen that the death rate of the McCollum group is lower than that of the bread and milk group, though not so low as were the rates previously observed when the complete McCollum formula, including the butter fat, was fed.[1]



TEXT-FIG. 2.

Experiment 2.—This test was planned to control Experiment 1 and in addition to try the effect of the various constituents of the McCollum diet, other than butter fat, when added separately to the routine bread and milk diet. The following diet groups were arranged, the substances used in the modified diets being thoroughly mixed with the bread and milk ration before feeding.

A. 20 Institute mice. Bread and milk diet plus 10 per cent powdered whole milk (Klim).
B. 18 Institute mice. Bread and milk diet plus 10 per cent commercial casein.
C. 20 " " " " " " " 10 " " whole wheat flour.
D. 47 " " " " " " only—controls.
E. 20 " " McCollum diet minus butter fat.

The mice were kept as before in small groups in cages. After 2 weeks on the various modified diets, each mouse was placed in a separate battery jar and re-

received *per os* the usual standard dose of mouse typhoid bacilli. The results of this experiment are given in Text-fig. 2.

It will be seen from Text-fig. 2 that the mortality rate in all groups, except the modified McCollum diet group, closely approximated that of the controls. Thus none of the constituents of the McCollum diet that were tested separately in this experiment had been capable of conferring a resistance equal to that of a combination of the same substances, to which was added a salt mixture.

It appeared from this experiment that Klim, casein, and whole wheat flour exerted by themselves little beneficial effect upon the mice. A third experiment was therefore planned to test the effect of the addition of butter fat, the chief remaining constituent of the McCollum diet, to the control bread and milk diet. Cod liver oil, a substance of somewhat similar dietary properties, was also tested.

Experiment 3.—Mice of the Institute strain were brought up from the breeding room on December 1, 1925. The following diet groups were arranged:

A. 22 Institute mice. Bread and milk diet plus 10 per cent butter fat.

B. 22 " " " " " " " 10 " " cod liver oil (Harris).
C. 24 " " " " " " only—controls.
D. 24 " " McCollum diet minus fat.

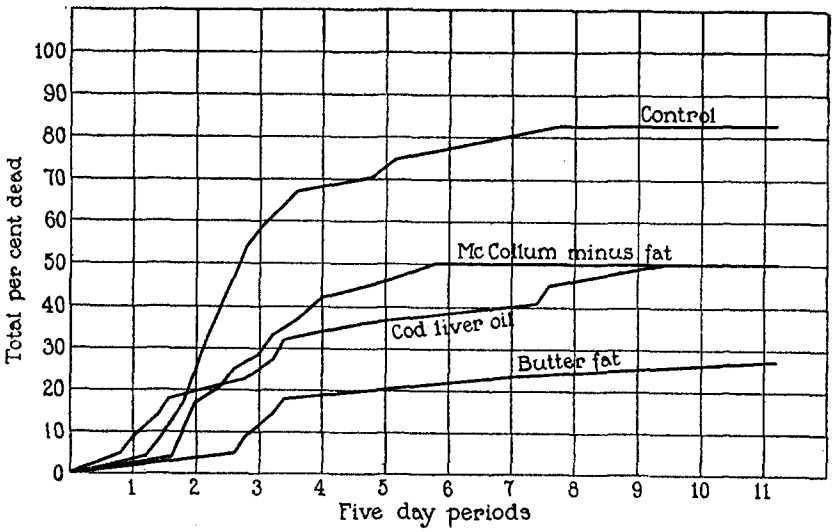
On December 15, each mouse received *per os* the usual standard dose of mouse typhoid bacilli, containing about 4,250,000 organisms. The results of this experiment are given in Text-fig. 3.

It will be seen from Text-fig. 3 that the mortality rates of all the groups on the modified diets were considerably lower than that of the control group. The inference drawn from these experiments is that butter fat is the most important single constituent of the McCollum "complete" diet in raising the resistance of mice of the Institute strain to infection with the mouse typhoid bacillus, and that the same protective action may perhaps be found in cod liver oil.

A series of experiments was now performed in an effort to determine the relative efficacy of various fat-containing substances in protecting our mice against experimental mouse typhoid infection. Keeping in mind the possibility, already mentioned, that observed seasonal fluctuations in resistance might be due in some way to seasonal

changes in diet, we employed for these experiments (*a*) a fat known to vary seasonally in its content of accessory food factors, (*b*) a fat known to be relatively constant in its content of such factors, and (*c*) a fat thought to be free of vitamins. They were represented by the following fats and oils:

1. Butter fat (from Borden's sweet butter). Subject to seasonal fluctuations in its content of fat-soluble vitamin.
2. Cod liver oil (Harris). A concentrated and relatively uniform source of fat-soluble vitamin.
3. Crisco. A hydrogenated vegetable oil thought to be lacking in vitamins.



TEXT-FIG. 3.

In addition two other diets were employed:

4. A bread and milk diet in which the milk had been subjected to the direct light of a small mercury vapor lamp, for 1 hour.
5. McCollum diet minus fat.

Experiments with several of these diets were carried out throughout the first half of the year 1926, from January to June inclusive, since previous studies had indicated that at this time of year our mortality rates were likely to be at their highest.[2,3] The milk employed

TABLE

Experiment started	Diet	Number of mice	Number of mice															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.15.26	Bread and milk + 10% butter fat	25									2	2	1	2	1			1	
	McCollum minus butter fat	24				1		1			3	1	1	1	1			2	
	Bread and milk—control	24						1			3	1	1	4	1	1		1	
2.16.26	Bread and milk + 5% butter fat	20									1	2	2		3				
	Bread and milk + 5% cod liver oil	20								1		2	1						
	Bread and milk + 5% Crisco	20									2		1	1	2			2	
	McCollum minus butter fat	20					1				2	1	1				1		
	Bread and milk—control	18									1	3	2	2	3	1			
3.16.26	Bread and milk + 5% butter fat	30				2		1	1		1	1			2	1	2	1	
	Bread and milk + 5% cod liver oil	29						1	2	2	3								
	Bread and milk + 5% Crisco	29				1			1	4	1	1			1			1	
	Bread and milk—control	31					1				2	1	2	1	4	1			
4.15.26	Bread and milk + 5% butter fat	29						1		1	1	3	2	1	2	1	1		
	Bread and milk + 5% cod liver oil	30						1			3	1	2	1					
	Bread and milk + 5% Crisco	29									1		3			2			
	Bread with rayed milk	30								2	2	2	1	1				1	
	Bread and milk—control	30					2			2	1	2	2	4		1		2	
5.14.26	Bread and milk + 5% butter fat	29							1		2	2		2					
	Bread and milk + 5% cod liver oil	30						2	1	2	1	1	2						
	Bread and milk + 5% Crisco	30					1			2	2	4	2	2	2	2		1	
	Bread with rayed milk	30							1		2	2	1			1			
	Bread and milk—control	30							1	3	1	1	1	1	2	2	1		
6.15.26	Bread and milk + 5% butter fat	30							1	2	1		1	1	1	2		2	
	Bread and milk + 5% cod liver oil	30							1	3	1	1	2	1		1	1		
	Bread and milk + 5% Crisco	29							1	2		1		2	1	1	2		
	Bread with rayed milk	30								2	2	2				2	3		
	Bread and milk—control	30								2	2	2		1		2			1
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17

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dying each day																																		Total dead	
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	Number	Per cent
							1														1	1																12	48
																																							11	46
2		1		1		1							1																										21	87
																																							8	40
			2																																				8	40
	1					1																																	10	50
1																																							8	40
		1																	1																				14	78
		1				1																																	14	47
	1		1				1																										1						12	41
2			1																																1				15	52
						1				1								1																					16	52
								1							1														1						1				17	59
		1			1	1			1														1	1	1													16	53
		1																				1																9	31
1					1							1						1																					13	43
2																																							19	63
				1	1	1																		1				1											12	41
					1																							1											11	37
	2				1																	1	1																23	77
	1								1	1		1				1	1											1											14	47
1	1			1								1									1	1																	19	63
			1			1					1						1		1					1															17	57
																		1			1					1													14	47
															1				1							1					1				1	1			16	55
	1	1																	1																			14	47
		1	1									1					1		1			1								1								17	57
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56		

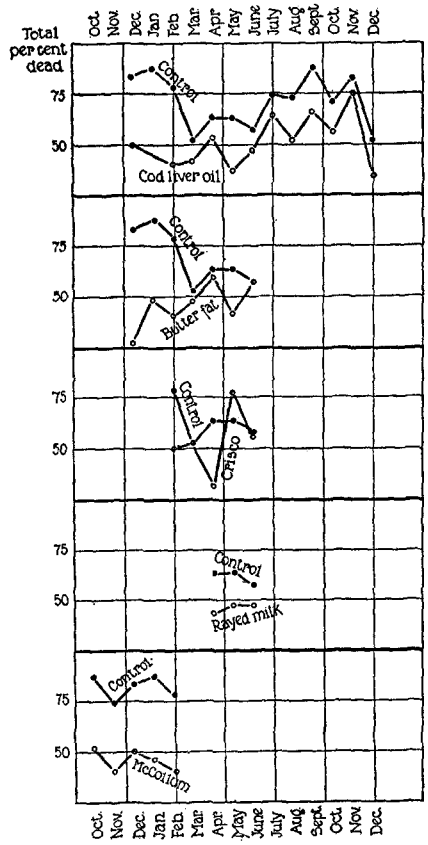
TABLE I

Experiment started	Diet	Number of mice	Number of mice															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
7.27.26	Bread and milk + 5% cod liver oil.....	50							1	2	2	1	3	3	1	1	2	3	
	Bread and milk—control.....	50							1	2	2	2	2	1	3	1		3	1
8.27.26	Bread and milk + 5% cod liver oil.....	50								1	3		4	1	5	1			?
	Bread and milk—control.....	48						1	2	2	1	2	3	1	6	3			
9.22.26	Bread and milk + 5% cod liver oil.....	50						1		4	1	5	2	3	2				
	Bread and milk—control.....	50						2	1	1	3	7		16	3		1	1	
10.20.26	Bread and milk + 5% cod liver oil.....	48						1	2		5	3		1	4		2	1	
	Bread and milk—control.....	48					2	1			1		1	2	5		3		
11.16.26	Bread and milk + 5% cod liver oil.....	48							1	2	3	2	1	2	7	6			
	Bread and milk—control.....	49						3	1		3	2	5	4	15	3	2		1
12.15.26	Bread and milk + 5% cod liver oil.....	50								1		2	2			3		1	
	Bread and milk—control.....	50									3	1	7	1	1	1	2	1	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17

—*Concluded.*

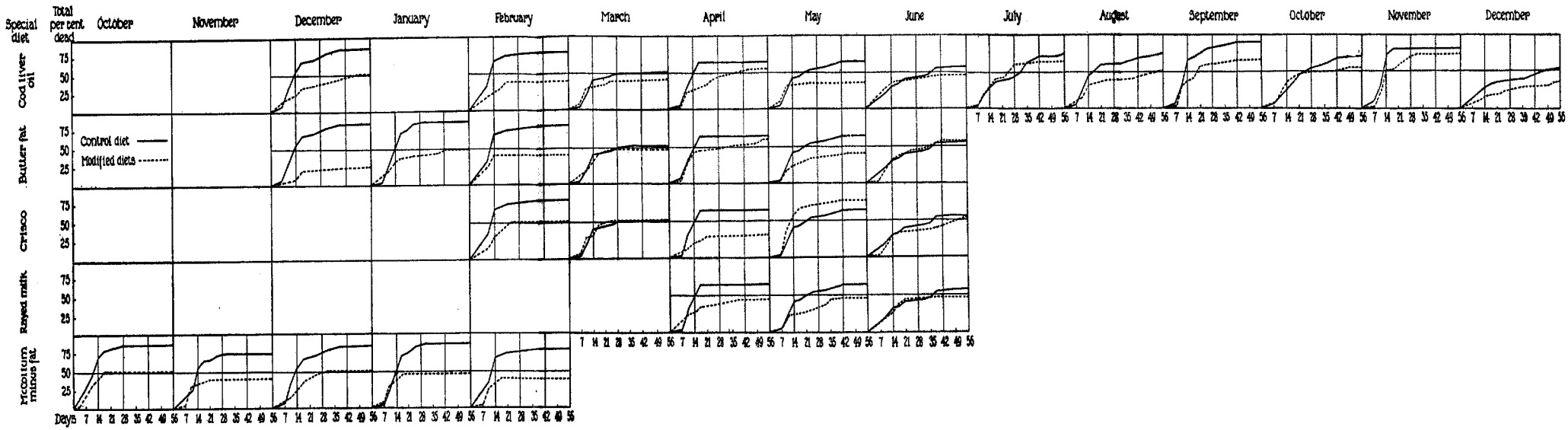
dying each day																																			Total dead	
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	Number	Per cent
1	1				2	1	1	1		3							1						1	1															32	64
				1		1		2			2	1				6						1	3											1			1		37	74
	1					1													1					2		1				1					1		1	26	52
		7				1							1										1	2						1							1	35	73
3	3	1	3				1			1	1										1		1															33	66
	1	2				1	1					2							1				1															44	88
	3					1		1														1				1				1								27	56
1	4	1	1					2	1	1					3							2	2								1							34	71
		2	1	1	1	1			1	2			1		1																							36	75
																					1																	40	82
	1			1			1								2														2		1							17	34
	1											1					1										4	1							1			26	52
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56		

for Diet 4 above was identical with that used for the control bread and milk diet except that it had been exposed for 1 hour, in a shallow glass vessel, at a distance of 4 or 5 inches, to the light of a small mercury vapor lamp, and was thoroughly stirred with a pipette at the end of the first half-hour. From the month of March on the modified Mc-



TEXT-FIG. 4.

Collum diet (minus butter fat) was abandoned, and only the bread and milk control diet together with its fat and rayed milk variants was employed. This was done in an effort to simplify the problem as much as possible and to permit the use of larger numbers of mice in each diet group. From February on only 5 per cent of fat was used in the



TEXT-FIG. 5.

modified diets, instead of the 10 per cent employed in the earlier experiments. This was done in order that the fat content of the modified bread and milk diets might be more nearly comparable to that of the whole McCollum diet, the original formula for which, as given us by Dr. E. V. McCollum, called for 5 per cent of butter fat. The results of all these experiments are condensed in Table I and in Text-figs. 4 and 5.

Text-fig. 4 shows the monthly mortality rate for each of the modified diet groups, and for the corresponding control groups, from October, 1925, to December, 1926, inclusive, thereby including all the experiments recorded in this paper. The mortality rate for any one month represents the total mortality of the mice used in the experiment begun in that month, all experiments being started on or near the 15th. Although the points on this chart are discontinuous, related points have been roughly connected into curves, in order to enable the reader to compare more easily the mortality rates of the mice on the various modified diets with the rate of the controls on the plain bread and milk diet. The total per cent mortality is indicated along the ordinate, while the successive months are arranged along the abscissa. In each division of the chart appear two curves—that of the diet group indicated, together with that of the control group for the same period. In October and November only the McCollum diet minus fat was used, in addition to the control bread and milk diet. From December on, several fat diets were included in the experiment and for each one a separate curve is plotted, in each case accompanied by the corresponding portion of the control curve. These diets were continued through June, 1926, after which time only the cod liver oil and the control diet groups were employed.

It will be seen that, almost without exception, the total mortality rate of the control group was higher than that of the groups on the various modified diets. The results presented here are somewhat fragmentary, since only the cod liver oil and control series were carried on throughout the year. Nevertheless, within the longer or shorter periods covered, the effects of all the modified diet groups, save one, were consistent. In every case except that of the group receiving the bread and milk diet plus 5 per cent Crisco, the mortality rate is consistently lower than in the control group on the unmodified bread and milk diet. They follow with considerable regularity the fluctuations

in mortality of the control group, a tendency to stabilization of the death rate at a level lower than that of the controls being indicated. The mortality rate of the Crisco series, on the other hand, was three times lower than, once higher than, and once equal to that of the control series.

Text-fig. 5, like Text-fig. 4, is compiled from the data given in Table I, and shows the separate mortality curves of each of the monthly diet experiments. In each division of the chart appear two curves, plotted as total mortality against time—that of the diet group indicated along the left margin of the chart together with the corresponding control curve. The curve for the control group in any one month is therefore repeated as many times as there were modified diet groups in the experiment for that month. In this chart it is easy to compare the separate diet curves throughout their course with the corresponding control curves. It will be seen that for the most part the various diet curves exhibit considerable similarity in their tendency to approximate or diverge from the corresponding control curve in any given month. In December, January, and February, the final mortality rate of all the modified diet groups was considerably below that of the controls. In March, however, the mortality rate in the control group dropped and closely approached the level usually attained by the groups on the modified diets. In April and May, the death rate in the control group increased somewhat and again diverged from the level usually attained by the modified diet groups; the only decided exception is in the May group receiving the modified diet plus Crisco, which showed a mortality rate above that of the May control group. In June, the mortality rate in the control group again dropped and approximated the level maintained, with fair regularity, by the modified diet groups.

In the period from July to December inclusive, only the control diet and the cod liver oil diet were employed, 50 mice being included monthly in each diet group. In July, both curves were surprisingly high for this time of year and closely approximated each other. Thereafter they showed the customary divergence, except in the month of November, when both were again high.

It seems to be clearly indicated by these experiments that butter fat is the most important single constituent of the McCollum diet in

protecting the mice against *per os* infection with the bacillus of mouse typhoid. The degree of protection afforded by butter fat, when added to the control diet, was about equal to that of all the other constituents of the McCollum diet combined, and was closely approximated by that of cod liver oil.

While seasonal fluctuations in resistance were not completely eliminated by the various modified diets, they were reduced. There was apparently a tendency for the various modified diets to stabilize the death rate at a point lower than that usually reached by the mice on the control diet.

DISCUSSION.

Recently a paper has appeared by Vaile[4] on the correlation between fat consumption and susceptibility to tuberculosis. Through a period of 7 years' country practice in England, he studied various families in which cases of tuberculosis had occurred, dividing up their members into two groups according to whether they were or were not normal fat eaters. He concluded that "when several persons are equally exposed to infection [with tuberculosis], those who habitually eat animal fat will probably escape—but may not, while those who avoid fat may escape—but probably will not." He could not see that forcing fat consumption above the level reached by the normal fat eater was profitable.

So far as I am aware, most of the published experiments on the effect of diet upon susceptibility to disease are concerned primarily with diets in which one or more of the accessory food substances is markedly deficient or wholly lacking. In the experiments here recorded, this was not the case. The "control" diet employed here was one which had been shown, through a period of years, to be adequate for the breeding and rearing of successive generations of mice, apparently without any loss of vigor. Though somewhat fragmentary, the experiments here recorded do suggest, however, that the addition of 5 per cent of an active animal fat to an apparently adequate diet can so improve its quality as to increase the resistance of mice fed on such a modified diet to *per os* infection with the bacillus of mouse typhoid. The reason for the beneficial effect of these modifications of our bread

[4] Vaile, W. B., *Lancet*, 1927, ccxii, 72.

and milk diet, aside from the probable promotion of better general health, is not known. The bread and milk food may be one of the borderline diets referred to by Cramer,[5] who observes that the deficiencies of an apparently adequate diet may manifest themselves only when the animal is subjected to a strain. It is difficult to say whether these deficiencies could be made good in a scant 2 weeks' feeding on the modified diets, before inoculation with the bacillus of mouse typhoid. The consistency of the results would seem to indicate, however, that the apparently increased resistance of the mice on the modified bread and milk diets containing butter fat and cod liver oil, as well as those on the modified McCollum diet, was not due to chance.

SUMMARY.

When 5 per cent of butter fat or cod liver oil is added to a bread and milk diet, in itself adequate to promote the breeding and rearing of a healthy stock of mice through a period of years, the resistance of these mice to *per os* infection with the paratyphoid mouse typhoid bacillus (*B. pestis caviæ*), as compared to that of mice on the unmodified diet, is definitely increased. A similar effect may be obtained with a McCollum "complete" diet, even when the butter fat is omitted, and with a bread and milk diet in which the milk used has been rayed with a mercury vapor lamp. When an inactive fat like Crisco is added to the bread and milk diet, the results obtained are not very clear-cut. While seasonal fluctuations in resistance to mouse typhoid were not completely eliminated by the various modified diets, they were nevertheless reduced, the modified diets tending to stabilize the death rate at a point lower than that usually reached by the mice on the control diet.

[5] Cramer, W., *Brit. J. Exp. Path.*, 1922, iii, 298.