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Increased Resistance of Rachitic Rats Fed Irradiated Food.

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(Introduced by F. F. Tisdall.)

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A number of albino rats 24 to 27 days old were separated into 2 groups, whenever possible by dividing litters, each week. The diet of the breeding rats had previously been regulated so that there was no excess of vitamin D. Group 1 was fed a modification of McCollum's¹ rachitogenic diet 3143 in which the usual 33% of whole wheat was replaced by 33% of non-irradiated muffets, which are made of whole wheat. The rats of this group were usually slightly heavier (1 to 2 gm.) at the outset than the rats in group 2. Group 2 was fed a diet identical in every respect except that the muffets had been irradiated. After 3 weeks on this diet the average weight of the group 1 rats (non-irradiated food) was 44 gm., and of the group 2 rats (irradiated food) 46 gm. In an individual experiment the variations in weight in the two groups were often the same. After the 3 weeks on the diet the rats were starved for 6 hours, then

TABLE I.

Group 1 (Non-irradiated Muffets)					Group 2 (Irradiated Muffets)		
Exp.	Infecting Dose of Broth Culture	No. of Rats	No. of Survivors	Ave. lgth. of life of rats dying	No. of Rats	No. of Survivors	Ave. lgth. of life of rats dying
	cc.			days			days
1	0.5	3	0	8.3	2	2	—
2	0.5	7	0	7.6	4	3	5.0
3	0.4	3	0	7.0	1	1	—
4	0.5	6	0	9.1	8	2	8.5
5	0.1-0.3	14	0	7.2	14	2	9.3
6*	0.4	6	0	7.0	9	3	12.0
7*	0.6	10	0	9.5	10	1	12.5
8*	0.4	5	0	8.4	4	1	7.0
9	0.1	6	1	9.0	7	2	8.4
10	0.05	7	2	7.6	13	8	19.2
11	0.05	10	0	10.9	13	2	10.2
12	0.05	12	1	8.9	9	1	13.5
Total		89	4	8.6	94	28	11.1

* Were given 30% muffets and 6% yeast.

¹ McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, xlvii, 507.

weighed and put in individual cages. Every rat in a single experiment was then fed the same small amount (see Table I, column 2) of an 18 hours' broth culture of *Salmonella muriotitis* on a small piece of dried bread. After the bread was eaten the previous diet was restored. This salmonella strain which was isolated from albino rats by McCordock,² can not be differentiated from *S. enteriditis* (*B. enteriditis*) by its biological reactions, but serologically it appears to be distinct. *S. enteriditis* is known to cause epidemics of severe and often fatal diarrhea in rats. The rats, therefore, were being infected with a rat disease by the most natural route (*per os*). The stock strain was subcultured weekly on plain agar slants and there was no evidence of decreasing virulence.

Death usually took place in 6 to 10 days, although occasionally a rat lived as long as 21 days. Cyanosis, blood stained discharge from the nose and eyes, weakness and diarrhea usually accompany the infection and convulsions frequently precede death. Of the rats which died, the group 1 rats (non-irradiated food) usually lived a shorter time than the group 2 rats (irradiated food), see Table I, columns 5 and 8. Post mortem examinations were made on all the dead rats and cultures were always made from the heart's blood and occasionally from the spleen. Cultures were identified as *S. muriotitis* if they were Lactose—, Dextrose+, and Saccharose— and if they were agglutinated in dilutions of 1:1000 or over with *S. muriotitis* immune serum.

Of 85 non-irradiated food rats which died, 80 yielded cultures of *S. muriotitis* from their heart's blood (mostly pure). Of the 66 irradiated food rats which died, 56 showed similar cultures. All spleen cultures yielded *S. muriotitis*. Practically all of the dead showed blood in the pyloric portion of the stomach and in the small intestine. Macroscopic ulcers were seen in the pyloric part of the stomach of 5 rats. Necrotic and hemorrhagic areas in the gastric and intestinal mucosa and in the liver were shown on microscopic section.

Survivors. Twenty-eight of the rats fed the irradiated food (group 2) withstood the infection as compared with 4 fed the non-irradiated food (group 1), see Table I, columns 7 and 4. The survivors were allowed to live 28 days, when they were killed with ether and examined. All of these heart's blood cultures were sterile.

Controls. As *S. enteriditis* is not infrequently found in normal rats, blood cultures were made on a series of rachitic rats. These

² McCordock, H. A., *Bull. Johns Hop. Hosp.*, 1925, xxxvii, 412.

animals were kept in a separate animal room and had no contact with our infected rats. Twelve blood cultures have been made up to the present and these have all been sterile. More of these controls will be examined.

Fecal cultures were made on 47 of the rats used in the resistance experiments the day before they were infected, and only 3 of these showed *S. muritidis*. Consequently it is thought that the recovery of *S. muritidis* from the blood of a dead rat, after it has been fed this organism suggests very strongly that the rat died of a murio-titis infection and it is not likely that the disease originated from the rat itself.

Two typical rats from each group were set aside for chemical controls, and were fed a small amount of sterile broth, instead of the culture, on a similar piece of bread. After a week in the individual cages, that is after 4 weeks of the diet, the whole blood phosphorus was determined by the method described by Tisdall³ and the bone ash percentage by that of Bethke, Steenbock, and Nelson.⁴ The results are shown in Table II.

TABLE II.

Group 1 (Non-irradiated Muffets)				Group 2 (Irradiated Muffets)		
Exp.	Blood Phos-phorus mgm. per 100 cc.	Bone Ash %	X-ray Showing Rickets	Blood Phos-phorus mgm. per 100 cc.	Bone Ash %	X-ray Showing
4	—	—	—	1.5	41.1	—
5	2.5	31.9	marked	3.7	42.6	no rickets
6	2.5	37.3	"	3.6	46.1	" "
7	1.4	37.5	moderate	3.1	49.3	" "
8	—	36.0	"	—	—	—
9	2.5	31.7	marked	2.4	42.2	slight rickets
10	1.4	39.8	"	3.2	47.9	no rickets
11	3.1	27.7	"	3.6	40.0	" "
12	2.8	37.4	moderate	3.5	48.8	slight rickets

The resistance-raising properties of cod liver oil, irradiated ergosterol and sunlight through vita glass are being investigated. Direct sunshine has been previously shown⁵ to raise the resistance of rachitic rats.

Summary. Of 89 rats fed a rachitic diet including non-irradiated muffets, 5% survived a *per os* enteriditis infection, as compared with 30% of 94 rats fed the same diet including irradiated muf-

³ Tisdall, F. F., *J. Biol. Chem.*, 1922, 1, 329.

⁴ Bethke, R. M., Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1923, lviii, 71.

⁵ Robertson, E. C., *Am. J. Hyg.*, 1929, ix, 75.

fets. Therefore we conclude that the use of irradiated whole wheat (muffets) in a rachitogenic diet fed to rats increases their resistance against infection.

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Experimental Edema—Further Observations on the Plasma Proteins and Blood Cholesterol.

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Since the first report, in 1928,¹ of the experimental production of edema in dogs by the method of plasmapheresis, further work has been carried out, the results of which are given in this preliminary report.

Edema has been produced in about 40 dogs, often several times in the same dog, whenever the plasma protein level has been maintained at about 3 gm. % or below. Above this level, edema disappeared promptly. There has been essentially no exception among the dogs used.

Several factors have been controlled. *Cardiac damage* has been ruled out by bleeding a series of dogs from the external jugular veins. *Starvation* alone, or with the daily administration of 1500 cc. of saline by stomach tube, gives rise to no significant change in the concentration of the plasma proteins nor to visible edema. *The alkalinity of the modified Locke's solution* used as a suspending medium for the erythrocytes injected into the dog plays no important rôle.

The *regeneration of the plasma globulin is much more rapid than that of the plasma albumin* and accounts for most of the sharp rise in the total protein when plasmapheresis is discontinued. This is shown best in the long experiments in which the plasma albumin level remains at about 1 or 1½% with relatively slight fluctuations up or down, while the plasma globulin curve shows striking variations depending upon whether or not plasma depletion is being carried out. The total protein curve parallels closely that for globulin. These results confirm the earlier work of Kerr, Hurwitz and Whipple.² It is easy to see, therefore, why the albumin/globulin ratio is

¹ Leiter, L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 173.

² Kerr, W. J., Hurwitz, J. H., and Whipple, G. H., *Am. J. Physiol.*, 1918, xlvii, 356.