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Increased Resistance of Rachitic Rats Exposed to Sunlight Through Vita Glass.*

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(Introduced by F. F. Tisdall.)

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Each week albino rats, 4 weeks old, were separated as evenly as possible into 2 groups. The diet of the breeding rats had previously been regulated, so that it contained no excess of vitamin D. The young rats were put on Steenbock's rachitogenic diet 2965.[1] One group was exposed to the sun under plain or ordinary window glass, and the other under Vita glass of the same thickness, for 2 hours daily, except Sunday, from 11 A. M. to 1 P. M. for 4 weeks. Groups of rats were started once a week during May and again in the last 2 weeks of August and the first week in September. For their daily exposures, the rats were put out on the roof of a 5 story building in round wire cages covered by a square glass box, open at the 2 ends. An extra empty ordinary glass box was fitted closely at one end and a wooden cover over an electric fan at the other, so that the rats in the center cages were protected from any unrefracted sunlight, although when the fan was turned on, there was a good current of air over the animals. At first the rats were put out under a box covered on all sides except the bottom with glass, with no provision for the circulation of air, but on a warm day many of them died after 40 minutes' exposure to the sun. After the fans were installed this trouble was not encountered.

After 4 weeks, 4 rats were killed in each experimental lot (2 plain glass and 2 Vita glass) to determine the degree of rickets. The blood phosphorus was estimated by the method described by Tisdall,[2] and the bone ash percentage by that of Bethke, Steenbock and Nelson.[3] The results are shown in Table I. The Vita glass prevented the development of rickets.

The remaining rats were starved for about 20 hours, then weighed and put in individual cages kept on wide wooden shelves, so that

* For the details of the technique of these experiments see the earlier paper by the same authors, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 999.

1 Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

2 Tisdall, F. F., *J. Biol. Chem.*, 1922, **50**, 329.

3 Bethke, R. M., Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1923, **58**, 71.

there was no possibility of any infected feces dropping into the cages below. The floors of the cages were sterilized 3 times a week. There was considerable variation in the weights of the rats in both groups, but those exposed under Vita glass weighed on the average slightly more (64 gm.) than those exposed under plain glass (59 gm.) by this time. Every rat was then given 1/20 of a cc. of an 18 hour culture of *Salmonella muriotitis* on a small piece of dried bread, except in the 2 experiments noted in Table II. After they were infected the exposures to sunshine were continued for another 4 weeks.

TABLE I.

Exp.	Plain Glass			Vita Glass		
	Blood P. mgm. per 100 cc.	Bone Ash %	X-ray Showing Rickets	Blood P. mgm. per 100 cc.	Bone Ash %	X-ray Showing
1	—	—	—	4.2	53.2	No rickets
2	1.6	24.3	Marked	3.0	40.1	Slight rickets
3	4.8	24.7	"	5.7	44.8	No rickets
4	3.2	31.4	Moderate	5.7	38.9	Slight rickets
5	1.7	30.9	Marked	—	44.3	No rickets
6	—	42.3	Moderate	4.0	50.6	" "
7	—	37.0	Slight	—	42.1	" "

TABLE II.

Exp.	Exposure to Sun Commenced	Plain Glass		Vita Glass	
		No. of Rats	No. of Survivors	No. of Rats	No. of Survivors
1*	May 5	2	1	5	3
2	May 12	6	2	4	3
3	May 19	7	4	8	7
4†	May 27	6	0	7	2
5	Aug. 13	4	0	6	3
6	Aug. 29	5	2	8	4
7	Sept. 4	2	0	3	3
Total		32	9	41	25

* Dose—0.01 cc. † Dose—0.1 cc.

As shown in Table II, considerably more of the rats exposed under Vita glass survived the infection; 25 of 41 such rats or 61% survived. Of those exposed under plain glass, only 9 of 32 or 28% survived. The survivors all lived at least 28 days after eating the infected bread. All of the rats which died yielded *S. muriotitis* from the heart's blood at post mortem, and showed characteristic gross pathological lesions. Only 3 of the rats which died lived more than 16 days after the infection.

Blood cultures were made on 50 rachitic rats which had not been infected, and only 2 of these showed *S. muriotitis*. Of 84 fecal cultures on similar rats 3 yielded *S. muriotitis*. In 9 rats the fecal cultures were repeated 3 times at intervals of a few days and were uniformly negative. A series of rachitic rats (McCollum's diet 3143[4] for 4 weeks) were fed ½ cc. of the *S. muriotitis* culture and killed at daily intervals. The organism was recovered from the mesenteric glands, liver and spleen in 48 hours and from the blood stream in 5 days.

Using the results in Tables I and II, it appears that the difference in the survival rate between the Vita and the plain glass rats in each experiment corresponds roughly with the difference in the bone ash between the corresponding rats. The difference in the bone ash indicates the difference in the degree of rickets. In other words, it seems probable that the degree of resistance varies approximately with the degree of rickets present.

Summary.—Of 41 rats fed a rachitic diet and exposed to sunshine through Vita glass, 61% survived a *per os* enteriditis infection, as compared with 28% of 32 similar rats exposed to sunshine through plain or ordinary glass.

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Effects of Ethyl Alcohol on Red Blood Cells.

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The observations present the microscopic changes occurring in human red blood cells when subjected to different dilutions of alcohol in saline. Approximately 50 specimens of blood from various patients were studied and the results obtained conform as a whole. Studies were made by the hanging drop method. Cells deposited in the serum of clotted blood were examined within 4 hours after withdrawal and whole blood was examined immediately following withdrawal. The red cells were diluted so that the suspension contained approximately 4000 per cu. mm.

When the red blood cells were found to be in rouleux formation the alcohol dilutions overcame this formation more quickly than

[4] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, **47**, 507.